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Optimisation of chemoenzymatic processes in asymmetric synthesis

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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Rebecca E. Deasy, B.Sc.

A thesis presented for the degree of

Doctor of Philosophy

to

THE NATIONAL UNIVERSITY OF IRELAND, CORK

Department of Chemistry

University College Cork

Supervisor: Prof. Anita R. Maguire

Head of Department: Prof. Michael Morris

September 2012

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Rebecca Deasy
September 2012

Abstract

This thesis describes the optimisation of chemoenzymatic methods in asymmetric synthesis. Modern synthetic organic chemistry has experienced an enormous growth in biocatalytic methodologies; enzymatic transformations and whole cell bioconversions have become generally accepted synthetic tools for asymmetric synthesis. Biocatalysts are exceptional catalysts, combining broad substrate scope with high regio-, enantio- and chemoselectivities enabling the resolution of organic substrates with superb efficiency and selectivity. In this study three biocatalytic applications in enantioselective synthesis were explored and perhaps the most significant outcome of this work is the excellent enantioselectivity achieved through optimisation of reaction conditions improving the synthetic utility of the biotransformations.

In the first chapter a summary of literature discussing the stereochemical control of baker's yeast (*Saccharomyces Cerevisae*) mediated reduction of ketones by the introduction of sulfur moieties is presented, and sets the work of Chapter 2 in context.

The focus of the second chapter was the synthesis and biocatalytic resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone. For the first time the practical limitations of this resolution have been addressed providing synthetically useful quantities of enantiopure synthons for application in the total synthesis of both enantiomers of 4-methyloctanoic acid, the aggregation pheromone of the rhinoceros beetles of the genus *Oryctes*. The unique aspect of this enantioselective synthesis was the overall regio- and enantioselective introduction of the methyl group to the octanoic acid chain. This work is part of an ongoing research programme in our group focussed on baker's yeast mediated kinetic resolution of 2-keto sulfones.

The third chapter describes hydrolase-catalysed kinetic resolutions leading to a series of 3-aryl alkanoic acids. Hydrolysis of the ethyl esters with a series of hydrolases was undertaken to identify biocatalysts that yield the corresponding acids in highly enantioenriched form. Contrary to literature reports where a complete disappearance of efficiency and, accordingly enantioselection, was described upon kinetic resolution of sterically demanding 3-arylalkanoic acids, the highest reported enantiopurities of these acids was achieved (up to >98% ee) in this study through optimisation of reaction conditions. Steric and electronic effects on the efficiency and enantioselectivity of the biocatalytic transformation were also explored. Furthermore, a novel approach to determine the absolute stereochemistry of the enantiopure 3-aryl alkanoic acids was investigated through combination of co-crystallisation and X-ray diffraction linked with chiral HPLC analysis.

The fourth chapter was focused on the development of a biocatalytic protocol for the asymmetric Henry reaction. Efficient kinetic resolution in hydrolase-mediated transesterification of *cis*- and *trans*- β-nitrocyclohexanol derivatives was achieved. Combination of a base-catalysed intramolecular Henry reaction coupled with the hydrolase-mediated kinetic resolution with the view to selective acetylation of a single stereoisomer was investigated. While dynamic kinetic resolution in the intramolecular Henry was not achieved, significant progress in each of the individual elements was made and significantly the feasibility of this process has been demonstrated.

The final chapter contains the full experimental details, including spectroscopic and analytical data of all compounds synthesised in this project, while details of chiral HPLC analysis are included in the appendix. The data for the crystal structures are contained in the attached CD.

To Mum and Dad

Contents

Chapter 1

Introduction.....1

Chapter 2

Baker's yeast mediated asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-**1** and (*S*)-**1**.....43

Chapter 3

Hydrolase-mediated kinetic resolutions of 3-arylalkanoic acids.....75

Chapter 4

Dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis.....137

Chapter 5

Experimental.....253

Appendices

Chiral HPLC data.....*ii*
List of abbreviations.....*xviii*
Publications.....*xx*

Chapter 1

Introduction

Contents

1.1 Introduction **4**
1.2 Baker's yeast mediated reduction of sulfur-containing compounds **6**
1.2.1 Baker's yeast mediated reduction of ketosulfides **7**
1.2.1.1 Reduction of β-ketoesters **7**
1.2.1.2 Reduction of ketones **11**
1.2.1.3 Reduction of dicarbonyls **15**
1.2.1.4 Reduction of olefins **17**
1.2.2 Baker's yeast mediated reduction of ketones bearing dithioacetals **20**
1.2.3 Baker's yeast mediated reduction of thiocarbonyls **23**
1.2.4 Baker's yeast mediated reduction of ketosulfoxides **24**
1.2.4.1 Asymmetric oxidation of sulfides by baker's yeast **27**
1.2.5 Baker's yeast mediated reduction of β-ketosulfones **28**
1.3 Conclusion **36**
1.4 References **37**

1.1 Introduction

The importance of ready access to biologically active pharmaceutical compounds in enantiomerically pure form is widely accepted, primarily due to the recognition that chirality plays a crucial role in potency, efficiacy and safety. In 2006, it was estimated that chiral compounds comprised more than half of drugs approved worldwide.[1] Furthermore, five of the six top-selling drugs in 2007, Lipitor®, Plavix®, Nexium®, Diovan® and Advair® were sold as single enantiomers.[2] While the predominant source of enantiopurity in the pharmaceutical industry resides in the starting material/intermediate purchased, alternative approaches of in-house generation of chirality exist, namely; resolution, employment of chiral auxiliaries as temporary tethers and asymmetric catalysis.[1] The latter process involves the application of enantiopure catalysts to transform prochiral and racemic substrates into enantiomerically enriched products. Two of the main strategies employed for catalytic enantioselective synthesis are biotransformations and transition metal catalysis. Biocatalytic methods in particular have been widely developed due to enzymes' intrinsic regio- and enantioselectivity, high catalytic activity and ability to function under mild reaction conditions.[3]

One of the most widely studied and commercially significant whole cell systems employed in biocatalysis is baker's yeast (*Saccharomyces cerevisiae*), which has been extensively utilised in the asymmetric reduction of a wide variety of ketones and carbon-carbon double bonds. This is largely due to the ready availability, ease of experimental procedures (especially for non-experts in microbiological techniques), and versatility of this microorganism.[4-8] Alcohol dehydrogenases (ADHs) have been identified to be the key enzymes responsible for catalytic activity in the reduction of the carbonyl functional group.[9] However, these enzymes require a nicotinamide cofactor, NAD(P)H, from which a hydride is transferred to the substrate carbonyl carbon. The major advantage of such whole cell systems is that *in situ* regeneration of the coenzyme is achieved *via* the metabolic pathways of the microorganism once an oxidisable co-substrate (generally glucose or ethanol) is provided (Figure 1.1).

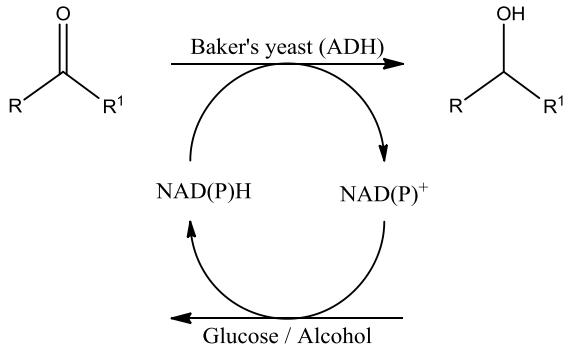


Figure 1.1

Stereoselectivity in reductions with baker's yeast is governed by the geometry of hydride addition, and is generally in accordance with Prelog's rule, with approach of the hydride from the *re*-face of the prochiral carbonyl to give the (*S*)-enantiomer of the alcohol (Figure 1.2).[10] Several exceptions have been found, and this can be due to the presence of competing dehydrogenases that interfere with or even dominate the desired transformation, as each cell contains an array of varied yeast reductases with conflicting stereoselectivities. More recent publications predominantly describe the use of genetically engineered

microorganisms with over-expressed enzymes for asymmetric reductions.[11-15] Also, isolated and purified enzymes are often employed to catalyse specific transformations, generally without the complication of competing reactions or tedious product recovery.[16] Nevertheless, baker's yeast is still a viable biocatalyst for the large-scale asymmetric reduction of ketones in the production of simple chiral building blocks. The overall economics of baker's yeast mediated reduction may well be competitive because not only is the biocatalyst less expensive and possesses a wide substrate range, but the enzymes within the cell are also more stable than single isolated and purified enzymes.[17] Significantly, in resolutions where the enzymes may require addition of exogenous enzyme co-factors, for example alcohol dehydrogenases, the natural regeneration of co-factors such as the nicotinamide cofactor [NAD(P)H] in the whole cell is a major advantage which should not be underestimated.[17]

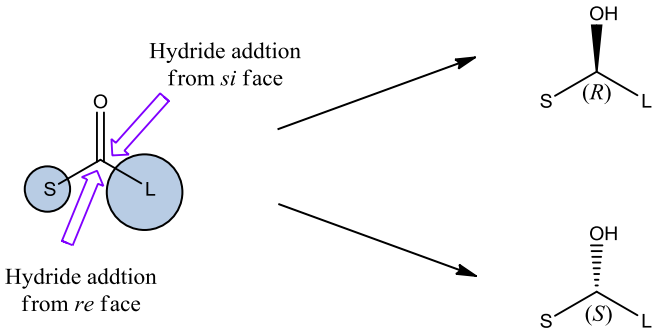


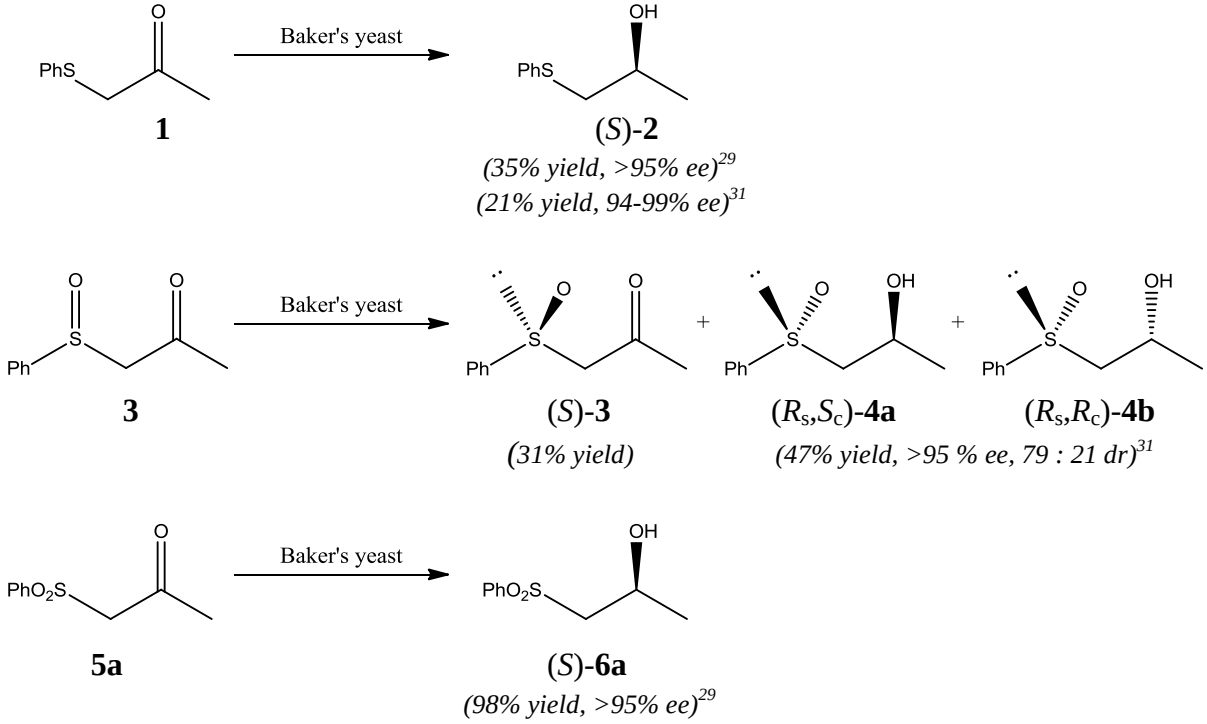
Figure 1.2

A number of strategies have been developed to improve the enantioselectivity of baker's yeast, namely: action of irreversible enzyme inhibitors,[17-20] addition of organic solvents,[21] cell immobilisation,[22-24] chemical modification of the substrate,[25] pre-treatment of the cellular mass[26] and addition of sulfur compounds to the reaction mixture.[27] Of these parameters, stereochemical control in the baker's yeast reduction of ketones by the introduction of sulfur functional groups has attracted considerable attention. Sulfides, sulfoxides and sulfones not only enhance the stereochemical course of the bioreduction by differentiating the steric bulk of the two substituents on the carbonyl group, but also possess great synthetic utility and may be employed as chiral building blocks for natural product synthesis *via* functional group transformation and carbon-carbon bond formation.[28]

The objective of this review is to provide an accurate summary of all developments to date of stereocontrol in baker's yeast reductions by introduction of sulfur-based functionality. This subject was previously extensively reviewed 22 years ago[28] and also has been partially covered in other reviews dealing with general baker's yeast mediated transformations in the preparation of enantiomerically pure chiral building blocks.[3,4,6,7]

1.2 Baker's yeast mediated reduction of sulfur-containing compounds

Pioneering work in the late 70's initiated by Ridley *et al*. reported that baker's yeast mediated reduction of α-sulfur functionalised acetone derivatives is critically dependent upon the substituents attached to the sulfur-containing group and to the carbonyl group.[29,30] In cases where these substituents are bulky, very little reduction occurs. The relative ease of reduction increases from β-ketosulfide to β-ketosulfoxide to β-ketosulfone. Thus, in the case of the β-ketosulfides, reduction of 1-(phenylthio)propan-2-one **1** proceeds with relative difficulty, generating the corresponding (*S*)-1-(phenylthio)propan-2-ol (*S*)-**2** with high optical purity (≥94%) but poor yield (21-35%).[29,31] Reduction of racemic (±)-1-(phenylsulfinyl)propan-2-one (±)-**3** results in kinetic resolution, affording the untransformed optically active ketone (*S*)-**3** and a mixture of diastereomeric alcohols (R_s,S_c)-**4a** and (R_s,R_c)-**4b**; the ratio of **3** to **4a**/**4b** is dependent on the supplied sucrose concentration.[31] The corresponding β-ketosulfone, 1-(phenylsulfonyl)propan-2-one **5a** is reduced to the (*S*)-alcohol (*S*)-**6a** in 98% yield with high optical purity (>95 % ee) (Scheme 1.1).[29]



Scheme 1.1

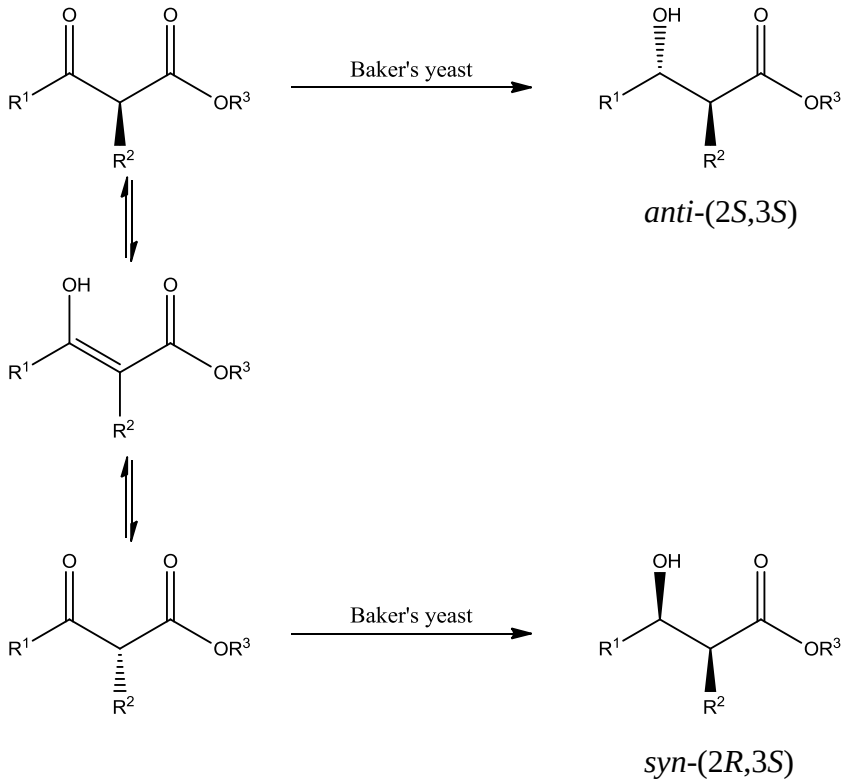
Fujisawa and co-workers later expanded this study and found that introduction of a sulfur atom into a substrate provides a facile method for controlling the stereochemical outcome of baker's yeast mediated reductions so that the enantioselectivity is improved, or alternatively, reversed.[32-34] Following this fundamental work the microbial reduction of sulfur-containing compounds attracted considerable interest. Furthermore these sulfur moieties can be easily manipulated chemically to either introduce a variety of functionalities,[35-37] or be cleaved with retention of configuration by reductive desulfonylation.[38] Therefore, the products of baker's yeast mediated reduction and/or kinetic resolution of sulfur-functionalised ketone derivatives are versatile synthetic intermediates and have been used extensively in the synthesis of a number of enantioenriched target molecules.[39]

1.2.1 Baker's yeast mediated reduction of ketosulfides

The synthetic utility of enantiomerically pure secondary alcohols has been extensively documented.[40-44] Furthermore, the presence of an additional heteroatom in the chiral alcohol, placed at an appropriate position from the hydroxyl group can provide further versatility to the compound. Chiral hydroxysulfides serve as valuable intermediates in the synthesis of naturally occurring spiroketal pheromones,[45] chiral oxiranes,[32,46,47] thiiranes,[48] tetrahydrofurans[49,50] and 4-acetoxyazetidinones.[51] Moreover, they are easily oxidised to hydroxysulfoxides[52-54] or sulfones[55] which also serve as extremely useful chiral building blocks.

1.2.1.1 Reduction of β-ketoesters

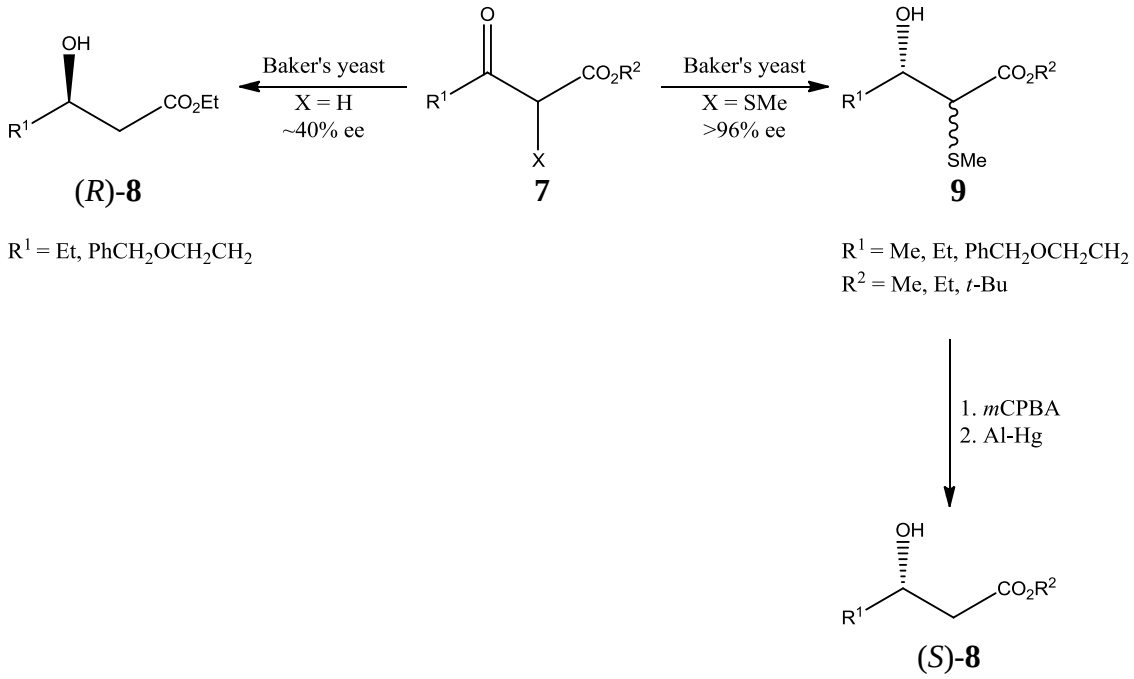
Optically active β-hydroxyesters or acids obtained by baker's yeast mediated reduction have been efficiently employed as intermediates in various natural product syntheses.[56-60] Chiral β-ketoester derivatives monosubstituted at C-2 spontaneously racemise at this position so that a diastereoselective reductase can convert an initially racemic β-ketoester to a single β-hydroxyester stereoisomer with total conversion (Scheme 1.2).



Scheme 1.2

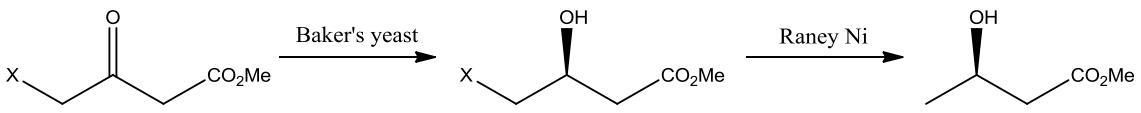
Although the stereochemical course of the baker's yeast reduction is generally predicted by Prelog's rule, several different oxidoreductases of *Saccharomyces cerevisiae* can compete for the same substrate to alter the course of hydrogen delivery and/or to reduce the enantioselectivity. For example, while reduction of ethyl acetoacetate **7** (R^1 = Me, X = H, R^2 = Et) provides the (*S*)-hydroxyester (*S*)-**8** with 97% ee,[61] ethyl 3-oxopentanoate **7** (R^1 = Et, X = H, R^2 = Et)[60] and ethyl 5-benzyloxy-3-oxopentanoate **7** (R^1 = $PhCH_2OCH_2CH_2$, X = H, R^2 = Et) are reduced to the corresponding (*R*)-hydroxyesters (*R*)-**8,** of opposite configuration with poor optical purities (~40% ee) (Scheme 1.3).[62]

Fujisawa reported that the stereochemical control of baker's yeast catalysed reduction of β-ketoesters can be strongly influenced by the introduction of a sulfur substituent at the α-position, effectively discriminating the *re*- and *si*- faces of the substrate to give enantiopure (*S*)-hydroxyesters (*S*)-**8**.[62] Enantioselectivity was observed to dramatically improve for the reduction of β-keto-α-(methylthio)esters **7** (X = SMe), giving exclusively (3*S*)-β-hydroxyesters **9** with >96% ee. The α-methylthio group of **9** was easily removed following enzymatic reduction by *m*CPBA oxidation to the corresponding sulfoxide and subsequent desulfonylation with Al-Hg to afford optically pure (*S*)-**8** (Scheme 1.3). A similar effect of an electronegative substituent α- to the ketone controlling stereoselectivity was observed in the reduction of α-hydroxy-β-ketoesters.[63]



Scheme 1.3

Interestingly, on introduction of a sulfur moiety into the γ-position of the β-ketoester, the absolute configuration of the resulting β-hydroxyester was inverted from (*S*) to (*R*). This *anti*-Prelog enantiopreference was observed in the enantioselective baker's yeast mediated reduction of β-keto-γ-phenylthio ester **10a**[64] and the γ-phenylsulfonyl ester derivative **10b** (Scheme 1.4).[25] The latter was reduced with greater enantioselectivity (98% ee *vs.* 73% ee) and subsequent sulfur group cleavage yields the (*R*)-β-hydroxyester **11**. Thus, stereochemical control of the baker's yeast mediated reduction to both enantiomeric series, (*S*)- and (*R*)-β-hydroxyesters is achievable through careful positioning of the sulfur functional groups.



10a X = PhS
10b X = $PhSO_2$

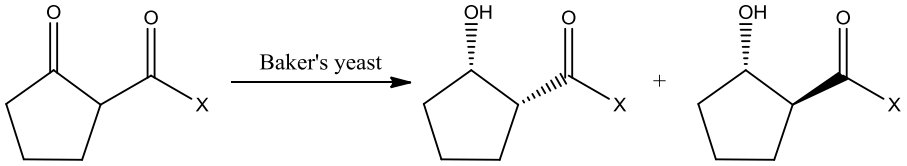
12a X = PhS *(73% ee)*
12b X = $PhSO_2$ *(98% ee)*

(*R*)-**11**

Scheme 1.4

As is evident from Table 1.1, good diastereo- and enantioselectivity was achieved in the baker's yeast mediated reduction of 2-cyclopentanonecarboxylates with a methyl **13a** (94% ee) and ethyl ester **13b** (89% ee) (entries 1 and 2, Table 1.1). However, on introduction of a sulfur atom instead of an oxygen atom into the ester moiety the *cis*-(1*R*,2*S*)-S-ethyl 2-hydroxycyclopentanecarbothioate (1*R*,2*S*)-**14d** was exclusively formed with enhanced enantioselectivity (>96% ee) (entry 4, Table 1.1).[65] Although the octyl ester derivative **13c** also gave optically pure *cis*-(1*R*,2*S*)-hydroxyester (1*R*,2*S*)-**14c** (entry 3, Table 1.1), the reduction required a significantly longer reaction time (6 d *vs*. 3.5 h). Such cyclic hydroxyesters are highly versatile chiral building blocks and for example have been utilised in the asymmetric synthesis of (2*R*,5*S*)-malynoglide, a biologically active antibiotic isolated from the marine blue-green algae (*Lyngbya majuscula*), and (1*S*,5*R*)-frontalin, the aggregation pheromone of the southern pine bark beetle (*Dendroctonus brevicomis*).[65]

*Table 1.1: The baker's yeast reduction of 2-cyclopentanonecarboxylate **13**[65]*

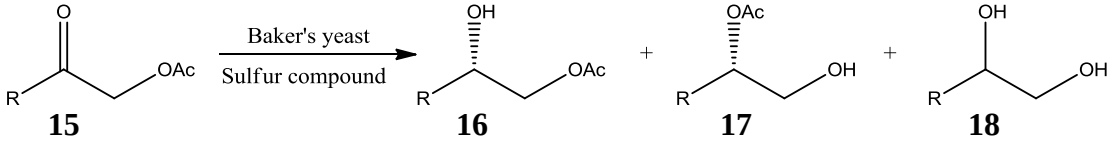


13 (1*R*,2*S*)-**14** (1*S*,2*S*)-**14**

Entry	Ketone	X	Time	Alcohol	Yield (%)	*cis* (1*R*,2*S*)-14 : *trans* (1*S*,2*S*)-14	ee (%) of (1*R*,2*S*)-14
1	**13a**	OCH_3	1 d	**14a**	35	93 : 7	94
2	**13b**	OC_2H_5	2 d	**14b**	50	97 : 3	89
3	**13c**	OC_8H_{17}	6 d	**14c**	62	100 : 0	>96
4	**13d**	SC_2H_5	3.5 h	**14d**	88	100 : 0	>96

A more recent development to increase the stereoselectivity and enhance the reactivity of yeast reductases is the addition of a sulfur compound. This stems from Fujisawa's work demonstrating that the introduction of a sulfur atom in the vicinity of the carbonyl group of the substrate improves the enantioselectivity and reactivity of the baker's yeast reduction. The first report of employing a sulfur compound as an additive in the baker's yeast reduction of 1-acetoxy-2-alkanones **15a-c** was described by Fujisawa in 1997.[66] While yeast reduction of these substrates in the absence of a sulfur additive generally results in compromised yields and enantiopurities, undesired migration of an acetyl group and hydrolysis of the acetoxy moiety are also competing factors. However, in the presence of a sulfur compound, this migration is suppressed (Table 1.2).

*Table 1.2: The baker's yeast reduction of 1-acetoxy-2-alkanones **15**[66]*



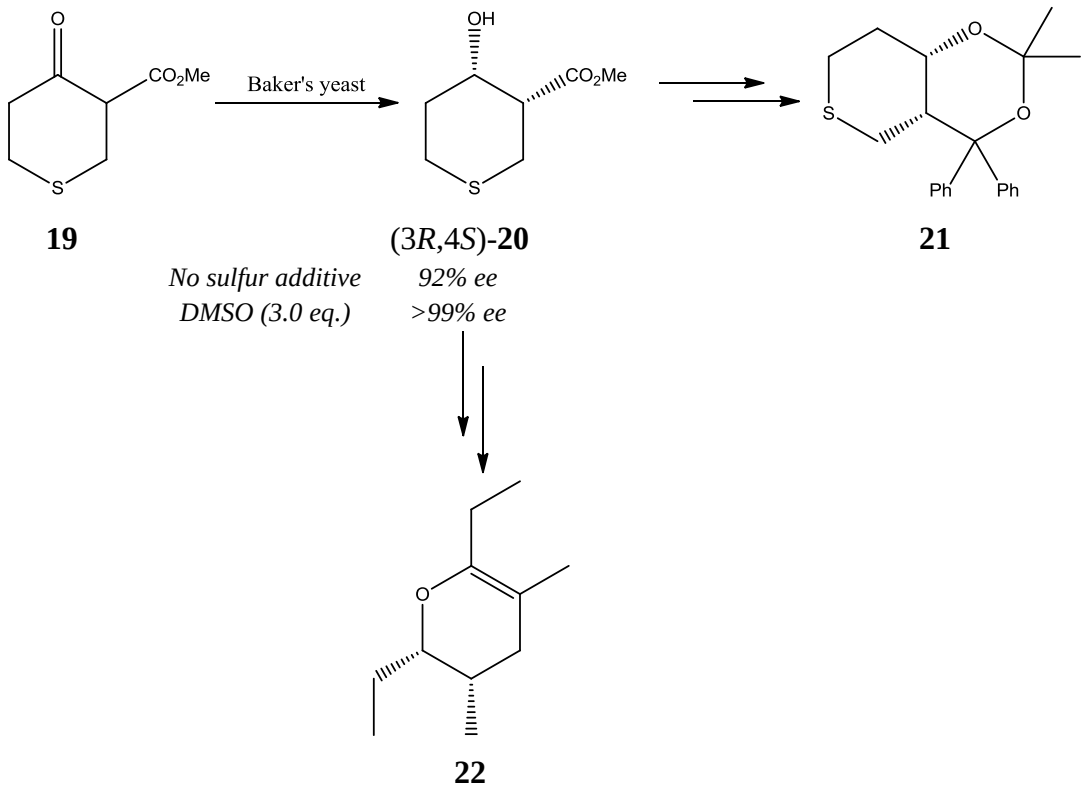
15 **16** **17** **18**

Entry	Ketone	R	Sulfur compound	eq.	Time	Yield (%)[a] 16 + 17	Yield (%)[a] 18	16 : 17 : 18 [ee (%)][b]
1	**15a**	Me	None	-	2 h	41	-	94 (>99) : 6 : -
2	**15a**	Me	Me_2S	1.0	2 h	46	-	>99 (>99) : 1 : -
3	**15b**	*n*-Bu	None	-	6 h	52	28	53 (98) : 12 (>99) : 35 (68)
4	**15b**	*n*-Bu	L-Cysteine	1.0	1 h	66	8	79 (96) : 10 (>99) : 11 (60)
5	**15c**	Ph	None	-	5 h	58	2	64 (90) : 33 (>99) : 3
6	**15c**	Ph	L-Cysteine	2.0	4 h	48	-	75 (>98) : 25 (>99) : -

a. Isolated yield.
b. Determined by HPLC (Hibar column, Merck) analysis and 500 MHz ^{1}H NMR of the corresponding (−)-MTPA ester derivative.

Reduction of the simplest substrate acetoxyacetone **15a** with the addition of 1.0 equivalent of dimethyl sulfide (Me_2S), results in enantiopure alcohol (*S*)-**16a** with limited migration of the acetyl group (entry 2, Table 1.2). Reduction of **15b** is also improved with the addition of a sulfur compound as additive (entry 4, Table 1.2). In this case L-cysteine suppresses not only the migration of the acetyl group but also the hydrolysis of the acetoxy group to give (*S*)-**16b** predominantly. Moreover, the reaction rate of the baker's yeast reduction is greatly accelerated in the presence of L-cysteine, 1 h *vs.* 6 h. In the case of the aromatic ketone acetoxyacetophenone **15c**, the addition of L-cysteine results in excellent enantiopurity of (*S*)-**16c** and complete suppression of the hydrolysis of the acetoxy group and reduced formation of the migration product **17c** (entry 6, Table 1.2). Fujisawa postulated that the improved results on addition of a sulfur additive may be due to the interaction of the sulfur compound with the active site of the reductase, altering the cavity.

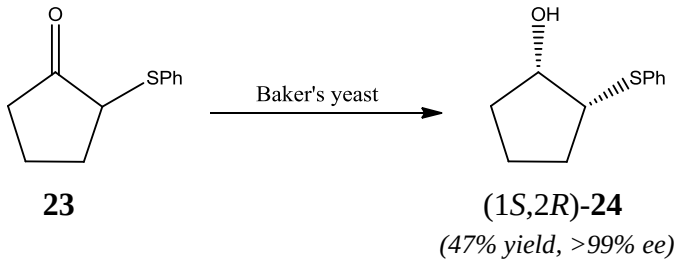
Shimizu later explored the baker's yeast reduction of tetrahydrothiofuran and pyran β-ketoester derivatives in the presence of a sulfur additive.[67] The resulting *cis*-β-hydroxyesters can be converted by Raney nickel into highly useful chiral building blocks that are not readily available with a similar degree of stereocontrol by yeast reduction of open chain β-ketoesters. Although the baker's yeast reduction of 2-methoxycarbonyl-tetrahydrothiopyran-4-one **19** had previously been reported the enantioselectivity was not satisfactory.[68,69] The β-ketoester **19** gave the β-hydroxyester derivative **20** with 92% ee. Thus, 3.0 equivalents of dimethyl sulfoxide (DMSO) as an additive was employed in the resolution. Improved enantioselectivity and acceleration of the reaction rate were observed, with recovery of the product **20** in >99% ee, which can be subsequently transformed to the sulfide **21** and employed in a catalytic asymmetric sulfur-ylide epoxidation of aldehydes[67,70] and also into anhydro-serricornine **22**, a sex pheromone of the cigarette beetle (Scheme 1.5).[68]



Scheme 1.5

1.2.1.2 Reduction of ketones

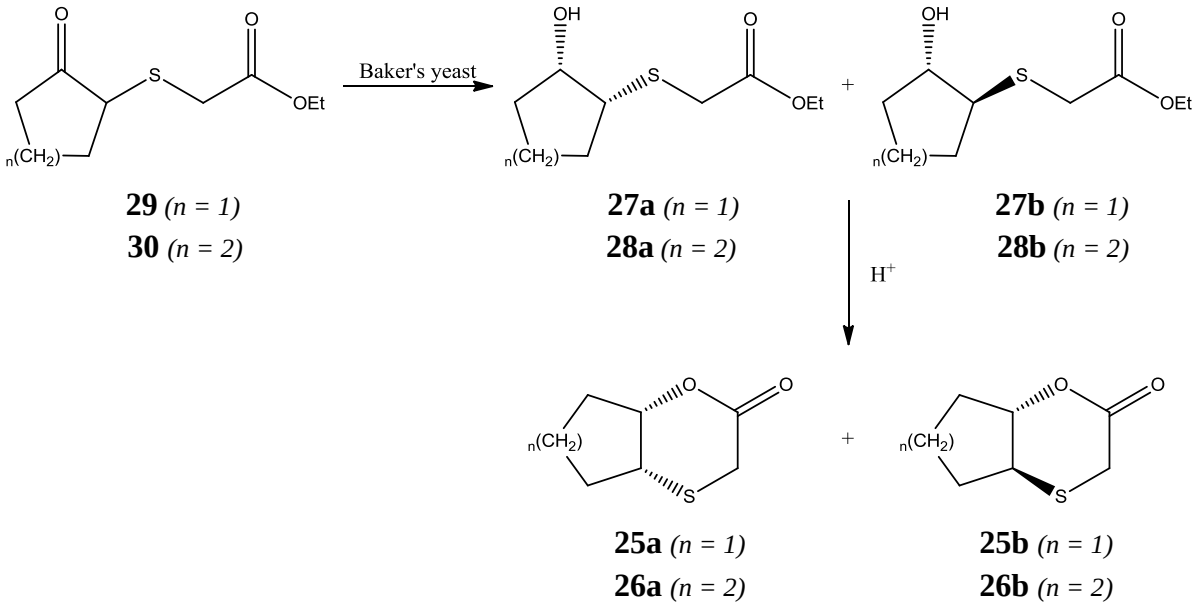
Baker's yeast can enantioselectively reduce aliphatic ketones with various functional groups which are generally difficult to obtain by chemical reduction methods using hydride reagents (see section 1.2.5, Table 1.14). In 1991, Fujisawa reported the efficient baker's yeast mediated reduction of 2-phenylthiocyclopentanone **23** which is more cleanly reduced than the corresponding cyclohexanone congener[29,71] and affords (1*S*,2*R*)-2-(phenylthio)cyclopentanol **24** in optically pure form (>99% ee) with the *cis* alcohol obtained exclusively (Scheme 1.6).[71]



Scheme 1.6

In the synthesis of sulfur-containing lactones **25b** and **26b**, Vankar and co-workers obtained the β-thiocyclopentanol **27b** and hexanol **28b** with *trans* stereochemistry on baker's yeast mediated reduction of **29** and **30** with poor yield, 16% and 17% respectively (Scheme 1.7).[72] Notably, although stereochemistry was assigned by spectral ^{1}H NMR data, the optical purity of these compounds was not reported. Recently Maguire and co-workers reinvestigated the asymmetric synthesis of the lactone **26**.[73] Significantly, mixtures of the *cis* and *trans* fused lactones **26a** and **26b** were isolated in ≥98% ee and the diastereoselectivity of the yeast reduction was determined to be sensitive to the reduction protocol, with the reaction

conditions for isolation of enantiopure **26a** and **26b** optimised. Presumably in Vankar's work both isomers were also formed but only **26b** was recovered following purification. Use of this methodology for the asymmetric synthesis of the α-diazosulfoxide **31** in enantiopure form (≥98% ee) has also been demonstrated (Figure 1.3).[73]



29 *(n = 1)*
30 *(n = 2)*

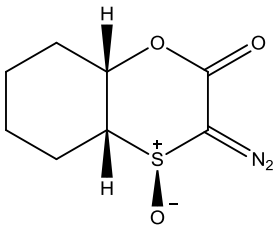
27a *(n = 1)*
28a *(n = 2)*

27b *(n = 1)*
28b *(n = 2)*

25a *(n = 1)*
26a *(n = 2)*

25b *(n = 1)*
26b *(n = 2)*

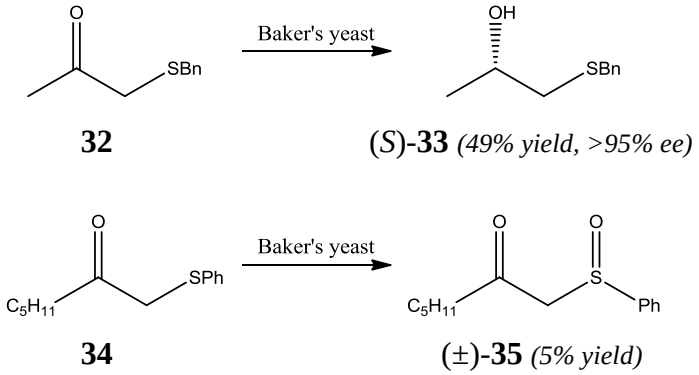
Scheme 1.7



31*(≥98% ee)*

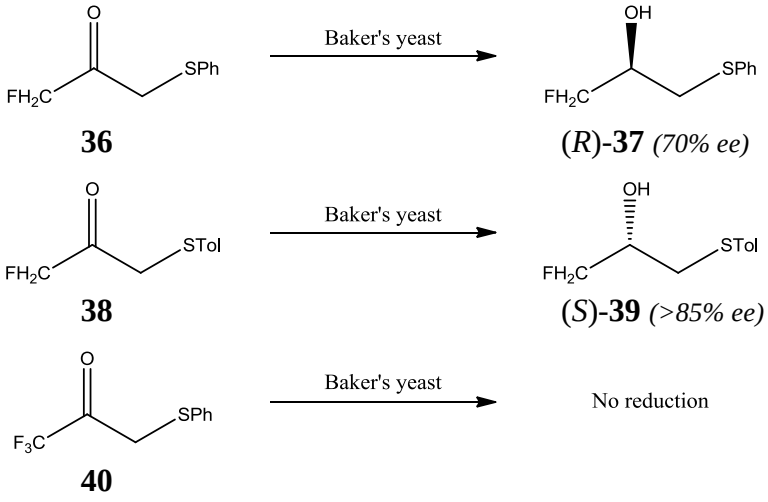
Figure 1.3

As mentioned previously (section 1.2), Ridley reported the reduction by baker's yeast of 1-(phenylthio)propan-2-one **1** affording (*S*)-hydroxysulfide (*S*)-**2** in high enantiomeric purity (≥94% ee) albeit with low yield (35%) (Scheme 1.1).[29] Similarly, the structural analogue 1-(benzylthio)propan-2-one **32** afforded the corresponding (*S*)-configured alcohol (*S*)-**33** in 49% yield and >95% ee (Scheme 1.8).[29] However, when the alkyl chain length was increased, the attempted reduction of 1-phenylthio-2-heptanone **34** yielded only the racemic β-ketosulfoxide **35**, formed by oxidation of the sulfide **34** in 5% yield (Scheme 1.8).[29] In general Ridley found that reduction of these β-ketosulfides proceeded with relative difficulty and only at low concentrations of the substrate.[29]



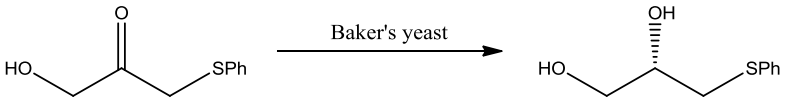
Scheme 1.8

The preparation of enantiomerically pure fluorine-containing compounds by means of enzymatic or microbial systems has garnered much interest due to their potential use as drugs and valuable tools for metabolic studies. Yamazaki reported the yeast reduction of fluorinated β-ketosulfides and found that the reduction of 1-fluoro-2-oxopropylphenylsulfide **36** gave the alcohol (*R*)-**37** with 70% ee but with the opposite configuration to the corresponding non-fluorinated sulfide,[74] while Ghiringhelli[75] and Moretti[76] later reported baker's yeast mediated reduction of the *p*-tolyl sulfide derivative **38** to give the alcohol (*S*)-**39** with >85% ee. Interestingly, 1,1,1-trifluoropropylsulfide **40** did not undergo reduction (Scheme 1.9).[74] Comparison of the data in Schemes 1.8 and 1.9 indicate that the stereochemical assignment of (*R*)-**37** warrants further investigation.



Scheme 1.9

The introduction of a hydroxyl group in the baker's yeast reduction of β-ketosulfides has also been investigated. When 1-hydroxy-3-(phenylthio)-2-propanone **41** was reduced the resulting diol, (*S*)-3-(phenylthio)-1,2-propanediol (*S*)-**42** was formed in 90% yield and 78% ee which was successfully employed for the synthesis of both enantiomers of the insect pheromone δ-*n*-hexadecanolide[32] and for the synthesis of the deoxy sugars L-rhodinose and D-amicetose (Scheme 1.10).

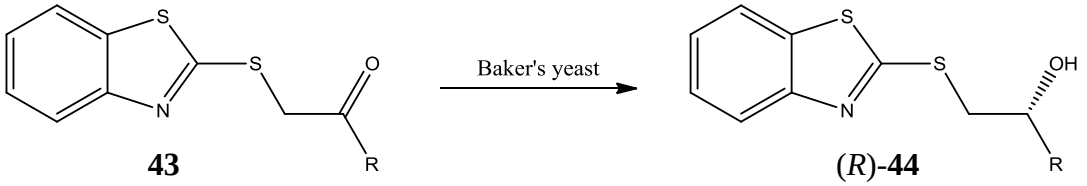


41 (*S*)-**42** *(90% yield, 78% ee)*

Scheme 1.10

The baker's yeast mediated asymmetric resolution of various α-ketosulfides bearing the benzothiazole-2-thiolic moiety has also been reported in good to excellent enantioselectivity and recovery (entries 1-4, Table 1.3).[48] However, in the case of the more sterically demanding substrates, no reduction was observed (entries 5 and 6, Table 1.3). Interestingly the stereoselectivity of the reduction in all cases was (*R*) even with the larger alkyl groups.

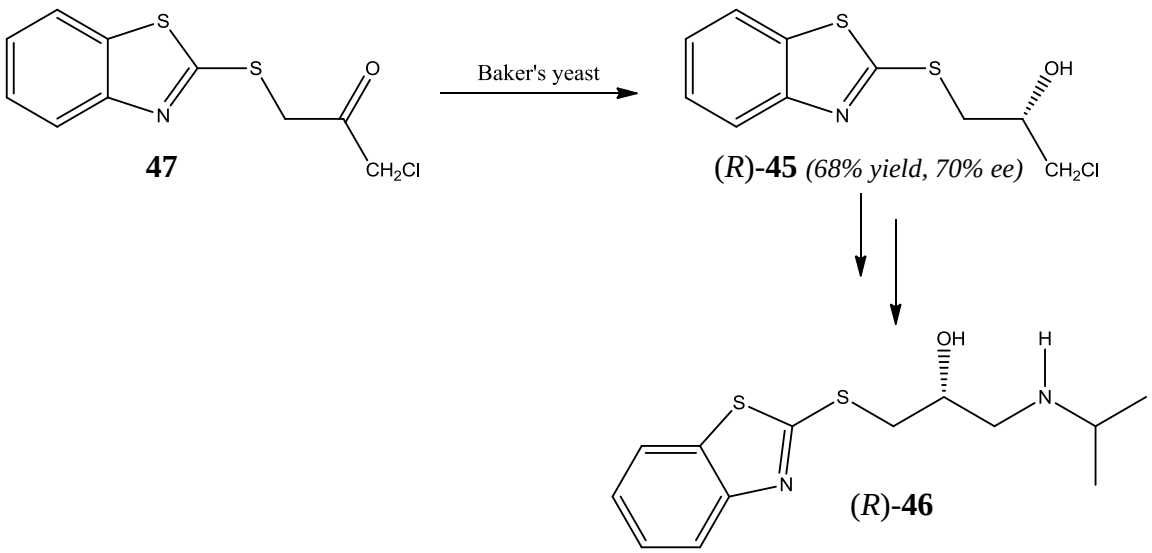
*Table 1.3: The baker's yeast reduction of α-ketosulfides **43**[48]*



43 (*R*)-**44**

Entry	Ketone	R	Time	Alcohol	Yield (%)	ee (%)
1	**43a**	CH_3	3 h	(*R*)-**44a**	91	>99
2	**43b**	C_2H_5	5 d	(*R*)-**44b**	89	74
3	**43c**	*n*-C_4H_9	3 d	(*R*)-**44c**	80	>99
4	**43d**	*n*-C_6H_{13}	6 d	(*R*)-**44d**	80	91
5	**43e**	*n*-$C_{10}H_{21}$	-	-	-	-
6	**43f**	Ph	-	-	-	-

Application of this chemoenzymatic methodology to the asymmetric synthesis of (*R*)-(benzothiazol-2-ylsulfanyl)-3-chloropropan-2-ol (*R*)-**45**, a precursor of potentially biologically active β-blockers (*R*)-**46** proceeded with 70% ee in a biphasic mixture of hexane and water (Scheme 1.11).[77]

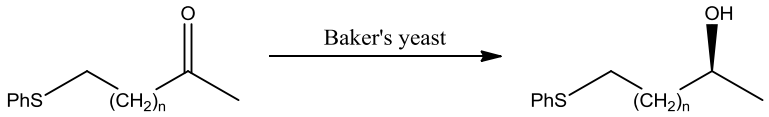


47 (*R*)-**45** *(68% yield, 70% ee)* (*R*)-**46**

Scheme 1.11

The baker's yeast mediated reduction of ketones bearing remote sulfide functional groups has been reported as a key step in the synthesis of a number of chiral target molecules. Cohen *et al.* described the efficient preparation of (*S*)-phenylthio-2-alkanols (*S*)-**48a-c** in excellent enantiopurity (≥96% ee) by enzymatic reduction of the corresponding ketones **49a-c** (Table 1.4).[45,78] These highly enantiomerically enriched alcohols were then transformed to furan and pyran cyclic systems, to substituted cycloalkane rings and to the spiroacetal bee pheromone.[78]

Table 1.4: The baker's yeast reduction of remote sulfide functional groups[45,78]



49 **48**

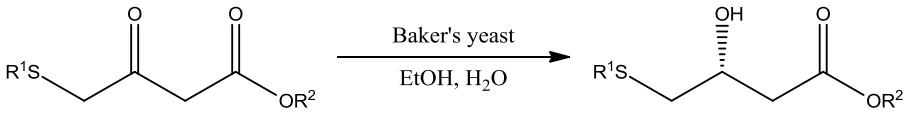
Entry	Ketone	n	Alcohol	Yield (%)	ee(%)
1[45]	**49a**	1	(*S*)-**48a**	70	96
2[45]	**49b**	2	(*S*)-**48b**	99	97
3[78]	**49c**	3	(*S*)-**48c**	97	97

1.2.1.3 Reduction of dicarbonyls

Although diketones are good substrates for baker's yeast, the selectivity of the reductions is rather low. Introduction of a bulky sulfur-containing moiety (which can be easily removed) is an effective way to stereochemically control these reductions.

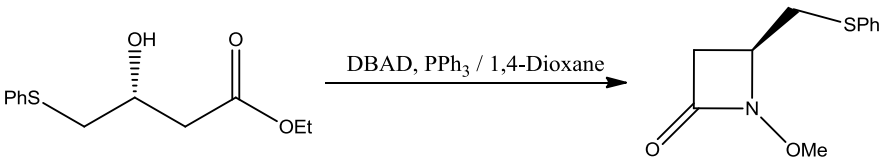
Fujisawa *et al.* investigated the baker's yeast catalysed reduction of various 4-sulfenylacetoacetic ester derivatives **50a-g**.[79] As evident from Table 1.5, enantioselectivity varied considerably, with ethyl (*R*)-3-hydroxy-4-phenylthiobutanoate (*R*)-**51d** recovered in excellent enantiopurity and good yield (entry 4, Table 1.5), while the corresponding methylthio or benylthio **50a-c** substrates were reduced with poor stereoselectivity (entries 1-3, Table 1.5). Furthermore, on substitution of the aromatic ring, a dramatic decrease in enantiopurity was observed (entries 5-7, Table 1.5). Despite the limited substrate specificity, enantiomerically pure ethyl (*R*)-3-hydroxy-4-phenylthiobutanoate (*R*)-**51d** was subsequently transformed into the β-lactam **52** *via* the oxamate derivative, without loss in enantiomeric purity (Scheme 1.12).

*Table 1.5: The baker's yeast reduction of 4-sulfenylacetoacetic ester derivatives **50***[79]



50 (*R*)-**51**

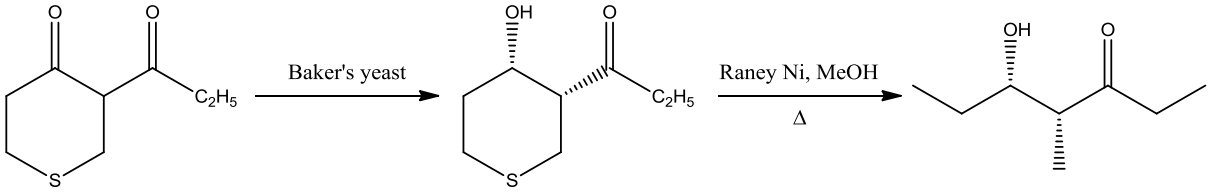
Entry	Ketone	R^1	R^2	Reaction time (h)	Alcohol	Yield (%)	ee (%)
1	**50a**	Me	Et	11	(*R*)-**51a**	47	26
2	**50b**	Bn	Et	4.5	(*R*)-**51b**	85	45
3	**50c**	Bn	*n*Bu	26	(*R*)-**51c**	70	72
4	**50d**	Ph	Et	13	(*R*)-**51d**	63	>99
5	**50e**	*m*-Tol	Et	2.5	(*R*)-**51e**	72	63
6	**50f**	*o*-Tol	Et	5	(*R*)-**51f**	68	5
7	**50g**	*p*-MeOPh	Et	13	(*R*)-**51g**	64	56



(*R*)-**51d** *(>99% ee)* **52**

Scheme 1.12

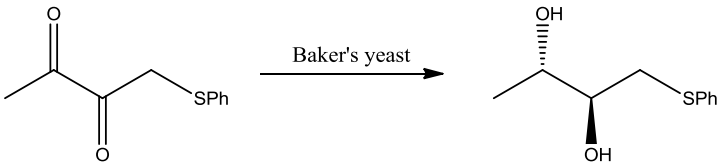
Fujisawa observed the baker's yeast mediated reduction of 3-propionyltetrahydrothiopyran-4-ones **53** to give exclusively the (3*R*,4*S*)-3-propionyl-4-hydroxytetrahydrothiopyrane **54** in 66% yield with excellent enantioselectivity (97% ee).[80] This highly regio- and stereoselective baker's yeast reduction was applied to the stereocontrolled synthesis of the aggregation pheromone of both the rice and maize weevils (4*R*,5*S*)-sitophilure **55** (Scheme 1.13).[80]



53 (3*R*,4*S*)-**54** (4*R*,5*S*)-**55**

Scheme 1.13

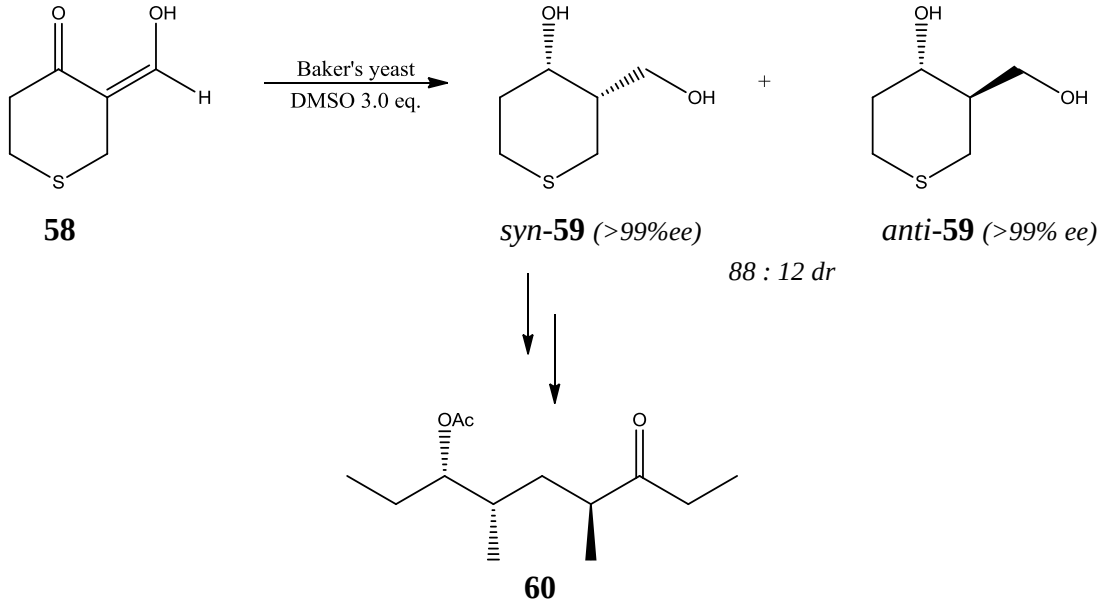
A further example of the versatility of the whole cell baker's yeast is reduction of the diketone **56** into the *anti*- and *syn*-isomeric diols **57** in a 86 : 14 ratio.[81] Optically pure diol (2*S*,3*S*)-**57** was obtained after crystallisation in 55-60% yield which can be employed as a precursor for the synthesis of γ-lactones of natural origin (Scheme 1.14).



56 (2*S*,3*S*)-**57**

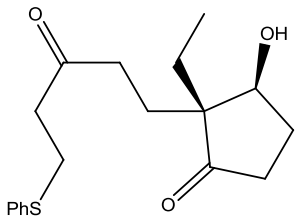
Scheme 1.14

Improved enantio- and diastereoselectivity was achieved in the baker's yeast reduction of β-keto aldehyde derivatives using a sulfur compound as an additive.[82] Reduction of 3-formyltetrahydrothiopyran-4-one **58** gave the sulfur-containing diol derivative **59** as a mixture of *syn* and *anti* diastereomers with the *syn* isomer being favoured (82 : 18 dr), but recovered in only 84% ee (Scheme 1.15). The *syn*-selectivity was improved using a sulfur compound such as L-cysteine or DMSO as an additive leading to a diastereomeric ratio of 90 : 10 or 88 : 12 respectively. The best enantioselectivity of the *syn*-isomer **59** was achieved using DMSO as additive, with an enantiomeric excess of >99% ee. The resulting enantiomerically pure 1,3-diol derivative was transformed into serricornin **60**, a sex pheromone of the cigarette beetle (*Lasioderma serricorne F.*).



Scheme 1.15

Various other chiral synthons have been obtained by resolution of di- or triketones, for example this methodology has been applied to the preparation of the intermediate **61** in the synthesis of an optically active steroid (Figure 1.4).[83]

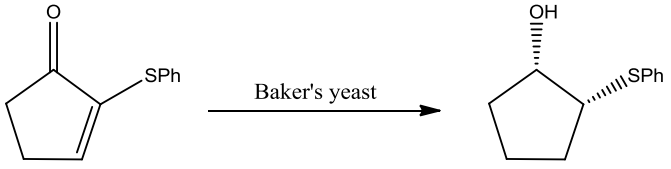


(2*R*,3*S*)-**61**

Figure 1.4

1.2.1.4 Reduction of olefins

Although not as widely investigated as carbonyl reduction, baker's yeast mediated reduction of carbon-carbon double bonds is a useful transformation. The baker's yeast specific hydrogenation of the olefin and carbonyl reduction in the sulfur-containing α,β-unsaturated cyclopentenone derivative **62** proceeds with excellent enantiopurity (>99% ee) affording (1*S*,2*R*)-**24** as the sole product in 47% yield (Scheme 1.16).[71] In contrast to the cyclopentenone **62**, incubation of the cyclohexenone derivative for 20 days led to recovery of the substrate.

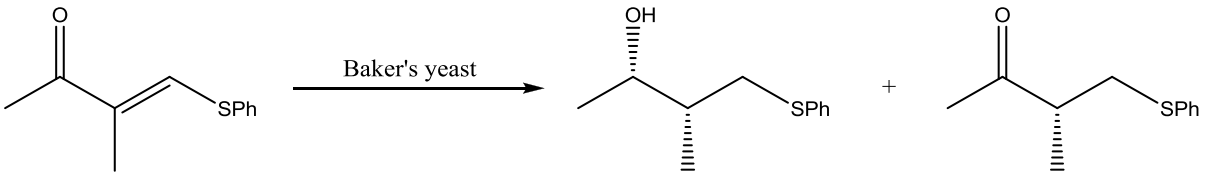


62

(1*S*,2*R*)-**24**
(47% yield, >99% ee)

Scheme 1.16

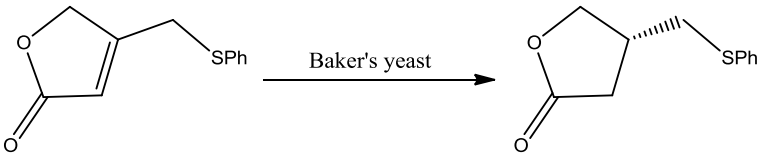
Crout and co-workers investigated the baker's yeast mediated reduction of a number of α,β-unsaturated carbonyl compounds with a γ-sulfide, sulfoxide and sulfone moiety attached (see Scheme 1.31 and Scheme 1.38).[84] Carbonyl bond reduction was always detected but significantly, olefin bond reduction was only observed with the sulfide. Reduction of the vinyl sulfide **63** by *Saccharomyces cerevisiae* gave the fully saturated alcohol **64** in 18% yield and of 90% de, with both diastereomers found to be enantiomerically pure (>96% ee), and also the ketosulfide **65** in 20% yield and 72% ee (Scheme 1.17).



63 **64** *(18% yield, 90% de, >96% ee)* **65** *(20% yield, 72% ee)*

Scheme 1.17

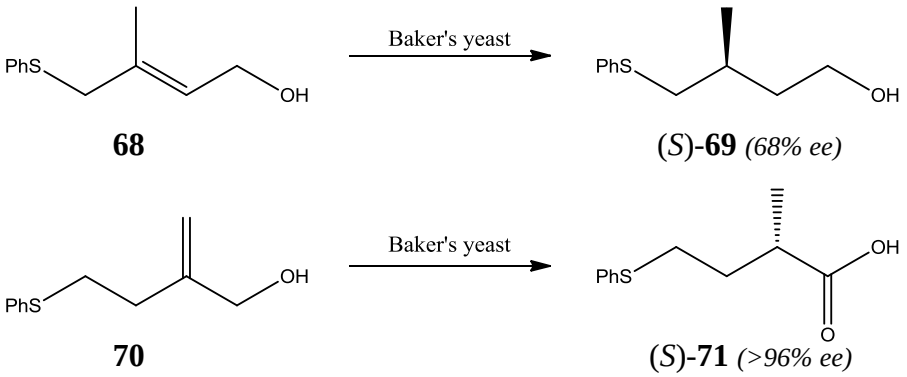
A further example of yeast reduction of a sulfide-functionalised compound is the reduction of 3-phenylthiomethyl-2-butenolide **66** reported by Takabe.[85] In this example the double bond is reduced in preference to the carbonyl group to afford 2-phenylthiomethylbutanolide **67** in 41% yield, 99% ee (Scheme 1.18).



66 **67** *(41% yield, 99% ee)*

Scheme 1.18

Allylic alcohol **68**, with a γ-methyl group, gave the (*S*)-alcohol (*S*)-**69** with a rather low enantiomeric excess of 68% ee.[86] Conversely, baker's yeast mediated reduction of the alcohol **70** proceeded with concomitant hydrogenation of the methylene double bond and oxidation of the alcohol **70** to the carboxylic acid (*S*)-**71** with >96% ee. Thus, Fujisawa reports changing the feature and position of the double bond results in the reversal of hydrogen delivery by the yeast (Scheme 1.19).

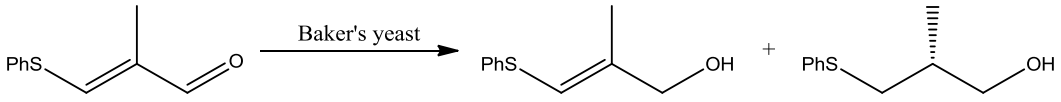


68 (*S*)-**69** *(68% ee)*

70 (*S*)-**71** *(>96% ee)*

Scheme 1.19

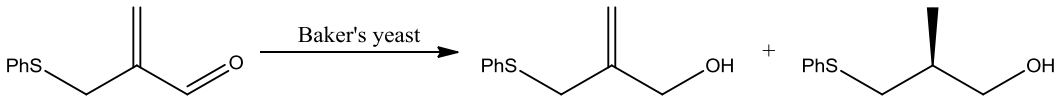
A similar trend of stereochemical behaviour was described by Serra *et al.* in the baker's yeast mediated reduction of sulfur-functionalised methacroleins.[87] After 4 days of fermentation aldehyde **72** was reduced to a mixture of the allylic alcohol **73** (64%), saturated (*R*)-alcohol (*R*)-**74** (25%) in 90% ee and starting aldehyde **72** (6%) (Scheme 1.20).



72 **73** (*R*)-**74** *(90% ee)*

Scheme 1.20

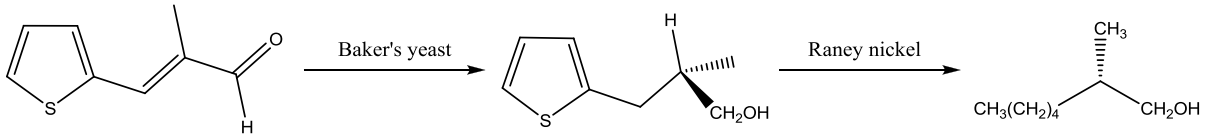
However, completely different behaviour both in terms of yield and enantioselectivity was observed on reduction of aldehyde **75**. The double bond of the methacrolein **75** was saturated readily and after four days of fermentation (*S*)-**74** (40%) was the main product together with allylic alcohol **76** (35%) and residual unsaturated aldehyde **75** (10%). In spite of the efficiency of the reduction step, the enantiomeric purity of the (*S*)-**74** was only 65% ee, whereas when fermentation was interrupted after only 48 h the isolated (*S*)-**74** had greater enantiopurity of 80% ee. The lower enantiopurity may be explained by considering that the heteroatom may assist the double bond isomerisation by conjugation. The following reduction of isomerised aldehyde affords the opposite enantiomer (*R*)-**74**, lowering the enantiomeric purity of the product (*S*)-**74** (Scheme 1.21).



75 **76** (*S*)-**74** *(65-89% ee)*

Scheme 1.21

Högberg reported the baker's yeast mediated enantioselective reduction of the thiophene derivative **77** to yield the (*S*)-alcohol (*S*)-**78** in 98% ee. Raney nickel reduction of the acetate of (*S*)-**78** followed by hydrolysis provided (*S*)-2-methyl-1-alkanol **79** of unchanged optical purity (Scheme 1.22).[88]



77 (*S*)-**78** *(98% ee)* (*S*)-**79**

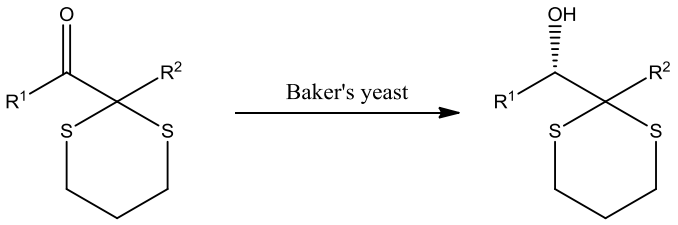
Scheme 1.22

While there are many examples of baker's yeast mediated hydrogenations of alkenes to yield the corresponding alkanes, the reverse process is reported to a much lesser extent.[89]

1.2.2 Baker's yeast mediated reduction of ketones bearing dithioacetals

Ketones possessing 1,3-dithiane at the α-position **80a-q** on treatment with baker's yeast provide the corresponding α-hydroxythioacetals **81a-q** with high enantiomeric excess and proceed predominantly to the (*S*)-enantiomer.[90-93] Notably, only reduction of the allyl derivative **80g** (entry 7, Table 1.6) afforded the (*R*)-enantiomer with high enantioselection.[90] The methyl ester **80r** (entry 18, Table 1.6) was rapidly hydrolysed by the esterase of baker's yeast whereas the *tert*-butyl ester **80q** (entry 17, Table 1.6) was stable and afforded **81q** in good yield and >97% enantiomeric purity.[92] These optically active α-hydroxythioacetals are the synthetic equivalent of chiral α-hydroxy aldehydes and ketones and have a wide applicability for the synthesis of many biologically active compounds, including the total synthesis of the anti-inflammatory agent leukotriene B_4 *via* the Wittig reaction.[92]

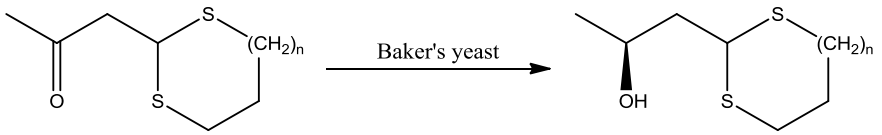
Table 1.6: The baker's yeast reduction of α-ketothioacetals ***80***[90-92]



80 **81**

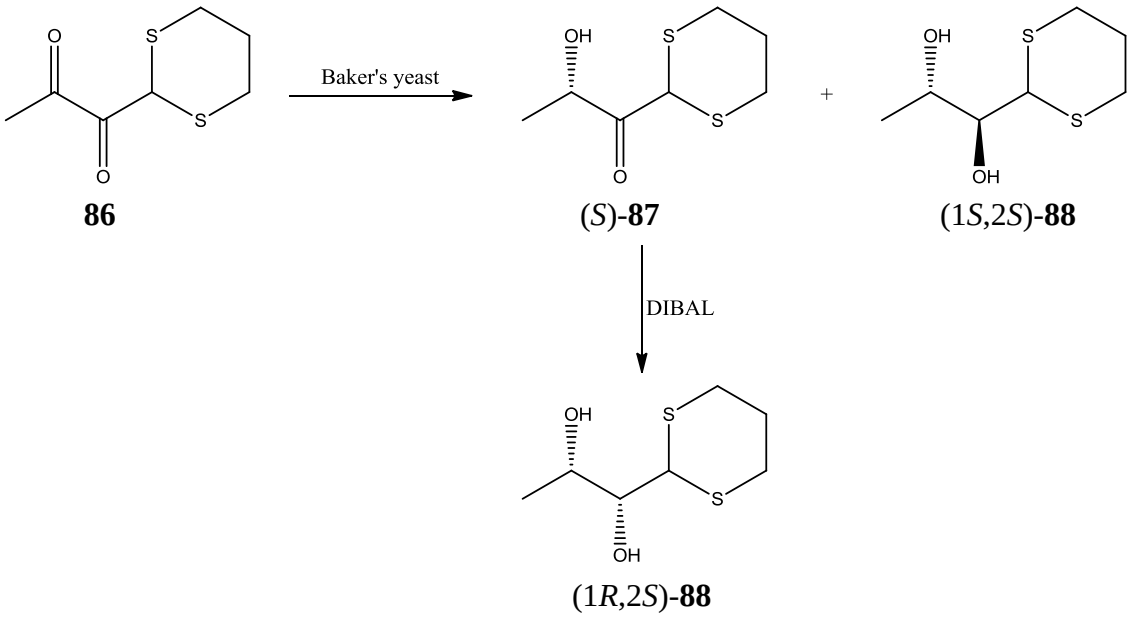
Entry	Ketone	R^1	R^2	Time	Alcohol	Yield (%)	ee (%)
1[90,91]	**80a**	CH_3	H	1 d	(*S*)-**81a**	84	>96
2[90,91]	**80b**	C_2H_5	H	2 d	(*S*)-**81b**	71	>96
3[90]	**80c**	*n*-C_3H_7	H	4 d	(*S*)-**81c**	92	>96
4[90]	**80d**	*n*-C_4H_9	H	3 d	(*S*)-**81d**	71	>96
5[90]	**80e**	$(CH_2)_3OH$	H	10 d	(*S*)-**81e**	74	>96
6[90]	**80f**	CH_3	CH_3	2.5 d	(*S*)-**81f**	50	>96
7[90]	**80g**	CH_3	CH_2CHCH_2	4 d	(*R*)-**81g**	31	>96
8[91]	**80h**	*n*-C_6H_{13}	H	8 d	(*S*)-**81h**	38	≥95
9[91]	**80i**	CF_3	H	2 h	(*S*)-**81i**	96	67
10[91]	**80j**	CH_2OH	H	5 d	(*S*)-**81j**	28	≥95
11[91]	**80k**	CH_2OAc	H	1 d	(*S*)-**81k**	58	87
12[91]	**80l**	CH_2OBn	H	5 d	(*S*)-**81l**	50	≥95
13[91]	**80m**	CH_2O(*p*-MeOPh)	H	6 d	(*S*)-**81m**	27	≥98
14[91]	**80n**	CH_2OTHP	H	3 d	(*S*)-**81n**	37	≥95
15[91]	**80o**	CH_2OCH_2OMe	H	1 d	(*S*)-**81o**	82	≥95
16[91]	**80p**	$CH_2OSi(CH_3)_2t$-Bu	H	7 d	(*S*)-**81p**	<10%	-
17[92]	**80q**	$(CH_2)_3CO_2t$-Bu	H	3 d	(*S*)-**81q**	65	>97
18[92]	**80r**	$(CH_2)_3CO_2CH_3$	H	-	-	-	-

In addition, the baker's yeast reduction of masked 1,3-dicarbonyl compounds affords secondary alcohols in optically active form. Ghiringhelli reports excellent enantiomeric excess on reduction of the ketone 1-(1,3-dithian-2-yl)-2-propanone **82**, to give the enantiomerically pure (*S*)-β-hydroxythioacetal (*S*)-**83** in 99% ee (Scheme 1.23), a key intermediate for the preparation of a variety of compounds with biological and pharmacological importance[94-97] including both enantiomers of the macrocyclic lactone lasiodiplodin, which have demonstrated significant antileukemic activity.[98] The corresponding 5-membered thioacetal 1-(1,3-dithiolan-2-yl)-2-propanone **84** was also reduced in >99% ee.[99]



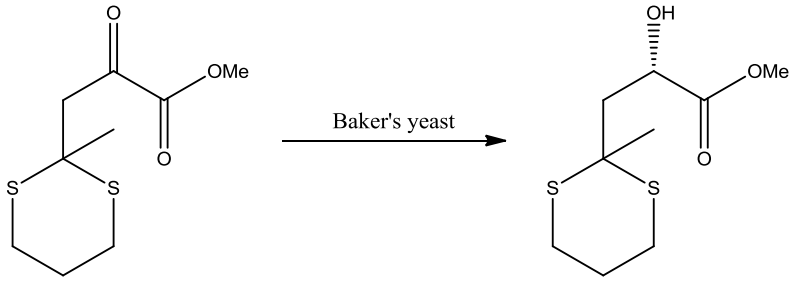
Scheme 1.23

The baker's yeast reduction of the diketone **86** proceeds with excellent selectivity depending on the reaction time. Reduction of the β-carbonyl group progresses much faster than that of the α-carbonyl group. (*S*)-Hydroxyketone (*S*)-**87** and (1*S*,2*S*)-*anti*-diol (1*S*,2*S*)-**88** are obtained in 60% and 82% yields after 2 and 48 h respectively.[33] The products were obtained enantiomerically pure following recrystallisation from hexane. The large differences between the reduction rates of the two carbonyl groups was attributed to the steric bulk around them. Reduction of the hydroxyketone (*S*)-**87** with diisobutylaluminium hydride gave the *syn*-diol (1*R*,2*S*)-**88** with high diastereoselectivity, while (1*S*,2*S*)-**88** is employed in the total synthesis of L-digitoxose, a rare sugar in nature.[33]



Scheme 1.24

Batyl alcohol, the key intermediate for the preparation of platelet-activating factor (PAF), was synthesised from the ketoester **89** which gave on baker's yeast treatment the corresponding (*R*)-alcohol **90** in high yield (80%), but only with moderate enantiomeric excess (64%) (Scheme 1.25).[100] Use of *Saccharomyces cerevisiae* Kisato Inst. improved the enantioselectivity (89%), although the yield dropped significantly (22%). The highest enantiomeric excess (99%) but very low yield (15%) were finally achieved, however, with *Torulopsis* sp. Jyoxo kyokai 17.

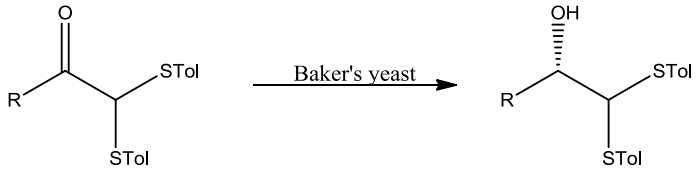


89 (*R*)-**90**

Scheme 1.25

Somewhat lower yields but still high enantiomeric excesses were achieved upon reduction of 1,1-bis-*p*-tolylthioalkan-2-ones **91a-g** to the corresponding alcohols (*S*)-**92a-g** (Table 1.7).[101] The rate of reduction was shown to depend on the length of the hydrocarbon chain and on the nature of the hetero-substituent.

*Table 1.7: The baker's yeast reduction of 1,1-bis-*p*-tolythioalkan-2-ones **91**[101]*

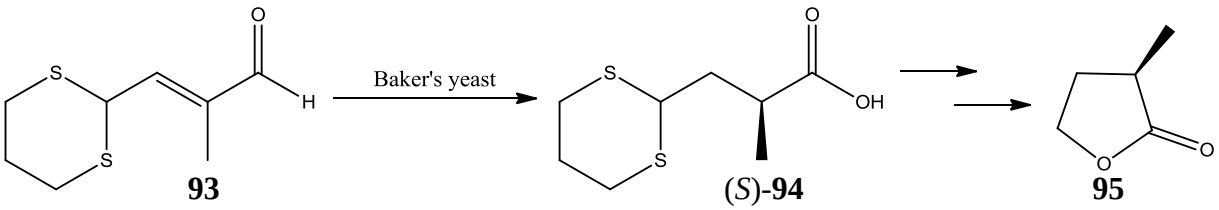


91 (*S*)-**92**

Entry	Ketone	R	Time	Alcohol	Yield (%)	ee (%)
1	**91a**	CH_3	3.5 d	(*S*)-**92a**	50	95
2	**91b**	CH_2F	4 d	(*S*)-**92b**	42	95
3	**91c**	$CH_3OCH_2OCH_2$	6 d	(*S*)-**92c**	50	95
4	**91d**	C_2H_5	7 d	(*S*)-**92d**	10	-
5	**91e**	CH_2Cl	7 d	(*S*)-**92e**	5	-
6	**91f**	CH_2OH	7 d	(*S*)-**92f**	5	-
7	**91g**	CF_3	3 d	(*S*)-**92g**	25	95

Notably, poor yields and enantioselectivities were also observed on the reduction of 2-acyl-thiazoles.[91] These results summarised in Table 1.6-1.7 and Scheme 1.23-1.25 indicate that 1,3-dithiane as a sulfur functional group instead of the bis(*p*-tolylthio)methane or thiazole derivatives permits the synthesis of a broader range of α-alkoxycarbonyl compounds.

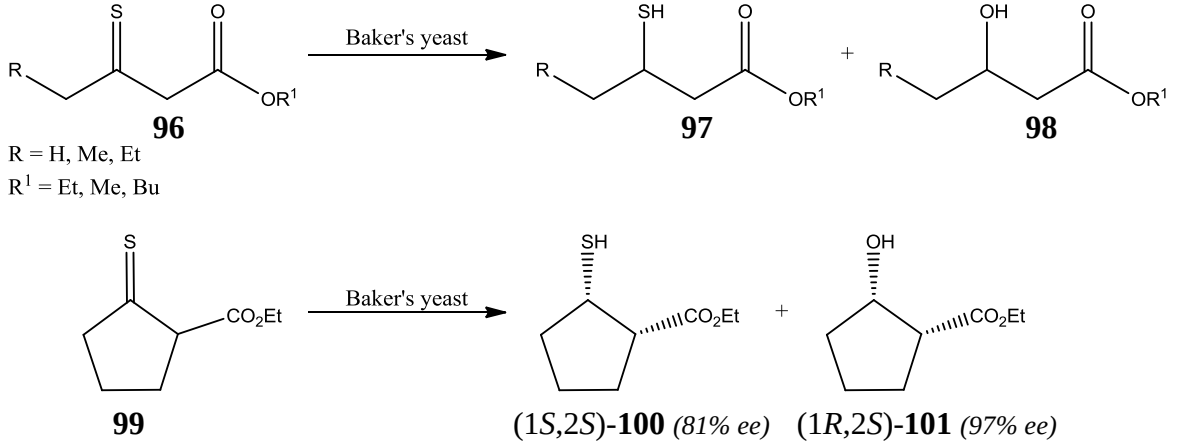
Finally, baker's yeast mediated reduction of carbon-carbon double bond possessing 1,3-dithiane moiety has been reported.[99,102] For example, the α,β-unsaturated aldehyde **93** was incubated with baker's yeast for 19 days to yield optically pure (*S*)-propanoic acid (*S*)-**94** derivative in 48% yield.[86] Absolute configuration is proved by conversion to the (*S*)-lactone **95**. Oxidation of the aldehyde to the carboxylic acid occurs after the reduction of the carbon-carbon double bond (Scheme 1.26).



Scheme 1.26

1.2.3 Baker's yeast mediated reduction of thiocarbonyls

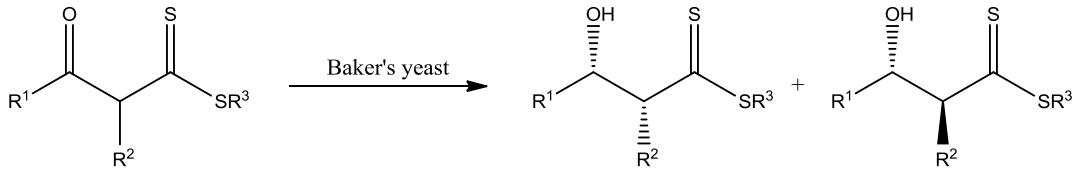
Madsen and Nielson reported that reduction of β-thioxoester derivatives **96** with baker's yeast gave mixtures of optically active β-mercaptoesters **97** and β-hydroxyesters **98** the latter formed by the hydrolysis of the thiocarbonyl group and the subsequent reduction of the hydrolysis product (Scheme 1.27).[103] The reductions parallel those of oxygen analogues in terms of rate, diastereo- and enantioselectivity, but the enantiomeric purities were generally lower. Optimisation of reaction conditions led to improvements in stereoselectivity. The cyclic β-thioxo ester **99** was also reduced to furnish the (1*S*,2*S*)-thiol **100** with 81% ee and the (1*R*,2*S*)-alcohol **101** with 97% ee.



Scheme 1.27

Highly *syn*-selective baker's yeast mediated reduction was observed of the β-ketodithioesters **102a-d** providing primarily the corresponding optically active (3*S*)-hydroxythioester *syn*-**103a-d**.[34] Reduction of methyl 3-oxo-2-methyldithiobutanoate **102c** yielded an easily separable 94 : 6 mixture of *syn*-**103c** and *anti*-**103c** (entry 3, Table 1.8), the latter was obtained as a low yield byproduct although with high enantiomeric excess (96% ee), while the reduction of methyl 2-oxocyclohexanedithiocarboxylate **102d** gave an optically pure single product of *syn*-(1*R*,2*S*)-**103d** (entry 4, Table 1.8). The *syn* to *anti* ratio is better than with the corresponding oxo isomers, a fact that appears to be due to the enhanced enolization of the β-keto groups by the thiocarbonyl moiety. Thus, changing the oxygen atoms in an ester group of a β-ketoester to sulfur atoms can control both the diastereo- and enantioselectivity of the reduction quite efficiently.

Table 1.8: The baker's yeast reduction of β-keto dithioester derivatives ***102***[34]



102 *syn*-**103** *anti*-**103**

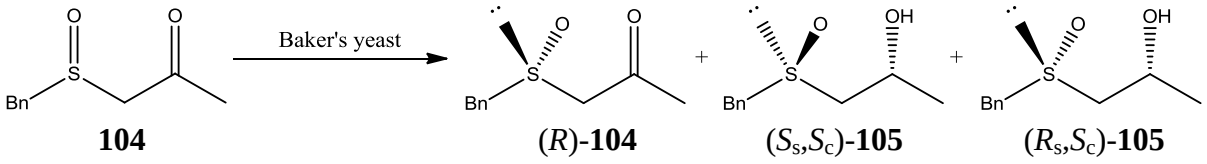
Entry	Ketone	R^1	R^2	R^3	Alcohol	Yield (%)	*syn*-**103** ee (%)
1	**102a**	CH_3	H	CH_3	*syn*-**103a**	50	>96
2	**102b**	CH_3	H	C_2H_5	*syn*-**103b**	30	>96
3	**102c**	CH_3	CH_3	CH_3	*syn*-**103c** : *anti*-**103c** (94 : 6)	65	>96
4	**102d**	-$(CH_2)_4$-		CH_3	*syn*-**103d:** *anti*-**103d** (100 : 0)	27	>96

1.2.4 Baker's yeast mediated reduction of ketosulfoxides

Optically pure sulfoxides have attracted a great deal of interest in the past three decades due to their use as chiral auxiliaries in a broad range of synthetic reactions; including carbon-carbon[104,105] and carbon-oxygen[53] bond forming reactions, in cycloaddition reactions,[106-108] radical addition reactions[109,110] and in asymmetric catalysis.[111] Furthermore, chiral sulfoxides are formed as metabolites of many sulfur-containing drugs,[112,113] and exhibit differential stereochemically-dependent metabolism[114,115] and enzyme inhibition.[116]

However, in contrast to the research described above for the reduction of various carbonyl groups bearing sulfide functionality, efficient baker's yeast mediated kinetic resolution of racemic β-ketosulfoxide derivatives with concomitant reduction of the ketone functionality has been reported to a lesser extent, as the maximum yield of enantioenriched β-ketosulfoxide and/or β-hydroxysulfoxide is at best 50%.

As described at the beginning of section 1.2, one of the earliest reports of kinetic resolution of sulfoxides was the reduction of (±)-1-(phenylsulfinyl)propan-2-one (±)-**3** affording a diastereomeric mixture of 1-(phenylsulfinyl)propan-2-ol (*R*s,*S*c)-**4** and (*R*s,*R*c)-**4** while the (*S*)-sulfoxide **3** was recovered in optically pure form (Scheme 1.1).[29,31] The optically active ketone (*S*)-**3** can be further reduced and alkylated to provide chiral building blocks for the asymmetric synthesis of disparlure.[117] Ridley and co-workers also monitored the reduction of the analogous benzylsulfinyl compound **104** over a period of four days and found that the reduction of the two enantiomers of the starting sulfoxide **104** can proceed at different rates (Scheme 1.28). For example, reduction of one enantiomer of 1-(benzylsulfinyl)propan-2-one **104** occurred readily to afford (S_s,S_c)-(+)-1-(benzylsulfinyl)propan-2-ol (S_s,S_c)-**105** (40%) after 5 h while after 4 d the reaction yielded the (*R*)-sulfoxide (*R*)-**104** (10%) and the alcohols (S_s,S_c)-**105** (30%) and (R_s,S_c)-**105** (25%).

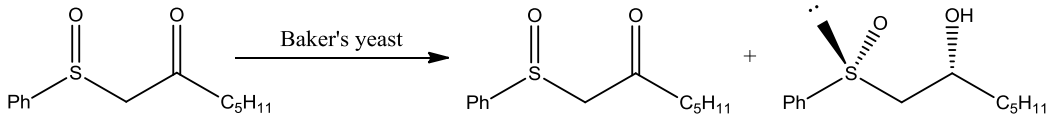


104 (*R*)-**104** (S_s,S_c)-**105** (R_s,S_c)-**105**

Reaction duration	Yield (%)	Yield (%)	Yield (%)
5 h	-	40	-
4 d	10	30	25

Scheme 1.28

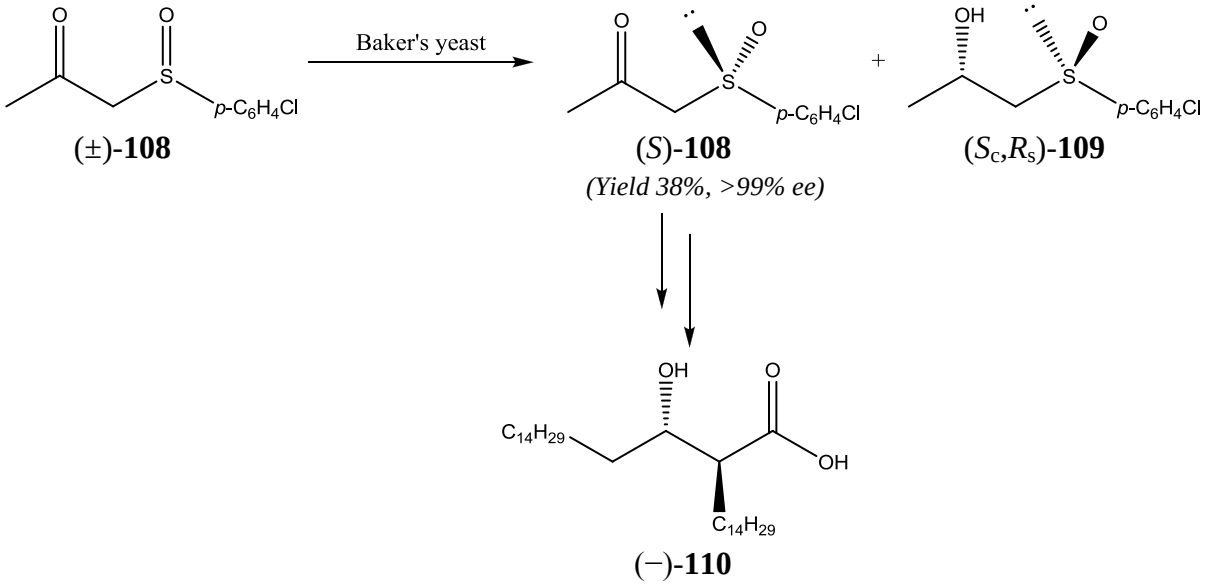
The reduction of 1-phenylsulfinylheptan-2-one **106** proceeded very slowly, yielding the racemic ketone **106** and the (R_s,S_c)-alcohol (R_s,S_c)-**107** in low yield (Scheme 1.29). Clearly extending the alkyl chain length of the β-ketosulfoxide reduces the efficiency of the yeast reductions; a similar trend was observed with the β-ketosulfides (see Scheme 1.8).[29]



106 (±)-**106** (R_s,S_c)-**107**

Scheme 1.29

Fujisawa *et al.* employed baker's yeast to resolve racemic (±)-1-(*p*-chlorophenylsulfinyl)propan-2-one (±)-**108** to provide access to (S_c,R_s)-2-hydroxypropyl *p*-chlorophenyl sulfoxide (S_c,R_s)-**109** while the optically pure (*S*)-ketosulfoxide (*S*)-**108** was recovered in a yield of 38% (Scheme 1.30).[118] This chiral sulfoxide was subsequently employed as a precursor for the preparation of both enantiomers of the antitumor and antibacterial agent corynomycolic acid (+)-**110** and (−)-**110**.



(±)-**108** (*S*)-**108** (S_c,R_s)-**109**

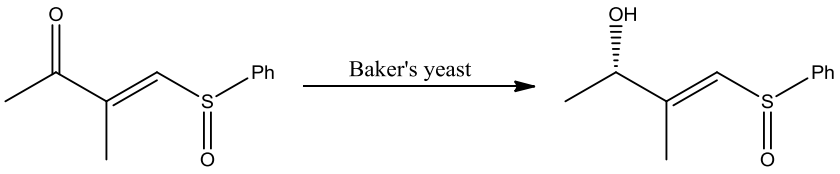
(Yield 38%, >99% ee)

(−)-**110**

Scheme 1.30

Crout *et al.* also investigated the baker's yeast reduction of α,β-unsaturated ketones with sulfinyl functionality.[84] In contrast to the corresponding vinyl sulfide **63** discussed earlier in section 1.2.1.4, the major product isolated when the corresponding sulfoxide **111**

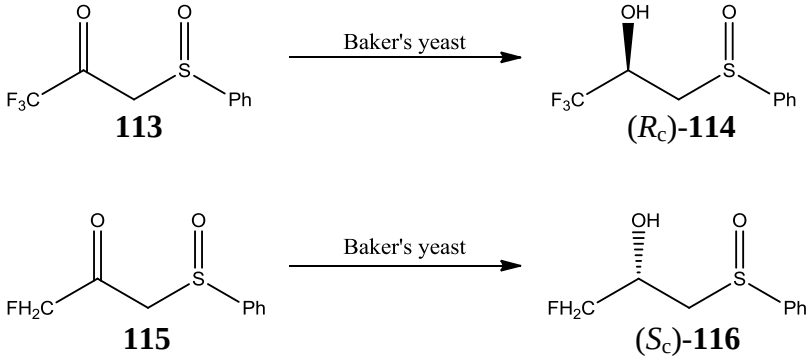
was reduced was the unsaturated alcohol **112**, in only 19% yield, though there were traces of both the corresponding sulfide and sulfone also isolated (Scheme 1.31). The alcohol **112** was isolated as a diastereomeric mixture (64% de) of enantiomerically pure compounds. Further studies showed that the reduction was stereoselective with respect to reduction of the carbonyl group and that there was partial discrimination between the two sulfoxide enantiomers.



(±)-**111** **112**

Scheme 1.31

Reduction of the β-ketosulfoxide with a trifluoromethyl moiety **113** afforded a 87 : 13 mixture of diastereomers with the major diastereomer (R_c)-**114** obtained with low enantiopurity of 28% ee.[74] Reduction of the fluorinated compound **115** afforded (S_c)-**116** in 53% ee (Scheme 1.32).[74] The stereochemistry of the sulfur atom was not assigned.

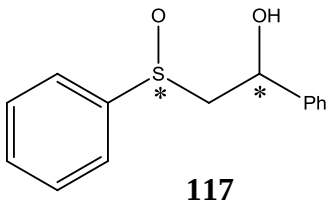


113 (R_c)-**114**

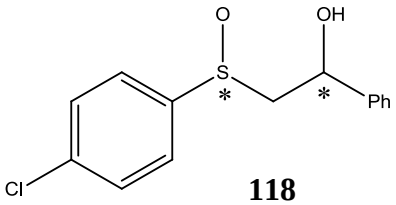
115 (S_c)-**116**

Scheme 1.32

Recently, all four stereoisomers of substituted phenylsulfinylpropan-2-ols **117** and **118** were prepared simultaneously for the first time from β-sulfinylketones based on the combination of baker's yeast and *Candida antarctica* lipase B with excellent enantioselectivities and high *syn*/*anti* ratios (Figure 1.5).[119] Advantageously this chemoenzymatic process employs a novel diisopropyl ether/limited water system with greatly simplified work-up relative to aqueous bioreductions.



117



118

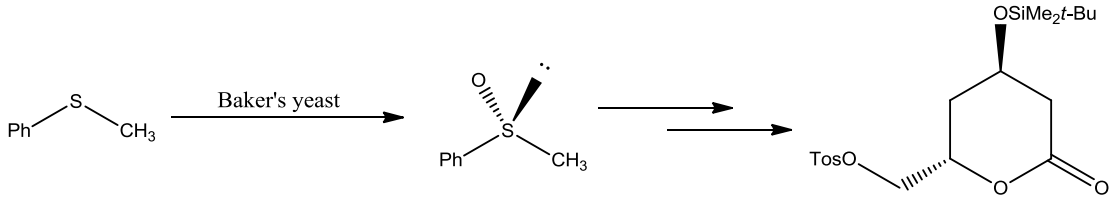
Product	ee (%)	*syn*/*anti*
(S_s,S_c)-**117**	>99	26 : 1
(S_s,R_c)-**117**	99	1 : 38
(R_s,S_c)-**117**	99	1 : 29
(S_s,S_c)-**117**	>99	12 : 1

Product	ee (%)	*syn*/*anti*
(S_s,S_c)-**118**	>99	53: 1
(S_s,R_c)-**118**	>99	1 : 19
(R_s,S_c)-**118**	93	1 : 8915
(S_s,S_c)-**118**	98	12 : 1

Figure 1.5

1.2.4.1 Asymmetric oxidation of sulfides by baker's yeast

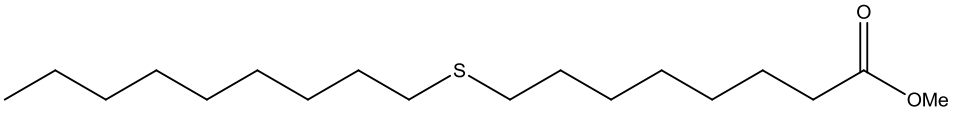
While asymmetric sulfur oxidation *via* the Kagan[120,121] and Modena[122] methods is well established, there have been some reports of biological asymmetric sulfide oxidation.[123-126] Although uncommon, a small number of baker's yeast mediated oxidation transformations of sulfides to sulfoxides have been reported.[89,127] Roberts obtained the (*R*)-sulfoxide (*R*)-**119** in good yield (60%) and high enantiopurity (92% ee) by baker's yeast mediated oxidation of the sulfide **120** under semi-anaerobic conditions (Scheme 1.33). This was subsequently employed in the synthesis of the hypocholestemic agent **121**.[128,129]



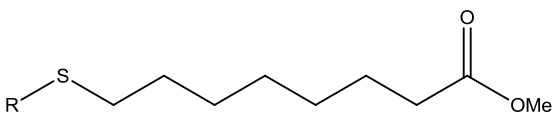
120 (*R*)-**119** *(60% yield, 92% ee)* **121**

Scheme 1.33

Under aerobic conditions, the thia-fatty acid **122** is converted to the (*R*)-sulfoxide by baker's yeast in >96% ee,[130-132] and the analogues **123** are similarly transformed (Figure 1.6).[133,134] Recently the effect of substrate chain length on the efficiency of the baker's yeast mediated sulfoxiation of 9-thia fatty acid methyl esters was investigated.[135]



122



123

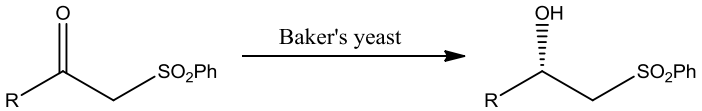
R = $C_6H_5CH_2$, $C_6H_5CH_2CH_2$, *p*-$CF_3C_6H_4CH_2$, *p*-$CF_3C_6H_4CH_2CH_2$

Figure 1.6

1.2.5 Baker's yeast mediated reduction of β-ketosulfones

Several examples of baker's yeast mediated reduction of β-ketosulfones have been reported, and these reactions generally proceed in a more efficient manner than their sulfide counterparts, giving synthetically versatile β-hydroxysulfones. Baker's yeast mediated reduction of simple acyclic β-ketosulfones has been reported to be dependent on the nature of the alkyl chain. Thus, as previously described 1-(phenylsulfonyl)propan-2-one **5a** afforded (*S*)-2-hydroxypropyl phenyl sulfone (*S*)-**6a** in 98% yield and >95% ee (Scheme 1.1 and entry 1, Table 1.9).[29] The rate of conversion was shown to depend on the ratio of substrate to sucrose.[29,31] However, it was found that the efficiency and stereoselectivity achieved in the baker's yeast reduction of β-ketosulfones **5a-f** decreased considerably as the alkyl chain length increased. Thus, **5b** afforded (*S*)-**6b** with 63% ee (entry 2, Table 1.9)[136] and gave (*S*)-**6c** with 46% ee (entry 3, Table 1.9),[137] whereas reduction of **5d** yielded only 10% of nearly racemic (*S*)-**6d (**entry 4, Table 1.9).[29] No reduction by baker's yeast was observed for **5e** (entry 5, Table 1.9). 1-Phenyl-2-(phenylsulfonyl)ethanone **5f** afforded upon reduction with baker's yeast (*R*)-**6f** (87% yield and 15% ee) (entry 6, Table 1.9).[25] Reduction with Sake yeast *(Saccharomyces cerevisiae)* kyokai-7, however, gave (*R*)-**6f** with 84% yield and 92% ee.[25]

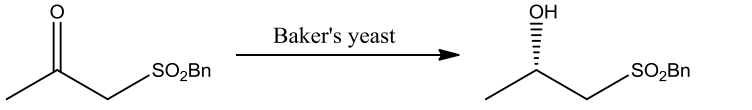
Table 1.9: The baker's yeast reduction of acyclic β-ketosulfones ***5***[25,29,136,137]



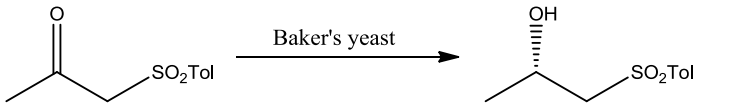
5 **6**

Entry	Ketone	R	Alcohol	Yield	ee (%)
1	**5a**	CH_3	(*S*)-**6a**	98	>95[29]
2	**5b**	CH_2CH_3	(*S*)-**6b**	90	63[136]
3	**5c**	*n*-C_3H_7	(*S*)-**6c**	53	46[137]
4	**5d**	*n*-C_5H_{11}	(*S*)-**6d**	10	10[29]
5	**5e**	*n*-C_6H_{13}	(*S*)-**6e**	-	-[137]
6	**5f**	C_6H_5	(*R*)-**6f**	87	15[25]

In addition to phenylsulfone, tolyl- and benzyl-substituted compounds have been investigated. The reduction of 1-(benzylsulfonyl)propan-2-one **124** gave the corresponding (*S*)-alcohol (*S*)-**125** in 38% yield, 95% ee while the *p*-tolyl analogue **126** was also reduced to afford the (*S*)-alcohol (*S*)-**127** in 97% ee (Scheme 1.34).[136]



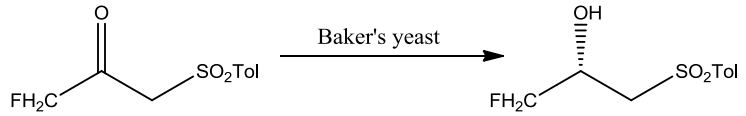
124 (*S*)-**125** *(38% yield, 95% ee)*



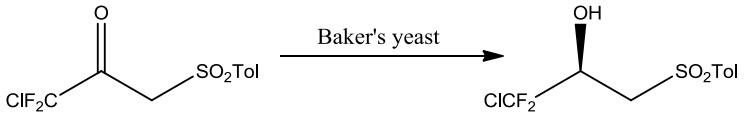
126 (*S*)-**127** *(84% yield, 97% ee)*

Scheme 1.34

The fluorinated derivative **128** was reduced by baker's yeast to the corresponding alcohol **129** with (*S*)-configuration and high enantioselectivity >97% ee[75] or >80% ee[76] (Scheme 1.35). However the reduction of 1-chloro-1,1-difluoro-3-(*p*-tolylsulfonyl)propan-2-one **130** afforded the alcohol **131** with predominantly (*R*)-configuration (78% ee) in 85% yield.[138]



128 (*S*)-**129** *(84% yield, >97% ee)*[75]



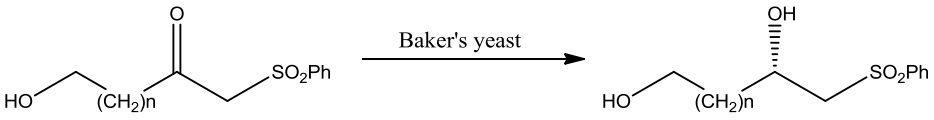
130 (*R*)-**131** *(85% yield, 78% ee)*

Scheme 1.35

It is evident from comparison of the baker's yeast mediated reduction of the *p*-tolylsulfonyl substituted 1-fluoropropan-2-one **128** with the analogous sulfoxides **115** (Scheme 1.32) and sulfides **38** (Scheme 1.9) that the presence of the electron withdrawing sulfone leads to much better selectivity. Furthermore, 1-chloro-1,1-difluoro-3-tosylpropan-2-one **130** was selectively reduced to provide the (*R*)-**131** alcohol in good enantiopurity (78% ee) while the closely related electron deficient sulfoxide **113** (Scheme 1.32) and sulfide **40** (Scheme 1.9) derivatives underwent limited to no reduction.

Introduction of a hydroxyl group at the terminal carbon of the alkyl chain not only improved the enantioselectivity relative to the unsubstituted analogues especially in the longer alkyl chain compounds but also simplified conversion of the products into optically active lactones (Table 1.10).[139] For example, 7-hydroxy-1-phenylsulfonylheptan-2-one **132e** afforded the corresponding (*S*)-diol (*S*)-**133e** in 84% yield and 72% ee compared to 1-phenylsulfonylheptan-2-one **5d** which on reduction gave the (*S*)-alcohol (*S*)-**6d** in 10% yield and 10% ee (entry 4, Table 1.9).[29]

Table 1.10: The baker's yeast reduction of acyclic β-ketosulfones ***132***[139]



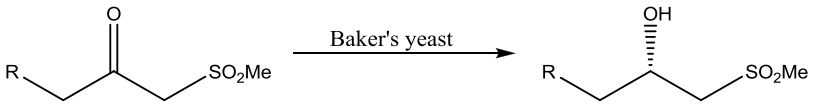
132 (*S*)-**133**

Entry	Ketone	n	Alcohol	Yield	ee (%)
1	**132a**	1	(*S*)-**133a**	87	99
2	**132b**	2	(*S*)-**133b**	42	94
3	**132c**	3	(*S*)-**133c**	74	93
4	**132d**	4	(*S*)-**133d**	39	96
5	**132e**	5	(*S*)-**133e**	84	72

In contrast, baker's yeast mediated reduction of the analogous methanesulfonyl derivatives **134a-c** exhibited greater stereoselectivity even with longer alkyl chains, leading to recovery of the corresponding (*S*)-alcohols **135a-c** with higher enantiopurity than the

corresponding benzenesulfonyl derivatives (Table 1.11).[140] However, the extent of reaction was generally low in the transformations, with poor recoveries of **135a-c** obtained.

*Table 1.11: The baker's yeast reduction of methanesulfonyl derivatives **134**[140]*

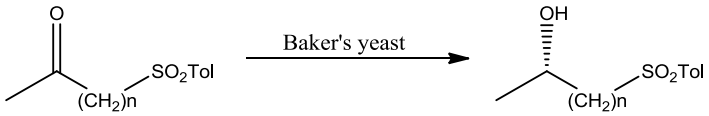


134 (*S*)-**135**

Entry	Ketone	R	Alcohol	Yield	ee (%)
1	**134a**	*n*-C_4H_9	(*S*)-**135a**	12	71
2	**134b**	*n*-C_5H_{11}	(*S*)-**135b**	26	87
3	**134c**	*n*-C_6H_{13}	(*S*)-**135c**	14	76

Baker's yeast mediated reduction of keto derivatives bearing sulfone functionalities in more remote positions have also been reported (Table 1.12). For example Gopalan found that the efficiency and selectivity of baker's yeast mediated reduction of acyclic ketosulfone derivatives **136a-c** decreased as distance between the keto and sulfone groups increased.[39] The hydroxysulfones (*S*)-**137a-b** obtained in these reactions were subsequently employed as chiral intermediates for the syntheses of enantiomerically pure parasorbic acid and the pheromone (*S*)-(+)-2-tridecanol.

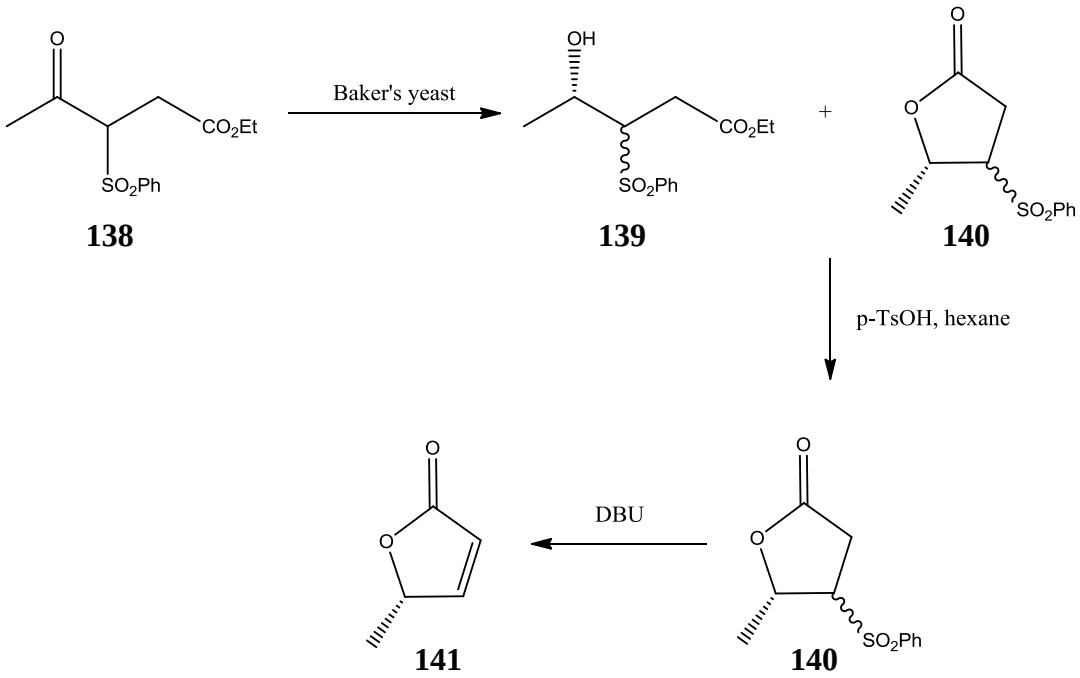
*Table 1.12: The baker's yeast reduction of remote sulfone functional groups **136**[39]*



136 (*S*)-**137**

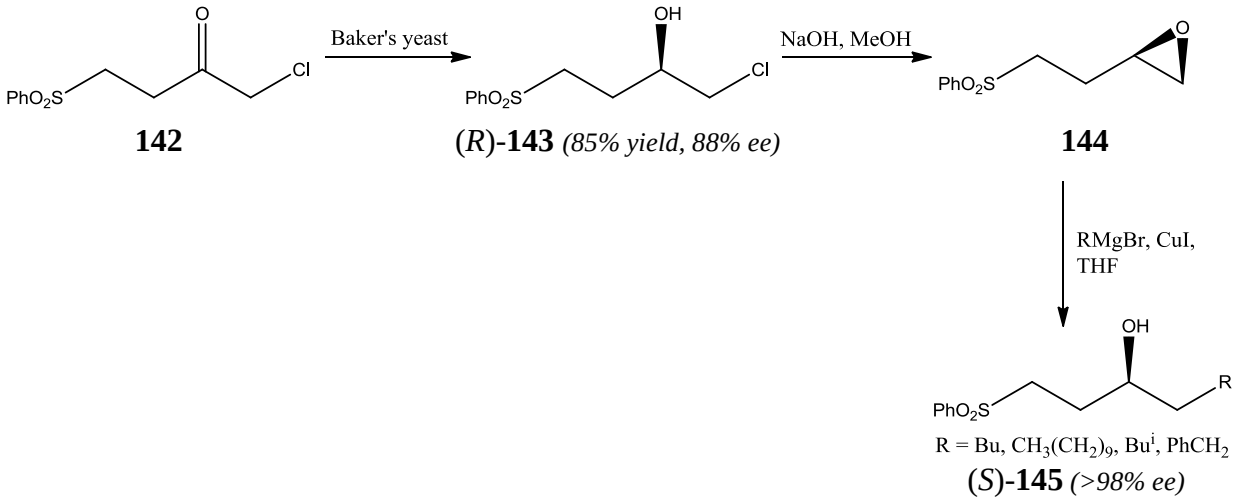
Entry	Ketone	n	Alcohol	Yield	ee (%)
1	**136a**	2	(*S*)-**137a**	68	98
2	**136b**	3	(*S*)-**137b**	44	98
3	**136c**	4	(*S*)-**137c**	5	81

Enantioselective baker's yeast mediated reduction of β-ketosulfone derivatives has also been employed in the synthesis of a number of useful chiral target molecules, for example, treatment of **138** with baker's yeast gave a complex mixture of products, including the alcohol **139** and the lactone **140**; treatment of the crude product mixture with *p*-toluenesulfonic acid gave **140** in good yield (Scheme 1.36).[37] This was subsequently employed in the synthesis of (*S*)-angelica lactone **141**, a versatile synthetic intermediate, which was obtained in high enantiomeric purity (% ee not recorded). Huet has also reported the preparation of several lactones in high enantiomeric excess from (*S*)-1-(phenylsulfonyl)-3-butanol obtained by baker's yeast mediated resolution.[141]



Scheme 1.36

Tanikaga and co-workers investigated the baker's yeast reduction of α-chloroketone derivatives such as **142**, to afford the corresponding alcohol (*R*)-**143** (Scheme 1.37).[137,142,143] The reduction proceeded cleanly with excellent recovery (85%) of the hydroxysulfone (*R*)-**143**. Though the enantiomeric purity of the isolated alcohol was only 88% ee, it could be recrystallised to give (*R*)-alcohol (*R*)-**143** in its enantiomerically pure state which can be transformed further by chemical methods to the epoxide **144** and subsequent alkylation with Grignard reagents provides the chiral alcohols (*S*)-**145** (>98% ee) without racemisation.

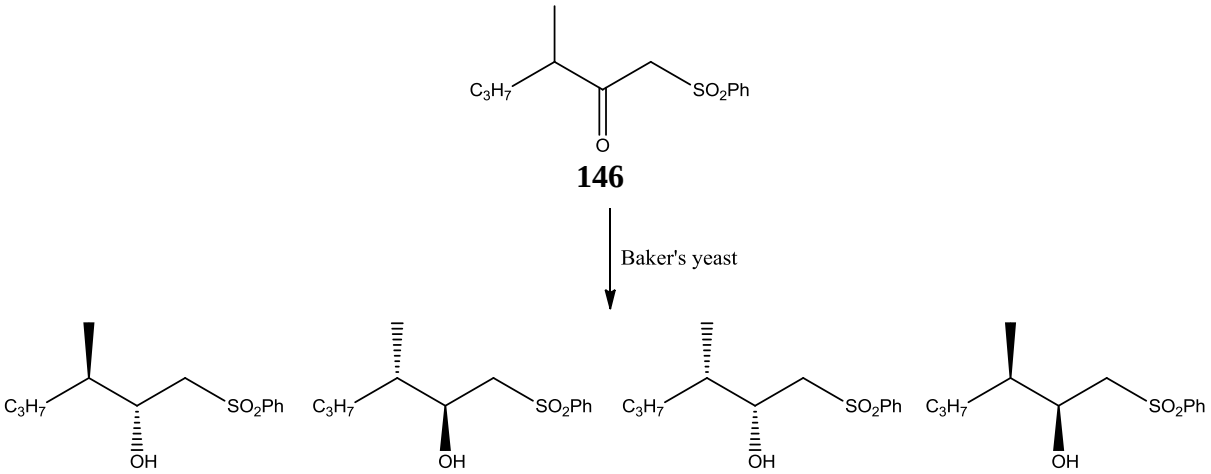


Scheme 1.37

Svatoš *et al.* recently investigated the microbial reduction of 3-methyl-1-(phenylsulfonyl)hexan-2-one **146** with more than 20 yeasts.[144] While *Saccharomyces cerevisiae* resulted in very poor conversion to the alcohol, 72% of starting material being recovered, other yeasts, *e.g. Candida guillermondii*, *C. zeylanoides* and *Kloeckera apiculata*

proceeded with excellent *re*-face stereoselectivity, >99% ee in each case, with the (*R*)-enantiomer of the starting material generally reacting faster as outlined in Table 1.13.

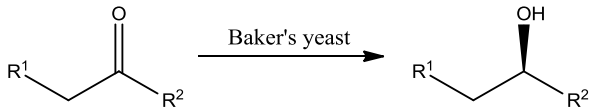
Table 1.13: The baker's yeast reduction of 3-methyl-1-(phenylsulfonyl)hexan-2-one ***146*** *by selected yeasts*[144]



Entry	Yeast	146	Recovered yields (%)			
			(2*R*,3*R*)-147	(2*S*,3*S*)-147	(2*R*,3*S*)-147	(2*S*,3*R*)-147
1	*Candida guillermondii*	46	0	10	0	43
2	*Candida zeylanoides*	4	0	50	0	45
3	*Hansenula anomala*	4	1	42	0	53
4	*Kloeckera apiculata*	34	0	10	0	55
5	*S. uvarum*	52	3	7	3	35
6	*S. cerevisiae*	72	9	3	4	13

Yuan *et al.* reported a facile method for the preparation of β-hydroxysulfones **148a-h** and β-hydroxysulfides **148i-n** in medium to high yields and excellent enantiopurity.[119] These bioresolutions were performed in a novel diisopropyl ether/limited water system as previously described for resolution of all four stereoisomers of phenylsulfinylpropan-2-ols **117** and **118** (Figure 1.5). As evident in Table 1.14 when R^2 was methyl, substituted phenylsulfonylpropan-2-ols (*S*)-**149a-e** were obtained with excellent enantioselectivities except for **148d,** due to the poor solubility of **148d** in diisopropyl ether. With the increasing steric hindrance of R^2, both the yield and enantiomeric purity decreased. Substituted phenylthiopropan-2-ones **148i-n** were also reduced, however longer reaction times were required presumably due to the poor electron withdrawing ability of the phenylthio group.

*Table 1.14: The baker's yeast reduction of sulfur-containing ketones **148**[119]*

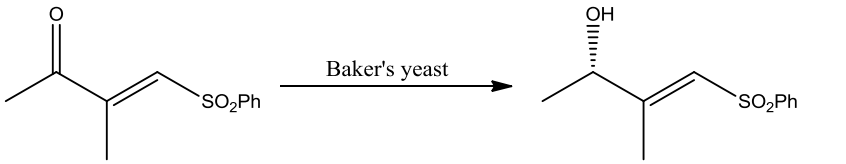


148 (*S*)-**149**

Entry	Ketone	R^1	R^2	Time	Alcohol	Yield (%)	ee (%)
1	**148a**	$C_6H_5SO_2$	CH_3	4 h	(*S*)-**149a**	99	99
2	**148b**	4-$CH_3C_6H_4SO_2$	CH_3	5 h	(*S*)-**149b**	95	99
3	**148c**	4-$CH_3OC_6H_4SO_2$	CH_3	5 h	(*S*)-**149c**	85	99
4	**148d**	4-$NO_2C_6H_4SO_2$	CH_3	4 h	(*S*)-**149d**	47	96
5	**148e**	4-$ClC_6H_5SO_2$	CH_3	4 h	(*S*)-**149e**	77	99
6	**148f**	$C_6H_5SO_2$	C_2H_5	10 h	(*S*)-**149f**	91	93
7	**148g**	$C_6H_5SO_2$	C_3H_7	17 h	(*S*)-**149g**	74	70
8	**148h**	$C_6H_5SO_2$	C_6H_5	20 h	**149h**	-	-
9	**148i**	C_6H_5S	CH_3	12 h	(*S*)-**149i**	97	95
10	**148i**[a]	C_6H_5S	-CH_3	24 h	**149i**	35	-
11	**148j**	4-$CH_3C_6H_4S$	CH_3	12 h	(*S*)-**149j**	96	99
12	**148k**	4-$CH_3OC_6H_4S$	CH_3	12 h	(*S*)-**149k**	86	99
13	**148l**	4-ClC_6H_5S	CH_3	18 h	(*S*)-**149l**	67	98
14	**148m**	4-$NO_2C_6H_4S$	CH_3	22 h	(*S*)-**149m**	92	96
15	**148n**	4-BrC_6H_4S	CH_3	17 h	(*S*)-**149n**	73	97

c. 0.5 g **148i** in 600 mL baker's yeast suspension (water).

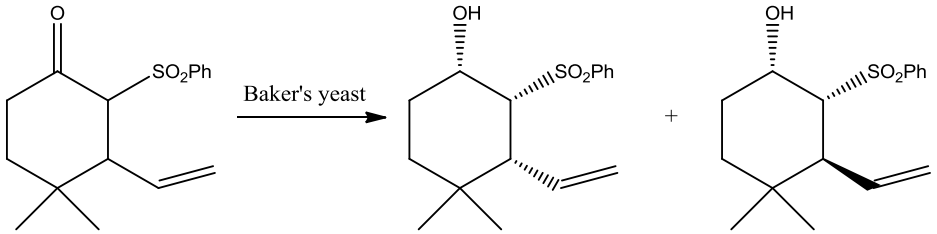
Crout *et al.* completed their investigation of the baker's yeast mediated reduction of α,β-unsaturated ketones with γ-sulfur functionalities with the vinyl sulfone **150** (Scheme 1.38).[84] The major product of the reduction was the corresponding alcohol (*S*)-**151** in excellent yield (75%) and in enantiopure form. In this case, the rate of reduction of the carbonyl functionality by the yeast oxidoreductases was much greater than that of the carbon-carbon double bond. As discussed earlier, the yeast reduction of a vinyl ketone (Scheme 1.17 and 1.31) is dependent on the substituents attached to the carbon-carbon double bond, however, Crout has shown here that it is also dependent on the oxidation level on the sulfur atom.



150 (*S*)-**151***(75% yield, >99% ee)*

Scheme 1.38

Fujisawa *et al.* reported one of the first baker's yeast mediated reduction of a cyclic β-ketosulfone.[145] The resolution of the highly substituted α-benzenesulfonylcyclohexanone derivative **152** proceeded with excellent efficiency (69% combined yield) to give a 45 : 55 mixture of the two diastereomers (1*S*,2*R*,3*S*)-**153** and (1*S*,2*R*,3*R*)-**153** (Scheme 1.39). Both isomers were isolated with excellent enantiopurity (>99% ee).

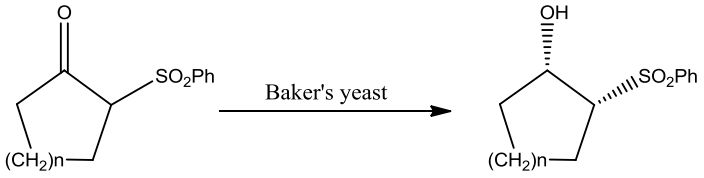


152 (1*S*,2*R*,3*S*)-**153** (1*S*,2*R*,3*R*)-**153**

Scheme 1.39

Recently, efficient dynamic kinetic resolution in the baker's yeast reduction of 2-benzenesulfonylcycloalkanones was reported (Table 1.15).[146] Although cyclopentanol and cyclohexanol derivatives were formed with excellent diastereo- and enantiocontrol, reduction of the 7- and 8-membered ring analogues was much less efficient. This is consistent with the trend observed for 2-carbethoxycycloalkanones, where decreased reduction efficiency with increasing ring size was reported.[29,71]

*Table 1.15: The baker's yeast reduction of 2-benzenesulfonylcycloalkanones **154**[146]*

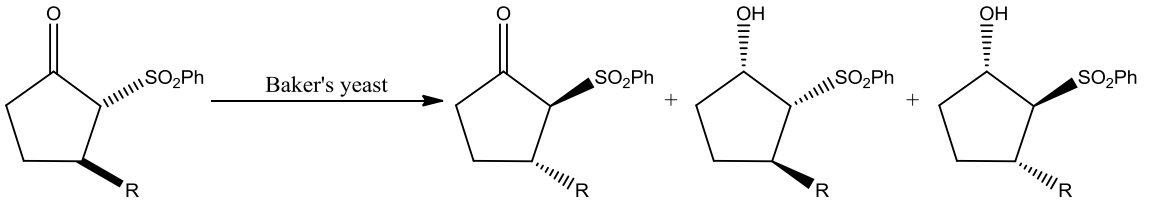


154 (*S*)-**155**

Entry	Ketone	n	Alcohol	Yield	ee (%)	dr
1	**154a**	1	(*S*)-**155a**	95	>98	98 : 2
2	**154b**	2	(*S*)-**155b**	78	>95	>98 : 2
3	**154c**	3	(*S*)-**155c**	8	-	>98 : 2
4	**154d**	4	(*S*)-**155d**	-	-	-

More complex ketosulfones are reduced just as efficiently; for example the β-substituted α-benzenesulfonylcyclopentanones **156a-g** resulted in extremely efficient kinetic resolution generating the (1*S*,2*R*)-cyclopentanols **157a-g** and recovery of the (2*S*)-cyclopentanones **156a-g** (Table 1.16).[147,148] By variation of the conditions employed for the yeast reduction the efficiency, enantio- and diastereoselectivity of the transformation have been optimised for each of the cyclopentanone derivatives in particular for the 3-methyl, ethyl and propyl substituted substrates **156b**, **c** and **d** respectively (entries 2-4, Table 1.16). However, extension of the alkyl chain resulted in dramatic decreases in both the efficiency and the enantioselectivity of the yeast mediated reduction.

Table 1.16: Efficient kinetic resolution of 2-benzenesulfonylcyclopentanone derivatives ***156*** via *baker's yeast mediated reduction*[147]



156a-g (2*S*,3*R*)-**156a-e** (2*S*,3*S*)-**156f-g** (*1S*,2*R*,3*S*)-**157a-e** (*1S*,2*R*,3*R*)-**157f-g** (1*S*,2*S*,3*R*)-**157a-e** (1*S*,2*S*,3*S*)-**157f-g**

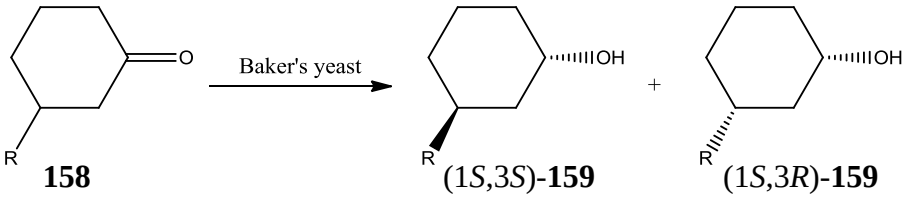
Major *Minor*

Entry	R	Cyclopentanone 156			Cyclopentanol 157			dr[a]
			Yield (%)	ee (%)		Yield (%)	ee (%)	
1	H	**156a**	~4	-	(1*S*,2*R*)-**157a**	79	>95	97 : 3
2	Me	(2*S*,3*R*)-**156b**	10	>95	(*1S*,2*R*,3*S*)-**157b**	29	>95	82 : 18
3	Et	(2*S*,3*R*)-**156c**	28	>95	(*1S*,2*R*,3*S*)-**157c**	40	>95	94 : 6
4	^{n}Pr	(2*S*,3*R*)-**156d**	26	95	(*1S*,2*R*,3*S*)-**157d**	36	>95	93 : 7
5	^{n}Bu	(2*S*,3*R*)-**156e**	34	60	**157e**	32	>95	>98 : 2
6	Ph	(2*S*,3*S*)-**156f**	20	86	(*1S*,2*R*,3*R*)-**157f**	31	>95	>98 : 2
7	CH_2Ph	(2*S*,3*S*)-**156g**	-	-	(*1S*,2*R*,3*R*)-**157g**	22	>95	95 : 5

a. Diastereomeric ratio (dr) refers to the ratio of major (1*S*,2*R*)-**157a-g**/minor (1*S*,2*S*)-**157a-g** diastereomeric cyclopentanols determined from ^{1}H NMR spectra.

Tanikaga *et al.* reported the reduction of cyclohexanones **158a-e**, containing a functional group at C-3 (Table 1.17).[149] Baker's yeast mediated reduction of 3-(nitromethyl)-, 3-(phenylsulfonyl)-, and 3-[(phenylsulfonyl)methyl]- cyclohexanones **158a**, **c** and **d** led to the delivery of a hydride to the *re*-face of the prochiral ketones to provide cyclohexanols (1*S*,3*S*)- and (1*S*,3*R*)-**159a**, **c** and **d** according to the Prelog rule, with high enantioselectivities and in good yield (entries 1, 3 and 4, Table 1.17). In contrast, 3-butylcyclohexanone **158e** (entry 5, Table 1.17) was found to be unreactive towards baker's yeast, and 3-(phenylthio)cyclohexanone **158b** (entry 2, Table 1.17) was reduced with poor enantioselectivity and with very low yield, indicating that the remote nitro and sulfonyl groups may play an important role in binding to an enzyme.

Table 1.17: The baker's yeast reduction of cyclohexanones ***158***[149]



158 (1*S*,3*S*)-**159** (1*S*,3*R*)-**159**

Entry	Ketone	R	(1*S*,3*S*)-159			(1*S*,3*R*)-159		
				Yield	ee (%)		Yield	ee (%)
1	**158a**	CH_2NO_2	(1*S*,3*S*)-**159a**	46	>99	(1*S*,3*R*)-**159a**	43	>98
2	**158b**	SPh	(1*S*,3*S*)-**159b**	6	5	(1*S*,3*R*)-**159b**	9	6
3	**158c**	SO_2Ph	(1*S*,3*S*)-**159c**	45	>99	(1*S*,3*R*)-**159c**	40	>99
4	**158d**	CH_2SO_2Ph	(1*S*,3*S*)-**159d**	41	90	(1*S*,3*R*)-**159d**	38	95
5	**158e**	*n*-C_4H_9	(1*S*,3*S*)-**159e**	-	-	(1*S*,3*R*)-**159e**	-	-

1.3 Conclusion

Both the efficiency and stereoselectivity in baker's yeast mediated reduction of ketones is strongly influenced by both the presence and the position of sulfur moieties or indeed by use of sulfur-containing additives (Scheme 1.5 and 1.15). The oxidation level of the sulfur substituent has a powerful impact on the outcome of the yeast reduction, and for example the reduction of 2-sulfonylketones is in general very successful and significantly more effective than the analogous sulfide and sulfoxide derivatives. This is clearly evident in the baker's yeast mediated reduction of α-sulfur functionalised acetone (Scheme 1.1) and 1-fluoropropan-2-one derivatives (Scheme 1.9, 1.32 and 1.35) where the highest yield and enantioselectivity was obtained in reduction of the β-ketosulfone substrates relative to the sulfide or sulfoxide derivatives. Furthermore, the synthetic application of the reduction of ketosulfoxides is limited by the kinetic resolution of the sulfur atom. Interestingly in all cases [β-ketosulfide (Scheme 1.8), β-ketosulfoxide (Scheme 1.29) and β-ketosulfone (Table 1.9)], very little reduction occurs on extension of the alkyl (R) chain length (Figure 1.6).

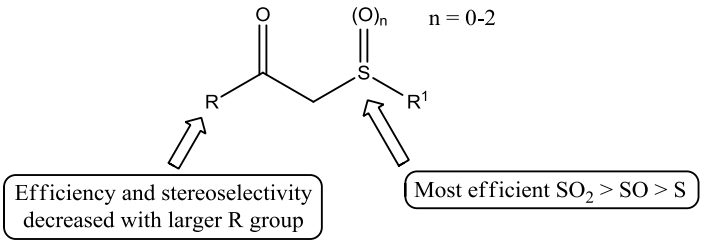


Figure 1.6

Reduction of sulfur-containing alkenes by yeast is also possible. Reduction of the α,β-unsaturated ketone with γ-sulfide, sulfoxide and sulfone moieties has been described (Figure 1.7). Notably, carbonyl bond reduction was observed in all substrates but olefin bond reduction was dependent on the oxidation state of the sulfur atom and only occurred with the sulfide. Thus, baker's yeast reduction of unsaturated sulfide (Scheme 1.17) and sulfoxide (Scheme 1.31) derivatives both lead to a mixture of diastereomers while the sulfone yielded the unsaturated alcohol as the sole product in excellent yield and enantiopurity (Scheme 1.38). Furthermore, the nature and position of the double bond was observed to affect the stereochemical course of the baker's yeast reduction of sulfides (Scheme 1.19-1.21).

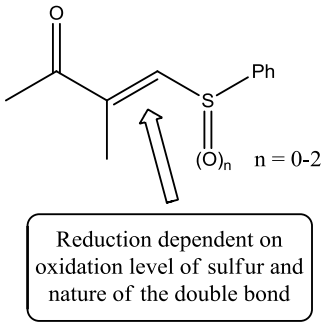


Figure 1.7

Thus, it is apparent that use of the sulfone moiety as a substituent to influence the efficiency and stereoselectivity in ketone reduction is substantially more effective than the analogous sulfide and sulfoxide moieties. Combination with chemical methods provides a powerful tool for the enantioselective synthesis of optically active natural products.

1.4 References

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Chapter 2

Baker's yeast mediated asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-1 and (*S*)-1

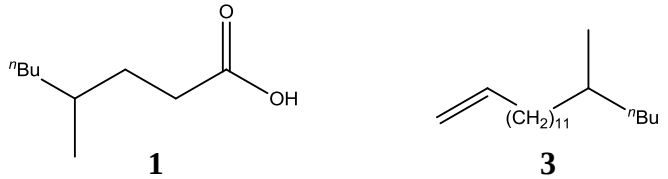
Contents

2.1 Background to the project **46**
2.2 Objectives of the project **47**
2.3 Synthesis of substrates for baker's yeast mediated reduction **47**
2.3.1 Synthesis of β-keto sulfone **48**
2.3.2 Synthesis of α-diazo-β-keto sulfone **49**
2.3.3 Synthesis of cyclopentanone **51**
2.3.4 Synthesis of cyclopentanol **53**
2.4 Kinetic resolution in the baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2 ..... **54**
2.5 Asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acids (*R*)-1 and (*S*)-1 ..... **59**
2.5.1 Access to the complimentary enantiomeric series of cyclopentanone *via* Dess-Martin oxidation . **61**
2.5.2 Base-induced ring cleavage of the cyclopentanone derivative **64**
2.5.3 Reductive desulfonylation **66**
2.6 Project conclusion **71**
2.7 References **73**

2.1 Background to the project

The synthesis and kinetic resolution of 2-benzenesulfonyl substituted cyclopentanone derivatives *via* baker's yeast (*Saccharomyces cerevisae*) mediated reduction was initially explored by Kelleher.[1] Over the last 15 years, significant developments within the research group have effectively extended this work to generate a wide variety of synthetically powerful, highly enantioenriched cyclopentanones and cyclopentanols, which have been employed in the asymmetric synthesis of insect pheromones.[2-7]

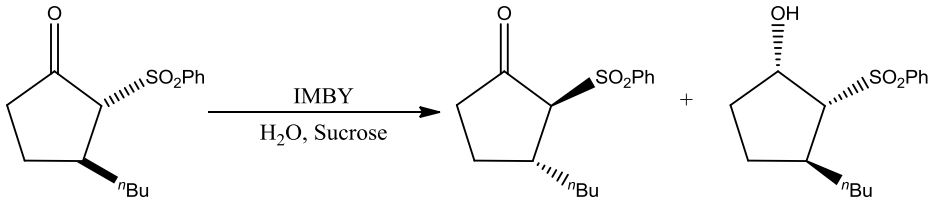
While the baker's yeast reductions of many racemic *trans*-3-substituted-2-sulfonyl cyclopentanones proceed with excellent diastereo- and enantioselectivity, poor efficiency and kinetic resolution were observed in the baker's yeast catalysed resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**.[1,2] The moderate enantioselectivity observed of this key chiral precursor limited the application of this kinetic resolution in the asymmetric synthesis of the aggregation pheromone of the rhinoceros beetles of the genus *Oryctes*, 4-methyloctanoic acid **1** and of the sex pheromone of the peach leafminer moth *Lyonetia clerkella* Linné, 14-methyloctadecene **3**, compounds for which the stereochemistry generally dictates the behavioural response of the insect (Figure 2.1).[1,2,8]



1 **3**

Figure 2.1

Significantly, preliminary investigations by Kelly[3] and later exploration by Milner[8] have demonstrated efficient immobilised baker's yeast (IMBY) mediated reduction of the (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** substrate in water with repeated sucrose additions, albeit with poor recovery of the enantiopure products (2*S*,3*R*)-**2** and (1*S*,2*R*,3*S*)-**4** (Scheme 2.1).



trans (±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4**

Scheme 2.1

Prompted by the success of the kinetic resolution of the substrate (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**, the primary aim of this work was to optimise the synthetic utility of this biocatalytic process on a preparative scale, thereby obtaining access to synthetically useful quantities of the enantiopure building blocks cyclopentanone (2*S*,3*R*)-**2** and cyclopentanol (1*S*,2*R*,3*S*)-**4**. Access to both enantiomeric series in high enantiomeric purity was envisaged, and application of this chemoenzymatic resolution to the asymmetric synthesis of the natural insect pheromone 4-methyloctanoic acid **1** would be subsequently explored (Scheme 2.2).

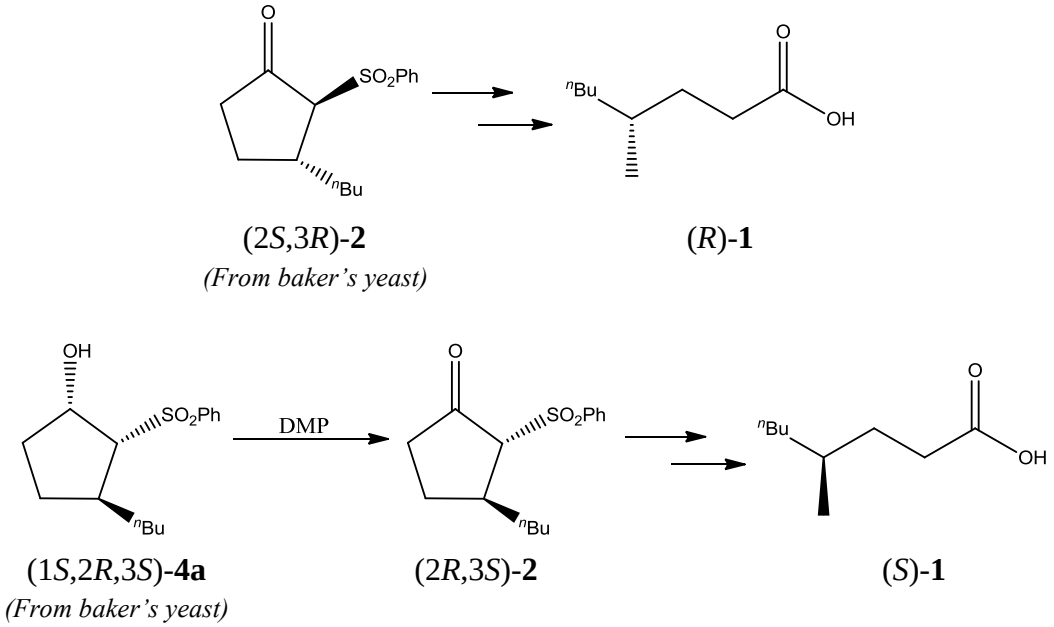
2.2 Objectives of the project

The specific aims of this project may be summarised as follows:

- To synthesise multi-gram quantities of the desired racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**, the targeted substrate for the biocatalytic study.

- To achieve efficient baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** (Scheme 2.1) to obtain synthetically useful quantities of the generated cyclopentanol (1*S*,2*R*,3*S*)-**4a** and the recovered cyclopentanone (2*S*,3*R*)-**2** in excellent enantiopurity.

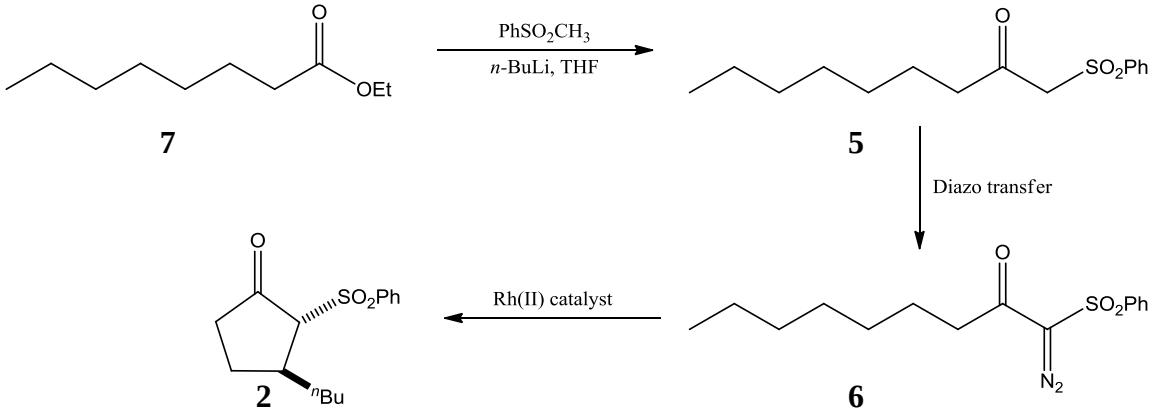
- To demonstrate the synthetic potential of this chemoenzymatic process in the asymmetric synthesis of both enantiomers of the aggregation pheromone of the rhinoceros beetles of the genus *Oryctes*, 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** (Scheme 2.2).



Scheme 2.2

2.3 Synthesis of substrates for baker's yeast mediated reduction

Highly regioselective carbenoid C-H insertion reactions of α-diazocarbonyl compounds mediated by rhodium(II) catalysts lead to very efficient cyclopentanone formation. This mild and effective C-H insertion was chosen as a synthetic route for the preparation of racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**, the desired substrate for baker's yeast reduction. The first synthetic challenge in this study involved preparation of the β-keto sulfone **5**, followed by diazo transfer to afford the analogous α-diazo-β-keto sulfone **6**, and subsequent cyclisation to the desired cyclopentanone (±)-**2** (Scheme 2.3).

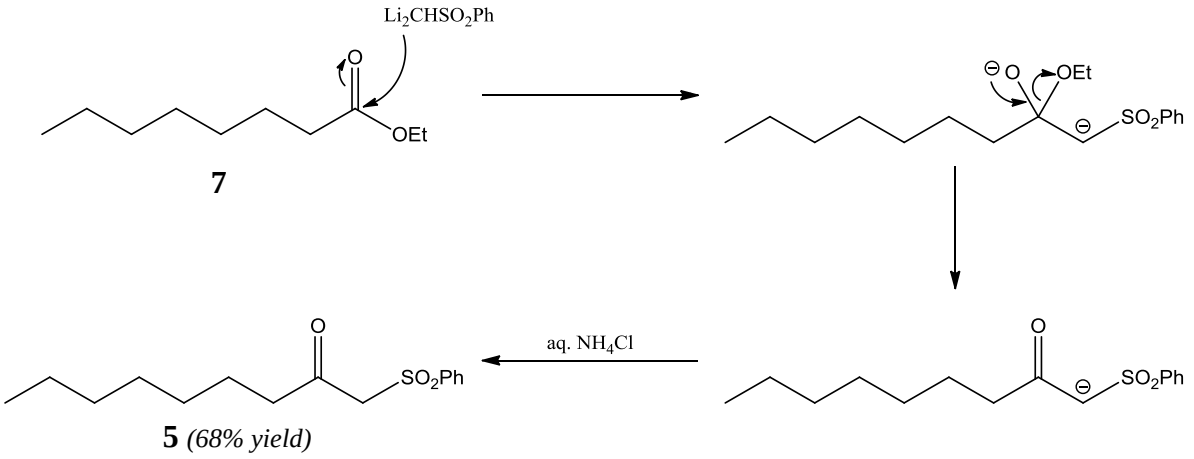


Scheme 2.3

2.3.1 Synthesis of β-keto sulfone

The synthesis of β-keto sulfone products has been achieved by a variety of methods within the research group including the Corey and Chaykovsky sodium hydride mediated procedure.[1,2,9,10] In this study, *n*-butyllithium was employed for the synthesis of the targeted 1-benzenesulfonylnonan-2-one **5**.[11-13] For this purpose, *n*-butyllithium (2.0 equivalents) was added to a solution of methyl phenyl sulfone **8** (readily prepared in high yield by oxidation of thioanisole **9** with hydrogen peroxide)[14], in tetrahydrofuran (THF) at 0 °C. The resulting yellow solution was stirred for 1.5 h at 0 °C before addition of ethyl octanoate **7** in THF to the reaction mixture.

Use of 2.0 equivalents of *n*-butyllithium in this reaction serves to generate the dilithio derivative of methyl phenyl sulfone **8**. Nucleophilic attack of the dilithio species at the ester carbonyl group, with the displacement of the ethoxy group, results in direct generation of the deprotonated β-keto sulfone derivative, which, upon protonation with aqueous ammonium chloride during work-up, provides the desired 1-benzenesulfonylnonan-2-one **5** (Scheme 2.4) This *n*-butyllithium-mediated procedure is highly advantageous relative to other methods employed previously within the research group.[1,2,9,10] This one-step protocol requires only 1.0 equivalent of methyl phenyl sulfone **8** in contrast to the 2.0 equivalents necessary in the Corey and Chaykovsky sodium hydride procedure and consequently chromatographic purification is simplified due to the lower quantities of unreacted methyl phenyl sulfone **8** remaining in the reaction mixture.



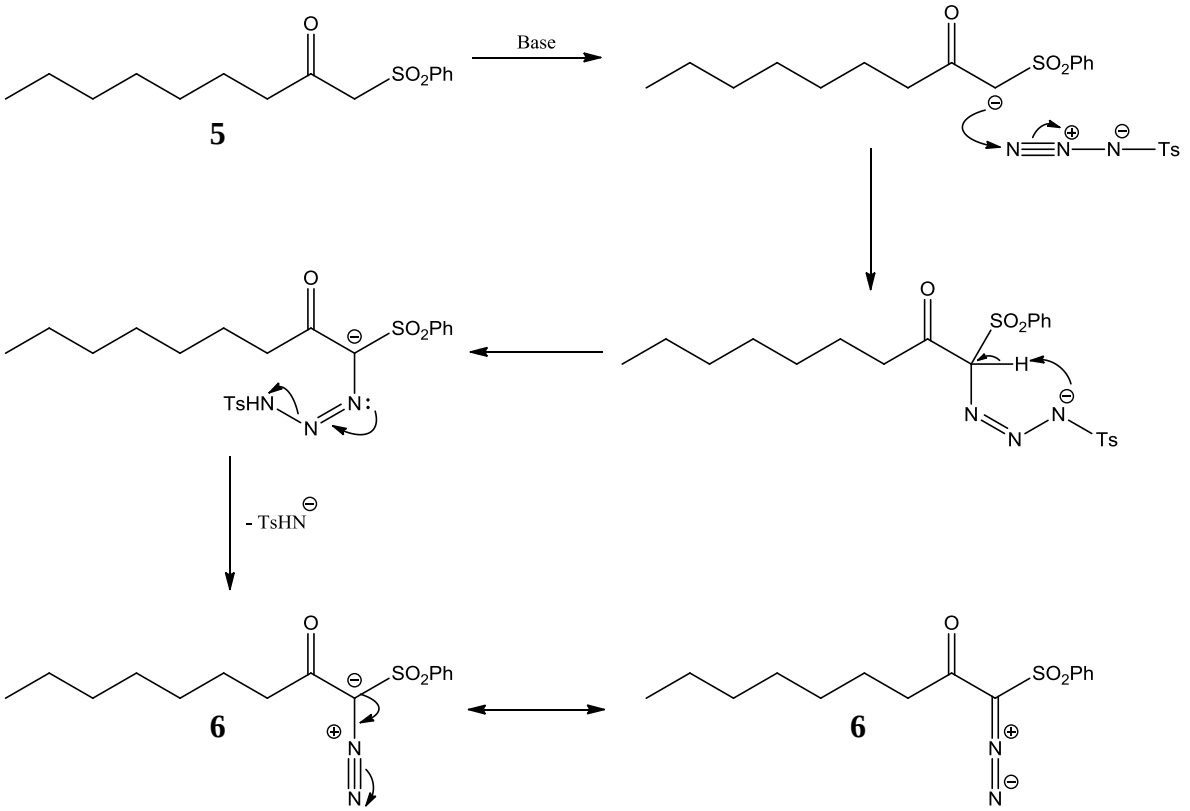
Scheme 2.4

This synthetic protocol allowed for ready access to multi-gram quantities of the desired 1-benzenesulfonylnonan-2-one **5**. The β-keto sulfone **5** was isolated by chromatographic purification as a white crystalline solid in good yield (68%) and excellent purity. The product is characterised by carbonyl and sulfonyl absorptions in the IR spectrum of the β-keto sulfone **5** at ν_{max} 1717 cm^{-1} and ν_{max} 1301 (symmetric stretch), 1153 (asymmetric stretch) cm^{-1}, respectively, and by the appearance of a characteristic singlet in the ^1H NMR spectrum at δ_H 4.14 ppm assigned to the $C(1)H_2$ methylene protons adjacent to both the carbonyl and sulfonyl groups. All spectral characteristics of **5** were consistent with those previously reported by Kelleher,[1] O'Keeffe[2] and Milner.[8]

2.3.2 Synthesis of α-diazo-β-keto sulfone

The application of α-diazocarbonyl compounds is now widely recognised as a valuable synthetic tool in modern organic synthesis.[15-18] These versatile reagents undergo a variety of transformations including insertion, cyclopropanation, ylide formation and Wolff rearrangement.[18] One of the fundamental methods of synthesising α-diazocarbonyl compounds, the diazo transfer reaction was developed by Regitz in 1967.[19] Such reactions involve the base-mediated transfer of the diazo moiety from a donor to the active α-methylene position of a suitable carbonyl derivative. A base of sufficient strength is required to deprotonate the substrate yet mild enough to avoid undesired side reactions, *e.g.* triethylamine, sodium hydroxide and potassium carbonate. To ensure that the α-methylene position is sufficiently acidic to allow efficient reaction, the presence of a second electron withdrawing group, in addition to the carbonyl group is required, thus β-diketones, β-keto esters and β-keto sulfones are generally suitable substrates.

The diazo transfer reagent of choice in this study was *p*-toluenesulfonyl (tosyl) azide **10** owing to its ease of preparation and high efficiency.[20] However, it is potentially explosive, possessing a high impact sensitivity and low initiation temperature, thus extreme caution must be exercised in its use.[21] Furthermore, difficulties have been experienced in the chromatographic separation of the desired α-diazo-β-keto sulfone from excess **10** and the *p*-toluenesulfonylamide byproduct following diazo transfer.[22] During this research, *p*-tosyl azide **10** was prepared in high yield (79%) according to the procedure outlined by Curphey using sodium azide and *p*-tosyl chloride in acetone at 0 °C and utilised without further purification.[20] It should be noted that spectral characterisation of *p*-tosyl azide **10** was not conducted during this study due to the hazardous nature of the diazo transfer reagent. The mechanism of the diazo transfer is illustrated in Scheme 2.5.



Scheme 2.5

During this research, efficient diazo transfer to the β-keto sulfone **5** was achieved employing potassium carbonate and *p*-tosyl azide **10** in acetonitrile according to the procedure first described by Koskinen.[23] A solution of *p*-tosyl azide **10** in acetonitrile was added dropwise to a mixture of potassium carbonate and 1-benzenesulfonylnonan-2-one **5** in acetonitrile at 0 °C. The reaction progress was monitored by TLC and diazo transfer was generally complete within 4 h of stirring at room temperature. The reported advantage of this method was that work-up consisted simply of the addition of a non-polar organic co-solvent to the crude reaction mixture and subsequent filtration to remove the inorganic salt and the sulfonamide byproduct. In practice however, the presence of residual *p*-tosyl amide necessitated further purification by column chromatography. The *p*-tosyl amide byproduct is evident in the ^{1}H NMR spectrum of the crude product **6** as a singlet at δ_H 2.43 ppm, indicative of the methyl protons of the toluene substituent and in the aromatic region at δ_H 7.31 ppm (d, *J* 8.4) and δ_H 7.81 ppm (d, *J* 8.4). The removal of the *p*-tosyl amide and any residual **10** is often a difficult task due to the close elution of these compounds and several rounds of chromatographic separation were often required.

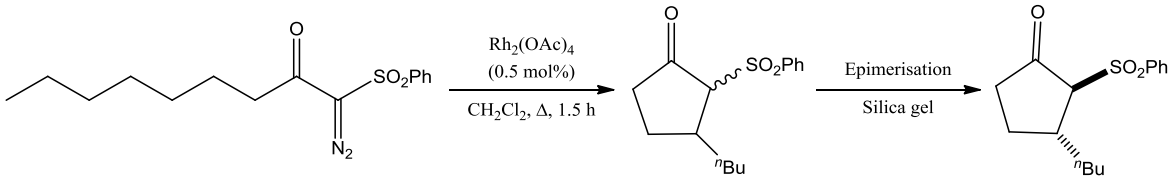
The α-diazo-β-keto sulfone **6** was isolated as a low-melting yellow solid (m.p. 44-45 °C) in high yield (89%) and excellent purity as evident by the new carbonyl stretch in the IR spectrum at ν_{max} 1662 cm^{-1}, a significant shift from the corresponding band in the spectra of the β-keto sulfone **5** (ν_{max} 1717 cm^{-1}). Characteristic absorptions are also observed in the IR spectrum at ν_{max} 2129 cm^{-1}and ν_{max} 1339 (symmetric stretch), ν_{max} 1153 (asymmetric stretch) cm^{-1} representing the diazo and sulfonyl groups, respectively. Spectral analysis by ^{1}H NMR indicated the absence of the singlet at δ_H 4.14 ppm indicative of the diazo functionality replacing the two C(1)H$_2$ protons of the β-keto sulfone **5**. Spectral characteristics of 1-diazo-1-benzenesulfonyl-nonan-2-one **6** were consistent with those reported by Kelleher[1], O'Keeffe[2] and Milner.[8] Multi-gram quantities of the desired α-diazo-β-keto sulfone **6** were

readily accessible *via* this synthetic methodology and the material can be stored for extended periods in the freezer without decomposition.

2.3.3 Synthesis of cyclopentanone

Intramolecular rhodium(II) catalysed carbenoid C-H insertion reactions of α-diazocarbonyl compounds lead to very efficient cyclopentanone formation, offering excellent regioselectivity in the remote functionalisation of an unactivated C-H bond.[15,16,18,24,25] This synthetic methodology was originally developed by Taber,[26-29] with α-diazo-β-keto esters, while Monteiro[30] later extended this work to include α-diazo-β-keto sulfones. Intramolecular metal-catalysed C-H insertions are known to lead to the preferential formation of five-membered rings and, when no more than one cyclopentane ring can form, these reactions proceed with excellent regioselectivity.[27] In terms of stereoselectivity, *trans*-cyclopentanone products are generally produced, and insertion into equatorial C-H bonds is usually favoured over axial C-H insertion.

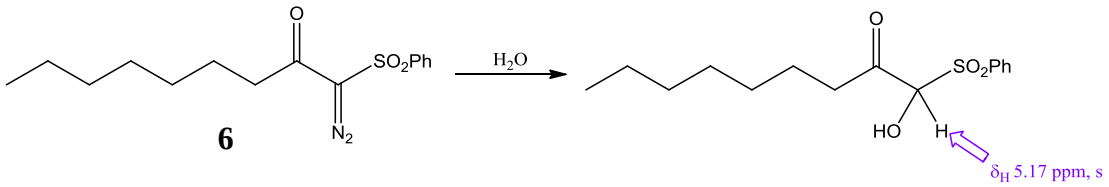
In this study, the racemic cyclopentanone **2** required for baker's yeast mediated reduction was prepared by rhodium(II) acetate catalysed intramolecular C-H insertion of the α-diazo-β-keto sulfone **6** in dichloromethane at reflux. This cyclisation involves insertion of an electrophilic metal carbene into a methylene site adjacent to an *n*-butyl moiety. Rhodium(II) acetate catalysed decomposition of α-diazo-β-keto sulfone **6** involved a single addition of 0.5 mol% of the catalyst providing the cyclopentanone **2** as an initial mixture 38 : 62 of *cis* : *trans* products and solely *trans* in 55% yield following column chromatography on silica gel (Scheme 2.6). The moderate yield may be attributable to low catalyst loading, Kelleher[1] and O'Keeffe[2] previously reported two 0.5 mol% additions of rhodium(II) acetate to the reaction mixture over a 48 h reaction period, while Slattery[10] described a single addition of 1.0 mol% of the catalyst and a reaction time of 0.5 h.



6 *38 : 62* *cis : trans* **2** *(55% yield)*

Scheme 2.6

A minor amount of competing O-H insertion into adventitious water was observed in the rhodium(II) acetate catalysed C-H insertion reaction. The O-H byproduct is characterised by the appearance of a 1H singlet at δ_H 5.17 ppm in the ^{1}H NMR spectrum of the crude product **2** (Scheme 2.7) in accordance with literature reports for similar hydroxyl compounds.[31,32] Significantly, this byproduct was no longer observed following chromatographic purification.



Scheme 2.7

Reactions were monitored by IR analysis, where reaction completion was indicated by the disappearance of peaks at ν_{max} 2129 cm^{-1} (CN_2) and ν_{max} 1662 cm^{-1} (CO) characteristic of the α-diazo-β-keto sulfone **6** and the appearance of the cyclopentanone **2** carbonyl absorption at ν_{max} 1751 cm^{-1}. The rhodium(II) catalysed C-H insertion reaction was complete within 1.5 h and the crude cyclopentanone **2** was isolated by evaporation of the reaction solvent dichloromethane.

While excellent regioselectivity was observed in these transformations stereocontrol was less efficient with the *cis* isomer accounting for 38% of the crude product. The two diastereomers were easily distinguished by ^{1}H NMR; the signal for the *trans* C(2)H proton adjacent to the sulfonyl substituent appeared as a doublet at δ_H 3.38 ppm (d, *J* 6.6). For the *cis* diastereomer of the cyclopentanone **2** the corresponding signal was observed at δ_H 3.73 ppm (d, *J* 7.8) (Figure 2.2)

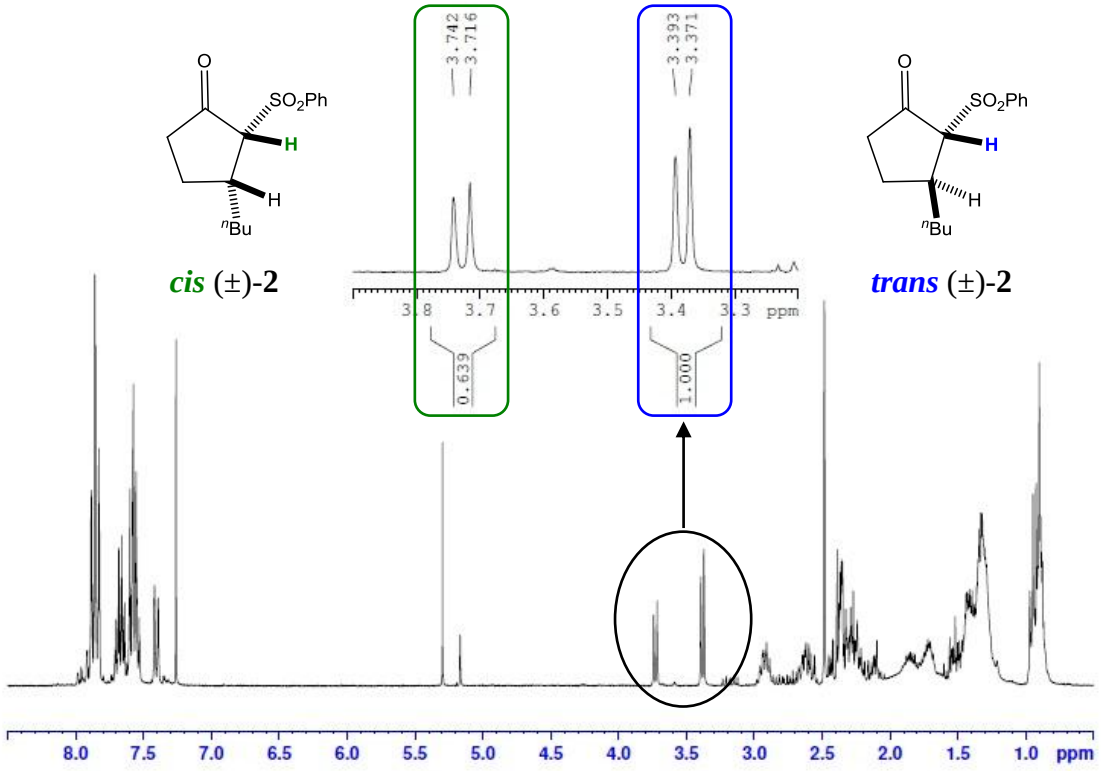
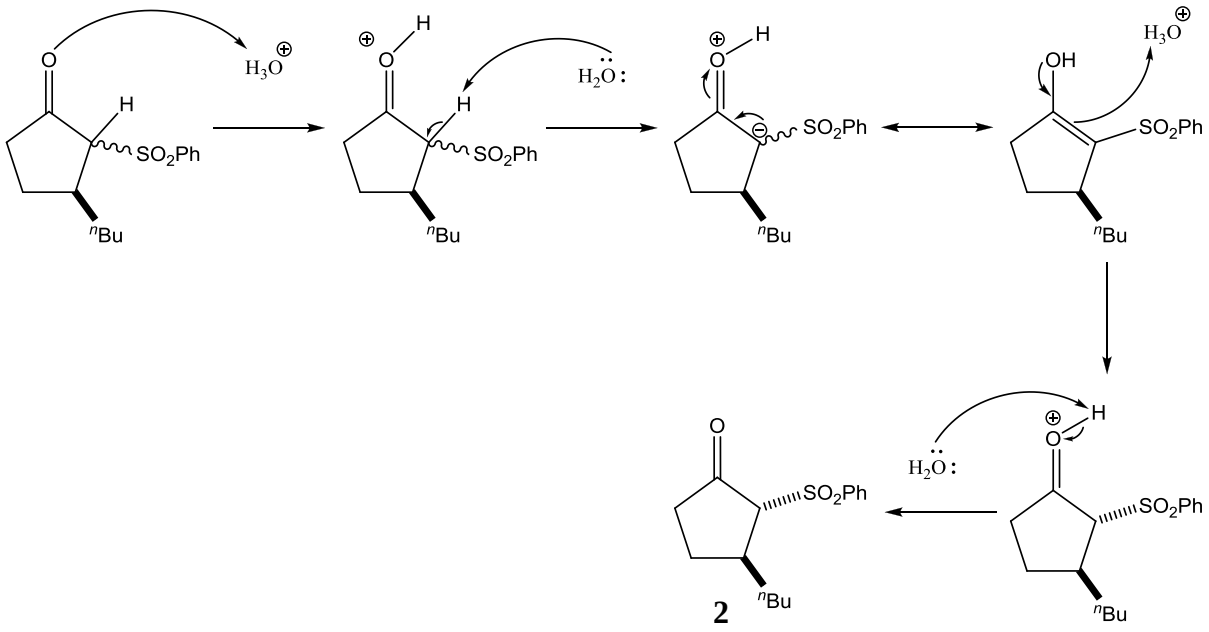


*Figure 2.2: ^{1}H NMR spectrum of crude (±)-*cis*- and (±)-*trans*-2-benzenesulfonyl-3-n-butylcyclopentanone (±)-**2** isolated from rhodium(II) catalysed C-H insertion reaction recorded in $CDCl_3$ at 300 MHz.*

While the crude product may contain a mixture of *cis* and *trans* isomers, epimerisation to form exclusively the thermodynamically more stable *trans* isomer is readily achieved owing to the acidity of the proton α to the benzenesulfonyl moiety (pK_a ~10) (Scheme 2.8). Hence, following column chromatography on silica gel, (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** was exclusively obtained.



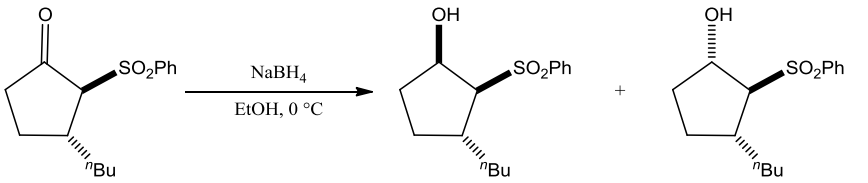
Scheme 2.8

Following chromatographic purification, the racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** was isolated as a light yellow solid in 55% yield. Spectral characteristics for the isolated cyclopentanone (±)-**2** are in agreement with previously reported data[i] and the *trans* isomer was assigned the relative stereochemistry (2*R**,3*S**).[1,2] Characteristic carbonyl and sulfonyl absorptions are observed in the IR spectrum at ν_{max} 1751 cm^{-1} and ν_{max} 1305 (symmetric stretch) and ν_{max} 1152 (asymmetric stretch) cm^{-1}, respectively. As mentioned earlier, the ^1H NMR spectrum of the *trans* cyclopentanone (±)-**2** includes a distinct doublet due to the C(2)H proton geminal to the sulfonyl group observed at δ_H 3.39 ppm. Coupling with the C(3)H proton with *J* 6.9 Hz is consistent with a *trans* arrangement of the C(2) and C(3) protons. The signal for the cyclopentanone proton at C(3) appeared as a multiplet in the region of δ_H 2.79-2.99 ppm. This reaction was readily conducted on a mult-gram scale, providing sufficient material for the subsequent baker's yeast mediated reduction.

2.3.4 Synthesis of cyclopentanol

A racemic sample of (±)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (±)-**4** was required for comparison of spectral characteristics with the enantiomerically enriched cyclopentanol **4** obtained by baker's yeast mediated reduction and also to aid in the development of methods for the determination of the enantiomeric purity of the enantioenriched cyclopentanol **4** by chiral HPLC. The racemic cyclopentanol (±)-**4** was readily prepared by sodium borohydride reduction of the corresponding racemic *trans* cyclopentanone (±)-**2**. A solution of the *trans* cyclopentanone (±)-**2** in distilled ethanol was added dropwise to 1.9 equivalents of sodium borohydride and ethanol at 0 °C to yield the desired cyclopentanol **4** as a white crystalline solid in high yield (75%) (Scheme 2.9).

[i] Significant difference in ^1H NMR chemical shift and multiplicity of the CH_3 signal is observed between Milner and spectral data obtained during this study. Milner reports, δ_H (400 MHz) 1.29 (3H, d, *J* 7, CH_3),[8] while in this research the methyl protons appear as a triplet further upfield, δ_H (300 MHz) 0.89 (3H, t, *J* 6.9, CH_3). Milner also does not describe the chemical shifts of the 3 x CH_2, *n*-butyl protons. ^1H NMR assignment in this study is consistent with O'Keeffe[2] and Kelleher.[1]



2 (1*R**,2*S**,3*R**)-**4a** (1*R**,2*R**,3*S**)-**4b**

Major *Minor*

dr 97 : 3

Scheme 2.9

The reduction proceeded with high stereocontrol as the bulky benzenesulfonyl functionality directs the approach of the reducing agent to the opposite, less hindered face, resulting in the newly formed hydroxyl group having a *cis* relationship to the sulfonyl moiety. The relative stereochemistry of the major diastereomer formed was assigned as (1*R**,2*S**,3*R**). This assignment was established unequivocally by Kelleher, by X-ray analysis of diastereomerically pure **4a**.[1] However small amounts (~3%) of the minor diastereomer **4b** was also detected in the ^{1}H NMR spectrum by the appearance of a multiplet at δ_H 4.63-4.66 ppm attributable to the C(1)H proton geminal to the hydroxyl group. The formation of the minor product **4b** with the hydroxyl group *trans* to the benzenesulfonyl moiety resulted from hydride approach from the more hindered face of the cyclopentanone **2**. Kelleher assigned the relative stereochemistry of the minor diastereomer **4b** as (1*R**,2*R**,3*S**).[1]

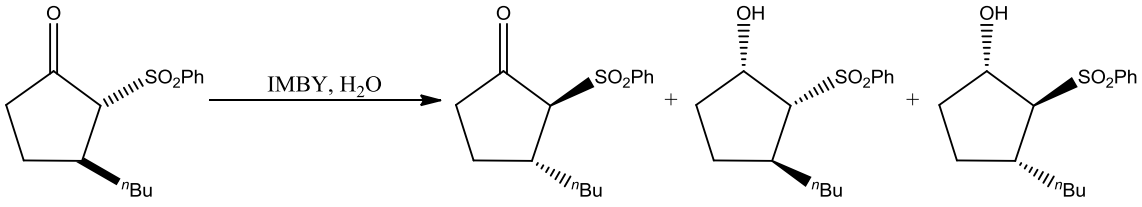
Successful product synthesis was indicated in the IR spectrum by the disappearance of the intense absorption band of the cyclopentanone (±)-**2** carbonyl stretch at ν_{max} 1751 cm^{-1} and observation of a distinct broad OH stretch at ν_{max} 3499 cm^{-1}, while the characteristic sulfonyl asymmetric and symmetric stretching vibrations appear at ν_{max} 1302 and 1141 cm^{-1}, respectively. In the ^{1}H NMR spectrum the C(2) proton geminal to the sulfonyl group appears as a doublet of doublets at δ_H 3.03-3.07 ppm. The coupling constants for this signal are consistent with a *cis* arrangement (*J* 4.5 Hz) between the protons C(1) and C(2), and the unchanged *trans* arrangement (*J* 9.0 Hz) of the protons at C(2) and C(3). The C(1) proton geminal to the hydroxyl group appear in the region δ_H 4.28-4.33 ppm. Spectral characteristics of cyclopentanol **4a** are consistent with those previously described for this compound.[1,2,8]

2.4 Kinetic resolution in the baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2

With a synthetic route to (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** established, the next step undertaken was investigation of the kinetic resolution process. The baker's yeast (*Saccharomyces cerevisae*) mediated biocatalytic reduction of the cyclopentanone (±)-**2** was initially explored by Kelleher[1] and O'Keeffe,[2] who observed significant reduction in diastereoselectivity and enantiopurity of the recovered (2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, indicating both inefficient reduction and kinetic resolution. Significantly, Kelly[3] discovered that the use of IMBY in an aqueous medium in conjunction with repeated sucrose addition over time yielded the desired (1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** in 37% yield and >98% ee, and critically, the unreacted (2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2** was recovered in 14% yield with >95% ee (entry 1, Table 2.1). Based on Kelly's[3]

promising one off result Milner[8] further explored the kinetic resolution under the IMBY optimised reaction conditions. Excellent enantiopurity of the generated cyclopentanol (1*S*,2*R*,3*S*)-**4a** (>98% ee) and the recovered cyclopentanone (2*S*,3*R*)-**2** (>98% ee) was attained, confirming Kelly's[3] preliminary observation. However, as evident from entry 2, Table 2.1 on increased scale of reaction (1.27 g), low recovery of the enantiopure products from the lipophilic beads, poor diastereoselectivity, and prolonged reaction times remained notable obstacles in the development of the synthetic utility of this biocatalytic resolution in the natural product asymmetric synthesis of 4-methyloctanoic acid **1**. Thus, one of the primary aims of this research was to address the practical limitations of the preparative scale-up of this biotransformation to obtain access to the enantio- and diastereomerically pure products (2*S*,3*R*)-**2** and (1*S*,2*R*,3*S*)-**4a** of the baker's yeast mediated kinetic resolution of (±)-**2** in synthetically useful quantities.

*Table 2.1: Baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-n-butyl cyclopentanone (±)-**2** with IMBY in water and with repeated addition of sucrose*[3,8,ii]



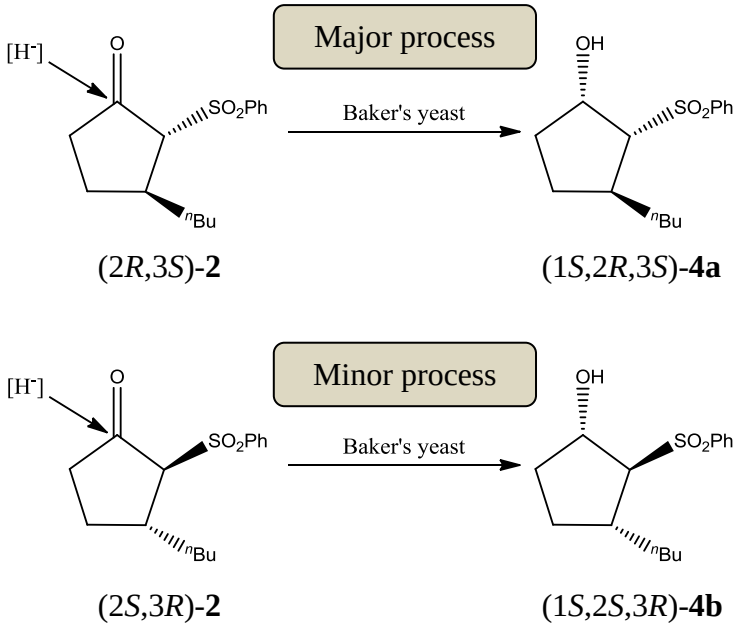
(±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4a** (1*S*,2*S*,3*R*)-**4b**

Entry	Reaction Conditions[a]	Scale (g)[b]	Reaction Time	Cyclopentanone (2*S*,3*R*)-2		Cyclopentanol (1*S*,2*R*,3*S*)-4a		Cyclopentanol (1*S*,2*S*,3*R*)-4b
				ee (%)	Isolated yield (%)[c]	ee (%)	Isolated yield (%)[c]	dr[f]
1[3]	IMBY-H_2O	0.40	84 h	>95[d]	14[g]	>98[d]	37[g]	-[h]
2[8]	IMBY-H_2O	1.27	74 h	>98[e]	5[g]	98[e]	16[g]	89 : 11

a. IMBY in water with repeated addition of sucrose over time at t = 0, 12, 18, 24, 36, 42 and 48 h.
b. Scale refers to the quantity of cyclopentanone (±)-**2** employed for the reaction.
c. Yields quoted are following chromatographic purification.
d. Enantiomeric excess (ee %) determined by chiral HPLC analysis using a Chiralcel® OD-H column.
e. Enantiomeric excess (ee %) determined (single injection) by chiral HPLC analysis using a Chiralcel® OJ-H column.
f. Diastereomeric ratio (dr) refers to the ratio of major (**4a**)/minor (**4b**) cyclopentanols determined from ^{1}H NMR spectra.
g. A maximum 50% yield is possible in the resolution.
h. Diastereomeric ratio (dr) was not reported.[3]

In the baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**, the (2*R*,3*S*)-enantiomer was selectively reduced and thus the major diastereomer formed was **4a** with the hydride approach to the carbonyl opposite to the bulky sulfonyl group in accordance with Prelog's prediction.[33] However, a minor amount of the diastereomeric cyclopentanol **4b** derived from partial reduction of the cyclopentanone (2*S*,3*R*)-**2** with the same absolute sense of hydride attack is also observed (Scheme 2.10). This minor reaction pathway is believed to be catalysed by a different yeast reductase to that which produces the major diastereomer (1*S*,2*R*,3*S*)-**4a** as Kelleher[1] and O'Keeffe[2] found that the amount of the minor diastereomer (1*S*,2*S*,3*R*)-**4b** present varied considerably with minor variation of the reaction conditions.

[ii] Milner recorded the results in the baker's yeast mediated resolution (entry 2, Table 2.1) as 10 and 32% respectively based on a maximum yield of 50%.[8] In this study, for consistency Milner's yields were recalculated based on actual recovery.

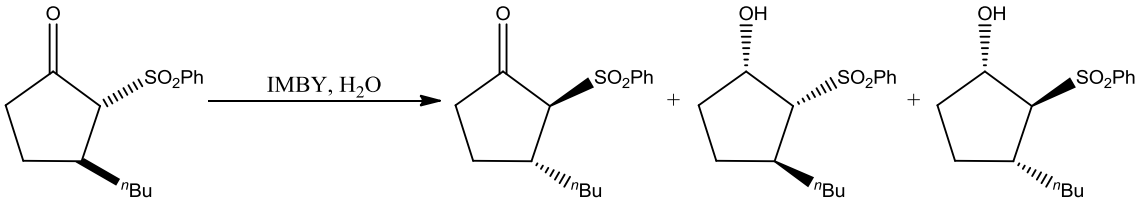


Scheme 2.10

Reductions with IMBY, particularly in conjunction with organic solvent systems, can offer significant synthetic advantages in terms of ease of product recovery from the reaction medium. Furthermore, immobilisation may also affect the efficiency and the stereochemical outcome of a reduction.[34-37] In order to prepare the IMBY utilised in this study, an aqueous suspension of sodium alginate was heated at 50 °C for 1 h, then allowed to cool to ambient temperature. Baker's yeast was added and the resultant mixture stirred for a further 2 h. Mechanical stirring was necessary, as magnetic stirring was rendered ineffective by the high viscosity of the alginate/yeast mixture. Dropwise addition of the resultant suspension, *via* a glass funnel of small outlet diameter (1-2 mm), to an aqueous solution (10%) of calcium chloride gave the required IMBY in the form of small beads (2-3 mm in diameter).[1-3,8,38] The IMBY could be stored for short periods in the fridge (~4 °C) but was generally utilised within 24 h.

The biocatalytic reduction procedure involved preparation of a suspension of IMBY and sucrose in tap water which was stirred at 28-30 °C for 30 minutes. The cyclopentanone substrate (±)-**2** in DMSO was then added dropwise over 1 minute, and the mixture was stirred at this temperature with repeated addition of sucrose over time to enable effective reduction. Reaction monitoring by chiral HPLC was conducted throughout the biotransformation, and on completion of the kinetic resolution, the beads were removed by filtration and washed with ethyl acetate. It was postulated that the poor yields observed by Kelly[3] and Milner[8] for this biotransformation were due to inadequate recovery of the crude reaction mixture trapped within the IMBY beads. Therefore, during this study the beads were firstly pressed and then sonicated at ~40 °C with ethyl acetate for 6-8 h. The beads were subsequently filtered, squeezed and ground with a pestle and mortar and then mechanically stirred in ethyl acetate overnight to ensure shearing of the IMBY beads. All organic extracts were combined and concentrated under reduced pressure.

*Table 2.2: Baker's yeast reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** – demonstrating the reproducibility of the biotransformation*



(±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4a** (1*S*,2*S*,3*R*)-**4b**

Entry	Reaction Conditions	Scale (g)[d]	Reaction Time	Cyclopentanone (2*S*,3*R*)-2		Cyclopentanol (1*S*,2*R*,3*S*)-4a		Cyclopentanol (1*S*,2*S*,*3R*)-4b
				ee (%)[e]	Isolated yield (%)	ee (%)[e]	Isolated yield (%)	dr
1	IMBY-H_2O[a]	2.07	23 h	99	25[f]	98	33	-[h]
2	IMBY-H_2O[b]	3.00	47 h 15 min	98	35	98	32	-[h]
3	IMBY-H_2O[c]	2.00	24 h	>98	12[g]	>98	18[g]	-[h]

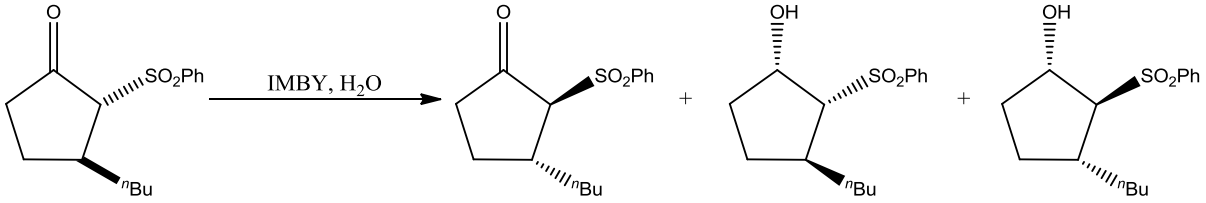
a. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
b. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h, 6 h, 8 h, 23 h 15 min, 26 h 15 min, 28 h 45 min and 41 h 45 min.
c. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
d. Scale refers to the quantity of cyclopentanone (±)-**2** employed for the reaction.
e. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
f. Purification by column chromatography was repeated, hence this contributed to a loss in yield.
g. Poor yield attributed to experimental error during work-up leading to physical loss.
h. The ^{1}H NMR of pure isolated (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** showed no evidence of the minor cyclopentanol (1*S*,2*S*,3*R*)-**4b** at δ_H (300 MHz) 4.63-4.66 (C***H***OH).

As evident from Table 2.2, efficient kinetic resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** was achieved during this research on a preparative scale [2.00-3.00 g of (±)-**2**]. The recovered (2*S*,3*R*)-cyclopentanone (2*S*,3*R*)-**2** and the generated (1*S*,2*R*,3*S*)-cyclopentanol (1*S*,2*R*,3*S*)-**4** were isolated with excellent enantiopurity (≥98% ee). In general, despite the removal of aliquots of reaction mixture for chiral HPLC reaction monitoring, a significant improvement in the yields of the enantiopure products was observed, with (2*S*,3*R*)-**2** and (1*S*,2*R*,3*S*)-**4** obtained in 35% and 32% respectively following chromatographic purification, owing to the implementation of the additional efficient extraction techniques (entry 2, Table 2.2). On increase of scale to 3.00 g of substrate (±)-**2** the kinetic resolution proved to be more sluggish requiring further additions of sucrose and prolonged reaction time of 47 h relative to the 2.00 g scale. The poor yield of (2*S*,3*R*)-**2** and (1*S*,2*R*,3*S*)-**4** observed in entry 3 Table 2.2 is attributed to experimental error during work-up.

As mentioned above, reaction monitoring was conducted throughout the biotransformations, for example Table 2.3 and Figure 2.3 correlate to chiral HPLC analysis of the preparative-scale (2.00 g) baker's yeast mediated resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** summarised in entry 3, Table 2.2. Aliquots of reaction mixture were withdrawn and following work-up chiral HPLC analysis was performed to determine enantiopurity and conversion. Chiral HPLC conditions were developed during this study to enable determination of the enantiomeric excess of the crude product mixture *i.e.* (2*S*,3*R*)-**2** and (1*S*,2*R*,3*S*)-**4a**, in a single reaction injection. As evident from entry 2, Table 2.3 the kinetic bioresolution is essentially complete within 9 h with excellent enantiopurity observed of both the recovered cyclopentanone (2*S*,3*R*)-**2** (>98% ee) and the generated cyclopentanol (1*S*,2*R*,3*S*)-**4a** (>98% ee). While extraction of the crude reaction mixture from the IMBY may be commenced at 9 h, in practice however, work-up of the baker's yeast media was only initiated at 24 h.

Significantly, the kinetic resolutions of (±)-**2** were completed in a much shorter reaction time relative to those reported by Milner[8] and Kelly[3] (Table 2.1). Critically, the minor cyclopentanol enantiomer (1*S*,2*S*,3*R*)-**4b** was not observed in the kinetic resolutions, by ^{1}H NMR or chiral HPLC analysis (Figure 2.3 and Figure 2.4). Thus, in this study excellent diastereoselectivity was achieved indicating that the shorter reaction time relative to previous researchers, and thus limited exposure to yeast decreased the extent of reduction of the (2*S*,3*R*)-cyclopentanone (2*S*,3*R*)-**2**.

*Table 2.3: Baker's yeast reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**: Monitoring over time*



(±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4a** (1*S*,2*S*,3*R*)-**4b**

Entry	Reaction Conditions[a]	Scale (g)[b]	Reaction Time	Cyclopentanone (2*S*,3*R*)-2		Cyclopentanol (1*S*,2*R*,3*S*)-4a		Cyclopentanol (1*S*,2*S*,3*R*)-4b
				ee (%)[c]	Isolated yield (%)	ee (%)[c]	Isolated yield (%)	dr
1	IMBY-H_2O	2.00	2 h	44	-	>98	-	-
2			4 h	98	-	>98	-	-
3			9 h	>98	-	>98	-	-
4			24 h	>98	12[d]	>98	18[d,e]	-[e]

a. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
b. Scale refers to the quantity of cyclopentanone (±)-**2** employed for the reaction.
c. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
d. A yield of 35% was obtained for pure (+)-(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, 98% ee and 32% for pure (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a**, 98% ee for a batch that was synthesised later (entry 2, Table 2.2).
e. The ^{1}H NMR of pure isolated (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** showed no evidence of the minor cyclopentanol (1*S*,2*S*,3*R*)-**4b** at δ_H (300 MHz) 4.63-4.66 (C***H***OH).

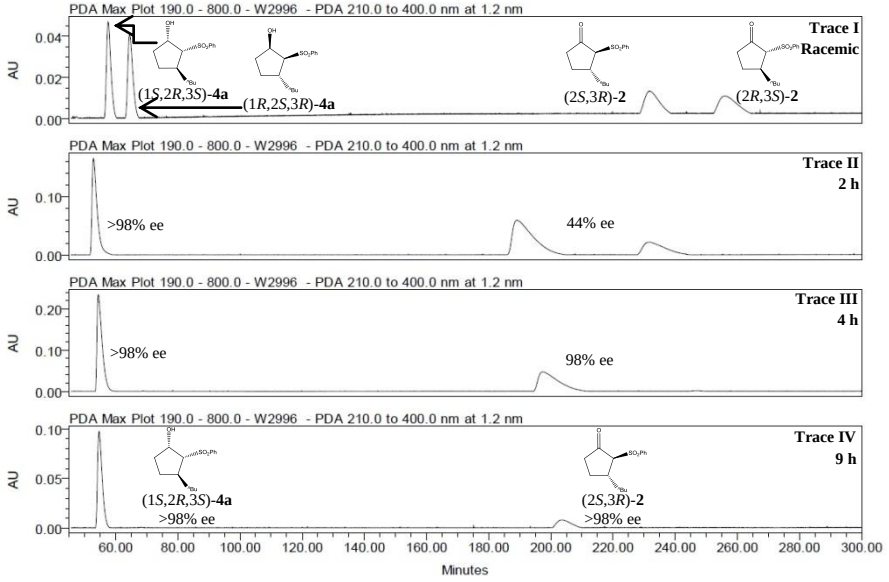


*Figure 2.3: HPLC **Trace I:** Racemic (±)-(1R*,2S*,3R*)-2-Benzenesulfonyl-3-*n*-butylcyclopentanol **4a** and (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**. **Trace II:** Reaction sampling 2 h. **Trace III:** Reaction sampling 4 h. **Trace IV:** Reaction sampling 9 h, (+)-(1S,2R,3S)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1S,2R,3S)-**4a**, >98% ee, (+)-(2S,3R)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2S,3R)-**2**, >98% ee. For HPLC conditions see appendix I.*

In summary, the baker's yeast mediated resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** was demonstrated on a preparative scale [2.00 – 3.00 g of precursor (±)-**2**], obtaining access to enantio- and diastereomerically pure (1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** and (2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2** in excellent purity (Figure 2.4), increased yield and with shorter reaction times. Thus, for the first time the practical limitations of the scale-up of this biotransformation have been addressed leading to synthetically useful quantities of the enantiopure building blocks (1*S*,2*R*,3*S*)-**4a** and and (2*S*,3*R*)-**2**.

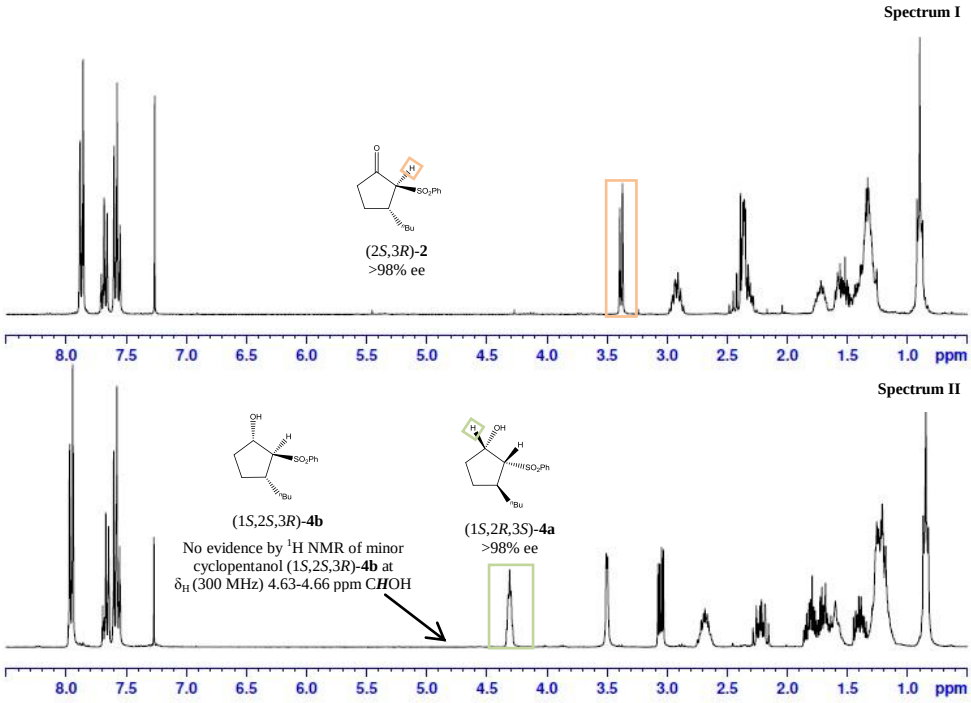


Figure 2.4: ¹H NMR ***Spectrum I:*** *(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, >98% ee.* ***Spectrum II:*** *(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a**, >98% ee. (All spectra recorded in $CDCl_3$ at 300 MHz).*

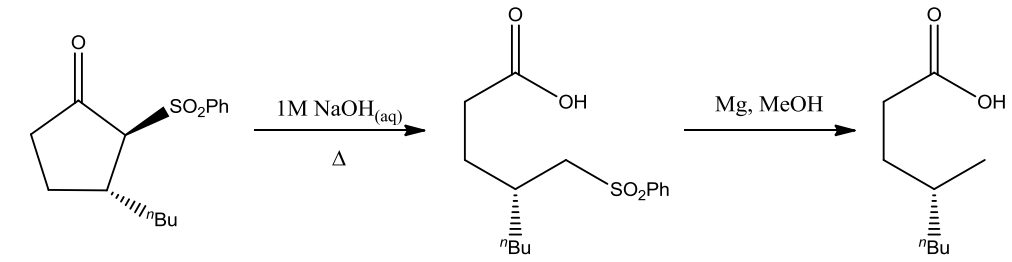
2.5 Asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acids (*R*)-1 and (*S*)-1

Rhinoceros beetles of the genus *Oryctes* are the main pests of coconut, oil and date palm plantations in Southeast Asia, North Africa, and some Pacific islands.[39-42] The adults burrow galleries into the growing points of palms killing the mature trees by defoliation or producing wounds that favour entry points for palm weevils or deadly diseases. Despite the use of high doses of insecticides (*e.g.*, carbofuran and cypermethrin) against most rhinoceros beetles, efficient and acceptable methods of controlling these insects are still lacking, because adults spend more of their life hidden in galleries and rapidly colonize new feeding and breeding sites, flying easily away from the initial colony.[43] The idea to manipulate adult populations by luring beetles into traps with specific attractants was investigated in the 1970s. The attractant chosen at that time, ethyl chrysanthemate, was rapidly abandoned because of insufficient catches. More recently, it was reported that the main pheromone emitted by males of most of the rhinoceros beetles of the genus *Oryctes* are 4-methyloctanoic acid **1** and its corresponding ethyl ester.[40] Both substances have been studied and proved to be powerful attractants in operational programs to control the major pest in oil palm plantations. Besides

its activity as a pheromone, 4-methyloctanoic acid **1** is also cited in the literature for its contribution to the aroma of various foods.[44-46] To date no information about the absolute configuration of naturally occurring 4-methyloctanoic acid **1** is available.

Several approaches have been reported for the synthesis of racemic 4-methyloctanoic acid **1**.[39,43,47-49] Conversely, the asymmetric synthesis of optically active (*R*)- and (*S*)-4-methyloctanoic acids (*R*)-**1** and (*S*)-**1** has been limited to the employment of citronellol as a chiral starting material,[39,50] liquid chromatographic separation of diastereomeric phenylglycinol amides[50] or phenylethylamides,[51] and induction of chirality using a pseudoepedrine amide as a chiral auxiliary.[52] Enzymatic resolution of 4-methyloctanoic acid **1** has been reported with modest success in the literature. Straathof describes the *Candida antarctica* lipase B catalysed enantioselective amidation of racemic (±)-4-methyloctanoic acid (±)-**1** in methyl isobutyl ketone.[53] After 3 days the (*R*)-enantiomer (*R*)-**1** was selectively transformed with 52% yield. The enantiomers of the generated amide were not sufficiently separated on GC and therefore the enantiomeric ratio (*E* = 76) was calculated from the enantiomeric excess of the remaining substrate (*S*)-4-methyloctanoic acid (*S*)-**1** (95% ee) *versus* degree of conversion and thus, should be interpreted with caution.[53] This enantioselectivity, called the enantiomeric ratio, *E*, measures the ability of the enzyme to distinguish between enantiomers (for a further detailed explanation of *E* value see chapter 3 page 78). Franssen *et al.* investigated use of Novozym 435® for esterification of (±)-4-methyloctanoic acid (±)-**1** (*E* = 54) and hydrolysis of the corresponding ethyl ester (*E* = 12) leading to modest enantioselectivity.[54,55] Novozym 435® mediated esterification of (±)-4-methyloctanoic acid (±)-**1** with polyethylene glycol as donor at different water concentrations was also described; conversion of 50% was achieved, however, no enantioselectivity data was reported.[56] More recently Boom reported the Novozym 435® catalysed esterification of (±)-4-methyloctanoic acid (±)-**1** with ethanol in a batch reactor resulting in the isolation of enantiomerically enriched product (*R*)-4-methyloctanoic ethyl ester (81% ee) and substrate (*S*)-4-methyloctanoic acid (*S*)-**1** (93% ee).[57]

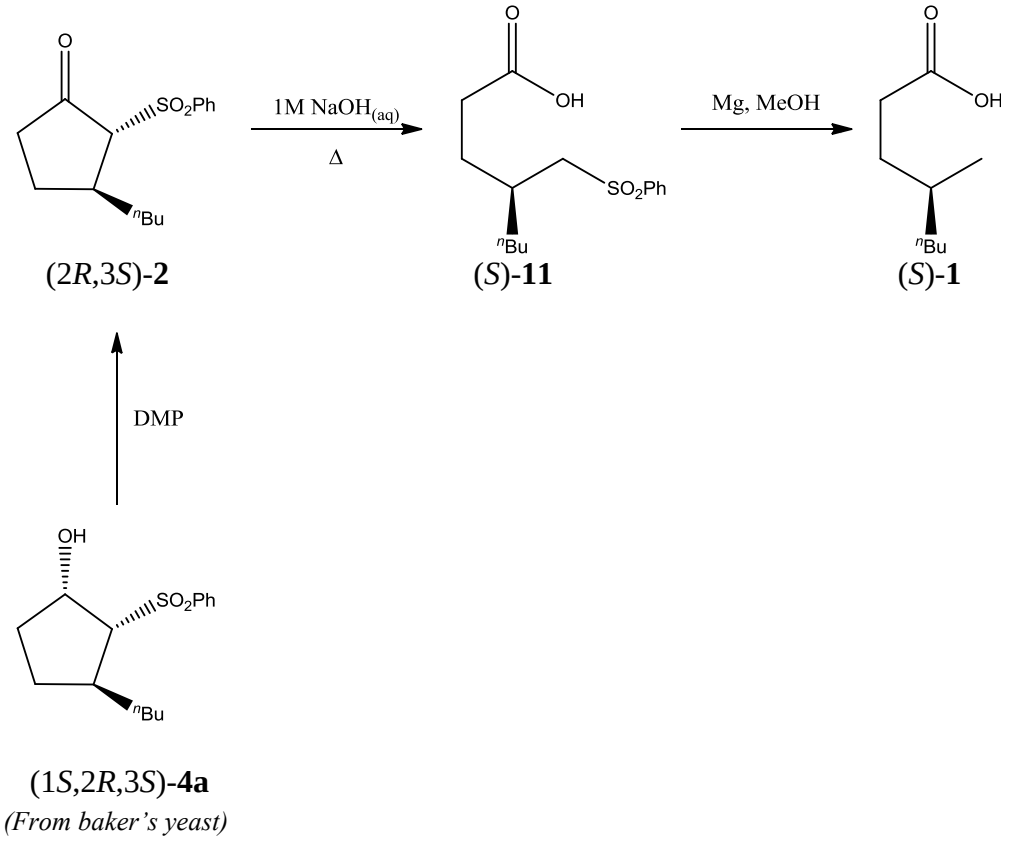
Having achieved efficient kinetic resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** it was envisaged that this could lead effectively to both enantiomers of the aggregation pheromone 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** as outlined in Scheme 2.11 and 2.12.



(2*S*,3*R*)-**2** (*R*)-**11** (*R*)-**1**

(From baker's yeast)

Scheme 2.11



(2*R*,3*S*)-**2** (*S*)-**11** (*S*)-**1**

(1*S*,2*R*,3*S*)-**4a**
(From baker's yeast)

Scheme 2.12

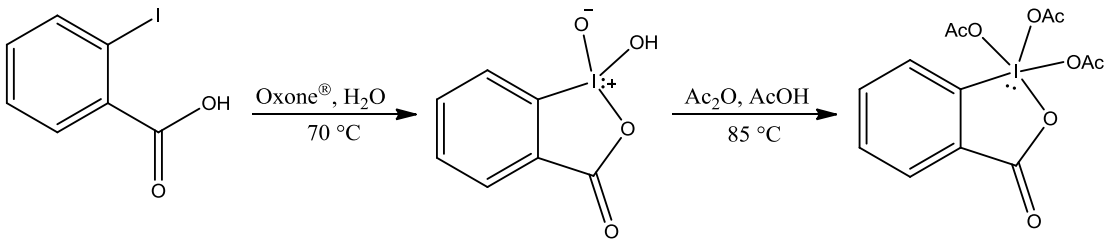
2.5.1 Access to the complimentary enantiomeric series of cyclopentanone *via* Dess-Martin oxidation

The baker's yeast mediated reduction and kinetic resolution of (±)-**2** allowed ready access to enantioenriched (2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2** (>98% ee) and (1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** (>98% ee) in high yields. To obtain access to both complimentary enantiomeric forms of the *trans*-cyclopentanone **2**, oxidation of (1*S*,2*R*,3*S*)-**4a** with retention of stereochemistry was performed.[1,2,8] Subsequently, this was exploited to provide access to both enantiomers of the natural product aggregation pheromone of the rhinoceros beetles of the genus *Oryctes*, 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1**.

Dess-Martin periodinane (DMP), 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one **12**, is one of the mildest and most convenient reagents available for the chemoselective oxidation of primary and secondary alcohols to aldehydes and ketones respectively.[58-60] Dess-Martin oxidation of (1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** was conducted to obtain the (2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, the complementary cyclopentanone enantiomer to that recovered from the baker's yeast mediated reduction of (±)-**2** with kinetic resolution. The periodinane **12** was synthesised in two steps (Scheme 2.13). The first step involved the preparation of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) **13** by oxidation of 2-iodobenzoic acid **14** with Oxone® ($2KHSO_5$-$KHSO_4$-K_2SO_4) in water at 70 °C according to Santagostino.[61] This procedure offers distinct advantages over the original $KBrO_3$ oxidation by Dess and Martin,[58] avoiding use of hot aqueous sulfuric acid and obnoxious bromine vapours. Furthermore, environmentally safe sulfate salts are the only byproducts. The adduct **13** was recovered by filtration as a white crystalline solid in good yield (75%). IBX **13** has

been reported to be explosive under impact or heating to >200 °C, thus **13** was handled with extreme care, but no difficulties were encountered in practice.[58]

The procedure for the second step is a variation of the Dess and Martin[58] protocol and is based heavily upon it.[62] The hydroxyiodinane oxide **13** was treated with a mixture of acetic anhydride and acetic acid at 85 °C until all the solids dissolve to afford a colourless to clear yellow solution. The product **12** was recovered by slow cooling of the reaction mixture and vacuum filtration of the resulting crystalline solids under a nitrogen atmosphere. It is essential that this filtration is carried out under an inert atmosphere as exposure to air leads to loss of activity due to hydrolysis to re-form the hydroxyiodinane oxide **13**.[58] The periodinane **12** was obtained as a white solid in high yield (78%) and can be stored in the freezer under nitrogen in the absence of light to avoid the possibility of hydrolysis and photolytic decomposition. Some variability was observed in the oxidation activity of different batches of Dess Martin periodinane **12**, with lower yields of the oxidised product (2*R*,3*S*)-**2** recovered. Irreproducibility in the synthesis of Dess-Martin periodinane has been well documented and a number of solutions have been put forward.[60,63,64] Stevenson *et al.* suggested that the yield and purity of the triacetoxyperiodinane **12** was dependent on the morphology of the batch of hydroxyiodine oxide **13** precursor employed.[64] This compound can be isolated in crystalline or powder form, however, isolation as the microcrystalline powder has proved much more reactive. A facile method for converting the crystalline 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide **13** to powder form has been described.[64] In general, however, high yields were observed in this study when **12** was employed immediately following filtration.

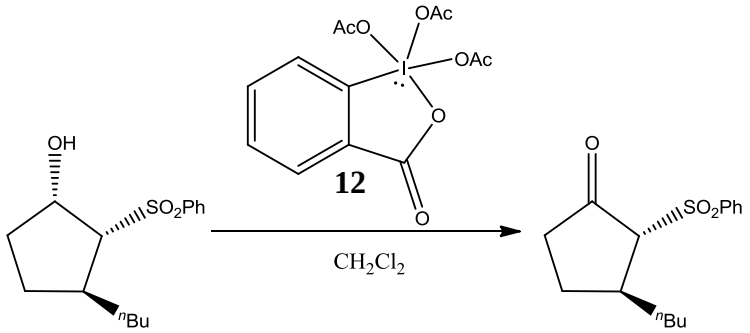


14 **13** *(75% yield)* **12** *(78% yield)*

Scheme 2.13

Dess-Martin oxidation of the (1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** involved the addition of a dichloromethane solution of the substrate (1*S*,2*R*,3*S*)-**4a** to a solution of Dess-Martin periodinane **12** in the same solvent. The reaction progress was monitored by TLC and the oxidation was complete within 4 h (Scheme 2.14). Reaction work-up involved extraction of the reaction mixture with saturated sodium bicarbonate solution containing sodium thiosulfate, enabling mild reduction of the byproduct acetoxyperiodinane to the even more water soluble 2-iodobenzoate and acetic acid. This led to the desired (2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R*,3*S*)-**2** in good yield (89%) and excellent purity warranting no further purification. Spectral analysis was identical to the previously synthesised racemic cyclopentanone (±)-**2** and enantiopure (2*S*,3*R*)-**2**. A slight reduction in enantioselectivity was observed of the obtained (2*R*,3*S*)-cyclopentanone (2*R*,3*S*)-**2** (96% ee) relative to that of the (1*S*,2*R*,3*S*)-cyclopentanol (1*S*,2*R*,3*S*)-**4a** (>98% ee) substrate. The slight decrease in enantiopurity may be due to oxidation of trace amounts of the minor diastereomer (1*S*,2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*S*,3*R*)-**4b**,

although (1*S*,2*S*,3*R*)-**4b** was not observed by ^{1}H NMR or chiral HPLC analysis of the substrate (1*S*,2*R*,3*S*)-**4a**.



(1*S*,2*R*,3*S*)-**4a** *(>98% ee)*

(2*R*,3*S*)-**2** *(89% yield, 96% ee)*

Scheme 2.14

As mentioned previously, ^{1}H NMR analysis of (2*R*,3*S*)-**2** was identical to that of the cyclopentanone (2*S*,3*R*)-**2** obtained by baker's yeast mediated kinetic resolution of (±)-**2**. The only differences observed were the opposite sign of the specific rotations of (−)-(2*R*,3*S*)-**2**, although the values were similar in magnitude to that recorded for (+)-(2*S*,3*R*)-**2** and also different retention times by chiral HPLC, which corresponded to the opposite enantiomer to that obtained by baker's yeast mediated kinetic resolution of (±)-**2** (Figure 2.5).

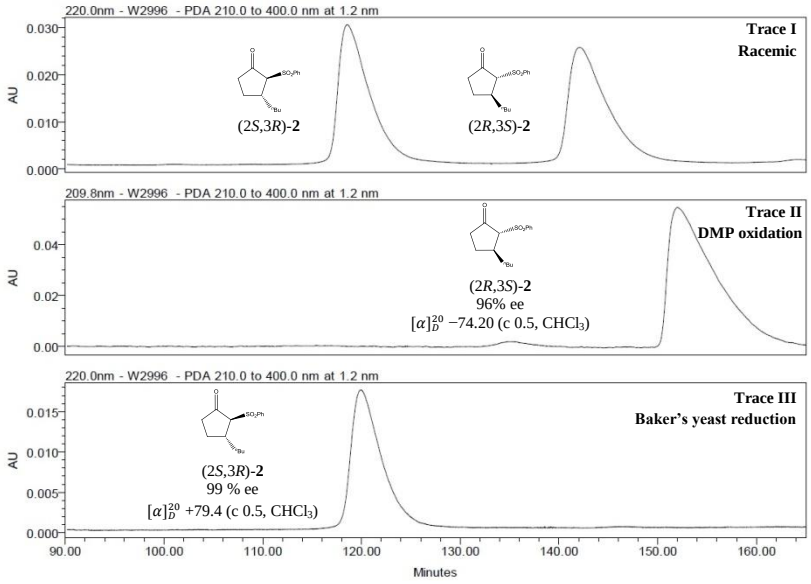


Figure 2.5: HPLC ***Trace I:*** *Racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**. **Trace II:** (−)-(2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R*,3*S*)-**2**, 96% ee (from DMP oxidation). **Trace III:** (+)-(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, 99% ee (from baker's yeast). For HPLC conditions see appendix I.*

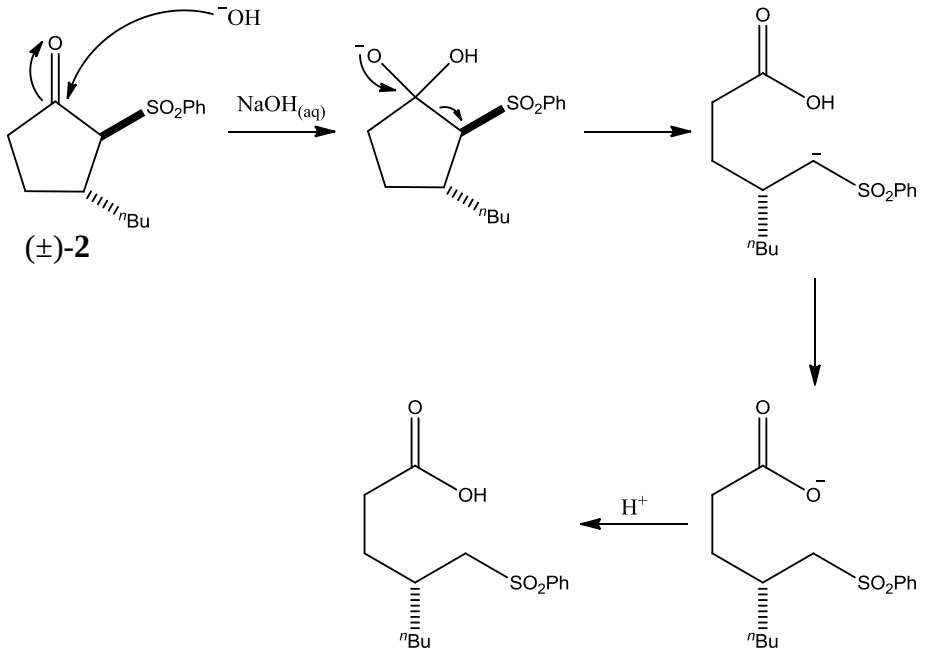
In summary, for the first time in our research group, synthetically useful quantities (~1.00 g) of each enantiomer of 2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** have been obtained in essentially enantiopure form (≥96% ee), signifying a major step forward in the application of baker's yeast methodology in the development of an asymmetric synthetic strategy to the natural product 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1**.

2.5.2 Base-induced ring cleavage of the cyclopentanone derivative

Stork and Ficini reported in 1965 that cyclic ketones bearing α-sulfonyl substituents can be cleaved under basic conditions in a retro-Claisen type process.[65] More recently, Monteiro reported the facile ring cleavage of α-sulfonylcyclopentanones in basic solution at reflux to give carboxylic acid derivatives.[66] Application of this methodology to the enantioenriched enantiomers of 2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** offered an effective route to carboxylic acids bearing a γ-stereogenic centre which, due to the versatility of both carboxylic acid and sulfone functionalities, represent an important synthetic step in the desired asymmetric synthesis of the aggregation pheromone 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1**. Both the racemic and the enantioenriched carboxylic acid **11** have been synthesised in previous work, however conducted on a limited scale and with poor enantioselectivity.[1,2,6,8]

In this research, the racemic cyclopentanone (±)-**2** was initially investigated and observed to undergo smooth ring cleavage in refluxing aqueous sodium hydroxide to give the carboxylic acid (±)-**11** in high yield (entry 1, Table 2.4). Ring cleavage occurs by a retro-Claisen type process *via* the mechanism depicted in Scheme 2.15. Nucleophilic attack of the hydroxide anion at the carbonyl carbon is followed by regiospecific ring cleavage, due to the activating influence of the benzenesulfonyl group, with formation of a sulfur-stabilised carbanion. Proton transfer results in formation of the carboxylate anion and subsequent acidification allows recovery of the carboxylic acid (±)-**11**, in high yield and without necessitating chromatographic purification. In general, reaction completion was achieved within 30 min.

Spectral characteristics of the isolated carboxylic acid are in agreement with previously reported data.[1,2,8] Characteristic broad hydroxyl and intense carbonyl absorptions are observed in the IR spectrum at ν_{max} 3066 cm^{-1} and ν_{max} 1710 cm^{-1} respectively. In the ^1H NMR spectrum, the methylene protons adjacent to the sulfonyl group appear as the AB portion of an ABX system which appears at δ_H 2.97-3.12 ppm.



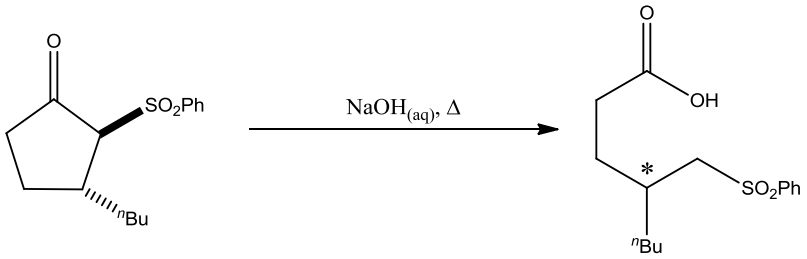
(±)-**2** (±)-**11**

Scheme 2.15

It has previously been established that the base-induced ring opening proceeds with retention of stereochemistry at C3 of the cyclopentanone, thus enabling either enantiomer of the 4-substituted carboxylic acid **11** to be formed, depending on which enantiomer of the cyclopentanone **2** is used as starting material.[1,2,8] Facile ring cleavage of both enantiomeric series of the highly enantioenriched cyclopentanone (2*S*,3*R*)-**2** and (2*R*,3*S*)-**2** was achieved. This led to the two complementary series of chiral carboxylic acids (*R*)-**11** and (*S*)-**11** with excellent enantiopurity and specific rotations of opposite sign (entries 2 and 3, Table 2.4). Notably, confidence in the accuracy of the optical rotations of (*R*)-**11** and (*S*)-**11** in this study were significantly improved relative to Kelleher's,[1] due to increased enantiomeric purity and sample size. The enantiopurities of the chiral carboxylic acids was determined by chiral HPLC analysis (see appendix I for details). Two sets of conditions were determined for separation of the enantiomers of (±)-**11** significantly the order of elution of (*R*)-**11** and (*S*)-**11** was the same in both (Figure 2.6 and 2.7). As anticipated, spectral characteristics of the enantioenriched carboxylic acid (*R*)-**11** and (*S*)-**11** are identical to the racemic (±)-**11**.

After fifteen years of investigation,[1-3,8] application of the base-induced ring cleavage of the enantioenriched 2-benzenesulfonyl-3-*n*-butylcyclopentanones (2*S*,3*R*)-**2** and (2*R*,3*S*)-**2** provided for the first time an effective route to essentially enantiopure 4-(benzenesulfonylmethyl)octanoic acid (*S*)-**11** and (*R*)-**11** (≥94% ee), important building blocks in the asymmetric synthesis of the insect pheromone 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1**. Significantly, in addition to the improved enantiomeric purity obtained in this study of (*S*)-**11** and (*R*)-**11**, the synthetic scale of the ring cleavage reaction has been substantially increased [1.16 g and 1.44 g of precursor (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** respectively] for the first time providing the desired (*S*)-**11** and (*R*)-**11** in synthetically useful quantities with excellent purity, high yield and critically with retention of stereochemistry, all of which represent important advances in the application of baker's yeast methodology to natural product asymmetric synthesis.

*Table 2.4: Synthesis of 4-(benzenesulfonylmethyl)octanoic acid **11***



2 **11**

Entry	Cyclopentanone[a]	ee (%)[b]	Carboxylic acid	Crude yield (%)[c]	ee (%)[b]	Optical rotation $[\alpha]_D^T$	
						Experimental	Literature
1	(±)-**2**	-	(±)-**11**	69	-	-	-
2	(2*R*,3*S*)-**2**	93	(*S*)-**11**	82	94	$[\alpha]_D^{20}$ −11.0 (c 1.2, CH_2Cl_2)	$[\alpha]_D^{20}$ −5.6 (c 2.7, CH_2Cl_2), 91% ee[1]
3	(2*S*,3*R*)-**2**	>98	(*R*)-**11**	73	>98	$[\alpha]_D^{20}$ +9.1 (c 1.2, CH_2Cl_2)	$[\alpha]_D^{20}$ +6.3 (c 1.2, CH_2Cl_2), 75% ee[1]

a. The base induced ring cleavage of enantioenriched cyclopentanones (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** providing the carboxylic acids (*S*)-**11** and (*R*)-**11** has been performed with 1.16 g and 1.44 g of precursor (2*R*,3*S*)-**2** and (2*S*,3*R*)-**2** respectively, with high yield (≥84%) and retention of stereochemistry (≥94% ee). The above experiments were performed with 170 mg of precursor (2*R*,3*S*)-**2** for entry 2 and 239 mg of precursor (2*S*,3*R*)-**2** for entry 3 and are the experiments described in section 5.2.3 as they represent the highest enantiomeric purity obtained of (*S*)-**11** and (*R*)-**11** in this study.

b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

c. 4-(Benzenesulfonylmethyl)octanoic acid **11** was of sufficient purity to warrant no further purification.

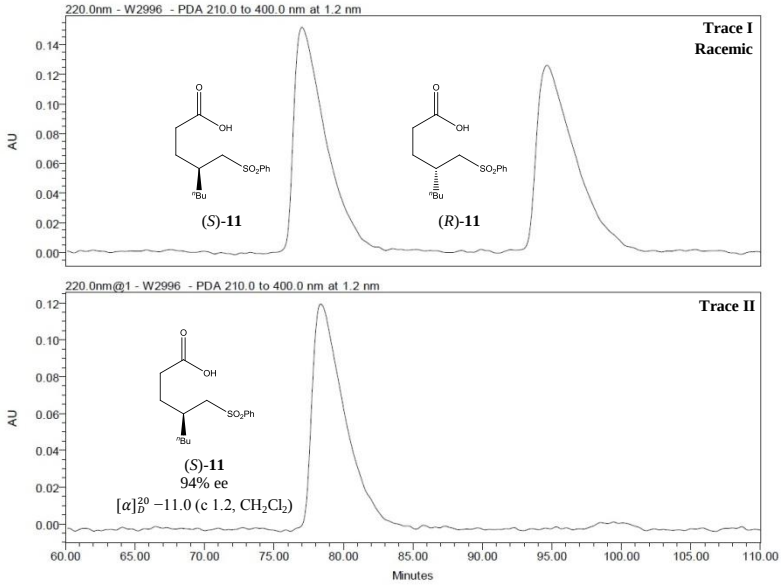


Figure 2.6: HPLC ***Trace I:*** *(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-****11****,* ***Trace II****: (−)-(*S*)-4 -(Benzenesulfonylmethyl)octanoic acid (*S*)-****11****, 94% ee. For HPLC conditions see appendix I. Note the above traces correlate to chiral HPLC conditions A for* ***11.***

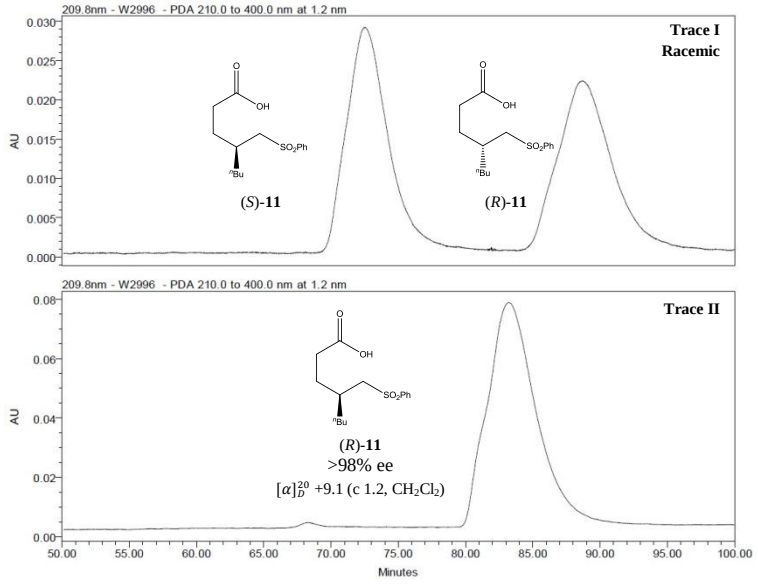


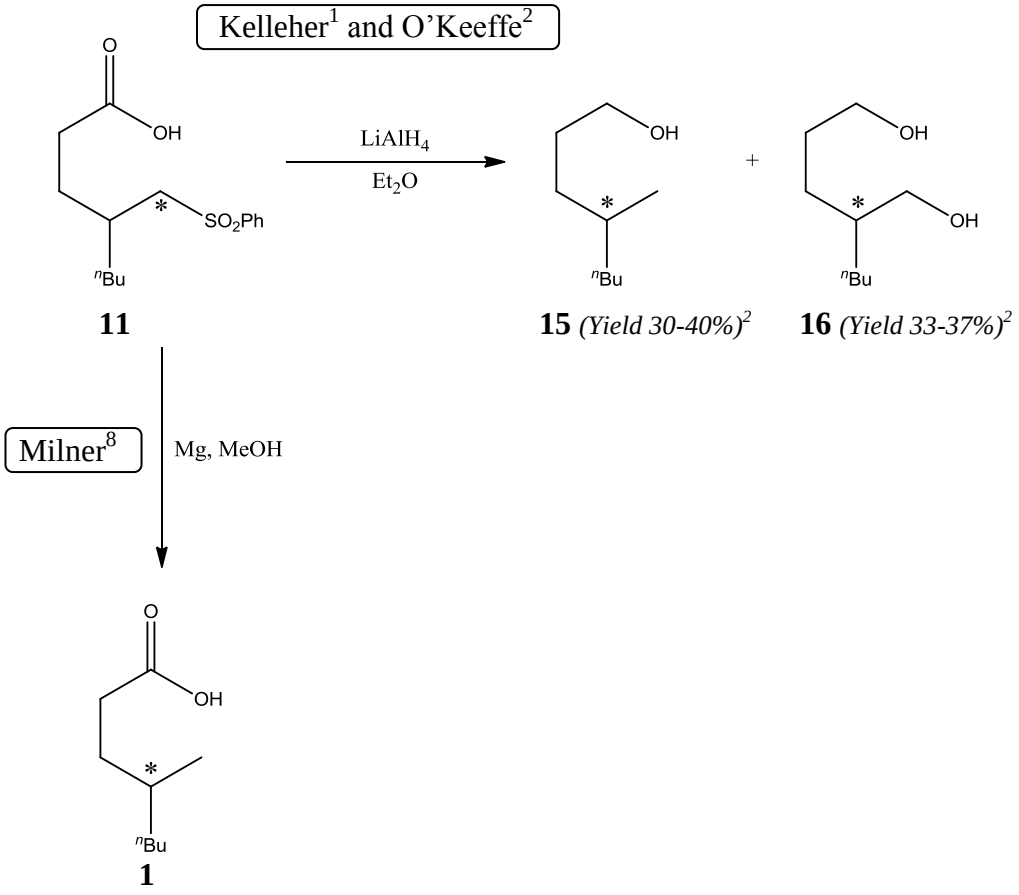
Figure 2.7: HPLC ***Trace I:*** *(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-****11****.* ***Trace II:*** *(+)-(*R*)-4 -(Benzenesulfonyl methyl)octanoic acid (*R*)-****11****, >98% ee. For HPLC conditions see appendix I. Note the above traces correlate to chiral HPLC conditions B for* ***11****.*

2.5.3 Reductive desulfonylation

In the preceding section 2.5.2, stereocontrolled functionalisation at the remote unactivated γ-carbon of 4-(benzenesulfonylmethyl)octanoic acid **11** has been successfully achieved in both enantiomeric forms and with high enantiomeric purity. One of the key synthetic steps to application of these chiral compounds (*R*)-**11** and (*S*)-**11** to asymmetric synthesis of the natural product 4-methyloctanoic acid **1** is efficient reductive desulfonylation to reveal the unsubstituted methyl substitutent, characteristic of many insect pheromones.

Kelleher[1] and O'Keeffe[2] have previously reported concomitant desulfonylation and carboxylic acid reduction of 4-(benzenesulfonylmethyl)octanoic acid (*R*)-**11** and (*S*)-**11** with retention of stereochemistry by prolonged treatment with excess lithium aluminium hydride

in diethyl ether. However, the lithium aluminium hydride mediated tandem desulfonylation/reduction reaction was problematic as it was difficult to attain reaction completion and unexpectedly led to a mixture of the desired 4-methyloctan-1-ol **15** and a diol derivative **16** (Scheme 2.16).[2] Although the products were separable by chromatographic purification, poor yields of **15** were achieved.[2]



Scheme 2.16

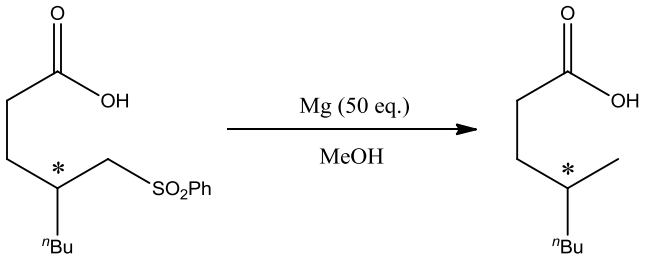
Recently, Milner reinvestigated the desulfonylation step, to improve efficiency and yield without the reduction of the carboxylic acid moiety to form 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** (Scheme 2.16).[8] Milner reported successful desulfonylation of (*R*)-**11** and (*S*)-**11** with a large excess of magnesium (50 equivalents) in methanol at room temperature to obtain 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** in poor to high yield, 45%[iii] and 94% respectively.[8] Notably, Milner did not achieve complete desulfonylation of (*S*)-**11** with ~2% of the sulfonylated carboxylic acid (*S*)-**11** evident by ^{1}H NMR analysis of (*S*)-4-methyloctanoic acid (*S*)-**1** following chromatographic purification. Significantly, the stereochemistry at C(4) was unaffected by this reaction, confirmed by chiral HPLC analysis of (*R*)-**1** (>98% ee), however, optical rotation data was not recorded due to the small sample size of (*R*)-**1**.[8] The enantiomeric excess of (*S*)-**1** was not determined by Milner as an analytically pure sample was not obtained, however, the sulfonylated carboxylic acid precursor to (*S*)-**1**, (*S*)-**11**, was of moderate enantiopurity (70% ee).[8]

[iii] Milner reports in chapter 3, results and discussion, 45% yield of (*R*)-**1** in the desulfonylation of (*R*)-**11** and attributes the low yield to the presence of the large excess of magnesium alkoxide and the small sample size [~ 30 mg of (*R*)-**11**], however in chapter 4, experimental a 75% yield of (*R*)-**1** is reported.[8]

Some difficulties were encountered in this research when desulfonylation of racemic (±)-**11** was attempted according to Milner, utilising an excess of magnesium in methanol.[8] Although complete desulfonylation of (±)-**11** was successfully achieved in this study, a moderate 51% yield was obtained following column chromatography. Numerous experiments were conducted in which the batch of magnesium turnings and purity of methanol employed to desulfonylate (±)-**11** as well as the reaction time were varied with negligible increase in yield of (±)-**1**. The ^{1}H NMR spectrum of the crude product (±)-**1** was relatively clean, with only the desired product (±)-**1** observed with just trace evidence of unassigned peaks in the aromatic region, indicating the absence of a substantive competing process. Thus, the low yield was attributed to poor extraction of 4-methyloctanoic acid (±)-**1** from the crude reaction mixture due to the large excess of magnesium alkoxide employed, despite acidification of the reaction mixture with aqueous hydrochloric acid (10%). Further studies are therefore warranted investigating the quantity of magnesium turnings employed in the desulfonylation step.

Reactions were monitored by IR analysis, where reaction completion was indicated by the disappearance of the symmetric and asymmetric stretches of the sulfonyl group at ν_{max} 1305 cm^{-1} and ν_{max} 1146 cm^{-1}, characteristic of the sulfonyl carboxylic acid **11**. The IR spectra of (±)-**1** displays strong characteristic absorptions evident at ν_{max} 3043 cm^{-1} and ν_{max} 1714 cm^{-1} attributable to the hydroxyl and carbonyl moieties. The ^{1}H NMR spectrum of racemic (±)-**1** is in accordance with the spectroscopic data of Milner,[8] with a distinct multiplet at δ_H 2.19-2.50 ppm attributable to the methylene C(2)H$_2$ protons α- to the carboxylic acid moiety.

*Table 2.4: Synthesis of 4-methyloctanoic acid **1***



Entry	Sulfonylated carboxylic acid	Scale (g)[a]	ee (%)	Desulfonylated carboxylic acid	Yield (%)[d]	ee[e] (%)[f]	Optical rotation $[\alpha]_D^T$	
							Experimental	Literature[g]
1	(±)-**11**	1.04	-	(±)-**1**	51	-	-	-
2	(*R*)-**11**	0.74	-[b]	(*R*)-**1**	53	>98	$[\alpha]_D^{20}$ −2.2 (c 1.4, $CHCl_3$)	$[\alpha]_D^{20}$ −1.5 (c 1.4, $CHCl_3$), 94% ee[52]
3	(*S*)-**11**	0.56	-[c]	(*S*)-**1**	57	97	$[\alpha]_D^{20}$ +1.6 (c 1.4, $CHCl_3$)	$[\alpha]_D^{20}$ +1.5 (c 1.4, $CHCl_3$), 93% ee[52]

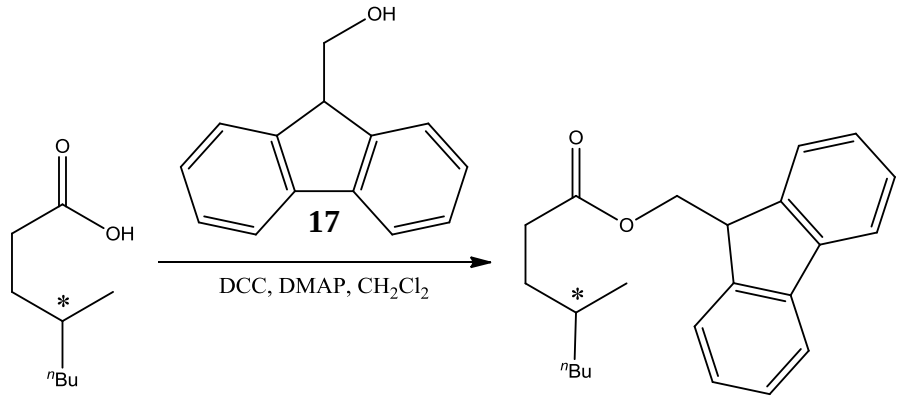
a. Scale refers to the quantity of 4-(Benzenesulfonylmethyl)octanoic acid **11** employed for the reaction.
b. Chiral HPLC and optical rotation analysis was not conducted on the batch of (*R*)-4-(benzenesulfonylmethyl)octanoic acid (*R*)-**11** utilised in this experiment. However, the enantiopurity of the precursor of (*R*)-**11**, (−)-(2*S*,3*R*)-cyclopentanone (2*S*,3*R*)-**2** was determined to be 99% ee by chiral HPLC analysis and base-catalysed ring cleavage to (*R*)-**11** proceeds with retention of stereochemistry (see section 2.5.2).
c. Chiral HPLC and optical rotation analysis was not conducted on the batch of (*S*)-4-(benzenesulfonylmethyl)octanoic acid (*S*)-**11** utilised in this experiment. However, the enantiopurity of the precursor of (*S*)-**11**, (+)-(2*R*,3*S*)-cyclopentanone (2*R*,3*S*)-**2** was determined to be 97% ee by chiral HPLC analysis and base-catalysed ring cleavage to (*S*)-**11** proceeds with retention of stereochemistry (see section 2.5.2).
d. Yields quoted are following chromatographic purification.
e. The highest enantiomeric purity of (*R*)-4-methyloctanoic acid (*R*)-**1** reported by Milner was >98% ee. Enantiomeric excess [ee (%)] of (*R*)-**1** was determined by chiral HPLC analysis using a Chiralcel® OJ-H column, however no optical rotation data was obtained due to small sample size.[8] The enantiomeric excess [ee (%)] of (*S*)-**1** was not determined by Milner as an analytically pure sample was not obtained, however, the sulfonylated carboxylic acid precursor to (*S*)-**1**, (*S*)-**11** was of moderate enantiopurity 70% ee.[8]
f. Due to the low UV absorption of 4-methyloctanoic acid (*R*)-**1** and (***S***)-**1**, chiral HPLC analysis was not possible with a PDA detector and enantiomeric excess [ee (%)] was therefore determined by derivatisation with 9-fluorenemethanol **17**.
g. The literature ee values were determined by chiral HPLC following derivatisation of the methyloctanoic acid derivatives (*S*)-**1** and (*R*)-**1** with (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol.[52]

Significantly, the desulfonylation reaction proceeds with retention of stereochemistry at C(4) and for the first time in our group complete desulfonylation of (*R*)-**11** and (*S*)-**11** was successfully achieved, providing both enantiomers of the aggregation pheromone of the rhinoceros beetles of the genus *Oryctes*, 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** with excellent purity following chromatographic purification (entries 2 and 3, Table 2.4). In addition, the magnesium/methanol mediated desulfonylation was performed on a preparative scale [0.56 g and 0.74 g of precursor (*S*)-**11** and (*R*)-**11** respectively] to provide substantial quantities of the pure natural product (*R*)-**1** and (*S*)-**1** for full characterisation including chiral HPLC and optical rotatory analysis, representing an important advance in the asymmetric synthesis of (*R*)-**1** and (*S*)-**1**. Notably, spectral characteristics of the enantioenriched natural product (*R*)-**1** and (*S*)-**1** were identical to the racemic (±)-**1** and literature reports.[51,52,67]

Milner reported chiral HPLC analysis of (*R*)-**1** utilising the Chiralcel® OJ-H column at room temperature with isopropanol/hexane (10 : 90), a flow rate of 1.0 mL/min and a detector wavelength of 220 nm.[8] However, when these conditions were applied utilising a photodiode array (PDA) detector examining a range of wavelengths simultaneously, the enantiomers of (±)-**1** were not observed, attributable to the low UV absorption of 4-methyloctanoic acid (±)-**1**. Increased concentration of the chiral HPLC samples of (±)-**1** were prepared (>1 mg/mL), however once again no trace of the enantiomers was observed. Therefore, unequivocal determination of the enantiomeric purity of acids (*R*)-**1** and (*S*)-**1** was obtained by derivatisation with 9-fluorenemethanol **17**, a highly conjugated polycyclic aromatic hydrocarbon. It was envisaged that chiral HPLC analysis with a PDA detector would resolve the enantiomers of the resulting ester **18**.

As evident from Table 2.5, derivatisation of racemic and enantiopure 4-methyloctanoic acid **1** occurred in moderate yields (61-79%) in the presence of 9-fluorenemethanol **17**, dicyclohexylcarbodiimide (DCC) and *N*,*N*-dimethylaminopyridine (DMAP) in dichloromethane and the resulting novel esters **18** were analysed by chiral HPLC. Significantly, no racemisation occurred during the derivatisation step and the enantiomeric purity of (*R*)-**18** and (*S*)-**18** was determined as 99 and 97% ee respectively (Figure 2.8). Thus, successful synthesis of both enantiomers and the racemic form of the naturally occurring target compound 4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** has been achieved in this work, with significant improvements in terms of synthetic scale, purity and enantiomeric excess relative to results reported by Milner.[8]

*Table 2.5: Synthesis of (9H-fluoren-9-yl)methyl 4-methyloctanoate **18***



1 **18**

Entry	Desulfonylated carboxylic acid	Ester	Yield (%)[a]	ee (%)[b]	Optical rotation $[\alpha]_D^T$	
					Experimental	Literature
1	(±)-**1**	(±)-**18**	61	-	-	-
2	(*S*)-**1**	(*S*)-**18**	69	97	$[\alpha]_D^{20}$ +1.5 (c 1.0, $CHCl_3$)	-
3	(*R*)-**1**	(*R*)-**18**	79	99	$[\alpha]_D^{20}$ -3.4 (c 1.0, $CHCl_3$)	-

a. Yields quoted are following chromatographic purification.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

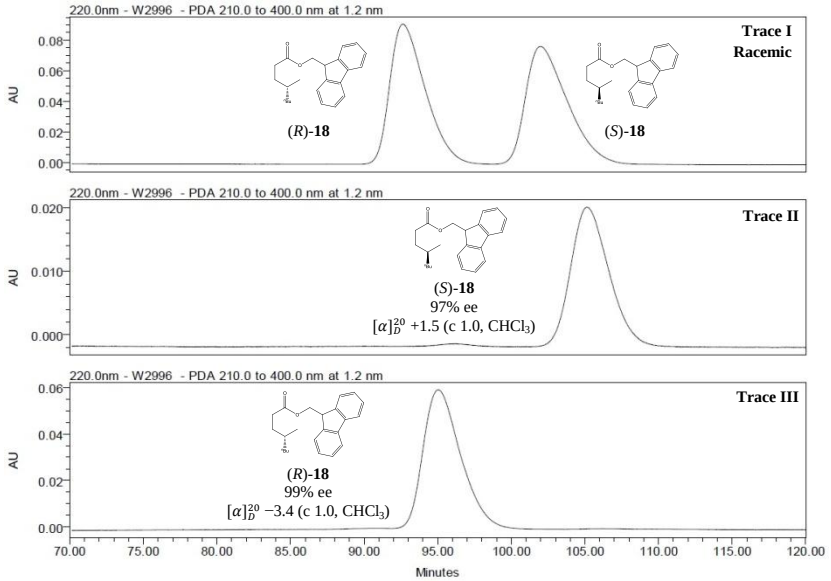
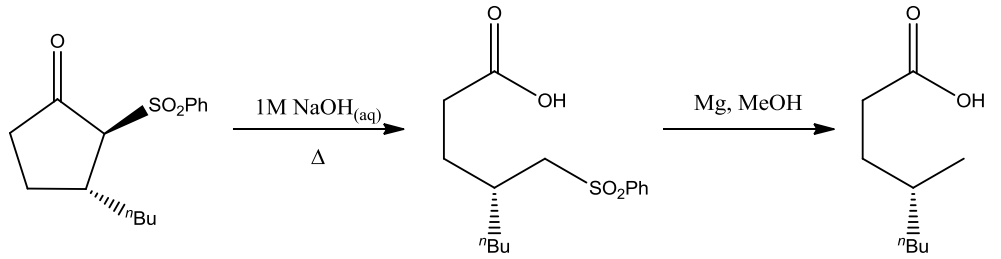


*Figure 2.8: HPLC **Trace I:** (±)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (±)-**18**. **Trace II:** (+)-(S)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (S)-**18**, 97% ee. **Trace III**: (−)-(R)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (R)-**18**, 99% ee. For HPLC conditions see appendix I.*

2.6 Project conclusion

In summary, access to both enantiomers of the natural occurring target compound (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** has been achieved in excellent enantiopurity (≥97% ee) for the first time *via* chemoenzymatic synthesis (Scheme 2.17 and 2.18), in addition to the racemic form (±)-**1**. This example illustrates the synthetic utility of the enantioenriched cyclopentanones (2*S*,3*R*)-**2** and (2*R*,3*S*)-**2** obtained from baker's yeast mediated biotransformation, as chiral building blocks in natural product synthesis. Both enantiomers (*R*)-**1** and (*S*)-**1** were obtained in three and four steps respectively from racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** and in a total of six and seven steps respectively from ethyl octanoate **7**. The overall yield of (*R*)-**1** (15%) and (*S*)-**1** (13%) was determined from racemic (±)-**2**. Furthermore, optical rotation data in conjunction with chiral HPLC analysis of derivatised **18** confirm the excellent enantiopurity obtained by induction of chirality through baker's yeast methodology and the retention of stereochemistry throughout the synthetic protocol.

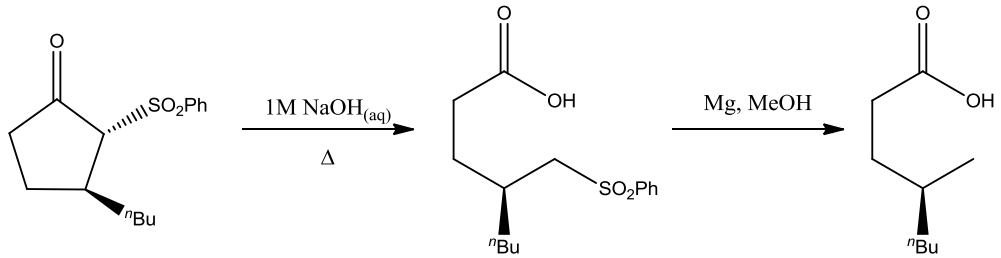


(2*S*,3*R*)-**2**
(From baker's yeast)
(99% ee, 31% yield)

(*R*)-**11**
(89% yield)

(*R*)-**1**
(99% ee, 53% yield)
*15% overall yield from (±)-**2***

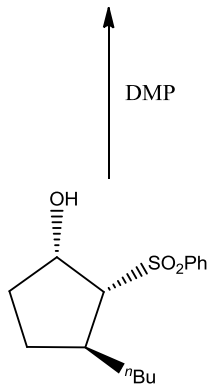
Scheme 2.17



(2*R*,3*S*)-**2**
(97% ee, 78% yield)

(*S*)-**11**
(99% yield)

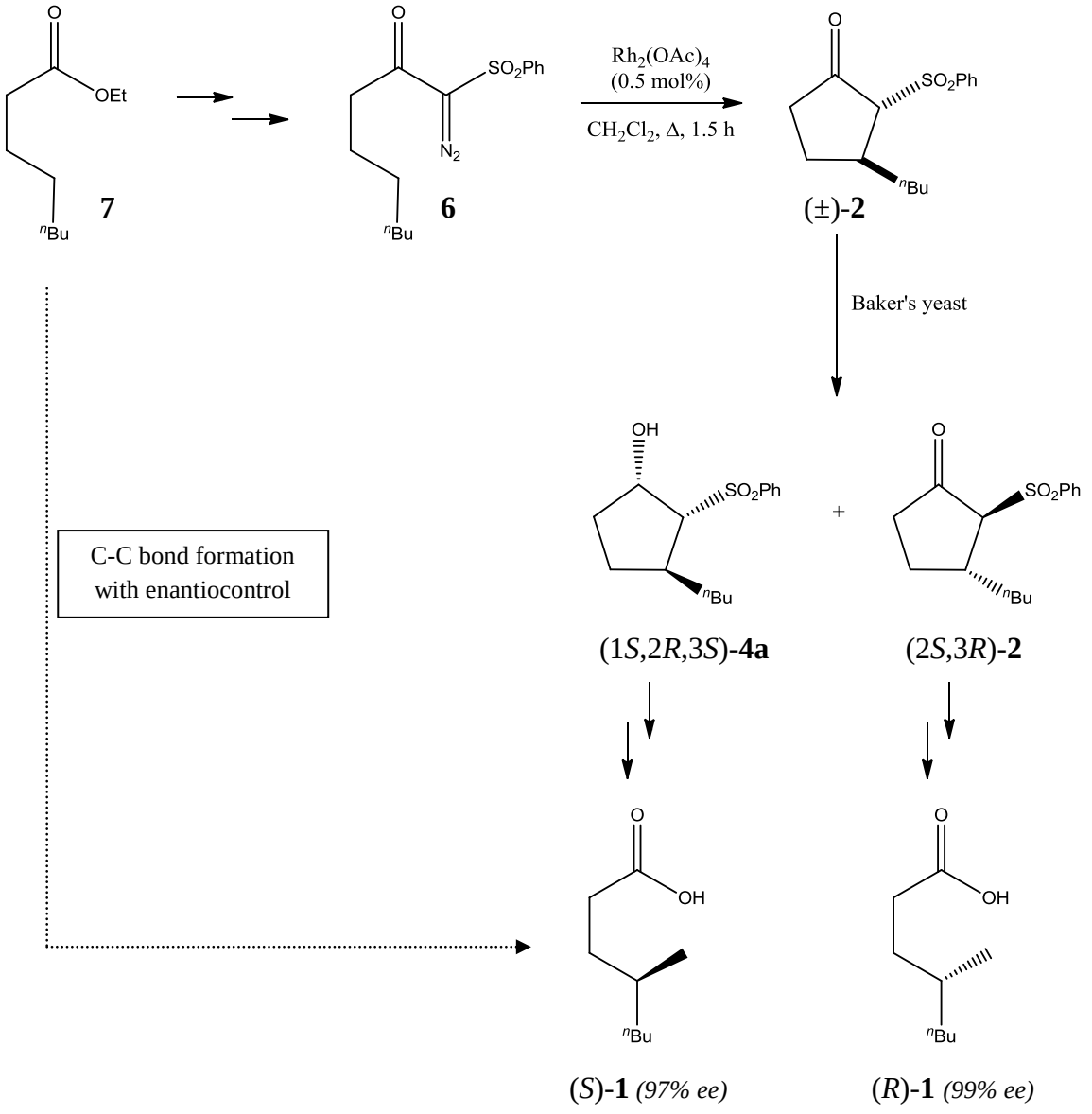
(*S*)-**1**
(97% ee, 57% yield)
*13% overall yield from (±)-**2***



(1*S*,2*R*,3*S*)-**4a**
(From baker's yeast)
(>98% ee, 29% yield)

Scheme 2.18

The key step in the asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-**1** and (*S*)-**1** is asymmetric C-C bond formation introducing the benzenesulfonylmethyl group at the unactivated γ-carbon of carboxylic ester **7** through a synthetically powerful combination of a regiospecific transition metal catalysed carbenoid insertion process (which activates an unactivated C-H bond at the γ-carbon) and an enantioselective kinetic resolution *via* biocatalysis (Scheme 2.19). Subsequent reductive desulfonylation reveals the unsubstituted methyl substituent characteristic of several insect pheromones. This distinctive moiety was readily obtained from the carboxylic ester **7** in both enantiomeric forms through application of this novel methodology.



Scheme 2.19

Thus, while asymmetric synthesis of (*R*)-**1** and (*S*)-**1** can be readily envisaged starting from a small enantiopure synthetic precursor from the chiral pool the novelty of this approach is that the methyl group is selectively introduced both in terms of regio- and stereochemistry on the achiral octanoic acid chain.

2.7 References

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Chapter 3

Hydrolase-mediated kinetic resolutions of 3-arylalkanoic acids

Contents

3.1 Introduction **78**
3.1.1 Importance of 3-arylalkanoic acids as key synthetic intermediates **80**
3.1.2 Background to the project **82**
3.2 Objectives of the project **86**
3.3 Synthesis of ethyl 3-arylalkanoates **87**
3.3.1 Esterification of commercially available 3-arylalkanoic acids **88**
3.3.2 Three-step synthesis of 3-arylalkanoates **90**
3.3.2.1 Addition of Grignard reagents to α,β-unsaturated acids **90**
3.3.2.1.1 Addition of alkyl Grignard reagents to α,β-unsaturated acids **90**
3.3.2.1.2 Addition of aryl Grignard reagents to α,β-unsaturated acids **91**
3.3.2.2 Synthesis of acid chlorides **93**
3.3.2.3 Synthesis of ethyl esters **94**
3.3.3 Horner-Wadsworth-Emmons reaction **95**
3.3.4 Synthesis of α-alkylated alkanoate **98**
3.4 Hydrolase-mediated kinetic resolution - analytical screens **99**
3.4.1 Chiral HPLC method development **99**
3.4.2 Screening protocol – aqueous hydrolysis of esters **100**
3.4.3 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C3 substituted alkanoic acids **101**
3.4.3.1 Hydrolase-catalysed kinetic resolution to provide enantioenriched 3-phenylbutanoic acid **23** **101**
3.4.3.2 Hydrolase-catalysed kinetic resolution to provide enantioenriched 3-phenylpentanoic acid **28**... **103**
3.4.3.3 Hydrolase-catalysed kinetic resolution to provide enantioenriched 4-methyl-3-phenylpentanoic acid 29 ..... **107**
3.4.3.4 Hydrolase-catalysed kinetic resolution to provide enantioenriched 4,4-dimethyl-3-phenylpentanoic acid **37** **108**
3.4.4 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C3 substituted phenyl butanoic acids **111**
3.4.5 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C2 substituted alkanoic acids **115**
3.4.5.1 Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-methyl-3-phenylpropanoic acid **32** **116**
3.4.5.2 Hydrolase-catalysed kinetic resolution to provide enantioenriched of 2-benzylbutanoic acid 45 ..... **118**
3.4.5.3 Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-benzyl-3,3-dimethylbutanoic acid 61 ..... **119**
3.5 Hydrolase-mediated kinetic resolution – preparative-scale **122**
3.5.1 Preparative-scale hydrolase-catalysed kinetic resolution of C3 substituted alkanoic esters **123**
3.5.2 Preparative-scale hydrolase-catalysed kinetic resolution of substituted phenyl butanoic esters **125**
3.5.3 Preparative-scale hydrolase-catalysed kinetic resolution of C2 substituted alkanoic esters **126**
3.5.4 The use of co-crystals for the determination of absolute stereochemistry of the products of preparative-scale reactions **127**
3.6 Project conclusion **131**
3.7 References **132**

3.1 Introduction

The use of biocatalysis in synthetic organic chemistry has grown enormously in popularity, from academic curiosity a century ago to a standard practise of industrial importance.[1,2] One rationale for this achievement is that biocatalysis is one of the greenest technologies for the synthesis of chiral molecules. Biotransformations are a key tool in Green Chemistry, offering environmentally benign, biodegradable catalysts, which are effective under mild reaction conditions, and which afford excellent selectivity.[3] Consequently, novel biocatalytic pharmaceutical processes are often more desirable than their conventional chemical counterparts from an environmental and economic standpoint.

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are ubiquitous enzymes belonging to the family of serine hydrolases and are unequivocally the most utilized biocatalysts, providing one of the most advantageous and versatile bioresolution methods in asymmetric synthesis.[4] Hydrolases combine wide substrate specificity with high regio- and enantioselectivity enabling the resolution of organic substrates with superb efficiency and selectivity. Furthermore lipases do not require the use of labile co-factors and can be used in both free and immobilised form. These favourable attributes make lipases especially attractive for the pharmaceutical and agrochemical areas, where the interest for enantiomerically pure and specifically functionalised compounds is continuously growing.

Kinetic resolution of racemic compounds is by far the most common transformation catalysed by lipases, in which the enzyme discriminates between the two enantiomeric constituents of a racemic mixture, so that one is more readily transformed into a product than the other. While a severe limitation of kinetic resolution is that the maximum yield obtainable is restricted to 50% and thus the resolution is usually accompanied by additional processing such as separation, racemisation and recycling of unwanted enantiomers this route has the advantage of easy preparation of both enantiomers by using a single lipase.[5]

To more conveniently compare kinetic resolutions, a series of equations were developed by Sih *et al.* to calculate their inherent enantioselectivity.[6] This laid the basis for the application of lipases and allows synthetic chemists to make highly useful predictions. This enantioselectivity, called the enantiomeric ratio, *E*, is a measure of comparing the ability of different enzymes to distinguish between enantiomers. In practical terms, the higher the *E* value, the more selective the bioresolution is, giving a higher yield of product at higher enantiomeric excess. A non-selective reaction has an *E* value of 1, while resolutions with an *E* value above 20 require further development. An *E* value of 200 is excellent and little optimisation will be necessary.

To calculate *E*, one measures two of the three variables: enantiomeric purity of the starting material (ee_s), enantiomeric purity of the product (ee_p), and extent of conversion (c) and uses one of the three equations below (Figure 3.1). Often, enantiomeric purities are more precisely measured than conversion; in these cases, the third equation is more accurate.

$$E = \frac{\ln[1 - c(1 + ee_p]}{\ln[1 - c(1 + ee_p)]} \qquad E = \frac{\ln[(1 - c)(1 - ee_s]}{\ln[(1 - c)(1 + ee_s)]} \qquad E = \frac{\ln\left[\frac{1 - ee_s}{1 + \frac{ee_e}{ee_p}}\right]}{\ln\left[\frac{1 + ee_s}{1 + \frac{ee_s}{ee_p}}\right]}$$

Figure 3.1

It should be noted that values >200 are less accurately measured than low or moderate *E* values because the enantiomeric ratio is a logarithmic function of the enantiomeric purity. At *E* >200 small changes in the measured enantiomeric purities give large variation in the enantiomeric ratio.

A simple program developed by Kroutil *et al.* and publicly available at *http://www.orgc.tugraz.at/* allows the enantiomeric ratio to be calculated using the above equations.[7] Though these equations include assumptions such as an irreversible reaction, one substrate and product, and no product inhibition, they are reliable in the vast majority of cases, especially for screening studies.

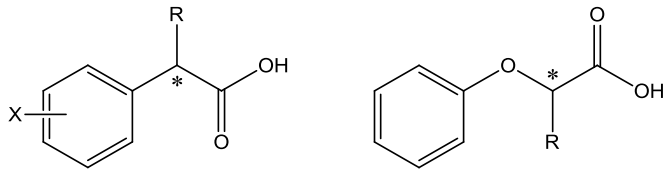
As the most versatile class of substrates for synthetic applications, alcohols have received the most attention in the context of lipase-catalysed kinetic resolution. In nature, lipases catalyse the reversible cleavage of the ester bonds of triacylglycerol thus lipases may be expected to be the most effective in conversions involving esters of chiral alcohols rather than chiral acids.[5] However, there are a number of examples in the literature of the successfully application of lipases for the resolution of highly enantioenriched chiral carboxylic acids.

The simplest conventional route to enantiopure carboxylic acids is the hydrolysis of the carboxylic acid ester in water or biphasic mixtures of water and an organic solvent. Use of lipases does not require a soluble substrate and the immiscible liquid ester substrate can act as an organic phase. The presence of a biphasic mixture is highly advantageous because it activates most lipases by 10 to 100-fold.[4] A lipid-induced change in the orientation of the lid (an amphiphilic α-helix peptide sequence preventing access of the substrate to the catalytic triad) exposes the active site and increases the catalytic power of the lipase, a phenomenon called interfacial activation.[4]

Studies over the past 25 years have revealed that enzymatic catalysis in non-aqueous media significantly extends the synthetic utility of enzymes far beyond conventional aqueous-based hydrolysis. Klibanov and co-workers recognised the immense synthetic potential of enzymes in organic solvents and their fundamental pioneering studies have generated great interest in these systems.[8-10] In such seemingly hostile environments enzymes can catalyse reactions unattainable in aqueous medium and the range of possible chemical reactions has broadened extensively. Carboxylic acid derivatives can be enzymatically resolved in organic solvents *via* hydrolase-catalysed esterification, transesterification and aminolysis reactions with suitable amines.

Biotransformations of nitriles as precursors to enantiopure amides and carboxylic acids have developed substantially in recent years. Biocatalytic hydrolysis of nitriles proceeds with excellent chemo-, regio- and stereoselectivity under very mild aqueous conditions and is now firmly established in both academia and industry as a viable synthetic route to chiral carboxylic acids and carboxamides.[11-13]

In the literature, the kinetic bioresolution of chiral carboxylic acids has been dominated by the successful resolution of commercially important 2-aryl **19** or 2-aryloxy-propionic acids **20**; the former are non-steroidal anti-inflammatory drugs NSAID's (such as naproxen and ibuprofen) and the latter an important class of herbicides (such as dichlorprop and mecoprop). The (*S*)-enantiomers of **19** display therapeutic activity, while the (*R*)-enantiomers of **20** are effective (Figure 3.2).



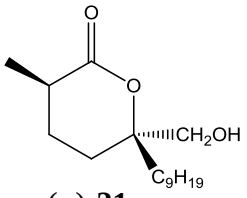
19 **20**

Figure 3.2

While the stereocentre at the α-position has been studied extensively in lipase resolutions of alkanoic acids, fewer reports exist where the stereocentre is further away from the reactive site. Therefore, the primary objective of this research was to explore the lipase-mediated kinetic resolution of β-substituted 3-arylalkanoic acids and compare the enantioselectivity to that reported for their α-substituted counterparts.

3.1.1 Importance of 3-arylalkanoic acids as key synthetic intermediates

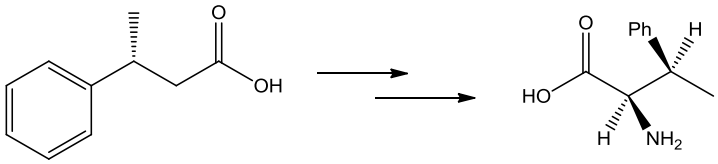
Enantiomerically pure 3-arylalkanoic acids are important synthetic intermediates for the preparation of a variety of compounds with biological and pharmacological importance including several drug candidates.[14-16] Optically pure 3-arylalkanoic acids are valuable chiral synthons in the asymmetric synthesis of antibacterial agents such as (−)-malyngolide (−)-**21**, a naturally occurring δ-lactone of algae origin (Figure 3.3).[17]



(−)-**21**

Figure 3.3

All four individual stereoisomers of amino acid β-methyl phenylalanine **22** have been prepared in excellent optical purity, from enantiopure (*R*)- and (*S*)-3-phenylbutanoic acid **23** (Scheme 3.1).[18]

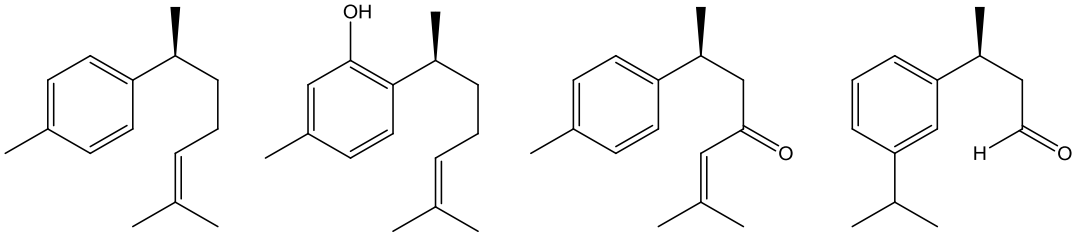


(*R*)-**23** (2*S*,3*R*)-**22**

Scheme 3.1

Enantiopure 3-arylalkanoic acids can be efficiently reduced to their corresponding primary alcohols, which are key intermediates in the enantiospecific synthesis of biologically important bisabolene sesquiterpenes. This class of compounds, showing a wide range of therapeutic activities, is characterised by a benzylic stereogenic centre often carrying a methyl moiety at this position. For example, enantiopure 3-arylalkanols have been reported as chiral synthons in the enantiospecific synthesis of the therapeutic agents curcumene **24** and

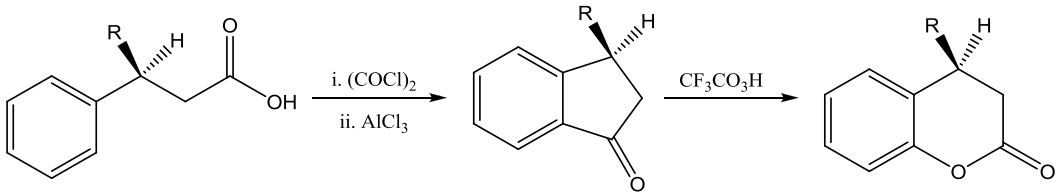
curcuphenol **25**,[19] the structurally related cytotoxic agent turmerone **26**,[20] and the synthetic fragrant florhydral **27**[21] (Figure 3.4).



(*S*)-**24** (*S*)-**25** (*S*)-**26** (*S*)-**27**

Figure 3.4

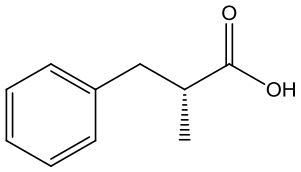
The enantiopure methyl, ethyl and isopropyl β-substituted 3-arylalkanoic acids (*S*)-**23**, (*S*)-**28** and (*R*)-**29** have been successfully cyclised *via* classical Friedel-Crafts reactions in the course of the preparation of chiral 3-alkylindanones **30**. The analogous optically active dihydrocoumarins **31** have been synthesised by Baeyer-Villiger peracid oxidation (Scheme 3.2).[22]



R = Me (*S*)-**23** **30** **31**
R = Et (*S*)-**28**
R = *i*-Pr (*R*)-**29**

Scheme 3.2

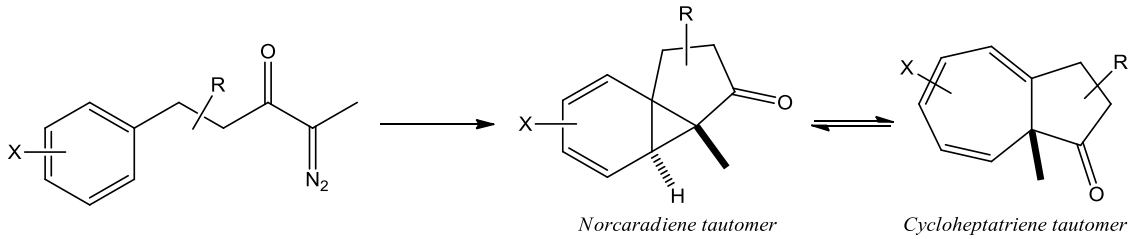
Furthermore, enantiopure (*R*)-2-methyl-3-phenylpropanoic acid (*R*)-**32** is a potentially useful compound for incorporating chiral recognition units in adenosine receptor agonists[23] and antagonists[24] (Figure 3.5).



(*R*)-**32**

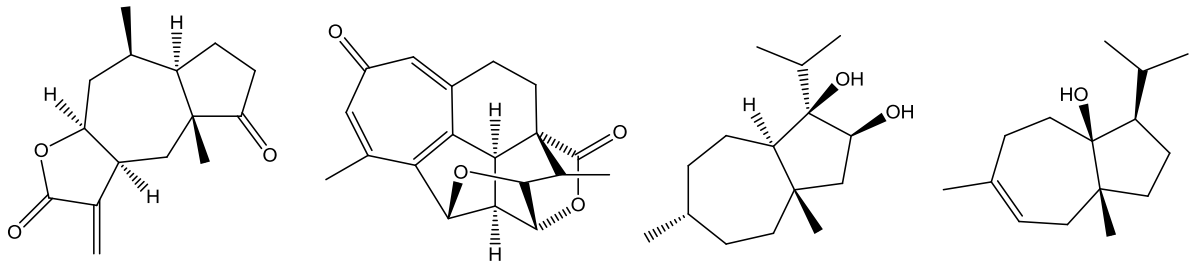
Figure 3.5

Within our own group, 3-arylalkanoic acids are utilised in the synthesis of aryl-diazoketone derivatives, which in turn have been employed in Buchner cyclisation reactions demonstrating excellent diastereoselectivity (Scheme 3.3).[25-29]



Scheme 3.3

This key transformation provides an extremely efficient asymmetric route to the bicyclo[5.3.0]decane skeleton, characteristic of daucane sesquiterpenoids. Synthetic approaches to this ring system have been extensively reviewed.[30,31] This important synthetic route has been exploited by McKervey *et al*.[32,33] in their work towards the synthesis of (±)-confertin **33** and Mander *et al*.[34-36] in their synthesis of harringtonolide **34**. Within our laboratory, O'Leary and Foley have investigated the synthesis of CAF-603 **35** using this methodology.[37,38] More recently Stack and McDowell applied the intramolecular aromatic addition to the synthesis of carotol **36** with several key advanced intermediates for its synthesis prepared[39-41] (Figure 3.6).



33 **34** **35** **36**

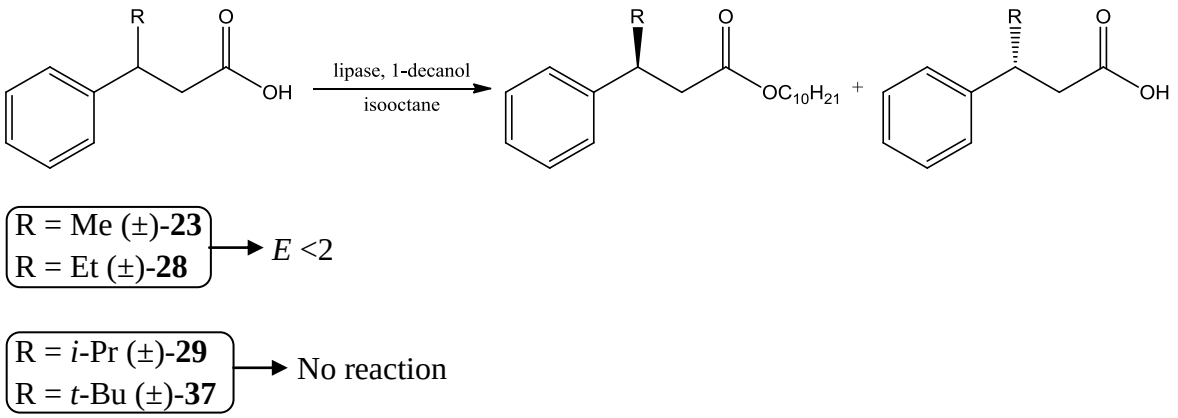
Figure 3.6

Thus, enantiopure 3-arylalkanoic acids are attractive synthetic targets and their enantioselective lipase-mediated resolution is the focus of this study.

3.1.2 Background to the project

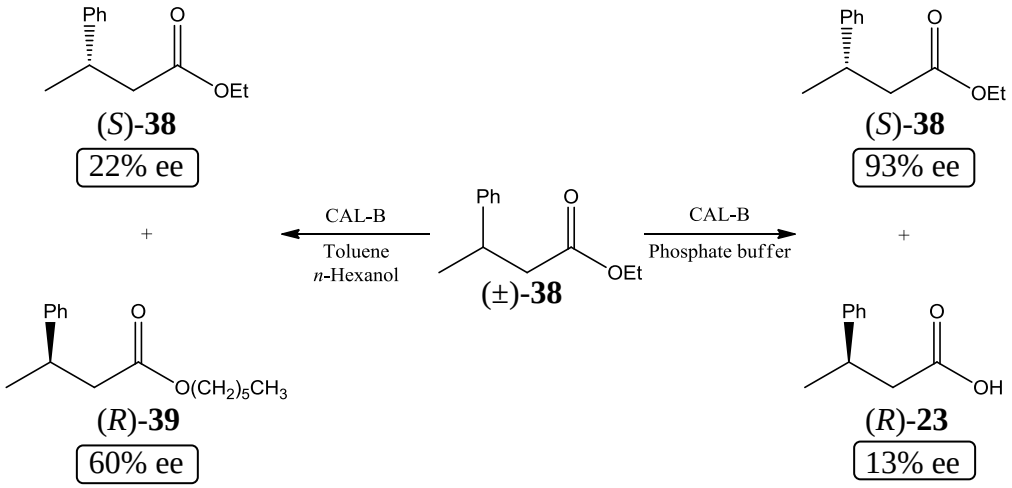
In this section, recent advances in the kinetic bioresolutions of 3-arylalkanoic acids will be summarised. Following a brief survey of enantioselective biotransformations in organic solvents and nitrile hydrolysis as routes to enantiopure 3-arylalkanoic acids, traditional aqueous based hydrolysis of the analogous esters will be discussed.

Attempted hydrolase-catalysed non-aqueous enantioselective esterification of methyl, ethyl, isopropyl and *tert*-butyl β-substituted 3-arylalkanoic acids, (±)-**23**, (±)-**28**, (±)-**29** and (±)-**37** has previously been investigated by Hedenström *et al.*[42] Resolution was not achieved with immobilised *Candida rugosa* lipase, *Burkholderia cepacia* (Amano PS) or *Candida antarctica* lipase B (Chirazyme® L-2). Substrate acids (±)-**23** and (±)-**28** were reported to esterify with a modest to slow rate with a minimal preference for the (*S*)-enantiomer, resulting in very low *E* values ($E < 2$). No ester was observed under any conditions for the acids (±)-**29** and (±)-**37**, with the larger substituents at the β-position despite extended reaction times (Scheme 3.4).



Scheme 3.4[42]

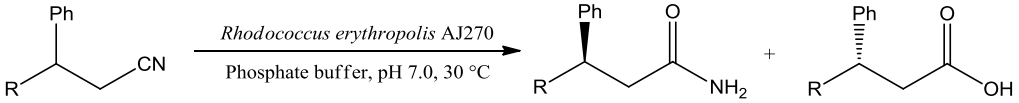
Bornscheuer *et al.* investigated *Candida antarctica* lipase B (CAL-B) catalysed transesterification and hydrolysis of vinyl and ethyl esters of racemic aryl aliphatic carboxylic acids.[43] Notably, poor enantioselectivity was observed for the transesterification (E = 13) and hydrolysis (E = 9) of 3-phenylbutanoic acid ethyl ester (±)-**38** (Scheme 3.5).



Scheme 3.5[43]

Numerous studies have demonstrated that biotransformations of nitriles complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives.[11-13] The enantioselective hydration of racemic 3-phenylbutanenitrile by *Mycobacterium strain* A277 afforded enantiomerically enriched (*S*)-3-phenylbutanoic acid (*S*)-**23**.[44] Furthermore, Wang *et al*. established that the amidase *Rhodococcus erythropolis* AJ270 showed greater activity and higher enantioselectivity against 3-arylpent-4-enoic acid amides, than 3-arylpentanoic acids amides.[45] Despite the high sensitivity of the amidase toward the structure of the amide substrate, (*S*)-3-phenylpentanoic acid (*S*)-**28** was isolated with good enantioselectivity (82% ee) after 26 h incubation (entry 2, Table 3.1).

*Table 3.1: Enantioselective biotransformations of racemic nitriles (±)-**40** and (±)-**41**[45]*



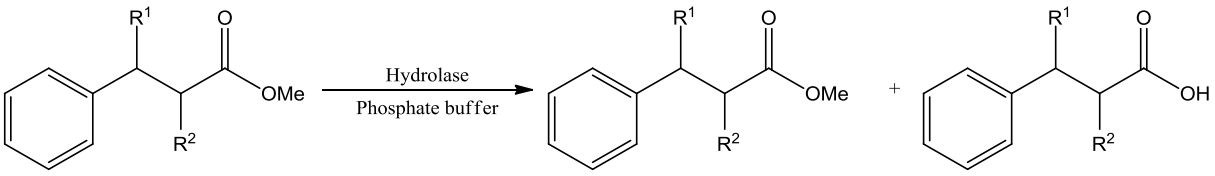
Entry	R	Nitrile	Time	Amide			Acid		
					Yield[a] (%)	ee[b] (%)		Yield[a] (%)	ee[b] (%)
1	Vinyl	(±)-**40**	15.5 h	(*S*)-**42**	47.5	95	(*R*)-**43**	47	95
2	Et	(±)-**41**	26 h	(*R*)-**44**	48	86	(*S*)-**28**	47	82

a. Isolated yield.
b. Determined by HPLC or GC analysis.

Traditional aqueous *Burkholderia cepacia* and *Pseudomonas sp.* catalysed ester hydrolysis has been described for the successful resolution of the β-methyl substituted (*S*)-3-phenylbutanoic acid (*S*)-**23**, 89% ee[44] (entry 1, Table 3.2) and the α-methyl substituted (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**32**, >95% ee,[23,46] (entry 2, Table 3.2) respectively, however significantly this work has not been expanded to include acid substrates encompassing more sterically demanding substituents at the C3 or C2 stereogenic centre.

Carrea *et al.*[46] (entry 3, Table 3.2) and Sih *et al.*[47] (entry 4, Table 3.2) broadened the scope of this hydrolysis to include α-ethyl substituted 3-aryl 2-alkyl alkanoic acid (±)-**45**, however an almost total disappearance of the enzyme capacity for stereoselection together with a drastic reduction of activity was observed on replacement of the methyl with the bulkier ethyl group at the C2 chiral centre.

Table 3.2: Enantioselective aqueous hydrolysis of 3-arylalkanoic acids[23,44,46,47]

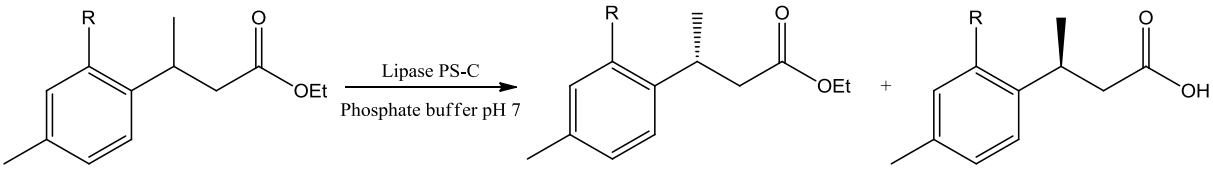


Entry	R^1	R^2	Hydrolase	Ester		Acid		*E* value
					ee (%)		ee (%)	
1	Me	H	*Burkholderia cepacia*	(*R*)-**46**	>98	(*S*)-**23**	89	>50[44]
2	H	Me	*Pseudomonas sp.*	(*R*)-**47**	>95	(*S*)-**32**	>95	145[23,46]
3	H	Et	Lipase PS	(*R*)-**48**	-[a]	(*S*)-**45**	7	1.2[46]
4	H	Et	Lipase N (Amano)	(*R*)-**48**	32	(*S*)-**45**	53	-[47]

a. Enantiopurity was not reported.[46]

Recently the lipase-catalysed hydrolysis of substituted ethyl butanoates (±)-**49** and (±)-**50** was performed with the lipase from *Pseudomonas cepacia* immobilised on ceramic particles (PS-C) in 0.1 M phosphate buffer (pH 7.0) at 30 °C (Table 3.3). The desired acids (*S*)-**51** and (*S*)-**52** were obtained in high enantiopurity (>99% ee).[19]

*Table 3.3: Lipase-mediated hydrolysis of (±)-**49** and (±)-**50**[19]*



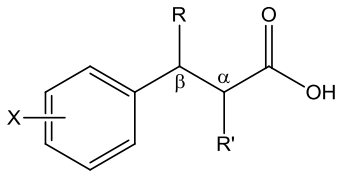
Entry	R	Substrate ester	Ester		Acid	
				ee (%)		ee (%)
1	H	(±)-**49**	(*R*)-**49**	92	(*S*)-**51**	>99
2	OMe	(±)-**50**	(*R*)-**50**	94	(*S*)-**52**	>99

Besides classical screening of microorganisms to find suitable enzymes, protein engineering using rational or directed evolution is a promising and often successful tool to create the desired biocatalyst.[48] Recent reports in the literature have screened metagenomic libraries as an efficient alternative to find novel biocatalysts for the enantioselective kinetic resolution of (*S*)-3-phenylbutanoic acid (*S*)-**23** with modest to high enantioselection.[49,50] The literature also reveals a 2004 patent filed in Japan titled "Manufacture of optically active 3-alkyl-3-arylpropionic acid and ester with lipase of *Burkholderia cepacia*", however limited experimental detail is supplied.[51]

In summary, an overview of the literature reveals modest enantioselection in the preparation of 3-arylalkanoic acids *via* enzymatic esterification, transesterification and nitrile hydrolysis. While promising results have been achieved by aqueous enantioselective hydrolysis of 3-arylalkanoic carboxylic esters, excellent enantioselection has been limited to the resolution of substrates with methyl substituents at the C2 or C3 chiral centre. Based on this precedent, the present study focused on aqueous hydrolysis for the resolution of a broad series of 3-arylalkanoic acids, expanding the range of alkyl substituents at the C2 and C3 stereocentres and the aromatic ring.

3.2 Objectives of the project

The overall objective of this work was to explore lipase-mediated kinetic resolutions as a practical synthetic route to a series of 3-arylalkanoic acids with a wider range of substrates than the butanoic acids (Figure 3.7).



R, R' = H, Me, Et, *i*-Pr, *t*-Bu
X = *p*-Me, *m*-Me, *o*-Me, *p*-OMe, *p*-F

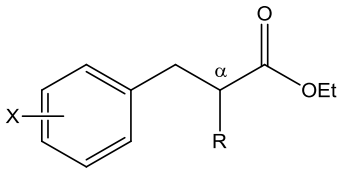
Figure 3.7

The specific objectives of this study can be summarised as follows:

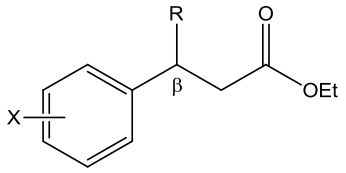
- To prepare a series of structurally related known and novel racemic ethyl 3-arylalkanoates and 3-arylalkanoic acids for the investigation of hydrolase-mediated kinetic resolution.
- To develop chiral HPLC conditions in which both enantiomers of the ethyl ester substrate and carboxylic acid product for each ester hydrolysis could be seen on a single trace, to facilitate analysis and optimisation.
- To perform screening assays of the ethyl 3-arylalkanoates against a series of hydrolases investigating the influence of each of the following on the outcome of the enantioselective hydrolysis both in terms of efficiency and enantioselectivity:
    - Variation of biocatalyst.
    - Alteration of reaction conditions *e.g.* temperature and co-solvent addition.
    - The impact of substituents at C3 and C2, specifically steric effect.
    - Both steric and electronic effects of substituents on the aromatic ring.
    - Effect of the position of the stereocentre relative to the reactive site.
- To conduct preparative-scale synthesis of each of the enantioenriched 3-arylalkanoic acids under optimised conditions to demonstrate their synthetic utility.
- To determine the absolute stereochemistry of each of the optically active 3-arylalkanoic acids.

3.3 Synthesis of ethyl 3-arylalkanoates

The initial task in this project was to synthesis a series of ethyl 3-arylalkanoates, bearing a range of substituents on both the carbon framework (α- and β-position) and on the aromatic ring, as summarised below in Figure 3.8. Compounds (±)-**48**, (±)-**53**, (±)-**54** and (±)-**55** are novel while each of the others have been previously characterised in the literature.[19,52-57]



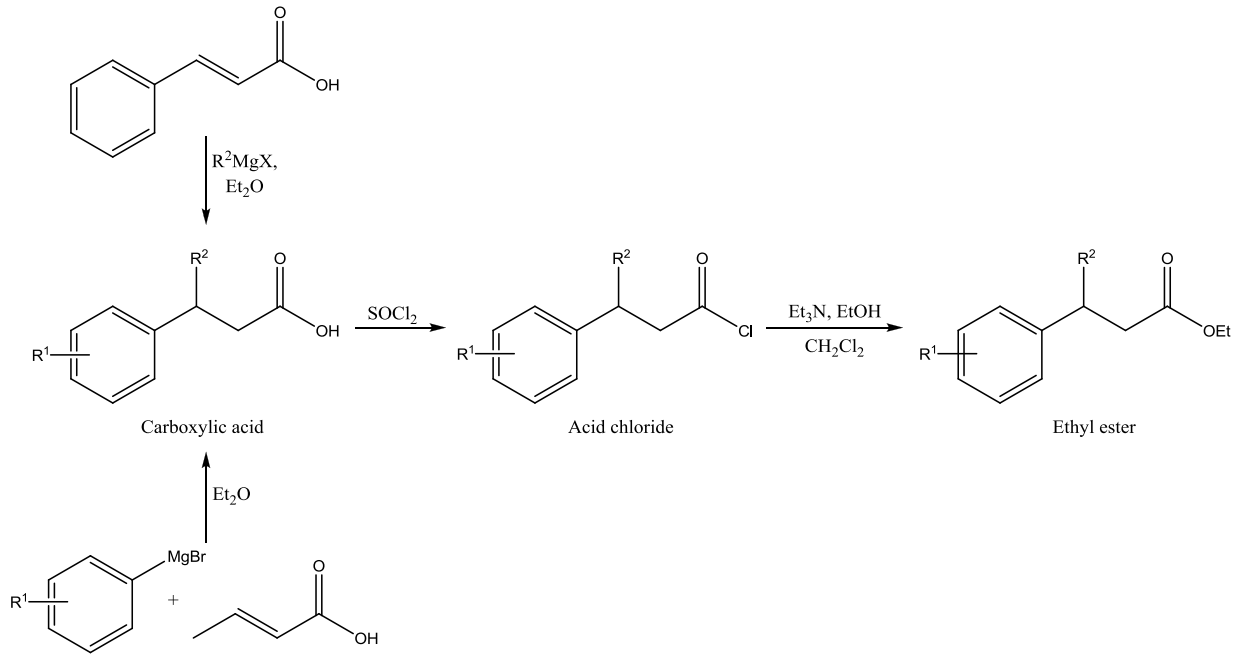
X	R	
H	Me	(±)-**47**[53]
H	Et	(±)-**48**
H	*t*-Bu	(±)-**57**[54]



X	R	
H	Me	(±)-**38**[52]
H	Et	(±)-**56**[55]
H	*i*-Pr	(±)-**58**[56]
H	*t*-Bu	(±)-**59**[54]
p-Me	CH_3	(±)-**49**[19]
o-Me	CH_3	(±)-**53**
m-Me	CH_3	(±)-**54**
p-F	CH_3	(±)-**55**
p-OMe	CH_3	(±)-**60**[57]

Figure 3.8

Four synthetic routes were employed for the preparation of the ethyl 3-arylalkanoates; (a) esterification of commercially available 3-arylalkanoic acids; (b) three-step synthesis, firstly conjugate addition of alkyl or aryl Grignard reagents to α,β-unsaturated acids, subsequent acid chloride formation and finally reaction of the acid chloride with ethanol in the presence of triethylamine to yield the desired ethyl ester (Scheme 3.6);



Scheme 3.6

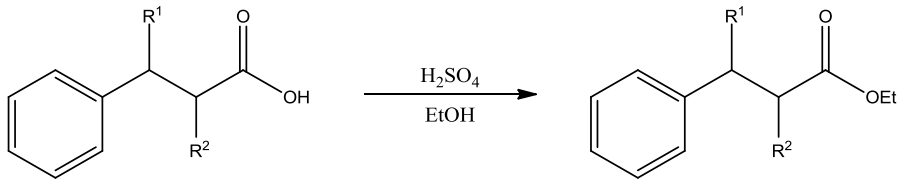
(c) Horner-Wadsworth-Emmons reaction to produce the α,β-unsaturated ester followed by hydrogenation (d) treatment of ethyl 3-arylalkanoates with lithium diisopropylamide and subsequent reaction of the resulting anion with aryl halides.

The method chosen for the preparation of each acid depended on the substituents required and position on the molecular framework; each will be addressed in turn.

3.3.1 Esterification of commercially available 3-arylalkanoic acids

The 3-arylalkanoic acids (±)-**23**, (±)-**32** and (±)-**61** were commercially available[58] and esterification yielded the desired 3-arylalkanoates (±)-**38**, (±)-**47** and (±)-**57**. Fischer esterification of the α- and β-methyl substituted carboxylic acids, (±)-**23** and (±)-**32** respectively, proceeded very smoothly to yield the analogous ethyl 3-arylalkanoates (±)-**47** and (±)-**38** in excellent purity and good yields (Table 3.4). The acid-catalysed esterification procedure employed in this study involved heating the commercially available acid in excess absolute ethanol under reflux with catalytic sulfuric acid overnight. The Fischer esterifications were not monitored for reaction completion and it may be possible that the reactions were complete in a shorter period of time. A slightly lower yield is observed for the α-methyl substituted ester (±)-**47**, however on analysis of the ^{1}H NMR spectrum of the crude product no starting material (±)-**32** was observed and the position of the methyl substituent on the carbon framework is understood to have little or no effect on the efficiency of the reaction. The purity of the racemic substrate for enzymatic catalysis is of utmost importance for the success of the asymmetric hydrolysis. Therefore, even though the ethyl esters appeared >99% pure on analysis of the ^{1}H NMR spectrum of the crude product, purification by column chromatography was conducted.

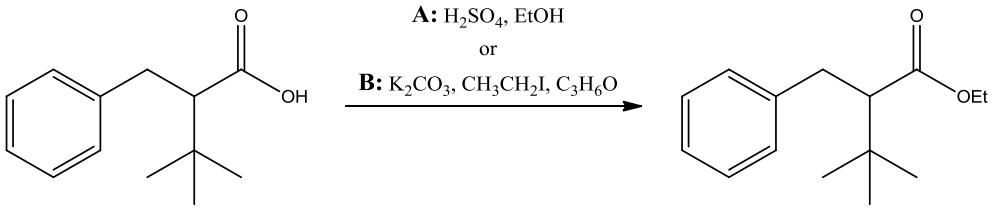
Table 3.4: Fischer esterification of 3-arylalkanoic acids



Entry	Alkanoic acid	R^1	R^2	Alkanoic ester	Yield (%)[a]
1	(±)-**23**	CH_3	H	(±)-**38**[52]	78
2	(±)-**32**	H	CH_3	(±)-**47**[53]	63

a. Yield following purification by column chromatography.

Esterification of the carboxylic acid (±)-**61** was attempted *via* Fischer esterification however <5% of the desired product (±)-ethyl 2-benzyl-3,3-dimethylbutanoate (±)-**57** was evident in the ^{1}H NMR spectrum of the crude product (Scheme 3.7). The poor conversion is most likely due to the steric hindrance of the bulky α-*tert*-butyl group, preventing nucleophilic attack of the alcohol. An alternative S_N2 approach was adopted. Thus, acid (±)-**61** was deprotonated using potassium carbonate in acetone, and the resulting carboxylate was alkylated with ethyl iodide to afford the desired ester (±)-**57**. This reaction did not proceed to completion with the carboxylic acid (±)-**61** (~13%) evident in the ^{1}H NMR spectrum of the crude product. The starting material (±)-**61** was easily removed upon chromatographic purification which yielded the pure ester (±)-**57** in 53% yield (Scheme 3.7).



(±)-**61** (±)-**57**[54]

A: *<5% evident in ^{1}H NMR of crude product.*

B: *53% isolated yield.*

Scheme 3.7

The ethyl esters (±)-**38**, (±)-**47** and (±)-**57** were characterised by a carbonyl absorption band in the IR spectra at ν_{max} 1729-33 cm^{-1}. In the ^{1}H NMR spectra of the 3-arylalkanoates (±)-**38** and (±)-**47** a distinctive triplet at δ_H 1.17-1.18 ppm and quartet at δ_H 4.08 ppm attributable to the ethyl ester moiety was observed. The 3-arylalkanoate (±)-**57** bearing the α-*tert*-butyl substituent displayed a triplet in the ^{1}H NMR spectrum at δ_H 1.04 ppm as anticipated, however interestingly the methylene resonance of the ethyl moiety appears as a symmetrical multiplet at δ_H 3.88-4.03 ppm reflecting their diastereotopic nature (Figure 3.9).

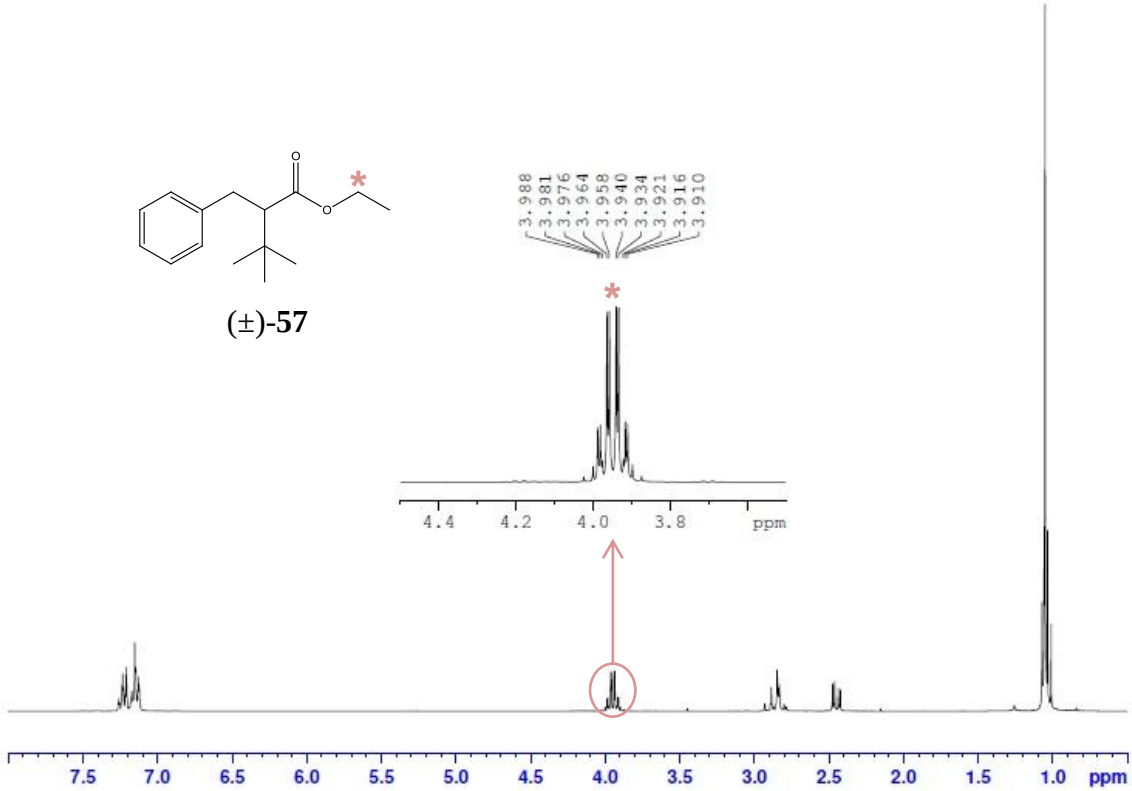


*Figure 3.9: ^{1}H NMR spectrum of (±)-ethyl 2-benzyl-3,3-dimethylbutanoate (±)-**57** (recorded in $CDCl_3$ at 300 MHz).*

In summary, the esterification of commercially available 3-arylalkanoic acids (±)-**23**, (±)-**32** and (±)-**61** proceeded efficiently with modest to good yields. Each of the ethyl esters (±)-**38**, (±)-**47** and (±)-**57** was isolated as a colourless oil. Significantly no decomposition of the ethyl esters (±)-**38**, (±)-**47** and (±)-**57** was identifiable by ^{1}H NMR spectroscopy on storage of the oils on the laboratory bench at room temperature for several months. Spectral

characteristics of each ethyl alkanoate (±)-**38**, (±)-**47** and (±)-**57** were in agreement with those previously described in the literature.[52-54]

3.3.2 Three-step synthesis of 3-arylalkanoates

Throughout the course of this work it was necessary to investigate 3-arylalkanoates bearing alkyl substituents at the β-position and substituents on the aromatic ring, the former to determine the impact of steric effects at C3 and the latter to explore both steric and electronic effects of the aromatic ring, on the efficiency of the kinetic resolution. The carboxylic acid precursors were not commercially available, therefore the first step was to synthesise a series of acids using the conjugate addition of alkyl or aryl Grignard reagents to α,β-unsaturated acids.

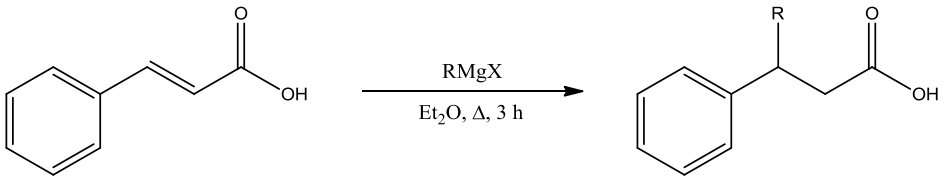
3.3.2.1 Addition of Grignard reagents to α,β-unsaturated acids

3.3.2.1.1 Addition of alkyl Grignard reagents to α,β-unsaturated acids

The primary method utilised in this study was first described by Wotiz[59] and has been extensively optimised within our own research group as a route to precursors for α-diazoketones.[37-39,60-63] This methodology was considered appealing due to the high yields obtained and the scalability of the reaction. The mechanism was described by Harrington.[61] All Grignard reagents were freshly prepared *in situ*, from magnesium turnings, the appropriate alkyl bromide in diethyl ether and a crystal of iodine as an initiator, with the exception of *tert*-butylmagnesium chloride which was commercially available as a 2.0 M solution in diethyl ether. The optimal conditions involved portionwise addition of 1.0 equivalent of the α,β-unsaturated acid over 30 min to a stirring ethereal solution of 3.0-4.0 equivalents of the Grignard reagent at 0 °C followed by heating under reflux for 3 h. After such time the solution was subjected to an acidic work-up.

The reactions were not followed by TLC analysis since the products tended to streak on the TLC plate, but, in general, reaction time did not exceed 3 h. Previous researchers within the group have utilised copper(I) chloride (0.05 eq.) to promote 1,4 addition,[37,60-62] however during this study and throughout the work by Stack[39] and McNamara[63] Cu(I)Cl was not employed with no adverse effects on both yield or quality of product. The 1,2-addition product was not identified in any case in the ^{1}H NMR spectrum of the crude product

*Table 3.5: Addition of alkyl Grignard reagents to cinnamic acid **62***



Entry	R	X	Propanoic acid	Crude yield (%)[a]
1	Et	Br	(±)-**28**[39,60-62]	92
2	*i*-Pr	Br	(±)-**29**[39,61,62]	83
3	*t*-Bu	Cl	(±)-**37**[39,60-62]	79

a. Products were not purified and were brought through as crude material to the subsequent acid chloride stage. For a more accurate representation of the efficiency of each reaction an examination of the yield of the acid chloride is required as this is the yield calculated over the two steps.

Attempts by Buckley,[60] O'Leary[37] and Harrington[61] to purify these acids by column chromatography were unsuccessful, leading to greatly diminished yields (30-45%) and no

other satisfactory technique identified. As a result of these difficulties all acids synthesised during this study were carried forward in their crude form and purified by distillation in the subsequent acid chloride stage. Therefore the pure yield of the acid chloride is more indicative of the efficiency of the conjugate addition.

Thus, use of conjugate addition of alkyl Grignard reagents to cinnamic acid **62** generated a series of crude β-substituted carboxylic acids very efficiently. Spectral characteristics for each of the β-substituted acids (±)-**28**, (±)-**29** and (±)-**37** agreed with those described by previous members of the group[39,60-62] as well as literature reports.[26,59,64]

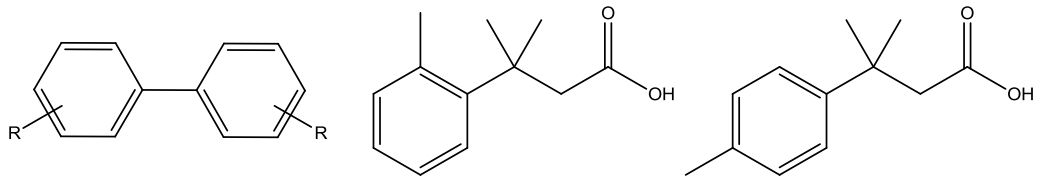
3.3.2.1.2 Addition of aryl Grignard reagents to α,β-unsaturated acids

Earlier efforts by Harrington found that when a methyl substituent was required at the β-position of the carboxylic acid, the standard methodology of conjugate addition of methylmagnesium bromide to cinnamic acid **62** was not effective and resulted in complex mixtures and reduced yields.[61] However conjugate addition of the appropriate aryl Grignard to crotonic acid **63** proceeds very efficiently and the desired product is obtained in good yield and purity.[39,61,62]

It is well-established that the preparation of Grignard reagents from alkyl halides is far more efficient than those from aryl halides. Specifically, low yields and poor conversions have been reported of Grignard reagents derived from electron-rich aryl halides, owing predominantly to competing formation of the biaryl side product (Wurtz coupling product).[65]

O'Leary and Harrington previously synthesised the *para*-methyl, *ortho*-methyl and *meta*-methyl substituted carboxylic acids (±)-**51**, (±)-**64** and (±)-**65**[37,61] In addition, O'Leary reported the preparation of the *para*-methoxy substituted butanoic acid (±)-**66**.[37] All 3-arylbutanoic acids were synthesised employing the conjugate addition methodology described above, neither O'Leary nor Harrington had discussed formation of the Wurtz coupling product.[37,61]

Recently, O'Keeffe synthesised (±)-3-(4-methoxyphenyl)butanoic acid (±)-**66** and found the formation of the desired acid (±)-**66** and Wurtz coupling product **67** in the ratio 44 : 56.[62] O'Keeffe also described the synthesis of (±)-3-(2-methylphenyl)butanoic acid (±)-**64** and notably reports no competing biaryl formation. However, when the same Grignard reagent was generated for the synthesis of **68**, anomalous peaks were detected in the ^{1}H NMR spectrum which were tentatively assigned as the Wurtz coupling product **69** (Figure 3.10).[62] Furthermore, during the synthesis of the β-disubstituted acid **70**, 3% of the coupling product **71** was identified (Figure 3.10).[62] Thus, the Wurtz coupling products **69** and **71** have previously only been formally identified when reacting acrylic acid with aryl Grignard reagents, however this does not discount their possible formation during the analogous conjugate addition of aryl Grignard reagents to crotonic acid **63**. O'Keeffe used a basic extraction in the work-up to ensure isolation of the acids free from the Wurtz coupling side products.[62] In this work, use of this protocol as a precautionary measure was undertaken in the synthesis of the 3-arylbutanoic acids (±)-**51**, (±)-**64-66**.



R = *p*-OMe **67** **68** **70**
R = *p*-Me **71**
R = *o*-Me **69**
R = *m*-Me **72**
Wurtz Coupling Product

Figure 3.10

During this study the exact ratio of (±)-3-(4-methylphenyl)butanoic acid (±)-**51** to Wurtz coupling **67** (85 : 15 respectively) was determined on only one occasion.[iv] In all subsequent reactions involving aryl Grignard reagents the crude product was not isolated from the reaction mixture but subjected to a basic extraction. The biaryl side product was readily removed by extraction of the required acid into aqueous sodium hydroxide, washing with ethyl acetate to remove the Wurtz coupling product followed by acidification of the remaining aqueous layer which led to isolation of the acid in good yield and purity. This method successfully diminished the amount of Wurtz coupling product, with only trace evidence of the biaryl side product evident on analysis of the ^{1}H NMR spectrum of the crude product.

*Table 3.6: Addition of aryl Grignard reagents to crotonic acid **63***

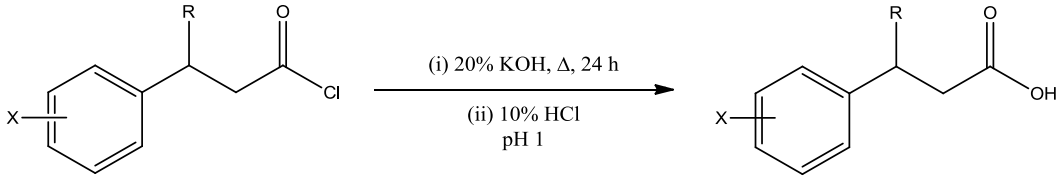
Entry	R	Product acid	Crude yield (%)[a]
1	*p*-OMe	(±)-**66**[37,62]	71
2	*o*-Me	(±)-**64**[37,61]	79
3	*p*-Me	(±)-**51**[37,61]	86
4	*m*-Me	(±)-**65**[37,61]	83

a. Products were not purified and were brought through as crude material to the subsequent acid chloride stage. For a more accurate representation of the efficiency of each reaction an examination of the yield of the acid chloride is required as this is the yield calculated over the two steps.

Analytically pure samples of each of the carboxylic acids synthesised were required for chiral HPLC method development, in which both enantiomers of the ester and acid could be seen on a single trace. As stated earlier, purification by column chromatography of these 3-arylalkanoic acids was not feasible, therefore in order to obtain material suitable for chiral HPLC analysis the corresponding acid chlorides which had been purified by vacuum distillation were subjected to basic hydrolysis (Table 3.7). The analytically pure acids (±)-**28, 29, 37, 51, 64-66** were regenerated in good yield and were sufficiently pure to warrant no further purification.

[iv] It should be noted that this reaction was carried out by a fourth year student.[66]

Table 3.7: Hydrolysis of distilled acid chloride



Entry	Acid chloride	R	X	Product acid	Yield[a] (%)
1	(±)-**73**	Et	H	(±)-**28**[26,59,64]	92
2	(±)-**74**	*i*-Pr	H	(±)-**29**[26,64]	99
3	(±)-**75**	*t*-Bu	H	(±)-**37**[26,59]	78
4	(±)-**76**	CH_3	*p*-OMe	(±)-**66**[27,67]	62
5	(±)-**77**	CH_3	*o*-Me	(±)-**64**[68]	78
6	(±)-**78**	CH_3	*p*-Me	(±)-**51**[64]	83
7	(±)-**79**	CH_3	*m*-Me	(±)-**65**[69]	69

a. The carboxylic acids were analytically pure and did not require further purification prior to chiral HPLC analysis.

In conclusion, conjugate addition of the appropriate aryl Grignard to crotonic acid **63** proceeded highly efficiently with a series of substituted aromatic, 3-arylbutanoic acids (±)-**51**, (±)-**64-66** successfully synthesised. All spectroscopic data compiled in this study agreed with those previously reported.[37,61,62] The Wurtz coupling product was easily removed with limited loss in yield. Analytically pure samples of each of the 3-arylalkanoic acids (±)-**28, 29, 37, 51, 64-66** were obtained by basic hydrolysis of the corresponding acid chlorides (±)-**73-79** and were of sufficient purity for chiral HPLC development.

3.3.2.2 Synthesis of acid chlorides

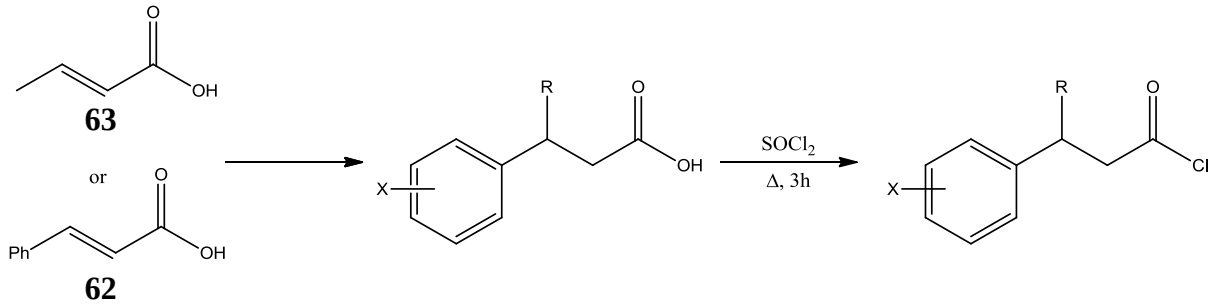
While the direct esterification of the crude carboxylic acid was attempted *via* Fischer esterification methodology, it was found that it was simpler to obtain the ethyl esters in analytically pure form by first transforming the carboxylic acid isolated from Grignard additions to the analogous acid chloride, which was readily purified by vacuum distillation.

Great care was taken to produce the acid chlorides in high purity, minimising impurities which could adversely affect the ester formation and subsequent hydrolase-mediated kinetic resolutions. In general, the crude acid and 8.0 equivalents of thionyl chloride were heated under reflux for 3 h. Following concentration of the solution under reduced pressure, and removal of residual thionyl chloride by azeotrope with toluene, the product was purified by vacuum distillation. It should be noted that (±)-3-(4-methylphenyl)butanoyl chloride (±)-**78** was synthesised with 10.0 equivalents of thionyl chloride, however there was no rationale to the increased equivalents of chlorinating agent and it is anticipated that the reaction would proceed smoothly in good yield with just 8.0 equivalents of thionyl chloride.[66]

In recent years Stack and O'Keeffe have incorporated the use of *N*,*N*-dimethylformamide (DMF) (3-5 drops) as a catalytic reagent in the preparation of acid chlorides with thionyl chloride, reporting that reaction times can be reduced to 1 h and thionyl chloride loading reduced to 5.0 equivalents.[39,62] This modified procedure was not applied in this study due to associated hazard. When DMF is exposed to thionyl chloride *N*,*N*-dimethylcarbamoyl chloride (DMCC) is formed, a potential carcinogen in humans.[70,71]

In all cases the acid chlorides (±)-**73-79** following distillation were of excellent purity as evident by the new carbonyl stretch in the IR spectrum at ν_{max} 1790-1801 cm^{-1} and ^1H NMR analysis. Yields were calculated over two steps from the respective starting cinnamic **62** or crotonic acids **63** and yields were moderate to low (29-54%).

Table 3.8: Preparation of acid chlorides from crude 3-arylalkanoic acids



Entry	Acid	R	X	Acid chloride	Yield[a] (%)
1	(±)-**28**	Et	H	(±)-**73**[26]	31
2	(±)-**29**	*i*-Pr	H	(±)-**74**[26]	36
3	(±)-**37**	*t*-Bu	H	(±)-**75**[26]	54
4	(±)-**66**	CH_3	*p*-OMe	(±)-**76**[27]	29
5	(±)-**64**	CH_3	*o*-Me	(±)-**77**[62]	41
6	(±)-**51**	CH_3	*p*-Me	(±)-**78**[72,b]	44
7	(±)-**65**	CH_3	*m*-Me	(±)-**79**[37,61]	40

a. % Yield is calculated over two steps and is quoted following distillation *in vacuo*. Yields of (±)-**73**, (±)-**74** and (±)-**75** are quoted from cinnamic acid **62** and yields of (±)-**76**, (±)-**77**, (±)-**78** and (±)-**79** are quoted from crotonic acid **63**.

b. 10.0 Equivalents of thionyl chloride was utilised in the synthesis of the acid chloride (±)-**78**. For the preparation of all other acid chlorides (±)-**73**-**77** and (±)-**79**, 8.0 equivalents of thionyl chloride was employed.

In order to obtain analytically pure samples of the 3-arylalkanoic acids which are not amenable to chromatographic purification, the distilled acid chloride (±)-**73-79** was hydrolysed regenerating each of the carboxylic acids as discussed in section 3.3.2.1.2.

In summary, multi-gram quantities of the acid chlorides (±)-**73-79** were prepared during this study. The acid chlorides were generally transformed to the corresponding ethyl esters within 24 h of preparation although they can be stored for short periods in the freezer without adverse effect.

3.3.2.3 Synthesis of ethyl esters

Acid chlorides are readily converted to esters when treated with the appropriate alcohol. A tertiary amine such as pyridine or triethylamine is used to scavenge the HCl by-product. The general procedure adopted during this study involved dropwise addition of the acid chloride to a stirring solution of triethylamine, distilled ethanol and dichloromethane at 0 °C. The addition of the acid chloride at room temperature proved quite vigorous and it was essential to cool the reaction mixture, however following addition the reaction mixture was allowed warm to room temperature and stirred overnight. Throughout this reaction precipitation of triethylamine hydrochloride was evident. Overall, this procedure proceeded extremely well with good yields and excellent purity of the desired 3-arylalkanoates following chromatographic purification.

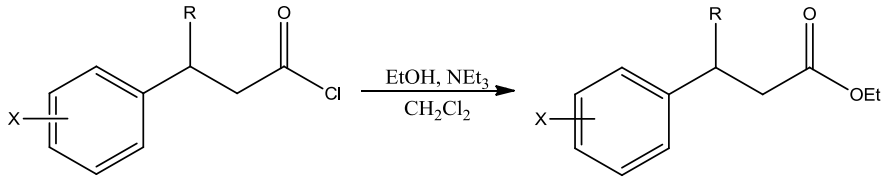
Employing this methodology, ethyl esters (±)-**49, 53, 54, 56, 58-60** were synthesised as summarised in Table 3.9. While ethyl esters (±)-**49** and (±)-**56, 58-60** have previously been reported in the literature, ethyl esters (±)-**53** and (±)-**54** are novel and were fully characterised during the course of this work. For the known ethyl esters, spectroscopic characteristics were in agreement with those described previously.[19,54-57]

Yields were reasonable in most cases ranging from 56-72% following purification by column chromatography. The only exception was in the case of the formation of ethyl ester

(±)-**53** which required additional purification by distillation following column chromatography leading to a slightly reduced yield of 44%.

As stated previously in section 3.3.2.1.2. by implementing the modified work-up outlined by O'Keeffe only trace evidence of the biaryl side product was observed on analysis of the ^{1}H NMR spectrum of the crude 3-arylbutanoic acids (±)-**51**, (±)-**64-66**.[62] Any residual Wurtz coupling product that was not removed by vacuum distillation of the analogous acid chlorides (±)-**76-79** was easily separated during column chromatography of the corresponding ethyl 3-arylbutanoates (±)-**49, 53, 54, 60**.

Table 3.9: Preparation of ethyl esters from acid chlorides



Entry	Acid chloride	R	X	Ethyl ester	Yield[a] (%)
1	(±)-**73**	Et	H	(±)-**56**[55]	72
2	(±)-**74**	*i*-Pr	H	(±)-**58**[56]	63
3	(±)-**75**	*t*-Bu	H	(±)-**59**[54]	56
4	(±)-**76**	CH_3	*p*-OMe	(±)-**60**[57]	56
5	(±)-**77**	CH_3	*o*-Me	(±)-**53**	44[b]
6	(±)-**78**	CH_3	*p*-Me	(±)-**49**[19]	62
7	(±)-**79**	CH_3	*m*-Me	(±)-**54**	67

a. % Yield is calculated following purification by column chromatography.
b. Required additional purification by vacuum distillation.

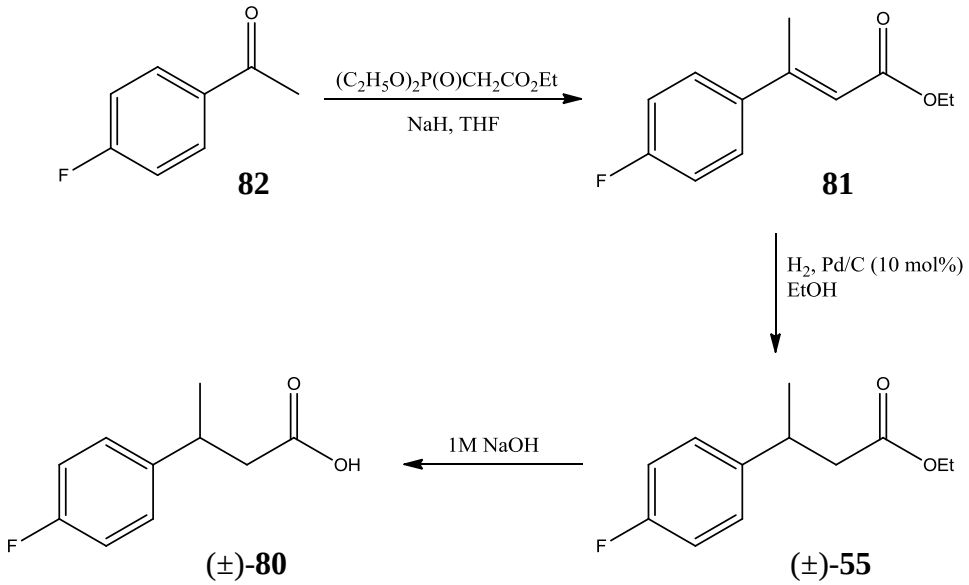
The ethyl esters were characterised by disappearance of the acid chloride carbonyl stretch at ν_{max} 1790-1801 cm^{-1} and the appearance of the new ester carbonyl stretch at the lower frequency of ν_{max} 1732-37 cm^{-1}. Analysis by ^{1}H NMR spectroscopy displays some interesting trends, with the methylene hydrogens of the ethyl moiety, which appears as a quartet between δ_H 3.95-4.07 ppm, providing the most characteristic evidence of ethyl ester formation. Once again for the ethyl ester substituted with a *tert*-butyl group at the β-position (±)-**59** the diastereotopic OCH_2 hydrogens were seen as a multiplet as discussed earlier for the α-*tert*-butyl substituted alkanoate (±)-**57** (see section 3.3.1).

Thus, this procedure proved highly successful with isolation of multi-gram quantities of the 3-arylalkanoates (±)-**49, 53, 54, 56, 58-60**, of sufficient purity for the hydrolase-mediated kinetic resolution screens.

3.3.3 Horner-Wadsworth-Emmons reaction

To introduce a methyl substituent β to the carbonyl group the general synthetic methodology employed was conjugate addition of aryl Grignard reagents to an α,β-unsaturated acid (described in section 3.3.2.1.2). McNamara employed this synthetic route in generating (±)-3-(4-fluorophenyl)butanoic acid (±)-**80** from 4-fluorophenylmagnesium bromide and crotonic acid **63**.[63] However, an unknown aromatic side product was observed in the ^{1}H NMR spectrum of the crude product and the desired product was obtained in poor yield (23%) following purification by column chromatography.[63] Therefore, an alternative method for the synthesis of the carboxylic acid (±)-**80** and the corresponding ethyl ester (±)-**55** was implemented in this research.

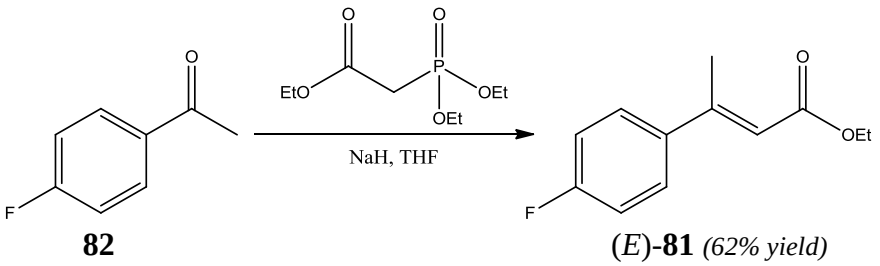
Marcantoni *et al.* reports utilising the Horner-Wadsworth-Emmons reaction to produce the α,β-unsaturated ester **81**, followed by catalytic hydrogenation to smoothly provide the required ethyl ester (±)-**55** which was subjected to basic hydrolysis to yield (±)-3-(4-fluorophenyl)butanoic acid (±)-**80**.[73] High yields (≥85%) and excellent purity were described for all synthetic steps (Scheme 3.8).[73] Therefore, this synthetic protocol was engaged in this study.



Scheme 3.8

Pioneering papers published by Wittig and co-workers in the early 1950's disclosed a means for the olefination of ketones and aldehydes, in which carbonyl compounds were treated with phosphonium ylides to form the desired olefin and phosphine oxide, exhibiting high selectivity for the *Z* or *E* isomer depending on reaction conditions.[74,75] In 1958, Horner[76] described a modified Wittig reaction employing phosphonate-stabilised carbanions and the scope of the reaction was further defined by Wadsworth and Emmons.[77] This adapted procedure possesses significant advantages over the traditional Wittig olefination; the phosphonate carbanions are more nucleophilic and undergo smooth alkylation, in contrast to the corresponding conventional phosphonium ylides. In addition the dialkylphosphate salt by-product is readily removed by aqueous extraction from the desired olefin.[77-80]

In this work the initial step of the Horner-Wadsworth-Emmons reaction involved abstraction of the acidic proton of triethyl phosphonoacetate with NaH to yield the phosphonate carbanion, which underwent nucleophilic addition to commercially available *p*-fluoroacetophenone **82** to form a betaine intermediate species. Irreversible decomposition of the betaine yielded the desired alkene **81** (Scheme 3.9).



Scheme 3.9

Marcantoni *et al.* reports almost exclusive formation of the *E* isomer of ethyl 3-(4-fluorophenyl)but-2-enoate **81** without any appreciable formation of the *Z* isomer evident by ^{1}H NMR analysis.[73] In this investigation, a 76 : 24 mixture of *E* : *Z* isomers was apparent on examination of the ^{1}H NMR spectrum of the crude product (Figure 3.11). The *E* and Z isomers were separated upon purification by column chromatography to give a pure sample of the *E* isomer in 62% yield.

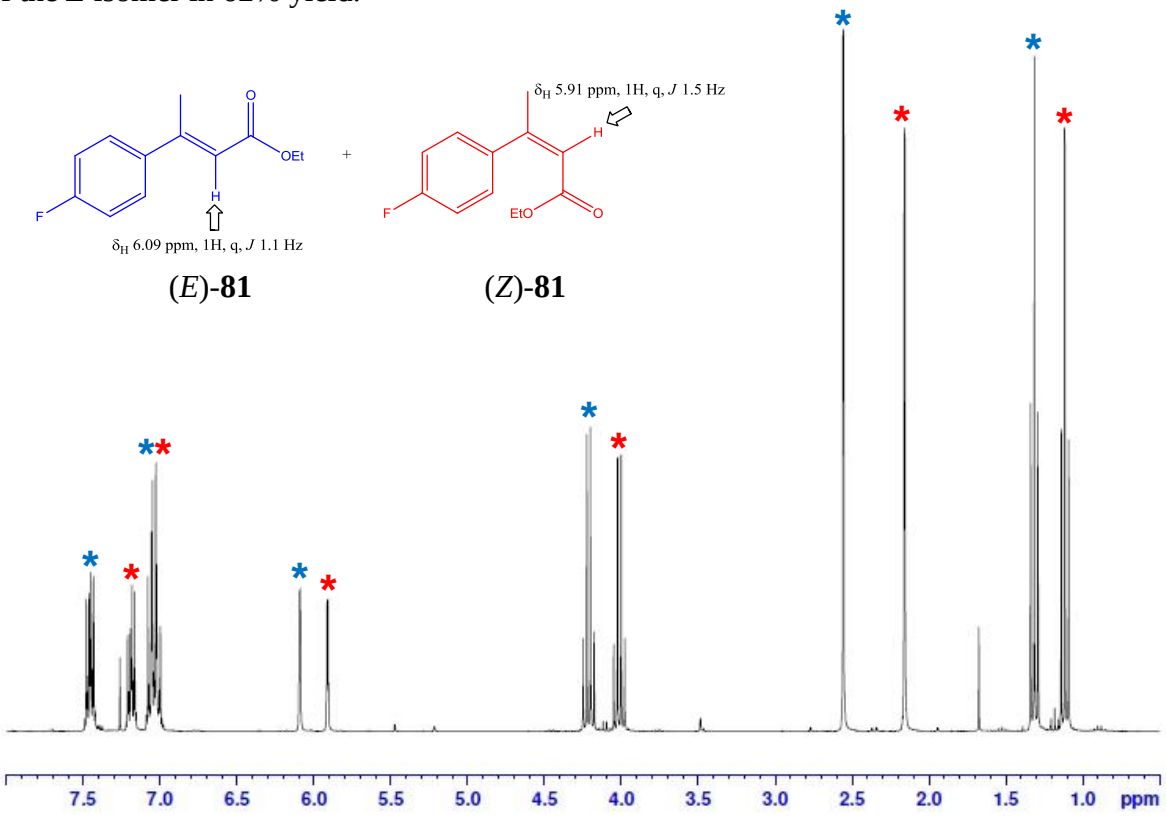
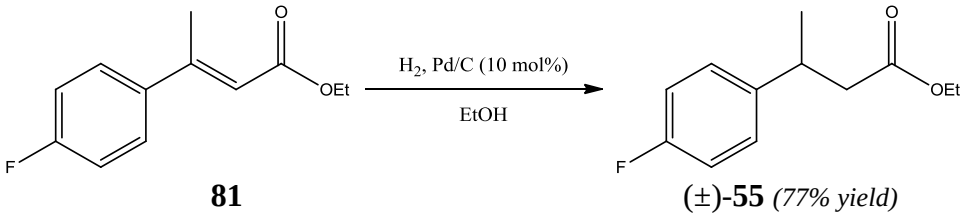


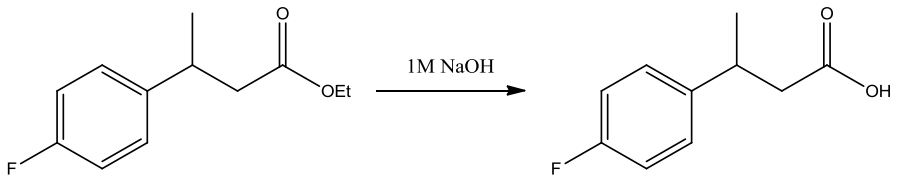
Figure 3.11: *^{1}H NMR spectrum of fraction isolated during column chromatography which contained 53 : 47 mixture of* E *:* Z *isomers of ethyl 3-(4-fluorophenyl)but-2-enoate* ***81*** *(recorded in $CDCl_3$ at 300 MHz).*

The next step in the synthesis was reduction of the olefinic bond of (*E*)-ethyl 3-(4fluorophenyl)but-2-enoate (*E*)-**81** by catalytic hydrogenation (Scheme 3.10). The α,β-unsaturated ester (*E*)-**81** was reacted under an atmosphere of hydrogen (15 psi) at room temperature for 15.5 h over 10% palladium on carbon as catalyst to yield the (±)-3-arylalkanoate (±)-**55**. The hydrogenation was carried out in undistilled absolute ethanol. The desired ester (±)-**55** was isolated in good yield (77%) following purification by column chromatography. (±)-Ethyl 3-(4-fluorophenyl)butanoate (±)-**55** was previously described in the literature, however, characterisation was limited to mass spectrometry[73], therefore full analysis was obtained in this study.



Scheme 3.10

To access analytically pure samples of the analogous carboxylic acid for chiral HPLC method development the ethyl ester (±)-**55** was hydrolysed under basic conditions (Scheme 3.11). (±)-Ethyl 3-(4-fluorophenyl)butanoate (±)-**55** was stirred overnight with aqueous sodium hydroxide (1M) to yield the carboxylic acid (±)-**80** in good yield (67%) and of sufficient purity to warrant no further purification. Spectral characteristics were in agreement with those reported by McNamara.[63]



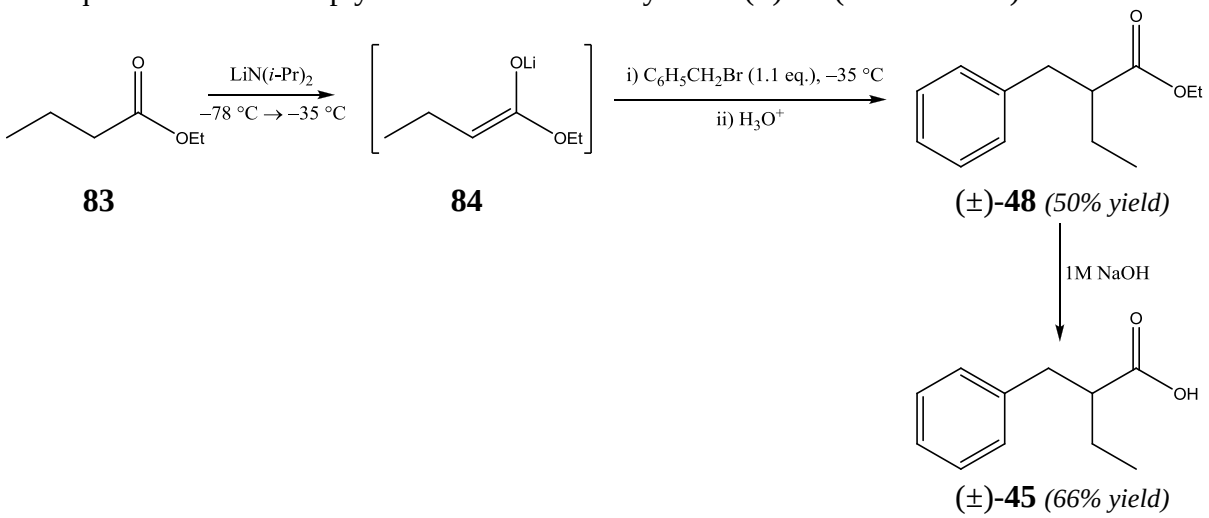
(±)-**55** (±)-**80** *(67% yield)*

Scheme 3.11

The Horner-Wadsworth-Emmons synthetic route demonstrated a viable alternative to conjugate addition of aryl Grignard reagents to α,β-unsaturated acids for the synthesis of analytically pure 3-arylbutanoic acids. Each step proceeded in good yield and high purity. Significantly this route circumvents the Wurtz coupling side product and represents a shorter synthetic methodology to ethyl 3-arylbutanoates.

3.3.4 Synthesis of α-alkylated alkanoate

As discussed in section 3.3.1, the α-substituted ethyl esters (±)-**47** and (±)-**57** were easily obtained through esterification of their commercially available carboxylic acids. However, the next challenge was to alkylate α to the ester moiety where the analogous carboxylic acids were not commercially accessible. O'Keeffe reported the synthesis of α-alkylated carboxylic acids, however purification was not straightforward and the crude acids were carried through to the acid chloride stage.[62] In this study, the methodology described by O'Keeffe for α-alkylation of carboxylic acids was adapted to α-alkylation of ethyl esters, therefore eliminating the need for synthesis of the acid chloride and the subsequent esterification step.[62] The addition of ethyl butyrate **83** to lithium diisopropylamide at −35 °C generated the enolate **84**. Alkylation was achieved using the appropriate benzyl halide and subsequent acidic work-up yielded the desired ethyl ester (±)-**48** (Scheme 3.12).



83 **84** (±)-**48** *(50% yield)*

(±)-**45** *(66% yield)*

Scheme 3.12

This modified procedure involved addition of ethyl butyrate **83** to 1.0 equivalent of lithium diisopropylamide, prepared from *n*-butyllithium and diisopropylamine in tetrahydrofuran at −78 °C. The reaction mixture was then warmed to −35 °C and stirred for 1 h before benzyl bromide was added in one portion. The reaction mixture was stirred overnight at −35 °C before warming and subjecting to an acidic work-up. The ^{1}H NMR spectrum of the crude product showed no evidence of residual starting ethyl butyrate **83** or the doubly alkylated product. The ethyl ester (±)-**48** was purified by flash chromatography and obtained in a 50% yield. (±)-Ethyl 2-benzylbutanoate (±)-**48** was previously mentioned in the literature however no spectral details were reported;[81] therefore full characterisation of (±)-**48** was conducted during this research.

The analogous α-alkylated carboxylic acid (±)-**45** was obtained in 66% yield by basic hydrolysis of (±)-ethyl 2-benzylbutanoate (±)-**48**. (±)-2-Benzylbutanoic acid (±)-**45** was of sufficient purity for chiral HPLC analysis and did not require further purification.

3.4 Hydrolase-mediated kinetic resolution - analytical screens

The next phase in the course of this work was hydrolase-mediated aqueous hydrolysis of the ethyl esters, synthesised in section 3.3. In the development of an efficient resolution procedure, selection of the hydrolase is critical. Consequently, screening assays were implemented, in which a series of lipases and esterases (kindly donated by Almac Sciences) was screened against each ethyl ester substrate to identify a biocatalyst that generated a high enantiomeric excess of both the substrate and product upon enantioselective hydrolysis. In order to fully determine the efficiency and stereoselectivity of these hydrolase-mediated biotransformations, a robust and efficient chiral HPLC technique was required.

3.4.1 Chiral HPLC method development

Ideally for efficient screening this project required the resolution of the enantiomeric pairs of both the ethyl ester substrate and carboxylic acid product of each kinetic resolution in one injection. A range of chiral columns were examined including the Chiralcel® OD-H and Chiralpak® IB exploring a variety of isopropanol/hexane solvent compositions, flow rates and temperatures for each kinetic resolution. Following a significant amount of method development, the Chiralcel® OJ-H column was found to provide complete baseline separation of the individual stereoisomers of the ethyl esters and corresponding acids of all kinetic resolutions (see for example Figure 3.12). The only exception was in the case of substrate (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-**55** and corresponding carboxylic acid (±)-**80** where the Chiralcel® AS-H provided enantioseparation. Appendix I describes all the established chiral HPLC conditions implemented in the resolution of each set of enantiomeric pairs on a single trace.

Thus, the conditions for the chiral HPLC analysis were developed to the point where a single injection of the reaction mixture enabled determination of the enantiopurity of the substrate and product. While quantification of the individual components was not undertaken in this work, this would be feasible if required to support the product ratio determined by ^{1}H NMR analysis. Basis for the assignment of absolute stereochemistry of each of the HPLC peaks will be discussed later in section 3.5.

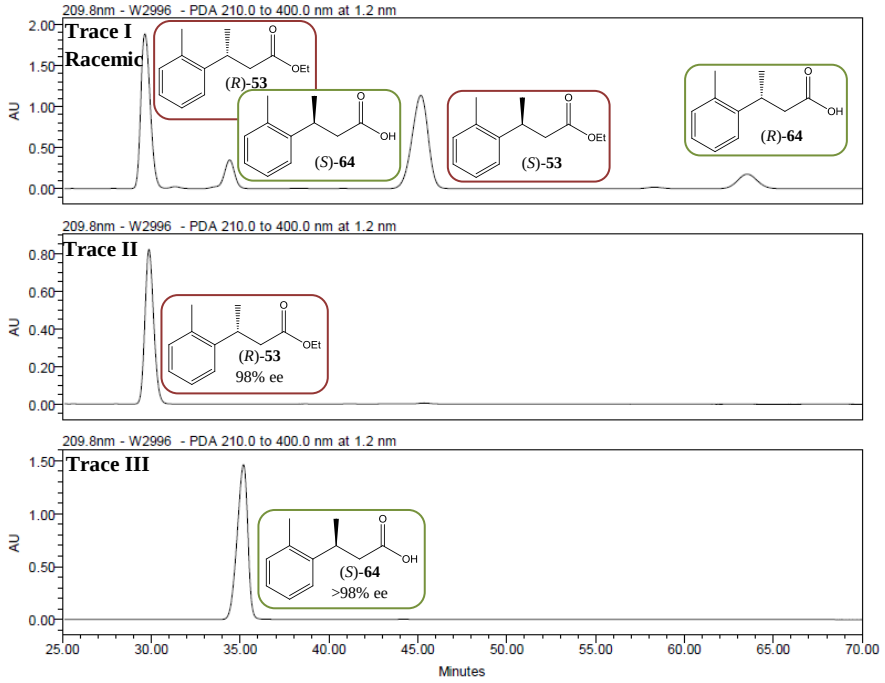


Figure 3.12: HPLC ***Trace I****: A racemic mixture of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-**53** and (±)-3-(2-methylphenyl)butanoic acid (±)-**64**. **Trace II**: (R)-Ethyl 3-(2-methylphenyl)butanoate (R)-**53**, 98% ee, from the preparative-scale enzymatic resolution (see section 3.5.2). **Trace III**: (S)-3-(2-Methylphenyl)butanoic acid (S)-**64**, >98% ee, from the preparative-scale enzymatic resolution (see section 3.5.2). For HPLC conditions see appendix I.*

3.4.2 Screening protocol – aqueous hydrolysis of esters

Once the analytical method was established, the next stage was to develop hydrolase-mediated resolution conditions for the enantioselective hydrolysis of the ethyl ester substrates. A suggested screening protocol was provided by Almac Sciences which described adding a spatula tip of enzyme, to ~50 mg of ethyl ester substrate in 4.5 mL of pH 7.0 phosphate buffer.[82] The resulting biphasic suspension was incubated on a shaking platform. The screening protocol advises sampling and assaying for conversion by TLC, however while during this work TLC analysis indicated if the desired product had formed, it was in reality difficult to estimate conversion *via* this method. Work-up involved initial filtration of the reaction mixture to remove the hydrolase and then extraction of the desired products with diethyl ether. Analysis of the crude product was conducted by ^{1}H NMR and chiral HPLC. Because of the convenient HPLC method which had been developed, enantiopurities of both the ester and acid components could be assessed through a single injection of the crude product without requiring chromatographic purification.

Conversion rate was determined by two means, analysis by ^{1}H NMR spectroscopy and by the *E*-value calculator. In theory, formation of the desired enantiopure acid is visible in the ^{1}H NMR by the appearance of an OH peak at δ_H 10.00-13.20 ppm. However, determination of conversion by integration of this singlet proves unreliable due to its broad nature attributable to the exchange rate of the acid. Owing to overlapping signals the desired acid has no characteristic distinct ^{1}H NMR signal relative to the ester substrate. Consequently conversion by ^{1}H NMR was determined by integrating the characteristic ester multiplet/quartet at δ_H 3.85-4.08 ppm of the methylene protons of the ethyl moiety against a neighbouring multiplet including protons from both the ester substrate and acid product.

While for many of the acids studied the conversion determined by ^{1}H NMR spectroscopy agreed very closely with those estimated based on the *E* value. In some instances **28**, **29**, **37** and **45** with a R group larger than a methyl it was observed that

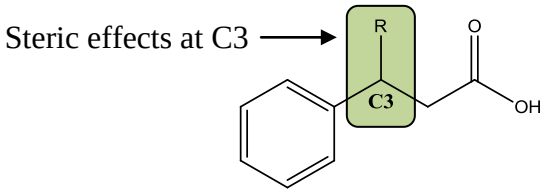
percentage conversion determined *via* ^{1}H NMR spectroscopy was not reliable, as the acid was recovered to a greater degree from the reaction mixture than the corresponding ethyl ester. This was especially evident in determination of conversions by ^{1}H NMR of the sterically bulky C2 and C3 substituted 3-arylalkanoic acids **28**, **29**, **37** and **45**. Consequently the conversions calculated by ^{1}H NMR analysis proved to be deceptively high. This was not anticipated, in fact if anything it was predicted that the acid would not be quantitatively recovered from the resolution due to the formation of its corresponding salt in the phosphate buffer.

All percentage conversions were therefore also calculated utilising the program developed by Kroutil *et al.* and dependent on the enantiomeric purity of the substrate and product.[7] However, conversions determined with the *E*-value calculator where limited enantioselectivity is observed should be interpreted with caution (see entry 16, Table 3.10).

As mentioned previously, all chiral HPLC conditions are detailed in appendix I. When the second enantiomer was absent, the enantiomeric excess was stated as >98% ee. Lipase-mediated resolutions where conversion was determined by ^{1}H NMR analysis to be <10% were not analysed by chiral HPLC.

3.4.3 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C3 substituted alkanoic acids

The focus of the kinetic resolution of the esters (±)-**38**, (±)-**56**, (±)-**58** and (±)-**59** was to obtain enantiopure samples of each of the methyl, ethyl, isopropyl and *tert*-butyl β-substituted 3-arylalkanoic acids, **23**, **28**, **29** and **37**, ideally in both *R* and *S* enantiopure forms (Figure 3.13). This series was designed to enable exploration of the steric effect of the R substituent on both the efficiency and enantioselectivity of the kinetic resolution.



R = Me **23**
R = Et **28**
R = *i*-Pr **29**
R = *t*-Bu **37**

Figure 3.13

3.4.3.1 Hydrolase-catalysed kinetic resolution to provide enantioenriched 3-phenylbutanoic acid 23

In total, 21 lipases and 1 esterase were screened in resolving racemic (±)-ethyl 3-phenylbutanoate (±)-**38** to provide enantioenriched 3-phenylbutanoic acid **23**. Each of the hydrolases investigated resulted in hydrolysis to a certain degree and the screening results are summarised in Table 3.10. Lipases *Pseudomonas cepacia*, *Alcaligenes spp*. and *Pseudomonas fluorescens*, entries 6, 11 and 15 respectively, provided (*S*)-3-phenylbutanoic acid (*S*)-**23** and (*R*)-ethyl 3-phenylbutanoate (*R*)-**38** in excellent enantiopurity.

Burholderia cepacia hydrolysis of the analogous methyl ester of (±)-**23** had previously been reported (*E* >50), providing access to the acid (*S*)-**23** with 89% ee.[44] Herein, *Alcaligenes spp*. yielded the acid (*S*)-**23** with excellent enhanced enantioselectivity of 97% ee

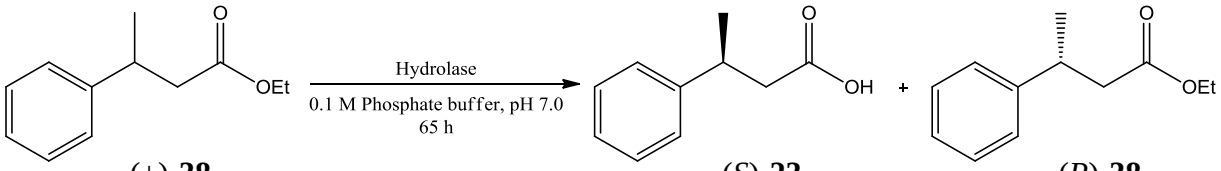
(*E* >200) by hydrolysis of the corresponding ethyl ester (±)-**38**. Unreacted (*R*)-**38** was retrieved in 98% ee providing access to both enantiomeric series in a single resolution.

From Table 3.10, it is apparent that certain hydrolases preferentially hydrolysed the (*R*)-enantiomer of the ethyl ester substrate (±)-**38**, providing access to the opposite enantiomer of the desired (*R*)-3-phenylbutanoic acid (*R*)-**23** albeit with modest enantiopurity. Resolution of the complementary enantiomer (*R*)-3-phenylbutanoic acid (*R*)-**23**, had previously been described *via Candida antarctica* lipase B catalytic hydrolysis of (±)-**38**, although at very low enantioselectivity (*E* <10).[43] *Candida antarctica* lipase B (free and immobilised) also selectively hydrolysed the (*R*) ester in this study (entries 16 and 19, Table 3.10) and resolution *via* this less common pathway has been successfully extended to include the hydrolases *Candida cyclindracea* C1, C2 and *Mucor meihei* (entries 1, 2 and 17, Table 3.10 respectively).

In practise, while use of *Candida antarctica* lipase B (free and immobilised), *Candida cyclindracea* C1, C2 and *Mucor meihei* all lead selectively to (*R*)-**23**, access to the enantiopure (*R*)-**23** is more effectively achieved *via* isolation of enantiopure (*R*)-**38** using *Pseudomonas cepacia*, *Alcaligenes spp*. and *Pseudomonas fluorescens*, followed by saponification.

In general the extent of conversion calculated by ^{1}H NMR compared favourably with that determined by the *E*-value calculator, with the exception of *Candida antarctica* lipase B hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-**38** (entry 16, Table 3.10). This resolution provided poor enantiopurity for the unresolved ester (*S*)-**38** (13% ee) and no enantiopurity for the desired acid (*R*)-**23**. The minimal enantioselectivity observed generated an unreliable conversion by the *E*-value calculator, and in this instance, conversion calculated by ^{1}H NMR analysis is presumed to be more accurate.

*Table 3.10: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-**38***



(±)-**38** (*S*)-**23** (*R*)-**38**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 38	Acid 23	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	30	11 (*S*)	58 (*R*)	16	15	4.2
2	*Candida cyclindracea* C2	Ambient	25 (*S*)	59 (*R*)	30	41	4.9
3	*Rhizopus oryzae*	30	-	-	-	<10	-
4	*Achromobacter spp.*	30	11 (*R*)	90 (*S*)	11	12	21
5	*Alcaligenes spp.* 1	30	27 (*R*)	95 (*S*)	22	24	50
6	*Pseudomonas cepacia* P1	30	>98 (*R*)	94 (*S*)	51	49	170
7	*Pseudomonas stutzeri*	Ambient	14 (*R*)	61 (*S*)	19	23	4.7
8	*Rhizopus spp.*	Ambient	-	-	-	<10	-
9	*Rhizopus niveus*	Ambient	-	-	-	<10	-
10	*Aspergillus niger*	Ambient	-	-	-	<10	-
11	*Alcaligenes spp.* 2	Ambient	98 (*R*)	97 (*S*)	50	54	>200
12	*Pseudomonas cepacia* P2	Ambient	96 (*R*)	75 (*S*)	56	59	26
13	*Mucor javanicus*	Ambient	-	-	-	<10	-
14	*Penicillium camembertii*	Ambient	-	-	-	<10	-
15	*Pseudomonas fluorescens*	30	>98 (*R*)	94 (*S*)	51	55	170
16	*Candida antarctica* B	Ambient	13 (*S*)	0 (*R*)	51	96	1.4
17	*Mucor meihei*	Ambient	3 (*S*)	24 (*R*)	11	<10	1.7
18	*Candida antarctica* A	Ambient	10 (*R*)	68 (*S*)	13	17	5.8
19	*Candida antarctica* B (immob)	Ambient	70 (*S*)	5 (*R*)	93	100	1.8
20	*Porcine pancrease* Type II	Ambient	15 (*R*)	93 (*S*)	14	19	31
21	*Porcine pancrease* Grade II	30	35 (*R*)	95 (*S*)	27	27	54
22	Pig liver esterase	Ambient	0[a]	0[a]	-[a]	100[a]	-[a]

a. Reaction went to 100% completion, no enantioselectivity observed.

3.4.3.2 Hydrolase-catalysed kinetic resolution to provide enantioenriched 3-phenylpentanoic acid 28

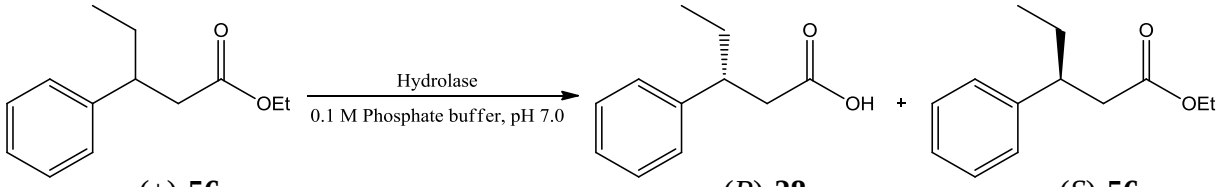
The enzymatic hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**56** proved to be significantly less facile than with (±)-**38**. Of the 16 hydrolases screened, many displayed no catalytic activity for hydrolysis of the substrate (±)-ethyl 3-phenylpentanoate (±)-**56** (~10 days incubation period). Thus replacement of the methyl with the slightly larger ethyl moiety at the stereogenic centre C3 resulted in a very significant reduction of enzymatic activity. Just 6 of the hydrolases resulted in conversion as summarised in Table 3.11.

Significantly, the biocatalysts which had yielded the most effective kinetic bioresolution with (±)-**38** were ineffective for enzymatic hydrolysis of (±)-**56**. For the 6 biocatalysts which resulted in ester hydrolysis the enantioselectivities were modest at best (Table 3.11). Interestingly, with entries 2, 3 and 5 the poor enantioselectivity is associated with lack of discrimination of the enantiomers by the biocatalyst with conversions ≥78% in each case, while *Candida antarctica* lipase B and *Mucor meihei* with (±)-**38** gave very limited reaction. The immobilised *Candida antarctica* lipase B provided the most promising

results in this instance (E = 25); this is in direct contrast to the poor enantioselection (E = 1.8) of (±)-**38** with *Candida antarctica* lipase B (immob).

The direction of enantioselectivity in the hydrolysis of (±)-**56** was consistent with that observed in the reactions of (±)-**38** with *Candida antarctica* lipase B, *Candida antarctica* lipase B (immob) and Pig liver esterase providing the (*R*) acid selectively. Based on the initial promising result with *Candida antarctica* lipase B (immob), the reaction conditions for the hydrolysis were varied to determine if the outcome could be optimised.

*Table 3.11: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**56***



(±)-**56** (*R*)-**28** (*S*)-**56**

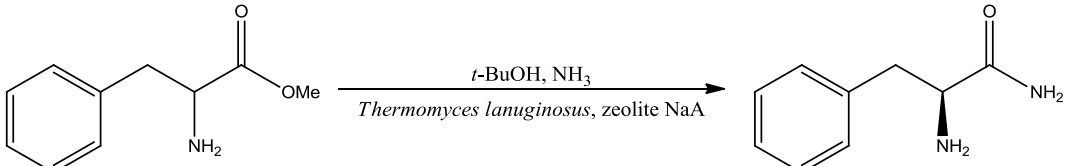
Entry	Enzyme source[a]	Time	Temp (°C)	ee (%)		Conversion (%)		*E* value
				Ester 56	Acid 28	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C2	120 h	Ambient	1	15	6	33	1.4
2	*Candida antarctica* B	65 h	Ambient	80 (*S*)	23 (*R*)	78	93	3.4
3	*Mucor meihei*	67 h	Ambient	0[b]	0[b]	-[b]	100[b]	-[b]
4	*Candida antarctica* A	67 h	Ambient	5 (*R*)	44 (*S*)	10	22	2.7
5	Pig liver esterase	65 h	Ambient	87 (*S*)	15 (*R*)	85	88	3.1
6	*Candida antarctica* B (immob)	65 h	Ambient	85 (*S*)	81 (*R*)	51	93	25
7	*Candida antarctica* B (immob)	72 h	4	62 (*S*)	86 (*R*)	42	87	24

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes spp.* 2, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger* and *Mucor javanicus*.

b. Reaction went to 100% completion, no enantioselectivity observed.

Temperature control in hydrolase-catalysed resolutions has been explored due to its simplicity and reliability for enhancement of enantioselectivity, albeit at the expense of longer reaction times.[83] Lowering of the reaction temperature to below 0 °C to enhance enantioselectivity was first investigated by Jones and co-workers in the Pig liver esterase catalysed hydrolyses of C3-substituted dimethyl glutarates.[84] The concept that enantioselectivity increases upon lowering the temperature was unequivocally established by Sheldon *et al.* in the ammoniolysis based kinetic resolution of phenylalanine methyl ester **85** catalysed by the lipase from *Thermomyces lanuginosus* (Table 3.12).[85] The selectivity factor E was observed to increase steadily by decreasing the temperature from 40 °C (E = 13) to −20 °C (E = 84).[85]

Table 3.12: Thermomyces lanuginosus *catalysed ammoniolysis of* ***85*** *at different temperatures*[85]



85 **86**

Temp (°C)[a]	Time	Conversion (%)	ee (%) (*S*)-**86**	*E* value
40	1 h	8	84	13
20	1 h	11	89	20
4	5 h	29	91	30
−10	24 h	40	93	52
−20	5 h	8	97	84

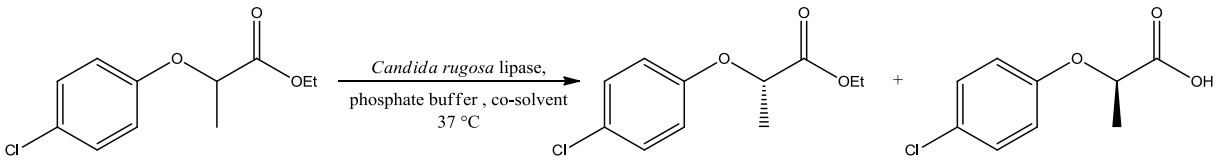
a. Reaction conditions: substrate 50 mM solution in ammonia saturated *tert*-butyl alcohol, 50 mg mL^{-1} zeolite NaA, 50 mg mL^{-1} enzyme. TBME (30%) was added in reactions below 20 °C.

Thus, investigation of *Candida antarctica* lipase B (immob) resolution of (±)-**56** at 4 °C, (entry 7, Table 3.11) was warranted. A decrease in the extent of conversion to (*R*)-**28** was observed despite an extended incubation period. In addition, no significant increase in the selectivity factor (*E* value) was recorded, therefore this approach was not pursued further.

Lipases bind to the water/lipid boundary and catalyse hydrolysis at this interface, exhibiting high interfacial activity.[4] Thus, utilisation of organic co-solvents or ionic liquids has been frequently shown to enhance the enantioselectivity of hydrolase-catalysed resolution of an extensive range of compounds.[86,87]

Amoroso *et al.* reported *Candida rugosa* lipase (CRL) catalysed hydrolysis of 2-substituted aryloxyacetic esters in the presence of dimethyl sulfoxide (DMSO) and isopropyl alcohol (IPA) from 0 to 80% v/v as additives in aqueous media, in an effort to improve enantioselectivity in enzymatic resolution (Table 3.13).[88] Hydrolysis of (±)-**87** in the absence of an organic co-solvent additive proceeded to >90% conversion, with minimal chiral discrimination (E = 0). The addition of DMSO 20% v/v to the reaction medium produced a dramatic enhancement of enantioselectivity (E >200).

*Table 3.13: Effect of addition of DMSO on the CRL mediated hydrolysis of ethyl 2-(4-chlorophenoxy)propanoate (±)-**87** in aqueous buffer*[88]



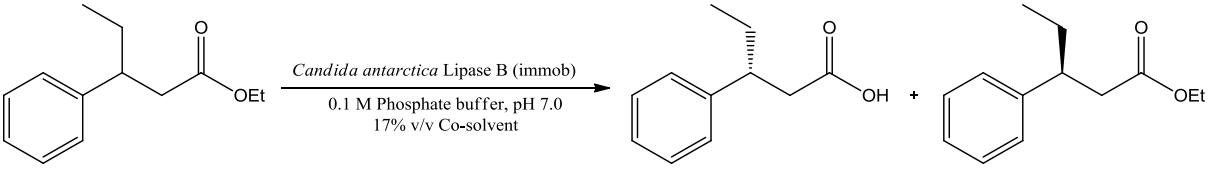
(±)-**87** (*S*)-**87** (*R*)-**88**

DMSO (% v/v)	Time	Conversion (%)	ee (%) (*S*)-**87**	*E* value
0	7 h	>90	0	-
10	2 h	50	58	4.8
20	1 h	55	96	>200
30	30 min	52	90	186
40	30 min	48	88	122
50	2 h	47	82	65
60-70	72 h	<20	0	-

Screening reactions were therefore performed to assess the effect of a series of co-solvents (at 17% v/v) on *Candida antarctica* lipase B (immob) resolution of (±)-**56** (Table 3.14). The majority of co-solvents investigated resulted in a decrease in the rate of hydrolysis, but notably, with the exception of *tert*-butyl methyl ether TBME, resulted in equivalent or improved enantiopurity of (*R*)-**28**. The utilisation of acetone as an additive, (entry 3, Table 3.14) resulted in recovery of (*R*)-**28** with 94% ee and E = 41 while with dioxane (entry 5, Table 3.14) E = 51. It should be noted that on one occasion the enantiomeric excess isolated from dioxane of (*R*)-**28** was 97% ee, however on repeating the enzymatic resolution the high enantioselection was non-reproducible and the enantioselectivity of (*R*)-**28** was determined at 92% ee.

Thus hydrolase-catalysed resolution can be effective as a route to enantioenriched (*R*)-**28** provided the biocatalyst and reaction conditions are carefully chosen. The only prior report of hydrolase-catalysed esterification of **28** describes very low activity and enantioselectivity (E <2).[42] Furthermore, the acid (*S*)-**28** has been resolved using amidase biocatalysis and again enantiopurity was lower (88% ee).[45]

Table 3.14: Investigation of co-solvent effect on Candida antarctica *lipase B (immob) hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**56***



(±)-**56** (*R*)-**28** (*S*)-**56**

Entry	Co-solvent	Time	Temp (°C)	ee (%)		Conversion (%)		*E* value
				Ester (*S*)-56	Acid (*R*)-28	*E* calc.	^{1}H NMR	
1	DMSO	64 h	Ambient	93	81	53	80	31
2	Acetonitrile[a]	64 h	Ambient	28	93	23	84	36
3	Acetone[a]	64 h	Ambient	25	94	21	76	41
4	THF	64 h	Ambient	6	88	6	24	16
5	Dioxane	64 h	Ambient	72	92[b]	44	71	51
6	TBME[a]	64 h	Ambient	24	57	30	90	4.6

a. HPLC grade solvent.
b. On one occasion the enantiomeric excess [ee (%)] isolated from dioxane of (*R*)-**28** was 97% ee.

It is evident in the hydrolase-catalysed kinetic resolution to provide enantioenriched (*R*)-3-phenylpentanoic acid (*R*)-**28** and all subsequent resolutions involving sterically hindered C3 substituted 3-arylalkanoic acids that the conversion determined by ^{1}H NMR is inaccurate due to greater recovery of the acid over the ester from the reaction mixture.

3.4.3.3 Hydrolase-catalysed kinetic resolution to provide enantioenriched 4-methyl-3-phenylpentanoic acid 29

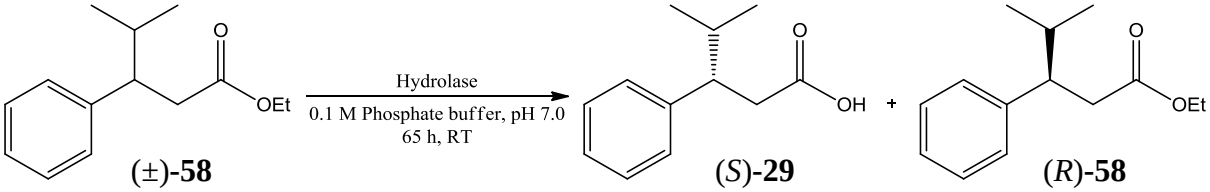
Given the decrease in biocatalytic activity on increasing the C3 substituent from methyl to ethyl it was anticipated that enzymatic hydrolysis to form enantiopure (*S*)-**29** and (*S*)-**37** with the more sterically demanding isopropyl and *tert*-butyl substituents at C3 would prove extremely challenging. Of the 19 hydrolases screened many displayed no hydrolytic activity towards the isopropyl β-substituted ethyl ester (±)-**58** and hydrolysis failed to occur even at elevated temperature and extended reaction periods. Significantly, the hydrolases that were identified to hydrolyse (±)-**56** were also found to hydrolyse (±)-**58** as depicted in Table 3.15, thus confirming that these biocatalysts can accommodate increased steric demand in the "C3 region" of the enzyme pocket.

Interestingly, the extent of reaction in entries 1, 2 and 4, Table 3.15, is decreased somewhat relatively to those seen with (±)-**56** in Table 3.11, resulting in improved enantiopurities of the recovered acid (*S*)-**29**. Thus discrimination between the phenyl and isopropyl groups in the active site of the enzymes is improved somewhat relative to that seen in (±)-**56** where discrimination between the ethyl and phenyl substituents is quite poor. While the *R, S* labels in the acid (*S*)-**29** are switched relative to acids (*R*)-**23** and (*R*)-**28** the sense of enantioselection is identical in hydrolysis of the ethyl and isopropyl esters (±)-**56** and (±)-**58** with the (*S*)-enantiomer isolated using enzymes *Candida antarctica* lipase B, *Mucor meihei* and *Candida antarctica* lipase B (immob). In this instance (*S*)-**29** was obtained in 99% ee using *Candida antarctica* lipase B; hence no further optimisation was required.

Once again, careful control of reaction conditions and selection of biocatalyst leads to efficient bioresolution of (*S*)-**29**, in direct contrast to the literature report which states it was not possible to resolve this acid using hydrolase catalysis.[42]

A solvent screen involving dioxane, acetone and TBME was conducted for *Candida antarctica* lipase A and *Candida antarctica* lipase B resolution of (±)-**58** to investigate the effect on enantiomeric excess, but resulted in a significant reduction in activity and therefore was not pursued further.

*Table 3.15: Hydrolase-mediated hydrolysis of (±)-ethyl 4-methyl-3-phenylpentanoate (±)-**58**.*



(±)-**58** (*S*)-**29** (*R*)-**58**

Entry	Enzyme source[a]	ee (%)		Conversion (%)		*E* value
		Ester 58	Acid 29	*E* calc.	^{1}H NMR	
1	*Candida antarctica* B	12 (*R*)	>98 (*S*)	11	24	>200
2	*Mucor meihei*	61 (*R*)	23 (*S*)	73	79	2.7
3	*Candida antarctica* A	10 (*S*)	64 (*R*)	14	31	5
4	*Candida antarctica* B (immob)	33 (*R*)	97 (*S*)	25	42	90
5	Pig liver esterase	-[b]	-[b]	-[b]	100	-[b]

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes spp.* 1, *Penicillium camembertii*, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Candida cyclindracea* C2, *Rhizopus spp*., *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger*, *Alcaligenes spp.* 2 and *Mucor javanicus.*

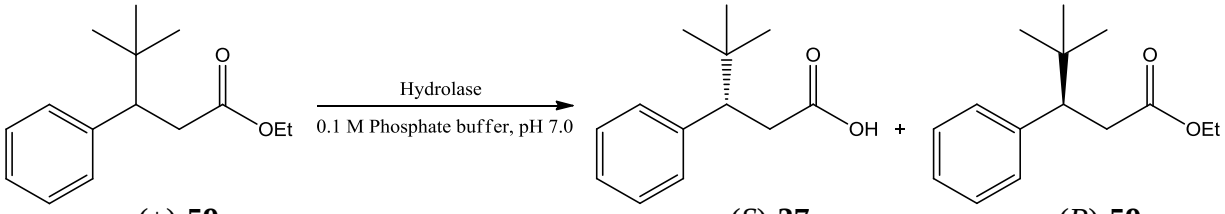
b. Reaction went to 100% completion, HPLC analysis was not conducted

3.4.3.4 Hydrolase-catalysed kinetic resolution to provide enantioenriched 4,4-dimethyl-3-phenylpentanoic acid 37

Hydrolase-catalysed resolution of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**59** was achieved using the same biocatalysts which catalysed reaction of (±)-**56** and (±)-**58**, albeit at much lower extent of conversion presumably due to the increased steric demand of the C3 substituent. However, the overall trends are very similar for (±)-**56**, (±)-**58** and (±)-**59** with the optimum results achieved with the immobilised or free *Candida antarctica* lipase B, (Figure 3.14 and 3.15). While the extent of reaction at room temperature was extremely limited, increasing the temperature improved the conversion, for example see entries 2 and 6, Table 3.16. The direction of enantioselectivities is consistent with earlier observations for *Candida antarctica* lipase B (immob) and *Candida antarctica* lipase A. Interestingly the sense of enantioselection in the Pig liver esterase hydrolase, resulting in selective hydrolysis of the (*R*)-enantiomer, is opposite to that seen in the hydrolysis of the corresponding ethyl derivative (±)-**56**. Thus, in the ethyl derivative (±)-**56** *Candida antarctica* lipase A provided the (*S*)-enantiomer of the acid selectively while Pig liver esterase provides the (*R*)-enantiomer selectively, whereas in the case of the *tert*-butyl derivative Pig liver esterase displays the same direction of enantioselection as *Candida antarctica* lipase A.

With both the free and immobilised *Candida antarctica* lipase B, while the extent of the hydrolysis is limited, the enantioselectivity is excellent, with the acid (*S*)-**37** isolated in enantiopure form. Increasing the temperature improved the conversion somewhat, thereby resulting in an increased enantiopurity of the unreacted ester (*R*)-**59**.

*Table 3.16: Hydrolase-mediated hydrolysis of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**59** at variable temperature.*



(±)-**59** (*S*)-**37** (*R*)-**59**

Entry	Enzyme source[a]	Temperature (°C)	ee (%)		Conversion (%)		*E* value
			Ester 59	Acid 37	*E* Calc.	^{1}H NMR	
1	*Candida antarctica* B	Ambient[b]	2 (*R*)	>98 (*S*)	2	<10	>200
2		35 – 40 °C[c]	23 (*R*)	>98 (*S*)	19	25	>200
3	*Candida antarctica* A	Ambient[b]	3 (*S*)	73 (*R*)	4	6	6.6
4		35 – 40 °C[d]	7 (*S*)	81 (*R*)	8	12	10
5	*Candida antarctica* B	Ambient[b]	1 (*R*)	>98 (*S*)	1	15	>200
6	(immob)	35 – 40 °C[c]	30 (*R*)	98 (*S*)	23	55	132
7	Pig liver esterase	Ambient[b]	32 (*S*)	34 (*R*)	48	51	2.7
8		35 – 40 °C[c]	0[e]	0[e]	-[e]	100[e]	-[e]

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P1, *Rhizopus niveus*, *Pseudomonas fluorescens*, *Candida cyclindracea* C1, *Pseudomonas cepacia* P2 and *Porcine Pancrease* Type II.
b. Time for ester hydrolysis was 66 h.
c. Time for ester hydrolysis was 64.5 h at 35 °C temperature increased to 40 °C for the final 24 h.
d. Time for ester hydrolysis was 72 h at 35 °C temperature increased to 40 °C for the final 24 h.
e. Reaction went to 100% completion, no enantioselectivity observed.

It is evident that once the alkyl group at the C3 stereogenic centre increases in size greater than a methyl substituent, a large decrease in the efficiency of the hydrolysis and thereby the kinetic bioresolution with regards to the enantiopurity of the ester is observed (Figure 3.14).

Despite the steric hindrance within the active site, 3-arylalkanoic carboxylic acids (*S*)-**23**, (*R*)-**28**, (*S*)-**29** and (*S*)-**37** can be obtained through optimisation of reaction conditions with excellent enantioselectivity. The acid (*S*)-**23** was obtained in 97% ee, through *Alcaligenes spp.* 2 catalysed hydrolysis of (±)-**38**, while acids (*R*)-**28**, (*S*)-**29** and (*S*)-**37** were obtained in ≥94% ee *via* immobilised or free *Candida antarctica* lipase B catalysed kinetic bioresolution (Figure 3.15).

*Comparison of reaction conversion (%) versus hydrolase for C3 substituted alkanoic acids **23**, **28**, **29** and **37***

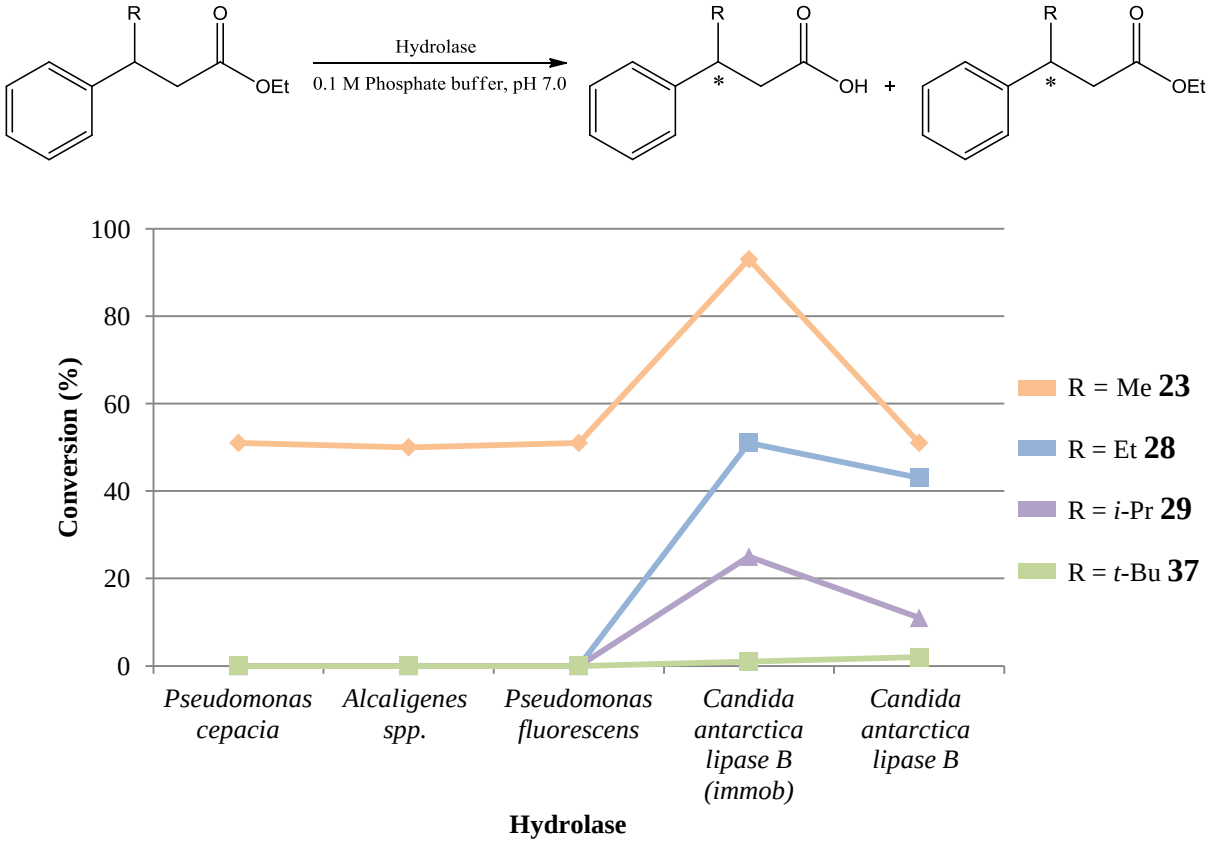


Figure 3.14

*Comparison of enantiomeric ratio (*E*-value) versus hydrolase for C3 substituted alkanoic acids **23**, **28**, **29** and **37***

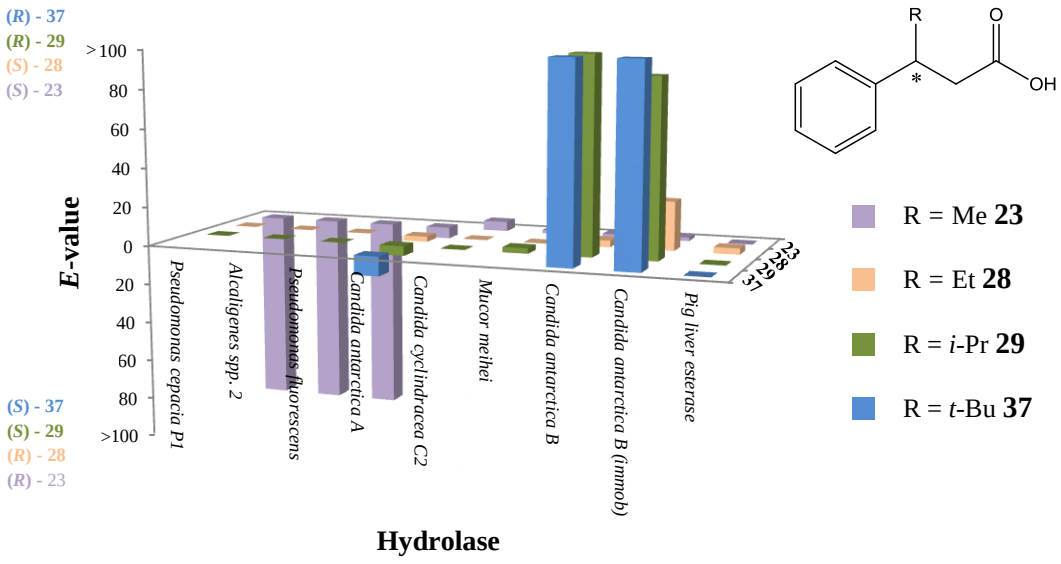
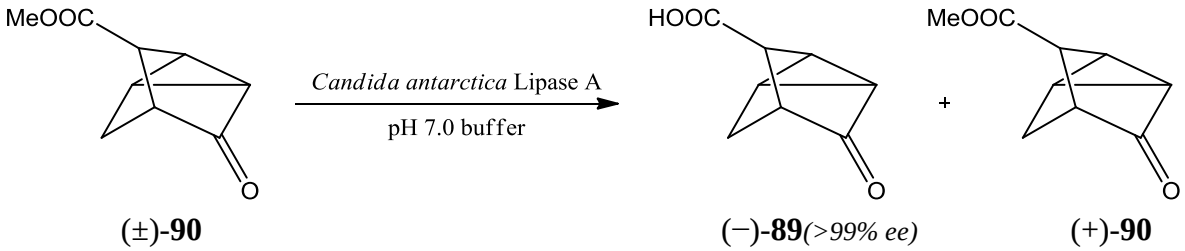


Figure 3.15

It is noteworthy that *Candida antarctica* lipase A provides a viable route to the complementary enantiomers (*S*)-**28,** (*R*)-**29** and (*R*)-**37**, circumventing the limitation of the modest enantiomeric excess of the esters achieved in the resolutions using the free and immobilised *Candida antarctica* lipase B. In addition, Kingery-Wood *et al.* demonstrated that *Candida antarctica* lipase A has a unique ability to accept very bulky, highly sterically hindered substrates in the *Candida antarctica* lipase A catalysed resolution of the sterically hindered, (−)-*anti*-3-oxotricyclo[2.2.1.0]heptane-7-carboxylic acid, (−)-**89** >99% ee (Scheme 3.13).[89]



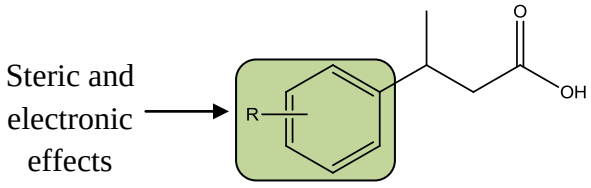
(±)-**90** (−)-**89***(>99% ee)* (+)-**90**

Scheme 3.13[89]

This unique ability of the *Candida antarctica* lipase A to accept sterically hindered substrates correlates with the observations herein whereby the enantiopurity of the acid obtained *via Candida antartica* lipase A catalysed resolution improved as the size of the alkyl substituent at C3 increased, the highest enantiopurity obtained being of (*R*)-**37** at 81% ee (entry 4, Table 3.16).

3.4.4 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C3 substituted phenyl butanoic acids

A series of substituted phenyl butanoic acids (±)-**51, 64-66, 80** were selected for investigation of both the steric and electronic effect of substituents on the aryl ring on the efficiency of the kinetic bioresolution process (Figure 3.16).

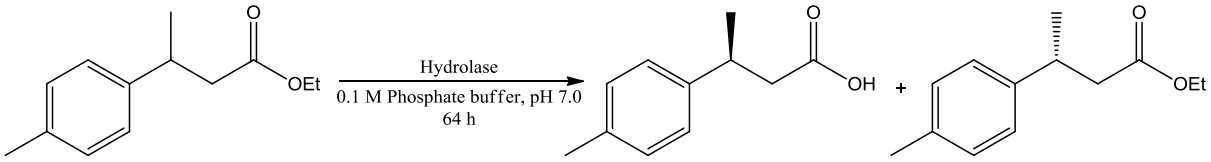


R = *p*-Me **51**
R = *o*-Me **64**
R = *m*-Me **65**
R = *p*-OMe **66**
R = *p*-F **80**

Figure 3.16

The results of the enzymatic screens are summarised in Tables 3.17-3.21. In each case, effective kinetic bioresolution was achieved with *Pseudomonas cepacia* P1, *Pseudomonas cepacia* P2, and *Pseudomonas fluorescens*, resulting in the successful hydrolysis of the (*S*)-enantiomer with very similar outcomes to those seen with (*S*)-3-phenylbutanoic acid (*S*)-**23**, indicating that the aryl substituent had little impact on the enzymatic hydrolysis.

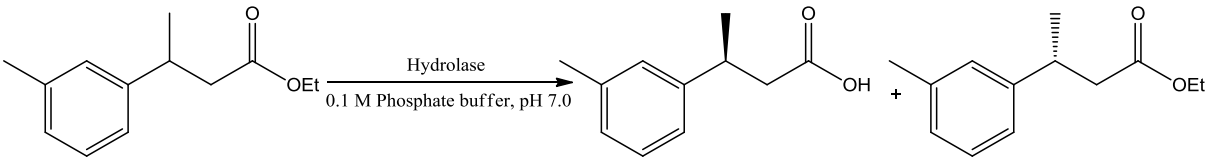
*Table 3.17: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methylphenyl)butanoate (±)-**49***



(±)-**49** (*S*)-**51** (*R*)-**49**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 49	Acid 51	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	98 (*R*)	>98 (*S*)	50	62	>200
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	96 (*S*)	51	51	>200
3	*Pseudomonas fluorescens*	30	>98 (*R*)	95 (*S*)	51	58	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* A	30	5 (*R*)	68 (*S*)	7	19	5.5
6	*Candida antarctica* B (immob)	30	6 (*S*)	5 (*R*)	55	43	1.2

*Table 3.18: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(3-methylphenyl)butanoate (±)-**54***

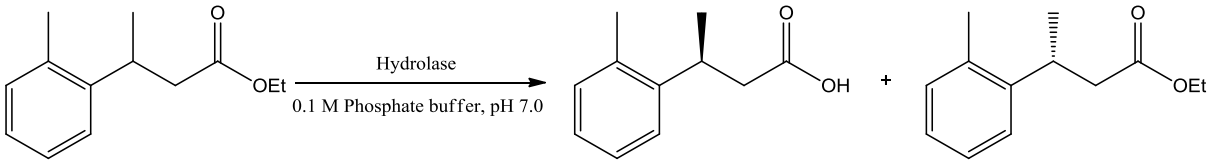


(±)-**54** (*S*)-**65** (*R*)-**54**

Entry	Enzyme source[a]	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 54	Acid 65	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	88 (*R*)	96 (*S*)	48	53	143
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	76 (*S*)	57	57	52
3	*Pseudomonas fluorescens*	30	96 (*R*)	97 (*S*)	50	69	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* B (immob)	30	>98 (*S*)	7 (*R*)	93	100	4.7

a. Time for ester hydrolysis was 65 h with the exception of *Candida cyclindracea* catalysed hydrolysis which was 64 h.

*Table 3.19: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-**53***

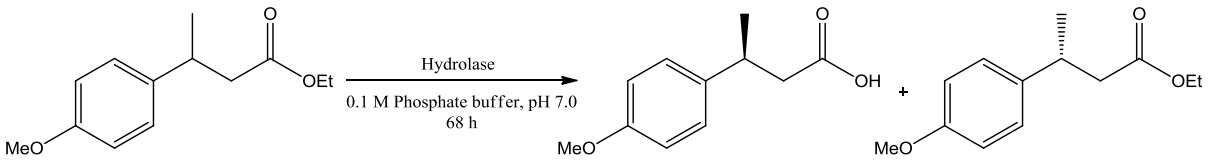


(±)-**53** (*S*)-**64** (*R*)-**53**

Entry	Enzyme source[a]	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 53	Acid 64	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	>98 (*R*)	>98 (*S*)	50	57	>200
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	80 (*S*)	56	53	65
3	*Pseudomonas fluorescens*	30	>98 (*R*)	>98 (*S*)	50	61	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* B (immob)	30	90 (*R*)	46 (*S*)	66	84	7.7

a. Time for ester hydrolysis was 67 h with the exception of *Pseudomonas fluorescens* catalysed hydrolysis which was 64 h.

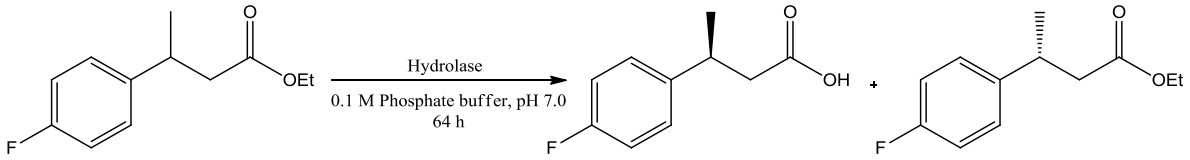
*Table 3.20: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-**60***



(±)-**60** (*S*)-**66** (*R*)-**60**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 60	Acid 66	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	98 (*R*)	86 (*S*)	53	48	60
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	88 (*S*)	53	54	81
3	*Pseudomonas fluorescens*	30	>98 (*R*)	97 (*S*)	51	57	>200
4	*Candida antarctica* A	30	4 (*R*)	48 (*S*)	8	9	3
5	*Candida cyclindracea*	30	-	-	-	<10	-
6	*Candida antarctica* B (immob)	30	66 (*S*)	7 (*R*)	90	96	1.9

*Table 3.21: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-**55***



(±)-**55** (*S*)-**80** (*R*)-**55**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 55	Acid 80	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	>98 (*R*)	84 (*S*)	54	55	59
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	69 (*S*)	84	84	27
3	*Pseudomonas fluorescens*	30	>98 (*R*)	94 (*S*)	62	62	170
4	*Candida cyclindracea*	30	3 (*S*)	25 (*R*)	11	<10	1.7
5	*Candida antarctica* B (immob)	30	>98 (*S*)	8 (*R*)	92	94	3.4

In all cases, highly enantioenriched samples of the (3*S*)-acids and the (3*R*)-esters are readily obtained using the *Pseudomonas* biocatalysts, see Figure 3.17. Previously, (±)-3-(4-methylphenyl)butanoic acid (±)-**51** has been resolved utilising *Pseudomonas cepacia* immobilised on ceramic particles to yield (*S*)-**51** in 99% ee.[19] The results utilising the free hydrolase, entry 1, Table 3.17 correlate strongly. The only significant effect of the substituent seen in this series of substituted phenyl butanoic acids was with the *para*-fluoro substrate (±)-**55** where the conversion is increased relative to the other substrates resulting in a slight decrease in enantiopurity of the recovered acids. Use of the *Candida cyclindracea* biocatalyst with the substituted substrates was also explored; while *Candida cyclindracea* had resulted in some hydrolysis with the parent compound (±)-**23**, very little conversion was seen with the substituted derivatives (Figure 3.17).

The use of *Candida antarctica* lipase B (immob) with the parent substrate (±)-**38** provided access to the opposite enantiomeric series (entry 19, Table 3.10), albeit with a very high extent of reaction. In general similar reaction patterns were seen with the substituted substrates resulting in recovery of the (*S*)-esters **49, 54, 60** and the (*R*)-acids **51, 65, 66**. Notably, with the *para*-methyl substrate (±)-**49** the extent of reaction was less, resulting in a decrease in enantiopurity of the recovered ester (*S*)-**49**, while with the *para*-fluoro substrate both enantiomers are indiscriminately hydrolysed. The sense of enantioselection in *Candida antarctica* lipase B (immob) resolution of the *ortho*-methyl substrate (±)-**53** was the same as that seen with the *Pseudomonas* biocatalysts. Interestingly this biocatalyst was the one that was able to accommodate increased steric demand at the C3 position. The switch in enantioselection must be due to combined steric effects of the *ortho*-methyl and 3-methyl substituents possibly *via* conformational changes (Figure 3.18).

*Comparison of enantiomeric ratio (*E*-value) versus hydrolase for C3 substituted phenyl butanoic acids **23, 51, 64-66** and **80***

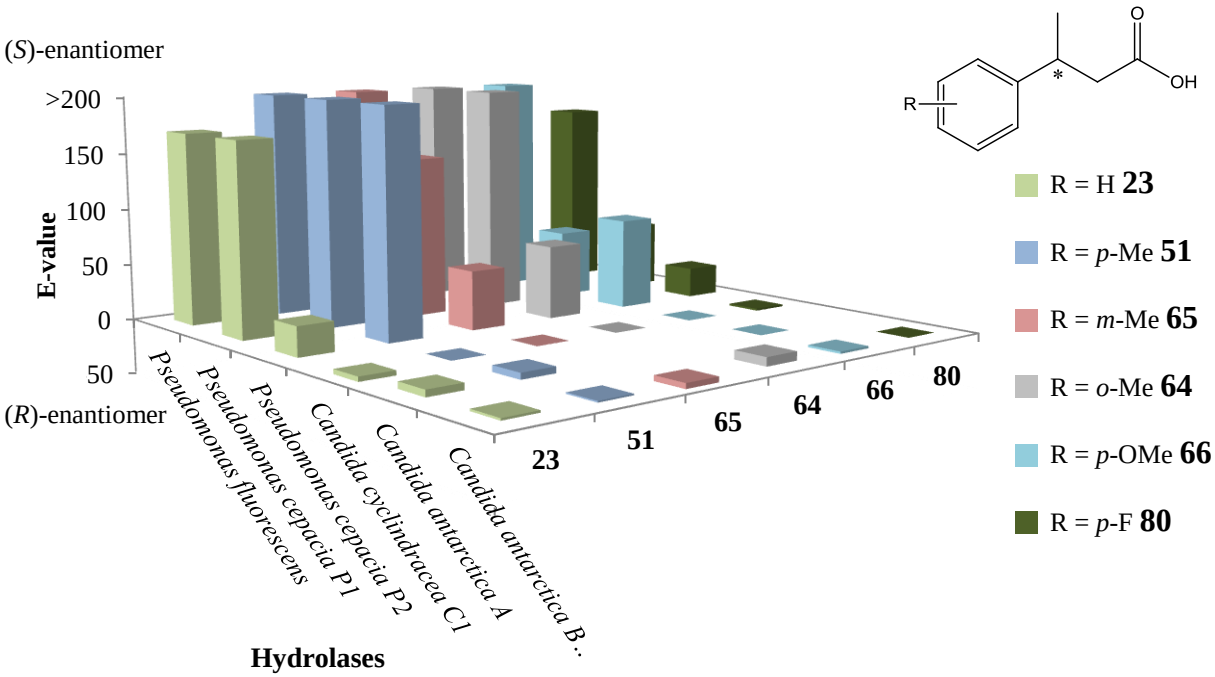


Figure 3.17

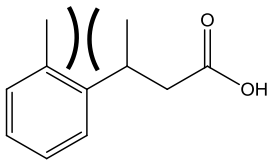
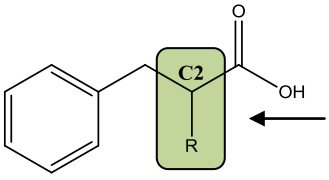


Figure 3.18

3.4.5 Hydrolase-catalysed kinetic resolution of esters to provide enantiopure C2 substituted alkanoic acids

The next step was to screen a series of substrates that were alkylated α to the ester moiety. The primary objective of this study was to assess the effect of the position of the stereocentre relative to the active site on the efficiency of the bioresolution. The steric effect of substituents at C2 in comparison to those at C3 on enantioselection was also examined. The C2 substituted methyl, ethyl and *tert*-butyl, α-substituted 3-arylalkanoic acids (±)-**32**, (±)-**45** and (±)-**61** were selected for investigation (Figure 3.19).



Steric effects at C2 and position of the stereocentre relative to reactive ester moiety.

R = Me **32**
R = Et **45**
R = *t*-Bu **61**

Figure 3.19

3.4.5.1 Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-methyl-3-phenylpropanoic acid 32

In total, 18 lipases were screened in the lipase-catalysed kinetic resolution to provide enantioenriched 2-methyl-3-phenylpropanoic acid **32** as summarised in Table 3.22. All enzymatic resolutions investigated resulted in at least partial hydrolysis of the (*S*)-enantiomer of (±)-ethyl 2-methyl-3-phenylpropanaote (±)-**47**. *Alcaligenes spp.* 2, *Pseudomonas cepacia* P2, and *Pseudomonas fluorescens* catalysed hydrolysis of (±)-**47** in 0.1 M phosphate buffer (pH 7.0) at ambient temperature, for 20 h (entries 9, 10a and 13a, Table 3.22 respectively), provided the desired acid (*S*)-**32** in excellent enantiomeric excess. The unreacted ester (*R*)-**47** was also recovered in high optical purity with the exception of *Alcaligenes spp.* 2 (entry 9, Table 3.22) (*R*)-**47** with just 67% ee obtained.

Margolin and co-workers previously reported *Pseudomonas* sp. (Amano) catalysed hydrolysis of the corresponding methyl ester of (±)-**32** providing the acid (*S*)-**32** with high enantiomeric excess (95% ee). Notably no lipase screening assays were reported.[23] Herein, a comprehensive screening protocol was implemented which identified *Alcaligenes spp.* 2 in addition to *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* for the enzymatic mediated hydrolysis of (±)-**47** providing highly enantioenriched (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**32**. The *Pseudomonas* lipases afforded the (*S*)-acid **32** (≥92% ee) and (*R*)-ester **47** (>98% ee) in excellent enantiopurity (entries 10a and 13a, Table 3.22). *Alcaligenes spp.* 2 generated the (*S*)-acid **32** with an improved enantioselectivity of 97% ee (entry 9, Table 3.22). However the rate of the resolution was decreased (conversion 41%) and therefore the enantiopurity of the recovered (*R*)-ester **47** (67% ee) was compromised.

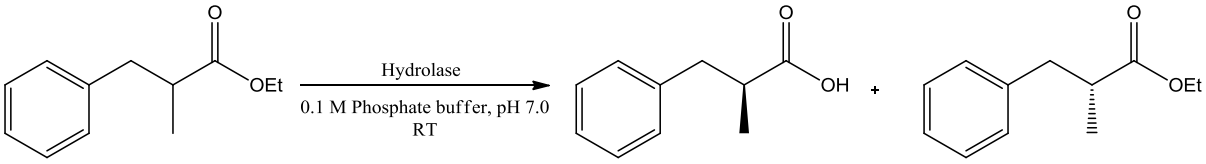
Candida cyclindracea C2 and *Candida antarctica* lipase B (free and immobilised) (entries 2, 14 and 16, Table 3.22 respectively) mediated hydrolysis proceeded with 100% conversion to racemic acid (±)-**32** exhibiting a lack of discrimination of enantiomers. Bornscheuer *et al.* reported that *Candida antarctica* lipase B usually displays low to moderate enantioselectivity toward carboxylic acids with a stereocentre at the α-position.[4] The acyl binding site of *Candida antarctica* lipase B is a shallow crevice. It is likely that the lower enantioselectivity toward stereocentres in the acyl part of an ester stems from fewer and/or weaker contacts between the acyl part and its binding site.[4]

Significantly, *Alcaligenes spp.* 2 and the *Pseudomonas* lipases (entries 6, 11 and 15, Table 3.10 respectively) also yielded the highest enantiopurity on resolution of the structurally related β-substituted 3-phenylbutanoic acid (*S*)-**23** (≥94% ee) and the analogous ethyl ester (*R*)-**38** (>98% ee) albeit at extended reaction times (65 h). Thus, the position of the chiral methyl substituent relative to the reactive ester moiety has limited effect on the choice of biocatalyst or high enantiopurity obtainable, however notably the reaction rate is altered with efficient resolution achieved within 20 h for (*S*)-**32** *vs.* 65 h for (*S*)-**23**.

Candida antarctica lipase B (free and immobilised), *Candida cyclindracea* (C1 and C2) and *Mucor meihei* hydrolysed the (*R*)-enantiomer in the resolution of the β-methyl substituted 3-phenylbutanoic acid (*R*)-**23**, providing access to the opposite enantiomer albeit with low to modest enantiopurity. In this study, *Candida antarctica* lipase B (free and immobilised) and *Candida cyclindracea* C2 mediated hydrolysis proceeded with no enantioselectivity. *Candida cyclindracea* C1 and *Mucor meihei* did display enantioselectivity albeit at low levels (entries 1 and 15, Table 3.22) however interestingly a switch in enantiopreference was not observed, with the (*S*)-enantiomer preferentially hydrolysed. Due to the low levels of enantiopurity the direction of enantioselection should be interpreted with caution.

Based on the screening results in Table 3.22 use of *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* (entries 10a and 13a respectively) were evidently the most attractive from the perspective of achieving enantioenriched samples of the acid (*S*)-**32**. It is clear from the extent of conversion (>50%) and the slightly low enantiopurity of the acid (*S*)-**32** (92 or 93% ee) that a small amount of the ester (*R*)-**47** is undergoing hydrolysis during the kinetic resolution. Accordingly, kinetic resolutions were undertaken with a shorter reaction time of 10 h, under otherwise identical reaction conditions, and significantly, as we anticipated, the enantiopurity of the recovered acids (*S*)-**32** was enhanced to 97 or 96% ee. This observation highlights that with optimisation, highly enantioenriched samples of (*S*)-**32** could be obtained.

*Table 3.22: Hydrolase-mediated hydrolysis of (±)-ethyl 2-methyl-3-phenylpropanoate (±)-**47***



(±)-**47** (*S*)-**32** (*R*)-**47**

Entry	Enzyme source	Time	ee (%)		Conversion (%)		*E* value
			Ester (*R*)-47	Acid (*S*)-32	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	20 h	3[a]	3[a]	50	25	1.1
2	*Candida cyclindracea* C2	20 h	0[b]	0[b]	-[b]	100	-[b]
3	*Alcaligenes spp.*1	72 h	2	11	15	12	1.3
4	*Pseudomonas cepacia* P1	72 h	53	68	44	55	6.4
5	*Pseudomonas stutzeri*	72 h	83	50	62	69	17
6	*Rhizopus spp.*	72 h	5	30	14	<10	1.9
7	*Rhizopus niveus*	72 h	20	21	49	55	1.8
8	*Aspergillus niger*	72 h	5	33	13	<10	2.1
9	*Alcaligenes spp.*2	20 h	67	97	41	41	132
10a	*Pseudomonas cepacia* P2	20 h	>98	93	52	79	>200
10b		*10 h*	*95*	*96*	*50*	*61*	*183*
11	*Mucor javanicus*	20 h	14	21	40	29	1.7
12	*Penicillium camembertii*	72 h	3	17	15	<10	1.5
13a	*Pseudomonas fluorescens*	20 h	>98	92	52	55	179
13b		*10 h*	*81*	*97*	*46*	*49*	*164*
14	*Candida antarctica* B	20 h	0[b]	0[b]	-[b]	100	-[b]
15	*Mucor meihei*	20 h	23	0	52	97	1.9
16	*Candida antarctica* B (immob)	20 h	0[b]	0[b]	-[b]	100	-[b]
17	*Porcine pancrease* Type II	72 h	58	90	39	69	34
18	*Porcine pancrease* Grade II	72 h	11	47	19	35	3.1

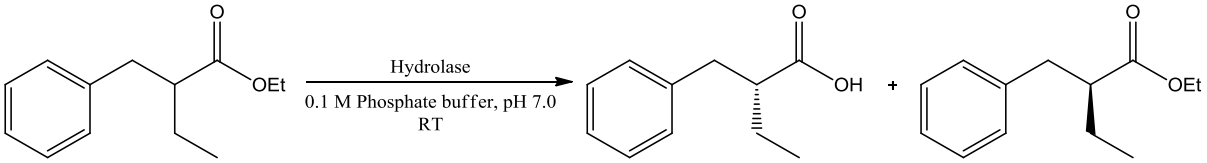
a. Limited enantiopurity observed, thus direction of enantioselection should be interpreted with caution.
b. Reaction went to 100% completion, no enantioselectivity observed.

3.4.5.2 Hydrolase-catalysed kinetic resolution to provide enantioenriched of 2-benzylbutanoic acid 45

On increasing the size of the methyl moiety at the C2 site to the bulkier ethyl group, a sharp decrease in both efficiency and enantioselection was observed, with a number of hydrolases displaying no hydrolysis. This correlated strongly with literature reports[46,47] and with the trends observed with the C3 substituted 3-arylalkanoic acids, (see section 3.4.3), demonstrating the dramatic dependence of the synthetic and stereochemical outcome of the reaction upon the size of the alkyl group at the stereogenic centre.

In this study, *Candida antarctica* lipase B (entry 3, Table 3.23) provided the highest enantiopurity of (*R*)-2-benzylbutanoic acid (*R*)-**45** (83% ee) *via* lipase-mediated hydrolysis. Previous to this result the highest reported lipase-mediated resolution of (*S*)-**45** was 53% ee.[47] Herein *Candida antarctica* lipase B (free and immobilised) provided the (*R*)-enantiomer selectively, all other reported hydrolases preferentially hydrolysed the (*S*)-enantiomer, albeit with low to modest enantioselectivity.

*Table 3.23: Hydrolase-mediated hydrolysis of (±)-ethyl 2-benzylbutanoate (±)-**48***



(±)-**48** (*R*)-**45** (*S*)-**48**

Entry	Enzyme source[a]	Time	ee (%)		Conversion (%)		*E* value
			Ester 48	Acid 45	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	43 h	3 (*R*)	13 (*S*)	19	28	1.3
2	*Candida cyclindracea* C2	17 h	20 (*R*)	4 (*S*)	83	89	1.3
3	*Candida antarctica B*	17 h	35 (*S*)	83 (*R*)	30	74	15
4	*Candida antarctica B*	43 h	74 (*S*)	71 (*R*)	49	79	14
5	*Candida antarctica B* (immob)	43 h	17 (*S*)	73 (*R*)	19	57	7.6
6	Pig Liver esterase	17 h	6 (*R*)[b]	3 (*S*)[b]	67	96	1.1

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Mucor javanicus*, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Pseudomonas stutzeri*, *Rhizopus niveus and Penicillium camembertii*
b. Limited enantiopurity observed, thus direction of enantioselection should be interpreted with caution.

Notably, 4 of the 5 hydrolases screened which resulted in conversion (Table 3.23) had also displayed enantioselection for the resolution of the β-ethyl substituted 3-phenylpentanoic acid (±)-**28**, the exception being *Candida cylindracea* C1. In addition, under similar reaction conditions the same source of enzyme *Candida antarctica* lipase B, although the immobilised version rather than the free enzyme, afforded the highest enantiopurity of (*R*)-**28** (81% ee) (entry 6, Table 3.11), comparable to the enantioselectivity obtained in this study for (*R*)-**45** (83% ee) (entry 3, Table 3.23).Thus, the position of the ethyl moiety had limited impact on choice of biocatalyst.

In this study the enantiomeric excess of the unreacted ester (*S*)-**48** was poor (35% ee) (entry 3, Table 3.23) relative to that of the β-substituted analogue (*S*)-**56** (85% ee) (entry 6, Table 3.11), owing to the decreased conversion rate (30% *vs.* 51%). Increasing reaction time from 17 h to 43 h (entry 4, Table 3.23) did result in a higher conversion rate (49%) and increased optical purity of (*S*)-**48** (74% ee), however, enantiopurity of the acid (*R*)-**45** was

compromised (71% ee) due to partial hydrolysis of the ester (*S*)-**48** at the extended reaction time.

Thus, the enantiodiscrimination in the hydrolysis to form the α-ethyl acid **45** was somewhat less efficient than that in the corresponding β-ethyl acid **28** despite the increased proximity of the stereocentre. This may be rationalised on examination of the structure whereby discrimination between the ethyl and benzyl substituents occurs at only one carbon removed from the stereogenic centre with similar methylene groups directly attached (Figure 3.20).

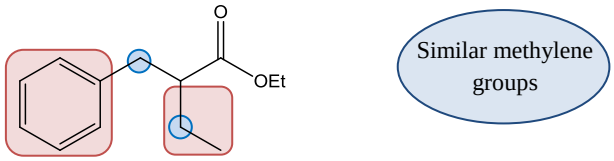


Figure 3.20

The direction of enantioselectivity of *Candida antarctica* lipase B (free and immobilised) was consistent with that observed for the β-substituted 3-phenylpentanoic acid (±)-**28**, with the (*R*)-enantiomer selectively hydrolysed. Interestingly, the opposite direction of enantioselection was observed in this study with the Pig liver esterase relative to that of the β-substituted analogue. Herein, Pig liver esterase and *Candida cyclindracea* (C1 and C2) selectively resolved the (*S*)-enantiomer. Notably Pig liver esterase mediated resolution of (±)-2-benzylbutanoic acid (±)-**45** proceeded with limited enantiopurity, therefore the direction of enantioselection should be interpreted with caution.

3.4.5.3 Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-benzyl-3,3-dimethylbutanoic acid 61

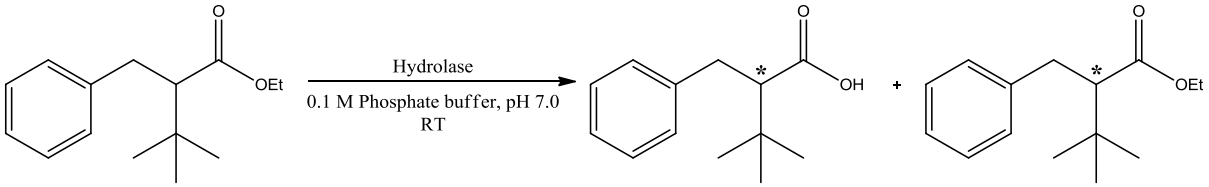
The next substrate investigated was the α-*tert*-butyl substituted ester (±)-**57**. Given the decrease in efficiency and enantioselectivity for the bulky α-ethyl substituted (*R*)-2-benzylbutanoic acid (*R*)-**45** relative to the α-methyl substituted (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**32** a similar lack of efficiency was anticipated in this study. In the screening assays, none of the lipases successfully catalysed hydrolysis of (±)-**57** to any extent, and thus, the sense of enantioselection could not be determined. This was particularly significant with regards to the lipase *Candida antarctica* lipase B. This lipase had previously demonstrated its unique ability to resolve sterically bulky α- and β-substituted substrates, proving to be the lipase of choice for the mediated resolution of (*R*)-**28**, (*S*)-**29**, (*S*)-**37** and (*R*)-**45.** As there was no evidence of any hydrolysis within 20 h, extended reaction times were not explored; use of increased reaction temperature may warrant investigation.

The steric bulk of the large *tert*-butyl group α to the carbonyl carbon clearly prevents hydrolysis occurring. A similar trend was observed when chemical esterification was attempted of the analogous acid (±)-**61** *via* Fischer esterification conditions (see section 3.3.1), the steric hindrance present prevented nucleophilic attack of the alcohol at the carbonyl carbon and thus <5% of the desired ester (±)-**57** was observed in the ^{1}H NMR of the crude product.

The presence of the *tert*-butyl substituent at either the α- or β-position dramatically reduced the efficiency of enzymatic hydrolysis in each case. However, with the β-substituted derivative, through careful optimisation of reaction conditions, isolation of enantiopure samples of the acid was achieved, albeit with low extent of biotransformation. Thus, the

proximity of the *tert*-butyl group to the ester is, in this instance, significant in reducing the hydrolysis efficiency.

*Table 3.24: Hydrolase-mediated hydrolysis of (±)-ethyl 2-benzyl-3,3-dimethylbutanoate (±)-**57***



(±)-**57** **61** **57**

Entry	Enzyme source	Time	ee (%)		Conversion (%)		*E* value
			Ester 57	Acid 61	*E* calc.	^{1}H NMR	
1	*Pseudomononas cepacia* P1	20 h	-	-	-	0	-
2	*Pseudomononas cepacia* P2	20 h	-	-	-	0	-
3	*Candida antarctica B*	20 h	-	-	-	0	-
4	*Candida antarctica* B (immob)	20 h	-	-	-	0	-
5	*Pseudomonas fluorescens*	20 h	-	-	-	0	-

Sinisterra *et al.* studied the influence of the alkyl chain on the efficiency of the *Candida cylindracea* (immob) mediated resolution of α-substituted (*S*)-2-arylpropionic carboxylic acids.[90] A large diminution in yield was reported on the resolution of ethyl 2-phenylbutanoate **91** compared to that observed with ethyl 2-phenylpropanoate **92** with the same immobilised lipase. In order to explain these results the conformers of the substrates were analysed by a molecular mechanics methodology and it was concluded that the reduction in yield was attributable to the presence of steric hindrance in the M subsite in the active site, due to the volume of the alkyl chain in the case of the ethyl 2-phenylbutanoate **91** (Figure 3.21)

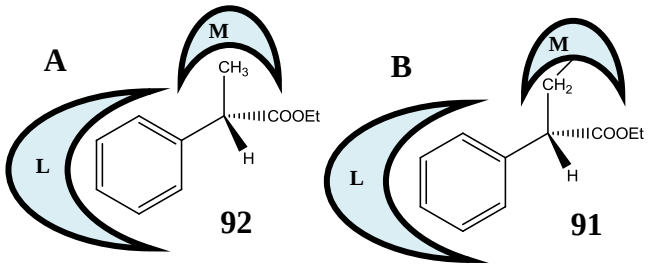


Figure 3.21: Steric hindrance produced in the subsite M due to the enhancement of the alkyl chain. ***A**: (S)- ethyl 2-phenylpropanoate **92** and **B**: (S)-ethyl 2-phenylbutanoate **91**.*[90]

Sinisterra's rationale may be applied to the substrates studied in this research where a similar trend of steric hindrance was observed at the α-position.[90] In this study when the methyl substituent increased in size to an ethyl or *tert*-butyl group a dramatic decrease in the efficiency of the hydrolysis was observed. This correlated with earlier reports in the literature[46,47] and with the results observed with the β-substituted 3-arylalkanoic acids (see section 3.4.3). Despite the steric hindrance within the active site, (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**32** was obtained in 97% ee, through *Alcaligenes spp. 2* catalysed hydrolysis of (±)-**47** while *Candida antarctica* lipase B was identified as resolving the α-

ethyl substituted 3-arylalkanoic acid (*R*)-**45** (83% ee) with improved enantioselection relative to that achieved by Sih *et al.* (53% ee) (Figure 3.22 and Figure 3.23).[47]

Interestingly we demonstrated that through variation of reaction time the enantiopurity of the recovered acids (*S*)-**32** and (*R*)-**45** can be optimised. Thus these biotransformations have the potential to be synthetically useful processes as the hydrolysis of the slower reacting enantiomer of the esters (*R*)-**47** and (*S*)-**48** can be minimised by careful reaction control.

*Comparison of enantiomeric ratio (E-value) versus hydrolase for C2 substituted alkanoic acids **32** and **45***

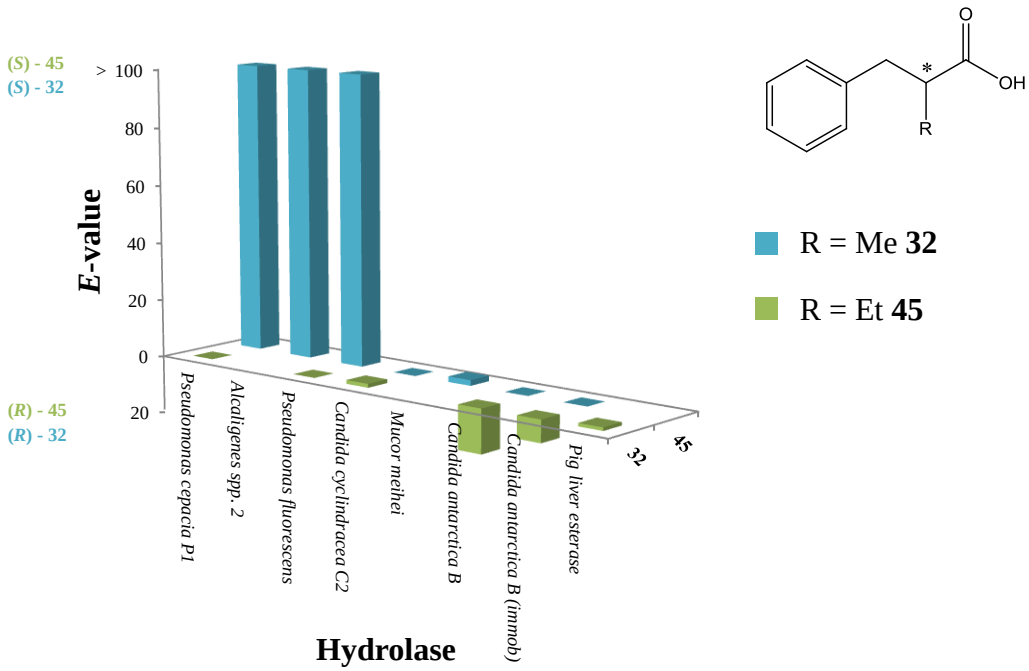


Figure 3.22

*Comparison of enantiomeric ratio (*E*-value) versus hydrolase for C2 substituted alkanoic acids **32** and **45** and C3 substituted acids **23** and **28***

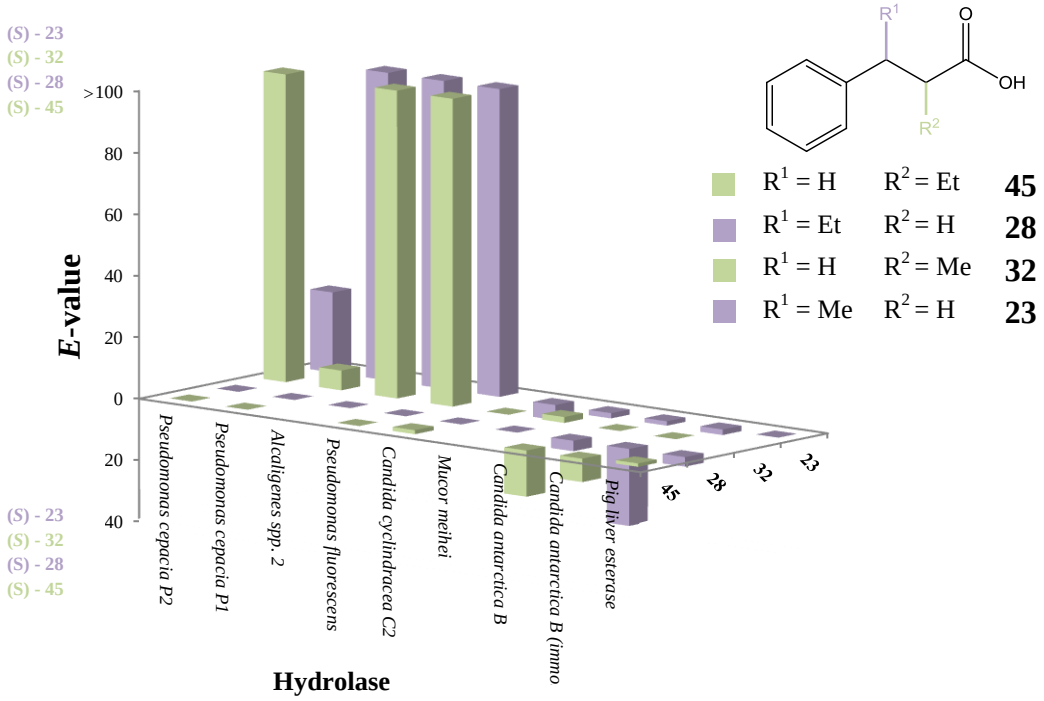


Figure 3.23

3.5 Hydrolase-mediated kinetic resolution – preparative-scale

The kinetic resolutions were next performed on a preparative-scale in order to confirm the absolute configuration of the products, demonstrate the synthetic utility of the bioresolution protocols and examine the practical viability of the lipase-mediated processes.

In general, the products of the biocatalysis were readily isolated by extraction with ethyl acetate followed by chromatographic separation on silica gel of the acid and ester. Column chromatography proved more effective than acid-base extraction for recovery of the esters (*R*)-**38, 47, 49, 53-55, 60** and acids (*S*)-**23, 32, 51, 64-66, 80** in this instance. For the bulkier 3-arylalkanoic substrates flash chromatography was less effective. In practice each of the esters (*S*)-**56**, (*R*)-**58,** (*R*)-**59** and (*S*)-**48** were recovered first by heptane extraction of the biotransformation mixture, then subsequent acidification[v] and extraction with ethyl acetate provided the acids (*R*)-**28**, (*S*)-**29**, (*S*)-**37** and (*R*)-**45**. Each of the products were characterised by ^{1}H NMR with spectroscopic details identical to those for the racemic materials previously prepared. Enantiopurity of each of the esters and acids was established by chiral HPLC. Conversion was determined by the *E*-value calculator and by analysis of the ^{1}H NMR of the crude reaction mixture.

The reaction times employed for the preparative resolutions were selected based on the analytical screens and were conducted in general without ongoing reaction monitoring. With the analytical screens pH adjustment was never an issue. In the preparative-scale reactions use of a pH stat to maintain the pH was explored during this work, but it was found to have no detectable impact on the outcome of the kinetic resolution, and therefore its use was discontinued, with clear practical advantages in terms of experimental setup.

[v] In retrospect the acidification may not be required but this was not explored during this work.

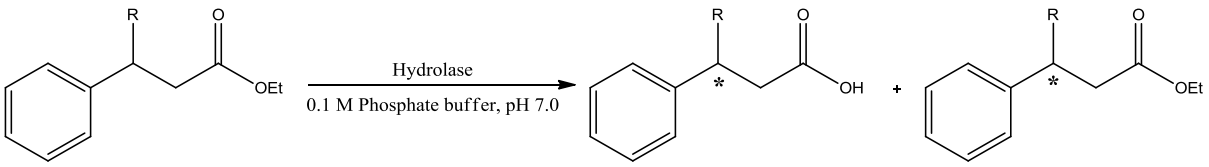
3.5.1 Preparative-scale hydrolase-catalysed kinetic resolution of C3 substituted alkanoic esters

Each of the hydrolyses were scaled up to synthetic batches (200-510 mg) leading to isolation of the β-substituted acids (*S*)-**23,** (*R*)-**28**, (*S*)-**29** and (*S*)-**37** in excellent enantiopurity. *Pseudomonas fluorescens* was selected for the preparative-scale hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-**38**, while *Candida antarctica* lipase B (immob) was the lipase of choice for the large scale hydrolysis of the sterically hindered (±)-ethyl 3-phenylpentanoate (±)-**56**, (±)-ethyl 4-methyl-3-phenylpentanoate (±)-**58**, and (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**59** (Table 3.25). The above mentioned lipases were selected due to the high enantiopurity observed in the analytical screens. Notably, on scale up, the efficiencies and selectivities of the resolutions mirrored quite closely the outcomes seen in the analytical scale reactions summarised in Tables 3.10 and 3.14-3.16.

Acids (*S*)-**23**,[91] (*S*)-**28**,[45] (*R*)-**29**,[92] (*S*)-**37**[93] and analogous esters (*S*)-**38**,[94] (*R*)-**56**,[95] (*S*)-**58**[95] were previously reported in the literature in enantioenriched form and therefore the assignment of the absolute stereochemistry for each of these compounds was made by comparison of specific rotation data. Although ethyl 4,4-dimethyl-3-phenylpentanoate **59** was not previously reported in enantiopure form, the absolute stereochemistry of the untransformed ester has to be (*R*)-**59** on the basis of recovery of the acid (*S*)-**37**.

The ^{1}H NMR spectra and chiral HPLC traces obtained of (*R*)-**38** and (*S*)-**23** during the work-up and purification of the *Pseudomonas fluorescens* mediated hydrolysis of (*R*)-**38** are shown below in Figure 3.24 and Figure 3.25 displaying the remarkably clean crude products obtained from the bioresolution.

Table 3.25: Preparative-scale hydrolase-catalysed kinetic resolution of C3 substituted alkanoic esters



R	Hydrolase	Conversion (%)		Acid	Ester	Yield (%)	ee (%)	Optical rotation $[\alpha]_D^T$	
		E calc.	^{1}H NMR					Experimental	Literature
Me	*P. fluorescens*	50	51	(*S*)-**23**		34	98	$[\alpha]_D^{20}$ +27.90 (c 1.0, EtOH)	$[\alpha]_D^{25}$ +24.50 (c 1.0, EtOH), (*S*)-isomer 97% ee[91]
					(*R*)-**38**	35	99	$[\alpha]_D^{20}$ −27.55 (c 1.1, $CHCl_3$)	$[\alpha]_D^{25}$ +19.00 (c 1.1, $CHCl_3$), (*S*)-isomer, 90% ee[94]
Et[a]	CAL-B (immob)	42	-[b]	(*R*)-**28**		22	90	$[\alpha]_D^{20}$ −33.73 (c 1.4, C_6H_6)	$[\alpha]_D^{25}$ +42.3 (c 8.0, C_6H_6), (*S*)-isomer, 83% ee[45]
					(*S*)-**56**	19	65	$[\alpha]_D^{20}$ +9.46 (c 0.6, $CHCl_3$)	$[\alpha]_D^{26}$ −18.3 (c 1.1, $CHCl_3$), (*R*)-isomer, 97% ee[95]
i-Pr	CAL-B (immob)	21	-[b]	(*S*)-**29**		24	98	$[\alpha]_D^{20}$ −24.35 (c 0.7, $CHCl_3$)	$[\alpha]_D^{23}$ +28.12 (c 1.9, $CHCl_3$), (*R*)-isomer, 96% ee[92]
					(*R*)-**58**	25	26	$[\alpha]_D^{20}$ +7.05 (c 1.0, $CHCl_3$)	$[\alpha]_D^{26}$ −25.4 (c 1.0, $CHCl_3$), (*S*)-isomer, 98% ee[95]
t-Bu	CAL-B (immob)	11	-[b]	(*S*)-**37**		13	>98	$[\alpha]_D^{20}$ −10.53 (c 0.1, $CHCl_3$)	$[\alpha]_D^{20}$ −20.4 (c 2.2, $CHCl_3$), (*S*)-isomer, 91% ee[93]
					(*R*)-**59**	39	12	$[\alpha]_D^{20}$ +0.80 (c 1.0, $CHCl_3$)	-

a. 17% v/v dioxane co-solvent was added, see experimental for further information.
b. During work-up the acid and ester are isolated separately from the biotransformation by sequential extraction thus conversion by ^{1}H NMR is not feasible.

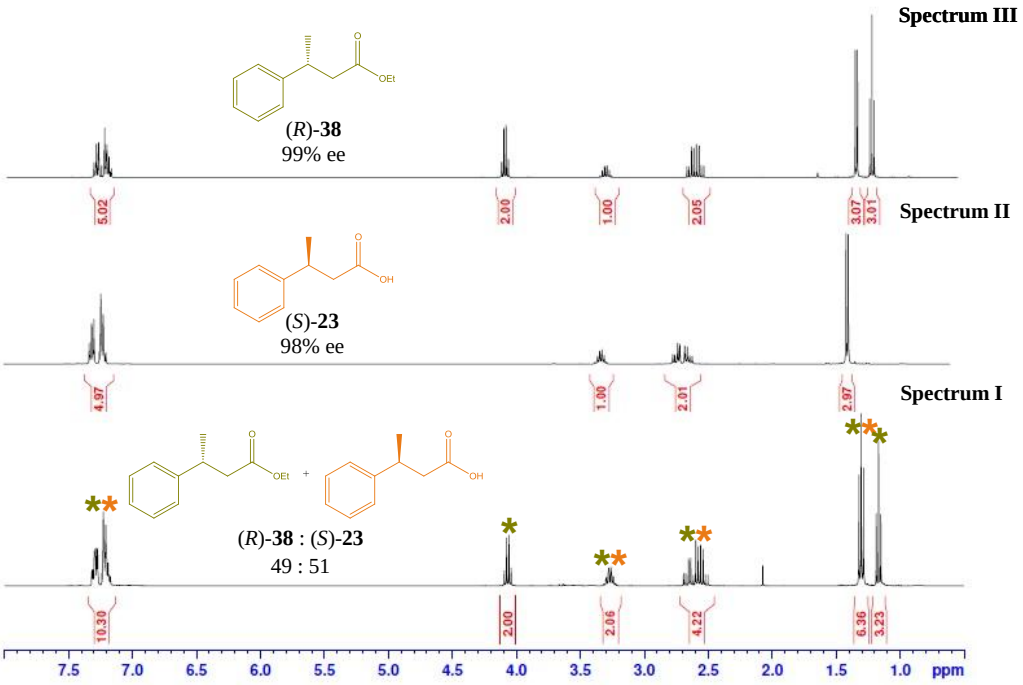


Figure 3.24: *^{1}H NMR* ***Spectrum I:*** *Crude product following* Pseudomonas fluorescens *hydrolysis containing a mixture of* (R)*-ethyl 3-phenylbutanoate* (R)***-38*** *and* (S)*-3-phenylbutanoic acid* (S)***-23****, 49 : 51 respectively.* ***Spectrum II:*** *Purified* (S)*-3-phenylbutanoic acid* (S)***-23****, 98% ee, following column chromatography.* ***Spectrum III:*** *Purified* (R)*-ethyl 3-phenylbutanoate* (R)***-38,*** *99% ee, following column chromatography (all spectra recorded in* $CDCl_3$ *at 400 MHz).*

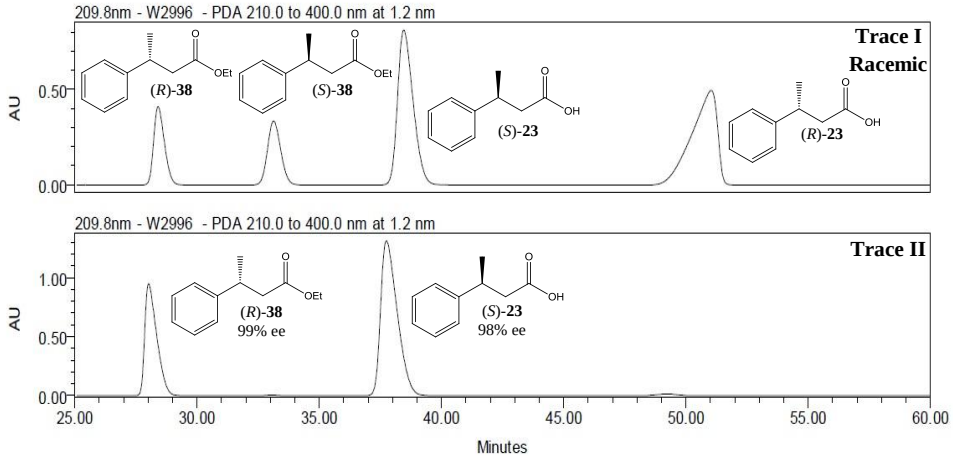


Figure 3.25: *HPLC* ***Trace I:*** *A racemic mixture of (±)-ethyl 3-phenylbutanoate (±)****-38*** *and (±)-3-phenylbutanoic acid (±)****-23****.* ***Trace II:*** *Crude product following* Pseudomonas fluorescens *hydrolysis containing* (R)*-ethyl 3-phenylbutanoate* (R)***-38*** *99% ee and* (S)*-3-phenylbutanoic acid* (S)***-23*** *98% ee.*

3.5.2 Preparative-scale hydrolase-catalysed kinetic resolution of substituted phenyl butanoic esters

The next step in the investigation was preparative-scale hydrolysis of the aromatic substituted ethyl phenyl butanoates (±)-**49**, (±)-**53-55, 60**. In the analytical screens excellent enantioselectivities of both the untransformed ester (>98% ee) and acid product (≥94% ee) were attainable, with ideal 50% conversion achieved utilising the *Pseudomonas* lipases. Thus, this series was selected as the most appropriate hydrolase for preparative-scale use.

Reaction monitoring was performed for the resolution of (*S*)-3-(4-methylphenyl)butanoic acid (*S*)-**51**. At 62 h, 1 mL of reaction mixture was removed, a mini work-up was performed and reaction progress determined by ^{1}H NMR and chiral HPLC. Excellent enantioselectivity of the ester (*R*)-**49** and acid (*S*)-**51** was observed at 62 h which reflected favourably the outcome of the analytical scale resolution, where the kinetic resolution achieved a desirable 50% conversion rate and high enantiopurity at 64 h. As the reaction time of the preparative-scale appears to mimic very closely that of the analytically scale each of the other resolutions in this series did not undergo chiral HPLC reaction monitoring. Ideal 50% conversion was achieved in all cases within the reaction time identified from the analytical screens (Table 3.26). This is hugely advantageous from a synthetic perspective as extracting reaction aliquots for analysis contributes significantly to overall loss of yield. Furthermore none of the hydrolase-mediated kinetic resolutions exceeded 50% conversion on scale-up, demonstrating excellent discrimination between the (*R*)- and (*S*)-enantiomers.

Significantly the enantioselectivities of the large scale hydrolysis compared very favourably with the analytical screens. The untransformed (*R*)-ester was obtained in ≥94% ee and the (*S*)-acid ≥97% ee (Table 3.26). Notably, with the *para*-fluoro series the acid (*S*)-**80** was recovered in excellent enantiopurity (97% ee) improved relative to that of the small scale reaction (94% ee) (entry 3, Table 3.21).

The absolute stereochemistry of each of the isolated acids (*S*)-**51**[19], (*S*)-**66**[91] and ester (*R*)-**49**[19] was identified by comparing the specific rotation data obtained in this study with those reported in the literature. While (*R*)-ethyl 3-(4-methoxyphenyl)butanoate (*R*)-**60** has not been described in enantioenriched form its absolute stereochemistry was assigned as (*R*)-**60** as it must be opposite to that of the recovered acid (*S*)-**66**.

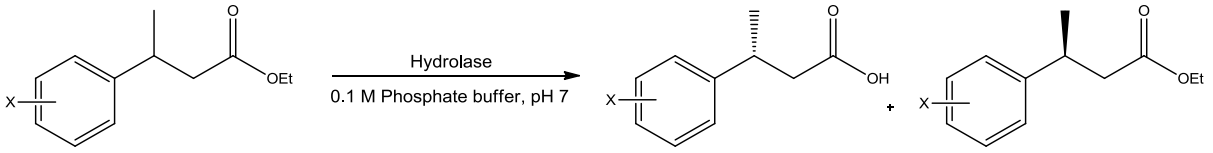
In contrast, as the enantiopure acids **65**, **64**, **80** and esters **54**, **53**, **55** have not previously been described, comparison of specific rotation data was not feasible. Each of the acids and esters were oils at room temperature and therefore absolute stereochemistry was difficult to determine. Attempts to solidify and crystallise the enantiopure acids by salt formation proved unsuccessful.

Co-crystallisation involves generating a crystalline material consisting of more than one neutral compound. The combination of co-crystallisation with both X-ray diffraction and chiral HPLC was particularly powerful in this study for overcoming the difficulties of assigning absolute stereochemistry to the enantiopure 3-arylbutanoic acids **65**, **64** and **80**. Co-crystallisation offers advantages over salt formation because co-crystals dissociate in solution, meaning identical HPLC conditions can be used for both the materials of interest and their co-crystals. The use of co-crystals for the determination of absolute stereochemistry of acids **65**, **64** and **80**, as an alternative to salt formation is further discussed in section 3.5.4.

As expected the sense of enantioselection of each of the biotransformations are the same, providing the (*R*)-ester and (*S*)-acid. The assignment of absolute stereochemistry through this new technique is supported by the specific rotation data where all the (*S*)-acids

are dextrorotatory and the (*R*)-acids levorotatory. As observed in the analytical screening, from a preparative perspective presence of substituents on the aromatic ring, even with the *ortho*-substituent, had no detectable impact on the outcome of the resolution.

Table 3.26: Preparative-scale hydrolase-catalysed kinetic resolution of C3 substituted alkanoic esters



X	Hydrolase	Conversion (%)		Acid	Ester	Yield (%)	ee (%)	Optical rotation $[\alpha]_D^T$	
		E calc.	^{1}H NMR					Experimental	literature
p-Me	*P. cepacia*	49	47	(*S*)-**51**		40[a]	>98	$[\alpha]_D^{20}$ +31.80 (c 1.0, $CHCl_3$)	$[\alpha]_D^{25}$ +34.2 (c 1.0, $CHCl_3$), (*S*)-isomer 99% ee[19]
					(*R*)-**49**	31[a]	97	$[\alpha]_D^{20}$ −28.67 (c 3.5, $CHCl_3$)	$[\alpha]_D^{25}$ −26.2 (c 3.5, $CHCl_3$), (*R*)-isomer 92% ee[19]
m-Me	*P. fluorescens*	49	-[b]	(*S*)-**65**		26	>98	$[\alpha]_D^{20}$ +32.32 (c 0.6, $CHCl_3$)	-
					(*R*)-**54**	22	94	$[\alpha]_D^{20}$ −24.40 (c 1.0, $CHCl_3$)	-
o-Me	*P. fluorescens*	50	59	(*S*)-**64**		28	>98	$[\alpha]_D^{20}$ +24.17 (c 1.4, $CHCl_3$)	-
					(*R*)-**53**	27	98	$[\alpha]_D^{20}$ −11.00 (c 1.0, $CHCl_3$)	-
p-OMe	*P. fluorescens*	51	50	(*S*)-**66**		23	97	$[\alpha]_D^{20}$ +26.25 (c 1.0, EtOH)	$[\alpha]_D^{25}$ +27.50 (c 1.0, EtOH) (*S*)-isomer 94% ee[91]
					(*R*)-**60**	43	>98	$[\alpha]_D^{20}$ −30.03 (c 1.0, $CHCl_3$),	-
p-F	*P. fluorescens*	51	53	(*S*)-**80**		35	97	$[\alpha]_D^{20}$ +30.51 (c 1.0, $CHCl_3$)	-
					(*R*)-**55**	32	>98	$[\alpha]_D^{20}$ −24.34 (c 1.0, $CHCl_3$)	-

a. Yield may be reduced due to reaction sampling.
b. Presence of ethyl acetate in the ^{1}H NMR spectrum overlaps with key characteristic ester peaks of (*R*)-**54** required for determination of conversion.

3.5.3 Preparative-scale hydrolase-catalysed kinetic resolution of C2 substituted alkanoic esters

The final series to be extended to preparative-scale was the C2 substituted 3-arylalkanoic acids. While the α-methyl (±)-**47** and α-ethyl (±)-**48** substituted esters demonstrated hydrolysis *via* hydrolase-mediated kinetic resolution, the α-*tert*-butyl ester (±)-**57** did not resolve to any extent; consequently, preparative-scale hydrolysis was performed on (±)-**47** and (±)-**48** only.

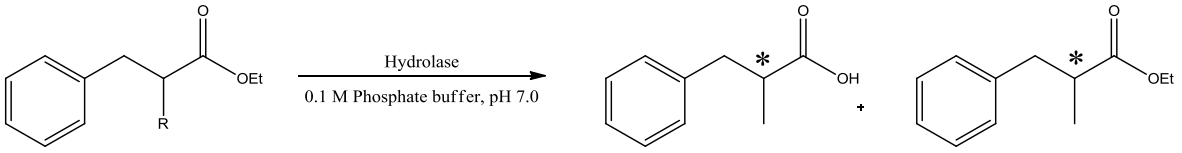
Pseudomonas fluorescens was selected for preparative-scale hydrolysis of (±)-**47**; this too was the biocatalyst of choice for the resolution of (±)-3-phenylbutanoic acid (±)-**23** and the analogous aromatic substituted (±)-3-phenylbutanoic acids (±)-**51** and (±)-**64**-**66**, exhibiting excellent enantioselectivities in all cases. Thus, as described previously in the analytical screens, the aryl substituent or position of the methyl moiety on the carbon framework had little impact on the enzymatic hydrolysis and this carried through to preparative-scale. Reaction sampling was conducted at 20 h and the reaction deemed complete by ^{1}H NMR and chiral HPLC analysis, thus work-up was undertaken at this time.

In the analytical screens, free *Candida antarctica* lipase B resulted in the highest enantioselectivity (83% ee) of the sterically hindered α-ethyl acid (*R*)-**45**. However, *Candida antarctica* lipase B (immob) was utilised in the preparative-scale as immobilised lipases offer significant advantages over their free counterpart in large scale resolutions. Immobilisation can enhance stability, enable repeated or continuous use, ease separation from the reaction mixture and modulate catalytic properties.[4] In this study *Candida antarctica* lipase B (immob) mediated resolution of the α-ethyl (*R*)-acid (*R*)-**45** on preparative-scale resulted in excellent enantiopurity (82% ee) improved relative to that from the small scale reaction (73% ee). From the perspective of process efficiency, the fact that the level of enantioselectivity

achieved on the preparative-scale with the immobilised biocatalyst was restored to the optimised level seen in the analytical screen with the free biocatalyst, is very significant.

The direction of enantioselection of each of the recovered acids (*S*)-**32**[96] and (*R*)-**45**[97] and ester (*R*)-**47**[52] was determined by comparing the specific rotation data obtained in this study with those reported in the literature. Although the α-ethyl substituted ester (*S*)-**48** has not previously been reported in enantioenriched form its absolute stereochemistry was assigned as (*S*)-**48** as it must be opposite to that of the recovered acid (*R*)-**45**.

Table 3.27: Preparative-scale hydrolase-catalysed kinetic resolution of C3 substituted alkanoic esters



R	Hydrolase	Conversion (%)		Acid	Ester	Yield (%)	ee (%)	Optical rotation $[\alpha]_D^T$	
		E calc.	^{1}H NMR					Experimental	literature
Me	*P. fluorescens*	51	-[a]	(*S*)-**32**		37[b]	96	$[\alpha]_D^{20}$ +28.0 (c 0.82, $CHCl_3$)	$[\alpha]_D^{20}$ +30.2 (c 0.82, $CHCl_3$), (*S*)-isomer 99% ee[96]
					(*R*)-**47**	27[b]	>98	$[\alpha]_D^{20}$ −36.4 (c 1.0, $CHCl_3$)	$[\alpha]_D^{20}$ +28.4 (c 1.0, $CHCl_3$), (*S*)-isomer, 82% ee[52]
Et	CAL-B (immob)	24	-[c]	(*R*)-**45**		19	82	$[\alpha]_D^{20}$ −43.8 (c 1.0, CH_2Cl_2)	$[\alpha]_D^{23}$ −40.0 (c 1.0, CH_2Cl_2), (*R*)-isomer >99% ee[97]
					(*S*)-**48**	43	26	$[\alpha]_D^{20}$ +6.8 (c 1.0, CH_2Cl_2)	-

a. Presence of ethyl acetate in the ^{1}H NMR spectrum overlaps with key characteristic ester peaks of (*R*)-**54** required for determination of conversion.
b. Yield may be reduced due to reaction sampling.
c. During work-up the acid and ester are isolated separately from the biotransformation by sequential extraction thus conversion by ^{1}H NMR is not feasible.

3.5.4 The use of co-crystals for the determination of absolute stereochemistry of the products of preparative-scale reactions

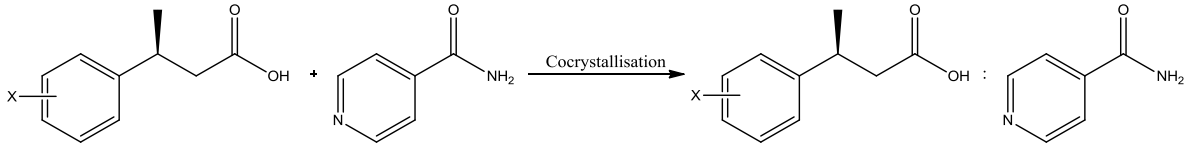
Despite major advances in asymmetric synthesis over the past 30 years, one of the major challenges that remains is definitively assigning absolute stereochemistry,[98] especially with materials which are not readily crystalline. This is increasingly important as new synthetic products are often difficult to crystallise,[99] and many can only be isolated as viscous oils.

The two main synthetic strategies which have been employed to circumvent these problems involve modification of the material, *via* either additional chemical transformations or salt formation.[100] Both of these strategies, however, have limitations. The use of chemical transformations can be limited by the availability of only small quantities of enantiopure material, and furthermore, additional reaction steps may affect the stereochemical integrity of the required compound. For salt formation, in addition to the limitations mentioned above, the compound also requires ionizable sites. For both strategies, chiral HPLC analysis has to be developed not only for the pure material but also for the derivatives, since in many cases the conditions are non-transferrable.[100]

As stated in section 3.5.2, the acids **65**, **64**, **80** and esters **54**, **53**, **55** were not previously reported in enantiopure form and therefore assignment of stereochemistry was not possible by comparison with previously reported specific rotation data. In addition, each of the acids and esters were isolated as oils at room temperature. The traditional approach of salt formation was initially explored: salts were formed with 4,4'-bipyridine, sodium and potassium hydroxide, which precipitated as white powders. However, single crystals of these salts were not attained. The materials produced were poorly crystalline and not suitable for X-

ray diffraction studies. Additional chemical transformations were not attempted, as the enantiopure samples were available in limited quantities.

Co-crystallisation, involving crystallising two (or more) neutral molecules together in one crystalline material, has recently garnered great interest as an alternative to salt formation for improving the physical properties of active pharmaceutical ingredients, without detrimental effects on the chemical properties.[101-105] Therefore, in collaboration with Dr. K. S. Eccles and Dr. S. E. Lawrence, Department of Chemistry, University College Cork, a study was conducted exploring if the absolute stereochemistry of the carboxylic acids **23**, **65**, **64** and **80** could be determined through co-crystals.[106] While (*S*)-**23** has previously been described in the literature, and thus the stereochemistry of the starting material for the co-crystal known, it was utilised in this study as a model system and the optimised procedure was then implemented for the three unknown samples **65**, **64** and **80**.



X = H	(*S*)-**23**	**93**	X = H	(*S*)-**94**
X = *o*-Me	(*S*)-**64**		X = *o*-Me	(*S*)-**95**
X = *m*-Me	(*S*)-**65**		X = *m*-Me	(*S*)-**96**
X = *p*-F	(*S*)-**80**		X = *p*-F	(*S*)-**97**

Scheme 3.14

A search of the Cambridge Structural Database[107] revealed that monocarboxylic acids often co-crystallise with nicotinamide **98** and isonicotinamide **93**.[108-111] Initial co-crystal screening was performed by neat grinding with the racemic acid, and isonicotinamide **93** was identified by powder X-ray diffraction as an appropriate co-crystal former for each of the acids (Scheme 3.14). Suitable conditions for growing single crystals from solution were identified by using the racemic acids and similar conditions were then applied to the enantiopure analogues. The advantage of this approach was that once the conditions were developed, a small sample of the enantiopure material (<8 mg) was sufficient. Each of the co-crystals, both enantiopure and racemic, were grown by slow evaporation of acetonitrile/acetone (70 : 30) solutions. Single crystal diffraction data, in combination with the chromatographic experiments, allowed the unambiguous assignment of absolute configuration.[112-114]

Each enantiopure co-crystal was formed with the (*S*)-enantiomer of the acid (Figure 3.26). Each of the co-crystals display a common set of intermolecular interactions (Figures 3.27) with amide-amide [N-H···O=C] and acid-pyridine [COOH···N] hydrogen bonds linking the co-crystal components.

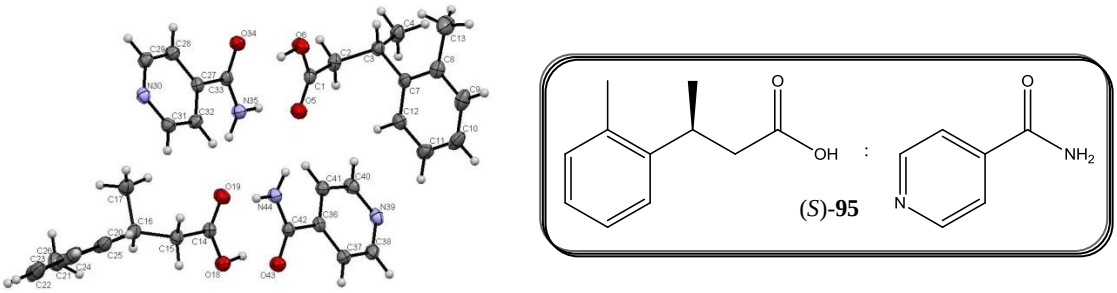


*Figure 3.26: A view of co-crystal (S)-**95** showing the structure and relative stereochemistry. The model has chirality C3 (S) and C16 (S). Anisotropic displacement parameters are drawn at the 50% probability level.*

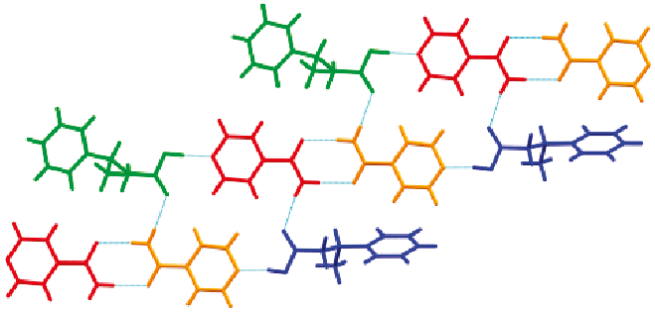


*Figure 3.27: Hydrogen bonding interactions in (S)-**94**. The different colours indicate unique, symmetry-independent (S)-**94** (green and blue) and isonicotinamide **93** (red and orange) molecules.*
*The same motif is formed in (S)-**96**, (S)-**97**, (±)-**95**, and (±)-**96**.*

.

Interestingly, irrespective of the chiral or enantiopure nature of the acid molecules, similar infinite one-dimensional hydrogen-bonded ribbons are formed in each of the co-crystals except (±)-**97**, which exhibits two-dimensional puckered sheets. Thus, the overall core hydrogen-bonded motif is retained across the structures, with the conformation of the acids varying. Presumably, this conformational variation is necessary to maintain both a close-packed arrangement of molecules with asymmetric shapes and a (topologically) symmetrical hydrogen bond network. Further details of the structural features present in these co-crystals are contained in the accompanying CD.

An important consideration was whether the diffraction experiment was a true representation of the bulk sample. To ensure this was the case, powder X-ray diffraction, PXRD, was performed on each of the ground materials **94-97** and compared to the theoretical PXRD patterns calculated from the single crystal data confirming that single crystals were representative of the bulk sample.

It was anticipated that the acid molecules, once dissolved, would have the same properties irrespective of whether they are obtained by dissolving the pure acid or the co-crystal, as co-crystallisation only affects the properties of the solid phase. Thus, co-crystallisation has the clear advantage that the conditions developed to separate the enantiomers of the pure compound by chiral HPLC can be applied directly for analysis of the co-crystal employed for X-ray crystallography, enabling direct correlation with the chiral HPLC peak for the enantiomer. The only potential concern is the impact of the co-crystal former. To check for possible interference, chiral HPLC of the racemic acids, in the presence of isonicotinamide **93**, was recorded, clearly showing that chromatographic behaviour of (±)-

23, **65**, **64** and **80** was unaffected, confirming the validity of the methodology employed. Thus, chiral HPLC were recorded on the individual crystals used for the X-ray diffraction experiments (for chiral HPLC conditions see appendix I). This enabled the use of chiral HPLC to directly monitor the direction of enantioselection in the enzyme-mediated resolutions (Figure 3.28). Thus, while (*S*)-**23** has been described in the literature,[91,115] this is the first time the absolute stereochemistry of this compound has been definitively determined by X-ray diffraction. Having validated the approach with the known (*S*)-**23** then the procedure was successfully employed to determine the absolute stereochemistry of the novel enantiopure acids (*S*)-**65**, (*S*)-**64** and (*S*)-**80**.

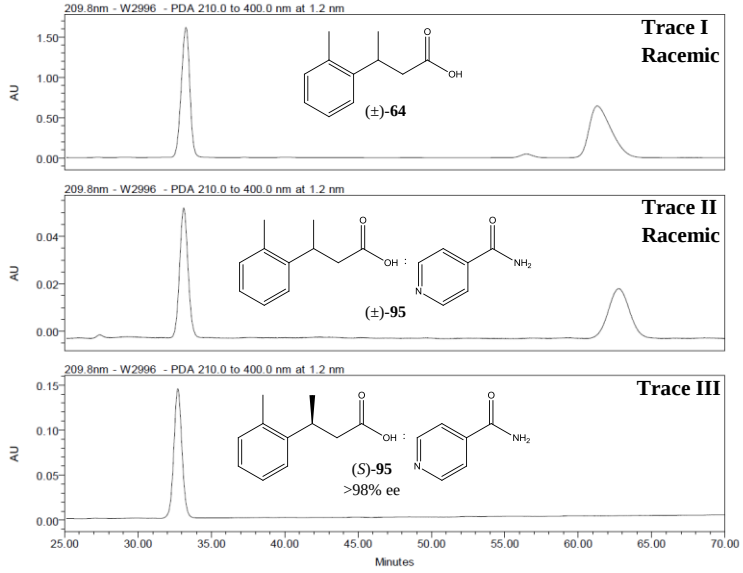


Figure 3.28: HPLC ***Trace I:*** *Racemic (±)-3-(2-methylphenyl)butanoic acid (±)-**64**.* ***Trace II:*** *Racemic (±)-3-(2-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-**95**.* ***Trace III:*** *(S)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (S)-**95** >98% ee. For HPLC conditions see appendix I.*

Co-crystallisation involving achiral compounds with enantiopure and racemic partners is known; in particular interest has centred on the different physical properties that are obtained for the racemic and enantiopure co-crystals, such as density and stability.[108,116-118] It has been used to introduce heavy atoms into a chiral structure, enabling absolute stereochemistry determination of known materials with Mo Kα radiation.[119] To date the stereochemistry of the starting material has always been known.

During these studies into developing co-crystallisation as a tool for determining the absolute stereochemistry of materials which are hard to crystallise, it was discovered that subtle changes in the solvent composition had a dramatic effect on the crystallisation outcome. As previously mentioned for the successful co-crystallisation of substituted 3-arylbutanoic acids with isonicotinamide, a 70 : 30 solvent mixture of acetonitrile and acetone respectively was employed. Interestingly the use of pure acetone as solvent gave rise to two new forms of isonicotinamide.[120]

This is the first time that the application of co-crystallisation methodology was demonstrated to be successful for determination of the absolute stereochemistry of compounds that are oils or viscous liquids at ambient conditions. The advantage of co-crystals over salt formation is that the chromatographic conditions developed to separate the enantiomers do not change on co-crystallisation, unless the co-crystal former interferes with the separation of the enantiomers. Furthermore, co-crystals do not require ionizable sites,

which in principle means that co-crystallisation should be more universally applicable than salt formation. In conclusion, co-crystallisation and X-ray diffraction, in combination with chiral HPLC, provides an effective method in synthetic research to identify absolute stereochemistry in novel compounds.

3.6 Project conclusion

At the outset of this project it was reported that 3-arylalkanoic acids with substituents larger than a methyl moiety at the C2 or C3 chiral centre could not be enzymatically resolved in high enantiopurity *via* lipase-mediated hydrolysis of 3-arylalkanoic carboxylic acid esters or transesterification.[42,46,47] The literature precedent reported a complete disappearance of efficiency and, accordingly enantioselection, upon kinetic resolution of the sterically demanding 3-alkylalkanoic acids. However, in this study, through optimisation of reaction conditions, the highest reported enantiopurities of 3-arylalkanoic acids were described *via* hydrolase-mediated kinetic hydrolysis of the corresponding ethyl esters.

The specific focus of this project was the investigation of the impact of steric effects at C2 and at C3 on the efficiency of the lipase-mediated kinetic hydrolysis. While resolution of C2 and C3 methyl substituted acids proved very straightforward, in general, the presence of a sterically demanding substituent larger than a methyl at the C2 or C3 stereogenic centre decreased the efficiency of the kinetic resolution, and therefore the enantioselectivity of the unreacted ethyl ester was compromised. Despite this, through comprehensive screening protocols and optimization of co-solvent and reaction temperature, the highest obtained enantiopurities of lipase-catalysed bioresolutions of 3-arylalkanoic acids were achieved, and in some cases, a viable route to both enantiomers has been identified. The only exception for which no hydrolysis was observed was with the very sterically demanding α-*tert*-butyl substituent, while reaction occurred with the β-*tert*-butyl substituent to a limited extent.

Furthermore, steric and electronic effects of substituents on the phenyl ring were explored and determined to have no detectable effect on the outcome of the kinetic resolution with excellent enantioselectivities attainable. Thus the hydrolases can tolerate increased steric demand in the aryl group even at the ortho position more readily than in the C3 or C2 position.

Significantly each of the successful bioresolutions were conducted on a synthetic scale leading to isolation of acids in excellent enantiopurity, demonstrating the synthetic potential of this chemoenzymatic approach. The practical aspect of the biocatalytic optimisation was greatly facilitated through development of HPLC conditions to enable analysis of the ester and acid through a single injection. A novel approach to determine the absolute stereochemistry of the acids which existed as oils was explored through combination of co-crystallisation and X-ray diffraction linked with chiral HPLC analysis.

3.7 References

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Chapter 4

Dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis

Contents

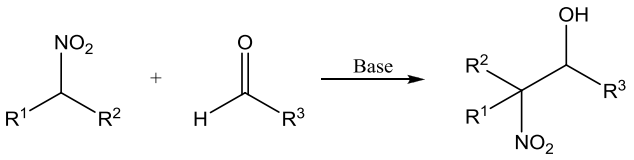
4.1 Introduction **141**
4.1.1 Henry reaction **141**
4.1.1.1 Enzymatic methods in the asymmetric Henry reaction **142**
4.1.1.1.1 Approach I – Direct enzyme-mediated Henry reaction **143**
4.1.1.1.2 Approach II – Enzymatic resolution of the products of the Henry reaction **146**
4.2 Dynamic kinetic resolution of the intramolecular Henry reaction of (±)-2-nitrocyclohexanol (±)-99 through lipase catalysis **151**
4.2.1 Background to the project **151**
4.2.2 Objectives of the project **154**
4.2.3 Synthesis of substrates **155**
4.2.3.1 Synthesis of 6-nitrohexanal 101 ..... **156**
*4.2.3.1.1 Route I – Ring opening of ε-caprolactam **109** and subsequent oxidation* **156**
4.2.3.1.2 Route II – Kornblum reaction **158**
4.2.3.2 Synthesis of (±)-2-nitrocyclohexanol (±)-**99** **161**
4.2.3.3 Synthesis of (±)-2-nitrocyclohexyl acetate (±)-**100** **164**
4.2.4 Chiral HPLC method development **166**
4.2.5 Evidence of a dynamic interconversion process – ^{1}H NMR analysis **169**
4.2.6 Hydrolase-mediated kinetic resolution – analytical screens **172**
4.2.6.1 Analytical screening protocol – vinyl acetate as both acyl donor and solvent **172**
4.2.6.2 Stereochemical assignment of the products of transesterification **173**
4.2.6.3 Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** **174**
4.2.6.4 Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** **176**
4.2.6.5 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** **177**
4.2.7 Evidence of a dynamic interconversion process – chiral HPLC **179**
4.2.8 Project conclusion **185**
4.3 Dynamic kinetic resolution of the intramolecular Henry reaction of (±)-2-methyl-2-nitrocyclohexanol (±)-117 through lipase catalysis **187**
4.3.1 Background to the project **187**
4.3.2 Objectives of the project **188**
4.3.3 Synthesis of substrates **189**
4.3.3.1 Synthesis of 2-methyl-2-nitrocyclohexanone **120** **189**
4.3.3.2 Synthesis of (±)-2-methyl-2-nitrocyclohexanol (±)-**117** **192**
4.3.3.3 Synthesis of (±)-2-methyl-2-nitrocyclohexyl acetate (±)-**119** **194**
4.3.3.4 Synthesis of 6-nitroheptanal **118** **196**
4.3.4 Hydrolase-mediated kinetic resolution – analytical screens **202**
4.3.4.1 Chiral HPLC method development **202**
4.3.4.2 Analytical screening protocol – vinyl acetate as both acyl donor and solvent **203**
4.3.4.3 Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** **204**
4.3.4.4 Hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** **206**
4.3.4.5 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** **207**
4.3.5 Hydrolase-mediated kinetic resolution – preparative-scale **210**
4.3.5.1 Preparative-scale hydrolase-catalysed transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** **210**
4.3.5.2 Preparative-scale hydrolase-catalysed transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** **212**
4.3.5.3 Stereochemical assignment of the products of preparative-scale reactions **214**
4.3.6 One-pot hydrolase-mediated dynamic resolution screens **216**
4.3.6.1 Triethylamine **217**
*4.3.6.1.1 Cyclisation of 6-nitroheptanal **118*** via *the intramolecular Henry reaction* **218**
4.3.6.1.2 Dynamic interconversion process **220**
4.3.6.1.3 Dynamic one-pot kinetic resolution process **222**

4.3.6.2 1,8-Diazabicycloundec-7-ene (DBU) **225**
*4.3.6.2.1 Cyclisation of 6-nitroheptanal **118** via the intramolecular Henry reaction* **225**
4.3.6.2.2 Dynamic interconversion process **230**
4.3.6.2.3 Dynamic one-pot kinetic resolution process **235**
4.3.6.3 Base screening **237**
*4.3.6.3.1 Cyclisation of 6-nitroheptanal **118** via the intramolecular Henry reaction* **237**
4.3.6.4 1,4-Diazabicyclo[2.2.2]octane (DABCO) **239**
4.3.6.4.1 Dynamic interconversion process **239**
4.3.6.4.2 Dynamic one-pot kinetic resolution process **239**
4.3.6.5 1,1,3,3-Tetramethylguanidine (TMG) **240**
4.3.6.5.1 Dynamic interconversion process **240**
4.3.6.5.2 Dynamic one-pot kinetic resolution process **241**
4.3.6.6 Polymer-bound 1,8-Diazabicycloundec-7-ene [DBU (immob)] **243**
*4.3.6.6.1 Cyclisation of 6-nitroheptanal **118** via the intramolecular Henry reaction* **243**
4.3.6.6.2 Dynamic interconversion process **243**
4.3.6.7 Influence of base strength **244**
4.3.7 Two-pot hydrolase-mediated dynamic resolution screens **245**
4.3.8 Project conclusion **247**
4.4 References **249**

4.1 Introduction

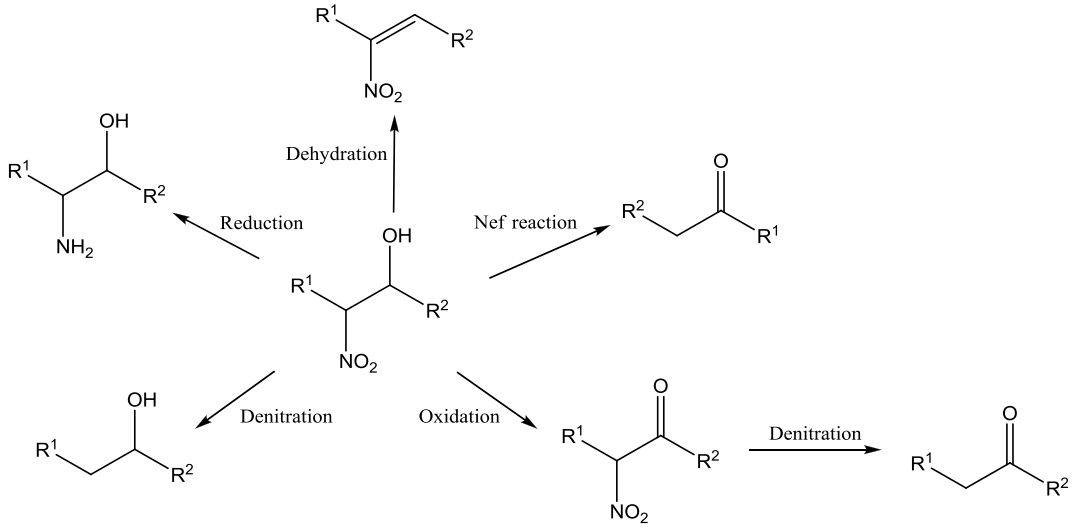
4.1.1 Henry reaction

The Henry or nitroaldol reaction is one of the classical named reactions in organic synthesis. Essentially a base-catalysed coupling reaction between a carbonyl compound and a nucleophilic nitroalkane bearing α hydrogens, (Scheme 4.1) it has proved to be a powerful carbon-carbon bond-forming process since its discovery in 1895.[1-3]



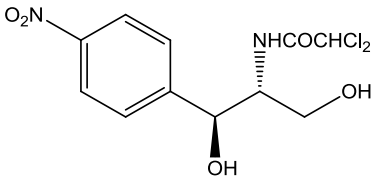
Scheme 4.1

This process represents an important and versatile tool for the synthesis of valuable β-nitroalcohols, providing efficient access to highly functionalised structural motifs *e.g.* dehydration to conjugated nitroalkenes, reduction to 1,2-amino alcohols, denitration, oxidation to nitro carbonyl compounds and α-hydroxy carbonyl compounds *via* the Nef reaction (Scheme 4.2).[4,5]

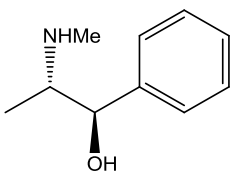


Scheme 4.2

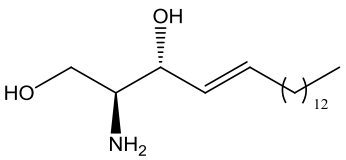
The Henry reaction is a useful tool in the elaboration of pharmacological important β-amino alcohol derivatives including chloramphenicol, ephedrine, norephedrine (Figure 4.1 and 4.2) highlighting the significance of this transformation as a source of chiral building blocks.[4]



Chloramphenicol[6]

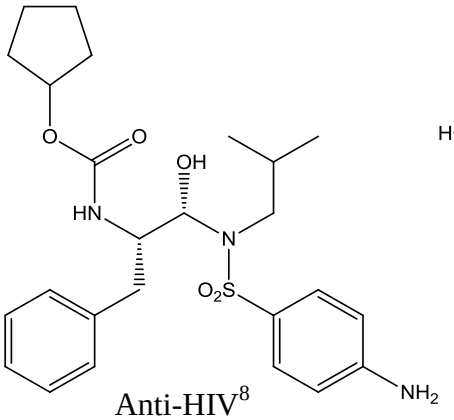


Ephedrine[6]

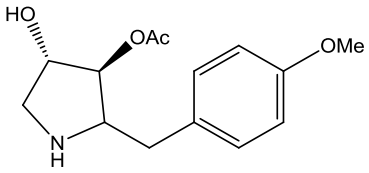


Sphingosine[7]

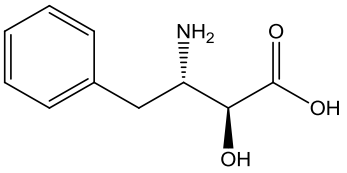
Figure 4.1



Anti-HIV[8]



Anisomycin[9]



Allophenylnorstatine[10]

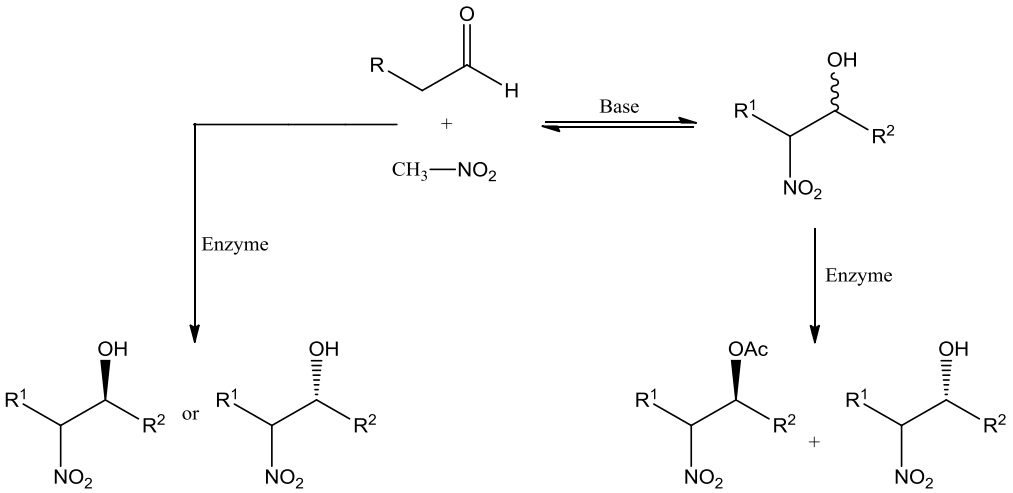
Figure 4.2

The Henry reaction is generally conducted under mild conditions at room temperature in the presence of typically about 10 mol% base to give the desired β-nitro alcohol in good yields. A vast array of basic catalysts have been utilised to perform this synthetic transformation; the most popular bases include carbonates, bicarbonates, alkali metal hydroxides, alkoxides and organic nitrogen bases. Recently considerable work concerning the Henry reaction mediated by unusual rare earth metal alkoxides, rare earth hexamethyldisilazides (HDMS) and binaphthol (BINOL)-rare earth metal complexes has been reported.[4,11,12] The Henry reaction is often accompanied by unwanted side reactions: i) β-nitroalcohols can undergo dehydration especially when aromatic aldehydes are used as substrates, ii) with sterically hindered carbonyl compounds a base-catalysed self condensation or Cannizzaro reaction may take place and iii) the *retro*-Henry reaction may prevent the reaction from going to completion.[13] More recent research has led to the development of mild reaction conditions which suppress these competitive reactions, *e.g.* solvent free[14,15] or in aqueous media.[16]

4.1.1.1 Enzymatic methods in the asymmetric Henry reaction

In general, the Henry reaction results in a mixture of diastereomers and enantiomers. This lack of selectivity is due to the reversibility of the reaction and the ease of epimerisation at the nitro substituted carbon atom.[4] The first catalytic asymmetric version of the Henry reaction was reported by Shibasaki in 1992.[11] Since then, interest in this area has expanded considerably and principal methods to the catalytic asymmetric Henry reaction include transition metal- and organo-catalysed methods and these have been reviewed in detail.[1,17,18]

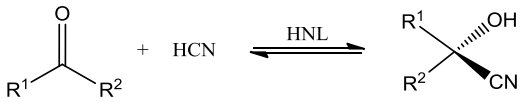
In the past decade however, there has been an emergence of biocatalytic protocols due to their mild reaction conditions and high selectivity.[19] There are two distinct biocatalytic approaches to enantioenriched products of the Henry reaction reported in the literature; direct enzyme-catalysed nitroaldol reaction or initial chemical formation of the β-nitroalcohol product followed by enzymatic kinetic resolution of the stereoisomers (Scheme 4.3).



Scheme 4.3

4.1.1.1.1 Approach I – Direct enzyme-mediated Henry reaction

Hydroxynitrile lyases (HNLs, also referred to as oxynitrilases) are important biocatalysts for the industrial scale enantioselective synthesis of optically pure cyanohydrins which represent important building blocks for the synthesis of various pharmaceuticals and agrochemicals.[20-22] HNLs can be isolated from a wide array of plant sources and in reversal of the *in vivo* reaction successfully catalyse the stereoselective addition of hydrogen cyanide to a variety of aliphatic, aromatic and heterocyclic carbonyl compounds, demonstrating superb efficiency and enantioselectivity (Scheme 4.4).[21,23] In order to expand the synthetic applicability of HNL mediated enantioselective carbon-carbon bond formation, the nucleophile hydrogen cyanide was replaced by a nitroalkane in the first reported biocatalytic asymmetric Henry reaction.[24]

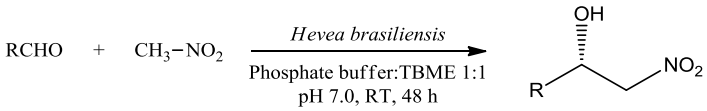


Scheme 4.4

The (*S*)-selective *Hevea brasiliensis* (*Hb*HNL) catalysed addition of nitromethane to a range of aromatic, heteroaromatic and aliphatic aldehydes yielded the desired enantiomerically enriched β-nitro alcohols (Table 4.1). However, low conversions, high enzyme loading, extended incubation periods and formation of the corresponding elimination product (10-15%) limit the development of this enzyme-catalysed nitoaldol reaction.[24]

The kinetics of the *Hb*HNL mediated Henry reaction have been demonstrated to fit the classical mechanistic model, Rapid Equilibrium Random Bi Uni with independent substrate binding, which implies that the bottleneck for this biotransformation is a very low turnover number of the enzyme, not the binding of the substrates.[25] The explanation of this has not yet been deciphered.

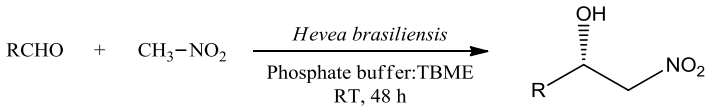
Table 4.1: H. brasiliensis (Hb*HNL*) *catalysed Henry reaction*[24]



Entry	R	Yield (%)	ee (%)
1	Ph	63	92
2	3-HOC_6H_4	46	18
3	4-$O_2NC_6H_4$	77	28
4	2-Furyl	57	72
5	CH_3-$(CH_2)_5$-	25	89

A number of reaction parameters were subsequently examined by Griengl *et al.* in order to optimise this enzymatic transformation.[26] Enantioselectivity increased significantly at pH 5.5 albeit with loss of yield (Table 4.2). In addition, a phase ratio aqueous/organic 1 : 2 increased conversion and enantiopurity. Notably, the HNL from *Manihot esculenta* is also able to catalyze this transformation but with reduced activity and selectivity. Furthermore, the (*R*)-selective HNL *Prunus amygdalus* displayed no enzymatic activity

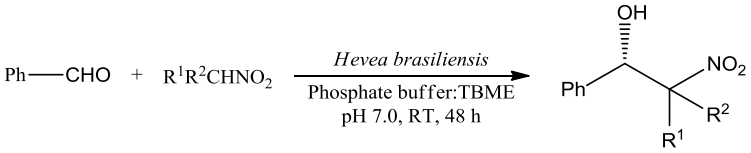
Table 4.2: H. brasiliensis (Hb*HNL*) *catalysed Henry reaction: pH investigation*[26]



Entry	R	pH 7.0		pH 5.5	
		Yield (%)	ee (%)	Yield (%)	ee (%)
1	Ph	63	93	32	97
2	4-O_2NC6H4	77	28	57	64
3	*n*-Hexyl	25	89	34	96
4	Ph$(CH_2)_2$	9	66	13	66
5	2-Furyl	57	72	43	88

The scope of the reaction with respect to the nitroalkane was explored, and it was found with increase in size of the nucleophile, activity was severely decreased, for example substitution of nitromethane with (nitromethyl)benzene led to complete loss of enzymatic activity (Table 4.3). In summary, while oxynitrilases provide an attractive system for the asymmetric Henry reaction, the narrow substrate range and low activity is a significant limitation of this process. Furthermore, although the HNL from *Manihot esculenta* is commercially available, the far more active analogue form *Hevea brasiliensis* (*Hb*HNL) is not currently available.

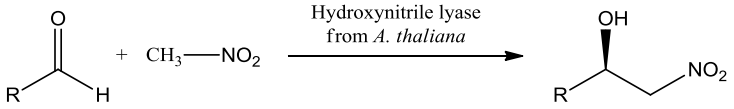
Table 4.3: Henry reaction of other nitroalkanes[26]



Entry	R^1	R^2	Yield (%)	ee (%)
1	H	H	63	92
2	H	CH_3	67	95
3	CH_3	CH_3	7	80
4	H	Ph	0	0

Recently Asano and Fuhshuku reported for the first time a (*R*)-selective hydroxynitrile lyase from *Arabidopsis thaliana*.[27] As evident from Table 4.4 excellent to moderate enantiopurity was achieved of the β-nitroalcohols. This reaction proceeded in an aqueous/*n*-butyl acetate biphasic system with an optimum aqueous phase content of 50% (v/v). However, the scope of this biotransformation is limited to aromatic aldehydes and a maximum yield of 34% was reported.

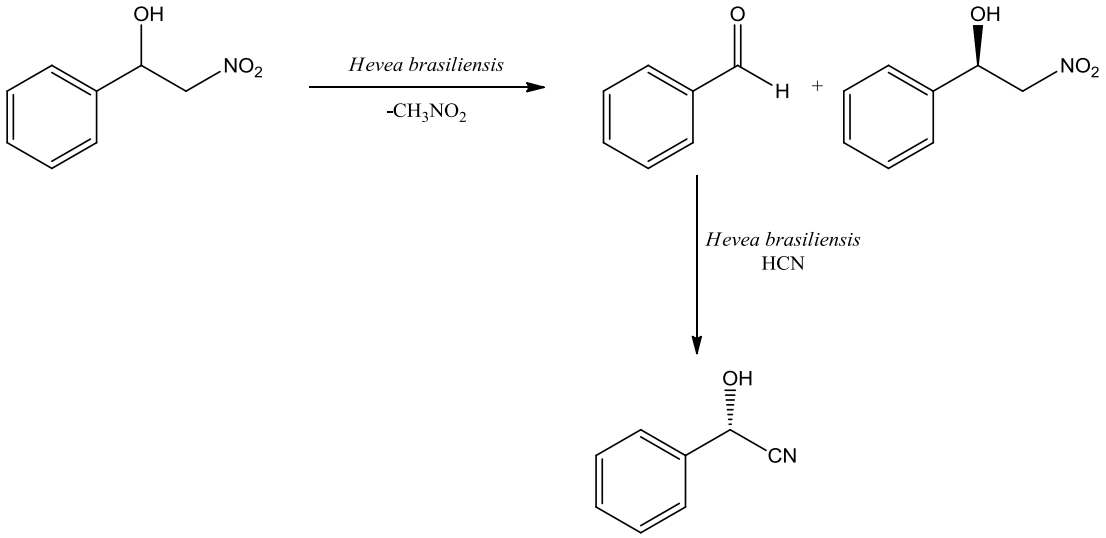
*Table 4.4: (*R*)-Selective hydroxyl nitrile lyase catalysed Henry reaction*[27]



Entry	R	Time	Yield (%)	ee (%)
1	Ph	2 h	30	91
2	Ph	4 h	26	86
3	2-MeC_6H_4	2 h	12	95
4	3-MeC_6H_4	2 h	12	96
5	4-MeC_6H_4	2 h	11	94
6	2-$MeOC_6H_4$	2 h	13	90
7	3-$MeOC_6H_4$	2 h	17	91
8	4-$MeOC_6H_4$	2 h	2	79
9	2-ClC_6H_4	2 h	34	68
10	3-ClC_6H_4	2 h	17	91
11	4-ClC_6H_4	2 h	9	87
12	4-FC_6H_4	2 h	20	81
13	4-BrC_6H_4	2 h	9	82
14	2-Naphthyl	2 h	7	>99.9
15	$Me(CH_2)_4$	2 h	Trace	>80
16	$Me(CH_2)_8$	2 h	No reaction	-

The hydroxylnitrile lyase from *H. brasiliensis* (*Hb*HNL) has also been described to catalyse the biocatalytic retro-Henry reaction, using the cleavage of 2-nitro-1-phenylethanol as a model system (Scheme 4.5).[28] This biotransformation suffered from low enantioselectivity and conversion due to product inhibition by benzaldehyde. Liese *et al.* overcame this product inhibition by performing the biocatalytic retro-Henry reaction in the presence of HCN, which reacts *in situ* with benzaldehyde and converts it to the less-inhibitive mandelonitrile. By using such a reaction cascade, it was possible to conduct the resolution practically to completion (95% ee, 49% conversion). Furthermore, the catalyst productivity

achieved during the resolution was ten times higher than that in the *Hb*HNL catalyzed synthesis of (*S*)-2-nitro-1-phenylethanol by condensation of benzaldehyde and nitromethane.



Scheme 4.5

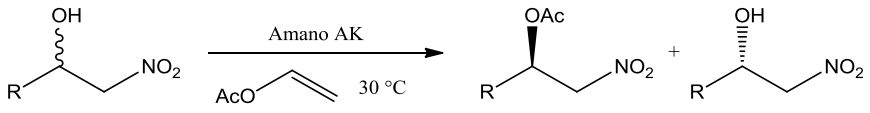
Notably, a number of other enzyme systems including a transglutaminase from *Streptoverticillium grisoverticillatum*, D-aminoacylase from *E.coli* and the hydrolase from bovine serum albumin have been reported to display nitroaldol activity however; no enantioselectivity data has been reported thus far.[29-32]

4.1.1.1.2 Approach II – Enzymatic resolution of the products of the Henry reaction

Enzyme-mediated asymmetric carbon-carbon bond formation is one approach to enantiopure β-nitroalcohols. Another enzymatic route is to employ a biocatalytic resolution step in conjunction with the Henry reaction. One such hydrolase-mediated protocol is kinetic resolution of the resulting β-nitroalcohols *via* acetylation.

In 1999, Kitayama and co-workers reported the *Pseudomonas sp.* (Amano AK) mediated stereoselective preparation of four β-nitroalcohols with vinyl acetate.[33] Furthermore the effect of organic solvents on the course of the biotransformation was investigated with moderate to good enantioselectivities achieved of all four substrates in the presence of *n*-propyl ether (Table 4.5).

Table 4.5: Effect of solvent on the E*-value of hydrolase-mediated acetylation*[33]

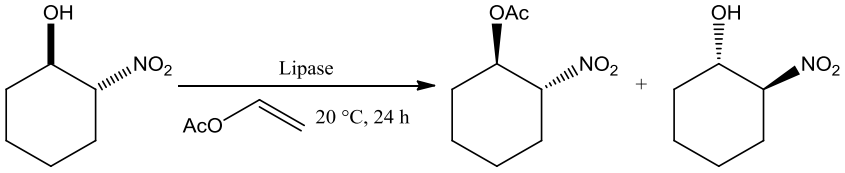


R	*E* value					
	Dioxane	**THF**	**Benzene**	**AcOEt**	**Hexane**	***n*-Propyl ether**
C_2H_9	4.8	4.1	5.6	21.9	9.6	20.9
C_3H_7	1.2	1.1	1.4	1.4	1.0	2.1
i-C_3H_7	1.3	1.9	2.4	1.7	6.7	12.5
C_4H_9	1.2	1.6	1.6	1.4	2.5	3.4

Barua *et al.* conducted screening studies with several lipases, investigating the resolution of optically pure (*S*)-2-nitroalcohols.[34] The lipase from *Pseudomonas fluorescens* demonstrated high enantioselection (66-98% ee) in the asymmetric transesterification of a series of aromatic, aliphatic and heterocyclic 2-nitroalcohols at 30 °C with vinyl acetate. The elimination product was observed in the majority of the aromatic systems.

The hydrolysis of *trans*-2-nitrocyclohexyl butyrate with *C. cyclindracea* lipase (CCL) to both enantiomers of 2-nitrocyclohexanol **99b** has been reported in high enantioselectivity.[35] While this preliminary study had limited scope, recent work within our own research group demonstrates potential access to all four enantiomers of 2-nitrocyclohexanol **99a** and **99b**.[36] A series of screening studies were conducted investigating both enantioselective transesterification and hydrolysis. Through appropriate choice of biocatalyst and reaction conditions, both enantiomers of *cis*- and *trans*-2-nitrocyclohexanol **99a** and **99b** can be acquired in high optical purity (Table 4.7 and 4.6).[36]

*Table 4.6: Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol in vinyl acetate as solvent and acyl donor*[36]

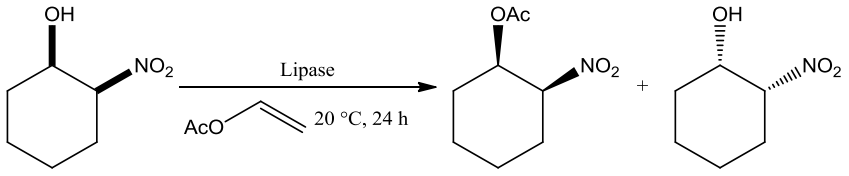


(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Entry[a]	Enzyme Strain	Conversion (%)	ee (%)		*E* value
			Acetate (1*R*,2*R*)	Alcohol (1*S*,2*S*)	
1	*C. cylindracea* C1	81	>98	>98	>400
2	*P. cepacia* P1	13	>98	16	232
3	*P. stutzeri*	53	>98	>98	>400
4	*Alcaligenes spp.*	47	>98	88	>400
5	*P. cepacia*	14	-	-	-
6	*P. fluorescens*	50	>98	>98	>400

a. Only biotransformations that resulted in some extent of conversion are reported here. See reference 36 for further details.

*Table 4.7: Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol in vinyl acetate as solvent and acyl donor*[36]

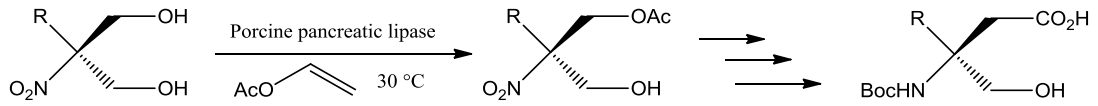


(±)-**99a** (1*R*,2*S*)-**100a** (1*S*,2*R*)-**99a**

Entry[a]	Enzyme Strain	Conversion (%)	ee (%)		*E* value
			Acetate (1*R*,2*S*)	Alcohol (1*S*,2*R*)	
1	*C. cylindracea* C1	45	>98	80	>200
2	*C. cy;indracea* C2	26	-	-	-
3	*Alcaligenes spp.*	37	98	53	168
4	*P. cepacia*	39	>98	46	156
5	*P. stutzeri*	59	69	89	15
6	*Rhizopus spp.*	7	-	-	-
7	*Alcaligenes spp.*	50	>98	91	>200
8	*P. cepacia P2*	8	>98	6	105
9	*Mucor javavicus*	2	-	-	-
10	*P. fluorescens*	50	>98	>98	>200
11	*Mucor meihei*	17	>98	32	>200
12	*C. antarctica*	49	>98	98	>200

a. Only biotransformations that resulted in some extent of conversion are reported here. See reference 36 for further details.

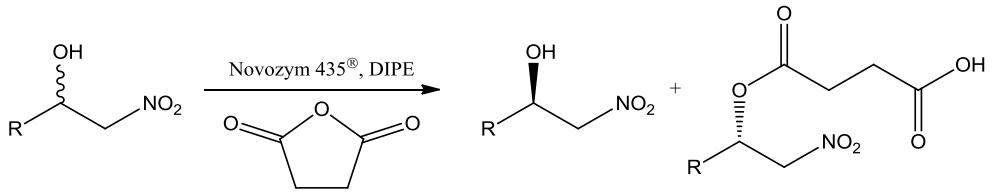
Hydrolase-mediated acylation and deacylation have been employed to gain access to enantio- and diastereomerically enriched β- and γ-nitroalcohols.[37-39] Enzymatic selective acetylation of quaternary 2-nitropropane-1,3-diols has been reported as an asymmetric synthetic route to α-substituted serine analogues with good enantioselectivity in some cases (<5-92% ee) (Scheme 4.6).[40]



Scheme 4.6

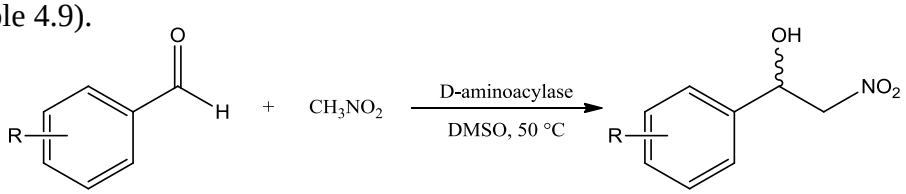
The first systematic study of a hydrolase-mediated resolution of β-nitroalcohols in conjunction with the Henry reaction was reported in 2004.[41] The lipase-catalysed enantioselective resolution of a range of alkyl- and phenylalkyl substituted nitroalcohols, adducts of the Henry reaction, have been described (Table 4.8). Several biotransformation parameters were investigated in this study including the nature of the lipase, acyl donor and solvent effect. Novozym 435® catalysed resolution of 1-nitro-2-alkanols in diisopropyl ether with succinic anhydride as acyl donor provided the highest conversion and selectivities of the recovered alcohol and acetylated product.

Table 4.8: Resolution of 1-nitro-2-alkanols with succinic anhydride in diisopropyl ether[41]



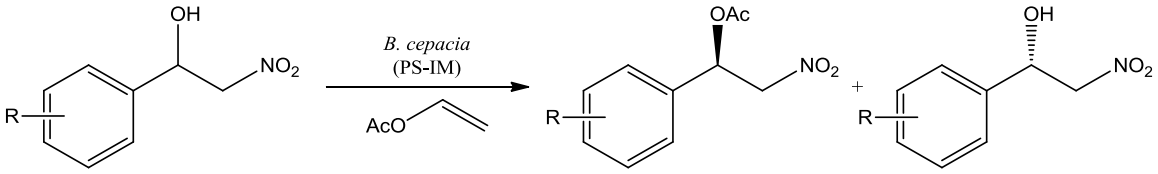
Entry	R	Conversion (%)	ee_s (%)	ee_p (%)	*E* value
1	CH_3	39	57	67	28 (*R*)
2	C_2H_5	47	44	43	4 (*R*)
3	C_3H_7	54	92	93	82 (*S*)
4	C_6H_5	4	3	75	7 (*S*)
5	$(C_6H_5)CH_2$	-	-	-	-
6	$(C_6H_5)C_3H_4$	42	71	97	96 (*S*)

Recently, an enzymatic method which combines a biocatalysed nitroaldol reaction and lipase-mediated acyl resolution of the resulting β-nitroalcohols in organic media has been reported.[42] Initial formation of the racemic β-nitroalcohols was effected by D-aminoacylase as catalyst and DMSO as solvent with yields being highly substrate dependent, ranging from 12-80% (Scheme 4.7). The subsequent *Burkholderia cepacia* (immob) mediated kinetic resolution step achieved excellent conversion and enantioselectivity with vinyl acetate as acyl donor (Table 4.9).



Scheme 4.7

Table 4.9: Kinetic resolution of β-nitroalcohols[42]

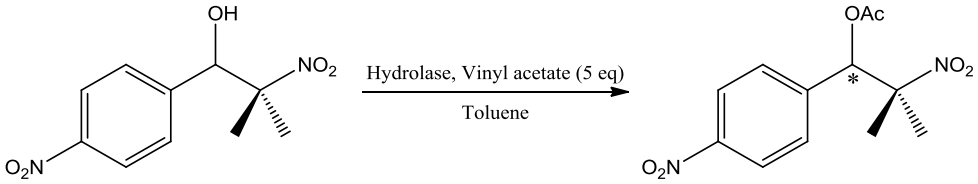


Entry	R	Conversion (%)	ee_s (%)	ee_p (%)	*E* value
1	*p*-NO_2	48	97	>99	>200
2	*m*-NO_2	49	95	49	>200
3	*o*-NO_2	<1	-	<1	-
4	*p*-Cl	46	97	46	>200
5	*m*-Cl	47	91	47	>200
6	*o*-Cl	<1	-	<1	-
7	H	47	95	47	155
8	*p*-CH_3	48	84	48	>200
9	*P*-OCH_3	49	84	49	>200

Ramström *et al.* reported the first one-pot dynamic kinetic resolution process, combining the base-catalysed nitroaldol reaction and lipase-catalysed transesterification of the corresponding adduct under mild reaction conditions.[43,44] The first step in this resolution protocol was to establish a suitable catalyst for the enzymatic transformation. The immobilised lipase *Pseudomonas cepacia* CI displayed transesterification activity with high

enantiospecificity (entry 4, Table 4.10). Moreover, in combination with increased temperature and enzyme loading almost complete kinetic resolution was achieved (46% yield) with retention of excellent enantiopurity (entry 7, Table 4.10).

Table 4.10: Effect of hydrolase source on the kinetic bioresolution of 2-methyl-2-nitro-1-(4-nitrophenyl)propan-1-ol[43]

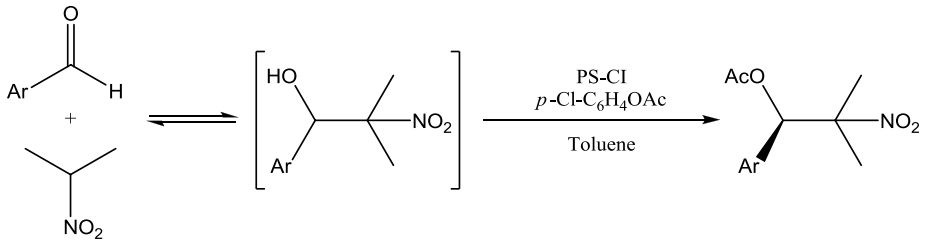


Entry	Enzyme Source	Conversion (%)	Acetate ee (%)	*E* value
1	*C. antarctica B*	5	78	8
2	*C. rugosa*	0	0	0
3	*P. cepacia*	10	0	1
4	*P. cepacia CI*	11	99	>200
5	*P. cepacia CII*	10	90	21
6	*P. fluorescens*	7	93	30
7[a]	*P. cepacia CI*	46	99	>200

a. 30 mg enzyme, the reaction was run at 40 °C for 24 h.

The one-pot dynamic kinetic resolution process was subsequently addressed, using PS-CI as enzyme, triethylamine as base and *p*-chlorophenyl acetate as acetylating agent (Table 4.11). A series of different aldehyde substrates was investigated, and, in general, aromatic aldehydes resulted in good yields and enantioselection after a reaction time of 2-4 days. Notably, aliphatic aldehydes lead to poor conversion and decreased enantiopurity even over extended incubation periods. While this paper clearly demonstrates the feasibility of a combination of a Henry reaction with a dynamic kinetic resolution, there are significant limitations to be overcome before this protocol has a broad synthetic utility.

Table 4.11: Hydrolase-mediated dynamic kinetic resolution of β-nitroalcohols[43]



Entry	R	Time	Yield (%)	ee (%)
1	4-O_2N-C_6H_4	2 d	90	99
2	4-NC-C_6H_4	2 d	89	91
3	4-F_3C-C_6H_4	3 d	89	97
4	3-O_2N-C_6H_4	3 d	90	91
5	4-CH_3-C_6H_4	4 d	35	93
6	Thiophene-2-yl	4 d	68	46

Thus, in recent years major improvements have been made in the biocatalytic approach to the asymmetric Henry reaction, especially in the kinetic resolution of the nitroaldol adduct with excellent enantioselectivity achieved, albeit with a maximum yield of

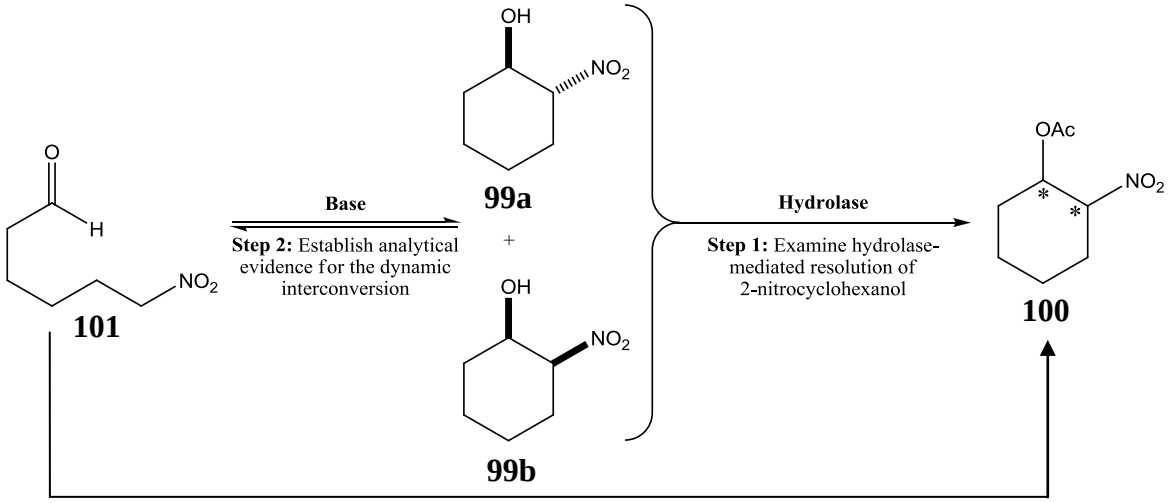
50%. The dynamic biocatalytic Henry reaction does provide an attractive alternative, however, at present further research is required to overcome the limited substrate scope. To date, the direct enzyme-mediated Henry reaction is restricted to the hydroxyl nitrile lyase *H. brasiliensis* which is limited in its availability.

4.2 Dynamic kinetic resolution of the intramolecular Henry reaction of (±)-2-nitrocyclohexanol (±)-99 through lipase catalysis

4.2.1 Background to the project

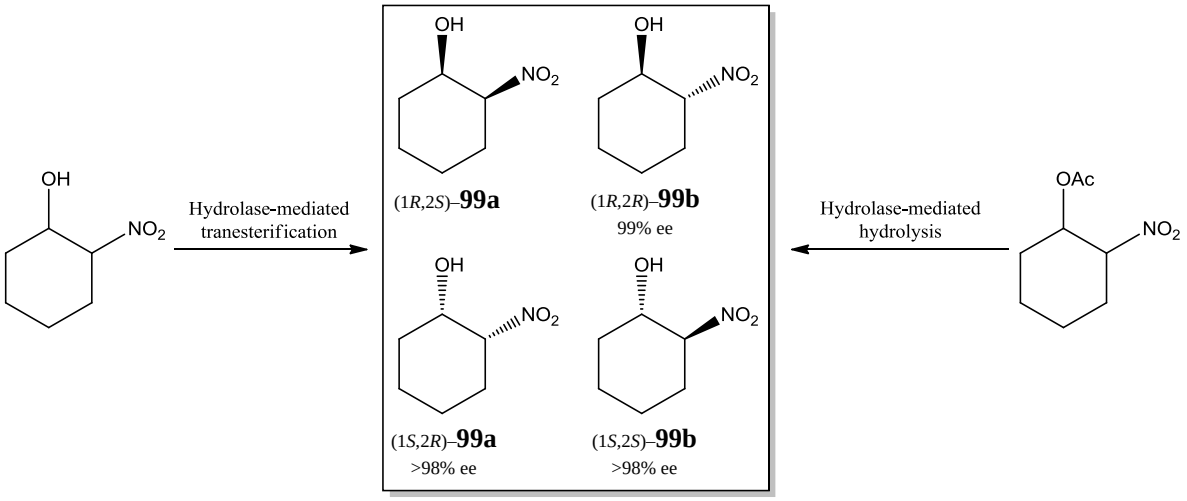
Previous work in the research group was focused on the development of a biocatalytic protocol for the asymmetric Henry reaction.[45] The concept involved an intramolecular Henry cyclisation process to form (±)-2-nitrocyclohexanol (±)-**99**, together with a one-pot hydrolase-mediated dynamic process, potentially leading to 2-nitrocyclohexyl acetate **100** with high diastero- and enantiopurity.[45] The strategy combined an intramolecular base-catalysed cyclisation of the nitroaldehyde **101** coupled with hydrolase-mediated acetylation, with a view to selective acetylation of a single stereoisomer. Ideally this process would operate in a dynamic fashion giving access to quantitative yields of a single stereoisomer of 2-nitrocyclohexyl acetate **100**.

This preliminary investigation was approached in a stepwise manner. The first step focused on the development of hydrolase-mediated acetylation conditions for the selective resolution of (±)-**99a** or (±)-**99b**. The second step involved the development of the dynamic interconversion process between the two diastereomers (±)-**99a** and (±)-**99b** *via* ring opening and closure of 6-nitrohexanal **101**. Finally, once the two individual steps had been established they were combined, potentially developing a one-pot dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis (Scheme 4.8).



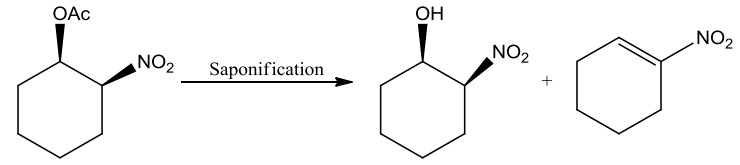
Scheme 4.8

Considerable progress has been made by Milner in the individual elements of the dynamic kinetic resolution process.[45] The first step in the resolution protocol was to establish a suitable catalyst for the enzymatic transformation. In this early study, efficient kinetic bioresolution has been effected for both the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols, (±)-**99a** and (±)-**99b**, *via* enzyme-mediated transesterification and ester hydrolysis processes (Scheme 4.9).



Scheme 4.9

Through the appropriate selection of biocatalyst, three of the four enantiomers were obtained directly in high optical purity, while hydrolysis of the acetate (1*R*,2*S*)-**100a** would potentially lead to the alcohol (1*R*,2*S*)-**99a** although this is complicated by competing elimination of the *cis*-alcohol **99a** to 1-nitrocyclohexene **102** *via* base-mediated dehydration (Scheme 4.10).[36] Furthermore, this dehydration byproduct **102** was observed throughout the development of this biocatalytic dynamic intramolecular Henry reaction, reducing the efficiency of the desired one-pot process.

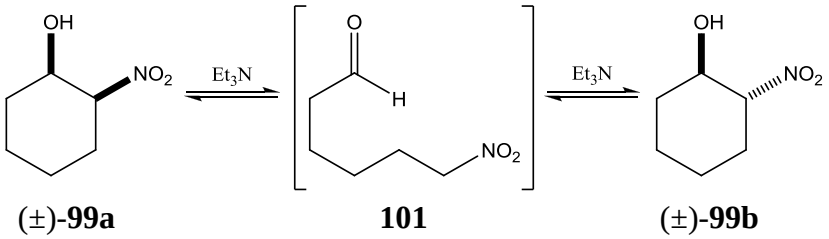


(1*R*,2*S*)-**100a** (1*R*,2*S*)-**99a** **102**

Scheme 4.10

In Milner's preliminary study, any hydrolase which transformed (±)-*cis*-2-nitrocyclohexanol (±)-**99a** with high enantioselectivity also performed efficiently for the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b**. This lack of diastereoselectivity of the examined hydrolases severely hampered the progress of the research. Fundamentally, for an efficient dynamic process, it is essential that one enantiomer of (±)-**99a** or (±)-**99b** can be efficiently and selectively acetylated; lack of selective enzymatic activity for (±)-**99a** or (±)-**99b** is a significant barrier.

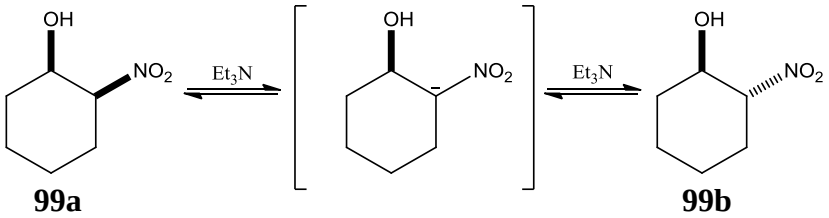
The second step in this investigation involved the development of the base-mediated dynamic interconversion process between (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol, (±)-**99a** and (±)-**99b**. Preliminary ^{1}H NMR results indicated that triethylamine accomplished the desired interconversion and it was assumed that this was *via* dynamic ring opening and closing (Scheme 4.11)



(±)-**99a** **101** (±)-**99b**

Scheme 4.11

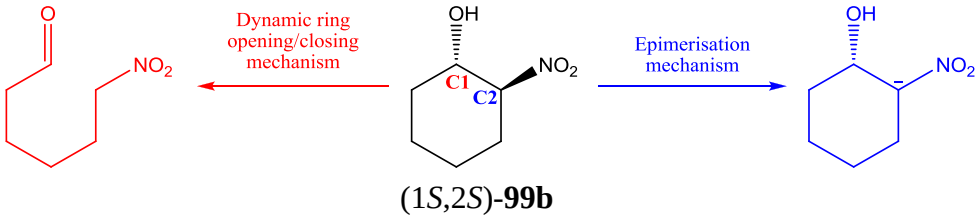
However, towards the end of Milner's work, when the base-mediated cyclisation of 6-nitrohexanal **101** and dynamic interconversion was combined with the hydrolase-mediated acetylation, it became evident that epimerisation was the predominant process, and not dynamic ring opening/closure *via* 6-nitrohexanal **101** (Scheme 4.12). These two competing processes led to a significant hurdle in the development of a dynamic kinetic resolution of the intramolecular nitroaldol reaction.



99a **99b**

Scheme 4.12

Towards the conclusion of Milner's study, an enantiopure sample of (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** was exposed to a series of bases and monitored closely by chiral HPLC.[45] A change in the stereochemistry at the C1 carbon, thus the appearance of either *cis*-(1*R*,2*S*)-**99a** or *trans*-(1*R*,2*R*)-**99b** enantiomers denoted a dynamic ring opening/closing interconversion (Scheme 4.13).



(1*S*,2*S*)-**99b**

Scheme 4.13

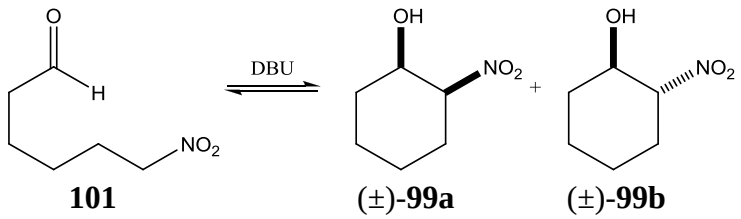
Base screening indicated that, while with triethylamine only epimerisation occurs, use of alternative bases, such as DBU, promotes the dynamic ring opening/closure *via* 6-nitrohexanal **101**, in addition to the epimerisation process. This preliminary observation augurs well for development of an effective dynamic kinetic resolution process, although substantial optimisation is required.

4.2.2 Objectives of the project

The previous work described by Milner indicated that a DBU-mediated dynamic kinetic resolution process was feasible, although a number of obstacles would first have to be circumvented.[45]

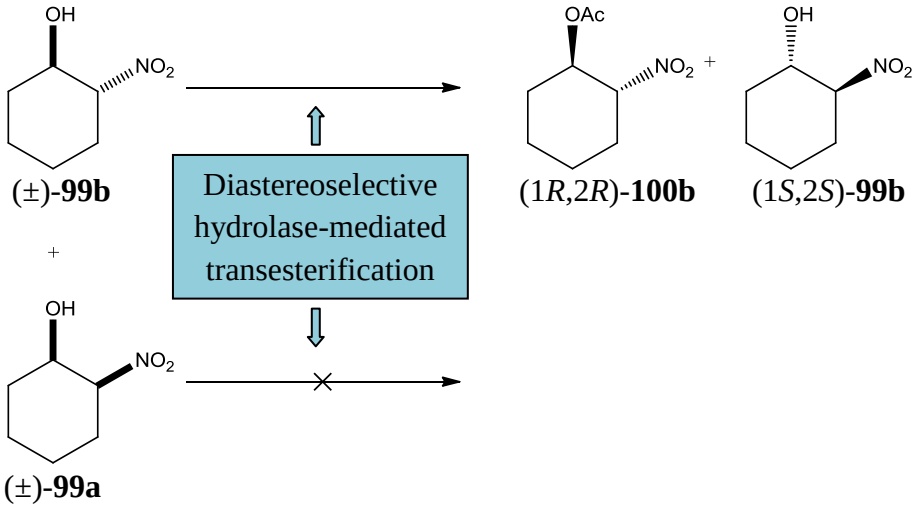
Therefore, the initial objectives of this study at the outset were:

- To prepare 6-nitrohexanal **101** and the racemic substrates (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** and (±)-*cis*- and (±)-*trans*-2-nitrocyclohexyl acetates (±)-**100a** and (±)-**100b** for the investigation of the dynamic hydrolase-mediated kinetic resolution of the Henry reaction.
- To investigate by ^{1}H NMR and chiral HPLC analysis the DBU-promoted intramolecular nitroaldol reaction and associated interconversion of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** (Scheme 4.14).



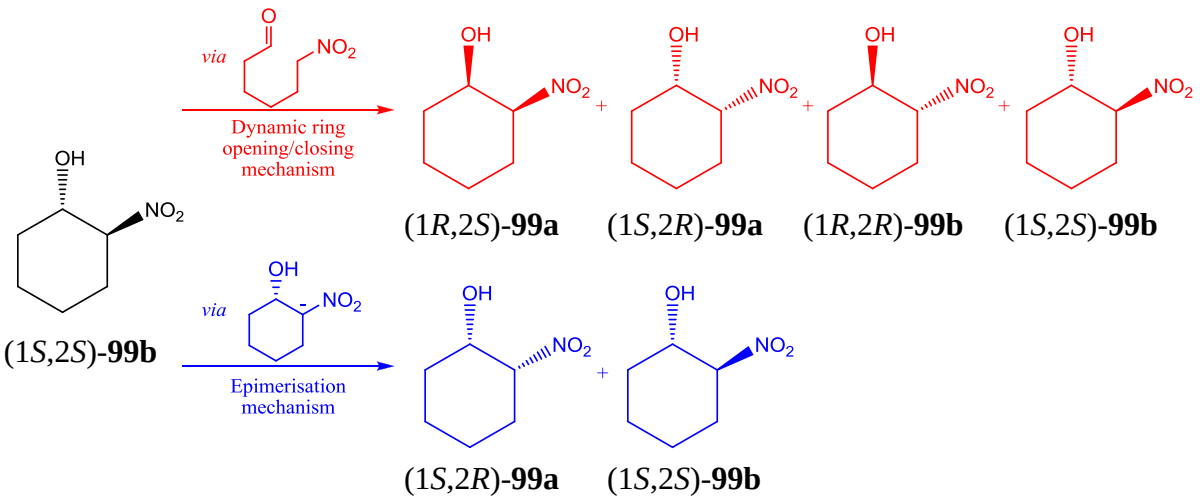
Scheme 4.14

- To expand the series of lipases screened for the kinetic resolution of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b**, to ultimately identify a suitable hydrolase for the selective acetylation of one diastereomer and one enantiomer of 2-nitrocyclohexanol **99** (Scheme 4.15).



Scheme 4.15

- To explore in detail the DBU-mediated interconversion of enantiopure (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, based on the promising one off preliminary result by Milner (Scheme 4.16).[45]



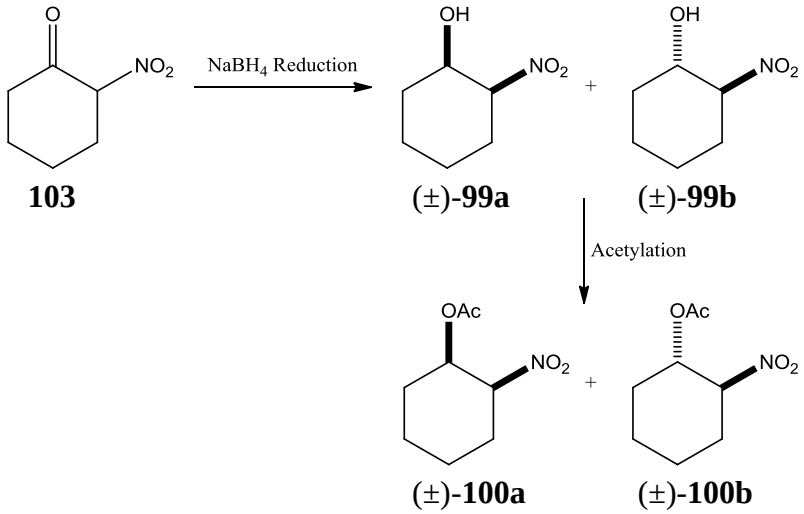
Scheme 4.16

- To develop calibration curves for the quantitative analysis of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** by chiral HPLC facilitating determination of product ratio and comparison with ^{1}H NMR studies.

4.2.3 Synthesis of substrates

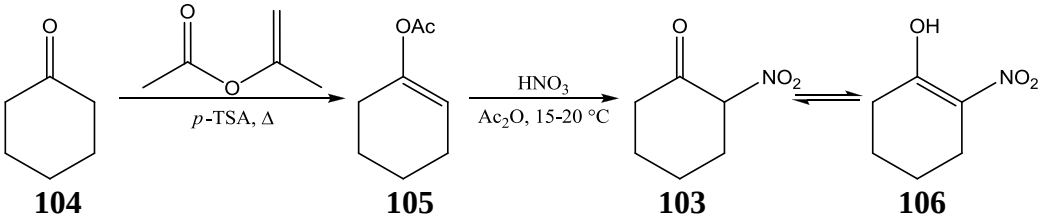
The investigation of a one-pot Henry reaction coupled with hydrolase-mediated resolution required the synthesis of a number of synthetic targets. The racemic synthesis of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**, and (±)-*cis*- and (±)-*trans*-2-nitrocyclohexyl acetate (±)-**100a** and (±)-**100b** was essential for investigation of the lipase-mediated kinetic resolution, and 6-nitrohexanal **101** was necessary as the precursor to the intramolecular Henry reaction.

Milner previously described the successful synthesis of the (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** by sodium borohydride ($NaBH_4$) reduction of 2-nitrocyclohexanone **103** and subsequent acetylation to form the acetates (±)-**100a** and (±)-**100b** in good yields (Scheme 4.17).[45]



Scheme 4.17

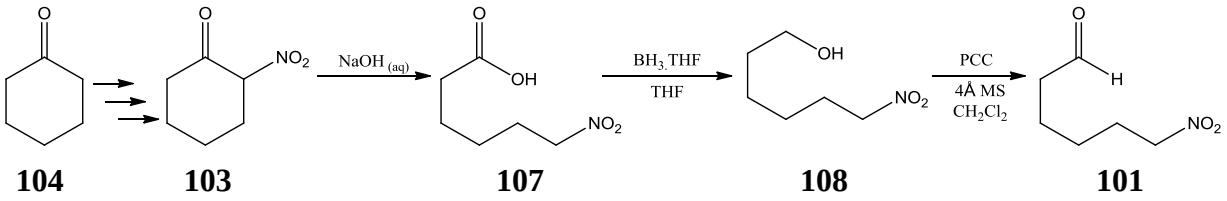
The desired starting material 2-nitrocyclohexanone **103** is commercially available,[46] however, it is somewhat expensive. Consequently, Milner reported the conversion of cyclohexanone **104** into its corresponding enol acetate **105**, followed by nitration with nitric acid, providing access to the desired 2-nitrocyclohexanone **103** and corresponding enol tautomer 2-nitrocyclohexen-1-ol **106** in a ratio of 1 : 0.9 respectively (Scheme 4.18). Early reports by Moloney[47] and Özbal[48] were used as a basis for this work.



Scheme 4.18

However, due to the potential explosion risk during distillation of 2-nitrocyclohexanone **103**, this synthetic route was deemed unsuitable and thus not employed in this study.

Milner also described utilising 2-nitrocyclohexanone **103** as a precursor to 6-nitrohexanal **101** *via* base-induced ring opening to the analogous carboxylic acid **107**, followed by subsequent reduction to the alcohol **108** and PCC oxidation to the synthetic target **101** (Scheme 4.19).[45]



Scheme 4.19

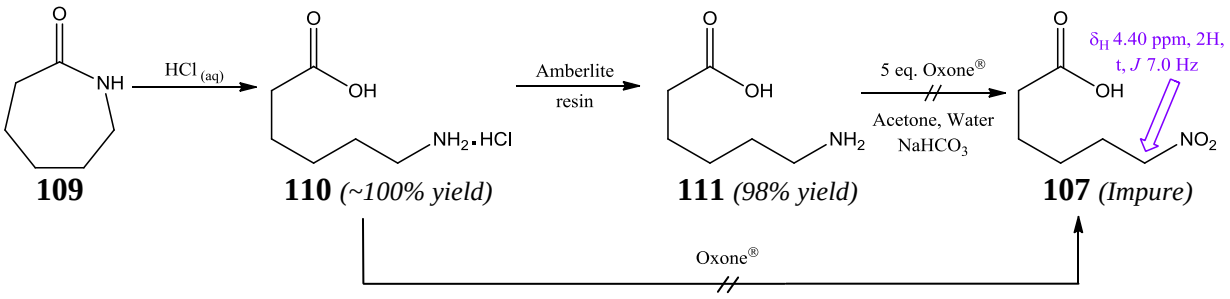
Milner's approach to the synthesis of **101** from **103**, while feasible, is not synthetically practical, due to the prohibitive cost of **103** on a commercial basis, and total number of steps from cyclohexanone **104** to aldehyde **101**. Therefore, an alternative synthetic route to the aldehyde **101** and racemic (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** that avoided the synthesis of 2-nitrocyclohexanone **103** was desirable.

4.2.3.1 Synthesis of 6-nitrohexanal 101

It was initially envisaged that an efficient route to multi-gram quantities of 6-nitrohexanal **101** would provide access to the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**, through the intramolecular Henry reaction, and subsequent acetylation would provide the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexyl acetates (±)-**100a** and (±)-**100b**.

*4.2.3.1.1 Route I – Ring opening of ε-caprolactam **109** and subsequent oxidation*

The first route to 6-nitrohexanal **101** investigated was acid-catalysed ring opening of ε-caprolactam **109** to the hydrochloride salt **110** and subsequent ion exchange to yield the free amino acid **111**. Oxidation of the primary amine **111** was explored to provide the desired nitro carboxylic acid derivative **107** (Scheme 4.20).

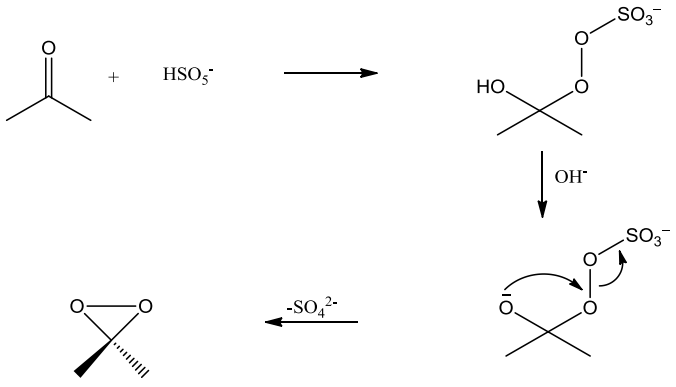


109 **110** *(~100% yield)* **111** *(98% yield)* **107** *(Impure)*

Scheme 4.20

The synthesis of 6-aminohexanoic acid **111** was adapted from a procedure described by Meyers and Miller.[49] A solution of ε-caprolactam **109** and aqueous hydrochloric acid was heated under reflux for 1 h, yielding the resulting moist hydrochloric salt **110** in quantitative yield. The ε-aminocaproic acid hydrochloride **110** was converted into the free amino acid **111** by means of an ion exchange column containing Amberlite IRA-400(OH) resin. The yield of 6-aminohexanoic acid **111** (98%) was calculated over two steps based on the starting material ε-caprolactam **109**.

The direct oxidation of primary amines into the corresponding nitro derivative is very useful for fundamental and industrial application because it provides nitro compounds which may otherwise be difficult to synthesise by direct nitration methods.[4] Oxone®, a stoichiometric commercially available oxidising reagent, was first reported by Kennedy and Stock in 1960 and consists of two moles of potassium peroxymonosulfate, one mole of potassium bisulfate and one mole of potassium sulfate.[50] Potassium peroxymonosulfate reacts with acetone to produce dimethyldioxirane **112**, which is a very efficient oxygen transfer reagent (Scheme 4.21).[51]



112

Scheme 4.21

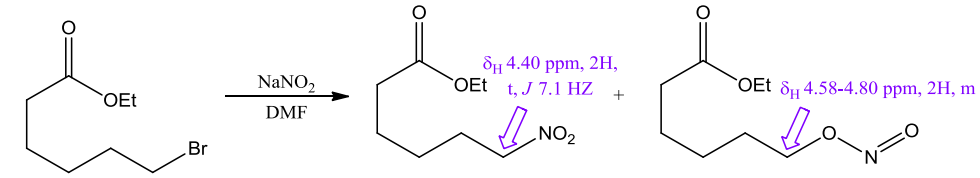
In this work oxidation of 6-aminohexanoic acid **111** was conducted by treatment of the primary amine **111** with 5.0 equivalents of Oxone® in acetone, sodium bicarbonate and water at 0 °C to room temperature for 16 hours (Scheme 4.20). The ^{1}H NMR spectrum of the crude product showed evidence of the oxidised nitro compound **107** with a characteristic triplet present at δ_H ~4.40 ppm attributable to the methylene protons geminal to the nitro moiety. However, a number of unidentifiable byproducts were also observed in the ^{1}H NMR which could not be removed by flash column chromatography or acid/base extraction. This oxidation procedure was thus repeated with the hydrochloric salt **110** to ascertain if this limited the production of undesired side reactions; again oxidation was achieved, however,

purification remained difficult. Thus, while this route provides an efficient protocol to 6-aminohexanoic acid **111** in excellent yield and purity, further optimisation is required in the oxidation step.

4.2.3.1.2 Route II – Kornblum reaction

The reaction of alkyl halides with metal nitrites is one of the most important methods for the preparation of nitroalkanes. As a metal nitrite, silver nitrite in diethyl ether (Victor-Meyer reaction), potassium nitrite, or sodium nitrite in *N*,*N*-dimethylformamide (DMF) or in dimethyl sulfoxide (DMSO) (Kornblum reaction)[52-59] have frequently been used.[4] The obtained products are usually a mixture of the desired nitroalkane together with the undesired alkyl nitrite, which are easily separated.

The procedure implemented in this study was previously described by Cobb and co-workers for the synthesis of **113**.[60] Sodium nitrite was added to a 0.1 M solution of the commercially available primary alkyl bromide **114** in anhydrous DMF (Scheme 4.22). The reaction was monitored by TLC and in general reaction completion was achieved within 16 h. A mixture of the desired ethyl 6-nitrohexanoate **113** and a byproduct believed to be the alkyl nitrite **115** (76 : 24 respectively) was evident in the ^{1}H NMR spectrum of the crude product. Cobb[60] had not described formation of **115** as a byproduct although as mentioned previously there is considerable precedent in the literature for competing formation of nitrites under these conditions.[4,58]

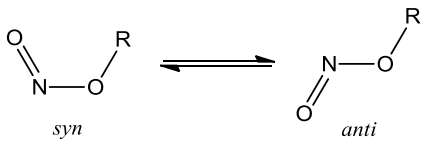


114 **113** **115**

Product Ratio by ^{1}H NMR *76* : *24*

Scheme 4.22

The alkyl nitrite **115** was characterised by a broad multiplet at δ_H 4.58-4.80 ppm representative of the methylene protons adjacent to the nitrite moiety and was not further analysed or characterised during this study. Alkyl nitrites exist as mixtures of *syn* and *anti* conformers which differ in the orientation of the alkyl group (Scheme 4.23). At room temperature alkyl nitrites undergo rapid interchange and the *syn* and *anti* methylene protons adjacent to the nitrite group are chemical shift equivalent, thus they appear as a broad multiplet in the ^{1}H NMR of the crude product.[61-64]



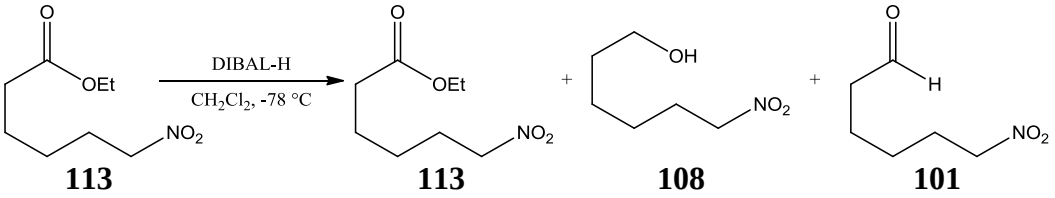
Scheme 4.23

The alkyl nitrite **115** is easily removed upon column chromatography to give the desired nitroalkane **113** as a light yellow oil with a yield of 49%, which is in accordance to literature reports.[60] Ethyl 6-nitrohexanoate **113** was characterised by a carbonyl absorption band at ν_{max} 1733 cm^{-1} and the asymmetrical and symmetrical stretching of the nitro group at

ν_{max} 1554 and 1377 cm^{-1}, while in the ^1H NMR spectrum, the principal signals include a distinctive quartet at δ_H 4.13 ppm attributable to the ethyl ester and triplet at δ_H 4.40 ppm due to the methylene protons alpha to the nitro moiety.

Thus, the reaction of alkyl halides with sodium nitrite provides a very useful synthetic method for nitroalkanes, although yield is slightly limited by competing formation of the undesired alkyl nitrite. All spectral characteristics agreed with those previously described.[60]

The DIBAL-H selective reduction of ethyl 6-nitrohexanoate **113** to the corresponding aldehyde has been described previously.[60] Following the literature procedure, DIBAL-H (1.1 equivalents) was added dropwise to a solution of the ethyl ester **113** in doubly distilled dichloromethane at −78 °C (Scheme 4.24).[60] After stirring for 1.5 h TLC analysis indicated the presence of starting material **113**, desired aldehyde 6-nitrohexanal **101** and reduction product 6-nitrohexanol **108**. An additional 0.2 equivalent of DIBAL-H was added at this point to encourage reaction completion and the reaction was stirred for a further 2 h. ^1H NMR analysis of the crude product showed a mixture of ethyl 6-nitrohexanoate **113**, 6-nitrohexanol **108** and 6-nitrohexanal **101** (41 : 9 : 50 respectively). This DIBAL-H reduction was repeated several times implementing strict anhydrous conditions and temperature control; however, each time a mixture of reduction products, including the desired aldehyde **101** and starting material **113**, was identified. Milner likewise was unable to achieve selective DIBAL-H reduction of methyl 6-nitrohexanoate **116**; a mixture of methyl 6-nitrohexanoate **116**, 6-nitrohexanol **108** and 6-nitrohexanal **101** (26 : 43 : 31 respectively) was reported.[45] The three products were separable by column chromatography, however, due to the poor reactions efficiency and lack of selectivity this route was no longer pursued.



113 **113** **108** **101**

DIBAL-H

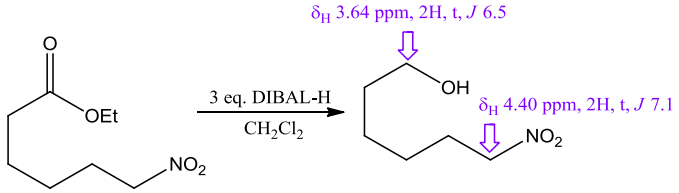
1.1 eq. *Mixture of **113**, **108** and **101** by TLC*

1.3 eq. *A mixture of **113**, **108** and **101**, 41 : 9 : 50 respectively by ^1H NMR*

Scheme 4.24

Due to the limited success in the selective reduction of the ethyl ester **113** to the aldehyde **101**, a new route was explored where the ethyl ester **113** would be reduced completely to the analogous alcohol **108** (Scheme 4.25). While lithium aluminium hydride reduction of the ester **113** to the alcohol **108** was considered, competing reduction of the nitro group by this powerful reducing agent was considered a significant risk, and therefore this route was not explored.

A number of mono-, di- and cyclic esters have been reduced to alcohols by Miller[65] and Ziegler[66] using DIBAL-H. They reported yields of 70-100%. Typically, 2.0 to 4.0 equivalents of DIBAL-H were required. Notably, in contrast to lithium aluminium hydride, DIBAL-H selectively reduces carbonyls in the presence of nitro substituents.



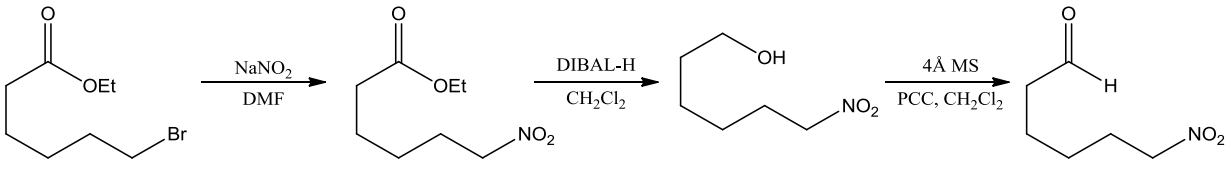
113 **108** *(79% yield)*

Scheme 4.25

In this work, DIBAL-H (3.0 equivalents) was added dropwise to a solution of ethyl 6-nitrohexanoate **113** in dichloromethane at −78 °C. The reaction was maintained at this temperature for 1 h before warming to −40 °C and stirring for an additional 1 h then quenched. A rapid increase in temperature was observed upon slow quenching of the reaction mixture with dilute hydrochloric acid at −10 °C. 6-Nitrohexanol **108** was found to be of a highly volatile nature; therefore removal of solvent was performed under reduced pressure in an ice bath. Purification by column chromatography produced the alcohol **108** in high yield 79% and excellent purity. A strong, broad OH peak at ν_{max} 3400 cm^{-1} and nitro stretching bands at ν_{max} 1552 and 1385 cm^{-1} were observed in the IR spectrum of 6-nitrohexanol **108**. The characteristic signals in the ^1H NMR were a broad OH singlet at δ_H 1.76 ppm and two triplets at δ_H 3.64 ppm and δ_H 4.40 ppm attributable to the methylene hydrogens alpha to the alcohol C(1)H_2 and nitro C(6)H_2 moiety respectively.[vi]

Pyridinium chlorochromate (PCC), first developed by Corey and co-workers in 1975, is used to oxidise primary alcohols to aldehydes and secondary alcohols to ketones; notably over-oxidation is rare.[69] It was envisaged in this study that the final step to gain access to the desired 6-nitrohexanal **101** would involve oxidation of the nitro alcohol **108** by PCC to the corresponding aldehyde **101**. The procedure adapted in this study was previously described by Milner and involved the addition of 6-nitrohexanol **108** to a solution of PCC and crushed 3Å molecular sieves in dichloromethane.[45] Stirring was continued for 4 h under nitrogen. The drawback of this method is the formation of viscous tarry residues that complicate product isolation and limit recovery. Addition of powdered molecular sieves simplified the work-up somewhat; the reduced chromium salts and other reagent derived byproducts are deposited onto these solids which can then be readily removed by filtration. The pure aldehyde **101** was isolated in 38% yield as a colourless oil following column chromatography. Significantly Milner reported a crude yield of 72% which did not require further purification.[45] It was envisaged that the decreased yield in this study was associated in part with the additional purification step and in part due to insufficient product recovery. Further optimisation of the PCC oxidation is warranted to improve the yield. However, for the needs of this study, ready access to multi-gram quantities of the desired aldehyde **101** was enabled by this method as outlined in Scheme 4.26.

[vi] Milner[45] reports the C(1)H_2 triplet at δ_H 3.24 ppm in the experimental data, however, in the results and discussion section the ^1H NMR spectrum is described at δ_H 3.64 ppm, which is in agreement with the ^1H NMR data reported in this study and literature reports.[67,68] Thus it is believed Milner erroneously reported δ_H 3.24 ppm for the C(1)H_2 protons in the experimental data.



114 **113** *(49% yield)* **108** *(79% yield)* **101** *(38% yield)*

Scheme 4.26

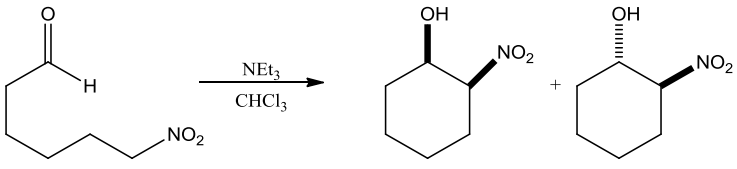
The aldehyde **101** is characterised in the IR spectrum by a carbonyl stretch at ν_{max} 1722 cm^{-1} and the symmetric and asymmetric bands of the nitro group at ν_{max} 1552 and 1386 cm^{-1}. The ^1H NMR spectrum of 6-nitrohexanal **101** has a distinct doublet of triplets (J 7.2, 1.5) at δ_H 2.47-2.52 ppm attributable to the methylene protons C(2)H_2 alpha to the carbonyl group. Coupling of the aldehyde proton C(1)H with the C(2)H_2 methylene protons provides a triplet (J 1.5) at δ_H 9.78 ppm. All spectral characteristics were as described in the literature.[60]

In summary a three-step synthetic route to 6-nitrohexanal **101** from inexpensive, commercially available ethyl 6-bromohexanoate **114** has been successfully devised. Multi-gram quantities of the pure desired aldehyde **101** were accessible *via* this route despite the low- yielding PCC oxidation step. This synthetic sequence to 6-nitrohexanal **101** reduces the number of synthetic steps from five to three relative to the procedure outlined by Milner,[45] due to the exclusion of the synthesis of 2-nitrocyclohexanone **103**, and in addition the overall safety of the synthetic protocol is increased.

4.2.3.2 Synthesis of (±)-2-nitrocyclohexanol (±)-99

The next step in this study was the synthesis of the (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**. Two methods were explored, the unreported intramolecular nitroaldol reaction of 6-nitrohexanal **101** and the literature procedure by Hönig,[35] later adapted by Milner,[45] which involved $NaBH_4$ reduction of 2-nitrocyclohexanone **103**. Both routes were anticipated to readily provide a mixture of diastereomeric nitroalcohols (±)-**99a** and (±)-**99b**.

As multi-gram quantities of 6-nitrohexanal **101** were now readily available, it was envisaged that access to the (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** could be achieved through the intramolecular variant of the Henry reaction. Triethylamine was added in one portion to a solution of 6-nitrohexanal **101** in chloroform (Scheme 4.27). Stirring was continued at room temperature until IR analysis indicated the disappearance of the aldehyde carbonyl stretch at ν_{max} 1722 cm^{-1}, signifying reaction completion. ^1H NMR analysis of the crude product revealed a mixture of diastereomeric nitroalcohols (±)-**99a** and (±)-**99b** (14 : 86 respectively).



101	(±)-**99a**	(±)-**99b**
Product ratio by ^1H NMR	*14*	*86*
Isolated yield	*4%*	*53%*

Scheme 4.27

The diastereomeric β-nitroalcohols (±)-**99a** and (±)-**99b**, have comparable polarity, but isolation of pure fractions of the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** was achievable by careful chromatographic purification. However, complete separation by this means was not possible, thus reduced yields are reported.

The assignment of the relative stereochemistry of the structures of (±)-**99a** and (±)-**99b** was determined by Milner through hydrolase-mediated acetylation of (±)-**99b** to enantiopure (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**100b** with subsequent crystallographic determination of the structure.[45] Hönig had previously described the synthesis of (±)-**99a** and (±)-**99b** by $NaBH_4$ reduction and assigned the ^{1}H NMR details of the *trans*-isomer (±)-**99b** which were consistent with our data.[35]

Spectral characteristics for the β-nitroalcohols (±)-**99a** and (±)-**99b** agreed with those described by Milner[45] as well as literature reports.[35] As anticipated, the IR spectra of the two diastereomers (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and the more polar (±)-*trans*-2-nitrocyclohexanol (±)-**99b** are very similar with a broad OH absorption at ν_{max} 3256-3427 cm^{-1} and the characteristic nitro stretches at ν_{max} 1551-1548 and 1376-1383 cm^{-1}.

Analysis by ^{1}H NMR identifies key spectroscopic features distinct to each diastereomer, (±)-**99a** or (±)-**99b** (Figure 4.3). The principal signals in the ^{1}H NMR of the (±)-*cis*-2-nitrocyclohexanol (±)-**99a** include the doublet at δ_H 2.56 ppm for the OH peak and the multiplet at δ_H 4.34-4.41 ppm representative of the proton alpha to the nitro moiety C(2)H. The proton geminal to the alcohol group C(1)H appears as a broad singlet at δ_H 4.52 ppm.

The characteristic hydroxyl signal of the corresponding (±)-*trans*-diastereomer (±)-**99b** appears as a doublet at δ_H 2.61 ppm. Significantly in the more polar *trans*-diastereomer (±)-**99b** the order of ^{1}H NMR signals for the protons alpha to the nitro C(2)H and alcohol moiety C(1)H are inverted relative to the *cis*-diastereomer (±)-**99a** characteristic of their relative stereochemistry. The C(1)H multiplet is found at δ_H 4.04-4.12 ppm while the C(2)H proton appears as a multiplet at δ_H 4.26-4.34 ppm.

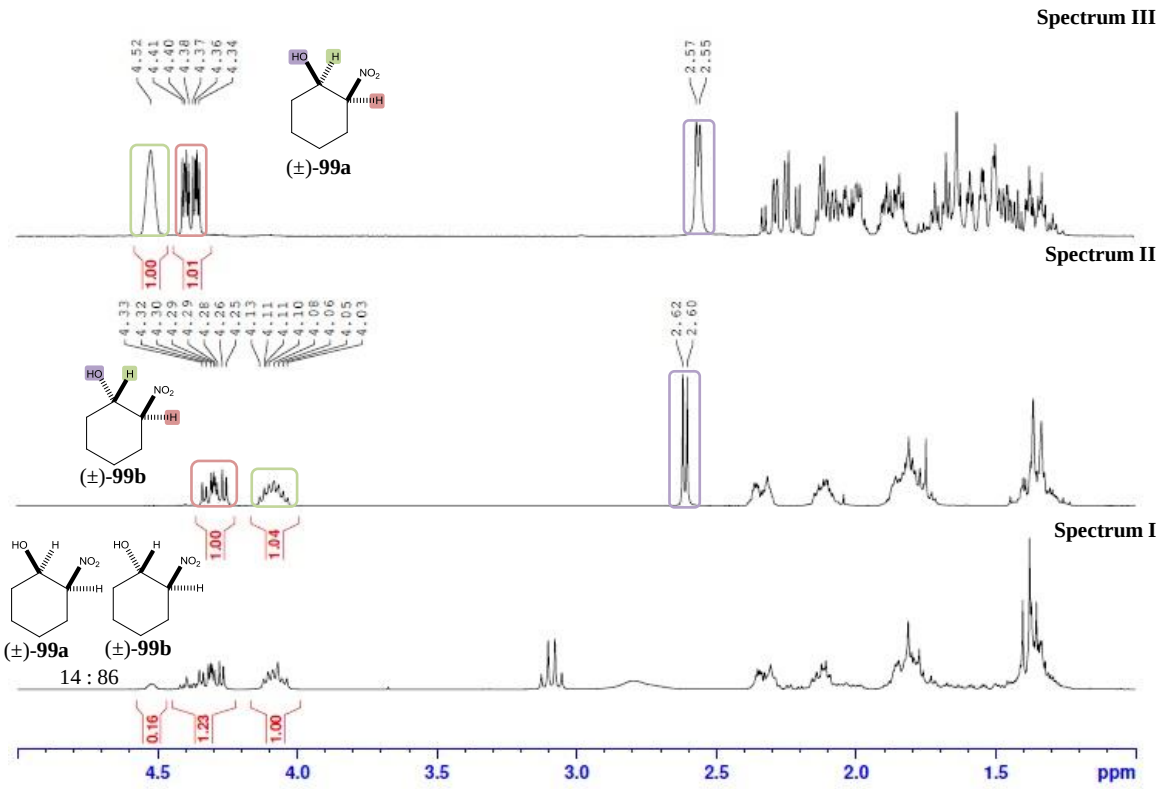
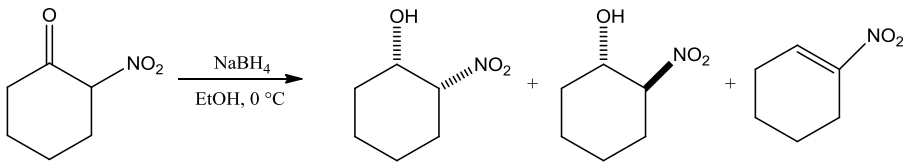


Figure 4.3: ^{1}*H NMR* ***Spectrum I:*** *Crude product following intramolecular Henry reaction containing a mixture of (±)-*cis- *and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**, 14 : 86 respectively.* ***Spectrum II:*** *Purified (±)-*trans*-2-nitrocyclohexanol (±)-**99b** following column chromatography.* ***Spectrum III:*** *Purified (±)-*cis*-2-nitrocyclohexanol (±)-**99a** following column chromatography (all spectra recorded in $CDCl_3$ at 300 MHz).*

As mentioned previously, the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** are also accessible *via* $NaBH_4$ reduction of 2-nitrocyclohexanone **103**. One of the initial synthetic objectives of this study was to limit utilisation of this β-nitro ketone **103** in the synthetic protocol due to its hazardous preparation and high commercial cost. However, the direct reduction of commercially available 2-nitrocyclohexanone **103** provides straightforward access to the desired β-nitroalcohols (±)-**99a** and (±)-**99b**, in good yield and high purity, thus this synthetic protocol was performed in conjunction with the development of a novel synthetic route to (±)-**99a** and (±)-**99b**.

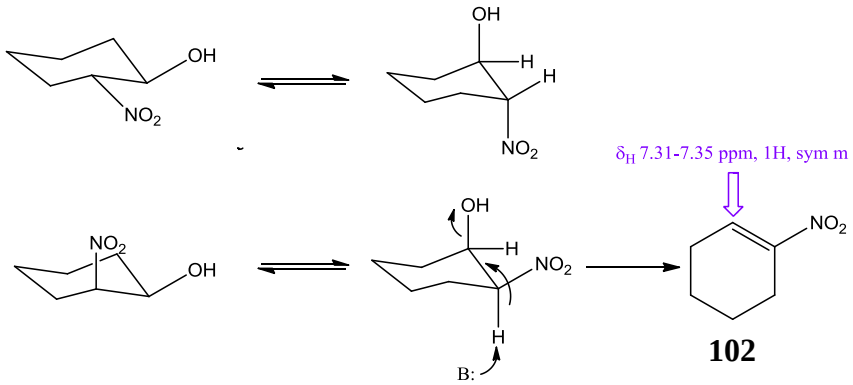
The reduction procedure was adapted from Hönig.[35] A solution of the ketone **103** in distilled ethanol was added dropwise to 1.0 equivalent of sodium borohydride and ethanol at 0 °C to yield a mixture of diastereomeric alcohols, (±)-**99a** and (±)-**99b** and the elimination product 1-nitrocyclohexene **102** (11 : 72 : 17 respectively) (Scheme 4.28). Milner reported a similar ratio of products on reduction of the 2-nitrocyclohexanone **103** (12 : 71 : 17 respectively).[45]



103	(±)-**99a**	(±)-**99b**	**102**
Product ratio by ^{1}H NMR	*11*	*72*	*17*
Isolated yield	*4%*	*36%*	*12%*

Scheme 4.28

The nitro alkene **102**, the most polar component of the reaction mixture was easily removed upon column chromatography and isolated as a yellow oil. 1-Nitrocyclohexene **102** is characterised in the IR spectrum by the alkene stretch at ν_{max} 1668 cm^{-1} and nitro absorption bands at ν_{max} 1515 and 1333 cm^{-1}. The ^{1}H NMR displays a distinctive multiplet at δ_H 7.31-7.35 ppm due to the vinylic proton. This side product is produced due to the relatively high acidity of the proton geminal to the nitro group of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** in conjunction with the necessary antiperiplanar conformation leading to dehydration (Scheme 4.29).



Scheme 4.29

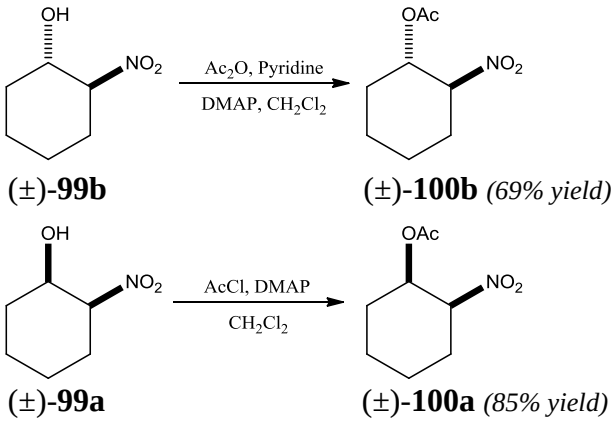
Notably this alkene by-product **102** is not evident in the triethylamine-catalysed intramolecular Henry reaction of 6-nitrohexanal **101** presumably due to less basic nature of triethylamine. Spectral characteristic of isolated diastereomeric alcohols (±)-**99a** and (±)-**99b**, were as previously described.

Thus, the new route to nitrocyclohexanols (±)-**99a** and (±)-**99b** *via* the intramolecular Henry reaction developed during this work offers distinct advantages relative to the literature procedure.[35,45] Firstly, it avoids use or synthesis of 2-nitrocyclohexanone **103** but more importantly, contamination with nitrocyclohexene **102** is avoided. The diastereomeric ratio of (±)-**99a** and (±)-**99b** from each of the routes is comparable.

4.2.3.3 Synthesis of (±)-2-nitrocyclohexyl acetate (±)-100

The separated (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**, were successfully converted to their corresponding acetates (±)-**100a** and (±)-**100b** employing the optimised conditions outlined by Milner (Scheme 4.30).[45]

Acetylation of (±)-*trans*-2-nitrocyclohexanol (±)-**100b** was achieved using acetic anhydride, pyridine and *N*,*N*-dimethylaminopyridine (DMAP). The *trans*-acetate (±)-**100b** was isolated in 69% yield as a clear oil which solidified on storage on the bench to a white crystalline solid. The *trans*-acetate (±)-**100b** was sufficiently pure to use for chiral HPLC development without further purification.



Scheme 4.30

When these conditions were applied by Milner to the acetylation of the *cis*-diastereomer (±)-**99a**, only starting material (±)-**99a** was recovered together with a trace of elimination product **102**. Thus, a new synthetic route was developed by Milner for the acetylation of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** which employed acetyl chloride and excess DMAP.[45] This adapted methodology was applied in this study and the *cis*-acetate (±)-**100a** was isolated as a clear oil in 85% yield (Scheme 4.30). Additional signals were apparent in the ^{1}H NMR spectrum of the crude product which were assigned to unreacted starting material (±)-**99a** and residual DMAP; this is in accordance with earlier reports where reaction completion was similarly not achieved.[45] Purification by column chromatography was not attempted due to the reported formation of the elimination product **102** on silica gel.[45] Thus, the crude *cis*-acetate (±)-**100a** was utilised for chiral HPLC method development.

IR analysis of the *cis*- and *trans*-diastereomers (±)-**100a** and (±)-**100b** display characteristic carbonyl stretches of the acetate moiety at ν_{max} 1745 and 1737 cm^{-1} respectively. The ^{1}H NMR spectra are illustrated in Figure 4.4. The two diastereomers are easily identifiable due to the characteristic multiplicity and chemical shift of the C(1)H hydrogen alpha to the acetate moiety. In (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a** the C(1)H hydrogen appears as a multiplet at δ_H 5.52-5.65 ppm. In the analogous (±)-*trans*-2-nitrocyclohexyl acetate this C(1)H proton appears as a distinctive doublet of doublet of doublets at δ_H 5.19-5.27 ppm.

Assignment of the relative stereochemistry of (±)-**100a** and (±)-**100b** was confirmed by X-ray crystallography of (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**100b**, acquired from a preparative-scale hydrolase-mediated acetylation.[45]

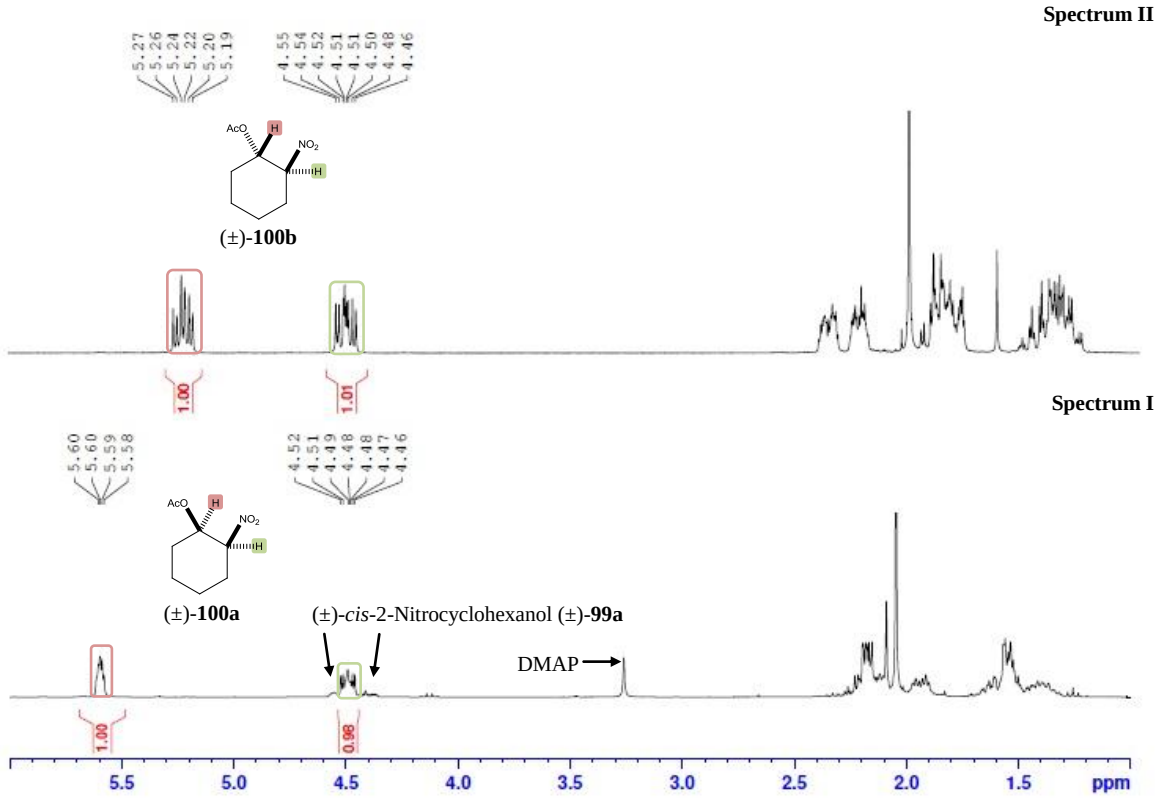


Figure 4.4: ^{1}H *NMR* ***Spectrum I:*** *Crude (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a**, additional signals present due to (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (12%) and* N,N*-dimethylaminopyridine (5%).* ***Spectrum II:*** *Crude (±)-*trans*-2-nitrocyclohexyl acetate (±)-**100b** (all spectra recorded in* $CDCl_3$ *at 300 MHz).*

4.2.4 Chiral HPLC method development

Once the racemic materials were prepared, the next step was to develop a chiral HPLC method where multiple stereoisomers could be eluted on a single trace to facilitate analysis and optimisation. In this study, resolution of the following enantiomeric pairs in a single injection was required for the determination of the enantiopurity of the hydrolase-mediated transesterification products and development of quantification calibration curves;

- (±)-*cis*-2-Nitrocyclohexanol (±)-**99a** and (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a**
- (±)-*trans*-2-Nitrocyclohexanol (±)-**99b** and (±)-*trans*-2-nitrocyclohexyl acetate (±)-**100b**
- (±)-*cis*-2-Nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b**

Previous work within the research group had successfully resolved the enantiomeric pairs of both the nitroacetates (±)-**100a** and (±)-**100b** and nitroalcohols (±)-**99a** and (±)-**99b** in one injection, utilising the Chiralcel® OJ-H column at room temperature with isopropanol/hexane (1 : 99), a flow rate of 0.9 mL/min and a detector wavelength of 220 nm.[45] On implementation of these conditions, resolution of each of the enantiomers of (±)-**99a**, (±)-**99b** and (±)-**100b** was achieved. However, baseline separation of (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a** was not attained. Thus, under these conditions two out of the three desired resolutions were developed. (±)-*trans*-2-Nitrocyclohexanol (±)-**99b** and (±)-*trans*-2-nitrocyclohexyl acetate (±)-**100b** were resolved in a single trace. Similarly (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b** were obtained in a single injection.

Resolution of the enantiomeric pairs of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a** was therefore explored utilising a series of chiral

columns including the Chiralcel® AS-H and Chiralpak® IB. A variety of isopropanol/hexane solvent composition, or flow rates and temperatures for each resolution was investigated. Following a significant amount of method development, the Chiralcel® OD-H column led to complete baseline separation of both sets of enantiomers (±)-**99a** and (±)-**100a** at room temperature with identical conditions for solvent and flow rate to those outlined for the aforementioned successful resolutions (Figure 4.5).

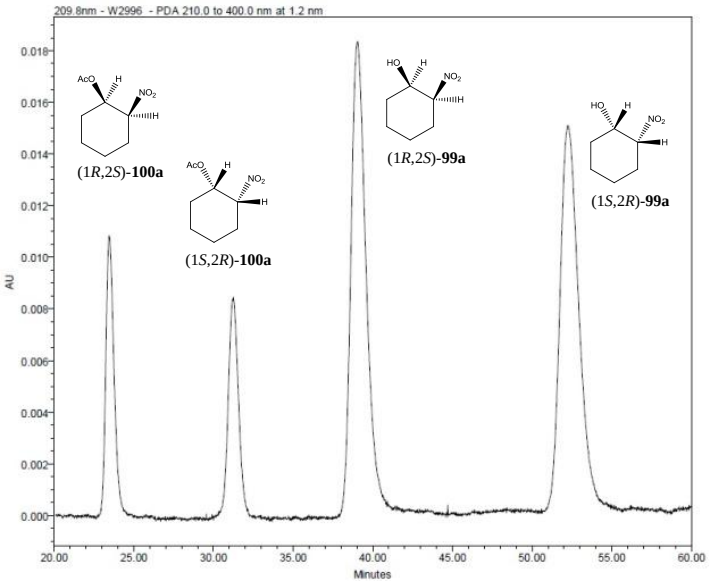


*Figure 4.5: HPLC trace of (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100a** and (±)-*cis*-2-nitrocyclohexanol (±)-**99a**. For HPLC conditions see appendix I.*

One of the primary objectives of this study was quantification of the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** by chiral HPLC analysis to allow determination of product ratios and comparison with ratios determined by ^{1}H NMR spectroscopy if feasible. Calibration curves of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** were generated by injecting standards of five known concentrations of (±)-**99a** or (±)-**99b** (ranging from 0.2 mg/mL to 1.2 mg/mL) and processing response factors based on peak area. The linear regression of a plot of peak area versus concentration demonstrates the direct proportionality between the variables. It depends upon the linear response of the detector and also upon the accurate preparation of the standards. A least squares regression is employed and the value of the coefficient of determination (r^2 value) is evaluated as a measure of acceptability. A linear fit with a r^2 value >0.990 is considered satisfactory and all calibration curves in this study achieve this standard signifying the high precision of the calculation of unknown concentration. The chiral HPLC peak area of the unknown concentration was measured and by interpolation with the relevant calibration curve the concentration of the *cis*- or *trans*-2-nitrocyclohexanols **99a** or **99b** analyte was determined. Calibration curves for both the *cis*- and *trans*-diastereomers (±)-**99a** and (±)-**99b** for 2μL and 10 μL injection volumes were prepared during this research (Figure 4.6 and 4.7).

The development of calibration curves to allow quantification of *cis*- and *trans*-2-nitrocyclohexanols **99a** and **99b** by chiral HPLC analysis denoted a significant advance in this study allowing for deeper understanding of the mechanistic detail of the dynamic interconversion process. Direct comparison of chiral HPLC and ^{1}H NMR experiments investigating the dynamic interconversion of the two diastereomers (±)-**99a** and (±)-**99b** could now be conducted.

Calibration curve: (±)-*cis*-2-Nitrocyclohexanol (±)-**99a** (2 µL injection volume)[vii]

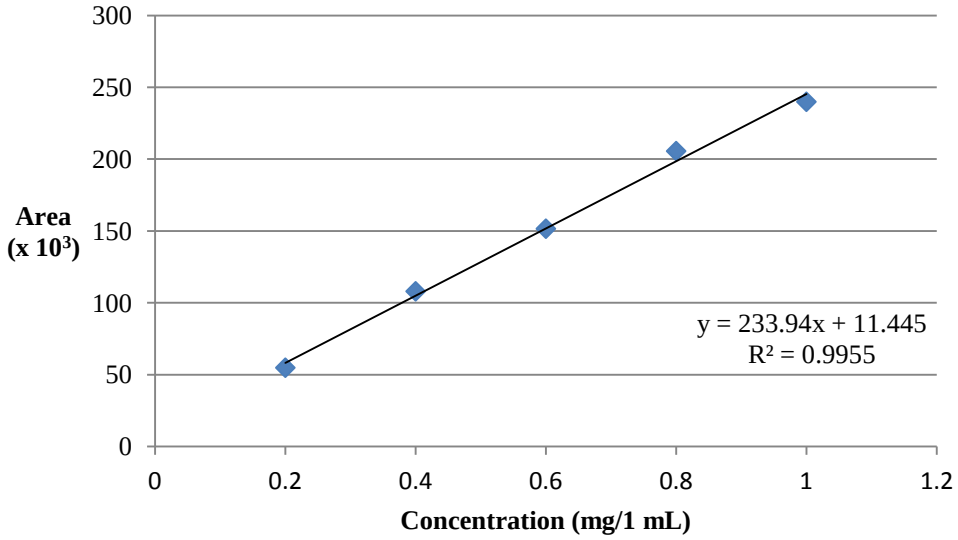


*Figure 4.6: Calibration curve (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (2 µL injection volume). Area (x 10^3) vs. concentration (mg/1 mL).*

Calibration curve: (±)-*trans*-2-Nitrocyclohexanol (±)-**99b** (2 µL injection volume)[IV]

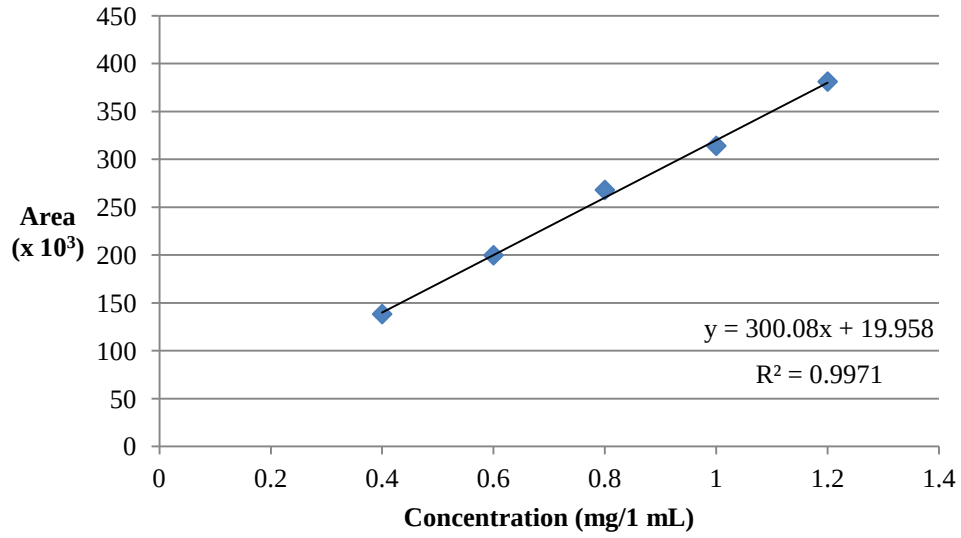


*Figure 4.7: Calibration curve (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (2 µL injection volume). Area (x 10^3) vs. concentration (mg/1 mL).*

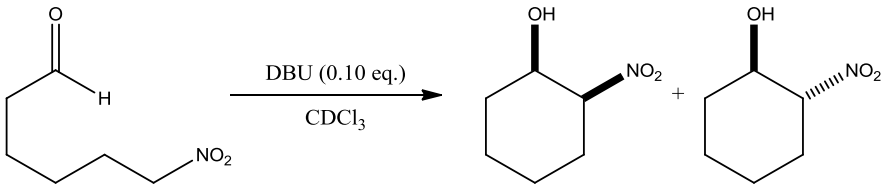
[vii] Calibration curves were conducted on a Waters alliance 2690 separations module with a PDA detector. The Chiralcel® OJ-H column was utilised at room temperature with isopropanol/hexane (1 : 99), a flow rate of 0.9 mL/min and λ_{max} at 209.8 nm.

4.2.5 Evidence of a dynamic interconversion process – ^{1}H NMR analysis

In Milner's preliminary study ^{1}H NMR analysis indicated that triethylamine-mediated ring closing of 6-nitrohexanal **101** to (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols, (±)-**99a** and (±)-**99b**, and subsequent dynamic interconversion between the two diastereomers (±)-**99a** and (±)-**99b** is possible.[45] However, this initial premise was challenged on exploration of the one-pot intramolecular nitroaldol reaction with lipase-mediated kinetic resolution. It became evident that the dynamic ring opening/closing of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** *via* 6-nitrohexanal **101** was not the predominant process taking place, but rather epimerisation; thus triethylamine is an ineffective base for this process. Towards the end of Milner's study, a series of base screening experiments was conducted monitored closely by chiral HPLC analysis, and, significantly, identified that both ring opening/closing and epimerisation mechanisms operate when DBU is employed.[45] Thus, this preliminary result suggested that optimisation of the base-mediated dynamic process may lead to an efficient dynamic resolution protocol.

The first step in this investigation was to employ ^{1}H NMR analysis to investigate the DBU-mediated ring closing of 6-nitrohexanal **101** to form the diastereomeric β-nitroalcohols (±)-**99a** and (±)-**99b**. Previous to this investigation only triethylamine had been explored. Two experiments involving 0.10 and 0.05 equivalent of DBU and the precursor aldehyde **101** were conducted in deuterated chloroform in a NMR tube in order to enable direct monitoring by ^{1}H NMR analysis.

*Table 4.12: Evidence for dynamic interconversion – 6-nitrohexanal **101**, $CDCl_3$ and DBU (0.10 eq.)*



101 (±)-**99a** (±)-**99b**

Reaction Time	101 (%)	(±)-99a (%)	(±)-99b (%)
0 min	21	19	60
17 min	20	18	62
27 min	14	21	65
1 h 27 min	4	18	78
3 h 29 min	0	18	82
9 h 29 min	0	17	83
15 h 29 min	0	17	83
20 h 22 min	0	15	85
22 h 37 min	0	16	84
20 days	0	14	86

As is evident from Table 4.12 and Figure 4.8 6-nitrohexanal **101** cyclises readily to the (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** in the presence of 0.10 equivalent of DBU. Furthermore, the thermodynamic ratio of (±)-**99a** and (±)-**99b** is achieved within 4 h and was determined to be 16 : 84 respectively. This correlates with the thermodynamic ratio obtained by Milner (15 : 85 respectively) when 1.00 and 5.00 equivalents of triethylamine were utilised in this protocol.[45] It is apparent from this study that the (±)-*trans*-2-nitrocyclohexanol

(±)-**99b** is the thermodynamically more stable diastereomer and the conversion to (±)-*cis*-2-nitrocyclohexanol (±)-**99a** never exceeded 21% *cis* in this investigation. The variation in the ratio of (±)-**99a** and (±)-**99b** over time may be indicative of dynamic interconversion *via* ring opening and closing of the aldehyde **101**. However, these results may also be interpreted as evidence of epimerisation at the C2 stereogenic centre, therefore they were interpreted with caution at this point in the study.

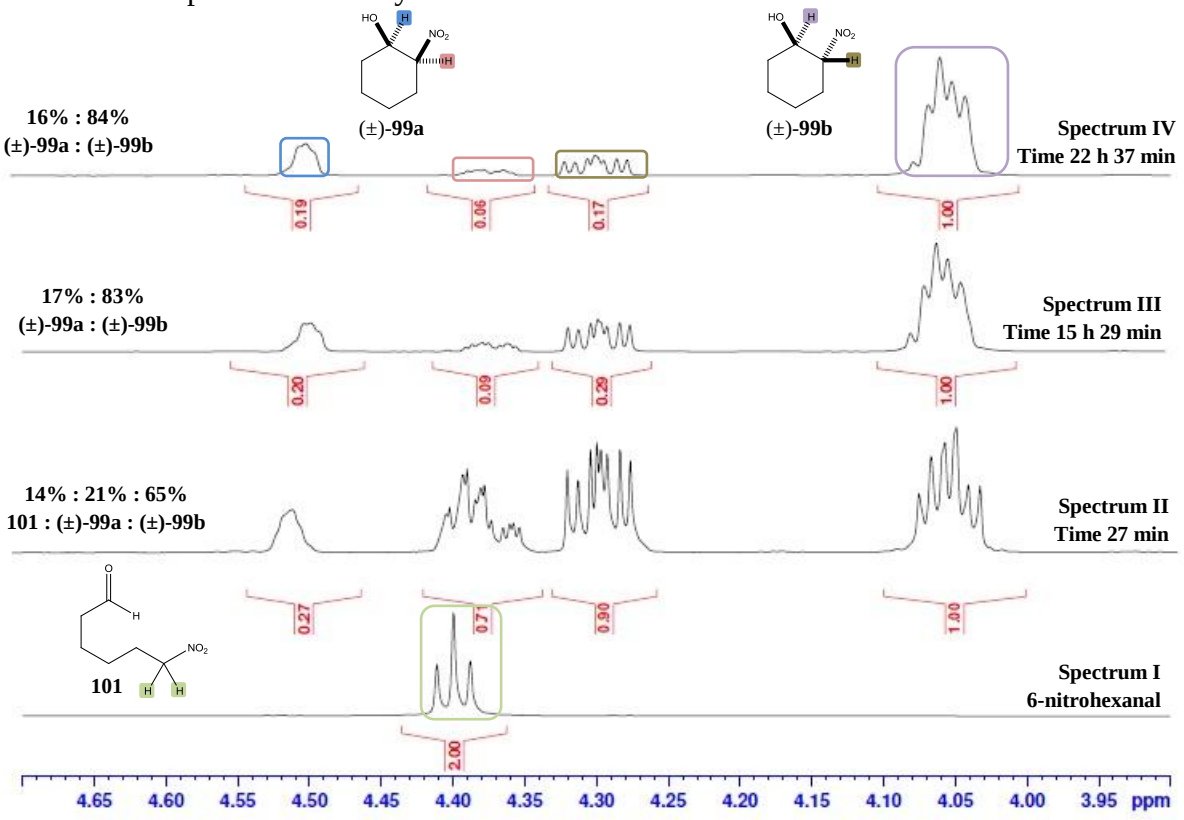


*Figure 4.8: Stacked ^{1}H NMR spectra - Evidence for dynamic interconversion, 6-nitrohexanal **101** and DBU (0.10 eq.).*

Significantly, integration of the C(2)HNO_2 signals was low in the ^{1}H NMR spectra from reaction time 1 h 27 min onwards. This may be rationalised by deuterium exchange at this acidic proton. If this is the case, this is suggestive of the epimerisation process.

Following a reaction time of 35 days the ^{1}H NMR sample was diluted in chloroform and the DBU removed by washing with saturated aqueous ammonium chloride solution. ^{1}H NMR analysis of the isolated products (±)-**99a** and (±)-**99b** indicated a thermodynamic ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b** of 11 : 89 respectively. The sample was then submitted to chiral HPLC analysis which determined both the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** to be racemic (Figure 4.9). Additionally quantification of (±)-**99a** and (±)-**99b** by chiral HPLC indicated a thermodynamic ratio of 15 : 85, supporting the ratio determined by ^{1}H NMR analysis. This correlation between the chiral HPLC and ^{1}H NMR data was very important as it enabled monitoring of the dynamic process by chiral HPLC.

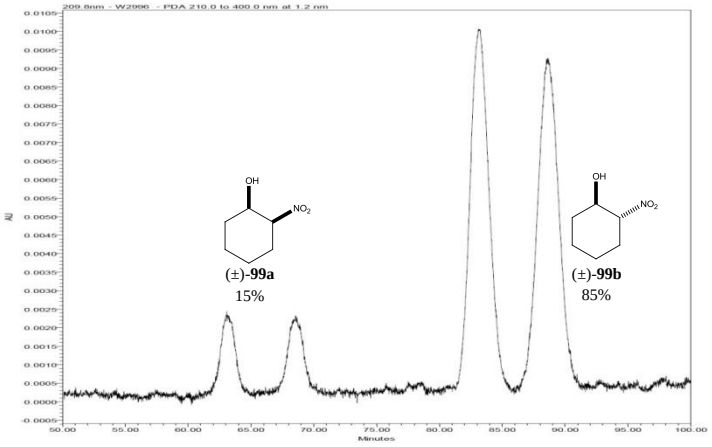
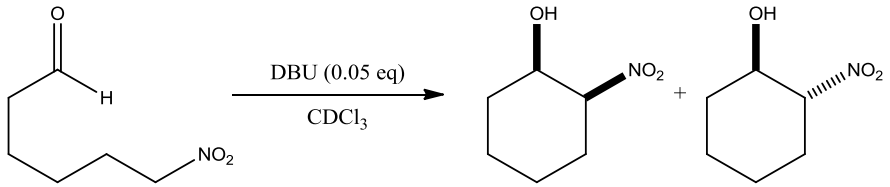


*Figure 4.9: HPLC trace (2 µL injection volume) of ^{1}H NMR sample DBU (0.10 eq.) and 6-nitrohexanal **101**, reaction time 35 days. For HPLC conditions see appendix I.*

In contrast, in the presence of 0.05 equivalent of DBU, complete cyclisation of 6-nitrohexanal **101** to the corresponding 2-nitrocyclohexanols (±)-**99a** and (±)-**99b** was not achieved within 27 h and furthermore the thermodynamic ratio was not achieved (Table 4.13). Notably, no decrease in integration of the C(2)HNO_2 signals was observed at 0.05 equivalent of DBU.

*Table 4.13: Evidence for dynamic interconversion – 6-nitrohexanal **101**, $CDCl_3$ and DBU (0.05 eq.)*



101 (±)-**99a** (±)-**99b**

Reaction Time	101 (%)	(±)-99a (%)	(±)-99b (%)
0 min	92	4	4
5 min	92	4	4
7 min	92	4	4
34 min	91	4.5	4.5
1 h 35 min	89	5	6
3 h 35 min	86.5	6.5	7
9 h 34 min	78	10	12
15 h 35 min	71	14	15
20 h 28 min	65	16	19
22 h 43 min	62	18	20
23 h 50 min	60	20	20
27 h 37 min	56	22	22

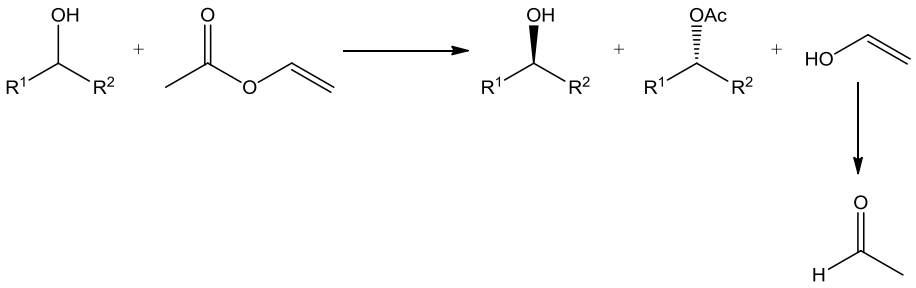
4.2.6 Hydrolase-mediated kinetic resolution – analytical screens

4.2.6.1 Analytical screening protocol – vinyl acetate as both acyl donor and solvent

Essential for the development of an efficient dynamic process is that one enantiomer of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** or (±)-*trans*-2-nitrocyclohexanol (±)-**99b** is efficiently and selectively acetylated. Milner conducted screening experiments with the specific aim to identify a hydrolase which would selectively transform one diastereomer of (±)-**99a** or (±)-**99b** enantioselectively.[45] The hydrolase-mediated kinetic resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b** were examined independently. In Milner's study, any hydrolase which transformed the (±)-*cis*-2-nitrocyclohexanol (±)-**99a** with high enantioselectivity and efficiency also performed efficiently for the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b**. Thus, lack of hydrolase diastereoselectivity severely hampered the early development of the hydrolase-mediated dynamic kinetic resolution process.

A primary objective of this study was to screen hydrolases which had not previously been investigated for the enzyme-mediated transesterification of (±)-*trans*- or/and (±)-*cis*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b**. Ideally identification of a lipase that selectively acetylated one diastereomer (±)-**99a** or (±)-**99b** efficiently and with excellent enantioselectivity would potentially overcome a significant limitation of the desired one-pot dynamic resolution process.

All hydrolases screened were kindly donated by Almac Sciences. An optimised analytical screening protocol developed by Milner for transesterification of alcohols (±)-**99a** and (±)-**99b** was implemented in this study.[45] A spatula tip of enzyme was added to ~20 mg of the (±)-2-nitrocyclohexanol substrate (±)-**99a** or (±)-**99b** in 1 mL of vinyl acetate and incubated on a shaking platform for 24 h at 24 °C and 3 h at 40 °C unless otherwise stated. Use of vinyl acetate as both acyl donor and solvent is highly advantageous as this enol ester irreversibly forces the enzymatic process in the forward direction preventing loss of enantioselectivity associated with the reversible nature of the transesterification.[70] The reaction is irreversible due to the formation of vinyl alcohol which tautomerises rapidly and essentially irreversibly to acetaldehyde (Scheme 4.31). Furthermore, vinyl acetate is inexpensive and volatile and thus easily removed upon work-up.



Scheme 4.31

The work-up of the analytical screens involved initial filtration of the reaction mixture through Celite® to remove the hydrolase. The Celite® was then washed with ethyl acetate and all organic extracts combined and concentrated under reduced pressure. The crude product was analysed by ^{1}H NMR spectroscopy to determine product ratio and extent of conversion. Significantly the conversion determined by ^{1}H NMR analysis agreed very closely with those

estimated based on the enantiomeric ratio. In this study the *E*-value was calculated using the program developed by Kroutil *et al.*[71]

For efficiency, chiral HPLC was only conducted for conversions >10% where the enantiopurities of both the substrate alcohol **99** and generated acetate **100** could be determined *via* a single injection of the crude reaction mixture without chromatographic separation. All chiral HPLC conditions are detailed in appendix I. When the second enantiomer was absent, the enantiomeric excess was stated as >98% ee.

4.2.6.2 Stereochemical assignment of the products of transesterification

The enantioselectivities of lipases are largely dependent on the structure of the substrate as formulated by Kazlauskas.[72,73] This empirical rule is highly effective for lipase action on secondary alcohols, predicting which enantiomer reacts faster based on the relative sizes of the substituents at the stereocentre.

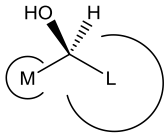
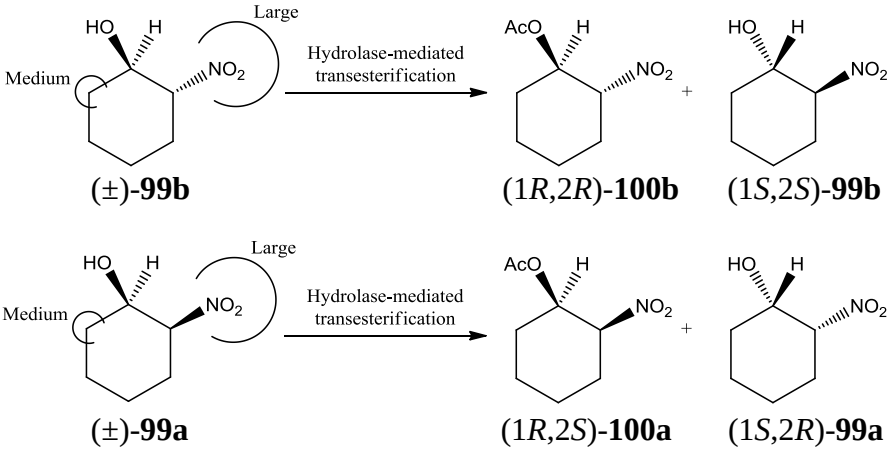


Figure 4.10

This rule generalizes the observed enantioselectivity of hydrolases in both hydrolysis and tranesterifications reactions. In transesterification reactions, the enantiomer shown in Figure 4.10 reacts faster; in hydrolysis reactions, the ester of the enantiomer shown is preferentially catalysed. Accordingly, the Kazlauskas's rule is useful as a guideline for predicting substrates that can be efficiently resolved by lipases as well as the stereochemistry of resolved substrates.

When this rule was applied to the hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** the following stereochemical outcome was predicted (Scheme 4.32).



Scheme 4.32

The predicted assignment by Kazlauskas's rule was in direct agreement with Milner's study.[45] In the preparative-scale *Pseudomonas fluorescens* mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b**, Milner isolated the (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**100b** in >99% ee. The absolute stereochemistry of enantiopure (1*R*,2*R*)-**100b**

was confirmed by single crystal X-ray diffraction and assigned as (1*R*,2*R*)-*trans*-2-nitrocylohexylacetate (1*R*,2*R*)-**100b**. The absolute stereochemistry of the untransformed alcohol therefore has to be (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**. Furthermore, this stereochemical outcome agreed with Hönig's assignment of absolute stereochemistry of the products of the *Candida cylindracea* mediated hydrolysis of a butyrate of (±)-**99b** providing access to the complementary enantiopure alcohol (1*R*,2*R*)-**99b**.[35]

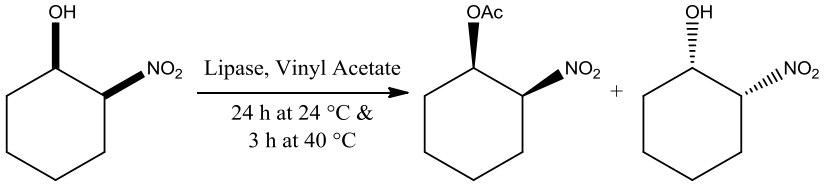
Due to the ease of formation of the elimination product **102** during purification Milner was unable to obtain an analytically pure sample of enantioenriched *cis*-2-nitrocyclohexyl acetate **100a** or *cis*-2-nitrocyclohexanol **99a**.[45] Thus, it should be noted that in this study the stereochemical assignment of the transesterification products of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** were tentatively assigned as (1*R*,2*S*)-*cis*-2-nitrocyclohexyl acetate (1*R*,2*S*)-**100a** and (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a** by analogy to the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** and in accordance to Kazlauskas's rule.[72,73]

*4.2.6.3 Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-99a*

The first substrate screened was (±)-*cis*-2-nitrocyclohexanol (±)-**99a** utilising enzymes that had not previously been investigated for this resolution. The primary objective of this screen was to compare efficiencies and enantioselectivities of the resolution of the (±)-*cis*-2-nitrocyclohexanol (±)-**99a** with that of the (±)-*trans*-2-nitrocyclohexanol (±)-**99b** to identify a hydrolase selective to one diastereomer. Notably, some of the lipases listed in Table 4.14 have previously been screened by Milner in the resolution of the *trans*-diastereomer (±)-**99b**[45] and thus comparison between the enantioselectivities obtained in the kinetic resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** in this study with Milner's results of the kinetic resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** was conducted.[36,45] In the case of *Candida antarctica* lipase B (immob), *Candida antarctica* lipase A, and *Achromobacter spp.*, screening was performed on both diastereomers (±)-**99a** or (±)-**99b** before efficiency and enantioselectivity was compared.

It is apparent from Table 4.14 that many of the hydrolases resulted in limited transesterification with conversions <10% recorded. Significantly some extent of acetylation was observed with each of the hydrolases *Candida antarctica* lipase B (immob), *Alcaligenes spp*. 1, and *Candida antarctica* lipase A (entries 1, 2 and 8, Table 4.14). The rate of conversions of *Candida antarctica* lipase B (immob) and *Alcaligenes spp*. 1 mediated kinetic resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** was deduced by ^{1}H NMR to be 19% and 18% respectively. The limited efficiency of the transesterification restricted the enantiopurity of the recovered alcohol (1*S*,2*R*)-**99a**, however, the generated acetate (1*R*,2*S*)-**100a** was obtained in high enantiomeric excess (≥92% ee). In the case of *Candida antarctica* lipase A a lack of discrimination was displayed of the enantiomers of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** with a conversion greater than the ideal 50% achieved, thus low enantiopurity of the generated acetate (1*R*,2*S*)-**100a** was observed. Notably no elimination product **102** was observed in the ^{1}H NMR spectrum of the crude products.

*Table 4.14: Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** in vinyl acetate*



(±)-**99a** (1*R*,2*S*)-**100a** (1*S*,2*R*)-**99a**

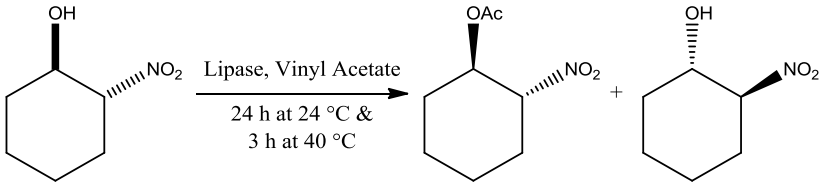
Entry	Enzyme Source	Ratio 99a : 99b[a]	Conversion (%)		ee (%)		*E* Value
			E Calc.	^{1}H NMR	Alcohol *cis*-99a (1*S*,2*R*)	Acetate *cis*-100a (1*R*,2*S*)	
1	*Candida antarctica* B (immob)[b]	96 : 4	23	19	29	95	51
2	*Alcaligenes spp.* 1	96 : 4	20	18	23	92	30
3	*Rhizopus spp.*	96 : 4	-	<10	-	-	-
4	*Aspergillus niger*	96 : 4	-	<10	-	-	-
5	*Mucor meihei*	96 : 4	-	<10	-	-	-
6	*Porcine pancrease* Type II	95 : 5	-	<10	-	-	-
7	Pig liver esterase	96 : 4	-	<10	-	-	-
8	*Candida antarctica* A	96 : 4	60	60[c]	80	54	7.8
9	*Achromobacter spp.*	96 : 4	-	<10	-	-	-

a. The ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in the starting material, determined by ^{1}H NMR spectroscopy.
b. Time for tranesterification of the nitroalcohol was 51 h at 24 °C.
c. Unknown impurity observed in the crude ^{1}H NMR.

Previous research by Milner had established that *Rhizopus spp.*, *Aspergillus niger*, *Porcine pancrease* Type II, and Pig liver esterase also proceeded with trace transesterification (<10% conversion) in the resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b.**[45] Thus, these lipases were concluded to be unsuitable for the development of a dynamic kinetic resolution process due to their lack of reactivity for both diastereomers.

Milner had also previously screened *Alcaligenes spp.* 1 and *Mucor meihei* for the transesterification of the (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (see Table 4.15).[45] Poor conversion (37% and 17% respectively) and thus low enantiopurity of the recovered alcohol (53% ee and 32% ee respectively) was reported.[45] In both cases the rate of acetylation was considered to be too similar to that observed in this study for the *Alcaligenes spp.* 1 and *Mucor meihei* mediated resolution of the (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (entries 2 and 5, Table 4.14) and this, together with the poor enantioselection observed, deemed these lipases unsuitable for implementation in a dynamic kinetic process.

*Table 4.15: Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in vinyl acetate*[36,45]



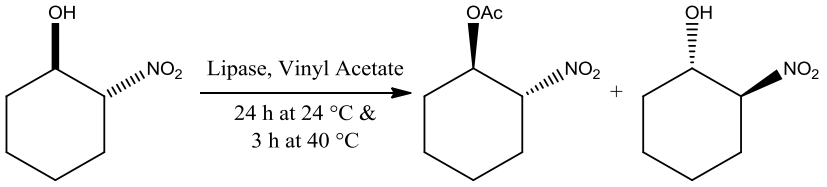
(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Entry	Enzyme Source	Ratio 99a : 99b[a]	Conversion (%)		ee (%)		*E* Value
			E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*,2*S*)	Acetate *trans*-100b (1*R*,2*R*)	
1	*Alcaligenes spp.* 1	0 : 100	-	37	53	98	168
2	*Mucor meihei*	0 : 100	-	17	32	>98	272

*4.2.6.4 Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-99b*

The transesterification of the (±)-*trans*-2-nitrocyclohexanol (±)-**99b** was next investigated employing the conditions utilised for the resolution of the *cis*-diastereomer (±)-**99a**. *Achromobacter spp.* and *Candida antarctica* lipase A (entries 2 and 3, Table 4.16) resulted in limited extent of conversion <10%. Excellent enantioselectivity was observed in the *Candida antarctica* lipase B (immob) mediated resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** with an ideal conversion of 50% achieved (entry 1, Table 4.16). This hydrolase had previously been screened by Milner with similar excellent enantioselectivity observed for both the alcohol (1*S*,2*S*)-**99b** and acetate (1*R*,2*R*)-**100b**.[45]

*Table 4.16: Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in vinyl acetate*



(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Entry	Enzyme Source	Ratio 99a : 99b[b]	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*,2*S*)	Acetate *trans*-100b (1*R*,2*R*)	
1	*Candida antarctica* B (immob)[a]	1 : 99	50	49	>98	>98	>200
2	*Candida antarctica* A	1 : 99	9	-[c]	8	77	8.3
3	*Achromobacter spp.*	1 : 99	-	<10	-	-	-

a. Time for tranesterification of the nitroalcohol was 51 h at 24 °C.
b. The ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in the starting material, determined by ^{1}H NMR spectroscopy.
c. Unknown impurity observed in the ^{1}H NMR of the crude product, conversion could not be obtained satisfactorily due to overlapping peaks.

In this study, *Achromobacter spp.* also displayed limited transesterification in the resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (entry 9, Table 4.14) and therefore was not considered suitable for the dynamic resolution process.

In direct contrast to the limited transesterification (<10% conversion) observed with the (±)-*trans*-2-nitrocyclohexanol (±)-**99b**, *Candida antarctica* lipase A mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (entry 8, Table 4.14) proceeded with 60% conversion albeit with poor stereocontrol. The substantial difference in relative rates of acetylation between the two diastereomers (±)-**99a** and (±)-**99b** supports further optimisation of *Candida antarctica* lipase A mediated transesterification to improve enantioselection of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and to extensively investigate the apparent diastereoselectivity.

Candida antarctica lipase B (immob) demonstrated excellent enantioselectivity in the resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** with a 50% optimum conversion obtained. This compared favourably with the resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** in this study with only 19% conversion reported under the same reaction conditions (entry 1, Table 4.14). This significant difference in rates of transesterification, together with the excellent enantioselection observed with *Candida antarctica* lipase B (immob) mediated resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b**, demonstrates the greatest potential for development of a hydrolase-mediated diastereoselective resolution process. In this research, the identification of *Candida antarctica* lipase B (immob) as a lipase that demonstrates apparent diastereoselectivity signifies a major advancement in the development of a dynamic kinetic resolution of 2-nitrocyclohexanol **99**.

*4.2.6.5 Diastereoselective hydrolase-mediated transesterification of (±)-***cis-** *and (±)-***trans-** *2-nitrocyclohexanol (±)-99a and (±)-99b*

Further investigation of the relative rates of acetylation of the *Candida antarctica* lipase B (immob) mediated biotransformation of a equimolar mixture of diastereomers (±)-**99a** and (±)-**99b** was warranted, with careful examination of the appearance of acetates **100a** and **100b** both by ^{1}H NMR and chiral HPLC analysis over time. This potential diastereoselective process would ideally lead to faster kinetic resolution of the *trans*-diastereomer (±)-**99b** in high enantioselectivity, leaving the *cis*-diastereomer (±)-**99a** unchanged.

A 50 : 50 mixture of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** was dissolved in vinyl acetate and *Candida antarctica* lipase B (immob) was charged to the reaction vessel. For the initial 15 h of the transesterification the reaction mixture was shaken at room temperature (~19 °C) however for the subsequent 33 h reaction temperature was set at 24 °C. Reaction monitoring by ^{1}H NMR analysis was conducted throughout the incubation period. Aliquots of reaction mixture (~1 mL) were removed at regular intervals, filtered through Celite® and concentrated prior to ^{1}H NMR analysis. Due to overlapping signals in the ^{1}H NMR spectrum quantification of *cis*-2-nitrocyclohexanol **99a** could only be determined by integration of the C(2)H multiplet (4.34-4.41 ppm) however this resonance was in very close proximity to the C(2)H multiplet of the *trans*-diastereomer **99b** (4.26-4.34 ppm) and thus accurate quantification of **99a** was difficult in the presence of the acetates **100a** and **100b**.

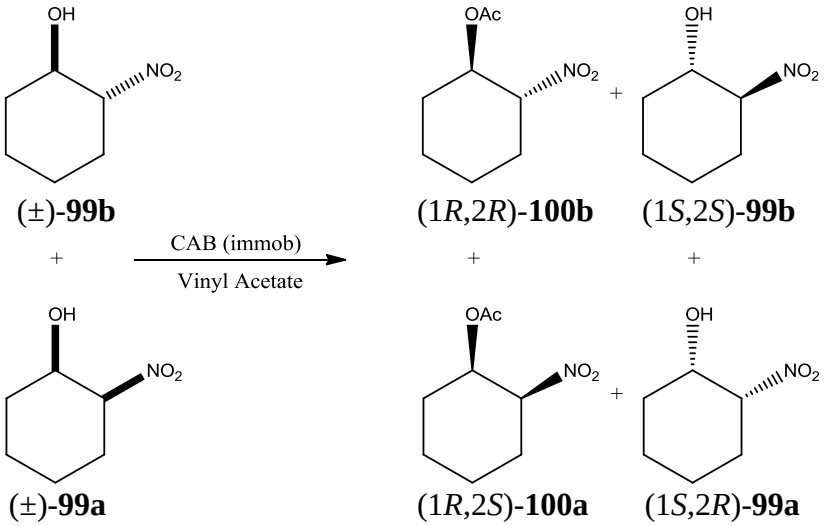
As anticipated from the preliminary analytical screens conducted on the individual diastereomers (±)-**99a** and (±)-**99b**, the dominant kinetic process was resolution of the (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (Table 4.17). After 15 h only 1% of the minor *cis*-acetate (1*R*,2*S*)-**100a** relative to 19% of the major *trans*-acetate (1*R*,2*R*)-**100b** was evident by ^{1}H NMR spectroscopy demonstrating the inherent diastereoselectivity of the lipase *Candida antarctica* lipase B (immob). As the resolution progressed partial transformation of the less favoured diastereomer (±)-*cis*-2-nitrocyclohexanol (±)-**99a** was observed with 8% of the *cis*-

acetate (1*R*,2*S*)-**100a** evident at 48 h, however this diastereomer still only constituted a minor component of the overall reaction mixture.

Furthermore, chiral HPLC analysis conducted on the final extraction (48 h) indicated excellent enantiopurity of the *trans*-alcohol (1*S*,2*S*)-**99b** (>98% ee) and *trans*-acetate (1*R*,2*R*)-**100b** (98% ee) demonstrating efficient kinetic resolution as observed in entry 1, Table 4.16. High enantioselectivity was also observed of the minor *cis*-acetate (1*R*,2*S*)-**100a** however enantiopurity of the *cis*-alcohol (1*S*,2*R*)-**99a** was poor due to limited conversion. Notably as chiral HPLC conditions were not developed to allow resolution of all eight enantiomers of (±)-**99a**, (±)-**99b**, (±)-**100a** and (±)-**100b** on a single trace (see section 4.2.4), the reaction mixture was analysed under two different sets of HPLC conditions (see appendix I), allowing determination of enantiomeric excess of all reaction components.

In summary, *Candida antarctica* lipase B (immob) exhibited significant potential as a biocatalyst in the dynamic kinetic resolution process demonstrating good diastereoselectivity with excellent enantioselection of (1*S*,2*S*)-**99b** especially if the transesterification reaction time was limited to 15 h. Most significantly the observation that (±)-**99b** can be selectively transformed in the presence of (±)-**99a** by the biocatalyst *Candida antarctica* lipase B indicates that with detailed process development and variation of the biotransformation conditions and/or enzyme evolution that the diastereocontrol could be further optimised leading to essentially exclusive transformation of (±)-**99b**.

*Table 4.17: Diastereoselective hydrolase-mediated transesterification of (±)-*cis- *and (±)-*trans-*2-nitrocyclohexanol (±)-**99a** and (±)-**99b** in vinyl acetate*



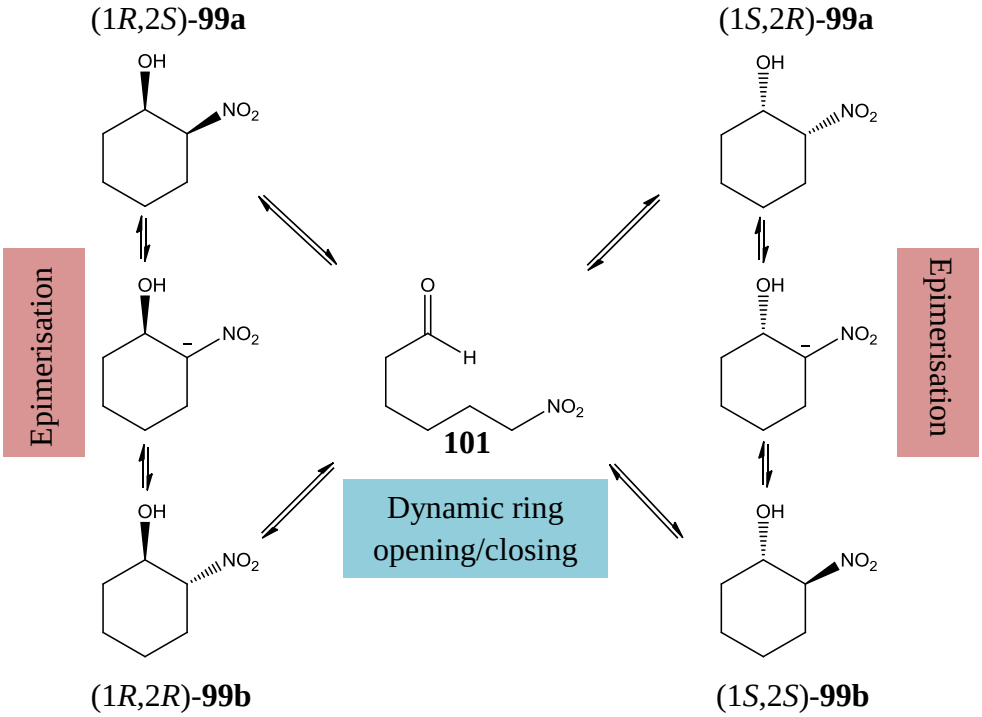
Enzyme Source	Reaction Time	Temp (°C)	Alcohol (±)-99		Acetate (±)-100	
			***cis*-99a (%)[a] [ee (%)][b]**	***trans*-99b (%)[a] [ee (%)][c]**	***cis*-100a (%)[a] [ee (%)][d]**	***trans*-100b (%)[a] [ee (%)][e]**
Candida antarctica B (immob)	15 h	19	45	35	1	19
	24 h	24	48	28	3	21
	39 h	24	43	29	6	22
	48 h	24	42 [23]	30 [>98]	8 [96]	20 [98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the spectrum of the mixture of the crude material not mass recovery.
b. The principal enantiomer was (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a**.
c. The principal enantiomer was (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**.
d. The principal enantiomer was (1*R*,2*S*)-*cis*-2-nitrocyclohexyl acetate (1*S*,2*R*)-**100a**.
e. The principal enantiomer was (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*) **100b**.

4.2.7 Evidence of a dynamic interconversion process – chiral HPLC

In Milner's preliminary study on combination of the triethylamine-mediated cyclisation of 6-nitrohexanal **101** with the *Pseudomonas fluorescens* catalysed transesterification it became apparent that kinetic resolution was occurring albeit with lack of diastereoselectivity (see section 4.2.1).[45] Both *cis*- and *trans*-enantiomers (1*R*,2*S*)-*cis*- and (1*R*,2*R*)-*trans*-2-nitrocyclohexanol (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** were acetylated with excellent enantioselectivity observed of the generated acetates (1*R*,2*S*)-**100a** and (1*R*,2*R*)-**100b**. Significantly this process failed to go to completion with residual (1*R*,2*S*)-*cis*- and (1*R*,2*R*)-*trans*-2-nitrocyclohexanol (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** clearly visible by chiral HPLC; thus a further addition of triethylamine was added to promote dynamic interconversion and reaction completion. Critically there was no change as seen by chiral HPLC in these minor enantiomers with no interconversion visible.[45]

Thus, it was postulated in this early study that the triethylamine *cis* and *trans* interconversion process, believed at the time of investigation to operate *via* a dynamic ring opening/closing of 6-nitrohexanal **101**, was in fact effected *via* epimerisation of the acidic proton α to the nitro moiety. While (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** enantiomers are interconverted, and similarly (1*S*,2*S*)-**99b** and (1*S*,2*R*)-**99a** are interconverted by epimerisation, interconversion between (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** with (1*S*,2*R*)-**99a** and (1*S*,2*S*)-**99b** is not possible as the epimerisation mechanism does not allow scrambling of the stereochemical integrity at C1 (Scheme 4.33).



Scheme 4.33

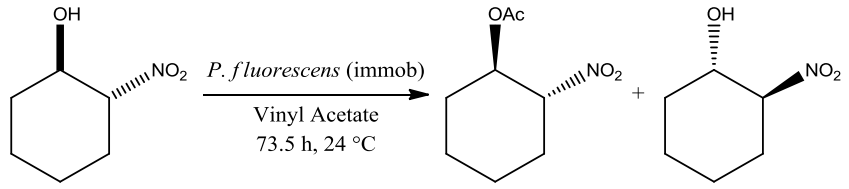
In order to confirm this theory, an investigation into the base-mediated interconversion process examining the stereochemistry at C1 and C2 by chiral HPLC was explored. Exposure of enantiopure (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** to a range of bases was investigated. Appearance of an enantiomer with variation of the stereochemistry at C1 *i.e.* appearance of the enantiomers (1*R*,2*R*)-**99b** and (1*R*,2*S*)-**99a** would indicate dynamic ring opening/closing process. Employing this protocol Milner identified DBU as a

potential base where both epimerisation and dynamic ring opening/closing mechanisms were tentatively observed.[45]

The objective in this study was to explore in detail the DBU-mediated interconversion of enantiopure (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, based on this promising one off preliminary result.[45] The first step in this investigation was preparative-scale hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** to access enantiopure (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** for the DBU-mediated chiral HPLC investigation. The large scale resolution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** was adapted from a procedure described from Milner.[45]

Pseudomonas fluorescens was the lipase of choice in Milner's study for preparative-scale demonstrating excellent efficiency and enantioselectivity in the analytical screens.[45] In this study, an immobilised version of *Pseudomonas fluorescens* obtained commercially[46] was analytically screened for the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (Table 4.18). It was envisaged that an immobilised lipase would enhance stability, enable repeated use and facilitate the separation of the enzyme catalyst easily from the reaction mixture during preparative-scale. However, in the analytical screen the *Pseudomonas fluorescens* (immob) demonstrated limited efficiency achieving only 38% conversion after 73.5 h. While high enantioselectivity of the *trans*-acetate (1*R*,2*R*)-**100b** was observed (>98% ee), the enantiopurity of the *trans*-alcohol (1*S*,2*S*)-**99b** suffered (61% ee) due to the poor conversion rate. Thus this immobilised lipase was considered unsuitable for preparative-scale and the free *Pseudomonas fluorescens* lipase employed by Milner was utilised instead.[45]

Table 4.18: Pseudomonas fluorescens *(immob) mediated transesterification of (±)-*trans*-2-nitrocyclohexanol(±)-**99b** in vinyl acetate*



(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Enzyme Source	Ratio 99a : 99b[b]	Conversion (%)		ee (%)		*E* value
		E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*, 2*S*)	Acetate *trans*-100b (1*R*, 2*R*)	
Pseudomonas fluorescens (immob)[a]	1 : 99	38	38	61	>98	185

a. *Pseudomonas fluorescens* (immob) was obtained commercially from Sigma-Aldrich.[46]
b. The ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in the starting material, determined by ^{1}H NMR spectroscopy.

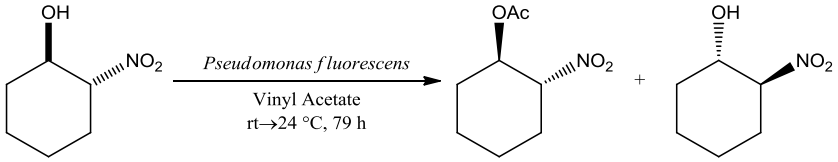
In this study reaction monitoring of the preparative-scale free *Pseudomonas fluorescens* mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** was conducted at 28 h and 55 h; an aliquot was withdrawn and following work-up, ^{1}H NMR and chiral HPLC analysis was performed to determine conversion and enantiopurity (Table 4.19). During the transesterification reaction, for the initial 28 h the reaction mixture was shaken at room temperature (<18 °C) however for the subsequent 51 h reaction temperature was fixed at 24 °C. An ideal 50% conversion estimated by the *E*-value calculator[71] was obtained after 79 h; significantly this was a far greater reaction time relative to Milner's study where 50%

conversion was achieved after 28 h at 20 °C.[45] It was postulated that denaturation and decrease in catalytic activity of the hydrolase *Pseudomonas fluorescens* may have occurred over prolonged storage resulting in the increased reaction time.

On reaction completion the reaction mixture was filtered through Celite® to remove the hydrolase, and the hydrolase washed with ethyl acetate. The combined organic extracts were concentrated under reduced pressure. The alcohol (1*S*,2*S*)-**99b** and acetate (1*R*,2*R*)-**100b** in the crude reaction mixture were isolated by column chromatography and analysed by ^{1}H NMR. Spectroscopic details were in agreement with those of the racemic material previously prepared (Figure 4.11 and 4.12).

While analysis by chiral HPLC of the crude product mixture showed 97% ee for (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, on chromatographic purification the enantiomeric excess of isolated pure (1*S*,2*S*)-**99b** was determined to be 95% ee obtained in a 45% yield. The enantiopure (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**100b** was isolated in 30% yield after purification with >98% enantiomeric excess. Although the enantioselectivity obtained in this study of the acetate (1*R*,2*R*)-**100b** was similar to that observed by Milner the enantioselectivity of the isolated alcohol (1*S*,2*S*)-**99b** was slightly lower (95% ee *vs.* >99% ee).[45] In addition, the yield of the isolated alcohol (1*S*,2*S*)-**99b** and acetate (1*R*,2*R*)-**100b** was slightly decreased relative to that reported by Milner. However this was anticipated due to the two aliquots of reaction mixture removed during reaction monitoring.[45]

Table 4.19: Large scale Pseudomonas fluorescens *mediated transesterification of (±)-*trans*-2 nitrocyclohexanol (±)-**99b** in vinyl acetate*



(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*,2*S*) [yield %][b]	Acetate *trans*-100b (1*R*,2*R*) [yield %][b]	
1	28 h	rt	24	25	31	>98	134
2	55 h	24	44	-	78	>98	>200
3	79 h	24	50	50	97[a] [45]	>98 [30]	>200

a. While analysis by chiral HPLC of the crude product mixture showed 97% ee for (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, on chromatographic purification the enantiomeric excess [ee (%)] of isolated (1*S*,2*S*)-**99b** was determined to be 95% ee.
b. Isolated yield following column chromatography.

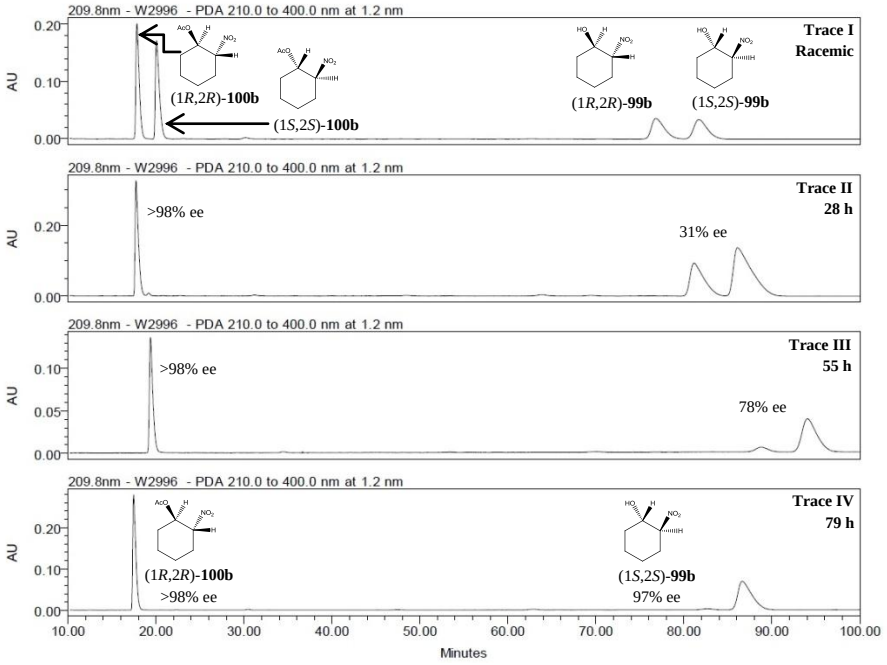


Figure 4.11: HPLC ***Trace I:*** *A racemic mixture of* (±)-trans-*2-nitrocyclohexyl acetate* (±)-***100b*** *and* (±)-trans-*2-nitrocyclohexanol* (±)-***99b***. ***Trace II:*** *Reaction sampling 28 h.* ***Trace III:*** *Reaction sampling 55 h.* ***Trace IV:*** *Reaction sampling 79 h,* (*1*R,*2*R)-trans-*2-nitrocyclohexyl acetate* (*1*R,*2*R)-***100b***, *>98% ee,* (*1*S,*2*S)-trans-*2-nitrocyclohexanol* (*1*S,*2*S)-***99b***, *97% ee. For HPLC conditions see appendix I.*

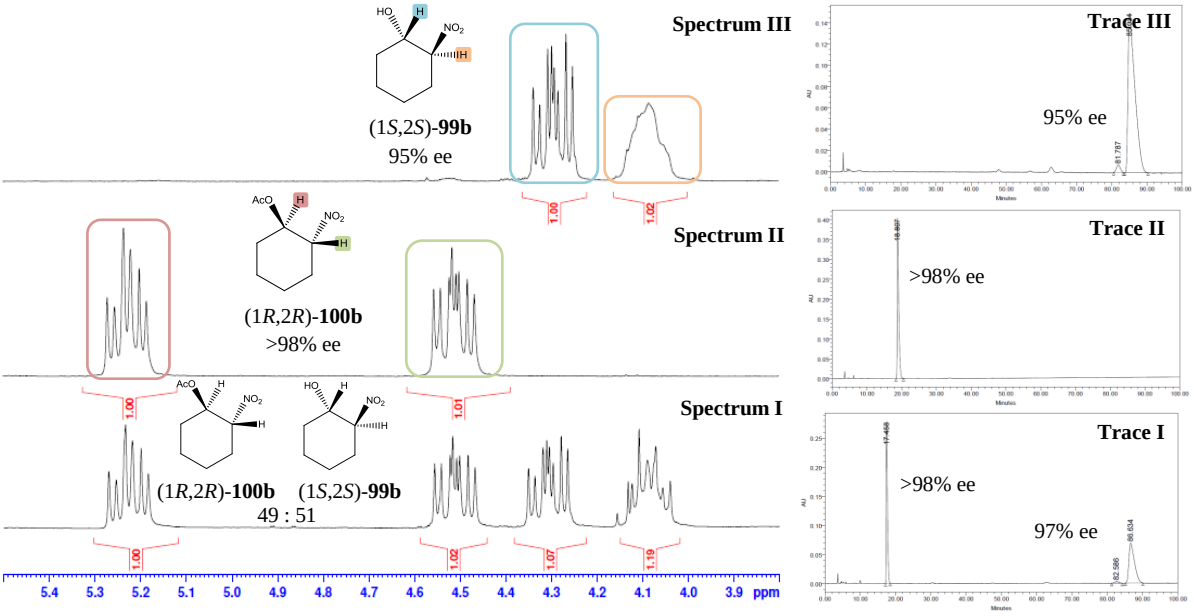


Figure 4.12: ^{1}*H NMR* ***Spectrum I*** *and HPLC* ***Trace I:*** *Crude product following preparative-scale transesterification containing a mixture of enantiopure* (*1*R,*2*R)-trans-*2-nitrocyclohexyl acetate* (*1*R,*2*R)-***100b***, *>98% ee and enantioenriched* (*1*S,*2*S)-trans-*2-nitrocyclohexanol* (*1*S,*2*S)-***99b,*** *97% ee, 49 : 51 respectively.* ^{1}*H NMR* ***Spectrum II*** *and HPLC* ***Trace II:*** *Purified* (*1*R,*2*R)-trans-*2-nitrocyclohexyl acetate* (*1*R,*2*R)-***100b*** *>98% ee following column chromatography.* ^{1}*H NMR* ***Spectrum III*** *and HPLC* ***Trace III:*** *Purified* (*1*S,*2*S)-trans-*2-nitrocyclohexanol* (*1*S,*2*S)-***99b*** *95% ee following column chromatography (*^{1}*H NMR recorded in* $CDCl_3$ *at 300 MHz)*

Once the enantioenriched (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** was obtained the next step was to investigate the progress of the DBU-mediated interconversion by chiral HPLC. Appearance of the enantiomer (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a** only would indicate an epimerisation process, whereas appearance of either enantiomers (1*R*,2*S*)-*cis*- or (1*R*,2*R*)-*trans*-2-nitrocyclohexanol (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** can only be rationalised *via* a dynamic ring opening/closing process. Two experiments were performed investigating 1.0 equivalent and 0.5 equivalent of DBU (Table 4.20). The base was added to the enantioenriched material (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** (50.0 mg, 95 % ee) in *tert*-butyl methyl ether (TBME) (10 mL) and the reaction mixture was assayed by chiral HPLC after 24 h and 48 h. Quantification of the *cis*- and *trans*-2-nitrocyclohexanol diastereomers **99a** and **99b** by chiral HPLC was conducted during this study (Figure 4.13 and 4.14). Peak area of the *cis*- and *trans*-2-nitrocyclohexanols **99a** and **99b** was measured, compared to the corresponding calibration curve of peak area versus concentration at the appropriate injection volume and concentration determined. This facilitated direct comparison of chiral HPLC analysis to the ^{1}H NMR studies investigating the dynamic ring opening/closing process.

*Table 4.20: Enantioenriched (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b,** 94% ee following exposure to 1.0 eq. and 0.5 eq. of DBU at 24 h and 48 h*

DBU (eq.)	**Reaction Time**	**Alcohol (±)-99**	
		***cis*-99a (%) [ee (%)]**[a]	***trans*-99b (%) [ee (%)]**[b]
1	0 h	-	100 [94]
	24 h	16 [31]	84 [34]
	48 h	17 [24]	83 [23]
0.5	0 h	-	100 [94]
	24 h	15 [2]	85 [3]
	48 h	13 [4]	87 [1]

a. The principal enantiomer was (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a**.
b. The principal enantiomer was (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**.

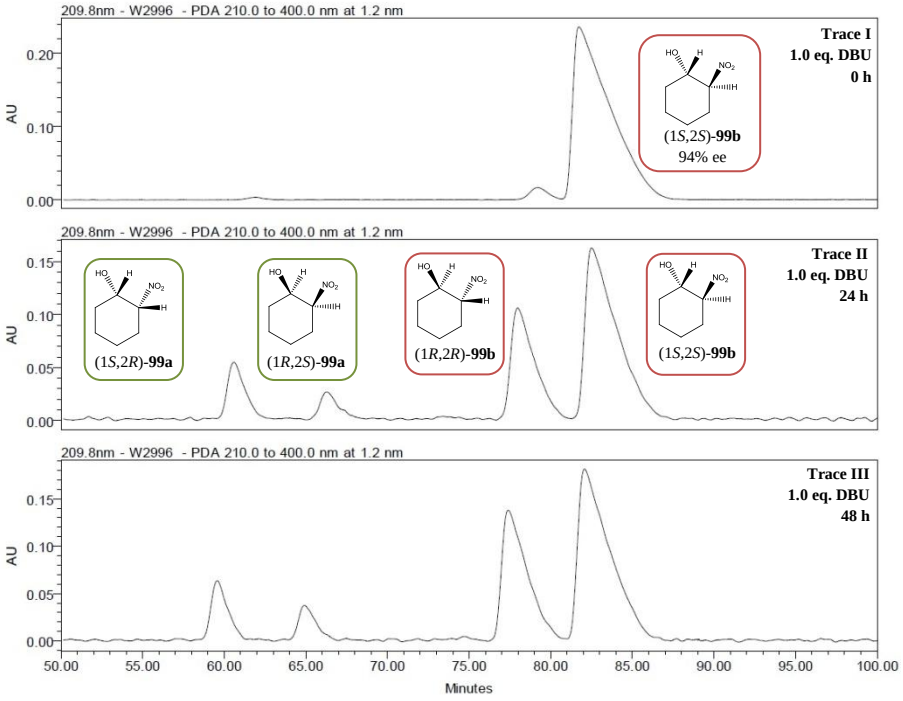


*Figure 4.13: (1S,2S)-*trans*-2-Nitrocyclohexanol (1S,2S)-**99b** 94% ee after exposure to 1.0 equivalent of DBU at 24 h and 48 h.*

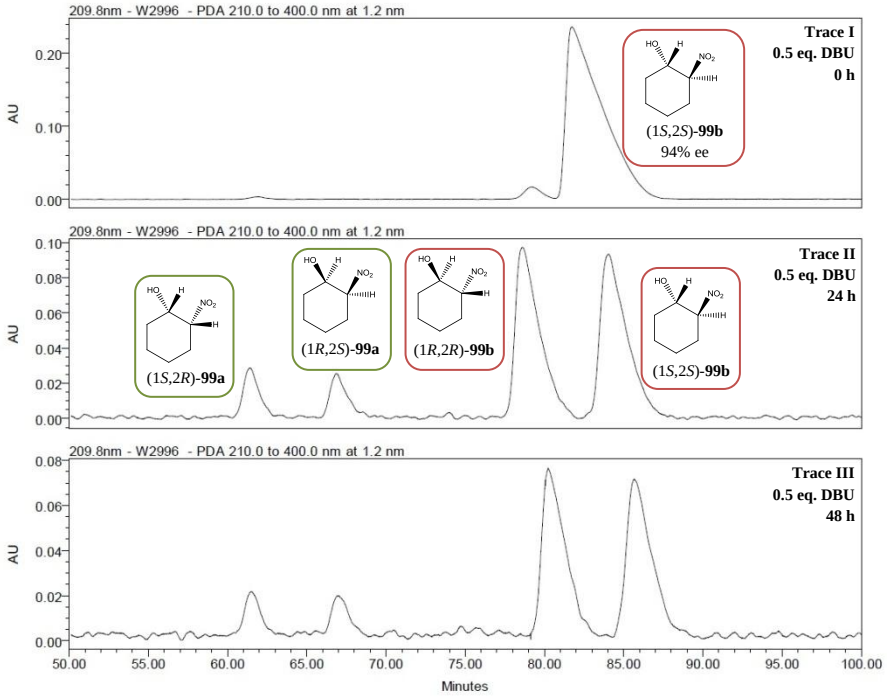


*Figure 4.14: (1S,2S)-*trans*-2-Nitrocyclohexanol (1S,2S)-**99b** 94% ee after exposure to 0.5 equivalent of DBU at 24 h and 48 h.*

In both studies chiral HPLC analysis demonstrates clear evidence for formation of the enantiomers (1*R*,2*S*)-*cis*- or (1*R*,2*R*)-*trans*-2-nitrocyclohexanol (1*R*,2*S*)-**99a** and (1*R*,2*R*)-**99b** with *R* stereochemistry at the C1 position; thus, dynamic ring opening/closing *via* 6-nitrohexanal **101** occurs at both DBU concentrations (0.5 and 1.0 equivalent).

When 1.0 equivalent of DBU was employed it appeared that the epimerisation process at C2 also operated in conjunction with the dynamic ring opening/closing mechanism, evident by the dominant enantiomers (1*S*,2*S*)-*trans*- and (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a** and (1*S*,2*S*)-**99b** at 24 h and 48 h. Furthermore, the prevalence of the (1*S*,2*R*)-**99a** and (1*S*,2*S*)-**99b** as the principal enantiomers over time suggests that the epimerisation process was in fact the leading contributor to the interconversion of (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** not dynamic ring opening/closing. Furthermore, complete racemisation of the *cis*- and *trans*-2-nitrocyclohexanols **99a** and **99b** was not achieved within 48 h. This postulation may be supported by the ^{1}H NMR experiments conducted where deuterium exchange of C(2)HNO_2 proton was observed at 0.1 equivalent of DBU which was tentatively taken as evidence of the epimerisation process. In addition the thermodynamic ratio of (±)-**99a** and (±)-**99b** (17 : 83 respectively) observed in this chiral HPLC study agrees with the ^{1}H NMR investigation of the DBU-mediated ring closing (16 : 84 respectively). The agreement in the thermodynamic ratios is particularly good considering different solvents ($CDCl_3$ *vs.* TBME), concentrations and amounts of DBU were employed. The rate at which the interconversion occurs from these experiments cannot be readily compared to the earlier data in the ^{1}H NMR studies due to these changes.

Surprisingly, at the lower concentration of DBU (0.5 equivalent) isomerisation and complete racemisation of the enantioenriched (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** was complete after 24 h with a thermodynamic ratio of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** 13 : 87 respectively. This agreed with the thermodynamic ratio achieved during ^{1}H NMR analysis of the DBU (0.1 equivalent) mediated ring closing of 6-nitrohexanal **101**. Additionally there was no clear evidence in this study to suggest that epimerisation was occurring in conjunction with the dynamic ring opening/closing process. However further investigation of the DBU (0.5 equivalent) mediated process incorporating shorter time intervals between assays is warranted due to the unexpected observation of more rapid racemisation in this study than with 1.0 equivalent of DBU. The difference in the studies illustrated in Figures 4.13 and 4.14 may reflect sensitivity to minor changes in the reaction conditions for example the presence of water. The data as shown were based on one off experiments and not reproduced.

In summary, while it is evident that DBU successfully mediated interconversion of (1*S*,2*S*)-**99b** *via* the dynamic ring opening and closing mechanism, epimerisation at the C2 position was also evident.

4.2.8 Project conclusion

In summary, considerable progress towards the mechanistic understanding of the DBU-mediated dynamic kinetic resolution process of (±)-2-nitrocyclohexanol (±)-**99** has been achieved. For the first time, two hydrolases *Candida antarctica* lipase B (immob) and *Candida antarctica* lipase A have been identified that demonstrate diastereoselectivity in the resolution of (±)-2-nitrocyclohexanol (±)-**99** signifying a major step forward in the development of a dynamic kinetic resolution process. Further investigation of the relative rates of *Candida antarctica* lipase B (immob) mediated transesterification of an equimolar mixture of diastereomers (±)-**99a** and (±)-**99b** with detailed monitoring by ^{1}H NMR and

chiral HPLC analysis demonstrated selective efficient acetylation of the *trans*-diastereomer (±)-**99b** with excellent enantioselection.

Interconversion of the (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** in the presence of DBU has been examined by both ^{1}H NMR and chiral HPLC analysis. Significantly, direct comparison of these two analytical methods was facilitated by the quantification of (±)-**99a** and (±)-**99b** by chiral HPLC. While interconversion of the diastereomers (±)-**99a** and (±)-**99b** was evident by ^{1}H NMR generating a thermodynamic ratio of 16 : 84 respectively, ^{1}H NMR analysis alone was unable to definitively define the mechanistic pathway.

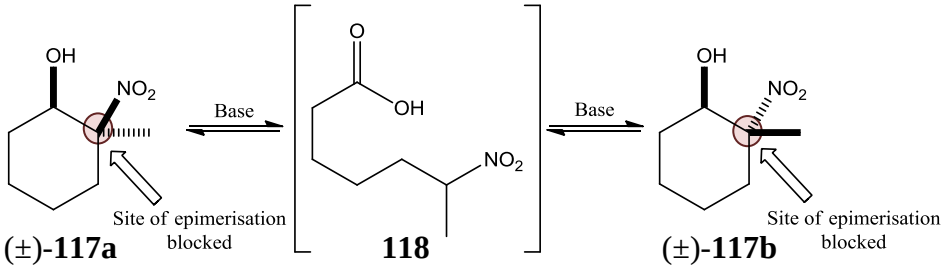
Thus, the DBU-mediated interconversion of enantioenriched (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** was monitored by chiral HPLC. Appearance of the enantiomers (1*R*,2*R*)-**99b** and (1*R*,2*S*)-**99a** with a change in the stereochemical integrity at C1, was evident by chiral HPLC, providing conclusive evidence that the ring opening/closing mechanism *via* 6-nitrohexanal **101** operated in the DBU-mediated interconversion. However, this investigation also provided evidence of the competing epimerisation process which was tentatively assigned as the dominant process of interconversion. Thus, although DBU operates through dynamic ring opening and closing, epimerisation is still a significant competing pathway and thereby complicates the development of an effective dynamic kinetic resolution process.

Therefore extension of this work to (±)-2-methyl-2-nitrocyclohexanol (±)-**117** was undertaken at this point as it was envisaged that the epimerisation mechanism for interconversion in this substrate would be blocked by a methyl moiety and accordingly *cis* (±)-**117a** and *trans* (±)-**117b** interconversion can only occur *via* ring opening/closing of 6-nitroheptanal **118** eliminating a significant complication in the development of a dynamic kinetic resolution process.

4.3 Dynamic kinetic resolution of the intramolecular Henry reaction of (±)-2-methyl-2-nitrocyclohexanol (±)-117 through lipase catalysis

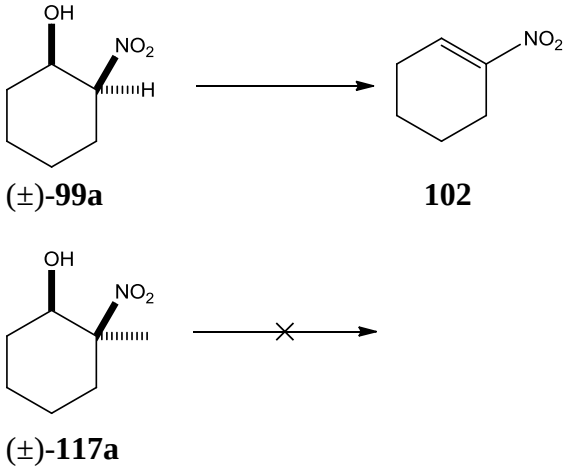
4.3.1 Background to the project

Significant progress has been achieved in this study towards the development of a one-pot intramolecular Henry reaction of 6-nitrohexanal **101** with dynamic kinetic lipase-mediated resolution of (±)-2-nitrocyclohexanol (±)-**99**. However, a significant complication in this process is competing epimerisation *via* deprotonation α to the nitro group (pK_a CH_3NO_2 ~10.2). Therefore investigation of a modified substrate (±)-2-methyl-2-nitrocyclohexanol (±)-**117** was envisaged that would avoid epimerisation at C2 due to the presence of a methyl moiety geminal to the nitro group and consequently interconversion of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** can only occur through ring opening and closing of the aldehyde 6-nitroheptanal **118** (Scheme 4.34).



Scheme 4.34

Furthermore, the absence of an acidic proton alpha to the nitro group removes the elimination pathway to form the dehydration product 1-nitrocyclohexene **102** which complicated the development of a dynamic kinetic resolution of (±)-2-nitrocyclohexanol (±)-**99** (Scheme 4.35).



Scheme 4.35

Finally, increasing the steric bulk at C2 was envisaged to assist hydrolase selectivity and increase diastereoselectivity (Figure 4.15).

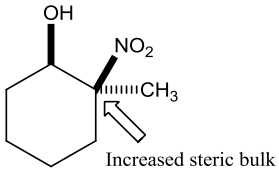


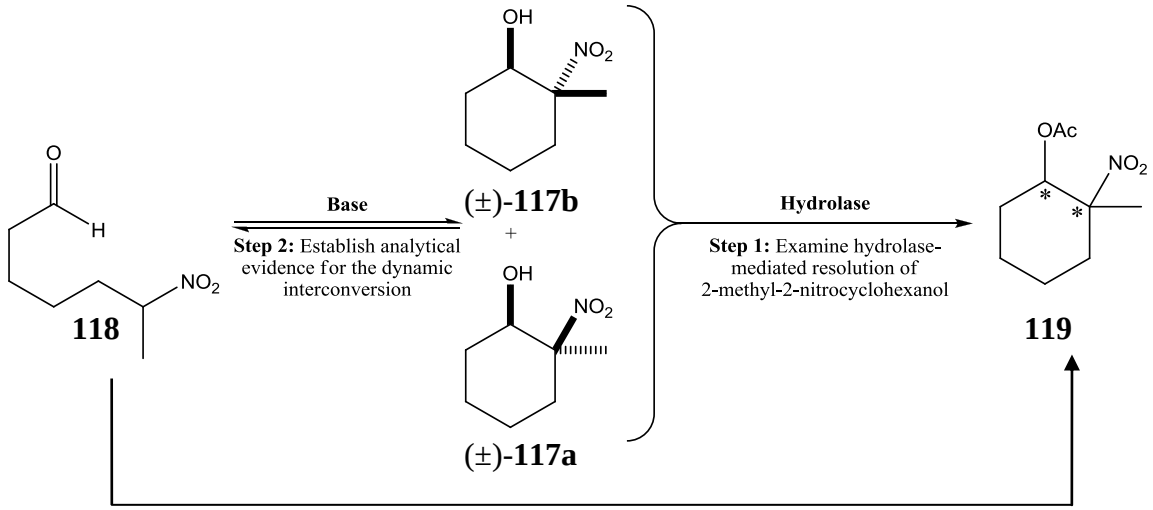
Figure 4.15

4.3.2 Objectives of the project

At the outset this project was investigated in a stepwise manner (Scheme 4.36). Similar to Milner's[45] approach towards the bioresolution of an intramolecular Henry reaction of 6-nitrohexanal **101**, the first step in this study involved examination of the lipase-mediated kinetic resolution of the racemic (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b**, establishing the most efficient hydrolase(s) to perform this biotransformation diastereoselectively.

The second step was examination by ^{1}H NMR analysis of the base-mediated intramolecular Henry reaction and associated interconversion process between the resultant (±)-*cis*- and (±)-*trans*-diastereomers (±)-**117a** and (±)-**117b** through ring opening and closing *via* the aldehyde 6-nitroheptanal **118**

Once the objectives of the two individual steps has been achieved it was envisaged that combination of the dynamic interconversion of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** with the lipase-mediated diastereoselective and enantioselective transesterification would selectively lead to quantitative yields of a single stereoisomer of 2-methyl-2-nitrocyclohexyl acetate **119**.



Scheme 4.36

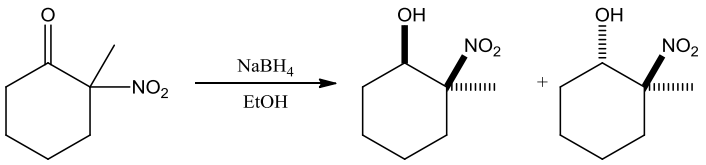
The specific objectives of this project can be summarised as follows;

- To prepare the racemic synthetic targets (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** and (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-**119b** for investigation of hydrolase-mediated kinetic resolution.

- To develop chiral HPLC conditions where all four enantiomeric pairs of the β-nitroalcohols (±)-**117a** and (±)-**117b** and nitro acetates (±)-**119a** and (±)-**119b** are resolved on a single trace, facilitating efficient analysis.
- To perform individual screening assays of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** against a series of hydrolases investigating optimal reaction conditions for transesterification and identifying a suitable hydrolase for the selective hydrolase-mediated acetylation of one diastereomer and one enantiomer of (±)-2-methyl-2-nitrocyclohexanol (±)-**117**.
- To prepare the aldehyde 6-nitroheptanal **118** and establish analytical evidence by ^{1}H NMR of the dynamic interconversion process between the two diastereomers (±)-**117a** and (±)-**117b** *via* ring opening and closing of 6-nitroheptanal **118**.
- To combine the intramolecular Henry reaction with the dynamic interconversion of the β-nitroalcohols (±)-**117a** and (±)-**117b** and the lipase-mediated transesterification in a dynamic kinetic resolution process.

4.3.3 Synthesis of substrates

The first objective in this investigation was to synthesise the following novel racemic synthetic targets: (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** and (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-**119b**. It was envisaged that synthesis of 2-methyl-2-nitrocyclohexanone **120** would provide access to the alcohol diastereomers (±)-**117a** and (±)-**117b** by sodium borohydride reduction (Scheme 4.37), as this synthetic protocol was successfully implemented in the synthesis of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** *via* the reduction of 2-nitrocyclohexanone **103**.

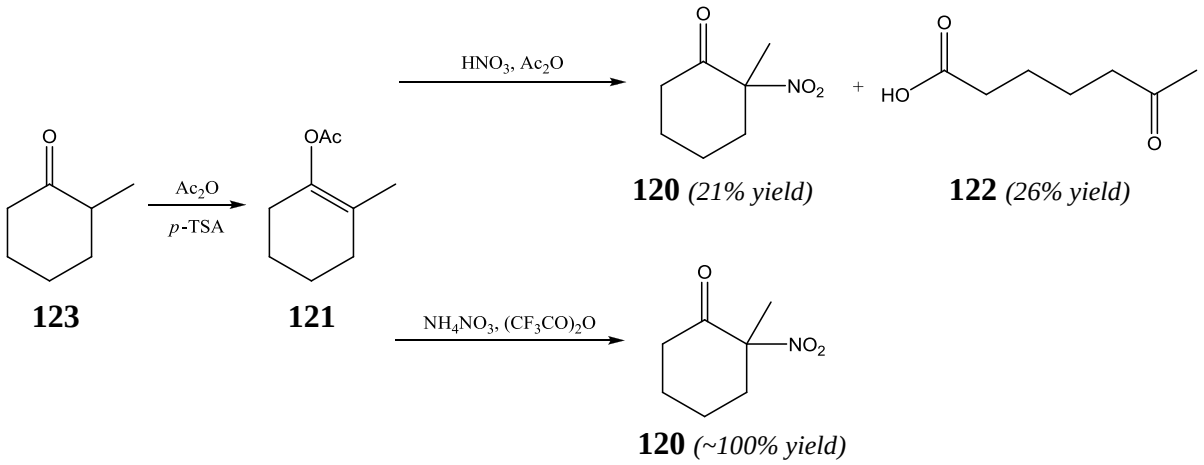


120 (±)-**117a** (±)-**117b**

Scheme 4.37

4.3.3.1 Synthesis of 2-methyl-2-nitrocyclohexanone 120

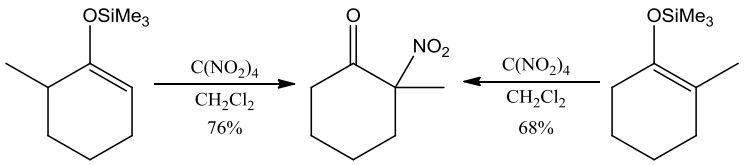
Direct electrophilic nitration of ketones with nitric acid as a synthetic method to α-nitroketones suffers from the formation of a variety of oxidised byproducts. Alternatively, initial activation of the ketones to their enolates, enol acetates and silyl enol ethers followed by nitration provides ready access to the desired α-nitroketones.[4] A number of synthetic routes have been reported in the literature to 2-methyl-2-nitrocyclohexanone **120**.[74-77] Zajac has described the preparation of the α-nitroketone **120** by nitration of the enol acetate 2-methyl-1-acetoxycyclohexene **121** with nitric acid in acetic anhydride (Scheme 4.38).[76] However, the ring cleavage product, 6-oxoheptanoic acid **122** was reported as the major component with low yields of the desired 2-methyl-2-nitrocyclohexanone **120**. Significantly, alternative use of the more powerful nitrating agent trifluoroacetyl nitrate prepared from ammonium nitrate and trifluoroacetic anhydride provided quantitative yield of 2-methyl-2-nitrocyclohexanone **120** (Scheme 4.38).[77]



123 **121** **120** *(21% yield)* **122** *(26% yield)* **120** *(~100% yield)*

Scheme 4.38[76,77]

Rathore reports a facile method for the nitration of kinetic and thermodynamic silyl enol ethers **124** and **125** with tetranitromethane to give 2-methyl-2-nitrocyclohexanone **120** in 76% and 68% yield respectively (Scheme 4.39).[74] The mechanism has been rationalised to incorporate an electron transfer pathway from the silyl enol ether **124** or **125** to tetranitromethane. A subsequent fast homolytic coupling of the resultant cation radical of the silyl enol ether **125** with $NO_2^{\bullet}$ leads to α-nitroketones.



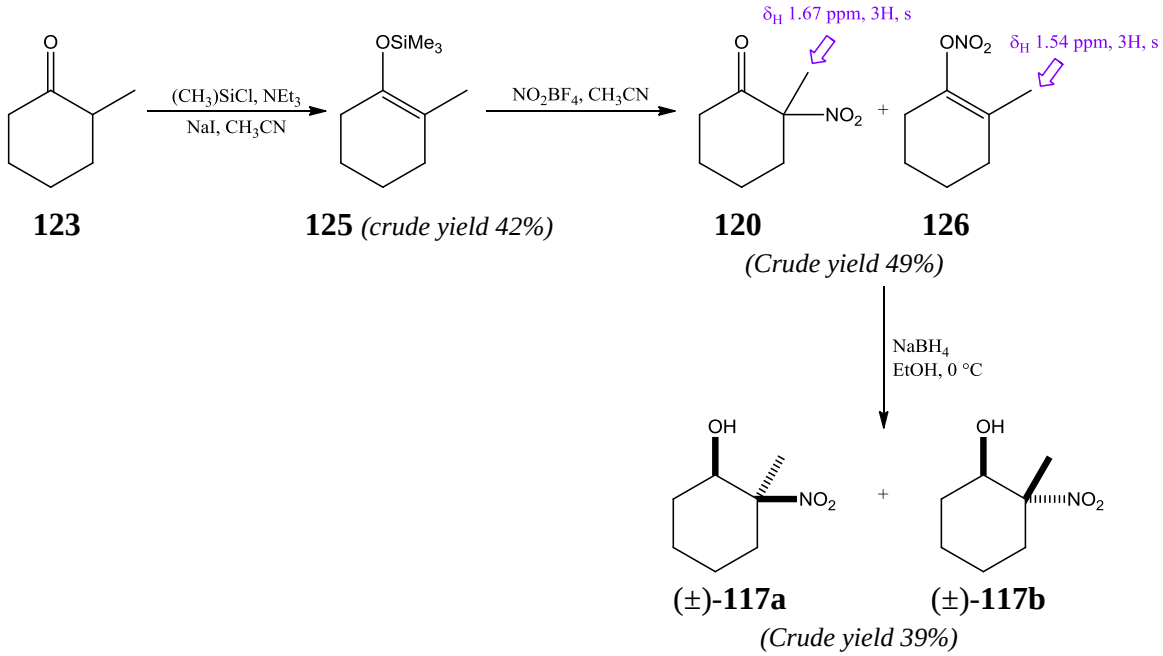
124 **120** **125**

Scheme 4.39[74]

In this study, the first approach investigated was the procedure adapted from Zajac involving nitration of the silyl enol ether **125** with nitronium tetrafluoroborate in acetonitrile.[76] 2-Methylcyclohexanone **123** was converted to the thermodynamic silyl enol ether **125** by means of chlorotrimethylsilane, triethylamine and pre-dried sodium iodide in distilled acetonitrile by the method of Zhang (Scheme 4.40).[78] Nitration of the crude silyl enol ether **125** with nitronium tetrafluoroborate in acetonitrile led to the desired 2-methyl-2-nitrocyclohexanone **120**; however, 2-methylcyclohexene-1-nitrate **126** (~33%) was also evident which could not be removed by column chromatography upon several attempts. Zajac also reports troublesome purification of 2-methyl-2-nitrocyclohexanone **120** with trace evidence of the nitrate ester byproduct **126** observed in the ^{1}H NMR spectrum, following attempted purification of **120** by vacuum distillation.[76]

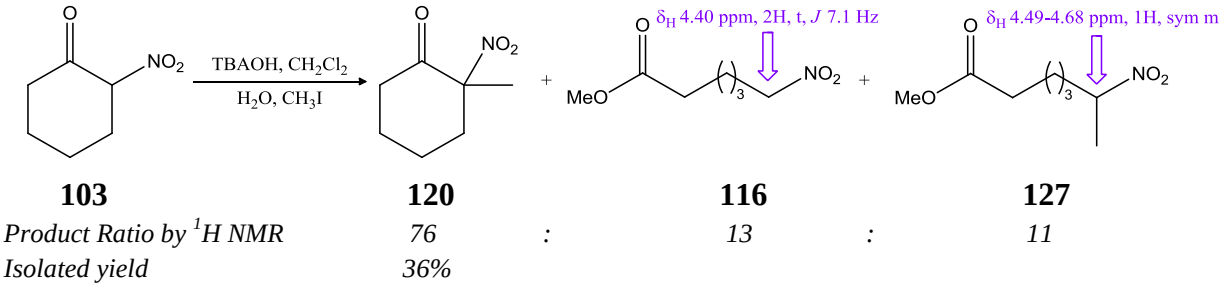
A fraction obtained from the attempted chromatographic purification contained a mixture of 2-methyl-2-nitrocyclohexanone **120** and the nitrate ester **126** (59 : 41 respectively) which was brought forward to the sodium borohydride reduction. The desired (±)-2-methyl-2-nitrocylohexanols (±)-**117a** and (±)-**117b** were successfully isolated following column chromatography, notably, with no trace of the nitrate ester **126**. However, the reduction proceeded in poor yield due to the impure starting material. Thus, this synthetic route

warranted further optimisation to minimise the formation of **126** to access analytically pure 2-methyl-2-nitrocyclohexanone **120**.



Scheme 4.40

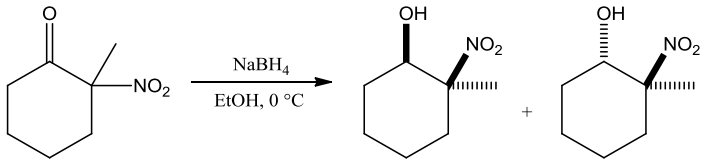
An alternative approach to the synthesis of 2-methyl-2-nitrocyclohexanone **120** was explored, involving the direct introduction of a methyl moiety by alkylation of the α-nitroketone **103**.[76] Initially, direct methylation of 2-nitrocyclohexanone **103** was attempted with methyl iodide and sodium hydride, resulting in recovery of starting material. The methylation was then investigated under phase-transfer conditions. 2-Nitrocyclohexanone **103** was successfully methylated by employing 40% aqueous tetrabutylammonium hydroxide (TBAOH) in dichloromethane and methyl iodide leading to a crude mixture of 2-methyl-2-nitrocyclohexanone **120**, methyl 6-nitrohexanoate **116** and methyl 6-nitroheptanoate **127** (76 : 13 : 11 respectively). The ring cleavage products methyl 6-nitroheptanoate **127** and methyl 6-nitrohexanoate **116** were identified in the ^{1}H NMR spectrum by the characteristic signals of the proton(s) alpha to the nitro moiety (Scheme 4.41). The desired 2-methyl-2-nitrocyclohexanone **120** was isolated as a colourless oil in moderate yield (36%) and excellent purity following purification by column chromatography. Spectral characteristics of **120** agreed with previous literature reports.[74-77]



Scheme 4.41

4.3.3.2 Synthesis of (±)-2-methyl-2-nitrocyclohexanol (±)-117

With a synthetic route to 2-methyl-2-nitrocyclohexanone **120** established, the next step was sodium borohydride reduction of the ketone **120** to the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** (Scheme 4.42). This was performed according to the procedure outlined for the reduction of 2-nitrocyclohexanone **103** and yielded a crude mixture of diastereomeric β-nitroalcohols (±)-**117a** and (±)-**117b** (41 : 59 respectively). The disappearance of the carbonyl stretch at ν_{max} 1732 cm^{-1} indicated reaction completion. The diastereomers are distinguishable in the ^{1}H NMR spectrum with two sets of signals observed. Significantly there is no evidence by ^{1}H NMR analysis of a competing dehydration side reaction, in contrast to the reduction of 2-nitrocyclohexanone **103**. As expected the presence of a methyl group geminal to the nitro moiety in 2-methyl-2-nitrocyclohexanone **120** prevents elimination occurring.



120	(±)-**117a**	(±)-**117b**
Product ratio by ^{1}H NMR	*41*	*59*
Isolated yield	*19%*	*28%*

Scheme 4.42

The (±)-*cis*- and (±)-*trans*-diastereomers (±)-**117a** and (±)-**117b** have similar polarity. Nevertheless, pure samples of each isomer (±)-**117a** and (±)-**117b** were obtained by careful column chromatography on silica gel. However, low yields were obtained of the isolated diastereomers (±)-**117a** and (±)-**117b** due to their persistent co-elution and hence difficult purification. In an effort to achieve better separation between the two diastereomers (±)-**117a** and (±)-**117b** purification by column chromatography on neutral alumina was investigated; however, the (±)-*cis*- and (±)-*trans*-diastereomers (±)-**117a** and (±)-**117b** were unresolved by this method.

The (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** had previously been reported in the literature.[79] Ranu successfully reduced 2-methyl-2-nitrocyclohexanone **120** employing zinc borohydride in 1,2-dimethoxyethane to give a crude mixture (55 : 45) of diastereomers (±)-**117a** and (±)-**117b**, however no stereochemical assignment or characterisation data was reported in this paper.[79] In this study, the assignment of the relative stereochemistry of (±)-**117a** and (±)-**117b** was initially investigated by a NOE experiment at 600 MHz on the major diastereomer (±)-**117b.** No enhancement of signals was observed after irradiation of C(2)CH_3 at 1.60 ppm, C(1)OH at 2.63 ppm and C(1)H at 4.28 ppm. However, relative stereochemistry was confirmed after derivatisation of (±)-**117a** to (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and subsequent crystallographic determination of the structure (see section 4.3.3.3).

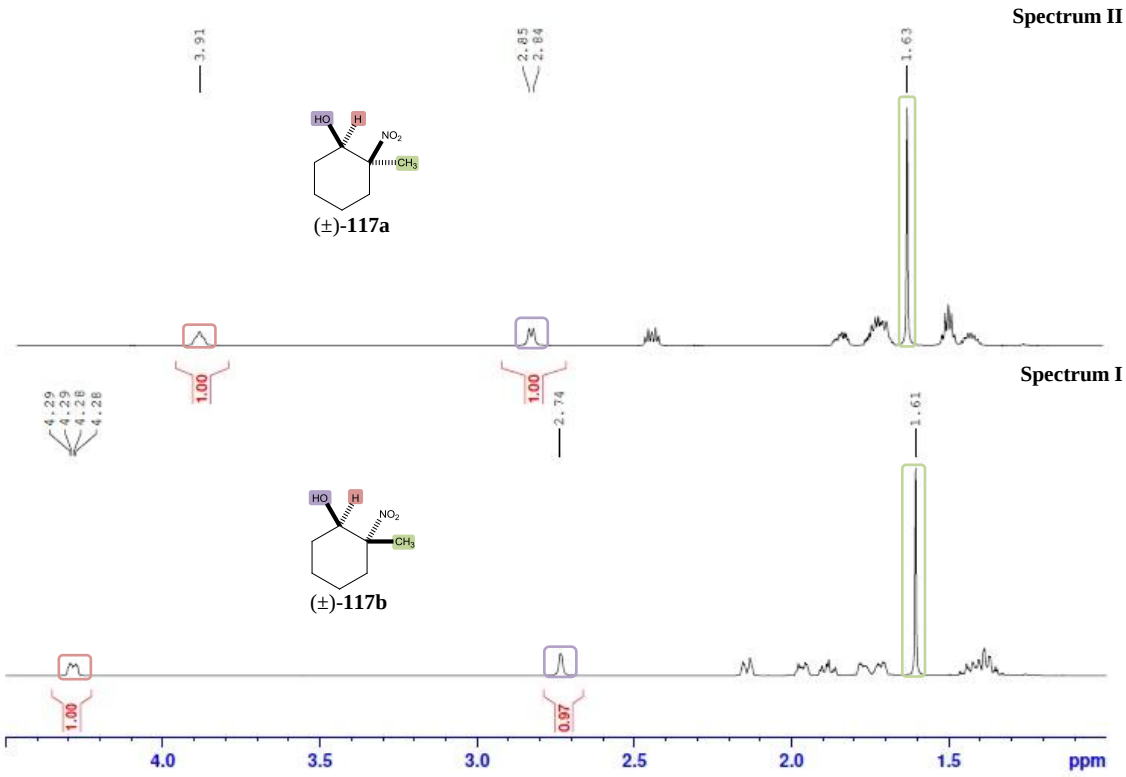


Figure 4.16: *^{1}H NMR* ***Spectrum I:*** *Purified (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** following column chromatography.* ***Spectrum II:*** *Purified (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** following column chromatography (all spectra recorded in $CDCl_3$ at 600 MHz).*

The IR spectra of the two diastereomers (±)-**117a** and (±)-**117b** are characterised by a broad OH peak at ν_{max} 3376-3445 cm^{-1} and asymmetrical and symmetrical stretching of the nitro group at ν_{max} 1538-1539 cm^{-1} and ν_{max} 1340-1352 cm^{-1}. Interestingly the aforementioned bands absorb at a higher frequency in (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** relative to the *trans*-diastereomer (±)-**117b**. Analysis by ^{1}H NMR identifies key spectroscopic features distinct to each diastereomer (±)-**117a** or (±)-**117b** (Figure 4.16). A larger deshielding effect is observed in (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** for the proton geminal to the hydroxyl group (δ_H 4.28-4.29 ppm) relative to the (±)-*cis*-diastereomer (±)-**117a** (δ_H 3.91 ppm) due to its proximity in space to the nitro group. Conversely the signal for the OH proton in (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** appears further downfield as a doublet at δ_H 2.84 ppm relative to the (±)-*trans*-diastereomer (±)-**117a** at δ_H 2.74 ppm.

Significant broadening of the C(5)H_2, C(3)H_2 and C(1)H signal is observed in the ^{13}C NMR spectrum for (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (Figure 4.17). The slow interconversion of the two chair forms may be contributing to this broadening effect. If the chair forms are interconverting, decreasing the temperature should slow down the interconversion and might be expected to lead to a splitting of the signals. Broadening is observed to a lesser extent for the signals C(2)***C***H_3 and C(3)H_2 in the ^{13}C NMR spectrum of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**.

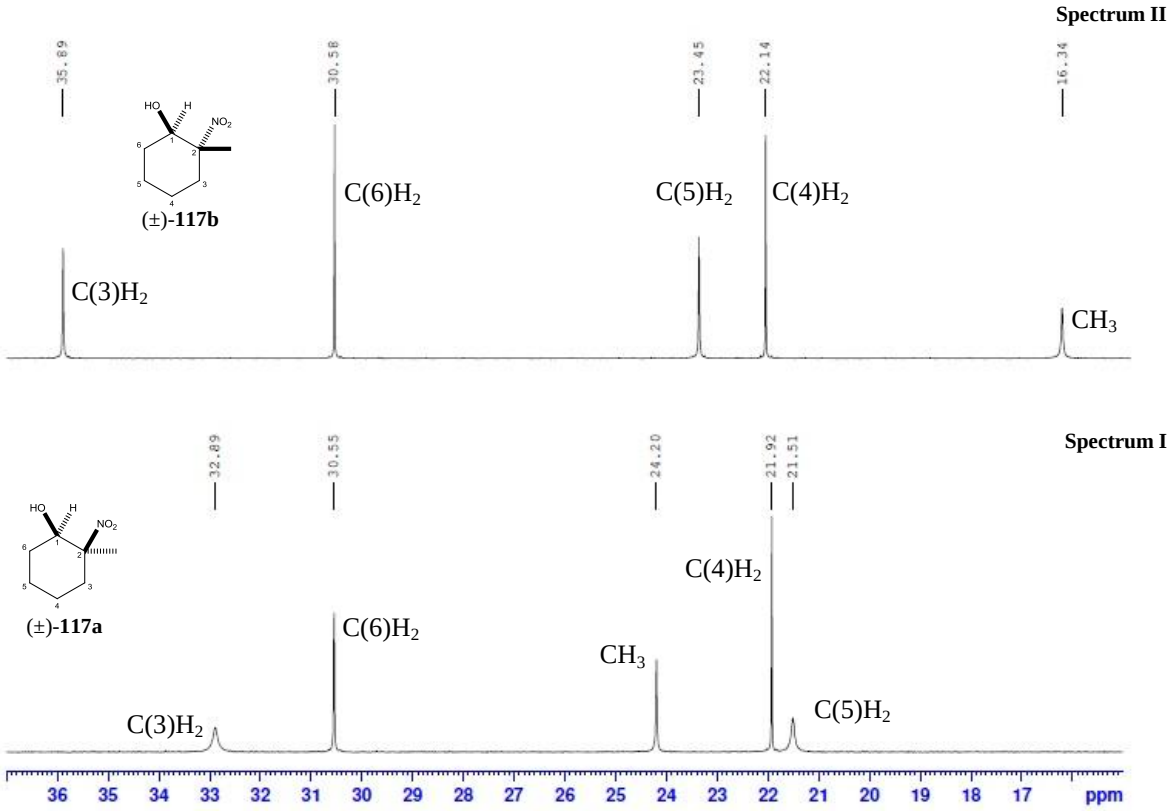
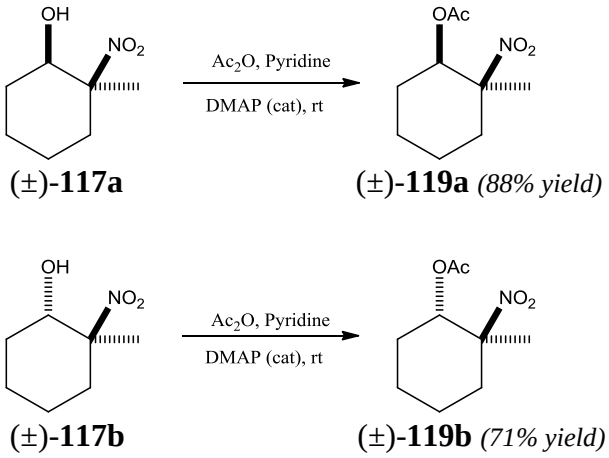


Figure 4.17: ^{13}C *NMR* ***Spectrum I:*** *Purified (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** following column chromatography.* ***Spectrum II:*** *Purified (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** following column chromatography (all spectra recorded in $CDCl_3$ at 600 MHz).*

4.3.3.3 Synthesis of (±)-2-methyl-2-nitrocyclohexyl acetate (±)-119

The procedure employed in the acetylation of the (±)-*cis*-2-nitrocyclohexanol (±)-**99a** utilising acetyl chloride and excess DMAP in dichloromethane was adapted for the acetylation of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**. This reaction failed to go to completion after stirring under nitrogen for 12 h. Starting material (±)-**117a** and the desired acetylated product (±)-**119a** (38 : 62 respectively) were evident in the ^{1}H NMR spectrum of the crude reaction mixture. Therefore the acetylation was repeated with acetic anhydride in pyridine with a catalytic amount of DMAP. This procedure had previously worked successfully for the acetylation of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** and in this study when applied for the acetylation of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** reaction completion was achieved after 16 h. The (±)-*cis*-acetate (±)-**119a** was obtained as a white solid in good yield (88%). Significantly, the crude (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** was of excellent purity and further purification was not required. This is in direct contrast to the crude (±)-*cis*-2-nitrocyclohexyl acetate (±)-**100b** in which starting material (±)-**99b** and DMAP were evident in the ^{1}H NMR spectrum and chromatographic purification was not feasible due to the ease of formation of the dehydration product **102** on silica gel.

The same reaction conditions were utilised for the acetylation of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and the *trans*-acetate (±)-**119b** was obtained as a light yellow oil in good yield (71%) and of sufficient purity to warrant no further purification (Scheme 4.43).



Scheme 4.43

The (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetates (±)-**119a** and (±)-**119b** had not previously been reported in the literature and thus full characterisation was obtained during this research. The molecular ion peak for an aliphatic mononitro compound is seldom observed and in this study the molecular ion of the novel acetates (±)-**119a** and (±)-**119b** was weak or absent in the nominal mass spectrum with the main peaks attributable to the hydrocarbon fragment minus the nitro moiety. High resolution mass spectrometry was successfully obtained of this hydrocarbon fragment. The relative stereochemistry of (±)-**119a** and (±)-**119b** was determined by single crystal X-ray diffraction on a crystalline sample of (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** recrystallised from deuterated chloroform (Figure 4.18).[80] Full structural details are contained on the accompanying CD.

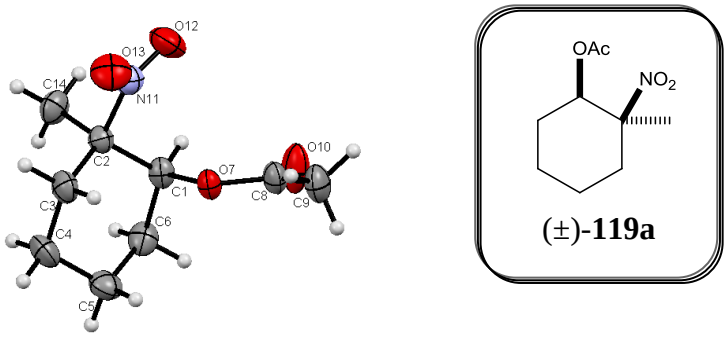


*Figure 4.18: A view of (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** showing the structure and relative stereochemistry. Anisotropic displacement parameters are drawn at the 30% probability level.*

IR analysis of the acetates (±)-**119a** and (±)-**119b** demonstrates a strong carbonyl stretch at ν_{max} 1738 and 1748 cm^{-1} respectively. ^1H NMR spectroscopy aided by COSY and HETCOR 2D NMR experiments identified characteristic peaks attributable to each diastereomer (±)-**119a** and (±)-**119b** (Figure 4.19). The signal for the C(1)H proton in (±)-**119b** appears further downfield (δ_H 5.50-5.55 ppm) relative to (±)-**119a** (δ_H 5.26-5.29 ppm) attributable to its proximity in space to the electron withdrawing nitro group.

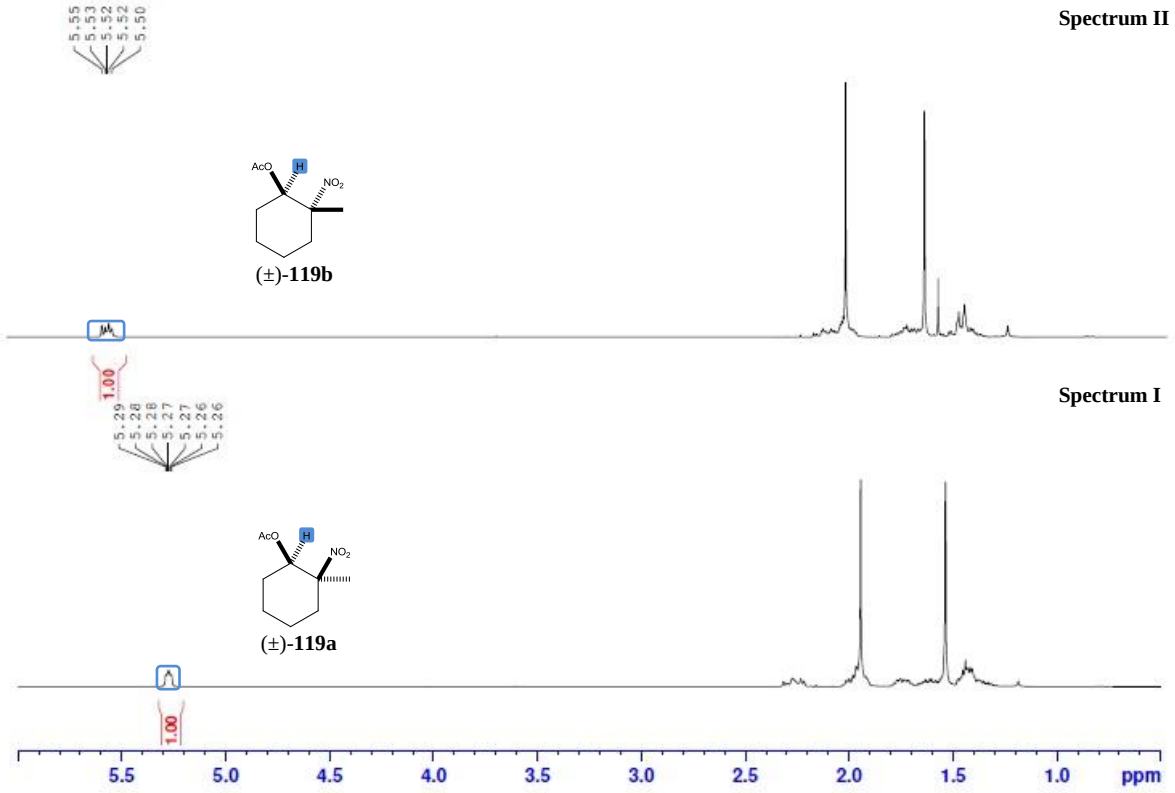
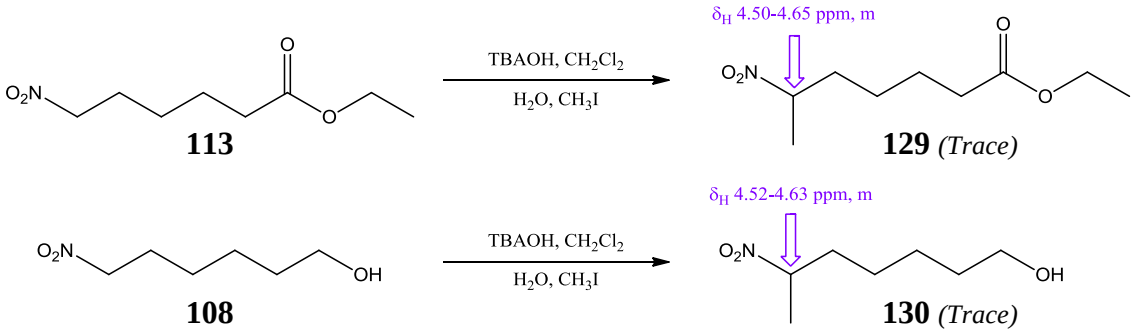


Figure 4.19: ^{1}*H NMR* ***Spectrum I:*** *Crude (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119b**.* ***Spectrum II:*** *Crude (±)-*cis*-2-methyl-2-nitrocyclohexyl aceate (±)-**119a** (all spectra recorded in $CDCl_3$ at 300 MHz).*

4.3.3.4 Synthesis of 6-nitroheptanal 118

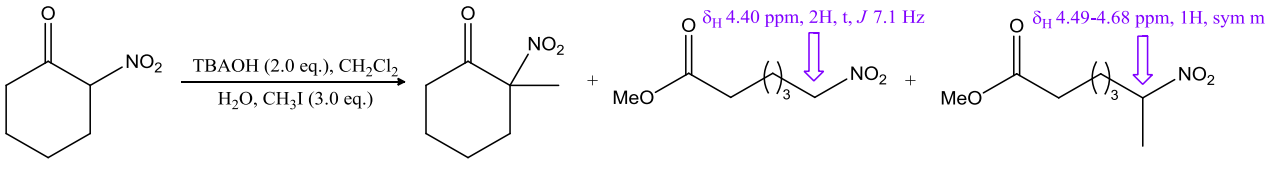
The next synthetic target in this study was preparation of the aldehyde 6-nitroheptanal **118** for investigation of the intramolecular nitroaldol reaction. Unlike the synthesis of 6-nitrohexanal **101** the parent secondary alkyl bromide ethyl 6-bromoheptanoate **128** was not commercially available for the synthesis of 6-nitroheptanal **118** thus the formation of the nitroalkane *via* the Kornblum reaction was not considered.

Direct alkylation of ethyl 6-nitrohexanoate **113** and 6-nitrohexanol **108** under phase-transfer conditions were initially explored. The optimum reaction conditions identified for the methylation of 2-nitrocyclohexanone **123** were applied. However on both occasions only trace evidence of the desired methylated product **129** or **130** was evident by ^{1}H NMR analysis, identified by the multiplet in the region of δ_H 4.50-4.65 ppm attributable to the C(6)H proton geminal to the nitro and methyl moiety (Scheme 4.44)



Scheme 4.44

The next synthetic route investigated treatment of 2-nitrocyclohexanol **103** with 2.0 equivalents of TBAOH and 3.0 equivalents of methyl iodide. Zajac had reported the ring cleavage product methyl 6-nitroheptanoate **127** as the major product under these reaction conditions (Scheme 4.45).[76]

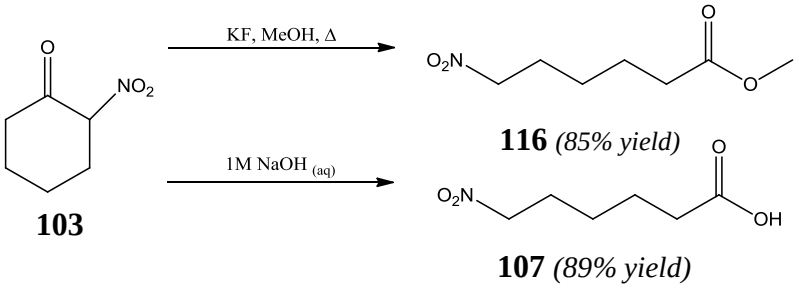


103	**120**		**116**		**127**
Product Ratio by ^{1}H NMR	*52*	:	*13*	:	*35*

Scheme 4.45

Although a larger percentage of methyl 6-nitroheptanoate **127** was evident in the ^{1}H NMR spectrum of the crude material relative to that observed when 1.0 equivalent of base was utilised (35 *vs.* 11%) (see section 4.3.3.1), 2-methyl-2-nitrocyclohexanol **120** remained the major product. Further optimisation of this protocol is warranted based on Zajac's report.[76]

Nucleophilic attack of aqueous base or alcohol to α-nitrocycloalkanones produces ring cleavage with the formation of ω-nitroacids and ω-nitroesters. Recently Milner reported treating 2-nitrocyclohexanone **103** with potassium fluoride in dry methanol to afford the nitroester **116** in high yield according to the procedure reported by Barua *et al.*[81] Milner also described the synthesis of 6-nitrohexanoic acid **107** in good yield *via* base-catalysed ring cleavage of 2-nitrocyclohexanone **103** (Scheme 4.46).



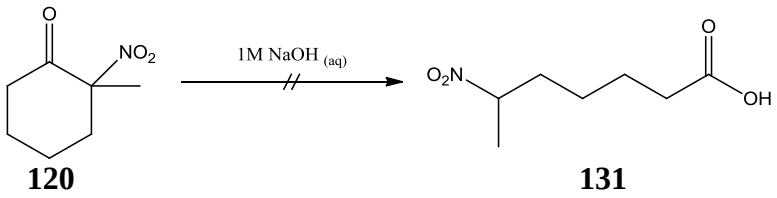
103

116 *(85% yield)*

107 *(89% yield)*

Scheme 4.46[45]

In this study, 2-methyl-2-nitrocyclohexanone **120** in sodium hydroxide (1M) was heated under reflux for 2 h according to Milner's synthetic protocol for the ring opening of 2-nitrocyclohexanone **103** (Scheme 4.47).[45] However, the anticipated ring cleavage product 6-nitroheptanoic acid **131** was not observed in the ^{1}H NMR spectrum. There were no signals evident in the region of ~4.40 ppm characteristic of a methine proton alpha to the nitro moiety, thus ring cleavage appears to have not occurred in the absence of an acidic proton geminal to the nitro group.



120 **131**

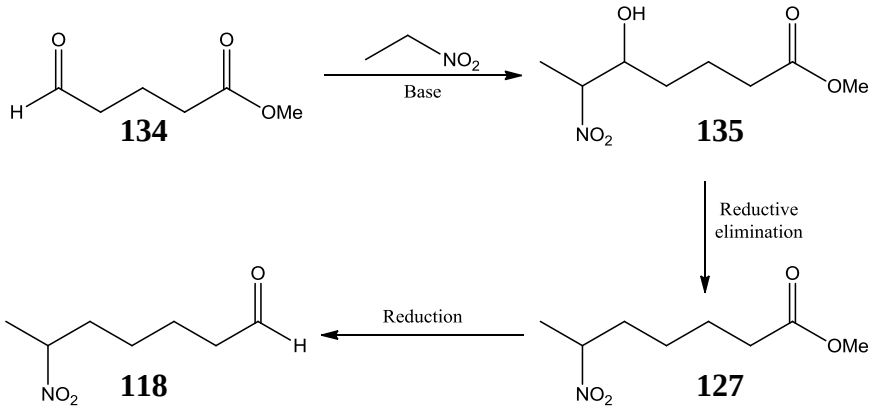
Scheme 4.47

Furthermore no residual starting material **120** was evident in the ^{1}H NMR spectrum of the crude product. While a compound was isolated which appeared to be a single product by ^{1}H NMR its structure was not determined (see section 5.6.1). Both cyclohexane-1,2-dione **132** and 2-hydroxy-2-methylcyclohexanone **133** were considered as possible products, however literature ^{1}H NMR data does not agree (Figure 4.20).[82,83]

132 **133**

Figure 4.20

Thus, a novel synthetic route to 6-nitroheptanal **118** was required. It was envisaged that a base-catalysed nitroaldol reaction with nitroethane and methyl 5-oxopentanoate **134** would successfully introduce the methyl moiety alpha to the nitro group and subsequent reductive elimination of the β-nitroalcohol **135** would generate the nitroalkane **127**. The desired aldehyde **118** would be readily accessible *via* reduction of the ester **127** (Scheme 4.48). While the sequence outlined in scheme 4.48 has not been previously described as a route to **118**, each of the individual synthetic steps is precedented in related compounds.

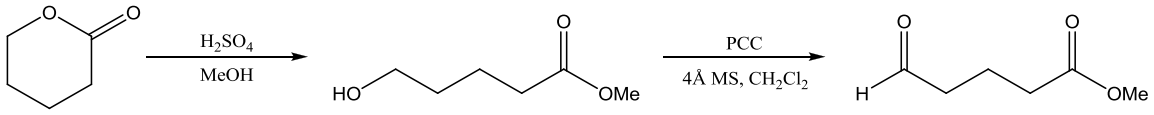


134 **135** **127** **118**

Scheme 4.48

According to literature procedure it was anticipated that ring opening of δ-valerolactone **136** by methanol and subsequent oxidation would provide the substrate methyl 5-oxopentanoate **134** (Scheme 4.49).[84,85] The methanolysis protocol employed in this study involved heating the commercially available δ-valerolactone **136**,[46] in excess methanol with catalytic sulfuric acid under reflux for 48 h, generating the parent straight-chain bifunctional

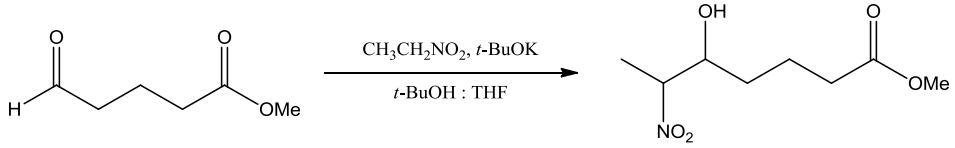
ester **137** as a milky white oil in excellent yield (97%) which required no further purification. This ring opening procedure was not monitored for reaction completion and it is envisaged that the starting lactone **136** was consumed in a shorter reaction time. Subsequent PCC oxidation of the primary alcohol **137** to the aldehyde methyl 5-oxopentanoate **134** proceeded in moderate yield (50%) and excellent purity following vacuum distillation. All spectral characteristics of **137** and **134** were in agreement with those previously reported.[84,85]



136 **137** *(97% yield)* **134** *(50% yield)*

Scheme 4.49

Critical to this synthetic strategy is the carbon-carbon bond forming Henry reaction allowing control of the position of the nitro group alpha to a methyl moiety. The nitrolaldol procedure described by Morrow in the synthesis of nitroalkene fatty acids was implemented in this research.[86] Condensation of methyl 5-oxopentanoate **134** with nitroethane and a catalytic amount of potassium *tert*-butoxide successfully afforded the β-nitro alcohol **135** as an essentially equimolar diastereomeric mixture in good yield (76%) and sufficient purity (>95% pure by ^{1}H NMR spectroscopy) to warrant no further purification (Scheme 4.50). No attempt was made to separate the diastereomers of **135** as both would lead to the same product **127**.



134 **135** *(76% yield)*
Equimolar diastereomeric mixture

Scheme 4.50

Methyl 5-hydroxy-6-nitroheptanoate **135** was previously identified in the literature, however spectral characterisation had not been conducted, thus in this study full analysis was obtained.[87] The *anti* and *syn* diastereomers of **135** were distinguishable in the ^{1}H NMR spectrum with two characteristic sets of signals observed. The signal for the proton geminal to the nitro group appears at δ_H 4.47–4.59 ppm for the two diastereomers whereas the proton geminal to the hydroxyl group appears as two distinct diastereomeric sets of signals at δ_H 3.90-3.98 ppm and δ_H 4.14-4.21 ppm due to hydrogen bonding between the hydroxyl and nitro group leading to a difference in the vicinal coupling constant.

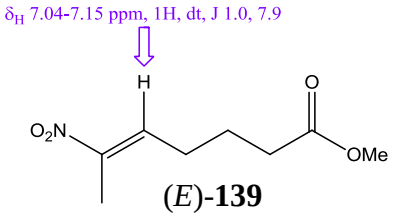
A viable synthetic route envisaged for the generation of the nitroalkane **127** from the β-nitro alcohol **135** was acylation followed by reductive elimination. The reduction of nitroalkyl acetates to nitroalkanes with sodium borohydride is well established and believed to proceed *via* elimination of acetic acid and formation and subsequent reduction of the corresponding nitro olefin.[88]

Early published reports on reductive elimination were not synthetically viable as they involved isolation of the acetate and alkene intermediates which lead to poor overall yields

(14-33%).[89,90] The low yields result in part from isolation steps and in part from the occurrence of side reactions leading to dimeric byproducts. In 1972, Bachman described a significant advance in the synthesis of nitroalkanes. A one-pot procedure was reported where the lower primary nitroalkanes may be converted to the higher nitroalkanes involving successive nitroaldol reaction, acylation and sodium borohydride reduction with significantly greater yields observed (75-80%).[88] Notably, this procedure involves no isolation of intermediates.

On the basis of Bachman's report the one-pot acetylation of the nitroalcohol **135** and mild reduction of the corresponding nitro acetate **138** with sodium borohydride was investigated. Acetylation of **135** proceeds smoothly with acetic anhydride using DMAP as a catalyst. In one instance, ^{1}H NMR analysis was obtained to confirm formation of the acetate **138**, again as an equimolar mixture, however, in general the crude acetate intermediate **138** was not isolated.

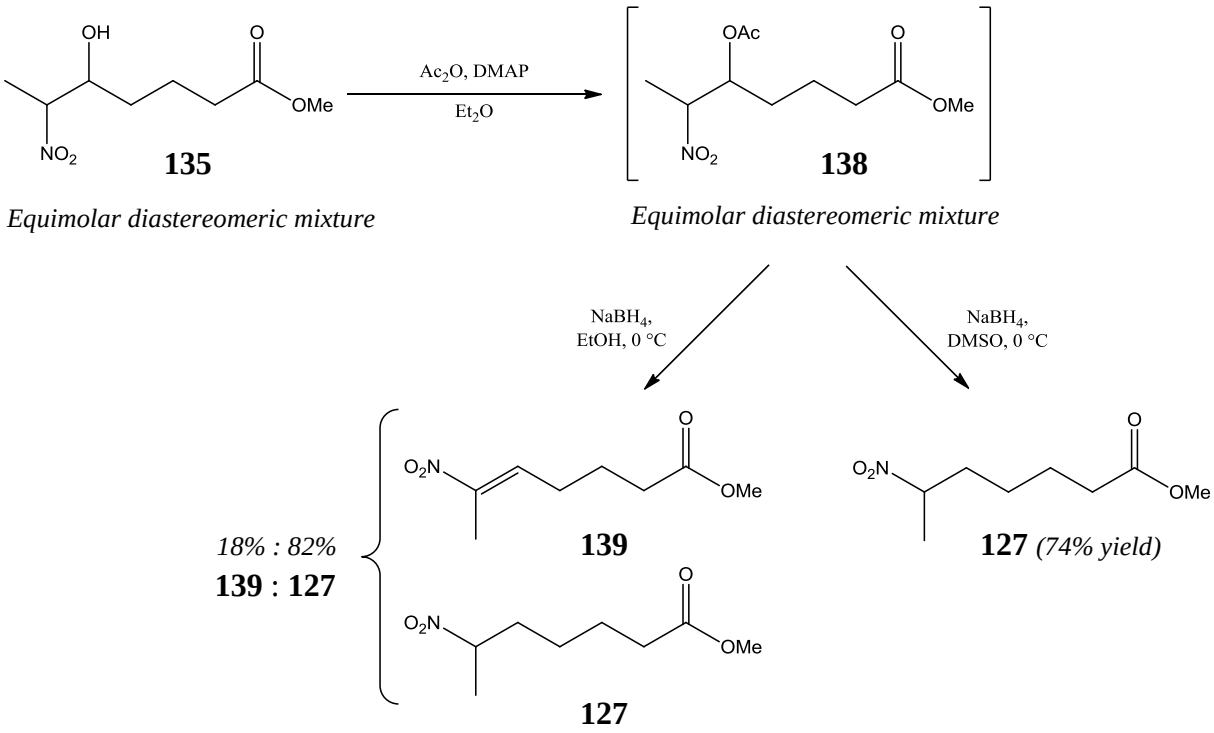
The reduction of the nitroalkyl acetate **138** to the corresponding nitroalkane **127** by the action of sodium borohydride was first conducted in ethanol at 0 °C.[91] However poor reduction to the nitroalkane **127** was observed with the nitroalkene product **139** evident in the crude ^{1}H NMR in ~18% (Figure 4.21). ^{1}H NMR analysis suggests the exclusive formation of the *E* isomer of **139** as the alkene proton displays a characteristic chemical shift of approximately δ_H 7.04-7.15 ppm (1H, dt, *J* 7.9, 1.0), in accordance with literature reports for similar *E*-nitroalkenes, while the alkene proton of *Z*-nitroalkenes has a distinct chemical shift of approximately δ_H 5.80 ppm.[91-93] Attempted separation of the undesired *E*-nitroalkene **139** from the nitroalkane **127** by column chromatography was unsuccessful.



(*E*)-**139**

Figure 4.21

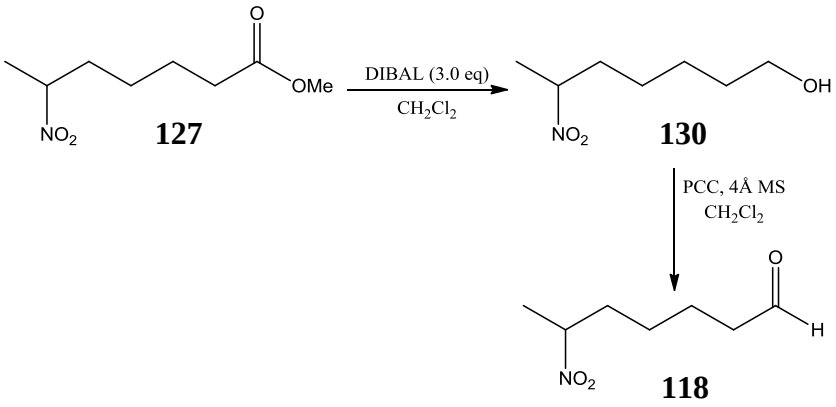
Bachman reports optimum conversions to nitroalkanes utilising sodium borohydride in DMSO.[88] In this study, to increase the efficiency of the reduction, Bachman's conditions were implemented at 0 °C and excellent conversion to the desired nitroalkane **127** was observed with no trace of the nitroalkene byproduct **139** (Scheme 4.51). Methyl 6-nitroheptanaote **127** was isolated in 74% yield for the two steps and of sufficient purity (>95% pure by ^{1}H NMR spectroscopy) to warrant no further purification. Spectral characteristics were in agreement with the literature report discussed earlier where **127** was obtained *via* ring cleavage and methylation of 2-nitrocyclohexanone **120** (Scheme 4.45).[76] This method described herein provides a convenient and high-yielding synthesis of nitroalkane **127** from the β-nitroalcohol **135**.



Scheme 4.51

Now that a synthetic route to methyl 6-nitroheptanoate **127** was developed, the next step was reduction of the ester functional group to the desired aldehyde **118**. Previous studies in this project investigated the selective DIBAL-H reduction of ethyl 6-nitrohexanoate **113** to 6-nitrohexanal **101**. However, following optimisation studies selective reduction was not achieved with a mixture of products 6-nitrohexanal **101**, ethyl 6-nitrohexanoate **113** and 6-nitrohexanol **108** evident by ^{1}H NMR spectroscopy (section 4.2.3.1.2, Scheme 4.24). Therefore, DIBAL-H reduction of methyl 6-nitroheptanoate **127** to aldehyde **118** was not examined, and alternatively the ester **127** was reduced successfully to the analogous alcohol **130** with 3.0 equivalents of DIBAL-H (Scheme 4.52). Purification by column chromatography produced the alcohol **130** in moderate yield (58%) and excellent purity. IR analysis reads a strong broad OH peak at ν_{max} 3365 cm^{-1} and nitro stretching bands at ν_{max} 1553 and 1391 cm^{-1} while in the ^{1}H NMR spectrum the characteristic multiplet at δ_H 4.52-4.63 ppm is indicative of the proton alpha to the nitro moiety.

Subsequent PCC oxidation of the primary alcohol **130** yielded the desired aldehyde **118** in 65% yield and excellent purity following chromatographic purification (Scheme 4.52). The aldehyde 6-nitroheptanal **118** was found to be unstable in the lab atmosphere and easily oxidised to the carboxylic acid **131**. Storage in the freezer at −20 °C was found to slow down the oxidation process but not halt it. Therefore 6-nitroheptanal **118** was freshly purified by column chromatography prior to use. 6-Nitroheptanal **118** had not previously been reported in the literature and full characterisation was obtained during this study. A characteristic carbonyl stretch is evident in the IR spectrum at ν_{max} 1724 cm^{-1} in addition to the asymmetric and symmetric bands of the nitro group at ν_{max} 1549 and 1392 cm^{-1}, while in the ^{1}H NMR spectrum a distinct triplet is seen downfield at δ_H 9.77 ppm indicative of the aldehyde proton C(1)H.



Scheme 4.52

In summary, although the synthetic route to 6-nitroheptanal **118** is rather long, multi-gram quantities of the aldehyde **118** were successfully obtained in good yield for investigation of the dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis

4.3.4 Hydrolase-mediated kinetic resolution – analytical screens

In order to develop a one-pot hydrolase-mediated dynamic process leading to 2-nitrocyclohexyl acetate **119** together with high diastero- and enantiopurity, a complete understanding of the hydrolase-mediated kinetic resolution of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** was required. The specific aim of this investigation was to identify a hydrolase which would selectively acetylate one diastereomer of (±)-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** with excellent enantioselectivity. Therefore, each diastereomer (±)-**117a** and (±)-**117b** was screened independently against a series of hydrolases. The efficiency and enantioselectivity of the hydrolase-mediated resolution of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** was then compared to that of the (±)-*trans*-diastereomer (±)-**117b** facilitating detection of diastereoselectivity. In order to efficiently assess enantiopurities from the screening protocol with minimal work-up, a reliable and robust analytical chiral HPLC technique was developed.

4.3.4.1 Chiral HPLC method development

Once the racemic starting materials (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** and (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** or (±)-**119b** were prepared, a chiral HPLC method was developed which resolved all four enantiomeric pairs with a single injection. This facilitated direct analysis of the crude reaction mixtures obtained from the lipase-mediated kinetic resolution screening assays and of the one-pot dynamic kinetic resolution process. A series of chiral columns were investigated for this resolution including the Chiralcel® OD-H, AS-H and Chiralpak® IB, examining a range of different isopropanol/hexane solvent compositions, flow rates and temperatures. Following a significant amount of method development the Chiralcel® OJ-H column was determined to achieve baseline separation of all four enantiomeric pairs under three sets of conditions:

i) room temperature with isopropanol/hexane (3 : 97), a flow rate of 0.50 mL/min and a detector wavelength of 209.8 nm (Appendix I, Conditions A) (see Figure 4.22).

ii) room temperature with isopropanol/hexane (0.5 : 99.5), a flow rate of 0.50 mL/min and a detector wavelength of 209.8 nm (Appendix I, Conditions B).

iii) room temperature with isopropanol/hexane (3 : 97), a flow rate of 0.75 mL/min and a detector wavelength of 209.8 nm (Appendix I, Conditions C).

It should be noted that the above chiral HPLC methods in general successfully resolved all four enantiomeric pairs, however, as eight enantiomers were eluted on a single trace with similar retention times small changes in column temperature or pressure may result in co-elution and thus the chiral HPLC method may need to be modified on a day to day basis. Further experimental details concerning chiral HPLC methods can be found in appendix I.

In summary, the conditions for chiral HPLC were developed to a point where a single injection of the reaction mixture enabled determination of enantiopurity of each of the components **117a**, **117b**, **119a** and **119b**. The assignment of stereochemistry of **117a**, **117b, 119a** and **119b** is discussed in section 4.3.5.3, notably the stereochemical assignment of **117b** and **119b** were determined by analogy to the transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** but not definitively confirmed. Quantification of the individual compounds by chiral HPLC was not undertaken in this study but, if required, would be determined by the protocol implemented in the quantification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** as discussed in section 4.2.4.

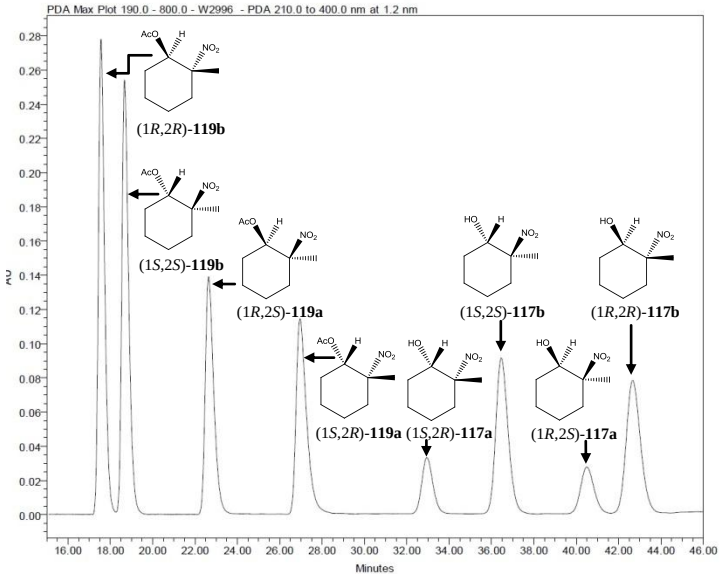


*Figure 4.22: HPLC trace of a racemic mixture of (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a**, (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119b**, (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**. For HPLC conditions see appendix I.*
Note the above traces correlate to chiral HPLC conditions A.

4.3.4.2 Analytical screening protocol – vinyl acetate as both acyl donor and solvent

Once the analytical method was developed the next step was to examine the hydrolase-mediated resolution for the enantioselective transesterification of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b**. The optimum reaction conditions developed by Milner for the kinetic resolution of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** employing vinyl acetate as both solvent and acyl donor were investigated in this study for the transesterification of (±)-2-methyl-2-nitrocyclohexanol diastereomers (±)-**117a** and (±)-**117b**.[45] All hydrolases screened were

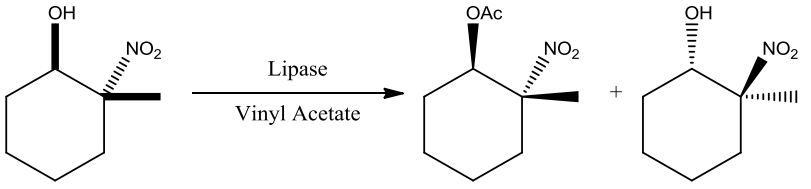
kindly donated by Almac Sciences. In this screening protocol the alcohol substrate (±)-**117a** or (±)-**117b** (~20 mg) was added to 1 mL of vinyl acetate with the biocatalyst and the resulting reaction mixture was incubated and agitated at 750 rpm at 24 °C. While TLC analysis indicated if the desired product had formed, no detailed chiral HPLC reaction monitoring was conducted during analytical screens. Work-up involved filtration of the reaction solution to remove the hydrolase and subsequent concentration of the organic phase. Analysis of the crude product was conducted by ^{1}H NMR spectroscopy and chiral HPLC. Significantly, the crude alcohol (±)-**117a** or (±)-**117b** and acetate (±)-**119a** or (±)-**119b** did not require separation prior to chiral HPLC analysis as the enantiopurities of both components can be conveniently assessed in a single injection.

Conversion was estimated by ^{1}H NMR analysis and by the *E*-value calculator.[71] Notably in some instances (entry 2, Table 4.22) slightly higher conversions were obtained by ^{1}H NMR spectroscopy relative to the *E*-value. For conversions determined by ^{1}H NMR spectroscopy to be <10%, chiral HPLC analysis was not conducted. In addition where the second enantiomer was not observed the enantiopurity is stated as >98% ee.

*4.3.4.3 Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-117b*

The hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** employing vinyl acetate as both acetylating donor and solvent was initially investigated. The hydrolases screened were selected on the basis that they had demonstrated conversion in the resolution of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** in some instances with excellent enantioselection.[45]

*Table 4.21: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1	*Candida cylindracea* C1	48 h	33	43	61	>98	185
2	*Pseudomonas cepacia* P2	48 h	-	<10	-	-	-
3	*Candida antarctica* B (immob)	72 h	49	48	96	>98	>200
4	*Pseudomonas stutzeri*	48 h	50	51	97	>98	>200
5	*Pseudomonas fluorescens*	113.5 h	-	<10	-	-	-

As is evident in Table 4.21 the vinyl acetate screen was very promising, with excellent enantioselection achieved *via Candida antarctica* lipase B (immob) and *Pseudomonas stutzeri* mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (entries 3 and 4, Table 4.21). Optimum conversions of ~50% were attained in both

resolutions and the generated acetate (1*R*,2*R*)-**119b** (>98% ee) and recovered alcohol (1*S*,2*S*)-**117b** (≥96% ee) were acquired with excellent enantiopurity. A lower rate of conversion was observed with *Candida cylindracea* C1 catalysed resolution (entry 1, Table 4.21) and although excellent enantiopurity of the acetate (1*R*,2*R*)-**119b** (>98% ee) was achieved the poor efficiency of the bioresolution resulted in a reduced enantiopurity of the alcohol (1*S*,2*S*)-**117b** (61% ee). Significantly, minimal transesterification was observed utilising the *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* biocatalysts (entries 2 and 5, Table 4.21) and chiral HPLC analysis was not conducted in these resolutions.

In the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** Milner reports high enantiopurity of both the nitrocyclohexanol (1*S*,2*S*)-**99b** and nitroacetate (1*R*,2*R*)-**100b** with *Pseudomonas fluorescens* and *Candida antarctica* lipase B (immob) (Figure 4.23). Significantly in the transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** *Candida antarctica* lipase B (immob) also demonstrated excellent enantioselection confirming that this biocatalyst can accommodate increased steric demand in the "C2 region" of the enzyme active site. In direct contrast *Pseudomonas fluorescens* demonstrated limited efficiency with the bulkier substrate (±)-**117b**. Furthermore, while *Pseudomonas stutzeri* mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** proceeded with excellent enantioselection (*E* >200), this hydrolase displayed poor enantiopreference in the transesterification of the less sterically hindered (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (*E* = 15).

*Comparison of enantioselection (*E*-value) for transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b** versus hydrolase*

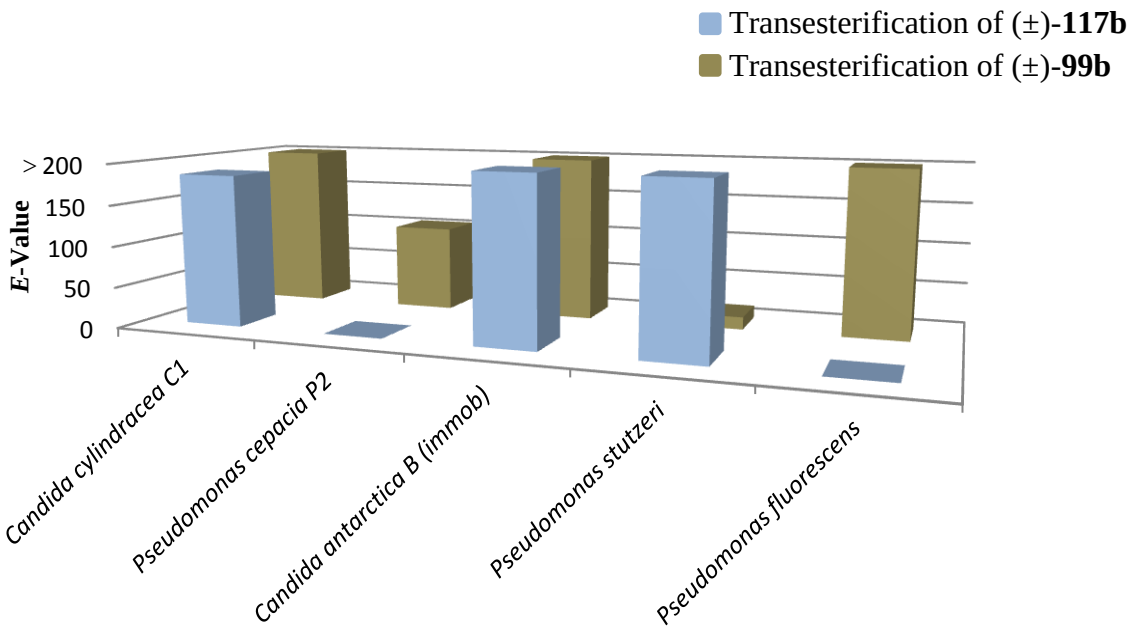
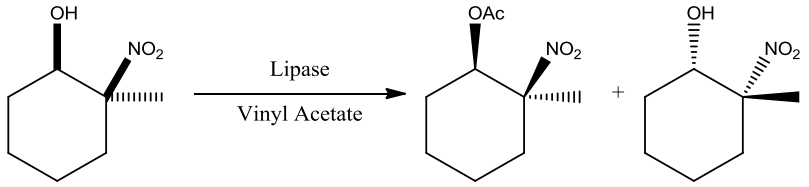


Figure 4.23

*4.3.4.4 Hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-117a*

Based on the efficient resolution achieved in the transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b,** attention was now focused on resolution of the (±)-*cis*-isomer (±)-**117a** employing the same optimised acetylation conditions developed for (±)-**117b**, that is vinyl acetate as both solvent and acyl donor.

*Table 4.22: Hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** in vinyl acetate*



(±)-**117a** (1*R*,2*S*)-**119a** (1*S*,2*R*)-**117a**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		*E* value
			E **Calc.**	**^{1}H NMR**	**Alcohol *cis*-117a (1*S*, 2*R*)**	**Acetate *cis*-119a (1*R*, 2*S*)**	
1	*Pseudomonas cepacia* P2	48 h	-	<10	-	-	-
2	*Candida antarctica* B (immob)	72 h	33	47	49	>98	159
3	*Pseudomonas stutzeri*	48 h	78	85	>98	27	6.4
4	*Pseudomonas fluorescens*	113.5 h	40	45	64	96	95

Of the four hydrolases screened, no hydrolase was identified which resolved both the *cis*-acetate (1*R*,2*S*)-**119a** and *cis*-alcohol (1*S*,2*R*)-**117a** with excellent enantioselectivity. Both *Candida antarctica* lipase B (immob) and *Pseudomonas fluorescens* (entries 2 and 3, Table 4.22) achieved high enantiopurity of the generated acetate (1*R*,2*S*)-**119a** (≥96% ee) however the enantiopurity of the alcohol (1*S*,2*R*)-**117a** was poor due to low rate of acetylation. Conversely, *Pseudomonas stutzeri* (entry 3, Table 4.22) mediated resolution proceeded with a high rate of conversion and limited enantiopreference with poor enantiopurity of the recovered acetate (1*R*,2*S*)-**119a** (27% ee). However, the recovered alcohol (1*S*,2*R*)-**117a** displayed excellent enantiopurity albeit with very low recovery due to over acetylation in this biotransformation. Finally *Pseudomonas cepacia* P2 demonstrated limited efficiency in the kinetic resolution of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (entry 1, Table 4.22) and no chiral HPLC analysis was conducted.

In the resolution of (±)-*cis*-2-nitrocyclohexanol (±)-**99a**, Milner reports efficient conversions and enantioselectivities with the *Pseudomonas stutzeri* and *Pseudomonas fluorescens* biocatalysts.[45] In this study these enzymes demonstrate poorer enantioselection towards the resolution of the more sterically demanding (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**, with inefficient conversion for acetylation catalysed by *Pseudomonas fluorescens* while over acetylation occurs in the reaction mediated by *Pseudomonas stutzeri* (Figure 4.24). Variation of the reaction conditions and times for these enzyme-mediated transformations may result in a better overall outcome.

*Comparison of enantioselection (*E*-value) for transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol* (±)-**117a** *and (±)-*cis*-2-nitrocyclohexanol* (±)-**99a** *versus hydrolase*

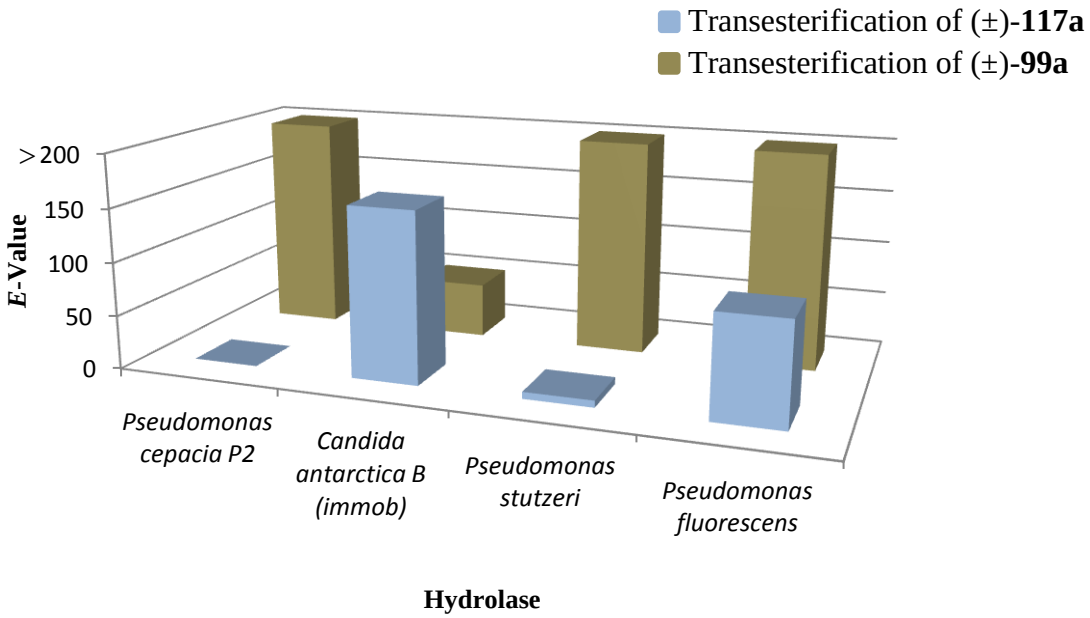


Figure 4.24

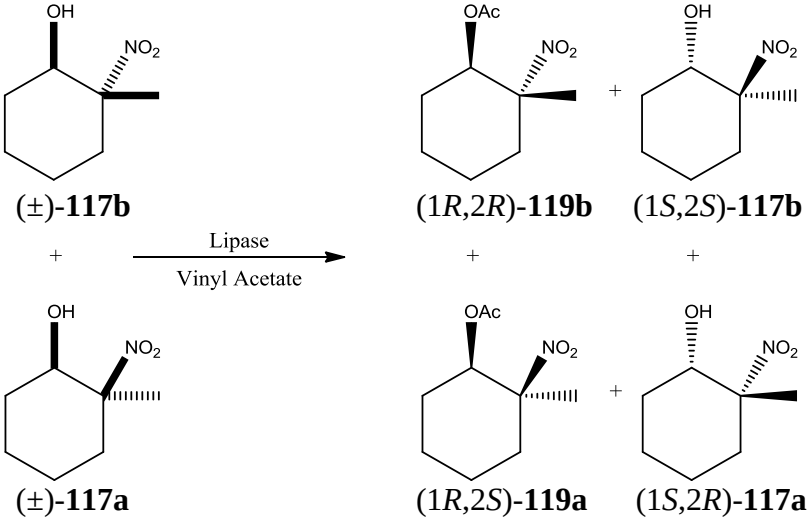
Significantly, on comparison of the efficiency and enantioselection of the resolution of the (±)-*trans*-2-methyl-2-nitrocyclohexanol with that of the (±)-*cis*-diastereomer both *Candida antarctica* lipase B (immob) and *Pseudomonas stutzeri* demonstrated potential diastereoselectivity. Excellent enantioselection in the resolution of the (±)-*trans*-alcohol (±)-**117b** was observed, and crucially, different rates of transesterification (as evidenced by extent of transformation at the same reaction time) were determined relative to the *cis*-isomer (±)-**117a**. Thus these hydrolases are possible biocatalysts for the diastereoselective resolution of (±)-2-methyl-2-nitrocyclohexanol (±)-**117**.

*4.3.4.5 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-117a or (±)-117b*

Further investigation of the potential diastereoselectivity of *Pseudomonas stutzeri* and *Candida antarctica* lipase B (immob) in the resolution of (±)-2-methyl-2-nitrocyclohexanol (±)-**117**, with particular examination of the rates of acetylation of the two diastereomers (±)-**117a** or (±)-**117b** was required. The objective was that the hydrolases would selectively acetylate one diastereomer (±)-**117a** or (±)-**117b** in high efficiency and excellent enantioselectivity while the opposite diastereomer (±)-**117a** or (±)-**117b** would remain unchanged. A 50 : 50 mixture of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** was dissolved in vinyl acetate and the appropriate hydrolase was charged to the reaction vessel. Reaction monitoring by ^{1}H NMR analysis was conducted throughout the incubation period. Aliquots of reaction mixture (0.5–1.0 mL) were isolated and filtered prior to concentration. The sample was then analysed by ^{1}H NMR spectroscopy and the relative conversions of the *cis*- and *trans*-alcohols **117a** and **117b** to their respective *cis*- and *trans*-acetates **119a** and **119b** was determined (Table 4.23). The diastereoselectivity is represented by the relative extent of transformation of the two diastereomers (±)-**117a** and (±)-**117b**.

The final extraction, in addition to ^{1}H NMR analysis, was also subjected to chiral HPLC and enantioselection determined. As an efficient and convenient chiral HPLC method had been developed, enantiopurities of all four enantiomeric pairs **117a**, **117b, 119a** and **119b** could be determined on a single trace, thus obviating prior separation of the acetates **119a** and **119b** and alcohols **117a** and **117b** by column chromatography.

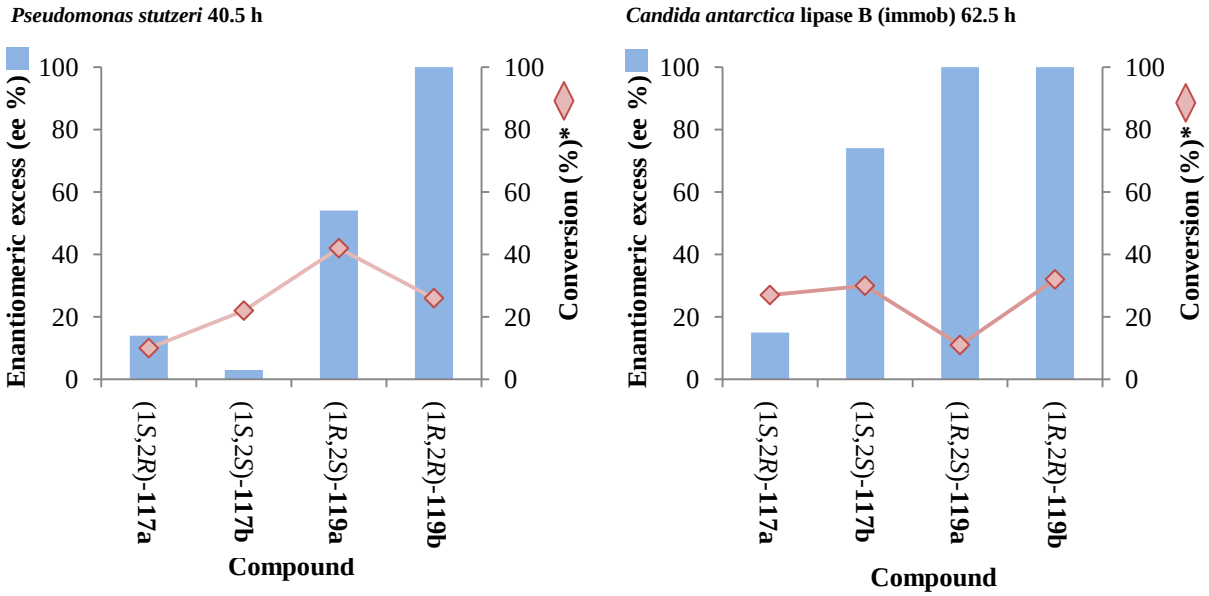
*Table 4.23: Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** in vinyl acetate*



Enzyme Source	Reaction Time	Alcohol (±)-117		Acetate (±)-119	
		***cis*-117a (%)[a] [ee (%)][b]**	***trans*-117b (%)[a] [ee (%)][c]**	***cis*-119a (%)[a] [ee (%)][d]**	***trans*-119b (%)[a] [ee (%)][e]**
Pseudomonas stutzeri	18.5 h	12	27	39	22
	40.5 h	10 [14]	22 [3]	42 [54]	26 [>98]
Candida antarctica B (immob)	12 h	44	38	3	15
	18.5 h	43	36	4	17
	40.5 h	41	30	6	23
	62.5 h	27 [15]	30 [74]	11 [>98]	32 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the spectrum of the mixture of the crude material not mass recovery.
b. The principal enantiomer was (1*S*,2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a**.
c. The principal enantiomer was (1*S*,2*S*)-*trans*-2-methyl-2-nitrocyclohexanol (1*S*,2*S*)-**117b**.
d. The principal enantiomer was (1*R*,2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetae (1*S*,2*R*)-**119a**.
e. The principal enantiomer was (1*R*,2*R*)-*trans*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*R*)-**119b**.

*Comparison of conversion (%) and enantiomeric excess (ee %) of **117a**, **117b**, **119a** and **119b** following diastereoselective hydrolase-mediated transesterification*



**Note: Conversion (%) illustrated as percentage of material in the crude product.*

Figure 4.25

As anticipated from the preliminary screens conducted on the individual diastereomers, *Pseudomonas stutzeri* demonstrated a higher rate of acetylation for the (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** relative to the *trans*-alcohol (±)-**117b** (Figure 4.25). However the difference in acetylation rates was minor and transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was also a significant process in the *Pseudomonas stutzeri* mediated resolution with the *trans*-acetate **119b** responsible for 22% of the overall product ratio after 18.5 h. Enantiopurity of the dominant *cis*-acetate (1*R*,2*S*)-**119a** was poor (54% ee) demonstrating the lipase's lack of enantiopreference towards (±)-**117a**. Thus, the poor enantiopurity of the faster generated acetate (1*R*,2*S*)-**119a** and limited diastereoselectivity observed deemed *Pseudomonas stutzeri* unsuitable for the development of a dynamic kinetic resolution process.

The hydrolase *Candida antarctica* lipase B (immob) mediated transesterification proceeded with high diastereoselectivity towards (±)-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** (Figure 4.25). The dominant process was resolution of the (±)-*trans*-alcohol (±)-**117b** with excellent enantiopurity observed of the acetate (1*R*,2*R*)-**119b** (>98% ee). After 18.5 h only 4% of the minor *cis*-acetate (1*R*,2*S*)-**119a** relative to 17% of the major *trans*-acetate (1*R*,2*R*)-**119b** was generated, demonstrating good diastereoselectivity. This was a marked improvement relative to the *Pseudomonas stutzeri* mediated transesterification of (±)-2-methyl-2-nitrocyclohexanol (±)-**117**.

In summary, *Candida antarctica* lipase B (immob) demonstrated the greatest potential for the development of a dynamic resolution process of (±)-2-methyl-2-nitrocyclohexanol (±)-**117**, with good diastereoselectivity and high enantiopurity observed in the preliminary screens. Optimisation through variation of reaction conditions may well lead to enhanced selectivity.

4.3.5 Hydrolase-mediated kinetic resolution – preparative-scale

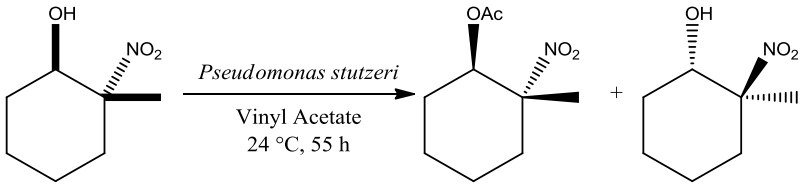
The kinetic resolutions were next undertaken on a preparative-scale to demonstrate the synthetic utility of the hydrolase-mediated transesterification and to determine the absolute stereochemistry of the enantiopure products.

*4.3.5.1 Preparative-scale hydrolase-catalysed transesterification of (±)-***trans***-2-methyl-2-nitrocyclohexanol (±)-117b*

A preparative-scale hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (1.30 mmol) was first investigated. The lipase *Pseudomonas stutzeri* was selected due to the optimum conversion observed and the excellent enantioselectivity achieved in the analytical kinetic resolution screens.

In this study, reaction monitoring of the preparative-scale *Pseudomonas stutzeri* mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was conducted at 48 h; an aliquot was removed and, following work-up, ^{1}H NMR and chiral HPLC analysis were performed to determine conversion and enantioselectivity (entry 1, Table 4.24). Due to the low enantiopurity of the alcohol (1*S*,2*S*)-**117b** (95% ee) the reaction mixture was incubated for a further 7 h at 24 °C and filtered at 55 h through Celite® to remove the hydrolase. The combined organic extracts were concentrated under reduced pressure. Purification by column chromatography separated the enantiopure *trans*-alcohol (1*S*,2*S*)-**117b** and *trans*-acetate (1*R*,2*R*)-**119b** as clear oils in 35% and 36% yield respectively with spectral characteristics identical to those for the racemic materials previously prepared (Figure 4.26 and 4.27). Due to the removal of a reaction aliquot, reaction monitoring may have led to a decreased yield. An optimum conversion rate of 50% was achieved during the large scale transesterification with excellent enantiopurity of the *trans*-alcohol (1*S*,2*S*)-**117b** (>98% ee) and the *trans*-acetate (1*R*,2*R*)-**119b** (>98% ee) obtained. Notably, on scale up the efficiencies and selectivities of the resolution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** mirrored the outcome seen in the analytical scale resolution in Table 4.21. The assignment of the absolute stereochemical outcome of this reaction is discussed further in section 4.3.5.3.

Table 4.24: Large scale Pseudomonas stutzeri *mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%) [yield (%)][c]		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1[a]	48 h	24	49	52	95	>98	>200
2[b]	52.5 h	24	50	50	>98 [35][c]	>98 [36][c]	>200

a. Chiral HPLC analysis was conducted on the crude reaction mixture aliquot acquired at 48 h.
b. Chiral HPLC analysis was conducted after purification by column chromatography and isolation of (1*S*,2*S*)-**117b** and (1*R*,2*R*)-**119b**.
c. Isolated yield following column chromatography.

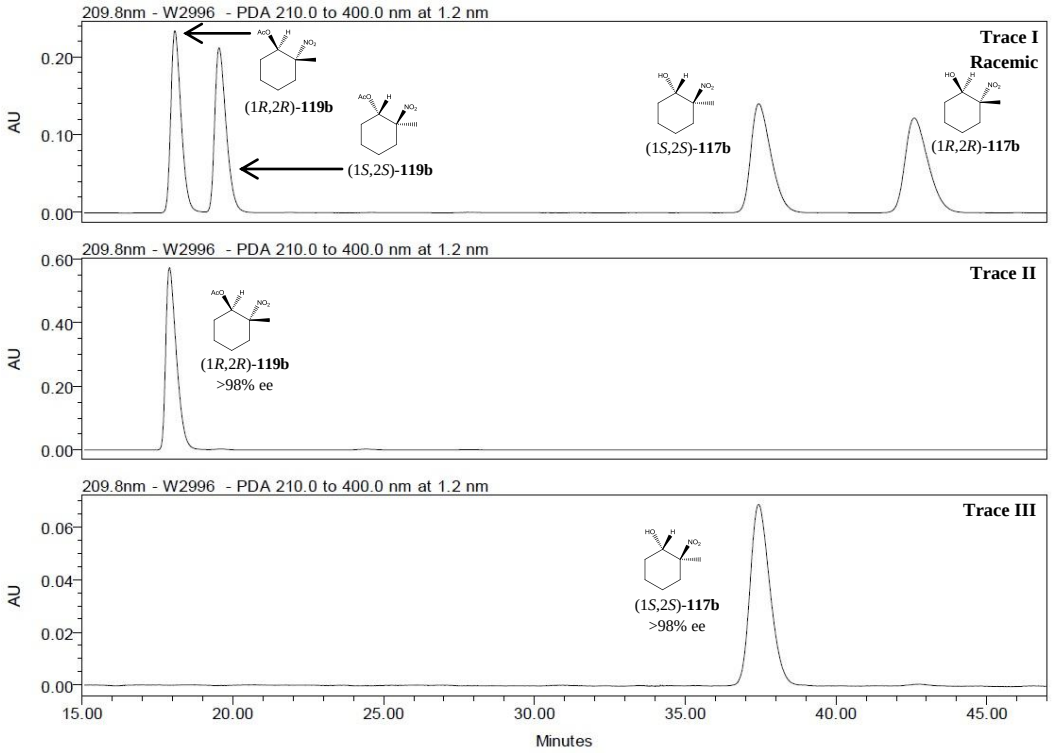


Figure 4.26: HPLC ***Trace I:*** *A racemic mixture of (±)*-trans-*2-methyl-2-nitrocyclohexyl acetate (±)-**119b** and (±)*-trans-*2-methyl-2-nitrocyclohexanol (±)-**117b**.* ***Trace II:*** *Purified (1*R,*2*R*)*-trans-*2-methyl-2-nitrocyclohexyl acetate (1*R,*2*R*)-**119b** >98% ee following column chromatography.* ***Trace III:*** *Purified (1*S,*2*S*)*-trans-*2-methyl-2-nitrocyclohexanol (1*S,*2*S*)-**117b** 95% ee following column chromatography. For HPLC conditions see appendix I.*

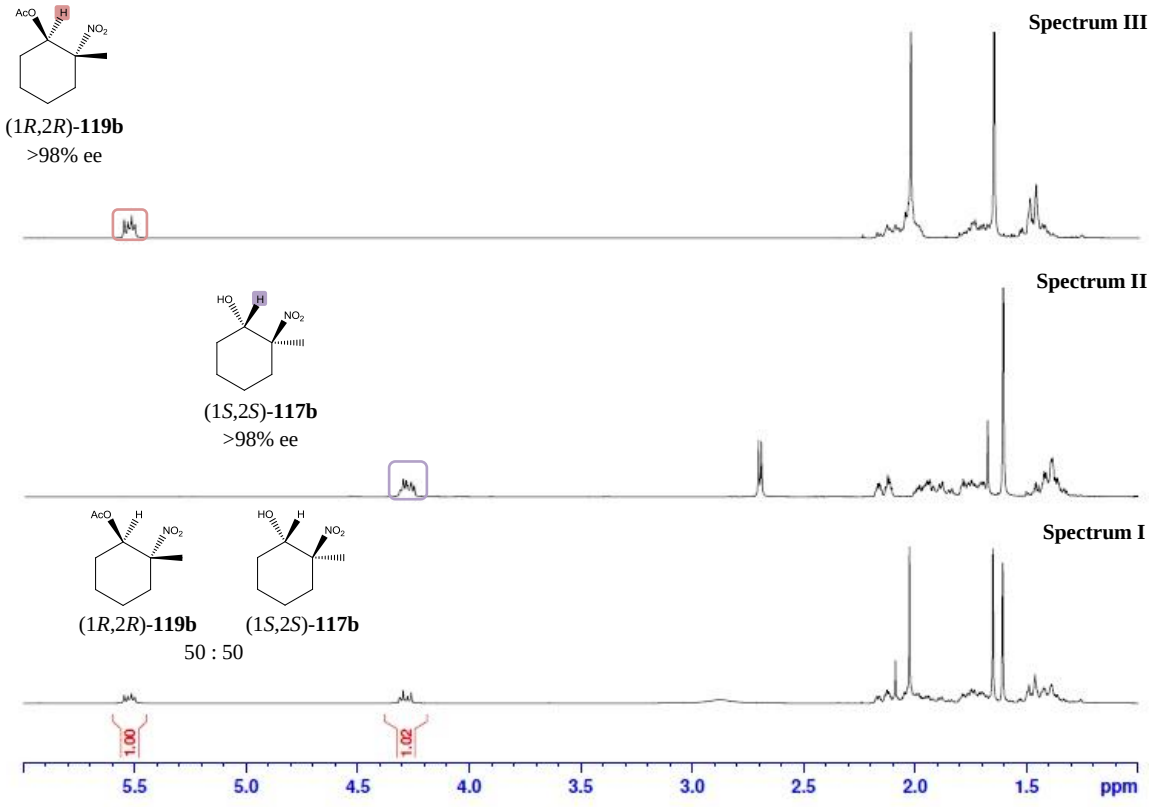


Figure 4.27: ^{1}H NMR ***Spectrum I:*** *Crude product following preparative-scale transesterification containing a mixture of enantioenriched (1*R,*2*R*)*-trans-*2-methyl-2-nitrocyclohexyl acetate (1*R,*2*R*)-**119b** and (1*S,*2*S*)*-trans-*2-methyl-2-nitro cyclohexanol (1*S,*2*S*)-**117b** 50 : 50 respectively.* ***Spectrum II:*** *Purified (1*S,*2*S*)*-trans-*2-methyl-2-nitrocyclohexanol (1*S,*2*S*)-**117b**, >98% ee following column chromatography.* ***Spectrum III:*** *Purified (1*R,*2*R*)*-trans-*2-methyl-2-nitrocyclohexyl acetate (1*R,*2*R*)-**119b**, >98% ee following column chromatography (all spectra recorded in $CDCl_3$ at 300 MHz).*

*4.3.5.2 Preparative-scale hydrolase-catalysed transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-117a*

The preparative-scale hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (0.51 mmol) was next investigated. The lipase *Candida antarctica* lipase B (immob) was selected as this lipase resulted in the highest observed enantioselectivity in the analytical screens (E = 159).

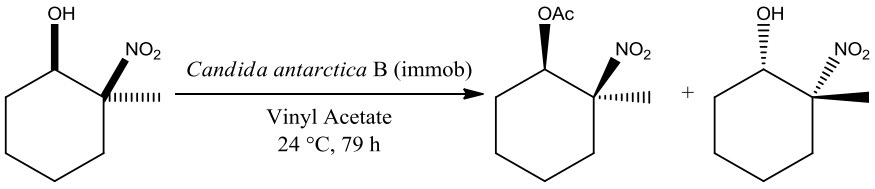
In this study, reaction monitoring of the enantioselective *Candida antarctica* lipase B (immob) catalysed acetylation of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** was conducted at 72 h. An aliquot (0.25 mL) was removed, filtered and concentrated under reduced pressure before conversion and enantioselectivity were determined by chiral HPLC analysis. It should be noted that ^{1}H NMR spectroscopy was not conducted at this point in the investigation. Poor conversion and low enantioselectivity of the recovered alcohol (1*S*,2*R*)-**117a** was observed (entry 1, Table 4.25) and thus, the bioresolution was shaken and incubated at 24 °C for a further 7 h before filtration of the reaction mixture through Celite® to remove the immobilised lipase. The lipase was washed with ethyl acetate and all combined organic extracts concentrated under reduced pressure.

The enantioenriched alcohol (1*S*,2*R*)-**117a** and acetate (1*R*,2*S*)-**119a** in the crude reaction mixture were isolated by column chromatography on silica gel in 33% and 30% yield respectively and analysed by ^{1}H NMR and chiral HPLC (Figure 4.28 and 4.29). ^{1}H NMR spectra were identical to those for the racemic materials previously prepared. The enantioselectivity of the preparative-scale (entry 2, Table 4.25) reflected very closely that of the analytical scale (entry 2, Table 4.22). While excellent enantiopurity of the (1*R*,2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*S*)-**119a** was observed (>98% ee), the enantiopurity of the (1*S*,2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a** was low (45% ee) due to the poor conversion rate (32%).

Significantly, in Milner's study, isolated enantiopure samples of (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a** and (1*R*,2*S*)-*cis*-2-nitrocyclohexylacetate (1*R*,2*S*)-**100a** were not obtainable due to the formation of the elimination product 1-nitrocyclohexene **102** on attempted purification of the enantioenriched products (1*S*,2*R*)-**99a** and (1*R*,2*S*)-**100a** on silica gel. Thus, the stereochemistry of the transesterification products of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** were tentatively assigned by analogy to the transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** and in accordance to Kazlauskas's rule (see section 4.2.6.2). In contrast, no competing dehydration process was evident in this study thus the absolute stereochemistry of the transesterification products of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**, (1*R*,2*S*)-**119a** and (1*S*,2*R*)-**117a** can be unequivocally assigned by single crystal X-ray diffraction on a crystalline sample of (1*R*,2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*S*)-**119a** (see section 4.3.5.3).

Thus, three of the four resolution products, (1*S*,2*S*)-**117b,** (1*R*,2*R*)-**119b** and (1*R*,2*S*)-**119a**, were obtained in excellent enantiopurity and reasonable yields while (1*S*,2*R*)-**117a** is obtained in only modest enantiopurity (45% ee). Interestingly, the preparative-scale reactions reproduced very closely the outcomes of the analytical scale reactions, even in the case of **117a** where the resolution does not easily proceed to the ideal 50% conversion, either overshooting or undershooting depending on the biocatalyst applied.

Table 4.25: Large scale Candida antarctica *lipase B (immob) mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** in vinyl acetate*



(±)-**117a** (1*R*,2*S*)-**119a** (1*S*,2*R*)-**117a**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%) [yield (%)][c]		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117a (1*S*,2*R*)	Acetate *trans*-119a (1*R*,2*S*)	
1[a]	72 h	24	27	-	37	>98	142
2[b]	79 h	24	31	32	45 [33][c]	>98 [30][c]	154

a. Chiral HPLC analysis was conducted on the crude reaction mixture aliquot acquired at 72 h.
b. Chiral HPLC analysis was conducted after purification by column chromatography and isolation of (1*R*,2*S*)-**119a** and (1*S*,2*R*)-**117a**.
c. Isolated yield following column chromatography.

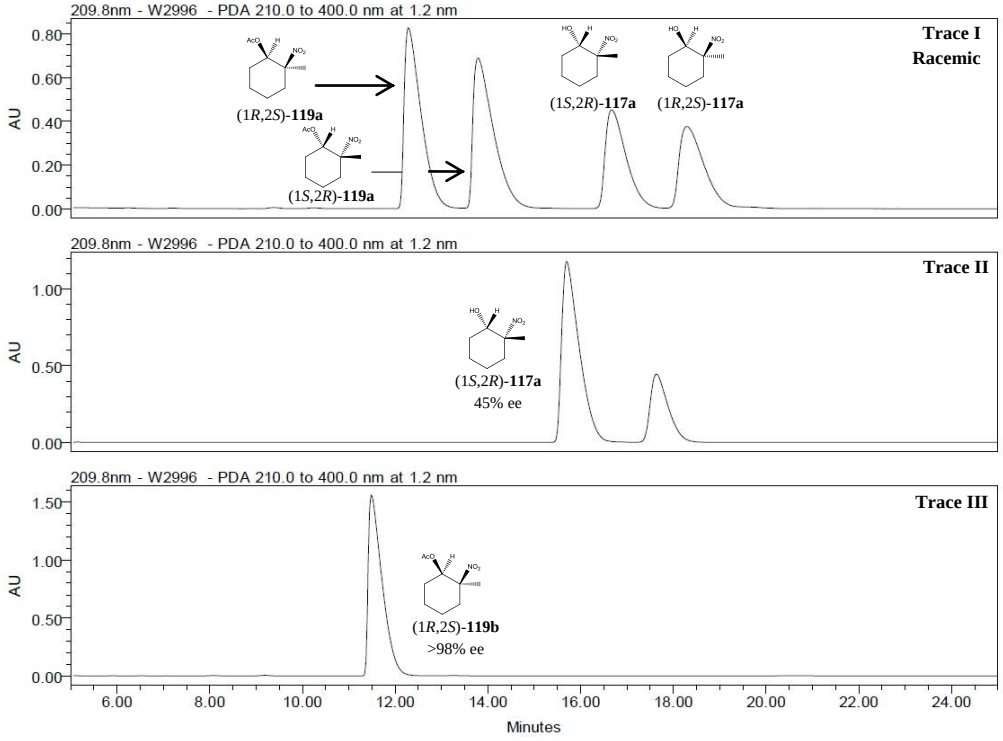


*Figure 4.28: HPLC **Trace I:** A racemic mixture of (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**. **Trace II:** Purified (1*S*,2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a**, 45% ee following column chromatography. **Trace III:** Purified (1*R*,2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*S*)-**119a**, >98% ee following column chromatography. For HPLC conditions see appendix I.*

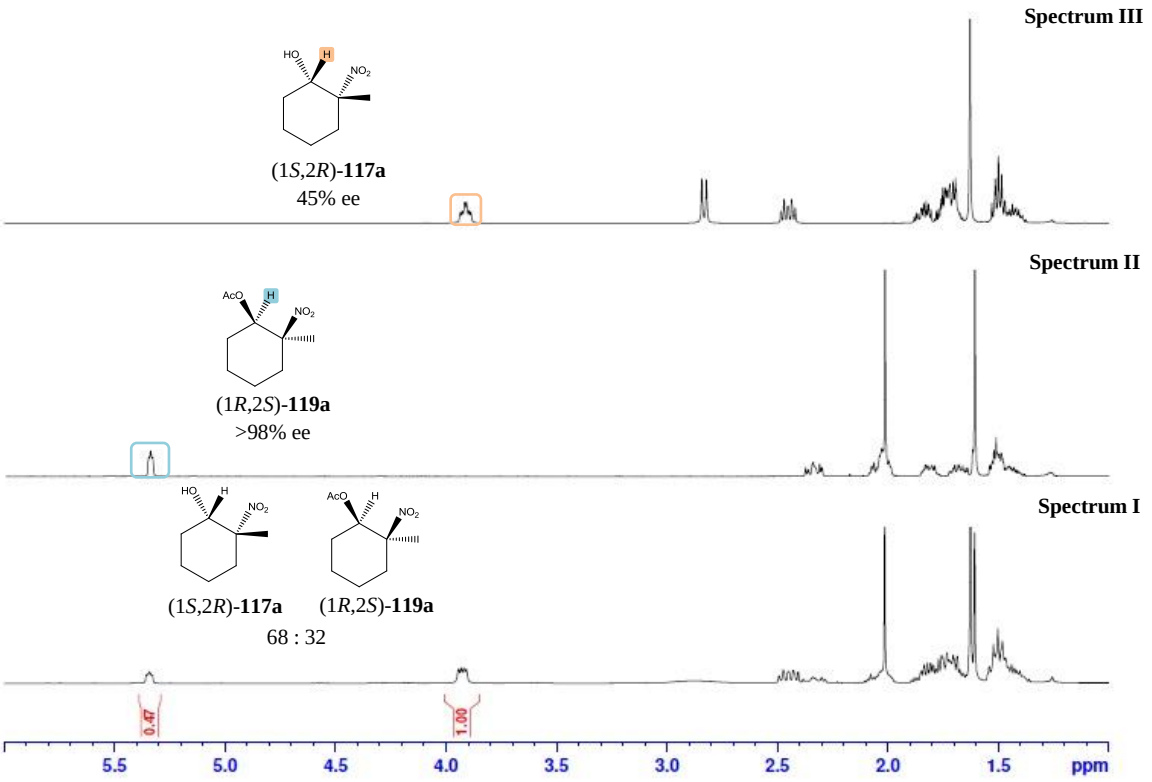
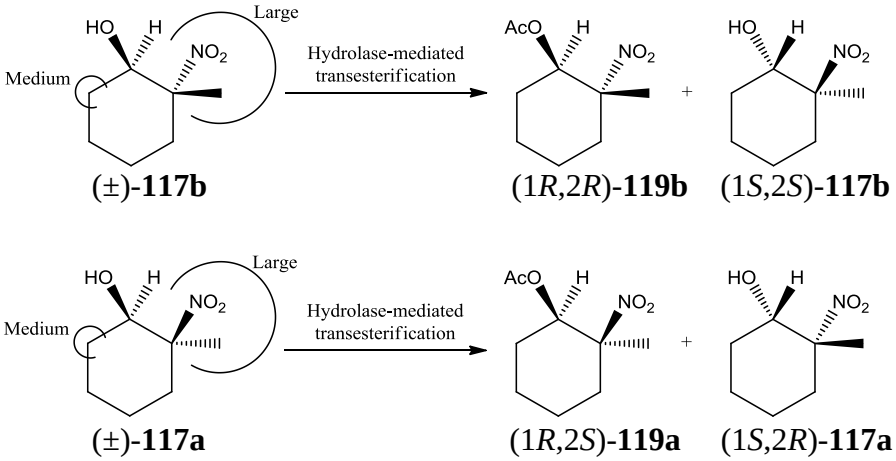


Figure 4.29: ^{1}*H NMR* ***Spectrum I:*** *Crude product following preparative-scale transesterification containing a mixture of enantioenriched (1*S,2R*)*-cis*-2-methyl-2-nitro cyclohexanol (1*S,2R*)-**117a** and (1*R,2S*)*-cis*-2-methyl-2-nitrocyclohexyl acetate (1*R,2S*)-**119a** 68 : 32 respectively (recorded in $CDCl_3$ at 300 MHz).* ***Spectrum II:*** *Purified (1*R,2S*)*-cis*-2-methyl-2-nitrocyclohexyl acetate (1*R,2S*)-**119a**, >98% ee following column chromatography (recorded in $CDCl_3$ at 400 MHz).* ***Spectrum III:*** *Purified (1*S,2R*)*-cis*-2-methyl-2-nitrocyclohexanol (1*S,2R*)-**117a**, 45% ee following column chromatography (recorded in $CDCl_3$ at 400 MHz).*

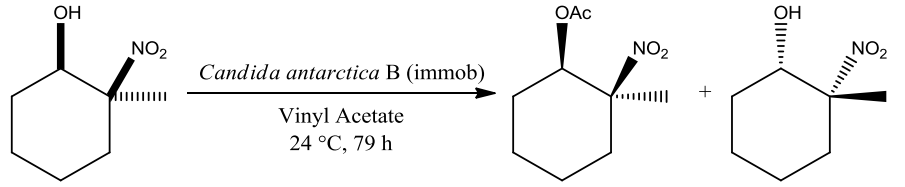
4.3.5.3 Stereochemical assignment of the products of preparative-scale reactions

As discussed in section 4.2.6.2, Kazlauskas's rule can be employed to predict which enantiomer of secondary alcohols reacts faster in reactions catalysed by hydrolases.[72,73] The stereochemical outcome of the reaction from the hydrolase-mediated transesterification of (±)-*cis* and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b**, were confirmed by Milner through single crystal X-ray diffraction and, significantly, agreed with Kazlauskas's rule.[45] When this rule was applied to the preparative-scale hydrolase-mediated bioresolutions of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** the following outcome was anticipated (Scheme 4.53).



Scheme 4.53

The absolute stereochemical assignment of the enantioenriched (1*S*,2*R*)-**117a** alcohol and enantiopure acetate (1*R*,2*S*)-**119a** isolated from the preparative-scale *Candida antarctica* lipase B (immob) mediated resolution of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** was initially examined (Scheme 4.54).



(±)-**117a**

(1*R*,2*S*)-**119a**

$[\alpha]_D^{20}$ −46.2 (c 0.5, $CHCl_3$)

>98% ee

(1*S*,2*R*)-**117a**

+0.7 (c 0.5, $CHCl_3$)

45% ee

Scheme 4.54

The absolute stereochemistry of (1*R*,2*S*)-**119a** was determined by single crystal X-ray diffraction on a crystalline sample of enantiopure **119a** recrystallised from HPLC grade acetonitrile (Figure 4.30).[80] Full structural details are contained on the accompanying CD. Significantly this assignment is in agreement with Kazlauskas's rule (Scheme 4.53). The absolute stereochemistry of the enantioenriched untransformed alcohol therefore is assigned as (1*S*,2*R*)-**117a**.

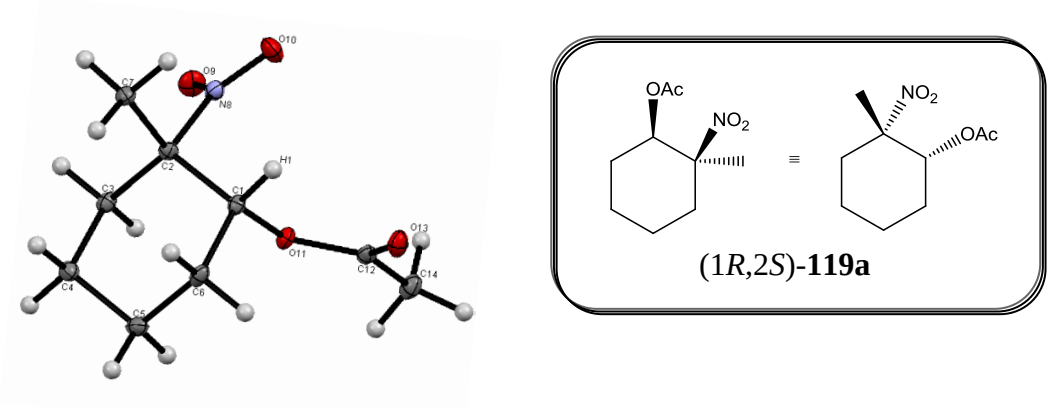
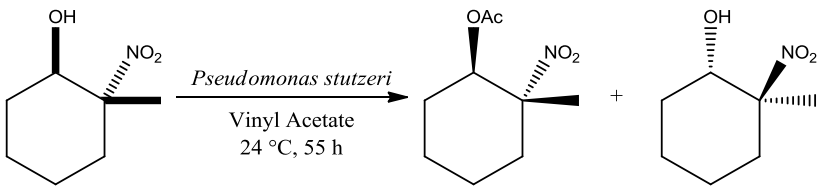


*Figure 4.30: A view of (1*R,*2*S*)-*cis-2-methyl-2-nitrocyclohexyl acetate (1*R,*2*S*)-**119a** showing the structure and relative stereochemistry. The model has chirality C1 (*R*) and C2 (*S*). Anisotropic displacement parameters are drawn at the 30% probability level.*

The stereochemical assignment of the products of the preparative-scale hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was next examined (Scheme 4.55).



(±)-**117b**

(1*R*,2*R*)-**119b**

$[\alpha]_D^{20}$ −32.6 (c 1.0, $CHCl_3$)

>98% ee

(1*S*,2*S*)-**117b**

+42.0 (c 1.0, $CHCl_3$)

>98% ee

Scheme 4.55

The absolute stereochemistry of the isolated enantiopure *trans*-acetate (1*R*,2*R*)-**119b** and *trans*-alcohol (1*S*,2*S*)-**117b** could not be determined by single crystal X-ray diffraction as both enantiopure products were obtained as oils. Thus, stereochemical assignment of the transesterification products of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** were tentatively assigned as (1*R*,2*R*)-*trans*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*R*)-**119b** and (1*S*,2*S*)-*trans*-2-methyl-2-nitrocyclohexanol (1*S*,2*S*)-**117b** by analogy to the transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and in accordance to Kazlauskas's rule.

Furthermore, the absolute stereochemistry of the products of the lipase-mediated transesterification of both the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and (±)-**117b** are in agreement with the stereochemical assignment of the products of preparative-scale transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b**.[36,45]

4.3.6 One-pot hydrolase-mediated dynamic resolution screens

Crucial to the success of the hydrolase-mediated dynamic kinetic resolution was selection of the correct base. One of the fundamental requirements of the selected base is catalysis of the dynamic interconversion process between the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b**. Once the dynamic process was confirmed by ^{1}H NMR spectroscopy it was then envisaged that combination with the established diastereoselective lipase-mediated transesterification would lead to a one-pot dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis.

The objective of this study was to identify a base that fulfils the following criteria:

- Effectively ring closes 6-nitroheptanal **118** *via* the intramolecular Henry reaction to form the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b**.
- Catalyses the dynamic interconversion process between (±)-**117a** and (±)-**117b** *via* ring opening and closing *via* 6-nitroheptanal **118**.
- Can be combined in the dynamic process with the diastereoselective lipase-mediated transesterification in a dynamic kinetic resolution protocol.

Conversions were determined throughout this study by ^{1}H NMR spectroscopy (Figure 4.31) and are derived from integration of;

- 1H, br s, C(1)**H**OH at 3.91 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**.
- 1H, m, C(1)**H**OH, at 4.28-4.29 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**
- 1H, m, C(6)HNO_2 at 4.51-4.63 ppm in 6-nitroheptanal **118**
- 1H, m, C(1)HOAc at 5.26-5.29 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119a**
- 1H, m, C(1)HOAc at 5.50-5.55 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119b**

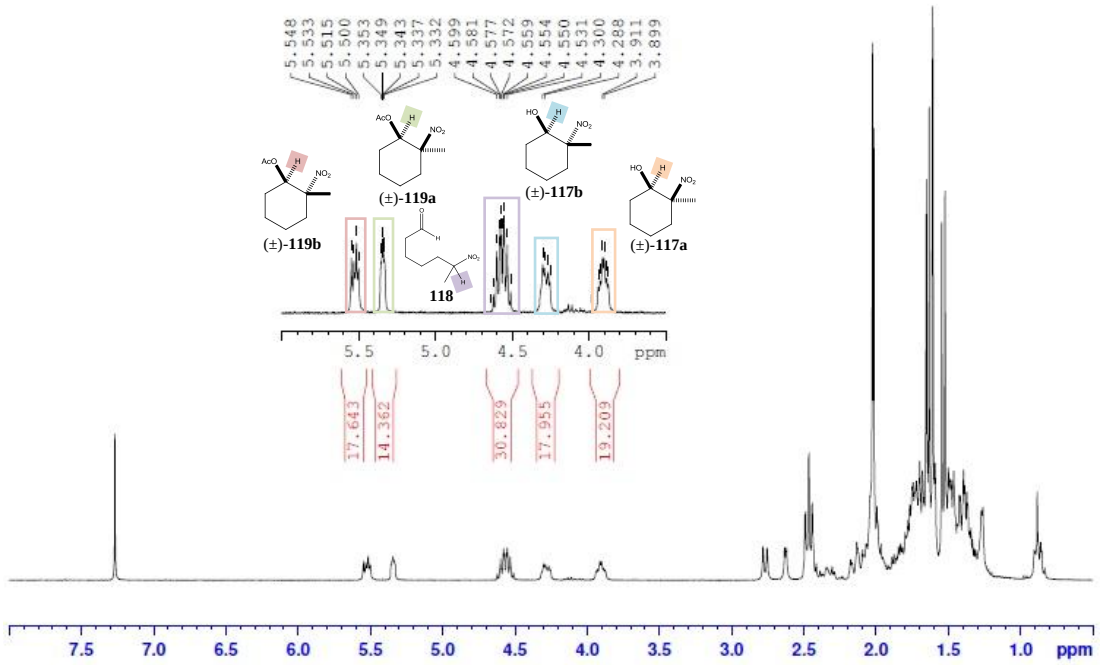
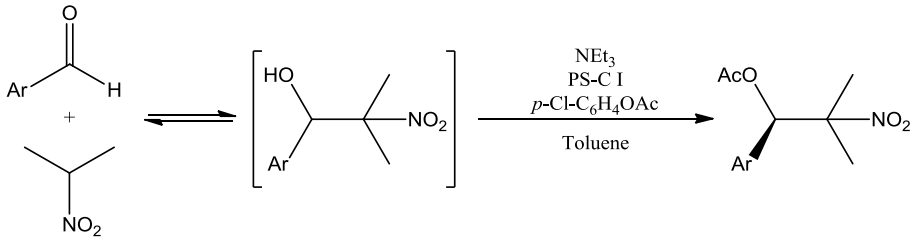


*Figure 4.31: ^{1}H NMR Spectrum: Mixture of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**, (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**, 6-nitroheptanal **118**, (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119a** and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119b** (19 : 18 : 31 : 14 : 18 respectively) recorded in $CDCl_3$ at 300 MHz.*

4.3.6.1 Triethylamine

As discussed in section 4.1.1.1.2, Ranström successfully synthesised highly enantioenriched β-nitroalkanol derivatives by an intermolecular Henry reaction between a series of aldehydes and nitropropane combined with an enzyme-mediated transesterification.[43] High yields and enantiopurities were achieved with a range of aromatic aldehydes. However, the bioresolution is restricted by a narrow substrate range with aliphatic aldehydes and electron donating *para* substituted aromatics (entries 5 and 6, Table 4.26) resolved with poor yields. In addition, limited enantioselectivity is observed with thiophene derivatives (entry 7, Table 4.26).

Table 4.26: Hydrolase-mediated dynamic kinetic resolution of β-nitroalcohols[43]



Entry	Ar	Time	Yield (%)	ee (%)
1	4-O_2N-C_6H_4	2 d	90	99
2	4-NC-C_6H_4	2 d	89	91
3	4-F_3C-C_6H_4	3 d	89	97
4	3-O_2N-C_6H_4	3 d	90	91
5	4-CH_3-C_6H_4	4 d	35	93
6	4-CH_3O-C_6H_4	4 d	28	99
7	Thiophene-2-yl	4 d	68	46

This one-pot procedure was performed at 40 °C in toluene with between 2.0 and 5.0 equivalents of triethylamine, and thus, based on this preceding report, initial experiments in this study were conducted investigating triethylamine as a suitable catalyst for dynamic interconversion of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b**.

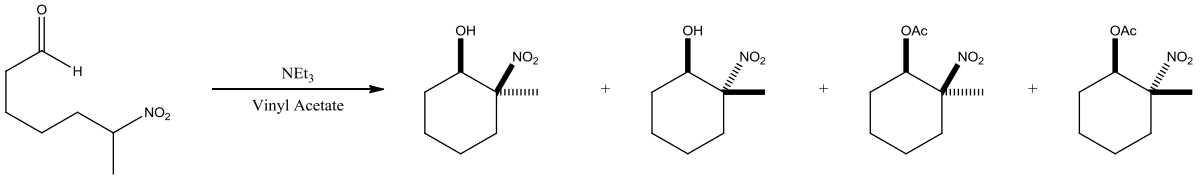
*4.3.6.1.1 Cyclisation of 6-nitroheptanal **118*** via *the intramolecular Henry reaction*

The objective of the initial series of experiments was to investigate if triethylamine could successfully effect ring closure of 6-nitroheptanal **118** *via* the intramolecular nitroaldol reaction to form the β-nitroalcohols (±)-**117a** and (±)-**117b** and subsequently determine the associated thermodynamic ratio between the diastereomeric alcohols (±)-**117a** and (±)-**117b**. As vinyl acetate had been applied for the lipase-mediated transesterification in both the analytical and preparative-scale, ideally the base-mediated investigation should be effected in the same solvent to ultimately enable combination of the two processes in a single pot. Four experiments were conducted, either at room temperature or 40 °C, with 6-nitroheptanal **118** in vinyl acetate together with varying amounts of triethylamine. Aliquots were removed at regular time intervals and concentrated under reduced pressure to enable direct monitoring of the formation of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** by ^{1}H NMR analysis.

While cyclisation of 6-nitroheptanal **118** by triethylamine in vinyl acetate can be achieved, as is evident from Table 4.27 and Figure 4.32 complete cyclisation of **118** to the diastereomeric alcohols (±)-**117a** and (±)-**117b** in the presence of between 1.0-2.0 equivalents of triethylamine was not observed. After 72 h at room temperature with 1.0, 1.5 and 2.0 equivalents of triethylamine (entries 2, 3 and 4, Table 4.27), 65-78% of the starting aldehyde **118** remained. The most promising result was achieved when the temperature was increased to 40 °C with 2.0 equivalents of triethylamine (entry 1, Table 4.27). Under these conditions only 9% of the aldehyde **118** was evident by ^{1}H NMR analysis at 72 h with the predominant products being the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b**, 24 : 67 respectively. Thus, the dynamic interconversion between (±)-**117a** and (±)-**117b** *via* ring closing and opening of 6-nitroheptanal **118** warrants investigation under these optimum reaction conditions.

In these screens, the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was clearly the more stable diastereomer, formed to a greater extent than the (±)-*cis*-diastereomer (±)-**117b** in all cases. In terms of our overall objective, the increased amount of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** fortuitously coincides with the observation of enhanced *Candida antarctica* lipase B (immob) mediated kinetic resolution of the *trans*-diastereomer (±)-**117b** when the biocatalysis was conducted with an equimolar mixture of (±)-**117a** and (±)-**117b** (see section 4.3.4.5). Indeed, with the thermodynamic ratio favouring (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** coupled with the enhanced enzyme-mediated transformation of this *trans*-diastereomer (±)-**117b** the potential to lead to a genuinely selective dynamic kinetic resolution is strengthened.

*Table 4.27: Triethylamine-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Entry	Triethylamine (eq.)	Temp (°C)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	2.0	40	24 h	62	14	24	-	-
			48 h	37	24	39	-	-
			72 h	9	24	67	-	-
2	2.0	Ambient	24 h	89	4	7	-	-
			48 h	76	9	15	-	-
			72 h	65	12	23	-	-
3	1.5	Ambient	24 h	90	3	7	-	-
			48 h	79	7	14	-	-
			72 h	67	11	22	-	-
			7 days	34	22	44	-	-
4	1.0	Ambient	24 h	92	3	5	-	-
			48 h	85	5	10	-	-
			72 h	78	7	15	-	-

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

From a practical prospective the absence of any significant side reactions in this study such as the aldol condensation augurs well for optimisation of this process. Significantly there was no evidence of acetylation with vinyl acetate in the absence of a lipase, which is crucial in terms of achieving a combined one-pot dynamic kinetic resolution process. In contrast to this study, Milner reports rapid cyclisation of 6-nitrohexanal **101** to the (±)-2-nitrocyclohexanols (±)-**99a** and (±)-**99b** with just 1.0 equivalent of triethylamine at room temperature.[45] Thus, the presence of a methyl moiety geminal to the nitro group appears to reduce the efficiency of the intramolecular Henry reaction.

*Comparison of conversion (%) of 6-nitrohetptanal **118**, (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** following exposure of 6-nitroheptanal **118** to different equivalents of triethylamine at room temperature or 40 °C at 72 h*

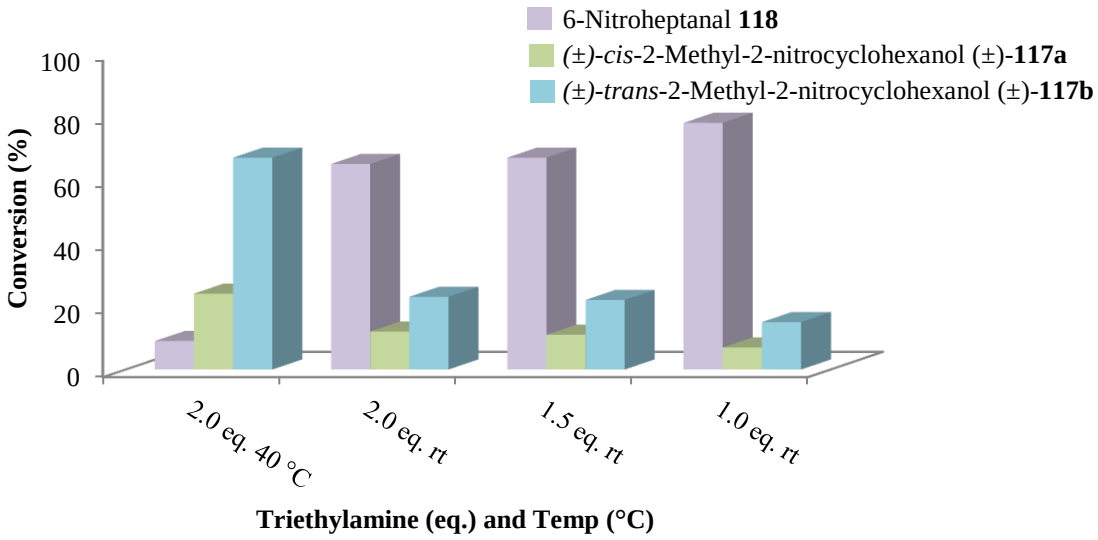
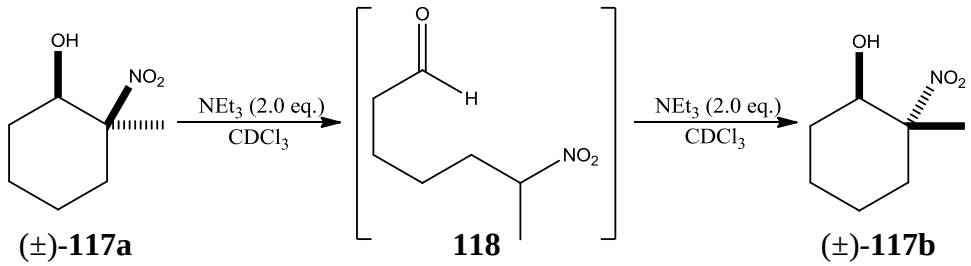


Figure 4.32

4.3.6.1.2 Dynamic interconversion process

The next step in this investigation was to examine the dynamic interconversion process between (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b**. The first study involved diastereomerically pure (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** dissolved in deuterated chloroform with 2.0 equivalents of triethylamine at room temperature (Table 4.28). This experiment was conducted in a NMR tube enabling direct analysis of the ratios of the β-nitroalcohols (±)-**117a** and (±)-**117b** over time. As is evident from Table 4.28 and Figure 4.33 limited dynamic interconversion was observed with only 28% of the more stable (±)-*trans*-diastereomer (±)-**117b** evident after 17 days. Thus, 2.0 equivalents of triethylamine in deuterated chloroform were ineffective in catalysing the dynamic interconversion process. This was not unexpected due to the limited cyclisation observed of 6-nitroheptanal **118** with 2.0 equivalents of triethylamine in vinyl acetate (entry 2, Table 4.27).

Table 4.28: Evidence for dynamic interconversion - (±)-cis-*2-methyl-2-nitrocyclohexanol (±)-**117a** and triethylamine (2.0 eq.) in $CDCl_3$.*



(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
10 min	100	-
1 h 23 min	100	-
5 h 46 min	100	-
11 h 30 min	100	-
3 days	95	5
7 days	90	10
17 days	72	28

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

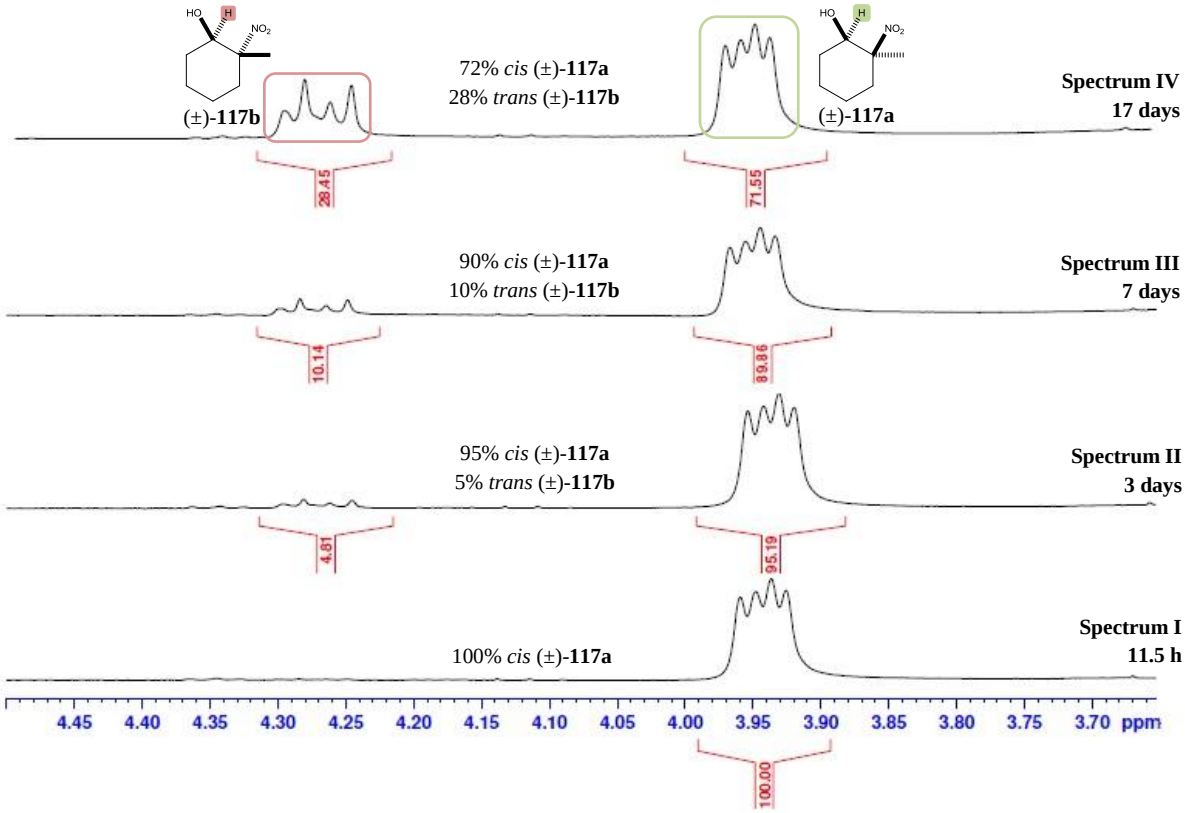


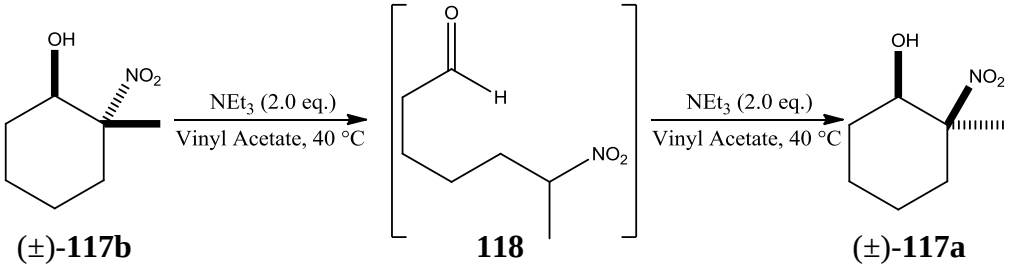
*Figure 4.33: Stacked ^{1}H NMR spectra: Investigation of dynamic interconversion – (±)-*cis-*2-methyl-2-nitrocyclohexanol (±)-**117a** and NEt_3 (2.0 eq.) (Recorded in $CDCl_3$ at 300 MHz).*

The next study investigated the dynamic interconversion process under the optimum reaction conditions identified in the base-mediated intramolecular nitroaldol reaction (entry 1, Table 4.27). Diastereomerically pure (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was dissolved in vinyl acetate with 2.0 equivalents of triethylamine and heated to 40 °C. Aliquots were removed at regular time intervals and analysed by ^{1}H NMR spectroscopy.

This dynamic interconversion would require transformation of the more thermodynamically stable diastereomer (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** to the *cis*-diastereomer (±)-**117a**. In this investigation, no interconversion to the (±)-*cis*-diastereomer (±)-**117a** was observed by ^{1}H NMR analysis even at the elevated temperature and prolonged reaction time. The dynamic interconversion process was briefly explored with a higher amount of triethylamine (8.0 equivalents) however there was negligible improvement in the efficiency of the dynamic interconversion process.

In direct contrast to this investigation, Milner reported interconversion between (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** in the presence of as little as 0.01 equivalent of triethylamine achieving a thermodynamic ratio of 15 : 85 over time. Significantly, this reported process was later determined to operate solely *via* an epimerisation mechanism and dynamic interconversion *via* ring opening and closing of 6-nitrohexanal **101** with triethylamine was not achieved. Accordingly these results seen with (±)-**117b** are consistent with Milner's results even though the outcome is different.

*Table 4.29: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and triethylamine (2.0 eq.)in vinyl acetate at 40 °C*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
1 day	-	100
2 days	-	100
3 days	-	100

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

The absence of interconversion of (±)-**117a** and (±)-**117b** on exposure to triethylamine and vinyl acetate represents a significant challenge to the objective of a dynamic kinetic resolution process as interconversion of the stereocentres at C1 and C2 *in situ* is an intrinsic requirement of the process. On the positive side the interconversion of (±)-**117a** and (±)-**117b** with triethylamine in deuterated chloroform (Table 4.28) does offer potential for the interconversion.

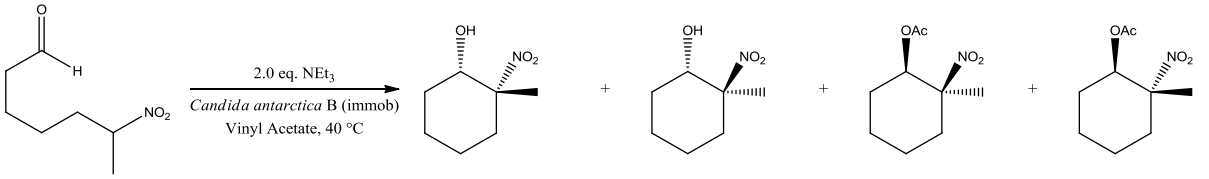
4.3.6.1.3 Dynamic one-pot kinetic resolution process

The combined one-pot dynamic kinetic resolution protocol was subsequently investigated with triethylamine. At the outset this investigation was complicated by the lack of evidence of a triethylamine-mediated dynamic interconversion process between (±)-**117a** and (±)-**117b**, see above section 4.3.6.1.2. The combined process was investigated with 2.0

equivalents of triethylamine at 40 °C as under these reaction conditions 6-nitroheptanal **118** was almost completely cyclised to the corresponding alcohol diastereomers (±)-**117a** and (±)-**117b** (entry 1, Table 4.27). *Candida antarctica* lipase B (immob) was selected, as this hydrolase had previously demonstrated excellent diastereoselectivity in the kinetic resolution of (±)-**117** (see section 4.3.4.5).

The aldehyde 6-nitroheptanal **118** was the predominant component after 72 h (Table 4.30), which was not anticipated as in the preliminary cyclisation screens only 9% of the aldehyde **118** was detected at 72 h with 2.0 equivalents of triethylamine in vinyl acetate at 40 °C (entry 1, Table 4.27). Therefore, the presence of the lipase, *Candida antarctica* lipase B (immob) in this study appears to inhibit the base and thus reduce the efficiency of the intramolecular Henry reaction signifying a further complication in the triethylamine-mediated dynamic kinetic resolution process. While no exploration of the mechanism of the base inactivation was undertaken, it is possible that the base is protonated and/or complexed to the surface of the protein.

*Table 4.30: Cyclisation of 6-nitroheptanal **118** with CAL-B (*immob*) catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with triethylamine (2.0 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Enzyme Source	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
			cis-117a (%)[a] [ee (%)][b]	*trans*-117b (%)[a] [ee (%)][b]	*cis*-119a (%)[a] [ee (%)][b]	*trans*-119b (%)[a] [ee (%)][b,f]
Candida antarctica B (immob)	72 h	31	16 [65]	23 [58]	10 [>98]	20 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

In the ^{1}H NMR spectrum of the crude product, in addition to the unreacted aldehyde **118**, the presence of the alcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** and the acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** were evident and critically, chiral HPLC analysis indicated that the acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** were essentially enantiopure while the alcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** displayed modest enantiopurity (Figure 4.34 and 4.35). Based on the investigation of each of the individual steps discussed earlier these results are entirely consistent with triethylamine-mediated ring closure of **118** *via* intramolecular Henry reaction to form (±)-**117a** and (±)-**117b** followed by kinetic resolution *via* enzyme-mediated transformation of each of the cyclohexanols (±)-**117a** and (±)-**117b**, independently. There is no evidence that the dynamic process for interconversion of (±)-**117a** and (±)-**117b** *via* ring opening and ring closure *via* **118** is operating. The differences in the outcome in terms of enantiopurity of the alcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** relative to the data in Table 4.23 can be rationalised based on the different starting ratios of (±)-**117a** and (±)-**117b**, thus in Table 4.23 the *Candida antarctica* lipase B (immob) mediated resolution was explored with a

50 : 50 mixture of (±)-**117a** and (±)-**117b**, while in the cyclisation of **118** it is envisaged that (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** is formed selectively (see Table 4.27) accordingly the higher enantiomeric excess of (1*S*,2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a** is simply due to more efficient transformation of (±)-**117a** at the reduced overall concentration.

In conclusion, while 2.0 equivalents of triethylamine at 40 °C catalysed the intramolecular nitroaldol reaction with 6-nitroheptanal **118** at least partially, dynamic interconversion was not observed under these reaction conditions and thus a dynamic kinetic resolution process was unattainable. Instead two parallel kinetic resolution processes with (±)-**117a** and (±)-**117b** independently were observed.

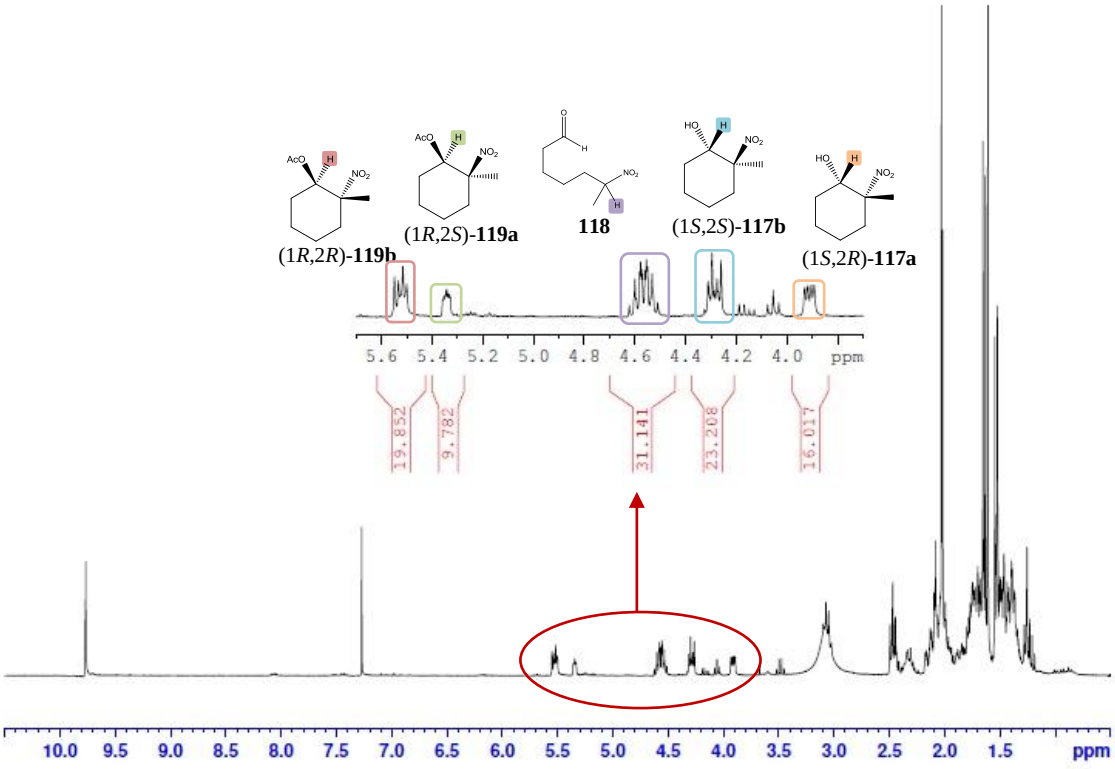


Figure 4.34: ^{1}H NMR Spectrum: Candida antarctica *lipase B (immob) and triethylamine-mediated dynamic kinetic resolution process. Mixture of* cis-*2-methyl-2-nitrocyclohexanol* ***117a***, trans-*2-methyl-2-nitrocyclohexanol* ***117b***, *6-nitroheptanal* ***118***, cis-*2-methyl-2-nitrocyclohexyl acetate* ***119a*** *and* trans-*2-methyl-2-nitrocyclohexyl acetate* ***119b*** *(16 : 23 : 31 : 10 : 20 respectively) recorded in $CDCl_3$ at 300 MHz.*

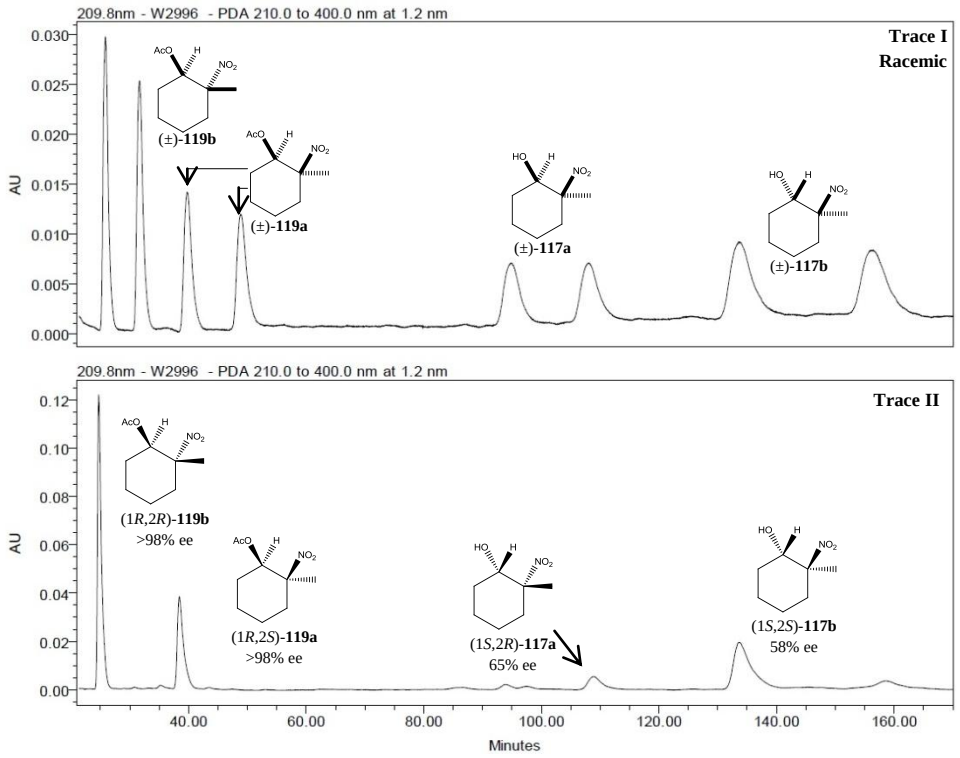


Figure 4.35: HPLC ***Trace I:*** *A racemic mixture of (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119b**, (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a**, (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**.* ***Trace II:*** Candida antarctica *lipase B (immob) and triethylamine-mediated dynamic kinetic resolution process. (1*R,*2*R*)-*trans*-2-Methyl-2-nitrocyclohexyl acetate (1*R,*2*R*)-**119b**, >98% ee, (1*R,*2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetate (1*R,*2*S*)-**119a**, >98% ee, (1*S,*2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S,*2*R*)-**117a**, 65% ee and (1*S,*2*S*)-*trans*-2-methyl-2-nitrocyclohexanol (1*S,*2*S*)-**117b**, 58% ee. For HPLC conditions see appendix I.*
Note the above traces correlate to chiral HPLC conditions B.

4.3.6.2 1,8-Diazabicycloundec-7-ene (DBU)

Due to the limited triethylamine-mediated interconversion between the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** *via* ring opening and closing of 6-nitroheptanal **118** especially in the presence of the biocatalyst, it was envisaged that a stronger base was required to catalyse the intramolecular Henry reaction together with the dynamic interconversion process. DBU was selected as its conjugate acid has a higher pK_a (pK_a 12.0 in H_2O)[94] relative to triethylamine (pK_a 10.75 in H_2O).[95]

*4.3.6.2.1 Cyclisation of 6-nitroheptanal **118*** via *the intramolecular Henry reaction*

The first series of experiments investigated the base-catalysed nitroaldol reaction. These studies were performed with 6-nitroheptanal **118** with DBU in vinyl acetate at room temperature. A range of amounts of DBU was investigated in order to examine the efficiency of this process. At regular time intervals aliquots were removed, concentrated under reduced pressure and analysed by ^{1}H NMR spectroscopy. As is evident from entries 1 and 2, Table 4.31, 1.00 and 0.50 equivalent of DBU result in complete cyclisation of 6-nitroheptanal **118** within 24 h. Clearly DBU-mediated cyclisation of **118** is more efficient than the corresponding reaction mediated by triethylamine, as expected. However, the anticipated alcohols (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** were not evident in the ^{1}H NMR spectrum as they were acetylated with vinyl acetate in the presence of DBU to form (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-

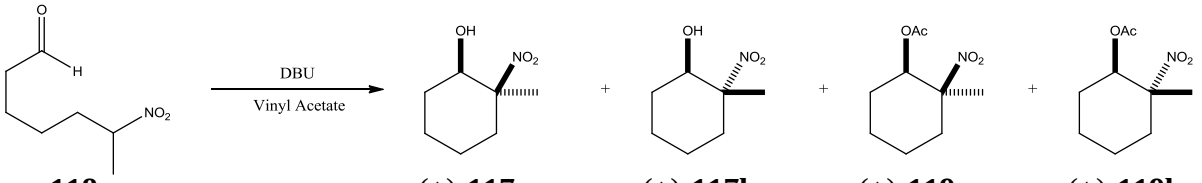
119b in a ratio of 40 : 60 respectively. Thus, in the absence of a lipase, chemical acetylation occurs under the aforementioned reaction conditions, leading to racemic acetates (±)-**119a** and (±)-**119b.** This result highlights yet another significant hurdle in this research in the development of a DBU-mediated one-pot dynamic kinetic resolution process to a single stereoisomer of 2-methyl-2-nitrocyclohexyl acetate **119**.

As the amount of DBU was reduced so did the efficiency of the chemical acetylation process. At 24 h with 0.10 equivalent of DBU (entry 3, Table 4.31) complete cyclisation of 6-nitroheptanal **118** was observed. Notably both the (±)-*cis*- and (±)-*trans*-alcohols (±)-**117a** and (±)-**117b** and (±)-*cis*- and (±)-*trans*-acetates (±)-**119a** and (±)-**119b** were evident in the ^{1}H NMR spectrum at 24 h. However, as the reaction proceeded, the *cis*-alcohol (±)-**117a** was completely acetylated over time and only 25% of the *trans*-alcohol (±)-**117b** remained after 7 days reaction time. Thus, chemical acetylation competes effectively with the intramolecular Henry under these reaction conditions.

Significantly, only trace evidence of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetates (±)-**119a** and (±)-**119b** were observed at 0.05 equivalent of DBU (entry 4, Table 4.31) and no chemical acetylation was determined at 0.01 equivalent of DBU (entry 5, Table 4.31). However, conversely at these low concentrations of DBU the efficiency of the desired cyclisation of 6-nitroheptanal **118** *via* the intramolecular Henry reaction was limited. Nevertheless, these results indicate that, in principle at least, the cyclisation to form (±)-**117a** and (±)-**117b** can be effected under these conditions which avoid competing chemical acetylation. This observation augurs well for the development of the dynamic kinetic resolution process.

In summary, while DBU in the presence of vinyl acetate efficiently ring closed 6-nitroheptanal **118** *via* the Henry reaction, a major complication was the observed chemical acetylation at 0.05 equivalent and greater of DBU. At 0.01 equivalent of DBU no chemical acetylation was detected, however, minimal ring closure of 6-nitroheptanal **118** was observed. Thus, a delicate balance must be achieved where the base DBU in the presence of vinyl acetate mediates the desired intramolecular nitroaldol reaction but does not catalyse the racemic chemical acetylation process.

*Table 4.31: DBU-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



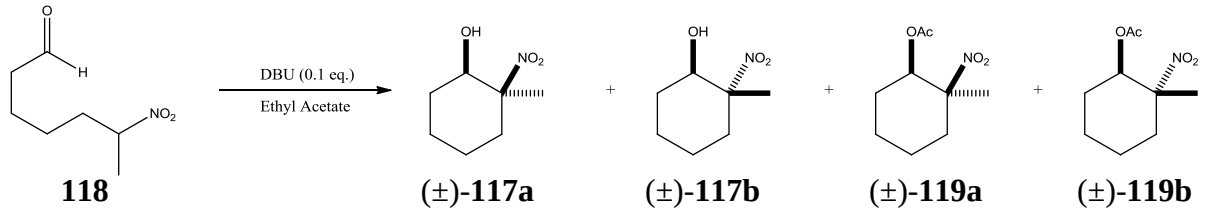
118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Entry	DBU (eq.)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
				cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	1.00	24 h	-	-	-	40	60
		48 h	-	-	-	40	60
		72 h	-	-	-	39	61
2	0.50	24 h	-	-	-	42	58
		48 h	-	-	-	40	60
		72 h	-	-	-	39	61
		7 days	-	-	-	39	61
3	0.10	24 h	-	11	52	22	15
		48 h	-	-	30	37	33
		72 h	-	-	28	37	35
		7 days	-	-	25	40	35
4	0.05	24 h	48	15	36	1	-
		48 h	50	16	31	2	1
		71 h	55.5	11	31	2	0.5
5	0.01	24 h	94	2	4	-	-
		48 h	93	2	5	-	-
		72 h	91	3	6	-	-

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

Due to the undesired chemical acetylation with vinyl acetate in the presence of DBU, ethyl acetate was investigated as an alternative solvent and less active acyl transfer agent (Table 4.32). Ethyl acetate has been employed as both solvent and acyl donor in lipase-mediated transesterification reactions.[96-98] The same protocol as previously described was employed in this study, with 6-nitroheptanal **118** and 0.1 equivalent of DBU in ethyl acetate. Reaction monitoring was conducted at 24 h and 48 h. Significantly, in direct contrast to the vinyl acetate study, ethyl acetate did not promote chemical acetylation with 0.1 equivalent of DBU. 6-Nitroheptanal **118** was successfully converted to the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** with a thermodynamic ratio of 29 : 71 achieved within 24 h with no evidence of the acetates (±)-**119a** and (±)-**119b** by ^{1}H NMR spectroscopy. Although this result is extremely promising, the lipase-mediated transesterification must first be established with ethyl acetate before this acetylating agent can be considered for the one-pot dynamic kinetic resolution process.

*Table 4.32: DBU (0.1 eq.) mediated cyclisation of 6-nitroheptanal **118** with ethyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

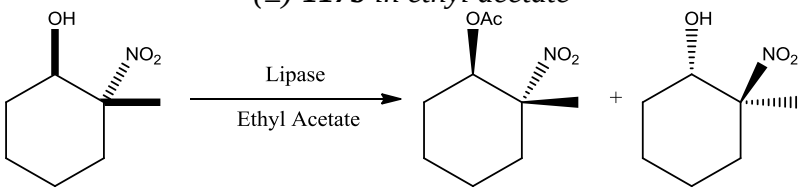
Acetylating agent	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
			cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
Ethyl Acetate	24 h	-	29	71	-	-
	48 h	-	30	70	-	-

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

Due to the complete absence of chemical acetylation with ethyl acetate in the presence of DBU, the transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was explored with ethyl acetate as both solvent and acetylating donor (Table 4.33). *Candida antarctica* lipase B (immob) and *Pseudomonas stutzeri* mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** with vinyl acetate as both solvent and acetylating agent achieved excellent enantioselection (Table 4.21); thus these lipases were selected for screening during the ethyl acetate study (Table 4.33). In the analytical screens, both resolutions resulted in poor conversions <19%. *Pseudomonas stutzeri* (entry 2, Table 4.33) reported <10% conversion and thus no HPLC analysis was conducted. Although excellent enantiopurity was observed of the acetate (1*R*,2*R*)-**119b** with *Candida antarctica* lipase B (immob) (entry 1, Table 4.33), the poor conversion resulted in minimal enantiopurity of the recovered alcohol (1*S*,2*S*)-**117b**.

Thus, DBU-mediated intramolecular nitroaldol cyclisation of 6-nitroheptanal **118** occurs readily in the presence of ethyl acetate without any evidence of chemical acetylation detected. If the excellent efficiency and enantioselectivity observed with vinyl acetate in the lipase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** can be achieved with ethyl acetate as the acyl donor through modification of conditions this would be a significant advance in this project.

*Table 4.33: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in ethyl acetate*



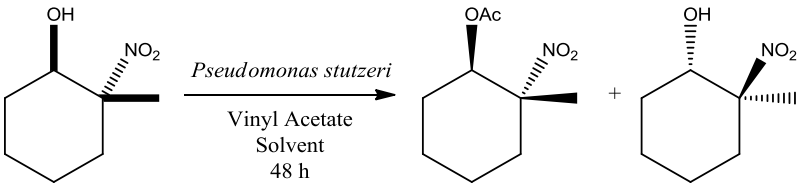
(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1	*Candida antarctica* B (immob)	72 h	16	18	19	>98	119
2	*Pseudomonas stutzeri*	48 h	-	<10	-	-	-

At this point in the study, the amount of DBU and an alternative acetylating agent have both been investigated in order to inhibit chemical acetylation. The next series of experiments examined the introduction of a solvent in the kinetic resolution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**, thereby reducing the amount of vinyl acetate. It was envisaged that by employing a solvent in the bioresolution, the non-enzymatic chemical acetylation might be avoided with the vinyl acetate (5.0 equivalents) only acting as an acyl donor in the lipase-mediated transesterification. However, this process is reliant on obtaining excellent efficiency and enantioselectivity in the kinetic resolution of the *trans*-alcohol (±)-**117b** in the presence of a solvent with vinyl acetate acting solely as acyl donor.

The hydrolase *Pseudomonas stutzeri* was selected for investigation in the analytical screens as this lipase had produced high enantioselectivity in the resolution of the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** with neat vinyl acetate as both solvent and acetylating agent (Table 4.21). A range of solvents of different properties in terms of polarity and hydrogen bonding potential was selected as summarised in Table 4.34. The substrate (±)-**117b** (~20 mg) was dissolved in the appropriate solvent (2.5 mL) with 5.0 equivalents of vinyl acetate and a spatula tip of lipase. The concentration of the substrate (±)-**117b** in the medium was unchanged to ensure comparability of the results with earlier screens. The reaction mixture was agitated for 48 h at 24 °C. Although excellent enantiopurity was obtained for the acetate (1*R*,2*R*)-**119b** in each of the *Pseudomonas stutzeri* mediated transesterifications, the resolutions failed to achieve the optimum 50% conversion rate and thus the enantiopurity of the alcohol (1*S*,2*S*)-**117b** was compromised. The highest enantioselectivity was observed with diisopropyl ether as solvent (entry 3, Table 4.34), however, even with this solvent the enantiopurity of the alcohol (1*S*,2*S*)-**117b** was reduced (84% ee) relative to that achieved when vinyl acetate was employed as both solvent and acetylating agent (>98% ee).

*Table 4.34: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate as acyl donor and alteration of solvents*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Enzyme Source	Solvent	Conversion (%)		ee (%)[a]		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1	*Pseudomonas stutzeri*	Heptane	40	41	66	>98	197
2	*Pseudomonas stutzeri*	Diethyl ether	46	44	82	>98	>200
3	*Pseudomonas stutzeri*	Diisopropyl ether	46	45	84	>98	>200
4	*Pseudomonas stutzeri*	Toluene	20	21	24	>98	125
5	*Pseudomonas stutzeri*	Acetonitrile	25	26	32	>98	135

a. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

In all cases, the precursor (±)-**117b** dissolved readily in the reaction medium and therefore the relative efficiencies in Table 4.34 appear to reflect varying enzymatic activity in these different solvents. Entries 2 and 3 in the ether solvents show a potential to lead to efficient kinetic resolution through variation of the biocatalysis conditions to enable complete conversion of (1*S*,2*S*)-**117b**.

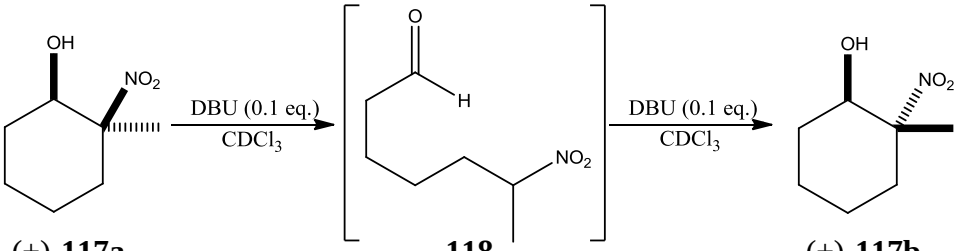
In summary, the introduction of a solvent to the hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** with vinyl acetate decreases the efficiency of the transesterification and accordingly the enantiopurity of the recovered alcohol (1*S*,2*S*)-**117b** is somewhat compromised. Critically the acetate (1*R*,2*R*)-**119b** is formed with excellent enantiopurity which is the key point required to enable the dynamic kinetic resolution process. Therefore, further studies are warranted investigating the cyclisation of 6-nitroheptanal **118** in the presence of DBU, diisopropyl ether and only 5.0 equivalents of vinyl acetate to determine if the lower concentration of vinyl acetate obviated chemical acetylation.

4.3.6.2.2 Dynamic interconversion process

The next series of experiments in this investigation explored the DBU-catalysed dynamic interconversion mechanism. Diastereomerically pure samples of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** were dissolved in deuterated chloroform and 0.1 and 0.5 equivalent of DBU were added to the appropriate NMR tube. Monitoring by ^{1}H NMR was conducted and the formation of the analogous diastereomer (±)-**117a** or (±)-**117b** examined over time. Significantly in each of the experiments summarised in Tables 4.35-4.37 conversion to the opposite diastereomer (±)-**117a** or (±)-**117b** was observed immediately. As indicated in Tables 4.35-4.37 below, the thermodynamic ratio of 35 : 65 (±)-**117a** : (±)-**117b** was attained in each of the experiments within 24 h irrespective of whether the experiment was conducted starting from the (±)-*cis*-(±)-**117a** or the (±)-*trans*-diastereomer (±)-**117b** and with 0.1 or 0.5 equivalent (Figure 4.36-4.38). Thermodynamic equilibrium was achieved in a similar timeframe with either 0.1 or

0.05 equivalent of DBU. Notably, chemical acetylation was not observed as these experiments were conducted free from an acetylating agent.

*Table 4.35: Evidence for dynamic interconversion – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.1 eq.) in $CDCl_3$*



(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
15 min	96	4
33 min	93	7
1 h 8 min	87	13
2 h 4 min	80	20
3 h 31 min	70	30
4 h 52 min	63	37
7 h 17 min	53	47
9 h	48	52
24 h 45 min	35	65
32 h 47 min	34	66
73 days	33	67

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

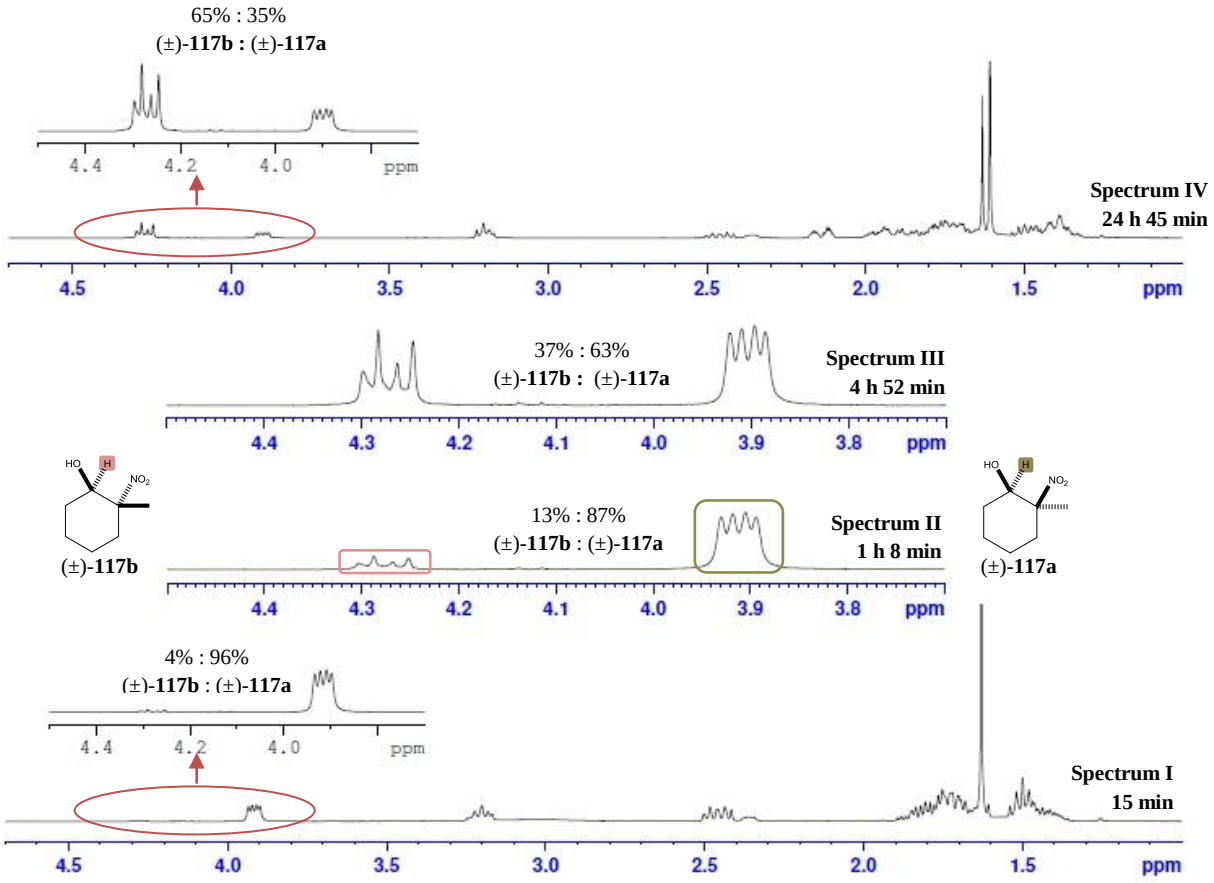
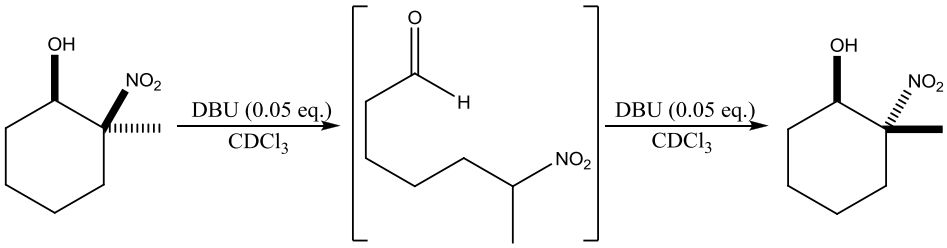


*Figure 4.36: Stacked ^{1}H NMR spectra: Evidence for dynamic interconversion - (±)-cis-2-methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.1 eq.) recorded in $CDCl_3$ at 300 MHz.*

*Table 4.36: Evidence for dynamic interconversion – (±)-cis-2-methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.05 eq.) in $CDCl_3$*

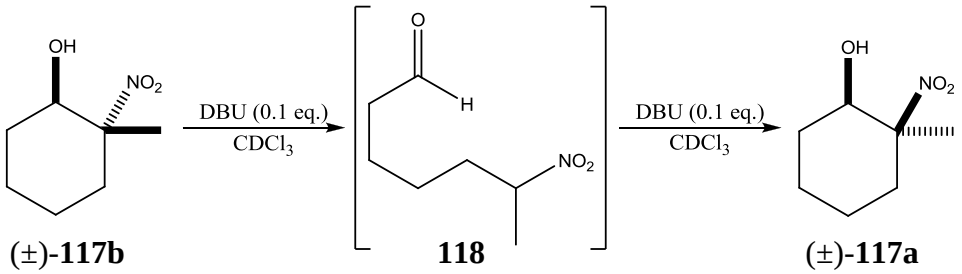


(±)-117a **118** **(±)-117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
2 h 11 min	78	22
18 h 14 min	37	63
25 h 49 min	34	66
7 days	35	65

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*Table 4.37: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) in $CDCl_3$*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
15 min	3	97
24 min	5	95
1 h 12 min	11	89
4 h 16 min	24	76
4 h 49 min	25	75
5 h 51 min	28	72
21 h 52 min	33	67
14 days	33	67

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

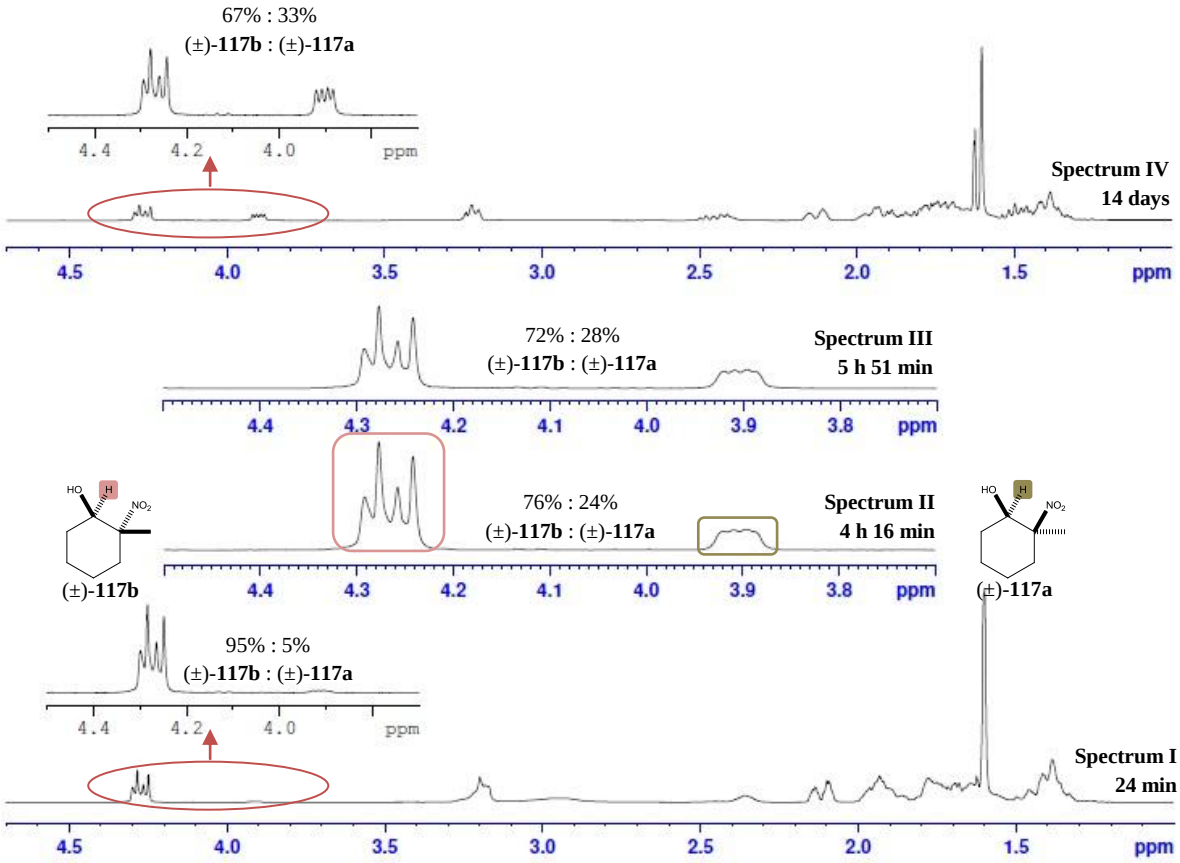
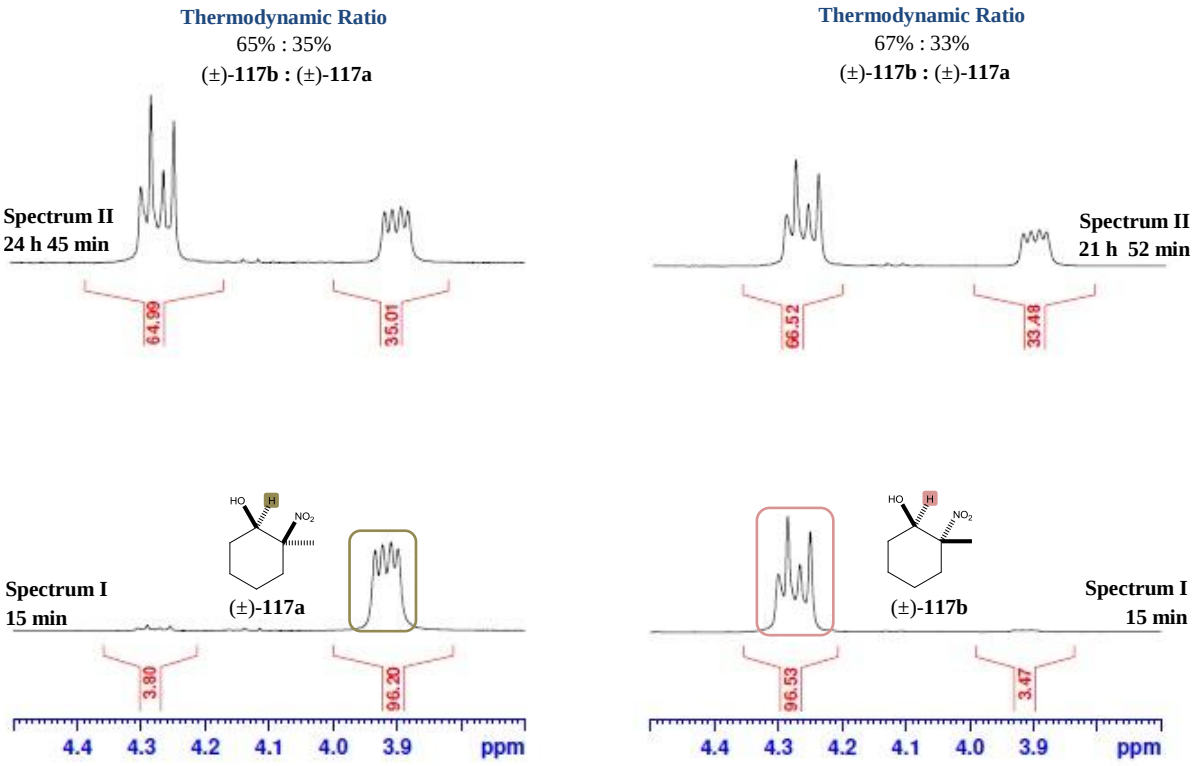


*Figure 4.37: Stacked ^{1}H NMR spectra: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) recorded in $CDCl_3$ at 300 MHz.*



(±)-*cis*-2-Methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.1 eq.) recorded in $CDCl_3$ at 300 MHz.

(±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) recorded in $CDCl_3$ at 300 MHz.

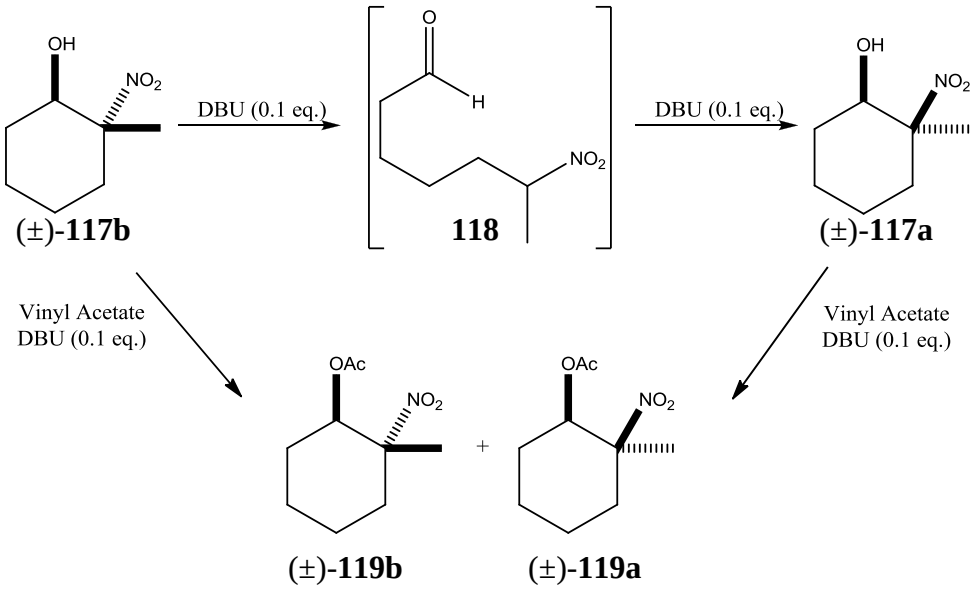
*Figure 4.38: Comparison of ^{1}H NMR spectra recorded and thermodynamic ratio achieved of cis (±)-**117a** on exposure to (0.1 eq.) DBU and trans (±)-**117b** on exposure to (0.1 eq.) DBU.*

It is evident from these studies that in the presence of DBU (just 0.05 equivalent), dynamic interconversion of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** occurs. The (±)-*trans*-diastereomer is the thermodynamically more stable diastereomer with a thermodynamic ratio of 35 : 65 (±)-**117a** : (±)-**117b** attained. As the site of epimerisation is blocked by a methyl moiety, the interconversion observed in this instance must occur *via* ring opening and closing, which is promising for the development of the dynamic hydrolase-mediated process. Notably, this is the first time the dynamic interconversion of nitrocyclohexanols (±)-**117a** and (±)-**117b**, exclusively v*ia* the reversible intramolecular Henry reaction, has been observed.

From the observations of the earlier studies conducted examining the intramolecular nitroaldol reaction with the base DBU and vinyl acetate as solvent (section 4.3.6.2.1), chemical acetylation was anticipated when 0.1 equivalent of DBU were employed in the presence of vinyl acetate in the dynamic interconversion process. To confirm this, DBU (0.1 equivalent) was added in one portion to a stirred solution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate at room temperature (Table 4.38). At 24 h and 48 h an aliquot was removed and concentrated under reduced pressure before ^{1}H NMR analysis was conducted. Dynamic interconversion to the *cis*-diastereomer (±)-**117a** was observed at 24 h however the *trans*-acetate (±)-**119b** was the predominant product (56%) indicating that chemical acetylation competes efficiently with the ring opening and closing process. At 48 h the *trans*-alcohol (±)-**117b** and *trans*-acetate (±)-**119b** were the only two species evident by ^{1}H NMR spectroscopy. Thus, although there was initial evidence for the dynamic

interconversion mechanism at 24 h through appearance of the (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** by ^{1}H NMR, chemical acetylation was evidently the dominant process at 48 h.

*Table 4.38: Evidence for chemical acetylation - (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) in vinyl acetate*



Reaction Time	Alcohol (±)-117[a]		Acetate (±)-119[a]	
	trans **(±)-117b (%)**	***cis*** **(±)-117a (%)**	***trans*** **(±)-119b (%)**	***cis*** **(±)-119a (%)**
24 h	32	13	56	-
48 h	31	-	69	-

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

4.3.6.2.3 Dynamic one-pot kinetic resolution process

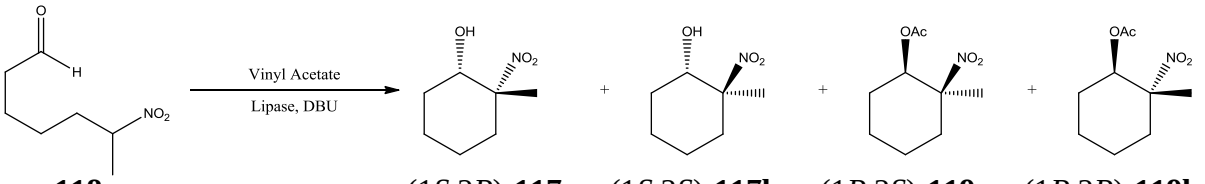
The dynamic kinetic resolution process was next investigated. From the preliminary studies conducted with DBU in the presence of vinyl acetate, this process was anticipated to be complicated by competing chemical acetylation. Initially a series of control experiments were performed and in the absence of both base and hydrolase, the aldehyde **118** was encouragingly determined not to cyclise or react with vinyl acetate over 48 h (entry 1, Table 4.39). When the hydrolase *Candida antarctica* lipase B (immob) was introduced to the vinyl acetate system in the absence of base, again gratifyingly no cyclisation was observed (entry 2, Table 4.39). The final control experiment involved DBU (0.1 equivalent) and vinyl acetate with no hydrolase; complete ring closure of 6-nitroheptanal **118** was achieved. However, significantly of the anticipated β-nitroalcohols (±)-**117a** and (±)-**117b** only the more stable *trans*-alcohol (±)-**117b** was evident by ^{1}H NMR spectroscopy (entry 3, Table 4.39). The predominant process observed was that of chemical acetylation evident by the *cis*- and *trans*-acetates (±)-**119a** and (±)-**119b** correlating with earlier results and signifying a major barrier in the development of a dynamic kinetic resolution process. The slightly different outcome when starting from the aldehyde **118** (entry 3, Table 4.39) in comparison to the outcome in Table 4.38 starting from the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** is interesting and not immediately rationalised. This was not pursued as it was not of value in achieving our overall objective.

When all four variables, DBU (0.05 or 0.10 equivalent), hydrolase, vinyl acetate and 6-nitroheptanal **118** were combined in a dynamic kinetic resolution process (entries 4-6, Table 4.39), unexpectedly no cyclisation of 6-nitroheptanal **118** was observed and thus no chemical acetylation detected. In the previous control experiment (entry 3, Table 4.39) when 0.10 equivalent of DBU with vinyl acetate and 6-nitroheptanal **118** were examined in the absence of a lipase cyclisation to (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** was evident with chemical acetylation. Thus, the addition of a hydrolase to the process appears to severely inhibit the activity of the base. In fact, while partial inhibition of the triethylamine-mediated process had been observed (see section 4.3.6.1.3), the DBU-catalysed process is essentially completely blocked by the presence of the enzyme in entries 4-6, Table 4.39.

On increasing the amount of DBU to 0.20 equivalent (entry 7, Table 4.39), promisingly cyclisation was observed albeit to a limited extent. Thus, the amount of base was further increased to 0.5 and 1.0 equivalent (entries 8 and 9, Table 4.39). Complete cyclisation of 6-nitroheptanal **118** was observed at the increased amount of DBU and any base inhibition by the hydrolase *Pseudomonas stutzeri*, significantly, did not affect the efficiency of the intramolecular Henry reaction. However, conversely, chemical acetylation predominated under these reaction conditions with racemic (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-**119b** the major products by ^{1}H NMR and by chiral HPLC analysis after 48 hours. While in both studies, the chiral HPLC indicates high enantiomeric excess for the recovered alcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b**, the very low levels present means that the accuracy of the data is questionable.

In summary, in contrast to the triethylamine-mediated transformations, DBU is clearly capable of effecting the interconversion of (±)-**117a** and (±)-**117b** *via* the reversible intramolecular Henry reaction. When conducted in the presence of vinyl acetate, chemical acetylation of the resulting alcohols (±)-**117a** and (±)-**117b** to form the racemic acetates (±)-**119a** and (±)-**119b** competes effectively with the cyclisation although the relative rate can be controlled by reducing the amount of DBU. However, use of DBU in the presence of the biocatalyst at least at the levels which give effective cyclisation is not possible due to complete deactivation of the base, and conversely, increased concentration of DBU promotes competing chemical acetylation.

*Table 4.39: Cyclisation of 6-nitroheptanal **118** with hydrolase-catalysed transesterification to 2-methyl-2-nitrocyclohexylacetate **119** in vinyl acetate with DBU as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	DBU (eq.)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis-117a (%)[a]	*trans*-117b (%)[a]	*cis*-119a (%)[a]	*trans*-119b (%)[a]
1	-	-	48 h	100	-	-	-	-
2	CAL-B (immob)	-	72 h	100	-	-	-	-
3	-	0.10	48 h	-	-	28 (±)	36 (±)	36 (±)
4	*P.stutzeri*	0.05	48 h	100	-	-	-	-
5	CAL-B (immob)	0.10	72 h	100	-	-	-	-
6	*P. stutzeri*	0.10	48 h	100	-	-	-	-
7	*P. stutzeri*	0.20	48 h	77	3	10	5	5
8	*P. stutzeri*	0.5	24 h	-	11	4	39	46
			48 h	-	6 [>98]	4 [>98]	37 [1]	53 [1]
9	*P. stutzeri*	1.0	48 h	-	6 [>98]	3 [>98]	53 [1]	38 [0]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

4.3.6.3 Base screening

*4.3.6.3.1 Cyclisation of 6-nitroheptanal **118*** via *the intramolecular Henry reaction*

At this juncture in the investigation, it was decided to screen a series of bases with 6-nitroheptanal **118** and vinyl acetate. The objective was to identify a base that cyclised 6-nitroheptanal **118** to (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** in the presence of vinyl acetate, with no chemical acetylation. These series of experiments were conducted with the aldehyde **118** in vinyl acetate and the appropriate base. Reaction monitoring was performed as previously described by ^{1}H NMR spectroscopy at regular time intervals. A number of the bases chosen have previously been employed in the Henry reaction and the selected bases allowed exploration of a range of pk_a values.[4]

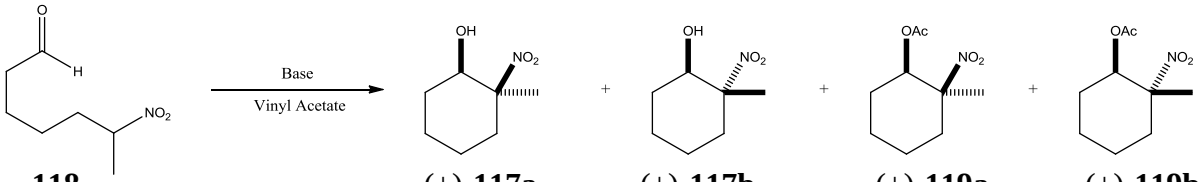
As is evident from Table 4.40 (entries 1, 2, 4 and 5), limited or no cyclisation to the diastereomeric alcohols (±)-**117a** and (±)-**117b** was observed when diethylamine, piperidine, Hünig's base and aqueous sodium hydroxide (1M) were investigated. As the aforementioned bases were unsuccessful in catalysing the intramolecular nitroaldol reaction, further investigation into their mediated dynamic interconversion process was not pursued.

The base 1,4-diazabicyclo[2.2.2]octane (DABCO) effectively mediated ring closing of 6-nitroheptanal **118** within 24 h forming the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** with a thermodynamic ratio of 40 : 60 respectively (entry 3, Table 4.40). Interestingly, this ratio differed slightly to that previously obtained when DBU was employed in the intramolecular Henry reaction (40 : 60 *vs.* 35 : 65). Significantly, no chemical acetylation was observed by ^{1}H NMR spectroscopy, vital for the development of a one-pot dynamic kinetic resolution process. Further studies were therefore

required to establish analytical evidence of the DABCO-mediated dynamic interconversion process before combination with the established diastereoselective kinetic resolution protocol.

The base 1,1,3,3-tetramethylguanidine (TMG) was also investigated (entry 6, Table 4.40). Initial studies explored 0.1 equivalent of TMG and limited cyclisation of 6-nitroheptanal **118** was observed by ^{1}H NMR analysis after 24 h. Thus, an additional 2.0 equivalents of base were added at this point in the study to promote the intramolecular Henry reaction. Notably, at 48 h with the increased concentration of base only 6% of the aldehyde **118** remained and significantly, chemical acetylation was evident only in trace amounts (≤ 5%). Thus, the TMG-mediated dynamic interconversion of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** was also further explored.

*Table 4.40: Base-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Entry	Base	pK_a	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	DEA (2 eq.)	11.3 (H_2O)[99]	24 h	83	8	9	-	-
			48 h	82	8	10	-	-
2	Piperidine (2 eq.)	11.3 (H_2O)[99]	24 h	100	-	-	-	-
			48 h	100	-	-	-	-
			72 h	100	-	-	-	-
3	DABCO (2 eq.)	8.82, 2.97 (H_2O)[95]	24 h	-	39	61	-	-
			48 h	-	40	60	-	-
			72 h	-	39	61	-	-
4	Hünigs Base (2 eq.)	11.4 (H_2O)[100]	48 h	85	5	10	-	-
			72 h	79	7	14	-	-
5	1M NaOH	~13.8	24 h	100	-	-	-	-
			48 h	100	-	-	-	-
6	TMG (0.1 eq.)	13.6 (H_2O)[101]	24 h	69	10	21	-	-
	(0.1 - 2.1 eq.)[b]		48 h	6	30	57	5	2

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery

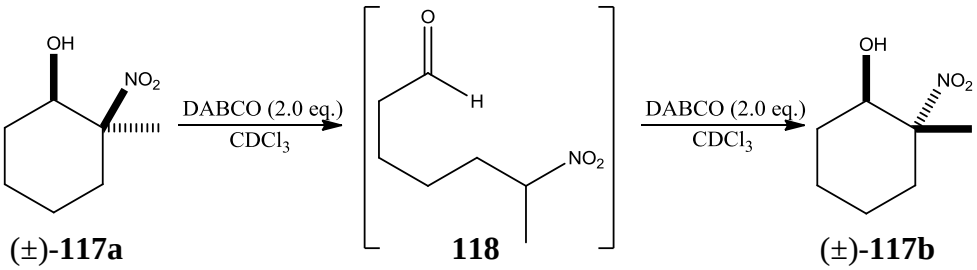
b. At 24 h after analysis by ^{1}H NMR spectroscopy an additional 2.0 equivalents of TMG (2.0 eq., 95 μL, 86.8 mg, 0.75 mmol) were added to the reaction vessel and stirred at room temperature for a further 24 h.

4.3.6.4 1,4-Diazabicyclo[2.2.2]octane (DABCO)

4.3.6.4.1 Dynamic interconversion process

As DABCO effectively catalysed the intramolecular nitroaldol reaction in the presence of vinyl acetate with no adverse chemical acetylation the dynamic interconversion process was next explored. A ^{1}H NMR sample was prepared with diastereomerically pure (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DABCO (2.0 equivalents) in deuterated chloroform. While this base successfully mediated the ring closure of 6-nitroheptanal **118** (entry 3, Table 4.40), significantly in this study no dynamic interconversion to the *trans*-diastereomer (±)-**117b** was observed over an extended reaction period of 10 days at room temperature (Table 4.41).

*Table 4.41: Evidence for dynamic interconversion – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DABCO (2.0 eq.) in $CDCl_3$*



(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
42 min	100	-
3 h 10 min	100	-
1 day	100	-
4 days	100	-
10 days	100	-

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra

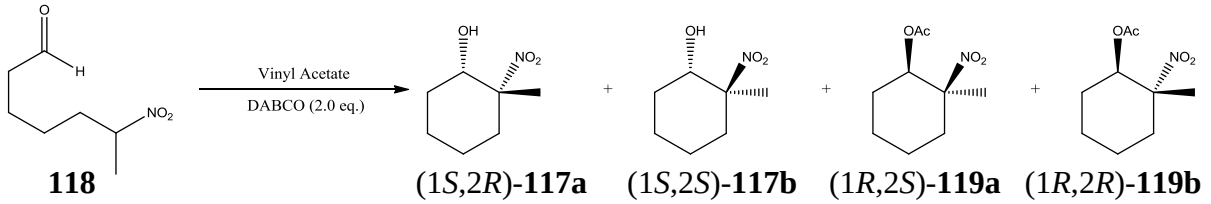
4.3.6.4.2 Dynamic one-pot kinetic resolution process

Although dynamic interconversion was not achieved, the kinetic resolution process was investigated with DABCO to determine if base inhibition in the presence of a lipase, as had been observed with DBU, was evident in the DABCO study. The initial investigation examined the dynamic kinetic resolution process with no lipase (entry 1, Table 4.42) and as anticipated from earlier studies complete ring closure was achieved within 48 h to the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** (40 : 60). However, on introduction of the hydrolase *Pseudomonas stutzeri* under similar reaction conditions (entry 2, Table 4.42) a significant reduction in the efficiency of the intramolecular nitroaldol reaction was observed with the aldehyde 6-nitroheptanal **118** evident as the major component by ^{1}H NMR spectroscopy (75%) after 48 h. Thus, base inhibition in the presence of a hydrolase is not restricted to DBU, but also observed with DABCO. Interestingly for the small amounts of the alcohol (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** and acetate (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** observed there is some evidence of kinetic resolution in line with the results seen in section 4.3.4.5.

Although DABCO favourably catalysed the intramolecular Henry reaction, a major limitation of this base was that it was unsuccessful in mediating the dynamic interconversion

process between the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b**, and, thus, DABCO was no longer considered in this investigation.

*Table 4.42: Cyclisation of 6-nitroheptanal **118** with* P. stutzeri *catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with DABCO (2.0 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
				***cis*-117a (%)[a] [ee (%)][b]**	***trans*-117b (%)[a] [ee (%)][b]**	***cis*-119a (%)[a] [ee (%)][b]**	***trans*-119b (%)[a] [ee (%)][b]**
1	-	48 h	-	40	60	-	-
2	*Pseudomonas stutzeri*	48 h	75	4 [77]	11 [24][c]	5 [72]	5 [>98]

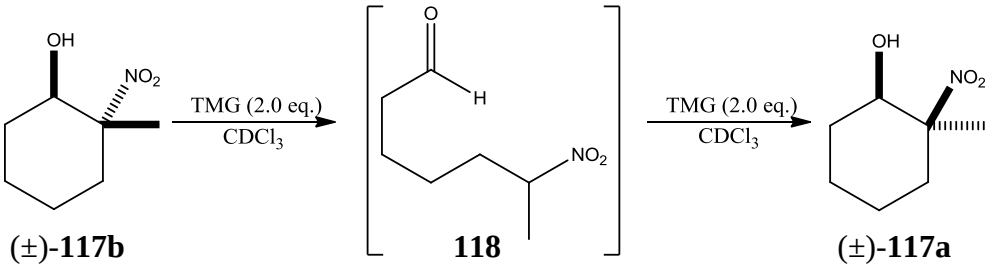
a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
c. The HPLC trace displays an unknown impurity which co-eluted with the *trans*-2-methyl-2-nitrocyclohexanol **117b**, therefore enantiomeric excess [ee (%)] is an estimation.

4.3.6.5 1,1,3,3-Tetramethylguanidine (TMG)

4.3.6.5.1 Dynamic interconversion process

The base TMG at 2.1 equivalents had previously been demonstrated to successfully catalyse the ring closure of 6-nitroheptanal **118** (entry 6, Table 4.40) with limited chemical acetylation in the presence of vinyl acetate and thus, the dynamic interconversion process between (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** was next explored. The more stable diastereomer (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and TMG (2.0 equivalents) were dissolved in deuterated chloroform in a NMR tube allowing for efficient analysis by ^{1}H NMR spectroscopy. The *cis*-diastereomer (±)-**117a** was evident within 50 min after the initial addition of TMG and a thermodynamic ratio of *cis*-alcohol (±)-**117a**/*trans*-alcohol (±)-**117b**, 28 : 72 was observed after 3 days (Table 4.43). Thus, TMG is effective in mediating the dynamic interconversion process and in addition this base successfully catalysed the intramolecular nitroaldol reaction with minimal chemical acetylation in the presence of vinyl acetate, therefore TMG holds the greatest potential for the development of a dynamic kinetic resolution process.

*Table 4.43: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and TMG (2.0 eq.) in $CDCl_3$*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
49 min	14	88
5 h 7 min	26	74
3 days	28	72

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

4.3.6.5.2 Dynamic one-pot kinetic resolution process

The combined dynamic one-pot kinetic resolution process was next explored with 6-nitroheptanal **118**, TMG (2.0 equivalents), and *Candida antarctica* lipase B (immob) or *Pseudomonas stutzeri* in vinyl acetate (Table 4.44). Significantly, there was limited effect of base inhibition observed in this study with efficient TMG-mediated intramolecular cyclisation of 6-nitroheptanal **118** achieved in the presence of both hydrolases *Candida antarctica* lipase B (immob) or *Pseudomonas stutzeri* (entries 1 and 2, Table 4.44).

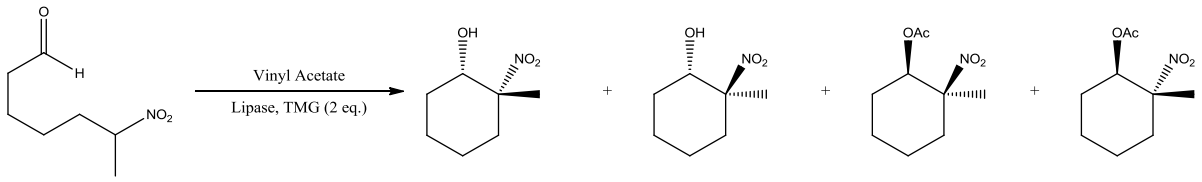
Surprisingly, the hydrolase *Candida antarctica* lipase B (immob) catalysed dynamic transesterification led to almost racemic samples of the *cis*- and *trans*-2-methyl-2-nitrocyclohexanol **117a** and **117b** with no trace of the *cis*- and *trans*-acetate **119a** or **119b** evident by ^{1}H NMR spectroscopy despite the extended reaction time of 5 days (entry 1, Table 4.44). It appears that the activity of the enzyme is completely switched off by the presence of TMG possibly due to protein denaturing. This is in direct contrast to the earlier *Candida antarctica* lipase B (immob) mediated diastereoselective study (see section 4.3.4.5) conducted with an equimolar mixture of the *cis*- and *trans*-alcohols (±)-**117a** and (±)-**117b** in the absence of TMG. The transesterification of the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was the predominant process operating in the earlier diastereoselective study, with excellent enantiopurity achieved for the (1*R*,2*R*)-*trans*-acetate (1*R*,2R)-**119b**.

The *Pseudomonas stutzeri* and TMG-catalysed dynamic kinetic resolution process provided the most promising results to date (entry 2, Table 4.44). The intramolecular nitroaldol reaction was efficiently mediated by TMG and the predominant products observed after 48 h were the (1*S*,2*R*)-*cis*- and (1*S*,2*S*)-*trans*-alcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** albeit with poor enantioselectivity (Figure 4.39). Significantly the (1*R*,2*S*)-*cis*- and (1*R*,2*R*)-*trans*-acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** displayed moderate to good enantiopurity (Figure 4.39) indicating for the first time that the base-mediated ring closure and kinetic resolution of (±)-**117a** and (±)-**117b** can be successfully conducted in a one pot process. While chemical acetylation may be competing to a minor extent it is clear from the enantiopurity of (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** that enzyme-mediated transformation is dominant.

In summary, TMG displayed for the first time the potential of a genuine dynamic kinetic resolution process, avoiding both competing chemical acetylation in the presence of

vinyl acetate and base inhibition in the presence of the hydrolase leading to the acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** in good enantiopurities. Limited efficiency and conversions and low diastereoselection are evident and therefore further investigation is warranted to optimise this process.

*Table 4.44: Cyclisation of 6-nitroheptanal **118** with hydrolase-catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with TMG (2 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	TMG	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
					cis-117a (%)[a] [ee (%)][b]	*trans*-117b (%)[a] [ee (%)][b]	*cis*-119a (%)[a] [ee (%)][b]	*trans*-119b (%)[a] [ee (%)][b]
1	*Candida antarctica B* (immob)	2 eq.	5 days	7	26 [2]	67 [1]	-	-
2	*Pseudomonas stutzeri*	2 eq.	48 h	15	22 [35]	50 [6]	9 [75]	4 [80]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery

b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

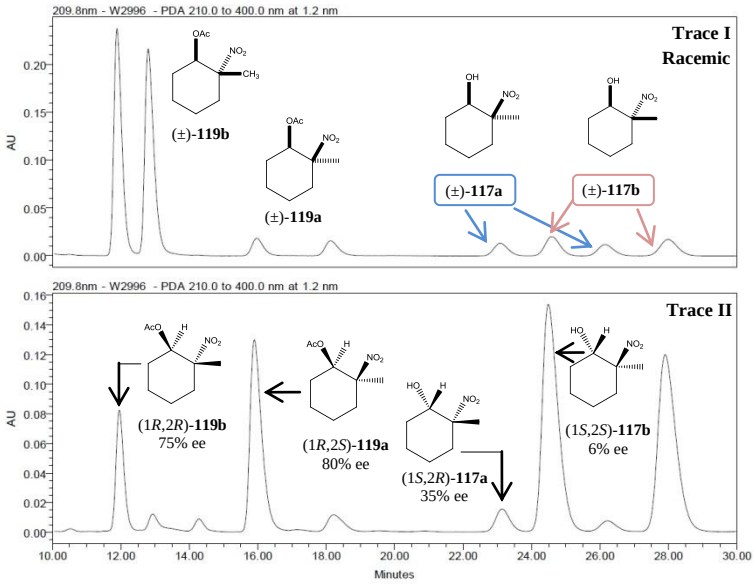


*Figure 4.39: HPLC **Trace I:** A racemic mixture of (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119b**, (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a**, (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**. **Trace II:** Pseudomonas stutzeri and TMG-mediated dynamic resolution process, (1*R*,2*R*)-trans-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*R*)-**119b**, 75% ee, (1*R*,2*S*)-cis-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*S*)-**119a**, 80% ee, (1*S*,2*R*)-cis-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a**, 35% ee and (1*S*,2*S*)-trans-2-methyl-2-nitrocyclohexanol (1*S*,2*S*)-**117b**, 6% ee. For HPLC conditions see appendix I. Note the above traces correlate to chiral HPLC conditions C.*

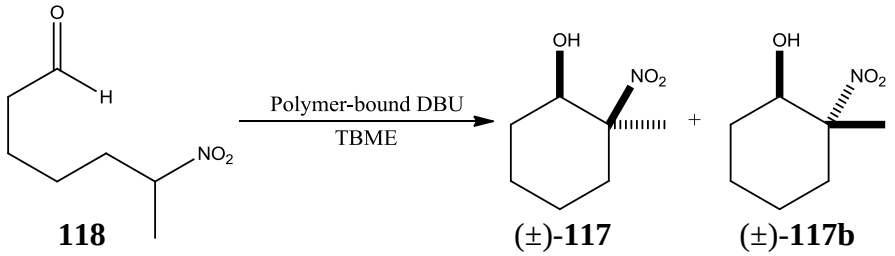
4.3.6.6 Polymer-bound 1,8-Diazabicycloundec-7-ene [DBU (immob)]

*4.3.6.6.1 Cyclisation of 6-nitroheptanal **118** via the intramolecular Henry reaction*

Use of polymer-bound DBU was next investigated as it was envisaged that the rate of chemical acetylation with the immobilised base would be reduced. Furthermore, deactivation of the immobilised base in the presence of the hydrolase may be avoided. However, on investigation of the intramolecular nitroaldol reaction in TBME, DBU (immob) was found to be far less effective in catalysing the cyclisation of 6-nitroheptanal **118** relative to its free counterpart. With 0.1 equivalent of DBU (immob) at room temperature, the diastereomeric alcohols (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** were not evident by ^{1}H NMR analysis after 13 h (entry 1, Table 4.45). In direct contrast the same amount of free base successfully mediated the intramolecular nitroaldol reaction, albeit with chemical acetylation in the presence of vinyl acetate.

The cyclisation of 6-nitroheptanal **118** was therefore investigated with an increased amount (1.0 equivalent) of DBU (immob) (entry 2, Table 4.45). At room temperature with 1.0 equivalent of base, limited cyclisation was observed after 22.5 h thus an additional 1.0 equivalent of DBU (immob) was charged to the reaction flask and the reaction temperature was increased to 40 °C. Gratifyingly, almost complete cyclisation was observed at the increased concentration of DBU (immob) and elevated reaction temperature, and thus the dynamic interconversion process was next studied under these optimised reaction conditions.

*Table 4.45: Polymer-bound DBU mediated cyclisation of 6-nitroheptanal **118** with TBME as solvent*



118 (±)-**117** (±)-**117b**

Entry	Polymer-bound DBU (eq.)	Reaction Time	Temp (°C)	Aldehyde 118 (%)[a]	Alcohol (±)-117	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]
1	0.1	13 h	Ambient	100	-	-
2	1.0	22.5 h	Ambient	92	3	5
	1.0 – 2.0[a]	44 h	40 °C	11	24	65

a. At 22.5 h after analysis by ^{1}H NMR spectroscopy an additional equivalent of polymer-bound DBU (1.0 eq., 273.7 mg, 0.31 mmol) was added to the reaction vessel and stirred at 40 °C for a further 21.5 h.

4.3.6.6.2 Dynamic interconversion process

To examine the dynamic interconversion process 2.0 equivalents of DBU (immob) were investigated with diastereomerically pure (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in TBME at 40 °C (Table 4.46). However, with such high catalyst loading of the polymer-bound base (1.15 mmol N per g) the ^{1}H NMR spectrum obtained after 19 h were too complex to decipher conversions, despite filtration to remove the beads.

Thus, although DBU (immob) did successfully ring close 6-nitroheptanal **118**, the increased loading of the immobilised base resulted in unknown impurities in the ^{1}H NMR

spectrum from the crude dynamic interconversion reaction mixture, and thus this base was no longer investigated.

*Table 4.46: Evidence for dynamic interconversion - (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and polymer-bound DBU in TBME at 40 °C*

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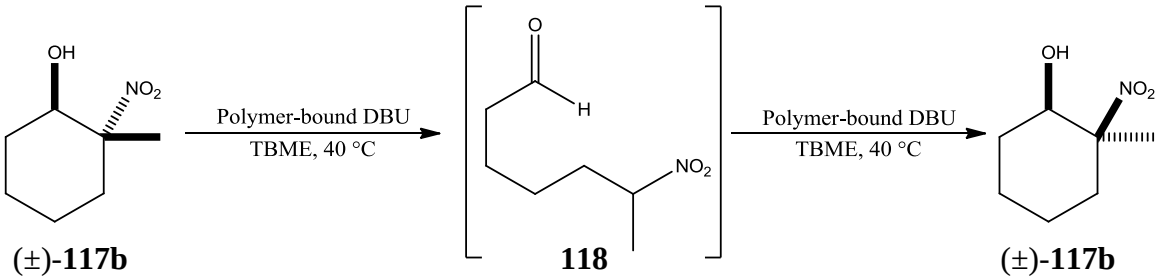


(±)-**117b** **118** (±)-**117b**

Entry	Polymer-bound DBU	Reaction Time	(±)-117b (%)	(±)-117a (%)
1	2 eq.	19 h	-[a]	-[a]
2	3 eq.	19 h	-[a]	-[a]

a. The crude ^{1}H NMR spectrum was too complex to decipher conversions.

4.3.6.7 Influence of base strength

The link between the pK_a of the conjugate acids of the bases and the efficiency of both the Henry nitroaldol and the enzyme-mediated acetylation was explored. Focussing initially on the ring closure the Henry nitroaldol was mediated by both strong and weak bases with no evidence of direct correlation between efficiency and base strength. However, the competing chemical acetylation with vinyl acetate is definitely linked with base strength, with increased chemical acetylation in the presence of DBU and TMG, while no chemical acetylation is seen with DABCO and NEt_3. In terms of base inhibition by the presence of the enzyme, it was envisaged that this might be linked to base strength, but as summarised below in Table 4.47, there was no direct correlation evident. In considering the data summarised in the table it should be noted that the pK_a values were determined in water and accordingly the outcome should be considered in the context that the base strengths in the organic media may differ somewhat.

Table 4.47: Influence of base strength

Entry	Base	pK_a (H_2O)	Cyclisation of 6-nitroheptanal 118 with vinyl acetate		Base inhibition with lipase
			Intramolecular Henry reaction	Chemical acetylation	
1	DABCO	8.7/4.2[95]	✔	x	x
2	NEt_3	10.8[95]	✔	x	✔ (partial)
3	Piperidine	11[99]	x	-	-
4	DEA	11[99]	✔ (partial)	-	-
5	Hünig's base	11.4[100]	✔ (partial)	-	-
6	DBU	12[94]	✔	✔	✔
7	TMG	13.6[101]	✔	✔ (partial)	✔ (partial)
8	NaOH (1M)	13.8	x	-	-

4.3.7 Two-pot hydrolase-mediated dynamic resolution screens

At this point in the investigation it was apparent that there were two major barriers in the development of a one-pot dynamic kinetic resolution process. Firstly, employment of a base in the presence of vinyl acetate can lead to competing racemic chemical acetylation. Secondly, base inhibition in the presence of the hydrolase can hinder the intramolecular nitroaldol cyclisation and thus result in poor dynamic interconversion. Therefore, a two-pot system was developed, where the dynamic interconversion process and diastereoselective kinetic resolution protocol were performed independently (Figure 4.40). Ultimately recycling of the reaction mixture between the two vessels in a flow system can be envisaged.

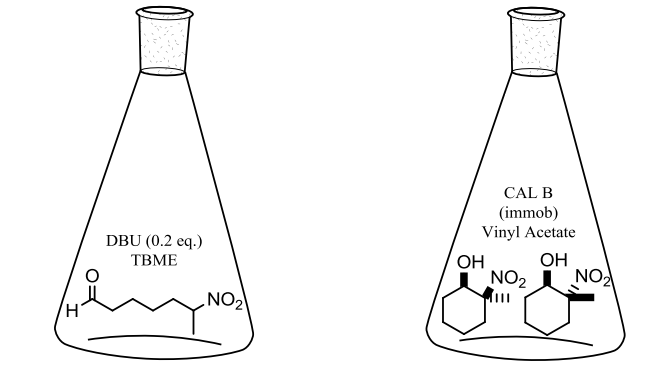


Figure 4.40

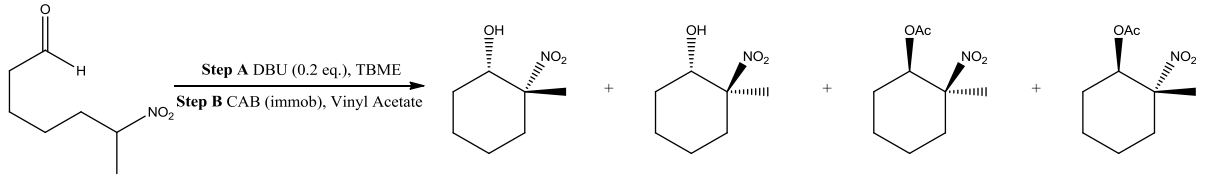
The intramolecular nitroaldol reaction and associated dynamic interconversion process (Step A) was first examined. The aldehyde 6-nitroheptanal **118** was dissolved in TBME with DBU (0.2 equivalent) and stirred at room temperature overnight. In this reaction vessel, vinyl acetate was not employed thus no chemical acetylation was observed. In addition, no base inhibition was detected due to the absence of a hydrolase. The DBU was subsequently removed by washing the reaction mixture with water. The organic layers were then concentrated under reduced pressure before analysis by ^{1}H NMR spectroscopy. Complete cyclisation of 6-nitroheptanal **118** to the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** was achieved with a thermodynamic ratio of 30 : 70.

The crude β-nitroalcohols (±)-**117a** and (±)-**117b** were then dissolved in vinyl acetate and treated with *Candida antarctica* lipase B (immob) (Step B). This lipase was selected as it had previously demonstrated excellent diastereoselectivity in the resolution of (±)-2-methyl-2-nitrocyclohexanol (±)-**117** (see section 4.3.4.5). The reaction mixture was shaken at 24 °C for 18 h, the hydrolase was then removed by filtration and the reaction mixture dried and concentrated under reduced pressure before ^{1}H NMR and chiral HPLC analysis. In this study, as anticipated, the lipase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was the dominant kinetic resolution process in step B, with the (1*R*,2*R*)-*trans*-acetate (1*R*,2*R*)-**119b** accounting for 33% of the overall product ratio. Excellent diastereoselectivity was also observed with limited transformation of (±)-*cis*-2-methyl-2-nitrocylohexanol (±)-**117a**.

The two individual steps, A and B were then continuously repeated. In the dynamic interconversion process, step A, the ratio of *trans*-alcohol **117b** was observed to increase relative to the ratio determined in the kinetic resolution protocol step B due to DBU-mediated

ring opening and closing of 6-nitroheptanol **118** to form the more stable *trans*-diastereomer **117b**. Significantly, the ratios of the *cis*- and *trans*-acetates **119a** and **119b** were not altered. In the kinetic resolution process step B, the enantioselective acetylation of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** was the major process in all cases. It is evident from Table 4.48 that the efficiency of both steps A and B, the dynamic interconversion and kinetic resolution process, was hindered significantly as the reaction progressed with minimal change in conversion observed between the last two processes. The (1*R*,2*R*)-*trans*-2-methyl-2-nitrocyclohexanol (1*R*,2*R*)-**119b** was the dominant product observed in the dynamic interconversion process prior to work-up (57%) highlighting the significant potential of this dynamic process to provide a single stereoisomer (1*R*,2*R*)-**119b**.

Table 4.48: Two pot dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Step	DBU (eq.)	Vinyl acetate (eq.)	*Candida antarctica* B (immob) (% w/w)	Alcohol 117		Acetate 119	
				***cis*-117a (%)[a] [ee (%)][b,c]**	***trans*-117b (%)[a] [ee (%)][b,d]**	***cis*-119a (%)[a] [ee (%)][b,e]**	***trans*-119b (%)[a] [ee (%)][b,f]**
A	0.2	-	-	30 (±)	70 (±)	-	-
B	-	138	18	25	37	5	33
A	0.2	-	-	19	41	6	34
B	-	138	18	16	29	8	47
A	0.2	-	-	13	30	8	49
B	-	138	18	11	22	10	57
A	0.2	-	-	10 [10]	22 [37]	10 [>98]	57 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix 1 for conditions.

The crude β-nitroalcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** and β-nitroacetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** were separated by column chromatography. The individual diastereomers of 2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** were not isolated in this case. However if separation was required the column chromatographic conditions as outlined in section 4.3.3.2 would be applied. Thus, chiral HPLC analysis was conducted on the *cis* and *trans* mixture **117a** and **117b**. Poor enantiopurity for (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** was observed, however this was anticipated due to the dynamic interconversion process which scrambles the stereochemistry at C1 and C2. A low yield was obtained of the β-nitroalcohols (1*S*,2*R*)-**117a** and (1*S*,2*S*)-**117b** attributable to the multiple work-ups required during this investigation

The (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**119b** was successfully separated from the (1*R*,2*S*)-*cis*-diastereomer (1*R*,2*S*)-**119a**, and chiral HPLC and optical rotation analysis was conducted on the enantiopure sample (1*R*,2*R*)-**119b**. Although the isolated yield was poor, excellent enantioselectivity was achieved (≥98% ee) demonstrating the significant potential of this process for the development of a formal dynamic kinetic resolution of the

intramolecular Henry reaction of 6-nitroheptanal **118** through *Candida antarctica* lipase B (immob) catalysis. While the cycling of the material through the two steps is tedious, development of this protocol through engineering of a flow system can be envisaged to provide an efficient process (Figure 4.41).

A pure sample of the *cis*-acetate (1*R*,2*S*)-**119a** was not obtained free from the *trans*-diastereomer (1*R*,2*R*)-**119b** and chiral HPLC analysis was conducted on the *cis* and *trans* mixture **119a** and **119b**. Although the enantioselective transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** was a minor process relative to the transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**, high enantiopurity was achieved for the generated acetate (1*R*,2*S*)-**119a** (≥98% ee). While the experiment summarised in Table 4.48 was commenced with 291.5 mg of **118**, inevitably product loss in the multiple steps and isolation meant that there was a limit to the total number of cycles which could be undertaken.

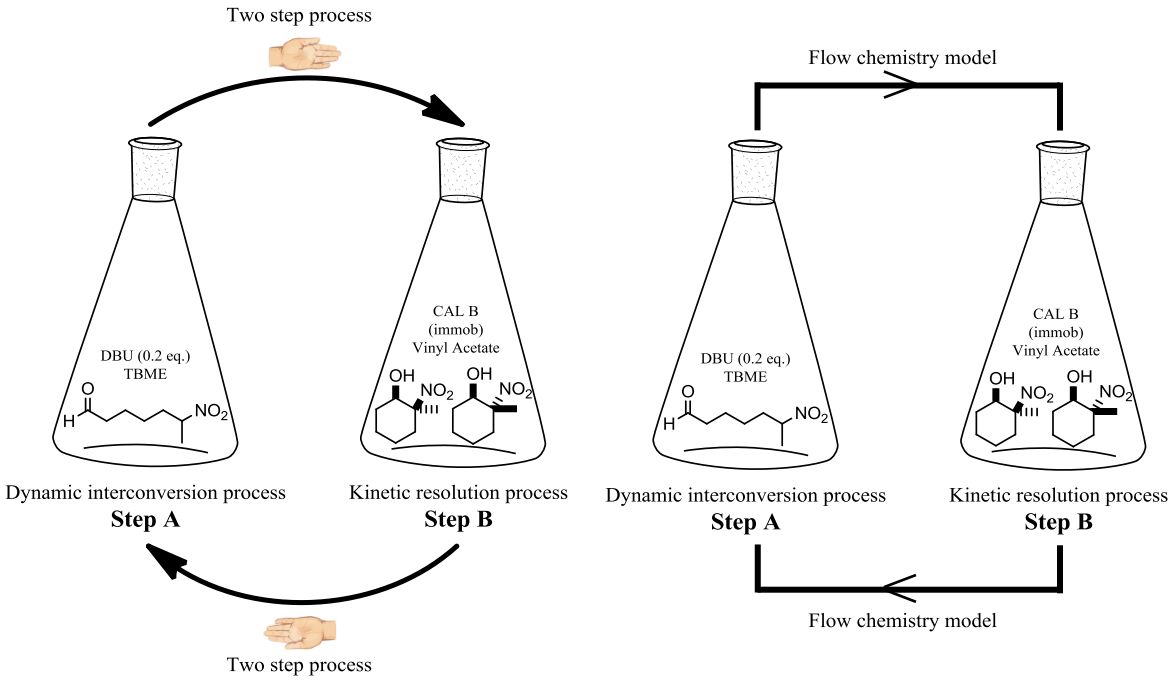


Figure 4.41

4.3.8 Project conclusion

Significant progress has been made in the individual elements of the dynamic resolution process as outlined in the original objectives. Efficient kinetic resolution has been effected for the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** through *Pseudomonas stutzeri* mediated transesterification. The enantioselective acetylation of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** has also been explored. Critically, *Candida antarctica* lipase B (immob) displayed high diastereoselectivity, selectively acetylating the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** diastereomer efficiently and with excellent enantioselectivity. Furthermore, both the lipase-mediated transesterification of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols have been performed on a preparative-scale accessing the *trans*-alcohol (1*S*,2*S*)-**117b** and *cis*- and *trans*-acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** in excellent enantioselectivity (≥98% ee).

A series of bases were investigated to mediate the intramolecular nitroaldol reaction and associated dynamic interconversion process. DBU and TMG were definitively

established by ^{1}H NMR spectroscopy to fulfil both criteria with interconversion of the (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanols (±)-**117a** and (±)-**117b** *via* ring opening and closing of 6-nitroheptanal **118**. However, chemical acetylation in the presence of vinyl acetate and lipase-mediated base inhibition presented significant barriers to the development of a one-pot dynamic kinetic resolution process. Despite the considerable limitations present, TMG displayed significant potential in the dynamic resolution process with high enantioselectivity obtained of the generated acetates (1*R*,2*S*)-**119a** and (1*R*,2*R*)-**119b** with negligible competing chemical acetylation or base inhibition evident. However, further investigation is required to improve the poor conversion and limited diastereoselectivity observed.

To circumvent the competing processes of chemical acetylation and base inhibition in the one-pot system, a two-pot dynamic kinetic resolution process was explored whereby the dynamic interconversion process was performed independently of the lipase-mediated kinetic resolution. In the dynamic interconversion process vinyl acetate was not employed thus no chemical acetylation was observed. In addition, no base inhibition was detected due to the physical separation of the hydrolase. Significantly, excellent diastereoselectivity was observed with *Candida antarctica* lipase B in the two-pot system with the transesterification of the (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** the dominant kinetic resolution process. Thus the *trans*–acetate (1*R*,2*R*)-**119b** was the major component (57%) of the reaction mixture and isolated in excellent enantioselectivity (≥98% ee) albeit in low yield, attributable to the multiple work-ups required during this investigation. This two-pot system augurs well for the development of a flow chemistry model where the intensive work-ups may be avoided and thus loss of yield minimised; however, the removal of vinyl acetate before the dynamic interconversion may prove problematic.

In summary, achieving dynamic kinetic resolution in the intramolecular Henry is extremely challenging due to the number of competing processes arising. By careful exploration of the conditions including variation of biocatalyst, base and solvent we have demonstrated for the first time the feasibility of this process. Introduction of the methyl group in (±)-**117a** and (±)-**117b** to avoid epimerisation *via* deprotonation as seen in (±)-**99a** and (±)-**99b** was critical to the success of this outcome.

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Chapter 5

Experimental

Contents

5.1 General procedures **257**
5.2 Baker's yeast mediated asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-1 and (*S*)-1 ..... **259**
5.2.1 Synthesis of cyclopentanone and cyclopentanol **259**
5.2.2 Kinetic resolution in the immobilised baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2 ..... **261**
5.2.3 Asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acids (*R*)-1 and (*S*)-1 ..... **265**
5.3 Kinetic resolutions of β-substituted 3-aryl alkanoic acids **272**
5.3.1 Synthesis of hydrolase substrates **272**
5.3.1.1 Synthesis of carboxylic acids **272**
5.3.1.2 Synthesis of acid chlorides **275**
5.3.1.3 Synthesis of ethyl esters **277**
5.3.1.4 Synthesis of fluorine substituted hydrolase substrates **280**
5.3.2 Hydrolase-mediated kinetic resolution - analytical screens **282**
5.3.3 Hydrolase-mediated kinetic resolution – preparative-scale **288**
5.3.4 Determination of absolute stereochemistry **292**
5.3.4.1 Synthesis of isonicotinamide co-crystals **292**
5.4 Kinetic resolutions of α-substituted 3-aryl alkanoic acids **295**
5.4.1 Synthesis of hydrolase substrates **295**
5.4.2 Hydrolase-mediated kinetic resolution - analytical screens **298**
5.4.3 Hydrolase-mediated kinetic resolution – preparative-scale **300**
5.5 Dynamic kinetic resolution of 2-nitrocyclohexanol (±)-99 ..... **301**
5.5.1 Synthesis of substrates **301**
5.5.2 Evidence of a dynamic interconversion process – ^{1}H NMR **307**
5.5.3 Hydrolase-mediated kinetic resolution - analytical screens **312**
5.5.3.1 Analytical screen – vinyl acetate as both acyl donor and solvent **312**
5.5.3.2 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** **314**
5.5.4 Evidence of a dynamic interconversion process - chiral HPLC **315**
5.6 Dynamic kinetic resolution of 2-methyl-2-nitrocyclohexanol (±)-117 ..... **318**
5.6.1 Synthesis of nitro substituted hydrolase substrates **318**
5.6.2 Hydrolase-mediated kinetic resolution – analytical screens **324**
5.6.2.1 Analytical screen – acetylating agent as both acyl donor and solvent **324**
5.6.2.2 Analytical screen – vinyl acetate as acyl donor and alteration of solvents **325**
5.6.2.3 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** **326**
5.6.3 Hydrolase-mediated kinetic resolution – preparative-scale **328**
5.6.4 Analytical dynamic kinetic resolution screens **330**
5.6.4.1 Analytical dynamic kinetic resolution screens: Triethylamine **331**
*5.6.4.1.1 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118*** **331**
5.6.4.1.2 ^{1}H NMR study - Evidence for dynamic interconversion **333**
*5.6.4.1.3 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117*** **335**
5.6.4.2 Analytical dynamic kinetic resolution screens: 1,8-Diazabicycle[5.4.0]undec-7-ene (DBU) **336**
5.6.4.2.1 ^{1}H NMR study - Evidence for dynamic interconversion **336**
*5.6.4.2.2 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118*** **338**
5.6.4.2.3 ^{1}H NMR study - Evidence for chemical acetylation in the dynamic interconversion process **339**
*5.6.4.2.4 ^{1}H NMR study - One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117*** **340**
5.6.4.3 Analytical dynamic kinetic resolution screens: Alteration of base **341**
5.6.4.4 Analytical dynamic kinetic resolution screens: 1,4-Diazabicyclo[2.2.2]octane (DABCO) **342**
5.6.4.4.1 ^{1}H NMR study - Evidence for dynamic interconversion **342**

*5.6.4.4.2 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117*** **343**
5.6.4.5 Analytical dynamic kinetic resolution screens: 1,1,3,3-Tetramethylguanidine (TMG) **344**
5.6.4.5.1 ^{1}H NMR study - Evidence for dynamic interconversion **344**
*5.6.4.5.2 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117*** **344**
5.6.4.6 Analytical dynamic kinetic resolution screens: Polymer bound 1,8-diazabicycle[5.4.0]undec-7-ene (DBU) **346**
*5.6.4.6.1 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118*** **346**
5.6.4.6.2 ^{1}H NMR study - Evidence for dynamic interconversion **346**
5.6.5 Two pot hydrolase-mediated dynamic resolution of (±)-2-methyl-2-nitrocyclohexanol (±)-117 .. **347**
5.7 References **349**

5.1 General procedures

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorus pentoxide and when doubly distilled dichloromethane was used it was further distilled from calcium hydride and stored over 4 Å molecular sieves. Ethyl acetate was distilled from potassium carbonate and ethanol was distilled from magnesium in the presence of iodine and stored over 3 Å molecular sieves.[1] Hexane was distilled prior to use. Tetrahydrofuran (THF) was distilled from sodium and benzophenone.[1] Molecular sieves were dried by heating at 150 ºC overnight. Organic phases were dried using anhydrous magnesium sulfate.

All commercial reagents were used without further purification unless otherwise stated. The rhodium(II) acetate dimer catalyst employed was kindly donated by Johnson Matthey. In the baker's yeast reductions, sucrose obtained from Siúcra Irish Granulated Sugar and Sigma type II baker's yeast (BY; *Saccharomyces cerivisae*) was employed. Ordinary tap water was used as solvent. All hydrolases were kindly donated by Almac Sciences.

Infrared spectra were recorded as thin films on sodium chloride plates for oils or as potassium bromide (KBr) discs for solids on a Perkin Elmer Paragon 1000 FT-IR spectrometer. Bulb to bulb distillations were carried out on an Aldrich Kugelrohr apparatus and the oven temperature is given as the boiling point of the substrate. Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus and are uncorrected.

^{1}H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. ^{1}H (400 MHz) NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. All spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$) unless otherwise stated using tetramethylsilane (TMS) as an internal standard. ^{1}H NMR spectra that were recorded in deuterated dimethylsulfoxide (DMSO-d_6) were assigned using the DMSO peak as the reference peak. Chemical shifts (δ_H & δ_C) are reported in parts per million (ppm) relative to TMS and coupling constants are expressed in Hertz (Hz).

Splitting patterns in ^{1}H spectra are designated as s (singlet), br s (broad singlet), br d (broad doublet), br t (broad triplet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets) and m (multiplet). ^{13}C NMR spectra were assigned with the aid of DEPT experiments. Compounds which were assigned with the aid of DEPT experiments were assigned by identifying both the carbon, (CH_3, CH_2, CH or C) and also the atom number of the carbon, for example [CH_3, *C*(3)H_3]. All spectroscopic details for compounds previously made were in agreement with those previously reported unless otherwise stated.

Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluant; samples were made up in acetonitrile. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionization (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluant; samples were made up in acetonitrile.

Elemental analysis were performed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers. Wet flash chromatography was performed using Kieselgel silica gel 60, 0.040-0.063 mm (Merck). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 PF_{254}). Visualisation was achieved by UV (254nm) light detection,

potassium permanganate, vanillin, phosphomolybdic acid (PMA) and bromocresol green staining.

Enantiomeric excess were measured by high performance liquid chromatography (HPLC), using a Chiralpak® AS-H, Chiralcel® OD-H and Chiralcel® OJ-H column. Details of the column condition and mobile phases are included in Appendix I. HPLC analysis was performed on a Waters alliance 2690 separations module. All chiral columns were purchased from Daicel Chemical Industries Limitied. All solvents employed were of HPLC grade. Low temperature chiral HPLC analysis was obtained using an Igloo-Cil® column cooler. PDA detection was used in all cases. When only one single enantiomer could be detected, the enantiomeric excess is quoted as >98%. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 589 nm in a 10 cm cell; concentrations (*c*) are expressed in g/100 mL. $[\alpha]_D^{20}$ is the specific rotation of a compound and is expressed in units of 10^{-1} deg cm^2 g^{-1}. All hydrolase enzymatic reactions were performed on a VWR Incubating Mini Shaker 4450.

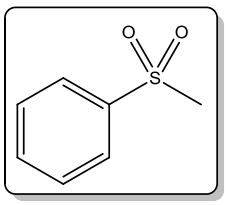
Mechanical grinding experiments were conducted in a Retsch MM400 Mixer mill, equipped with stainless steel 5 mL grinding jars and one 2.5 mm stainless steel grinding ball per jar. The mill was operated at a rate of 30 Hz for 30 min. Powder X-ray diffraction (PXRD) data were collected using a STÖE STADI MP diffractometer with Cu Kα_1 radiation (λ = 1.5406 Å), 40 kV, 40mAusing a linear PSD over the 2θ range (3.5 – 60°) with a step size equal to 0.5 and step time of 60 s.

Single crystal X-ray data was collected by Dr. S. E. Lawrence and Dr. K. S. Eccles, Department of Chemistry, University College Cork on a Bruker APEX II DUO diffractometer at temperature 100 K using graphite monochromatic Mo Kα (λ = 0.7107 Å) radiation fitted with an Oxford Cryosystems Cobra low-temperature device.. The structures were solved using direct methods and refined on F^2 using SHELXL-97. Analysis was undertaken with the SHELX suite of programs[2] and diagrams prepared with Mercury 3.0.[3] All non-hydrogen atoms were located and refined with anisotropic thermal parameters. Hydrogen atoms were included in calculated positions or they were located and refined with isotropic thermal parameters.

5.2 Baker's yeast mediated asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acid (*R*)-1 and (*S*)-1

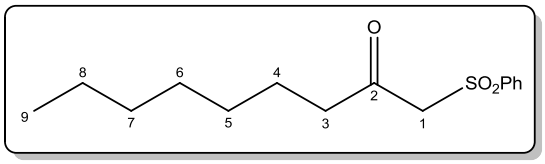
5.2.1 Synthesis of cyclopentanone and cyclopentanol

Methyl phenyl sulfone 8[4-7]



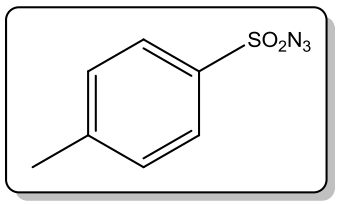
Hydrogen peroxide (30%, 150 mL) was added over 5 min to a stirring solution of thioanisole **9** (19 mL, 161.66 mmol) in acetic acid (100 mL) at room temperature. The reaction mixture was then stirred at reflux for 30 min, following which it was cooled by addition of water (100 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with a saturated aqueous solution of sodium bicarbonate (3 × 50 mL) followed by brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *sulfone* **8** (21.64 g, 86%) as a white crystalline solid; m.p. 84-87 °C (lit.,[4] 85-88 °C); ν_{max}/cm^{-1} (KBr) 1285, 1147 (SO_2); δ_H (300 MHz) 3.06 (3H, s, CH_3), 7.55-7.61 (2H, m, ArH), 7.64-7.70 (1H, m, ArH), 7.94-7.98 (2H, m, ArH).

1-Benzenesulfonylnonan-2-one 5[4,5,7,8]



Methyl phenyl sulfone **8** (4.53 g, 29.00 mmol) was placed under an atmosphere of nitrogen in a flame dried round bottom flask in an ice bath. THF (175 mL) was added to the flask followed by the addition of *n*-butyllithium (2.3 M solution in hexanes; 25.3 mL, 58.19 mmol) over a 20 minute period. The resulting cloudy yellow solution was stirred for 1.5 h at 0 °C after which a solution of ethyl octanoate **7** (6.0 mL, 30.20 mmol) in THF (15 mL) was added over 15 min producing a light orange solution. The reaction mixture was stirred overnight at room temperature under a blanket of nitrogen. The solution was quenched with saturated ammonium chloride solution (75 mL). The organic layer was isolated and the aqueous layer washed with diethyl ether (3 × 50 mL). The organic layers were combined and washed with brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *β-ketosulfone* **5** (7.32 g) as a yellow oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 80/20 as eluent gave the pure *β-ketosulfone* **5** (5.83 g, 68%) as a white crystalline solid; m.p. 54-56 °C (lit.,[8] 55-56 °C); ν_{max}/cm^{-1} (KBr) 1717 (CO), 1301, 1153 (SO_2); δ_H (300 MHz) 0.89 [3H, t, *J* 6.8, C(9)H_3], 1.14-1.38 [8H, m, C(5), (6), (7) and (8)H_2], 1.46-1.63 [2H, m, C(4)H_2], 2.69 [2H, t, *J* 7.2, C(3)H_2CO], 4.14 [2H, s, C(1)H_2SO_2], 7.53-7.64 (2H, m, ArH), 7.66-7.72 (1H, m, ArH), 7.85-7.93 (2H, m, ArH).

p-Toluenesulfonyl azide (*p*-Tosyl azide) 10[4,5,7,9]

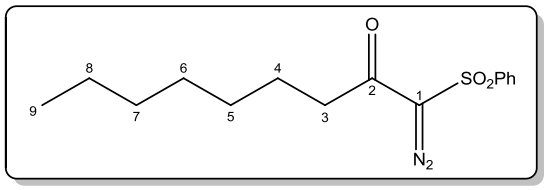


A solution of *p*-toluenesulfonyl chloride (5.61 g, 29.43 mmol) in acetone (15 mL) was added over 5 min to a solution of sodium azide (2.11 g, 32.46 mmol) in water (10 mL) and acetone (15 mL) at 0 °C. The reaction mixture was stirred for 2 h and then concentrated under reduced pressure until no acetone remained. Dichloromethane (15 mL) was added and the layers separated. The organic layer was washed with water (2 × 15 mL), dried, filtered and concentrated under reduced pressure to give the *azide* **10** (4.57 g, 79%) as a colourless oil which solidifies upon storage at low temperature.

Note:

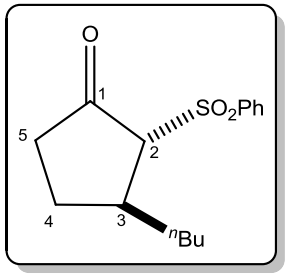
1. *Previous researchers have reported recrystallising* p-*toluenesulfonyl chloride before use.*[4,5,7] *Recrystallisation was not conducted in this research with no impact on yield or quality of* p-*tosyl azide* ***10****.*
2. *As all azides are potentially explosive caution was exercised in their handling.* p-*Tosyl azide* ***10*** *was stored in the freezer and transferred as an oil due to its high impact sensitivity.*

1-Benzenesulfonyl-l-diazononan-2-one 6[4,5,7]



Potassium carbonate (2.82 g, 20.40 mmol) was added to a solution of 1-benzenesulfonylnonan-2-one **5** (4.40 g, 15.58 mmol) in acetonitrile (75 mL). The mixture was stirred at room temperature under an atmosphere of nitrogen and a solution of *p*-tosyl azide **10** (3.10 g, 15.72 mmol) in acetonitrile (11 mL) added over 2 min. The reaction mixture was stirred for 4 h, after which diethyl ether (10 mL) and hexane (20 mL) was added to precipitate the amide salts and the resultant mixture was filtered through a short pad of Celite® and the filtrate concentrated under reduced pressure gave the crude *α-diazo-β-keto sulfone* **6** (8.45 g) as an orange solid. Purification by column chromatography on silica gel using hexane/ethyl acetate 65/35 as eluent gave the pure *α-diazo-β-keto sulfone* **6** (4.29 g, 89%) as a yellow crystalline solid; m.p. 44-45 °C (lit.,[4] 39-41 °C); ν_{max}/cm^{-1} (KBr) 2129 (CN_2), 1662 (CO), 1339, 1153 (SO_2); δ_H (300 MHz) 0.86 [3H, t, *J* 6.8, C(9)H_3], 1.11-1.34 [8H, m, C(5), (6), (7) and (8)H_2], 1.44-1.65 [2H, m, C(4)H_2], 2.54 [2H, t, *J* 7.4, C(3)H_2CO], 7.56-7.62 (2H, m, ArH), 7.65-7.72 (1H, m, ArH), 7.97-8.01 (2H, m, Ar*H*).

(±)-*trans*-2-Benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2[4,5,7]



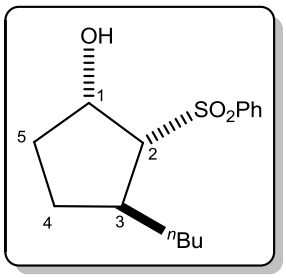
A solution of 1-benzenesulfony-l-diazononan-2-one **6** (2.00 g, 6.49 mmol) in doubly distilled dichloromethane (115 mL) was added dropwise over 1.5 h to a refluxing solution of rhodium(II) acetate (14.9 mg, 0.5 mol%) in doubly distilled dichloromethane (233 mL) while stirring under nitrogen. The mixture was maintained at reflux for 1 h then allowed cool to room temperature and concentrated under reduced pressure to give the crude cyclopentanones (2.26 g) as a dark brown oil in a 38 : 62 mixture of *cis* : *trans* isomers. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure *trans cyclopentanone* **2** (1.00 g, 55%) as a yellow oil. The pure *trans cyclopentanone* **2** was obtained as a light yellow solid for a batch that was synthesised later; m.p. 33-35 °C (lit.,[7] 34-35 °C); ν_{max}/cm^{-1} (KBr) 1751 (CO), 1305, 1152 (SO_2); δ_H (300 MHz) 0.89 [3H, t, *J* 6.9, CH_3], 1.18-1.43 [5H, m, (C***H***$_2$)$_2$$CH_3$ and one of C***H***$_2$(CH_2)$_2$$CH_3$], 1.46-1.60 [1H, m, one of

C(4)H_2], 1.63-1.80 [1H, m, one of C***H***$_2$(CH_2)$_2$CH_3], 2.21-2.50 [3H, m, C(5)H_2, one of C(4)H_2], 2.79-2.99 [1H, m, C(3)H^{n}Bu], 3.39 [1H, d, *J* 6.9, C(2)HSO_2Ph], 7.55-7.60 (2H, m, ArH), 7.65-7.72 (1H, m, ArH), 7.85-7.88 (2H, m, Ar***H***). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note:

1. *A signal for the* cis *isomer of* cis-***2*** *was detected in the ^{1}H NMR of the crude product spectrum at δ_H (300MHz) 3.73 [1H, d, J 7.8 C(2)HSO_2Ph].*
2. *Significant difference in ^{1}H NMR chemical shift and multiplicity of the CH_3 signal is observed between Milner[5] and data described above. Milner reports; δ_H (400 MHz) 1.29 (3H, d, J 7, CH_3). Milner also does not report the chemical shifts of the 3 x CH_2,* n*-butyl protons. ^{1}H NMR assignment in this study is consistent with O'Keeffe[7] and Kelleher.[4]*

(±)-(1*R**,2*S**,3*R**)-2-Benzenesulfonyl-3-*n*-butylcyclopentanol 4a[4,5,7]



A solution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone **2** (0.55 g, 1.98 mmol) in distilled ethanol (10 mL) was added dropwise over 10 min to a stirred suspension of $NaBH_4$ (0.14 g, 3.81 mmol) in distilled ethanol (25 mL) at 0 °C under nitrogen and stirring was continued for 5 h at 0 °C. The ice bath was then removed and aqueous hydrochloric acid (10%) was added to adjust to pH 1. The solution was concentrated under reduced pressure and the resulting residue was partitioned between water (10 mL) and dichloromethane (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined organic extracts were washed with brine (30 mL), dried, filtered and concentrated under reduced pressure to give the crude *cyclopentanol* **4a** (0.42 g, 75%) as a white crystalline solid which was sufficiently pure to use without further purification; m.p. 47-49 °C (lit.,[7] 47-49 °C); ν_{max}/cm^{-1} (KBr) 3499 (OH), 1302, 1141 (SO_2); δ_H (300 MHz) 0.85 (3H, t, *J* 6.6, CH_3), 1.08-1.49 [6H, m, one of C(4)H_2, one of C***H***$_2$(CH_2)$_2$CH_3 and (C***H***$_2$)$_2$CH_3], 1.51-1.89 [3H, m, one of C(5)H_2, one of C(4)H_2, and one of C***H***$_2$(CH_2)$_2$CH_3], 2.16-2.29 [1H, m, one of C(5)H_2], 2.61-2.77 [1H, m, C(3)H^{n}Bu], 3.03-3.07 [1H, dd, *J* 9.0, 4.5, C(2)HSO_2Ph], 3.51 (1H, d, *J* 2.7, OH), 4.28-4.33 (1H, sym m, C***H***OH), 7.54-7.72 (3H, m, ArH), 7.89-8.01 (2H, m, ArH).

Note:

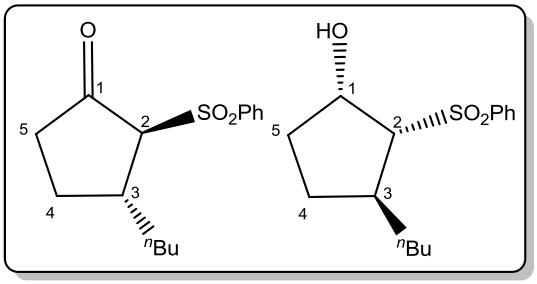
1. *Signals due to the minor diastereomer (±)-(1*R*,*2*R*,*3*S*)-***4b*** *(~3%) could be detected in the ^{1}H NMR spectrum at δ_H (300 MHz) 4.63-4.66 (C***H***OH).*
2. *Previous researchers have reported a broad singlet for the OH peak, in this study this was observed as a finely split doublet. [4,5,7]*
3. *Previous researchers have reported a ddd for the C***H***OH peak, in this study it was assigned as a multiplet. [4,5,7]*

5.2.2 Kinetic resolution in the immobilised baker's yeast mediated reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2

Immobilisation of baker's yeast on alginate[5,10-12]

Sodium alginate (20.00 g) was added portionwise to stirring tap water (1.0 L) at 50 °C. The reaction mixture was stirred using an overhead mechanical stirrer for 1 h at 50 °C. The oil bath was then removed and the reaction mixture allowed to slowly cool to room temperature while stirring. Following this, baker's yeast (80.00 g) was added portionwise and the mixture stirred for a further 2 h at room temperature, then poured into a stirring aqueous solution 10% calcium chloride (1.0 L) *via* a glass funnel with a small diameter hole (1-2 mm). The resultant beads were filtered and washed with water to give 975 mL of immobilised baker's yeast (IMBY) beads. It was then stored in the fridge at 4 °C and used the following day.

IMBY mediated reduction with kinetic resolution of racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-2[5,13]



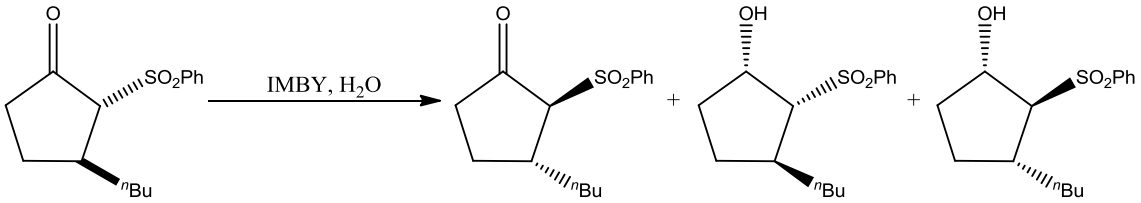
A mixture of IMBY (975 mL, which contained approximately 80.00 g free baker's yeast), sucrose (81.00 g), and tap water (406 mL) was stirred using an overhead mechanical stirrer at 28-30 °C for 30 min. A solution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** (2.07 g, 7.39 mmol) in DMSO (8 mL) was added dropwise over 1 min and the mixture stirred for 2 h. Sucrose (4 × 25.00 g) was then added at 2, 4, 6 and 9 h, the mixture was then stirred for a further 14 h (overnight) at 28-30 °C. Reaction sampling was performed at 23 h as follows: 15 mL of reaction solution was withdrawn with a plastic syringe and transferred to a separation funnel containing 10 mL of ethyl acetate, the organic layer was removed and the aqueous layer was extracted with (3 × 10 mL) of ethyl acetate. The combined organic layers were washed with brine (15 mL), dried, filtered and concentrated under reduced pressure. The crude product was dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC, (see appendix I for conditions).

Upon reaction completion, the mixture was filtered at 23 h to remove the IMBY beads and the beads were washed and pressed with ethyl acetate (3 × 100 mL). The pressed beads were placed in a beaker, covered with ethyl acetate and sonicated at ~40 °C for 6-8 h. The mixture was then filtered and the sonciated beads were washed with ethyl acetate (3 × 100 mL). The beads were then compressed with a pestle and mortor and stirred quickly with an overhead stirrer in ethyl acetate overnight. The solution was once again filtered and the compressed beads washed with ethyl acetate (3 × 100 mL). The combined aqueous filtrate was extracted with ethyl acetate (3 × 100 mL) and the combined organic extracts dried, filtered and concentrated under reduced pressure. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the *pure (+)-(1*S,*2*R,*3*S*)-2-benzenesulfonyl-3*-n-*butylcyclopentanol (1*S,*2*R,*3*S*)-**4a*** (0.69 g, 33%) as a white solid $[\alpha]_D^{20}$ +33.1 (c 0.5, $CHCl_3$), 98% ee, lit[4] $[\alpha]_D^{20}$ +22.4 (c 11.5, CH_2Cl_2), >95% ee; >98 : 2 dr and the crude *ketone (2*S,*3*R*)-**2*** which required further purification by column chromatography on silica gel using hexane/ethyl acetate 97/3 as eluent to give the *pure (+)-(2*S,*3*R*)-2-benzenesulfonyl-3*-n-*butylcyclopentanone (2*S,*3*R*)-**2*** (0.53 g, 25%) as a light yellow oil $[\alpha]_D^{20}$ +79.4 (c 0.5, $CHCl_3$), 99% ee, lit[4] $[\alpha]_D^{20}$ +21.3 (c 8.6, CH_2Cl_2), 33% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. *The ^{1}H NMR of pure (+)-(1*S,*2*R,*3*S*)-2-benzenesulfonyl-3*-n-*butylcyclopentanol (1*S,*2*R,*3*S*)-**4a** shows no evidence of the minor cyclopentanol (1*S,*2*S,*3*R*)-**4b** at δ_H (300 MHz) 4.63-4.66 (C**H**OH).*
2. *As it was necessary to repeat flash chromatography in order to obtain a pure sample of (+)-(2*S,*3*R*)-2-benzenesulfonyl-3*-n-*butylcyclopentanone (2*S,*3*R*)-**2** this contributed to a loss in yield. A yield of 35% was obtained for pure (+)-(2*S *3*R*)-2-benzenesulfonyl-3*-n-*butylcyclopentanone (2*S,*3*R*)-**2**,* 98% ee *and 32% for pure (+)-(1*S,*2*R,*3*S*)-2-benzenesulfonyl-3*-n-*butylcyclopentanol (1*S,*2*R,*3*S*)-**4a**,* 98% ee *for a batch that was synthesised later that required purification by column chromatography only once (entry 2, Table 5.1).*

*Table 5.1: Baker's yeast reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** – demonstrating the reproducibility of the biotransformation*



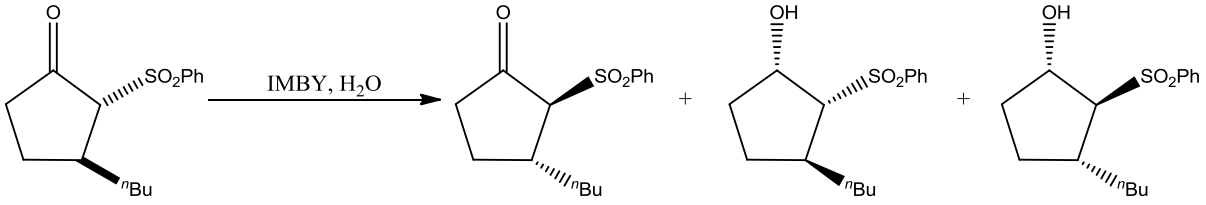
(±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4a** (1*S*,2*S*,3*R*)-**4b**

Entry	Reaction Conditions	Scale (g)[d]	Reaction Time	Cyclopentanone (2*S*,3*R*)-2		Cyclopentanol (1*S*,2*R*,3*S*)-4a		Cyclopentanol (1*S*,2*S*,3*R*)-4b
				ee (%)[e]	Isolated yield (%)	ee (%)[e]	Isolated yield (%)	dr
1	IMBY-H_2O[a]	2.07	23 h	99	25[f]	98	33	-[h]
2	IMBY-H_2O[b]	3.00	47 h 15 min	98	35	98	32	-[h]
3	IMBY-H_2O[c]	2.00	24 h	>98	12[g]	>98	18[g]	-[h]

a. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
b. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h, 6 h, 8 h, 23 h 15 min, 26 h 15 min, 28 h 45 min and 41 h 45 min.
c. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
d. Scale refers to the quantity of cyclopentanone (±)-**2** employed for the reaction.
e. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
f. Purification by column chromatography was repeated, hence this contributed to a loss in yield.
g. Poor yield attributed to experimental error during work-up leading to physical loss.
h. The ^{1}H NMR of pure isolated (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** showed no evidence of the minor cyclopentanol (1*S*,2*S*,3*R*)-**4b** at δ_H (300 MHz) 4.63-4.66 (C***H***OH).

Reaction monitoring was conducted throughout the biotransformations, for example Table 5.2 and Figure 5.1 and 5.2 correlate to chiral HPLC analysis of the preparative-scale (2.00 g) baker's yeast mediated resolution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** summarised in entry 3, Table 5.1.

*Table 5.2: Baker's yeast reduction of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**: Monitoring over time*



(±)-**2** (2*S*,3*R*)-**2** (1*S*,2*R*,3*S*)-**4a** (1*S*,2*S*,3*R*)-**4b**

Entry	Reaction Conditions[a]	Scale (g)[b]	Reaction Time	Cyclopentanone (2*S*,3*R*)-2		Cyclopentanol (1*S*,2*R*,3*S*)-4a		Cyclopentanol (1*S*,2*S*,3*R*)-4b
				ee (%)[c]	Isolated yield (%)	ee (%)[c]	Isolated yield (%)	dr
1	IMBY-H_2O	2.00	2 h	44	-	>98	-	-
2			4 h	98	-	>98	-	-
3			9 h	>98	-	>98	-	-
4			24 h	>98	12[d]	>98	18[d,e]	-[e]

a. IMBY-H_2O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
b. Scale refers to the quantity of cyclopentanone (±)-**2** employed for the reaction.
c. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
d. A yield of 35% was obtained for pure (+)-(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, 98% ee and 32% for pure (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a**, 98% ee for a batch that was synthesised later (entry 2, Table 5.1).
e. The ^{1}H NMR of pure isolated (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a** showed no evidence of the minor cyclopentanol (1*S*,2*S*,3*R*)-**4b** at δ_H (300 MHz) 4.63-4.66 (C***H***OH).

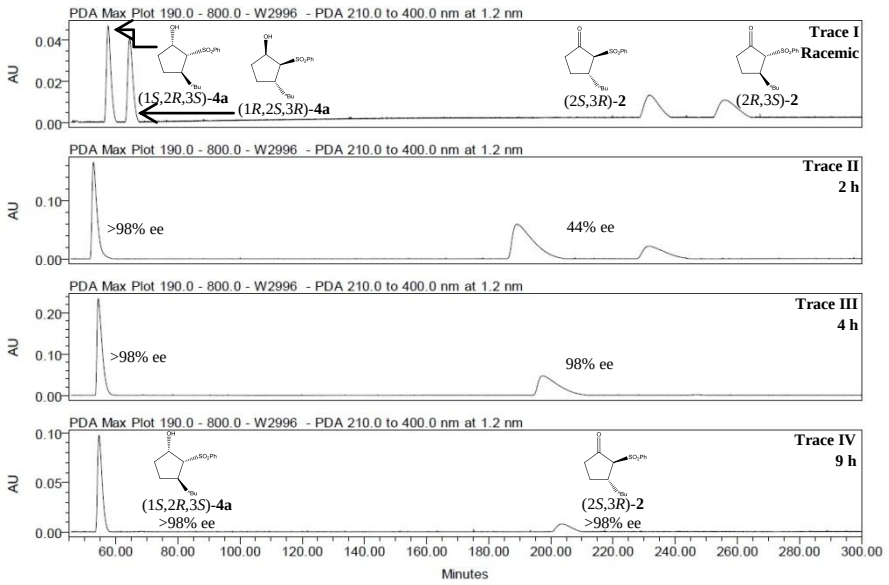


Figure 5.1: HPLC ***Trace I:*** *Racemic (±)-(1R*,2S*,3R*)-2-Benzenesulfonyl-3-*n*-butylcyclopentanol* ***4a*** *and (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**. **Trace II:** Reaction sampling 2 h. **Trace III:** Reaction sampling 4 h. **Trace IV:** Reaction sampling 9 h, (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanol (1*S*,2*R*,3*S*)-**4a**, >98% ee, (+)-(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2**, >98% ee. For HPLC conditions see appendix I.*

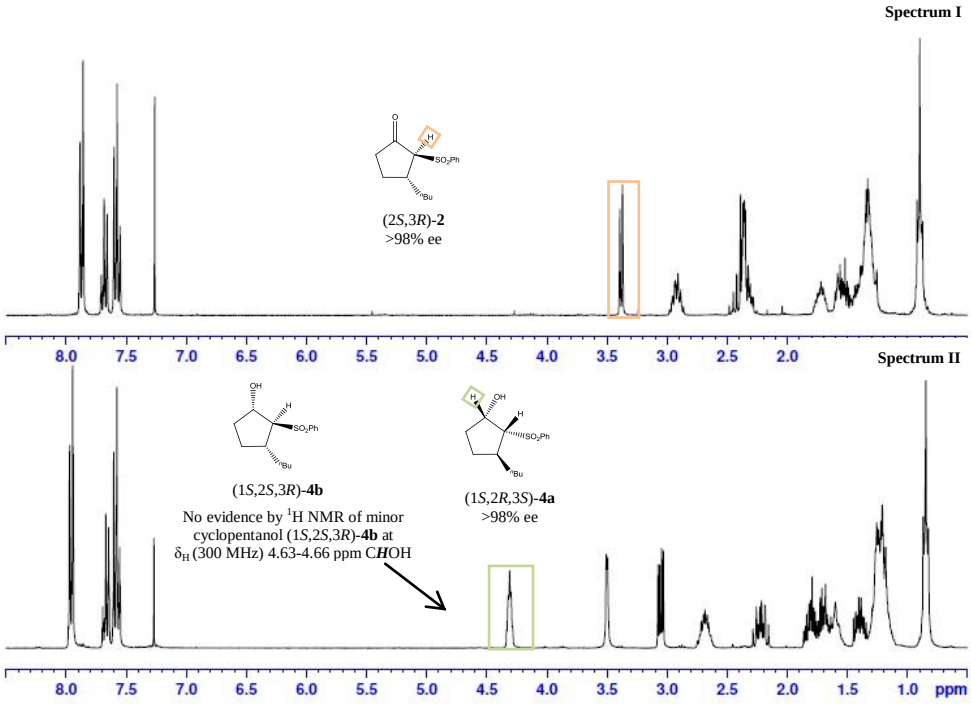
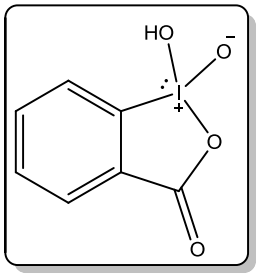


*Figure 5.2: ^{1}H NMR **Spectrum I:** (2S,3R)-2-benzenesulfonyl-3-n-butylcyclopentanone (2S,3R)-**2**, >98% ee.*
***Spectrum II:** (1S,2R,3S)-2-benzenesulfonyl-3-n-butylcyclopentanol (1S,2R,3S)-**4a**, >98% ee.*
(All spectra recorded in $CDCl_3$ at 300 MHz).

5.2.3 Asymmetric synthesis of (*R*)- and (*S*)-4-methyloctanoic acids (*R*)-1 and (*S*)-1

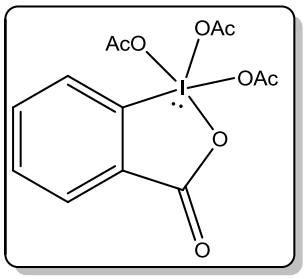
1-Hydroxy-1,2-benziodoxol-3-(1H)-one-1-oxide (IBX) 13[14-16]



2-Iodobenzoic acid (10.00 g, 40.32 mmol) was added all at once to a solution of Oxone® (36.20 g, 58.89 mmol) in water (130 mL) in a 500 mL 2-necked round bottomed flask. The reaction mixture was warmed to 70-73 °C (internal temperature) over 20 min and stirred using an overhead mechanical stirrer at this temperature for 3h. The initial thick slurry coating the walls of the flask eventually became a finely dispersed easy to stir suspension of a small amount of solid that sedimented easily upon stopping the stirring The suspension was then cooled to ~5 °C with an ice bath and left at this temperature for 1.5 h with slow magnetic stirring. The mixture was filtered through a sintered glass funnel and the solid was repeatedly rinsed with cold water (6 × 20 mL) and acetone (2 × 20 mL) to give the pure *IBX* **13** (8.42 g, 75%) as a moist white crystalline solid; m.p. 232-233 °C (violent decomposition) (lit.,[15] 232-233 °C); δ_H (300 MHz, DMSO) 7.84 (1H, t, *J* 6.6, ArH), 7.96-8.07 (2H, m, ArH), 8.15 (1H, d, *J* 8.1, ArH).

Note: Literature ^{1}H NMR data reports, δ_H (DMSO-d_6) 8.01 (1H, d), 7.98 (1H, t), however in this study the aromatic protons in this region are reported as a multiplet due to overlapping peaks.[16]

1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3-(1H)-one (Dess-Martin periodinane) 12[17]

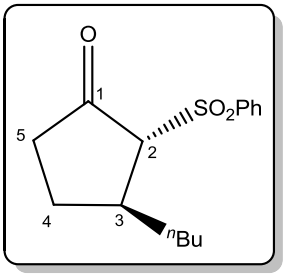


1-Hydroxy-1,2-benziodoxol-3-(1H)-one-1-oxide (IBX) **13** (8.42 g, 30.06 mmol) was added to a stirring mixture of acetic anhydride (29 mL, 307.36 mmol) and glacial acetic acid (14 mL, 244.56 mmol) under nitrogen. The reaction mixture was heated to 85 °C (internal temperature) over 30 min and kept at this temperature until all the solids dissolved (~20 min) to afford a colourless to clear yellow solution. Heating and stirring were discontinued and the reaction mixture was allowed to cool slowly to room temperature in the oil bath overnight. A large quantity of colourless crystals separate during this time. The resulting crystalline solids are isolated by careful vacuum Schlenk filtration under a nitrogen atmosphere, followed by washing of the solids with diethyl ether (3 × 10 mL). The solids were allowed to dry under vaccum within the Schlenk filter for 2 h to give the crude *Dess-Martin periodinane* **12** (10.00 g, 78%) as a white free-flowing crystalline solid which was used immediately; m.p. 133-134 °C (lit.,[15] 133-134 °C); δ_H (300 MHz, $CDCl_3$) 2.01 (6H, s, 2 x CH_3), 2.34 (3H, s, CH_3), 7.86-8.37 (4H, m, ArH).

Note:

1. *Literature states that the Dess-Martin periodinane **12** can be stored in a dark bottle under nitrogen at −20 °C in a freezer, however best results were obtained in this study if **12** was used immediately following filtration.[17]*
2. *During this research it was found that the Dess-Martin periodinane **12** did not fully dissolve when an opened bottle of deuterated chloroform ($CDCl_3$) was used, and filtration of the ^{1}H NMR sample was essential. This correlates with literature reports that a freshly opened bottle of $CDCl_3$ was required to dissolve **12**.[17]*
3. *Signals and multiplicity for the aromatic protons were difficult to decipher due to overlap with aromatic proton signals of the incompletely acetylated products. Integration of signals was slightly higher than anticipated due to these impurity peaks and the presence of acetic acid ~62 mol%, ~19 mass%.*

(−)-(2*R*,3*S*)-2-Benzenesulfonyl-3-*n*-butylcyclopentanone (2R,3S)-2[4,5,7]



A solution of (+)-(1*S*,2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butyl cyclopentanol (1*S*,2*R*,3*S*)-**4** (1.38 g, 4.63 mmol), 98% ee in dichloromethane (60 mL) was added to a solution of 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3-(1H)-one **12** (3.93 g, 9.27 mmol) in dichloromethane (80 mL) and the mixture was stirred for 4 h. The mixture was poured onto a saturated aqueous solution of sodium bicarbonate (300 mL) containing sodium thiosulfate (14.00 g) and stirred for ~15 min. The layers were separated and the organic layer was washed with a saturated aqueous solution of sodium bicarbonate (2 × 150 mL) and water (100 mL), dried, filtered and concentrated under reduced pressure to give the pure *(−)-(2*R,*3*S*)-cyclopentanone* (2*R*,3*S*)-**2** (1.16 g, 89%) as a yellow oil $[\alpha]_D^{20}$ −74.20 (c 0.5, $CHCl_3$), 96% ee, lit[4] $[\alpha]_D^{20}$ −46.3 (c 4.3, CH_2Cl_2), 75% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

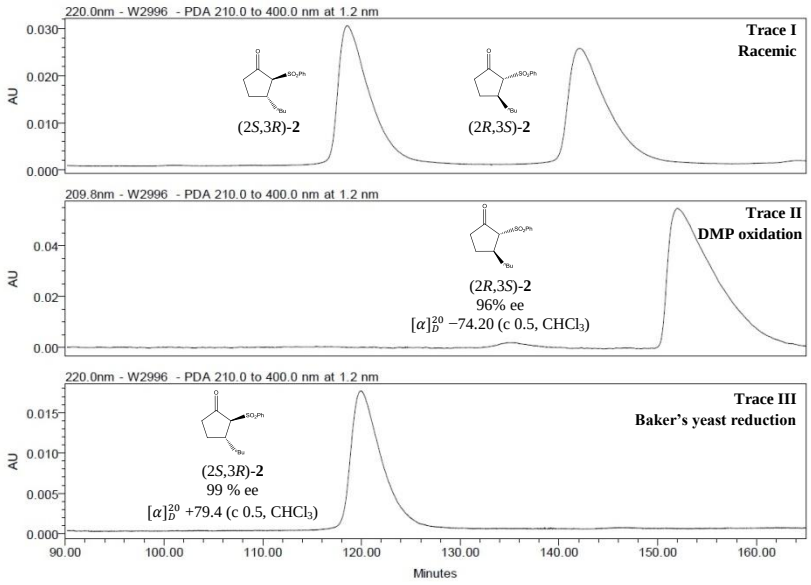
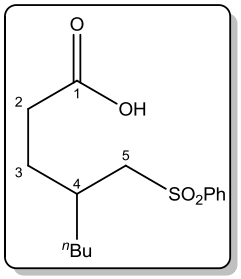


Figure 5.3: HPLC ***Trace I:*** *Racemic (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2**.* ***Trace II:*** *(-)-(2*R,3S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R,3S*)-**2**, 96% ee (from DMP oxidation).* ***Trace III:*** *(+)-(2*S,3R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S,3R*)-**2**, 99% ee (from baker's yeast). For HPLC conditions see appendix I.*

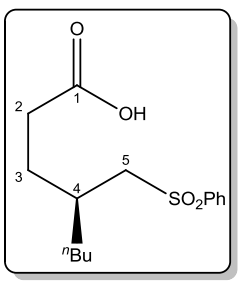
(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-11[4,5,7]



A solution of (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone **2** (1.42 g, 5.07 mmol) in aqueous sodium hydroxide (1M, 150 mL) was heated under reflux for 30 min. The reaction mixture was cooled to room temperature and washed with diethyl ether (50 mL). The aqueous layer was acidified to pH 2 with aqueous hydrochloric acid (10%) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (100 mL), dried, filtered and concentrated under reduced pressure to give the pure *carboxylic acid* (±)-**11** (1.04 g, 69%) as a viscous light orange oil; ν_{max}/cm^{-1} (film) 3066 (OH), 1710 (CO), 1305, 1146 (SO_2); δ_H (300 MHz) 0.85 (3H, t, *J* 6.9, CH_3), 1.06-1.48 [6H, m, C***H***$_2$(C***H***$_2$)$_2$CH$_3$], 1.66-1.92 [1H, m, C(3)H$_2$], 1.98-2.12 [1H, sym m, *J* 6.2 C(4)H^{n}Bu], 2.34 [2H, t, *J* 7.8, C(2)H$_2$], 3.00 [1H, dd, A of ABX, J_{AB} 14.4, J_{AX} 6.3, one of C(5)H$_2$], 3.08 [1H, dd, B of ABX, J_{AB} 14.4, J_{BX} 5.7, one of C(5)H$_2$], 7.51-7.72 (3H, m, ArH), 7.87-7.96 (2H, m, ArH), 9.05 (1H, br s, OH).

*Note: Following work-up it was found to be difficult to fully remove the ethyl acetate due to the viscous nature of (±)-**11**, therefore (±)-**11** was analysed with ethyl acetate present ~7% and integration was slightly higher for peaks that overlaped with residual ethyl acetate.*

(−)-(*S*)-4-(Benzenesulfonylmethyl)octanoic acid (*S*)-11[4,5,7]



This was prepared following the procedure described for racemic (±)-**11** from (−)-(2*R*,3*S*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*R*,3*S*)-**2** (170.7 mg, 0.61 mmol), 93% ee and aqueous sodium hydroxide (1M, 20 mL) to give the pure *(−)-(*S*)-carboxylic acid* (−)-(*S*)-**11** (149.1 mg, 82%) as a viscous light yellow oil $[\alpha]_D^{20}$ −11.0 (c 1.2, CH_2Cl_2), 94% ee, lit[4] $[\alpha]_D^{20}$ −5.6 (c 2.7, CH_2Cl_2), 91% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note: Ethyl acetate (~3%) was evident in the ^{1}H NMR spectrum.

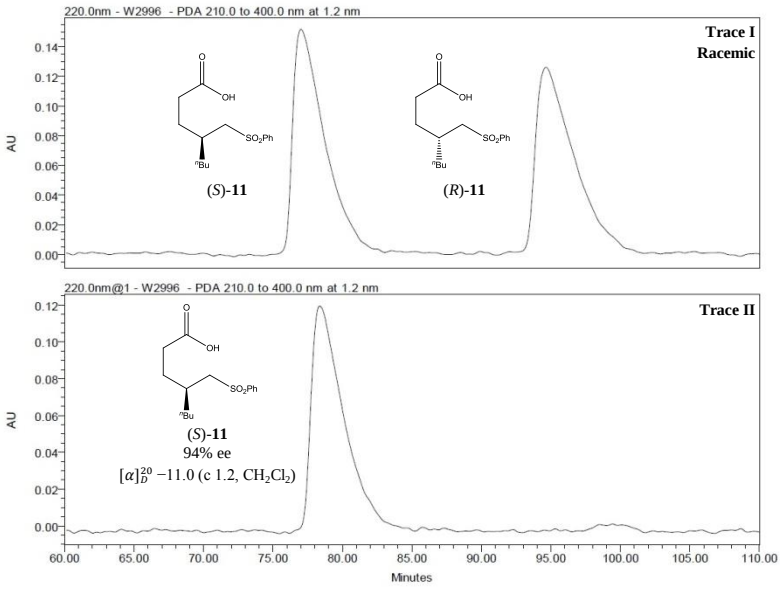
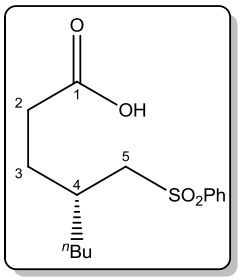


Figure 5.4: HPLC ***Trace I:*** *(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-**11**,*
Trace II*: (−)-(*S*)-4 -(Benzenesulfonylmethyl)octanoic acid (*S*)-**11**, 94% ee. For HPLC conditions see appendix I.*
Note the above traces correlate to chiral HPLC conditions A for ***11.***

(+)-(*R*)-4-(Benzenesulfonylmethyl)octanoic acid (*R*)-11[4,5,7]



This was prepared following the procedure described for racemic (±)-**11** from (+)-(2*S*,3*R*)-2-benzenesulfonyl-3-*n*-butylcyclopentanone (2*S*,3*R*)-**2** (239.1 mg, 0.85 mmol), >98% ee and aqueous sodium hydroxide (1M, 22 mL) to give the pure *(+)-(*R*)-carboxylic acid* (-)-(*R*)-**11** (185.0 mg, 73%) as a viscous light yellow oil $[\alpha]_D^{20}$ +9.1 (c 1.2, CH_2Cl_2), 99% ee, lit[4] $[\alpha]_D^{20}$ +6.3 (c 1.2, CH_2Cl_2), 75% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note: Ethyl acetate (~5%) was evident in the ^{1}H NMR spectrum.

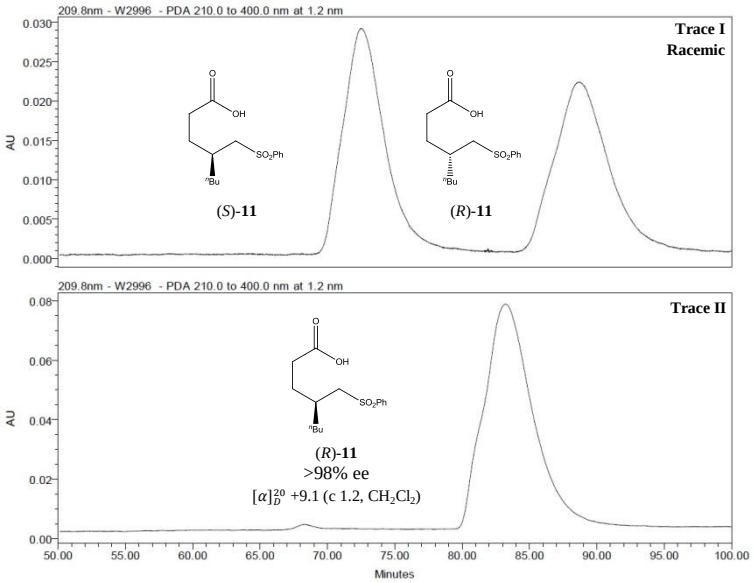
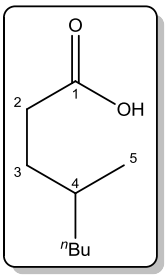


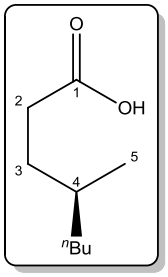
Figure 5.5: HPLC ***Trace I:*** *(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-**11**.*
Trace II: *(+)-(*R*)-4 -(Benzenesulfonyl methyl)octanoic acid (*R*)-**11**, >98% ee. For HPLC conditions see appendix I.*
Note the above traces correlate to chiral HPLC conditions B for ***11****.*

(±)-4-Methyloctanoic acid (±)-1[5,18,19]



Magnesium turnings (2.15 g, 89.54 mmol) were added to a stirred solution of (±)-4-(benzenesulfonylmethyl)octanoic acid (±)-**11** (1.04 g, 3.48 mmol) in methanol (150 mL) and the mixture was stirred for 10 h after which a further addition of magnesium (2.15 g, 89.54 mmol) was made and the slurry was stirred for an additional 18 h at room temperature under nitrogen. The reaction mixture was then carefully poured onto aqueous hydrochloric acid (10%, ~300 mL) and ice (~100 g). The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate (3 × 50 mL), brine (100 mL), dried, filtered and concentrated under reduced pressure to give the crude *desulfonylated acid* (±)-**1** (0.64 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *desulfonylated acid* (±)-**1** (0.28 g. 51%) as a clear oil; ν_{max}/cm^{-1} (film) 3043 (OH), 1714 (CO); δ_H (300 MHz) 0.78-0.98 (6H, m, 2 x CH_3), 1.04-1.36 [6H, m, C***H***$_2$(C***H***$_2$)$_2$CH$_3$], 1.37-1.55 [2H, m, one of C(3)H$_2$ and C(4)H], 1.57-1.81 [1H, m, one of C(3)H$_2$], 2.19-2.50 [2H, sym m, C(2)H$_2$], 10.55 (1H, br s, OH). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

(+)-(*S*)-4-Methyloctanoic acid (*S*)-1[5,18,19]

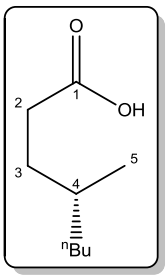


This was prepared following the procedure described for racemic (±)-**1** from (*S*)-4-(benzenesulfonylmethyl)octanoic acid (*S*)-**11** (0.56 g, 1.88 mmol), magnesium turnings (2.26 g, 94.17 mmol) and methanol (75 mL) to give the crude *desulfonylated acid* (*S*)-**1** (0.28 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *desulfonylated acid* (*S*)-**1** (0.17 g. 57%) as a clear oil $[\alpha]_D^{20}$ +1.6 (c 1.4, $CHCl_3$), lit[20] $[\alpha]_D^{20}$ +1.5 (c 1.4, $CHCl_3$), 93% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. *Chiral HPLC and optical rotation analysis was not conducted on the batch of (*S*)-4-(benzenesulfonylmethyl)octanoic acid (*S*)-**11** utilised in this experiment. However, the enantiopurity of the precursor (-)-(2*R*,3*S*)-cyclopentanone (2*R*,3*S*)-**2** was determined to be 97% ee by chiral HPLC analysis and base-catalysed ring cleavage to the (*S*)-carboxylic acid (*S*)-**11** proceeds with retention of enantioselectivity.*
2. *Due to the low UV absorption of (+)-(*S*)-4-methyloctanoic acid (*S*)-**1**, chiral HPLC analysis was not possible with a PDA detector and enantiomeric excess [ee (%)] was determined as 97% ee by derivatisation with 9-fluorenemethanol **17**.*
3. *The literature ee values were determined by derivatisation of (+)-(*S*)-methyloctanoic acid (*S*)-**1** with (R)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol.[20]*

(−)-(*R*)-4-Methyloctanoic acid (*R*)-1[5,18,19]

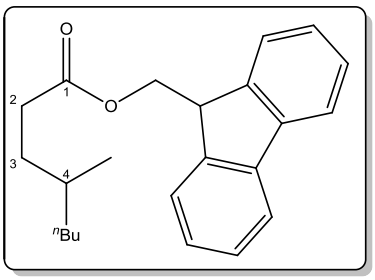


This was prepared following the procedure described for racemic (±)-**1** from (*R*)-4-(benzenesulfonylmethyl)octanoic acid (*R*)-**11** (0.74 g, 2.49 mmol), magnesium turnings (2.98 g, 124.17 mmol) and methanol (100 mL) to give the *crude desulfonylated acid* (*R*)-**1** (0.35 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *desulfonylated acid* (*R*)-**1** (0.21 g. 53%) as a clear oil $[\alpha]_D^{20}$ −2.2 (c 1.4, $CHCl_3$), lit[20] $[\alpha]_D^{20}$ −1.5 (c 1.4, $CHCl_3$), 94% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. *Chiral HPLC and optical rotation analysis was not conducted on the batch of (*R*)-4-(benzenesulfonylmethyl)octanoic acid (*R*)-**11** utilised in this experiment. However, the enantiopurity of the precursor (−)-(2*S*,3*R*)-cyclopentanone (2*S*,3*R*)-**2** was determined to be 99% ee by chiral HPLC analysis and base-catalysed ring cleavage to the (*R*)-carboxylic acid (*R*)-**11** proceeds with retention of enantioselectivity.*
2. *Due to the low UV absorption of (−)-(*R*)-4-methyloctanoic acid (*R*)-**1**, chiral HPLC analysis was not possible with a PDA detector and enantiomeric excess [ee (%)] was determined as 99% ee by derivatisation with 9-fluorenemethanol **17**.*
3. *The literature ee values were determined by derivatisation of (−)-(*R*) methyloctanoic acid (R)-**1** with (R)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol.[20]*

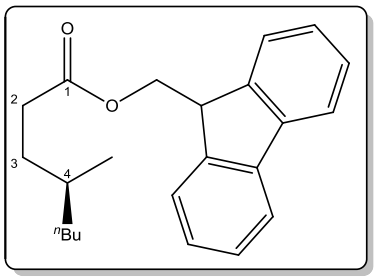
(±)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (±)-18



9-Fluorenemethanol **17** (431.0 mg, 2.20 mmol) in doubly distilled dichloromethane (3 mL) was added to a solution of (±)-4-methyloctanoic acid (±)-**1** (86.8 mg, 0.55 mmol), dicyclohexylcarbodiimide (DCC) (340.0 mg, 1.65 mmol) and *N*,*N*-dimethylaminopyridine (67.0 mg, 0.55 mmol) in doubly distilled dichloromethane (5 mL). The resulting suspension was stirred for 72 h at room temperature under nitrogen. The reaction mixture was then concentrated under reduced pressure to give the crude *ester* (±)-**18** (928.4 mg) as a light yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *ester* (±)-**18** (113.6 mg. 61%) as a clear oil; (Found C, 81.75; H 8.35. $C_{23}H_{28}O_2$ requires C, 82.10; H, 8.39%); ν_{max}/cm^{-1} (film) 2956, 2827 (CH), 1737 (CO), 1450 (CH_2), 1167 (C-O); δ_H (300 MHz) 0.78-0.97 (6H, m, 2 x CH_3), 1.03-1.53 [8H, m, C***H***$_2$(C***H***$_2$)$_2$CH$_3$, C(4)H and one of C(3)H$_2$], 1.57-1.76 [1H, m, one of C(3)H$_2$], 2.25-2.50 [2H, sym m, C(2)H$_2$], 4.20 (1H, t, *J* 7.1, OCH$_2$C***H***), 4.39 (2H, d, *J* 6.9, OCH$_2$), 7.29-7.34 (2H, dt, *J* 1.2, 7.5, ArH), 7.40 (2H, t, *J* 7.2 ArH), 7.60 (2H, d, *J* 7.5, ArH), 7.76 (2H, d, *J* 7.2, ArH); δ_C (75.5 MHz) 14.1, 19.3 (2 x CH_3), 23.0, 29.1 [2 x CH_2, two of ***C***H$_2$(***C***H$_2$)$_2$CH$_3$], 31.9 [CH_2, C(3)H$_2$], 32.1 [CH_2, C(2)H$_2$], 32.3 [CH, C(4)H], 36.3 [CH_2, one of ***C***H$_2$(***C***H$_2$)$_2$CH$_3$], 46.9 (CH, OCH$_2$***C***H), 66.2 (CH_2, O***C***H$_2$CH), 120.0, 125.0, 127.1, 127.8 (8 x CH, ArCH), 141.3, 143.9 (4 x C, ArC), 174.0 [C, C(1)]; m/z (ES−) 336.5 (M^-, tentative). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments. m/z (ES+) 64.2 [(SO2)+, 100%], 239.1 [(M+H)+,

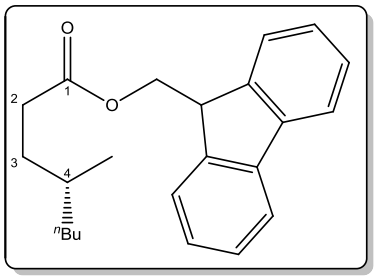
Note: Water (~13%) was evident in the ^{1}H NMR spectrum.

(+)-(*S*)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (*S*)-18



This was prepared following the procedure described for racemic (±)-**18** from 9-fluorenemethanol **17** (235.0 mg, 1.20 mmol) in doubly distilled dichloromethane (1.6 mL) was added to a solution of (*S*)-4-methyloctanoic acid (*S*)-**1** (47.3 mg, 0.30 mmol), [sample from experiment described above, $[\alpha]_D^{20}$ +1.6 (c 1.4, $CHCl_3$)], dicyclohexylcarbodiimide (DCC) (186.0 mg, 0.90 mmol), *N*,*N*-dimethylaminopyridine (36.0 mg, 0.29 mmol) in doubly distilled dichloromethane (3 mL) to give the crude *ester* (*S*)-**18** (50.0 mg) as a light yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *ester* (*S*)-**18** (69.7 mg. 69%) as a clear oil $[\alpha]_D^{20}$ +1.5 (c 1.0, $CHCl_3$), 97% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

(−)-(*R*)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (*R*)-18



This was prepared following the procedure described for racemic (±)-**18** from 9-fluorenemethanol **17** (245.0 mg, 1.25 mmol) in doubly distilled dichloromethane (1.6 mL) was added to a solution of (*R*)-4-methyloctanoic acid (*R*)-**1** (49.4 mg, 0.31 mmol), [sample from experiment described above, $[\alpha]_D^{20}$ −2.2 (c 1.4, $CHCl_3$)], dicyclohexylcarbodiimide (DCC) (194.0 mg, 0.94 mmol), *N*,*N*-dimethylaminopyridine (38.0 mg, 0.31 mmol) in doubly distilled dichloromethane (3 mL) to give the crude *ester* (*R*)-**18** (50.7 mg) as a light yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *ester* (*R*)-**18** (82.2 mg. 79%) as a clear oil $[\alpha]_D^{20}$ −3.4 (c 1.0, $CHCl_3$), >98% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

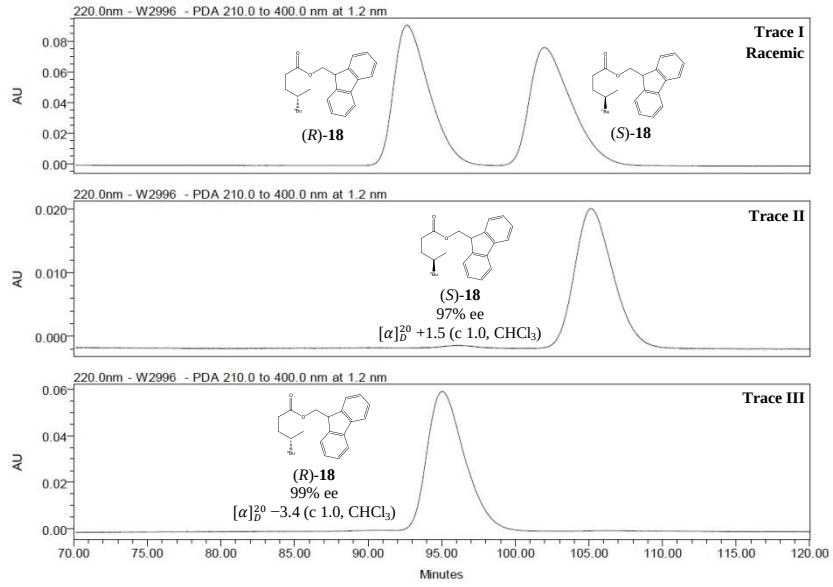


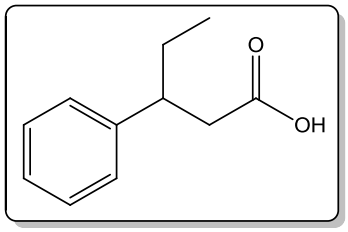
Figure 5.6: HPLC ***Trace I:*** *(±)-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (±)-**18**. **Trace II:** (+)-*(S)*-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (*S*)-**18**, 97% ee. **Trace III**: (−)-*(R)*-(9H-Fluoren-9-yl)methyl 4-methyloctanoate (*R*)-**18**, 99% ee. For HPLC conditions see appendix I.*

5.3 Kinetic resolutions of β-substituted 3-aryl alkanoic acids

5.3.1 Synthesis of hydrolase substrates

5.3.1.1 Synthesis of carboxylic acids

(±)-3-Phenylpentanoic acid (±)-28[21-23]

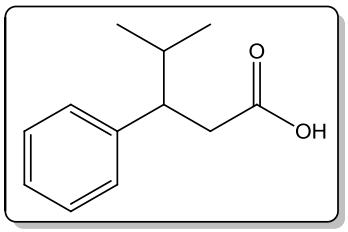


Ethyl magnesium bromide was freshly prepared from magnesium (2.85 g, 117.23 mmol), iodine (one crystal) in diethyl ether (30 mL) and ethyl bromide (8.7 mL, 116.56 mmol) in diethyl ether (30 mL) at 0 °C under nitrogen and the mixture was stirred for 30 min at 0 ºC. Cinnamic acid **62** (5.00 g, 33.75 mmol) was added portionwise while stirring at 0 °C, then the reaction mixture was heated under reflux for 3 h. The reaction mixture was subsequently cooled to room temperature and carefully poured onto aqueous hydrochloric acid (10%, ~100 mL) and ice (~65 g). The layers were separated and the aqueous layer was washed with diethyl ether (3 × 50 mL). The combined organic layer was washed with aqueous hydrochloric acid (10%, 50 mL), water (50 mL), brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *acid* (±)-**28** (5.54 g, 92%) as an orange oil which was used without further purification; ν_{max}/cm^{-1} (film) 2965 (OH), 1708 (CO), 1603, 1495, 1454; δ_H (400 MHz) 0.79 [3H, t, *J* 7.4, C(5)H_3], 1.46-1.83 [2H, m, C(4)H_2], 2.60 [1H, dd, A of ABX, J_{AB} 15.6, J_{AX} 7.9, one of C(2)H_2], 2.68 [1H, dd, B of ABX, J_{AB} 15.6, J_{BX} 7.1, one of C(2)H_2], 2.89-3.08 [1H, m, X of ABX, C(3)H], 7.09-7.38 (5H, m, ArH).

*Preparation of the analytically pure acid (±)-**28** by basic hydrolysis of the corresponding acid chloride (±)-**73**.*

Aqueous potassium hydroxide (20%, 35 mL) was added to a sample of 3-phenylpentanoyl chloride (±)-**73** (0.51 g, 2.60 mmol) under nitrogen. The reaction mixture was heated to reflux and stirred under reflux overnight. The reaction mixture was then cooled to room temperature and acidified to pH 1 with aqueous hydrochloric acid (10%), which resulted in a white precipitate. The suspension was then extracted with dichloromethane (3 × 50 mL), and the combined organic layers where washed with brine (100 mL), dried, filtered and concentrated under reduced pressure to give the pure *acid* (±)-**28** (428 mg, 92%) as a cream solid, m.p. 59-62 ºC (Lit.,[24] 60-61 ºC) and with spectral characteristics identical to those described above.

(±)-4-Methyl-3-phenylpentanoic acid (±)-29[21,23]

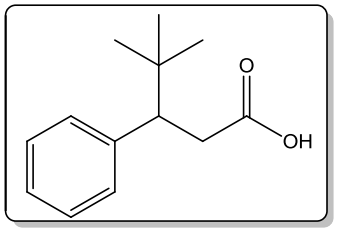


This was prepared following the procedure described for (±)-**28** from isopropyl magnesium bromide [freshly prepared from magnesium (2.70 g, 111.07 mmol), iodine (one crystal) in diethyl ether (30 mL), and isopropyl bromide (11.3 mL, 119.82 mmol) in diethyl ether (30 mL)] and cinnamic acid **62** (5.00 g, 33.75 mmol) to give the crude *acid* (±)-**29** (5.38 g, 83%) as an orange oil, which was used without further purification; ν_{max}/cm^{-1} (film) 2963 (OH), 1709 (CO), 1602, 1495, 1454; δ_H (400 MHz) 0.74, 0.92 [2 × 3H, 2 × d, *J* 6.7, *J* 6.7, C(4)HC$\boldsymbol{H_3}$ and C(5)H_3], 1.79-1.91 [1H, m, C(4)H], 2.60 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 9.5, one of C(2)H_2], 2.72-2.92 [2H, m, BX of ABX, one of C(2)H_2 and C(3)H], 7.10-7.28 (5H, m, ArH).

*Preparation of the analytically pure acid (±)-**29** by basic hydrolysis of the corresponding acid chloride (±)-**74**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 50 mL) and 4-methyl-3-phenylpentanoyl chloride (±)-**74** (1.00 g, 4.75 mmol) to give the pure *acid* (±)-**29** (0.90 g, 99%) as a cream, white solid, m.p. 48-50 °C (Lit.,[21] 46-48 ºC) and with spectral characteristics identical to those described above.

(±)-4,4-Dimethyl-3-phenylpentanoic acid (±)-37[21,22]

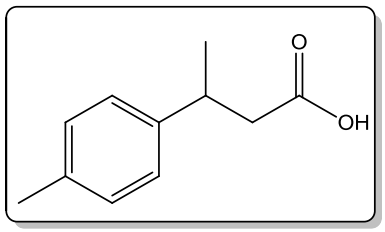


This was prepared following the procedure described for (±)-**28**, from *tert*-butyl magnesium chloride (2 M in diethyl ether, 106 mL, 212.00 mmol) and cinnamic acid **62** (7.85 g, 52.98 mmol) to give the crude *acid* (±)-**37** (8.63 g, 79%) as a yellow solid, which was used without further purification. ν_{max}/cm^{-1} (KBr) 2955 (OH), 1726 (CO), 1638, 1453; δ_H (400 MHz) 0.87 [9H, s, $C(CH_3)_3$], 2.73 [1H, dd, A of ABX, J_{AB} 15.8, J_{AX} 10.8, one of $C(2)H_2$], 2.81 [1H, dd, B of ABX, J_{AB} 15.8, J_{BX} 4.5, one of $C(2)H_2$], 2.93 [1H, dd, X of ABX, J_{AX} 10.8, J_{BX} 4.5, C(3)H], 7.07-7.33 (5H, m, ArH).

*Preparation of the analytically pure acid (±)-**37** by basic hydrolysis of the corresponding acid chloride (±)-**75**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 8 mL) and 4,4-dimethyl-3-phenylpentanoyl chloride (±)-**75** (214 mg, 0.95 mmol) to give the pure *acid* (±)-**37** (153 mg, 78%) as a white solid, m.p. 108-110 °C (Lit.,[21] 114–116 °C) and with spectral characteristics identical to those described above.

(±)-3-(4-Methylphenyl)butanoic acid (±)-51[23]

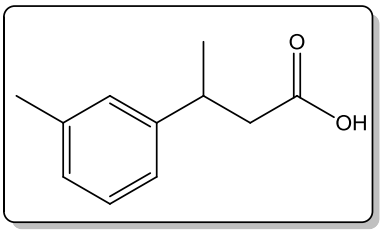


p-Tolyl magnesium bromide was freshly prepared from magnesium (10.00 g, 411.44 mmol), iodine (one crystal) in diethyl ether (80 mL) and 4-bromotoluene (51.6 mL, 419.34 mmol) in diethyl ether (60 mL) at 0 °C under nitrogen and the mixture was stirred for 30 min. Crotonic acid **63** (12.00 g, 139.39 mmol) was added portionwise while stirring at 0 °C, then the reaction was heated under reflux for 3 h. The reaction mixture, containing the product (±)-**51** and the Wurtz coupling product **71**, was acidified to pH 2 and the aqueous layer washed with diethyl ether (2 × 100 mL). The combined diethyl ether extracts were washed with sodium hydroxide (20%, 2 × 100 mL) the aqueous layer was acidified to pH 1 with conc. hydrochloric acid and extracted with diethyl ether (3 × 100 mL). The organic layer was dried, filtered and concentrated under reduced pressure to give the crude *acid* (±)-**51** as a viscous yellow oil (21.19 g, 86%) which was used without further purification; ν_{max}/cm^{-1} (film) 2926 (OH), 1704 (CO), 1515, 1455, 1416; δ_H (300 MHz) 1.30 [3H, d, *J* 7.0, $C(4)H_3$], 2.31 [3H, s, $C(4')CH_3$], 2.55 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 8.2, one of $C(2)H_2$], 2.65 [1H, dd, B of ABX, J_{AB} 15.5, J_{BX} 6.9, one of $C(2)H_2$], 3.18-3.30 [1H, m, X of ABX, C(3)H], 7.11 (4H, s, ArH).

*Preparation of the analytically pure acid (±)-**51** by basic hydrolysis of the corresponding acid chloride (±)-**78**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 8 mL) and 3-(4-methylphenyl)butanoyl chloride (±)-**78** (200 mg, 1.02 mmol) to give the pure *acid* (±)-**51** (152 mg, 83%) as a white solid, m.p. 91-92 °C (Lit.,[23] 92-93 °C) and with spectral characteristics identical to those described above.

(±)-3-(3-Methylphenyl)butanoic acid (±)-65[25]

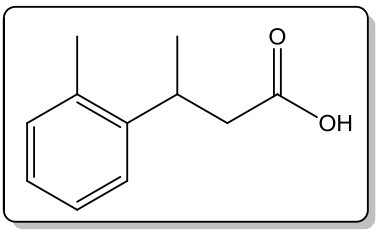


This was prepared following the procedure described for (±)-**51** from 3-tolyl magnesium bromide [freshly prepared from magnesium (4.24 g, 174.45 mmol) and iodine (one crystal) in diethyl ether (40 mL), and 3-bromotoluene (21 mL, 172.99 mmol) in diethyl ether (40 mL)] and crotonic acid **63** (5.00 g, 58.08 mmol) to give the crude *acid* (±)-**65** (8.55 g, 83%) as an orange oil, which was used without further purification; ν_{max}/cm^{-1} (film) 2971 (OH), 1718 (CO), 1608, 1490, 1455; δ_H (300 MHz) 1.30 [3H, d, *J* 7.0, C(4)H_3], 2.33 [3H, s, C(3')CH_3], 2.55 [1H, dd, A of ABX, J_{AB} 15.5, J_{AX} 8.4, one of C(2)H_2], 2.66 [1H, dd, B of ABX, J_{AB} 15.5, J_{BX} 6.7, one of C(2)H_2], 3.14-3.31 [1H, m, X of ABX, C(3)H], 6.91-7.22 (4H, m, ArH).

*Preparation of the analytically pure acid (±)-**65** by basic hydrolysis of the corresponding acid chloride (±)-**79**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 150 mL) and 3-(3-methylphenyl)butanoyl chloride (±)-**79** (4.59 g, 23.36 mmol) to give the pure *acid* (±)-**65** (2.87 g, 69%) as a clear oil and with spectral characteristics identical to those described above.

(±)-3-(2-Methylphenyl)butanoic acid (±)-64[26]

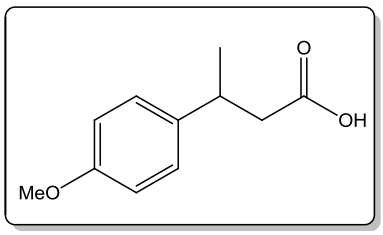


This was prepared following the procedure described for (±)-**51** from 2-tolyl magnesium bromide [freshly prepared from magnesium (4.22 g, 173.59 mmol) and iodine (one crystal) in diethyl ether (40 mL), and 2-bromotoluene (21 mL, 174.59 mmol) in diethyl ether (40 mL)] and crotonic acid **63** (5.00 g, 58.08 mmol) to give the crude *acid* (±)-**64** (8.21 g, 79%) as an orange oil, which was used without further purification; ν_{max}/cm^{-1} (film) 2973 (OH), 1712 (CO), 1605, 1492, 1460; δ_H (400 MHz) 1.27 [3H, d, *J* 6.9, C(4)H_3], 2.36 [3H, s, C(2')CH_3], 2.55 [1H, dd, A of ABX, J_{AB} 15.7, J_{AX} 8.6, one of C(2)H_2], 2.67 [1H, dd, B of ABX, J_{AB} 15.6, J_{BX} 6.3, one of C(2)H_2], 3.45-3.58 [1H, m, X of ABX, C(3)H], 7.06-7.27 (4H, m, ArH).

*Preparation of the analytically pure acid (±)-**64** by basic hydrolysis of the corresponding acid chloride (±)-**77**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 100 mL) and 3-(2-methylphenyl)butanoyl chloride (±)-**77** (3.10 g, 15.75 mmol) to give the pure *acid* (±)-**64** (2.20 g, 78%) as a yellow solid, m.p. 46-48 ºC (Lit.,[26] 46-47 °C) and with spectral characteristics identical to those described above.

(±)-3-(4-Methoxyphenyl)butanoic acid (±)-66[27,28]



This was prepared following the procedure described for (±)-**51** from *para*-methoxyphenyl magnesium bromide [freshly prepared from magnesium (10.22 g, 420.49 mmol) and iodine (one crystal) in diethyl ether (80 mL), and 4-bromoanisole (52 mL, 415.36 mmol) in diethyl ether (100 mL)] and crotonic acid **63** (12.00 g, 139.39 mmol) to give the crude *acid* (±)-**66** (19.17 g, 71%) as a yellow oil, which was used without further purification; ν_{max}/cm^{-1} (film) 2963 (OH), 1711 (CO), 1611, 1511, 1458; δ_H (400 MHz) 1.29 [3H, d, *J* 7.0, C(4)H_3], 2.54 [1H, dd, A of ABX, J_{AB} 15.4, J_{AX} 8.1, one of C(2)H_2], 2.62 [1H, dd, B of ABX, J_{AB} 15.4, J_{BX} 7.0, one of C(2)H_2], 3.17-3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH_3), 6.79-6.88 [2H, m, C(3')H, C(5')H], 7.11-7.16 [2H, m, C(2')H, C(6')H].

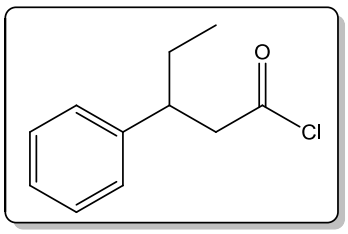
*Preparation of the analytically pure acid (±)-**66** by basic hydrolysis of the corresponding acid chloride (±)-**76**.*

This was prepared following the procedure described for (±)-**28**, from aqueous potassium hydroxide (20%, 8 mL) and 3-(4-methoxyphenyl)butanoyl chloride (±)-**76** (200 mg, 0.94 mmol) to give the pure *acid* (±)-**66** (114 mg, 62%) as a cream solid, m.p. 66-68 °C (Lit.,[27] 67-69 °C) and with spectral characteristics identical to those described above.

5.3.1.2 Synthesis of acid chlorides

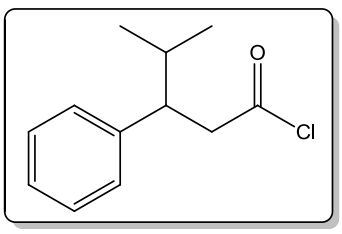
The acid chlorides were prepared from crude acids. The yield given below is the yield of acid chloride over two steps calculated from the unsaturated precursor for the acid.

(±)-3-Phenylpentanoyl chloride (±)-73[21]



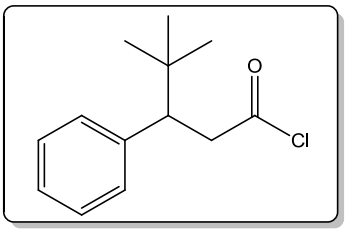
3-Phenylpentanoic acid (±)-**28** (5.01 g, 28.13 mmol) in thionyl chloride (16 mL, 219.34 mmol) was heated under reflux for 3 h while stirring under nitrogen. Excess thionyl chloride was evaporated under reduced pressure to give the crude *acid chloride* (±)-**73** as a brown oil. Purification by vacuum distillation gave the pure *acid chloride* (±)-**73** (2.07 g, 31%) as a bright yellow oil, b.p. 72-76°C at 0.09 mmHg (Lit.,[21] 113-115 °C at 0.5 mmHg); ν_{max}/cm^{-1} (film) 1799 (CO), 1604, 1495, 1454; δ_H (400 MHz) 0.80 [3H, t, *J* 7.4, C(5)H_3], 1.58-1.80 [2H, m, C(4)H_2], 3.04-3.23 [3H, m, C(2)H_2 and C(3)H], 7.16-7.34 (5H, m, ArH).

(±)-4-Methyl-3-phenylpentanoyl chloride (±)-74[21]



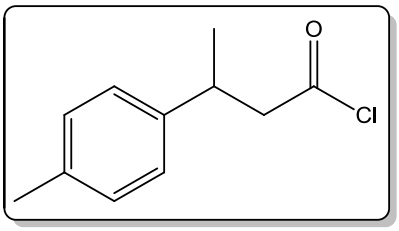
This was prepared following the procedure described for (±)-**73**, from crude 4-methyl-3-phenylpentanoic acid (±)-**29** (5.28 g, 27.46 mmol) and thionyl chloride (16 mL, 219.34 mmol) to give the crude *acid chloride* (±)-**74** as a brown oil. Purification by distillation gave the pure *acid chloride* (±)-**74** (2.54 g, 36%) as a clear oil, b.p. 78-80 °C at 0.15 mmHg (Lit.,[21] 90-94 °C at 0.08 mmHg); ν_{max}/cm^{-1} (film) 1799 (CO), 1602, 1495, 1454; δ_H (400 MHz) 0.76, 0.97 [2 × CH_3, 2 × d, *J* 6.7, *J* 6.7, C(4)HC***H*****$_3$** and C(5)H_3], 1.82-1.94 [1H, m, C(4)H], 2.92-2.98 [1H, m, X of ABX, C(3)H], 3.18 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 9.7, one of C(2)H_2], 3.34 [1H, dd, B of ABX, J_{AB} 16.4, J_{AX} 5.2, one of C(2)H_2], 7.09-7.35 (5H, m, ArH)

(±)-4,4-Dimethyl-3-phenylpentanoyl chloride (±)-75[21]



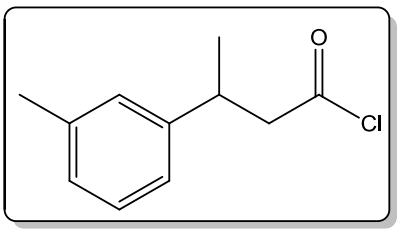
This was prepared following the procedure described for (±)-**73**, from crude 4,4-dimethyl-3-phenylpentanoic acid (±)-**37** (8.63 g 41.84 mmol) and thionyl chloride (24 mL, 329.02 mmol) to give the crude *acid chloride* (±)-**75** as a brown oil. Purification by distillation gave the pure *acid chloride* (±)-**75** (5.04 g, 54%) as a bright yellow solid, b.p. 84-86 °C at 0.12 mmHg (Lit.,[21] 123-125 °C at 0.1 mmHg); ν_{max}/cm^{-1} (KBr) 1793 (CO), 1601, 1494, 1453; δ_H (400 MHz) 0.91 [9H, s, C(CH_3)$_3$], 3.04 [1H, dd, X of ABX, *J* 8.9, 6.1, C(3)H], 3.26-3.39 [2H, m, C(2)H_2], 7.14-7.31 (5H, m, ArH).

(±)-3-(4-Methylphenyl)butanoyl chloride (±)-78[29]



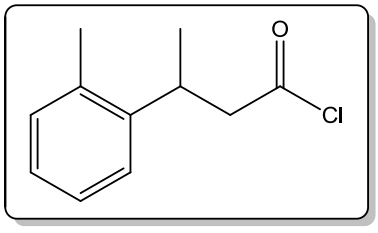
This was prepared following the procedure described for (±)-**73**, from crude 3-(4-methylphenyl)butanoic acid (±)-**51** (21.19 g, 118.89 mmol) and thionyl chloride (86 mL, 1179.00 mmol) to give the crude *acid chloride* (±)-**78** as a black oil. Purification by distillation gave the pure *acid chloride* (±)-**78** (12.09 g, 44%) as a dark orange oil, b.p. 66-68 °C at 0.2 mmHg (Lit.,[29] 127 °C at 0.20 mmHg); ν_{max}/cm^{-1} (film) 1800 (CO), 1649, 1516, 1455; δ_H (300 MHz) 1.32 [3H, d, *J* 7.0, C(4)H_3], 2.32 [3H, s, C(4')CH_3], 3.07 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 7.9, one of C(2)H_2], 3.17 [1H, dd, B of ABX, J_{AB} 16.4, J_{BX} 6.4, one of C(2)H_2], 3.27-3.38 [1H, m, X of ABX, C(3)H], 7.04-7.21 (4H, m, ArH).

(±)-3-(3-Methylphenyl)butanoyl chloride (±)-79[30,31]



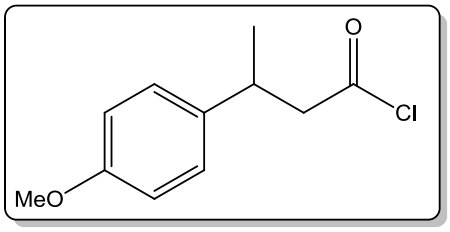
This was prepared following the procedure described for (±)-**73**, from crude 3-(3-methylphenyl)butanoic acid (±)-**65** (8.55 g, 47.97 mmol) and thionyl chloride (28 mL, 383.86 mmol) to give the crude *acid chloride* (±)-**79** as a black oil. Purification by distillation gave the pure *acid chloride* (±)-**79** (4.59 g, 40%) as a bright yellow oil, b.p. 77-80 °C at 0.2 mmHg (Lit.,[30] 60 °C at 0.01 mmHg); ν_{max}/cm^{-1} (film) 1800 (CO), 1608, 1491, 1456; δ_H (300 MHz) 1.32 [3H, d, *J* 6.9, C(4)H_3], 2.33 [3H, s, C(3')CH_3], 3.06 [1H, dd, A of ABX, J_{AB} 16.5, J_{AX} 8.0, one of C(2)H_2], 3.17 [1H, dd, B of ABX, J_{AB} 16.5, J_{BX} 6.5, one of C(2)H_2], 3.25-3.36 [1H, m, X of ABX, C(3)H], 6.98-7.05 [3H, m, C(4')H, C(5')H and C(6')H, ArH], 7.17-7.22 [1H, m, C(2')H, ArH].

(±)-3-(2-Methylphenyl)butanoyl chloride (±)-77[32]



This was prepared following the procedure described for (±)-**73**, from crude 3-(2-methylphenyl)butanoic acid (±)-**64** (8.21 g, 46.04 mmol) and thionyl chloride (27 mL, 370.15 mmol) to give the crude *acid chloride* (±)-**77** as a black oil. Purification by distillation gave the pure *acid chloride* (±)-**77** (4.68 g, 41%) as a bright yellow oil, b.p. 64-66 °C at 0.09 mmHg (Lit.,[30] 55-56 °C at 0.007 mmHg); ν_{max}/cm^{-1} (film) 1801 (CO), 1605, 1492, 1459; δ_H (400 MHz) 1.27 [3H, d, *J* 7.0, C(4)H_3], 2.35 [3H, s, C(2')CH_3], 3.03 [1H, dd, A of ABX, J_{AB} 16.6, J_{AX} 8.2, one of C(2)H_2], 3.16 [1H, dd, B of ABX, J_{AB} 16.6, J_{BX} 6.3, one of C(2)H_2], 3.54-3.63 [1H, m, X of ABX, C(3)H], 7.07-7.28 (4H, m, ArH).

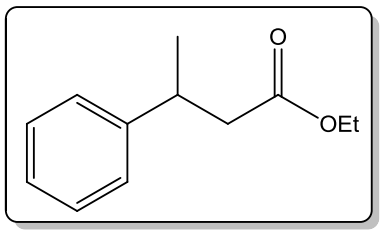
(±)-3-(4-Methoxyphenyl)butanoyl chloride (±)-76[27]



This was prepared following the procedure described for (±)-**73**, from crude 3-(4-methoxyphenyl)butanoic acid (±)-**66** (19.17 g, 98.70 mmol), thionyl chloride (57 mL, 781.43 mmol) to give the crude *acid chloride* (±)-**76** as a black oil. Purification by distillation gave the pure *acid chloride* (±)-**76** (8.69 g, 29%) as a orange brown oil, b.p. 102-110 °C at 0.35 mmHg (Lit.,[27] 100 °C at 0.5 mmHg); ν_{max}/cm^{-1} (film) 1790 (CO), 1614, 1515, 1463; δ_H (400 MHz) 1.32 [3H, d, *J* 7.0, C(4)H_3], 3.07 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 7.8, one of C(2)H_2], 3.15 [1H, dd, B of ABX, J_{AB} 16.4, J_{BX} 6.8, one of C(2)H_2], 3.27-3.36 [1H, m, X of ABX, C(3)H], 3.78 (3H, s, OCH_3), 6.84-6.87 [2H, m, C(3')H and C(5')H, ArH], 7.11-7.15 [2H, m, C(2')H and C(6')H, ArH].

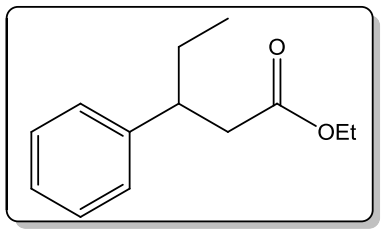
5.3.1.3 Synthesis of ethyl esters

(±)-Ethyl 3-phenylbutanoate (±)-38[33]



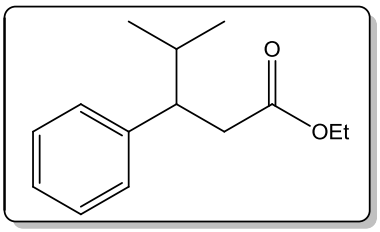
Sulfuric acid (conc. 95-97%, 1.0 mL, 18.76 mmol) was added to a solution of 3-phenylbutanoic acid **(±)-23** (0.99 g, 6.08 mmol) in absolute ethanol (20 mL) and refluxed overnight. Excess ethanol was evaporated under reduced pressure. The crude product was dissolved in dichloromethane (45 mL) and washed with water (2 × 45 mL), a saturated aqueous solution of sodium bicarbonate (2 × 45 mL), brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *ester* (±)-**38** (972 mg) as a clear oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 60/40 as eluent gave the pure *ester* (±)-**38** (0.91 g, 78%) as a clear oil; ν_{max}/cm^{-1} (film) 2969 (CH), 1733 (CO), 1604, 1495, 1454 (Ar), 1174 (C-O); δ_H (400 MHz) 1.17 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.30 [3H, d, *J* 7.0, C(4)H_3], 2.53 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H_2], 2.61 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.0, one of C(2)H_2], 3.23-3.32 [1H, sym. m, X of ABX, C(3)H], 4.08 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 7.17-7.34 (5H, m, ArH).

(±)-Ethyl 3-phenylpentanoate (±)-56[34]



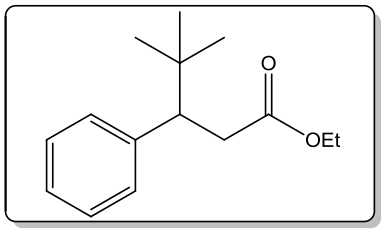
3-Phenylpentanoyl chloride **(±)-73** (1.96 g, 9.96 mmol) in dichloromethane (10 mL) was added dropwise to a solution of triethylamine (1.7 mL, 11.98 mmol), dichloromethane (10 mL) and distilled ethanol (1.5 mL, 24.83 mmol), at 0 °C. The reaction mixture was stirred at room temperature overnight. The crude product was dissolved in dichloromethane (30 mL) and washed with water (2 × 50 mL), aqueous hydrochloric acid (10%, 2 × 50 mL), brine (100 mL), dried, filtered and concentrated under reduced pressure to give the crude *ester* (±)-**56** (1.64 g) as a deep orange oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *ester* (±)-**56** (1.49 g, 72%) as a clear oil; ν_{max}/cm^{-1} (film) 2967 (CH), 1735 (CO), 1603, 1493, 1454 (Ar), 1167 (C-O); δ_H (400 MHz) 0.79 [3H, t, *J* 7.4, C(5)H_3], 1.13 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.55-1.76 [2H, m, C(4)H_2], 2.55 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H_2], 2.63 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.1, one of C(2)H_2], 2.96-3.04 [1H, m, X of ABX, C(3)H], 4.02 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 7.17-7.30 (5H, m, ArH).

(±)-Ethyl 4-methyl-3-phenylpentanoate (±)-58[35]



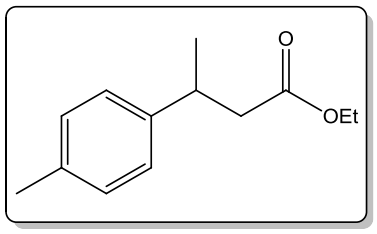
This was prepared following the procedure described for (±)-**56**, from 4-methyl-3-phenylpentanoyl chloride (±)-**74** (2.43 g, 11.52 mmol), triethylamine (1.9 mL, 13.82 mmol), dichloromethane (10 mL) and distilled ethanol (1.7 mL, 28.79 mmol) to yield the crude *ester* (±)-**58** (2.02 g) as a yellow - orange oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *ester* (±)-**58** (1.60 g, 63%) as a clear oil; ν_{max}/cm^{-1} (film) 2961 (CH), 1736 (CO), 1602, 1494, 1453 (Ar), 1162 (C-O); δ_H (400 MHz) 0.75, 0.95 [2 × 3H, 2 × d, *J* 6.7, *J* 6.7, C(4)HC***H*****$_3$** and C(5)H_3], 1.05 (3H, t, *J* 7.1, OCH_2C***H*****$_3$**) 1.79-1.91 [1H, sym m, C(4)H], 2.58 [1H, dd, A of ABX, J_{AB} 14.9, J_{AX} 9.9, one of C(2)H_2], 2.77 [1H, dd, B of ABX, J_{AB} 14.9, J_{BX} 5.6, one of C(2)H_2], 2.85-2.91 [1H, m, X of ABX, C(3)H], 3.95 (2H, q, *J* 7.1, OC***H*****$_2$**CH_3), 7.14-7.28 (5H, m, ArH).

(±)-Ethyl 4,4-dimethyl-3-phenylpentanoate (±)-59[36]



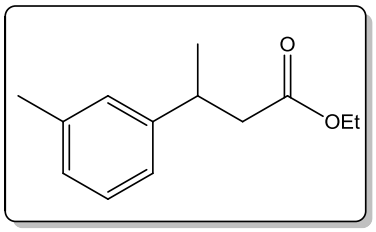
This was prepared following the procedure described for (±)-**56**, from 4,4-dimethyl-3-phenylpentanoyl chloride (±)-**75** (5.04 g, 22.44 mmol), triethylamine (3.8 mL, 26.93 mmol), dichloromethane (30 mL) and distilled ethanol (3.3 mL, 56.17 mmol) to yield the crude *ester* (±)-**59** (3.81 g) as a bright yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *ester* (±)-**59** (2.94 g, 56%) as pale yellow oil; ν_{max}/cm^{-1} (film) 2965 (CH), 1737 (CO), 1602, 1473, 1454 (Ar), 1152 (C-O); δ_H (400 MHz) 0.89 [9H, s, $C(CH_3)_3$], 0.99 (3H, t, *J* 7.1 OCH_2C***H*****$_3$**) 2.71 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 10.9, one of C(2)H_2], 2.79 [1H, dd, B of ABX, J_{AB} 15.2, J_{BX} 5.0, one of C(2)H_2], 2.98 [1H, dd, X of ABX, J_{AX} 10.9, J_{BX} 5.0, C(3)H], 3.85-3.97 (2H, m, OC***H*****$_2$**CH_3), 7.13-7.26 (5H, m, ArH).

(±)-Ethyl 3-(4-methylphenyl)butanoate (±)-49[37]



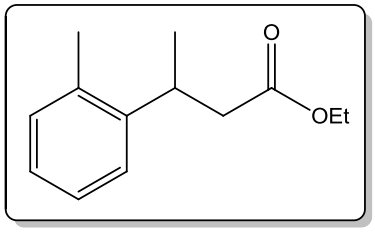
This was prepared following the procedure described for (±)-**56**, from 3-(4-methylphenyl)butanoyl chloride (±)-**78** (12.09 g, 61.47 mmol), triethylamine (10.3 mL, 73.76 mmol), dichloromethane (25 mL) and distilled ethanol (8.9 mL, 152.42 mmol) to yield the crude *ester* (±)-**49** (9.41 g) as a deep orange oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *ester* (±)-**49** (7.84 g, 62%) as a clear oil; ν_{max}/cm^{-1} (film) 2967 (CH), 1732 (CO), 1516, 1456 (Ar), 1166 (C-O); δ_H (400 MHz) 1.19 (3H, t, *J* 7.1, OCH_2C***H*****$_3$**), 1.28 [3H, d, *J* 7.0, C(4)H_3], 2.32 [3H, s, C(4')CH_3], 2.51 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H_2], 2.59 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.0, one of C(2)H_2], 3.20-3.29 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, *J* 7.2, OC***H*****$_2$**CH_3), 7.11 (4H, s, ArH).

(±)-Ethyl 3-(3-methylphenyl)butanoate (±)-54



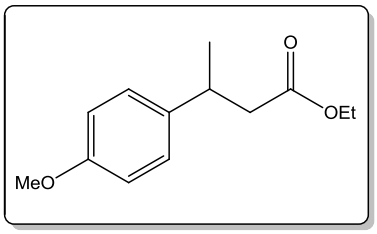
This was prepared following the procedure described for (±)-**56**, from 3-(3-methylphenyl)butanoyl chloride (±)-**79** (7.32 g, 37.20 mmol), triethylamine (6.2 mL, 44.64 mmol), dichloromethane (30 mL) and distilled ethanol (5.4 mL, 92.99 mmol) to yield the crude *ester* (±)-**54** (6.53 g) as a dark brown oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *ester* (±)-**54** (5.16 g, 67%) as a clear oil; (Found C, 75.13; H 8.77. $C_{13}H_{18}O_2$ requires C, 75.69; H, 8.80%); ν_{max}/cm^{-1} (film) 2968 (CH), 1733 (CO), 1608, 1490, 1461 (Ar), 1177 (C-O); δ_H (400 MHz) 1.18 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.28 [3H, d, *J* 7.0, C(4)H_3], 2.32 [3H, s, C(3')CH_3], 2.51 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.3, one of C(2)H_2], 2.59 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 6.9, one of C(2)H_2], 3.19-3.28 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 6.97-7.05 [3H, m, C(4')H, C(5')H and C(6')H, ArH], 7.13-7.19 [1H, m, C(2')H, ArH]; δ_C (75.5 MHz) 14.2 (CH_3, $OCH_2\boldsymbol{C}H_3$), 21.5 [CH_3, C(3')CH_3] 21.8 [CH_3, C(4)H_3], 36.5 [CH, C(3)H], 43.0 [CH_2, C(2)H_2], 60.3 [CH_2, $O\boldsymbol{C}H_2CH_3$], 123.7, 127.1, 127.6 [3 x CH, C(4')H, C(5')H and C(6')H, ArCH] 128.4 [CH, C(2')H, ArCH], 138.0, 145.8 (2 x C, ArC), 172.5 [C, C(1)]; HRMS (ES+): Exact mass calculated for $C_{13}H_{19}O_2$ $[M+H]^+$ 207.1385. Found 207.1390; m/z (ES+) 207.1 $[(M+H)^+$, 57%], 202.1 (100%), 161.1 $[(M-C_2H_5O)^+$, 60%], 151.0 (23%), 141.0 (7%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

(±)-Ethyl 3-(2-methylphenyl)butanoate (±)-53



This was prepared following the procedure described for (±)-**56**, from 3-(2-methylphenyl)butanoyl chloride (±)-**77** (4.68 g, 23.79 mmol), triethylamine (4.0 mL, 28.54 mmol), dichloromethane (20 mL) and distilled ethanol (3.5 mL, 59.46 mmol) to yield the crude *ester* (±)-**53** (2.85 g) as a yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the semi pure *ester* (±)-**53** (2.50 g, 67%) as a clear oil. Further purification by distillation gave the pure *ester* (±)-**53** (2.14 g, 44%) as a clear oil; b.p. 96 °C at 0.06 mmHg; (Found C, 75.43; H 8.67. $C_{13}H_{18}O_2$ requires C, 75.69; H, 8.80%); ν_{max}/cm^{-1} (film) 2975 (CH), 1733 (CO), 1605, 1492, 1462 (Ar), 1175 (C-O); δ_H (400 MHz) 1.17 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.25 [3H, d, *J* 6.9, C(4)H_3], 2.37 [3H, s, C(2')CH_3], 2.52 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 8.5, one of C(2)H_2], 2.61 [1H, dd, B of ABX, J_{AB} 15.2, J_{BX} 6.6, one of C(2)H_2], 3.46-3.61 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 7.07-7.19 (4H, m, ArH); δ_C (75.5 MHz) 14.2 (CH_3, $OCH_2\boldsymbol{C}H_3$) 19.5 [CH_3, C(2')CH_3], 21.3 [CH_3, C(4)H_3], 31.5 [CH, C(3)H], 42.2 [CH_2, C(2)H_2], 60.3 [CH_2, $O\boldsymbol{C}H_2CH_3$], 125.1, 126.1, 126.3, 130.4 (4 x CH, ArCH), 135.3, 143.9 (2 x C, ArC), 172.6 [C, C(1)]; HRMS (ES+): Exact mass calculated for $C_{13}H_{19}O_2$ $[M+H]^+$ 207.1385. Found 207.1393; m/z (ES+) 207.1 $[(M+H)^+$, 71%], 202.1 (100%), 161.1 $[(M-C_2H_5O)^+$, 48%], 151.0 (68%), 141.0 (24%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

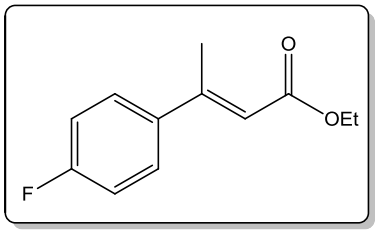
(±)-Ethyl 3-(4-methoxyphenyl)butanoate (±)-60[38]



This was prepared following the procedure described for (±)-**56**, from 3-(4-methoxyphenyl)butanoyl chloride (±)-**76** (8.49 g, 39.90 mmol), triethylamine (6.7 mL, 47.88 mmol), dichloromethane (50 mL) and distilled ethanol (5.8 mL, 99.75 mmol) to yield the crude *ester* (±)-**60** (6.68 g) as an orange oil. Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 0-10% ethyl acetate) gave the *pure* ester (±)-**60** (5.32 g, 56%) as a clear oil; ν_{max}/cm^{-1} (film) 2966 (CH), 1732 (CO), 1613, 1513, 1461 (Ar), 1174 (C-O); δ_H (400 MHz) 1.18 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.27 [3H, d, *J* 7.0, $C(4)H_3$], 2.50 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 8.0, one of $C(2)H_2$], 2.57 [1H, dd, B of ABX, J_{AB} 14.8, J_{BX} 7.2, one of $C(2)H_2$], 3.17-3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH_3), 4.07 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 6.81-6.85 [2H, m, C(3')H and C(5')H, ArH], 7.12-7.16 [2H, m, C(2')H and C(6')H, ArH].

5.3.1.4 Synthesis of fluorine substituted hydrolase substrates

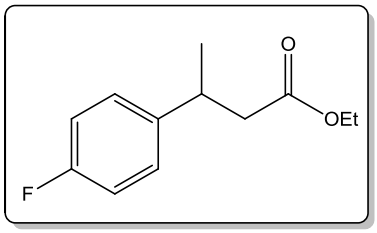
(*E*)-Ethyl 3-(4-fluorophenyl)but-2-enoate 81[39-41]



Sodium hydride (1.47 g of 60% dispersion in mineral oil, 36.75 mmol) was placed in a 3-necked round bottomed flask under an atmosphere of nitrogen. Following washing with hexane (3 × 20 mL), dry tetrahydrofuran (90 mL) was added and the resulting suspension was stirred for 10 min. A solution of triethyl phosphonoacetate (7.58 g, 33.81 mmol) in dry tetrahydrofuran (10 mL) was added dropwise over 30 min to the reaction mixture. The reaction mixture was stirred for 30 min, then a solution of 4-fluoroacetophenone **82** (4.00 g, 28.96 mmol) in dry tetrahydrofuran (25 mL) was added dropwise over 20 min. The mixture was refluxed while stirring for 24 h and then cooled with a water bath. A saturated aqueous ammonium chloride solution (20 mL) was added dropwise to the cold mixture. The aqueous phase was extracted with diethyl ether (4 × 100 mL), and the combined organic phase was washed with brine (3 × 50 mL), dried, filtered and concentrated under reduced pressure to give the crude *ester* **81** (6.69 g) as an orange oil in a 76 : 24 mixture of *E* : *Z* isomers. Purification by column chromatography on silica gel using hexane/ethyl acetate 95/5 as eluent gave the pure *E ester* **81** (3.67 g, 62%) as a clear oil; ν_{max}/cm^{-1} (film) 2982 (CH), 1714 (CO), 1632, 1602, 1510 (Ar), 1161 (C-O); δ_H (300 MHz) 1.31 (3H, t, *J* 7.2, $OCH_2C\boldsymbol{H_3}$), 2.55 [3H, d, *J* 1.2, $C(4)H_3$], 4.21 (2H, q, *J* 7.2, $OC\boldsymbol{H_2}CH_3$), 6.09 [1H, q, *J* 1.2, C(2)H], 7.00-7.08 [2H, m, C(3')H and C(5')H, ArH], 7.42-7.48 [2H, m, C(2')H and C(6')H, ArH].

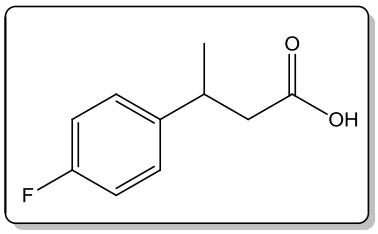
*Note: During purification by column chromatography of the crude ester **81** a fraction was isolated which contained 53 : 47 mixture of* E *:* Z *isomers. Spectral characterisation of the minor* Z *isomer of **81**; δ_H (300 MHz) 1.12 (3H, t,* J *7.1, $OCH_2C\mathbf{H_3}$), 2.16 [3H, d,* J *1.5, $C(4)H_3$], 4.01 (2H, q,* J *7.2, $OC\mathbf{H_2}CH_3$), 5.91 [1H, q,* J *1.5, C(2)H], 6.96-7.12 obscured by signals for the major* E *isomer [2H, m, C(3')H and C(5')H, ArH], 7.13-7.24 [2H, m, C(2')H and C(6')H, ArH].*

(±)-Ethyl 3-(4-fluorophenyl)butanoate (±)-55[40]



(*E*)-Ethyl 3-(4-fluorophenyl)but-2-enoate **81** (3.00 g, 14.41 mmol) in absolute ethanol (25 mL) was hydrogenated at 15 psi over palladium on activated carbon (10%, 0.70 g) for 15.5 h. The crude reaction mixture was filtered through Celite® with absolute ethanol as the eluent to remove the hydrogenation catalyst. The filtrate was evaporated under reduced pressure to produce the crude *ester* (±)-**55** (2.74 g, 91% yield) as a yellow oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 95/5 as eluent gave the pure *ester* (±)-**55** (2.34 g, 77%) as a clear oil; (Found C, 68.34; H 6.82. $C_{12}H_{15}FO_2$ requires C, 68.55; H, 7.19%); ν_{max}/cm^{-1} (film) 2970 (CH), 1733 (CO), 1605, 1512, 1462 (Ar), 1160 (C-O); δ_H (300 MHz) 1.17 (3H, t, *J* 7.2, $OCH_2C\boldsymbol{H_3}$), 1.28 [3H, d, *J* 7.2, $C(4)H_3$], 2.51 [1H, overlapping dd, A of ABX, J_{AB} 15.0, J_{AX} 7.8, one of $C(2)H_2$], 2.57 [1H, overlapping dd, B of ABX, J_{AB} 15.0, J_{AX} 7.5, one of $C(2)H_2$], 3.27 [1H, apparent sextet, X of ABX, *J* 7.2 C(3)H], 4.06 (2H, q, *J* 7.1 $OC\boldsymbol{H_2}CH_3$), 6.93-7.01 [2H, m, C(3')H and C(5')H, ArH], 7.14-7.21 [2H, m, C(2')H and C(6')H, ArH]; δ_C (75.5 MHz) 14.1 (CH_3, $OCH_2\boldsymbol{C}H_3$) 21.9 [CH_3, $C(4)H_3$], 35.8 [CH, C(3)H], 43.0 [CH_2, $C(2)H_2$], 60.2 [CH_2, $O\boldsymbol{C}H_2CH_3$], 115.2 [CH, d, $^2J_{CF}$ 21.2, C(3')H and C(5')H, ArCH], 128.2 [CH, d, $^3J_{CF}$ 7.8, C(2')H and C(6')H, ArCH], 141.3 [C, d, $^4J_{CF}$ 3.2, C(1'), ArC], 161.4 [C, d, $^1J_{CF}$ 243.9, C(4')F], 172.1 [C, C(1)]; HRMS (ES+): Exact mass calculated for $C_{12}H_{16}FO_2$ $[M+H]^+$ 211.1134 Found 211.1127; m/z (ES+) 211.3 [$(M+H)^+$, 45%], 236.1 (100%), 238.1 (53%), 111.3 (58%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

3-(4-Fluorophenyl)butanoic acid (±)-80[40,42,43]



Aqueous sodium hydroxide (1M, 20 mL) was added to ethyl 3-(4-fluorophenyl)butanoate (±)-**55** (0.52 g, 2.45 mmol) and the emulsion was stirred overnight at room temperature. The reaction mixture was extracted with diethyl ether (2 × 30 mL) and the ethereal solution was discarded. The aqueous phase was acidified to pH 1 with aqueous hydrochloric acid (10%) and then extracted with diethyl ether (3 × 30 mL) and the combined organic extracts were washed with brine (50 mL), dried, filtered and concentrated under reduced pressure to give the pure *acid* (±)-**80** (300 mg, 67%) as a white solid, m.p. 68-69 °C (Lit.,[42] 53-57 ºC); ν_{max}/cm^{-1} (KBr) 2979 (OH), 1705 (CO), 1607, 1511, 1432 (Ar); δ_H (300 MHz) 1.30 [3H, d, *J* 7.2, $C(4)H_3$], 2.57 [1H, overlapping dd, A of ABX, J_{AB} 15.6, J_{AX} 7.8, one of $C(2)H_2$], 2.64 [1H, overlapping dd, B of ABX, J_{AB} 15.6, J_{AX} 7.8, one of $C(2)H_2$], 3.27 [1H, apparent sextet, X of ABX, *J* 7.2 C(3)H], 6.95-7.03 [2H, m, C(3')H and C(5')H, ArH], 7.14-7.22 [2H, m, C(2')H and C(6')H, ArH].

5.3.2 Hydrolase-mediated kinetic resolution - analytical screens

General procedure for the hydrolase-mediated kinetic resolution of the β-substituted 3-aryl alkanoic ethyl esters (±)-38, 49, 53-56, 58-60

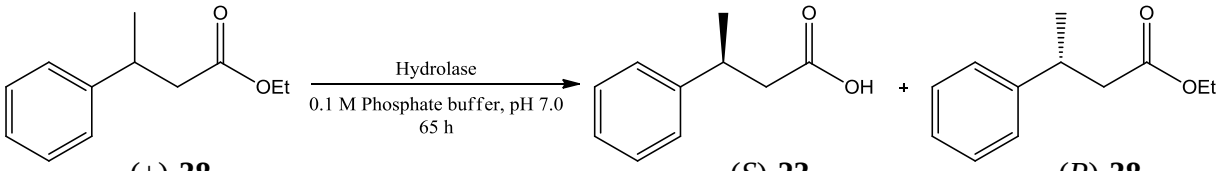
Procedure followed was kindly supplied by Almac Sciences[44]

A spatula tip of enzyme (~5-10 mg, amount not critical) was added to the ester substrate (±)-**38, 49, 53-56, 58-60** (~50 mg) in 0.1 M phosphate buffer, pH 7 (4.5 mL). Co-solvents (17% v/v) were added if required as indicated in Table 5.5. The small test tubes were sealed and agitated at 700-750 rpm and incubated for the appropriate length of time and temperature. The aqueous layer was extracted with diethyl ether (3 × 5 mL) and the combined organic extracts were filtered through Celite® and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screens are summarised in the Tables 5.3-5.12.

Note:

1. *Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.*
2. *Conversion (%) and enantiomeric ratio (*E*-value) was calculated from enantiomeric excess [ee (%)] of substrate ester (ee_s) and product acid (ee_P) employing the* E*-value calculator.*[45] *Values >200 should be interpreted with caution, due to the inaccuracies associated with the determination of enantiomeric excess [ee (%)] by chiral HPLC, GC, NMR; a small variation in the enantiomeric excess [ee (%)] of the substrate and or the product may result in a significant change in the* E*-value.*
3. *As stated in the general procedures when the second enantiomer is not observed enantiomeric excess [ee (%)] is stated as >98% ee.*
4. *In all cases spectroscopic characteristics were in agreement with previously reported data.*
5. *For conversions determined by ^{1}H NMR spectroscopy of the crude products to be <10%, chiral HPLC analysis was not conducted.*

*Table 5.3: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutanoa*te *(±)-**38***

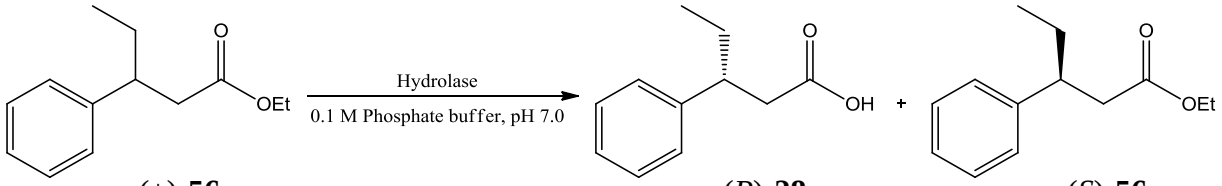


(±)-**38** (*S*)-**23** (*R*)-**38**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 38	Acid 23	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	30	11 (*S*)	58 (*R*)	16	15	4.2
2	*Candida cyclindracea* C2	Ambient	25 (*S*)	59 (*R*)	30	41	4.9
3	*Rhizopus oryzae*	30	-	-	-	<10	-
4	*Achromobacter spp.*	30	11 (*R*)	90 (*S*)	11	12	21
5	*Alcaligenes spp.* 1	30	27 (*R*)	95 (*S*)	22	24	50
6	*Pseudomonas cepacia* P1	30	>98 (*R*)	94 (*S*)	51	49	170
7	*Pseudomonas stutzeri*	Ambient	14 (*R*)	61 (*S*)	19	23	4.7
8	*Rhizopus spp.*	Ambient	-	-	-	<10	-
9	*Rhizopus niveus*	Ambient	-	-	-	<10	-
10	*Aspergillus niger*	Ambient	-	-	-	<10	-
11	*Alcaligenes spp.* 2	Ambient	98 (*R*)	97 (*S*)	50	54	>200
12	*Pseudomonas cepacia* P2	Ambient	96 (*R*)	75 (*S*)	56	59	26
13	*Mucor javanicus*	Ambient	-	-	-	<10	-
14	*Penicillium camembertii*	Ambient	-	-	-	<10	-
15	*Pseudomonas fluorescens*	30	>98 (*R*)	94 (*S*)	51	55	170
16	*Candida antarctica* B	Ambient	13 (*S*)	0 (*R*)	51	96	1.4
17	*Mucor meihei*	Ambient	3 (*S*)	24 (*R*)	11	<10	1.7
18	*Candida antarctica* A	Ambient	10 (*R*)	68 (*S*)	13	17	5.8
19	*Candida antarctica* B (immob)	Ambient	70 (*S*)	5 (*R*)	93	100	1.8
20	*Porcine pancrease* Type II	Ambient	15 (*R*)	93 (*S*)	14	19	31
21	*Porcine pancrease* Grade II	30	35 (*R*)	95 (*S*)	27	27	54
22	Pig liver esterase	Ambient	0[a]	0[a]	-[a]	100[a]	-[a]

a. Reaction went to 100% completion, no enantioselectivity observed.

*Table 5.4: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**56***



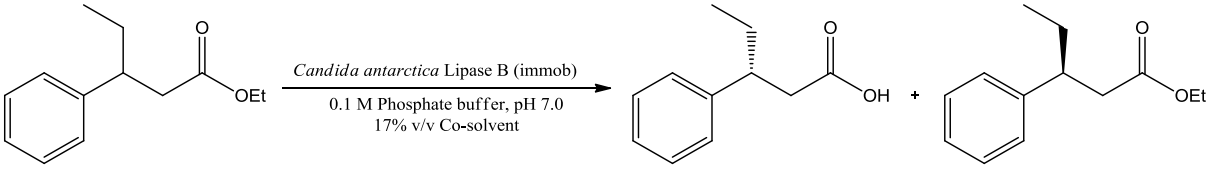
(±)-**56** (*R*)-**28** (*S*)-**56**

Entry	Enzyme source[a]	Time	Temp (°C)	ee (%)		Conversion (%)		*E* Value
				Ester **56**	Acid **28**	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C2	120 h	Ambient	1	15	6	33	1.4
2	*Candida antarctica* B	65 h	Ambient	80 (*S*)	23 (*R*)	78	93	3.4
3	*Mucor meihei*	67 h	Ambient	0[b]	0[b]	-[b]	100[b]	-[b]
4	*Candida antarctica* A	67 h	Ambient	5 (*R*)	44 (*S*)	10	22	2.7
5	Pig liver esterase	65 h	Ambient	87 (*S*)	15 (*R*)	85	88	3.1
6	*Candida antarctica* B (immob)	65 h	Ambient	85 (*S*)	81 (*R*)	51	93	25
7	*Candida antarctica* B (immob)	72 h	4	62 (*S*)	86 (*R*)	42	87	24

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes spp.* 2, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger* and *Mucor javanicus*.

b. Reaction went to 100% completion, no enantioselectivity observed.

Table 5.5: Investigation of co-solvent effect on Candida antarctica *lipase B (immob) hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-**56***



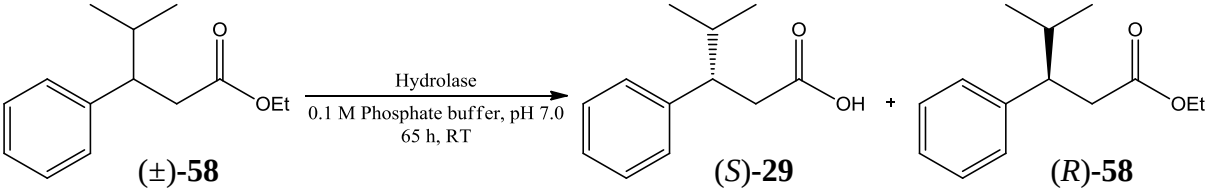
(±)-**56** (*R*)-**28** (*S*)-**56**

Entry	Co-solvent	Time	Temp (°C)	ee (%)		Conversion (%)		*E* value
				Ester (*S*)-**56**	Acid (*R*)-**28**	*E* calc.	^{1}H NMR	
1	DMSO	64 h	Ambient	93	81	53	80	31
2	Acetonitrile[a]	64 h	Ambient	28	93	23	84	36
3	Acetone[a]	64 h	Ambient	25	94	21	76	41
4	THF	64 h	Ambient	6	88	6	24	16
5	Dioxane	64 h	Ambient	72	92[b]	44	71	51
6	TBME[a]	64 h	Ambient	24	57	30	90	4.6

a. HPLC grade solvent.

b. On one occasion the enantiomeric excess [ee (%)] isolated from dioxane of (*R*)-**28** was 97% ee.

*Table 5.6: Hydrolase-mediated hydrolysis of (±)-ethyl 4-methyl-3-phenylpentanoate (±)-**58**.*



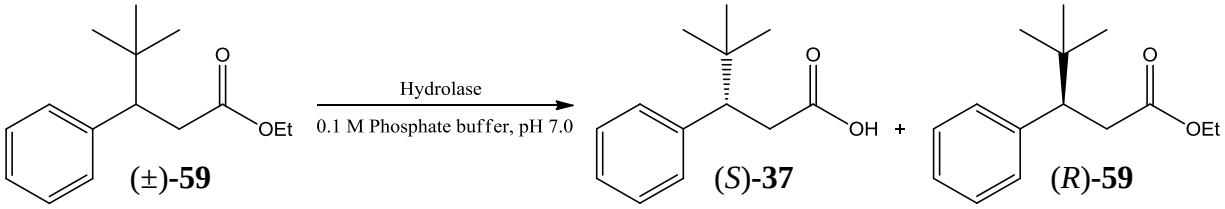
(±)-**58** (*S*)-**29** (*R*)-**58**

Entry	Enzyme source[a]	ee (%)		Conversion (%)		*E* value
		Ester 58	Acid 29	*E* calc.	^{1}H NMR	
1	*Candida antarctica* B	12 (*R*)	>98 (*S*)	11	24	>200
2	*Mucor meihei*	61 (*R*)	23 (*S*)	73	79	2.7
3	*Candida antarctica* A	10 (*S*)	64 (*R*)	14	31	5
4	*Candida antarctica* B (immob)	33 (*R*)	97 (*S*)	25	42	90
5	Pig liver esterase	-[b]	-[b]	-[b]	100	-[b]

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Alcaligenes spp.* 1, *Penicillium camembertii*, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Candida cyclindracea* C2, *Rhizopus spp*., *Pseudomonas stutzeri*, *Rhizopus niveus*, *Candida cyclindracea* C1, *Aspergillus niger*, *Alcaligenes spp.* 2 and *Mucor javanicus.*

b. Reaction went to 100% completion, HPLC analysis was not conducted

*Table 5.7: Hydrolase-mediated hydrolysis of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**59** at variable temperature.*



(±)-**59** (*S*)-**37** (*R*)-**59**

Entry	Enzyme source[a]	Temperature (°C)	ee (%)		Conversion (%)		*E* value
			Ester 59	Acid 37	*E* Calc.	^{1}H NMR	
1	*Candida antarctica* B	Ambient[b]	2 (*R*)	>98 (*S*)	2	<10	>200
2		35 – 40 °C[c]	23 (*R*)	>98 (*S*)	19	25	>200
3	*Candida antarctica* A	Ambient[b]	3 (*S*)	73 (*R*)	4	6	6.6
4		35 – 40 °C[d]	7 (*S*)	81 (*R*)	8	12	10
5	*Candida antarctica* B	Ambient[b]	1 (*R*)	>98 (*S*)	1	15	>200
6	(immob)	35 – 40 °C[c]	30 (*R*)	98 (*S*)	23	55	132
7	Pig liver esterase	Ambient[b]	32 (*S*)	34 (*R*)	48	51	2.7
8		35 – 40 °C[c]	0[e]	0[e]	-[e]	100[e]	-[e]

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P1, *Rhizopus niveus*, *Pseudomonas fluorescens*, *Candida cyclindracea* C1, *Pseudomonas cepacia* P2 and *Porcine Pancrease* Type II.

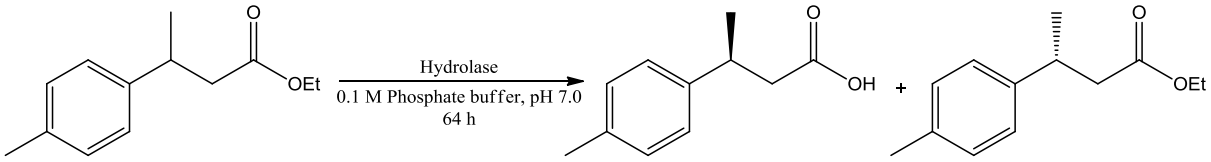
b. Time for ester hydrolysis was 66 h.

c. Time for ester hydrolysis was 64.5 h at 35 °C temperature increased to 40 °C for the final 24 h.

d. Time for ester hydrolysis was 72 h at 35 °C temperature increased to 40 °C for the final 24 h.

e. Reaction went to 100% completion, no enantioselectivity observed.

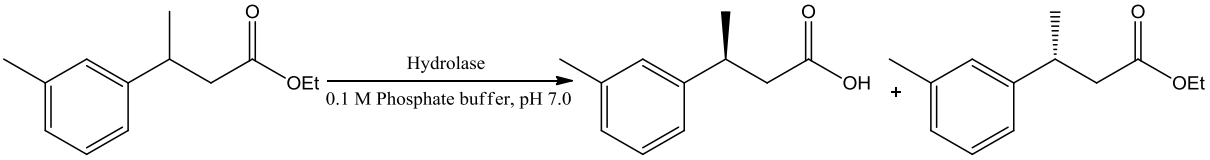
*Table 5.8: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methylphenyl)butanoate (±)-**49***



(±)-**49** (*S*)-**51** (*R*)-**49**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 49	Acid 51	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	98 (*R*)	>98 (*S*)	50	62	>200
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	96 (*S*)	51	51	>200
3	*Pseudomonas fluorescens*	30	>98 (*R*)	95 (*S*)	51	58	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* A	30	5 (*R*)	68 (*S*)	7	19	5.5
6	*Candida antarctica* B (immob)	30	6 (*S*)	5 (*R*)	55	43	1.2

*Table 5.9: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(3-methylphenyl)butanoate (±)-**54***

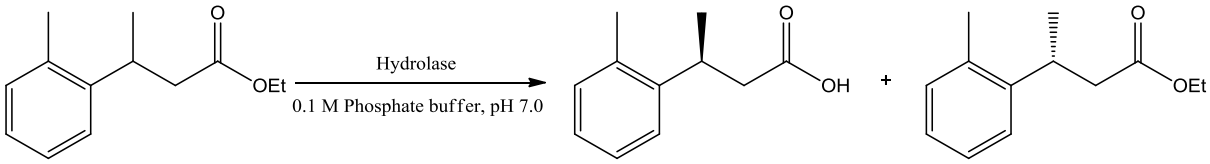


(±)-**54** (*S*)-**65** (*R*)-**54**

Entry	Enzyme source[a]	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 54	Acid 65	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	88 (*R*)	96 (*S*)	48	53	143
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	76 (*S*)	57	57	52
3	*Pseudomonas fluorescens*	30	96 (*R*)	97 (*S*)	50	69	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* B (immob)	30	>98 (*S*)	7 (*R*)	93	100	4.7

a. Time for ester hydrolysis was 65 h with the exception of *Candida cyclindracea* catalysed hydrolysis which was 64 h.

*Table 5.10: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-**53***

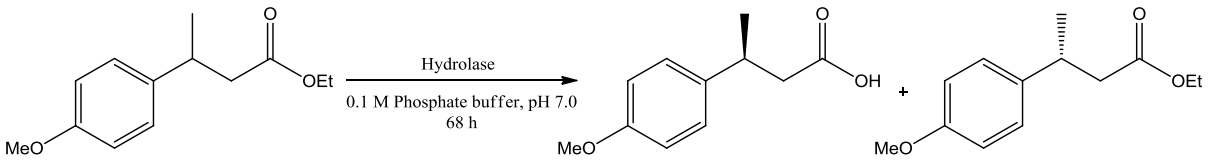


(±)-**53** (*S*)-**64** (*R*)-**53**

Entry	Enzyme source[a]	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 53	Acid 64	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	>98 (*R*)	>98 (*S*)	50	57	>200
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	80 (*S*)	56	53	65
3	*Pseudomonas fluorescens*	30	>98 (*R*)	>98 (*S*)	50	61	>200
4	*Candida cyclindracea*	30	-	-	-	<10	-
5	*Candida antarctica* B (immob)	30	90 (*R*)	46 (*S*)	66	84	7.7

a. Time for ester hydrolysis was 67 h with the exception of *Pseudomonas fluorescens* catalysed hydrolysis which was 64 h.

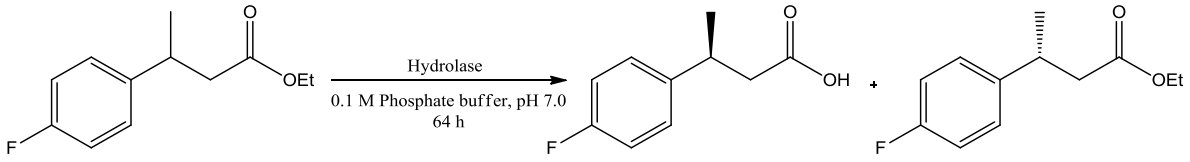
*Table 5.11: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-**60***



(±)-**60** (*S*)-**66** (*R*)-**60**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 60	Acid 66	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	98 (*R*)	86 (*S*)	53	48	60
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	88 (*S*)	53	54	81
3	*Pseudomonas fluorescens*	30	>98 (*R*)	97 (*S*)	51	57	>200
4	*Candida antarctica* A	30	4 (*R*)	48 (*S*)	8	9	3
5	*Candida cyclindracea*	30	-	-	-	<10	-
6	*Candida antarctica* B (immob)	30	66 (*S*)	7 (*R*)	90	96	1.9

*Table 5.12: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-**55***

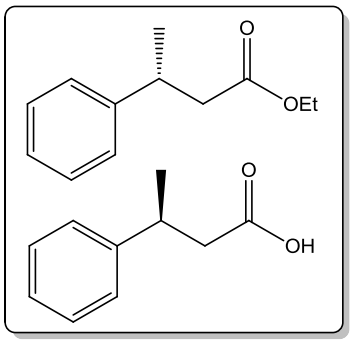


(±)-**55** (*S*)-**80** (*R*)-**55**

Entry	Enzyme source	Temp (°C)	ee (%)		Conversion (%)		*E* value
			Ester 55	Acid 80	*E* calc.	^{1}H NMR	
1	*Pseudomonas cepacia* P1	30	>98 (*R*)	84 (*S*)	54	55	59
2	*Pseudomonas cepacia* P2	30	>98 (*R*)	69 (*S*)	84	84	27
3	*Pseudomonas fluorescens*	30	>98 (*R*)	94 (*S*)	62	62	170
4	*Candida cyclindracea*	30	3 (*S*)	25 (*R*)	11	<10	1.7
5	*Candida antarctica* B (immob)	30	>98 (*S*)	8 (*R*)	92	94	3.4

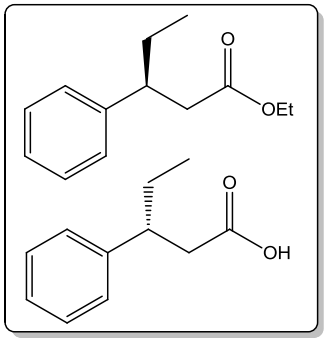
5.3.3 Hydrolase-mediated kinetic resolution – preparative-scale

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-38



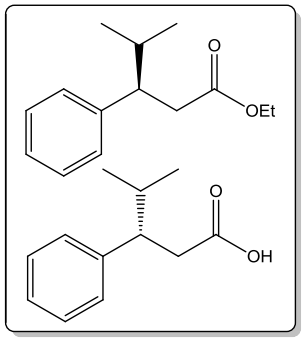
Pseudomonas fluorescens (108 mg) was added to ethyl 3-phenylbutanoate (±)-**38** (510 mg, 2.65 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL) and this was shaken at 750 rpm for 64 h at 30 ºC. The solution was filtered through a pad of Celite® and the hydrolase washed with water (2 × 20 mL) and ethyl acetate (10 × 10 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 30 mL) and then acidified with aqueous hydrochloric solution (10%) and extracted with a further (3 × 30 mL) ethyl acetate. The combined organic layers were washed with brine (1 × 100 mL) dried, filtered and concentrated under reduced pressure to produce a clear oil (395 mg). Conversion estimated by *E*-value calculator at 50%.[45] Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**38** (178 mg, 35%) as a clear oil $[\alpha]_D^{20}$ −27.55 (c 1.1, $CHCl_3$), >98% ee, lit[46] $[\alpha]_D^{25}$ +19.00 (c 1.1, $CHCl_3$), (*S*)-isomer, 90% ee and pure *acid* (*S*)-**23** (147 mg, 34%) as a clear oil $[\alpha]_D^{20}$ +27.90 (c 1.0, EtOH), 98% ee, lit[43] $[\alpha]_D^{25}$ +24.50 (c 1.0, EtOH), 97% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-56



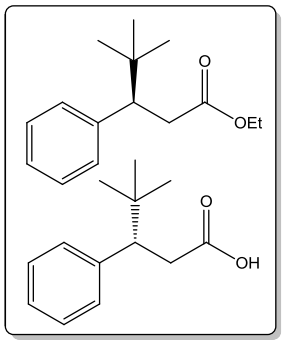
Candida antarctica lipase B (immob) (410 mg) was added to ethyl 3-phenylpentanoate (±)-**56** (408 mg, 1.98 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL) and dioxane (17% v/v, 4 mL). The reaction mixture was shaken at 750 rpm for 62 h at 30 ºC, the solution was filtered through a pad of Celite® and the hydrolase washed with water (2 × 20 mL) and heptane (10 × 10 mL). The layers were separated and the aqueous layer was extracted with heptane (3 × 30 mL). The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the pure *ester* (*S*)-**56** (79.2 mg, 19%) as a light yellow oil. $[\alpha]_D^{20}$ +9.46 (c 0.6, $CHCl_3$), 65% ee, lit[47] $[\alpha]_D^{26}$ −18.3 (c 1.1, $CHCl_3$), (*R*)-isomer, 97% ee. The aqueous layer was acidified with aqueous hydrochloric solution (10%) and extracted with (3 × 30 mL) ethyl acetate. The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the pure *acid* (*R*)-**28** (77.6 mg, 22%) as a yellow oil. $[\alpha]_D^{20}$ −33.73 (c 1.4, C_6H_6), 90% ee, lit[48] $[\alpha]_D^{25}$ +42.3 (c 8.0, C_6H_6), (*S*)-isomer, 83% ee. Conversion estimated by *E*-value calculator at 42%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 4-methyl-3-phenylpentanoate (±)-58



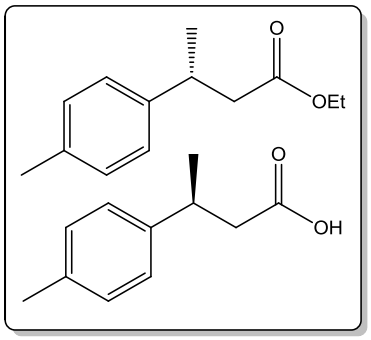
This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**56** from *Candida antarctica* lipase B (immob) (426 mg) and ethyl 4-methyl-3-phenylpentanoate (±)-**58** (428 mg, 1.94 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 63 h at 30 ºC to produce the pure *ester* (*R*)-**58** (107 mg, 25%) as a clear oil $[\alpha]_D^{20}$ +7.05 (c 1.0, $CHCl_3$), 26% ee, lit[30] $[\alpha]_D^{26}$ −25.4 (c 1.0, $CHCl_3$), (*S*)-isomer, 98% ee, and the pure *acid* (*S*)-**29** (88 mg, 24%) as a clear oil $[\alpha]_D^{20}$ −24.35 (c 0.655, $CHCl_3$), 98% ee, lit[49] $[\alpha]_D^{23}$ +28.12 (c 1.855, $CHCl_3$), (*R*)-isomer, 96% ee. Conversion estimated by *E*-value calculator at 21%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 4,4-dimethyl-3-phenylpentanoate (±)-59



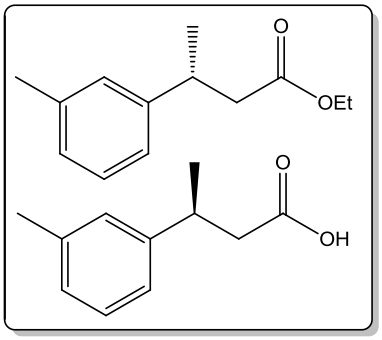
This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**56** from *Candida antarctica* lipase B (immob) (200 mg) and ethyl 4,4-dimethyl-3-phenylpentanoate (±)-**59** (200 mg, 0.85 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for ~65 h at 35 ºC and at 40 ºC for the final 24 h to produce the pure *ester* (*R*)-**59** (79 mg, 39%) as a clear oil. $[\alpha]_D^{20}$ +0.80 (c 1.0, $CHCl_3$), 12% ee and the pure *acid* (*S*)-**37** (23 mg, 13%) as a yellow oil which solidified overnight $[\alpha]_D^{20}$ −10.53 (c 0.1, $CHCl_3$), >98% ee, lit[50] $[\alpha]_D^{20}$ −20.4 (c 2.2, $CHCl_3$), 91% ee. Conversion estimated by *E*-value calculator at 11%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methylphenyl)butanoate (±)-49



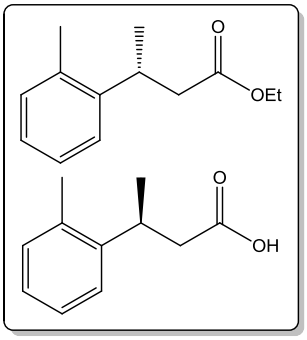
This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas cepacia* P1 (95 mg) and ethyl 3-(4-methylphenyl)butanoate (±)-**49** (446 mg, 2.26 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm at 30 ºC. An aliquot of reaction mixture (1 mL) was withdrawn at 62 h. Following a mini work-up chiral HPLC analysis was conducted. Conversion estimated by *E*-value calculator at 49%.[45] The reaction mixture was filtered at 62 h to produce a yellow oil (361 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**49** (145 mg, 31%) as a clear oil $[\alpha]_D^{20}$ −28.67 (c 3.5, $CHCl_3$), 97% ee, lit[37] $[\alpha]_D^{25}$ −26.2 (c 3.5, $CHCl_3$), 92% ee and the pure *acid* (*S*)-**51** (163 mg, 40%) as a yellow oil which solidified overnight $[\alpha]_D^{20}$ +31.80 (c 1.0, $CHCl_3$), >98% ee, lit[37] $[\alpha]_D^{25}$ +34.2 (c 1.0, $CHCl_3$), 99% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of ethyl 3-(3-methylphenyl)butanoate (±)-54



This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas fluorescens* (94 mg) and ethyl 3-(3-methylphenyl)butanoate (±)-**54** (471 mg, 2.28 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 62 h at 30 ºC to produce a yellow oil (232 mg). Conversion estimated by *E*-value calculator at 49%.[45] Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**54** (105 mg, 22%) as a yellow oil $[\alpha]_D^{20}$ −24.40 (c 1.0, $CHCl_3$), 94% ee and acid (*S*)-**65** (107 mg, 26%) as a clear oil $[\alpha]_D^{20}$ +32.32 (c 0.6, $CHCl_3$), >98% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of ethyl 3-(2-methylphenyl)butanoate (±)-53



This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas fluorescens* (74 mg) and ethyl 3-(2-methylphenyl)butanoate (±)-**53** (371 mg, 1.80 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 66 h at 30 ºC to produce a yellow oil (268 mg). Conversion estimated by *E*-value calculator at 50%.[45] Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**54** (100 mg, 27%) as a clear oil $[\alpha]_D^{20}$ −11.00 (c 1.0, $CHCl_3$), 98% ee and the pure *acid* (*S*)-**65** (90 mg, 28%) as a yellow oil $[\alpha]_D^{20}$ +24.17 (c 1.4, $CHCl_3$), >98% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

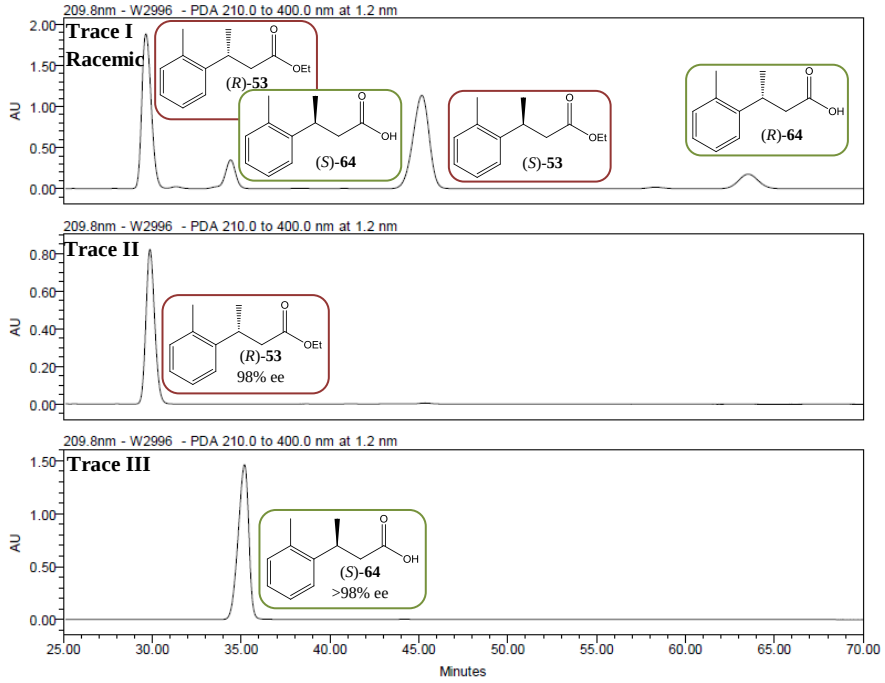
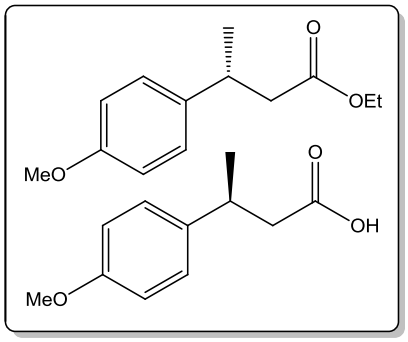


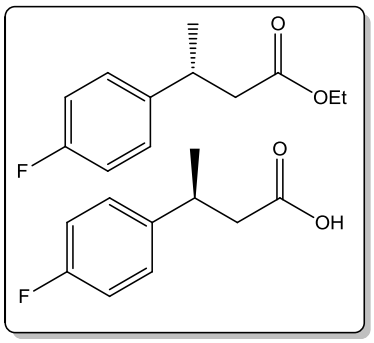
Figure5.7: HPLC ***Trace I****: A racemic mixture of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-****53*** *and (±)-3-(2-methylphenyl)butanoic acid (±)-****64****.* ***Trace II****: (R)-Ethyl 3-(2-methylphenyl)butanoate (R)-****53****, 98% ee, from the preparative-scale enzymatic resolution (see section 3.5.2).* ***Trace III****: (S)-3-(2-Methylphenyl)butanoic acid (S)-****64****, >98% ee, from the preparative-scale enzymatic resolution (see section 3.5.2). For HPLC conditions see appendix I.*

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-60



This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas fluorescens* (100 mg) and ethyl 3-(4-methoxyphenyl)butanoate (±)-**60** (498 mg, 2.24 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 64 h at 30 ºC to produce an orange oil (428 mg). Conversion estimated by *E*-value calculator at 51%.[45] Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**60** (212 mg, 43%) as a clear oil $[\alpha]_D^{20}$ −30.03 (c 1.0, $CHCl_3$), >98% ee and the pure *acid* (*S*)-**66** (99 mg, 23%) as a yellow oil $[\alpha]_D^{20}$ +26.25 (c 1.0, EtOH), 97% ee, lit[43] $[\alpha]_D^{25}$ +27.50 (c 1.0, EtOH) 94% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-55



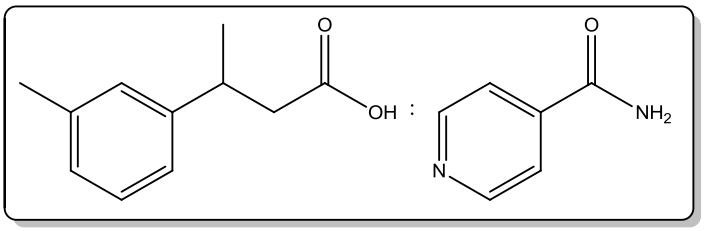
This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas fluorescens* (45 mg) and ethyl 3-(4-fluorophenyl)butanoate (±)-**55** (221 mg, 1.05 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 64 h at 30 ºC to produce a yellow oil (171 mg). Conversion estimated by *E*-value calculator at 51%.[45] Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 10-40% ethyl acetate) gave the pure *ester* (*R*)-**55** (71 mg, 32%) as a clear oil $[\alpha]_D^{20}$ −24.34 (c 1.0, $CHCl_3$), >98% ee, and the pure *acid* (*S*)-**80** (67 mg, 35%) as a brown oil $[\alpha]_D^{20}$ +30.51 (c 1.0, $CHCl_3$), 97% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

5.3.4 Determination of absolute stereochemistry

5.3.4.1 Synthesis of isonicotinamide co-crystals

Grinding and crystal growth experiments, PXRD and single crystal X-ray analysis was conducted in partnership with Eccles.[51,52]

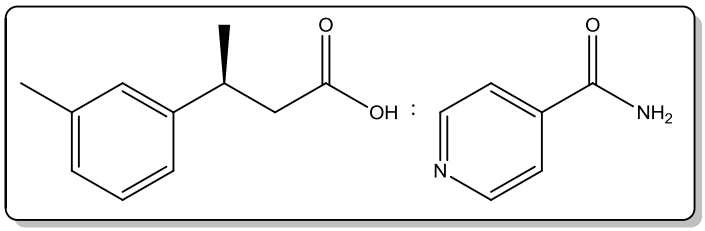
(±)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96



(±)-3-(3-Methylphenyl) butanoic acid (±)-**65** (26 mg, 0.15 mmol) and isonicotinamide **93** (17 mg, 0.14 mmol) were placed in the grinding jars and the mill was operated at 30 Hz for 30 min. The material obtained was analyzed by PXRD. It was then dissolved in a minimum amount of acetonitrile. Acetone was added until the solvent ratio was 70 : 30 acetonitrile : acetone. The solution was allowed to stand at ambient conditions and crystals suitable for single crystal diffraction were obtained by slow evaporation over 3-4 days. After single crystal analysis, chiral HPLC analysis was performed on the co-crystal used in the diffraction experiment; mp 71-73 °C. Crystal data: $C_{17}H_{20}N_2O_3$, M = 300.35, triclinic, a = 5.5847(9) Å, b = 7.8959(12) Å, c = 17.784(3) Å, α = 84.990(4)°, β = 85.314(4)°, γ = 83.194(4)°, V = 773.7(2) Å^3, T = 100.(2) K, space group *P*-1, Z = 2, 16279 reflections measured, 2987 unique (R_{int} = 0.0600). The final R_1 values were 0.0656 ($I > 2\sigma(I)$) and 0.0868 (all data). The final $wR(F^2)$ values were 0.1833 ($I > 2\sigma(I)$) and 0.1980 (all data). Full details are given on the accompanying CD.

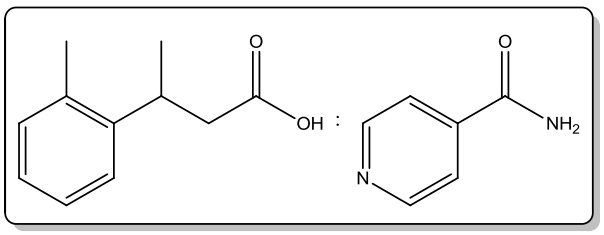
*Note: That spontaneous resolution occurs for some of the sample during grinding and thus there is also a mixture of enantiopure (*S*)-**65** and (*R*)-**65** present, as evidenced by the DSC and PXRD data.*

(*S*)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (*S*)-96



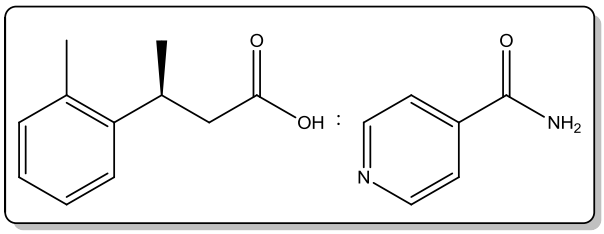
Isonicotinamide **93** (8 mg, 0.07 mmol) and (*S*)-3-(3 methylphenyl) butanoic acid (*S*)-**65** (11 mg, 0.06 mmol) were dissolved in a minimum amount of acetonitrile. Acetone was added until the solvent ratio was 70 : 30 acetonitrile : acetone. The solution was allowed stand at ambient conditions and crystals suitable for single crystal diffraction were obtained by slow evaporation over 3-4 days. After single crystal analysis, chiral HPLC analysis was performed on the co-crystal used in the diffraction experiment; m.p. 89-91°C. Crystal data: $C_{17}H_{20}N_2O_3$, M = 300.35, triclinic, a = 6.0360(2) Å, b = 9.4615(4) Å, c = 14.6013(5) Å, α = 83.438(2)°, β = 84.291(2)°, γ = 71.750(2)°, V = 784.95(5) Å^3, T = 100.(2) K, space group P1, Z = 2, 17372 reflections measured, 4835 unique (R_{int} = 0.0361). The final R_i values were 0.0317 ($I > 2\sigma(I)$) and 0.0323 (all data). The final $wR(F^2)$ values were 0.0831 ($I > 2\sigma(I)$) and 0.0840 (all data). Flack parameter = 0.06(14), Hooft y parameter = 0.02(8). Full details are given on the accompanying CD. Found C, 68.45; H, 6.80; N, 9.06, $C_{17}H_{20}N_2O_3$ requires C, 67.98; H, 6.71; N, 9.33%.

(±)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95



This was prepared following the procedure described for (±)-**96** from (±)-3-(2-methyl phenyl)butanoic acid (±)-**64** (40 mg, 0.22 mmol) and isonicotinamide **93** (27 mg, 0.22 mmol), m.p. 84-86 °C. Crystal data: $C_{17}H_{20}N_2O_3$, M = 300.35, triclinic, a = 9.6495(16) Å , b = 12.915(2) Å , c = 13.390(2) Å, α = 102.007(3)°, β = 103.616(4)°, γ = 92.047(4)°, V = 1580.0(4)Å^3, T = 100.(2) K, space group P-1, Z = 4, 43120 reflections measured, 7818 unique (R_{int} = 0.0647). The final R_1 values were 0.0402 ($I > 2\sigma(I)$) and 0.0570 (all data). The final $wR(F^2)$ values were 0.1009 ($I > 2\sigma(I)$) and 0.1070 (all data). Full details are given on the accompanying CD.

(*S*)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (*S*)-95



This was prepared following the procedure described for (*S*)-**96** from (*S*)-3-(2-methyl phenyl)butanoic acid (*S*)-**64** (7 mg, 0.05 mmol) and isonicotinamide **93** (5 mg, 0.06 mmol), m.p. 89-91 °C. Crystal data: $C_{17}H_{20}N_2O_3$, M = 300.35, triclinic, a = 7.1279(4) Å, b = 8.8202(5) Å, c = 13.1405(8) Å, α = 102.542(3)º, β = 92.659(3)º, γ = 95.430(3)º, V = 800.90(8) Å^3, T = 100.(2) K, space group P1, Z = 2, 18797 reflections measured, 5028 unique (R_{int} = 0.0238). The final R_1 values were 0.0278 ($I > 2\sigma(I)$) and 0.0285 (all data). The final $wR(F^2)$ values were 0.0773 ($I > 2\sigma(I)$) and 0.0781 (all data). Flack parameter = -0.10(9), Hooft y parameter = 0.05(6). Full details are given on the accompanying CD.

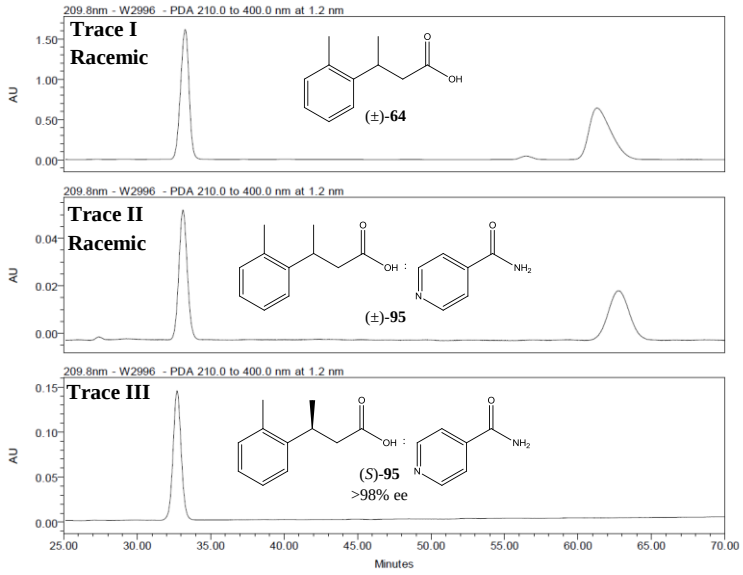
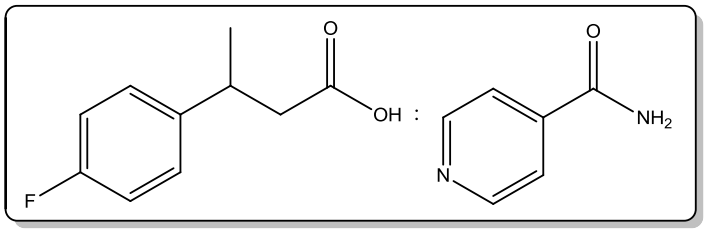


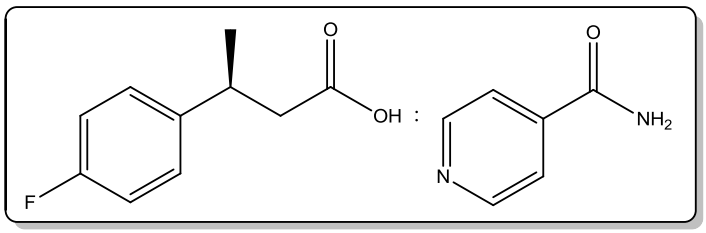
Figure 5.8: HPLC ***Trace I:*** *Racemic (±)-3-(2-methylphenyl)butanoic acid (±)-**64**.* ***Trace II:*** *Racemic (±)-3-(2-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-**95**.* ***Trace III:*** *(S)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (S)-**95** >98% ee. For HPLC conditions see appendix I.*

(±)-3-(4-Fluorophenyl)butanoic acid : isonicotinamide co-crystal (±)-97



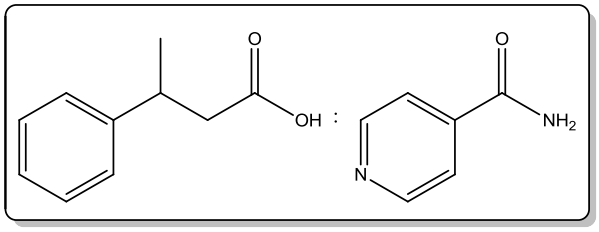
This was prepared following the procedure described for (±)-**96** from isonicotinamide **93** (11 mg, 0.09 mmol) and (±)-3-(4-fluorophenyl) butanoic acid (±)-**80** (16 mg, 0.09 mmol), m.p. 100-102 °C. Crystal data: $C_{16}H_{17}FN_2O_3$, M = 304.32, monoclinic, a = 13.397(5) Å, b = 9.956(3) Å, c = 12.273(4) Å, β = 110.812(8)°, V = 1530.2(9) Å^3, T = 100.(2) K, space group $P2_1/c$, Z = 4, 14546 reflections measured, 2660 unique (R_{int} = 0.0594). The final R_1 values were 0.0615 ($I > 2\sigma(I)$) and 0.0830 (all data). The final $wR(F^2)$ values were 0.1601 ($I > 2\sigma(I)$) and 0.1842 (all data). Full details are given on the accompanying CD. Found C, 62.99; H, 5.67; N, 9.08, $C_{16}H_{17}FN_2O_3$ requires C, 63.15; H, 5.63; N, 9.21%.

(*S*)-3-(4-Fluorophenyl)butanoic acid : isonicotinamide co-crystal (*S*)-97



This was prepared following the procedure described for (*S*)-**96** from isonicotinamide **93** (5 mg, 0.04 mmol) and (*S*)-3-(4-fluoro phenyl) butanoic acid (*S*)-**80** (8 mg, 0.04 mmol), m.p. 91-93 °C. Crystal data: $C_{16}H_{17}FN_2O_3$, M = 304.32, a = 8.8898(4) Å, b = 12.0610(5) Å, c = 15.2167(7) Å, α = 72.416(2)°, β = 81.661(3)°, γ = 75.268(3)°, V = 1500.05(11) Å^3, T = 100.(2) K, space group $P1$, Z = 4, 23873 reflections measured, 8800 unique (R_{int} = 0.0334). The final R_1 values were 0.0356 ($I > 2\sigma(I)$) and 0.0398 (all data). The final $wR(F^2)$ values were 0.0890 ($I > 2\sigma(I)$) and 0.0928 (all data). Flack parameter = -0.10(9), Hooft y parameter = -0.01(6). Full details are given on the accompanying CD.

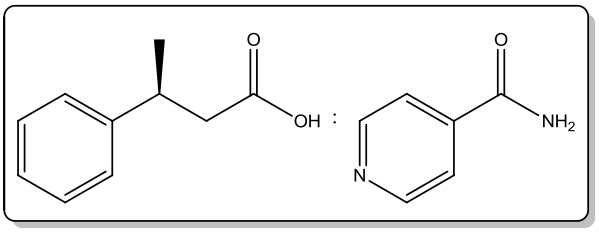
(±)-3-Phenylbutanoic acid : isonicotinamide co-crystal (±)-94



This was prepared following the procedure described for (±)-**96** from isonicotinamide **93** (17 mg, 0.14 mmol) and (±)-3-phenylbutanoic acid **(±)-23** (24 mg, 0.15 mmol), m.p. 84-86 °C. Crystal data: $C_{16}H_{18}N_2O_3$, M = 286.32, triclinic, a = 9.684(2) Å, b = 12.086(3) Å, c = 13.299(3) Å, α = 81.860(6)°, β = 82.501(5)°, γ = 74.533(4)°, V = 1478.0(6) Å^3, T = 100.(2) K, space group P-1, Z = 4, 30199 reflections measured, 5266 unique (R_{int} = 0.0390). The final R_1 values were 0.0544 ($I > 2\sigma(I)$) and 0.0683 (all data). The final $wR(F^2)$ values were 0.1340 ($I > 2\sigma(I)$) and 0.1450 (all data). Full details are given on the accompanying CD.

*Note: The crystal of **(±)-94** was disordered and only of sufficient quality to confirm its identity.*

(*S*)-3-Phenylbutanoic acid : isonicotinamide co-crystal (*S*)-94

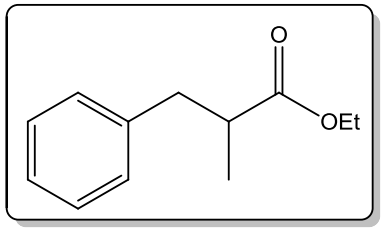


This was prepared following the procedure described for (*S*)-**140** from isonicotinamide **93** (7 mg, 0.06 mmol) and (*S*)-3-phenylbutanoic acid (*S*)-**23** (9 mg, 0.05 mmol), m.p. 78-80 °C. Crystal data: $C_{16}H_{18}N_2O_3$, M = 286.32, triclinic, a = 5.9580(2) Å, b = 9.2476(3) Å, c = 14.1993(5) Å, α = 91.391(2)°, β = 97.183(2)°, γ = 107.512(2)°, V = 738.64(4) Å^3, T = 100.(2)K, space group P1, Z = 2, 16675 reflections measured, 4575 unique (R_{int} = 0.0270). The final R_1 values were 0.0292 ($I > 2\sigma(I)$) and 0.0297 (all data). The final $wR(F^2)$ values were 0.0833 ($I > 2\sigma(I)$) and 0.0837 (all data). Flack parameter = 0.01(11). Hooft y parameter, as calculated by Platon = 0.08(6). Full details are given on the accompanying CD. Found C, 67.34; H, 6.40; N, 9.77, $C_{16}H_{18}N_2O_3$ requires C, 67.12; H, 6.34; N, 9.78%.

5.4 Kinetic resolutions of α-substituted 3-aryl alkanoic acids

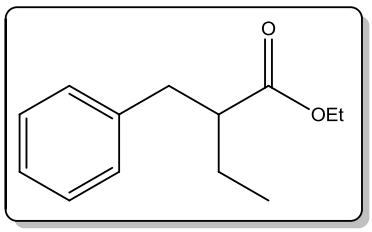
5.4.1 Synthesis of hydrolase substrates

(±)-Ethyl 2-methyl-3-phenylpropanoate (±)-47[53]



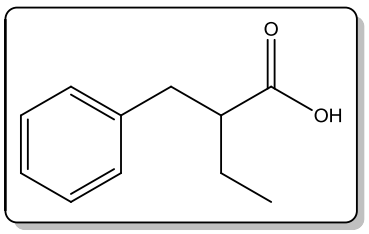
This was prepared following the procedure described for (±)-**38** from sulfuric acid (conc. 95-97%, 2.1 mL, 39.4 mmol), 2-methyl-3-phenylpropanoic acid (±)-**32** (2.21 g, 13.46 mmol) and absolute ethanol (40 mL) to give the crude *ester* (±)-**47** (1.67 g) as a clear oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 60/40 as eluent gave the pure *ester* (±)-**47** (1.62 g, 63%) as a clear oil; ν_{max}/cm^{-1} (film) 2979 (CH), 1733 (CO), 1605, 1496, 1455 (Ar), 1176 (C-O); δ_H (400 MHz) 1.15 [3H, d, J 6.8, C(2)CH_3], 1.18 (3H, t, J 7.2, $OCH_2C\boldsymbol{H_3}$), 2.63-2.76 (2H, m, AB of ABX, $C\boldsymbol{H_2}$Ph), 3.01 [1H, dd, X of ABX, J_{AX} 6.4, J_{BX} 12.8, C(2)H], 4.08 (2H, q, J 7.2, $OC\boldsymbol{H_2}CH_3$), 7.15–7.21 [3H, m, C(3')H, C(4')H and C(5')H, ArH], 7.25-7.28 [2H, m, C(2')H and C(6')H, ArH].

(±)-Ethyl 2-benzylbutanoate (±)-48[54]



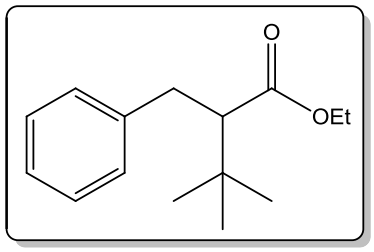
n-Butyllithium (1.9 M in hexanes, 27 mL, 51.45 mmol) was added dropwise to freshly distilled diisopropylamine (8.5 mL, 60.65 mmol) in freshly distilled tetrahydrofuran (40 mL) at −78 ºC under an atmosphere of nitrogen. Once the addition was complete the reaction mixture was warmed to −35 ºC. Ethyl butyrate **83** (6.5 mL, 49.13 mmol) in tetrahydrofuran (35 mL) was then added dropwise to the solution and once addition was complete the reaction mixture was stirred for 1.5 h at −35 °C. Benzyl bromide (6.4 mL, 53.81 mmol) was then added in one portion. The reaction mixture was stirred overnight at −35 °C. The reaction was quenched by pouring the mixture onto aqueous hydrochloric acid (10%, 400 mL) and diethyl ether (200 mL). The layers were separated and the aqueous layer extracted with diethyl ether (2 × 100 mL). The combined organic layer was washed with water (100 mL), brine (100 mL), dried, filtered and concentrated under reduced pressure to give the crude *ester* (±)-**48** (10.14 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure *ester* (±)-**48** (5.08 g, 50%) as a clear oil; (Found C, 74.80; H 8.73. $C_{13}H_{18}O_2$ requires C, 75.69; H, 8.80%); ν_{max}/cm^{-1} (film) 2966 (CH), 1732 (CO), 1605, 1496, 1456 (Ar), 1163 (C-O); δ_H (300 MHz) 0.92 [3H, t, *J* 7.4, C(4)H_3], 1.15 (3H, t, *J* 7.2, $OCH_2C\boldsymbol{H_3}$), 1.44-1.77 [2H, m, C(3)H_2], 2.53-2.62 [1H, m, X of ABX, C(2)H], 2.74 [1H, dd, A of ABX, J_{AB} 13.5, J_{AX} 6.6, one of $C\boldsymbol{H_2}Ph$], 2.93 [1H, dd, B of ABX, J_{AB} 13.5, J_{BX} 8.4, one of $C\boldsymbol{H_2}Ph$], 4.06 (2H, q, *J* 7.1, $OC\boldsymbol{H_2}CH_3$), 7.15-7.29 (5H, m, ArH); δ_C (75.5 MHz) 11.7 [CH_3, C(4)H_3],14.2 (CH_3, $OCH_2\boldsymbol{C}H_3$), 25.2 [CH_2, C(3)H_2] 38.2 (CH_2, $\boldsymbol{C}H_2Ph$), 49.2 [CH, C(2)H], 60.1 (CH_2, $O\boldsymbol{C}H_2CH_3$), 126.2 [CH, C(4')H, ArCH], 128.3, 128.9 [4 x CH, C(2')H, C(6')H, C(3')H and C(5')H, ArCH], 139.6 [C, C(1'), ArC], 175.5 [C, C(1)]; HRMS (ES+): Exact mass calculated for $C_{13}H_{18}O_2$ $[M+H]^+$ 207.1385 Found 207.1388; m/z (ES+) 207.3 $[(M+H)^+$, 30%], 277.2 (26%), 161.3 (18%), 248.3 (17%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

(±)-2-Benzylbutanoic acid (±)-45[55]



Aqueous sodium hydroxide (1M, 6 mL) was added to (±)-ethyl 2-benzylbutanoate (±)-**48** (88.5 mg, 0.43 mmol). The reaction mixture was maintained at reflux while stirring overnight then allowed cool to room temperature and extracted with diethyl ether (2 × 5 mL). The ethereal solution was discarded. The aqueous phase was acidified to pH 1 with aqueous hydrochloric acid (10%) and then extracted with diethyl ether (3 × 5 mL) and the combined organic extracts were washed with brine (10 mL), dried, filtered and concentrated under reduced pressure to give the pure *acid* **(±)-45** (50.3 mg, 66%) as a light orange oil; ν_{max}/cm^{-1} (film) 2966 (OH), 1705 (CO), 1605, 1496, 1456 (Ar); δ_H (300 MHz) 0.96 [3H, t, *J* 7.5, C(4)H_3], 1.50-1.77 [2H, m, C(3)H_2], 2.57-2.66 [1H, m, X of ABX, C(2)H], 2.75 [1H, dd, A of ABX, J_{AB} 13.8, J_{AX} 6.9, one of $C\boldsymbol{H_2}Ph$], 2.98 [1H, dd, B of ABX, J_{AB} 13.5, J_{BX} 7.8, one of $C\boldsymbol{H_2}Ph$], 7.09-7.34 (5H, m, ArH).

(±)-Ethyl 2-benzyl-3,3-dimethylbutanoate (±)-57

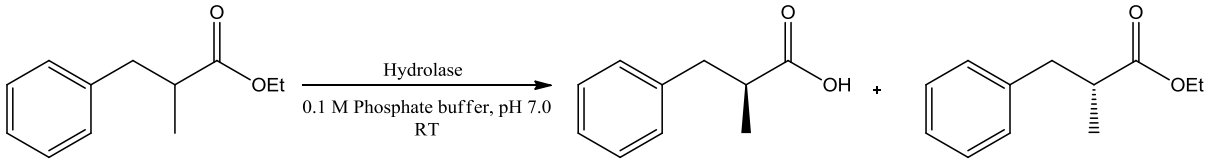


Potassium carbonate (0.63 g 4.58 mmol) was added to a solution of 2-benzyl-3,3-dimethylbutanoic acid **61** (0.94 g, 4.58 mmol) in HPLC grade acetone (40 mL). Once the addition was complete the reaction mixture was stirred for 10 min before iodoethane (1.53 g, 9.81 mmol) was added in one portion. The reaction mixture was stirred at room temperature overnight, and then filtered to remove the potassium carbonate. Acetone was evaporated under reduced pressure and at this point further filtration was performed to remove excess potassium carbonate. The crude product was dissolved in dichloromethane (50 mL) and washed with water (2 × 20 mL), a saturated aqueous solution of sodium bicarbonate (2 × 20 mL), aqueous hydrochloric acid (5%, 2 × 25 mL) and brine (30 mL). The organic extract was dried, filtered and concentrated under reduced pressure to give a mixture of 2-benzyl-3,3-dimethylbutanoic acid (±)-**61** and ethyl 2-benzyl-3,3-dimethylbutanoate **(±)-57** (0.67 g) as a clear oil in the ratio 13 : 87. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure *ester* (±)-**57** (0.57 g, 53%) as a clear oil; ν_{max}/cm^{-1} (film) 2963 (CH), 1729 (CO), 1605, 1496, 1456 (Ar), 1152 (C-O); δ_H (300 MHz) 1.04 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.05 [9H, s, $C(CH_3)_3$], 2.45 [1H, dd, X of ABX, J_{AX} 11.4, J_{BX} 3.9, C(2)H], 2.79-2.93 (2H, m, AB of ABX, $C\boldsymbol{H_2}Ph$), 3.88-4.03 (2H, sym. m, $OC\boldsymbol{H_2}CH_3$), 7.13–7.27 (5H, m, ArH).

5.4.2 Hydrolase-mediated kinetic resolution - analytical screens

The general procedure outlined in section 5.3.2 for the hydrolase-mediated kinetic resolution of the β-substituted 3-aryl alkanoic ethyl esters also applies to the resolution of the α-substituted esters.

*Table 5.13: Hydrolase-mediated hydrolysis of (±)-ethyl 2-methyl-3-phenylpropanoate (±)-**47***

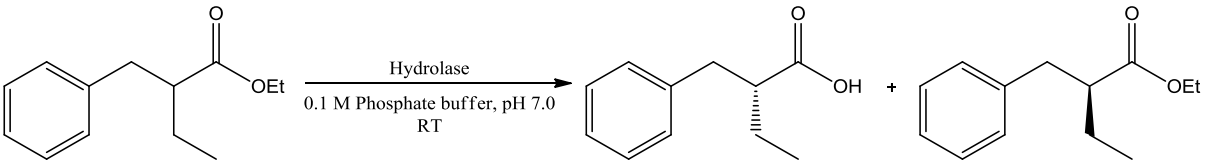


(±)-**47** (*S*)-**32** (*R*)-**47**

Entry	Enzyme source	Time	ee (%)		Conversion (%)		*E* value
			Ester (*R*)-47	Acid (*S*)-32	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	20 h	3[a]	3[a]	50	25	1.1
2	*Candida cyclindracea* C2	20 h	0[b]	0[b]	-[b]	100	-[b]
3	*Alcaligenes spp.*1	72 h	2	11	15	12	1.3
4	*Pseudomonas cepacia* P1	72 h	53	68	44	55	6.4
5	*Pseudomonas stutzeri*	72 h	83	50	62	69	17
6	*Rhizopus spp.*	72 h	5	30	14	<10	1.9
7	*Rhizopus niveus*	72 h	20	21	49	55	1.8
8	*Aspergillus niger*	72 h	5	33	13	<10	2.1
9	*Alcaligenes spp.*2	20 h	67	97	41	41	132
10a	*Pseudomonas cepacia* P2	20 h	>98	93	52	79	>200
10b		*10 h*	*95*	*96*	*50*	*61*	*183*
11	*Mucor javanicus*	20 h	14	21	40	29	1.7
12	*Penicillium camembertii*	72 h	3	17	15	<10	1.5
13a	*Pseudomonas fluorescens*	20 h	>98	92	52	55	179
13b		*10 h*	*81*	*97*	*46*	*49*	*164*
14	*Candida antarctica* B	20 h	0[b]	0[b]	-[b]	100	-[b]
15	*Mucor meihei*	20 h	23	0	52	97	1.9
16	*Candida antarctica* B (immob)	20 h	0[b]	0[b]	-[b]	100	-[b]
17	*Porcine pancrease* Type II	72 h	58	90	39	69	34
18	*Porcine pancrease* Grade II	72 h	11	47	19	35	3.1

a. Limited enantiopurity observed, thus direction of enantioselection should be interpreted with caution.
b. Reaction went to 100% completion, no enantioselectivity observed.

*Table 5.14: Hydrolase-mediated hydrolysis of (±)-ethyl 2-benzylbutanoate (±)-**48***



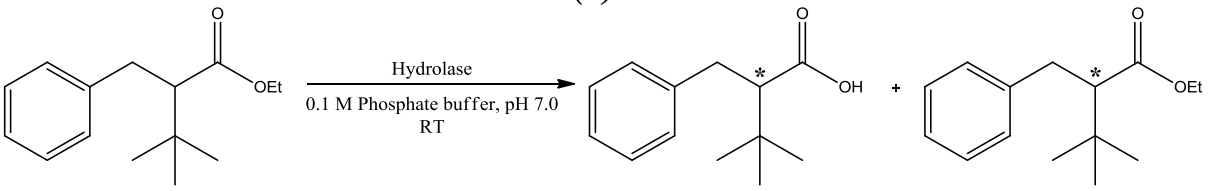
(±)-**48** (*R*)-**45** (*S*)-**48**

Entry	Enzyme source[a]	Time	ee (%)		Conversion (%)		*E* value
			Ester 48	Acid 45	*E* calc.	^{1}H NMR	
1	*Candida cyclindracea* C1	43 h	3 (*R*)	13 (*S*)	19	28	1.3
2	*Candida cyclindracea* C2	17 h	20 (*R*)	4 (*S*)	83	89	1.3
3	*Candida antarctica B*	17 h	35 (*S*)	83 (*R*)	30	74	15
4	*Candida antarctica B*	43 h	74 (*S*)	71 (*R*)	49	79	14
5	*Candida antarctica B* (immob)	43 h	17 (*S*)	73 (*R*)	19	57	7.6
6	Pig Liver esterase	17 h	6 (*R*)[b]	3 (*S*)[b]	67	96	1.1

a. The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Mucor javanicus, Pseudomonas fluorescens, Porcine Pancrease* Type II, *Pseudomonas stutzeri, Rhizopus niveus and Penicillium camembertii*

b. Limited enantiopurity observed, thus direction of enantioselection should be interpreted with caution.

*Table 5.15: Hydrolase-mediated hydrolysis of (±)-ethyl 2-benzyl-3,3-dimethylbutanoate (±)-**57***

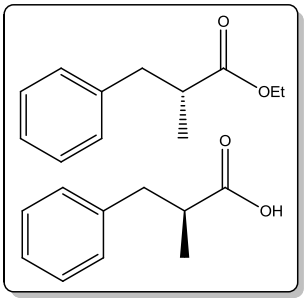


(±)-**57** **61** **57**

Entry	Enzyme source	Time	ee (%)		Conversion (%)		*E* value
			Ester 57	Acid 61	*E* calc.	^{1}H NMR	
1	*Pseudomononas cepacia* P1	20 h	-	-	-	0	-
2	*Pseudomononas cepacia* P2	20 h	-	-	-	0	-
3	*Candida antarctica B*	20 h	-	-	-	0	-
4	*Candida antarctica* B (immob)	20 h	-	-	-	0	-
5	*Pseudomonas fluorescens*	20 h	-	-	-	0	-

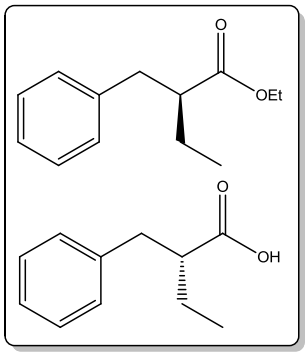
5.4.3 Hydrolase-mediated kinetic resolution – preparative-scale

Large scale hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate (±)-47



This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**38** from *Pseudomonas fluorescens* (48.0 mg) and (±)-ethyl 2-methyl-3-phenylpropanoate (±)-**47** (232.0 mg, 1.21 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm at 24 ºC. An aliquot of reaction mixture (1 mL) was withdrawn at 20 h. Following a mini work-up chiral HPLC analysis was conducted. Conversion estimated by *E*-value calculator at 51%.[45] The reaction mixture was filtered at 20 h to produce a light yellow oil (186.8 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure *ester* (*R*)-**47** (63.7 mg, 27%) as a clear oil $[\alpha]_D^{20}$ −36.4 (c 1.0, $CHCl_3$), >98% ee, lit[33] $[\alpha]_D^{20}$ +28.4 (c 1.0, $CHCl_3$), (*S*)-isomer, 82% ee, and the pure *acid* (*S*)-**32** (76.6 mg, 37%) as a clear oil $[\alpha]_D^{20}$ +28.0 (c 0.82, $CHCl_3$), 96% ee, lit[56] $[\alpha]_D^{20}$ +30.2 (c 0.82, $CHCl_3$), 99% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Large scale hydrolase-mediated hydrolysis of ethyl 2-benzylbutanoate (±)-48

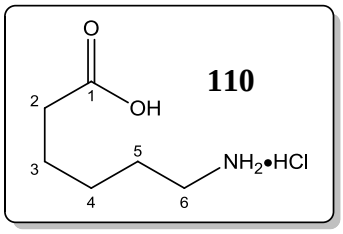


This was prepared following the procedure described for the hydrolase-mediated hydrolysis of (±)-**56** from *Candida antarctica* lipase B (immob) (407.8 mg) and (±)-ethyl 2-benzylbutanoate (±)-**48** (401.4 mg, 1.95 mmol) in 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 45 h at 24 ºC followed by solvent partitioning with heptane and ethyl acetate to produce the pure *ester* (*S*)-**48** (172.1 mg, 43%) as a clear oil $[\alpha]_D^{20}$ +6.8 (c 1.0, CH_2Cl_2), 26% ee and the pure *acid* (*R*)-**45** (66.2 mg, 19%) as a clear oil $[\alpha]_D^{20}$ −43.8 (c 1.0, CH_2Cl_2), 82% ee, lit[57] $[\alpha]_D^{23}$ −40.0 (c 1.0, CH_2Cl_2), >99% ee. Conversion estimated by *E*-value calculator at 24%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

5.5 Dynamic kinetic resolution of 2-nitrocyclohexanol (±)-99

5.5.1 Synthesis of substrates

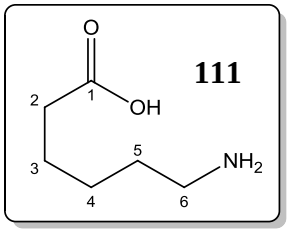
6-Aminohexanoic hydrochloride 110 and 6-aminohexanoic acid 111[58]



110

ε-Caprolactam **109** (10.00 g, 88.37 mmol) was added to a solution of conc. hydrochloric acid (9 mL) in water (30 mL) and refluxed for 1 h. The crude product was decolurised with charcoal and concentrated under reduced pressure to give the moist *hydrochloric salt* **110** (16.34 g, >100%) as a white solid, m.p. 112-115 °C (Lit.,[59] 132-133 °C); ν_{max}/cm^{-1} (KBr) 3367 (OH) 2945 (CH), 1725 (CO), 1216; δ_H (400 MHz, D_2O) 1.25-1.35 [2H, m, C(4)H_2], 1.45-1.65 [4H, sym m, C(3)H_2 and C(5)H_2], 2.29 [2H, t, *J* 7.4, C(2)H_2], 2.89 [2H, t, *J* 7.6, C(6)H_2].

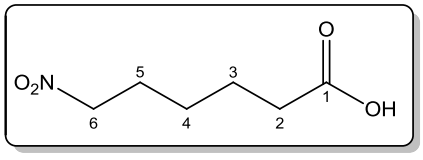
*Note: The experimental melting point of **110** differs significantly to the literature as the hydrochloric salt was not dried prior to analysis. However, the experimental melting point remains significant as it is a reference for the formation of the free amino acid **111**.*



111

The resulting hydrochloric salt **110** was coverted into the amino acid **111** by means of an ion-exchange column containing Amberlite IRA-400(OH) resin (6 inches). Aqueous hydrochloric acid (1%) was passed through the column until the pH of the solution leaving the column decreased from 5.5-6.5 to ~2.0. An aqueous sodium hydroxide solution (1%) was then passed through the column until the solution leaving the column was strongly alkaline. The resin was then washed with water (1 L) to remove all salts and the pH of the washings were 5.6-6.6. The column was now ready for use. The solid **110** (16.34 g) was dissolved in water (200 mL) and drawn onto the column. The column was then eluted with water (~800 mL) and detection of the amino acid **111** was by ninhydrin stain. The fractions containing the product were combined and concentrated under reduced pressure to a volume of about 20 mL. Absolute ethanol (60 mL) and diethyl ether (100 mL) were then added. Following vigourous shaking the pure *amino acid* **111** [11.36 g, 98% (from ε-caprolactam **109**)] formed and was collected by filtration as a white solid, m.p. 202-205 °C (Lit.,[58] 202-203 °C); ν_{max}/cm^{-1} (KBr) 3030 (OH), 2944 (CH), 1729 (CO), 1229; δ_H (400 MHz, D_2O) 1.21-1.29 [2H, m, C(4)H_2], 1.43-1.51 [2H, q, *J* 7.5 C(3)H_2 or C(5)H_2], 1.51-1.59 [2H, q, *J* 7.6 C(3)H_2 or C(5)H_2], 2.07 [2H, t, *J* 7.3, C(2)H_2], 2.88 [2H, t, *J* 7.5, C(6)H_2].

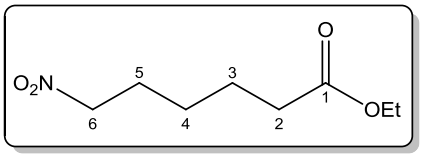
Attempted oxidation of 6-aminohexanoic acid 111



A saturated aqueous solution of sodium bicarbonate (25 mL) was added to a solution of 6-aminohexanoic acid **111** (0.50 g, 3.81 mmol) in acetone (50 mL) and the resulting white precipitate was cooled to 0 °C for 1 h under nitrogen. A solution of Oxone® (11.72 g, 19.06 mmol) in water (30 mL) was then added dropwise to the solution and the mixture was stirred overnight.The reaction mixture was filtered through a sintered glass funnel containing a layer of Celite® and washed with diethyl ether (2 × 100 mL). The filtrate was transferred to a separating funnel and the layers separated. The aqueous solution was extracted with diethyl ether (100 mL) and the combined organic extracts were washed with water (100 mL) and brine (100 mL), dried, filtered and concentrated at reduced pressure to give the crude product (0.08 g) as yellow/orange viscous oil. A ^{1}H NMR spectrum

of the crude product was recorded, which showed the presence of signals pertaining to 6-nitrohexanoic acid **107** δ_H (400 MHz) 2.39 [2H, t, *J* 7.4, C(2)H_2], 4.40 [2H, t, *J* 7.0, C(6)H_2], 10.98 (1H, br s, OH). Numerous attempts of purification by column chromatography on silica gel and acid/base extraction were unsuccessful in removing unidentifiable byproducts.

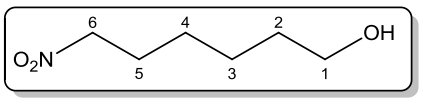
Ethyl 6-nitrohexanoate 113[60]



Sodium nitrite (4.60 g, 67.23 mmol) was added to a 0.1 M solution of ethyl 6-bromohexanoate **114** (10.00 g, 44.82 mmol) in dry DMF (450 mL). The reaction mixture was stirred overnight at room temperature under a blanket of nitrogen, then poured onto water (100 mL) and ice (~80 g). The aqueous phase was extracted with diethyl ether (3 × 100 mL). The combined organic layer was washed with brine (100 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (10.74 g) of ethyl 6-nitrohexanoate **113** and ethyl 6-(nitrosooxy)hexanoate **115** (76 : 24 respectively) as a yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether 85/15 as eluent gave the pure *nitro ester* **113** (4.17 g, 49%) as a light yellow oil; ν_{max}/cm^{-1} (film) 2940 (CH), 1733 (CO), 1554 (NO_2), 1377 (NO_2), 1187; δ_H (300 MHz) 1.26 (3H, t, *J* 7.2, $OCH_2C\boldsymbol{H_3}$), 1.38-1.49 [2H, m, C(3)H_2], 1.64-1.74 [2H, m, C(4)H_2], 1.99-2.09 [2H, m, C(2)H_2], 2.33 [2H, t, *J* 7.4, C(5)H_2], 4.13 (2H, q, *J* 4.1, $OC\boldsymbol{H_2}CH_3$), 4.40 [2H, t, *J* 7.1, C(1)H_2].

*Note: Signals for ethyl 6-(nitrosooxy)hexanoate **115** was detected in the ^1H NMR of the crude product spectrum at δ_H (300MHz) 4.58-4.80 (2H, br m, CH_2ONO).*

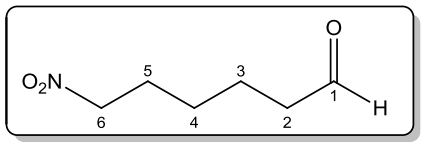
6-Nitrohexan-1-ol 108[5,61,62]



DIBAL-H (54 mL, 54.00 mmol, 1M solution in hexanes) was added dropwise to a solution of ethyl 6-nitrohexanonate **113** (3.30 g, 17.44 mmol) in doubly distilled dichloromethane (135 mL) at −78 ºC under nitrogen. The reaction mixture was stirred at -78 ºC for 1 h, then warmed to −40 ºC and stirred for a further 1 h. The temperature was then increased to -10 ºC and the reaction carefully quenched with aqueous hydrochloric acid (1M, 20 mL). Vigorous stirring and further addition of aqueous hydrochloric acid (1M, 20 mL) was required to degrade the gelatinous nature of the aluminium salt precipitate. The solution was transferred to a separating funnel and the layers were separated. The aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layer was washed with aqueous hydrochloric acid (1M, 100 mL), brine (100 mL), dried, filtered and concentrated under reduced pressure in an ice cold water bath to give the crude *nitro alcohol* **108** (2.31 g) as a light yellow oil. Purification by column chromatography on silica gel using dichloromethane/methanol 90/10 as eluent gave the pure *nitro alcohol* **108** (2.02 g, 79%) as a clear oil; ν_{max}/cm^{-1} (film) 3400 (OH), 2936 (CH), 1552 (NO_2), 1385 (NO_2), 1198; δ_H (300 MHz) 1.30-1.50 [4H, m, C(3)H_2 and C(4)H_2], 1.52-1.66 [2H, m, C(2)H_2], 1.76 (1H, br s, OH), 1.92-2.12 [2H, m, C(5)H_2], 3.64 [2H, t, *J* 6.5, C(1)H_2], 4.40 [2H, t, *J* 7.1, C(6)H_2]. ^1H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note: Significant difference in ^1H NMR chemical shift of the C(1)H_2 triplet is observed between Milner[5] and data described above. Milner[5] reports; δ_H (400 MHz) 3.24 [2H, t, J 6.4, C(1)H_2]. Literature reference Ballini[61] reports; δ_H (300 MHz) 3.65 (2H, t, J 6.2) with spectral characteristics consistent with the experimental data obtained during this project.

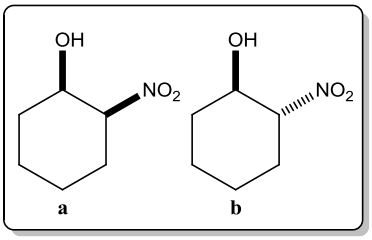
6-Nitrohexanal 101[5,60]



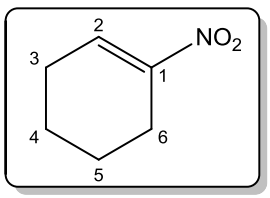
Pyridinium chlorochromate (PCC) (10.64 g, 49.36 mmol) and crushed 3Å molecular sieves (3.00 g) were added to a solution of 6-nitrohexan-1-ol **108** (3.56 g, 24.19 mmol) in doubly distilled dichloromethane (100 mL) under nitrogen at room temperature. Stirring was continued for 4 h. The reaction mixture was diluted with diethyl ether (100 mL) and filtered through a sintered glass funnel containing a layer each of Celite® and silica gel. The filtrate was concentrated under reduced pressure. The resulting oil was diluted with diethyl ether (40 mL), washed with water (2 × 50 mL), brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *aldehyde* **101** (1.88 g) as a light green oil (discolouration due to presence of residual PCC). Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure *aldehyde* **101** (1.33 g, 38%) as a clear oil; ν_{max}/cm^{-1} (film) 2938 (CH), 1722 (CO), 1552 (NO_2), 1386 (NO_2); δ_H (300 MHz) 1.34-1.51 [2H, m, C(4)H_2], 1.60-1.77 [2H, m, C(3)H_2], 1.95-2.13 [2H, m, C(5)H_2], 2.47-2.52 [2H, dt, *J* 1.5, 7.2, C(2)H_2], 4.41 [2H, t, *J* 6.9 C(6)H_2], 9.78 [1H, t, *J* 1.5, C(1)H].

(±)-2-Nitrocyclohexanol (±)-99[5,63,64]

Method I: Sodium borohydride reduction of 2-nitrocyclohexanone 103



A solution of 2-nitrocyclohexanone **103** (1.00 g, 6.99 mmol) in distilled ethanol (10 mL) was added dropwise over 10 min to a stirred suspension of $NaBH_4$ (264 mg, 6.99 mmol) in distilled ethanol (30 mL) at 0 °C under nitrogen and stirring was continued for 5 h at 0 °C. The ice bath was then removed and aqueous hydrochloric acid (10%) was added to adjust to pH 1. The solution was concentrated under reduced pressure and the resulting residue was partitioned between water (10 mL) and dichloromethane (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined organic extracts were washed with brine (30 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (0.78 g) of *nitroalcohols* (±)-**99a**, (±)-**99b** and *nitroalkene* **102** (11 : 72 : 17 respectively) as an orange oil. Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 3-10% ethyl acetate) gave four fractions.



The first (least polar) fraction was the pure *1-nitrocyclohex-1-ene* **102** (107.0 mg, 12%) as a yellow oil; ν_{max}/cm^{-1} (film) 1668 (C=C), 1515 (NO_2), 1333 (NO_2); δ_H (300 MHz) 1.57-1.70 [2H, m, C(3)H_2 or C(4)H_2 or C(5)H_2], 1.72-1.82 [2H, m, C(3)H_2 or C(4)H_2 or C(5)H_2], 2.30-2.38 [2H, sym m, C(3)H_2 or C(4)H_2 or C(5)H_2], 2.54-2.61 [2H, sym m, C(6)H_2], 7.31-7.35 [1H, sym m, C(2)H]; δ_C (75.5 MHz) 20.7, 21.8, 23.9, 24.8 [4 x CH_2, C(3)H_2, C(4)H_2, C(5)H_2 and C(6)H_2], 134.4 [CH, C(2)H], 149.7 [C, C(1)]. ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

The second fraction was the pure *(±)*-cis-*2-nitrocyclohexanol* (±)-**99a** (41.7 mg, 4%) as a clear oil; ν_{max}/cm^{-1} (film) 3427 (OH), 2943 (CH), 1548 (NO_2), 1383 (NO_2); δ_H (300 MHz) 1.19-2.35 [8H, m, C(3)H_2, C(4)H_2, C(5)H_2 and C(6)H_2], 2.63 (1H, d, *J* 3.6, OH), 4.34-4.41 [1H, m, C(2)H], 4.51 [1H, br s, C(1)H]; δ_C (75.5 MHz) 18.8, 23.5, 24.6, 31.4 [4 x CH_2,

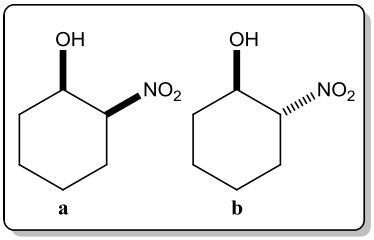
$C(3)H_2$, $C(4)H_2$, $C(5)H_2$ and $C(6)H_2$], 67.1 [CH, C(1)H], 86.9 [CH, C(2)H]. ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

The third fraction was a 17 : 83 mixture of *cis* : *trans (±)-2-nitrocyclohexanols*, (±)-**99a** and (±)-**99b** (135.9 mg, 13%) as a clear oil.

The fourth (most polar) fraction was the pure *(±)*-trans-*2-nitrocyclohexanol* (±)-**99b** (367.0 mg, 36%) as a clear oil which solidified upon cooling to a white crystalline solid, m.p. 46-48 °C (Lit.,[65] 46-47 °C); ν_{max}/cm^{-1} (KBr) 3256 (OH), 2952 (CH), 1551 (NO_2), 1376 (NO_2); δ_H (300 MHz) 1.20-1.46 [3H, m, one of $C(4)H_2$, one of $C(5)H_2$ and one of $C(6)H_2$], 1.68-1.93 [3H, m, one of $C(4)H_2$, one of $C(5)H_2$ and one of $C(3)H_2$], 2.03-2.18 [1H, m, one of $C(6)H_2$], 2.27-2.41 [1H, m, one of $C(3)H_2$], 2.70 (1H, d, *J* 4.5, OH), 4.04-4.12 [1H, m, C(1)H], 4.26-4.34 [1H, m, C(2)H]; δ_C (75.5 MHz) 23.6, 24.0 [2 x CH_2, $C(4)H_2$ and $C(5)H_2$], 30.4 [CH_2, $C(3)H_2$], 32.8 [CH_2, $C(6)H_2$], 71.1 [CH, C(1)H], 91.3 [CH, C(2)H]. ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note: Milner reports (±)-trans-*2-nitrocyclohexanol (±)-**99b**; δ_H (300 MHz) 1.12-1.46 [3H, m, one of $C(6)H_2$ and $C(5)H_2$], 1.70-1.89 [3H, m, one of $C(3)H_2$ and $C(4)H_2$].[5] In the experimental data described above the $C(4)H_2$ and the $C(5)H_2$ protons are split between the two multiplets. All remaining spectroscopic characteristics were in agreement with previously reported data.*

Method II: Henry reaction



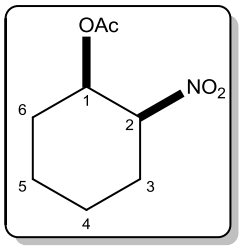
Triethylamine (1.6 mL, 11.35 mmol) was added in one portion to a stirring solution of 6-nitrohexanal **101** (0.82 g, 5.68 mmol) in chloroform (20 mL). Stirring was continued for 3 h at room temperature. Aqueous hydrochloric acid (10%) was added dropwise to the reaction mixture to adjust to pH 2. The solution was transferred to a separating funnel and the layers were separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The combined organic layer was washed with brine (20 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (0.78 g) of *nitro alcohols* (±)-**99a** and (±)-**99b** (14 : 86 respectively) as a clear oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave three fractions.

The first (least polar) fraction gave the pure *(±)*-cis-*2-nitrocyclohexanol* (±)-**99a** (32.6 mg, 4%) as a clear oil.

The second fraction gave a 15 : 85 mixture of *cis* : *trans (±)-2-nitrocyclohexanols*, (±)-**99a** and (±)-**99b** (7.4 mg, 1%) as a clear oil.

The third (most polar) fraction gave the pure *(±)*-trans-*2-nitrocyclohexanol* (±)-**99b** (436.6 mg, 53%) as a clear oil which solidified upon cooling to a white crystalline solid. Spectral characteristics were consistent with data described above.

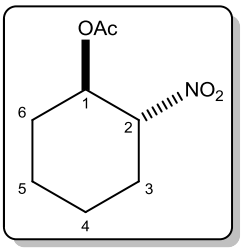
(±)-*cis*-2-Nitrocyclohexyl acetate (±)-100a[5,63,66]



(±)-*cis*-2-Nitrocyclohexanol (±)-**99a** (451.0 mg, 3.11 mmol) and acetyl chloride (2.3 mL, 32.35 mmol) were stirred in dichloromethane (15 mL) under nitrogen. *N*,*N*-Dimethylaminopyridine (0.54 g, 4.42 mmol) was added and stirring continued at room temperature for 12 h. A saturated aqueous solution of sodium bicarbonate (10 mL) was added and the mixture was transferred to a separating funnel. The aqueous phase was extracted with dichloromethane (3 × 20 mL) and the combined organic layers were washed with brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude *acetate* (±)-**100a** (0.49 g, 85%) as a clear oil; ν_{max}/cm^{-1} (film) 2947 (CH), 1745 (CO), 1549 (NO_2), 1381 (NO_2); δ_H (300 MHz) 1.28–1.71 [4H, m, one of C(4)H_2, one of C(6)H_2 and C(5)H_2], 1.87–1.99 [1H, m, one of C(4)H_2], 2.05 (3H, s, COC***H*****$_3$**), 2.10–2.23 [3H, m, one of C(6)H_2 and C(3)H_2], 4.46–4.52 [1H, m, C(2)H], 5.52–5.65 [1H, m, C(1)H]; δ_C (75.5 MHz) 19.5 [CH_2, C(5)H_2], 20.9 (CH_3, CO***C***H_3), 22.8 [CH_2, C(4)H_2], 24.9 [CH_2, C(3)H_2], 28.7 [CH_2, C(6)H_2], 69.5 [CH, C(1)H], 84.0 [CH, C(2)H],169.8 (C, ***C***OCH_3). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

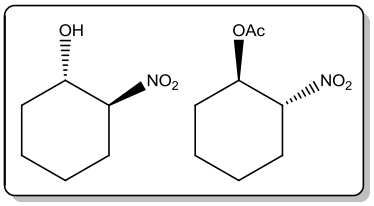
*Note: Additional signals present due to unreacted starting material (±)-*cis*-2-nitrocyclohexanol (±)-**99a** 12% and residual* N,N*-dimethylaminopyridine 5% were evident in the crude ^{1}H NMR spectrum. Attempted purification by column chromatography resulted in the formation of the elimination product 1-nitrocyclohex-1-ene **102**.*

(±)-*trans*-2-Nitrocyclohexyl acetate (±)-100b[5,63,66]



N,*N*-Dimethylaminopyridine (2.0 mg, 0.02 mmol) was added to a stirring solution of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (250.0 mg, 1.72 mmol), acetic anhydride (1.1 mL, 11.66 mmol) and pyridine (0.6 mL, 7.45 mmol) in dichloromethane (6 mL). The reaction mixture was stirred at room temperature for 21 h under nitrogen. A saturated aqueous solution of sodium bicarbonate (10 mL) was added and the mixture stirred for 30 min. The solution was transferred to a separating funnel and washed with a saturated aqueous solution of $CuSO_4$ (20 mL), water (20 mL), a saturated aqueous solution of sodium bicarbonate (20 mL) and brine (20 mL). The organic extract was dried, filtered and concentrated under reduced pressure to give the crude *acetate* (±)-**100b** (221.7 mg, 69%) as a clear oil which solidified on cooling to a white crystalline solid, which was sufficiently pure to use without further purification, m.p. 44-46 °C (Lit.,[5] 40-42 °C); ν_{max}/cm^{-1} (KBr) 2954 (CH), 1737 (CO), 1548 (NO_2), 1376 (NO_2); δ_H (300 MHz) 1.24–1.55 [3H, m, one of C(4)H_2, one of C(5)H_2 and one of C(6)H_2], 1.74–1.94 [3H, m, one of C(3)H_2, one of C(4)H_2 and one of C(5)H_2], 2.02 (3H, s, $COCH_3$), 2.18–2.28 [1H, m, one of C(6)H_2], 2.34–2.42 [1H, m, one of C(3)H_2], 4.47–4.55 [1H, m, C(2)H], 5.19–5.27 [1H, ddd appears as a dt, *J* 4.7, 10.5, C(1)H]; δ_C (75.5 MHz) 20.9 (CH_3, CO***C***H_3), 23.2, 23.7 [2 x CH_2, C(4)H_2 and C(5)H_2], 29.9 [CH_2, C(6)H_2], 30.8 [CH_2, C(3)H_2], 72.6 [CH, C(1)H], 87.7 [CH, C(2)H], 169.7 (C, ***C***OCH_3). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

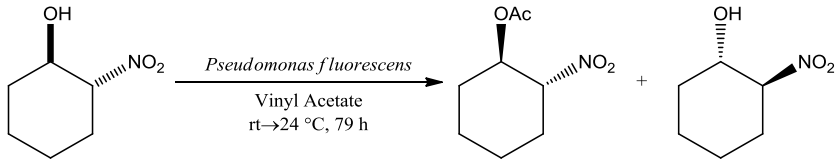
Large scale hydrolase-mediated transesterfication of (±)-*trans*-2-nitrocyclohexanol (±)-99b[5]



Pseudomonas fluorescens (41.0 mg) was added to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (423.0 mg, 2.91 mmol) in vinyl acetate (5 mL) and this was shaken at 750 rpm for 28 h at room temperature and 51 h at 24 °C. Aliquots of reaction mixture (0.5 mL) were withdrawn at 28 h and 55 h. Following a mini work-up, ^{1}H NMR and chiral HPLC analysis was conducted. The solution was filtered at 79 h through a pad of Celite® and the hydrolase washed with ethyl acetate (4 × 5 mL). Conversion estimated by *E*-value calculator at 50%.[45] The organic extracts were concentrated under reduced pressure to produce a light orange oil (215.8 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 3-25% ethyl acetate) gave the pure *(1R,2R)-trans-2-nitrocyclohexyl acetate* (1*R*,2*R*)-**100b** (81.1 mg, 30%) as a clear oil which solidified on cooling to a white crystalline solid $[\alpha]_D^{20}$ -41.0 (c 1.0, CH_2Cl_2), >98% ee, lit[5] $[\alpha]_D^{20}$ −40.6 (c 1.0, CH_2Cl_2), >98% ee and the pure *(1S,2S)-trans-2-nitrocyclohexanol* (1*S*,2*S*)-**99b** (96.0 mg, 45%) as a clear oil $[\alpha]_D^{20}$ +42.6 (c 0.3, CH_2Cl_2), 95% ee, lit[63] $[\alpha]_D^{20}$ +48.6 (c 1.0, CH_2Cl_2), >98% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note: Yield may be reduced due to samples being withdrawn during reaction monitoring.

Table 5.16: Large scale Pseudomonas fluorescens *mediated transesterfication of (±)-*trans*-2 nitrocyclohexanol (±)-**99b** in vinyl acetate*



(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*,2*S*) [yield %][b]	Acetate *trans*-100b (1*R*,2*R*) [yield %][b]	
1	28 h	rt	24	25	31	>98	134
2	55 h	24	44	-	78	>98	>200
3	79 h	24	50	50	97[a] [45]	>98 [30]	>200

a. While analysis by chiral HPLC of the crude product mixture showed 97% ee for (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, on chromatographic purification the enantiomeric excess [ee (%)] of isolated (1*S*,2*S*)-**99b** was determined to be 95% ee.
b. Isolated yield following column chromatography.

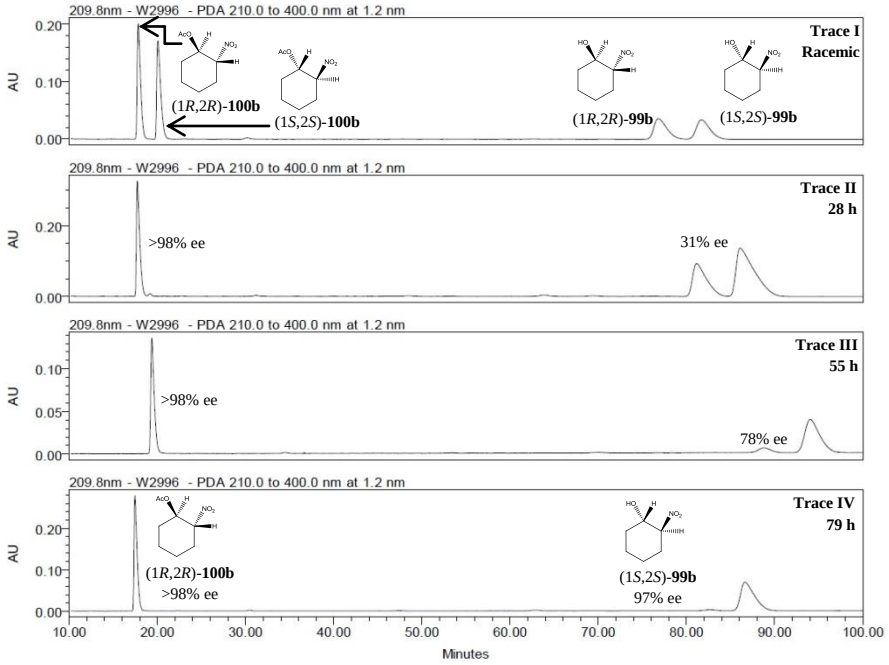
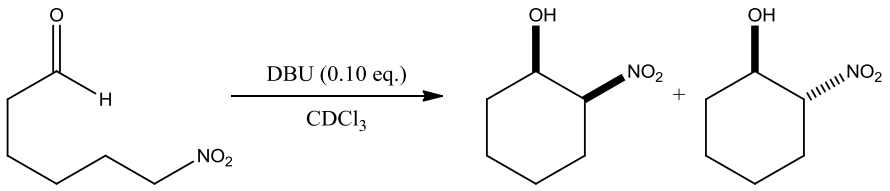


Figure 5.9: HPLC ***Trace I:*** *A racemic mixture of (±)-*trans*-2-nitrocyclohexyl acetate (±)-**100b** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b**. **Trace II:** Reaction sampling 28 h. **Trace III:** Reaction sampling 55 h. **Trace IV:** Reaction sampling 79 h, (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*)-**100b**, >98% ee, (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**, 97% ee. For HPLC conditions see appendix I.*

5.5.2 Evidence of a dynamic interconversion process – ^{1}H NMR

A solution of 6-nitrohexanal **101** (80.4 mg, 0.55 mmol) in deuterated chloroform, $CDCl_3$ (1.6 mL) was prepared. An aliquot (400 μL, 20.1 mg or 0.14 mmol of **101**) was dispensed into each NMR tube. A second solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (6.29 mg, 0.04 mmol) in deuterated chloroform, $CDCl_3$ (0.6 mL) was prepared. Aliquots of (0.10 eq, 200 μL, 0.014 mmol of DBU) or (0.05 eq, 100 μL, 0.007 mmol of DBU) were dispensed into the appropriate NMR tube with the 6-nitrohexanal **101** solution, agitated and analysed by ^{1}H NMR (600 MHz) spectroscopy at regular time intervals. The ratio of *trans* relative to *cis* was calculated by analysis of the integrals of the 1H, m, C(1)***H***OH, at 4.04-4.12 ppm in (±)-*trans*-2-nitrocyclohexanol (±)-**99b** and the 1H, br s, C(1)***H***OH, at 4.51 ppm in (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (Figure 5.10). The results of the spectroscopic analysis are summarised in Table 5.17 and 5.18.

*Table 5.17: Evidence for dynamic interconversion – 6-nitrohexanal **101**, $CDCl_3$ and DBU (0.10 eq.)*



101 (±)-**99a** (±)-**99b**

Reaction Time	101 (%)	(±)-99a (%)	(±)-99b (%)
0 min	21	19	60
17 min	20	18	62
27 min	14	21	65
1 h 27 min	4	18	78
3 h 29 min	0	18	82
9 h 29 min	0	17	83
15 h 29 min	0	17	83
20 h 22 min	0	15	85
22 h 37 min	0	16	84
20 days	0	14	86

Note: Integration of the C(2)HNO_2 signals was low in the ^{1}H NMR spectra from reaction time 1 h 27 min onwards. This may be due to deuterium exchange of this acidic C(2)HNO_2 proton of cis *and* trans *2-nitrocyclohexanol (±)-**99a** and (±)-**99b**.*

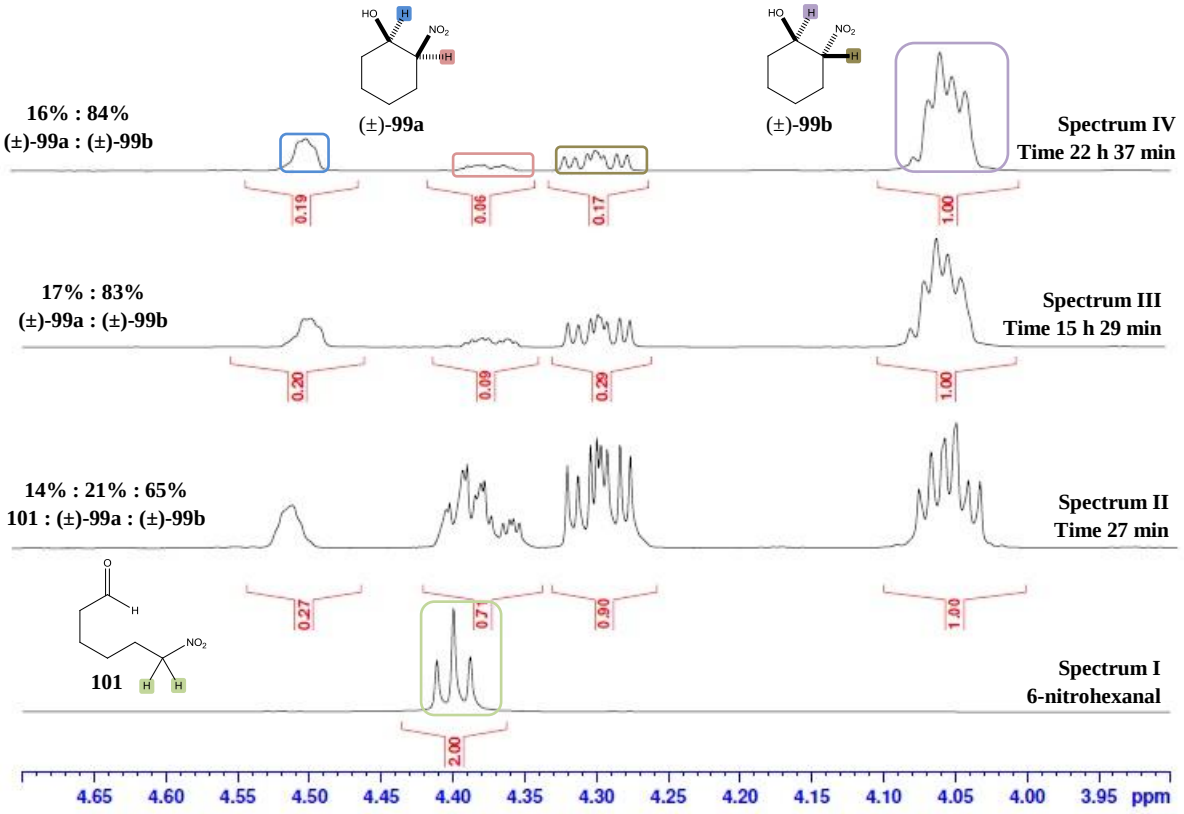


*Figure 5.10: Stacked ^{1}H NMR spectra - Evidence for dynamic interconversion, 6-nitrohexanal **101** and DBU (0.10 eq.).*

Following spectroscopic analysis over time (reaction time 35 days), the ^{1}H NMR sample (DBU 0.10 eq.) was dissolved in chloroform (2 mL) and washed with saturated aqueous ammonium chloride solution (3 × 3 mL) to remove the DBU. The organic layer was then washed with brine (3 mL), dried, filtered and concentrated under reduced pressure to yield a clear oil. The sample was analysed by ^{1}H NMR spectroscopy and gave a crude mixture of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b**, 11 : 89 respectively. The sample was reconcentrated and dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and analysed by chiral HPLC (Figure 5.11) in conjunction with 2 μL injection volume calibration curves (Figure 5.12 and 5.13) to give (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and (±)-*trans*-2-nitrocyclohexanol (±)-**99b** 15 : 85 respectively.

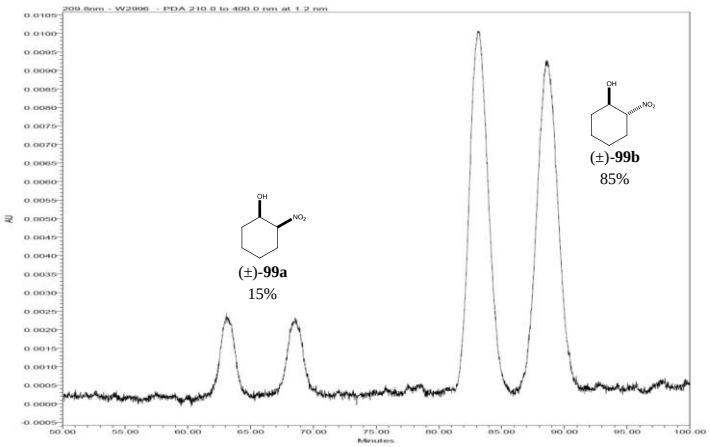


*Figure 5.11: HPLC trace (2 μL injection volume) of ^{1}H NMR sample DBU (0.10 eq.) and 6-nitrohexanal **101**, reaction time 35 days. For HPLC conditions see appendix I.*

Calibration curve: (±)-*cis*-2-Nitrocyclohexanol (±)-**99a** (2 μL injection volume)

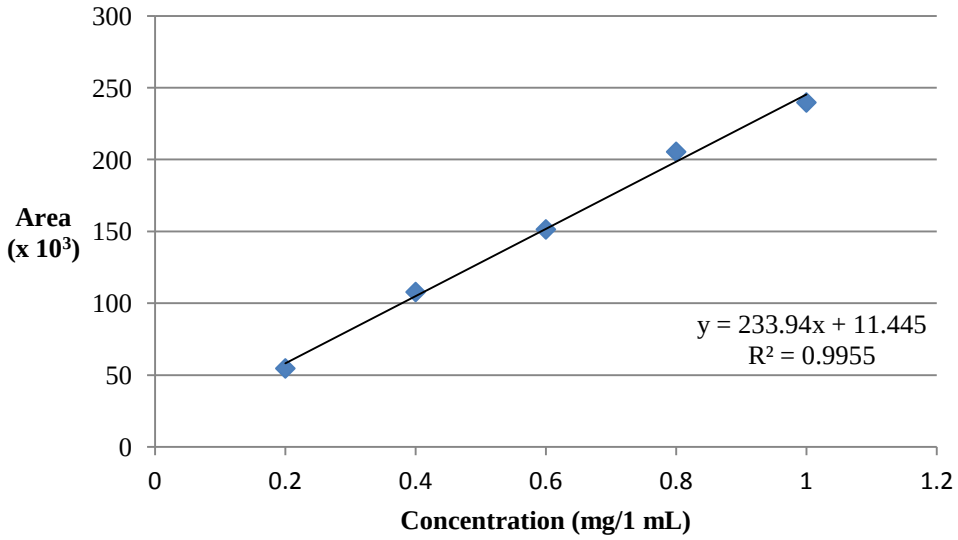


*Figure 5.12: Calibration curve (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (2 μL injection volume). Area (x 10^3)* vs. *concentration (mg/1 mL).*

Calibration curve: (±)-*trans*-2-Nitrocyclohexanol (±)-**99b** (2 μL injection volume)

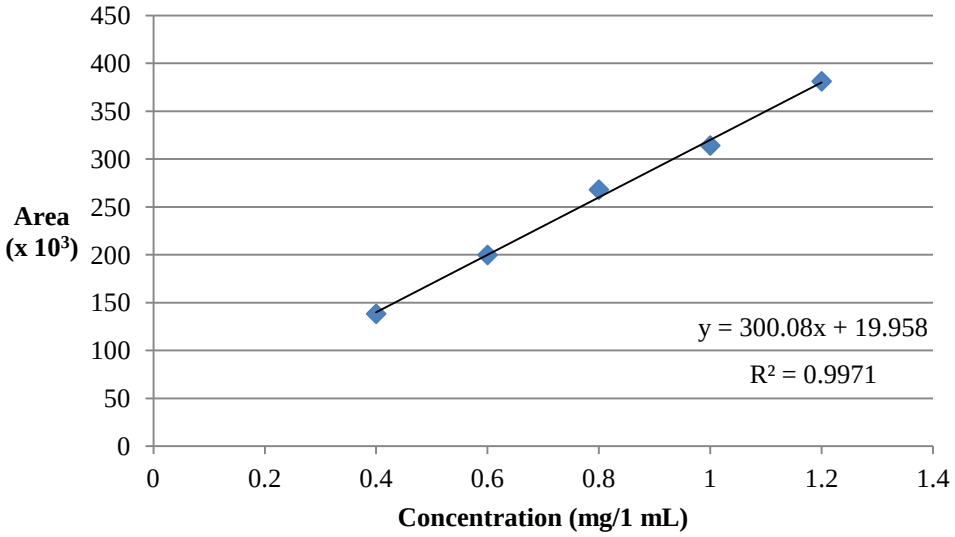
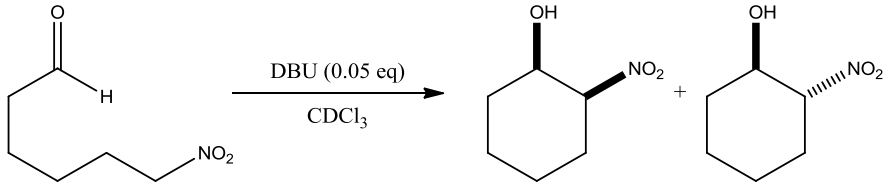


*Figure 5.13: Calibration curve (±)-*trans*-2-nitrocyclohexanol (±)-**99b** (2 μL injection volume). Area (x 10^3)* vs. *concentration (mg/1 mL).*

*Table 5.18: Evidence for dynamic interconversion – 6-nitrohexanal **101**, $CDCl_3$ and DBU (0.05 eq.)*



101 (±)-**99a** (±)-**99b**

Reaction Time	101 (%)	(±)-99a (%)	(±)-99b (%)
0 min	92	4	4
5 min	92	4	4
7 min	92	4	4
34 min	91	4.5	4.5
1 h 35 min	89	5	6
3 h 35 min	86.5	6.5	7
9 h 34 min	78	10	12
15 h 35 min	71	14	15
20 h 28 min	65	16	19
22 h 43 min	62	18	20
23 h 50 min	60	20	20
27 h 37 min	56	22	22

5.5.3 Hydrolase-mediated kinetic resolution - analytical screens

5.5.3.1 Analytical screen – vinyl acetate as both acyl donor and solvent

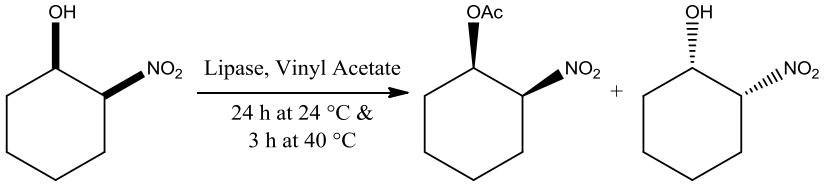
General procedure for the hydrolase-mediated transesterification of the (±)-2-nitrocyclohexanol (±)-99a or (±)-99b with vinyl acetate as both acyl donor and solvent.[5]

A spatula tip of enzyme (~5-10 mg, amount not critical) was added to the alcohol substrate (±)-**99a** or (±)-**99b** (~20 mg) in vinyl acetate (1 mL). The small test tubes were sealed and agitated at 750 rpm for 24 h at 24 °C and for 3 h at 40 °C, unless otherwise stated. The solution was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screen are summarised in Table 5.19 and 5.20.

Note:

1. *Notes 1-5 in section 5.3.2 (General procedure for the hydrolase-mediated kinetic resolution of the β-substituted 3-aryl alkanoic ethyl esters) apply to this screening protocol.*
2. *^{1}H NMR conversion was calculated for the hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** by analysis of the integrals 1H, br s, C(1)**H**OH, at 4.51 ppm in (±)-*cis*-2-nitrocyclohexanol (±)-**99a** and the 1H, m, C(1)HOAc at 5.58–5.60 ppm in (±)-*cis*-2-nitrocyclohexylacetate (±)-**100a.***
3. *^{1}H NMR conversion was calculated for the hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** by analysis of the integrals 1H, m, C(1)**H**OH, at 4.04-4.12 ppm in (±)-*trans*-2-nitrocyclohexanol (±)-**99b** and the 1H, ddd appears as a dt, C(1)HOAc at 5.19–5.27 ppm in (±)-*trans*-2-nitrocyclohexylacetate (±)-**100b**.*

*Table 5.19: Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** in vinyl acetate*

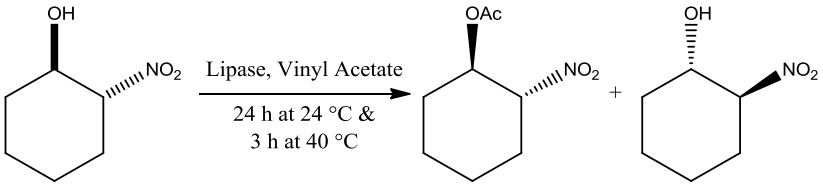


(±)-**99a** (1*R*,2*S*)-**100a** (1*S*,2*R*)-**99a**

Entry	Enzyme Source	Ratio 99a : 99b[a]	Conversion (%)		ee (%)		*E* Value
			E Calc.	^{1}H NMR	Alcohol *cis*-99a (1*S*,2*R*)	Acetate *cis*-100a (1*R*,2*S*)	
1	*Candida antarctica* B (immob)[b]	96 : 4	23	19	29	95	51
2	*Alcaligenes spp.* 1	96 : 4	20	18	23	92	30
3	*Rhizopus spp.*	96 : 4	-	<10	-	-	-
4	*Aspergillus niger*	96 : 4	-	<10	-	-	-
5	*Mucor meihei*	96 : 4	-	<10	-	-	-
6	*Porcine pancrease* Type II	95 : 5	-	<10	-	-	-
7	Pig liver esterase	96 : 4	-	<10	-	-	-
8	*Candida antarctica* A	96 : 4	60	60[c]	80	54	7.8
9	*Achromobacter spp.*	96 : 4	-	<10	-	-	-

a. The ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in the starting material, determined by ^{1}H NMR spectroscopy.
b. Time for tranesterification of the nitroalcohol was 51 h at 24 °C.
c. Unknown impurity observed in the crude ^{1}H NMR.

*Table 5.20: Hydrolase-mediated transesterification of (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in vinyl acetate*



(±)-**99b** (1*R*,2*R*)-**100b** (1*S*,2*S*)-**99b**

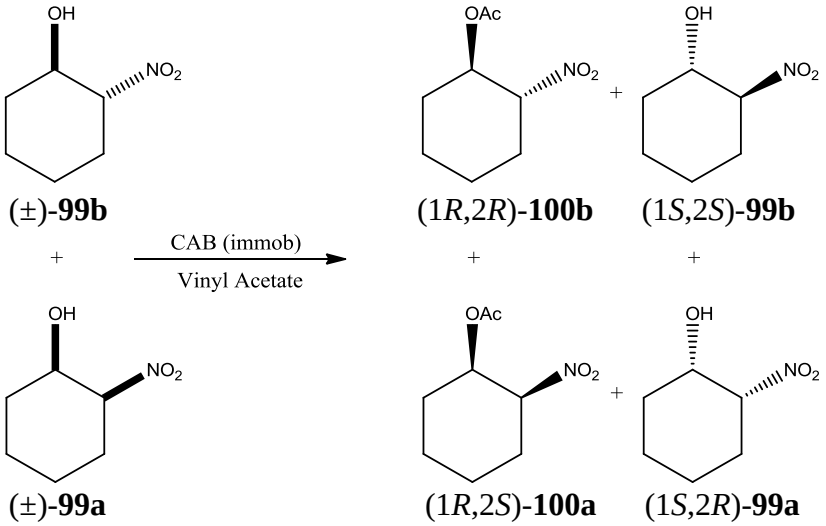
Entry	Enzyme Source	Ratio 99a : 99b[b]	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-99b (1*S*,2*S*)	Acetate *trans*-100b (1*R*,2*R*)	
1	*Candida antarctica* B (immob)[a]	1 : 99	50	49	>98	>98	>200
2	*Candida antarctica* A	1 : 99	9	-[c]	8	77	8.3
3	*Achromobacter spp.*	1 : 99	-	<10	-	-	-
4	*Pseudomonas fluorescens* (immob)[d]	1 : 99	38	38	61	>98	185

a. Time for tranesterification of the nitroalcohol was 51 h at 24 °C.
b. The ratio of (±)-*cis*-2-nitrocyclohexanol (±)-**99a** to (±)-*trans*-2-nitrocyclohexanol (±)-**99b** in the starting material, determined by ^{1}H NMR spectroscopy.
c. Unknown impurity observed in the ^{1}H NMR of the crude product, conversion could not be obtained satisfactorily due to overlapping peaks.
d. *Pseudomonas fluorescens* (immob) was obtained commercially from Sigma-Aldrich.

*5.5.3.2 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-99a and (±)-99b*

Candida antarctica lipase B (immob) (130.0 mg) was added to a equimolar mixture of (±)-*cis* and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** (154.8 mg, 1.07 mmol) dissolved in vinyl acetate (7.3 mL). The reaction mixture was shaken at 750 rpm at 19 °C for the first 15 h and then 24 °C for 33 h. Reaction monitoring was conducted as follows; an aliquot (1 mL) of reaction mixture was isolated and filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy. The final extraction following ^{1}H NMR spectroscopy was dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screens are summarised in Table 5.21.

*Table 5.21: Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-nitrocyclohexanol (±)-**99a** and (±)-**99b** in vinyl acetate*



Enzyme Source	Reaction Time	Temp (°C)	Alcohol (±)-99		Acetate (±)-100	
			***cis*-99a (%)[a] [ee (%)][b]**	***trans*-99b (%)[a] [ee (%)][c]**	***cis*-100a (%)[a] [ee (%)][d]**	***trans*-100b (%)[a] [ee (%)][e]**
Candida antarctica B (immob)	15 h	19	45	35	1	19
	24 h	24	48	28	3	21
	39 h	24	43	29	6	22
	48 h	24	42 [23]	30 [>98]	8 [96]	20 [98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the spectrum of the mixture of the crude material not mass recovery.
b. The principal enantiomer was (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a**.
c. The principal enantiomer was (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**.
d. The principal enantiomer was (1*R*,2*S*)-*cis*-2-nitrocyclohexyl acetate (1*S*,2*R*)-**100a**.
e. The principal enantiomer was (1*R*,2*R*)-*trans*-2-nitrocyclohexyl acetate (1*R*,2*R*) **100b**.

5.5.4 Evidence of a dynamic interconversion process - chiral HPLC

A solution of (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b** (100.0 mg, 0.69 mmol, 94% ee) in *tert*-butyl methyl ether (TBME) (20 mL) was prepared and aliquots [10 mL, 50.0 mg or 0.34 mmol of (1*S*,2*S*)-**99b**] were dispensed into two round bottom flasks. DBU (1.0 eq., 52 μL, 52.4 mg, 0.34 mmol) or (0.5 eq., 24 μL, 24.4 mg, 0.16 mmol) was added to the appropriate round bottom flask and stirred under nitrogen at room temperature. Reaction sampling was performed at 24 h as follows: 5 mL of reaction solvent was withdrawn and acidified to pH 1 with aqueous hydrochloric acid (10%). The mixture was extracted with TBME (3 × 5 mL), dried, filtered and concentrated under reduced pressure. The crude product was dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and analysed by chiral HPLC in conjunction with 10 μL injection volume calibration curves. The remaining reaction mixture was stirred for a further 24 h. Following work-up as per reaction sampling, analysis was again conducted by chiral HPLC in conjunction with 10 μL injection volume calibration curves (Figures 5.14 - 5.17).

*Table 5.22: Enantioenriched (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b,** 94% ee following exposure to 1.0 eq. and 0.5 eq. of DBU at 24 h and 48 h*

DBU (eq.)	**Reaction Time**	**Alcohol (±)-99**	
		***cis*-99a (%) [ee (%)][a]**	***trans*-99b (%) [ee (%)][b]**
1	0 h	-	100 [94]
	24 h	16 [31]	84 [34]
	48 h	17 [24]	83 [23]
0.5	0 h	-	100 [94]
	24 h	15 [2]	85 [3]
	48 h	13 [4]	87 [1]

a. The principal enantiomer was (1*S*,2*R*)-*cis*-2-nitrocyclohexanol (1*S*,2*R*)-**99a**.
b. The principal enantiomer was (1*S*,2*S*)-*trans*-2-nitrocyclohexanol (1*S*,2*S*)-**99b**.

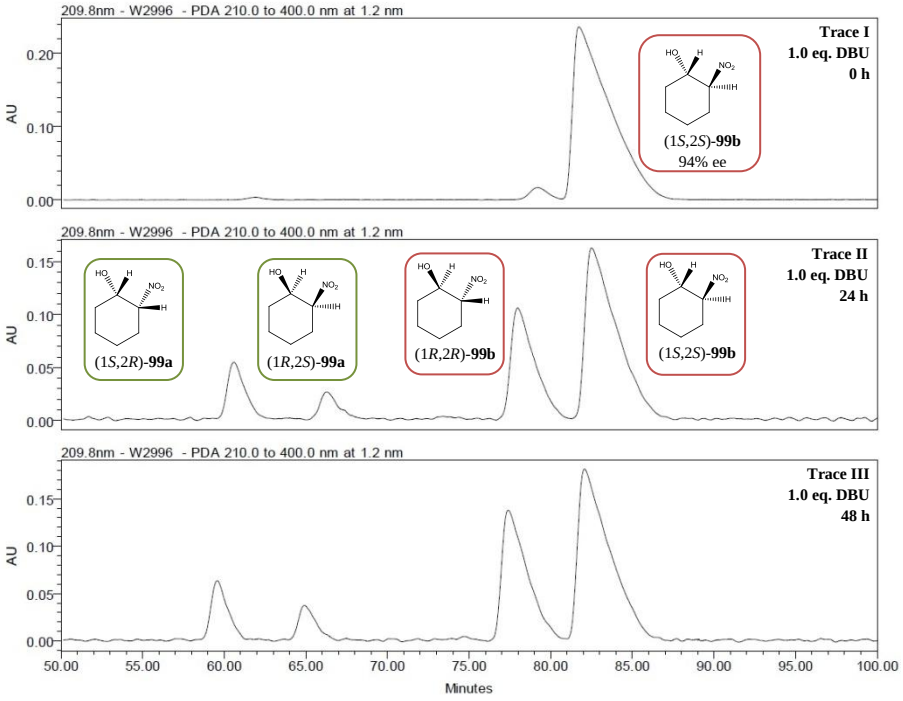


*Figure 5.14: (1S,2S)-*trans*-2-Nitrocyclohexanol (1S,2S)-**99b** 94% ee after exposure to 1.0 equivalent of DBU at 24 h and 48 h.*

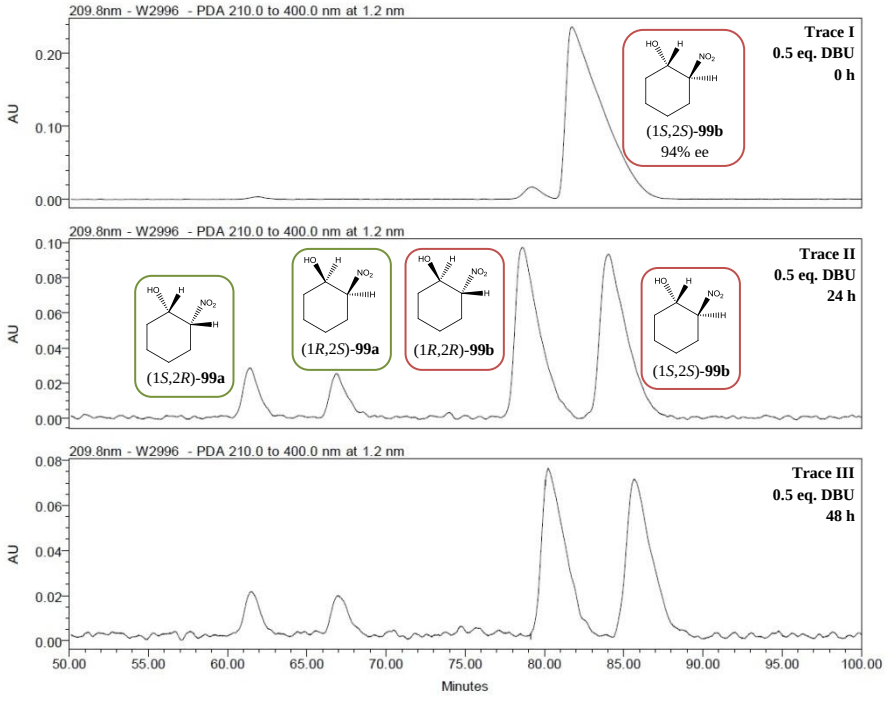


*Figure 5.15: (1S,2S)-*trans*-2-Nitrocyclohexanol (1S,2S)-**99b** 94% ee after exposure to 0.5 equivalent of DBU at 24 h and 48 h.*

Calibration curve: (±)-*cis*-2-Nitrocyclohexanol (±)-**99a (**10 µL injection volume)

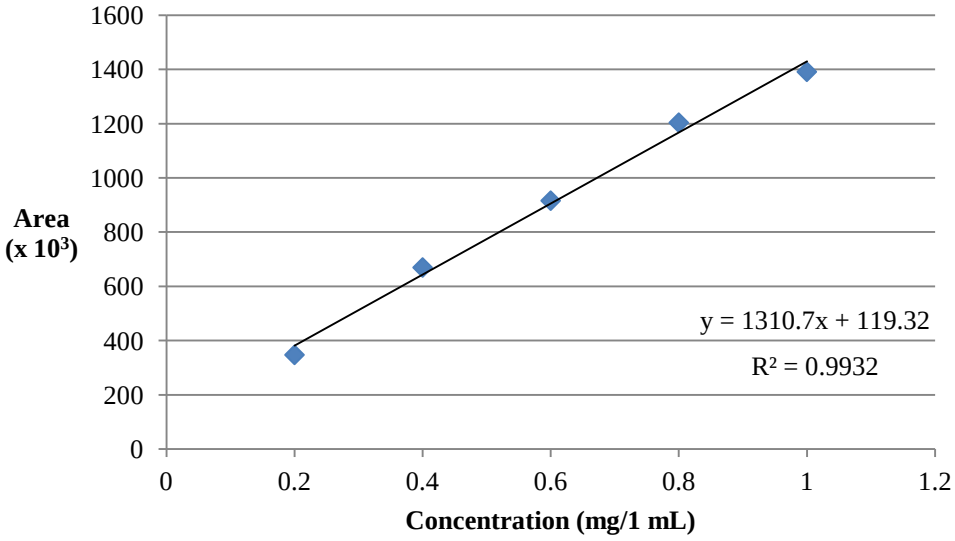


*Figure 5.16: Calibration curve (±)-*cis*-2-nitrocyclohexanol (±)-**99a** (10 µL injection). Area (x 10^3)* vs. *concentration (mg/1 mL).*

Calibration curve: (±)-*trans*-2-Nitrocyclohexanol (±)-**99b** (10 µL injection volume)

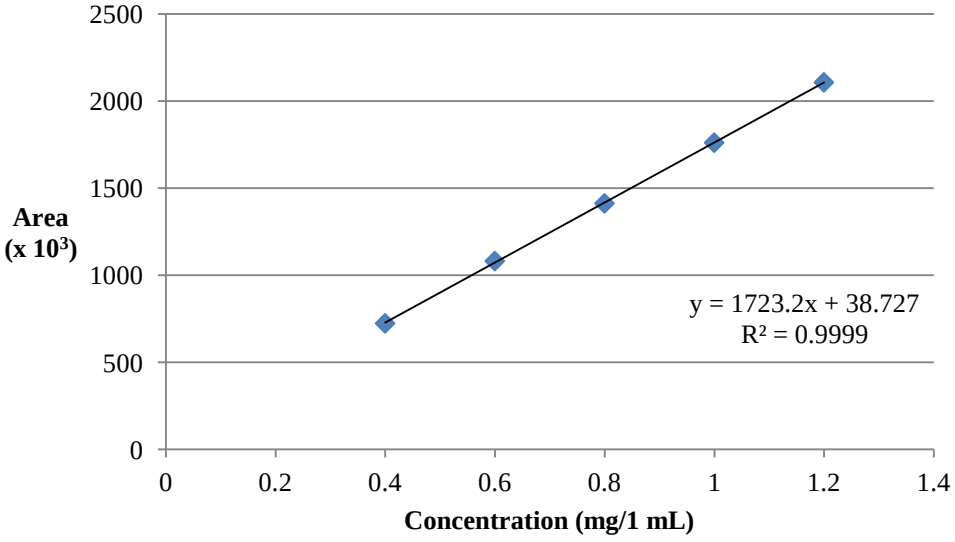


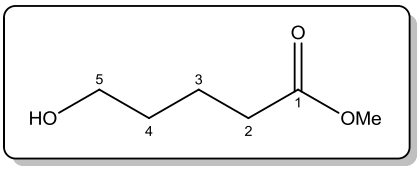
*Figure 5.17: Calibration curve (±)-*trans*-2-nitrocyclohexanol (±)-**99a** (10 µL injection). Area (x 10^3)* vs. *concentration (mg/1 mL).*

5.6 Dynamic kinetic resolution of 2-methyl-2-nitrocyclohexanol (±)-117

5.6.1 Synthesis of nitro substituted hydrolase substrates

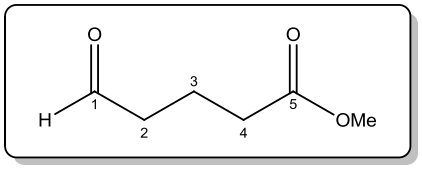
The molecular ion peak for an aliphatic mononitro compound is seldom observed. The main peaks are attributable to the hydrocarbon fragments up to M – NO_2.[67,68] Therefore where the nitro compound is novel and nominal mass spectrometry shows the molecular ion peak to be weak or absent, high resolution mass spectrometry is obtained where possible of the hydrocarbon fragment.

Methyl 5-hydroxypentanoate 137[69]



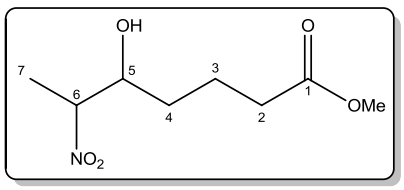
Sulfuric acid (conc. 95-97%, 1 mL, 18.76 mmol) was added to a stirred solution of δ-valerolactone **136** (15.00 g, 149.82 mmol) in methanol (269 mL). The reaction mixture was maintained at reflux while stirring for 48 h and then cooled to 0 °C and $NaHCO_3$ (1.90 g) added. The mixture was stirred for 10 min at 0 °C, then it was filtered through Celite® and the solvent removed under reduced pressure to give the crude *methyl ester* **137** (19.12 g, 97%) as a milky white oil which was used without further purification; ν_{max}/cm^{-1} (film) 3418 (OH), 2954 (CH), 1732 (CO), 1441; δ_H (300 MHz) 1.53-1.62 [2H, m, C(3)H_2], 1.64-1.76 [2H, m, C(4)H_2], 2.36 [2H, t, *J* 7.4, C(2)H_2], 3.52 (1H, br s, OH), 3.61 [2H, t, *J* 6.3, C(5)H_2], 3.67 (3H, s, OCH_3).

Methyl 5-oxopentanoate 134[69,70]



Methyl 5-hydroxypentanoate **137** (13.00 g, 98.36 mmol) was added to a solution of pyridinium chlorochromate (PCC) (31.50 g, 146.39 mmol) and crushed 4Å molecular sieves (4.80 g) in dichloromethane (473 mL) at 0 °C under nitrogen and allowed to warm slowly to room temperature. The mixture was stirred for 2 h and then diluted with diethyl ether (250 mL). The mixture was filtered through a sintered glass funnel containing a layer each of silica gel and Celite®. The filtrate was concentrated under reduced pressure and the residue redissolved in diethyl ether and the filtration process repeated to remove the remaining chromium residues. The resultant solution was concentrated under reduced pressure to produce the crude *aldehyde* **134** (9.96 g) as a light yellow oil. Purification by vacuum distillation gave the *aldehyde* **134** (6.44 g, 50%) as a clear oil; b.p. 34-49 °C at 0.1 mmHg (Lit.,[71] 80 °C at 0.2 mmHg); ν_{max}/cm^{-1} (film) 2936 (CH), 1733 (CO), 1198; δ_H (300 MHz) 1.91-2.01 [2H, quintet, *J* 7.2, C(3)H_2], 2.38 [2H, t, *J* 7.2, C(2)H_2], 2.54 [2H, dt, *J* 1.2, 7.2, C(4)H_2], 3.68 (3H, s, OCH_3), 9.78 [1H, t, *J* 1.2, C(1)H].

Methyl 5-hydroxy-6-nitroheptanoate 135[72]



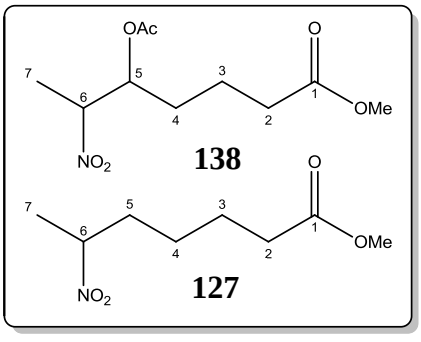
Powdered potassium *tert*-butoxide (0.92 g, 8.21 mmol) was added to a solution of methyl 5-oxopentanoate **134** (5.36 g, 41.22 mmol) and nitroethane (8.8 mL, 123.83 mmol) in *tert*-butanol : tetrahydrofuran (1 : 1, 50 mL) at 0 °C under nitrogen. The reaction mixture was stirred at room temperature overnight, and then diluted with diethyl ether (100 mL) and water (100 mL). The solution was transferred to a separating funnel and the layers were separated. The organic layer was washed with a saturated aqueous solution of sodium bicarbonate (100 mL) and

brine (100 mL). The combined aqueous layers were extracted with diethyl ether (2 × 200 mL) and the combined organic layers were dried, filtered, and concentrated under reduced pressure to give a crude equimolar mixture of *nitro alcohol* diastereomers **135** (10.05 g) as a yellow oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave a equimolar mixture of pure *nitro alcohol* diastereomers **135** (5.25 g, 62%) as a light yellow oil; (Found C, 46.89; H 7.37; N 6.56. $C_8H_{15}NO_5$ requires C, 46.82; H, 7.37; N, 6.83%); ν_{max}/cm^{-1} (film) 3444 (OH), 2955 (CH), 1728 (CO), 1557 (NO_2), 1393; δ_H (300 MHz) 1.38-1.62 {5H, m containing 2 × d, 1.54 [1.5H, d, *J* 6.9, C(7)H_3 of 1 diastereomer], 1.55 [1.5H, d, *J* 6.9, C(7)H_3 of 1 diastereomer] and [2H, m, C(4)H_2 of 2 diastereomers]}, 1.63-1.96 [2H, m, C(3)H_2 of 2 diastereomer], 2.39 [2H, t, *J* 7.1, C(2)H_2 of 2 diastereomers], 2.91-2.95 (1H, OH, of 2 diastereomers), 3.68 (3H, s, OCH_3, of 2 diastereomers), 3.90-3.98 [0.5H, m, C(5)H, of 1 diastereomer], 4.14-4.21 [0.5H, m, C(5)H, of 1 diastereomer], 4.47-4.59 [1H, m, C(6)H of 2 diasteromers]. δ_C (75.5 MHz) 12.5, 16.1 [2 x CH_3, C(7)H_3 of 2 diastereomer], 20.3, 21.0 [2 x CH_2, C(3)H_2 of 2 diastereomer], 32.1, 32.3 [2 x CH_2, C(4)H_2 of 2 diastereomer], 33.3 [CH_2, C(2)H_2 of 2 diastereomers], 51.7 (CH_3, OCH_3 of 2 diastereomers), 71.7, 72.4 [2 x CH, C(5)H, of 2 diastereomers], 86.4, 87.7 [2 x CH, C(6)H, of 2 diastereomers], 174.0 [C, C(1) of 2 diastereomers]; HRMS (ES+): Exact mass calculated for $C_8H_{16}NO_5$ $(M+H)^+$ 206.1028 Found 206.1024; m/z (ES+) 206.3 [$(M+H)^+$, 8%], 152.5 (14%), 233.2 (9%), 269.2 (8%), 391.3 (18%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note:

1. *Potassium* tert-*butoxide and nitroethane were obtained commercially from Sigma-Aldrich and used without further purification.*
2. *An additional signal present due to* tert-*butanol ~51%, δ_H (300MHz) 1.27 [9H, s, C(CH_3)$_3$] was evident in the crude ^{1}H NMR spectrum which lead to an increased crude yield (10.05 g, ~119%)*
3. *Purification by column chromatography on silica gel was conducted in the above procedure in order to obtain analytically pure nitro alcohol **135** for full characterisation. In all subsequent reactions the crude nitro alcohol **135** (typical crude yield 76%, >95% pure by ^{1}H NMR spectroscopy) was used without further purification.*

Methyl 5-acetoxy-6-nitroheptanoate 138[72] and methyl 6-nitroheptanoate 127[73]



A solution of an equimolar mixture of methyl 5-hydroxy-6-nitroheptanoate **135** diastereomers (5.00 g, 24.37 mmol), *N*,*N*-dimethylaminopyridine (150 mg, 1.23 mmol), acetic anhydride (2.5 mL, 26.71 mmol) and diethyl ether (62 mL) was stirred for 4 h at room temperature under nitrogen and concentrated under reduced pressure to yield the crude equimolar mixture of *nitro acetate* diastereomers **138** as a bright green oil; δ_H (300 MHz) 1.548 [1.5H, d, *J* 6.9, C(7)H_3 of 1 diastereomer] 1.554 [1.5H, d, *J* 6.9, C(7)H_3 of 1 diastereomer], 1.58-1.80 [4H, m, C(3)H_2 and C(4)H_2 of 2 diastereomers], 2.06 (1.5H, s, $COCH_3$ of 1 diastereomer) 2.09 (1.5H, s, $COCH_3$ of 1 diastereomer), 2.25-2.47 [2H, m, C(2)H_2 of 2 diastereomers], 3.68 (3H, s, OCH_3 of 2 diastereomers), 4.65-4.77 [1H, m, C(5)H of 2 diasteromers], 5.29-5.35 [1H, m, C(6)H of 2 diasteromers].

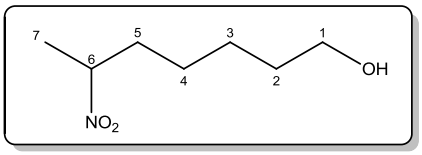
Note:

1. *Integration of the 3H, s, $COCH_3$ of 1 diastereomer at δ_H (300MHz) 2.09 is slightly higher due to overlap with acetic acid.*
2. *To distinguish between the diastereomers, signals are reported to three decimal places for the C(7)H_3 doublet.*

A suspension of $NaBH_4$ (0.68 g, 17.98 mmol) in dimethylsulfoxide (63 mL) was added dropwise to the crude *nitro acetates* **138** at 0 °C under nitrogen and the solution was stirred overnight at room temperature. The reaction mixture was then acidified with aqueous hydrochloric acid (1M), extracted with diethyl ether (3 × 100mL), dried, filtered and concentrated under reduced pressure to yield the crude *methyl ester* **127** (3.42 g, 74%) as a clear oil which was used without further purification (>95% pure by ^{1}H NMR spectroscopy); ν_{max}/cm^{-1} (film) 2953 (CH), 1738 (CO), 1552 (NO_2), 1361 (NO_2); δ_H (300 MHz) 1.26-1.44 [2H, m, C(4)H_2], 1.53 [3H, d, *J* 6.9, C(7)H_3], 1.61-1.80 [3H, m. C(3)H_2 and one of C(5)H_2], 1.92-2.13 [1H, m, one of C(5)H_2], 2.32 [2H, t, *J* 7.4, C(2)H_2], 3.67 (3H, s, OCH_3), 4.51-4.62 [1H, sym m, C(6)H]. ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

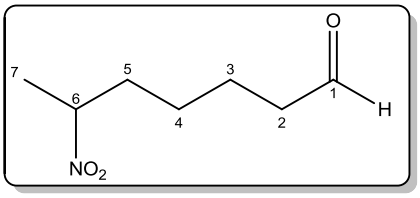
Note: This procedure was adapted from Morrow[74]

6-Nitroheptan-1-ol 130



This was prepared following the procedure described for **108** from methyl 6-nitroheptanoate **127** (2.54 g, 13.45 mmol), doubly distilled dichloromethane (100 mL) and DIBAL-H (38 mL, 38.00 mmol, 1M solution in hexanes) to give the crude *nitro alcohol* **130** (1.67 g) as a cloudy yellow oil (~95% pure by ^{1}H NMR spectroscopy). Purification by chromatography on silica gel using hexane/ethyl acetate 80/20 as eluent gave the pure *nitro alcohol* **130** (1.26 g, 58%) as a clear oil; (Found C, 52.18; H 9.21; N 8.33. $C_7H_{15}NO_3$ requires C, 52.16; H, 9.38; N, 8.69%); ν_{max}/cm^{-1} (film) 3365 (OH), 2940 (CH), 1553 (NO_2), 1391 (NO_2); δ_H (300 MHz) 1.26-1.48 [4H, m, C(3)H_2 and C(4)H_2], 1.52-1.61 {5H, m containing 1.53 [3H, d, *J* 6.6, C(7)H_3] and [2H, m, C(2)H_2]2, 1.64-1.79 [1H, m, one of C(5)H_2], 1.86 (1H, br s, OH), 1.96-2.08 [1H, m, one of C(5)H_2], 3.63 [2H, t, *J* 6.5, C(1)H_2], 4.52-4.63 [1H, sym m, C(6)H]; δ_C (75.5 MHz) 19.2 [CH_3, C(7)H_3], 25.2, 25.5 [2 x CH_2, C(3)H_2 and C(4)H_2], 32.2 [CH_2, C(2)H_2], 35.1 [CH_2, C(5)H_2], 62.5 [CH_2, C(1)H_2], 83.5 [CH, C(6)H]; Exact mass calculated for $C_7H_{16}NO_3$ $(M+H)^+$ 162.1130 Found 162.1122; m/z (ES+) 102 (86%), 143 (59%), 284 (52%), 334 (100%), 336 (60%), 466 (50%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

6-Nitroheptanal 118

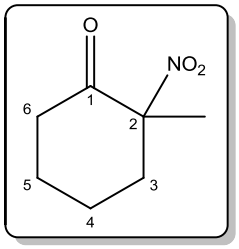


This was prepared following the procedure described for **101** from pyridinium chlorochromate (PCC) (2.00 g, 9.28 mmol), crushed 3Å molecular sieves (0.42 g), 6-nitrohexan-1-ol **108** (0.68 g, 4.19 mmol) and doubly distilled dichloromethane (24 mL) to yield the crude *aldehyde* **118** (0.58 g) as a light brown oil (~72% pure by ^{1}H NMR spectroscopy). Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure *aldehyde* **118** (0.44 g, 65%) as a clear oil; (Found C, 52.55; H 8.20; N 8.71. $C_7H_{13}NO_3$ requires C, 52.82; H, 8.23; N, 8.80%); ν_{max}/cm^{-1} (film) 2943 (CH), 1724 (CO), 1549 (NO_2), 1392 (NO_2); δ_H (300 MHz) 1.27-1.47 [2H, m, C(4)H_2], 1.53 [3H, d, *J* 6.6, C(7)H_3], 1.59-1.82 [3H, m. C(3)H_2 and one of C(5)H_2], 1.94-2.12 [1H, m, one of C(5)H_2], 2.44-2.50 [2H, dt, *J* 1.2, 7.2, C(2)H_2], 4.51-4.63 [1H, m, C(6)H], 9.77 [1H, t, *J* 1.4 C(1)H]; δ_C (75.5 MHz) 19.2 [CH_3, C(7)H_3], 21.3 [CH_2, C(3)H_2], 25.2 [CH_2, C(4)H_2], 34.8 [CH_2, C(5)H_2], 43.4 [CH_2, C(2)H_2], 83.2 [CH, C(6)H], 201.8 [CH, C(1)H]; Exact mass calculated for $C_7H_{14}NO_3$ $(M+H)^+$

160.0974 Found 160.0972; m/z (ES+) 135 (48%), 143 (100%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

*Note: 6-Nitroheptanal **118** was found to be unstable in the lab atmosphere and easily oxidised to the carboxylic acid. Storage in the freezer at -20 °C was found to slow down the oxidation process but not to halt it. Therefore 6-nitroheptanal **118** was freshly purified by column chromatography prior to use.*

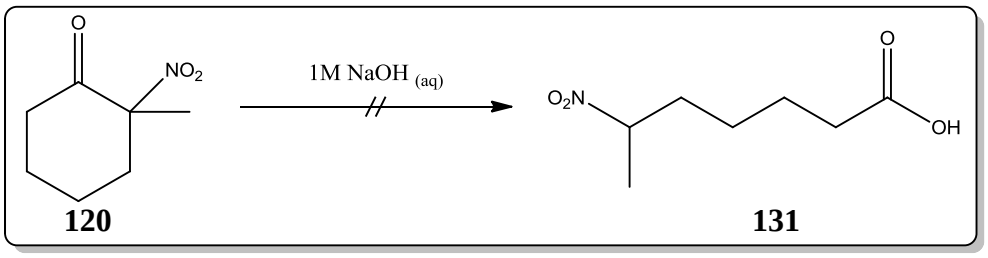
2-Methyl-2-nitrocyclohexanone 120[73]



2-Nitrocyclohexanone **103** (2.50 g, 17.47 mmol) in dichloromethane (19 mL) was added in one portion to a stirred solution of tetrabutylammonium hydroxide (11.33 g, of a 40% aq. solution, 17.47 mmol) in water (17 mL) under nitrogen. The reaction mixture was stirred for 10 min, and then methyl iodide (4.4 mL, 69.87 mmol) was added in one portion. The reaction mixture was stirred vigorously for 36 h at room temperature. The reaction mixture was then transferred to a separating funnel and layers separated. The organic layer was washed with water (20 mL). The aqueous phase was extracted with dichloromethane (2 × 30 mL) and the combined organic fractions were dried, filtered and concentrated under reduced pressure in an ice cold water bath. Diethyl ether (60 mL) was added to precipitate the tetrabutylammonium iodide salt, the solution was filtered and concentrated under reduced pressure in an ice cold water bath to give a crude mixture (1.54 g) of 2-methyl-2-nitrocyclohexanone **120** and ring cleavage products, methyl 6-nitrohexanoate **116** and methyl 6-nitroheptanoate **127** (76 : 13 : 11 respectively) as an orange oil. Purification by column chromatography on silica gel using hexane/diethyl ether 97/3 as eluent gave the pure *α-nitro ketone* **120** (0.78 g, 36%) as a colourless oil; ν_{max}/cm^{-1} (film) 2950, 2873 (CH), 1732 (CO), 1549 (NO_2); δ_H (600 MHz) 1.67 [3H, s, C(2)CH_3], 1.69-1.85 (4H, m, ring protons), 1.98-2.10 (1H, m, ring protons), 2.55-2.64 (2H, m, ring protons), 2.86-2.90 (1H, m, ring protons).

*Note: Signals for methyl 6-nitroheptanoate **127** and methyl 6-nitrohexanoate **116** were detected in the ^{1}H NMR of the crude product spectrum at δ_H (300MHz) 4.49-4.68 (1H, sym m, $CHNO_2$) and at δ_H (300MHz) 4.40 (2H, t, J 7.1 CH_2NO_2) respectively.*

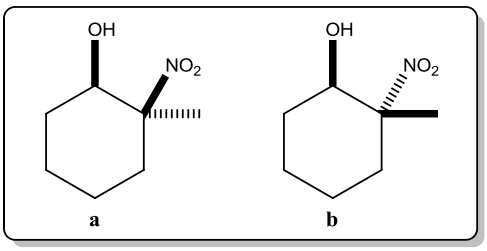
Attempted ring opening of 2-methyl-2-nitrocyclohexanone 120[5]



A solution of 2-methyl-2-nitrocyclohexanone **120** (166.5 mg, 1.06 mmol) in aqueous sodium hydroxide (20 mL, 1.0 M) was heated under reflux for 2 h. The reaction mixture was allowed to cool to room temperature and washed with diethyl ether (20 mL). The aqueous layer was acidified to pH 1-2 with aqueous hydrochloric acid (10%) and subsequently extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water (20 mL), brine (20 mL) and the combined organic fractions were dried, filtered and concentrated under reduced pressure to give the crude product (94.7 mg) as a bright blue oil. A ^{1}H, ^{13}C NMR and IR spectrum of the crude product were recorded, with signals characteristic of a single product however the signals did not correspond to 6-nitroheptanoic acid **131** and the structure

of the crude product was not determined; ν_{max}/cm^{-1} (film) 3421, 2937, 1711 (CO), 1568 (NO_2), 1412 (NO_2), 1199; δ_H (300 MHz) 1.52-1.76 (~6H, m), 2.15 (~3H, s), 2.33-2.40 (3H, m), 2.42-2.51 (2H, m), 9.61 (br s); δ_C (75.5 MHz) 23.0, 24.1 (2 × CH_2), 29.8 (CH_3), 33.7, 43.2 (2 × CH_2), 179.2, 208.9 (2 × C).

(±)-2-Methyl-2-nitrocyclohexanol (±)-117[75]



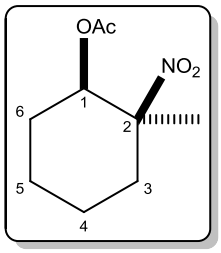
This was prepared following the procedure described for **99** from 2-methyl-2-nitrocyclohexanone **120** (0.98 g, 6.24 mmol) in distilled ethanol (10 mL) and $NaBH_4$ (0.24 g, 6.24 mmol) in distilled ethanol (25 mL) to give a crude mixture (0.72 g) of *nitroalcohols* (±)-**117a** and (±)-**117b** (41 : 59 respectively) as a yellow oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 97/3 as eluent gave three fractions.

The first (least polar) fraction was the pure *(±)*-cis-*2-methyl-2-nitrocyclohexanol* (±)-**117a** (186.6 mg, 19%) as a light yellow low melting solid, m.p. 33-35 °C; (Found C, 52.30; H 8.02; N 8.50. $C_7H_{13}NO_3$ requires C, 52.82; H, 8.23; N, 8.80%); ν_{max}/cm^{-1} (film) 3445 (OH), 2945 (CH), 1539 (NO_2), 1352 (NO_2); δ_H (600 MHz) 1.39-1.46 [1H, m, one of C(5)H_2], 1.48-1.52 [2H, m, C(4)H_2], 1.63 [3H, s, C(2)CH_3], 1.68-1.77 [3H, m, one of C(5)H_2, one of C(3)H_2 and one of C(6)H_2], 1.82-1.87 [1H, m, one of C(6)H_2], 2.43-2.48 [1H, m, one of C(3)H_2], 2.84 [1H, d, *J* 8.4, OH], 3.91 [1H, br s, C(1)H]; δ_C (150 MHz) 21.5 [CH_2, br, C(5)H_2], 21.9 [CH_2, C(4)H_2], 24.2 [CH_3, C(2)***C***H_3], 30.6 [CH_2, C(6)H_2], 32.9 [CH_2, br, C(3)H_2], 73.1 [CH, br, C(1)H], 91.5 [C, C(2)]; HRMS (ES+): Exact mass calculated for $C_7H_{14}NO_3$ $[M+H]^+$ 160.0974 Found 206.0974; m/z (ES+) 118 (22%), 242 (48%), 243 (32%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

The second fraction was a 28 : 72 mixture of *cis : trans (±)-2-methyl-2-nitrocyclohexanol*, (±)-**117a** and (±)-**117b** (38.5 mg, 4%) as a clear oil.

The third (most polar) fraction was the pure *(±)*-trans-*2-methyl-2-nitrocyclohexanol* (±)-**117b** (274.6 mg, 28%) was isolated as a white crystalline solid, m.p. 38-41 °C; (Found C, 53.06; H 8.17; N 8.62. $C_7H_{13}NO_3$ requires C, 52.82; H, 8.23; N, 8.80%); ν_{max}/cm^{-1} (KBr) 3376 (OH), 2947 (CH), 1538 (NO_2), 1340 (NO_2); δ_H (600 MHz) 1.33-1.47 [3H, m, one of C(4)H_2, one of C(5)H_2 and one of C(6)H_2], 1.61 [3H, s, C(2)CH_3], 1.70-1.73 [1H, m, one of C(4)H_2] 1.76-1.79 [1H, m, one of C(5)H_2], 1.86-1.91 [1H, m, one of C(3)H_2], 1.95-1.99 [1H, m, one of C(6)H_2], 2.13-2.16 [1H, m, one of C(3)H_2], 2.74 [1H, s, OH], 4.28-4.29 [1H, m, C(1)H]; δ_C (150 MHz) 16.3 [CH_3, br, C(2)***C***H_3], 22.1 [CH_2, C(4)H_2], 23.5 [CH_2, C(5)H_2], 30.6 [CH_2, C(6)H_2], 35.9 [CH_2, br, C(3)H_2], 72.4 [CH, C(1)H], 93.0 [C, C(2)];]; HRMS (ES+): Exact mass calculated for $C_7H_{12}NO_2$ $[(M+H)^+-H_2O]^+$ 142.0868 Found 142.0864; m/z (ES+) 141 {$[(M+H)^+-H_2O]^+$, 100%}, 245.3 (28%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

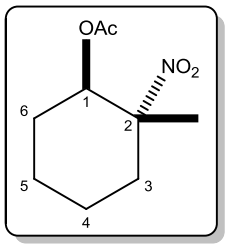
(±)-*cis*-2-Methyl-2-nitrocyclohexyl acetate (±)-119a



This was prepared following the procedure for (±)-**100b**, from *N*,*N*-dimethylaminopyridine (2 mg, 0.02 mmol), (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (131.8 mg, 0.83 mmol), acetic anhydride (0.5 mL, 5.80 mmol) and pyridine (0.3 mL, 3.39 mmol) in dichloromethane (5 mL) to give the crude *acetate* (±)-**119a** (145.8 mg, 88%) as a clear oil. which solidified on cooling to a white crystalline solid which was sufficiently pure to use without further purification, m.p. 46-48 °C; (Found C, 54.04; H 7.52; N 6.00. $C_9H_{15}NO_4$ requires C, 53.72; H, 7.51; N, 6.96%); ν_{max}/cm^{-1} (KBr) 2960 (CH), 1738 (CO), 1544 (NO_2), 1362 (NO_2); δ_H (300 MHz) 1.26–1.48 [3H, m, C(5)H_2 and one of C(4)H_2], 1.54-1.67 {4H, m containing 1.54 [3H, s, C(2)CH_3] and [1H, m, one of C(6)H_2]}, 1.70-1.82 [1H, m, one of C(4)H_2], 1.88-2.05 {5H, m containing 1.95 [3H, s, $COCH_3$] and [2H, m, one of C(6)H_2 and one of C(3)H_2]}, 2.22-2.32 [1H, m, one of C(3)H_2], 5.26-5.29 [1H, m, C(1)H]; δ_C (75.5 MHz) 18.6 [CH_2, C(5)H_2], 19.9 [CH_3, C(2)***C***H_3], 20.9 [CH_2, C(4)H_2], 22.1 [CH_3, CO***C***H_3], 25.8 [CH_2, C(6)H_2], 29.7 [CH_2, C(3)H_2], 72.4 [CH, C(1)H], 87.5 [C, C(2)], 168.6 [C, ***C***OCH_3];]; HRMS (ES+): Exact mass calculated for $C_9H_{15}O_2$ $(M\text{-}NO_2)^+$ 155.1072 Found 155.1065; m/z (ES+) 94.2 (100%), 155.4 [$(M\text{-}NO_2)^+$, 18%], 242.5 (90%), 243.5 (20%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

The structure of (±)-**119a** was determined by single crystal X-ray diffraction on a crystalline sample of (±)-**119a** recrystallised from slow evaporation of deuterated solvent $CDCl_3$.[76] Crystal data: $C_9H_{15}NO_4$, M = 201.22, orthorhombic, a = 20.418(8) Å, b = 25.919(16) Å, c =8.013(2) Å, V = 4241.(3)Å^3, T = 296.(2) K, space group *Fdd*2, Z = 16, 11831 reflections measured, 2148 unique (*Rint* = 0.0597). The final *R1* values were 0.0396 ($I > 2\sigma(I)$) and 0.0643 (all data). The final *wR*(*F*2) values were 0.0912 ($I > 2\sigma(I)$) and 0.1022 (all data). Full structural details are contained on the accompanying CD.

(±)-*trans*-2-Methyl-2-nitrocyclohexyl acetate (±)-119b



This was prepared following the procedure for (±)-**100b**, from *N*,*N*-dimethylaminopyridine (2 mg, 0.02 mmol), (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (152.8 mg, 0.96 mmol), acetic anhydride (0.6 mL, 6.68 mmol) and pyridine (0.3 μL, 3.97 mmol) in dichloromethane (5 mL) to give the crude *acetate* (±)-**119b** (137.5 mg, 71%) as a light yellow oil which was sufficiently pure to use without further purification; (Found C, 54.34; H 7.62; N 6.78. $C_9H_{15}NO_4$ requires C, 53.72; H, 7.51; N, 6.96%); ν_{max}/cm^{-1} (film) 2950 (CH), 1748 (CO), 1538 (NO_2), 1361 (NO_2); δ_H (300 MHz) 1.33–1.52 [3H, m, one of C(4)H_2, one of C(5)H_2 and one of C(6)H_2], 1.62-1.81 {5H, m containing 1.65 [3H, s, C(2)CH_3] and [2H, m, one of C(5)H_2 and one of C(4)H_2]}, 1.95-2.19 {6H, m containing 2.03 (3H, s, $COCH_3$) and [3H, m, C(3)H_2 and one of C(6)H_2]}, 5.50-5.55 [1H, m, C(1)H]; δ_C (75.5 MHz) 17.6 [CH_3, C(2)***C***H_3], 20.9 [CH_3, CO***C***H_3], 21.9, 22.7 [CH_2, C(5)H_2, and C(4)H_2], 28.1 [CH_2, C(6)H_2], 36.5 [CH_2, C(3)H_2], 73.9 [CH, C(1)H], 90.4 [C, C(2)], 169.5 (C, ***C***OCH_3); Exact mass calculated for $C_9H_{15}O_2$ $(M\text{-}NO_2)^+$ 155.1072 Found 155.1072; m/z (ES+) 141 (78%), 242 (54%), 300 (43%), 344 (100%), 388 (86%). ^{1}H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

5.6.2 Hydrolase-mediated kinetic resolution – analytical screens

Note:

1. *Notes 1-5 in section 5.3.2 (General procedure for the hydrolase-mediated kinetic resolution of the β-substituted 3-aryl alkanoic ethyl esters) apply to all screening protocols.*
2. *^{1}H NMR conversion was calculated for the hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** by analysis of the integrals 1H, br s, C(1)**H**OH at 3.91 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and the 1H, m, C(1)HOAc at 5.26-5.29 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexylacetate (±)-**119a.***
3. *^{1}H NMR conversion was calculated for the hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** by analysis of the integrals 1H, m, C(1)**H**OH, at 4.28-4.29 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and the 1H, m, C(1)HOAc at 5.50-5.55 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexylacetate (±)-**119b**.*

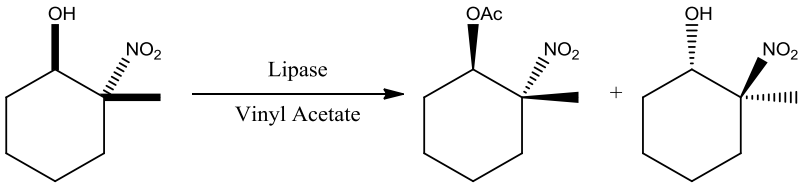
5.6.2.1 Analytical screen – acetylating agent as both acyl donor and solvent

General procedure for the hydrolase-mediated transesterification of (±)-2-methyl-2-nitrocyclohexanol (±)-117a or (±)-117b with an acyl donor as both acetylating agent and solvent.

Procedure followed was kindly supplied by Almac Sciences[44]

A spatula tip of enzyme (~5-10 mg, amount not critical) was added to the alcohol substrate (±)-**117a** or (±)-**117b** (~20 mg) dissolved in the appropriate acyl donor, vinyl acetate (1 mL), vinyl pivalate (1 mL) or ethyl acetate (1 mL). The small test tubes were sealed and agitated at 750 rpm at 24 °C. The solution was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screens are summarised in Table 5.23 - 5.25.

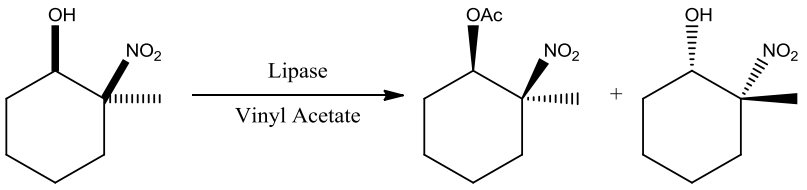
*Table 5.23: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1	*Candida cylindracea* C1	48 h	33	43	61	>98	185
2	*Pseudomonas cepacia* P2	48 h	-	<10	-	-	-
3	*Candida antarctica* B (immob)	72 h	49	48	96	>98	>200
4	*Pseudomonas stutzeri*	48 h	50	51	97	>98	>200
5	*Pseudomonas fluorescens*	113.5 h	-	<10	-	-	-

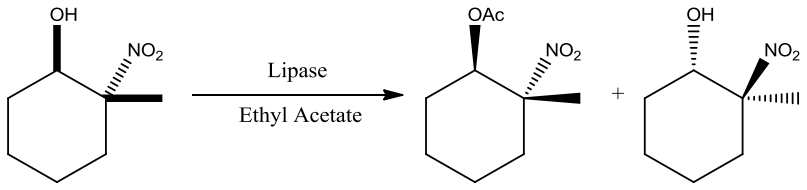
*Table 5.24: Hydrolase-mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** in vinyl acetate*



(±)-**117a** (1*R*,2*S*)-**119a** (1*S*,2*R*)-**117a**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		*E* value
			E Calc.	^{1}H NMR	Alcohol *cis*-117a (1*S*, 2*R*)	Acetate *cis*-119a (1*R*, 2*S*)	
1	*Pseudomonas cepacia* P2	48 h	-	<10	-	-	-
2	*Candida antarctica* B (immob)	72 h	33	47	49	>98	159
3	*Pseudomonas stutzeri*	48 h	78	85	>98	27	6.4
4	*Pseudomonas fluorescens*	113.5 h	40	45	64	96	95

*Table 5.25: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in ethyl acetate*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Enzyme Source	Reaction Time	Conversion (%)		ee (%)		E Value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*, 2*S*)	Acetate *trans*-119b (1*R*, 2*R*)	
1	*Candida antarctica* B (immob)	72 h	16	18	19	>98	119
2	*Pseudomonas stutzeri*	48 h	-	<10	-	-	-

5.6.2.2 Analytical screen – vinyl acetate as acyl donor and alteration of solvents

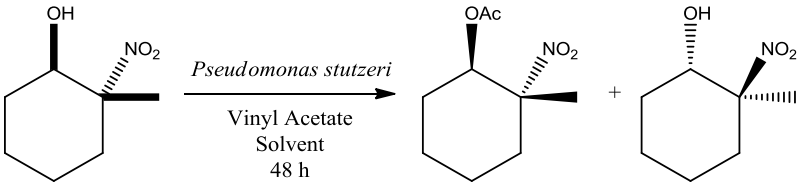
General procedure for the hydrolase-mediated transesterification of (±)-2-methyl-2-nitrocyclohexanol (±)-117a or (±)-117b with vinyl acetate as acyl donor and alteration of solvents.

Procedure followed was kindly supplied by Almac Sciences[44]

A spatula tip of *Pseudomonas stutzeri* (~5-10 mg, amount not critical) was added to the alcohol substrate (±)-**117a** or (±)-**117b** (~20 mg, 0.13 mmol) dissolved in the appropriate solvent (2.5 mL). Vinyl acetate (5 eq., 58 μL, 54.1 mg, 0.63 mmol) was added to each test tube. The small test tubes were sealed and agitated at 750 rpm at 24 °C for 48 h. The solution was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy, reconcentrated and dissolved

in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screens are summarised in Table 5.26.

*Table 5.26: Hydrolase-mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate as acyl donor and alteration of solvents*



(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

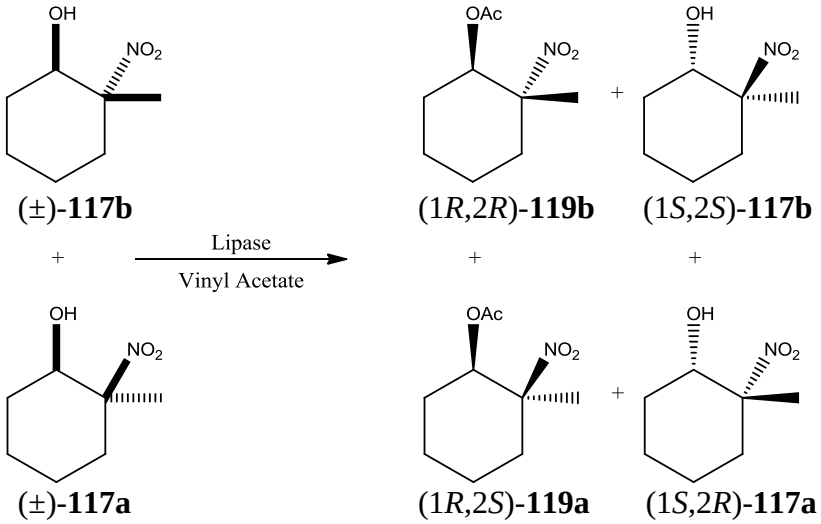
Entry	Enzyme Source	Solvent	Conversion (%)		ee (%)[a]		*E* value
			E **Calc.**	**^{1}H NMR**	**Alcohol *trans*-117b (1*S*,2*S*)**	**Acetate *trans*-119b (1*R*,2*R*)**	
1	*Pseudomonas stutzeri*	Heptane	40	41	66	>98	197
2	*Pseudomonas stutzeri*	Diethyl ether	46	44	82	>98	>200
3	*Pseudomonas stutzeri*	Diisopropyl ether	46	45	84	>98	>200
4	*Pseudomonas stutzeri*	Toluene	20	21	24	>98	125
5	*Pseudomonas stutzeri*	Acetonitrile	25	26	32	>98	135

a. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

*5.6.2.3 Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-117a or (±)-117b*

Pseudomonas stutzeri (39.4 mg) or *Candida antarctica* lipase B (immob) (88.6 mg) was added to a equimolar mixture of (±)-*cis* and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** (105.4 mg, 0.66 mmol) dissolved in vinyl acetate (5 mL). The reaction mixture was shaken at 750 rpm at 24 °C. Reaction monitoring was conducted as follows; an aliquot (0.5 - 1 mL) of reaction mixture was isolated and filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy. The final extraction following ^{1}H NMR spectroscopy was dissolved in a mixture of isopropanol/hexane [10 : 90 (HPLC grade)] and enantioselectivity determined by chiral HPLC. The results of the screens are summarised in Table 5.27 and Figure 5.18.

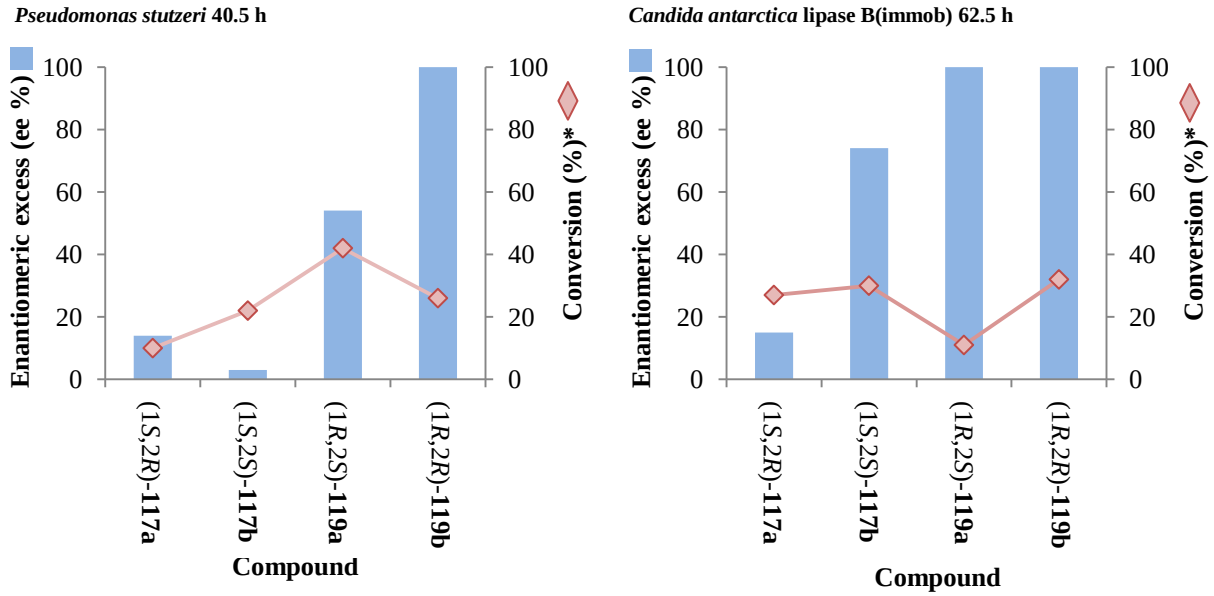
*Table 5.27: Diastereoselective hydrolase-mediated transesterification of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** or (±)-**117b** in vinyl acetate*



Enzyme Source	Reaction Time	Alcohol (±)-117		Acetate (±)-119	
		***cis*-117a (%)[a] [ee (%)][b]**	***trans*-117b (%)[a] [ee (%)][c]**	***cis*-119a (%)[a] [ee (%)][d]**	***trans*-119b (%)[a] [ee (%)][e]**
Pseudomonas stutzeri	18.5 h	12	27	39	22
	40.5 h	10 [14]	22 [3]	42 [54]	26 [>98]
Candida antarctica B (immob)	12 h	44	38	3	15
	18.5 h	43	36	4	17
	40.5 h	41	30	6	23
	62.5 h	27 [15]	30 [74]	11 [>98]	32 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the spectrum of the mixture of the crude material not mass recovery.
b. The principal enantiomer was (1*S*, 2*R*)-*cis*-2-methyl-2-nitrocyclohexanol (1*S*,2*R*)-**117a**.
c. The principal enantiomer was (1*S*, 2*S*)-*trans*-2-methyl-2-nitrocyclohexanol (1*S*,2*S*)-**117b**.
d. The principal enantiomer was (1*R*, 2*S*)-*cis*-2-methyl-2-nitrocyclohexyl acetae (1*S*,2*R*)-**119a**.
e. The principal enantiomer was (1*R*, 2*R*)-*trans*-2-methyl-2-nitrocyclohexyl acetate (1*R*,2*R*)-**119b**.

*Comparison of conversion (%) and enantiomeric excess (ee %) of **117a**, **117b**, **119a** and **119b** following diastereoselective hydrolase-mediated transesterification*

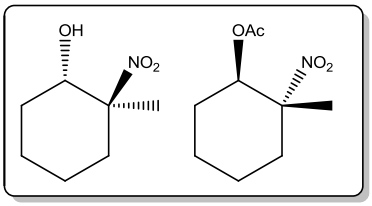


**Note: Conversion (%) illustrated as percentage of material in the crude product.*

Figure 5.18

5.6.3 Hydrolase-mediated kinetic resolution – preparative-scale

Large scale hydrolase-mediated transesterfication of (±)-*trans*-2-methyl-2-nitro cyclohexanol (±)-117b

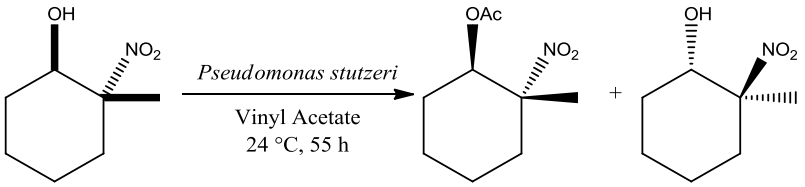


This was prepared following the procedure described for the hydrolase-mediated tranesterification of (±)-**99b** from *Pseudomonas stutzeri* (60.0 mg) and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (207.6 mg, 1.30 mmol) in vinyl acetate (7 mL). The reaction mixture was shaken at 750 rpm at 24 °C. An aliquot of reaction mixture (0.5 mL) was withdrawn at 48 h. Following a mini work-up, ^{1}H NMR and chiral HPLC analysis was conducted. The solution was filtered at 52.5 h to produce a clear oil (156.3 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 3-25% ethyl acetate) gave the pure *(1*R,*2*R*)*-trans-*2-methyl-2-nitrocyclohexyl acetate* (1*R*,2*R*)-**119b** (95.5 mg, 36%) as a clear oil $[\alpha]_D^{20}$ −32.6 (c 1.0, $CHCl_3$), >98% ee, and the pure *(1*S,*2*S*)*-trans-*2-methyl-2-nitrocyclohexanol* (1*S*,2*S*)-**117b** (72.1 mg, 35%) as a clear oil $[\alpha]_D^{20}$ +42.0 (c 1.0, $CHCl_3$), 98% ee. Conversion estimated by *E*-value calculator at 50%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. *Yield may be reduced due to sample being withdrawn during reaction monitoring at 48 h.*
2. *Chiral HPLC analysis was not conducted on the crude reaction mixture at 52.5 h, but rather on the isolated products (1*S,*2*S*)-**117b** and (1*R,*2*R*)-**119b** following purification by column chromatography.*

Table 5.28: Large scale Pseudomonas stutzeri *mediated transesterification of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** in vinyl acetate*

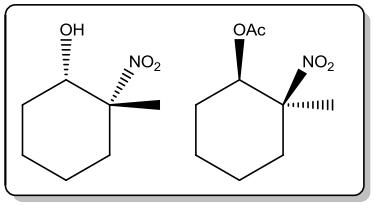


(±)-**117b** (1*R*,2*R*)-**119b** (1*S*,2*S*)-**117b**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%) [yield (%)][c]		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117b (1*S*,2*S*)	Acetate *trans*-119b (1*R*,2*R*)	
1[a]	48 h	24	49	52	95	>98	>200
2[b]	52.5 h	24	50	50	>98 [35][c]	>98 [36][c]	>200

a. Chiral HPLC analysis was conducted on the crude reaction mixture aliquot acquired at 48 h.
b. Chiral HPLC analysis was conducted after purification by column chromatography and isolation of (1*S*,2*S*)-**117b** and (1*R*,2*R*)-**119b**.
c. Isolated yield following column chromatography.

Large scale hydrolase-mediated transesterfication of (±)-*cis*-2-methyl-2-nitro cyclohexanol (±)-117a



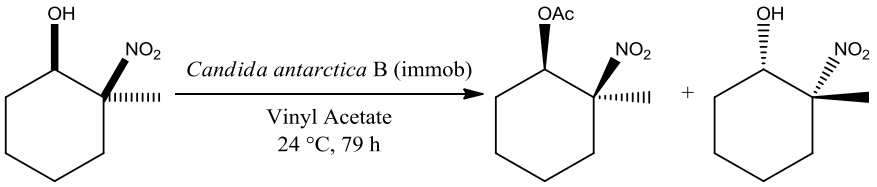
This was prepared following the procedure described for the hydrolase-mediated tranesterification of (±)-**99b** from *Candida antarctica* lipase B (immob) (70.0 mg) and (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (80.5 mg, 0.51 mmol) in vinyl acetate (3 mL). The reaction mixture was shaken at 750 rpm at 24 °C. An aliquot of reaction mixture (0.25 mL) was withdrawn at 72 h. Following a mini work-up, chiral HPLC analysis was conducted. The solution was filtered at 79 h to produce a clear oil (91.1 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate 97/3 as eluent gave the pure *(1*R,*2*S*)*-cis-*2-methyl-2-nitrocyclohexyl acetate* (1*R*,2*S*)-**119a** (30.9 mg, 30%) as a clear oil $[\alpha]_D^{20}$ –46.2 (c 0.5, $CHCl_3$), >98% ee, and the pure *(1*S*,2*R*)*-cis-*2-methyl-2-nitrocyclohexanol* (1*S*,2*R*)-**117a** (26.7 mg, 33%) as a clear oil $[\alpha]_D^{20}$ +0.7 (c 0.5, $CHCl_3$), 45% ee. Conversion estimated by *E*-value calculator at 31%.[45] ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. *Yield may be reduced due to sample being withdrawn during reaction monitoring at 72 h.*
2. *Chiral HPLC analysis was not conducted on the crude reaction mixture at 79 h, but rather on the isolated products (1*S*,2*R*)-**117a** and (1*R*,2*S*)-**119a** following purification by column chromatography.*

The absolute stereochemical assignment of the enantiopure acetate (1*R*,2*S*)-**119a** was determined by single X-ray diffraction on a crystalline sample of (1*R*,2*S*)-**119a** recyrstallised from slow evaporation of acetonitrile (HPLC grade).[76] Crystal data: $C_9H_{15}NO_4$, M = 201.22, orthorhombic, space group $P2_12_12_1$, a = 6.0502(2), b = 10.5734(3), c = 15.6769(5) Å, V = 1002.87(5)Å^3, Z = 4, D_c = 1.333 g cm^{-3}, F_{000} = 432, Cu-Ka radiation, λ = 1.54178 Å, T = 100(2) K, $2\theta_{max}$ = 66.09°, μ = 0.881 mm^{-1}, 6459 reflections collected, 1706 unique (R_{int} = 0.027). Final GooF = 1.329, R_1 = 0.0260, wR_2 = 0.0657 (obs. data: $I > 2\sigma(I)$, R_1 = 0.0268, wR_2 = 0. 0.0661 (add data). Full structural details are contained on the accompanying CD.

Table 5.29: Large scale Candida antarctica *lipase B (immob) mediated transesterification of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** in vinyl acetate*



(±)-**117a** (1*R*,2*S*)-**119a** (1*S*,2*R*)-**117a**

Entry	Reaction Time	Temperature (°C)	Conversion (%)		ee (%) [yield (%)][c]		*E* value
			E Calc.	^{1}H NMR	Alcohol *trans*-117a (1*S*, 2*R*)	Acetate *trans*-119a (1*R*, 2*S*)	
1[a]	72 h	24	27	-	37	>98	142
2[b]	79 h	24	31	32	45 [33][c]	>98 [30][c]	154

a. Chiral HPLC analysis was conducted on the crude reaction mixture aliquot acquired at 72 h.
b. Chiral HPLC analysis was conducted after purification by column chromatography and isolation of (1*R*,2*S*)-**119a** and (1*S*,2*R*)-**117a**.
c. Isolated yield following column chromatography.

5.6.4 Analytical dynamic kinetic resolution screens

Conversions were determined throughout this study by ^{1}H NMR spectroscopy (Figure 5.19) and are derived from integration of;

- 1H, br s, C(1)**H**OH at 3.91 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**.
- 1H, m, C(1)**H**OH, at 4.28-4.29 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**
- 1H, m, C(6)HNO_2 at 4.51-4.63 ppm in 6-nitroheptanal **118**
- 1H, m, C(1)HOAc at 5.26-5.29 ppm in (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119a**
- 1H, m, C(1)HOAc at 5.50-5.55 ppm in (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119b**

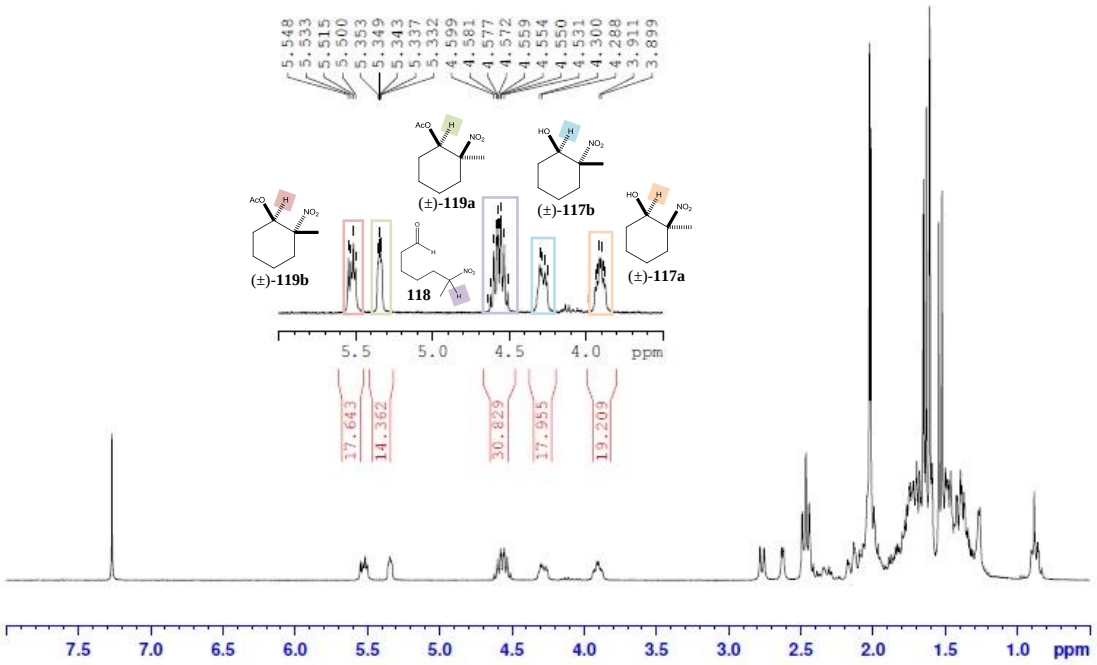


*Figure 5.19: ^{1}H NMR Spectrum: Mixture of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a**, (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b**, 6-nitroheptanal **118**, (±)-*cis*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119a** and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate, (±)-**119b** (19 : 18 : 31 : 14 : 18 respectively) recorded in $CDCl_3$ at 300 MHz.*

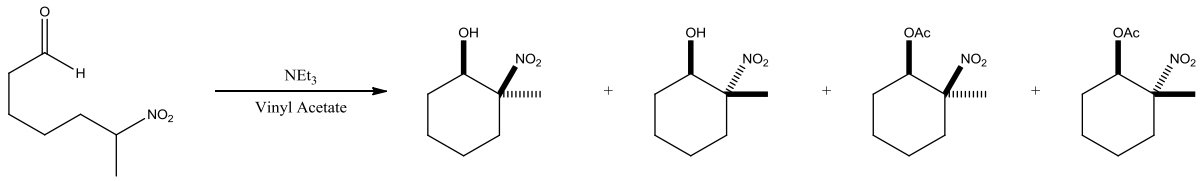
5.6.4.1 Analytical dynamic kinetic resolution screens: Triethylamine

*5.6.4.1.1 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118***

^{1}H NMR spectroscopic investigation: Triethylamine-mediated cyclisation of 6-nitroheptanal 118 with vinyl acetate as solvent

Four solutions of 6-nitroheptanal **118** (45.8 mg, 0.29 mmol) in vinyl acetate (2.3 mL, 24.95 mmol) were prepared. Triethylamine (2.0 eq., 80 µL, 58.2 mg, 0.58 mmol), (1.5 eq., 60 µL, 43.7 mg, 0.43 mmol) or (1.0 eq., 40 µL, 29.1 mg, 0.29 mmol) was added to the appropriate round bottom flask and stirred at room temperature or 40 °C. At regular time intervals an aliquot of reaction mixture was removed and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.30 and Figure 5.20.

*Table 5.30: Triethylamine-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Entry	Triethylamine (eq.)	Temp (°C)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	2.0	40	24 h	62	14	24	-	-
			48 h	37	24	39	-	-
			72 h	9	24	67	-	-
2	2.0	Ambient	24 h	89	4	7	-	-
			48 h	76	9	15	-	-
			72 h	65	12	23	-	-
3	1.5	Ambient	24 h	90	3	7	-	-
			48 h	79	7	14	-	-
			72 h	67	11	22	-	-
			7 days	34	22	44	-	-
4	1.0	Ambient	24 h	92	3	5	-	-
			48 h	85	5	10	-	-
			72 h	78	7	15	-	-

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

*Comparison of conversion (%) of 6-nitrohetptanal **118**, (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** following exposure of 6-nitroheptanal **118** to different equivalents of triethylamine at room temperature or 40 °C at 72 h*

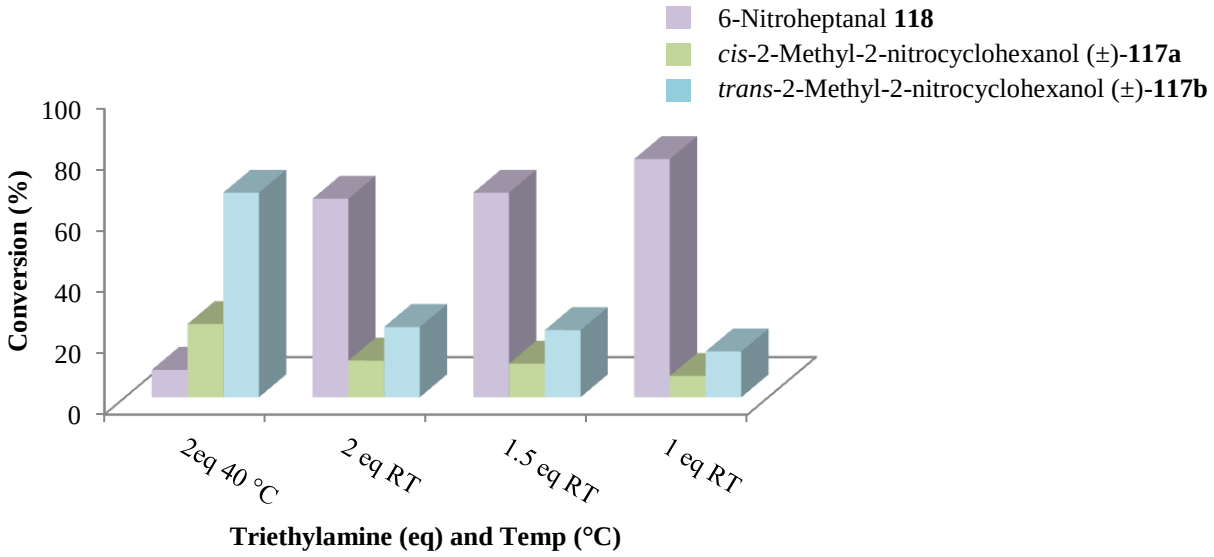


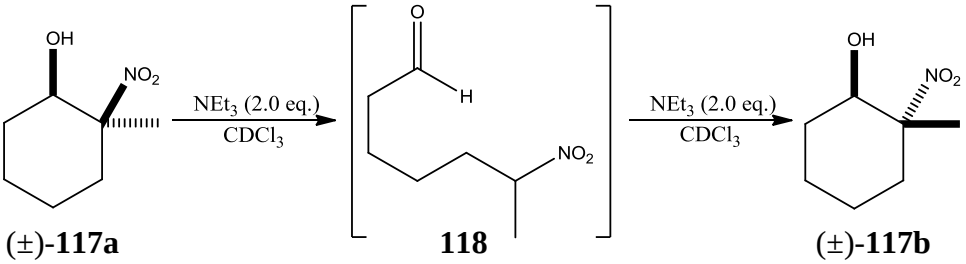
Figure 5.20

5.6.4.1.2 ^{1}H NMR study - Evidence for dynamic interconversion

^{1}H NMR spectroscopic investigation: (±)-*cis*-2-Methyl-2-nitrocyclohexanol (±)-117a and triethylamine (2.0 eq.) in $CDCl_3$.

A ^{1}H NMR sample of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (20.2 mg, 0.13 mmol) in deuterated chloroform, $CDCl_3$ (0.6 mL) was prepared. Triethylamine (2.0 eq., 35 μL, 25.7 mg, 0.25 mmol) was dispensed into the NMR tube, agitated and analysed by ^{1}H NMR (300 MHz) spectroscopy at regular time intervals. The results of the spectroscopic analysis are summarised in Table 5.31 and Figure 5.21.

Table 5.31: Evidence for dynamic interconversion - (±)-cis-*2-methyl-2-nitrocyclohexanol (±)-**117a** and triethylamine (2.0 eq.) in $CDCl_3$*



(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
10 min	100	-
1 h 23 min	100	-
5 h 46 min	100	-
11 h 30 min	100	-
3 days	95	5
7 days	90	10
17 days	72	28

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

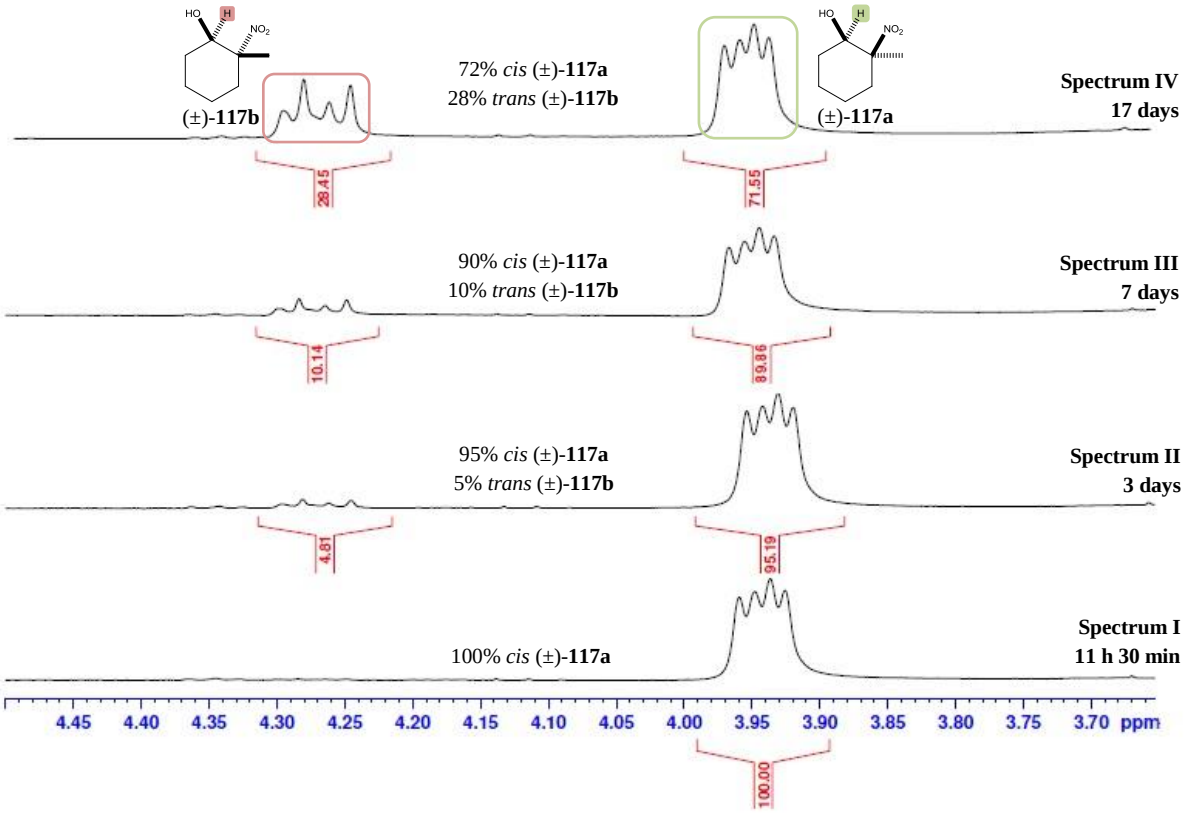
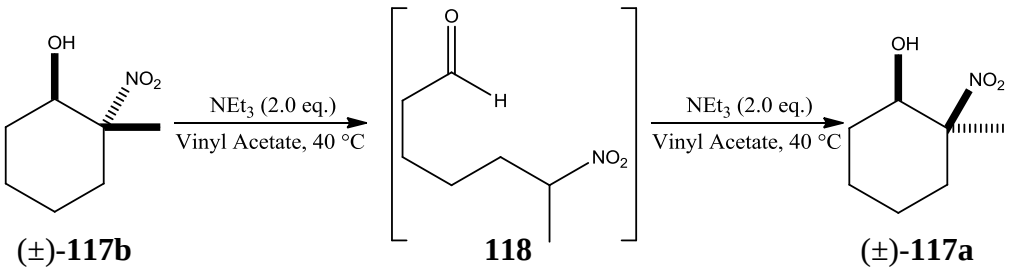


*Figure 5.21: Stacked ^{1}H NMR spectra: Investigation of dynamic kinetic resolution – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and NEt_3 (2.0 eq.). Recorded in $CDCl_3$ at 300 MHz.*

^{1}H NMR spectroscopic investigation: (±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-117b and triethylamine (2.0 eq.) in vinyl acetate at 40 °C.

Triethylamine (2.0 eq., 105 µL, 76.3 mg, 0.75 mmol) was added in one portion to a stirred solution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (60.0 mg, 0.38 mmol) in vinyl acetate (3 mL, 32.55 mmol) at 40 °C. At regular time intervals an aliquot of reaction mixture was removed and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results are summarised in Table 5.32.

*Table 5.32: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and triethylamine (2.0 eq.)in vinyl acetate at 40 °C*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
1 day	-	100
2 days	-	100
3 days	-	100

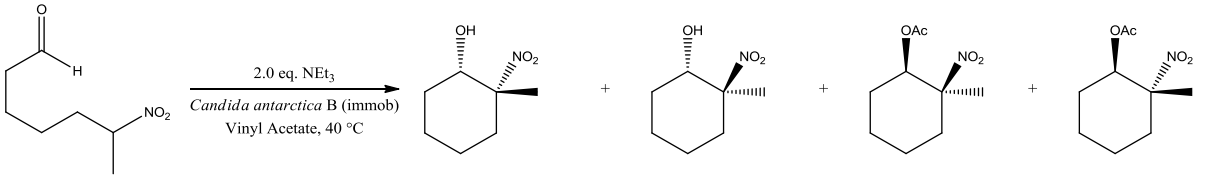
a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*5.6.4.1.3 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117***

^{1}H NMR and chiral HPLC spectroscopic investigation: Cyclisation of 6-nitroheptanal 118 with CAL-B (immob) catalysed transesterification to 2-methyl-2-nitrocyclohexylacetate 119 in vinyl acetate with triethylamine (2.0 eq.) as catalyst.

A spatula tip of *Candida antarctica* lipase B (immob) (~5-10 mg) was added to a solution of 6-nitroheptanal **118** (20.0 mg, 0.13 mmol), triethylamine (2.0 eq., 35 µL, 25.4 mg, 0.25 mmol) in vinyl acetate (1 mL, 10.85 mmol). The solution was magnetically stirred for 72 h at 40 °C. Water (1 mL) was added, the layers were separated, and the organic layer was filtered and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy (300 MHz) and chiral HPLC. The results are summarised in Table 5.33.

*Table 5.33: Cyclisation of 6-nitroheptanal **118** with CAL-B (*immob*) catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with triethylamine (2.0 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Enzyme Source	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
			***cis*-117a (%)[a] [ee (%)][b]**	***trans*-117b (%)[a] [ee (%)][b]**	***cis*-119a (%)[a] [ee (%)][b]**	***trans*-119b (%)[a] [ee (%)][b,f]**
Candida antarctica B (immob)	72 h	31	16 [65]	23 [58]	10 [>98]	20 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

5.6.4.2 Analytical dynamic kinetic resolution screens: 1,8-diazabicycle[5.4.0]undec-7-ene (DBU)

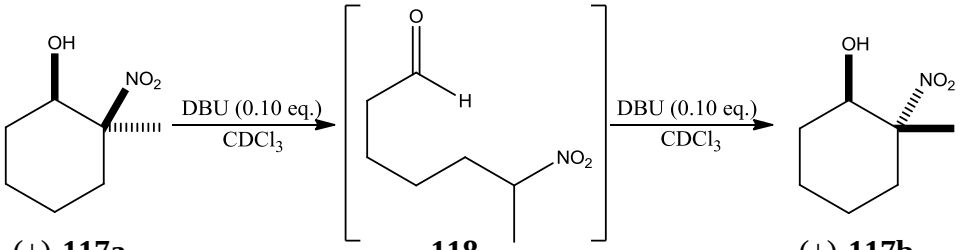
Note: Aliquots of DBU above 10 μL were added directly to the reaction mixture via *a micropipette. To accurately dispense concentrations requiring aliquots of DBU <10 μL a standard solution of DBU in $CDCl_3$ or TBME was prepared.*

5.6.4.2.1 ^{1}H NMR study - Evidence for dynamic interconversion

^{1}H NMR spectroscopic investigation: (±)-*cis*-2-Methyl-2-nitrocyclohexanol (±)-117a and DBU in $CDCl_3$.

Two ^{1}H NMR samples of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (20.3 mg, 0.13 mmol) in deuterated chloroform, $CDCl_3$ (0.6 mL) were prepared. DBU (0.10 eq., 2 μL, 1.94 mg, 0.013 mmol) or (0.05 eq., 1 μL, 0.97 mg, 0.006 mmol) was dispensed where appropriate to the NMR tube, agitated and analysed by ^{1}H NMR (300 MHz) spectroscopy at regular time intervals. The results of the spectroscopic analysis are summarised in Table 5.34 and 5.35.

*Table 5.34: Evidence for dynamic interconversion – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.10 eq.) in $CDCl_3$*

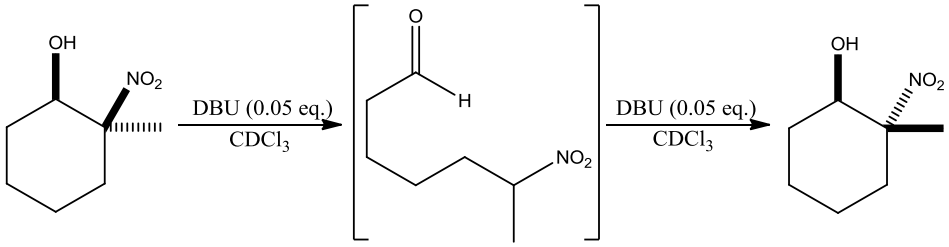


(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
15 min	96	4
33 min	93	7
1 h 8 min	87	13
2 h 4 min	80	20
3 h 31 min	70	30
4 h 52 min	63	37
7 h 17 min	53	47
9 h	48	52
24 h 45 min	35	65
32 h 47 min	34	66
73 days	33	67

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*Table 5.35: Evidence for dynamic interconversion – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DBU (0.05 eq.) in $CDCl_3$*



(±)-**117a** **118** (±)-**117b**

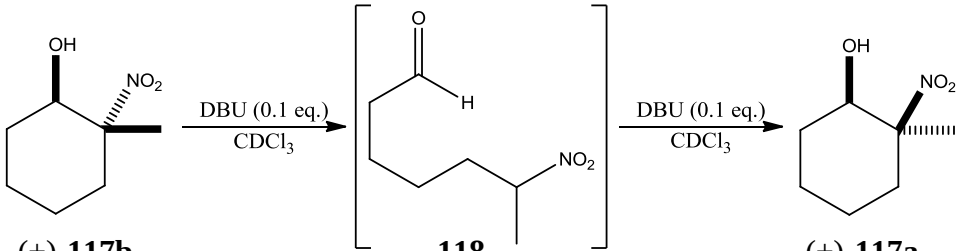
Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
2 h 11 min	78	22
18 h 14 min	37	63
25 h 49 min	34	66
7 days	35	65

a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

^{1}H NMR spectroscopic investigation: (±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-117b and DBU (0.1 eq.) in $CDCl_3$.

A ^{1}H NMR sample of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (22.2 mg, 0.14 mmol) in deuterated chloroform, $CDCl_3$ (0.6 mL) was prepared. DBU (0.1 eq., 2 μL, 2.12 mg, 0.014 mmol) was dispensed to the NMR tube, agitated and analysed by ^{1}H NMR (300 MHz) spectroscopy at regular time intervals and the results are summarised in Table 5.36.

*Table 5.36: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) in $CDCl_3$*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
15 min	3	97
24 min	5	95
1 h 12 min	11	89
4 h 16 min	24	76
4 h 49 min	25	75
5 h 51 min	28	72
21 h 52 min	33	67
14 days	33	67

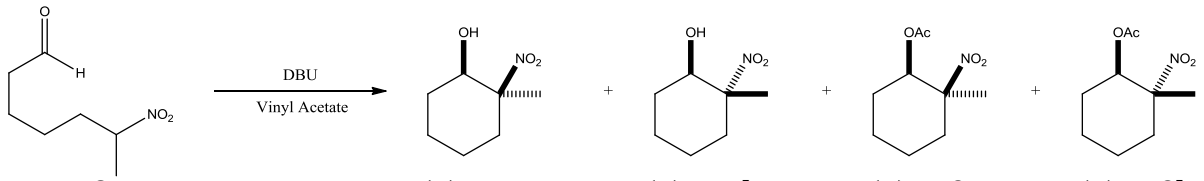
a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*5.6.4.2.2 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118***

^{1}H NMR spectroscopic investigation: DBU-mediated cyclisation of 6-nitroheptanal 118 with vinyl acetate as solvent.

Five solutions of 6-nitroheptanal **118** (45.8 mg, 0.29 mmol) in vinyl acetate (2.3 mL, 24.95 mmol) were prepared. DBU (1.00 eq., 43 μL, 43.8 mg, 0.29 mmol), (0.50 eq., 22 μL, 21.9 mg, 0.14 mmol), (0.10 eq., 4 μL, 4.38 mg, 0.03 mmol), (0.05 eq., 2 μL, 2.19 mg, 0.02 mmol) or (0.01 eq., 0.4 μL, 0.44 mg, 0.003 mmol) was added to the appropriate round bottom flask and stirred at room temperature. At regular time intervals an aliquot of reaction mixture was removed and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.37.

*Table 5.37: DBU-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

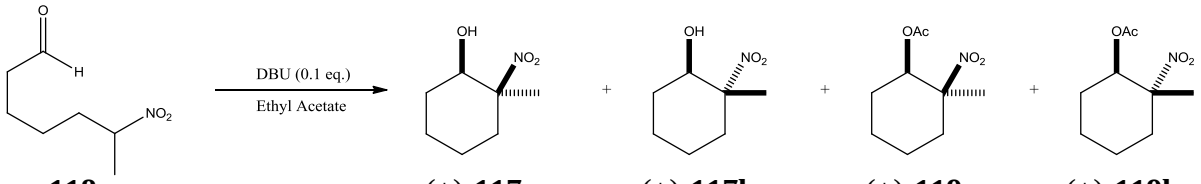
Entry	DBU (eq.)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
				cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	1.00	24 h	-	-	-	40	60
		48 h	-	-	-	40	60
		72 h	-	-	-	39	61
2	0.50	24 h	-	-	-	42	58
		48 h	-	-	-	40	60
		72 h	-	-	-	39	61
		7 days	-	-	-	39	61
3	0.10	24 h	-	11	52	22	15
		48 h	-	-	30	37	33
		72 h	-	-	28	37	35
		7 days	-	-	25	40	35
4	0.05	24 h	48	15	36	1	-
		48 h	50	16	31	2	1
		71 h	55.5	11	31	2	0.5
5	0.01	24 h	94	2	4	-	-
		48 h	93	2	5	-	-
		72 h	91	3	6	-	-

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

^{1}H NMR spectroscopic investigation: DBU (0.1 eq.) mediated cyclisation of 6-nitroheptanal 118 with ethyl acetate as solvent.

A solution of 6-nitroheptanal **118** (60.0 mg, 0.38 mmol) in ethyl acetate (3.2 mL, 32.58 mmol) was prepared. DBU (0.1 eq., 5.6 μL, 5.74 mg, 0.04 mmol) was added to the reaction mixture and stirred at room temperature. At regular time intervals an aliquot of reaction mixture was removed and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.38.

*Table 5.38: DBU (0.1 eq.) mediated cyclisation of 6-nitroheptanal **118** with ethyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Acetylating agent	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
			cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
Ethyl Acetate	24 h	-	29	71	-	-
	48 h	-	30	70	-	-

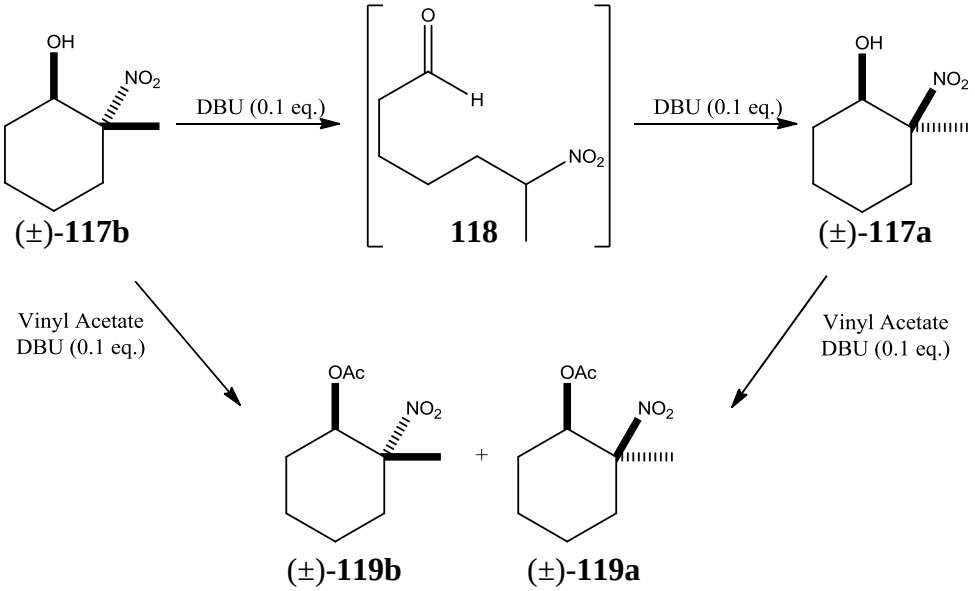
a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

5.6.4.2.3 ^{1}H NMR study - Evidence for chemical acetylation in the dynamic interconversion process

^{1}H NMR spectroscopic investigation: (±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-117b and DBU (0.1 eq.) in vinyl acetate

DBU (0.1 eq., 3.9 μL, 4.02 mg, 0.03 mmol) was added in one portion to a stirred solution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (42.0 mg, 0.26 mmol) in vinyl acetate (2 mL, 21.70 mmol) at room temperature. At regular time intervals an aliquot of reaction mixture was removed and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.39.

*Table 5.39: Evidence for chemical acetylation - (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and DBU (0.1 eq.) in vinyl acetate*



Reaction Time	Alcohol (±)-117[a]		Acetate (±)-119[a]	
	trans **(±)-117b (%)**	***cis*** **(±)-117a (%)**	***trans*** **(±)-119b (%)**	***cis*** **(±)-119a (%)**
24 h	32	13	56	-
48 h	31	-	69	-

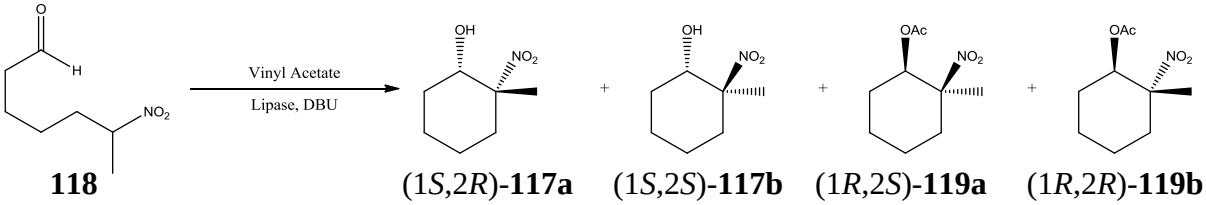
a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*5.6.4.2.4 ^{1}H NMR study - One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117***

^{1}H NMR and chiral HPLC spectroscopic investigation: Cyclisation of 6-nitroheptanal 118 with hydrolase-catalysed tranesterification to 2-methyl-2-nitrocyclohexylacetate 119 in vinyl acetate with DBU as catalyst.

Nine solutions of 6-nitroheptanal (0.20 mg, 0.13 mmol) in vinyl acetate (1 mL, 10.85 mmol) were prepared. DBU (0.05 eq., 1 μL, 0.96 mg, 0.006 mmol), (0.10 eq., 2 μL, 1.9 mg, 0.01 mmol), (0.20 eq., 4 μL, 3.8 mg, 0.03 mmol), (0.50 eq., 9 μL, 9.6 mg, 0.063 mmol) or (1.00 eq., 19 μL, 19.1 mg, 0.13 mmol) and a spatula tip (~5-10 mg) of hydrolase were added to the appropriate test tubes and the mixture was agitated for the required length of time at 24 °C. In the case of the samples which contained DBU, water (1 mL) was added, the layers were separated and the organic layer was filtered and concentrated under reduced pressure. When DBU was not used, the organic layer was filtered and concentrated under reduced pressure. All samples were analysed by ^{1}H NMR (300 MHz) spectroscopy and entries 8 and 9, Table 5.40 by chiral HPLC. The results of the screen are summarised in Table 5.40.

*Table 5.40: Cyclisation of 6-nitroheptanal **118** with hydrolase-catalysed transesterification to 2-methyl-2-nitrocyclohexylacetate **119** in vinyl acetate with DBU as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	DBU (eq.)	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis-117a (%)[a]	*trans*-117b (%)[a]	*cis*-119a (%)[a]	*trans*-119b (%)[a]
1	-	-	48 h	100	-	-	-	-
2	CAL-B (immob)	-	72 h	100	-	-	-	-
3	-	0.10	48 h	-	-	28 (±)	36 (±)	36 (±)
4	*P.stutzeri*	0.05	48 h	100	-	-	-	-
5	CAL-B (immob)	0.10	72 h	100	-	-	-	-
6	*P. stutzeri*	0.10	48 h	100	-	-	-	-
7	*P. stutzeri*	0.20	48 h	77	3	10	5	5
8	*P. stutzeri*	0.50	24 h	-	11	4	39	46
			48 h	-	6 [>98]	4 [>98]	37 [1]	53 [1]
9	*P. stutzeri*	1.00	48 h	-	6 [>98]	3 [>98]	53 [1]	38 [0]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

5.6.4.3 Analytical dynamic kinetic resolution screens: Alteration of base

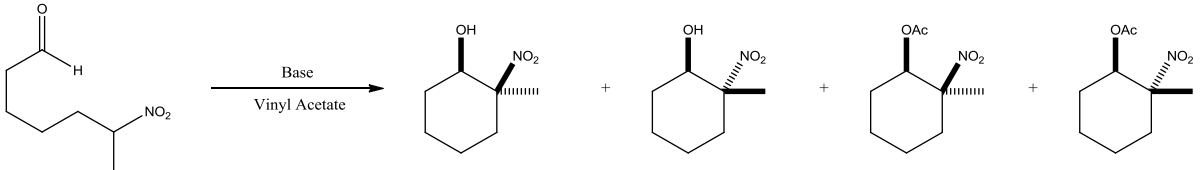
^{1}H NMR spectroscopic investigation: Base-mediated cyclisation of 6-nitroheptanal 118 with vinyl acetate as solvent.

Six solutions of 6-nitroheptanal **118** (60.0 mg, 0.38 mmol) in vinyl acetate (3 mL, 32.55 mmol) were prepared. The following bases were added to the appropriate round bottom flask and stirred at room temperature;

- Diethylamine (DEA) (2.0 eq., 78 μL, 55.1 mg, 0.75 mmol)
- Piperidine (2.0 eq., 74 μL, 64.2 mg, 0.75 mmol)
- 1,4-Diazabicyclo[2.2.2]octane (DABCO) (2.0 eq., 84.6 mg, 0.75 mmol)
- *N*,*N*-Diisopropylethylamine (Hünigs base) (2.0 eq., 131 μL, 97.4 mg, 0.75 mmol)
- Aqueous sodium hydroxide (1M, 1mL)
- 1,1,3,3-Tetramethylguanidine (TMG) (0.1 eq., 5 μL, 4.3 mg, 0.04 mmol)

At regular time intervals an aliquot of reaction mixture was removed. In the case of the 1M NaOH trial the layers were separated and the aqueous phase was extracted with diethyl ether (3 × 1 mL) and then concentrated under reduced pressure. In all other case the reaction aliquot was concentrated under reduced pressure with no work-up. The samples were analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.41.

*Table 5.41: Base-mediated cyclisation of 6-nitroheptanal **118** with vinyl acetate as solvent*



118 (±)-**117a** (±)-**117b** (±)-**119a** (±)-**119b**

Entry	Base	pK_a	Reaction Time	Aldehyde 118 (%)[a]	Alcohol (±)-117		Acetate (±)-119	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]	*cis* (±)-119a (%)[a]	*trans* (±)-119b (%)[a]
1	DEA (2.0 eq.)	11.3 (H_2O)[77]	24 h	83	8	9	-	-
			48 h	82	8	10	-	-
2	Piperidine (2.0 eq.)	11.3 (H_2O)[77]	24 h	100	-	-	-	-
			48 h	100	-	-	-	-
			72 h	100	-	-	-	-
3	DABCO (2.0 eq.)	8.82, 2.97 (H_2O)[78]	24 h	-	39	61	-	-
			48 h	-	40	60	-	-
			72 h	-	39	61	-	-
4	Hünigs Base (2.0 eq.)	11.4 (H_2O)[79]	48 h	85	5	10	-	-
			72 h	79	7	14	-	-
5	1M NaOH	~13.8	24 h	100	-	-	-	-
			48 h	100	-	-	-	-
6	TMG (0.1 eq.) (0.1 - 2.1 eq.)[b]	13.6 (H_2O)[80]	24 h	69	10	21	-	-
			48 h	6	30	57	5	2

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery

b. At 24 h after analysis by ^{1}H NMR spectroscopy an additional 2.0 equivalents of TMG (2.0 eq., 95 µL, 86.8 mg, 0.75 mmol) were added to the reaction vessel and stirred at room temperature for a further 24 h.

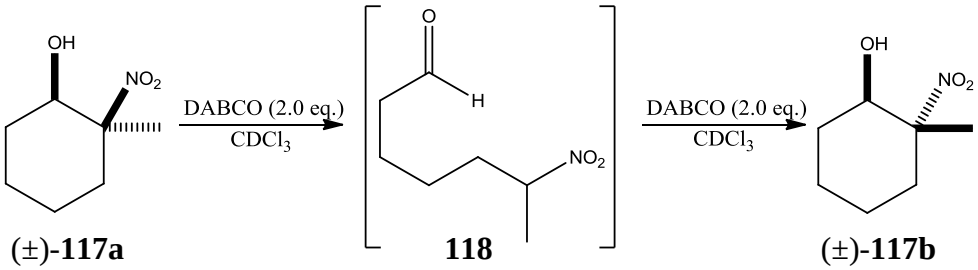
5.6.4.4 Analytical dynamic kinetic resolution screens: 1,4-diazabicyclo[2.2.2]octane (DABCO)

5.6.4.4.1 ^{1}H NMR study - Evidence for dynamic interconversion

^{1}H NMR spectroscopic investigation: (±)-*cis*-2-Methyl-2-nitrocyclohexanol (±)-117a and DABCO (2.0 eq.) in $CDCl_3$.

A ^{1}H NMR sample of (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** (21.4 mg, 0.13 mmol) in deuterated chloroform, $CDCl_3$ (0.3 mL) was prepared. A solution of DABCO (2.0 eq., 30.2 mg, 0.27 mmol) in deuterated chloroform, $CDCl_3$ (0.3 mL) was dispensed to the NMR tube agitated and analysed by ^{1}H NMR (300 MHz) spectroscopy at regular time intervals. The results of the spectroscopic analysis are summarised in Table 5.42.

*Table 5.42: Evidence for dynamic interconversion – (±)-*cis*-2-methyl-2-nitrocyclohexanol (±)-**117a** and DABCO (2.0 eq.) in $CDCl_3$*



(±)-**117a** **118** (±)-**117b**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
42 min	100	-
3 h 10 min	100	-
1 day	100	-
4 days	100	-
10 days	100	-

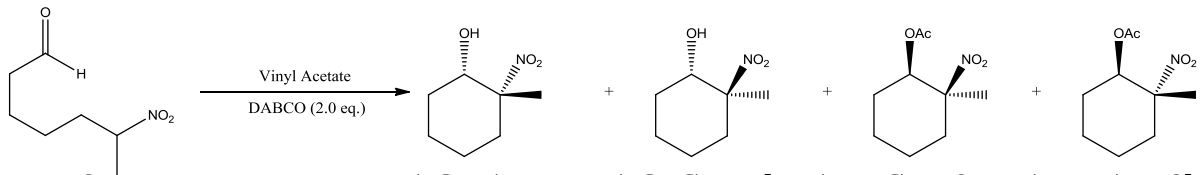
a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

*5.6.4.4.2 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-**117***

^{1}H NMR and chiral HPLC spectroscopic investigation: Cyclisation of 6-nitroheptanal 118 with *P. stutzeri* catalysed tranesterification to 2-methyl-2-nitrocyclohexylacetate 119 in vinyl acetate with DABCO (2.0 eq.) as catalyst

DABCO (2.0 eq., 28.2 mg, 0.25 mmol) was added to two solutions of 6-nitroheptanal (0.20 mg, 0.13 mmol) in vinyl acetate (1 mL, 10.85 mmol). A spatula tip (~5-10 mg) of *Pseudomonas stutzeri* was added to the appropriate test tube and the reaction mixtures were agitated for 48 h at 24 °C. Water (1 mL) was added, the layers were separated and the organic layer was filtered and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and chiral HPLC. The results of the screen are summarised in Table 5.43.

*Table 5.43: Cyclisation of 6-nitroheptanal **118** with* P. stutzeri *catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with DABCO (2.0 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
				cis-117a (%)[a] [ee (%)][b]	*trans*-117b (%)[a] [ee (%)][b]	*cis*-119a (%)[a] [ee (%)][b]	*trans*-119b (%)[a] [ee (%)][b]
1	-	48 h	-	40	60	-	-
2	*Pseudomonas stutzeri*	48 h	75	4 [77]	11 [24][c]	5 [72]	5 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.
c. The HPLC trace displays an unknown impurity which co-eluted with the *trans*-2-methyl-2-nitrocyclohexanol **117b**, therefore enantiomeric excess [ee (%)] is an estimation.

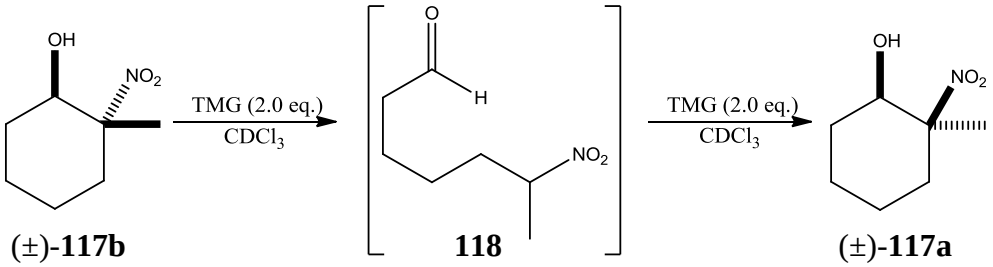
5.6.4.5 Analytical dynamic kinetic resolution screens: 1,1,3,3-Tetramethylguanidine (TMG)

5.6.4.5.1 ^{1}H NMR study - Evidence for dynamic interconversion

^{1}H NMR spectroscopic investigation: (±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-117b and TMG (2.0 eq.) in $CDCl_3$.

A ^{1}H NMR sample of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (15.6 mg, 0.10 mmol) in deuterated chloroform, $CDCl_3$ (0.6 mL) was prepared. TMG (2.0 eq., 25 µL, 22.6 mg, 0.20 mmol) was dispensed to the NMR tube agitated and analysed by ^{1}H NMR (300 MHz) spectroscopy at regular time intervals. The results of the spectroscopic analysis are summarised in Table 5.44.

*Table 5.44: Evidence for dynamic interconversion – (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and TMG (2.0 eq.) in $CDCl_3$*



(±)-**117b** **118** (±)-**117a**

Reaction Time	(±)-117a (%)[a]	(±)-117b (%)[a]
49 min	14	88
5 h 7 min	26	74
3 days	28	72

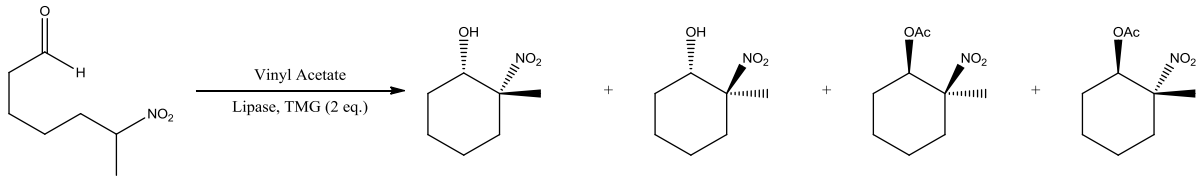
a. 6-Nitroheptanal **118** was not detected in the ^{1}H NMR spectra.

5.6.4.5.2 ^{1}H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-117

^{1}H NMR and chiral HPLC spectroscopic investigation: Cyclisation of 6-nitroheptanal 118 with hydrolase-catalysed transesterification to 2-methyl-2-nitrocyclohexylacetate 119 in vinyl acetate with TMG (2 eq.) as catalyst.

TMG (2 eq., 32 µL, 28.9 mg, 0.25 mmol) was added to two solutions of 6-nitroheptanal **118** (20.0 mg, 0.13 mmol) in vinyl acetate (1 mL, 10.85 mmol). A spatula tip of *Pseudomonas stutzeri* or *Candida antarctica* lipase B (immob) (~5-10 mg) were added to the appropriate test tubes and the mixture was agitated for the required length of time at 24 °C. Water (1 mL) was added, the layers were separated, and the organic layer was filtered and concentrated under reduced pressure. The sample was analysed by ^{1}H NMR spectroscopy (300 MHz) and chiral HPLC. The results summarised in Table 5.45 and Figure 5.22.

*Table 5.45: Cyclisation of 6-nitroheptanal **118** with hydrolase-catalysed transesterification to 2-methyl-2-nitrocyclohexyl acetate **119** in vinyl acetate with TMG (2 eq.) as catalyst*



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Entry	Enzyme Source	TMG	Reaction Time	Aldehyde 118 (%)[a]	Alcohol 117		Acetate 119	
					cis-117a (%)[a] [ee (%)][b]	*trans*-117b (%)[a] [ee (%)][b]	*cis*-119a (%)[a] [ee (%)][b]	*trans*-119b (%)[a] [ee (%)][b]
1	*Candida antarctica B* (immob)	2 eq.	5 days	7	26 [2]	67 [1]	-	-
2	*Pseudomonas stutzeri*	2 eq.	48 h	15	22 [35]	50 [6]	9 [75]	4 [80]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

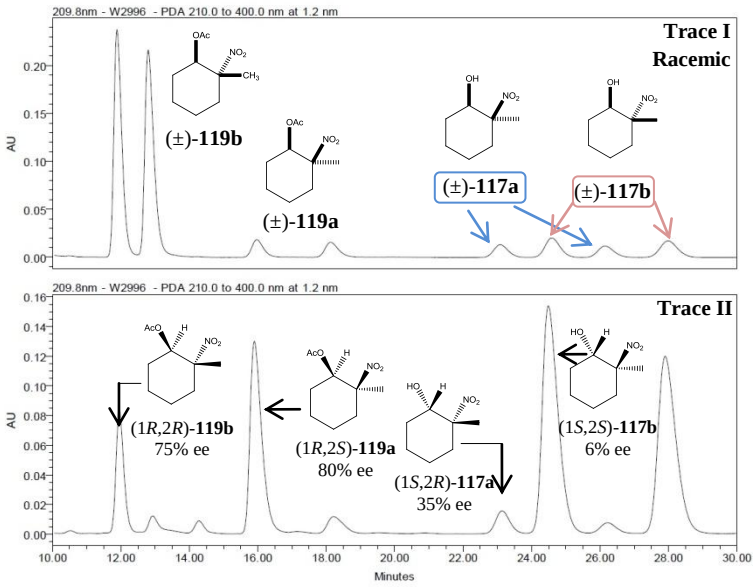


*Figure 5.22: HPLC **Trace I:** (±)-trans-2-methyl-2-nitrocyclohexyl acetate (±)-**119b**, (±)-cis-2-methyl-2-nitrocyclohexyl acetate (±)-**119a**, (±)-cis-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-trans-2-methyl-2-nitrocyclohexanol (±)-**117b**. **Trace II:** Pseudomonas stutzeri and TMG-mediated dynamic resolution process, (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-**119b**, 75% ee, (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-**119a**, 80% ee, (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-**117a**, 35% ee and (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-**117b**, 6% ee. For HPLC conditions see appendix I. Note the above traces correlate to chiral HPLC conditions C.*

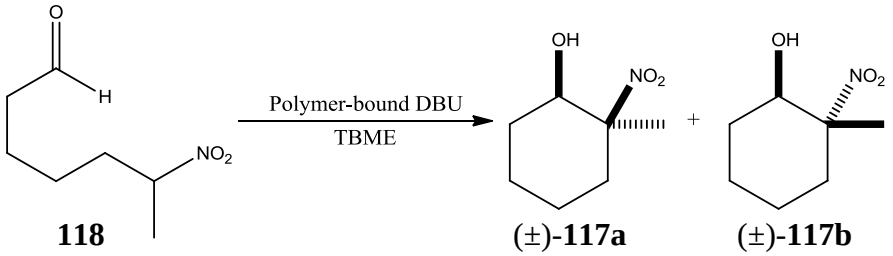
5.6.4.6 Analytical dynamic kinetic resolution screens: Polymer-bound 1,8-diazabicycle[5.4.0]undec-7-ene (DBU)

*5.6.4.6.1 ^{1}H NMR study - Investigation of the cyclisation of 6-nitroheptanal **118***

^{1}H NMR spectroscopic investigation: Polymer-bound DBU-mediated cyclisation of 6-nitroheptanal 118 with TBME as solvent.

Two solutions of 6-nitroheptanal **118** (50.1 mg, 0.31 mmol) in TBME (5 mL) were prepared. Polymer-bound DBU, 1.15 mmol N per g loading, (0.1 eq., 27.4 mg, 0.03 mmol) or (1.0 eq., 273.7 mg, 0.31 mmol) was added to the appropriate round bottom flask and stirred at room temperature or 40 °C. At regular time intervals an aliquot of reaction mixture was removed, filtered to remove the immobilised beads which were washed with TBME and combined organic extracts concentrated under reduced pressure. The sample was analysed by ^{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.46.

*Table 5.46: Polymer-bound DBU-mediated cyclisation of 6-nitroheptanal **118** with TBME as solvent*



118 (±)-**117a** (±)-**117b**

Entry	Polymer-bound DBU (eq.)	Reaction Time	Temp (°C)	Aldehyde 118 (%)[a]	Alcohol (±)-117	
					cis (±)-117a (%)[a]	*trans* (±)-117b (%)[a]
1	0.1	13 h	Ambient	100	-	-
2	1.0	22.5 h	Ambient	92	3	5
	1.0 – 2.0[a]	44 h	40 °C	11	24	65

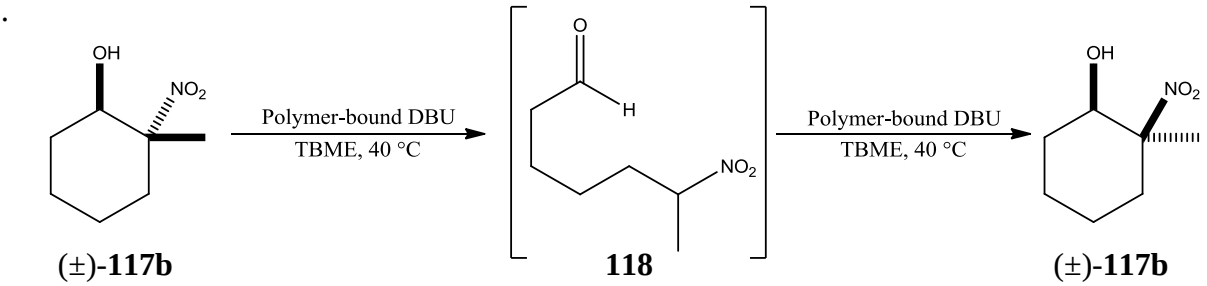
a. At 22.5 h after analysis by ^{1}H NMR spectroscopy an additional equivalent of polymer-bound DBU (1.0 eq., 273.7 mg, 0.31 mmol) was added to the reaction vessel and stirred at 40 °C for a further 21.5 h.

5.6.4.6.2 ^{1}H NMR study - Evidence for dynamic interconversion

^{1}H NMR spectroscopic investigation: (±)-*trans*-2-Methyl-2-nitrocyclohexanol (±)-117b and polymer-bound DBU in TBME at 40 °C.

To a solution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (49.2 mg, 0.31 mmol) in TBME (2 mL), polymer-bound DBU (2 eq., 537.5 mg, 0.62 mmol) was added. To a solution of (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** (49.2 mg, 0.31 mmol) in TBME (5 mL), polymer-bound DBU (3 eq., 806.3 mg, 0.93 mmol) was added. Both reaction mixtures were stirred at 40 °C for 19 h. The reaction mixture was filtered to remove the immobilised beads which were washed with TBME. The combined organic extracts were concentrated under reduced pressure and analysed by ^{1}H NMR (300 MHz) spectroscopy. The results of the spectroscopic analysis are summarised in Table 5.47.

*Table 5.47: Evidence for dynamic interconversion - (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117b** and polymer-bound DBU in TBME at 40 °C*



(±)-**117b** **118** (±)-**117b**

Entry	Polymer-bound DBU	Reaction Time	(±)-117b (%)	(±)-117a (%)
1	2 eq.	19 h	-[a]	-[a]
2	3 eq.	19 h	-[a]	-[a]

a. The crude ^{1}H NMR spectrum was too complex to decipher conversions.

5.6.5 Two pot hydrolase-mediated dynamic resolution of (±)-2-methyl-2-nitrocyclohexanol (±)-117

DBU-mediated dynamic interconversion

DBU (0.2 eq., 55 µL, 55.8 mg, 0.37 mmol) was added to a solution of 6-nitroheptanal **118** (291.5 mg, 1.83 mmol) in TBME (3 mL) and stirred at room temperature overnight. The reaction mixture was washed with water (3 × 2 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (258.0 mg, 89%) of nitroalcohols (±)-**117a** and (±)-**117b** as a clear oil The sample was analysed by ^{1}H NMR spectroscopy and conversions to *(±)*-cis-*2-methyl-2-nitrocyclohexanol* (±)-**117a** and *(±)*-trans-*2-methyl-2-nitrocyclohexanol* (±)-**117b** determined by integration of the crude material (Table 5.48). The ^{1}H NMR sample was then reconcentrated for lipase-mediated kinetic resolution.

***Candida antarctica* lipase B (immob) mediated kinetic resolution.**

Candida antarctica lipase B (immob) (228.0 mg, 88% w/w) was added to a solution of the crude nitroalcohols (±)-**117a** and (±)-**117b** (258.0 mg, 1.62 mmol) in vinyl acetate (12.9 mL, 12.05 g, 139.95 mmol) and shaken at 750 rpm at 24 °C for 18 h. The solution was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure to give a crude mixture (274.6 mg) of nitroalcohols **117a**, **117b** and nitroacetates **119a**, **119b** as a clear oil. The sample was analysed by ^{1}H NMR spectroscopy and conversions to cis-*2-methyl-2-nitrocyclohexyl acetate* **119a** and trans-*2-methyl-2-nitrocyclohexyl acetate* **119b** determined by integration of the crude material (Table 5.48). The ^{1}H NMR sample was then reconcentrated for base-mediated dynamic resolution.

The DBU-mediated dynamic interconversion procedure followed by *Candida antarctica* lipase B (immob) mediated kinetic resolution was repeated as per Table 5.48 to give a crude mixture of nitroalcohols **117a**, **117b** and nitroacetates **119a**, **119b** (88.5 mg) as a clear oil.

Purification by column chromatography on silica gel using hexane/ethyl acetate as eluent (gradient elution 1-25% ethyl acetate) gave four fractions.

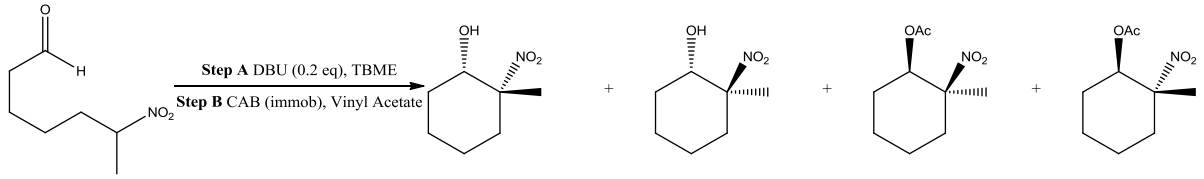
The first (least polar) fraction was the pure *(1R,2R)*-trans-*2-methyl-2-nitrocyclohexyl acetate* (1*R*,2*R*)-**119b** (10.4 mg, 3%) as a clear oil $[\alpha]_D^{20}$ +56.4 (c 0.4, $CHCl_3$), >98% ee. ^{1}H NMR spectra were identical to those for the racemic materials previously prepared.

The second fraction was a 22 : 78 mixture of *(1R,2S)*-cis-*2-methyl-2-nitrocyclohexyl aceate* (1R,2S)- **119a**, >98% ee and *(1R,2R)*-trans-*2-methyl-2-nitrocyclohexyl acetate* (1*R*,2*R*)-**119b**, >98% ee (19.9 mg, 5%) as a clear oil.

The third fraction was a 83 : 17 mixture of *(1R,2S)*-cis-*2-methyl-2-nitrocyclohexyl aceate* (1R,2S)-**119a**, >98% ee and *(1R,2R)*-trans-*2-methyl-2-nitrocyclohexyl acetate* (1*R*,2*R*)-**119b**, >98% ee (3.1 mg, 0.8%) as clear oil.

The third most polar fraction was a 24 : 76 mixture of *(1*S,*2*R*)*-cis-*2-methyl-2-nitrocyclohexanol* (1*S*,2*R*)-**117a**, 10% ee and *(1*S,*2*S*)*-trans-*2-methyl-2-nitrocyclohexanol* (1*S*,2*S*)-**117b**, 37% ee (15.6 mg, 5%) as clear oil.

Table 5.48: Two pot dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis



118 (1*S*,2*R*)-**117a** (1*S*,2*S*)-**117b** (1*R*,2*S*)-**119a** (1*R*,2*R*)-**119b**

Step	DBU (eq.)	Vinyl acetate (eq.)	*Candida antarctica* B (immob) (% w/w)	Alcohol 117		Acetate 119	
				***cis*-117a (%)[a] [ee (%)][b,c]**	***trans*-117b (%)[a] [ee (%)][b,d]**	***cis*-119a (%)[a] [ee (%)][b,e]**	***trans*-119b (%)[a] [ee (%)][b,f]**
A	0.2	-	-	30 (±)	70 (±)	-	-
B	-	138	18	25	37	5	33
A	0.2	-	-	19	41	6	34
B	-	138	18	16	29	8	47
A	0.2	-	-	13	30	8	49
B	-	138	18	11	22	10	57
A	0.2	-	-	10 [10]	22 [37]	10 [>98]	57 [>98]

a. The conversions were determined by ^{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
b. Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix 1 for conditions.

5.7 References

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Appendices

Appendix I *Chiral HPLC data*

- Samples for chiral HPLC analysis were prepared at a concentration of ~1 mg/mL.

- The retention times can change per injection (particularly for long run times), however, in general the elution sequence of enantiomers remains the same.

- In the resolution of all four enantiomeric pairs of (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexanol (±)-**117a** and (±)-**117b** and (±)-*cis*- and (±)-*trans*-2-methyl-2-nitrocyclohexyl acetate (±)-**119a** and (±)-**119b** in a single trace three sets of conditions are described. Notably under conditions A and C the sequence of elution of all eight enantiomers remains the same. However, under conditions B elution of the enantiomeric pairs of the diastereomeric alcohol differs relative to A and C.

- Stereochemical assignments of known enantioenriched products were made by comparison to previously reported polarimetry, and chiral HPLC data (where known).

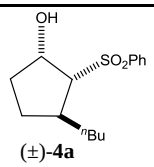
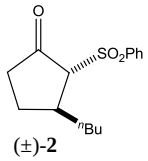
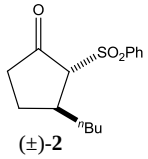
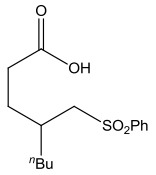
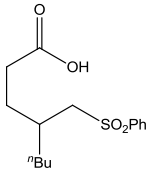
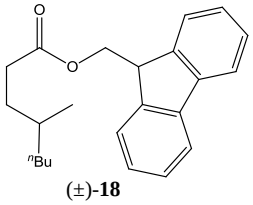
- In general, the absolute stereochemistry of novel enantioenriched products was assigned by single crystal analysis and chiral HPLC analysis of the isolated single crystal.

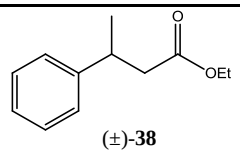
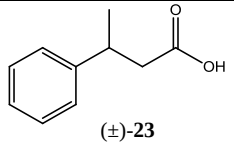
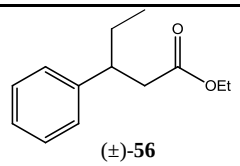
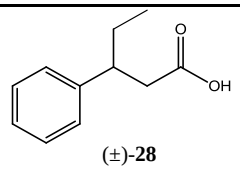
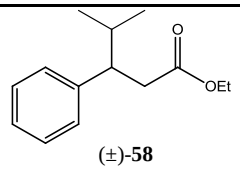
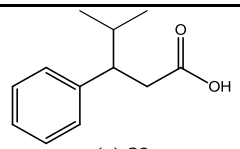
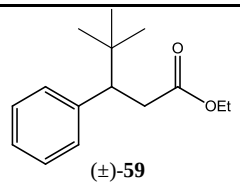
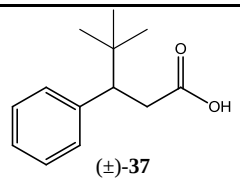
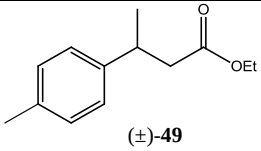
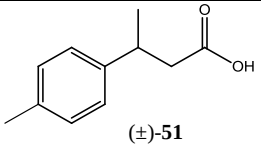
- Wherever feasible, the crystals employed for X-ray diffraction to determine absolute stereochemistry were subsequently analysed by chiral HPLC to unambiguously confirm the absolute stereochemistry corresponding to each HPLC peak. In some instances the detection of the weak signals from the single crystal proved challenging.

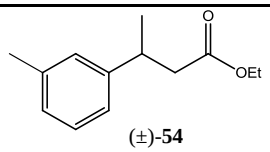
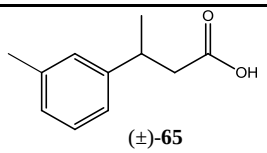
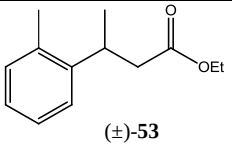
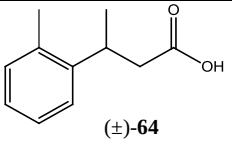
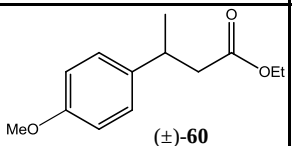
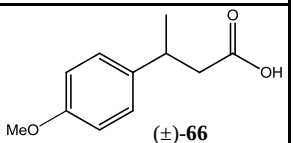
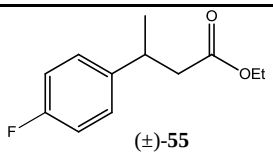
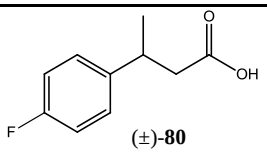
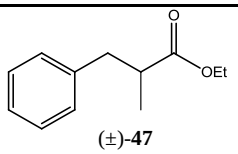
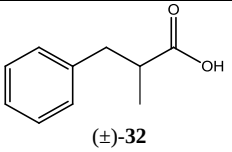
- In this study for some compounds there are more than one set of chiral HPLC conditions described. What conditions were employed was dependent on the requirements of the resolution *i.e.* if multiple compounds were required to be resolved in a single injection *e.g.* (±)-*trans*-2-benzenesulfonyl-3-*n*-butylcyclopentanone (±)-**2** page iii.

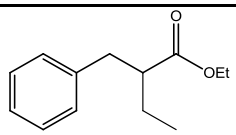
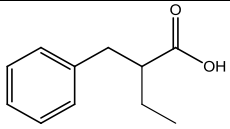
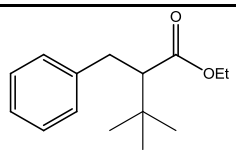
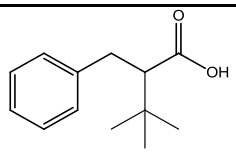
- Notably chiral HPLC conditions of known compounds are not all identical to previous workers in the group.

Baker's yeast mediated asymmetric synthesis of
*(*R*)- and (*S*)-4-methyloctanoic acid (*R*)-**1** and (*S*)-**1***

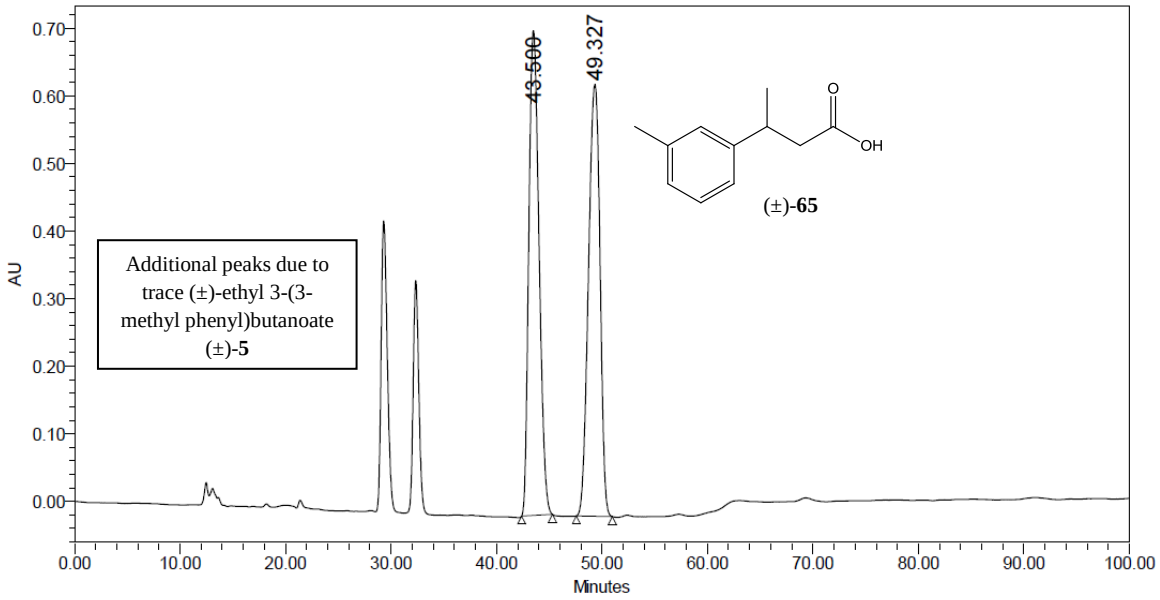
Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**4a**	Chiralcel OJ-H	2	0.40	220.0	*Gradient* **0 min:** 96 : 4 **30 min:** 99 : 1	Rt	(1*S*,2*R*,3*S*)-**4a**	58
							(1*R*,2*S*,3*R*)-**4a**	64
 (±)-**2**							(2*S*,3*R*)-**2**	232
							(2*R*,3*S*)-**2**	256
 (±)-**2**	Chiralcel OJ-H	10	0.80	220.0	99 : 1	rt	(2*S*,3*R*)-**2**	119
							(2*R*,3*S*)-**2**	142
Conditions A  (±)-**11**	Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 93 : 7	rt	(*S*)-**11**	78
							(*R*)-**11**	99
Conditions B  (±)-**11**	Chiralcel OJ-H	10	0.50	209.8	93 : 7	rt	(*S*)-**11**	68
							(*R*)-**11**	83
 (±)-**18**	Chiralcel OD-H	8	0.50	220	98 : 2	rt	(*R*)-**18**	96
							(*S*)-**18**	105

Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**38**	Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 95 : 5	0	(*R*)-**38**	28
							(*S*)-**38**	33
 (±)-**23**							(*S*)-**23**	38
							(*R*)-**23**	51
 (±)-**56**	Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 99 : 1	0	(*S*)-**56**	12
							(*R*)-**56**	20
 (±)-**28**							(*S*)-**28**	61
							(*R*)-**28**	86
 (±)-**58**	Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 98 : 2	0	(*R*)-**58**	22
							(*S*)-**58**	28
 (±)-**29**							(*R*)-**29**	35
							(*S*)-**29**	58
 (±)-**59**	Chiralcel OJ-H	10	0.25	209.8	*IPA mobile phase consists of 3% TFA* 96 : 4	20	(*S*)-**59**	26
							(*R*)-**59**	32
 (±)-**37**							(*R*)-**37**	45
							(*S*)-**37**	58
 (±)-**49**	Chiralcel OJ-H	10	0.50	211.0	*IPA mobile phase consists of 3% TFA* 99.5 : 0.5	0	(*S*)-**49**	30
							(*R*)-**49**	32
 (±)-**51**							(*S*)-**51**	120
							(*R*)-**51**	155

Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**54**	Chiralcel OJ-H	10	0.25	209.8	*IPA mobile phase consists of 3% TFA* 98 : 2	0	(*R*)-**54**	36
							(*S*)-**54**	40
 (±)-**65**							(*S*)-**65**	73
							(*R*)-**65**	84
 (±)-**53**	Chiralcel OJ-H	10	0.25	209.8	*IPA mobile phase consists of 3% TFA* 94 : 6	0	(*R*)-**53**	30
							(*S*)-**53**	45
 (±)-**64**							(*S*)-**64**	34
							(*R*)-**64**	64
 (±)-**60**	Chiralcel OJ-H	10	0.25	216.9	*IPA mobile phase consists of 3% TFA* 82 : 18	0	(*R*)-**60**	45
							(*S*)-**60**	51
 (±)-**66**							(*S*)-**66**	42
							(*R*)-**66**	59
 (±)-**55**	Chiralcel AS-H	10	*Gradient* **0 min:** 1.00 **30 min:** 0.25	256.0	*IPA mobile phase consists of 3% TFA Gradient* **0 min:** 99.7 : 0.3 **30 min:** 94 : 6	0	(*R*)-**55**	9
							(*S*)-**55**	11
 (±)-**80**							(*S*)-**80**	34
							(*R*)-**80**	49
 (±)-**47**	Chiralcel OJ-H	2	*Gradient* **0 min:** 0.20 **50 min:** 1.00	209.8	*IPA mobile phase consists of 3% TFA Gradient* **0 min:** 99.5 : 0.5 **50 min:** 95 : 5	45	(*R*)-**47**	38
							(*S*)-**47**	41
 (±)-**32**							(*R*)-**32**	57
							(*S*)-**32**	58

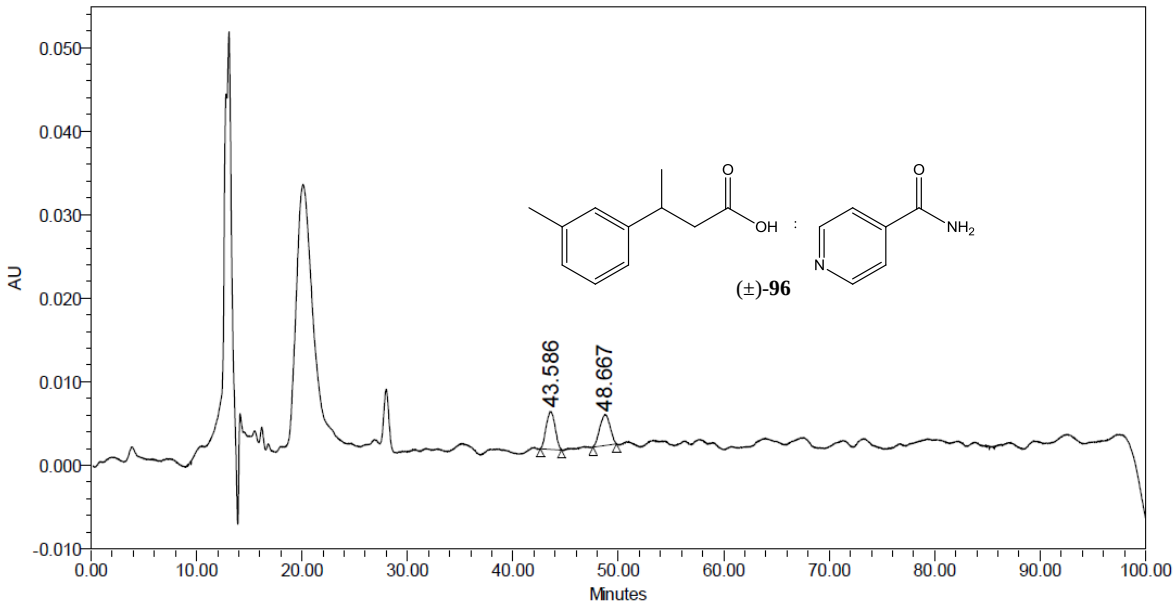
Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**48**	Chiralcel OJ-H	2	0.5	209.8	*IPA mobile phase consists of 3% TFA* 95 : 5	rt	(*R*)-**48**	11
							(*S*)-**48**	13
 (±)-**45**							(*R*)-**45**	14
							(*S*)-**45**	16
 (±)-**57**	Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 99.5 : 0.5	rt	**57**	9
							57	10
 (±)-**61**							**61**	40
							61	50

(±)-3-(3-Methylphenyl)butanoic acid (±)-65



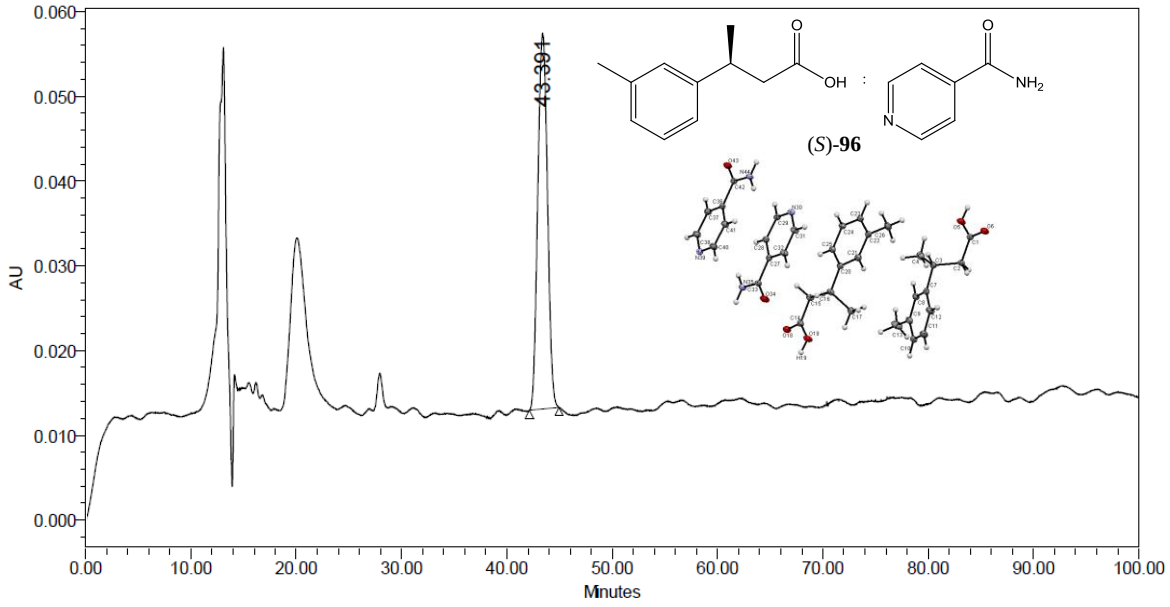
	RT	Area	% Area	Height
1	43.500	48183022	49.89	716956
2	49.327	48388711	50.11	638637

(±)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96



	RT	Area	% Area	Height
1	43.586	267189	51.73	4515
2	48.667	249270	48.27	3711

(*S*)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (*S*)-96

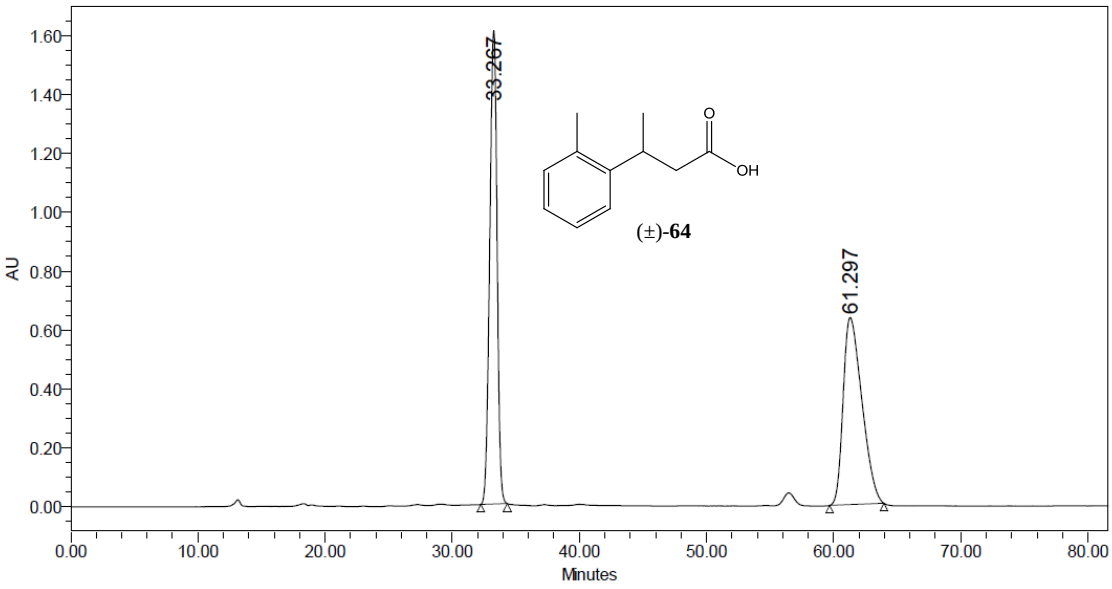


	RT	Area	% Area	Height
1	43.391	2835826	100.00	44306

Chiral HPLC conditions for (±)-3-(3-methylphenyl)butanoic acid (±)-65 and (±)-3-(3-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96

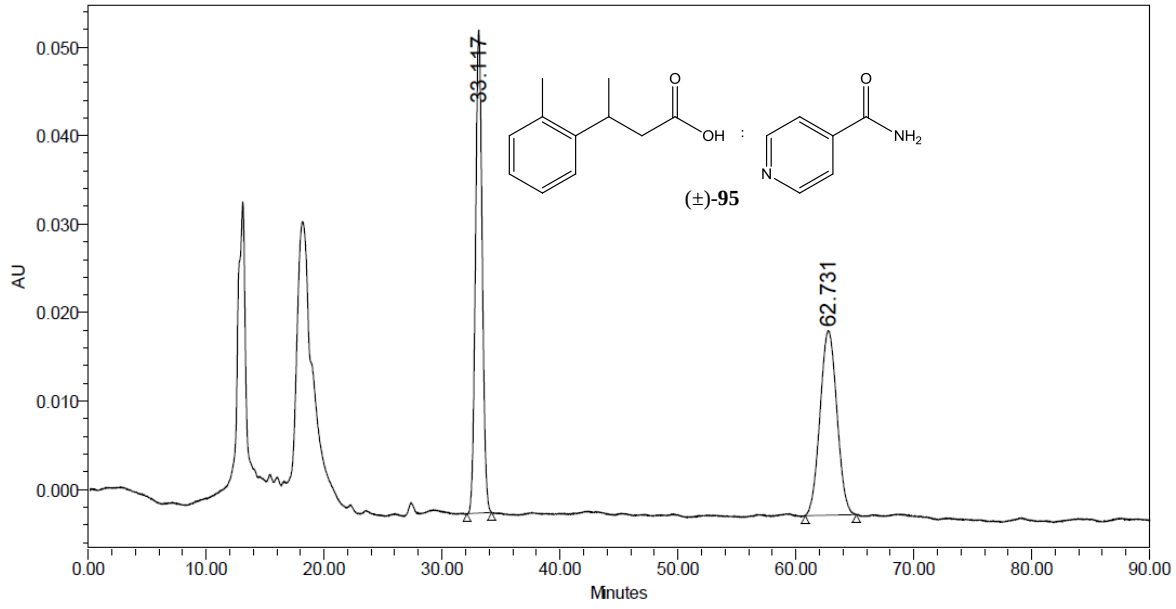
Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
						Enantiomer	min
Chiralcel OJ-H	10	0.25	209.8	*IPA mobile phase consists of 3% TFA* 96 : 4	0	(*S*)-**65** / **96**	44
						(*R*)-**65** / **96**	49

(±)-3-(2-Methylphenyl)butanoic acid (±)-64



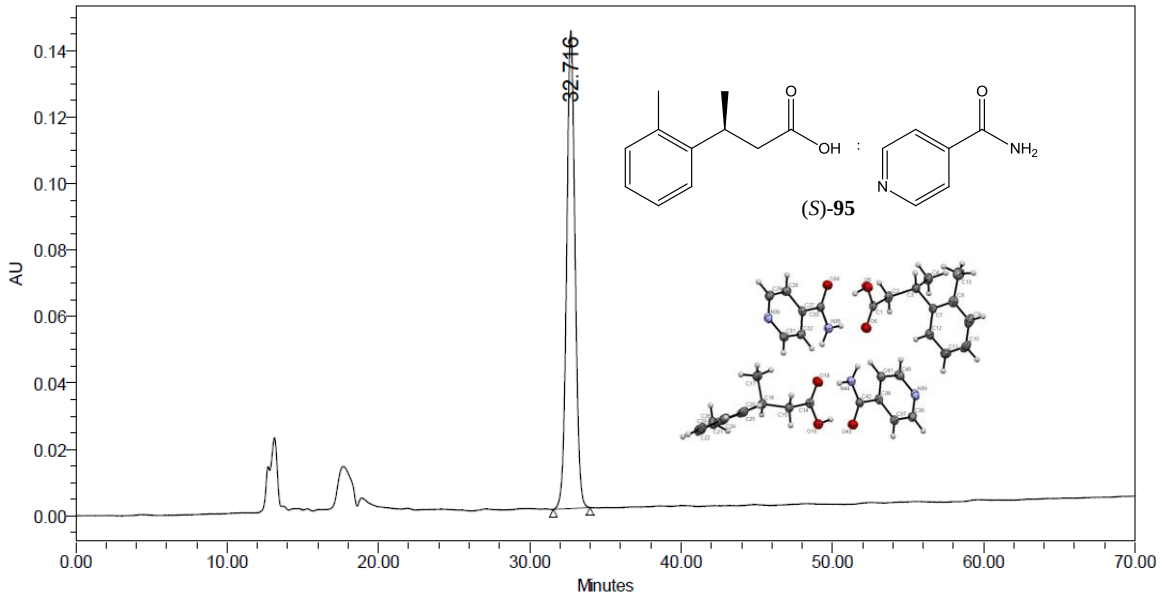
	RT	Area	% Area	Height
1	33.267	64417732	49.75	1607444
2	61.297	65059067	50.25	634969

(±)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95



	RT	Area	% Area	Height
1	33.117	2164888	51.08	54578
2	62.731	2073588	48.92	20854

(*S*)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (*S*)-95

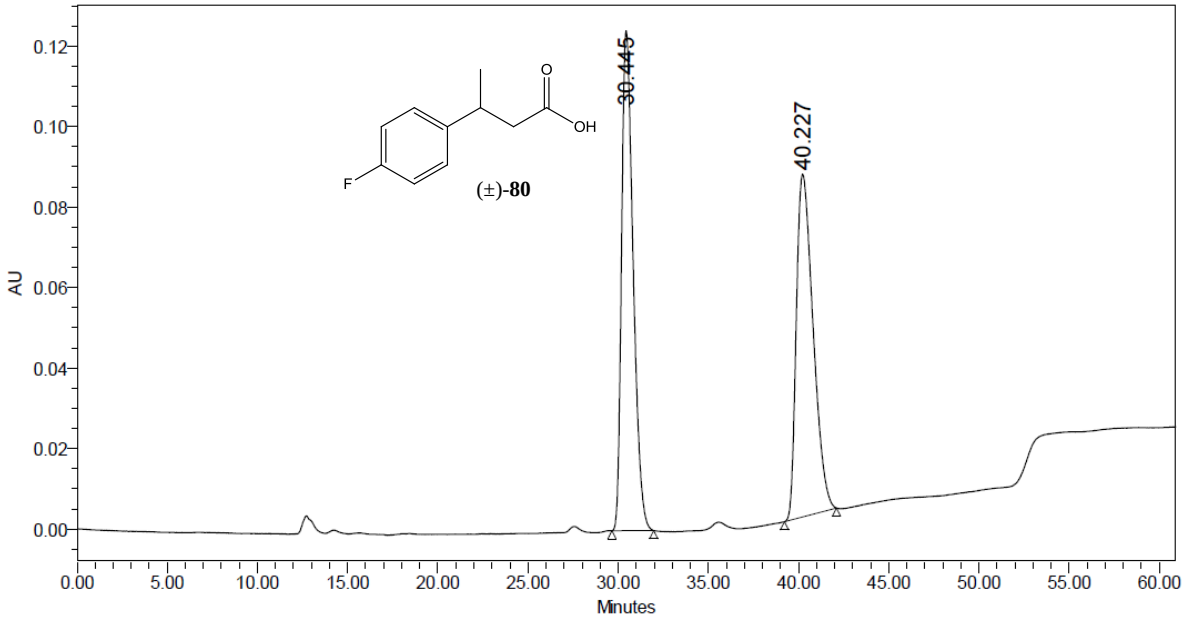


	RT	Area	% Area	Height
1	32.716	5534028	100.00	143764

Chiral HPLC conditions for (±)-3-(2-methylphenyl)butanoic acid (±)-64 and (±)-3-(2-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95

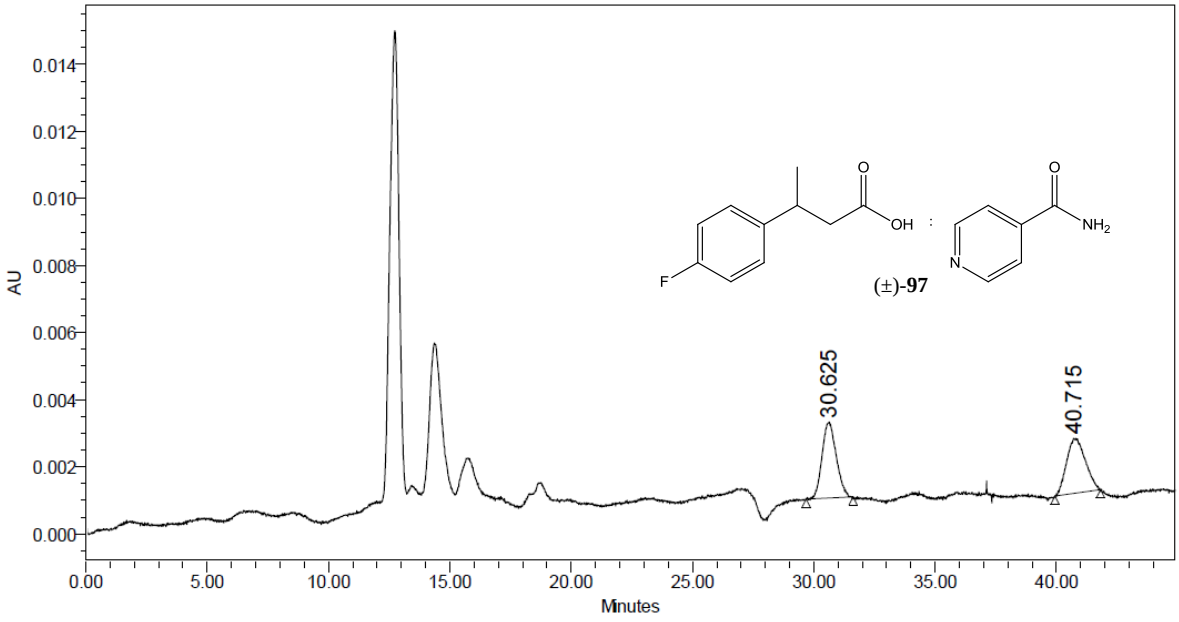
Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
						Enantiomer	min
Chiralcel OJ-H	10	0.25	209.8	*IPA mobile phase consists of 3% TFA* 96 : 4	0	(*S*)-**64** / **95**	33
						(*R*)-**64** / **95**	61

(±)-3-(4-Fluorophenyl)butanoic acid (±)-80



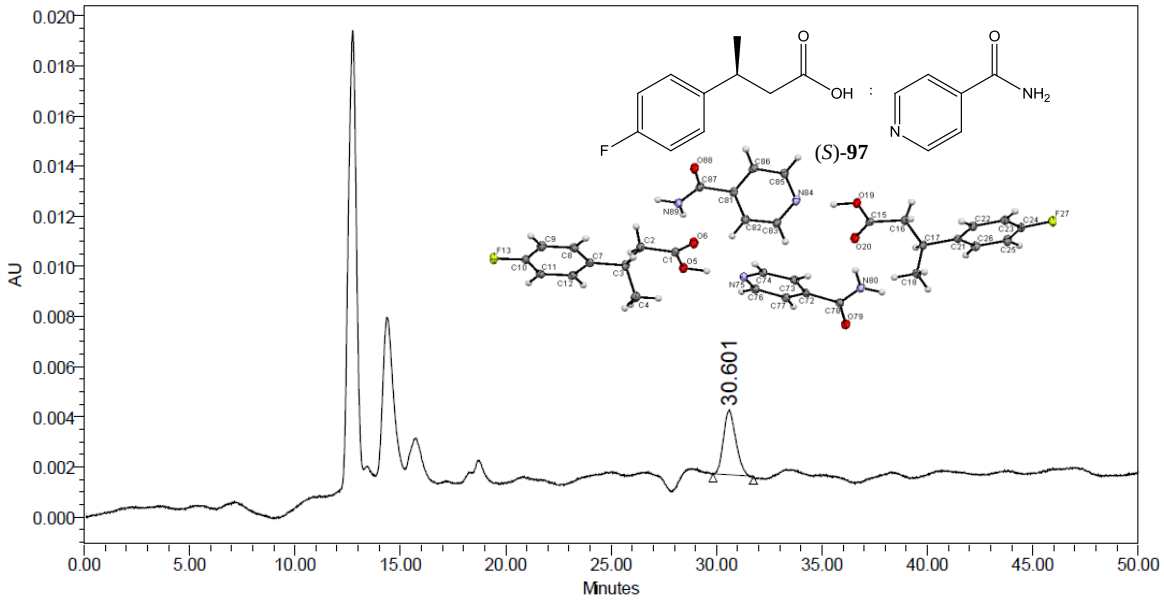
	RT	Area	% Area	Height
1	30.445	5533539	50.17	124234
2	40.227	5495619	49.83	85137

(±)-3-(4-Fluorophenyl)butanoic acid : isonicotinamide co-crystal (±)-97



	RT	Area	% Area	Height
1	30.625	91991	51.14	2267
2	40.715	87903	48.86	1639

(*S*)-3-(4-Fluorophenyl)butanoic acid: isonicotinamide co-crystal (*S*)-97

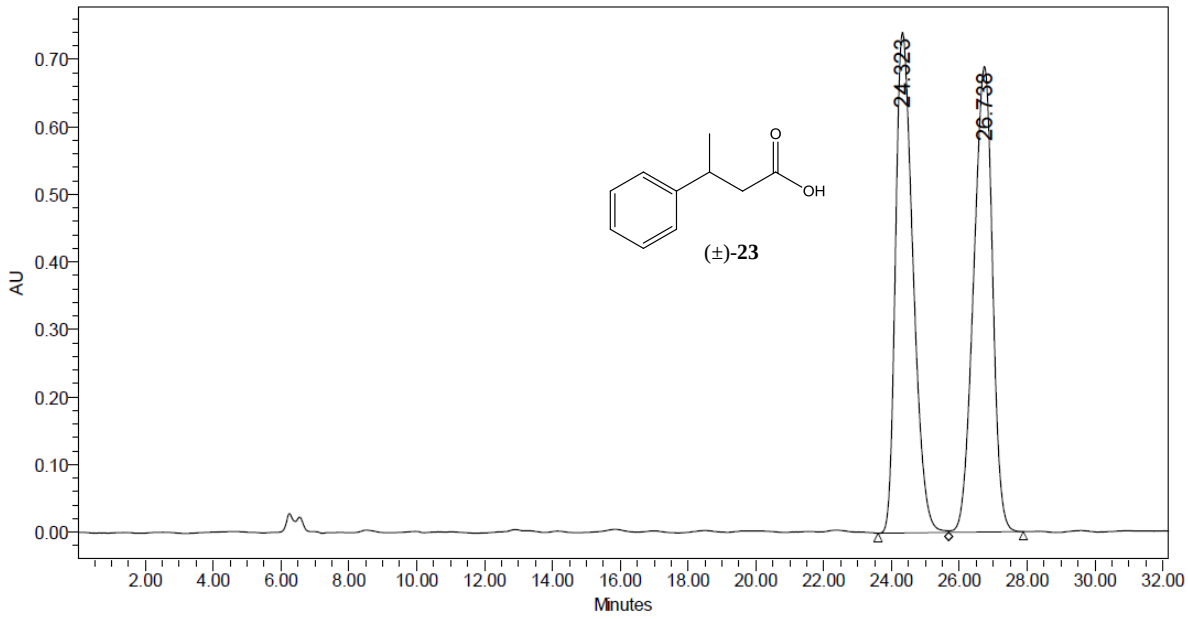


	RT	Area	% Area	Height
1	30.601	105340	100.00	2568

Chiral HPLC conditions for (±)-3-(4-fluorophenyl)butanoic acid (±)-80 and (±)-3-(4-fluoro phenyl)butanoic acid: isonicotinamide co-crystal (*S*)-97

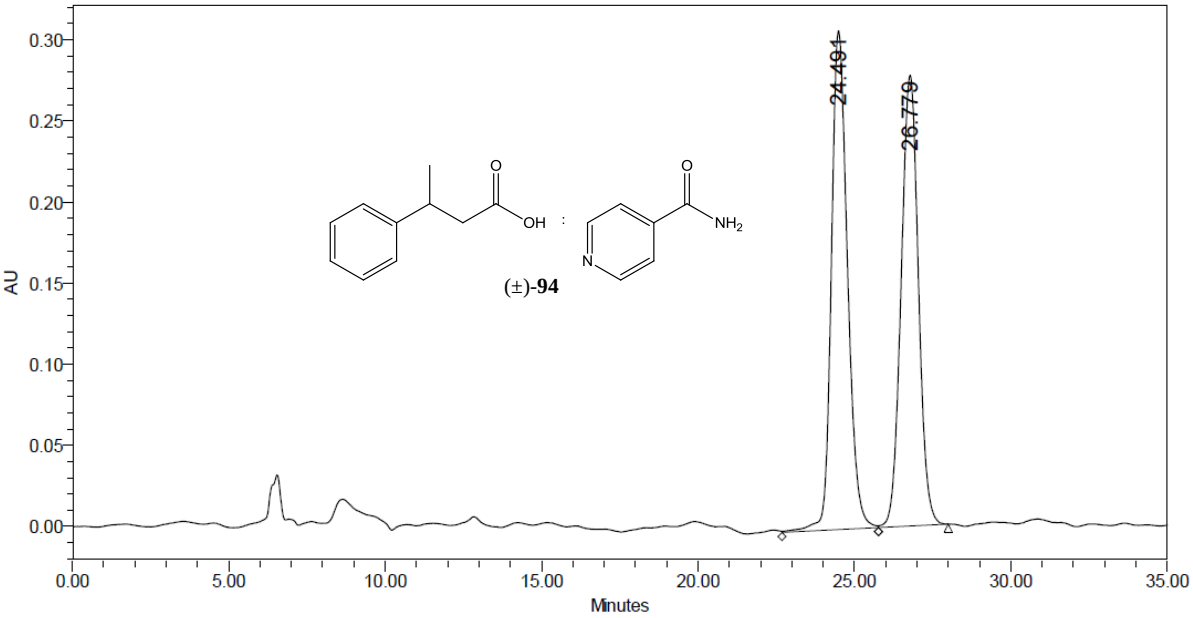
Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
						Enantiomer	min
Chiralcel OJ-H	10	0.25	256.0	*IPA mobile phase consists of 3% TFA* 96 : 4	0	(*S*)-**80** / **97**	30
						(*R*)-**80** / **97**	40

(±)-3-Phenylbutanoic acid (±)-23



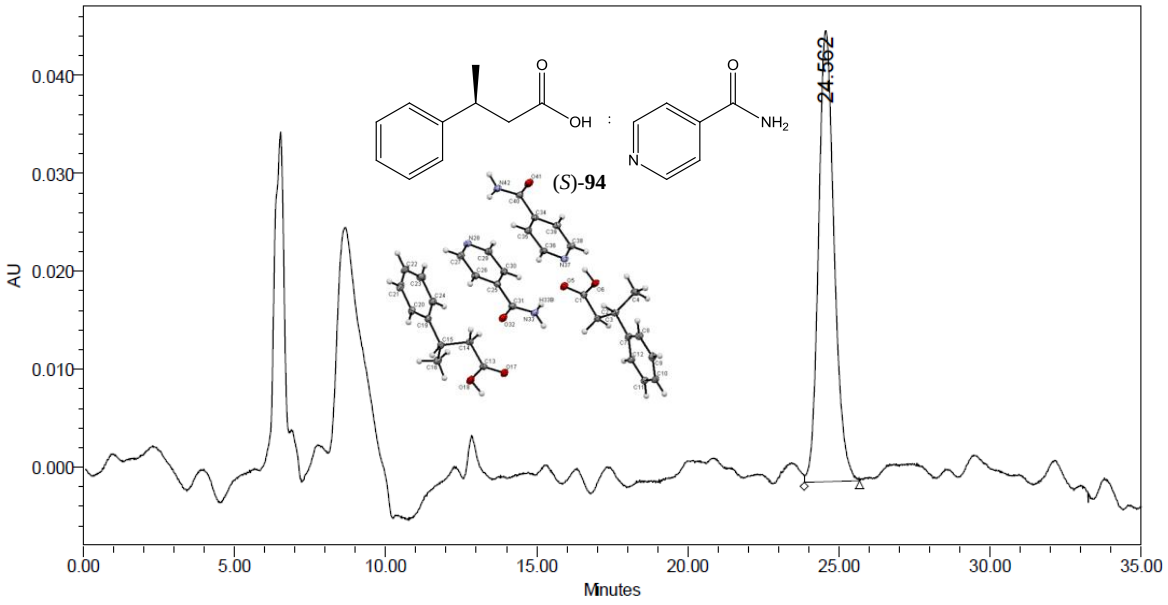
	RT	Area	% Area	Height
1	24.323	26556985	49.97	740472
2	26.738	26590236	50.03	688786

(±)-3-Phenylbutanoic acid : isonicotinamide co-crystal (±)-94



	RT	Area	% Area	Height
1	24.491	11035229	50.91	307660
2	26.779	10642441	49.09	277947

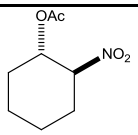
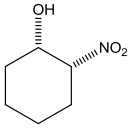
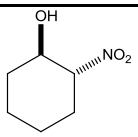
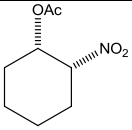
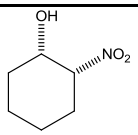
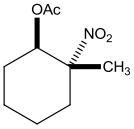
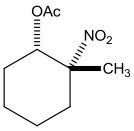
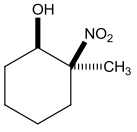
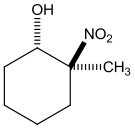
(*S*)-3-Phenylbutanoic acid : isonicotinamide co-crystal (*S*)-94

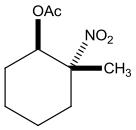
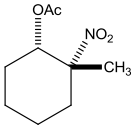
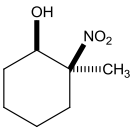
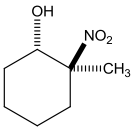
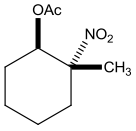
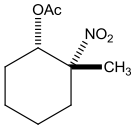
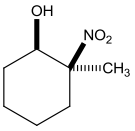
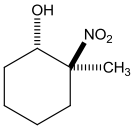


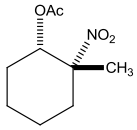
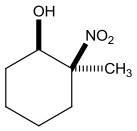
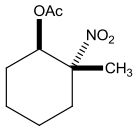
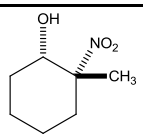
	RT	Area	% Area	Height
1	24.562	1658536	100.00	46096

Chiral HPLC conditions for (±)-3-Phenylbutanoic acid (±)-23 and (±)-3-Phenylbutanoic acid : isonicotinamide co-crystal (±)-94

Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
						Enantiomer	min
Chiralcel OJ-H	10	0.50	209.8	*IPA mobile phase consists of 3% TFA* 95 : 5	0	(*S*)-**23** / **94**	24
						(*R*)-**23** / **94**	27

Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**100b**	Chiralcel OJ-H	10	0.90	209.8	99 : 1	rt	(1*R*,2*R*)-**100b**	17
							(1*S*,2*S*)-**100b**	19
 (±)-**99a**							(1*S*,2*R*)-**99a**	58
							(1*R*,2*S*)-**99a**	65
 (±)-**99b**							(1*R*,2*R*)-**99b**	74
							(1*S*,2*S*)-**99b**	78
 (±)-**100a**	Chiralcel OD-H	10	0.90	209.8	99 : 1	rt	(1*R*,2*S*)-**100a**	23
							(1*S*,2*R*)-**100a**	30
 (±)-**99a**							(1*R*,2*S*)-**99a**	39
							(1*S*,2*R*)-**99a**	51
Conditions A  (±)-**119b**	Chiralcel OJ-H	10	0.50	209.8	97 : 3	rt	(1*R*,2*R*)-**119b**	18
							(1*S*,2*S*)-**119b**	19
Conditions A  (±)-**119a**							(1*R*,2*S*)-**119a**	23
							(1*S*,2*R*)-**119a**	27
Conditions A  (±)-**117a**							(1*S*,2*R*)-**117a**	33
							(1*R*,2*S*)-**117a**	40
Conditions A  (±)-**117b**							(1*S*,2*S*)-**117b**	36
							(1*R*,2*R*)-**117b**	43

Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
Conditions B  (±)-**119b**	Chiralcel OJ-H	10	0.50	209.8	99.5 : 0.5	rt	(1*R*,2*R*)-**119b**	26
							(1*S*,2*S*)-**119b**	32
Conditions B  (±)-**119a**							(1*R*,2*S*)-**119a**	40
							(1*S*,2*R*)-**119a**	49
Conditions B  (±)-**117a**							(1*R*,2*S*)-**117a**	95
							(1*S*,2*R*)-**117a**	108
Conditions B  (±)-**117b**							(1*S*,2*S*)-**117b**	134
							(1*R*,2*R*)-**117b**	156
Conditions C  (±)-**119b**	Chiralcel OJ-H	10	0.75	209.8	97 : 3	rt	(1*R*,2*R*)-**119b**	12
							(1*S*,2*S*)-**119b**	13
Conditions C  (±)-**119a**							(1*R*,2*S*)-**119a**	16
							(1*S*,2*R*)-**119a**	18
Conditions C  (±)-**117a**							(1*S*,2*R*)-**117a**	23
							(1*R*,2*S*)-**117a**	26
Conditions C  (±)-**117b**							(1*S*,2*S*)-**117b**	25
							(1*R*,2*R*)-**117b**	28

Compound	Column	Injection volume (µl)	Flow (mL/min)	λ Max (min)	Mobile phase (hexane : IPA)	Temp (°C)	Retention time	
							Enantiomer	min
 (±)-**119a**	Chiralcel OJ-H	10	0.90	209.8	96 : 4	rt	(1*R*,2*S*)-**119a**	12
							(1*S*,2*R*)-**119a**	14
 (±)-**117a**							(1*S*,2*R*)-**117a**	17
							(1*R*,2*S*)-**117a**	18
 (±)-**119b**	Chiralcel OJ-H	2	0.50	209.8	97 : 3	rt	(1*R*,2*R*)-**119b**	18
							(1*S*,2*S*)-**119b**	20
 (±)-**117b**							(1*S*,2*S*)-**117b**	37
							(1*R*,2*R*)-**117b**	43

Appendix II *List of abbreviations*

aq.	aqueous
Ar	aryl
Bn	benzyl
bp	boiling point
br s	broad singlet
Bu	butyl
BuLi	butyllithium
COSY	correlation spectroscopy
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
dd	doublet of doublets
DEPT	distortionless enhancement of polarisation transfer
DMAP	(dimethylamino)pyridine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
ee	enantiomeric excess
eq.	equivalents
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
E-value	enantiomeric value
g	gram
h	hour(s)
HETCOR	heteronuclear correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Hertz
i	*iso*
IMBY	immobilised baker's yeast
IPA	*iso*-propyl alcohol
IR	infrared
LDA	lithium diisopropyl amide
lit	literature
M	molar
m	multiplet
Me	methyl
mg	milligram
MHz	megahertz
min	minute(s)
mL	millilitre
mmol	millilitre
mol	mole
mp	melting point
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
OAc	acetate
PCC	pyridinium chlorochromate
Ph	phenyl

Pr	propyl
psi	pounds per square inch
rt	room temperature
t	*tert*
t	triplet
TBME	*tert*-butyl methyl ether
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMG	tetramethylguanidine
TMS	tetramethylsilane
tosyl	toluenesulfonyl
UV	ultraviolet

Appendix III *Publications*

Lipase catalysed kinetic resolutions of 3-aryl alkanoic acids

Rebecca E. Deasy, Maude Brossat, Thomas S. Moody, Anita R. Maguire

Tetrahedron: Asymmetry, **2011,** *22,* 47–61.

The Use of Co-crystals for the Determination of Absolute Stereochemistry: An Alternative to Salt Formation

Kevin S. Eccles, Rebecca E. Deasy, László Fábián, Anita R. Maguire, Simon E. Lawrence

J. Org. Chem., **2011**, *76*, 1159-1162.

Expanding the crystal landscape of isonicotinamide: concomitant polymorphism and co-crystallisation

Kevin S. Eccles, Rebecca E. Deasy, László Fábián, Doris E. Braun, Anita R. Maguire, Simon E. Lawrence

CrystEngComm, **2011**, *13*, 6923-6925.