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Optimisation of chemoenzymatic processes in asymmetric synthesis

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
Acknowledgements

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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
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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, efficacy and safety. In 2006, it was estimated that chiral compounds comprised more than half of drugs approved worldwide. Furthermore, five of the six top-selling drugs in 2007, Lipitor®, Plavix®, Nexium®, Diovan® and Advair® were sold as single enantiomers. 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. 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.

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. Alcohol dehydrogenases (ADHs) have been identified to be the key enzymes responsible for catalytic activity in the reduction of the carbonyl functional group. 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](image1.png)

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). 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. Also, isolated and purified enzymes are often employed to catalyse specific transformations, generally without the complication of competing reactions or tedious product recovery. 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. 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.

A number of strategies have been developed to improve the enantioselectivity of baker’s yeast, namely: action of irreversible enzyme inhibitors, addition of organic solvents, cell immobilisation, chemical modification of the substrate, pre-treatment of the cellular mass and addition of sulfur compounds to the reaction mixture. 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.

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 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.
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. 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 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%). 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)-4a and (R,R)-4b; the ratio of 3 to 4a/4b is dependent on the supplied sucrose concentration. 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).

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. 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, or be cleaved with retention of configuration by reductive desulfonylation. 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.

Scheme 1.1
1.2.1 Baker’s yeast mediated reduction of ketosulfides

The synthetic utility of enantiomerically pure secondary alcohols has been extensively documented.\textsuperscript{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,\textsuperscript{45} chiral oxiranes,\textsuperscript{32,46,47} thiranes,\textsuperscript{48} tetrahydrofurans\textsuperscript{49,50} and 4-acetoxyazetidinones.\textsuperscript{51} Moreover, they are easily oxidised to hydroxsulfoxides\textsuperscript{52-54} or sulfones\textsuperscript{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.\textsuperscript{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](image)

Although the stereochemical course of the baker’s yeast reduction is generally predicted by Prelog’s rule, several different oxidoreductases of \textit{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 \textsuperscript{7} (R\textsubscript{1} = Me, X = H, R\textsubscript{2} = Et) provides the (S)-hydroxyester (S)-8 with 97% ee,\textsuperscript{61} ethyl 3-oxopentanoate \textsuperscript{7} (R\textsubscript{1} = Et, X = H, R\textsubscript{2} = Et)\textsuperscript{60} and ethyl 5-benzyloxy-3-oxopentanoate \textsuperscript{7} (R\textsubscript{1} = PhCH\textsubscript{2}OCH\textsubscript{2}CH\textsubscript{2}, X = H, R\textsubscript{2} = Et) are reduced to the corresponding (R)-hydroxyesters (R)-8, of opposite configuration with poor optical purities (\textasciitilde 40% ee) (Scheme 1.3).\textsuperscript{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 \(7\) \((X = \text{SMe})\), giving exclusively \((3S)\)-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.\(^{63}\)

\[\text{Scheme 1.3}\]

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.

\[\text{Scheme 1.4}\]
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-(1R,2S)-S-ethyl 2-hydroxycyclopentanecarbothioate (1R,2S)-14d was exclusively formed with enhanced enantioselectivity (>96% ee) (entry 4, Table 1.1). Although the octyl ester derivative 13c also gave optically pure cis-(1R,2S)-hydroxyester (1R,2S)-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 (2R,5S)-malynoglide, a biologically active antibiotic isolated from the marine blue-green algae (Lyngbya majuscula), and (1S,5R)-frontalin, the aggregation pheromone of the southern pine bark beetle (Dendroctonus brevicomis).

Table 1.1: The baker’s yeast reduction of 2-cyclopentanonecarboxylate 13

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>X</th>
<th>Time</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>cis (1R,2S)-14 : trans (1S,2S)-14</th>
<th>ee (%) of (1R,2S)-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13a</td>
<td>OCH₃</td>
<td>1 d</td>
<td>14a</td>
<td>35</td>
<td>93 : 7</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>13b</td>
<td>OC₂H₅</td>
<td>2 d</td>
<td>14b</td>
<td>50</td>
<td>97 : 3</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>13c</td>
<td>OC₈H₁₇</td>
<td>6 d</td>
<td>14c</td>
<td>62</td>
<td>100 : 0</td>
<td>&gt;96</td>
</tr>
<tr>
<td>4</td>
<td>13d</td>
<td>SC₂H₅</td>
<td>3.5 h</td>
<td>14d</td>
<td>88</td>
<td>100 : 0</td>
<td>&gt;96</td>
</tr>
</tbody>
</table>

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. 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Sulfur compound</th>
<th>eq.</th>
<th>Time</th>
<th>Yield (%)a</th>
<th>Yield (%)b</th>
<th>16 : 17 : 18 [ee (%)]b</th>
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<tr>
<td>1</td>
<td>15a</td>
<td>Me</td>
<td>None</td>
<td>-</td>
<td>2 h</td>
<td>41</td>
<td>-</td>
<td>94 (&gt;99) : 6 : -</td>
</tr>
<tr>
<td>2</td>
<td>15a</td>
<td>Me</td>
<td>Me₂S</td>
<td>1.0</td>
<td>2 h</td>
<td>46</td>
<td>-</td>
<td>&gt;99 (&gt;99) : 1 : -</td>
</tr>
<tr>
<td>3</td>
<td>15b</td>
<td>n-Bu</td>
<td>None</td>
<td>-</td>
<td>6 h</td>
<td>52</td>
<td>28</td>
<td>53 (98) : 12 (99) : 35 (68)</td>
</tr>
<tr>
<td>4</td>
<td>15b</td>
<td>n-Bu</td>
<td>L-Cysteine</td>
<td>1.0</td>
<td>1 h</td>
<td>66</td>
<td>8</td>
<td>79 (96) : 10 (99) : 11 (60)</td>
</tr>
<tr>
<td>5</td>
<td>15c</td>
<td>Ph</td>
<td>None</td>
<td>-</td>
<td>5 h</td>
<td>58</td>
<td>2</td>
<td>64 (90) : 33 (99) : 3</td>
</tr>
<tr>
<td>6</td>
<td>15c</td>
<td>Ph</td>
<td>L-Cysteine</td>
<td>2.0</td>
<td>4 h</td>
<td>48</td>
<td>-</td>
<td>75 (98) : 25 (99) : -</td>
</tr>
</tbody>
</table>

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

Reduction of the simplest substrate acetoxyacetone 15a with the addition of 1.0 equivalent of dimethyl sulfide (Me₂S), 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 acetoxyacetoephonene 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 tetrahydrothiophuran and pyran β-ketoester derivatives in the presence of a sulfur additive. 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. 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 and also into anhydro-serricornine 22, a sex pheromone of the cigarette beetle (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 and affords (1S,2R)-2-(phenylthio)cyclopentanol 24 in optically pure form (99% ee) with the cis alcohol obtained exclusively (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). Notably, although stereochemistry was assigned by spectral 1H NMR data, the optical purity of these compounds was not reported. Recently Maguire and co-workers reinvestigated the asymmetric synthesis of the lactone 26. 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

![Diagram of Scheme 1.7](image)

Scheme 1.7

![Diagram of Figure 1.3](image)

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)-hydroxy sulfide (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
Chapter 1

Introduction

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, \((S)-3-(\text{phenylthio})-1,2\)-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 \(\delta\)-\(n\)-hexadecanolid\(^e\) 32 and for the synthesis of the deoxy sugars L-rhodinose and D-amicetose (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). 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.

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).
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). These highly enantiomerically enriched alcohols were then transformed to furan and pyran cyclic systems, to substituted cycloalkane rings and to the spiroacetal bee pheromone.

Table 1.4: The baker’s yeast reduction of remote sulfide functional groups

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>n</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{45})</td>
<td>49a</td>
<td>1</td>
<td>(S)-48a</td>
<td>70</td>
<td>96</td>
</tr>
<tr>
<td>2(^{45})</td>
<td>49b</td>
<td>2</td>
<td>(S)-48b</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>3(^{78})</td>
<td>49c</td>
<td>3</td>
<td>(S)-48c</td>
<td>97</td>
<td>97</td>
</tr>
</tbody>
</table>

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-sulfenylacetooacetic ester derivatives 50a-g. 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 benzylthio 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-sulfenylacetooacetic ester derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>Reaction time (h)</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50a</td>
<td>Me</td>
<td>Et</td>
<td>11</td>
<td>(R)-51a</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>50b</td>
<td>Bn</td>
<td>Et</td>
<td>4.5</td>
<td>(R)-51b</td>
<td>85</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>50c</td>
<td>Bn</td>
<td>nBu</td>
<td>26</td>
<td>(R)-51c</td>
<td>70</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>50d</td>
<td>Ph</td>
<td>Et</td>
<td>13</td>
<td>(R)-51d</td>
<td>63</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>50e</td>
<td>m-Tol</td>
<td>Et</td>
<td>2.5</td>
<td>(R)-51e</td>
<td>72</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>50f</td>
<td>o-Tol</td>
<td>Et</td>
<td>5</td>
<td>(R)-51f</td>
<td>68</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>50g</td>
<td>p-MeOPh</td>
<td>Et</td>
<td>13</td>
<td>(R)-51g</td>
<td>64</td>
<td>56</td>
</tr>
</tbody>
</table>
Fujisawa observed the baker’s yeast mediated reduction of 3-propionyltetrahydrothiopyran-4-ones 53 to give exclusively the (3R,4S)-3-propionyl-4-hydroxytetrahydrothiopyrane 54 in 66% yield with excellent enantioselectivity (97% ee). 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 (4R,5S)-sitophilure 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. Optically pure diol (2S,3S)-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).

Improved enantio- and diastereoselectivity was achieved in the baker’s yeast reduction of β-keto aldehyde derivatives using a sulfur compound as an additive. 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.).
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).\textsuperscript{83}

\begin{center}
\textbf{Figure 1.4}
\end{center}

\begin{center}
\textbf{1.2.1.4 Reduction of olefins}
\end{center}

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 (1S,2R)-24 as the sole product in 47% yield (Scheme 1.16).\textsuperscript{71} In contrast to the cyclopentenone 62, incubation of the cyclohexenone derivative for 20 days led to recovery of the substrate.

\begin{center}
\textbf{Scheme 1.16}
\end{center}
Crout and co-workers investigated the baker’s yeast mediated reduction of a number of \( \alpha,\beta \)-unsaturated carbonyl compounds with a \( \gamma \)-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 \textit{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).

![Scheme 1.17](image)

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).

![Scheme 1.18](image)

Allylic alcohol \( 68 \), with a \( \gamma \)-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).

![Scheme 1.19](image)
A similar trend of stereochemical behaviour was described by Serra et al. in the baker’s yeast mediated reduction of sulfur-functionalised methacroleins. 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).

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).

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).

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.
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. Notably, only reduction of the allyl derivative 80g (entry 7, Table 1.6) afforded the (R)-enantiomer with high enantioselection. 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. 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 B₄ via the Wittig reaction.

Table 1.6: The baker’s yeast reduction of α-ketothioacetals 80₉₀,₉₁

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R¹</th>
<th>R²</th>
<th>Time</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1₉₀,₉₁</td>
<td>80a</td>
<td>CH₃</td>
<td>H</td>
<td>1 d</td>
<td>(S)-81a</td>
<td>84</td>
<td>&gt;96</td>
</tr>
<tr>
<td>2₉₀,₉₁</td>
<td>80b</td>
<td>C₂H₅</td>
<td>H</td>
<td>2 d</td>
<td>(S)-81b</td>
<td>71</td>
<td>&gt;96</td>
</tr>
<tr>
<td>3₉₀</td>
<td>80c</td>
<td>n-C₃H₇</td>
<td>H</td>
<td>4 d</td>
<td>(S)-81c</td>
<td>92</td>
<td>&gt;96</td>
</tr>
<tr>
<td>4₉₀</td>
<td>80d</td>
<td>n-C₄H₉</td>
<td>H</td>
<td>3 d</td>
<td>(S)-81d</td>
<td>71</td>
<td>&gt;96</td>
</tr>
<tr>
<td>5₉₀</td>
<td>80e</td>
<td>(CH₂)OH</td>
<td>H</td>
<td>10 d</td>
<td>(S)-81e</td>
<td>74</td>
<td>&gt;96</td>
</tr>
<tr>
<td>6₉₀</td>
<td>80f</td>
<td>CH₃</td>
<td>CH₃</td>
<td>2.5 d</td>
<td>(S)-81f</td>
<td>50</td>
<td>&gt;96</td>
</tr>
<tr>
<td>7₉₀</td>
<td>80g</td>
<td>CH₃</td>
<td>CH₂CHCH₂</td>
<td>4 d</td>
<td>(R)-81g</td>
<td>31</td>
<td>&gt;96</td>
</tr>
<tr>
<td>8₉₁</td>
<td>80h</td>
<td>n-C₆H₁₃</td>
<td>H</td>
<td>8 d</td>
<td>(S)-81h</td>
<td>38</td>
<td>≥95</td>
</tr>
<tr>
<td>9₉₁</td>
<td>80i</td>
<td>CF₃</td>
<td>H</td>
<td>2 h</td>
<td>(S)-81i</td>
<td>96</td>
<td>67</td>
</tr>
<tr>
<td>10₉₁</td>
<td>80j</td>
<td>CH₂OH</td>
<td>H</td>
<td>5 d</td>
<td>(S)-81j</td>
<td>28</td>
<td>≥95</td>
</tr>
<tr>
<td>11₉₁</td>
<td>80k</td>
<td>CH₂OAc</td>
<td>H</td>
<td>1 d</td>
<td>(S)-81k</td>
<td>58</td>
<td>87</td>
</tr>
<tr>
<td>12₉₁</td>
<td>80l</td>
<td>CH₂OBn</td>
<td>H</td>
<td>5 d</td>
<td>(S)-81l</td>
<td>50</td>
<td>≥95</td>
</tr>
<tr>
<td>13₉₁</td>
<td>80m</td>
<td>CH₂O(p-MeOPh)</td>
<td>H</td>
<td>6 d</td>
<td>(S)-81m</td>
<td>27</td>
<td>≥98</td>
</tr>
<tr>
<td>14₉₁</td>
<td>80n</td>
<td>CH₂OTHP</td>
<td>H</td>
<td>3 d</td>
<td>(S)-81n</td>
<td>37</td>
<td>≥95</td>
</tr>
<tr>
<td>15₉₁</td>
<td>80o</td>
<td>CH₂OCH₂OMe</td>
<td>H</td>
<td>1 d</td>
<td>(S)-81o</td>
<td>82</td>
<td>≥95</td>
</tr>
<tr>
<td>16₉₁</td>
<td>80p</td>
<td>CH₂OSi(CH₂)₂Bu</td>
<td>H</td>
<td>7 d</td>
<td>(S)-81p</td>
<td>&lt;10%</td>
<td>-</td>
</tr>
<tr>
<td>17₉₂</td>
<td>80q</td>
<td>(CH₂)₂CO₂f-Bu</td>
<td>H</td>
<td>3 d</td>
<td>(S)-81q</td>
<td>65</td>
<td>≥97</td>
</tr>
<tr>
<td>18₉₂</td>
<td>80r</td>
<td>(CH₂)₂CO₂CH₃</td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 including both enantiomers of the macrocyclic lactone lasiodiplodin, which have demonstrated significant antileukemic activity. The corresponding 5-membered thioacetal 1-(1,3-dithiolan-2-yl)-2-propanone 84 was also reduced in >99% ee.
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 (1S,2S)-anti-diol (1S,2S)-88 are obtained in 60% and 82% yields after 2 and 48 h respectively. 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 (1R,2S)-88 with high diastereoselectivity, while (1S,2S)-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.
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). 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-tolylthioalkan-2-ones 91<sup>101</sup>

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Time</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91a</td>
<td>CH₃</td>
<td>3.5 d</td>
<td>(S)-92a</td>
<td>50</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>91b</td>
<td>CH₂F</td>
<td>4 d</td>
<td>(S)-92b</td>
<td>42</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>91c</td>
<td>CH₃OCH₂OCH₂</td>
<td>6 d</td>
<td>(S)-92c</td>
<td>50</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>91d</td>
<td>C₂H₅</td>
<td>7 d</td>
<td>(S)-92d</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>91e</td>
<td>CH₂Cl</td>
<td>7 d</td>
<td>(S)-92e</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>91f</td>
<td>CH₂OH</td>
<td>7 d</td>
<td>(S)-92f</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>91g</td>
<td>CF₃</td>
<td>3 d</td>
<td>(S)-92g</td>
<td>25</td>
<td>95</td>
</tr>
</tbody>
</table>

Notably, poor yields and enantioselectivities were also observed on the reduction of 2-acyl-thiazoles. 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. 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. 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).
1.2.3 Baker’s yeast mediated reduction of thiocarbonyls

Madsen and Nielson reported that reduction of β-thioester 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). 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 (1S,2S)-thiol 100 with 81% ee and the (1R,2S)-alcohol 101 with 97% ee.

Highly syn-selective baker’s yeast mediated reduction was observed of the β-ketodithioesters 102a-d providing primarily the corresponding optically active (3S)-hydroxythioester syn-103a-d. 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-(1R,2S)-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.
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\textsuperscript{104,105} and carbon-oxygen\textsuperscript{53} bond forming reactions, in cycloaddition reactions,\textsuperscript{106-108} radical addition reactions\textsuperscript{109,110} and in asymmetric catalysis.\textsuperscript{111} Furthermore, chiral sulfoxides are formed as metabolites of many sulfur-containing drugs,\textsuperscript{112,113} and exhibit differential stereochemically-dependent metabolism\textsuperscript{114,115} and enzyme inhibition.\textsuperscript{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 \(\beta\)-keto sulfoxide derivatives with concomitant reduction of the ketone functionality has been reported to a lesser extent, as the maximum yield of enantioenriched \(\beta\)-keto sulfoxide and/or \(\beta\)-hydroxy sulfoxide is at best 50%.

1.2.4 Baker’s yeast mediated reduction of ketosulfoxides

As described at the beginning of section 1.2, one of the earliest reports of kinetic resolution of sulfoxides was the reduction of (\(\pm\))-1-(phenylsulfinyl)propan-2-one (\(\pm\))-3 affording a diastereomeric mixture of 1-(phenylsulfinyl)propan-2-ol (Rs,Sc)-4 and (Rs,Rc)-4 while the (S)-sulfoxide 3 was recovered in optically pure form (Scheme 1.1)\textsuperscript{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.\textsuperscript{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,Sc)-(+-)-1-(benzylsulfinyl)propan-2-ol (S,Sc)-105 (40%) after 5 h while after 4 d the reaction yielded the (R)-sulfoxide (R)-104 (10%) and the alcohols (S,Sc)-105 (30%) and (R,Sd)-105 (25%).

To view this table as an image, please click on the image.
Scheme 1.28

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

Scheme 1.29

Fujisawa et al. employed baker’s yeast to resolve racemic \((\pm)-1-((p\text{-chlorophenylsulfinyl})propan-2-one \((\pm)-108\) to provide access to \((S_cR_s)-2\text{-hydroxypropyl } p\text{-chlorophenyl sulfoxide } (S_cR_s)-109\) 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 \((-)-110\).

Scheme 1.30

Crout et al. also investigated the baker’s yeast reduction of \(\alpha,\beta\)-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.

![Scheme 1.31](image)

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

![Scheme 1.32](image)

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). Advantageously this chemoenzymatic process employs a novel diisopropyl ether/limited water system with greatly simplified work-up relative to aqueous bioreductions.
1.2.4.1 Asymmetric oxidation of sulfides by baker’s yeast

While asymmetric sulfur oxidation via the Kagan\textsuperscript{120,121} and Modena\textsuperscript{122} methods is well established, there have been some reports of biological asymmetric sulfide oxidation.\textsuperscript{123-126} Although uncommon, a small number of baker’s yeast mediated oxidation transformations of sulfides to sulfoxides have been reported.\textsuperscript{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 \((R)-120\) under semi-anaerobic conditions (Scheme 1.33). This was subsequently employed in the synthesis of the hypocholesteremic agent \((R)-121\).\textsuperscript{128,129}

![Scheme 1.33](image-url)

**Scheme 1.33**

Under aerobic conditions, the thia-fatty acid \((R)-122\) is converted to the \((R)-\) sulfoxide by baker’s yeast in \(>96\%\) ee,\textsuperscript{130-132} and the analogues \((R)-123\) are similarly transformed (Figure 1.6).\textsuperscript{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.\textsuperscript{135}

![Figure 1.5](image-url)

**Figure 1.5**

\begin{tabular}{|c|c|c|}
\hline
\textbf{Product} & \textbf{ee (\%)} & \textbf{syn/anti} \\
\hline
\((S,S)-117\) & \textgreater 99 & 26 : 1 \\
\((S,R)-117\) & 99 & 1 : 38 \\
\((R,S)-117\) & 99 & 1 : 29 \\
\((S,S)-117\) & \textgreater 99 & 12 : 1 \\
\hline
\end{tabular}
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). The rate of conversion was shown to depend on the ratio of substrate to sucrose. 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) and gave (S)-6c with 46% ee (entry 3, Table 1.9), whereas reduction of 5d yielded only 10% of nearly racemic (S)-6d (entry 4, Table 1.9). 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). Reduction with Sake yeast (Saccharomyces cerevisiae) kyokai-7, however, gave (R)-6f with 84% yield and 92% ee.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Alcohol</th>
<th>Yield</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5a</td>
<td>CH₃</td>
<td>(S)-6a</td>
<td>98</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>2</td>
<td>5b</td>
<td>CH₂CH₃</td>
<td>(S)-6b</td>
<td>90</td>
<td>63%</td>
</tr>
<tr>
<td>3</td>
<td>5c</td>
<td>n-C₃H₇</td>
<td>(S)-6c</td>
<td>53</td>
<td>46%</td>
</tr>
<tr>
<td>4</td>
<td>5d</td>
<td>n-C₅H₁₁</td>
<td>(S)-6d</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>5</td>
<td>5e</td>
<td>n-C₆H₁₃</td>
<td>(S)-6e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>5f</td>
<td>C₆H₆</td>
<td>(R)-6f</td>
<td>87</td>
<td>15%</td>
</tr>
</tbody>
</table>

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).

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\(^7\) or >80% ee\(^2\) (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.\(^1\)

\[
\begin{align*}
\text{128} & \quad \xrightarrow{\text{Baker’s yeast}} \quad \text{129} & \quad \text{(S)-129 (84% yield, >97% ee)}\(^7\) \\
\text{130} & \quad \xrightarrow{\text{Baker’s yeast}} \quad \text{131} & \quad \text{(R)-131 (85% yield, 78% ee)}
\end{align*}
\]

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 sulfoxide 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).\(^1\) For example, 7-hydroxy-1-phenylsulfonylheptan-2-one 132 afforded the corresponding (S)-diol (S)-133 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).\(^2\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>n</th>
<th>Alcohol</th>
<th>Yield</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>132a</td>
<td>1</td>
<td>(S)-133a</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>132b</td>
<td>2</td>
<td>(S)-133b</td>
<td>42</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>132c</td>
<td>3</td>
<td>(S)-133c</td>
<td>74</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>132d</td>
<td>4</td>
<td>(S)-133d</td>
<td>39</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>132e</td>
<td>5</td>
<td>(S)-133e</td>
<td>84</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 1.10: The baker’s yeast reduction of acyclic β-ketosulfoxones 132\(^3\)

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). 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Alcohol</th>
<th>Yield</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>134a</td>
<td>n-C₄H₉</td>
<td>(S)-135a</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>134b</td>
<td>n-C₅H₁₁</td>
<td>(S)-135b</td>
<td>26</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>134c</td>
<td>n-C₆H₁₃</td>
<td>(S)-135c</td>
<td>14</td>
<td>76</td>
</tr>
</tbody>
</table>

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. 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>n</th>
<th>Alcohol</th>
<th>Yield</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136a</td>
<td>2</td>
<td>(S)-137a</td>
<td>68</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>136b</td>
<td>3</td>
<td>(S)-137b</td>
<td>44</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>136c</td>
<td>4</td>
<td>(S)-137c</td>
<td>5</td>
<td>81</td>
</tr>
</tbody>
</table>

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). 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.
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).\textsuperscript{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.

Svatoš et al. recently investigated the microbial reduction of 3-methyl-1-(phenylsulfonyl)hexan-2-one 146 with more than 20 yeasts.\textsuperscript{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 Kloekera 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

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

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. 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 R² 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 R², 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R¹</th>
<th>R²</th>
<th>Time</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>148a</td>
<td>C₆H₅SO₂</td>
<td>CH₃</td>
<td>4 h</td>
<td>(S)-149a</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>148b</td>
<td>4-CH₂C₆H₅SO₂</td>
<td>CH₃</td>
<td>5 h</td>
<td>(S)-149b</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>148c</td>
<td>4-C₆H₄C₆H₅SO₂</td>
<td>CH₃</td>
<td>5 h</td>
<td>(S)-149c</td>
<td>85</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>148d</td>
<td>4-NO₂C₆H₄SO₂</td>
<td>CH₃</td>
<td>4 h</td>
<td>(S)-149d</td>
<td>47</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>148e</td>
<td>4-ClC₆H₄SO₂</td>
<td>CH₃</td>
<td>4 h</td>
<td>(S)-149e</td>
<td>77</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td>148f</td>
<td>C₆H₅SO₂</td>
<td>C₂H₅</td>
<td>10 h</td>
<td>(S)-149f</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>148g</td>
<td>C₆H₅SO₂</td>
<td>C₅H₅</td>
<td>12 h</td>
<td>(S)-149g</td>
<td>74</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>148h</td>
<td>C₆H₅SO₂</td>
<td>C₆H₆</td>
<td>20 h</td>
<td>149h</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>148i</td>
<td>C₆H₆</td>
<td>CH₃</td>
<td>12 h</td>
<td>(S)-149i</td>
<td>97</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td>148i¹</td>
<td>C₆H₆</td>
<td>-CH₃</td>
<td>24 h</td>
<td>149i²</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>148j</td>
<td>4-CH₂C₆H₆</td>
<td>CH₃</td>
<td>12 h</td>
<td>(S)-149j</td>
<td>96</td>
<td>99</td>
</tr>
<tr>
<td>12</td>
<td>148k</td>
<td>4-C₆H₄C₆H₆</td>
<td>CH₃</td>
<td>12 h</td>
<td>(S)-149k</td>
<td>86</td>
<td>99</td>
</tr>
<tr>
<td>13</td>
<td>148l</td>
<td>4-ClC₆H₆</td>
<td>CH₃</td>
<td>18 h</td>
<td>(S)-149l</td>
<td>67</td>
<td>98</td>
</tr>
<tr>
<td>14</td>
<td>148m</td>
<td>4-NO₂C₆H₆</td>
<td>CH₃</td>
<td>22 h</td>
<td>(S)-149m</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>15</td>
<td>148n</td>
<td>4-BrC₆H₆</td>
<td>CH₃</td>
<td>17 h</td>
<td>(S)-149n</td>
<td>73</td>
<td>97</td>
</tr>
</tbody>
</table>

*0.5 g 148 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). 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.

Scheme 1.38

Fujisawa et al. reported one of the first baker’s yeast mediated reduction of a cyclic β-ketosulfone. 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 (1S,2R,3S)-153 and (1S,2R,3R)-153 (Scheme 1.39). Both isomers were isolated with excellent enantiopurity (>99% ee).
Recently, efficient dynamic kinetic resolution in the baker’s yeast reduction of 2-benzenesulfonylcycloalkanones was reported (Table 1.15).\textsuperscript{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.\textsuperscript{29,71}

\textit{Table 1.15: The baker’s yeast reduction of 2-benzenesulfonylcycloalkanones 154}\textsuperscript{146}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>n</th>
<th>Alcohol</th>
<th>Yield</th>
<th>ee (%)</th>
<th>dr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154a</td>
<td>1</td>
<td>(S)-155a</td>
<td>95</td>
<td>&gt;98</td>
<td>98:2</td>
</tr>
<tr>
<td>2</td>
<td>154b</td>
<td>2</td>
<td>(S)-155b</td>
<td>78</td>
<td>&gt;95</td>
<td>&gt;98:2</td>
</tr>
<tr>
<td>3</td>
<td>154c</td>
<td>3</td>
<td>(S)-155c</td>
<td>8</td>
<td>-</td>
<td>&gt;98:2</td>
</tr>
<tr>
<td>4</td>
<td>154d</td>
<td>4</td>
<td>(S)-155d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 (1S,2R)-cyclopentanols 157a-g and recovery of the (2S)-cyclopentanones 156a-g (Table 1.16).\textsuperscript{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.
Chapter 1

Table 1.16: Efficient kinetic resolution of 2-benzenesulfonylcyclopentanone derivatives 156 via baker’s yeast mediated reduction

Table 1.17: The baker’s yeast reduction of cyclohexanones 158

Introduction

Tanikaga et al. reported the reduction of cyclohexanones 158a-e, containing a functional group at C-3 (Table 1.17). 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 (1S,3S)- and (1S,3R)-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 1.16: Efficient kinetic resolution of 2-benzenesulfonylcyclopentanone derivatives 156 via baker’s yeast mediated reduction

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Cyclopentanone 156 Yield (%)</th>
<th>ee (%)</th>
<th>Cyclopentanol 157 Yield (%)</th>
<th>ee (%)</th>
<th>dr&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>156a</td>
<td>4</td>
<td>(1S,2R)-157a</td>
<td>79</td>
<td>&gt;95</td>
</tr>
<tr>
<td>2</td>
<td>Me</td>
<td>(2S,3R)-156b</td>
<td>10</td>
<td>(1S,2R,3S)-157b</td>
<td>29</td>
<td>&gt;95</td>
</tr>
<tr>
<td>3</td>
<td>Et</td>
<td>(2S,3R)-156c</td>
<td>28</td>
<td>(1S,2R,3S)-157c</td>
<td>40</td>
<td>&gt;95</td>
</tr>
<tr>
<td>4</td>
<td>&quot;Pr</td>
<td>(2S,3R)-156d</td>
<td>26</td>
<td>(1S,2R,3S)-157d</td>
<td>36</td>
<td>&gt;95</td>
</tr>
<tr>
<td>5</td>
<td>&quot;Bu</td>
<td>(2S,3R)-156e</td>
<td>34</td>
<td></td>
<td>32</td>
<td>&gt;95</td>
</tr>
<tr>
<td>6</td>
<td>Ph</td>
<td>(2S,3S)-156f</td>
<td>20</td>
<td>(1S,2R,3S)-157f</td>
<td>31</td>
<td>&gt;95</td>
</tr>
<tr>
<td>7</td>
<td>CH₂Ph</td>
<td>(2S,3S)-156g</td>
<td>-</td>
<td>(1S,2R,3S)-157g</td>
<td>22</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

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

Table 1.17: The baker’s yeast reduction of cyclohexanones 158

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>(1S,3S)-159 Yield (%)</th>
<th>ee (%)</th>
<th>(1S,3R)-159 Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>158a</td>
<td>CH₂NO₂</td>
<td>(1S,3S)-159a</td>
<td>46</td>
<td>&gt;99</td>
<td>(1S,3R)-159a</td>
</tr>
<tr>
<td>2</td>
<td>158b</td>
<td>SPh</td>
<td>(1S,3S)-159b</td>
<td>6</td>
<td>5</td>
<td>(1S,3R)-159b</td>
</tr>
<tr>
<td>3</td>
<td>158c</td>
<td>SO₂Ph</td>
<td>(1S,3S)-159c</td>
<td>45</td>
<td>&gt;99</td>
<td>(1S,3R)-159c</td>
</tr>
<tr>
<td>4</td>
<td>158d</td>
<td>CH₂SO₂Ph</td>
<td>(1S,3S)-159d</td>
<td>41</td>
<td>90</td>
<td>(1S,3R)-159d</td>
</tr>
<tr>
<td>5</td>
<td>158e</td>
<td>n-C₅H₁₀</td>
<td>(1S,3S)-159e</td>
<td>-</td>
<td>-</td>
<td>(1S,3R)-159e</td>
</tr>
</tbody>
</table>
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).

![Efficiency and stereoselectivity decreased with larger R group](image)

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).

![Reduction dependent on oxidation level of sulfur and nature of the double bond](image)

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

43. Seyden-Penne, J. In *Chiral Auxiliaries and Ligands in Asymmetric Synthesis*; Wiley: New York, **1995**.

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Chapter 1: Introduction


Chapter 2

Baker’s yeast mediated asymmetric synthesis of (R)- and (S)-4-methyloctanoic acid (R)-1 and (S)-1
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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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{1} and of the sex pheromone of the peach leafminer moth Lyonetia clerkella Linné, 14-methyloctadecene\textsuperscript{3}, compounds for which the stereochemistry generally dictates the behavioural response of the insect (Figure 2.1).\textsuperscript{1,2,8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_1.png}
\caption{Figure 2.1}
\end{figure}

Significantly, preliminary investigations by Kelly\textsuperscript{3} and later exploration by Milner\textsuperscript{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 (2S,3R)-2 and (1S,2R,3S)-4 (Scheme 2.1).

\begin{scheme}[h]
\centering
\includegraphics[width=\textwidth]{scheme2_1.png}
\caption{Scheme 2.1}
\end{scheme}

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 (2S,3R)-2 and cyclopentanol (1S,2R,3S)-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 \((\pm)-\text{trans-2}-\text{benzenesulfonyl-3}-n\)-butylcyclopentanone \((\pm)-2\), the targeted substrate for the biocatalytic study.

- To achieve efficient baker’s yeast mediated reduction of \((\pm)-\text{trans-2}-\text{benzenesulfonyl-3}-n\)-butylcyclopentanone \((\pm)-2\) (Scheme 2.1) to obtain synthetically useful quantities of the generated cyclopentanol \((1S,2R,3S)-4a\) and the recovered cyclopentanone \((2S,3R)-2\) 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 \textit{Oryctes}, \(4\)-methyloctanoic acid \((R)-1\) and \((S)-1\) (Scheme 2.2).

![Scheme 2.2](image)

2.3 Synthesis of substrates for baker’s yeast mediated reduction

Highly regioselective carbenoid C-H insertion reactions of \(\alpha\)-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 \((\pm)-\text{trans-2}-\text{benzenesulfonyl-3}-n\)-butylcyclopentanone \((\pm)-2\), the desired substrate for baker’s yeast reduction. The first synthetic challenge in this study involved preparation of the \(\beta\)-keto sulfone \(5\), followed by diazo transfer to afford the analogous \(\alpha\)-diao-\(\beta\)-keto sulfone \(6\), and subsequent cyclisation to the desired cyclopentanone \((\pm)-2\) (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 2.3](image)

![Scheme 2.4](image)
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 $\nu_{\text{max}}$ 1717 cm$^{-1}$ and $\nu_{\text{max}}$ 1301 (symmetric stretch), 1153 (asymmetric stretch) cm$^{-1}$, respectively, and by the appearance of a characteristic singlet in the $^1$H NMR spectrum at $\delta_H$ 4.14 ppm assigned to the C(1)H methylene protons adjacent to both the carbonyl and sulfonyl groups. All spectral characteristics of 5 were consistent with those previously reported by Kelleher, O’Keeffe and Milner.

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. These versatile reagents undergo a variety of transformations including insertion, cyclopropanation, ylide formation and Wolff rearrangement. One of the fundamental methods of synthesising α-diazo carbonyl compounds, the diazo transfer reaction was developed by Regitz in 1967. 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. However, it is potentially explosive, possessing a high impact sensitivity and low initiation temperature, thus extreme caution must be exercised in its use. 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. 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. 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.
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.\textsuperscript{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 $\delta_H$ 2.43 ppm, indicative of the methyl protons of the toluene substituent and in the aromatic region at $\delta_H$ 7.31 ppm (d, $J$ 8.4) and $\delta_H$ 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 $\nu_{\text{max}}$ 1662 cm$^{-1}$, a significant shift from the corresponding band in the spectra of the β-keto sulfone 5 ($\nu_{\text{max}}$ 1717 cm$^{-1}$). Characteristic absorptions are also observed in the IR spectrum at $\nu_{\text{max}}$ 2129 cm$^{-1}$ and $\nu_{\text{max}}$ 1339 (symmetric stretch), $\nu_{\text{max}}$ 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 $\delta_H$ 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\textsuperscript{1}, O’Keeffe\textsuperscript{2} and Milner.\textsuperscript{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. This synthetic methodology was originally developed by Taber, with α-diazo-β-keto esters, while Monteiro 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. 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 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 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 involved a single addition of 0.5 mol% of the catalyst providing the cyclopentanone as an initial mixture 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 and O’Keeffe previously reported two 0.5 mol% additions of rhodium(II) acetate to the reaction mixture over a 48 h reaction period, while Slattery described a single addition of 1.0 mol% of the catalyst and a reaction time of 0.5 h.

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 δH 5.17 ppm in the 1H NMR spectrum of the crude product (Scheme 2.7) in accordance with literature reports for similar hydroxyl compounds. Significantly, this byproduct was no longer observed following chromatographic purification.
Reactions were monitored by IR analysis, where reaction completion was indicated by the disappearance of peaks at $\nu_{\text{max}}$ 2129 cm$^{-1}$ (CN$_2$) and $\nu_{\text{max}}$ 1662 cm$^{-1}$ (CO) characteristic of the $\alpha$-diazo-$\beta$-keto sulfone 6 and the appearance of the cyclopentanone 2 carbonyl absorption at $\nu_{\text{max}}$ 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 $\delta_H$ 3.38 ppm (d, $J$ 6.6). For the cis diastereomer of the cyclopentanone 2 the corresponding signal was observed at $\delta_H$ 3.73 ppm (d, $J$ 7.8) (Figure 2.2).

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 $\alpha$ to the benzenesulfonyl moiety ($pK_a \sim 10$) (Scheme 2.8). Hence, following column chromatography on silica gel, (±)-trans-2-benzenesulfonyl-3-n-butylcyclopentanone (±)-2 was exclusively obtained.
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 and the trans isomer was assigned the relative stereochemistry (2R*,3S*). Characteristic carbonyl and sulfonyl absorptions are observed in the IR spectrum at \( \nu_{\text{max}} \) 1751 cm\(^{-1} \) and \( \nu_{\text{max}} \) 1305 (symmetric stretch) and \( \nu_{\text{max}} \) 1152 (asymmetric stretch) cm\(^{-1} \), respectively. As mentioned earlier, the \(^1\)H 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 \( \delta_{\text{H}} \) 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 \( \delta_{\text{H}} \) 2.79-2.99 ppm. This reaction was readily conducted on a multi-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).

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1. Significant difference in \(^1\)H NMR chemical shift and multiplicity of the CH\(_3\) signal is observed between Milner and spectral data obtained during this study. Milner reports, \( \delta_{\text{H}} \) (400 MHz) 1.29 (3H, d, \( J \) 7, CH\(_3\))\(^8\), while in this research the methyl protons appear as a triplet further upfield, \( \delta_{\text{H}} \) (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. \(^1\)H NMR assignment in this study is consistent with O’Keeffe\(^2\) and Kelleher.\(^3\)

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53
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 *1R*,2*S*,3*R*-4a. This assignment was established unequivocally by Kelleher, by X-ray analysis of diastereomerically pure 4a. However small amounts (~3%) of the minor diastereomer 4b was also detected in the 1H NMR spectrum by the appearance of a multiplet at δH 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 *1R*,2*R*,3*S*-.

Successful product synthesis was indicated in the IR spectrum by the disappearance of the intense absorption band of the cyclopentanone (±)-2 carbonyl stretch at νmax 1751 cm⁻¹ and observation of a distinct broad OH stretch at νmax 3499 cm⁻¹, while the characteristic sulfonyl asymmetric and symmetric stretching vibrations appear at νmax 1302 and 1141 cm⁻¹, respectively. In the 1H NMR spectrum the C(2) proton geminal to the sulfonyl group appears as a doublet of doublets at δH 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 δH 4.28-4.33 ppm. Spectral characteristics of cyclopentanol 4a are consistent with those previously described for this compound.

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

With a synthetic route to (±)-*trans*-2-benzenesulfonyl-3-n-butylocyclopentanone (±)-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 and O’Keeffe, who observed significant reduction in diastereoselectivity and enantiopurity of the recovered (2S,3R)-2-benzenesulfonyl-3-n-butylocyclopentanone (2S,3R)-2, indicating both inefficient reduction and kinetic resolution. Significantly, Kelly discovered that the use of IMBY in an aqueous medium in conjunction with repeated sucrose addition over time yielded the desired (1S,2R,3S)-2-benzenesulfonyl-3-n-butylocyclopentanol (1S,2R,3S)-4a in 37% yield and >98% ee, and critically, the unreacted (2S,3R)-2-benzenesulfonyl-3-n-butylocyclopentanone (2S,3R)-2 was recovered in 14% yield with >95% ee (entry 1, Table 2.1). Based on Kelly’s
promising one off result Milner\(^8\) further explored the kinetic resolution under the IMBY optimised reaction conditions. Excellent enantiopurity of the generated cyclopentanol (1S,2R,3S)-4a (>98% ee) and the recovered cyclopanone (2S,3R)-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 enanti- and diastereomerically pure products (2S,3R)-2 and (1S,2R,3S)-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\(^3,8,ii\)**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions(^a)</th>
<th>Scale (g)(^b)</th>
<th>Reaction Time</th>
<th>Cyclopentanone (2S,3R)-2</th>
<th>Cyclopentanol (1S,2R,3S)-4a</th>
<th>Cyclopentanol (1S,2S,3R)-4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^1)</td>
<td>IMBY-H(_2)O</td>
<td>0.40</td>
<td>84 h</td>
<td>&gt;95(^d)</td>
<td>14(^f)</td>
<td>&gt;98(^e)</td>
</tr>
<tr>
<td>2(^8)</td>
<td>IMBY-H(_2)O</td>
<td>1.27</td>
<td>74 h</td>
<td>&gt;98(^d)</td>
<td>5(^f)</td>
<td>98(^e)</td>
</tr>
</tbody>
</table>

- 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® OD-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.\(^i\)

In the baker’s yeast mediated reduction of (±)-trans-2-benzenesulfonyl-3-n-butylcyclopentanone (±)-2, the (2R,3S)-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 (2S,3R)-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 (1S,2R,3S)-4a as Kelleher\(^1\) and O’Keeffe\(^2\) found that the amount of the minor diastereomer (1S,2S,3R)-4b present varied considerably with minor variation of the reaction conditions.

\(^i\) 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%.\(^i\) In this study, for consistency Milner’s yields were recalculated based on actual recovery.
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.\textsuperscript{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).\textsuperscript{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\textsuperscript{3} and Milner\textsuperscript{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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction</th>
<th>Scale</th>
<th>Reaction</th>
<th>Cyclopentanone</th>
<th>Cyclopentanol</th>
<th>Cyclopentanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conditions</td>
<td>(g)</td>
<td>Time</td>
<td>(2S,3R)-2</td>
<td>(1S,2R,3S)-4a</td>
<td>(1S,2S,3R)-4b</td>
</tr>
<tr>
<td>1</td>
<td>IMBY-(\text{H}_2\text{O})(^a)</td>
<td>2.07</td>
<td>23 h</td>
<td>99</td>
<td>25(^f)</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>IMBY-(\text{H}_2\text{O})(^b)</td>
<td>3.00</td>
<td>47 h 15 min</td>
<td>98</td>
<td>35</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>IMBY-(\text{H}_2\text{O})(^c)</td>
<td>2.00</td>
<td>24 h</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td>18(^g)</td>
</tr>
</tbody>
</table>

a. IMBY-\(\text{H}_2\text{O}\) with repeated addition of sucrose over time at \(t = 0\) h, 2 h, 4 h and 6 h. 
b. IMBY-\(\text{H}_2\text{O}\) 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-\(\text{H}_2\text{O}\) 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 (+)-(1S,2R,3S)-2-benzenesulfonyl-3-n-butylcyclopentanol (1S,2R,3S)-4a showed no evidence of the minor cyclopentanol (1S,2S,3R)-4b at \(\delta_n(300\text{ MHz})\) 4.63-4.66 (\text{CH}_2OH).

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 (2S,3R)-cyclopentanone (2S,3R)-2 and the generated (1S,2R,3S)-cyclopentanol (1S,2R,3S)-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 (2S,3R)-2 and (1S,2R,3S)-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 (2S,3R)-2 and (1S,2R,3S)-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. (2S,3R)-2 and (1S,2R,3S)-4a, in a single reaction injection. As evident from entry 2, Table 2.3 the kinetic bioresolution is essentially complete with 9 h with excellent enantiopurity observed of both the recovered cyclopentanone (2S,3R)-2 (>98% ee) and the generated cyclopentanol (1S,2R,3S)-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\textsuperscript{5} and Kelly\textsuperscript{5} (Table 2.1). Critically, the minor cyclopentanol enantiomer (1S,2S,3R)-4b was not observed in the kinetic resolutions, by \textsuperscript{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 (2S,3R)-cyclopentanone (2S,3R)-2.

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions\textsuperscript{a}</th>
<th>Scale (g)\textsuperscript{b}</th>
<th>Reaction Time</th>
<th>Cyclopetanone (2S,3R)-2</th>
<th>Cyclopetanol (1S,2R,3S)-4a</th>
<th>Cyclopetanol (1S,2S,3R)-4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IMBY-H\textsubscript{2}O</td>
<td>2.00</td>
<td>2 h</td>
<td>44 \textsuperscript{c}</td>
<td>&gt;98 \textsuperscript{c}</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>IMBY-H\textsubscript{2}O</td>
<td>2.00</td>
<td>4 h</td>
<td>98 \textsuperscript{c}</td>
<td>&gt;98 \textsuperscript{c}</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>IMBY-H\textsubscript{2}O</td>
<td>2.00</td>
<td>9 h</td>
<td>&gt;98 \textsuperscript{c}</td>
<td>&gt;98 \textsuperscript{c}</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>IMBY-H\textsubscript{2}O</td>
<td>2.00</td>
<td>24 h</td>
<td>&gt;98 \textsuperscript{c}</td>
<td>12\textsuperscript{d}</td>
<td>18\textsuperscript{d,e}</td>
</tr>
</tbody>
</table>

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

Figure 2.3: HPLC Trace I: Racemic (+)-(1R\textsuperscript{*},2S\textsuperscript{*},3R\textsuperscript{*})-2-Benzensulfonyl-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 (1S,2R,3S)-2-benzenesulfonyl-3-n-butylcyclopentanol (1S,2R,3S)-4a and (2S,3R)-2-benzenesulfonyl-3-n-butylcyclopentanone (2S,3R)-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 (1S,2R,3S)-4a and (2S,3R)-2.

Figure 2.4: 1H 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 CDCl₃ 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 (\(\pm\))-4-methyloctanoic acid (\(\pm\))-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\(^{50}\) for esterification of (\(\pm\))-4-methyloctanoic acid (\(\pm\))-1 (\(E = 54\)) and hydrolysis of the corresponding ethyl ester (\(E = 12\)) leading to modest enantioselectivity.\(^{54,55}\) Novozym 435\(^{50}\) mediated esterification of (\(\pm\))-4-methyloctanoic acid (\(\pm\))-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\(^{50}\) catalysed esterification of (\(\pm\))-4-methyloctanoic acid (\(\pm\))-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 (\(\pm\))-trans-2-benzenesulfonyl-3-\(n\)-butylecyclopentanone (\(\pm\))-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.

![Scheme 2.11](image-url)
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 (2S,3R)-2-benzenesulfonyl-3-n-butylocyclopentanone (2S,3R)-2 (>98% ee) and (1S,2R,3S)-2-benzenesulfonyl-3-n-butylocyclopentanol (1S,2R,3S)-4a (>98% ee) in high yields. To obtain access to both complimentary enantiomeric forms of the trans-cyclopentanone 2, oxidation of (1S,2R,3S)-4a with retention of stereochemistry was performed. 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(1H)-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. Dess-Martin oxidation of (1S,2R,3S)-2-benzenesulfonyl-3-n-butylocyclopentanol (1S,2R,3S)-4a was conducted to obtain the (2S,3R)-2-benzenesulfonyl-3-n-butylocyclopentanone (2S,3R)-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(1H)-one 1-oxide (IBX) 13 by oxidation of 2-iodobenzoic acid 14 with Oxone® (2KHSO5-KHSO4-K2SO4) in water at 70 °C according to Santagostino. This procedure offers distinct advantages over the original KBrO3 oxidation by Dess and Martin, 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.\textsuperscript{58}

The procedure for the second step is a variation of the Dess and Martin\textsuperscript{58} protocol and is based heavily upon it.\textsuperscript{58} 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.\textsuperscript{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 (2R,3S)-2 recovered. Irreproducibility in the synthesis of Dess-Martin periodinane has been well documented and a number of solutions have been put forward.\textsuperscript{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 hydroxyiodinane oxide 13 precursor employed.\textsuperscript{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(1H)-one 1-oxide 13 to powder form has been described.\textsuperscript{64} In general, however, high yields were observed in this study when 12 was employed immediately following filtration.

\begin{center}
\textbf{Scheme 2.13}
\end{center}

Dess-Martin oxidation of the (1S,2R,3S)-2-benzensulfonyl-3-n-butylcyclopentanol (1S,2R,3S)-4a involved the addition of a dichloromethane solution of the substrate (1S,2R,3S)-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 (2R,3S)-2-benzensulfonyl-3-n-butylcyclopentanone (2R,3S)-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 (2S,3R)-2. A slight reduction in enantioselectivity was observed of the obtained (2R,3S)-cyclopentanone (2R,3S)-2 (96% ee) relative to that of the (1S,2R,3S)-cyclopentanone (1S,2R,3S)-4a (>98% ee) substrate. The slight decrease in enantiopurity may be due to oxidation of trace amounts of the minor diastereomer (1S,2S,3R)-2-benzensulfonyl-3-n-butylcyclopentanol (1S,2S,3R)-4b,
although (1S,2S,3R)-4b was not observed by $^1$H NMR or chiral HPLC analysis of the substrate (1S,2R,3S)-4a.

![Scheme 2.14](image)

**Scheme 2.14**

As mentioned previously, $^1$H NMR analysis of (2R,3S)-2 was identical to that of the cyclopentanone (2S,3R)-2 obtained by baker’s yeast mediated kinetic resolution of (±)-2. The only differences observed were the opposite sign of the specific rotations of (−)-(2R,3S)-2, although the values were similar in magnitude to that recorded for (+)-(2S,3R)-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](image)

**Figure 2.5: HPLC Trace I:** Racemic (±)-trans-2-benzensulfonyl-3-n-butylylcyclopentanone (±)-2. **Trace II:** (−)-(2R,3S)-2-benzensulfonyl-3-n-butylylcyclopentanone (2R,3S)-2, 96% ee (from DMP oxidation). **Trace III:** (+)-(2S,3R)-2-benzensulfonyl-3-n-butylylcyclopentanone (2S,3R)-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-benzensulfonyl-3-n-butylylcyclopentanone (2R,3S)-2 and (2S,3R)-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. More recently, Monteiro reported the facile ring cleavage of α-sulfonylcyclopentanones in basic solution at reflux to give carboxylic acid derivatives. Application of this methodology to the enantioenriched enantiomers of 2-benzenesulfonyl-3-n-butylcyclopentanone (2R,3S)-2 and (2S,3R)-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.

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. Characteristic broad hydroxyl and intense carbonyl absorptions are observed in the IR spectrum at $\nu_{\text{max}}$ 3066 cm$^{-1}$ and $\nu_{\text{max}}$ 1710 cm$^{-1}$ respectively. In the $^1$H NMR spectrum, the methylene protons adjacent to the sulfonyl group appear as the AB portion of an ABX system which appears at $\delta$H 2.97-3.12 ppm.

\[
\text{Scheme 2.15}
\]
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.\textsuperscript{1,2,8} Facile ring cleavage of both enantiomeric series of the highly enantioenriched cyclopentanone (2S,3R)-2 and (2R,3S)-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,\textsuperscript{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,\textsuperscript{1-3,8} application of the base-induced ring cleavage of the enantioenriched 2-benzenesulfonyl-3-n-butylcyclopentanones (2S,3R)-2 and (2R,3S)-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 (2R,3S)-2 and (2S,3R)-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.

\textit{Table 2.4: Synthesis of 4-(benzenesulfonylmethyl)octanoic acid 11}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cyclopentanone\textsuperscript{a}</th>
<th>ee (%)\textsuperscript{b}</th>
<th>Carboxylic acid</th>
<th>Crude yield (%)\textsuperscript{c}</th>
<th>ee (%)\textsuperscript{b}</th>
<th>Optical rotation [α]\textsubscript{D}</th>
<th>Experimental</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(±)-2</td>
<td>-</td>
<td>(±)-11</td>
<td>69</td>
<td>-</td>
<td>[α]\textsuperscript{11} = -11.0  (c 1.2, CHCl\textsubscript{3})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>(2R,3S)-2</td>
<td>93</td>
<td>(S)-11</td>
<td>82</td>
<td>94</td>
<td>[α]\textsuperscript{11} = +6.3  (c 1.2, CHCl\textsubscript{3})</td>
<td>75% ee\textsuperscript{e}</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>(2S,3R)-2</td>
<td>&gt;98</td>
<td>(R)-11</td>
<td>&gt;98</td>
<td>-</td>
<td>[α]\textsuperscript{11} = +9.1  (c 1.2, CHCl\textsubscript{3})</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a. The base induced ring cleavage of enantioenriched cyclopentanones (2R,3S)-2 and (2S,3R)-2 providing the carboxylic acids (S)-11 and (R)-11 has been performed with 1.16 g and 1.44 g of precursor (2R,3S)-2 and (2S,3R)-2 respectively, with high yield (≥84%) and retention of stereochemistry (≥94% ee). The above experiments were performed with 170 mg of precursor (2R,3S)-2 for entry 2 and 239 mg of precursor (2S,3R)-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.
Chapter 2

Results and Discussion

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-(Benzenesulfonylmethyl)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 I is efficient reductive desulfonylation to reveal the unsubstituted methyl substituent, characteristic of many insect pheromones.

Kelleher1 and O’Keeffe2 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

\[ \text{Kelleher}^1 \text{ and O'Keeffe}^2 \]

\[ \text{Milner}^3 \]

\[ \text{Mg, MeOH} \]

\[ 15 \text{ (Yield 30-40\%) }^2 \]

\[ 16 \text{ (Yield 33-37\%) }^2 \]

\[ \]

\[ 1 \]

\[ \]

\[ \text{Scheme 2.16} \]

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%³ and 94% respectively.³ 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)-1.³ 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).³

² 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. 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 $v_{\text{max}}$ 1305 cm$^{-1}$ and $v_{\text{max}}$ 1146 cm$^{-1}$, characteristic of the sulfonyl carboxylic acid 11. The IR spectra of (±)-1 displays strong characteristic absorptions evident at $v_{\text{max}}$ 3043 cm$^{-1}$ and $v_{\text{max}}$ 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, with a distinct multiplet at $\delta_H$ 2.19-2.50 ppm attributable to the methylene C(2)H$_2$ protons $\alpha$- to the carboxylic acid moiety.
Table 2.4: Synthesis of 4-methyloctanoic acid \( \text{I} \)

\[
\text{R} \quad \text{H} \quad \text{O} \\
\text{Bu} \quad \text{SO}_2 \text{Ph} \\
\text{nBu} \\
\text{Mg (50 eq.)} \\
\text{MeOH} \\
\text{S} \quad \text{OH} \\
\text{R} \quad \text{OH} \\
\text{nBu}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sulfonlated carboxylic acid</th>
<th>Scale (g)(^a)</th>
<th>ee (%)(^b)</th>
<th>Desulfonlated carboxylic acid</th>
<th>Yield (%)(^d)</th>
<th>ee (%)(^e)</th>
<th>Optical rotation ([\alpha]_D^0)</th>
<th>Experimental</th>
<th>Literature(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>((\pm))-\text{I1}</td>
<td>1.04</td>
<td>-</td>
<td>((\pm))-\text{I}</td>
<td>51</td>
<td>-</td>
<td>([\alpha]_D^0 = -2.2 (c 1.4, CHCl_3))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>((R))-\text{I1}</td>
<td>0.74</td>
<td>\pm</td>
<td>((R))-\text{I}</td>
<td>53</td>
<td>&gt;98</td>
<td>([\alpha]_D^0 = -1.5 (c 1.4, CHCl_3), 94% ee(^d))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>((S))-\text{I1}</td>
<td>0.56</td>
<td>\pm</td>
<td>((S))-\text{I}</td>
<td>57</td>
<td>97</td>
<td>([\alpha]_D^0 = +1.6 (c 1.4, CHCl_3), 93% ee(^d))</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\text{a.}\) Scale refers to the quantity of 4-(benzenesulfonylmethyl)octanoic acid \(\text{I1}\) employed for the reaction.

\(\text{b.}\) Chiral HPLC and optical rotation analysis was not conducted on the batch of (\(R\))-4-(benzenesulfonylmethyl)octanoic acid (\(\text{I}\))-\text{I1} utilised in this experiment. However, the enantiopurity of the precursor of (\(R\))-\text{I1}, (\(\pm\))-(2S,3R)-cyclopentanone (2S,3R)-2 was determined to be 99\% ee by chiral HPLC analysis and base-catalyzed ring cleavage to (\(R\))-\text{I} proceeds with retention of stereochemistry (see section 2.5.2).

\(\text{c.}\) Chiral HPLC and optical rotation analysis was not conducted on the batch of (\(S\))-4-(benzenesulfonylmethyl)octanoic acid (\(S\))-\text{I1} utilised in this experiment. However, the enantiopurity of the precursor of (\(S\))-\text{I1}, (+)-(2R,3S)-cyclopentanone (2R,3S)-2 was determined to be 97\% ee by chiral HPLC analysis and base-catalyzed ring cleavage to (\(S\))-\text{I} proceeds with retention of stereochemistry (see section 2.5.2).

\(\text{d.}\) Yields quoted are following chromatographic purification.

\(\text{e.}\) The highest enantiomeric purity of (\(R\))-4-methyloctanoic acid (\(R\))-\text{I} reported by Milner was >98\% ee. Enantiomeric excess [ee (%)] of (\(R\))-\text{I} was determined by chiral HPLC analysis using a Chiralcel\textsuperscript{®} OJ-H column, however no optical rotation data was obtained due to small sample size.\(^f\) The enantiometric excess [ee (%)] of (\(S\))-\text{I} was not determined by Milner as an analytically pure sample was not obtained, however, the sulfonlated carboxylic acid precursor to (\(S\))-\text{I}, (\(S\))-\text{I1} was of moderate enantiopurity 70\% ee.\(^g\)

\(\text{f.}\) Due to the low UV absorption of 4-methyloctanoic acid (\(R\))-\text{I} and (\(S\))-\text{I}, chiral HPLC analysis was not possible with a PDA detector and enantiomeric excess (ee [%]) was therefore determined by derivatisation with 9-fluorenemethanol 17.

\(\text{g.}\) The literature ee values were determined by chiral HPLC following derivatisation of the methylocyclooctanoic acid derivatives (\(S\))-\text{I} and (\(R\))-\text{I} with (\(R\))-(\(-\))2,2,2-trifluoro-1-(9-anthryl)ethanol.\(^5\)

Significantly, the desulfonylation reaction proceeds with retention of stereochemistry at C(4) and for the first time in our group complete desulfonylation of (\(R\))-\text{I1} and (\(S\))-\text{I1} was successfully achieved, providing both enantiomers of the aggregation pheromone of the rhinoceros beetles of the genus \textit{Oryctes}, 4-methyloctanoic acid (\(R\))-\text{I} and (\(S\))-\text{I} 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 (\(S\))-\text{I1} and (\(R\))-\text{I1} respectively] to provide substantial quantities of the pure natural product (\(R\))-\text{I} and (\(S\))-\text{I} for full characterisation including chiral HPLC and optical rotatory analysis, representing an important advance in the asymmetric synthesis of (\(R\))-\text{I} and (\(S\))-\text{I}. Notably, spectral characteristics of the enantioenriched natural product (\(R\))-\text{I} and (\(S\))-\text{I} were identical to the racemic (\(\pm\))-\text{I} and literature reports.\(^51,52,67\)

Milner reported chiral HPLC analysis of (\(R\))-\text{I} utilising the Chiralcel\textsuperscript{®} 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 (\(\pm\))-\text{I} were not observed, attributable to the low UV absorption of 4-methyloctanoic acid (\(\pm\))-\text{I}. Increased concentration of the chiral HPLC samples of (\(\pm\))-\text{I} were prepared (>1 mg/mL), however once again no trace of the enantiomers was observed. Therefore, unequivocal determination of the enantiomeric purity of acids (\(R\))-\text{I} and (\(S\))-\text{I} 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Desulfonylated carboxylic acid</th>
<th>Ester</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Optical rotation [α]D²⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(±)-1</td>
<td>(±)-18</td>
<td>61</td>
<td>-</td>
<td>[α]D²⁰ +1.5 (c 1.0, CHCl₃)</td>
</tr>
<tr>
<td>2</td>
<td>(S)-1</td>
<td>(S)-18</td>
<td>69</td>
<td>97</td>
<td>[α]D²⁰ -3.4 (c 1.0, CHCl₃)</td>
</tr>
<tr>
<td>3</td>
<td>(R)-1</td>
<td>(R)-18</td>
<td>79</td>
<td>99</td>
<td>[α]D²⁰ +1.5 (c 1.0, CHCl₃)</td>
</tr>
</tbody>
</table>

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 (2S,3R)-2 and (2R,3S)-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.

![Scheme 2.17](image1)

(From baker’s yeast)

(99% ee, 31% yield)

(2S,3R)-2

(89% yield)

(R)-11

(99% yield)

15% overall yield from (±)-2

![Scheme 2.18](image2)

(97% ee, 78% yield)

(2R,3S)-2

(99% yield)

(S)-11

(97% ee, 57% yield)

13% overall yield from (±)-2

(DMP)

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

(From baker’s yeast)

(>98% ee, 29% yield)
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 \(\gamma\)-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 \(\gamma\)-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.

\[ \begin{align*}
&\text{C-C bond formation} \\
&\text{with enantiocontrol}
\end{align*} \]

\[ \begin{align*}
&\text{C-C bond formation} \\
&\text{with enantiocontrol}
\end{align*} \]

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


Chapter 3

Hydrolase-mediated kinetic resolutions of 3-arylalkanoic acids
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Chapter 3

Results and Discussion

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.

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

*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. 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. 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. A lipid-induced change in the orientation of the lid (an amphiphilic $\alpha$-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.

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. 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.

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-aryloxypropionic 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 $\text{(S)}$-enantiomers of 19 display therapeutic activity, while the $\text{(R)}$-enantiomers of 20 are effective (Figure 3.2).
While the stereocentre at the $\alpha$-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 $\beta$-substituted 3-arylalkanoic acids and compare the enantioselectivity to that reported for their $\alpha$-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 $\delta$-lactone of algae origin (Figure 3.3).\(^{17}\)

![Figure 3.3](image)

All four individual stereoisomers of amino acid $\beta$-methyl phenylalanine 22 have been prepared in excellent optical purity, from enantiopure ($R$)- and ($S$)-3-phenylbutanoic acid 23 (Scheme 3.1).\(^{18}\)

![Scheme 3.1](image)

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-arylkanols 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).

\[
\text{(S)-24} \quad \text{(S)-25} \quad \text{(S)-26} \quad \text{(S)-27}
\]

Figure 3.4

The enantiopure methyl, ethyl and isopropyl \(\beta\)-substituted 3-arylalkanoic acids \((S)\)-23, \((S)\)-28 and \((R)\)-29 have been successfully cyclised \textit{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).

\[
\begin{align*}
\text{R = Me (S)-23} \\
\text{R = Et (S)-28} \\
\text{R = i-Pr (R)-29}
\end{align*}
\]

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\textsuperscript{23} and antagonists\textsuperscript{24} (Figure 3.5).

\[
(R)\text{-32}
\]

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).\textsuperscript{25-29}
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.\textsuperscript{30,31} This important synthetic route has been exploited by McKervey \textit{et al}.\textsuperscript{32,33} in their work towards the synthesis of (±)-confertin 33 and Mander \textit{et al}.\textsuperscript{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.\textsuperscript{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\textsuperscript{39-41} (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 hydrolyase-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 \textit{et al}.\textsuperscript{42} Resolution was not achieved with immobilised \textit{Candida rugosa} lipase, \textit{Burkholderia cepacia} (Amano PS) or \textit{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).
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. Notably, poor enantiomeric selectivity was observed for the transesterification ($E = 13$) and hydrolysis ($E = 9$) of 3-phenylbutanoic acid ethyl ester ($\pm$)-38 (Scheme 3.5).

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. The enantioselective hydration of racemic 3-phenylbutanenitrile by *Mycobacterium strain* A277 afforded enantiomerically enriched ($S$)-3-phenylbutanoic acid ($S$)-23. 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. 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).

\[ R = \text{Me (±)-23} \quad R = \text{Et (±)-28} \]  
\[ E < 2 \]

\[ R = \text{i-Pr (±)-29} \quad R = \text{t-Bu (±)-37} \]  
No reaction

**Scheme 3.4**

**Scheme 3.5**
Table 3.1: Enantioselective biotransformations of racemic nitriles (±)-40 and (±)-41

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Nitrile</th>
<th>Time</th>
<th>Amide</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vinyl</td>
<td>(±)-40</td>
<td>15.5 h</td>
<td>(S)-42 47.5 95</td>
<td>(R)-43 47 95</td>
</tr>
<tr>
<td>2</td>
<td>Et</td>
<td>(±)-41</td>
<td>26 h</td>
<td>(R)-44 48 86</td>
<td>(S)-28 47 82</td>
</tr>
</tbody>
</table>

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 (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. (entry 3, Table 3.2) and Sih et al. (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

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>Hydrolyase</th>
<th>Ester ee (%)</th>
<th>Acid ee (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me</td>
<td>H</td>
<td><em>Burkholderia cepacia</em></td>
<td>(R)-46 &gt;98</td>
<td>(S)-23 89</td>
<td>&gt;5044</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>Me</td>
<td><em>Pseudomonas sp.</em></td>
<td>(R)-47 &gt;95</td>
<td>(S)-32 &gt;95</td>
<td>14523,46</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>Et</td>
<td>Lipase PS</td>
<td>(R)-48 -a</td>
<td>(S)-45 7</td>
<td>1.246</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>Et</td>
<td>Lipase N (Amano)</td>
<td>(R)-48 32</td>
<td>(S)-45 53</td>
<td>-47</td>
</tr>
</tbody>
</table>

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 \((\pm)-49\) and \((\pm)-50\)^{19}

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Substrate ester</th>
<th>Ester</th>
<th>Acid</th>
<th>ee (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>((\pm)-49)</td>
<td>((R)-49)</td>
<td>((S)-51)</td>
<td>92</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>OMe</td>
<td>((\pm)-50)</td>
<td>((R)-50)</td>
<td>((S)-52)</td>
<td>94</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

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).

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

- To prepare a series of structurally related known and novel racemic ethyl 3-aryllalkanoates 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.\textsuperscript{19,52-57}

![Figure 3.8](image_url)

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Me</td>
<td>(±)-47\textsuperscript{53}</td>
</tr>
<tr>
<td>H</td>
<td>Et</td>
<td>(±)-48</td>
</tr>
<tr>
<td>H</td>
<td>t-Bu</td>
<td>(±)-57\textsuperscript{54}</td>
</tr>
<tr>
<td>H</td>
<td>Me</td>
<td>(±)-38\textsuperscript{52}</td>
</tr>
<tr>
<td>H</td>
<td>Et</td>
<td>(±)-56\textsuperscript{55}</td>
</tr>
<tr>
<td>H</td>
<td>i-Pr</td>
<td>(±)-58\textsuperscript{56}</td>
</tr>
<tr>
<td>H</td>
<td>t-Bu</td>
<td>(±)-59\textsuperscript{54}</td>
</tr>
<tr>
<td>p-Me</td>
<td>CH\textsubscript{3}</td>
<td>(±)-49\textsuperscript{19}</td>
</tr>
<tr>
<td>o-Me</td>
<td>CH\textsubscript{3}</td>
<td>(±)-53</td>
</tr>
<tr>
<td>m-Me</td>
<td>CH\textsubscript{3}</td>
<td>(±)-54</td>
</tr>
<tr>
<td>p-F</td>
<td>CH\textsubscript{3}</td>
<td>(±)-55</td>
</tr>
<tr>
<td>p-OMe</td>
<td>CH\textsubscript{3}</td>
<td>(±)-60\textsuperscript{57}</td>
</tr>
</tbody>
</table>

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](image_url)
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>
<thead>
<tr>
<th>Entry</th>
<th>Alkanoic acid</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>Alkanoic ester</th>
<th>Yield (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(±)-23</td>
<td>CH(_3)</td>
<td>H</td>
<td>(±)-38(^{58})</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>(±)-32</td>
<td>H</td>
<td>CH(_3)</td>
<td>(±)-47(^{53})</td>
<td>63</td>
</tr>
</tbody>
</table>

\(^a\) 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\(_{N}\)2 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).
The ethyl esters (±)-38, (±)-47 and (±)-57 were characterised by a carbonyl absorption band in the IR spectra at $\nu_{\text{max}}$ 1729-33 cm$^{-1}$. In the $^1$H NMR spectra of the 3-arylalkanoates (±)-38 and (±)-47 a distinctive triplet at $\delta_H$ 1.17-1.18 ppm and quartet at $\delta_H$ 4.08 ppm attributable to the ethyl ester moiety was observed. The 3-arylalkanoate (±)-57 bearing the $\alpha$-tert-butyl substituent displayed a triplet in the $^1$H NMR spectrum at $\delta_H$ 1.04 ppm as anticipated, however interestingly the methylene resonance of the ethyl moiety appears as a symmetrical multiplet at $\delta_H$ 3.88-4.03 ppm reflecting their diastereotopic nature (Figure 3.9).

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 Wotiz59 and has been extensively optimised within our own research group as a route to precursors for α-diazoalketones.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 Stack39 and McNamara63 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 1H NMR spectrum of the crude product

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>X</th>
<th>Propanoic acid</th>
<th>Crude yield (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et</td>
<td>Br</td>
<td>(±)-289.50-62</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>i-Pr</td>
<td>Br</td>
<td>(±)-299.61-62</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>t-Bu</td>
<td>Cl</td>
<td>(±)-379.60-62</td>
<td>79</td>
</tr>
</tbody>
</table>

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’Leary37 and Harrington61 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 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 as well as literature reports.

### 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 was not effective and resulted in complex mixtures and reduced yields. However conjugate addition of the appropriate aryl Grignard to crotonic acid proceeds very efficiently and the desired product is obtained in good yield and purity.

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).

O’Leary and Harrington previously synthesised the para-methyl, ortho-methyl and meta-methyl substituted carboxylic acids (±)-51, (±)-64 and (±)-65. In addition, O’Leary reported the preparation of the para-methoxy substituted butanoic acid (±)-66. 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.

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. 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 1H NMR spectrum which were tentatively assigned as the Wurtz coupling product 69 (Figure 3.10). Furthermore, during the synthesis of the β-disubstituted acid 70, 3% of the coupling product 71 was identified (Figure 3.10). 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. O’Keeffe used a basic extraction in the work-up to ensure isolation of the acids free from the Wurtz coupling side products. In this work, use of this protocol as a precautionary measure was undertaken in the synthesis of the 3-arylbutanoic acids (±)-51, (±)-64-66.
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. 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 1H NMR spectrum of the crude product.

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product acid</th>
<th>Crude yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-OMe</td>
<td>(±)-66</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>o-Me</td>
<td>(±)-64</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>p-Me</td>
<td>(±)-51</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>m-Me</td>
<td>(±)-65</td>
<td>83</td>
</tr>
</tbody>
</table>

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.

It should be noted that this reaction was carried out by a fourth year student.66
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-arylbutoanoic 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 hydrolyase-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 νmax 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>R</th>
<th>X</th>
<th>Acid chloride</th>
<th>Yield(^a)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(±)-28</td>
<td>Et</td>
<td>H</td>
<td>(±)-73(^{26})</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>(±)-29</td>
<td>i-Pr</td>
<td>H</td>
<td>(±)-74(^{26})</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>(±)-37</td>
<td>i-Bu</td>
<td>H</td>
<td>(±)-75(^{26})</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>(±)-66</td>
<td>CH(_3)</td>
<td>p-OMe</td>
<td>(±)-76(^{27})</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>(±)-64</td>
<td>CH(_3)</td>
<td>o-Me</td>
<td>(±)-77(^{62})</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>(±)-51</td>
<td>CH(_3)</td>
<td>p-Me</td>
<td>(±)-78(^{72.6})</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>(±)-65</td>
<td>CH(_3)</td>
<td>m-Me</td>
<td>(±)-79(^{77.61})</td>
<td>40</td>
</tr>
</tbody>
</table>

\(\text{a.}\) \% Yield is calculated over two steps and is quoted following distillation \textit{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.  
\(\text{b.}\) 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 \(^{\circ}\)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 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid chloride</th>
<th>R</th>
<th>X</th>
<th>Ethyl ester</th>
<th>Yielda (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(±)-73</td>
<td>Et</td>
<td>H</td>
<td>(±)-56</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>(±)-74</td>
<td>i-Pr</td>
<td>H</td>
<td>(±)-58</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>(±)-75</td>
<td>t-Bu</td>
<td>H</td>
<td>(±)-59</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>(±)-76</td>
<td>CH3</td>
<td>p-Ome</td>
<td>(±)-60</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>(±)-77</td>
<td>CH3</td>
<td>o-Me</td>
<td>(±)-53</td>
<td>44b</td>
</tr>
<tr>
<td>6</td>
<td>(±)-78</td>
<td>CH3</td>
<td>p-Me</td>
<td>(±)-49</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>(±)-79</td>
<td>CH3</td>
<td>m-Me</td>
<td>(±)-54</td>
<td>67</td>
</tr>
</tbody>
</table>

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 \( \nu_{\text{max}} \) 1790-1801 cm\(^{-1}\) and the appearance of the new ester carbonyl stretch at the lower frequency of \( \nu_{\text{max}} \) 1732-37 cm\(^{-1}\). Analysis by 1H NMR spectroscopy displays some interesting trends, with the methylene hydrogens of the ethyl moiety, which appears as a quartet between \( \delta_H \) 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. However, an unknown aromatic side product was observed in the 1H 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 $\alpha,\beta$-unsaturated ester 81, followed by catalytic hydrogenation to smoothly provide the required ethyl ester ($\pm$)-55 which was subjected to basic hydrolysis to yield ($\pm$)-3-(4-fluorophenyl)butanoic acid ($\pm$)-80. High yields ($\geq 85\%$) and excellent purity were described for all synthetic steps (Scheme 3.8). Therefore, this synthetic protocol was engaged in this study.

![Scheme 3.8](image1)

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. In 1958, Horner described a modified Wittig reaction employing phosphonate-stabilised carbanions and the scope of the reaction was further defined by Wadsworth and Emmons. 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.

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](image2)
Marcantoni et al. reports almost exclusive formation of the \( E \) isomer of ethyl 3-(4-fluorophenyl)but-2-enolate \( 81 \) 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.

![Chemical structures](image)

**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-enolate \( 81 \) (recorded in CDCl\(_3\) at 300 MHz).

The next step in the synthesis was reduction of the olefinic bond of \((E)\)-ethyl 3-(4-fluorophenyl)but-2-enolate \((E)-81\) by catalytic hydrogenation (Scheme 3.10). The \( \alpha,\beta \)-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 \((\pm)\)-3-aryllkanoate \((\pm)-55\). The hydrogenation was carried out in undistilled absolute ethanol. The desired ester \((\pm)-55\) was isolated in good yield (77\%) following purification by column chromatography. \((\pm)\)-Ethyl 3-(4-fluorophenyl)butanoate \((\pm)-55\) 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](image)
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

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).

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 \(-78^\circ\) C. The reaction mixture was then warmed to \(-35^\circ\) C and stirred for 1 h before benzyl bromide was added in one portion. The reaction mixture was stirred overnight at \(-35^\circ\) 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 (\(\pm\))-48 was purified by flash chromatography and obtained in a 50% yield. (\(\pm\))-Ethyl 2-benzylbutanoate (\(\pm\))-48 was previously mentioned in the literature however no spectral details were reported;\(^81\) therefore full characterisation of (\(\pm\))-48 was conducted during this research.

The analogous \(\alpha\)-alkylated carboxylic acid (\(\pm\))-45 was obtained in 66% yield by basic hydrolysis of (\(\pm\))-ethyl 2-benzylbutanoate (\(\pm\))-48. (\(\pm\))-2-Benzylbutanoic acid (\(\pm\))-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\textsuperscript{®} OD-H and Chiralpak\textsuperscript{®} 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\textsuperscript{®} 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 (\(\pm\))-ethyl 3-(4-fluorophenyl)butanoate (\(\pm\))-55 and corresponding carboxylic acid (\(\pm\))-80 where the Chiralcel\textsuperscript{®} 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. 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 $\delta_H$ 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 $\delta_H$ 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-arylkanoic 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. 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-arylkanoic 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.

**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 enantioselectivity. 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).\textsuperscript{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 cylindracea C1, C2 and Mucor mehei (entries 1, 2 and 17, Table 3.10 respectively).

In practise, while use of Candida antarctica lipase B (free and immobilised), Candida cylindracea C1, C2 and Mucor mehei 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 \textsuperscript{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 \textsuperscript{1}H NMR analysis is presumed to be more accurate.
The enzymatic hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-38 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.

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>3H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cyclindracea C1</td>
<td>30</td>
<td>11 (S)</td>
<td>58 (R)</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Candida cyclindracea C2</td>
<td>Ambient</td>
<td>25 (S)</td>
<td>59 (R)</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Rhizopus oryzae</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Achromobacter spp.</td>
<td>30</td>
<td>11 (R)</td>
<td>9 (S)</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>Alcaligenes spp. 1</td>
<td>30</td>
<td>27 (R)</td>
<td>95 (S)</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas cepacia P1</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>94 (S)</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>Pseudomonas stutzeri</td>
<td>Ambient</td>
<td>14 (R)</td>
<td>61 (S)</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>Rhizopus spp.</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Rhizopus niveus</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Aspergillus niger</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Alcaligenes spp. 2</td>
<td>Ambient</td>
<td>98 (R)</td>
<td>97 (S)</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>Pseudomonas cepacia P2</td>
<td>Ambient</td>
<td>96 (R)</td>
<td>75 (S)</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td>Mucor javanicus</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Penicillium camembertii</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Pseudomonas fluorescens</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>94 (S)</td>
<td>51</td>
</tr>
<tr>
<td>16</td>
<td>Candida antarctica B</td>
<td>Ambient</td>
<td>13 (S)</td>
<td>0 (R)</td>
<td>51</td>
</tr>
<tr>
<td>17</td>
<td>Mucor mehei</td>
<td>Ambient</td>
<td>3 (S)</td>
<td>24 (R)</td>
<td>11</td>
</tr>
<tr>
<td>18</td>
<td>Candida antarctica A</td>
<td>Ambient</td>
<td>10 (R)</td>
<td>68 (S)</td>
<td>13</td>
</tr>
<tr>
<td>19</td>
<td>Candida antarctica B (immob)</td>
<td>Ambient</td>
<td>70 (S)</td>
<td>5 (R)</td>
<td>93</td>
</tr>
<tr>
<td>20</td>
<td>Porcine pancrease Type II</td>
<td>Ambient</td>
<td>15 (R)</td>
<td>93 (S)</td>
<td>14</td>
</tr>
<tr>
<td>21</td>
<td>Porcine pancrease Grade II</td>
<td>Ambient</td>
<td>35 (R)</td>
<td>95 (S)</td>
<td>27</td>
</tr>
<tr>
<td>22</td>
<td>Pig liver esterase</td>
<td>Ambient</td>
<td>0a</td>
<td>0a</td>
<td>-a</td>
</tr>
</tbody>
</table>

a. Reaction went to 100% completion, no enantioreactivity 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 bioreolution 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 mehei 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 \((\pm)-38\) with Candida antarctica lipase B (immob).

The direction of enantioselectivity in the hydrolysis of \((\pm)-56\) was consistent with that observed in the reactions of \((\pm)-38\) 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 \((\pm)\)-ethyl 3-phenylpentanoate \((\pm)-56\)**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source (^a)</th>
<th>Time</th>
<th>Temp (^\circ)C</th>
<th>Ester 56</th>
<th>Acid 28</th>
<th>Conversion (^b)</th>
<th>ee (%)</th>
<th>Conversion (^b)</th>
<th>ee (%)</th>
<th>(E) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cyclindracea C2</td>
<td>120 h</td>
<td>Ambient</td>
<td>1</td>
<td>15</td>
<td>6</td>
<td>33</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Candida antarctica B</td>
<td>65 h</td>
<td>Ambient</td>
<td>80 ((S))</td>
<td>23 ((R))</td>
<td>78</td>
<td>93</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mucor meihei</td>
<td>67 h</td>
<td>Ambient</td>
<td>0 (^b)</td>
<td>0 (^b)</td>
<td>10 (^b)</td>
<td>100 (^b)</td>
<td>2 (^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica A</td>
<td>67 h</td>
<td>Ambient</td>
<td>5 ((R))</td>
<td>44 ((S))</td>
<td>10</td>
<td>22</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pig liver esterase</td>
<td>65 h</td>
<td>Ambient</td>
<td>87 ((S))</td>
<td>15 ((R))</td>
<td>85</td>
<td>88</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Candida antarctica B</td>
<td>65 h</td>
<td>Ambient</td>
<td>85 ((S))</td>
<td>81 ((R))</td>
<td>51</td>
<td>93</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Candida antarctica B  ((\text{immob}))</td>
<td>72 h</td>
<td>4</td>
<td>62 ((S))</td>
<td>86 ((R))</td>
<td>42</td>
<td>87</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 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 \(^\circ\)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 Thermomycys lanuginosus (Table 3.12).\(^{85}\) The selectivity factor \(E\) was observed to increase steadily by decreasing the temperature from 40 \(^\circ\)C \((E = 13)\) to −20 \(^\circ\)C \((E = 84)\).\(^{85}\)
Table 3.12: Thermomyces lanuginosus catalysed ammoniolysis of 85 at different temperatures

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Temp (°C)}^a & \text{Time} & \text{Conversion (%)} & \text{ee (%)} & \text{E value} \\
\hline
40 & 1 h & 8 & 84 & 13 \\
20 & 1 h & 11 & 89 & 20 \\
4 & 5 h & 29 & 91 & 30 \\
-10 & 24 h & 40 & 93 & 52 \\
-20 & 5 h & 8 & 97 & 84 \\
\hline
\end{array}
\]

\(^a\) 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).
Chapter 3

Results and Discussion

Table 3.13: Effect of addition of DMSO on the CRL mediated hydrolysis of ethyl 2-(4-chlorophenoxy)propanoate (±)-87 in aqueous buffer\textsuperscript{88}

\begin{table}[h]
\centering
\begin{tabular}{cccc}
\hline
DMSO (% v/v) & Time & Conversion (%) & ee (%) \textsuperscript{(S)-87} & \textit{E} value \\
\hline
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 & - \\
\hline
\end{tabular}
\end{table}

Screening reactions were therefore performed to assess the effect of a series of co-solvents (at 17% v/v) on \textit{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 \textit{E} = 41 while with dioxane (entry 5, Table 3.14) \textit{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 (\textit{E}<2).\textsuperscript{42} Furthermore, the acid (S)-28 has been resolved using amidase biocatalysis and again enantiopurity was lower (88% ee).\textsuperscript{45}
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. 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.\(^\text{42}\)
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.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ester 58</td>
<td>Acid 29</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida antarctica</em> B</td>
<td>12 (R)</td>
<td>&gt;98 (S)</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td><em>Mucor meihei</em></td>
<td>61 (R)</td>
<td>23 (S)</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida antarctica</em> A</td>
<td>10 (S)</td>
<td>64 (R)</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>33 (R)</td>
<td>97 (S)</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td><em>Pig liver esterase</em></td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
</tbody>
</table>

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.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Temperature (°C)</th>
<th>ee (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Conversion (%)</th>
<th>E&lt;sup&gt;c&lt;/sup&gt; value&lt;br&gt;Calc.</th>
<th>H&lt;sup&gt;d&lt;/sup&gt; NMR</th>
<th>E&lt;sup&gt;e&lt;/sup&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;em&gt;Candida antarctica&lt;/em&gt; B</td>
<td>Ambient&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2 (R) &gt;98 (S)</td>
<td>2 &lt;10</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&lt;em&gt;Candida antarctica&lt;/em&gt; A</td>
<td>35 – 40 °C&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3 (S) 73 (R)</td>
<td>4 6</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&lt;em&gt;Candida antarctica&lt;/em&gt; B (immob)</td>
<td>Ambient&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1 (R) &gt;98 (S)</td>
<td>1 15</td>
<td>&gt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pig liver esterase</td>
<td>Ambient&lt;sup&gt;f&lt;/sup&gt;</td>
<td>32 (S) 34 (R)</td>
<td>48 51</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The following hydrolases gave no conversion: <em>Pseudomonas cepacia</em> P1, <em>Rhizopus niveus</em>, <em>Pseudomonas fluorescens</em>, <em>Candida cyclindracea</em> C1, <em>Pseudomonas cepacia</em> P2 and <em>Porcine Pancrease</em> Type II.

<sup>b</sup> Time for ester hydrolysis was 66 h.

<sup>c</sup> Time for ester hydrolysis was 64.5 h at 35 °C temperature increased to 40 °C for the final 24 h.

<sup>d</sup> Time for ester hydrolysis was 72 h at 35 °C temperature increased to 40 °C for the final 24 h.

<sup>e</sup> 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 <em>Alcaligenes</em> spp. 2 catalysed hydrolysis of (±)-38, while acids (R)-28, (S)-29 and (S)-37 were obtained in ≥94% ee via immobilised or free <em>Candida antarctica</em> lipase B catalysed kinetic bioresolution (Figure 3.15).
Comparison of reaction conversion (%) versus hydrolase for C3 substituted alkanoic acids 23, 28, 29 and 37

\[\text{Hydrolase} \quad 0.1 \text{ M Phosphate buffer, pH 7.0} \]

Figure 3.14

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

\[\text{Hydrolase} \]

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

![Scheme 3.13](image)

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 antarctica* 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).

![Figure 3.16](image)

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

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

---

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>98 (R)</td>
<td>98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>98 (R)</td>
<td>96 (S)</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>98 (R)</td>
<td>95 (S)</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>98 (R)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida antarctica</em> A</td>
<td>30</td>
<td>80 (R)</td>
<td>68 (S)</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30</td>
<td>6 (S)</td>
<td>5 (R)</td>
<td>55</td>
</tr>
</tbody>
</table>

---

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>88 (R)</td>
<td>96 (S)</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>98 (R)</td>
<td>76 (S)</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>96 (R)</td>
<td>97 (S)</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30</td>
<td>&gt;98 (S)</td>
<td>7 (R)</td>
<td>93</td>
</tr>
</tbody>
</table>

---

**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

![Hydrolase-mediated hydrolysis of (±)-ethyl 3-(2-methylphenyl)butanoate (±)-53](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source(^a)</th>
<th>Temp (^\circ\text{C})</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>(E) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 53</td>
<td>Acid 64</td>
<td>(E) calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>&gt;98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>80 (S)</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>&gt;98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>90 (R)</td>
<td>46 (S)</td>
<td>66</td>
</tr>
</tbody>
</table>

\(a\) 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

![Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate (±)-60](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (^\circ\text{C})</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>(E) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 60</td>
<td>Acid 66</td>
<td>(E) calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>98 (R)</td>
<td>86 (S)</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>88 (S)</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>97 (S)</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida antarctica</em> A</td>
<td>30</td>
<td>4 (R)</td>
<td>48 (S)</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30</td>
<td>66 (S)</td>
<td>7 (R)</td>
<td>90</td>
</tr>
</tbody>
</table>

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

```
(±)-55
```

```
0.1 M Phosphate buffer, pH 7.0
64 h
```

```
(S)-80
(R)-55
```

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 55</td>
<td>Acid 80</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td>Pseudomonas cepacia P1</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>84 (S)</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>69 (S)</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas fluorescens</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>94 (S)</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>Candida cyclindracea</td>
<td>30</td>
<td>3 (S)</td>
<td>25 (R)</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>Candida antarctica B (immob)</td>
<td>30</td>
<td>&gt;98 (S)</td>
<td>8 (R)</td>
<td>92</td>
</tr>
</tbody>
</table>

In all cases, highly enantioenriched samples of the (3S)-acids and the (3R)-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.\(^1\) 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

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-aryalkanoic acids (±)-32, (±)-45 and (±)-61 were selected for investigation (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-phenylpropanoate (±)-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 excellentenantipurity (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 mehei 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 mehei 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**

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

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source(^a)</th>
<th>Time</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cylindracea C1</td>
<td>43 h</td>
<td>3 (R)</td>
<td>13 (S)</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Candida cylindracea C2</td>
<td>17 h</td>
<td>20 (R)</td>
<td>4 (S)</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>Candida antarctica B</td>
<td>17 h</td>
<td>35 (S)</td>
<td>83 (R)</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica B</td>
<td>43 h</td>
<td>74 (S)</td>
<td>71 (R)</td>
<td>49</td>
</tr>
<tr>
<td>5</td>
<td>Candida antarctica B</td>
<td>43 h</td>
<td>17 (S)</td>
<td>73 (R)</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>Pig Liver esterase</td>
<td>17 h</td>
<td>6 (R)</td>
<td>b</td>
<td>67</td>
</tr>
</tbody>
</table>

\(^a\) 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).

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 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Time</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomononas cepacia P1</em></td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomononas cepacia P2</em></td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida antarctica B</em></td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida antarctica B</em> (immob)</td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

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. 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.

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. 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 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 \textit{et al.} (53% ee) (Figure 3.22 and Figure 3.23).\textsuperscript{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.

\textit{Comparison of enantiomeric ratio (E-value) versus hydrolase for C2 substituted alkanoic acids 32 and 45}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure322.png}
\caption{Figure 3.22}
\end{figure}
Chapter 3

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)-3793 and analogous esters (S)-38,94 (R)-56,95 (S)-5895 were previously reported in the literature in enantiopure 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 1H 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 bioreaction.

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

<table>
<thead>
<tr>
<th>R</th>
<th>Hydrolase</th>
<th>Conversion (%)</th>
<th>Acid</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Optical rotation [α]D H NMR</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td><em>P. fluorescens</em></td>
<td>50</td>
<td>(S)-23</td>
<td>34</td>
<td>98</td>
<td>[α]D +27.90 (c 1.0, EtOH)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)-38</td>
<td>35</td>
<td>99</td>
<td>[α]D −27.55 (c 1.1, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td>Et</td>
<td>CAL-B (immob)</td>
<td>42</td>
<td>(R)-28</td>
<td>22</td>
<td>90</td>
<td>[α]D +33.73 (c 1.4, C₆H₆)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(S)-56</td>
<td>19</td>
<td>65</td>
<td>[α]D +9.46 (c 0.6, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td>i-Pr</td>
<td>CAL-B (immob)</td>
<td>21</td>
<td>(S)-29</td>
<td>24</td>
<td>98</td>
<td>[α]D −24.35 (c 0.7, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)-58</td>
<td>25</td>
<td>26</td>
<td>[α]D +7.05 (c 1.0, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td>t-Bu</td>
<td>CAL-B (immob)</td>
<td>11</td>
<td>(S)-37</td>
<td>13</td>
<td>&gt;98</td>
<td>[α]D −10.53 (c 0.1, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)-59</td>
<td>39</td>
<td>12</td>
<td>[α]D +0.80 (c 1.0, CHCl₃)</td>
<td></td>
</tr>
</tbody>
</table>

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 1H 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 1H 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 to 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)-5119, (S)-6691 and ester (R)-4919 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 hydrolyase-catalysed kinetic resolution of C3 substituted alkanoic esters

<table>
<thead>
<tr>
<th>X</th>
<th>Hydrolase</th>
<th>Conversion (%)</th>
<th>Acid</th>
<th>Ester</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Optical rotation [α]D</th>
<th>Experimental literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Me</td>
<td>P. cepacia</td>
<td>49</td>
<td>(S)-51</td>
<td>40*</td>
<td>&gt;98</td>
<td></td>
<td>[α]D30 -31.80 (c 1.0, CHCl3); [α]D30 -26.2 (c 3.5, CHCl3)</td>
<td>(R)-isomer 92% ee</td>
</tr>
<tr>
<td>m-Me</td>
<td>P. fluorescens</td>
<td>49</td>
<td>(S)-65</td>
<td>26</td>
<td>&gt;98</td>
<td></td>
<td>[α]D30 +32.32 (c 0.6, CHCl3)</td>
<td>-</td>
</tr>
<tr>
<td>o-Me</td>
<td>P. fluorescens</td>
<td>50</td>
<td>(S)-64</td>
<td>28</td>
<td>&gt;98</td>
<td></td>
<td>[α]D30 +24.17 (c 1.0, CHCl3)</td>
<td>-</td>
</tr>
<tr>
<td>p-Ome</td>
<td>P. fluorescens</td>
<td>51</td>
<td>(S)-66</td>
<td>23</td>
<td>97</td>
<td></td>
<td>[α]D30 +31.36 (c 1.0, CHCl3)</td>
<td>-</td>
</tr>
<tr>
<td>p-F</td>
<td>P. fluorescens</td>
<td>51</td>
<td>(S)-80</td>
<td>35</td>
<td>97</td>
<td></td>
<td>[α]D30 +30.51 (c 1.0, CHCl3)</td>
<td>-</td>
</tr>
</tbody>
</table>

a. Yield may be reduced due to reaction sampling.

3.5.3 Preparative-scale hydrolyase-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 hydrolyase-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 1H 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. 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 and (R)-45 and ester (R)-47 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 alkanolic esters

<table>
<thead>
<tr>
<th>R</th>
<th>Hydrolase</th>
<th>Conversion (%)</th>
<th>Acid</th>
<th>Ester</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Optical rotation [α] D</th>
<th>Experimental</th>
<th>literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td>P. fluorescens</td>
<td>51 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>(S)-32</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96</td>
<td>[α]&lt;sup&gt;D&lt;/sup&gt; = +28.0 (c 0.82, CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>(S)-isomer 99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(S)-isomer 99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R)-47</td>
<td>27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;98</td>
<td>[α]&lt;sup&gt;D&lt;/sup&gt; = +30.2 (c 0.82, CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>(S)-isomer 99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(S)-isomer 99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Et</td>
<td>CAL-B (immob)</td>
<td>24 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>(R)-45</td>
<td>19</td>
<td>82</td>
<td>[α]&lt;sup&gt;D&lt;/sup&gt; = −43.8 (c 1.0, CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>(R)-isomer &gt;99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(R)-isomer &gt;99% ee&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(S)-48</td>
<td>43</td>
<td>26</td>
<td>[α]&lt;sup&gt;D&lt;/sup&gt; = +6.8 (c 1.0, CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Presence of ethyl acetate in the <sup>1</sup>H NMR spectrum overlaps with key characteristic ester peaks of (R)-54 required for determination of conversion.
- Yield may be reduced due to reaction sampling.
- During work-up the acid and ester are isolated separately from the biotransformation by sequential extraction thus conversion by <sup>1</sup>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, especially with materials which are not readily crystalline. This is increasingly important as new synthetic products are often difficult to crystallise, 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. 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.

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-
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. 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. 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.

![Scheme 3.14](image)

A search of the Cambridge Structural Database revealed that monocarboxylic acids often co-crystallise with nicotinamide 98 and isonicotinamide 93. 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.

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 \([\text{N-H} \cdots \text{O=C}]\) and acid-pyridine \([\text{COOH} \cdots \text{N}]\) 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.

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,

---

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.
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 \(\alpha\)-tert-butyl substituent, while reaction occurred with the \(\beta\)-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

Chapter 3

Results and Discussion

Chapter 4

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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.\(^1\)\(^-\)\(^3\)

\[
\begin{array}{c}
\text{NO}_2 \quad \text{O} \\
\text{R}^1 \quad \text{R}^2 \\ + \\
\text{Base} \\
\text{OH} \\
\text{R}^2 \quad \text{NO}_2 \\
\text{R}^1 \quad \text{R}^3
\end{array}
\]

*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).\(^4\)\(^,\)\(^5\)

\[
\begin{array}{c}
\text{NO}_2 \\
\text{R}^1 \\
\text{OH} \\
\text{R}^2 \\
\text{NH}_2 \\
\text{Dehydration} \\
\text{Reduction} \\
\text{Nef reaction} \\
\text{O} \\
\text{R}^2 \\
\text{OH} \\
\text{Denitration} \\
\text{Oxidation} \\
\text{Denitration} \\
\text{O}
\end{array}
\]

*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.\(^4\)

*Figure 4.1*
Chapter 4

Results and Discussion

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 free14,15 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).

Figure 4.2
4.1.1.1 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.\textsuperscript{20-22} HNLs can be isolated from a wide array of plant sources and in reversal of the \textit{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).\textsuperscript{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.\textsuperscript{24}

The (S)-selective \textit{Hevea brasiliensis} (\textit{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 nitroaldol reaction.\textsuperscript{24}

The kinetics of the \textit{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.\textsuperscript{25} The explanation of this has not yet been deciphered.
Table 4.1: H. brasiliensis (HbHNL) catalysed Henry reaction\(^{24}\)

\[
\text{RCHO} + \text{CH}_3\text{NO}_2 \xrightarrow{\text{Hevea brasiliensis}} \text{OH} \quad \text{NO}_2
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>63</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>3-\text{HOC}_6\text{H}_4</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>4-\text{O}_2\text{NC}_6\text{H}_4</td>
<td>77</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>2-\text{Furyl}</td>
<td>57</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>CH\textsubscript{3}-(CH\textsubscript{2})\textsubscript{5}</td>
<td>25</td>
<td>89</td>
</tr>
</tbody>
</table>

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 (HbHNL) catalysed Henry reaction: pH investigation\(^{26}\)

\[
\text{RCHO} + \text{CH}_3\text{NO}_2 \xrightarrow{\text{Hevea brasiliensis}} \text{OH} \quad \text{NO}_2
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>pH 7.0 Yield (%)</th>
<th>pH 7.0 ee (%)</th>
<th>pH 5.5 Yield (%)</th>
<th>pH 5.5 ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>63</td>
<td>93</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>4-\text{O}_2\text{NC}_6\text{H}_4</td>
<td>77</td>
<td>28</td>
<td>57</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>n-\text{Hexyl}</td>
<td>25</td>
<td>89</td>
<td>34</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>Ph(CH\textsubscript{2})\textsubscript{2}</td>
<td>9</td>
<td>66</td>
<td>13</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>\text{2-Furyl}</td>
<td>57</td>
<td>72</td>
<td>43</td>
<td>88</td>
</tr>
</tbody>
</table>

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 (HbHNL) is not currently available.
Recently Asano and Fuhshuku reported for the first time a (R)-selective hydroxynitrile lyase from Arabidopsis thaliana. As evident from Table 4.4 excellent to moderate enantio-purity 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.

The hydroxynitrile lyase from H. brasiliensis (HbHN) 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). 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 HbHNL catalyzed synthesis of (S)-2-nitro-1-phenylethanol by condensation of benzaldehyde and nitromethane.

\[
\text{OH} \quad \begin{array}{c}
\text{NO}_2 \\
\text{Hevea brasiliensis} \\
\cdot \text{CH}_3\text{NO}_2
\end{array} \rightarrow \begin{array}{c}
\text{OH} \\
\text{Hevea brasiliensis} \\
\cdot \text{HCN}
\end{array}
\]

\[
\text{OH} \quad \begin{array}{c}
\text{CN}
\end{array}
\]

Scheme 4.5

Notably, a number of other enzyme systems including a transglutaminase from *Streptovercillium grisovercillatum*, 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.\textsuperscript{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.\textsuperscript{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).

\[
\begin{array}{l}
\text{OH} \\
\text{R} \\
\text{NO}_2 \\
\text{Amano AK} \\
\text{AcO} \\
30 \degree \text{C}
\end{array} \rightarrow \begin{array}{c}
\text{OAc} \\
\text{R} \\
\text{NO}_2 \\
\text{OH} \\
\text{R} \\
\text{NO}_2
\end{array}
\]

**Table 4.5: Effect of solvent on the E-value of hydrolase-mediated acetylation**\textsuperscript{33}

<table>
<thead>
<tr>
<th>R</th>
<th>Dioxane</th>
<th>THF</th>
<th>Benzene</th>
<th>AcOEt</th>
<th>Hexane</th>
<th>n-Propyl ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{2}H\textsubscript{5}</td>
<td>4.8</td>
<td>4.1</td>
<td>5.6</td>
<td>21.9</td>
<td>9.6</td>
<td>20.9</td>
</tr>
<tr>
<td>C\textsubscript{3}H\textsubscript{7}</td>
<td>1.2</td>
<td>1.1</td>
<td>1.4</td>
<td>1.4</td>
<td>1.0</td>
<td>2.1</td>
</tr>
<tr>
<td>i-C\textsubscript{3}H\textsubscript{7}</td>
<td>1.3</td>
<td>1.9</td>
<td>2.4</td>
<td>1.7</td>
<td>6.7</td>
<td>12.5</td>
</tr>
<tr>
<td>C\textsubscript{4}H\textsubscript{9}</td>
<td>1.2</td>
<td>1.6</td>
<td>1.6</td>
<td>1.4</td>
<td>2.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Barua et al. conducted screening studies with several lipases, investigating the resolution of optically pure (S)-2-nitroalcohols. 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. 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. 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).

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

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Strain</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetate (1R,2R)</td>
<td>Alcohol (1S,2S)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>C. cylindracea</em> C1</td>
<td>81</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td><em>P. cepacia</em> P1</td>
<td>13</td>
<td>&gt;98</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td><em>P. stutzeri</em></td>
<td>53</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
<tr>
<td>4</td>
<td><em>Alcaligenes</em> spp.</td>
<td>47</td>
<td>&gt;98</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td><em>P. cepacia</em></td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>P. fluorescens</em></td>
<td>50</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

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

![Chemical structure diagram]

Hydrolase-mediated acylation and deacylation have been employed to gain access to enantio- and diastereomerically enriched β- and γ-nitroalcohols. 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).

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. 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\textsuperscript{41}

![Resolution of 1-nitro-2-alkanols with succinic anhydride in diisopropyl ether]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conversion (%)</th>
<th>$ee_s$ (%)</th>
<th>$ee_p$ (%)</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_3$</td>
<td>39</td>
<td>57</td>
<td>67</td>
<td>28 (R)</td>
</tr>
<tr>
<td>2</td>
<td>C$_2$H$_5$</td>
<td>47</td>
<td>44</td>
<td>43</td>
<td>4 (R)</td>
</tr>
<tr>
<td>3</td>
<td>C$_3$H$_7$</td>
<td>54</td>
<td>92</td>
<td>93</td>
<td>82 (S)</td>
</tr>
<tr>
<td>4</td>
<td>C$_4$H$_9$</td>
<td>4</td>
<td>3</td>
<td>75</td>
<td>7 (S)</td>
</tr>
<tr>
<td>5</td>
<td>(C$_6$H$_5$)$_2$CH$_2$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>(C$_6$H$_5$)$_3$CH$_4$</td>
<td>42</td>
<td>71</td>
<td>97</td>
<td>96 (S)</td>
</tr>
</tbody>
</table>

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.\textsuperscript{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\textsuperscript{42}

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conversion (%)</th>
<th>$ee_s$ (%)</th>
<th>$ee_p$ (%)</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$p$-NO$_2$</td>
<td>48</td>
<td>97</td>
<td>&gt;99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>2</td>
<td>$m$-NO$_2$</td>
<td>49</td>
<td>95</td>
<td>49</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>$o$-NO$_2$</td>
<td>&lt;1</td>
<td>-</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>$p$-Cl</td>
<td>46</td>
<td>97</td>
<td>46</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>$m$-Cl</td>
<td>47</td>
<td>91</td>
<td>47</td>
<td>&gt;200</td>
</tr>
<tr>
<td>6</td>
<td>$o$-Cl</td>
<td>&lt;1</td>
<td>-</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>47</td>
<td>95</td>
<td>47</td>
<td>155</td>
</tr>
<tr>
<td>8</td>
<td>$p$-CH$_3$</td>
<td>48</td>
<td>84</td>
<td>48</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9</td>
<td>$P$-OCH$_3$</td>
<td>49</td>
<td>84</td>
<td>49</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

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.\textsuperscript{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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Conversion (%)</th>
<th>Acetate ee (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C. antarctica B</td>
<td>5</td>
<td>78</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>C. rugosa</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>P. cepacia</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>P. cepacia CI</td>
<td>11</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>P. cepacia CII</td>
<td>10</td>
<td>90</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>P. fluorescens</td>
<td>7</td>
<td>93</td>
<td>30</td>
</tr>
<tr>
<td>7^a</td>
<td>P. cepacia CI</td>
<td>46</td>
<td>99</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

^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-Cl 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Time</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-O2N-C6H4</td>
<td>2 d</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>4-NC-C6H4</td>
<td>2 d</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>4-F3C-C6H4</td>
<td>3 d</td>
<td>89</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>3-O2N-C6H4</td>
<td>3 d</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>4-CH3-C6H4</td>
<td>4 d</td>
<td>35</td>
<td>93</td>
</tr>
<tr>
<td>6</td>
<td>Thiophene-2-yl</td>
<td>4 d</td>
<td>68</td>
<td>46</td>
</tr>
</tbody>
</table>

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. 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. 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](image)

Considerable progress has been made by Milner in the individual elements of the dynamic kinetic resolution process. The first step in the resolution protocol was to establish a suitable catalyst for the enzymatic transformation. In this early study, efficient kinetic bioreolution 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).
Through the appropriate selection of biocatalyst, three of the four enantiomers were obtained directly in high optical purity, while hydrolysis of the acetate (1R,2S)-100a would potentially lead to the alcohol (1R,2S)-99a although this is complicated by competing elimination of the cis-alcohol 99a to 1-nitrocyclohexene 102 via base-mediated dehydration (Scheme 4.10). 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.

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 1H 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).
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.

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

- 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).

- To explore in detail the DBU-mediated interconversion of enantiopure (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, based on the promising one off preliminary result by Milner (Scheme 4.16).
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 (NaBH₄) reduction of 2-nitrocyclohexanone 103 and subsequent acetylation to form the acetates (±)-100a and (±)-100b in good yields (Scheme 4.17).
The desired starting material 2-nitrocyclohexanone 103 is commercially available, 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 and Özbal were used as a basis for this work.

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).

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).
Chapter 4

Results and Discussion

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).\(^{51}\)

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 \(\delta_H \sim 4.40\) 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-aminoehexanoic 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)\textsuperscript{52-59} have frequently been used.\textsuperscript{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.\textsuperscript{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 \textsuperscript{1}H NMR spectrum of the crude product. Cobb\textsuperscript{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.\textsuperscript{4,58}

![Scheme 4.22](image)

The alkyl nitrite 115 was characterised by a broad multiplet at \(\delta_H 4.58-4.80 \text{ ppm}\) 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 \textsuperscript{1}H NMR of the crude product.\textsuperscript{61-64}

![Scheme 4.23](image)

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.\textsuperscript{60} Ethyl 6-nitrohexanoate 113 was characterised by a carbonyl absorption band at \(\nu_{\text{max}} 1733 \text{ cm}^{-1}\) and the asymmetrical and symmetrical stretching of the nitro group at
$\nu_{\text{max}}$ 1554 and 1377 cm$^{-1}$, while in the $^1$H NMR spectrum, the principal signals include a distinctive quartet at $\delta_H$ 4.13 ppm attributable to the ethyl ester and triplet at $\delta_H$ 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.\textsuperscript{60}

The DIBAL-H selective reduction of ethyl 6-nitrohexanoate 113 to the corresponding aldehyde has been described previously.\textsuperscript{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 $-78^\circ\text{C}$ (Scheme 4.24).\textsuperscript{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. $^1$H 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.\textsuperscript{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.

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\textsuperscript{65} and Ziegler\textsuperscript{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.
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 \( \nu_{\text{max}} \) 3400 cm\(^{-1}\) and nitro stretching bands at \( \nu_{\text{max}} \) 1552 and 1385 cm\(^{-1}\) were observed in the IR spectrum of 6-nitrohexanol 108. The characteristic signals in the \(^1\)H NMR were a broad OH singlet at \( \delta_H \) 1.76 ppm and two triplets at \( \delta_H \) 3.64 ppm and \( \delta_H \) 4.40 ppm attributable to the methylene hydrogens alpha to the alcohol C(1)H and nitro C(6)H\(_2\) moiety respectively.\(^{\text{vi}}\)

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.

\(^{\text{vi}}\) Milner\(^{45}\) reports the C(1)H\(_2\) triplet at \( \delta_H \) 3.24 ppm in the experimental data, however, in the results and discussion section the \(^1\)H NMR spectrum is described at \( \delta_H \) 3.64 ppm, which is in agreement with the \(^1\)H NMR data reported in this study and literature reports.\(^{67,68}\) Thus it is believed Milner erroneously reported \( \delta_H \) 3.24 ppm for the C(1)H\(_2\) protons in the experimental data.
The aldehyde 101 is characterised in the IR spectrum by a carbonyl stretch at $\nu_{\text{max}}$ 1722 cm$^{-1}$ and the symmetric and asymmetric bands of the nitro group at $\nu_{\text{max}}$ 1552 and 1386 cm$^{-1}$. The $^1$H NMR spectrum of 6-nitrohexanal 101 has a distinct doublet of triplets ($J$ 7.2, 1.5) at $\delta_{H}$ 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 $\delta_{H}$ 9.78 ppm. All spectral characteristics were as described in the literature.

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, 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, later adapted by Milner, 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 $\nu_{\text{max}}$ 1722 cm$^{-1}$, signifying reaction completion. $^1$H NMR analysis of the crude product revealed a mixture of diastereomeric nitroalcohols (±)-99a and (±)-99b (14 : 86 respectively).
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 (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b with subsequent crystallographic determination of the structure. Höng had previously described the synthesis of (±)-99a and (±)-99b by NaBH₄ reduction and assigned the ¹H NMR details of the trans-isomer (±)-99b which were consistent with our data.

Spectral characteristics for the β-nitroalcohols (±)-99a and (±)-99b agreed with those described by Milner as well as literature reports. 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 νmax 3256-3427 cm⁻¹ and the characteristic nitro stretches at νmax 1551-1548 and 1376-1383 cm⁻¹.

Analysis by ¹H NMR identifies key spectroscopic features distinct to each diastereomer, (±)-99a or (±)-99b (Figure 4.3). The principal signals in the ¹H NMR of the (±)-cis-2-nitrocyclohexanol (±)-99a include the doublet at δH 2.56 ppm for the OH peak and the multiplet at δH 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 δH 4.52 ppm.

The characteristic hydroxyl signal of the corresponding (±)-trans-diastereomer (±)-99b appears as a doublet at δH 2.61 ppm. Significantly in the more polar trans-diastereomer (±)-99b the order of ¹H 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 δH 4.04-4.12 ppm while the C(2)H proton appears as a multiplet at δH 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. 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).
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 $\nu_{\text{max}}$ 1668 cm$^{-1}$ and nitro absorption bands at $\nu_{\text{max}}$ 1515 and 1333 cm$^{-1}$. The $^1$H NMR displays a distinctive multiplet at $\delta_{H}$ 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).

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.
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. 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. Purification by column chromatography was not attempted due to the reported formation of the elimination product 102 on silica gel. 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 $\nu_{\text{max}}$ 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 $\delta_H$ 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 $\delta_H$ 5.19-5.27 ppm.

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

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 1H 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 (r² value) is evaluated as a measure of acceptability. A linear fit with a r² 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 1H 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)\textsuperscript{vii}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{calibration_circuit CIS}
\caption{Calibration curve (±)-cis-2-nitrocyclohexanol (±)-99a (2 μL injection volume). Area (x 10\textsuperscript{3}) vs. concentration (mg/1 mL).}
\end{figure}

Calibration curve: (±)-trans-2-Nitrocyclohexanol (±)-99b (2 μL injection volume)\textsuperscript{IV}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{calibration_circuit TRANS}
\caption{Calibration curve (±)-trans-2-nitrocyclohexanol (±)-99b (2 μL injection volume). Area (x 10\textsuperscript{3}) vs. concentration (mg/1 mL).}
\end{figure}

\textsuperscript{vii} Calibration curves were conducted on a Waters alliance 2690 separations module with a PDA detector. The Chiralcel\textsuperscript{®} OJ-H column was utilised at room temperature with isopropanol/hexane (1 : 99), a flow rate of 0.9 mL/min and $\lambda_{\text{max}}$ 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. 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. 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, CDCl₃ and DBU (0.10 eq.)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>101 (%)</th>
<th>(±)-99a (%)</th>
<th>(±)-99b (%)</th>
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<tr>
<td>0 min</td>
<td>21</td>
<td>19</td>
<td>60</td>
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<tr>
<td>17 min</td>
<td>20</td>
<td>18</td>
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<td>78</td>
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<tr>
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<td>82</td>
</tr>
<tr>
<td>9 h 29 min</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>15 h 29 min</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>20 h 22 min</td>
<td>0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>22 h 37 min</td>
<td>0</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>20 days</td>
<td>0</td>
<td>14</td>
<td>86</td>
</tr>
</tbody>
</table>

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. 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 1H NMR spectra - Evidence for dynamic interconversion, 6-nitrohexanal 101 and DBU (0.10 eq.).](image)

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.
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)HNO₂ signals was observed at 0.05 equivalent of DBU.

Table 4.13: Evidence for dynamic interconversion – 6-nitrohexanal 101, CDCl₃ and DBU (0.05 eq.)

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

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 1H NMR spectroscopy to determine product ratio and extent of conversion. Significantly the conversion determined by 1H 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.\textsuperscript{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.\textsuperscript{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.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4.10.png}
\caption{Figure 4.10}
\end{figure}

This rule generalizes the observed enantioselectivity of hydrolases in both hydrolysis and transesterification 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 (±)-\textit{cis}-and (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{a} and (±)-99\textit{b} the following stereochemical outcome was predicted (Scheme 4.32).

\begin{scheme}[h]
\centering
\includegraphics[width=0.8\textwidth]{scheme4.32.png}
\caption{Scheme 4.32}
\end{scheme}

The predicted assignment by Kazlauskas’s rule was in direct agreement with Milner’s study.\textsuperscript{45} In the preparative-scale \textit{Pseudomonas fluorescens} mediated transesterification of (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b}, Milner isolated the (1\textit{R},2\textit{R})-\textit{trans}-2-nitrocyclohexyl acetate (1\textit{R},2\textit{R})-100\textit{b} in >99% ee. The absolute stereochemistry of enantiopure (1\textit{R},2\textit{R})-100\textit{b}
was confirmed by single crystal X-ray diffraction and assigned as (1R,2R)-trans-2-nitrocyclohexylacetate (1R,2R)-100b. The absolute stereochemistry of the untransformed alcohol therefore has to be (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 (1R,2R)-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 (1R,2S)-cis-2-nitrocyclohexyl acetate (1R,2S)-100a and (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a by analogy to the transesterification of (±)-trans-2-nitrocyclohexanol (±)-99b and in accordance to Kazlauska’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 (±)-99b45 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 1H NMR to be 19% and 18% respectively. The limited efficiency of the transesterification restricted the enantiopurity of the recovered alcohol (1S,2R)-99a, however, the generated acetate (1R,2S)-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 (1R,2S)-100a was observed. Notably no elimination product 102 was observed in the 1H NMR spectrum of the crude products.
Table 4.14: Hydrolase-mediated transesterification of (±)-cis-2-nitrocyclohexanol (±)-99a in vinyl acetate

![Chemical Structure]

OH

\[
\text{Lipase, Vinyl Acetate} \quad \text{24 h at 24 °C & 3 h at 40 °C}
\]

(±)-99a

\[
\begin{align*}
\text{OH} & \rightarrow \text{OAc} \\
\text{NO}_2 & \rightarrow \text{NO}_2
\end{align*}
\]

(1R,2S)-100a

(1S,2R)-99a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Ratio 99a : 99b</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>Alcohol cis-99a (1S,2R)</th>
<th>Acetate cis-100a (1R,2S)</th>
<th>E Calc.</th>
<th>E NMR</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>96 : 4</td>
<td>23</td>
<td>19</td>
<td>29</td>
<td>95</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Alcaligenes</em> spp. 1</td>
<td>96 : 4</td>
<td>20</td>
<td>18</td>
<td>23</td>
<td>92</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Rhizopus</em> spp.</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Aspergillus niger</em></td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Mucor mehei</em></td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Porcine pancrease</em> Type II</td>
<td>95 : 5</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pig liver esterase</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Candida antarctica</em> A</td>
<td>96 : 4</td>
<td>60</td>
<td>60</td>
<td>80</td>
<td>54</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Achromobacter</em> spp.</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. The ratio of (±)-cis-2-nitrocyclohexanol (±)-99a to (±)-trans-2-nitrocyclohexanol (±)-99b in the starting material, determined by \( ^1H \) NMR spectroscopy.

b. Time for transesterification of the nitroalcohol was 51 h at 24 °C.

c. Unknown impurity observed in the crude \( ^1H \) 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 mehei* 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 mehei* 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\textsuperscript{36,45}

![Chemical structure diagram]

(±)-99b  (1R,2R)-100b  (1S,2S)-99b

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Ratio 99a : 99b</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E Calc. 1\textsuperscript{H} NMR</td>
<td>Alcohol trans-99b (1S,2S)</td>
<td>Acetate trans-100b (1R,2R)</td>
</tr>
<tr>
<td>1</td>
<td>Alcaligenes spp. 1</td>
<td>0 : 100</td>
<td>-</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Mucor mehei</td>
<td>0 : 100</td>
<td>-</td>
<td>17</td>
<td>-</td>
</tr>
</tbody>
</table>

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 (1S,2S)-99b and acetate (1R,2R)-100b.\textsuperscript{45}

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

![Chemical structure diagram]

(±)-99b  (1R,2R)-100b  (1S,2S)-99b

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Ratio 99a : 99b</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E Calc. 1\textsuperscript{H} NMR</td>
<td>Alcohol trans-99b (1S,2S)</td>
<td>Acetate trans-100b (1R,2R)</td>
</tr>
<tr>
<td>1</td>
<td>Candida antarctica B</td>
<td>1 : 99</td>
<td>50</td>
<td>49</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td>Candida antarctica A</td>
<td>1 : 99</td>
<td>9</td>
<td>c</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Achromobacter spp.</td>
<td>1 : 99</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a.} Time for transesterification of the nitroalcohol was 51 h at 24 °C.
\textsuperscript{b.} The ratio of (±)-cis-2-nitrocyclohexanol (±)-99a to (±)-trans-2-nitrocyclohexanol (±)-99b in the starting material, determined by \textsuperscript{1}H NMR spectroscopy.
\textsuperscript{c.} Unknown impurity observed in the \textsuperscript{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 an equimolar mixture of diastereomers (±)-99a and (±)-99b was warranted, with careful examination of the appearance of acetates 100a and 100b both by 1H 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 1H 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 1H NMR analysis. Due to overlapping signals in the 1H 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 (1R,2S)-100a relative to 19% of the major trans-acetate (1R,2R)-100b was evident by 1H 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 (1R,2S)-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 (1S,2S)-99b (>98% ee) and trans-acetate (1R,2R)-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 (1R,2S)-100a however enantiopurity of the cis-alcohol (1S,2R)-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 (1S,2S)-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

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Temp (°C)</th>
<th>Alcohol (±)-99</th>
<th>Acetate (±)-100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cis-99a (%)</td>
<td>trans-99b (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[ee (%)]b</td>
<td>[ee (%)]c</td>
</tr>
<tr>
<td>Candida</td>
<td>15 h</td>
<td>19</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>antarctica</td>
<td>24 h</td>
<td>24</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td>B (immob)</td>
<td>39 h</td>
<td>24</td>
<td>43</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>24</td>
<td>42</td>
<td>30</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 3H 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 (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a.
c. The principal enantiomer was (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b.
d. The principal enantiomer was (1R,2S)-cis-2-nitrocyclohexyl acetate (1S,2R)-100a.
e. The principal enantiomer was (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-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 (1R,2S)-cis- and (1R,2R)-trans-2-nitrocyclohexanol (1R,2S)-99\(_a\) and (1R,2R)-99\(_b\) were acetylated with excellent enantioselectivity observed of the generated acetates (1R,2S)-100\(_a\) and (1R,2R)-100\(_b\). Significantly this process failed to go to completion with residual (1R,2S)-cis- and (1R,2R)-trans-2-nitrocyclohexanol (1R,2S)-99\(_a\) and (1R,2R)-99\(_b\) 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 \(\alpha\) to the nitro moiety. While (1R,2S)-99\(_a\) and (1R,2R)-99\(_b\) enantiomers are interconverted, and similarly (1S,2S)-99\(_b\) and (1S,2R)-99\(_a\) are interconverted by epimerisation, interconversion between (1R,2S)-99\(_a\) and (1R,2R)-99\(_b\) with (1S,2R)-99\(_a\) and (1S,2S)-99\(_b\) is not possible as the epimerisation mechanism does not allow scrambling of the stereochemical integrity at C1 (Scheme 4.33).

![Scheme 4.33](image)

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 (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99\(_b\) to a range of bases was investigated. Appearance of an enantiomer with variation of the stereochemistry at C1 i.e. appearance of the enantiomers (1R,2R)-99\(_b\) and (1R,2S)-99\(_a\) 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.\textsuperscript{45}

The objective in this study was to explore in detail the DBU-mediated interconversion of enantiopure (1S,2S)-\textit{trans}-2-nitrocyclohexanol (1S,2S)-99\textit{b}, based on this promising one off preliminary result.\textsuperscript{45} The first step in this investigation was preparative-scale hydrolase-mediated transesterification of (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b} to access enantiopure (1S,2S)-\textit{trans}-2-nitrocyclohexanol (1S,2S)-99\textit{b} for the DBU-mediated chiral HPLC investigation. The large scale resolution of (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b} was adapted from a procedure described from Milner.\textsuperscript{45}

\textit{Pseudomonas fluorescens} was the lipase of choice in Milner’s study for preparative-scale demonstrating excellent efficiency and enantioselectivity in the analytical screens.\textsuperscript{45} In this study, an immobilised version of \textit{Pseudomonas fluorescens} obtained commercially\textsuperscript{46} was analytically screened for the transesterification of (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b} (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 \textit{Pseudomonas fluorescens} (immob) demonstrated limited efficiency achieving only 38\% conversion after 73.5 h. While high enantioselectivity of the \textit{trans}-acetate (1R,2R)-100\textit{b} was observed (>98\% ee), the enantiopurity of the \textit{trans}-alcohol (1S,2S)-99\textit{b} suffered (61\% ee) due to the poor conversion rate. Thus this immobilised lipase was considered unsuitable for preparative-scale and the free \textit{Pseudomonas fluorescens} lipase employed by Milner was utilised instead.\textsuperscript{45}

\textbf{Table 4.18:} \textit{Pseudomonas fluorescens} (immob) mediated transesterification of (±)-\textit{trans}-2-nitrocyclohexanol(±)-99\textit{b} in vinyl acetate

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Ratio 99\textit{a} : 99\textit{b}</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>Alcohol Acetate (%)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pseudomonas fluorescens} (immob)\textsuperscript{a}</td>
<td>1 : 99</td>
<td>38</td>
<td>38</td>
<td>61</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

\textsuperscript{a} \textit{Pseudomonas fluorescens} (immob) was obtained commercially from Sigma-Aldrich.\textsuperscript{45} 
\textsuperscript{b} The ratio of (±)-\textit{cis}-2-nitrocyclohexanol (±)-99\textit{a} to (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b} in the starting material, determined by \textsuperscript{1}H NMR spectroscopy.

In this study reaction monitoring of the preparative-scale free \textit{Pseudomonas fluorescens} mediated transesterification of (±)-\textit{trans}-2-nitrocyclohexanol (±)-99\textit{b} was conducted at 28 h and 55 h; an aliquot was withdrawn and following work-up, \textsuperscript{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 \textit{E}-value calculator\textsuperscript{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.\textsuperscript{45} It was postulated that denaturation and decrease in catalytic activity of the hydrolase \textit{Pseudomonas fluorescens} may have occurred over prolonged storage resulting in the increased reaction time.

On reaction completion the reaction mixture was filtered through Celite\textsuperscript{®} to remove the hydrolase, and the hydrolase washed with ethyl acetate. The combined organic extracts were concentrated under reduced pressure. The alcohol (1\text{S},2\text{S})-99\text{b} and acetate (1\text{R},2\text{R})-100\text{b} in the crude reaction mixture were isolated by column chromatography and analysed by \textsuperscript{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\text{S},2\text{S})-\textit{trans}-2-nitrocyclohexanol (1\text{S},2\text{S})-99\text{b}, on chromatographic purification the enantiomeric excess of isolated pure (1\text{S},2\text{S})-99\text{b} was determined to be 95% ee obtained in a 45% yield. The enantiopure (1\text{R},2\text{R})-\textit{trans}-2-nitrocyclohexyl acetate (1\text{R},2\text{R})-100\text{b} was isolated in 30% yield after purification with >98% enantiomeric excess. Although the enantioselectivity obtained in this study of the acetate (1\text{R},2\text{R})-100\text{b} was similar to that observed by Milner the enantioselectivity of the isolated alcohol (1\text{S},2\text{S})-99\text{b} was slightly lower (95% ee \textit{vs.} >99% ee).\textsuperscript{45} In addition, the yield of the isolated alcohol (1\text{S},2\text{S})-99\text{b} and acetate (1\text{R},2\text{R})-100\text{b} 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.\textsuperscript{45}

\begin{table}
\centering
\caption{Large scale \textit{Pseudomonas fluorescens} mediated transesterification of (±)-\textit{trans}-2 nitrocyclohexanol (±)-99\text{b} in vinyl acetate\label{tab:4.19}}
\begin{tabular}{llllll}
\hline
\textbf{Entry} & \textbf{Reaction Time} & \textbf{Temperature (°C)} & \multicolumn{2}{c}{\textbf{Conversion (\%)}} & \textbf{ee (\%)} \\
 & & & \textbf{E Calc.} & \textbf{\textsuperscript{1}H NMR} & \\
\hline
1 & 28 h & rt & 24 & 25 & 31 \textsuperscript{b} & >98 \textsuperscript{a} & 134 \\
2 & 55 h & 24 & 44 & - & 78 & >98 & >200 \\
3 & 79 h & 24 & 50 & 50 & 97 \textsuperscript{a} \textsuperscript{[45]} & >98 & >200 \\
\hline
\end{tabular}
\textsuperscript{a} While analysis by chiral HPLC of the crude product mixture showed 97% ee for (1\text{S},2\text{S})-\textit{trans}-2-nitrocyclohexanol (1\text{S},2\text{S})-99\text{b}, on chromatographic purification the enantiomeric excess [ee (\%)] of isolated (1\text{S},2\text{S})-99\text{b} was determined to be 95% ee.

\textsuperscript{b} Isolated yield following column chromatography.
\end{table}
Chapter 4

Results and Discussion

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, (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b, >98% ee, (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b, >98% ee and enantioenriched (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, 97% ee, 49 : 51 respectively. $^1$H NMR Spectrum II and HPLC Trace II: Purified (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b >98% ee following column chromatography. $^1$H NMR Spectrum III and HPLC Trace III: Purified (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b 95% ee following column chromatography ($^1$H NMR recorded in CDCl$_3$ at 300 MHz)
Once the enantioenriched (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b was obtained the next step was to investigate the progress of the DBU-mediated interconversion by chiral HPLC. Appearance of the enantiomer (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a only would indicate an epimerisation process, whereas appearance of either enantiomers (1R,2S)-cis- or (1R,2R)-trans-2-nitrocyclohexanol (1R,2S)-99a and (1R,2R)-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 (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 1H NMR studies investigating the dynamic ring opening/closing process.

### Table 4.20: Enantioenriched (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, 94% ee following exposure to 1.0 eq. and 0.5 eq. of DBU at 24 h and 48 h

<table>
<thead>
<tr>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Alcohol (±)-99</th>
<th>cis-99a (%)</th>
<th>trans-99b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>100</td>
<td>-</td>
<td>84</td>
</tr>
<tr>
<td>1</td>
<td>24 h</td>
<td>16 [31]</td>
<td>84 [34]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>17 [24]</td>
<td>83 [23]</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0 h</td>
<td>100</td>
<td>-</td>
<td>85</td>
</tr>
</tbody>
</table>

a. The principal enantiomer was (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a.
b. The principal enantiomer was (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 (1R,2S)-cis- or (1R,2R)-trans-2-nitrocyclohexanol (1R,2S)-99a and (1R,2R)-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 (1S,2S)-trans- and (1S,2R)-cis-2-nitrocyclohexanol (1S,2S)-99a and (1S,2S)-99b at 24 h and 48 h. Furthermore, the prevalence of the (1S,2R)-99a and (1S,2S)-99b as the principal enantiomers over time suggests that the epimerisation process was in fact the leading contributor to the interconversion of (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 1H NMR experiments conducted where deuterium exchange of C(2)HNO₂ 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 1H NMR investigation of the DBU-mediated ring closing (16 : 84 respectively). The agreement in the thermodynamic ratios is particularly good considering different solvents (CDCl₃ 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 1H NMR studies due to these changes.

Surprisingly, at the lower concentration of DBU (0.5 equivalent) isomerisation and complete racemisation of the enantioenriched (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 1H 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 (1S,2S)-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 1H 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 1H 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 1H NMR generating a thermodynamic ratio of 16 : 84 respectively, 1H NMR analysis alone was unable to definitively define the mechanistic pathway.

Thus, the DBU-mediated interconversion of enantioenriched (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b was monitored by chiral HPLC. Appearance of the enantiomers (1R,2R)-99b and (1R,2S)-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 (pKa CH₃NO₂ ~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).

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).

Finally, increasing the steric bulk at C2 was envisaged to assist hydrolase selectivity and increase diastereoselectivity (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\textsuperscript{45} approach towards the bioresolution of an intramolecular Henry reaction of 6-nitrohexanal \textsuperscript{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 (±)-\textsuperscript{117}a and (±)-\textsuperscript{117}b, establishing the most efficient hydrolase(s) to perform this biotransformation diastereoselectively.

The second step was examination by \textsuperscript{1}H NMR analysis of the base-mediated intramolecular Henry reaction and associated interconversion process between the resultant (±)-cis- and (±)-trans-diastereomers (±)-\textsuperscript{117}a and (±)-\textsuperscript{117}b through ring opening and closing \textit{via} the aldehyde 6-nitroheptanal \textsuperscript{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 (±)-\textsuperscript{117}a and (±)-\textsuperscript{117}b with the lipase-mediated diastereoselective and enantioselective transesterification would selectively lead to quantitative yields of a single stereoisomer of 2-methyl-2-nitrocyclohexyl acetate \textsuperscript{119}.

\begin{center}
\textbf{Scheme 4.36}
\end{center}

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

- To prepare the racemic synthetic targets (±)-cis- and (±)-trans-2-methyl-2-nitrocyclohexanol (±)-\textsuperscript{117}a and (±)-\textsuperscript{117}b and (±)-cis- and (±)-trans-2-methyl-2-nitrocyclohexyl acetate (±)-\textsuperscript{119}a and (±)-\textsuperscript{119}b 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-nitrocyclohexanone (±)-117.
• To prepare the aldehyde 6-nitroheptanal 118 and establish analytical evidence by 1H 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.

\[ \text{Scheme 4.37} \]

### 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. A number of synthetic routes have been reported in the literature to 2-methyl-2-nitrocyclohexanone 120. 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). 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).
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). 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$_2^-$ leads to $\alpha$-nitroketones.

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. 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). 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 126 observed in the $^1$H NMR spectrum, following attempted purification of 120 by vacuum distillation.

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-nitrocyclohexanols (±)-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.

![Chemical Structures]

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 \( \alpha \)-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.\(^{74-77}\)

![Chemical Structures]

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 \( \nu_{\text{max}} \) 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.

![Scheme 4.42](image)

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. 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. 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).
The IR spectra of the two diastereomers \( (\pm) - 117a \) and \( (\pm) - 117b \) are characterised by a broad OH peak at \( \nu_{\text{max}} \) 3376-3445 cm\(^{-1}\) and asymmetrical and symmetrical stretching of the nitro group at \( \nu_{\text{max}} \) 1538-1539 cm\(^{-1}\) and \( \nu_{\text{max}} \) 1340-1352 cm\(^{-1}\). Interestingly the aforementioned bands absorb at a higher frequency in \( (\pm) - \text{cis}-2\)-methyl-2-nitrocyclohexanol \( (\pm) - 117a \) relative to the trans-diastereomer \( (\pm) - 117b \). Analysis by \(^1\)H NMR identifies key spectroscopic features distinct to each diastereomer \( (\pm) - 117a \) or \( (\pm) - 117b \) (Figure 4.16). A larger deshielding effect is observed in \( (\pm) - \text{cis}-2\)-methyl-2-nitrocyclohexanol \( (\pm) - 117a \) for the proton geminal to the hydroxyl group (\( \delta_H \) 4.28-4.29 ppm) relative to the \( (\pm) - \text{cis}-\)diastereomer \( (\pm) - 117a \) (\( \delta_H \) 3.91 ppm) due to its proximity in space to the nitro group. Conversely the signal for the OH proton in \( (\pm) - \text{cis}-2\)-methyl-2-nitrocyclohexanol \( (\pm) - 117a \) (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)CH\(_3\) and C(3)H\(_2\) in the \(^{13}\)C NMR spectrum of \( (\pm) - \text{trans}-2\)-methyl-2-nitrocyclohexanol \( (\pm) - 117b \).
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).
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).\textsuperscript{80} Full structural details are contained on the accompanying CD.

IR analysis of the acetates (±)-119a and (±)-119b demonstrates a strong carbonyl stretch at $\nu_{\text{max}}$ 1738 and 1748 cm$^{-1}$ respectively. $^1$H 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 ($\delta_H$ 5.50-5.55 ppm) relative to (±)-119a ($\delta_H$ 5.26-5.29 ppm) attributable to its proximity in space to the electron withdrawing nitro group.
Chapter 4

Results and Discussion

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 $\delta_H$ 4.50-4.65 ppm attributable to the C(6)H proton geminal to the nitro and methyl moiety (Scheme 4.44).

Figure 4.19: $^1$H NMR Spectrum I: Crude (±)-trans-2-methyl-2-nitrocyclohexyl acetate (±)-119b. Spectrum II: Crude (±)-cis-2-methyl-2-nitrocyclohexyl acetate (±)-119a (all spectra recorded in CDCl$_3$ at 300 MHz).

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).  

![Scheme 4.45](image)

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.  

Nucleophilic attack of aqueous base or alcohol to \(\alpha\)-nitrocycloalkanones produces ring cleavage with the formation of \(\omega\)-nitroacids and \(\omega\)-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.  

![Scheme 4.46](image)

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).  

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).  

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.
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).

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 $\beta$-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 precedent in related compounds.

According to literature procedure it was anticipated that ring opening of $\delta$-valerolactone 136 by methanol and subsequent oxidation would provide the substrate methyl 5-oxopentanoate 134 (Scheme 4.49). The methanolysis protocol employed in this study involved heating the commercially available $\delta$-valerolactone 136, 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

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 nitroaldol process 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 1H 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.

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 1H NMR spectrum with two characteristic sets of signals observed. The signal for the proton geminal to the nitro group appears at δH 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 δH 3.90-3.98 ppm and δH 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.
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%). 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. 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. Attempted separation of the undesired E-nitroalkene 139 from the nitroalkane 127 by column chromatography was unsuccessful.

Bachman reports optimum conversions to nitroalkanes utilising sodium borohydride in DMSO. 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-nitroheptanoate 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). This method described herein provides a convenient and high-yielding synthesis of nitroalkane 127 from the β-nitroalcohol 135.
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 $\nu_{\text{max}}$ 3365 cm$^{-1}$ and nitro stretching bands at $\nu_{\text{max}}$ 1553 and 1391 cm$^{-1}$ while in the $^1$H NMR spectrum the characteristic multiplet at $\delta_{\text{H}}$ 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 $\nu_{\text{max}}$ 1724 cm$^{-1}$ in addition to the asymmetric and symmetric bands of the nitro group at $\nu_{\text{max}}$ 1549 and 1392 cm$^{-1}$, while in the $^1$H NMR spectrum a distinct triplet is seen downfield at $\delta_{\text{H}}$ 9.77 ppm indicative of the aldehyde proton C(1)H.
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 coelution 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.](image)

### 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. All hydrolases screened were
kindly donated by Almac Sciences. In this screening protocol the alcohol substrate \((\pm)-117a\) or \((\pm)-117b\) (~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 \(^1\text{H} \text{NMR}\) spectroscopy and chiral HPLC.

Significantly, the crude alcohol \((\pm)-117a\) or \((\pm)-117b\) and acetate \((\pm)-119a\) or \((\pm)-119b\) 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 \(^1\text{H} \text{NMR}\) analysis and by the \(E\)-value calculator. Notably in some instances (entry 2, Table 4.22) slightly higher conversions were obtained by \(^1\text{H} \text{NMR}\) spectroscopy relative to the \(E\)-value. For conversions determined by \(^1\text{H} \text{NMR}\) 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 \((\pm)-\text{trans-2-methyl-2-nitrocyclohexanol} \ (\pm)-117b\)

The hydrolase-mediated transesterification of \((\pm)-\text{trans-2-methyl-2-nitrocyclohexanol} \ (\pm)-117b\) 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 \((\pm)-\text{cis-}\) and \((\pm)-\text{trans-2-nitrocyclohexanol} \ (\pm)-99a\) and \((\pm)-99b\) in some instances with excellent enantioselection.

**Table 4.21: Hydrolase-mediated transesterification of \((\pm)-\text{trans-2-methyl-2-nitrocyclohexanol} \ (\pm)-117b\) in vinyl acetate**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Conversion (%)*</th>
<th>ee (%)</th>
<th>(E) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cylindracea C1</td>
<td>48 h</td>
<td>33</td>
<td>43</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia P2</td>
<td>48 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Candida antarctica B (immob)</td>
<td>72 h</td>
<td>49</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas stutzeri</td>
<td>48 h</td>
<td>50</td>
<td>51</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas fluorescens</td>
<td>113.5 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
</tbody>
</table>

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 \((\pm)-\text{trans-2-methyl-2-nitrocyclohexanol} \ (\pm)-117b\) (entries 3 and 4, Table 4.21). Optimum conversions of ~50% were attained in both
resolutions and the generated acetate (1R,2R)-119b (>98% ee) and recovered alcohol (1S,2S)-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 (1R,2R)-119b (>98% ee) was achieved the poor efficiency of the bioresolution resulted in a reduced enantiopurity of the alcohol (1S,2S)-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 (1S,2S)-99b and nitroacetate (1R,2R)-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 enantioselectivity 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

![Comparison of enantioselection (E-value) for transesterification of (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b and (±)-trans-2-nitrocyclohexanol (±)-99b versus hydrolase](image.png)

**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

![Diagram of transesterification reaction]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas cepacia P2</td>
<td>48 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Candida antarctica B (immob)</td>
<td>72 h</td>
<td>33</td>
<td>47</td>
<td>49 &gt;98</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas stutzeri</td>
<td>48 h</td>
<td>78</td>
<td>85</td>
<td>&gt;98 27</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas fluorescens</td>
<td>113.5 h</td>
<td>40</td>
<td>45</td>
<td>64 96</td>
</tr>
</tbody>
</table>

Of the four hydrolases screened, no hydrolase was identified which resolved both the cis-acetate (1R,2S)-119a and cis-alcohol (1S,2R)-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 (1R,2S)-119a (≥96% ee) however the enantiopurity of the alcohol (1S,2R)-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 enantipreference with poor enantiopurity of the recovered acetate (1R,2S)-119a (27% ee). However, the recovered alcohol (1S,2R)-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. 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

![Bar chart showing comparison of enantioselection (E-value) for transesterification of different hydrolases.](image)

**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 1H 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

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Alcohol (±)-117</th>
<th>Acetate (±)-119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cis-117a (%)</td>
<td>trans-117b (%)</td>
<td>cis-119a (%)</td>
</tr>
<tr>
<td></td>
<td>[ee (%)]b</td>
<td>[ee (%)]c</td>
<td>[ee (%)]d</td>
</tr>
<tr>
<td><em>Pseudomonas stutzeri</em></td>
<td>18.5 h</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>40.5 h</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td><em>Candida antarctica</em> B (immob)</td>
<td>18.5 h</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>40.5 h</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>62.5 h</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 1H 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 (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a.
c. The principal enantiomer was (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b.
d. The principal enantiomer was (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1S,2R)-119a.
e. The principal enantiomer was (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-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 (1R,2S)-119a was poor (54% ee) demonstrating the lipase’s lack of enantiopreference towards (±)-117a. Thus, the poor enantiopurity of the faster generated acetate (1R,2R)-119b 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 (1R,2R)-119b (>98% ee). After 18.5 h only 4% of the minor cis-acetate (1R,2S)-119a relative to 17% of the major trans-acetate (1R,2R)-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 (1S,2S)-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 (1S,2S)-117b and trans-acetate (1R,2R)-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 (1S,2S)-117b (>98% ee) and the trans-acetate (1R,2R)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>Alcohol [yield (%)] E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$^1$H NMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>48 h</td>
<td>24</td>
<td>49</td>
<td>95</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2b</td>
<td>52.5 h</td>
<td>24</td>
<td>50</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

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 (1S,2S)-117b and (1R,2R)-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 (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b >98% ee following column chromatography. **Trace III:** Purified (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b 95% ee following column chromatography. For HPLC conditions see appendix I.

**Figure 4.27: 'H NMR Spectrum I:** Crude product following preparative-scale transesterification containing a mixture of enantioenriched (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b and (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b 50 : 50 respectively. **Spectrum II:** Purified (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b >98% ee following column chromatography. **Spectrum III:** Purified (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b >98% ee following column chromatography (all spectra recorded in CDCl₃ 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 (1S,2R)-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 (1S,2R)-117a and acetate (1R,2S)-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 enantipurity of the (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a was observed (>98% ee), the enantipurity of the (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a was low (45% ee) due to the poor conversion rate (32%).

Significantly, in Milner’s study, isolated enantiopure samples of (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a and (1R,2S)-cis-2-nitrocyclohexylacetate (1R,2S)-100a were not obtainable due to the formation of the elimination product 1-nitrocyclohexene 102 on attempted purification of the enantioenriched products (1S,2R)-99a and (1R,2S)-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, (1R,2S)-119a and (1S,2R)-117a can be unequivocally assigned by single crystal X-ray diffraction on a crystalline sample of (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a (see section 4.3.5.3).

Thus, three of the four resolution products, (1S,2S)-117b, (1R,2R)-119b and (1R,2S)-119a, were obtained in excellent enantiopurity and reasonable yields while (1S,2R)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>[yield (%)]</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E Calc.</td>
<td>$^1$H NMR</td>
<td>Alcohol trans-117a (1S,2R)</td>
<td>Acetate trans-119a (1R,2S)</td>
</tr>
<tr>
<td>1a</td>
<td>72 h</td>
<td>24</td>
<td>27</td>
<td>-</td>
<td>37</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2b</td>
<td>79 h</td>
<td>24</td>
<td>31</td>
<td>32</td>
<td>45</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

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 (1R,2S)-119a and (1S,2R)-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 (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a, 45% ee following column chromatography. Trace III: Purified (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a, >98% ee following column chromatography. For HPLC conditions see appendix I.
Chapter 4

Results and Discussion

Figure 4.29: ‘H NMR Spectrum I: Crude product following preparative-scale transesterification containing a mixture of enantioenriched (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a and (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a 68 : 32 respectively (recorded in CDCl₃ at 300 MHz). Spectrum II: Purified (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a, >98% ee following column chromatography (recorded in CDCl₃ at 400 MHz). Spectrum III: Purified (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a, 45% ee following column chromatography (recorded in CDCl₃ 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. 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. 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).
The absolute stereochemical assignment of the enantioenriched (1S,2R)-117a alcohol and enantiopure acetate (1R,2S)-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).

![Scheme 4.54](image)

The absolute stereochemistry of (1R,2S)-119a was determined by single crystal X-ray diffraction on a crystalline sample of enantiopure 119a recrystallised from HPLC grade acetonitrile (Figure 4.30). 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 (1S,2R)-117a.

![Figure 4.30](image)

*Figure 4.30: A view of (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-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).

![Scheme 4.55](image)
The absolute stereochemistry of the isolated enantiopure trans-acetate (1R,2R)-119b and trans-alcohol (1S,2S)-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 (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b and (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-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 1H 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 1H NMR spectroscopy (Figure 4.31) and are derived from integration of:

- 1H, br s, C(1)HOH at 3.91 ppm in (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a.
- 1H, m, C(1)HOH, at 4.28-4.29 ppm in (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b.
- 1H, m, C(6)HNO₂ 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 ($\pm$)-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$^{43}$

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Time</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-O$_2$N-C$_6$H$_4$</td>
<td>2 d</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>4-NC-C$_6$H$_4$</td>
<td>2 d</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>4-F$_3$C-C$_6$H$_4$</td>
<td>3 d</td>
<td>89</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>3-O$_2$N-C$_6$H$_4$</td>
<td>3 d</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>4-CH$_3$-C$_6$H$_4$</td>
<td>4 d</td>
<td>35</td>
<td>93</td>
</tr>
<tr>
<td>6</td>
<td>4-CH$_3$O-C$_6$H$_4$</td>
<td>4 d</td>
<td>28</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>Thiophene-2-yl</td>
<td>4 d</td>
<td>68</td>
<td>46</td>
</tr>
</tbody>
</table>
Chapter 4  Results and Discussion

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 1H 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 1H 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

![Diagram of cyclisation reaction](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Triethylamine (eq.)</th>
<th>Temp (°C)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alcohol (±)-117 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Acetate (±)-119 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis</td>
<td>trans</td>
<td>cis</td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>40</td>
<td>24 h</td>
<td>62</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>37</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>9</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>Ambient</td>
<td>24 h</td>
<td>89</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>76</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>65</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>Ambient</td>
<td>24 h</td>
<td>90</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>79</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>67</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 days</td>
<td>34</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>Ambient</td>
<td>24 h</td>
<td>92</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>85</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>78</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

<sup>a</sup> The conversions were determined by <sup>1</sup>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. 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-nitroheptanal 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 CDCl₃.

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1 h 23 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>5 h 46 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>11 h 30 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>3 days</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>7 days</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>17 days</td>
<td>72</td>
<td>28</td>
</tr>
</tbody>
</table>

a. 6-Nitroheptanal 118 was not detected in the ¹H NMR spectra.

Figure 4.33: Stacked ¹H NMR spectra: Investigation of dynamic interconversion – (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a and NEt₃ (2.0 eq.) (Recorded in CDCl₃ 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 1H 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 1H 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

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(±)-117b (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>2 days</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>3 days</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> 6-Nitroheptanal 118 was not detected in the 1H 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

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida antarctica B (immob)</td>
<td>72 h</td>
<td>31</td>
<td>cis-117a (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>trans-117b (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(ee (%))&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(ee (%))&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[65]</td>
<td>[58]</td>
<td>[&gt;98]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by <sup>1</sup>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 <sup>1</sup>H NMR spectrum of the crude product, in addition to the unreacted aldehyde 118, the presence of the alcohols (1S,2R)-117a and (1S,2S)-117b and the acetates (1R,2S)-119a and (1R,2R)-119b were evident and critically, chiral HPLC analysis indicated that the acetates (1R,2S)-119a and (1R,2R)-119b were essentially enantiopure while the alcohols (1S,2R)-117a and (1S,2S)-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 (1S,2R)-117a and (1S,2S)-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 (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-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.
Chapter 4

4.3.5: 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. (1R,2R)-trans-2-Methyl-2-nitrocyclohexyl acetate (1R,2R)-119b, >98% ee, (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a, >98% ee, (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a, 65% ee and (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b, 58% ee. For HPLC conditions see appendix I.

Note the above traces correlate to chiral HPLC conditions B.

4.3.6.1 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 pKₐ (pKₐ 12.0 in H₂O)⁹⁴ relative to triethylamine (pKₐ 10.75 in H₂O)⁹⁵.

4.3.6.1.1 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 ¹H 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 ¹H 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 (±)-
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 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alcohol (±)-117</th>
<th>Acetate (±)-119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis (±)-117a (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>trans (±)-117b (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>24 h</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>24 h</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
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<td>48 h</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>-</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>52</td>
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<tr>
<td></td>
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<td>7 days</td>
<td>-</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>24 h</td>
<td>48</td>
<td>15</td>
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<tr>
<td></td>
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<td>48 h</td>
<td>50</td>
<td>16</td>
<td>31</td>
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<tr>
<td></td>
<td></td>
<td>71 h</td>
<td>55.5</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>24 h</td>
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<td>2</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>93</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>91</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> The conversions were determined by '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. 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 '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

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 acetylation agent achieved excellent enantioselectivity (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 (1R,2R)-119b with Candida antarctica lipase B (immob) (entry 1, Table 4.33), the poor conversion resulted in minimal enantiopurity of the recovered alcohol (1S,2S)-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

![Diagram of hydrolase-mediated transesterification](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>Alcohol</th>
<th>Acetate</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida antarctica B (immob)</td>
<td>72 h</td>
<td>16</td>
<td>18</td>
<td>19</td>
<td>&gt;98</td>
<td>119</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas stutzeri</td>
<td>48 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 enantioselectivity was obtained for the acetate (1R,2R)-119b in each of the Pseudomonas stutzeri mediated transesterifications, the resolutions failed to achieve the optimum 50% conversion rate and thus the enantioselectivity of the alcohol (1S,2S)-117b was compromised. The highest enantioselectivity was observed with diisopropyl ether as solvent (entry 3, Table 4.34), however, even with this solvent the enantioselectivity of the alcohol (1S,2S)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Solvent</th>
<th>Conversion (%)</th>
<th>ee (%) ( ^a )</th>
<th>Alcohol ( ^{1} \text{H} ) NMR</th>
<th>Acetate ( ^{1} \text{H} ) NMR</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas stutzeri</td>
<td>Heptane</td>
<td>40</td>
<td>41</td>
<td>66</td>
<td>&gt;98</td>
<td>197</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas stutzeri</td>
<td>Diethyl ether</td>
<td>46</td>
<td>44</td>
<td>82</td>
<td>&gt;98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas stutzeri</td>
<td>Disisopropyl ether</td>
<td>46</td>
<td>45</td>
<td>84</td>
<td>&gt;98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas stutzeri</td>
<td>Toluene</td>
<td>20</td>
<td>21</td>
<td>24</td>
<td>&gt;98</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas stutzeri</td>
<td>Acetonitrile</td>
<td>25</td>
<td>26</td>
<td>32</td>
<td>&gt;98</td>
<td>135</td>
</tr>
</tbody>
</table>

\( ^a \) 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 (1S,2S)-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 (1S,2S)-117b is somewhat compromised. Critically the acetate (1R,2R)-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, disisopropyl 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 \(^{1}\text{H} \) 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 CDCl₃

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)ᵃ</th>
<th>(±)-117b (%)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>33 min</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>1 h 8 min</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>2 h 4 min</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3 h 31 min</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>4 h 52 min</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>7 h 17 min</td>
<td>53</td>
<td>47</td>
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<tr>
<td>9 h</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>24 h 45 min</td>
<td>35</td>
<td>65</td>
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<td>32 h 47 min</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>73 days</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>

ᵃ. 6-Nitroheptanal 118 was not detected in the ¹H NMR spectra.
Figure 4.36: Stacked $^1$H NMR spectra: Evidence for dynamic interconversion - $(\pm)$-cis-2-methyl-2-nitrocyclohexanol $(\pm)$-117a and DBU (0.1 eq.) recorded in CDCl$_3$ at 300 MHz.

Table 4.36: Evidence for dynamic interconversion – $(\pm)$-cis-2-methyl-2-nitrocyclohexanol $(\pm)$-117a and DBU (0.05 eq.) in CDCl$_3$

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>$(\pm)$-117a (%)$^a$</th>
<th>$(\pm)$-117b (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h 11 min</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>18 h 14 min</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>25 h 49 min</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>7 days</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

$^a$ 6-Nitroheptanal 118 was not detected in the $^1$H NMR spectra.
Table 4.37: Evidence for dynamic interconversion – $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117b and DBU (0.1 eq.) in CDCl$_3$

Table 4.37:

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)$^a$</th>
<th>(±)-117b (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>24 min</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>1 h 12 min</td>
<td>11</td>
<td>89</td>
</tr>
<tr>
<td>4 h 16 min</td>
<td>24</td>
<td>76</td>
</tr>
<tr>
<td>4 h 49 min</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>5 h 51 min</td>
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<td>72</td>
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<td>21 h 52 min</td>
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<td>67</td>
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<tr>
<td>14 days</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>

$^a$ 6-Nitroheptanal 118 was not detected in the $^1$H NMR spectra.

Figure 4.37: Stacked $^1$H NMR spectra: Evidence for dynamic interconversion – $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117b and DBU (0.1 eq.) recorded in CDCl$_3$ at 300 MHz.
It is evident from these studies that in the presence of DBU (just 0.05 equivalent), dynamic interconversion of \((\pm)-\text{cis}\) and \((\pm)-\text{trans}\)-2-methyl-2-nitrocyclohexanol \((\pm)-117a\) and \((\pm)-117b\) occurs. The \((\pm)-\text{trans}\)-diastereomer is the thermodynamically more stable diastereomer with a thermodynamic ratio of 35 : 65 \((\pm)-117a : (\pm)-117b\) 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 \((\pm)-117a\) and \((\pm)-117b\), exclusively via 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 \((\pm)-\text{trans}\)-2-methyl-2-nitrocyclohexanol \((\pm)-117b\) 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 \(^1\text{H}\) NMR analysis was conducted. Dynamic interconversion to the \(\text{cis}\)-diastereomer \((\pm)-117a\) was observed at 24 h however the \(\text{trans}\)-acetate \((\pm)-119b\) was the predominant product (56%) indicating that chemical acetylation competes efficiently with the ring opening and closing process. At 48 h the \(\text{trans}\)-alcohol \((\pm)-117b\) and \(\text{trans}\)-acetate \((\pm)-119b\) were the only two species evident by \(^1\text{H}\) NMR spectroscopy. Thus, although there was initial evidence for the dynamic

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**Figure 4.38:** Comparison of \(^1\text{H}\) NMR spectra recorded and thermodynamic ratio achieved of \((\pm)-117a\) on exposure to (0.1 eq.) DBU and \((\pm)-117b\) on exposure to (0.1 eq.) DBU.
interconversion mechanism at 24 h through appearance of the (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a by 1H 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

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>Alcohol (±)-117a (%)</th>
<th>Acetate (±)-119a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>32 trans (±)-117b</td>
<td>56 trans (±)-119b</td>
</tr>
<tr>
<td>48 h</td>
<td>31 cis (±)-117a</td>
<td>69 cis (±)-119a</td>
</tr>
</tbody>
</table>

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 1H 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 1H NMR and by chiral HPLC analysis after 48 hours. While in both studies, the chiral HPLC indicates high enantiomeric excess for the recovered alcohols (1S,2R)-117a and (1S,2S)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alcohol (±)-117 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Acetate (±)-119 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>48 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>CAL-B (immob)</td>
<td>-</td>
<td>72 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.10</td>
<td>48 h</td>
<td>-</td>
<td>28 (±)</td>
<td>36 (±)</td>
</tr>
<tr>
<td>4</td>
<td>P. stutzeri</td>
<td>0.05</td>
<td>48 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>CAL-B (immob)</td>
<td>0.10</td>
<td>72 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>P. stutzeri</td>
<td>0.10</td>
<td>48 h</td>
<td>77</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>P. stutzeri</td>
<td>0.20</td>
<td>48 h</td>
<td>11</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>P. stutzeri</td>
<td>0.5</td>
<td>48 h</td>
<td>6</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>P. stutzeri</td>
<td>1.0</td>
<td>48 h</td>
<td>[&gt;98]</td>
<td>[&gt;98]</td>
<td>[&gt;98]</td>
</tr>
</tbody>
</table>

<sup>a</sup> The conversions were determined by <sup>1</sup>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 <sup>1</sup>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<sub>a</sub> values.<sup>4</sup>

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 <sup>1</sup>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 ($\leq$ 5%). Thus, the TMG-mediated dynamic interconversion of $(\pm)$-cis- and $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117a and $(\pm)$-117b was also further explored.

Table 4.40: Base-mediated cyclisation of 6-nitroheptanal 118 with vinyl acetate as solvent

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>$pK_a$</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)$^a$</th>
<th>Alcohol $(\pm)$-117</th>
<th>Acetate $(\pm)$-119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis</td>
<td>trans</td>
<td>cis</td>
</tr>
</tbody>
</table>
|       |                       |        |               | $(\pm)$-117a (%)$^a$| $(\pm)$-117b (%)$^a$| $(\pm)$-119a (%)$^a$| $(\pm)$-119b (%)$^a$ |%
|       |                       |        |               | cis                  | trans              | cis              | trans            |
| 1     | DEA (2 eq.)           | 11.3   | 24 h          | 83                   | 8                  | 9                | -                |
|       | (H$_2$O)             | 99     | 48 h          | 82                   | 8                  | 10               | -                |
| 2     | Piperidine (2 eq.)    | 11.3   | 24 h          | 100                  | -                  | -                | -                |
|       | (H$_2$O)             | 99     | 48 h          | 100                  | -                  | -                | -                |
|       |                      |        | 72 h          | 100                  | -                  | -                | -                |
| 3     | DABCO (2 eq.)         | 8.82   | 24 h          | -                    | 39                 | 61               | -                |
|       | (H$_2$O)             | 95     | 48 h          | -                    | 40                 | 60               | -                |
|       |                      |        | 72 h          | -                    | 39                 | 61               | -                |
| 4     | Hünigs Base (2 eq.)   | 11.4   | 48 h          | 85                   | 5                  | 10               | -                |
|       | (H$_2$O)             | 100    | 72 h          | 79                   | 7                  | 14               | -                |
| 5     | 1M NaOH               | ~13.8  | 24 h          | 100                  | -                  | -                | -                |
|       |                      |        | 48 h          | 100                  | -                  | -                | -                |
| 6     | TMG (0.1 eq.)         | 13.6   | 24 h          | 69                   | 10                 | 21               | -                |
|       | (0.1 - 2.1 eq.$^b$)  |        | 48 h          | 6                    | 30                 | 57               | 5                |
|       | (H$_2$O)             | 101    |               |                      |                    |                  |                  |

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$_3$

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)$^a$</th>
<th>(±)-117b (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>3 h 10 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1 day</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>4 days</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>10 days</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ 6-Nitroheptan 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-nitroheptan 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 (1S,2R)-117a and (1S,2S)-117b and acetate (1R,2S)-119a and (1R,2R)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme</th>
<th>Source</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alcohol 117 cis-117a (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>cis-117a (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>trans-117b (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>trans-117b (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Acetate 119 cis-119a (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>cis-119a (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>trans-119b (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>trans-119b (%)&lt;sup&gt;a&lt;/sup&gt; [ee (%)]&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>48 h</td>
<td>-</td>
<td>40</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas stutzeri</td>
<td>48 h</td>
<td>75</td>
<td>4</td>
<td>[77]</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a. The conversions were determined by <sup>1</sup>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 <sup>1</sup>H NMR spectroscopy. The cis-diestereomer (±)-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.
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 (1R,2R)-trans-acetate (1R,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 (1S,2R)-cis- and (1S,2S)-trans-alcohols (1S,2R)-117a and (1S,2S)-117b albeit with poor enantioselectivity (Figure 4.39). Significantly the (1R,2S)-cis- and (1R,2R)-trans-acetates (1R,2S)-119a and (1R,2R)-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 (1R,2S)-119a and (1R,2R)-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

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**Table 4.43: Evidence for dynamic interconversion – (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b and TMG (2.0 eq.) in CDCl$_3$**

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)$^a$</th>
<th>(±)-117b (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>49 min</td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>5 h 7 min</td>
<td>26</td>
<td>74</td>
</tr>
<tr>
<td>3 days</td>
<td>28</td>
<td>72</td>
</tr>
</tbody>
</table>

* a. 6-Nitroheptanal 118 was not detected in the $^1$H NMR spectra.
vinyl acetate and base inhibition in the presence of the hydrolase leading to the acetates (1R,2S)-119a and (1R,2R)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>TMG</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)</th>
<th>Alcohol 117 cis-117a (%)</th>
<th>trans-117b (%)</th>
<th>cis-119a (%)</th>
<th>trans-119b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida antarctica B (immob)</td>
<td>2 eq.</td>
<td>5 days</td>
<td>7</td>
<td>26 [2]</td>
<td>67 [11]</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 1H 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, (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.
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 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer-bound DBU (eq.)</th>
<th>Reaction Time</th>
<th>Temp (°C)</th>
<th>Aldehyde 118 (%)a</th>
<th>Alcohol (±)-117 cis (%)a</th>
<th>Alcohol (±)-117 trans (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>13 h</td>
<td>Ambient</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>22.5 h</td>
<td>Ambient</td>
<td>92</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.0 – 2.0</td>
<td>44 h</td>
<td>40 °C</td>
<td>11</td>
<td>24</td>
<td>65</td>
</tr>
</tbody>
</table>

a. At 22.5 h after analysis by 1H 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 1H 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 1H 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**

![Diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer-bound DBU</th>
<th>Reaction Time (±)-117b (%)</th>
<th>(±)-117a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 eq.</td>
<td>19 h</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>3 eq.</td>
<td>19 h</td>
<td>-</td>
</tr>
</tbody>
</table>

a. The crude ¹H NMR spectrum was too complex to decipher conversions.

### 4.3.6.7 Influence of base strength

The link between the pKₐ 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 NEt₃. 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 pKₐ 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**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>pKₐ (H₂O)</th>
<th>Cyclisation of 6-nitroheptanal 118 with vinyl acetate</th>
<th>Base inhibition with lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intramolecular Henry reaction</td>
<td>Chemical acetylation</td>
</tr>
<tr>
<td>1</td>
<td>DABCO</td>
<td>8.7/4.2²⁹⁵</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>NEt₃</td>
<td>10.8²⁵</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>Piperidine</td>
<td>11²⁹⁹</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td>DEA</td>
<td>11²⁹⁹</td>
<td>✓ (partial)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Hünig’s base</td>
<td>11.4²⁰⁰</td>
<td>✓ (partial)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>DBU</td>
<td>12²⁰⁴</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>7</td>
<td>TMG</td>
<td>13.6²⁰¹</td>
<td>✓</td>
<td>✓ (partial)</td>
</tr>
<tr>
<td>8</td>
<td>NaOH (1M)</td>
<td>13.8</td>
<td>x</td>
<td>-</td>
</tr>
</tbody>
</table>
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 $(\pm)$-cis- and $(\pm)$-trans-2-methyl-2-nitrocyclohexanols $(\pm)$-117a and $(\pm)$-117b was achieved with a thermodynamic ratio of 30 : 70.

The crude $\beta$-nitroalcohols $(\pm)$-117a and $(\pm)$-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 $(\pm)$-2-methyl-2-nitrocyclohexanol $(\pm)$-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 $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117b was the dominant kinetic resolution process in step B, with the $(1R,2R)$-trans-acetate $(1R,2R)$-119b accounting for 33% of the overall product ratio. Excellent diastereoselectivity was also observed with limited transformation of $(\pm)$-cis-2-methyl-2-nitrocyclohexanol $(\pm)$-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 (1R,2R)-trans-2-methyl-2-nitrocyclohexanol (1R,2R)-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 (1R,2R)-119b.

Table 4.48: Two pot dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis

![Diagram of the reaction](image_url)

<table>
<thead>
<tr>
<th>Step</th>
<th>DBU (eq.)</th>
<th>Vinyl acetate (eq.)</th>
<th>Candida antarctica B (immob)</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>cis-117a (%)</td>
<td>trans-117b (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>[ee (±)]b</td>
<td>[ee (±)]b</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>138</td>
<td>18</td>
<td>30 (±)</td>
<td>70 (±)</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>8</td>
<td>47</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>138</td>
<td>18</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>[10]</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(% w/w)</td>
<td>[&gt;98]</td>
<td>[&gt;98]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 1H 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 (1S,2R)-117a and (1S,2S)-117b and β-nitroacetates (1R,2S)-119a and (1R,2R)-119b were separated by column chromatography. The individual diastereomers of 2-methyl-2-nitrocyclohexanol (1S,2R)-117a and (1S,2S)-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 (1S,2R)-117a and (1S,2S)-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 (1S,2R)-117a and (1S,2S)-117b attributable to the multiple work-ups required during this investigation.

The (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-119b was successfully separated from the (1R,2S)-cis-diastereomer (1R,2S)-119a, and chiral HPLC and optical rotation analysis was conducted on the enantiopure sample (1R,2R)-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 (1R,2S)-119a was not obtained free from the *trans*-diastereomer (1R,2R)-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 (1R,2S)-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](image_url)

**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 (1S,2S)-117b and *cis*- and *trans*-acetates (1R,2S)-119a and (1R,2R)-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 (1R,2S)-119a and (1R,2R)-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 (1R,2R)-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.
4.4 References

45. Milner, S. *Ph.D. Thesis, National University of Ireland, Cork* **2011**.
46. Aldrich Chemical Catalogue **2011**.
80. X-ray structure determination was conducted by Dr. S. E. Lawrence and Dr. K. S. Eccles, Deparment of Chemistry, UCC. 2012.
Chapter 5

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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. Hexane was distilled prior to use. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. 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 cerevisae*) 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 ºC) 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 ($\delta$$_H$ & $\delta$$_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 Limited. 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. \([\alpha]_D^2\) 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 STOE STADI MP diffractometer with Cu K\(\alpha_1\) radiation \((\lambda = 1.5406 \text{ Å})\), 40 kV, 40mA using 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 K\(\alpha\) (\(\lambda = 0.7107 \text{ Å}\)) 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 and diagrams prepared with Mercury 3.0. 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

Hydrogen peroxide (30%, 150 mL) was added over 5 min to a stirring solution of thioanisole (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 (21.64 g, 86%) as a white crystalline solid; m.p. 84-87 °C (lit., 485-88 °C); \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr) 1285, 1147 (SO\(_2\)); \( \delta_H \) (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-Benzencesulfonylnonan-2-one

Methyl phenyl sulfone (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 (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 \( \beta \)-ketosulfone (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 \( \beta \)-ketosulfone (5.83 g, 68%) as a white crystalline solid; m.p. 54-56 °C (lit., 8 55-56 °C); \( \nu_{\text{max}}/\text{cm}^{-1} \) (KBr) 1717 (CO), 1301, 1153 (SO\(_2\)); \( \delta_H \) (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\(_2\)CO], 4.14 [2H, s, C(1)H\(_2\)SO\(_2\)], 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**

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. 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**

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., 39-41 °C); \(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr) 2129 (C=O), 1662 (CO), 1339, 1153 (SO\(_2\)); \(\delta_{\text{H}}\) (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\(_2\)CO], 7.56-7.62 (2H, m, ArH), 7.65-7.72 (1H, m, ArH), 7.97-8.01 (2H, m, ArH).

(±)-trans-2-Benzenesulfonyl-3-n-butylcyclopentanone (±)-2

A solution of 1-benzenesulfonyl-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., 34-35 °C); \(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr) 1751 (CO), 1305, 1152 (SO\(_2\)); \(\delta_{\text{H}}\) (300 MHz) 0.89 [3H, t, J 6.9, CH\(_3\)], 1.18-1.43 [5H, m, (CH\(_2\))\(_2\)CH\(_3\) and one of CH\(_2\)(CH\(_2\))\(_2\)CH\(_3\)], 1.46-1.60 [1H, m, one of
C(4)H₂, 1.63-1.80 [1H, m, one of CH₃(CH₂)₂CH₃], 2.21-2.50 [3H, m, C(5)H₂, one of C(4)H₂], 2.79-2.99 [1H, m, C(3)H⁵Bu], 3.39 [1H, d, J 6.9, C(2)HSO₂Ph], 7.55-7.60 (2H, m, ArH), 7.65-7.72 (1H, m, ArH), 7.85-7.88 (2H, m, ArH). ¹H 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 ¹H NMR of the crude product spectrum at δₚ(300 MHz) 3.73 [1H, d, J 7.8 C(2)HSO₂Ph].
2. Significant difference in ¹H NMR chemical shift and multiplicity of the CH₃ signal is observed between Milner⁵ and data described above. Milner reports; δₚH (400 MHz) 1.29 (3H, d, J 7, CH₃). Milner also does not report the chemical shifts of the 3 x CH₂ n-butyl protons. ¹H NMR assignment in this study is consistent with O’Keeffe⁶ and Kelleher.⁷

(±)-(1R*,2S*,3R*)-2-Benzencesulfonyl-3-n-butylcyclopentanol 4a⁴,⁵,⁷

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 NaBH₄ (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 x 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); νmax/cm⁻¹ (KBr) 3499 (OH), 1302, 1141 (SO₂); δₚH (300 MHz) 0.85 (3H, t, J 6.6, CH₃), 1.08-1.49 [6H, m, one of C(4)H₂, one of CH₃(CH₂)₂CH₃ and (CH₂)₂CH₃], 1.51-1.89 [3H, m, one of C(5)H₂, one of C(4)H₂, and one of CH₃(CH₂)₂CH₃]; 2.16-2.29 [1H, m, one of C(5)H₂], 2.61-2.77 [1H, m, C(3)H⁵Bu], 3.03-3.07 [1H, dd, J 9.0, 4.5, C(2)HSO₂Ph], 3.51 (1H, d, J 2.7, OH), 4.28-4.33 (1H, sym m, CHOH), 7.54-7.72 (3H, m, ArH), 7.89-8.01 (2H, m, ArH).

Note:
1. Signals due to the minor diastereomer (±)-(1R*,2R*,3S*)-4b (~3%) could be detected in the ¹H NMR spectrum at δₚH (300 MHz) 4.63-4.66 (CHOH).
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 CHOH 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 alginate⁵,¹⁰-¹²

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.
Chapter 5

IMBY mediated reduction with kinetic resolution of racemic (+)-\textit{trans}-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanone (+)-2\textsuperscript{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 (+)-\textit{trans}-2-benzenesulfonyl-3-\textit{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 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 sonicated beads were washed with ethyl acetate (3 × 100 mL). The beads were then compressed with a pestle and mortar and stirred quickly with an overhead stirrer in ethyl acetate overnight. The solution was once again filtered and the compressed beads were 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 (+)-(1S,2R,3S)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanol (1S,2R,3S)-4a (0.69 g, 33%) as a white solid \([\alpha]_D^{20} +33.1 \text{ (c 0.5, CHCl}_3\text{)}\), 98% ee, lit\textsuperscript{4} [\(\alpha]_D^{20} +22.4 \text{ (c 11.5, CH}_2\text{Cl}_2\)] \(>95\% \text{ ee}; >98 : 2 \text{ dr and the crude ketone (2S,3R)-2 which required further purification by column chromatography on silica gel using hexane/ethyl acetate 97/3 as eluent to give the pure (+)-(2S,3R)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanone (2S,3R)-2 (0.53 g, 25%) as a light yellow oil [\(\alpha]_D^{20} +79.4 \text{ (c 0.5, CHCl}_3\)] \(99\% \text{ ee, lit\textsuperscript{4} [\(\alpha]_D^{20} +21.3 \text{ (c 8.6, CH}_2\text{Cl}_2\)} \(33\% \text{ ee.} \textsuperscript{1}H NMR spectra were identical to those for the racemic materials previously prepared.

Note:

1. The \textsuperscript{1}H NMR of pure (+)-(1S,2R,3S)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanol (1S,2R,3S)-4a shows no evidence of the minor cyclopentanol (1S,2S,3R)-4b at \(\delta\text{H}(300 \text{ MHz}) 4.63-4.66 \text{ (CHOH).}

2. As it was necessary to repeat flash chromatography in order to obtain a pure sample of (+)-(2S,3R)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanone (2S,3R)-2 this contributed to a loss in yield. A yield of 35% was obtained for pure (+)-(2S,3R)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanone (2S,3R)-2, 98% ee and 32% for pure (+)-(1S,2R,3S)-2-benzenesulfonyl-3-\textit{n}-butylcyclopentanol (1S,2R,3S)-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-benzensulfonyl-3-n-butylicyclopentanone (±)-2 – demonstrating the reproducibility of the biotransformation

![Chemical structures of (±)-2, (2S,3R)-2, (1S,2R,3S)-4a, and (1S,2S,3R)-4b]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions</th>
<th>Scale (g)d</th>
<th>Reaction Time</th>
<th>Cyclopentanone (2S,3R)-2</th>
<th>Cyclopentanol (1S,2R,3S)-4a</th>
<th>Cyclopentanol (1S,2S,3R)-4b</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ee (%)e</td>
<td>Isolated yield (%)</td>
<td>Isolated yield (%)</td>
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<tr>
<td>1</td>
<td>IMBY-H₂O⁴a</td>
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<td>23 h</td>
<td>99</td>
<td>25f</td>
<td>98</td>
</tr>
<tr>
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<td>IMBY-H₂O³b</td>
<td>3.00</td>
<td>47 h 15 min</td>
<td>98</td>
<td>35f</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>IMBY-H₂O³c</td>
<td>2.00</td>
<td>24 h</td>
<td>&gt;98</td>
<td>12g</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

a. IMBY-H₂O with repeated addition of sucrose over time at t = 0 h, 2 h, 4 h and 6 h.
b. IMBY-H₂O 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-H₂O 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 ¹H NMR of pure isolated (+)-(1S,2S,3S)-2-benzensulfonyl-3-n-butylicyclopentanol (1S,2R,3S)-4a showed no evidence of the minor cyclopentanol (1S,2S,3R)-4b at δH (300 MHz) 4.63-4.66 (CH₂OH).

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-benzensulfonyl-3-n-butylicyclopentanone (±)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions(\text{a})</th>
<th>Scale (g)(\text{b})</th>
<th>Reaction Time</th>
<th>Cyclopentane ((2S,3R)-2)</th>
<th>Cyclopentanol ((1S,2R,3S)-4a)</th>
<th>Cyclopentanol ((1S,2S,3R)-4b)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>2 h</td>
<td>ee (%)</td>
<td>Isolated yield (%)</td>
<td>ee (%)</td>
</tr>
<tr>
<td>2</td>
<td>IMBY-H(_2)O</td>
<td>2.00</td>
<td>4 h</td>
<td>98</td>
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<tr>
<td>3</td>
<td></td>
<td></td>
<td>9 h</td>
<td>&gt;98</td>
<td>-</td>
<td>&gt;98</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>24 h</td>
<td>&gt;98</td>
<td>12(\text{c})</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

\(\text{a}\) IMBY-H\(_2\)O with repeated addition of sucrose over time at \(t = 0\) h, 2 h, 4 h and 6 h.

\(\text{b}\) Scale refers to the quantity of cyclopentanone \((±)-2\) employed for the reaction.

\(\text{c}\) Enantiomeric excess [ee (%)] was determined by chiral HPLC, see Appendix I for conditions.

\(\text{d}\) A yield of 35% was obtained for pure \((+)-(2S,3R)-2\)-benzenesulfonyl-3-n-butylcyclopentanone \((2S,3R)-2\), 98% ee and 32% for pure \((+)-(1S,2R,3S)-2\)-benzenesulfonyl-3-n-butylcyclopentanol \((1S,2R,3S)-4a\), 98% ee for a batch that was synthesised later (entry 2, Table 5.1).

\(\text{e}\) The \(1^\text{H}\) NMR of pure isolated \((+)-(1S,2R,3S)-2\)-benzenesulfonyl-3-n-butylcyclopentanol \((1S,2R,3S)-4a\) showed no evidence of the minor cyclopentanol \((1S,2S,3R)-4b\) at \(t_0\) (300 MHz) 4.65-4.66 (\(\text{CH}_2\text{OH}\)).

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. \((+)-(1S,2R,3S)-2\)-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.
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 \(\times\) 10 mL). The solids were allowed to dry under vacuum 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); \(\delta_H\) (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%.

\((-\rangle-(2R,3S)-2\)-Benzenesulfonyl-3-\(n\)-butylocyclopentanone (2R,3S)-\(4^{4,5,7}\)

\((-\rangle-(1S,2R,3S)-2\)-benzenesulfonyl-3-\(n\)-butylcyclopentanol (1S,2R,3S)-\(4\) (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 \(\times\) 150 mL) and water (100 mL), dried, filtered and concentrated under reduced pressure to give the pure \((-\rangle-(2R,3S)-cyclopentanone (2R,3S)-\(2\) (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\(_2\)Cl\(_2\)), 75% ee. \(^1\)H NMR spectra were identical to those for the racemic materials previously prepared.
Chapter 5

Experimental

Figure 5.3: HPLC Trace I: Racemic (±)-trans-2-benzensulfonyl-3-n-butylcyclopentanone (±)-2. Trace II: (+)-(2R,3S)-2-benzensulfonyl-3-n-butylcyclopentanone (2R,3S)-2, 96% ee (from DMP oxidation). Trace III: (+)-(2S,3R)-2-benzensulfonyl-3-n-butylcyclopentanone (2S,3R)-2, 99% ee (from baker’s yeast). For HPLC conditions see appendix I.

(±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-114,5,7

A solution of (±)-trans-2-benzensulfonyl-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; νmax/cm⁻¹ (film) 3066 (OH), 1710 (CO), 1305, 1146 (SO₂); δH (300 MHz) 0.85 (3H, t, J 6.9, CH₃), 1.06-1.48 [6H, m, CH₅(CH₂)₂CH₃], 1.66-1.92 [1H, m, C(3)H₂], 1.98-2.12 [1H, sym m, J 6.2 C(4)H₄Bu], 2.34 [2H, t, J 7.8, C(2)H₂], 3.00 [1H, dd, A of ABX, JₐB 14.4, JₓA 6.3, one of C(5)H₂], 3.08 [1H, dd, B of ABX, JAB 14.4, JBₓ 5.7, one of C(5)H₂], 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 overlapped with residual ethyl acetate.

(−)-(S)-4-(Benzenesulfonylmethyl)octanoic acid (S)-114,5,7

This was prepared following the procedure described for racemic (±)-11 from (−)-(2R,3S)-2-benzensulfonyl-3-n-butylcyclopentanone (2R,3S)-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 [α]D⁺²⁰ −11.0 (c 1.2, CH₂Cl₂), 94% ee, lit [α]D⁺²⁰ −5.6 (c 2.7, CH₂Cl₂), 91% ee. ¹H NMR spectra were identical to those for the racemic materials previously prepared.

Note: Ethyl acetate (~3%) was evident in the ¹H 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

This was prepared following the procedure described for racemic (±)-11 from (+)-(2S,3R)-2-benzenesulfonyl-3-n-butylcyclopentanone (2S,3R)-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 [α]D20 +9.1 (c 1.2, CH2Cl2), 99% ee, lit4 [α]D20 +6.3 (c 1.2, CH2Cl2), 75% ee. 1H NMR spectra were identical to those for the racemic materials previously prepared.

Note: Ethyl acetate (~5%) was evident in the 1H NMR spectrum.

Figure 5.5: HPLC Trace I: (±)-4-(Benzenesulfonylmethyl)octanoic acid (±)-11. Trace II: (+)-(R)-4-(Benzenesulfonylmethyl)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 (±)-15,18,19

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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3043 (OH), 1714 (CO); \( \delta_{\text{H}} \) (300 MHz) 0.78-0.98 (6H, m, 2 x CH\(_3\)), 1.04-1.36 [6H, m, CH\(_2\)(CH\(_2\))\(_2\)CH\(_3\)], 1.37-1.55 [2H, m, one of C(3)H\(_2\) and C(4)H\(_2\)], 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)-15,18,19

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 [\( \alpha \rmb{D}^{20} +1.6 \) (c 1.4, CHCl\(_3\)), lit\(^{20} \) [\( \alpha \rmb{D}^{20} +1.5 \) (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 (2R,3S)-cyclopentanone (2R,3S)-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)-15,18,19

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 desulfonlated acid (R)-1 (0.35 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure desulfonlated 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 (-)-(2S,3R)-cyclopentanone (2S,3R)-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) methyl octanoic 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$_{26}$O$_2$ requires C, 82.10; H, 8.39%); $\nu_{\text{max}}$/cm$^{-1}$ (film) 2956, 2827 (CH), 1737 (CO), 1450 (CH$_2$), 1167 (C-O); $\delta_H$ (300 MHz) 0.78-0.97 (6H, m, 2 x CH$_3$), 1.03-1.53 [8H, m, CH$_2$(CH)$_2$CH$_3$, C(4)H and one of C(3)H$_3$], 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$_2$CH), 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); $\delta_C$ (75.5 MHz) 14.1, 19.3 (2 x CH$_3$), 23.0, 29.1 [2 x CH$_2$, two of CH$_2$(CH)$_2$CH$_3$], 31.9 [CH$_2$, C(3)H$_3$], 32.1 [CH$_2$, C(2)H$_2$], 32.3 [CH, C(4)H], 36.3 [CH$_2$, one of CH$_2$(CH)$_2$CH$_3$], 46.9 (CH, OCH$_2$CH), 66.2 (CH$_2$, OCH$_2$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 [SO$_2$]+, 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, $[\alpha]^20_D +1.6$ (c 1.4, CHCl$_3$)], dicyclohexylcarbodiimide (DCC) (186.0 mg, 0.90 mmol), N,N-dimethylanilinopyridine (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 $[\alpha]^20_D +1.5$ (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, $[\alpha]^20_D -2.2$ (c 1.4, CHCl$_3$)], dicyclohexylcarbodiimide (DCC) (194.0 mg, 0.94 mmol), N,N-dimethylanilinopyridine (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 $[\alpha]^20_D -3.4$ (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

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 °C. 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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2965 (OH), 1708 (CO), 1603, 1495, 1454; \( \delta_H \) (400 MHz) 0.79 [3H, t, \( J_{7.4} \), C(5)H\text{3}], 1.46-1.83 [2H, m, C(4)H\text{2}], 2.60 [1H, dd, A of ABX, \( J_{AB} \) 15.6, \( J_{AX} \) 7.9, one of C(2)H\text{2}], 2.68 [1H, dd, B of ABX, \( J_{AB} \) 15.6, \( J_{BX} \) 7.1, one of C(2)H\text{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 °C (Lit., 24 60-61 °C) and with spectral characteristics identical to those described above.

(±)-4-Methyl-3-phenylpentanoic acid (±)-29

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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2963 (OH), 1709 (CO), 1602, 1495, 1454; \( \delta_H \) (400 MHz) 0.74, 0.92 [2 × 3H, 2 × d, J 6.7, J 6.7, C(4)HCH\text{3} and C(5)H\text{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\text{2}], 2.72-2.92 [2H, m, BX of ABX, one of C(2)H\text{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 °C) and with spectral characteristics identical to those described above.

(±)-4,4-Dimethyl-3-phenylpentanoic acid (±)-37\textsuperscript{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. \(\nu_{\text{max}}/\text{cm}^{-1}\) (KBr) 2955 (OH), 1726 (CO), 1638, 1453; \(\delta_{\text{H}}\) (400 MHz) 0.87 [9H, s, C(CH\textsubscript{3})\textsubscript{3}], 2.73 [1H, dd, A of ABX, \(J_{AB}\) 15.8, \(J_{AX}\) 10.8, one of C(2)H\textsubscript{2}], 2.81 [1H, dd, B of ABX, \(J_{AB}\) 15.8, \(J_{BX}\) 4.5, one of C(2)H\textsubscript{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\textsuperscript{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; \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 2926 (OH), 1704 (CO), 1515, 1455, 1416; \(\delta_{\text{H}}\) (300 MHz) 1.30 [3H, d, J 7.0, C(4)H\textsubscript{3}], 2.31 [3H, s, C(4')CH\textsubscript{3}], 2.55 [1H, dd, A of ABX, \(J_{AB}\) 15.5, \(J_{AX}\) 8.2, one of C(2)H\textsubscript{2}], 2.65 [1H, dd, B of ABX, \(J_{AB}\) 15.5, \(J_{BX}\) 6.9, one of C(2)H\textsubscript{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

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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2971 (OH), 1718 (CO), 1608, 1490, 1455; \( \delta_{\text{H}} \) (300 MHz) 1.30 [3H, d, \( J = 7.0 \), C(4)H], 2.33 [3H, s, C(3')CH₃], 2.55 [1H, dd, A of ABX, \( J_{AB} = 15.5 \), \( J_{AX} = 8.4 \), one of C(2)H₂], 2.66 [1H, dd, B of ABX, \( J_{AB} = 15.5 \), \( J_{BX} = 6.7 \), one of C(2)H₂], 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

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 a yellow solid, m.p. 46-48 ºC (Lit.,26 46-47 °C) and with spectral characteristics identical to those described above.

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 (±)-6627,28

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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2963 (OH), 1711 (CO), 1611, 1511, 1458; \( \delta_H \) (400 MHz) 1.29 [3H, d, J 7.0, C(4)H3], 2.54 [1H, dd, A of ABX, \( J_{AB} \) 15.4, \( J_{AX} \) 8.1, one of C(2)H3], 2.62 [1H, dd, B of ABX, \( J_{AB} \) 15.4, \( J_{BX} \) 7.0, one of C(2)H2], 3.17-3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH3), 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 (±)-7321

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); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 1799 (CO), 1604, 1495, 1454; \( \delta_H \) (400 MHz) 0.80 [3H, t, J 7.4, C(5)H3], 1.58-1.80 [2H, m, C(4)H2], 3.04-3.23 [3H, m, C(2)H2 and C(3)H], 7.16-7.34 (5H, m, ArH).

(±)-4-Methyl-3-phenylpentanoyl chloride (±)-7421

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); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 1799 (CO), 1602, 1495, 1454; \( \delta_H \) (400 MHz) 0.76, 0.97 [2 x CH3, 2 x d, J 6.7, J 6.7, C(4)HC\textsubscript{H}3 and C(5)H\textsubscript{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)H2], 3.34 [1H, dd, B of ABX, \( J_{AB} \) 16.4, \( J_{AX} \) 5.2, one of C(2)H2], 7.09-7.35 (5H, m, ArH).
(±)-4,4-Dimethyl-3-phenylpentanoyl chloride (±)-75

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); νmax/cm⁻¹ (KBr) 1793 (CO), 1601, 1494, 1453; δH (400 MHz) 0.91 [9H, s, C(CH₃)₃], 3.04 [1H, dd, X of ABX, J 8.9, 6.1, C(3)H], 3.26-3.39 [2H, m, C(2)H₂], 7.14-7.31 (5H, m, ArH).

(±)-3-(4-Methylphenyl)butanoyl chloride (±)-78

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); νmax/cm⁻¹ (film) 1800 (CO), 1649, 1516, 1455; δH (300 MHz) 1.32 [3H, d, J 7.0, C(4)H₃], 2.32 [3H, s, C(4)CH₃], 3.07 [1H, dd, A of ABX, JAB 16.4, JAX 7.9, one of C(2)H₂], 3.17 [1H, dd, B of ABX, JAB 16.4, JBX 6.4, one of C(2)H₂], 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

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); νmax/cm⁻¹ (film) 1800 (CO), 1608, 1491, 1456; δH (300 MHz) 1.32 [3H, d, J 6.9, C(4)H₃], 2.33 [3H, s, C(3)CH₃], 3.06 [1H, dd, A of ABX, JAB 16.5, JAX 8.0, one of C(2)H₂], 3.17 [1H, dd, B of ABX, JAB 16.5, JBX 6.5, one of C(2)H₂], 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

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); νmax/cm⁻¹ (film) 1801 (CO), 1605, 1492, 1459; δH (400 MHz) 1.27 [3H, d, J 7.0, C(4)H₃], 2.35 [3H, s, C(2)CH₃], 3.03 [1H, dd, A of ABX, JAB 16.6, JAX 8.2, one of C(2)H₂], 3.16 [1H, dd, B of ABX, JAB 16.6, JBX 6.3, one of C(2)H₂], 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\textsuperscript{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 an orange brown oil, b.p. 102-110 °C at 0.35 mmHg (Lit.,\textsuperscript{27} 100 °C at 0.5 mmHg); \nu_{\text{max}}/\text{cm}^{-1} (film) 1790 (CO), 1614, 1515, 1463; \delta_{H} (400 MHz) 1.32 [3H, d, J 7.0, C(4)H], 3.07 [1H, dd, A of ABX, J_{AB} 16.4, J_{AX} 7.8, one of C(2)H], 3.15 [1H, dd, B of ABX, J_{AB} 16.4, J_{BX} 6.8, one of C(2)H], 3.27-3.36 [1H, m, X of ABX, C(3)H], 3.78 (3H, s, OCH\textsubscript{3}), 4.02 [2H, m, C(3)\textsuperscript{'H} and C(5)\textsuperscript{'H}, ArH], 7.11-7.15 [2H, m, C(2)\textsuperscript{'H} and C(6)\textsuperscript{'H}, ArH].

5.3.1.3 Synthesis of ethyl esters

(±)-Ethyl 3-phenylbutanoate (±)-38\textsuperscript{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; \nu_{\text{max}}/\text{cm}^{-1} (film) 2969 (CH), 1733 (CO), 1604, 1495, 1454 (Ar), 1174 (C-O); \delta_{H} (400 MHz) 1.17 (3H, t, J 7.1, OCH\textsubscript{2}CH\textsubscript{3}), 1.30 [3H, d, J 7.0, C(4)H\textsubscript{3}], 2.53 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H\textsubscript{2}], 2.61 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.0, one of C(2)H\textsubscript{2}], 3.23-3.32 [1H, sym. m, X of ABX, C(3)H], 4.08 (2H, q, J 7.1, OCH\textsubscript{2}CH\textsubscript{3}), 7.17-7.34 (5H, m, ArH).

(±)-Ethyl 3-phenylpentanoate (±)-56\textsuperscript{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; \nu_{\text{max}}/\text{cm}^{-1} (film) 2967 (CH), 1735 (CO), 1603, 1493, 1454 (Ar), 1167 (C-O); \delta_{H} (400 MHz) 0.79 [3H, t, J 7.4, C(5)H\textsubscript{3}], 1.13 (3H, t, J 7.1, OCH\textsubscript{2}CH\textsubscript{3}), 1.55-1.76 [2H, m, C(4)H\textsubscript{2}], 2.55 [1H, dd, A of ABX, J_{AB} 15.0, J_{AX} 8.2, one of C(2)H\textsubscript{2}], 2.63 [1H, dd, B of ABX, J_{AB} 15.0, J_{BX} 7.1, one of C(2)H\textsubscript{2}], 2.96-3.04 [1H, m, X of ABX, C(3)H], 4.02 (2H, q, J 7.1, OCH\textsubscript{2}CH\textsubscript{3}), 7.17-7.30 (5H, m, ArH).
(±)-Ethyl 4-methyl-3-phenylpentanoate (±)-58\textsuperscript{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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2961 (CH), 1736 (CO), 1602, 1494, 1453 (Ar), 1162 (C-O); \( \delta_H \) (400 MHz) 0.75, 0.95 [2 \times 3H, 2 \times 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\( 2\)CH\( 3 \)) 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, OCH\( 2\)CH\( 3 \)), 7.14-7.28 (5H, m, ArH).

(±)-Ethyl 4,4-dimethyl-3-phenylpentanoate (±)-59\textsuperscript{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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2965 (CH), 1737 (CO), 1602, 1473, 1454 (Ar), 1152 (C-O); \( \delta_H \) (400 MHz) 0.89 [9H, s, C(CH\( 3 \))\( 3 \)], 0.99 (3H, t, J 7.1 OCH\( 2\)CH\( 3 \)) 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, OCH\( 2\)CH\( 3 \)), 7.13-7.26 (5H, m, ArH).

(±)-Ethyl 3-(4-methylphenyl)butanoate (±)-49\textsuperscript{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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2967 (CH), 1732 (CO), 1516, 1456 (Ar), 1166 (C-O); \( \delta_H \) (400 MHz) 1.19 (3H, t, J 7.1, OCH\( 2\)CH\( 3 \)), 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, OCH\( 2\)CH\( 3 \)), 7.11 (4H, s, ArH).

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Chapter 5

Experimental
(±)-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, OCH₂CH₃), 1.28 [3H, d, J 7.0, C(4)H₃], 2.32 [3H, s, C(3')CH₃], 2.51 [1H, dd, B of ABX, J_{AB} 15.0, J_{AX} 8.3, one of C(2)H₂], 2.59 [1H, dd, A of ABX, J_{AB} 15.0, J_{BX} 6.9, one of C(2)H₂], 3.19-3.28 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, J 7.1, OCH₂CH₃), 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 (CH₃), 21.5 [CH₃, C(3')CH₃] 21.8 [CH₃, C(4)H₃], 36.5 [CH, C(3)H], 43.0 [CH₂, C(2)H₂], 60.3 [CH₂, OCH₂CH₃], 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}O₂ [M+H]⁺ 207.1385. Found 207.1390; m/z (ES+) 207.1 [(M+H)⁺, 57%], 202.1 (100%), 161.1 [(M-C₂H₅O)⁺, 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. 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, OCH₂CH₃), 1.25 [3H, d, J 6.9, C(4)H₃], 2.37 [3H, s, C(2')CH₃], 2.52 [1H, dd, A of ABX, J_{AB} 15.2, J_{AX} 8.5, one of C(2)H₂], 2.61 [1H, dd, B of ABX, J_{AB} 15.2, J_{BX} 6.6, one of C(2)H₂], 3.46-3.61 [1H, m, X of ABX, C(3)H], 4.07 (2H, q, J 7.1, OCH₂CH₃), 7.07-7.19 (4H, m, ArH); δ_{C} (75.5 MHz) 14.2 (CH₃, OCH₂CH₃), 19.5 [CH₃, C(2')CH₃], 21.3 [CH₂, C(4)H₃], 31.5 [CH, C(3)H], 42.2 [CH₂, C(2)H₂], 60.3 [CH₂, OCH₂CH₃], 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}O₂ [M+H]⁺ 207.1385. Found 207.1393; m/z (ES+) 207.1 [(M+H)⁺, 71%], 202.1 (100%), 161.1 [(M-C₂H₅O)⁺, 48%], 151.0 (68%), 141.0 (24%). NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.
Experimental

(±)-Ethyl 3-(4-methoxyphenyl)butanoate (±)-60

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; v_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, OCH₂C₃H₃), 1.27 [3H, d, C(4)H₃], 2.50 [1H, dd, A of ABX, J_AB 15.2, J_AX 8.0, one of C(2)H₂], 2.57 [1H, dd, B of ABX, J_AB 14.8, J_BX 7.2, one of C(2)H₂], 3.17-3.29 [1H, m, X of ABX, C(3)H], 3.77 (3H, s, OCH₃), 4.07 (2H, q, J 7.1, OC₃H₂CH₃), 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

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 x 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 x 100 mL), and the combined organic phase was washed with brine (3 x 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; v_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, OCH₂C₃H₃), 2.55 [3H, d, J 1.2, C(4)H₃], 4.21 (2H, q, J 7.2, OCH₂C₃H₃), 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, OCH₂C₃H₃), 2.16 [3H, d, J 1.5, C(4)H₃], 4.01 (2H, q, J 7.2, OCH₂C₃H₃), 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

(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. C₁₂H₁₅FO₂ requires C, 68.55; H, 7.19%); νmax/cm⁻¹ (film) 2970 (CH), 1733 (CO), 1605, 1512, 1462 (Ar), 1160 (C=O); δH (300 MHz) 1.17 (3H, t, J 7.2, OCH₂C₃H₃), 1.28 [3H, d, J 7.2, C(4)H₃], 2.51 [1H, overlapping dd, A of ABX, JAB 15.0, JAX 7.8, one of C(2)H₂], 3.27 [1H, apparent sextet, X of ABX, J 7.2 C(3)H], 4.06 (2H, q, J 7.1 OCH₂CH₃), 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]; δC (75.5 MHz) 14.1 (CH₃, OCH₂C₃H₃) 21.9 [CH₃, C(4)H₃], 35.8 [CH, C(3)H], 43.0 [CH₂, C(2)H₂], 60.2 [CH₂, OCH₂CH₃], 115.2 [CH, d, JCF 21.2, C(3')H and C(5')H, ArCH], 128.2 [CH, d, JCF 7.8, C(2')H and C(6')H, ArCH], 141.3 [C, d, JCF 3.2, C(1'), ArC], 161.4 [C, d, JCF 243.9, C(4)F], 172.1 [C, C(1)]; HRMS (ES+): Exact mass calculated for C₁₂H₁₅FO₂ [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

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., 53-57 °C); νmax/cm⁻¹ (KBr) 2979 (OH), 1705 (CO), 1607, 1511, 1432 (Ar); δH (300 MHz) 1.30 [3H, d, J 7.2, C(4)H₃], 2.57 [1H, overlapping dd, A of ABX, JAB 15.6, JAX 7.8, one of C(2)H₂], 2.64 [1H, overlapping dd, B of ABX, JAB 15.6, JAX 7.8, one of C(2)H₂], 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

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 1H 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 (eeₐ) and product acid (eeₚ) employing the E-value calculator. 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 1H NMR spectroscopy of the crude products to be <10%, chiral HPLC analysis was not conducted.
Table 5.3: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutan-2-oate (±)-38

![Diagram of hydrolase-mediated hydrolysis]  

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

\(^a\) Reaction went to 100% completion, no enantioselectivity observed.
Table 5.4: Hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylpentanoate (±)-56

\[
\text{Hydrolysis of (±)-56} \\
\text{Hydrolase} \\
0.1 \text{ M Phosphate buffer, pH 7.0} \\
\text{(±)-56} \rightarrow \text{(R)-28} + \text{(S)-56}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Time (h)</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cyclindracea C2</td>
<td>120</td>
<td>Ambient</td>
<td>1</td>
<td>6</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>Candida antarctica B</td>
<td>65</td>
<td>Ambient</td>
<td>80 (S)</td>
<td>78</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>Mucor mehei</td>
<td>67</td>
<td>Ambient</td>
<td>0$^b$</td>
<td>85</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica A</td>
<td>67</td>
<td>Ambient</td>
<td>5 (R)</td>
<td>10</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>Pig liver esterase</td>
<td>65</td>
<td>Ambient</td>
<td>87 (S)</td>
<td>85</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>Candida antarctica B (immob)</td>
<td>65</td>
<td>Ambient</td>
<td>85 (S)</td>
<td>51</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>Candida antarctica B (immob)</td>
<td>72</td>
<td>4</td>
<td>62 (S)</td>
<td>42</td>
<td>24</td>
</tr>
</tbody>
</table>

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

\[
\text{Candida antarctica Lipase B (immob)} \\
0.1 \text{ M Phosphate buffer, pH 7.0} \\
17\% \text{ v/v Co-solvent} \\
\text{(±)-56} \rightarrow \text{(R)-28} + \text{(S)-56}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Co-solvent</th>
<th>Time (h)</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>$E$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>64</td>
<td>Ambient</td>
<td>93</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Acetonitrile$^a$</td>
<td>64</td>
<td>Ambient</td>
<td>28</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Acetone$^a$</td>
<td>64</td>
<td>Ambient</td>
<td>25</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>THF</td>
<td>64</td>
<td>Ambient</td>
<td>6</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Dioxane</td>
<td>64</td>
<td>Ambient</td>
<td>72$^b$</td>
<td>44</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>TBME</td>
<td>64</td>
<td>Ambient</td>
<td>24</td>
<td>30</td>
<td>4.6</td>
</tr>
</tbody>
</table>

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.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source(^a)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ester 58</td>
<td>Acid 29</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida antarctica</em> B</td>
<td>12 (R)</td>
<td>&gt;98 (S)</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td><em>Mucor meihei</em></td>
<td>61 (R)</td>
<td>23 (S)</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida antarctica</em> A</td>
<td>10 (S)</td>
<td>64 (R)</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>33 (R)</td>
<td>97 (S)</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Pig liver esterase</td>
<td>-b</td>
<td>-b</td>
<td>-b</td>
</tr>
</tbody>
</table>


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.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source(^a)</th>
<th>Temperature ((\text{°C}))</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 59</td>
<td>Acid 37</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida antarctica</em> B</td>
<td>Ambient(^b)</td>
<td>2 (R)</td>
<td>&gt;98 (S)</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td><em>Candida antarctica</em> A</td>
<td>35 – 40 °C(^c)</td>
<td>23 (R)</td>
<td>&gt;98 (S)</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>3 (S)</td>
<td>73 (R)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida antarctica</em> B</td>
<td>35 – 40 °C(^d)</td>
<td>1 (R)</td>
<td>&gt;98 (S)</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30 (R)</td>
<td>98 (S)</td>
<td>23</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>Pig liver esterase</td>
<td>Ambient(^b)</td>
<td>32 (S)</td>
<td>34 (R)</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>Pig liver esterase</td>
<td>35 – 40 °C(^e)</td>
<td>0°</td>
<td>100°</td>
<td>0°</td>
</tr>
</tbody>
</table>

\(^a\) The following hydrolases gave no conversion: *Pseudomonas cepacia* P1, *Rhizopus niveus*, *Pseudomonas fluorescens*, *Candida cylindracea* C1, *Pseudomonas cepacia* P2 and Porcine Pancrease Type II.

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

c. Time for ester hydrolysis was 66 h.

d. Time for ester hydrolysis was 64.5 h at 35 °C temperature increased to 40 °C for the final 24 h.

e. Time for ester hydrolysis was 72 h at 35 °C temperature increased to 40 °C for the final 24 h.
Table 5.8: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methylphenyl)butanoate (±)-49

![Diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>Acid 51</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pseudomonas cepacia P1</td>
<td>30</td>
<td>98 (R)</td>
<td>&gt;98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>96 (S)</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas fluorescens</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>95 (S)</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Candida cyclindracea</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>Candida antarctica A</td>
<td>30</td>
<td>5 (R)</td>
<td>68 (S)</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Candida antarctica B (inmob)</td>
<td>30</td>
<td>6 (S)</td>
<td>5 (R)</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 5.9: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(3-methylphenyl)butanoate (±)-54

![Diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 54</td>
<td>Acid 65</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pseudomonas cepacia P1</td>
<td>30</td>
<td>88 (R)</td>
<td>96 (S)</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>76 (S)</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas fluorescens</td>
<td>30</td>
<td>96 (R)</td>
<td>97 (S)</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Candida cyclindracea</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>Candida antarctica B (inmob)</td>
<td>30</td>
<td>&gt;98 (S)</td>
<td>7 (R)</td>
<td>93</td>
</tr>
</tbody>
</table>

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

![Hydrolase-mediated hydrolysis of (±)-ethyl 3-(2-methylphenyl)butanoate](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 53</td>
<td>Acid 64</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>&gt;98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>80 (S)</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>&gt;98 (S)</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30</td>
<td>90 (R)</td>
<td>46 (S)</td>
<td>66</td>
</tr>
</tbody>
</table>

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

![Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-methoxyphenyl)butanoate](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Temp (°C)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ester 60</td>
<td>Acid 66</td>
<td>E calc.</td>
</tr>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P1</td>
<td>30</td>
<td>98 (R)</td>
<td>86 (S)</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>88 (S)</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>30</td>
<td>&gt;98 (R)</td>
<td>97 (S)</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida antarctica</em> A</td>
<td>30</td>
<td>4 (R)</td>
<td>48 (S)</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida cyclindracea</em></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>30</td>
<td>66 (S)</td>
<td>7 (R)</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 5.12: Hydrolase-mediated hydrolysis of (±)-ethyl 3-(4-fluorophenyl)butanoate (±)-55

\[
\begin{align*}
\text{Hydrolase} & \quad 0.1 \text{ M Phosphate buffer, pH 7.0} \\
64 \text{ h} & \\
\end{align*}
\]

(±)-55 \hspace{1cm} (S)-80 \hspace{1cm} (R)-55

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<td>&gt;98 (S)</td>
<td>8 (R)</td>
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5.3.3 Hydrolase-mediated kinetic resolution – preparative-scale

Large scale hydrolase-mediated hydrolysis of (±)-ethyl 3-phenylbutanoate (±)-38

\[
\begin{align*}
\text{Pseudomonas fluorescens} \quad (108 \text{ mg}) & \text{ was added to ethyl 3-}
\end{align*}
\]

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 °C. 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 \([\alpha]_{D}^{25} +19.00\) (c 1.1, CHCl₃), >98% ee, \(lit^{46}\) \([\alpha]_{D}^{25} +27.55\) (c 1.1, CHCl₃), (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. \(^1H\) 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 °C, 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 \text{ (c 0.6, CHCl}_3\text{), 65% ee, lit}^{47} \text{[} [\alpha]_{D}^{26} \text{ = } -18.3 \text{ (c 1.1, CHCl}_3\text{), (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 \text{ (c 1.4, C}_6\text{H}_4\text{), 90% ee, lit}^{48} \text{[} [\alpha]_{D}^{25} \text{ = +42.3 \text{ (c 8.0, C}_6\text{H}_4\text{), (S)-isomer, 83% ee. Conversion estimated by } E \text{-value calculator at 42%.}^{45} \text{^1H 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 °C to produce the pure ester (R)-58 (107 mg, 25%) as a clear oil \([\alpha]_{D}^{20} +7.05 \text{ (c 1.0, CHCl}_3\text{), 26% ee, lit}^{30} \text{[} [\alpha]_{D}^{26} \text{ = -25.4 \text{ (c 1.0, CHCl}_3\text{), (S)-isomer, 98% ee, and the pure acid (S)-29 (88 mg, 24%) as a clear oil [} [\alpha]_{D}^{20} -24.35 \text{ (c 0.655, CHCl}_3\text{), 98% ee, lit}^{49} \text{[} [\alpha]_{D}^{23} \text{ = +28.12 \text{ (c 1.855, CHCl}_3\text{), (R)-isomer, 96% ee. Conversion estimated by } E \text{-value calculator at 21%.}^{45} \text{^1H 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 °C and at 40 °C for the final 24 h to produce the pure ester (R)-59 (79 mg, 39%) as a clear oil \([\alpha]_{D}^{20} +0.80 \text{ (c 1.0, CHCl}_3\text{), 12% ee and the pure acid (S)-37 (23 mg, 13%) as a yellow oil which solidified overnight [} [\alpha]_{D}^{20} -10.53 \text{ (c 0.1, CHCl}_3\text{), >98% ee, lit}^{50} \text{[} [\alpha]_{D}^{20} -20.4 \text{ (c 2.2, CHCl}_3\text{), 91% ee. Conversion estimated by } E \text{-value calculator at 11%.}^{45} \text{^1H NMR spectra were identical to those for the racemic materials previously prepared.}

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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 °C. 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%. 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 \([\alpha]^{20}_D -28.67 (c 3.5, \text{CHCl}_3), 97\%\) ee, lit\([\alpha]^{25}_D -26.2 (c 3.5, \text{CHCl}_3), 92\%\) ee and the pure acid (S)-51 (163 mg, 40%) as a yellow oil which solidified overnight \([\alpha]^{20}_D +31.80 (c 1.0, \text{CHCl}_3), >98\%\) ee, lit\([\alpha]^{25}_D +34.2 (c 1.0, \text{CHCl}_3), 99\%\) 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 °C to produce a yellow oil (232 mg). Conversion estimated by E-value calculator at 49%. 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 \([\alpha]^{20}_D -24.40 (c 1.0, \text{CHCl}_3), 94\%\) ee and acid (S)-65 (107 mg, 26%) as a clear oil \([\alpha]^{20}_D +32.32 (c 0.6, \text{CHCl}_3), >98\%\) 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 °C to produce a yellow oil (268 mg). Conversion estimated by E-value calculator at 50%. 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 \([\alpha]^{20}_D -11.00 (c 1.0, \text{CHCl}_3), 98\%\) ee and the pure acid (S)-65 (90 mg, 28%) as a yellow oil \([\alpha]^{20}_D +24.17 (c 1.4, \text{CHCl}_3), >98\%\) ee. \(^1\)H NMR spectra were identical to those for the racemic materials previously prepared.
**Figure 5.7:** 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 °C to produce an orange oil (428 mg). Conversion estimated by E-value calculator at 51%. 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 \([\alpha]_D^{20} = -30.03 \text{ (c 1.0, CHCl}_3\), >98% ee and the pure acid (S)-66 (99 mg, 23%) as a yellow oil \([\alpha]_D^{20} = +26.25 \text{ (c 1.0, EtOH), 97% ee, lit}^{45} [\alpha]_D^{25} = +27.50 \text{ (c 1.0, EtOH) 94% ee.}^{1} \text{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%.\(^\text{45}\) 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 \([\alpha]_D^{20} -24.34 (c 1.0, \text{CHCl}_3), >98\% \text{ ee},\) and the pure acid (S)-80 (67 mg, 35%) as a brown oil \([\alpha]_D^{20} +30.51 (c 1.0, \text{CHCl}_3), 97\% \text{ ee}.\) \(^1\)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.\(^\text{51,52}\)

(±)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96

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 ºC. Crystal data: C\(_{17}\)H\(_{20}\)N\(_2\)O\(_3\), \(M = 300.35,\) triclinic, \(a = 5.5847(9) \text{ Å}, b = 7.8959(12) \text{ Å}, c = 17.784(3) \text{ Å}, \alpha = 84.990(4)^\circ, \beta = 85.314(4)^\circ, \gamma = 83.194(4)^\circ, V = 773.7(2) \text{ Å}^3, T = 100.2) K,\) space group P-1, \(Z = 2, 16279 \) reflections measured, 2987 unique \((R_{\text{int}} = 0.0600).\) The final \(R_f\) 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_{2}O_{3}, M = 300.35, triclinic, \(a = 6.0360(2) \text{ Å}, b = 9.4615(4) \text{ Å}, c = 14.6013(5) \text{ Å}, \alpha = 83.438(2)°, \beta = 84.291(2)°, \gamma = 71.750(2)°, V = 784.95(5) \text{ Å}^3\), \(T\) = 100.(2) K, space group \(P1\), \(Z\) = 2, 17372 reflections measured, 4835 unique (\(R_{int} = 0.0361\)). The final \(R\) 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_{2}O_{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_{2}O_{3}, M = 300.35, triclinic, \(a = 9.6495(16) \text{ Å}, b = 12.915(2) \text{ Å}, c = 13.390(2) \text{ Å}, \alpha = 102.007(3)°, \beta = 103.616(4)°, \gamma = 92.047(4)°, V = 1580.0(4)\text{Å}^3\), \(T\) = 100.(2) K, space group \(P1\), \(Z\) = 4, 43120 reflections measured, 7818 unique (\(R_{int} = 0.0647\)). The final \(R\) 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_{2}O_{3}, M = 300.35, triclinic, \(a = 7.1279(4) \text{ Å}, b = 8.8202(5) \text{ Å}, c = 13.1405(8) \text{ Å}, \alpha = 102.542(3)°, \beta = 92.659(3)°, \gamma = 95.430(3)°, V = 800.90(8) \text{ Å}^3\), \(T\) = 100.(2) K, space group \(P1\), \(Z\) = 2, 18797 reflections measured, 5028 unique (\(R_{int} = 0.0238\)). The final \(R\) 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.
(±)-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: C₁₆H₁₇FN₂O₃, 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) Å³, T = 100.(2) K, space group P2₁/c, Z = 4, 14546 reflections measured, 2660 unique (R_int = 0.0594). The final R_f values were 0.0615 (I > 2σ(I)) and 0.0830 (all data). The final wR(F²) values were 0.1601 (I > 2σ(I)) and 0.1842 (all data). Full details are given on the accompanying CD.

(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: C₁₆H₁₇FN₂O₃, 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) Å³, T = 100.(2) K, space group P1, Z = 4, 23873 reflections measured, 8800 unique (R_int = 0.0334). The final R_f values were 0.0356 (I > 2σ(I)) and 0.0398 (all data). The final wR(F²) values were 0.0890 (I > 2σ(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_{2}O_{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) Å³, T = 100.(2) K, space group P-1, Z = 4, 30199 reflections measured, 5266 unique (R_{int} = 0.0390). The final R_{I} values were 0.0544 (I > 2σ(I)) and 0.0683 (all data). The final wR(F^2) values were 0.1340 (I > 2σ(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_{2}O_{3}, M = 286.32, triclinic, a = 14.1993(5) Å, α = 91.391(2)°, β = 97.183(2)°, γ = 107.512(2)°, V = 738.64(4) Å³, T = 100.(2) K, space group P1, Z = 2, 16675 reflections measured, 4575 unique (R_{int} = 0.0270). The final R_{I} values were 0.0292 (I > 2σ(I)) and 0.0297 (all data). The final wR(F^2) values were 0.0833 (I > 2σ(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_{2}O_{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

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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2979 (CH), 1733 (CO), 1605, 1496, 1455 (Ar), 1176 (C-O); \( \delta_{\text{H}} \) (400 MHz) 1.15 [3H, d, J 6.8, C(2)CH_{3}], 1.18 (3H, t, J 7.2, OCH_{2}CH_{3}), 2.63-2.76 (2H, m, AB of ABX, CH_{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, OCH_{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

*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 °C under an atmosphere of nitrogen. Once the addition was complete the reaction mixture was warmed to −35 °C. 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. Benzyldi 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, 8.80%; νmax/cm−1 (film) 2966 (CH), 1732 (CO), 1605, 1496, 1456 (Ar); δH (300 MHz) 0.92 [3H, t, J 7.4, C(4)H3], 1.15 (3H, t, J 7.2, OCH2CH3), 1.44-1.77 [2H, m, C(3)H2], 2.53-2.62 [1H, m, X of ABX, C(2)H], 2.74 [1H, dd, A of ABX, JAB 13.5, JAX 6.6, one of CH2Ph], 2.93 [1H, dd, B of ABX, JAB 13.5, JBX 8.4, one of CH2Ph], 4.06 (2H, q, J 7.1, OCH2CH3), 7.15-7.29 (5H, m, ArH); δC (75.5 MHz) 11.7 [CH3, C(4)H3], 14.2 [CH3, OCH2CH3], 25.2 [CH2, C(3)H2], 38.2 [CH2, CH2Ph], 49.2 [CH, C(2)H], 60.1 [CH2, OCH2CH3], 126.2 [CH, C(4)H3], 128.3, 128.9 [4 x CH, C(2)H], 139.6 [C, C(1)], 175.5 [C, C(1)]; HRMS (ES+): Exact mass calculated for C13H18O2 [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

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; νmax/cm−1 (film) 2966 (OH), 1705 (CO), 1605, 1496, 1456 (Ar); δH (300 MHz) 0.96 [3H, t, J 7.5, C(4)H3], 1.50-1.77 [2H, m, C(3)H2], 2.57-2.66 [1H, m, X of ABX, C(2)H], 2.75 [1H, dd, A of ABX, JAB 13.8, JAX 6.9, one of CH2Ph], 2.98 [1H, dd, B of ABX, JAB 13.5, JBX 7.8, one of CH2Ph], 7.09-7.34 (5H, m, ArH).
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; \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2963 (CH), 1729 (CO), 1605, 1496, 1456 (Ar), 1152 (C-O); \( \delta_{H} \) (300 MHz) 1.04 (3H, t, \( J = 7.1 \), OCH\(_2\)CH\(_3\)), 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, CH\(_2\)Ph), 3.88-4.03 (2H, sym. m, OCH\(_2\)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**

![Diagram of reaction]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Time</th>
<th>Ester (%)</th>
<th>Acid (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
<th>E calc.</th>
<th>1H NMR</th>
</tr>
</thead>
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<td>25</td>
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<td>0</td>
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<td>93</td>
<td>1.9</td>
<td>183</td>
</tr>
<tr>
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<td>Alcaligenes spp.1</td>
<td>72 h</td>
<td>2</td>
<td>11</td>
<td>15</td>
<td>12</td>
<td>1.3</td>
<td></td>
</tr>
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<td>Pseudomonas cepacia P1</td>
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<td>68</td>
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<td>55</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
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<td>Pseudomonas stutzeri</td>
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<td>68</td>
<td>44</td>
<td>55</td>
<td>6.4</td>
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<tr>
<td>6</td>
<td>Rhizopus spp.</td>
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<td>33</td>
<td>13</td>
<td>&lt;10</td>
<td>1.9</td>
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<td>93</td>
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<td>79</td>
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<tr>
<td>10b</td>
<td>10 h</td>
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<td>50</td>
<td>61</td>
<td>183</td>
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<td>1.9</td>
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</tr>
<tr>
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<td>93</td>
<td>1.9</td>
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<tr>
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<td>11</td>
<td>47</td>
<td>19</td>
<td>35</td>
<td>3.1</td>
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</tbody>
</table>

---

### Notes

- **a.** Limited enantioptuney 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

\[
\begin{align*}
\text{Hydrolase} & \quad 0.1 \text{ M Phosphate buffer, pH 7.0} \\
\text{Enzyme} & \quad \text{Time} \\
\text{Candida cyclindracea C1} & \quad 43 \text{ h} \\
\text{Candida cyclindracea C2} & \quad 17 \text{ h} \\
\text{Candida antarctica B} & \quad 43 \text{ h} \\
\text{Candida antarctica B (immob)} & \quad 43 \text{ h} \\
\text{Pig Liver esterase} & \quad 17 \text{ h} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Time</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cyclindracea C1</td>
<td>43 h</td>
<td>3 (R)</td>
<td>13 (S)</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Candida cyclindracea C2</td>
<td>17 h</td>
<td>20 (R)</td>
<td>4 (S)</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>Candida antarctica B</td>
<td>43 h</td>
<td>35 (S)</td>
<td>83 (R)</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica B (immob)</td>
<td>43 h</td>
<td>74 (S)</td>
<td>71 (R)</td>
<td>49</td>
</tr>
<tr>
<td>5</td>
<td>Pig Liver esterase</td>
<td>17 h</td>
<td>17 (S)</td>
<td>73 (R)</td>
<td>19</td>
</tr>
</tbody>
</table>

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 camemberti.

b. Limited enantipurity observed, thus direction of enantioselection should be interpreted with caution.

Table 5.15: Hydrolase-mediated hydrolysis of (±)-ethyl 2-benzyl-3,3-dimethylbutanoate (±)-57

\[
\begin{align*}
\text{Hydrolase} & \quad 0.1 \text{ M Phosphate buffer, pH 7.0} \\
\text{Enzyme} & \quad \text{Time} \\
\text{Pseudomononas cepacia P1} & \quad 20 \text{ h} \\
\text{Pseudomononas cepacia P2} & \quad 20 \text{ h} \\
\text{Candida antarctica B} & \quad 20 \text{ h} \\
\text{Candida antarctica B (immob)} & \quad 20 \text{ h} \\
\text{Pseudomonas fluorescens} & \quad 20 \text{ h} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme source</th>
<th>Time</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
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<td>2</td>
<td>Pseudomononas cepacia P2</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>3</td>
<td>Candida antarctica B</td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica B (immob)</td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas fluorescens</td>
<td>20 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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 °C. 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%. 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, \text{CHCl}_3), >98\%\text{ ee, lit}^{33} [\alpha]_D^{20} +28.4 (c 1.0, \text{CHCl}_3), (S)-isomer, 82\%\text{ ee, and the pure acid (S)-32 (76.6 mg, 37\%)} as a clear oil \([\alpha]_D^{20} +28.0 (c 0.82, \text{CHCl}_3), 96\%\text{ ee, lit}^{56} [\alpha]_D^{20} +30.2 (c 0.82, \text{CHCl}_3), 99\%\text{ ee.} \)\(^1\)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 °C followed by solvent partitioning with heptane and ethyl acetate to produce the pure ester (S)-48 (172.1 mg, 43\%) as a clear oil \([\alpha]_D^{20} +6.8 (c 1.0, \text{CH}_2\text{Cl}_2), 26\%\text{ ee and the pure acid (R)-45 (66.2 mg, 19\%) as a clear oil [\alpha]_D^{20} -43.8 (c 1.0, \text{CH}_2\text{Cl}_2), 82\%\text{ ee, lit}^{57} [\alpha]_D^{23} -40.0 (c 1.0, \text{CH}_2\text{Cl}_2), >99\%\text{ ee.} \) Conversion estimated by E-value calculator at 24%.\(^4\)\(^5\)\(^1\)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 \textit{110} and 6-aminohexanoic acid \textit{111}\textsuperscript{58}

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{110}
\caption{E-Caprolactam \textit{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 decolourised with charcoal and concentrated under reduced pressure to give the moist hydrochloric salt \textit{110} (16.34 g, >100%) as a white solid, m.p. 112-115 °C (Lit.,\textsuperscript{59} 132-133 °C); \textit{v}_{\text{max}}/\text{cm}^{-1} (\text{KBr}) 3367 (\text{OH}), 2945 (\text{CH}), 1725 (\text{CO}), 1216; \textit{\delta}H (400 MHz, D\textsubscript{2}O) 1.25-1.35 [2H, m, C(4)H\textsubscript{2}], 1.45-1.65 [4H, sym m, C(3)H\textsubscript{2} and C(5)H\textsubscript{2}], 2.29 [2H, t, J 7.4, C(2)H\textsubscript{2}], 2.89 [2H, t, J 7.6, C(6)H\textsubscript{2}].}
\end{figure}

Note: The experimental melting point of \textit{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 \textit{111}.

The resulting hydrochloric salt \textit{110} was converted into the amino acid \textit{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 was 5.6-6.6. The column was now ready for use. The solid \textit{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 \textit{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 vigorous shaking the pure amino acid \textit{111} \textit{[11.36 g, 98\% (from e-caprolactam \textit{109})]} formed and was collected by filtration as a white solid, m.p. 202-205 °C (Lit.,\textsuperscript{58} 202-203 °C); \textit{v}_{\text{max}}/\text{cm}^{-1} (\text{KBr}) 3030 (\text{OH}), 2944 (\text{CH}), 1729 (\text{CO}), 1229; \textit{\delta}H (400 MHz, D\textsubscript{2}O) 1.21-1.29 [2H, m, C(4)H\textsubscript{2}], 1.43-1.51 [2H, q, J 7.5 C(3)H\textsubscript{2} or C(5)H\textsubscript{2}], 1.51-1.59 [2H, q, J 7.6 C(3)H\textsubscript{2} or C(5)H\textsubscript{2}], 2.07 [2H, t, J 7.3, C(2)H\textsubscript{2}], 2.88 [2H, t, J 7.5, C(6)H\textsubscript{2}].

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{111}
\caption{Attempted oxidation of 6-aminohexanoic acid \textit{111} \textit{A} saturated aqueous solution of sodium bicarbonate (25 mL) was added to a solution of 6-aminohexanoic acid \textit{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\textsuperscript{®} (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\textsuperscript{®} 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 \textit{\textsuperscript{1}H NMR spectrum}}
of the crude product was recorded, which showed the presence of signals pertaining to 6-
nitrohexanoic acid $^{107}$ $\delta_H$ (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$^{60}$**

![Ethyl 6-nitrohexanoate 113](image)

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; $\nu_{\text{max}}$/cm$^{-1}$ (film) 2940 (CH), 1733 (CO), 1554 (NO$_2$), 1377 (NO$_2$), 1187; $\delta_H$ (300 MHz) 1.26
(3H, t, $J$ 7.2, OCH$_2$CH$_3$), 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, OCH$_2$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 $^1$H NMR of the crude product spectrum at $\delta_H$
(300MHz) 4.58-4.80 (2H, br m, CH$_2$ONO).

**6-Nitrohexan-1-ol 108$^{5,61,62}$**

![6-Nitrohexan-1-ol 108](image)

DIBAL-H (54 mL, 54.00 mmol, 1M solution in hexanes) was added dropwise to a solution of ethyl 6-
nitrohexanolate $^{113}$ (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; $\nu_{\text{max}}$/cm$^{-1}$ (film) 3400 (OH), 2936 (CH), 1552 (NO$_2$), 1385 (NO$_2$), 1198; $\delta_H$
(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$_2$]. $^1$H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

**Note:** Significant difference in $^1$H NMR chemical shift of the C(1)H$_2$ triplet is observed between Milner$^5$ and data described
above. Milner$^5$ reports; $\delta_H$ (400 MHz) 3.24 [2H, t, $J$ 6.4, C(1)H$_2$]. Literature reference Ballini$^61$ reports; $\delta_H$
(300 MHz) 3.65 (2H, t, $J$ 6.2) with spectral characteristics consistent with the experimental data obtained during this project.

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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; \(\nu_{\text{max}} \text{ cm}^{-1}\) (film) 2938 (CH), 1722 (CO), 1552 (NO\(_2\)), 1386 (NO\(_2\)); \(\delta_{\text{H}}\) (300 MHz) 1.34-1.51 [2H, m, C(2)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\(_2\)], 7.35 [1H, sym m, C(2)H], 1.19 [1H, br s, 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; \(\nu_{\text{max}} \text{ cm}^{-1}\) (film) 1668 (C=C), 1515 (NO\(_2\)), 1333 (NO\(_2\)); \(\delta_{\text{H}}\) (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]; \(\delta_{\text{C}}\) (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; \(\nu_{\text{max}} \text{ cm}^{-1}\) (film) 3427 (OH), 2943 (CH), 1548 (NO\(_2\)), 1383 (NO\(_2\)); \(\delta_{\text{H}}\) (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]; \(\delta_{\text{C}}\) (75.5 MHz) 18.8, 23.5, 24.6, 31.4 [4 x CH\(_2\), C(3)H\(_2\),...
C(3)H₂, C(4)H₂, C(5)H₂ and C(6)H₂, 67.1 [CH, C(1)H], 86.9 [CH, C(2)H]. ¹H 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); νmax/cm⁻¹ (KBr) 3256 (OH), 2952 (CH), 1551 (NO₂), 1376 (NO₂); δH (300 MHz) 1.20-1.46 [3H, m, one of C(4)H₂, one of C(5)H₂ and one of C(6)H₂], 1.68-1.93 [3H, m, one of C(4)H₂, one of C(5)H₂ and one of C(3)H₂], 2.03-2.18 [1H, m, one of C(6)H₂], 2.27-2.41 [1H, m, one of C(3)H₂], 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]; δC (75.5 MHz) 23.6, 24.0 [2 x CH₂, C(4)H₂ and C(5)H₂], 30.4 [CH₂, C(3)H₂], 32.8 [CH₂, C(6)H₂], 71.1 [CH, C(1)H], 91.3 [CH, C(2)H]. ¹H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note: Milner reports (±)-trans-2-nitrocyclohexanol (±)-99b; δH (300 MHz) 1.12-1.46 [3H, m, one of C(6)H₂ and C(5)H₂], 1.70-1.89 [3H, m, one of C(3)H₂ and C(4)H₂]. In the experimental data described above the C(4)H₂ and the C(5)H₂ 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

(±)-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; v_max/cm⁻¹ (film) 2947 (CH), 1745 (CO), 1549 (NO₂), 1381 (NO₂); δ_H (300 MHz) 1.28–1.71 [4H, m, one of C(4)H₂, one of C(6)H₂ and C(5)H₂], 1.87–1.99 [1H, m, one of C(4)H₂], 2.05 (3H, s, COCH₃), 2.10–2.23 [3H, m, one of C(6)H₂ and C(3)H₂], 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 [CH₂, C(5)H₂], 20.9 (CH₃, COCH₃), 22.8 [CH₂, C(4)H₂], 24.9 [CH₂, C(3)H₂], 28.7 [CH₂, C(6)H₂], 69.5 [CH, C(1)H], 84.0 [CH, C(2)H], 169.8 (C, COCH₃). ¹H 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 ¹H 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

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 CuSO₄ (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); v_max/cm⁻¹ (KBr) 2954 (CH), 1737 (CO), 1548 (NO₂), 1376 (NO₂); δ_H (300 MHz) 1.24–1.55 [3H, m, one of C(4)H₂, one of C(5)H₂ and one of C(6)H₂], 1.74–1.94 [3H, m, one of C(3)H₂, one of C(4)H₂ and one of C(5)H₂], 2.02 (3H, s, COCH₃), 2.18–2.28 [1H, m, one of C(6)H₂], 2.34–2.42 [1H, m, one of C(3)H₂], 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 (CH₃, COCH₃), 23.2, 23.7 [2 x CH₂, C(4)H₂ and C(5)H₂], 29.9 [CH₂, C(6)H₂], 30.8 [CH₂, C(3)H₂], 72.6 [CH, C(1)H], 87.7 [CH, C(2)H], 169.7 (C, COCH₃). ¹H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.
Experimental

Large scale hydrolase-mediated transesterfication of (±)-trans-2-nitrocyclohexanol (±)-99b

*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, 1H 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 (1R,2R)-100b (81.1 mg, 30%) as a clear oil which solidified on cooling to a white crystalline solid (c 1.0, CH2Cl2), >98% ee, lit5 [α]D20 −40.6 (c 1.0, CH2Cl2), >98% ee and the pure (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b (96.0 mg, 45%) as a clear oil [α]D20 +42.6 (c 0.3, CH2Cl2), 95% ee, lit63 [α]D20 +48.6 (c 1.0, CH2Cl2), >98% ee. 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>Alcohol (1,2S) [yield %]</th>
<th>Acetate (1,2R) [yield %]</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28 h</td>
<td>rt</td>
<td>24</td>
<td>25</td>
<td>31</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td>55 h</td>
<td>24</td>
<td>44</td>
<td>-</td>
<td>78</td>
<td>&gt;98</td>
</tr>
<tr>
<td>3</td>
<td>79 h</td>
<td>24</td>
<td>50</td>
<td>50</td>
<td>97a</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

a. While analysis by chiral HPLC of the crude product mixture showed 97% ee for (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, on chromatographic purification the enantiomeric excess [ee (%)] of isolated (1S,2S)-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, (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b, >98% ee, (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, 97% ee. For HPLC conditions see appendix I.

5.5.2 Evidence of a dynamic interconversion process – ¹H NMR

A solution of 6-nitrohexanal 101 (80.4 mg, 0.55 mmol) in deuterated chloroform, CDCl₃ (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, CDCl₃ (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 ¹H 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)HOH, at 4.04-4.12 ppm in (±)-trans-2-nitrocyclohexanol (±)-99b and the 1H, br s, C(1)HOH, 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, CDCl₃ and DBU (0.10 eq.)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>101 (%)</th>
<th>(±)-99a (%)</th>
<th>(±)-99b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>21</td>
<td>19</td>
<td>60</td>
</tr>
<tr>
<td>17 min</td>
<td>20</td>
<td>18</td>
<td>62</td>
</tr>
<tr>
<td>27 min</td>
<td>14</td>
<td>21</td>
<td>65</td>
</tr>
<tr>
<td>1 h 27 min</td>
<td>4</td>
<td>18</td>
<td>78</td>
</tr>
<tr>
<td>3 h 29 min</td>
<td>0</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>9 h 29 min</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>15 h 29 min</td>
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<td>17</td>
<td>83</td>
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<tr>
<td>20 h 22 min</td>
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</tr>
<tr>
<td>22 h 37 min</td>
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<td>16</td>
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</tr>
<tr>
<td>20 days</td>
<td>0</td>
<td>14</td>
<td>86</td>
</tr>
</tbody>
</table>

Note: Integration of the C(2)HNO₂ signals was low in the ¹H NMR spectra from reaction time 1 h 27 min onwards. This may be due to deuterium exchange of this acidic C(2)HNO₂ proton of cis and trans 2-nitrocyclohexanol (±)-99a and (±)-99b.

Figure 5.10:(Stacked ¹H 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)

![Graph](image)

\[ y = 233.94x + 11.445 \]

\[ R^2 = 0.9955 \]

**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)

![Graph](image)

\[ y = 300.08x + 19.958 \]

\[ R^2 = 0.9971 \]

**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, CDCl₃ and DBU (0.05 eq.)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>101 (%)</th>
<th>(±)-99a (%)</th>
<th>(±)-99b (%)</th>
</tr>
</thead>
<tbody>
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<td>4</td>
<td>4</td>
</tr>
<tr>
<td>7 min</td>
<td>92</td>
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</tr>
<tr>
<td>34 min</td>
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<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>1 h 35 min</td>
<td>89</td>
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<td>6</td>
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<td>3 h 35 min</td>
<td>86.5</td>
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<td>9 h 34 min</td>
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<tr>
<td>15 h 35 min</td>
<td>71</td>
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<td>15</td>
</tr>
<tr>
<td>20 h 28 min</td>
<td>65</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>22 h 43 min</td>
<td>62</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>23 h 50 min</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>27 h 37 min</td>
<td>56</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>
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. 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)HOH, 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)HOH, 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

![Chemical structure diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Ratio 99a : 99b</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida antarctica B (immob)</td>
<td>96 : 4</td>
<td>23</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Alcaligenes spp. I</td>
<td>96 : 4</td>
<td>20</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Rhizopus spp.</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Aspergillus niger</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Mucor meihei</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Porcine pancrease Type II</td>
<td>95 : 5</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Pig liver esterase</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Candida antarctica A</td>
<td>96 : 4</td>
<td>60</td>
<td>60³</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>Achromobacter spp.</td>
<td>96 : 4</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
</tbody>
</table>

a. The ratio of (±)-cis-2-nitrocyclohexanol (±)-99a to (±)-trans-2-nitrocyclohexanol (±)-99b in the starting material, determined by ¹H NMR spectroscopy.

b. Time for transesterification of the nitroalcohol was 51 h at 24 °C.

c. Unknown impurity observed in the crude ¹H NMR. Conversion could not be obtained satisfactorily due to overlapping peaks.

d. Pseudomonas fluorescens (immob) was obtained commercially from Sigma-Aldrich.

### Table 5.20: Hydrolase-mediated transesterification of (±)-trans-2-nitrocyclohexanol
(±)-99b in vinyl acetate

![Chemical structure diagram](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Ratio 99a : 99b</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida antarctica B (immob)</td>
<td>1 : 99</td>
<td>50</td>
<td>49</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td>Candida antarctica A</td>
<td>1 : 99</td>
<td>9</td>
<td>²</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Achromobacter spp.</td>
<td>1 : 99</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas fluorescens (immob)</td>
<td>1 : 99</td>
<td>38</td>
<td>38</td>
<td>61</td>
</tr>
</tbody>
</table>

a. Time for transesterification 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 ¹H NMR spectroscopy.

c. Unknown impurity observed in the ¹H 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 an 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 ¹H NMR spectroscopy. The final extraction following ¹H 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*

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Temp (°C)</th>
<th>Alcohol (±)-99</th>
<th>Acetate (±)-100</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> antarctica lipase B (immob)</td>
<td>15 h</td>
<td>19</td>
<td>cis-99a (%)</td>
<td>trans-99b (%)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>24</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>39 h</td>
<td>24</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>24</td>
<td>43</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[23]</td>
<td>[&gt;98]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by ¹H 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 (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a.
c. The principal enantiomer was (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b.
d. The principal enantiomer was (1R,2S)-cis-2-nitrocyclohexyl acetate (1R,2S)-100a.
e. The principal enantiomer was (1R,2R)-trans-2-nitrocyclohexyl acetate (1R,2R)-100b.
5.5.4 Evidence of a dynamic interconversion process - chiral HPLC

A solution of (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-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 (1S,2S)-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 (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b, 94% ee following exposure to 1.0 eq. and 0.5 eq. of DBU at 24 h and 48 h

<table>
<thead>
<tr>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Alcohol (±)-99</th>
<th>cis-99a (%)</th>
<th>cis-99a [ee (%)]a</th>
<th>trans-99b (%)</th>
<th>trans-99b [ee (%)]b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>16</td>
<td>[31]</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>17</td>
<td>[24]</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0 h</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>15</td>
<td>[2]</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>13</td>
<td>[4]</td>
<td>87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. The principal enantiomer was (1S,2R)-cis-2-nitrocyclohexanol (1S,2R)-99a.
b. The principal enantiomer was (1S,2S)-trans-2-nitrocyclohexanol (1S,2S)-99b.
Figure 5.14: (1S,2S)-trans-2-Nitrocyclohexanol (1S,2S)-\textit{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)-\textit{99b} 94\% ee after exposure to 0.5 equivalent of DBU at 24 h and 48 h.
**Calibration curve:** \((\pm)-\text{cis-2-Nitrocyclohexanol (±)-99a}\) (10 \(\mu \text{L injection volume})

![Graph showing calibration curve for (±)-cis-2-Nitrocyclohexanol (±)-99a](image)

*Figure 5.16: Calibration curve (±)-cis-2-nitrocyclohexanol (±)-99a (10 \(\mu \text{L injection}). Area (x 10^3) vs. concentration (mg/1 mL).*

**Calibration curve:** \((\pm)-\text{trans-2-Nitrocyclohexanol (±)-99b}\) (10 \(\mu \text{L injection volume})

![Graph showing calibration curve for (±)-trans-2-Nitrocyclohexanol (±)-99b](image)

*Figure 5.17: Calibration curve (±)-trans-2-nitrocyclohexanol (±)-99a (10 \(\mu \text{L 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 – NO₂. 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**

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 NaHCO₃ (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; νmax/cm⁻¹ (film) 3418 (OH), 2954 (CH), 1732 (CO), 1441; δH (300 MHz) 1.53-1.62 [2H, m, C(3)H₂], 1.64-1.76 [2H, m, C(4)H₂], 2.36 [2H, t, J 7.4, C(2)H₂], 3.52 (1H, br s, OH), 3.61 [2H, t, J 6.3, C(5)H₃], 3.67 (3H, s, OCH₃).

**Methyl 5-oxopentanoate 134**

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); νmax/cm⁻¹ (film) 2936 (CH), 1733 (CO), 1198; δH (300 MHz) 1.91-2.01 [2H, m, C(3)H₂], 2.38 [2H, t, J 7.2, C(2)H₂], 2.54 [2H, dt, J 1.2, 7.2, C(4)H₂], 3.68 (3H, s, OCH₃), 9.78 [1H, t, J 1.2, C(1)H].

**Methyl 5-hydroxy-6-nitroheptanoate 135**

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 an 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. C₈H₁₅NO₅ requires C, 46.82; H, 7.37; N, 6.83%); νmax/cm⁻¹ (film) 3444 (OH), 2955 (CH), 1728 (CO), 1557 (NO₂), 1393; δH (300 MHz) 1.38-1.62 [5H, m containing 2 × d, 1.54 [1.5H, d, J 6.9, C(7)H₃ of 1 diastereomer], 1.55 [1.5H, d, J 6.9, C(7)H₃ of 1 diastereomer] and [2H, m, C(4)H₂ of 2 diastereomers]], 1.63-1.96 [2H, m, C(3)H₂ of 2 diastereomers], 2.39 [2H, t, J 7.1, C(2)H₂ of 2 diastereomers], 2.91-2.95 (1H, OH, of 2 diastereomers), 3.68 (3H, s, OCH₃, 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 diastereomers]. δC (75.5 MHz) 12.5, 16.1 [2 × CH₃, C(7)H₃ of 2 diastereomer], 20.3, 21.0 [2 × CH₂, C(3)H₂ of 2 diastereomers], 32.1, 32.3 [2 × CH₂, C(4)H₂ of 2 diastereomers], 33.3 [CH₂, C(2)H₂ of 2 diastereomers], 51.7 (CH₃, OCH₃ of 2 diastereomers), 71.7, 72.4 [2 × CH, C(5)H, of 2 diastereomers], 86.4, 87.7 [2 × CH, C(6)H, of 2 diastereomers], 174.0 [C, C(1) of 2 diastereomers]; HRMS (ES+): Exact mass calculated for C₈H₁₅NO₅ (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% δH (300MHz) 1.27 [9H, s, C(CH₃)₃] was evident in the crude ¹H 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 ¹H NMR spectroscopy) was used without further purification.

Methyl 5-acetoxy-6-nitroheptanoate 138 and methyl 6-nitroheptanoate 127

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; δH (300 MHz) 1.548 [1.5H, d, J 6.9, C(7)H₃ of 1 diastereomer] 1.554 [1.5H, d, J 6.9, C(7)H₃ of 1 diastereomer], 1.58-1.80 [4H, m, C(3)H₂ and C(4)H₂ of 2 diastereomers], 2.06 (1.5H, s, COCH₃ of 1 diastereomer) 2.09 (1.5H, s, COCH₃ of 1 diastereomer), 2.25-2.47 [2H, m, C(2)H₂ of 2 diastereomers], 3.68 (3H, s, OCH₃ of 2 diastereomers), 4.65-4.77 [1H, m, C(5)H of 2 diastereomers], 5.29-5.35 [1H, m, C(6)H of 2 diastereomers].

Note:
1. Integration of the 3H, s, COCH₃ of 1 diastereomer at δH (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)H₃ doublet.
A suspension of NaBH₄ (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 ¹H NMR spectroscopy); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2953 (CH), 1738 (CO), 1552 (NO₂), 1361 (NO₂); \( \delta_H \) (300 MHz) 1.26-1.44 [2H, m, C(4)H₂], 1.53 [3H, d, \( J \) 6.9, C(7)H₃], 1.61-1.80 [3H, m, C(3)H₂ and one of C(5)H₂], 1.92-2.13 [1H, m, one of C(5)H₂], 2.32 [2H, t, \( J \) 7.4, C(2)H₂], 3.67 (3H, s, OCH₃), 4.51-4.62 [1H, sym m, C(6)H]. ¹H NMR spectral assignment was aided by COSY and HETCOR 2D NMR experiments.

Note: This procedure was adapted from Morrow²⁴

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 ¹H 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. C₇H₁₅NO₃ requires C, 52.16; H, 9.38; N, 8.69%); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 3365 (OH), 2940 (CH), 1553 (NO₂), 1391 (NO₂); \( \delta_H \) (300 MHz) 1.26-1.48 [4H, m, C(3)H₂ and C(4)H₂], 1.52-1.61 [5H, m containing 1.53 [3H, d, \( J \) 6.6, C(7)H₃] and [2H, m, C(2)H₂] 2, 1.64-1.79 [1H, m, one of C(5)H₂], 1.86 [1H, br s, OH], 1.96-2.08 [1H, m, one of C(5)H₂], 3.63 [2H, t, \( J \) 6.5, C(1)H₃], 4.52-4.63 [1H, sym m, C(6)H]; \( \delta_C \) (75.5 MHz) 19.2 [CH₃, C(7)H₃], 25.2, 25.5 [2 x CH₂, C(3)H₂ and C(4)H₂], 32.2 [CH₂, C(2)H₂], 35.1 [CH₂, C(5)H₂], 62.5 [CH₂, C(1)H₃], 83.5 [CH, C(6)H]; Exact mass calculated for C₇H₁₅NO₃ (M+H)+ 162.1130 Found 162.1122; m/z (ES+) 102 (86%), 143 (59%), 284 (52%), 334 (100%), 336 (60%), 466 (50%). ¹H 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 ¹H 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. C₇H₁₃NO₃ requires C, 52.82; H, 8.23; N, 8.80%); \( \nu_{\text{max}}/\text{cm}^{-1} \) (film) 2943 (CH), 1724 (CO), 1549 (NO₂), 1392 (NO₂); \( \delta_H \) (300 MHz) 1.27-1.47 [2H, m, C(4)H₂], 1.53 [3H, d, \( J \) 6.6, C(7)H₃], 1.59-1.82 [3H, m, C(3)H₂ and one of C(5)H₂], 1.94-2.12 [1H, m, one of C(5)H₂], 2.44-2.50 [2H, dt, \( J \) 1.2, 7.2, C(2)H₃], 4.51-4.63 [1H, m, C(6)H], 9.77 [1H, t, \( J \) 1.4 C(1)H]; \( \delta_C \) (75.5 MHz) 19.2 [CH₃, C(7)H₃], 21.3 [CH₃, C(3)H₂], 25.2 [CH₂, C(4)H₂], 34.8 [CH₂, C(5)H₂], 43.4 [CH₂, C(2)H₂], 83.2 [CH, C(6)H], 201.8 [CH, C(1)H]; Exact mass calculated for C₇H₁₃NO₃ (M+H)+

320
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) 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 \(\alpha\)-nitro ketone 120 (0.78 g, 36%) as a colourless oil; \(\nu_{\text{max}}/\text{cm}^{-1}\) (film) 2950, 2873 (CH), 1732 (CO), 1549 (NO\(_2\)); \(\delta\)\(^{1}\)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 \(\delta\)\(^{1}\)H (300MHz) 4.49-4.68 (1H, sym m, CHNO\(_2\)) and at \(\delta\)\(^{1}\)H (300MHz) 4.40 (2H, t, J 7.1 CH\(_2\)NO\(_2\)) 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; $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 3421, 2937, 1711 (CO), 1568 (NO$_2$), 1412 (NO$_2$), 1199; $\delta_{\text{H}}$ (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); $\delta_{\text{C}}$ (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$^5$

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 ºC; (Found C, 52.30; H 8.02; N 8.80%); $\nu_{\text{max}}/\text{cm}^{-1}$ (film) 3445 (OH), 2945 (CH), 1539 (NO$_2$), 1352 (NO$_2$); $\delta_{\text{H}}$ (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]; $\delta_{\text{C}}$ (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)CH$_3$], 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$_7$H$_4$NO$_3$ [M+H]$^+$ 160.0974 Found 160.0974; 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 ºC; (Found C, 53.06; H 8.17; N 8.62; C$_7$H$_6$NO$_3$ requires C, 52.82; H, 8.23; N, 8.80%); $\nu_{\text{max}}/\text{cm}^{-1}$ (KBr) 3376 (OH), 2947 (CH), 1538 (NO$_2$), 1340 (NO$_2$); $\delta_{\text{H}}$ (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 (C3)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]; $\delta_{\text{C}}$ (150 MHz) 16.3 [CH$_3$, br, C(2)CH$_3$], 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$_7$H$_4$NO$_2$ [(M+H)$^+$H$_2$O]$^+$ 142.0868 Found 142.0864; m/z (ES+) 141 [(M+H)$^+$H$_2$O]$^+$, 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. C₇H₁₃NO₄ requires C, 53.72; H, 7.51; N, 6.96%); v\textsubscript{max}/cm\textsuperscript{-1} (KBr) 2960 (CH), 1738 (CO), 1544 (NO\textsubscript{2}), 1362 (NO\textsubscript{2}); δ\textsubscript{H} (300 MHz) 1.26–1.48 [3H, m, (C(5)H₂ and one of C(4)H₂], 1.54-1.67 [4H, m containing 1.54 [3H, s, C(2)CH₃] and [1H, m, one of C(6)H₂]), 1.70-1.82 [1H, m, one of C(4)H₂], 1.88-2.05 [5H, m containing 1.95 [3H, s, COCH₃] and [2H, m, one of C(6)H₂ and one of C(3)H₂]), 2.22-2.32 [1H, m, one of C(3)H₂], 5.26-5.29 [1H, m, C(1)H]; δ\textsubscript{C} (75.5 MHz) 18.6 [CH₂, C(5)H₂], 19.9 [CH₃, C(2)CH₃], 20.9 [CH₂, C(4)H₂], 22.1 [CH₃, COCH₃], 25.8 [CH₂, C(6)H₂], 29.7 [CH₂, C(3)H₂], 72.4 [CH, C(1)H], 87.5 [C, C(2)], 168.6 [C, COCH₃]; HRMS (ES+): Exact mass calculated for C₁₇H₂₃O₂ (M-NO₂)\textsuperscript{+} 295.1066 m/z (ES+) 94.2 (100%), 155.4 [(M-NO₂)\textsuperscript{+}, 18%], 242.5 (90%), 243.5 (20%). \textsuperscript{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 CDCl₃.\textsuperscript{76}

Crystal data: C₁₀H₁₅NO₄, M = 201.22, orthorhombic, a = 20.418(8) Å, b = 25.919(16) Å, c =8.013(2) Å, V = 4241.(3)Å\textsuperscript{3}, T = 296.(2) K, space group Fdd2, Z = 16, 11831 reflections measured, 2148 unique (R\textsubscript{int} = 0.0597). The final R1 values were 0.0396 (I > 2σ(I)) and 0.0643 (all data). The final wR(F2) values were 0.0912 (I > 2σ(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. C₇H₁₃NO₄ requires C, 53.72; H, 7.51; N, 6.96%); v\textsubscript{max}/cm\textsuperscript{-1} (film) 2950 (CH), 1748 (CO), 1538 (NO\textsubscript{2}), 1361 (NO\textsubscript{2}); δ\textsubscript{H} (300 MHz) 1.33–1.52 [3H, m, one of C(4)H₂, one of C(5)H₂ and one of C(6)H₂], 1.62-1.81 [5H, m containing 1.65 [3H, s, C(2)CH₃] and [2H, m, one of C(5)H₂ and one of C(4)H₂], 1.95-2.19 [6H, m containing 2.03 (3H, s, COCH₃) and [3H, m, C(3)H₂ and one of C(6)H₂]), 5.50-5.55 [1H, m, C(1)H]; δ\textsubscript{C} (75.5 MHz) 17.6 [CH₃, C(2)CH₃], 20.9 [CH₃, COCH₃], 21.9, 22.7 [CH₂, C(5)H₂, and C(4)H₂], 28.1 [CH₂, C(6)H₂], 36.5 [CH₂, C(3)H₂], 73.9 [CH, C(1)H], 90.4 [C, C(2)], 169.5 (C, COCH₃); Exact mass calculated for C₁₀H₁₅O₂ (M-NO₂)\textsuperscript{+} 155.1072 Found 155.1072; m/z (ES+) 141 (78%), 242 (54%), 300 (43%), 344 (100%), 388 (86%). \textsuperscript{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 \(^1\)H, br s, \(C(1)\)HOH at 3.91 ppm in (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a and the \(^1\)H, 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 \(^1\)H, m, \(C(1)\)HOH at 4.28-4.29 ppm in (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b and the \(^1\)H, 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

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.

**Table 5.23: Hydrolase-mediated transesterification of (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b in vinyl acetate**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>Alcohol trans-117b (1S,2S)</th>
<th>Acetate trans-119b (1R,2R)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida cylindracea C1</td>
<td>48 h</td>
<td>33</td>
<td>43</td>
<td>61</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia P2</td>
<td>48 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Candida antarctica B (inmob)</td>
<td>72 h</td>
<td>49</td>
<td>48</td>
<td>96</td>
<td>&gt;98</td>
<td>&gt;98</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas stutzeri</td>
<td>48 h</td>
<td>50</td>
<td>51</td>
<td>97</td>
<td>&gt;98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas fluorescens</td>
<td>113.5 h</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5.24: Hydrolase-mediated transesterification of (±)-cis-2-methyl-2-nitrocyclohexanol
(±)-117a in vinyl acetate

(±)-117a

\[
\begin{align*}
 & \text{Lipase} \\
 & \text{Vinyl Acetate} \\
 & \text{Conversion}\% \\
 & \text{ee}\% \\
 & E\text{ Value} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Alcohol</th>
<th>Acetate</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomonas cepacia</em> P2</td>
<td>48 h</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>72 h</td>
<td>33</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;98</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas stutzeri</em></td>
<td>48 h</td>
<td>78</td>
<td>85</td>
<td>&gt;98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>113.5 h</td>
<td>40</td>
<td>45</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
</tr>
</tbody>
</table>

Table 5.25: Hydrolase-mediated transesterification of (±)-trans-2-methyl-2-nitrocyclohexanol
(±)-117b in ethyl acetate

(±)-117b

\[
\begin{align*}
 & \text{Lipase} \\
 & \text{Ethyl Acetate} \\
 & \text{Conversion}\% \\
 & \text{ee}\% \\
 & E\text{ Value} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Alcohol</th>
<th>Acetate</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Candida antarctica</em> B (immob)</td>
<td>72 h</td>
<td>16</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas stutzeri</em></td>
<td>48 h</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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. 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 1H 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**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Solvent</th>
<th>Conversion (%)</th>
<th>en (%)a</th>
<th>Alcohol trans-117b (1S,2S)</th>
<th>Acetate trans-119b (1R,2R)</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas stutzeri</td>
<td>Heptane</td>
<td>40</td>
<td>41</td>
<td>66</td>
<td>&gt;98</td>
<td>197</td>
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<tr>
<td>2</td>
<td>Pseudomonas stutzeri</td>
<td>Diethyl ether</td>
<td>46</td>
<td>44</td>
<td>82</td>
<td>&gt;98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas stutzeri</td>
<td>Diisopropyl ether</td>
<td>46</td>
<td>45</td>
<td>84</td>
<td>&gt;98</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas stutzeri</td>
<td>Toluene</td>
<td>20</td>
<td>21</td>
<td>24</td>
<td>&gt;98</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas stutzeri</td>
<td>Acetonitrile</td>
<td>25</td>
<td>26</td>
<td>32</td>
<td>&gt;98</td>
<td>135</td>
</tr>
</tbody>
</table>

a. Enantiomeric excess [en (%)] 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

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Alcohol (±)-117</th>
<th>Acetate (±)-119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cis-117a (%)</td>
<td>trans-117b (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ee (%)]^b</td>
<td>[ee (%)]^c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cis-119a (%)</td>
<td>trans-119b (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ee (%)]^d</td>
<td>[ee (%)]^e</td>
</tr>
<tr>
<td>Pseudomonas stutzeri</td>
<td>18.5 h</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[14]</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>40.5 h</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td></td>
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<td>[54]</td>
<td>[&gt;98]</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td></td>
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<td>[15]</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>40.5 h</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td></td>
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<td>[15]</td>
<td>[&gt;98]</td>
</tr>
<tr>
<td></td>
<td>62.5 h</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[15]</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>18.5 h</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[15]</td>
<td>[&gt;98]</td>
</tr>
<tr>
<td></td>
<td>40.5 h</td>
<td>41</td>
<td>30</td>
</tr>
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<td></td>
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<td>[15]</td>
<td>[&gt;98]</td>
</tr>
<tr>
<td></td>
<td>62.5 h</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[15]</td>
<td>[74]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by '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 (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a.

c. The principal enantiomer was (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b.

d. The principal enantiomer was (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a.

e. The principal enantiomer was (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b.
Chapter 5

Comparison of conversion (%) and enantiomeric excess (ee %) of 117a, 117b, 119a and 119b following diastereoselective hydrolase-mediated transesterification

![Graph of conversion vs enantiomeric excess for Pseudomonas stutzeri and Candida antarctica lipase B(immob)]

*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 transesterification of (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b

This was prepared following the procedure described for the hydrolase-mediated transesterification 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, 1H 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 (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b (95.5 mg, 36%) as a clear oil [α]D20^20 = -32.6 (c 1.0, CHCl3), >98% ee, and the pure (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-117b (72.1 mg, 35%) as a clear oil [α]D20^20 = +42.0 (c 1.0, CHCl3), 98% ee. Conversion estimated by E-value calculator at 50%. 1H 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 (1S,2S)-117b and (1R,2R)-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>ee (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48 h</td>
<td>24</td>
<td>49</td>
<td>95</td>
<td>0.0661</td>
</tr>
<tr>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.5 h</td>
<td>24</td>
<td>50</td>
<td>&gt;98&lt;sup&gt;e&lt;/sup&gt;</td>
<td>[35]&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Chiral HPLC analysis was conducted on the crude reaction mixture aliquot acquired at 48 h.
<sup>b</sup> Chiral HPLC analysis was conducted after purification by column chromatography and isolation of (13,2S)-117b and (1R,2R)-119b.
<sup>c</sup> Isolated yield following column chromatography.

Large scale hydrolase-mediated transesterification of (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a

This was prepared following the procedure described for the hydrolase-mediated transesterification 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 (1R,2S)-cis-2-methyl-2-nitrocyclohexyl acetate (1R,2S)-119a (30.9 mg, 30%) as a clear oil [α]<sub>D</sub><sup>20</sup> = 46.2 (c 0.5, CHCl<sub>3</sub>), >98% ee, and the pure (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a (26.7 mg, 33%) as a clear oil [α]<sub>D</sub><sup>20</sup> = +0.7 (c 0.5, CHCl<sub>3</sub>), 45% ee. Conversion estimated by E-value calculator at 31%.<sup>45</sup> <sup>1</sup>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 (1S,2R)-117a and (1R,2S)-119a following purification by column chromatography.

The absolute stereochemical assignment of the enantiopure acetate (1R,2S)-119a was determined by single X-ray diffraction on a crystalline sample of (1R,2S)-119a recrystallised from slow evaporation of acetonitrile (HPLC grade).<sup>76</sup> Crystal data: C<sub>8</sub>H<sub>15</sub>NO<sub>4</sub>, M = 201.22, orthorhombic, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, a = 6.0502(2), b = 10.5734(3), c = 15.6769(5) Å, V = 1002.87(5)Å<sup>3</sup>, Z = 4, D<sub>c</sub> = 1.333 g cm<sup>-3</sup>, F<sub>000</sub> = 432, Cu-Kα radiation, λ = 1.54178 Å, T = 100(2) K, 2θ<sub>max</sub> = 66.09°, μ = 0.881 mm<sup>-1</sup>, 6459 reflections collected, 1706 unique (R<sub>int</sub> = 0.027). Final GoF = 1.329, R<sub>1</sub> = 0.0260, wR<sub>2</sub> = 0.0657 (obs. data: I > 2σ(I) , R<sub>1</sub> = 0.0268, wR<sub>2</sub> = 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

![Diagram of the reaction](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Time (h)</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
<th>[yield (%)]c</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>72 h</td>
<td>24</td>
<td>27</td>
<td>-</td>
<td>37</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2b</td>
<td>79 h</td>
<td>24</td>
<td>31</td>
<td>32</td>
<td>45</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

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 (1R,2S)-119a and (1S,2R)-117a.
c. Isolated yield following column chromatography.

5.6.4 Analytical dynamic kinetic resolution screens

Conversions were determined throughout this study by 1H NMR spectroscopy (Figure 5.19) and are derived from integration of;

- 1H, br s, C(1)OH at 3.91 ppm in (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a.
- 1H, m, C(1)OH, at 4.28-4.29 ppm in (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b
- 1H, m, C(6)NO₂ at 4.51-4.63 ppm in 6-nitroheptanal 118
- 1H, m, C(1)OAc at 5.26-5.29 ppm in (±)-cis-2-methyl-2-nitrocyclohexyl acetate, (±)-119a
- 1H, m, C(1)OAc at 5.50-5.55 ppm in (±)-trans-2-methyl-2-nitrocyclohexyl acetate, (±)-119b
Chapter 5
Experimental

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

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Triethylamine (eq.)</th>
<th>Temp (°C)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)</th>
<th>Alcohol (±)-117 cis (%)</th>
<th>Alcohol (±)-117 trans (%)</th>
<th>Acetate (±)-119 cis (%)</th>
<th>Acetate (±)-119 trans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>40</td>
<td>24 h</td>
<td>62</td>
<td>14</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>37</td>
<td>24</td>
<td>39</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>9</td>
<td>24</td>
<td>67</td>
<td>-</td>
<td>-</td>
</tr>
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<td>24 h</td>
<td>89</td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
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<td></td>
<td>48 h</td>
<td>76</td>
<td>9</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>65</td>
<td>12</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>Ambient</td>
<td>24 h</td>
<td>90</td>
<td>3</td>
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<td>-</td>
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<td></td>
<td>48 h</td>
<td>79</td>
<td>7</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>67</td>
<td>11</td>
<td>22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 days</td>
<td>34</td>
<td>22</td>
<td>44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>Ambient</td>
<td>24 h</td>
<td>92</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>85</td>
<td>5</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>78</td>
<td>7</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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-nitroheptanal 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 CDCl₃.

A $^1$H NMR sample of (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a (20.2 mg, 0.13 mmol) in deuterated chloroform, CDCl₃ (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 CDCl₃

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)⁴</th>
<th>(±)-117b (%)⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1 h 23 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>5 h 46 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>11 h 30 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>3 days</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>7 days</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>17 days</td>
<td>72</td>
<td>28</td>
</tr>
</tbody>
</table>

⁴ 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 – $(\pm)$-cis-2-methyl-2-nitrocyclohexanol $(\pm)$-117a and NEt$_3$ (2.0 eq.). Recorded in CDCl$_3$ at 300 MHz.

$^1$H NMR spectroscopic investigation: $(\pm)$-trans-2-Methyl-2-nitrocyclohexanol $(\pm)$-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 $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-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 – $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117b and triethylamine (2.0 eq.) in vinyl acetate at 40 °C

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>$(\pm)$-117a (%)$^a$</th>
<th>$(\pm)$-117b (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>3 days</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ 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

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)$^a$</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida antarctica B (immob)</td>
<td>72 h</td>
<td>31</td>
<td>cis-117a (%)$^a$</td>
<td>trans-117b (%)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[ee (%)]$^b$</td>
<td>[ee (%)]$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[65]</td>
<td>[58]</td>
</tr>
</tbody>
</table>

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-diazabicyclo[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 CDCl₃ or TBME was prepared.

5.6.4.2.1 ¹H NMR study - Evidence for dynamic interconversion

¹H NMR spectroscopic investigation: (±)-cis-2-Methyl-2-nitrocyclohexanol (±)-117a and DBU in CDCl₃.

Two ¹H NMR samples of (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a (20.3 mg, 0.13 mmol) in deuterated chloroform, CDCl₃ (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 ¹H 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 CDCl₃

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>33 min</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>1 h 8 min</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>2 h 4 min</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3 h 31 min</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>4 h 52 min</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>7 h 17 min</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>9 h</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>24 h 45 min</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>32 h 47 min</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>73 days</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>

a. 6-Nitroheptanal 118 was not detected in the ¹H NMR spectra.
Table 5.35: Evidence for dynamic interconversion – (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a and DBU (0.05 eq.) in CDCl₃

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h 11 min</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>18 h 14 min</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>25 h 49 min</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>7 days</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>

a. 6-Nitroheptanal 118 was not detected in the ¹H NMR spectra.

¹H NMR spectroscopic investigation: (±)-trans-2-Methyl-2-nitrocyclohexanol (±)-117b and DBU (0.1 eq.) in CDCl₃.
A ¹H NMR sample of (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b (22.2 mg, 0.14 mmol) in deuterated chloroform, CDCl₃ (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 ¹H 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 CDCl₃

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>24 min</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>1 h 12 min</td>
<td>11</td>
<td>89</td>
</tr>
<tr>
<td>4 h 16 min</td>
<td>24</td>
<td>76</td>
</tr>
<tr>
<td>4 h 49 min</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>5 h 51 min</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>21 h 52 min</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>14 days</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>

a. 6-Nitroheptanal 118 was not detected in the ¹H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)*</th>
<th>Alcohol (±)-117</th>
<th>Acetate (±)-119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis (±)-117a (%)*</td>
<td>trans (±)-117b (%)*</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>24 h</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>-</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>24 h</td>
<td>-</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>-</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>-</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>24 h</td>
<td>-</td>
<td>11</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>-</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>-</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>24 h</td>
<td>48</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>50</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71 h</td>
<td>55.5</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>24 h</td>
<td>13</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 h</td>
<td>93</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 h</td>
<td>91</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

* The conversions were determined by $^1$H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.
\textbf{1H 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 \mu L, 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 \textsuperscript{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.38.

<table>
<thead>
<tr>
<th>Acetylating agent</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)\textsuperscript{a}</th>
<th>Alcohol (\pm)-117</th>
<th>Acetate (\pm)-119</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Acetate</td>
<td>24 h</td>
<td>-</td>
<td>29 (\pm)-117a</td>
<td>48 (\pm)-119a</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>-</td>
<td>30 (\pm)-117b</td>
<td>70 (\pm)-119b</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The conversions were determined by \textsuperscript{1}H NMR spectroscopy and are derived from integration of the mixture of the crude material not mass recovery.

5.6.4.2.3 \textsuperscript{1}H NMR study - Evidence for chemical acetylation in the dynamic interconversion process

\textbf{1H NMR spectroscopic investigation: (\pm)-trans-2-Methyl-2-nitrocyclohexanol (\pm)-117b and DBU (0.1 eq.) in vinyl acetate}

DBU (0.1 eq., 3.9 \mu L, 4.02 mg, 0.03 mmol) was added in one portion to a stirred solution of (\pm)-trans-2-methyl-2-nitrocyclohexanol (\pm)-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 \textsuperscript{1}H NMR (300 MHz) spectroscopy and the results summarised in Table 5.39.
5.6.4.2.4 $^1$H NMR study - One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol ($\pm$)-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 5.39: Evidence for chemical acetylation - $(\pm)$-trans-2-methyl-2-nitrocyclohexanol $(\pm)$-117b and DBU (0.1 eq.) in vinyl acetate

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>Alcohol $(\pm)$-117$^b$</th>
<th>Acetate $(\pm)$-119$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trans ($\pm$)-117b (%)</td>
<td>cis ($\pm$)-117a (%)</td>
</tr>
<tr>
<td>24 h</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>48 h</td>
<td>31</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) 6-Nitroheptanal 118 was not detected in the $^1$H NMR spectra.
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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>DBU (eq.)</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)</th>
<th>Alcohol (±)-117 cis-117a (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>trans-117b (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Acetate (±)-119 cis-119a (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>trans-119b (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>48 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>CAL-B (immob)</td>
<td>-</td>
<td>72 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.10</td>
<td>48 h</td>
<td>-</td>
<td>28 (±)</td>
<td>36 (±)</td>
<td>36 (±)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>P. stutzeri</td>
<td>0.05</td>
<td>48 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>CAL-B (immob)</td>
<td>0.10</td>
<td>72 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>P. stutzeri</td>
<td>0.10</td>
<td>48 h</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>P. stutzeri</td>
<td>0.20</td>
<td>48 h</td>
<td>77</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>P. stutzeri</td>
<td>0.50</td>
<td>48 h</td>
<td>-</td>
<td>6 [&gt;98]</td>
<td>4 [&gt;98]</td>
<td>37 [1]</td>
<td>53 [1]</td>
</tr>
<tr>
<td>9</td>
<td>P. stutzeri</td>
<td>1.00</td>
<td>48 h</td>
<td>-</td>
<td>6 [&gt;98]</td>
<td>3 [&gt;98]</td>
<td>53 [0]</td>
<td>38 [0]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by <sup>1</sup>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

<sup>1</sup>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 <sup>1</sup>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

![Chemical structure](image)

| Entry | Base                | pK<sub>a</sub> | Reaction Time | Aldehyde 118 (%)<sup>a</sup> | Alcohol (±)-117 (%)<sup>a</sup> | Acetate (±)-119 (%)<sup>a</sup> |
|-------|---------------------|----------------|---------------|------------------------------|---------------------------------|---------------------------------
| 1     | DEA (2.0 eq.)       | 11.3           | 24 h          | 83                           | 8 (±)-117a (%)<sup>a</sup>      | 9 (±)-119a (%)<sup>a</sup>     |
|       |                     | (H<sub>2</sub>O)<sup>77</sup> | 48 h          | 82                           | 10 (±)-117b (%)<sup>a</sup>    | -                               |
| 2     | Piperidine (2.0 eq.)| 11.3           | 24 h          | 100                          | -                               | -                               |
|       |                     | (H<sub>2</sub>O)<sup>77</sup> | 48 h          | 100                          | -                               | -                               |
|       |                     |                 | 72 h          | 100                          | -                               | -                               |
| 3     | DABCO (2.0 eq.)     | 8.82, 2.97     | 24 h          | -                            | 39 (±)-117a (%)<sup>a</sup>    | 61 (±)-119a (%)<sup>a</sup>    |
|       |                     | (H<sub>2</sub>O)<sup>78</sup> | 48 h          | -                            | 40 (±)-117b (%)<sup>a</sup>    | 60 (±)-119b (%)<sup>a</sup>    |
|       |                     |                 | 72 h          | -                            | 39 (±)-117a (%)<sup>a</sup>    | 61 (±)-119a (%)<sup>a</sup>    |
| 4     | Hünigs Base (2.0 eq.) | 11.4          | 48 h          | 85                           | 5 (±)-117a (%)<sup>a</sup>     | 10 (±)-119a (%)<sup>a</sup>    |
|       |                     | (H<sub>2</sub>O)<sup>79</sup> | 72 h          | 79                           | 7 (±)-117b (%)<sup>a</sup>     | 14 (±)-119b (%)<sup>a</sup>    |
| 5     | 1M NaOH             | ~13.8          | 24 h          | 100                          | -                               | -                               |
|       |                     |                 | 48 h          | 100                          | -                               | -                               |
| 6     | TMG (0.1 eq.)       | 13.6           | 24 h          | 69                           | 10 (±)-117a (%)<sup>a</sup>    | 21 (±)-119a (%)<sup>a</sup>    |
|       |                     | (H<sub>2</sub>O)<sup>80</sup> | 48 h          | 6                            | 30 (±)-117b (%)<sup>a</sup>    | 57 (±)-119b (%)<sup>a</sup>    |

a. The conversions were determined by <sup>1</sup>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 <sup>1</sup>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 <sup>1</sup>H NMR study - Evidence for dynamic interconversion

<sup>1</sup>H NMR spectroscopic investigation: (±)-cis-2-Methyl-2-nitrocyclohexanol (±)-117a and DABCO (2.0 eq.) in CDCl<sub>3</sub>

A <sup>1</sup>H NMR sample of (±)-cis-2-methyl-2-nitrocyclohexanol (±)-117a (21.4 mg, 0.13 mmol) in deuterated chloroform, CDCl<sub>3</sub> (0.3 mL) was prepared. A solution of DABCO (2.0 eq., 30.2 mg, 0.27 mmol) in deuterated chloroform, CDCl<sub>3</sub> (0.3 mL) was dispensed to the NMR tube agitated and analysed by <sup>1</sup>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 CDCl3

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>3 h 10 min</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>1 day</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>4 days</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>10 days</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

a. 6-Nitroheptanal 118 was not detected in the 1H NMR spectra.

5.6.4.4.2 1H NMR study – One pot hydrolase-mediated dynamic resolution of 2-methyl-2-nitrocyclohexanol (±)-117

1H NMR and chiral HPLC spectroscopic investigation: Cyclisation of 6-nitroheptanal 118 with P. stutzeri catalysed transesterification 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 1H 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)*</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis-117a (%)</td>
<td>trans-117b (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[ee (%)]a</td>
<td>[ee (%)]b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>48 h</td>
<td>75</td>
<td>4 [77]</td>
<td>11 [24]</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 1H 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 CDCl₃.

A $^1$H NMR sample of (±)-trans-2-methyl-2-nitrocyclohexanol (±)-117b (15.6 mg, 0.10 mmol) in deuterated chloroform, CDCl₃ (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 CDCl₃

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>(±)-117a (%)</th>
<th>(±)-117b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49 min</td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>5 h 7 min</td>
<td>26</td>
<td>74</td>
</tr>
<tr>
<td>3 days</td>
<td>28</td>
<td>72</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme Source</th>
<th>TMG</th>
<th>Reaction Time</th>
<th>Aldehyde 118 (%)</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Candida antarctica B</strong> (immob)</td>
<td>2 eq.</td>
<td>5 days</td>
<td>7</td>
<td>26</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td><strong>Pseudomonas stutzeri</strong></td>
<td>2 eq.</td>
<td>48 h</td>
<td>15</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[35]</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[9]</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[4]</td>
<td>[80]</td>
</tr>
</tbody>
</table>

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]jundec-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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer-bound DBU (eq.)</th>
<th>Reaction Time</th>
<th>Temp (°C)</th>
<th>Aldehyde 6-nitroheptanal 118 (%)(^a)</th>
<th>Alcohol (±)-117cis (%)(^a)</th>
<th>Alcohol (±)-117trans (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>13 h</td>
<td>Ambient</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>22.5 h</td>
<td>Ambient</td>
<td>92</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.0 – 2.0(^a)</td>
<td>44 h</td>
<td>40 °C</td>
<td>11</td>
<td>24</td>
<td>65</td>
</tr>
</tbody>
</table>

\(^a\) 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer-bound DBU</th>
<th>Reaction Time</th>
<th>(±)-117b (%)</th>
<th>(±)-117a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 eq.</td>
<td>19 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 eq.</td>
<td>19 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. The crude 1H 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 1H 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 1H 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 1H 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 1H 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 (1R,2R)-119b (10.4 mg, 3%) as a clear oil [α]D20 +56.4 (c 0.4, CHCl3), >98% ee. 1H 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 acetate (1R,2S)-119a, >98% ee and (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-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 acetate (1R,2S)-119a, >98% ee and (1R,2R)-trans-2-methyl-2-nitrocyclohexyl acetate (1R,2R)-119b, >98% ee (3.1 mg, 0.8%) as clear oil.

The third most polar fraction was a 24 : 76 mixture of (1S,2R)-cis-2-methyl-2-nitrocyclohexanol (1S,2R)-117a, 10% ee and (1S,2S)-trans-2-methyl-2-nitrocyclohexanol (1S,2S)-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

<table>
<thead>
<tr>
<th>Step</th>
<th>DBU (eq.)</th>
<th>Vinyl acetate (eq.)</th>
<th>Candida antarctica B (immob) (% w/w)</th>
<th>Alcohol 117</th>
<th>Acetate 119</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cis-117a (%)</td>
<td>trans-117b (%)</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>30 (±)</td>
<td>70 (±)</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>138</td>
<td>18</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>41</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>138</td>
<td>18</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>138</td>
<td>18</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>22</td>
</tr>
</tbody>
</table>

a. The conversions were determined by 1H 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

42. McNamara, O. *Ph.D. Thesis, National University of Ireland, Cork* **2009**.
44. Moody, T. "Almac Sciences: Hydrolase Enzymes - Kinetic resolution of esters, acids and alcohols" **2008**.
76. X-ray structure determination was conducted by Dr. S. E. Lawrence and Dr. K. S. Eccles, Deparment of Chemistry, UCC. **2012**.
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)-I and (S)-I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column</th>
<th>Injection volume (μL)</th>
<th>Flow (mL/min)</th>
<th>λ Max (min)</th>
<th>Mobile phase (hexane : IPA)</th>
<th>Temp (°C)</th>
<th>Retention time Enantiomer</th>
<th>min</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>Chiralcel OJ-H</td>
<td>2</td>
<td>0.40</td>
<td>220.0</td>
<td>Gradient 0 min: 96 : 4 30 min: 99 : 1</td>
<td>Rt</td>
<td>(1S,2R,3S)-4a</td>
<td>58</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.80</td>
<td>220.0</td>
<td></td>
<td>rt</td>
<td>(2S,3R)-2</td>
<td>232</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 93 : 7</td>
<td>rt</td>
<td>(S)-11</td>
<td>78</td>
</tr>
<tr>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td></td>
<td>rt</td>
<td>(R)-11</td>
<td>99</td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>Chiralcel OD-H</td>
<td>8</td>
<td>0.50</td>
<td>220</td>
<td></td>
<td>rt</td>
<td>(R)-18</td>
<td>96</td>
</tr>
<tr>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>Chiralcel OD-H</td>
<td>8</td>
<td>0.50</td>
<td>220</td>
<td></td>
<td>rt</td>
<td>(S)-18</td>
<td>105</td>
</tr>
</tbody>
</table>
Hydrolase-mediated kinetic resolutions of 3-aryllalkanoic acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column</th>
<th>Injection volume (μl)</th>
<th>Flow (mL/min)</th>
<th>λ Max (min)</th>
<th>Mobile phase (hexane : IPA)</th>
<th>Temp (°C)</th>
<th>Retention time Enantiomer min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-38</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 95 : 5</td>
<td>0</td>
<td>(R)-38 28</td>
</tr>
<tr>
<td>(±)-23</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 99 : 1</td>
<td>0</td>
<td>(S)-38 33</td>
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<tr>
<td>(±)-56</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 99 : 1</td>
<td>0</td>
<td>(R)-56 20</td>
</tr>
<tr>
<td>(±)-28</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 98 : 2</td>
<td>0</td>
<td>(S)-28 61</td>
</tr>
<tr>
<td>(±)-58</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 98 : 2</td>
<td>0</td>
<td>(R)-58 28</td>
</tr>
<tr>
<td>(±)-29</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 96 : 4</td>
<td>20</td>
<td>(S)-29 58</td>
</tr>
<tr>
<td>(±)-37</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 96 : 4</td>
<td>20</td>
<td>(R)-37 45</td>
</tr>
<tr>
<td>(±)-49</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.50</td>
<td>211.0</td>
<td>IPA mobile phase consists of 3% TFA 99.5 : 0.5</td>
<td>0</td>
<td>(S)-49 30</td>
</tr>
<tr>
<td>(±)-51</td>
<td>Chiralcel OJ-H</td>
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<td>0.50</td>
<td>211.0</td>
<td>IPA mobile phase consists of 3% TFA 99.5 : 0.5</td>
<td>0</td>
<td>(R)-49 30</td>
</tr>
<tr>
<td>Compound</td>
<td>Column</td>
<td>Injection volume (μl)</td>
<td>Flow (mL/min)</td>
<td>λ Max (min)</td>
<td>Mobile phase (hexane : IPA)</td>
<td>Temp (°C)</td>
<td>Retention time</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>-----------------------</td>
<td>---------------</td>
<td>-------------</td>
<td>-----------------------------</td>
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</tr>
<tr>
<td>(±)-54</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 98 : 2</td>
<td>0</td>
<td>(R)-54 36</td>
</tr>
<tr>
<td>(±)-65</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 94 : 6</td>
<td>0</td>
<td>(S)-54 40</td>
</tr>
<tr>
<td>(±)-53</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 82 : 18</td>
<td>0</td>
<td>(S)-53 45</td>
</tr>
<tr>
<td>(±)-64</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>216.9</td>
<td>IPA mobile phase consists of 3% TFA 99.7 : 0.3</td>
<td>0</td>
<td>(R)-64 34</td>
</tr>
<tr>
<td>(±)-60</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>256.0</td>
<td>IPA mobile phase consists of 3% TFA 94 : 6</td>
<td>0</td>
<td>(S)-60 51</td>
</tr>
<tr>
<td>(±)-66</td>
<td>Chiralcel OJ-H</td>
<td>10</td>
<td>0.25</td>
<td>256.0</td>
<td>IPA mobile phase consists of 3% TFA 99.7 : 0.3</td>
<td>0</td>
<td>(S)-66 42</td>
</tr>
<tr>
<td>(±)-55</td>
<td>Chiralcel AS-H</td>
<td>10</td>
<td>0.25</td>
<td>256.0</td>
<td>IPA mobile phase consists of 3% TFA 94 : 6</td>
<td>0</td>
<td>(R)-80 49</td>
</tr>
<tr>
<td>(±)-80</td>
<td>Chiralcel AS-H</td>
<td>10</td>
<td>0.25</td>
<td>256.0</td>
<td>IPA mobile phase consists of 3% TFA 99.7 : 0.3</td>
<td>0</td>
<td>(S)-80 34</td>
</tr>
<tr>
<td>(±)-47</td>
<td>Chiralcel OJ-H</td>
<td>2</td>
<td>0.20 - 1.00</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 99.5 : 0.5</td>
<td>45</td>
<td>(R)-47 38</td>
</tr>
<tr>
<td>(±)-32</td>
<td>Chiralcel OJ-H</td>
<td>2</td>
<td>0.20 - 1.00</td>
<td>209.8</td>
<td>IPA mobile phase consists of 3% TFA 99.5 : 0.5</td>
<td>45</td>
<td>(S)-32 57</td>
</tr>
<tr>
<td>Compound</td>
<td>Column</td>
<td>Injection volume (μL)</td>
<td>Flow (mL/min)</td>
<td>λ Max (min)</td>
<td>Mobile phase (hexane : IPA)</td>
<td>Temp (°C)</td>
<td>Retention time</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-----------------------</td>
<td>--------------</td>
<td>-------------</td>
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IPA mobile phase consists of 3% TFA 95 : 5
IPA mobile phase consists of 3% TFA 99.5 : 0.5
IPA mobile phase consists of 3% TFA 99.5 : 0.5
The use of co-crystals for the determination of absolute stereochemistry of the products of preparative-scale reactions

(±)-3-(3-Methylphenyl)butanoic acid (±)-65

Additional peaks due to trace (±)-ethyl 3-(3-methyl phenyl)butanoate (±)-5

(±)-3-(3-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96

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Chiral HPLC conditions for (±)-3-(3-methylphenyl)butanoic acid (±)-65 and (±)-3-(3-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-96

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(±)-3-(2-Methylphenyl)butanoic acid (±)-64

![Graph for (±)-3-(2-Methylphenyl)butanoic acid (±)-64]

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(±)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95

![Graph for (±)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95]

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(S)-3-(2-Methylphenyl)butanoic acid : isonicotinamide co-crystal (S)-95

Chiral HPLC conditions for (±)-3-(2-methylphenyl)butanoic acid (±)-64 and (±)-3-(2-methylphenyl)butanoic acid : isonicotinamide co-crystal (±)-95

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(±)-3-(4-Fluorophenyl)butanoic acid (±)-80

(±)-3-(4-Fluorophenyl)butanoic acid : isonicotinamide co-crystal (±)-97
Chiral HPLC conditions for (±)-3-(4-fluorophenyl)butanoic acid (±)-80 and (±)-3-(4-fluoro phenyl)butanoic acid: isonicotinamide co-crystal (S)-97

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(±)-3-Phenylbutanoic acid (±)-23

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\text{\textcopyright} & \quad \text{\textcopyright} \\
\text{Phenylbutanoic acid} & \quad \text{Phenylbutanoic acid}
\end{align*}
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(±)-3-Phenylbutanoic acid : isonicotinamide co-crystal (±)-94

\[
\begin{align*}
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(S)-3-Phenylbutanoic acid : isonicotinamide co-crystal (S)-94

Chiral HPLC conditions for (±)-3-Phenylbutanoic acid (±)-23 and (±)-3-Phenylbutanoic acid : isonicotinamide co-crystal (±)-94

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**Dynamic kinetic resolution of the intramolecular nitroaldol reaction through lipase catalysis**

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(±)-119a: (1R,2S)-119a 12 min
(±)-117a: (1S,2R)-117a 17 min
(±)-119b: (1R,2S)-119b 18 min
(±)-117b: (1S,2S)-117b 37 min
(±)-117b: (1R,2S)-117b 43 min
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
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  millimole
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
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<tr>
<td>tosyl</td>
<td>toluenesulfonyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
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</table>

Appendix III Publications

Lipase catalysed kinetic resolutions of 3-aryl alkanoic acids
Rebecca E. Deasy, Maude Brossat, Thomas S. Moody, Anita R. Maguire

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

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