# Biocatalysis in organic synthesis


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This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

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Aoife M. Foley

January 2019

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*To my family*

# Abstract

The first chapter provides an overview of the use of biocatalysis for the synthesis of pharmaceutical intermediates and natural products. This review focuses, in particular, on the impact of recent developments in technologies which enable the increased use of biocatalysts. These developments include:

- the immobilisation of biocatalysts to enhance stability and ease of use, and enabling use in combination with metal and organocatalysts leading to dynamic kinetic resolution, as well as in continuous flow.

- discovery and development of novel enzymes using molecular biology for enzyme engineering and metagenomics.

The second chapter describes the lipase-mediated kinetic resolution of 2-phenylalkanols by tuning the steric properties of the acyl group to control the efficiency and selectivity of the resolution. In contrast to literature reports, efficient resolution was achieved using short-chain acyl groups through careful process control and substrate modification. The effect of increased steric demand at the stereocentre was also explored.

Chapter three describes proof of concept that a hydrolase-catalysed dynamic kinetic resolution of a lactol is possible. By taking advantage of the spontaneous racemisation of the unreacted starting material, the dynamic kinetic resolution was carried out without the need for a separate racemisation catalyst. While the kinetic resolution was effective in a model system, the biotransformation was inhibited by the introduction of a second, remote stereocentre.

Chapter four describes a dynamic kinetic resolution of synthetically versatile nitroalcohols by combination of a lipase-mediated resolution and the reversible intramolecular nitroaldol (Henry) reaction. Significant challenges in effecting the combination of the base-mediated racemisation step and the lipase-mediated resolution step were encountered. Reaction engineering allowed design of a sequential one-pot reaction system which furnished the products with excellent enantioselectivity, and good diastereoselectivity.

Chapter five describes the use of novel transaminases in the kinetic resolution of model amine substrates and pharmaceutical intermediates by oxidative deamination, including exploration of the substrate scope of these novel biocatalysts. While transaminase-mediated reductive amination is an attractive method for asymmetric synthesis of chiral amines, the reductive amination is

generally thermodynamically disfavoured. Preliminary investigation of approaches to favour the reductive amination is described.

The final chapter contains the full experimental details, including spectroscopic and analytical data of all the compounds synthesised in this project; details of chiral phase HPLC analysis are included in the appendix.

"Creativity is combining facts no one else has connected before."

Christiane Nüsslein-Volhard, 1995 Nobel Prize in Physiology or Medicine.

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# Chapter 1

# Introduction

# Contents

# Overview

This literature review will cover various topics. The use of biocatalysts is particularly well suited to the synthesis of pharmaceutical intermediates because of their excellent selectivity and their ability to work under mild conditions.

Recent technological developments facilitate the increased use of biocatalysis, for example immobilisation, which will be discussed here as an enabling technology, which can impart increased stability and potential for reuse of biocatalysts. The potential for the use of biocatalysis in continuous flow is very significant and their combination will be discussed here. Dynamic kinetic resolution using immobilised enzymes in combination with metal racemisation catalysts, and with enzymatic racemisation catalysts will be discussed.

The other major technological advance which has impacted the use of biocatalysis is the use of molecular biology techniques for biocatalyst discovery and modification to lead to designer biocatalysts with optimised outcomes relative to use of wild-type enzymes.

In any particular instance, when a biotransformation step is embedded in a pharmaceutical process, the background of the researchers will have a large effect on the biocatalytic development route chosen: molecular biology, changing the biocatalyst to suit the substrate or reaction conditions for making the catalyst better from the start, or process development, changing the substrates or reaction conditions to suit the enzyme e.g. immobilisation, temperature change, reaction mode or solvents. In each case, the recent advances in technology have enabled biocatalysis to be an efficient tool for large scale synthesis.

The use of enzyme engineering, and recombinant enzymes has led to multiple enzymes expressed in the same cell system. Some recent examples of biocascades will briefly be discussed.

## 1.1 Introduction

Enzymes, by their nature, have excellent chemo-, regio- and enantioselectivity, which, if utilised with the right substrate can give superior results to chemical catalysis.[1-3] This makes them especially attractive in the pharmaceutical industry where a growing number of drugs are sold as single enantiomers.[3-5] In addition to this, enzyme-catalysed reactions are normally carried out under mild conditions, usually around room temperature, and neutral pH, and can avoid the use of metal catalysts, although they can also be combined with metal catalysis in a dynamic system.[6-8] For these reasons biocatalytic processes can be less hazardous, less polluting, and less energy consuming than traditional reactions. Enzymes can be very efficient catalysts, in fact some of the fastest known reactions are those catalysed by enzymes.[1] This allows reactions which would otherwise be too slow to be useful to be utilised in synthesis. The native environment for most enzymes is aqueous which moves them even further into the realm of green catalysts.

The resolution of racemic compounds can broadly be divided into 4 categories: preferential crystallisation; diastereomeric crystallisation; chromatography; and kinetic resolution. Kinetic resolution can be described as a process where the two enantiomers of a racemic mixture are transformed to products at different rates and is usually achieved by interaction with a chiral molecule (a catalyst, ligand or biocatalyst). Kinetic resolution is effective when the rate of reaction of one enantiomer is much faster than that of the other. A disadvantage of this is the theoretical maximum yield of 50%. A more desirable form of kinetic resolution is dynamic kinetic resolution (DKR). In DKR the slow reacting compound is eventually converted into the desired enantiomer (see Scheme 1.26). This can be achieved by several methods: in situ racemisation; stereoinversion; and substrate recycling. There are many examples of dynamic kinetic resolution, some of which use metal catalysts or chemical means to racemise the unreacted enantiomer.[7] A major consideration is the effect of the racemisation conditions on the performance and stability of the biocatalyst under the racemisation conditions.

### 1.1.1 Biocatalysis

Biocatalysis employs enzymes in semi-purified, purified, or immobilised forms, or as a whole cell system to promote chemical reactions. Enzymes themselves are extraordinary catalysts and as previously mentioned they usually operate at mild conditions. Their excellent regioselectivity means that in many cases there is no need for protecting groups during the

transformation. Many enzymes can accept unnatural substrates, and the use of protein engineering means the substrate scope can be expanded even further. Biocatalysis complies with the 12 Principles of Green Chemistry and rate enhancements of up to $10^{12}$ are possible.[1,9-11] However, like all proteins, under conditions that deviate from the optimum they can become unstable, and denature. Although hydrolases do not require a cofactor, a drawback of some other classes of enzymes (e.g. oxidoreductases) is the need for a cofactor, and the necessity of cofactor regeneration which adds complexity to their use in synthesis.

### 1.1.2 Classes of enzymes

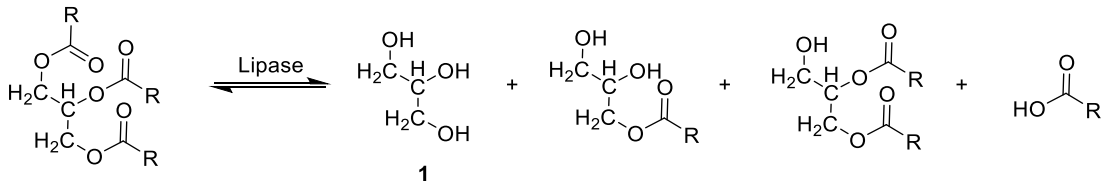
Enzymes are highly complex molecules, and the structures of only a small number are known; through sequence homology and molecular modelling it is possible to rationalise reasonable models where the structure is not known. For this reason, enzymes are classified according to the reaction they catalyse using an Enzyme Commission (EC) number. There are six common classes of enzymes, and it must be noted that enzymes may fall into more than one of these classes, as a result of being able to carry out multiple transformations, and similarly enzymes in the same class may differ vastly in their structure. Enzymes can catalyse both the forward and reverse reactions with both the substrate and the product fitting into the active site. For example, lipases have been known to catalyse both esterification and hydrolysis. The six main classifications of enzymes are: oxidoreductases (EC 1.X), transferases (EC 2.X), hydrolases (EC 3.X), lyases (EC 4.X), isomerases (EC 5.X) and ligases (EC 6.X). Oxidoreductase enzymes catalyse the transfer of electrons from one molecule to another, i.e. redox reactions; these enzymes usually require NAD(P)H or $NAD^+$ as cofactors. Transferases, such as transaminases, catalyse the transfer of a specific group from one molecule (the donor) to another molecule (the acceptor). Hydrolases catalyse the hydrolysis of compounds. Lyases catalyse bond breaking within molecules by means other than hydrolysis. Isomerases interconvert molecules to their isomers, a common example of this is racemase, which can be used in the racemisation of compounds. Ligases catalyse the formation of bonds between molecules.[2]

Hydrolases are by far the most commonly employed biocatalysts for industrial biotransformations.[12,13] Hydrolases have the classification EC 3 and can be further subdivided according to the type of bond being hydrolysed. EC 3.1 enzymes hydrolyse ester bonds, and enzymes can be sub classified to give a 4-digit EC number. Lipases, also called triacylglycerol ester hydrolases, have the classification EC 3.1.1.3.

Different classes of enzymes have been shown to employ different amino acid residues in catalysis, and the amino acid function in the active site has been classified into seven types.[14,15] The seven functions are: stabilization; steric roles; activation by affecting the $pK_a$; proton shuttling; electron shuttling; and covalent catalysis. Depending on the specific reaction being catalysed, different residues are more likely to be present in the active site.

### 1.1.2.1 Lipases

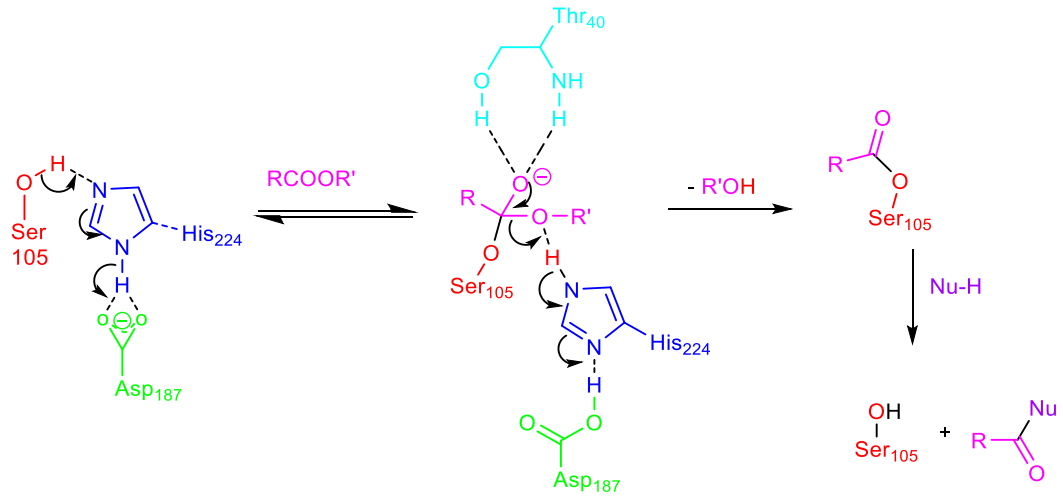
Lipases are ubiquitous enzymes belonging to the family of serine hydrolases and can be found in animals, plants, bacteria and fungi.[16] Lipases naturally catalyse the reversible hydrolysis (cleavage) of the ester bonds in triacylglycerol producing the free fatty acids, glycerol **1**, monoacylglycerol, and diacylglycerol (Scheme 1.1). Lipases are the most frequently used enzymes in biocatalysis, due in part to being inexpensive, commercially available, and stable, but also due to their wide substrate scope and high regio-, stereo- and chemo selectivity; they are also very useful because they work on the lipid-water interface, a phenomenon known as interfacial activation, thus increasing their substrate scope beyond water soluble compounds.[17-20] This affinity for hydrophobic media is what differentiates lipases from other hydrolytic enzymes. Hydrolases are commonly identified by the so-called catalytic triad; three residues (serine, aspartate/glutamate and histidine) which are highly conserved in the active site which are responsible for the hydrolytic activity.[21-24] This is in spite of the fact that the structures, chain lengths etc. can vary greatly. For example, *C. rugosa* lipase has more than 500 amino acids and *C. antarctica* lipase B has 317 amino acids but they both have the catalytic triad present in their core. Another major advantage of hydrolases, in comparison to other enzymes, such as oxidases and transferases, is that hydrolases do not require a cofactor; this makes them more attractive than other enzyme classes due to their ease of use.




*Scheme 1.1 Lipases naturally catalyse the reversible hydrolysis of triacylglycerols*

The hydrolase 3D structure shows a characteristic alpha/beta hydrolase fold which is a beta sheet (central) surrounded by a variable number of alpha helices.[20,25,26] As well as the catalytic triad, there is thought to be oxanion-stabilising residues in the active site. The catalytic triad passes protons between its constituent parts and the substrate involved and essentially

immobilises and activates it for attack by a nucleophile. The absence of one of these residues can dramatically reduce the reactivity of the active site, and thus enzyme activity.[21,22] The reactant ester forms a tetrahedral acyl-enzyme intermediate (Scheme 1.2). The tetrahedral intermediate collapses to form a serinate ester, resulting in loss of the alcohol by-product. The acyl-enzyme intermediate then reacts with the nucleophile and affords the product. The excess of negative charge following the first acyl transfer step is stabilized by an oxyanion hole. The oxyanion hole stabilizes the transition state and the anionic intermediate, and is made up of a special arrangement of three hydrogen bond donors: Thr40, which contributes through its backbone and side chain, and Gl106, which contributes through the backbone only (Scheme 1.2).[23,24]



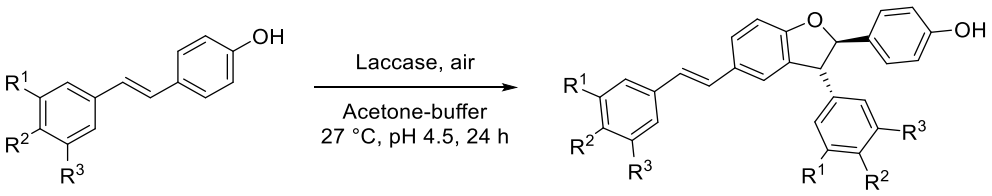

*Scheme 1.2 Mechanism of acylation by the catalytic triad. Adapted from van Rantwijk & Sheldon[27]*

## 1.1.3 Examples of enzymes in pharmaceutical & natural product synthesis

There have been several reviews in the past 10 years of the applications of biocatalysts in the synthesis of drug products and intermediates.[5,11,28-35]

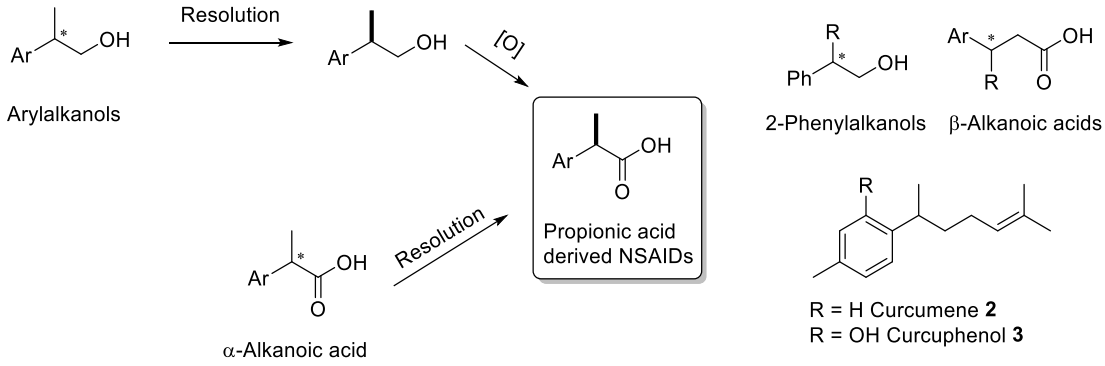
Biocatalytic retrosynthesis is a relatively new concept, pioneered by Turner et al., which recognises the need for the integration of biocatalysis in retrosynthetic analysis, choosing disconnections with consideration for reactions which can be catalysed by enzymes.[36-41] There have been a number of reported total syntheses using biocatalysis either as a single resolution step, or a multistep sequence. More importantly, the use of biocatalysts in the drug discovery stage has significant potential. For example recently the use of a laccase-mediated enantioselective oxidation was reported in the synthesis of a series of dihydrobenzofurans for in vitro testing (Scheme 1.3).[42] Biocatalysis is particularly suited to this use as the essentially

by-product free reactions means that a lengthy purification is not necessary; a biological oxidant means that the use of costly metal and/or chiral oxidants can be avoided.[43]

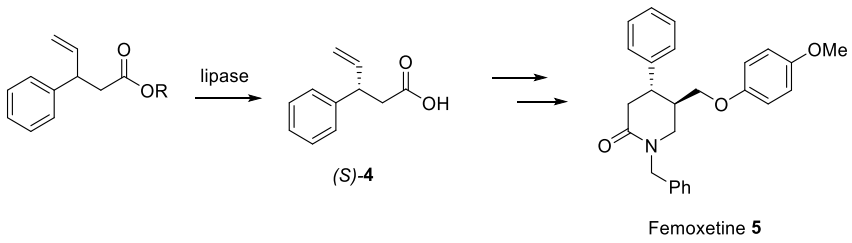



*Scheme 1.3 Biocatalysed medicinal chemistry route to highly substituted dihydrobenzofurans*

Lipases have been used for the resolution of propanoic acid derived non-steroidal anti-inflammatory drugs through both the resolution of the 2-arylalkylalcohol precursors and the resolution of the α-alkyl carboxylic acids themselves (Scheme 1.4).[44-46] Structurally related compounds, 2-phenylalkanols and 3-arylalkanoic acids (also called β-aryl alkanoic acids) have previously been resolved using lipases by our group.[47-49] Their resolution and use for the synthesis of natural products *(R)*- and *(S)*-curcumene **2** and curcuphenol **3** has also been reported (Scheme 1.4)[50] A related compound, alkenoic acid **4** has been resolved and used in the chemoenzymatic synthesis of femoxetine **5** (Scheme 1.5), with one of the two stereocentres installed by lipase-catalysed resolution.[51] A tandem metal-lipase dynamic kinetic resolution was also developed for the resolution of alkenoic acid **4**.[52]

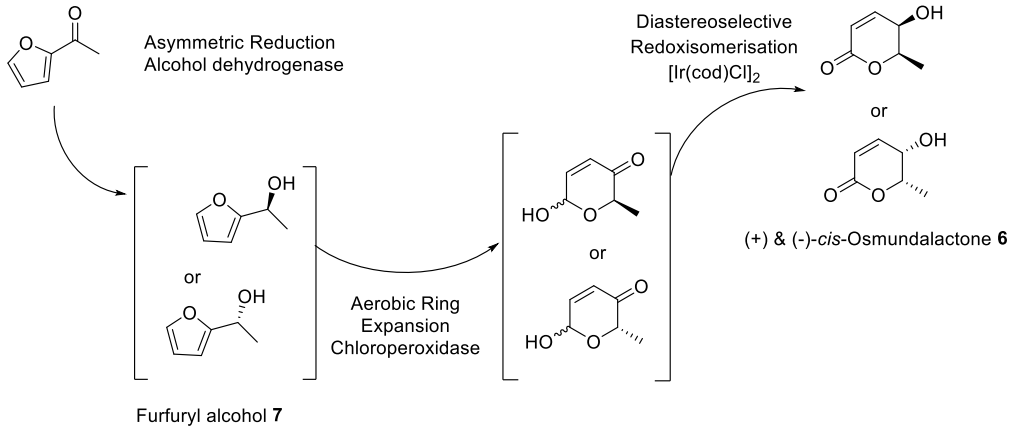



*Scheme 1.4 Use of arylalkanols and arylalkanoic acids in synthesis*



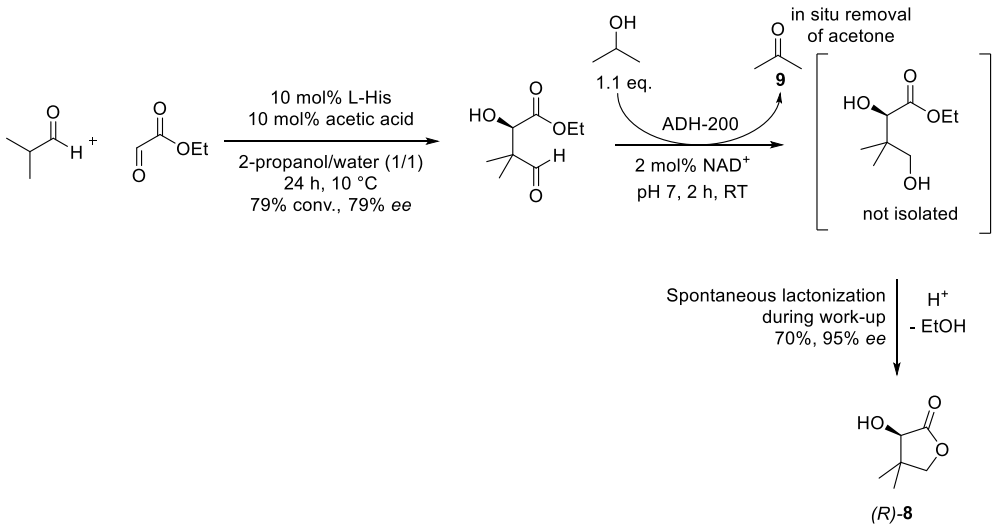

*Scheme 1.5 Synthesis of femoxetine **5***

Recently, the total synthesis of δ-lactones (+)- and (−)-*cis*-osmundalactone **6** by a three step chemoenzymatic synthesis from a readily available starting material has been described (Scheme 1.6).[53] By selecting an *(S)*- or *(R)*- selective alcohol dehydrogenase, both enantiomers of the furfuryl alcohol **7** can be accessed, which are then subjected to a biocatalysed-aerobic ring expansion using a chloroperoxidase, which can be carried out as a one-pot transformation. The lactone intermediates are then diastereoselectively reduced using a transition metal catalyst.



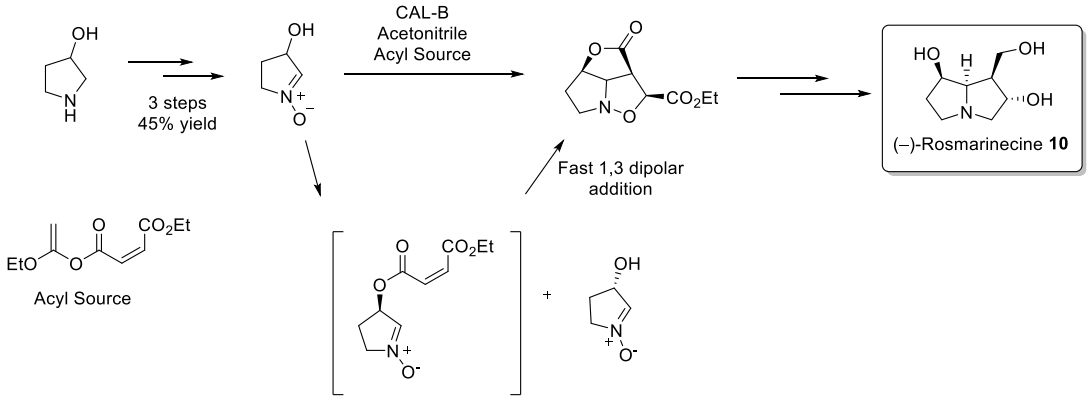

*Scheme 1.6 One-pot three enzyme cascade for the synthesis of osmundalactone **6***

The use of lipase-catalysed transformations for the synthesis of vitamins has been reviewed. The existing enzyme mediated synthesis of *(R)*-pantolactone was deemed to be inferior to chemical method described.[54] A "one-pot-like" synthesis of vitamin B6 intermediate *(R)*-pantolactone *(R)*-**8** was reported, combining an organocatalysed aldol reaction and a biocatalysed reduction, with in situ removal of the volatile by-products before the biocatalysed step and continuous (in situ) removal of the acetone **9** by-product from the biocatalysed reaction (Scheme 1.7).[55] In contrast to the other reactions discussed in this section, the stereochemistry is installed by the organocatalyst, and further enhanced by the biocatalyst.




*Scheme 1.7 Synthesis of (R)-**8** by combined chemo- and biocatalysis*

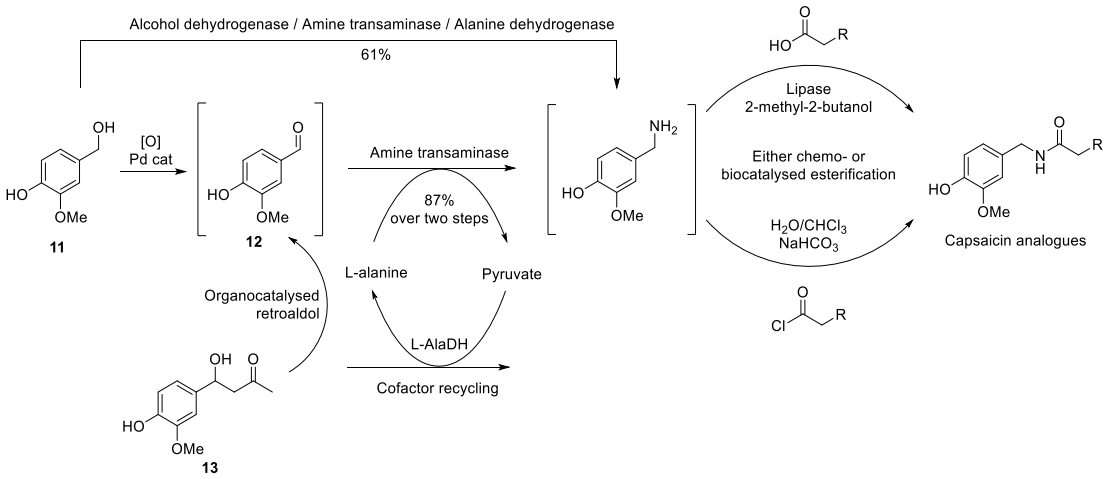
Akai et al. reported a one-pot domino biocatalytic transesterification/1,3-dipolar cycloadditon; the biocatalytic resolution is the key diastereoselective step in the synthesis of (−)-rosmarinecine **10**.[56] (Scheme 1.8) It is also the first example of a domino transesterification/1,3-dipolar cycloaddition. This is the first example of the total synthesis of (−)-**10**, which does not use chiral molecules either as starting materials or as chiral auxiliaries.




*Scheme 1.8 Synthesis of (−)-rosmarinecine **10** via a tandem transesterification/ dipolar cycloaddition*

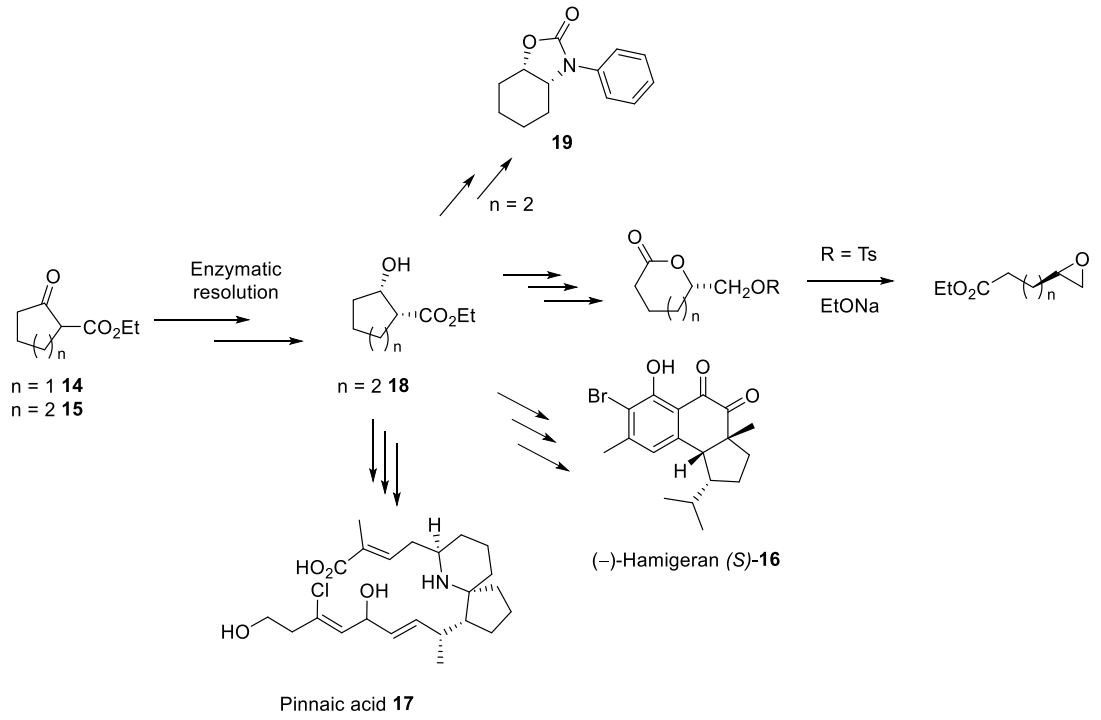
In an excellent example of the flexible use of multiple biocatalysts, Berglund et al. reported a total synthesis of capsaicin analogues from a primary alcohol **11** using a metal-catalysed oxidation to produce aldehyde **12**, followed by a one-pot amine transaminase-catalysed reductive amination and a lipase-catalysed amidification, using a fatty acid as the acyl donor (Scheme 1.9).[57] The authors also proposed the use of a biocatalysed oxidation, which could be coupled with the amine transaminase; the alcohol dehydrogenase could function as a redox partner for the regeneration of cofactors for the L-alanine dehydrogenase (L-AlaDH) as well as

the oxidation of the alcohol **11**. However, this step was less efficient than the palladium catalysed oxidation, giving 61% conversion rather than 87% conversion. In the development of this route, both the chemocatalysed steps and the biotransformations were considered, and the two methods were combined in different ways. This is an excellent example of the combination of biocatalysts and chemocatalysts in the synthesis of a natural product and its analogues. Another step considered was the retroaldol reaction of **13** to produce aldehyde **12** in situ, catalysed by the L-alanine which would already be present in the reaction mixture as it is used as a cofactor for the amine transaminase reaction.



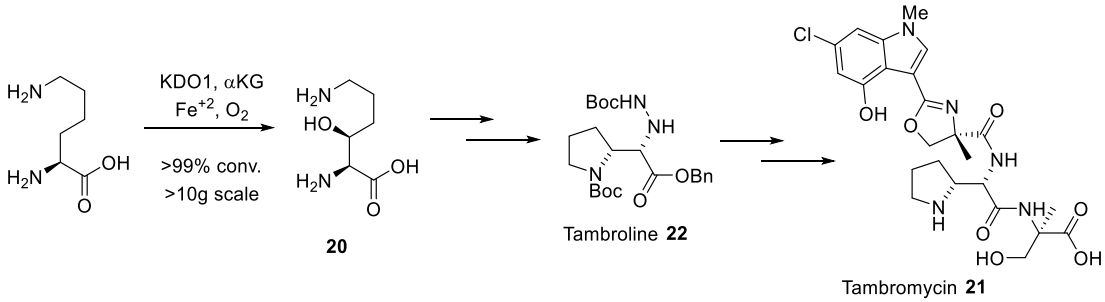

*Scheme 1.9 Combined chemo- and biocatalysed routes to capsaicin analogues*

The resolution of β-ketoesters **14** and **15** has been reported by many biocatalytic methods, including Baker's yeast and oxidoreductase reduction of the ketone, and resolution of the acetates or alcohols by lipases (Scheme 1.10).[58-68] These useful intermediates have been used in the synthesis of various pharmaceutically important compounds and intermediates and natural products such as (–)-hamigeran *(S)*-**16** [65,68] and pinnaic acid **17**.[61] The enantiopure hydroxyester **18** (n = 2) has also been used in the synthesis of 2-oxazolidinone **19** and the hydroxy esters have been used in the synthesis of lactones which can be further reacted to give chiral epoxides.[58,59,69,70]




*Scheme 1.10 Synthesis of natural products from 14 & 15*

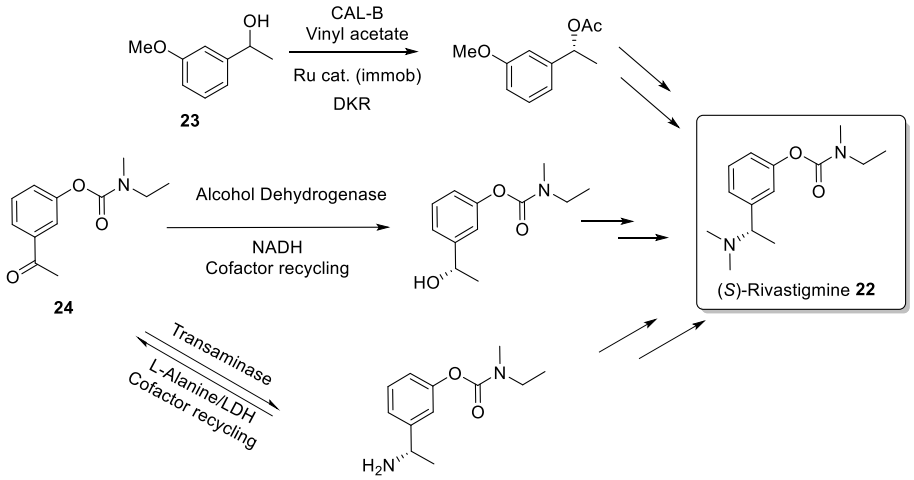
Renata et al. report the use of an overexpressed lysine hydroxylase as a clarified cell lysate, eliminating the need for costly protein purification.[71] The use of co-expressed chaperone proteins increased the expression and stability of the protein of interest. The lysine hydroxylase was used to make multigram quantities of the chiral material **20** which was further reacted to give natural product tambromycin **21** via the protected tambroline **22** (Scheme 1.11).




*Scheme 1.11 Synthesis of natural product tambromycin **21***

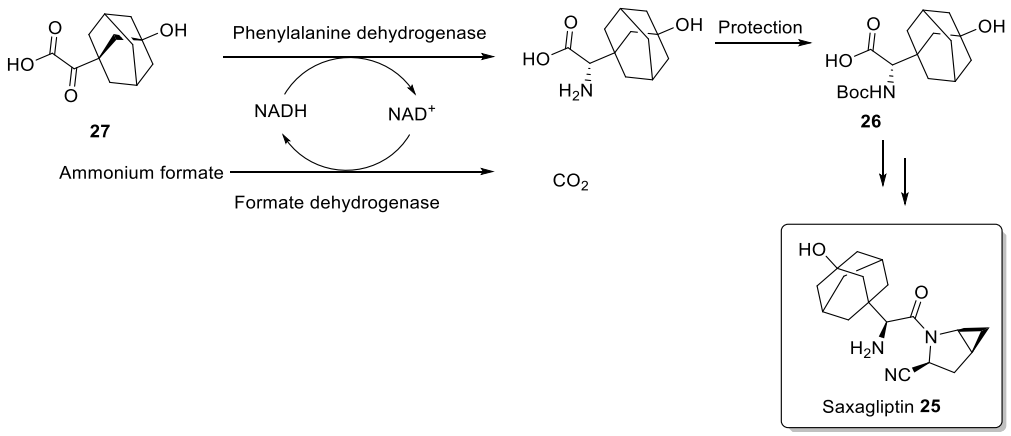
The chemoenzymatic synthesis of *(S)*-rivastigmine **22** was reported, using a lipase to impart the stereochemistry, through CAL-B-mediated transesterification of intermediate **23** (Scheme 1.12).[72] The reuse of the CAL-B in this process was also explored, where the enzyme was shown to retain activity through 6 reaction cycles. The racemisation of the alcohol **23** was carried out

using a ruthenium catalyst for a dynamic kinetic resolution, further enhancing the value of this synthesis.[73] The use of baker's yeast-derived alcohol dehydrogenase for the stereoselective reduction of the ketone **24**, has also been reported, where the biocatalyst was identified by screening a large range of commercially available biocatalysts.[74,75] A transaminase has also been used for the reductive amination of **24** and this is a more direct route to *(S)*-**22** than the alcohol dehydrogenase.[76]

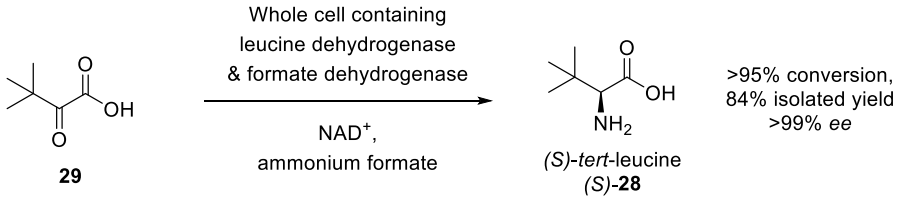



*Scheme 1.12 Synthesis of* (S)*-rivastigmine* ***22***

Saxagliptin **25** was developed by Bristol-Meyers Squibb as a treatment for diabetes.[77] A key intermediate in the synthesis of **25** is the *N*-protected glycine derivative **26**, which was prepared by reductive amination of the keto acid **27** using a mutated phenylalanine dehydrogenase, a NAD dependant transferase (Scheme 1.13).[78] The process was coupled with formate dehydrogenase for cofactor recycling. A whole cell catalyst, which co-expresses multiple enzymes can be useful for processes which require NAD dependant enzymes; the redox coupling partners can be produced in the same cell system, negating the need for an external enzyme to be added. *(S)-tert*-Leucine *(S)*-**28**, an intermediate in the synthesis of atazanavine, boceprevir and telaprevir, has been synthesised in a whole cell system producing both the required leucine dehydrogenase, which carries out the reductive amination on the keto acid **29**, and the formate dehydrogenase, for cofactor recycling (Scheme 1.14).[79]

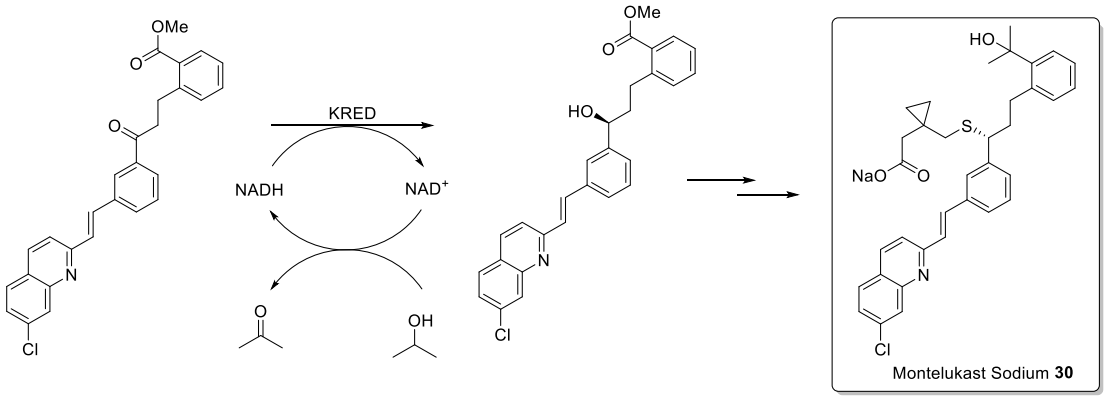



*Scheme 1.13 Synthesis of Saxagliptin **25***

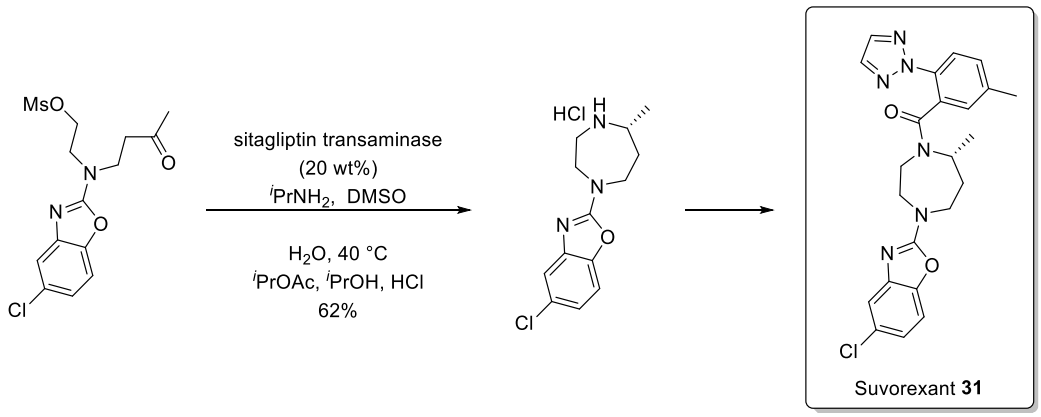



*Scheme 1.14 Synthesis of* (S)-tert *Leucine **28***

The key enantioselective step in the synthesis of montelukast **30** (Singulair®, Merck & Co.) has been achieved using an engineered ketoreductase in place of (–)-DIP-Cl and was run on a >200 kg scale (Scheme 1.15).[80,81] Another drug developed by Merck & Co., suvorexant **31**, requires only a short synthesis involving a tandem transamination and ring annulation using a mutated transaminase (Scheme 1.16) replacing the classical resolution in the previous synthetic route.[82,83]

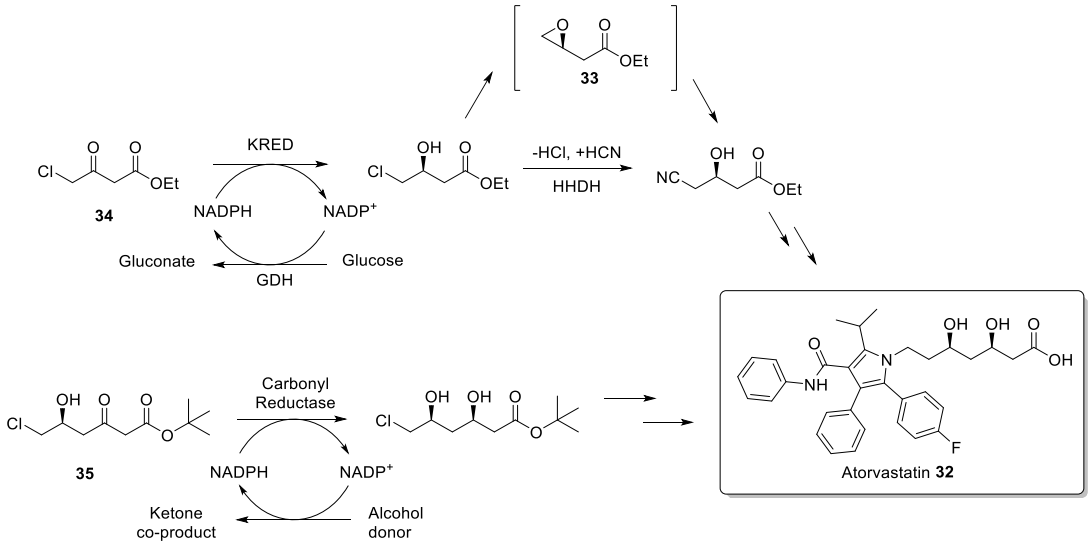



*Scheme 1.15 Synthesis of montelukast sodium **30***



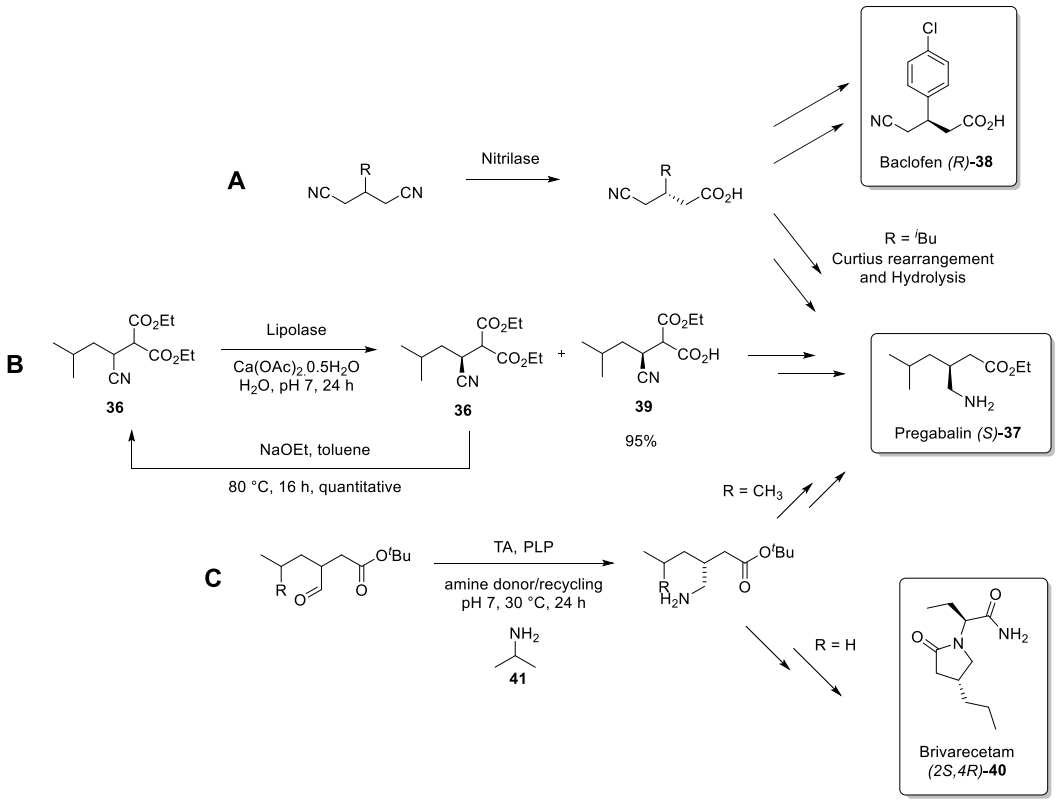

*Scheme 1.16 Synthesis of Suvorexant **31***

For atorvastatin **32** (Lipitor®) there are several intermediates which can be produced in enantiomerically pure forms using biocatalysed reactions and this area has been reviewed.[84] Since this review a two-step three enzyme process has been reported using a ketoreductase (KRED) and glucose dehydrogenase (GDH) in a redox coupling step, and a halohydrin dehydrogenase (HHDH) to carry out a functional group conversion via an intermediate epoxide **33** (Scheme 1.17).[85] The use of reductase to resolve the intermediate **34** can suffer from product inhibition, i.e. the product can slow down the reaction; Wang et al. reported the use of a functionalised resin in the reduction of **34**, which adsorbs the product, preventing product inhibition, allowing increased substrate loading.[86] Recently, the biocatalysed synthesis of intermediate ester **35** has been reported using a carbonyl reductase, identified through directed evolution.[87-89]




*Scheme 1.17 Chemoenzymatic resolution of two intermediates of atorvastatin **32***

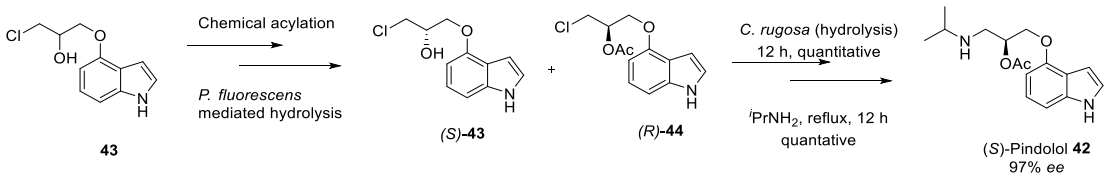
The diester intermediate **36** in the synthesis of pregabalin **37** was resolved using a commercially available lipase.[90] A recycling step was also developed, where the unreacted ester **36** from the resolution could be recycled to enhance the efficiency of the reaction (Scheme 1.18, **B**). The route is particularly well set up for the potential transfer to continuous flow, for the resolution/recycling. Another route to **37** utilised nitrilases for an initial desymmetrisation step, followed by a Curtius rearrangement and hydrolysis (Scheme 1.18, **A**).[91] This route allowed access to both pregabalin **37** and baclofen **38**. The use of a mutated lipase from *Thermomyces lanuginosus* has also been reported in the resolution of **36**, giving increased activity against **39**.[92] Recently, the use of transaminases has been applied to the resolution of pregabalin **37** and brivaracetam **40** intermediate α-chiral aldehydes (Scheme 1.18 **C**).[93] The aldehydes racemise under the biotransformation conditions, specifically in the presence of the amine donor isopropylamine **41**, furnishing an effective DKR.




*Scheme 1.18 Resolution of pregabalin **39** precursors and synthesis of related compounds **38** & **40***

Synthesis of *(S)*-pindolol **42** has been reported using two lipase-catalysed steps (Scheme 1.19).[94] After a chemical acylation of alcohol **43**, the first lipase-catalysed step hydrolyses the acetate substrate **44** stereoselectively. Once the resolution has been carried out the enantiopure alcohol *(S)*-**43** and ester *(R)*-**44** are separated, and a second lipase is used to

hydrolyse the unreacted ester *(R)*-**44**, to give alcohol *(R)*-**43** which can be further reacted to give *(S)*-**42**. *(S)*-Metoprolol **45**, atenolol **46** and propranolol **47** have also been resolved by lipase-catalysed transesterification. The mechanism of the reaction of **47** has been studied by computational methods.[95-98] As well as this, the 5′-hydroxy analogue **48** has been synthesised by a P450 enzyme for its use in toxicity studies.[99] The *Pseudomonas fluorescens*-mediated kinetic resolution of metoprolol intermediate **49** was also studied; the reaction was modelled and the in silico results agreed with the experimental results, showing that a molecular docking study could potentially be used in the identification of suitable enzymes for the biotransformation (structures shown in Figure 1.1).[100] *(R)*-Bufuralol **50** was also prepared by lipase-catalysed resolution; the addition of a ruthenium based racemisation catalyst increased the efficiency for the resolution step, furnishing the intermediate acetate *(S)*-**51** in excellent yield and enantiopurity (96%, >99% *ee*) (Scheme 1.20).[101] Several approaches were taken to the synthesis of the sulfur-containing betablocker analogue **52** (Figure 1.1), including the use of enantiopure *(R)*-(–)-epichlorohydrin **53**, Baker's yeast-mediated reduction and lipase-mediated resolution.[102] The use of enantiopure epichlorohydrin **53** would avoid the need for a subsequent resolution; the biocatalysed resolution of epichlorohydrin **53** has previously been reviewed.[103] Since this review, **53** has been resolved using an immobilised halohydrin dehalogenase in both aqueous and non-aqueous media (Scheme 1.21).[104,105] A novel immobilisation technique was used and product inhibition was avoided by continuous removal of the product.




*Scheme 1.19 Synthesis of* (S)*-pindolol* ***42***

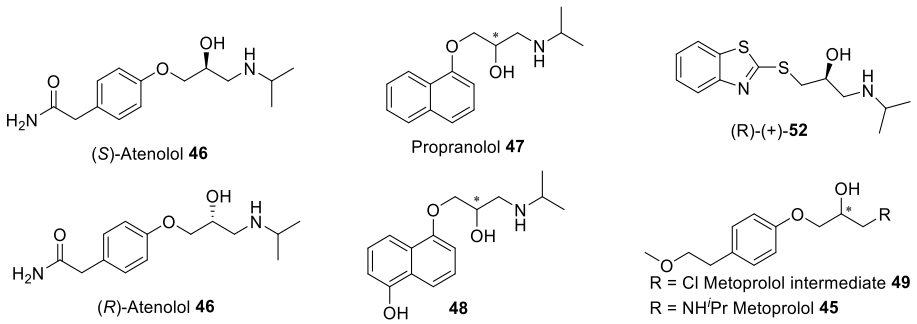
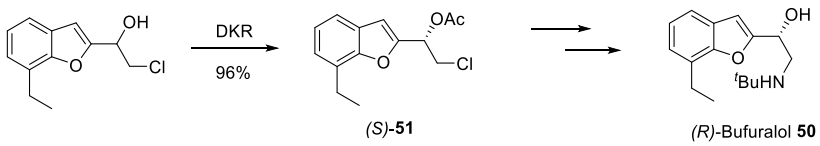
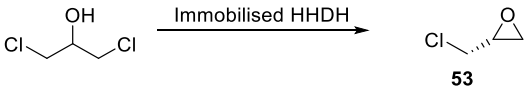



*Figure 1.1 Structures of some* β*-blockers whose intermediates have been resolved by lipase-mediated reactions*

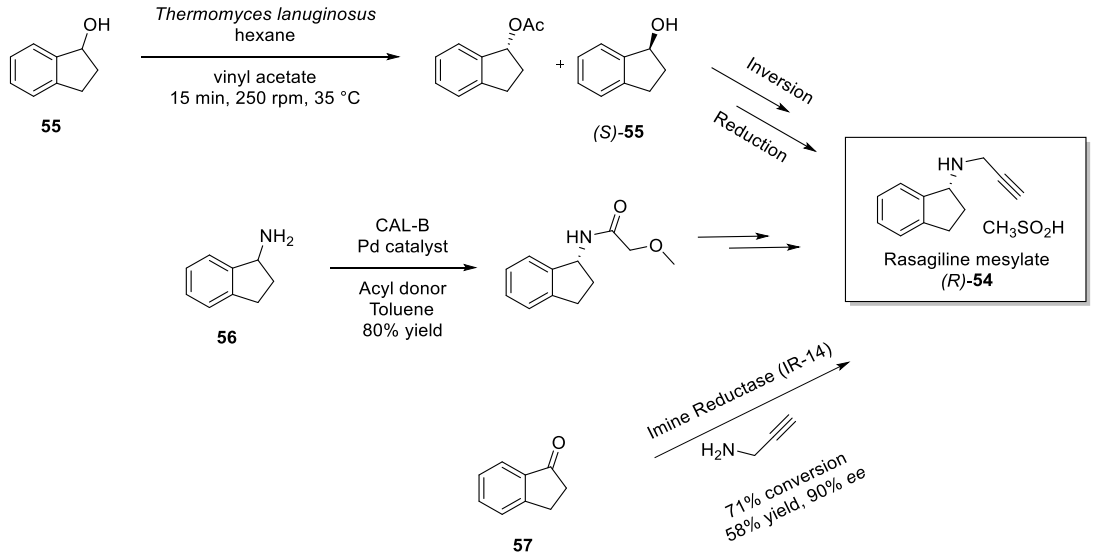



*Scheme 1.20 Synthesis of* (R)-*Burfuralol* ***50***



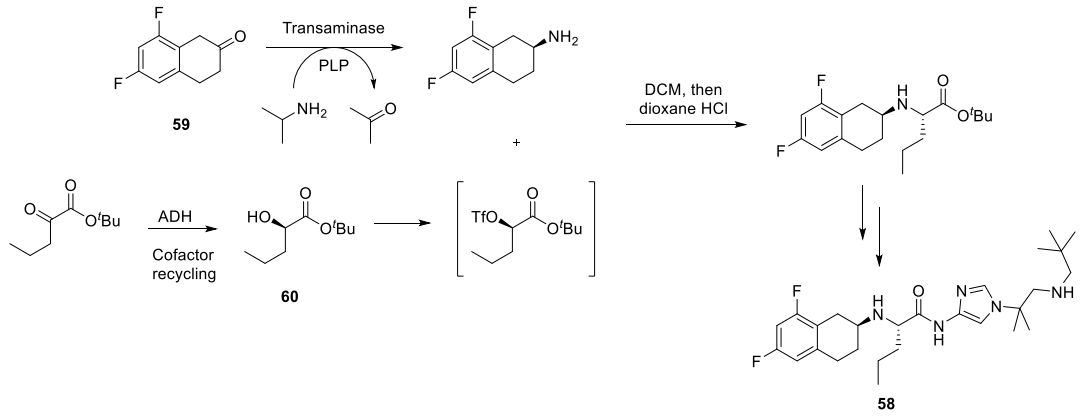

*Scheme 1.21 Resolution of epichlorohydrin* ***53***

Several biocatalytic routes have been developed to access rasagiline mesylate *(R)*-**54**, a commercially marketed treatment for Parkinson's disease (Scheme 1.22). The resolution of *(S)*-1-indanol **55**, followed by a stereoinversion using Mitsunobu chemistry and a Staudinger reduction gives the enantiopure amine **56**.[106] The resolution of the amine has also been developed, combining the CAL-B-catalysed transesterification of amine **56** with an in situ racemisation step.[107] A route to rasagiline **54** has also been reported using imine reductases (IREDs) to convert the ketone **57** directly to the target compound *(R)*-**54**.[108] The enantiomer *(S)*-**54**, can also be accessed through the use of *(S)*-selective imine reductase. While the IRED route seems the most logical route to use, factors such as scalability and knowledge of the chemistry involved would need to be carefully considered; CAL-B is a very well-studied lipase, and has been used in industrial processes previously, thus, may be a better choice than the lesser known biocatalyst.



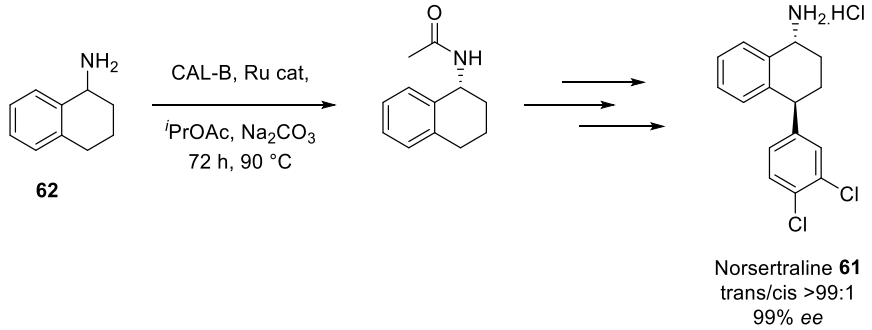

*Scheme 1.22 Enzymatic routes to rasagiline mesylate* ***54***

Enzymatic reactions have been used in the enantioselective synthesis of two enantiopure advanced intermediates of γ-secretase inhibitor **58**, on a multikilogram scale (Scheme 1.23).[109] The transaminase was used in conjunction with isopropylamine to effect enantioselective reductive amination of tetralone **59**; while an additional cofactor recycling was required for the alcohol dehydrogenase-mediated reaction to produce the enantiopure ester **60**.




*Scheme 1.23 Synthesis of γ-secretase inhibitor **58***

A chemoenzymatic route to norsertraline **61**, an API structurally related to the Pfizer antidepressant sertraline, has been reported through the resolution of 1-aminotetralin **62** by transesterification, and retention of the acyl group throughout the synthesis as a protecting group (Scheme 1.24).[110] The use of CAL-B at elevated temperatures in conjunction with a racemisation catalyst is the first step in a six- step synthesis from commercially available **62**.




*Scheme 1.24 Dynamic kinetic resolution of 1-aminotetralin **62***

## 1.2 Enabling technology

Biocatalysis is a useful tool in chemical synthesis, and advances in technology give us the resources to expand their use beyond the natural substrates, as well as to enhance the stability and scope of biocatalysts. This can be achieved through making existing enzymes physically more stable, such as by immobilisation, and through developing modified, more robust enzymes, using molecular biology techniques to discover new biocatalysts, and to modify existing biocatalysts.[111]

Because of the extraordinary selectivity of biocatalysts, they can be particularly suited to use in cascade systems, where the product from one reaction is further reacted without the need for isolation; the biocatalysts could be used both with other biocatalysts or with cheomocatalysts.[112-116] With the availability of a cornucopia of immobilisation techniques, biocatalysts can be made more stable to harsher reaction conditions, and can be used for dynamic kinetic resolution. As well as this, the immobilisation of enzymes opens up the possibilities for use and reuse, especially in continuous processing.

Immobilisation of enzymes and their use in dynamic kinetic resolutions, as well as the use of lipases in cascade or one-pot systems, and their integration into continuous processing, will be discussed in this section. Molecular biology techniques such as metagenomics and biocatalyst modification will also be discussed briefly, as well as the use of multiple enzymes in parallel, or biocascades.

### 1.2.1 Immobilisation

The use of commercially available immobilised biocatalysts is obviously the easiest approach as little specialised expertise is required. However, the immobilisation of free enzymes, or whole cells, can allow the use of a much wider range of biocatalysts by utilizing immobilisation media with desired attributes e.g. extra stability, ease of separation, and reusability; there are many recent reviews on this topic.[117-123] Immobilisation of biocatalysts can protect them from by-product inhibition, increasing temperatures or pH; it also adds economic value to the biocatalysts by allowing recovery and reuse.[122-127] The immobilisation of enzymes directly from the whole cell preparation without purification is even more advantageous as it combines the immobilisation step with the purification step.[120,128] There are three types of immobilisation: binding to a support, entrapment (also called encapsulation), and cross linking (Figure 1.2, A, B and C, respectively).[129,130] There are many different supports for the immobilisation of

biocatalysts examples include chitin and chitosan, which are biodegradable, magnetic supports, silica, and several preparations which are commercially available.[131-135] The focus of this section will be immobilisation by binding to a support.

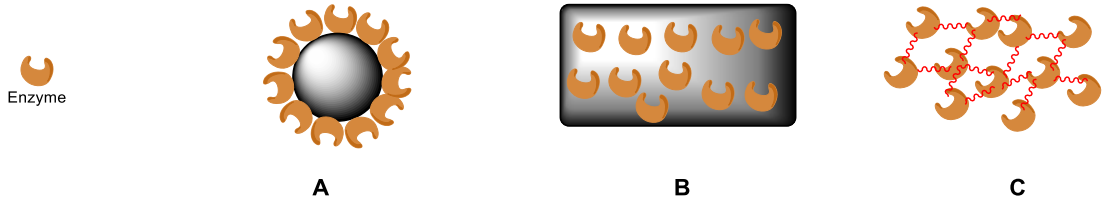



*Figure 1.2 Modes of immobilisation of biocatalysts*

Lipases are commonly immobilised on hydrophobic supports; this can increase their activity by holding them in the open or active conformation (Figure 1.3).[136-138] Non-specific immobilisation e.g. through ionic interaction of the enzyme with the support, can lead to loss in activity as there is little control over the interactions; a recent review discusses the interaction of proteins with surfaces.[139] Specific interactions, for example the use of fusion enzymes, allows better control over the activity of the enzyme, as the part of the protein interacting with the support is essentially separate from the protein. Fusion proteins involve the addition of a sequence for a binding peptide to the enzyme to allow specific immobilisation, and is highly useful.[140] For some varieties of tags and immobilising supports, similar results for enzyme activity can be attained when immobilising the pure protein and when using the crude cell extract, thus eliminating the need for pre-purification of the enzyme (Scheme 1.25).[141,142] The use of magnetic supports coated with ligands or peptides for interaction with the tagged proteins allows facile separation of the supported catalyst for reuse. His tags (histidine tags) are a short sequence which adds a short poly-histidine chain to one end of the protein. Histidine binds to many metal ions, for example nickel, cobalt, and copper; the use of supports with metal chelating groups can be used to combine the immobilisation and purification steps.[142,143]

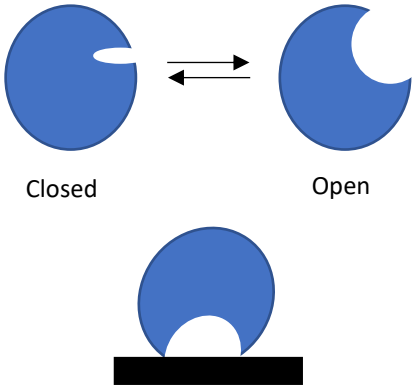
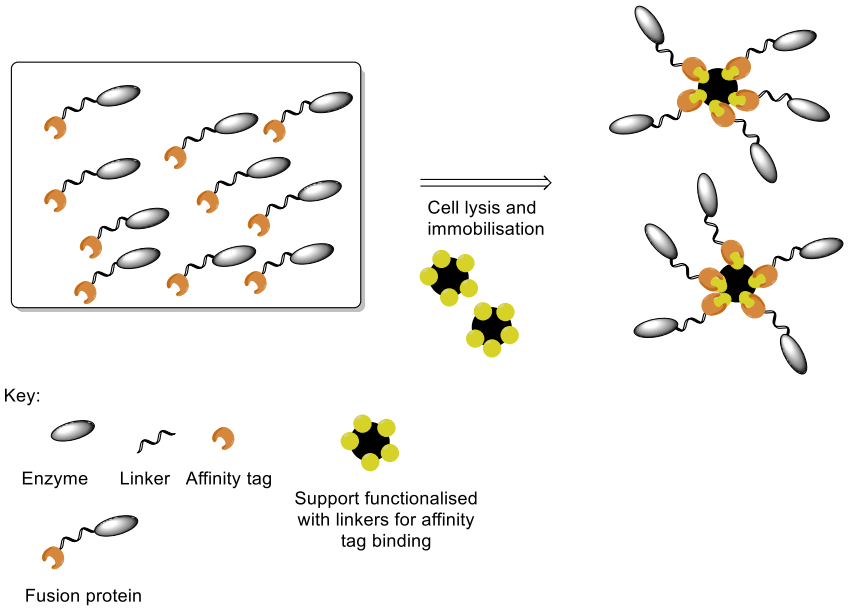



*Figure 1.3 Representation of immobilisation of lipases on a hydrophobic support*




*Scheme 1.25 Simultaneous purification and immobilisation of fusion proteins*

The availability of specialised immobilisation carriers designed for enzymes is growing. EziG™ utilises the iron-His-tag affinity to immobilise the enzymes.[144] Covalent immobilisation of HaloTag® modified proteins directly from the whole cell has also been reported.[145] Here tagged fusion enzymes are immobilised directly from the crude cell extract onto a resin pre-packed into a flow reactor, eliminating a costly separate purification step by combination with the immobilisation step (Scheme 1.25). Using this approach, two biocatalysts were effectively combined in a cascade reactor. The concept of a one-step purification immobilisation is potentially very useful and has been reviewed previously.[120]

Recently, *Thermomyces lanuginosus* lipase has been immobilised on metal chelated magnetic nanoparticles with retention of activity and increase in stability relative to the free enzyme; this also allowed the facile separation and recovery of the enzyme.[146] Marszałł et al.

immobilised lipases onto magnetic chitosan nanoparticles, improving both the resolution of (*RS*)-atenolol **46** and the recoverability and reusability of the lipase.[147,148] The immobilised lipases were reused for five reaction cycles, totalling 1200 h operating time. Lipases have also previously been immobilised on magnetic lauric acid-stabilised particles for the resolution of menthol.[149] Horseradish peroxidase has been immobilised on iron oxide nanospheres on chemically reduced graphene oxide which has been shown to be an effective immobilisation medium.[150] The advantage of these systems is that the immobilised biocatalysts can be readily recovered by magnetic separation, removing the need for a filtration step. Lipases immobilised on magnetic nanoparticles have also been used in microtube reactors.[151]

Zhu et al. reported the use of a pH sensitive support for *Candida rugosa* lipase used in the hydrolysis of ketoprofen ester.[152] In this case, once the reaction was completed, a simple adjustment of the pH precipitated the support and allowed facile recovery (Figure 1.4). The immobilised enzyme survived several precipitation and dissolution cycles while retaining activity (46% activity after 8 cycles) and increased the enantioselectivity relative to the free enzyme by 1.5-8.7 times.

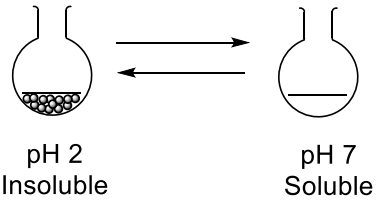


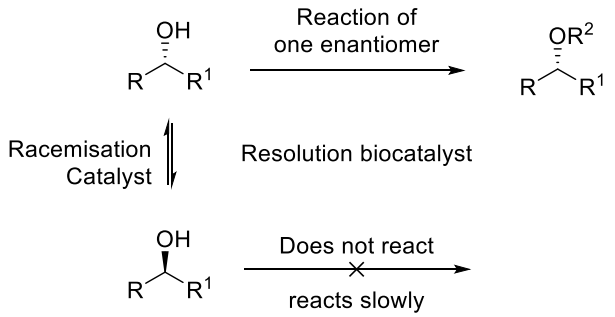

*Figure 1.4 pH-Controlled solubility of enzyme support*

Wever et al. reported a method for covalent immobilisation of an acid phosphatase, which retained the activity and the majority of the efficiency of the enzyme.[153] The immobilised enzyme was shown to be stable after a year of storage in buffer. The catalyst was also used in a continuous flow system to produce a range of phosphorylated alcohols at a gram scale, using a cheap phosphate donor. The methodology could allow the facile integration of the reaction in a large, multi-enzyme continuous system.

The use of epoxy functionalised resins is advantageous because of their ease of use. They have been used to immobilise halohydrin dehydrogenases directly from the cell lysate, without need for prior purification.[105] Epoxy functionalised silica has been used as an efficient support for lipases in the resolution of ibuprofen esters.[154]

### 1.2.1.1 Dynamic kinetic resolution and combining chemo- and biocatalysts

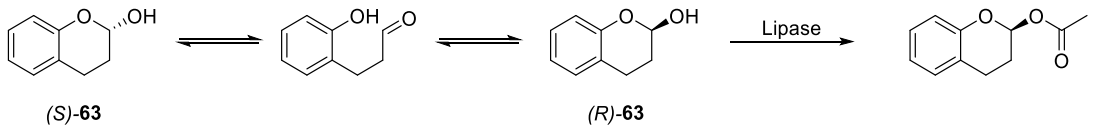
The use of multiple biocatalysts or a combination of chemo- and biocatalysts in the same reaction vessel is an attractive method of synthesis, since it can reduce the reaction time and product purification required.[114,113,155-157] The use of lipases is widespread in biocatalysis but their use alone can be disadvantageous as the maximum theoretical yield of a kinetic resolution is 50% without the use of a racemisation catalyst.[158,159] Dynamic kinetic resolution is an attractive method of increasing the yield of kinetic resolution reactions (Scheme 1.26), and can be particularly well suited to continuous processing, as the conditions under which biocatalysts and racemisation catalysts work can be very different.[7,8,159-161] This is especially useful for lipases; they are very well studied, so are an attractive option for those inexperienced in the use of biocatalysts.[159] The dynamic kinetic resolution of alcohols using metal-based racemisation catalysts in combination with lipases is well reported and there have been several reviews on the use of metals such as iron, ruthenium, and iridium, and complexes thereof.[7,8,159,161-164] Acids and bases, zeolite, even light activated catalysts have also been used as racemisation catalysts for the dynamic kinetic resolution of alcohols.[165-169]



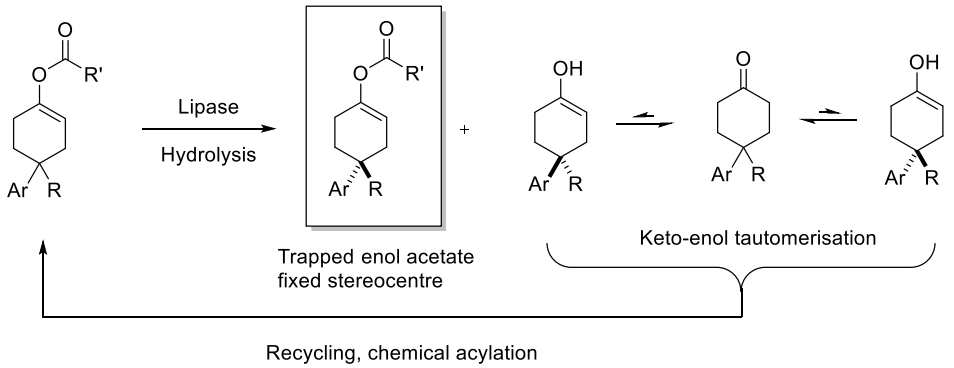

*Scheme 1.26 Dynamic kinetic resolution, using alcohols as an example*

The lipase-catalysed resolution of the hemiacetal moiety of sugars is widely reported and utilises the dynamic ring opening/closing of the hemiacetal in place of the addition of an external catalyst.[170-175] The same ring-opening and ring-closing was utilised within our group for the dynamic kinetic resolution of 2-chromanol **63** (Scheme 1.28).[176] The use of such systems takes advantage of the instability of the ring structure, or the reversibility of reaction that occurs naturally, without the need for an external catalyst. Another example of this is the use of prochiral ketones (Scheme 1.28). The enol can be trapped by chemical acylation, taking advantage of the keto-enol tautomerization, and the resulting enol acetate can be selectively hydrolysed using a hydrolytic enzyme.[177,178] Spontaneous racemisation has also been applied to the synthesis of 1,2-diaminopentane derivatives and 3,4-dihydroisocoumarins (Figure

1.5).[179,180] The use of prochiral substrates (desymmetrisation) is an attractive alternative to kinetic resolution; some examples of such compounds which have been used as intermediates in the synthesis of pharmaceutical compounds are shown below (Scheme 1.29).[181-183]

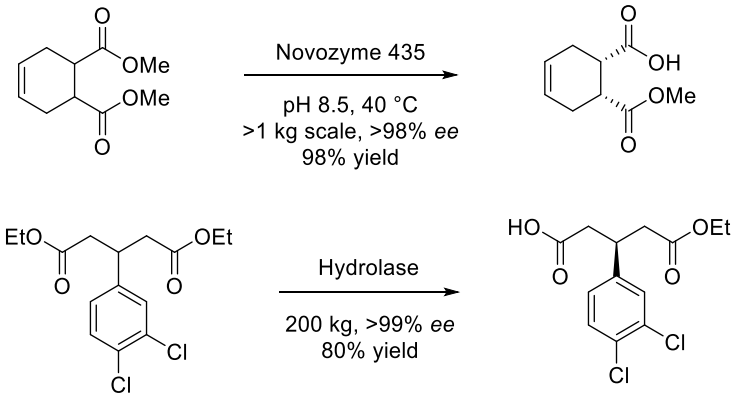



*Scheme 1.27 Spontaneous racemisation*




*Scheme 1.28 Dynamic kinetic resolution with no external catalysts: using equilibria for racemisation*

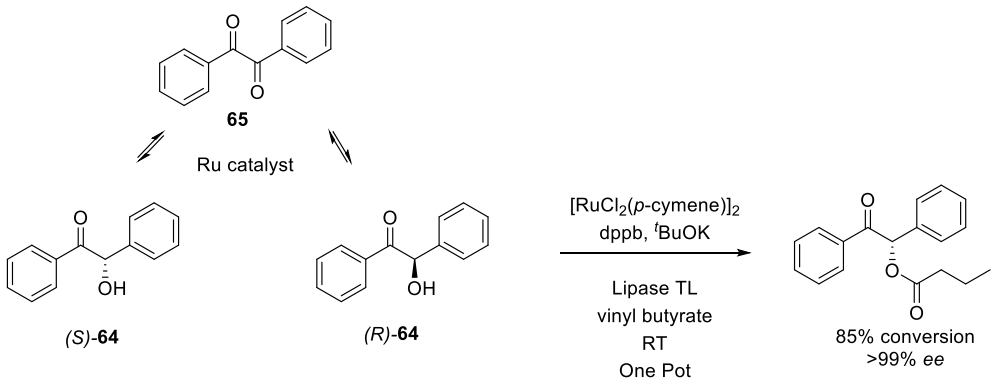

*Figure 1.5 1,2-Diaminopentane derivatives, and 3,4-dihydroisocoumarin derivatives*




*Scheme 1.29 Lipase-mediated resolution of prochiral compounds*

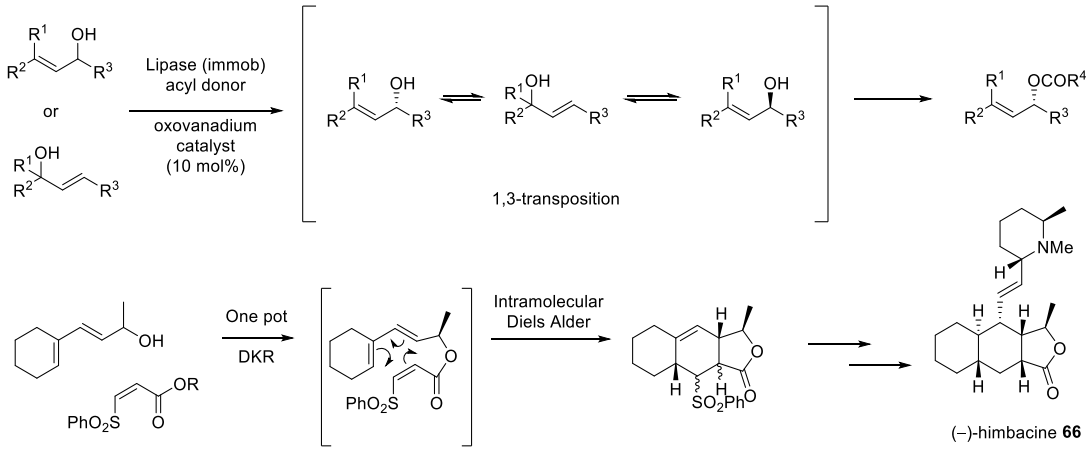
The dynamic kinetic resolution of α-hydroxy ketones, such as benzoin **64**, via a diketone intermediate **65** has been reported (Scheme 1.41).[184] This dynamic kinetic resolution has been carried out in both batch and continuous mode by variation of support used for lipases in combination with various chemocatalysts, as well as examination of solvent effect, to make the reaction greener.[185-189] The use of a readily available ruthenium catalyst in combination

with a base was initially considered at 50 °C, however, it was shown that the right combination of catalyst and ligand allowed the reaction to be carried out at a lower temperature (room temperature).




*Scheme 1.30 Dynamic kinetic resolution of benzoin **64***

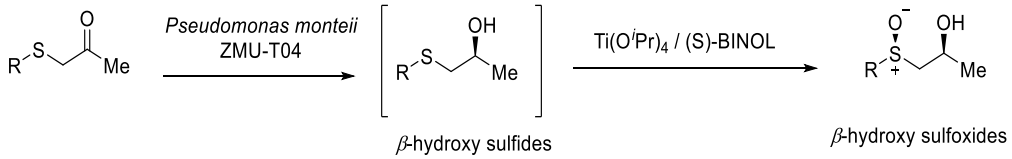
Akai et al. reported the lipase/metal-catalysed dynamic kinetic resolution of racemic allylic alcohols (Scheme 1.31).[190] The acyl group installed in the lipase-catalysed transesterification was carefully chosen so that it would undergo further reaction, making the reaction more atom economical, as the acyl group did not require removal after the resolution step.[191,192] The oxo vanadium catalyst carried out a racemisation/isomerisation of the allylic alcohol starting material. As an illustrative example this methodology was applied to the total synthesis of (−)-himbacine (−)-66, furnishing the product with excellent enantioselectivity.[193]



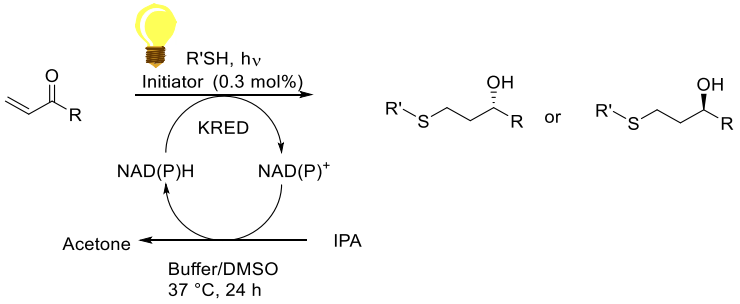

*Scheme 1.31 Combining lipase-catalysed resolution and an intramolecular Diels-Alder reaction*

The combination of a biocatalysed reduction and a chemical oxidation was also reported. The bioreduction was carried out first, followed by sulfur oxidation using a chiral chemocatalyst (Scheme 1.32).[194] Potentially, the bioreduction could be combined with a biocatalytic sulfur

oxidation step in place of the chemocatalysed step. Biocatalysed sulfur oxidation can avoid the over oxidation to the sulfone which can be a problem for chemical catalysis.[195] The synthesis of sulfur containing ketones and subsequent bioreduction to give the corresponding alcohols has also been reported, in a one-pot photobiocatalytic cascade (Scheme 1.33).[196] The initial Michael reaction is light activated, forming a sulfide from a thiol and an α,β-unsaturated ketone. Varying the choice of ketoreductase gave access to both enantiomers of the 1,3-mercaptoalcohol products.




*Scheme 1.32 Combining a bioreduction and a chemical oxidation*




*Scheme 1.33 One-pot photobiocatalytic cascade*

These selected examples from a wide array highlight the potential of combining biotransformations and other transformations as a powerful synthetic strategy.

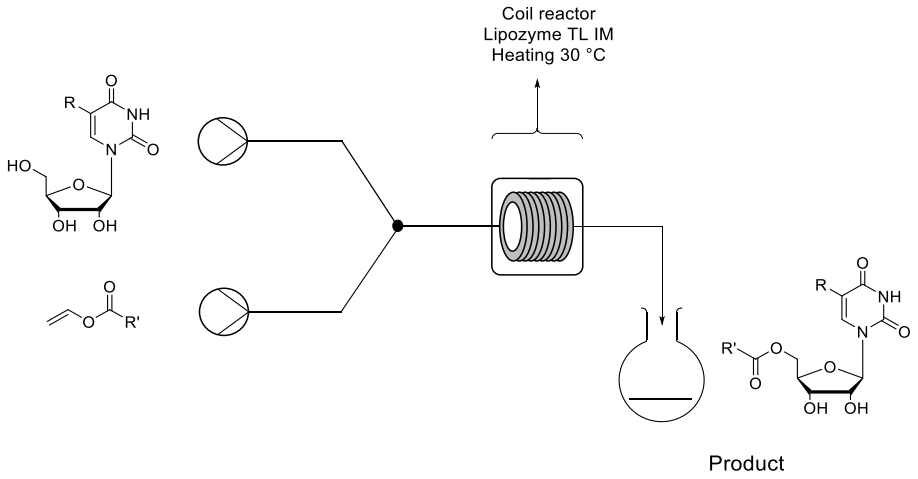
### 1.2.1.2 Flow chemistry

Flow chemistry or continuous processing is an important and rapidly growing area of research and has been used in the synthesis of pharmaceutical compounds.[197,198] The main advantages of flow chemistry are the small footprint from the point of view of equipment, and the ease of scale-up. Another advantage is that hazardous intermediates, which can hamper the scale-up of many synthetically useful processes, can be utilised as they are produced, avoiding the build-up of and requirement to store these hazardous agents. Previous work in our group has applied flow chemistry to the synthesis of the diazo transfer agent mesyl azide, which is too hazardous to use on an industrial scale, but through the use of flow chemistry the hazardous agent is synthesised and utilised in situ, negating the need for isolation.[199]

Another advantage of flow chemistry is that the reagents can be somewhat protected from product inhibition, which is common with biocatalytic reactions. By continually removing the inhibitors of the enzymes, the productivity, and thus value, of these biocatalysts can be increased. Other problems which can be addressed by continuous flow technology are those associated with scale-up of processes, such as heat and mass transfer and sufficient mixing of reagents, which can limit the utility of some reactions; oxidases can be limited in their utility by the rate of oxygen uptake, a problem which has been addressed by flow technology.[200] Biocatalysts used in combination with continuous flow technology has been recently reviewed by a number of groups.[201-204]

Microreactors allow precise control of mixing and temperature, and their use, in conjunction with biocatalysts has been recently reviewed. They are well suited to integration in a continuous flow system. However, their use requires the immobilisation of the biocatalyst.[205,206]

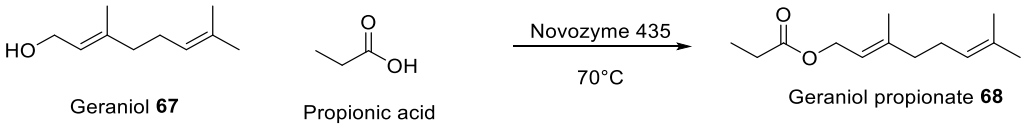
Recently, the enzymatic synthesis of nucleoside analogues using immobilised Lipozyme® catalysed transesterification has been reported.[207] The regioselective reaction was carried out using a microflow reactor (Scheme 1.34). The use of microflow technology allowed the reduction in the amount of DMSO required to solubilise the uridine derivatives, as well as mild temperature and short reaction time. The use of this technology enabled large libraries of compounds to be generated quickly without the use of protecting groups.




*Scheme 1.34 Synthesis of nucleoside analogues using immobilised Lipozyme®*

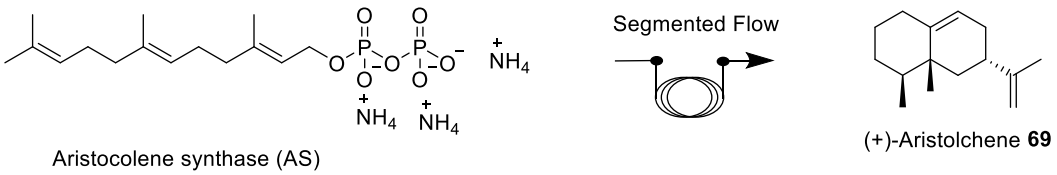
Using various carboxylic acids, esters of geraniol **67** were synthesised by lipase-catalysed transesterification in a flow system (Scheme 1.35).[208] The process optimisation was carried

out in flow; a batch reaction mimicking the optimised conditions for the reaction was carried out to compare the efficiencies in both systems. Commercially available immobilised lipases were used in a packed bed reactor. Commercially available enzymes have also been immobilised, with the nature of the support being varied to optimise the esterification reaction.[209] The ability to change the enzyme support is useful as the nature of the support can be altered to enhance the stability of the biocatalyst, the stability of the immobilised enzyme in different solvent systems, or can be changed to enhance separation or stability in the presence of other catalysts. The continuous synthesis of geranyl propionate **68** has been carried out.[210] The lipase for the transformation was identified through the use of batch reactions and a range of supports were screened to maximise the immobilisation efficiency in order to facilitate the transfer of the reaction to a continuous system. As well as this, the support system chosen increased the activity of the lipase by almost 30%.




*Scheme 1.35 Continuous synthesis of geranyl propionate **68***

Flow chemistry has been used to overcome the slow release of products in the synthesis of sesquiterpene **69** from farnesyldiphosphate using a terpene synthase as biocatalyst (Scheme 1.36).[211] Continuous extraction of the product from the aqueous layer is necessary to make the biocatalytic process viable. When compared to batch, the flow system offered many advantages. The use of high speed mixing, which would be necessary in batch conditions to achieve continuous extraction of the terpene products from the aqueous layer was avoided; this can lead to denaturation of the enzyme by shearing. The flow system offered more control over the interactions of the biocatalyst with the solvent; correct solvent choice was crucial, as emulsion formation would impede phase separation and complicate product recovery.

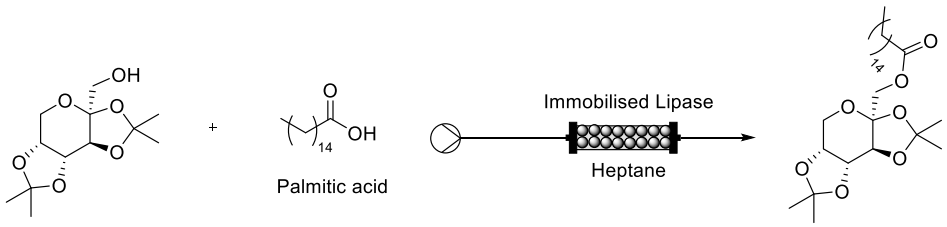



*Scheme 1.36 Synthesis of (+)-**69***

The combination of flow chemistry and biocatalysis has also been applied to the production of fats for the food industry, as an alternative to hydrogenation and chemical

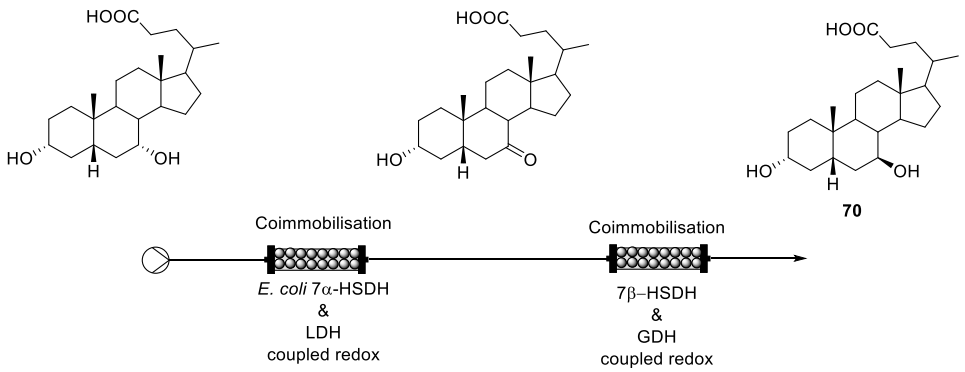
(trans)esterification.[212] An immobilised preparation of lipase from *Thermomyces lanuginosus* was used to convert soybean oil into semi-solid fats such as margarine and shortening, which are higher value products than the liquid oil starting materials. Immobilised CAL-B has been used in a continuous flow system for the synthesis of ceramides, important compounds in both the pharmaceutical and cosmetic industries.[213]

Biodiesel and other materials derived from vegetable oils or biobased oils are economically important, as they can be used as an alternative to petroleum based products.[214,215] Lipases can be used in the synthesis of biofuels from oil through (trans)esterification.[216-219] Commercially available immobilised biocatalysts were evaluated for the synthesis of ketal protected fatty acid esters of fructose (Scheme 1.37).[220] The best conditions for the reaction were identified in batch reactors, and used as a starting point for the development of continuous flow conditions.



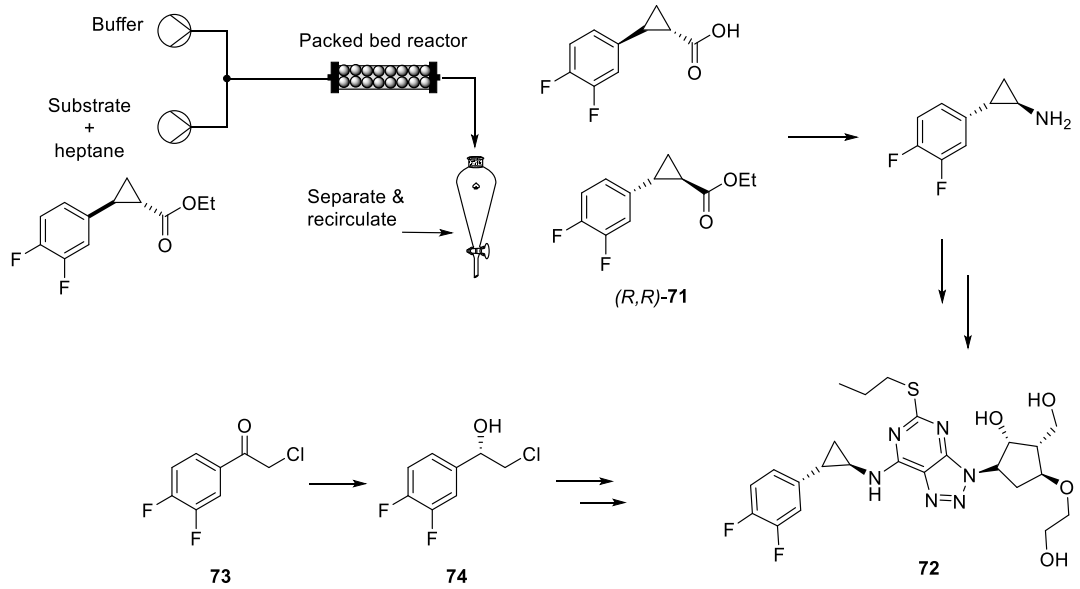

*Scheme 1.37 Synthesis of ketal protected fatty acids*

Another example of the use of biocatalysts in flow is the synthesis of ursodeoxycholic acid **70** (Scheme 1.38).[221] The process in batch involved the use of four enzymes in a two-step one-pot system. After the first step in batch, the enzymes had to be inactivated by heat treatment to avoid a reverse reaction occurring; a potentially costly and time-consuming step. The system was transferred to flow to spatially separate the enzymes required for the first and second steps. The two enzymes for each step were co-immobilised. A number of different immobilisation media were screened after the initial selection of the enzymes based on the batch process. Furthermore, the performance of the system was compared for the two-pot process in batch with and without the immobilisation of the enzymes and in flow. This resulted in a continuous process which, by spatially separating the enzymes involved in the separate steps, avoided the need for heat treatment to denature the enzymes, and allowed reuse of the enzymes.




*Scheme 1.38 Coimmobilisation and combination of multiple enzymatic steps*

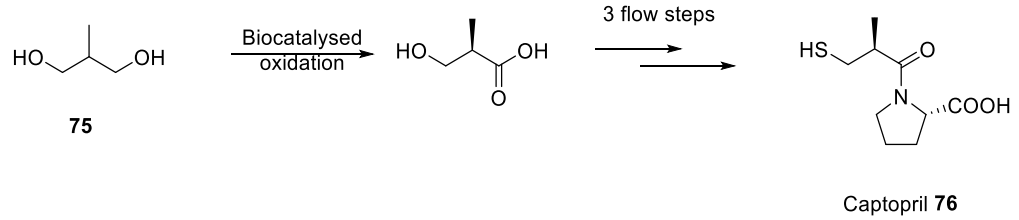
Recently, Turner et al. have shown the use of flow chemistry for the lipase-catalysed resolution of the cyclopropyl subunit **71** for the synthesis of ticagrelor **72** (Scheme 1.39), the biphasic mixture of product and starting material was separated, and the organic material recirculated to increase the conversion.[222] The asymmetric reduction of the chloroethyl precursor **73** was also reported, in this case using a ketoreductase from a metagenomic library.[223] The process for the synthesis of alcohol **74** was optimized to give up to 500 g/L substrate concentration.




*Scheme 1.39 Synthesis of ticagrelor **72***

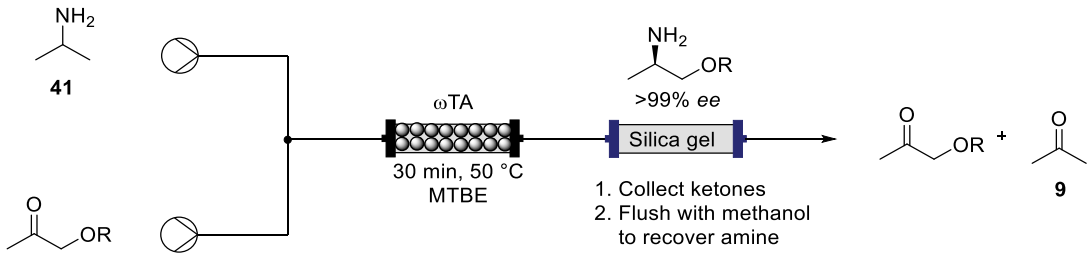
Enzymatic desymmetrisation of the prochiral diol **75** in continuous flow has recently been reported, as the first step in an entirely flow-based synthetic route to Captopril **76** (Scheme 1.40).[224] In this system, not only was the entire synthesis carried out using flow chemistry, but the chemoenzymatic step is carried out using immobilised whole cells, instead of purified

protein, which decreases the overall cost of the product, as the expense associated with purification of proteins is eliminated.




*Scheme 1.40 Synthesis of captopril **76***

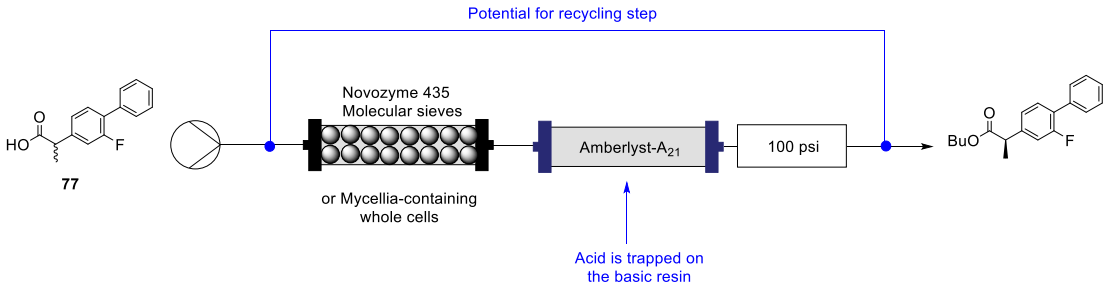
Purified transaminase from *Halomonas elongata* containing a poly His-tag was immobilised on a metal derivatised epoxy resin and the substrate scope explored through the use of flow chemistry;[225] although this required purification of the enzyme, it had the advantage of being able to withstand higher flow rates than the previously reported immobilisation of a similar whole cell system on a methyl acrylate polymer (0.78 vs. 0.02 mL $min^{-1}$).[226] The ability to use a higher flow rate is important as it allows higher productivity. When the immobilisation procedure was applied to the crude cell extract, the transaminase was also successfully immobilised, eliminating the need for a separate purification step. As well as this, a "catch-and-release" in-line product purification was added, by trapping the amine products on either a column of silica gel or an acidic resin allowing facile separation of the product amine from unreacted starting material, as well as the potential to reuse the unreacted material again (Scheme 1.41).[225,226]




*Scheme 1.41 Using continuous processing and in-line purification*

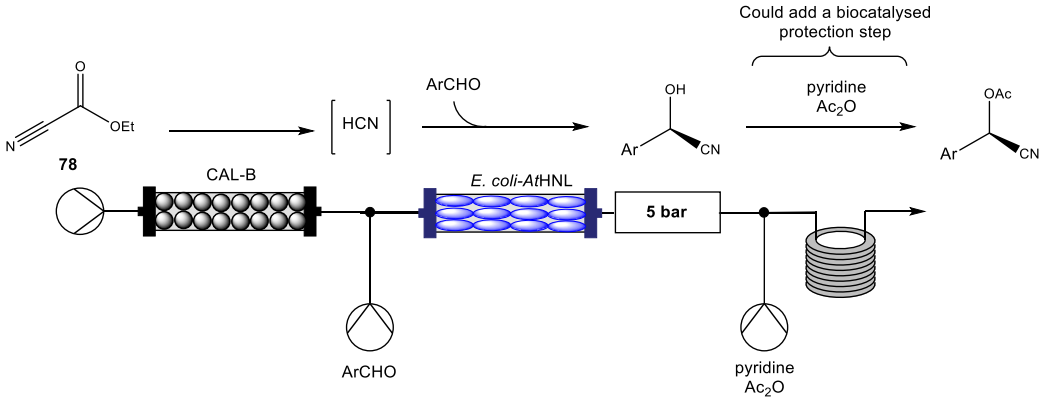
The lipase-catalysed resolution of flurbiprofen **77** has been carried out in a flow system, using the commercially available immobilised lipase, Novozyme 435, using molecular sieves to absorb excess water (Scheme 1.42).[227] The same transformation was carried out using mycelium-containing whole cells, in both batch and flow reactors.[227-230] When using either immobilised lipase, or mycelia, an in-line purification step was added, using a basic resin to separate the unreacted acid from the ester product in the continuous flow system. This

allowed a racemisation step to be added so that the unreacted acid could be reused. When using the commercially available catalyst the excess water was absorbed by including molecular sieves. As well as removing water, the cell mycelia can be separated from the reaction mixture without the need for immobilisation.[230,231] When using both the commercially available catalyst, and the whole cell systems, the product was obtained in excellent enantiopurity and high conversion; the use of flow reactors reduced the reaction time in both cases.




*Scheme 1.42 Biocatalysed continuous synthesis of flurbiprofen*

Continuous flow has been utilised in the synthesis of cyanoacetates using two distinct biocatalysts, CAL-B and a hydroxynitrile lyase (HNL) in a linked multistep flow process (Scheme 1.43).[232] The CAL-B hydrolyses a masked HCN group, ethyl cyanoformate **78**, which is then utilised by the lyase in the synthesis of cyanohydrins, which are chemically acylated before isolation. The advantage of this continuous flow system is that it avoids the need to use hydrogen cyanide gas. The use of a surrogate which is reacted in situ, means that there should only ever be a very small amount of the HCN in the system. The use of a flow-system also allowed an additional protecting group to be easily added to improve the overall stability of the target cyanohydrins. This could also potentially be replaced with a biocatalysed transesterification step to add the protecting group, rather than the chemical step; lipases have previously been used to resolve cyanohydrins via both hydrolysis and transesterification.[233,234]




*Scheme 1.43 In situ generation of HCN gas for asymmetric synthesis of cyanohydrins*

It is evident that the use of biocatalysis in continuous flow opens enormous possibilities which will no doubt be widely exploited by chemists in the coming years.

### 1.2.2 Biotechnology: metagenomics, directed evolution and rational design

The modification of reaction conditions to optimise the performance of enzymes is widely used, but the use of molecular biology techniques, such as directed evolution, rational design and genome mining can increase the potential library of biocatalysts enormously. Protein engineering can provide access to new biocatalysts, but ideally a good biocatalyst needs to be identified first. The larger the library of genetic material there is to work from, the better the chances of finding an enzyme which will carry out novel reactions or which has interesting substrate specificity.[235,236]

As genome sequencing becomes cheaper, it has resulted in more genetic material becoming available. The amount of organisms which can be cultured under laboratory conditions is limited to approximately 1–2% of all organisms, meaning that traditional methods for accessing genetic diversity are limited to those organisms which can be cultured (Figure 1.6).[237] Genetic sequencing and metagenomic techniques can be used to identify genes which encode for proteins, which otherwise would be inaccessible to researchers via a culture independent method.[238] The study of genetic material from unusual environments, such as high salt, high temperature, or low or high pH environments can give rise to biocatalysts which are suited to these environments i.e. high salt concentration, extreme temperatures, and pH values. Metagenomics allows us to look at the sequences which have evolved over millions of years. There are two types of analysis associated with metagenomic data: functional screening where a clone with the desired functionality is identified, or sequence-driven analysis, where a conserved sequence is used as a probe or hook to identify a potential biocatalyst. Functional

screening involves the cloning of the metagenomic DNA into a host organism, whereby the protein of interest needs to be expressed by the host cell.[239] Several factors can affect the success of this method, the major one being that the host cell may not express the proteins of interest; additionally a high throughput screen is usually required and a suitable screen is not always available.[240-242] Sequence-driven analysis uses a conserved sequence as a "hook" or "probe" for picking out DNA which may code for a protein of interest. The catalytic triad is an example of a conserved motif which can be used in the identification of novel hydrolases.[243]

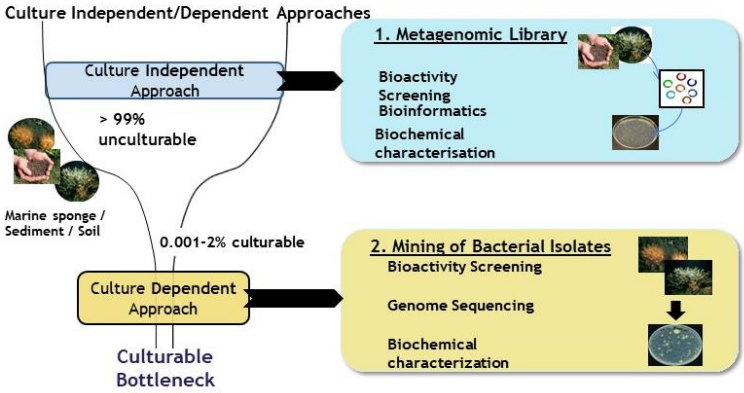
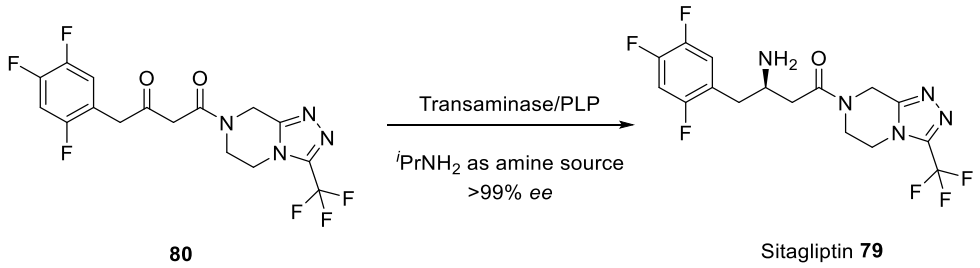



*Figure 1.6 Adapted from Bandh et al.[244]*

The identification of novel enzymes alone may not suffice in the context of finding an enzyme with desired activity/selectivity; for this, genetic mutation, either random or by design, can offer greater opportunities to tailor the enzymes for the required functionality. Directed evolution is a technique which has been successfully applied to the expression of modified biocatalysts.[245-247] By examination of the structure of the biocatalyst through molecular docking techniques, as well as comparison to biocatalysts of known function and structure through homology modelling, desired mutations can be identified to improve activity of the biocatalyst through (semi)rational design and using site directed mutagenesis.[248] As well as this, random mutations can be used to give information on the effect of changing various residues in the active site.

An excellent example of directed evolution is the mutation of a transaminase for the asymmetric reduction step in the synthesis of Sitagliptin **79**. Sitagliptin **79** (marketed as Januvia) is an antidiabetic drug developed and marketed by Merck & Co. In conjunction with Codexis, a biocatalyst was identified for the reductive amination of the intermediate **80**.[249] Through the use of directed evolution, the poorly performing wild-type enzyme was

extensively mutated until a biocatalyst with the desired activity was identified. In addition to this, the solvent tolerability and substrate scope were expanded beyond what is normally found in wild type transaminases, especially in the ability of the enzyme to accept a substrate with two bulky groups adjacent to the reaction site. Following this Bornscheuer et al. reported the resolution of bulky substrates **81** and **82** (Figure 1.7) through rational design of a wild-type enzyme, requiring only four mutations.[250]




*Scheme 1.44 Synthesis of sitagliptin **79***

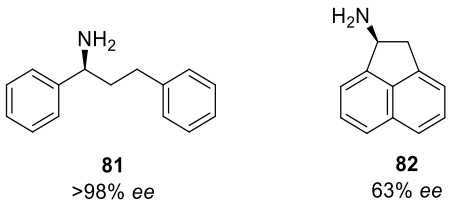
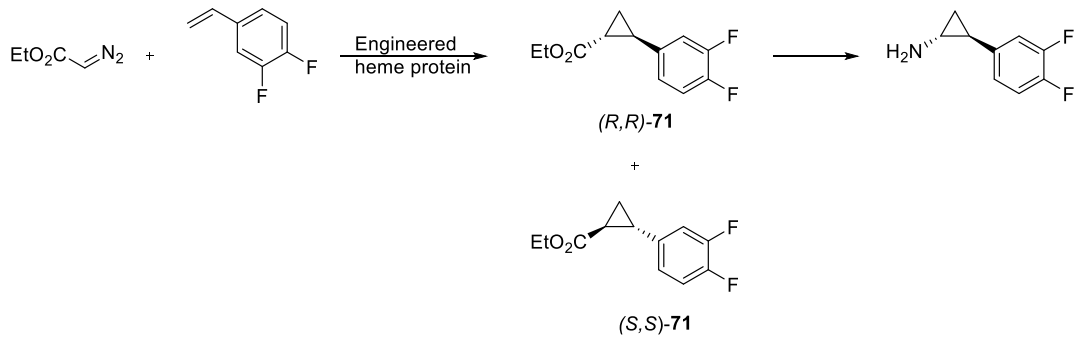
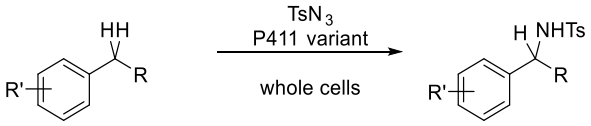



*Figure 1.7 Bulky substrates*

The use of mutated enzymes for non-natural substrates is a growing area of interest, and has recently been reviewed;[251,252] it is potentially a very useful enabling strategy, and as the techniques available for enzyme modification and evolution are continuously improving, the possibilities are growing.[251,252] Arnold et al. have reported the use of mutated P450 enzymes for olefin transformations, including cyclopropanation via carbene transfer, using the transformation for the synthesis of cyclopropane intermediate **71** in the synthesis of ticagrelor **72** (Scheme 1.45).[253-255] As well as this, workers from the same group have reported the functionalisation of benzylic C–H bonds by a engineered iron-heme enzyme (Scheme 1.46).[256]




*Scheme 1.45 Cyclopropanation catalysed by engineered heme protein*

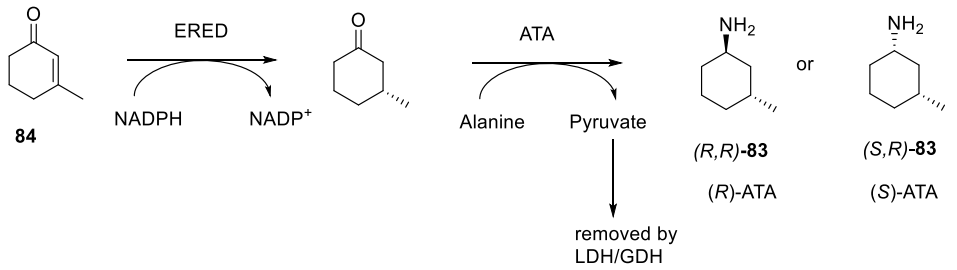



*Scheme 1.46 Enzymatic C–H amination*

### 1.2.2.1 Engineering cells for coexpression of multiple enzymes & cascade biocatalysis

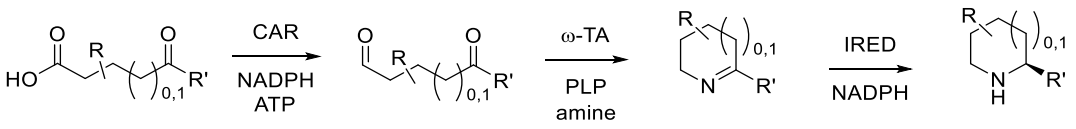
*E. coli* and other host organisms can be used to coexpress multiple enzymes in a cascade reaction. All of the enzymes can be produced by the same cell by modification of the plasmid(s) which are inserted into the expression vector. The topic of multi-enzymatic cascades has been reviewed recently.[257-262] The use of multiple enzymes in a one-pot reaction can be attractive, because of the generally similar conditions under which enzymes operate, and the selectivity of enzymes.

The combination of coexpressed enoate reductase (ERED) and amine transaminase (ATA) has been recently reported (Scheme 1.47).[263] The stereochemistry at the β-position is "set" by the enoate reductase, using either an *(S)*- or *(R)*- selective enoate reductase and the use of the appropriate transaminase gives either *(S)*-or *(R)*- stereochemistry at the amine. All four diastereomers of amine **83** can be accessed by use of the appropriate combination of biocatalysts. Initially the diastereoselectivity of the transaminases was poor (14% *de*). The proteins were modified using directed mutation; two small changes gave improved diastereoselectivity (from 14% *de* to 97% *de*). Following this, the enoate reductase was modified to give better enantioselectivity in the reduction of **84**.[264] The stereochemistry of the beta position is "set" by the ene-reductase, and the amine stereochemistry is selected by the transaminase. Although this is a good example of coexpression and engineering of enzymes, lactate dehydrogenase and glucose dehydrogenase required for cofactor regeneration still had to be added separately.



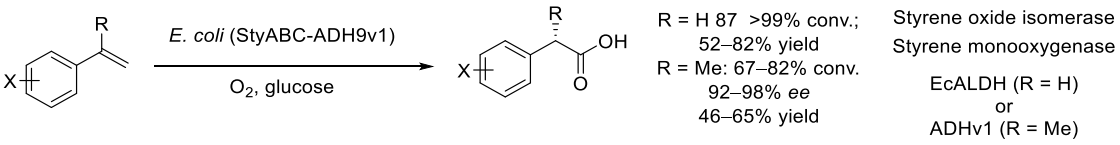

*Scheme 1.47 Coexpression of multiple enzymes for the synthesis of cyclic amines*

Turner et al. used a three enzyme one-pot cascade for the synthesis of substituted piperidines and pyrrolidines (Scheme 1.48). Reduction of the carboxylic acid by carboxylic acid reductase (CAR), gave an aldehyde substrate for the transaminases (ω-TA); the resulting imine was then reduced by an imine reductase (IRED). By changing the position of the substituent on the substrate, isomeric products were obtained.[265] Further work by the same group carried out the same reactions, but in this case coexpressing the required biocatalysts in the same cell expression system.[266]

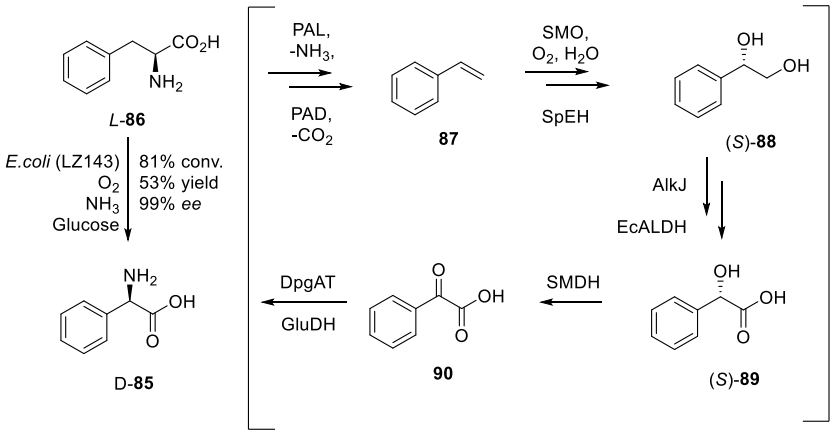



*Scheme 1.48 Synthesis of disubstituted pyrrolidines and piperidines*

A one-pot epoxidation-isomerisation-oxidation to make terminal acids from terminal alkenes was recently reported (Scheme 1.49).[267] Workers from the same group, not satisfied with a three enzyme cascade, coexpressed up to nine enzymes in one host.[268] The eight-step nine enzyme reaction produced D-phenylglycine **85** from L-phenylalanine **86** in a cyanide-free synthesis (Scheme 1.50), using only ammonia, glucose and atmospheric oxygen. Phenylalanine ammonia ligase (PAL) and phenylacrylic acid dehydrogenase (PAD) catalyse the loss of ammonia and carbon dioxide, respectively to produce styrene **87** which is epoxidized using styrene monooxygenase (SMO), and hydrolysed using epoxide hydrolase (SpEH) to diol **88**. The diol **88** undergoes two oxidation steps, catalysed by an alcohol dehydrogenase and an aldehyde dehydrogenase (AlkJ and EcALDH respectively) to furnish *(S)*-mandelic acid *(S)*-**89**, which is further oxidised to the alpha-keto acid **90** by *(S)*-mandelate dehydrogenase (SMDH). Ammonia transfer is the final step in the eight-step cascade, catalysed by D-phenylglycine ammonia transferase (DpgAT); glutamate dehydrogenase (GluDH) is used to regenerate the amine donor using ammonia. The specificity of each of these enzymes means that the product of one reaction is used by another enzyme, with the enzymes selected to avoid cross reaction.

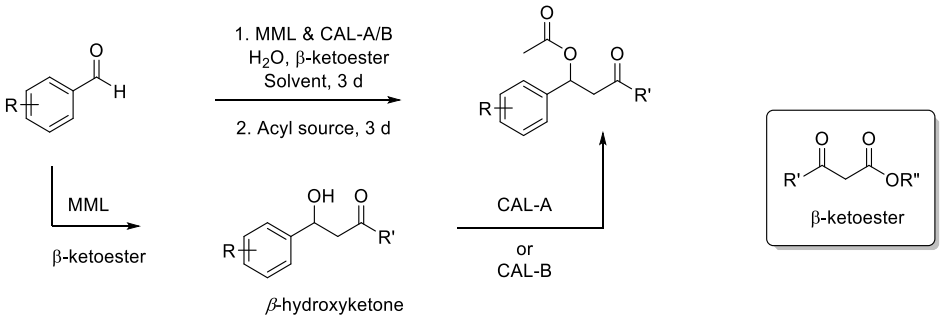



*Scheme 1.49 Whole cell cascades: from alkenes to carboxylic acids*




*Scheme 1.50 Whole cells coexpressing nine enzymes, the synthesis of D-phenyl glycine **85***

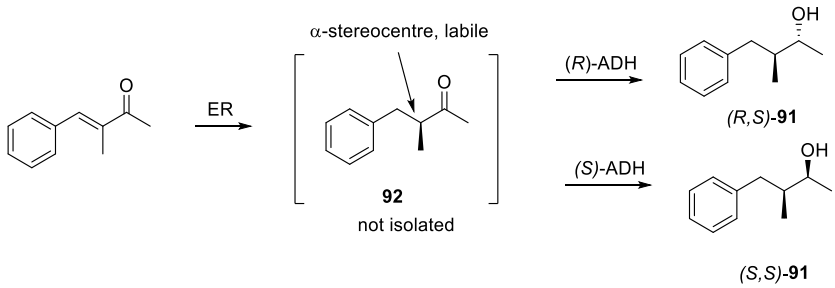
A series of β-hydroxy ketones and the acylated β-hydroxy ketones were synthesised by combination of two lipases (Scheme 1.51).[269] The first step is the decarboxylative aldol reaction in the presence of immobilised *Mucor miehei* lipase, followed by the kinetic resolution of the resulting β-hydroxy ketones. The catalysts were recycled up to three times, and some of the reactions were carried out at a >1 g scale.



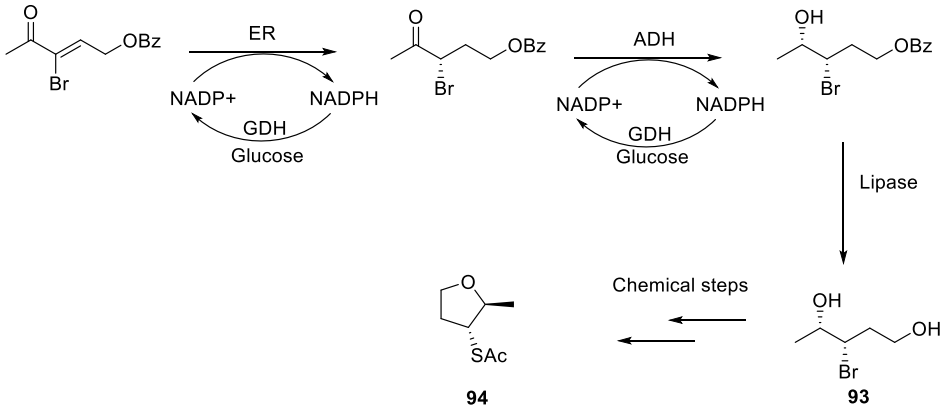

*Scheme 1.51 Use of multiple lipases*

Use of two immobilised enzymes, which are coexpressed by *E. coli*, in the synthesis of diastereomers of fragrance molecules has been described. Two reductions are carried out, initial reduction of the alkene, followed by reduction of the ketone (Scheme 1.52).[270] One of the main advantages of this is it is an economical route to the fragrance molecules **91**; another is that the unstable ketone **92** is not isolated. In fact, the reduction of the ketone proceeds with higher diastereoselectivity when combined with the ene-reductase in a one-pot reaction

than when the reactions are carried out sequentially; this is assumed to be because the compound with the labile α-stereocentre is not isolated when the two enzymes are used together. Workers from the same group also combined four different enzymes, this time in addition to the ADH and ene-reductase, GDH and a lipase were used in the synthesis of bromodiol **93**, a precursor to the tetrahydrofuran **94**, which is also a fragrance molecule (Scheme 1.53).[271] The substrate was modified, so that after reaction with the ene-reductase and alcohol dehydrogenase, it would give a suitable substrate for the lipase, as well as furnishing enantiopure products from the bioreductions. Again, the one-pot strategy works better than sequential addition of the biocatalysts.



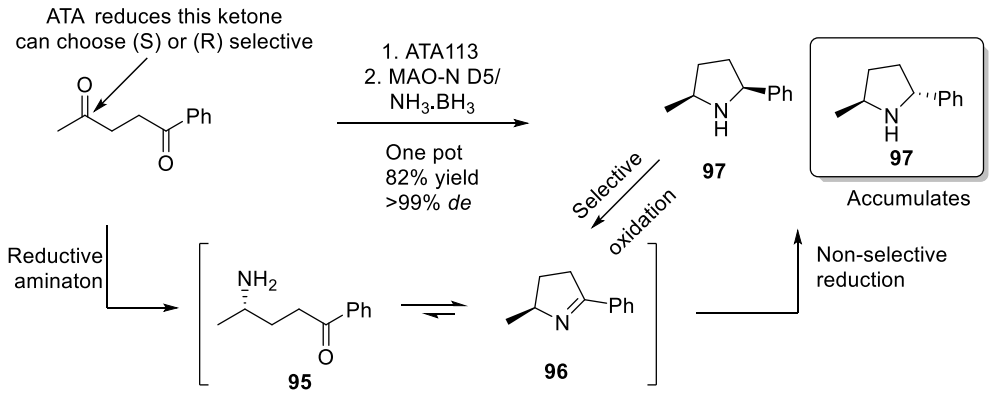

*Scheme 1.52 Combination of an ene-reductase (ERED) with either* (R)- *or* (S)*-selective alcohol dehydrogenase (ADH)*



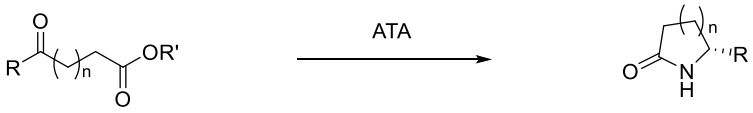

*Scheme 1.53 Four enzyme cascade for the synthesis of **93**, an intermediate in the synthesis of odourant **94***

The coupling of reduction and oxidation reactions can be useful for a dynamic system, Turner et al. reported the use of a chemical reductant in conjunction with an oxidative biocatalyst (Scheme 1.54).[272] In this system, the initial step is mediated by either an *(S)*- or *(R)*-selective amine transaminase (ATA), and the resulting amine **95** can cyclise to give the cyclic imine **96.** The imine **96** is non-selectively reduced to **97**; only one diastereomer is oxidised back to **96**,

eventually only one product is isolated, as the monoamine oxidase-favoured diastereomer is continually removed. Gotor-Fernández et al. reported a related transamination, using γ- and δ- ketoesters, the ketone moiety is reduced by a transaminase (Scheme 1.55); the cyclisation of the resulting amino esters result in γ- and δ- lactams.[273] The use of amino alcohols has been recently reported in the synthesis of β-, γ- or δ-lactams, in a bienzymatic cascade.[274]




*Scheme 1.54 Using a chemical reductant in combination with a biocatalyst*




*Scheme 1.55 Biocatalytic reduction , followed by spontaneous cyclisation*

## 1.3 Conclusions

The number of synthetically useful biocatalysts is rapidly expanding due to a number of key technological advances:

- An increasing number of biocatalysts are becoming available through modification of existing biocatalysts. These modifications can range from simply changing the preparation of the enzymes (immobilisation) or by modifying the protein itself (mutation). The increased availability of diverse materials for the immobilisation of biocatalysts, and the evolution of molecular biology techniques means that there are more possibilities for the modification of the biocatalyst.

- The push towards greener processes, and the development of continuous flow processing means that the use of biocatalysis is becoming more accessible and more desirable. The ease of use and mild conditions needed for biocatalytic reactions, as well as their excellent selectivity makes them a more attractive alternative to transition metal- or organocatalysed reactions. Flow chemistry and biocatalysis have been successfully combined previously, and their combination is helped enormously by the immobilisation of enzymes.

- The integration of biocatalyst into dynamic reactions, or reaction cascades further increases their utility, by reducing the overall steps involved i.e. less purification and fewer work ups.

- Molecular biology techniques give rise to more enzymes by genomic mining, where all the sequences from an environment can be explored. The use of directed evolution is also useful as poorly performing biocatalysts can be improved. This helps our understanding of how protein structure effects function leading on to rational design, where specific mutations can be made, rather than random mutations.

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# Chapter 2

# Resolution of 2-phenylalkanols

# Contents

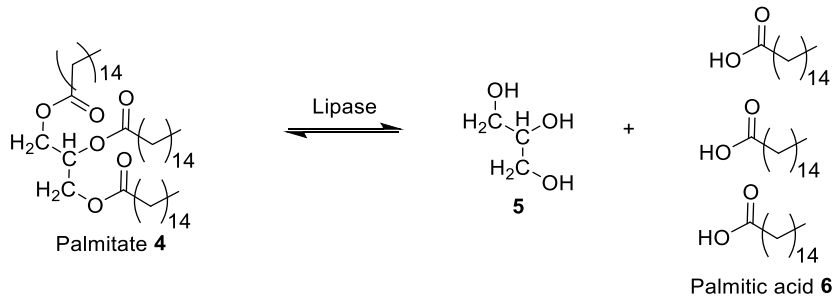
## Overview

By tuning the steric properties of the acyl group to control the efficiency and selectivity of the resolution, 2-phenyl-1-propanol **1** was prepared by lipase-catalysed hydrolysis using a short-chain acyl group, with E-values of up to 66 (*ee* up to 95%). 2-Phenylbutan-1-ol **2** was similarly resolved (up to 86% *ee*) using the optimised conditions, while the ester of the more sterically demanding 3-methyl-2-phenylbutan-1-ol **3** proved resistant to enzymatic hydrolysis under these conditions.

## 2.1 Introduction

The use of biocatalysts in organic synthesis is a growing and attractive method for achieving chemical transformations.[1] Enzymes, by their nature, have excellent chemo-, regio- and enantioselectivity, which, if utilised correctly can give superior results to chemical catalysts.[2]

Hydrolases are the most studied biocatalysts and have found application in many industrial processes.[1] Lipases are ubiquitous enzymes belonging to the family of serine hydrolases and can be found in animals, plants, bacteria, and fungi.[3] Lipases naturally catalyse the reversible hydrolysis of the ester bonds in triacylglycerols for example **4**, producing glycerol **5** and the free fatty acids **6** (Scheme 2.1) and are highly enantioselective in the kinetic resolution of carboxylic acids, alcohols, and related derivatives. Lipases are the most frequently used enzymes in organic synthesis, due in part to being inexpensive, commercially available, and stable, but also due to their wide substrate scope and high regio-, stereo-, and chemoselectivity. The fact that lipases do not require cofactors is a significant advantage when used for biocatalysis. They are also very useful because they naturally act on the lipid water interface, thus increasing their substrate scope beyond water soluble compounds.

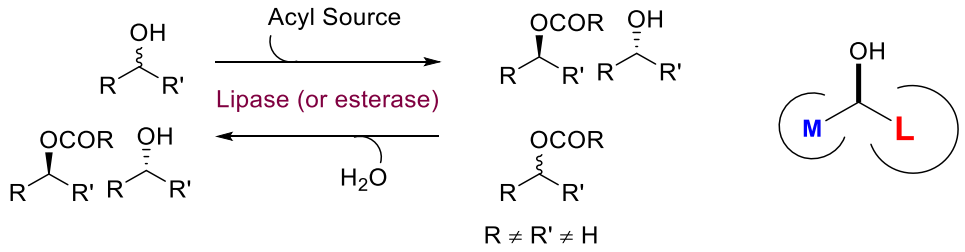



*Scheme 2.1 Lipases naturally catalyse the reversible hydrolysis of triacylglycerols such as palmitate **5***

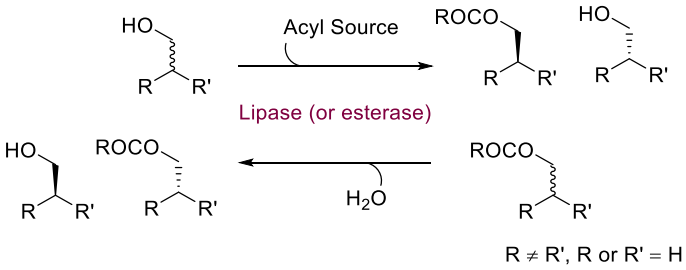
Many different strategies can be employed to increase the selectivity of the lipases including, but not limited to: addition of organic solvents,[4-6] changing temperature,[7,8] immobilisation and modification of the enzyme,[9,10] the presences of additives,[11-13] and, in the case of (trans)esterification, modification of the acyl donor.[14-20]

Studies on the selectivity of lipases usually focus on compounds where the chiral centre is adjacent to the site of reaction, e.g. α-alkyl carboxylic acids or secondary alcohols (Scheme 2.2). Secondary alcohols are so well studied that there are size based rules for predicting stereochemical outcome with well-known lipases.[1] Extension of these systems, where the

chiral centre is more remote, e.g β-or γ-alkyl carboxylic acids or primary alcohols (Scheme 2.3), can lead to challenges in terms of efficiency and selectivity, and they are therefore less studied than their secondary alcohol counterparts.[21-24]



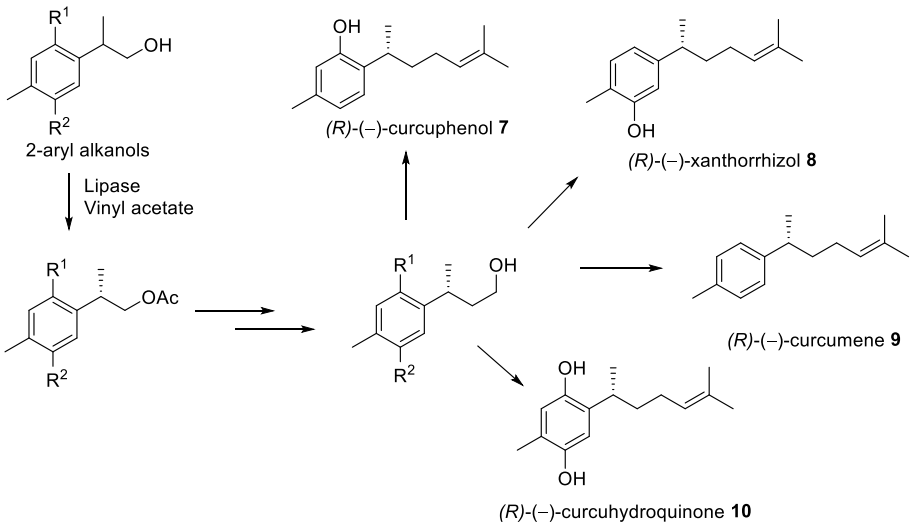

*Scheme 2.2 Hydrolysis and transesterification of chiral secondary alcohols (left), size-based rules for predicting stereochemical outcome (right)*




*Scheme 2.3 Hydrolysis and transesterification of primary alcohols*

### 2.1.1 Importance of 2-phenylalkanols as synthetic intermediates

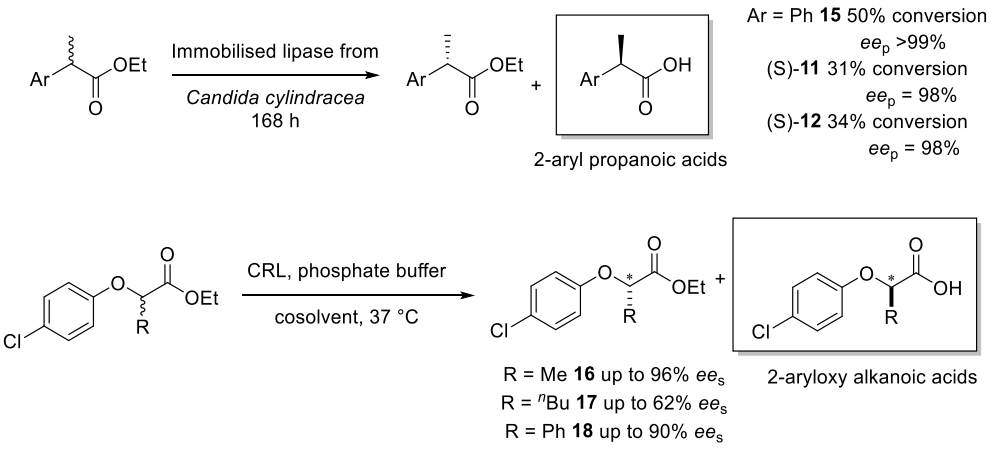
Chiral primary alcohols can be used as building blocks in many drugs, such as the profenols, but are also important in the flavour industry and for the synthesis of natural products including **7–10** (Scheme 2.4).[25]




*Scheme 2.4 Synthesis of natural products from 2-aryl alkanols*

Chiral primary alcohols, having a benzylic stereocentre, can be used in the synthesis of 2-arylpropionic acids, a common class of non-steroidal anti-inflammatory drugs (NSAIDs) (Scheme 2.5), [26,27] as well as in the synthesis of fragrance molecules,[28,29] and substituted coumarins.[25] In most cases, only one form (*R* or *S*) of the drug is active, but the drug may still be produced and sold racemically. For example, *(S)*-ibuprofen *(S)*-**11** is pharmacologically active but it is sold as a racemic mixture. *(S)*-Naproxen *(S)*-**12** was the first NSAID marketed as a single enantiomer.[30] For fragrance or flavour molecules, the different enantiomers can have different smells or tastes e.g *(R)*-(–)-carvone (spearmint) **13** and *(S)*-(+)-carvone (caraway) **13**.[31] The fragrance of Pamplefleur **14** changes only when the stereocentre at C2 changes.[28,29]

Previous work involving 2-aryl and 2-aryloxy propionic acids (being classes of non-steroidal anti-inflammatory drugs, and herbicides, respectively, (Scheme 2.5) achieved the resolution of esters **11**, **12** and **15-18** bearing the stereocentre at the α-position,[32-37] where the conditions were optimised using cosolvents, and immobilising the lipases, as well as mechanistic studies to determine the best conditions for the reactions.

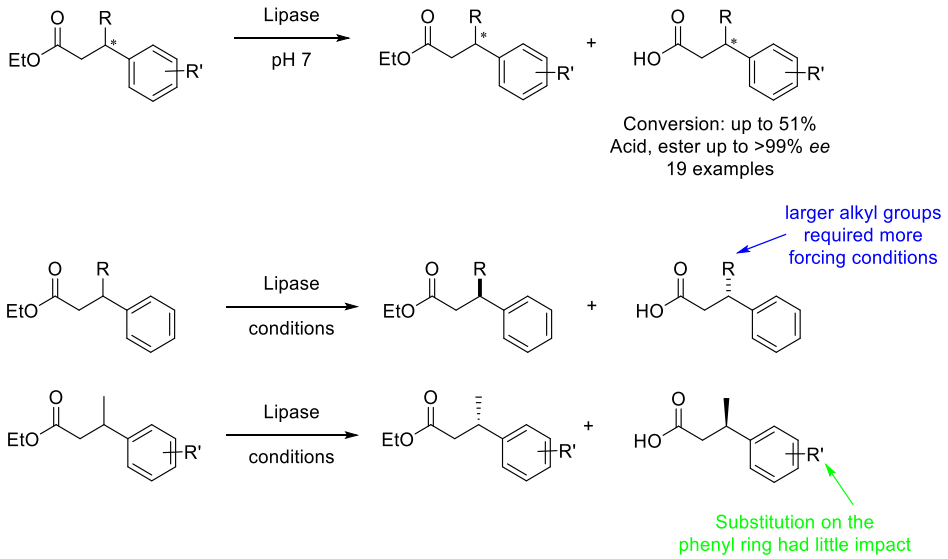



*Scheme 2.5 Resolution of 2-aryl and 2-aryloxyalkanoic acid esters*

## 2.1.2 Previous work in our group

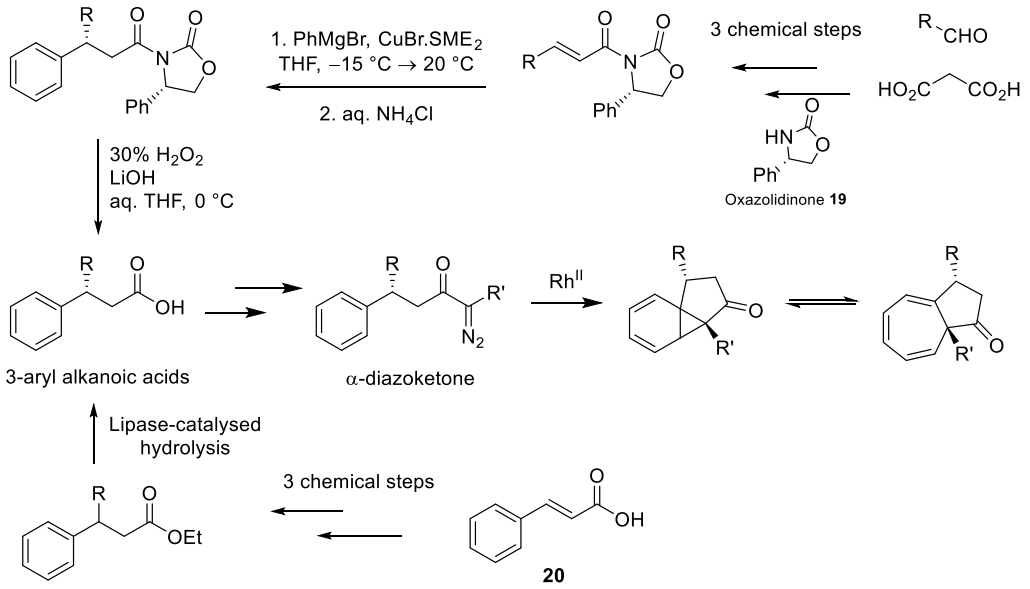
Previous work within our group on the lipase mediated kinetic resolution of chiral 3-aryl alkanoic acids, bearing the stereocentre on the carboxylic acid moiety, showed hydrolase recognition of stereocentres β to the reacting site (Scheme 2.6).[38,39] While under standard conditions, the outcomes were modest, excellent enantioselectivities were achieved for both the ester substrate and acid product through extensive process optimisation. This study showed the effect of changing substitution on the chiral centre, by systematically varying first the alkyl group, followed by the aryl group; as the size of the alkyl group was increased, the

resolution required more forcing conditions in order to hydrolyse the ester.[38,39] The electronic effects through substitution of the aromatic ring were also investigated;[39] this showed that the effect of the aryl substitution on the resolution was minimal, having little effect on the outcome of the resolution.



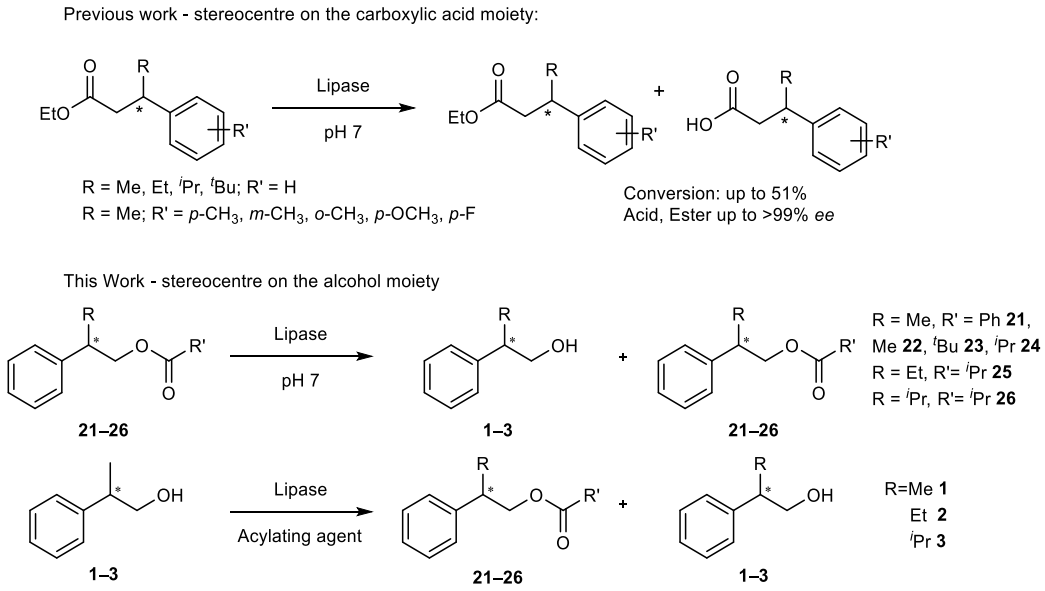

*Scheme 2.6 Resolution of carboxylic acids containing remote stereocentres – the effect of substitution.*

These compounds are synthetically important within our group; enantiopure 3-aryl alkanoic acids are precursors to α-diazoketones, which are used as substrates in the Buchner cyclisation reaction (Scheme 2.7). The reaction has been shown to proceed with very high diastereoselectively when using an enantiopure carboxylic acid starting material (Scheme 2.7).[40,41] The development of enzymatic methods to resolve compounds of this type,[38,39] could replace the use of the chiral oxazolidinone **19** directing group when accessing these compounds. Advantages of this include the use of a readily available carboxylic acid starting material **20**, the removal of the need to add and remove the chiral auxilary, as well as the use of mild conditions (Scheme 2.7).




*Scheme 2.7 Buchner cyclisation*

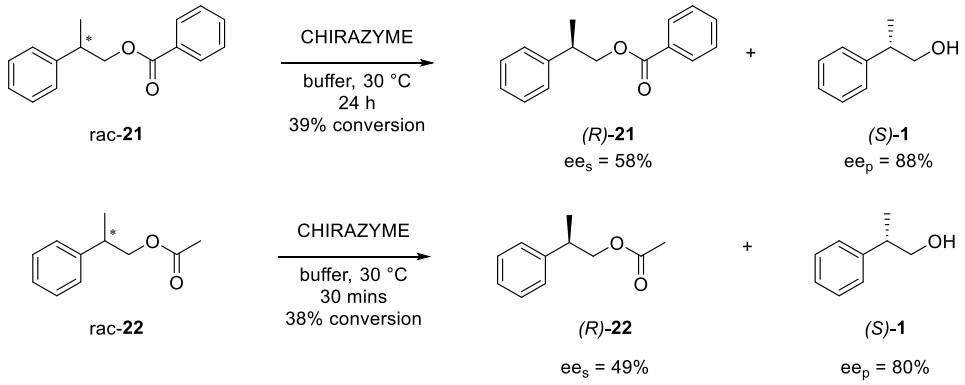
Extension to this work, using derivatives with the chiral centre on the alcohol moiety, removed from the reacting site is the focus of this project. Both the hydrolysis and transesterification reactions can potentially provide access to a series of enantioenriched alcohols and esters (Scheme 2.8). This work focuses on the exploitation of the excellent chemo, regio- and enantioselectivity associated with lipases in the synthesis of 2-phenyl-1-propanol **1**, studying both transesterification and hydrolysis reactions of 2-phenyl-1-propanol **1** and derivative esters **21–24**, respectively, as well as derivative alcohols containing larger alkyl groups **2** and **3** and esters thereof **25** and **26** (Scheme 2.8).




*Scheme 2.8 Hydrolysis and transesterification of chiral primary alcohols and esters*

### 2.1.3 Previous enzymatic resolution of 2-phenyl-1-propanol **1**

The resolution of 2-phenyl-1-propanol **1** has previously been reported, where good selectivities and conversions were attained, using both 2-phenylpropyl benzoate **21** (up to 88% $ee_p$, E = 29) (Scheme 2.9), and 2-phenylpropyl acetate **22** (up to 80% *ee,* E = 14).[17] In general, high enantioselectivity can be achieved in the hydrolysis of 2-phenyl-1-propanol **1** but with the disadvantage of using larger acyl chains (some examples **27–29** shown in Figure 2.8).[17,18,25,42-45] While these chains have the advantage of conferring excellent selectivity, removing the carboxylic acid by-products could lead to problems. The study of short chain acyl groups, aside from acetate, is uncommon in this specific resolution; very few examples are reported.[24,46,47] In the case of smaller carboxylic acid groups, they are usually removed in the work-up and are left in the aqueous layer, leaving only a two-component system in the organic layer, giving an easier separation. In this work small, alkyl groups are used, which is more atom economical in addition to the more convenient work-up.




*Scheme 2.9 Lipase-catalysed resolution of **21** and **22***

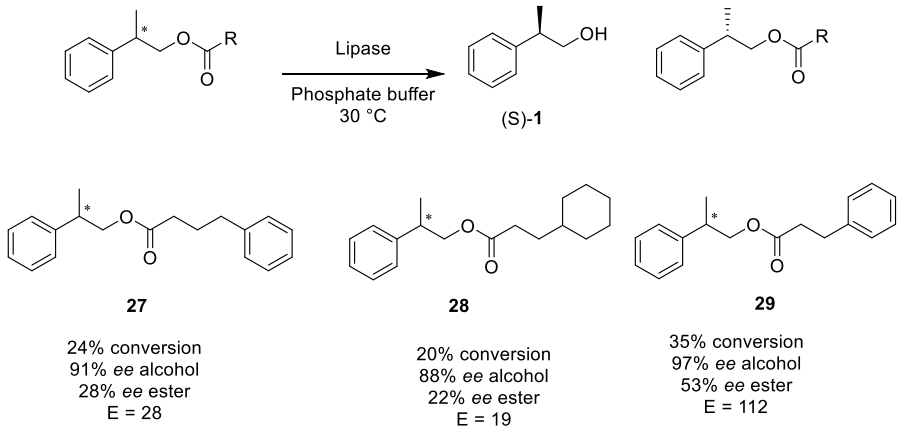
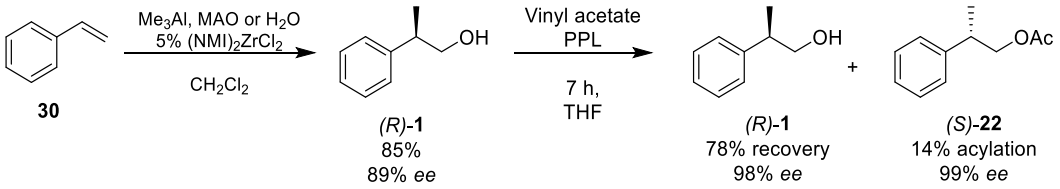



*Figure 2.8 Product esters from the transesterification of 2-phenyl-1-propanol **1** using long chain acyl chains*

For transesterification of 2-phenyl-1-propanol **1**, the acylating agent has been extensively studied, including the effect of a phenyl group on the resolution (phenyl group at β-position is favourable), the length of the alkyl chains (longer chains tend to give better selectivity), and the electronic effects of the acyl chain (with isomeric pentenoic acids as acyl donors it was shown that the position of the double bond is important).[14,18]

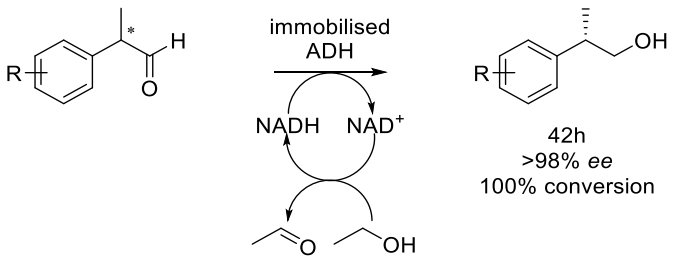
### 2.1.4 Enantioselective synthesis of 2-phenyl-1-propanol **1** – other chemoenzymatic methods

2-Phenyl-1-propanol **1** has previously been prepared enantioselectively from styrene, followed by a lipase catalysed transesterification to further enhance *ee* (Scheme 2.10).[48]




*Scheme 2.10 Use of porcine pancreas lipase (PPL) to enhance* ee

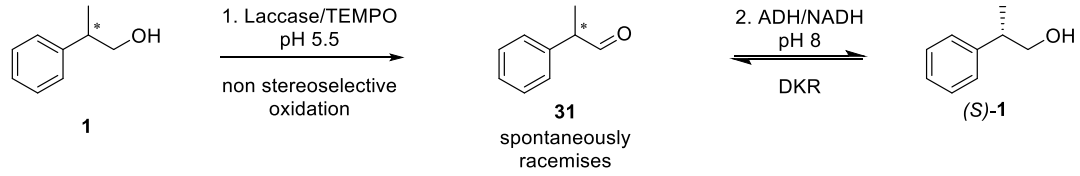
There have been several reports of resolution of racemic alcohols using biocatalysts as shown in Scheme 2.11.[49,50] The mixture is racemised on oxidation, and resolved on reduction. However, while this is a dynamic kinetic resolution, theoretically giving 100% conversion, a disadvantage is that a cofactor is required, and with that, a cofactor regeneration system.




*Scheme 2.11 Resolution of chiral primary alcohols using alcohol dehydrogenase (ADH) as biocatalyst*

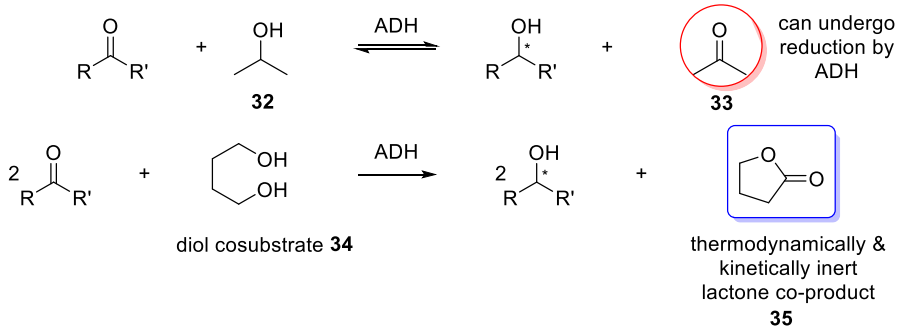
Gotor et al. reported the deracemisation of 2-phenyl-1-propanol **1** by non-selective oxidation of the racemic alcohol to the aldehyde, which undergoes spontaneous racemization, using laccase from *T. versicolor* and TEMPO as an oxidant, followed by enantioselective reduction using an immobilized alcohol dehydrogenase (ADH) (Scheme 2.12).[51] Laccase is a biocatalyst which utilizes molecular oxygen as an oxidant. The enzymes used are active at different pHs.

Both enantiomers were accessible (>94% *ee*) through the use of stereocomplimentary enzymes.




*Scheme 2.12 One-pot stereoselective synthesis of 2-phenyl-1-propanol **1***

The advantage of this synthesis is the use of a cheap racemic starting material. However, because an oxidoreductase (ADH) is employed a cofactor is also required, which is regenerated by the addition of ethanol or 2-propanol **32** as a co-substrate (Scheme 2.11). Smart cosubstrate selection, such as diol **34**, can improve the efficiency of reactions of this type by producing an inert by-product **35** (Scheme 2.13).[52]

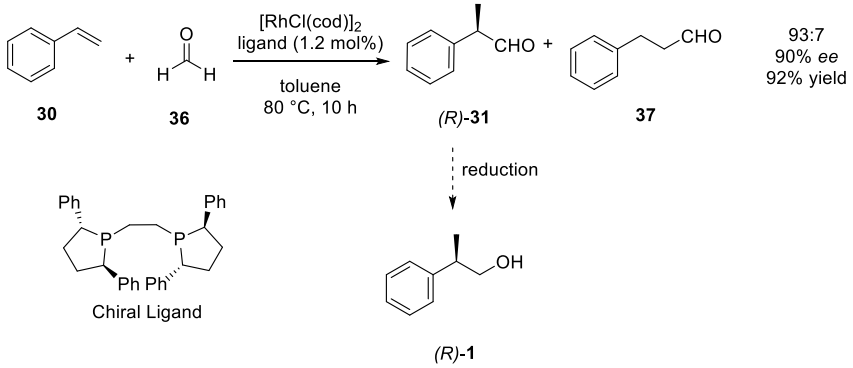



*Scheme 2.13 Smart co-substrate selection can improve the conversion of the reactions*

### 2.1.5 Non-chemoenzymatic synthesis of enantiopure 2-phenyl-1-propanol **1**

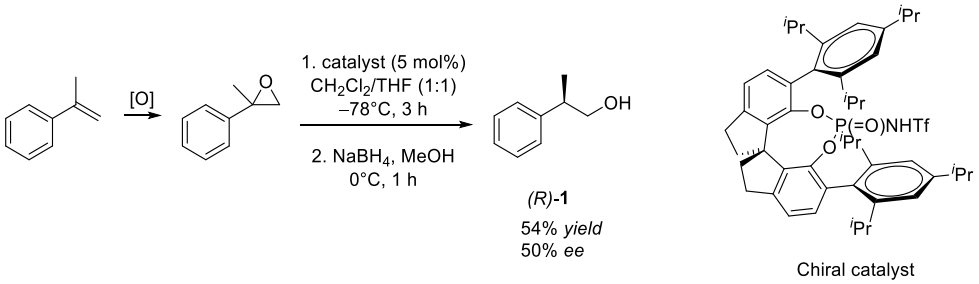
There are a number of recently reported routes to substituted β-aryl alcohols. There are several reports of non-stereoselective reduction of the corresponding aldehydes, catalysed by iron, immobilised ruthenium, and silver.[53-55] Beller et al. reported the synthesis using two ruthenium catalysts cooperatively.[56] Ley et al. prepared a series of racemic alcohols from terminal alkenes through a continuous flow hydroboration-oxidation reaction.[57]

Stereoselective synthesis of aldehydes from terminal alkenes has been reported, giving the internal aldehyde *(R)*-**31** in a stereoselective fashion (up to 95% *ee*) with only minor amounts (7%) of the regioisomer **37**. Reduction of *(R)*-**31** could allow access to the alcohol *(R)*-**1** (Scheme 2.14).[58]



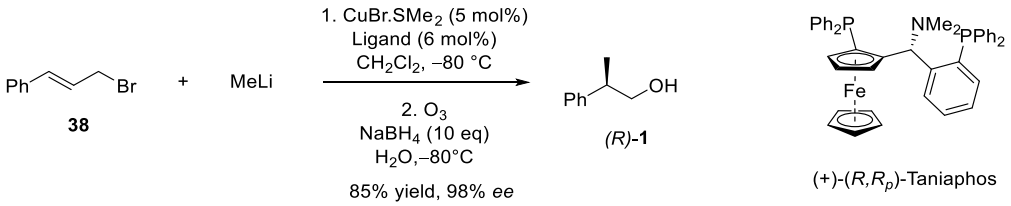

*Scheme 2.14 Asymmetric hydroformylation to give aldehyde (R)-**31***

Zhuang and Du reported a one pot, stereoselective synthesis of 2,2-substituted alcohols from alkenes through a sequential epoxidation, Brønsted-acid catalysed rearrangement to a chiral aldehyde, and reduction with retention of stereochemistry, which gave up to 50% *ee* (Scheme 2.15).[59]



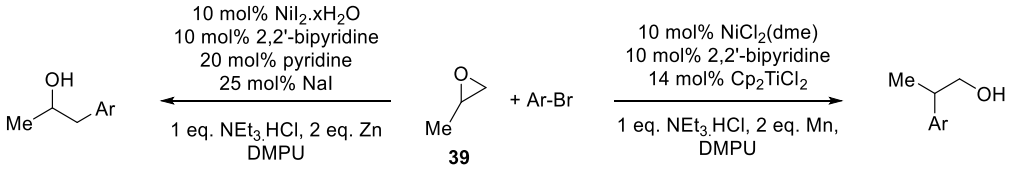

*Scheme 2.15 Asymmetric rearrangement of racemic epoxides*

Feringa et al. reported a copper catalysed asymmetric allylic alkylation of **38**, using organolithium reagents, followed by either reductive ozonolysis, which furnished the 2-substituted alcohols (up to 99% *ee*) (Scheme 2.16), or hydroboration-oxidation, which extended the chain by one carbon, and gave 3-substituted alcohols.[60]




*Scheme 2.16 Copper-catalysed asymmetric allylic alkylation*

Zhao and Weix reported the nickel catalysed coupling of epoxide **39** and arylhalides. The regiochemical outcome of this coupling can be controlled by an additive, either iodide or titanium (Scheme 2.17).[61]




*Scheme 2.17 Regiochemistry controlled by iodine or titanium additives*

Each of the above stereoselective reactions involved the use of complex chiral ligands to induce stereoselectivity. Biocatalytic routes to enantiopure or enantioenriched compounds would avoid the use of complex chiral ligands or metal catalysts, as well as extremes of temperature and the use of organic solvents.

## 2.2 Objectives of this project

The overall objective of this project is to explore the effect of variation of the acyl group on the resolution of 2-phenyl-1-propanol **1**, focusing specifically on the effect of smaller acyl chains which are poorly represented in the literature.

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

- To prepare a series of esters of 2-phenyl-1-propanol **1**.
- To develop chiral HPLC conditions which will separate both enantiomers of the alcohol and the ester
- To screen these esters against a library of lipases in order to assess their activity in the resolution of 2-phenyl-1-propanol **1** by variation of the following factors:
    - Variation of biocatalyst.
    - Alteration of the ester group.
    - Changing the mode of reaction.
    - Alteration of the reaction conditions (e.g. temperature, cosolvent addition, reaction time).
- To conduct a preparative scale synthesis of 2-phenyl-1-propanol **1**.
- To confirm the absolute stereochemistry of 2-phenyl-1-propanol **1**.

## 2.3 Synthesis of substrates

The aim of this section of the project was to synthesize a series of chiral esters to test against lipase enzymes. The compounds varied by having different ester groups (R′), as well as different substituents (R) at the β-position of that ester (Figure 2.9)

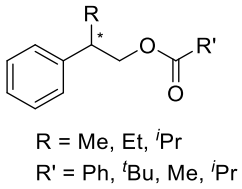



*Figure 2.9 General structure of the target ester substrates **21 – 26***

The key starting materials for the synthesis of the ester substrates are chiral primary alcohols, 2-phenylpropan-1-ol **1**, 2-phenylbutan-1-ol **2** and 3-methyl-2-phenylbutan-1-ol **3**, Figure 2.10, of which two are commercially available, **1** and **2**. Alcohol **3** is not commercially available, and was synthesised for this investigation

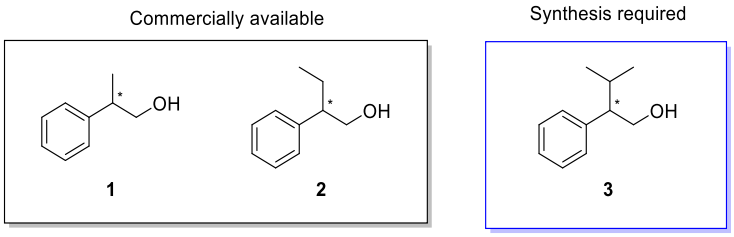
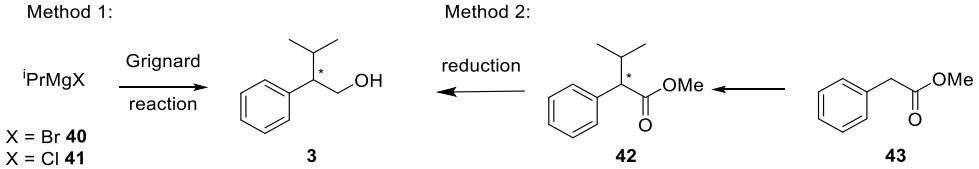



*Figure 2.10 Starting alcohols **1–3**.*

### 2.3.1 Alcohol synthesis

3-Methyl-2-phenyl-1-butanol **3** was synthesized by two methods (Scheme 2.18). The first method used a Grignard reaction, and the second approach used a substitution reaction followed by a reduction.




*Scheme 2.18 Synthesis of 3-methyl-2-phenylbutan-1-ol **3***

#### 2.3.1.1 Method 1 – Grignard reaction

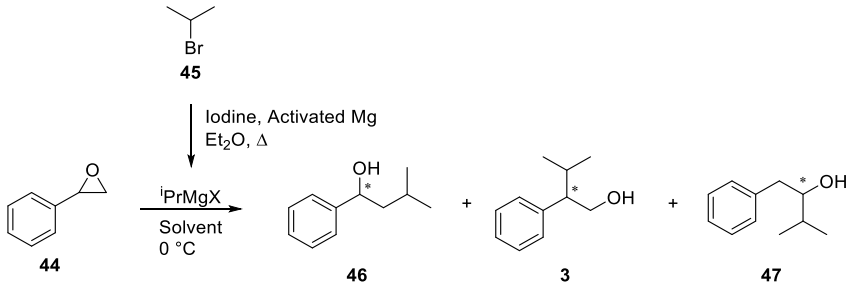
The first attempted route was through a Grignard reaction, using styrene oxide **44** as the electrophile, and in situ generated isopropyl magnesium bromide **40** as the nucleophile.[62]

The reaction uses approx. 1.2 equivalents of magnesium and 1.1 eq. of the alkyl halide to prepare the Grignard reagent. The disappearance of styrene oxide **44** by TLC is used to monitor the reaction progress and is generally complete within 30 minutes.

There are many reported methods to enhance the efficiency of the initiation of the Grignard reaction.[63-65] In this case the magnesium was activated before use by vigorous stirring in ~1 mL of solvent in the presence of a crystal of iodine for 1–1.5 h. To initiate the reaction, the neat alkyl halide **45** was added slowly to the solution, until the reaction had started, which is evident by a colour change from brown to grey. Once the reaction was initiated, the alkyl halide was diluted with the required amount of solvent for the reaction and added at such a rate as to maintain reflux without external heating. As the presence of even minor amounts of water has a detrimental effect on Grignard reagents freshly opened bottles of diethyl ether were used in this reaction.[63,66-69]

The addition of the Grignard reagent to the styrene oxide can give rise to two isomeric products. However, the second expected product **46**, was not observed; instead a rearrangement product **47** was isolated (Table 2.1). The reaction was also carried out using commercially available isopropyl magnesium chloride **41** (2.0 M in THF), in this case the ratios of products formed were different, this is due to solvent induced change in the Schlenk equilibrium. This reactivity has been previously reported and the product ratios can be altered by adding the Grignard reagent to the epoxide instead of adding the epoxide to the Grignard reagent.[70,71]

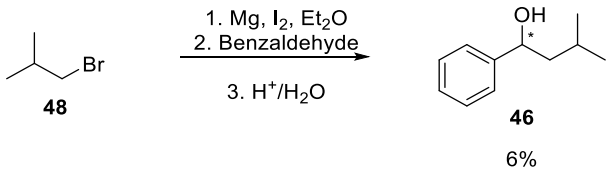
*Table 2.1 Preparation of alcohol **3***




| Entry | X | Solvent | Ratio 46:3:47 | Yield 3 (%) | Yield 47 (%) |
|---|---|---|---|---|---|
| 1 | Br (in situ generated) | $Et_2O$ | 0:45:55 | 21 | 21 |
| 2[a] | *Cl* (commercially available, 2.0M in THF) | THF, distilled | 0:66:34 | 30 | 12[b] |

[a]Carried out by fourth year student; [b]material was recovered from the column, made up of compound **3** and **47** (63:37) coeluting, and was equivalent to approximately 25% yield, it was not separated as sufficient material was isolated.

Figure 2.11 shows the $^1$H NMR spectrum of the crude material from the Grignard reaction using the commercially available Grignard reagent **41**. The spectra of the two expected products **46** and **3** are also shown. Alcohol **46** was synthesised independently by a Grignard reaction which would not give rise to isomeric products (Scheme 2.19); the reaction was low-yielding due to incomplete formation of the Grignard reagent; a freshly opened bottle of benzaldehyde was used but it was not distilled. Sufficient material was isolated for analysis. The absence of the 1H dd at 4.75 ppm, corresponding to the proton at the chiral centre of **46** (C**H**OH) indicates that this product was not formed in the Grignard reaction using styrene oxide **44**.




*Scheme 2.19 Synthesis of **46***

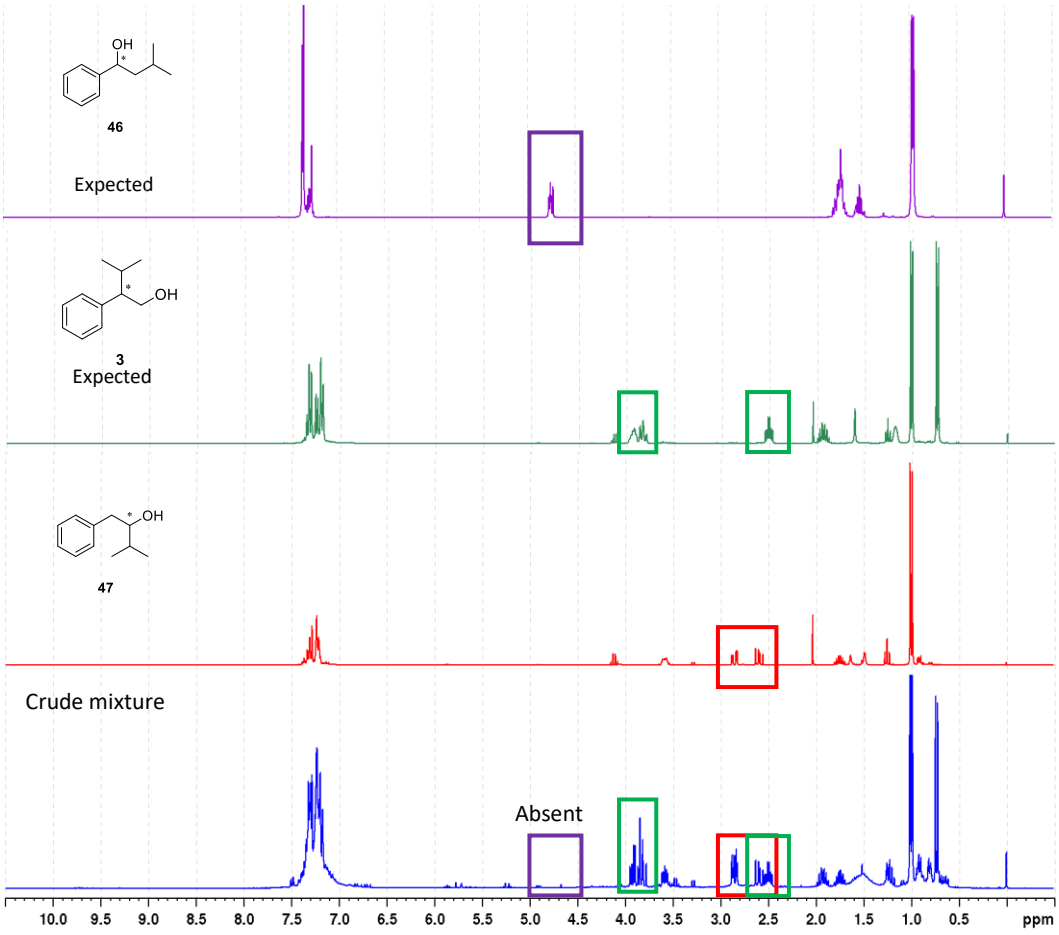
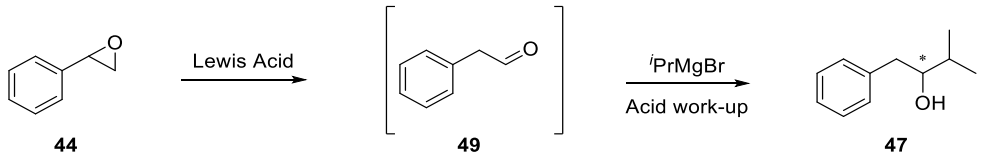



*Figure 2.11 $^{1}$H NMR spectra, showing the crude material (blue), the rearrangement product **47** (red), the target alcohol **3** (green), and the expected regioisomer **46** (purple). ($CDCl_3$, 300 MHz)*

The product formed was analysed by $^{1}$H and $^{13}$C NMR and DEPT experiments; 2D experiments were carried out in order to identify the product **47**. It is thought that the product arose from a Lewis acid catalysed ($MgBr_2$) Meinwald rearrangement, via aldehyde intermediate **49** (Scheme 2.20).[70] Data for **47** matches previously reported data.[72] All alcohol products are known compounds and spectroscopic data is consistent with previous reports.




*Scheme 2.20 Meinwald rearrangement*

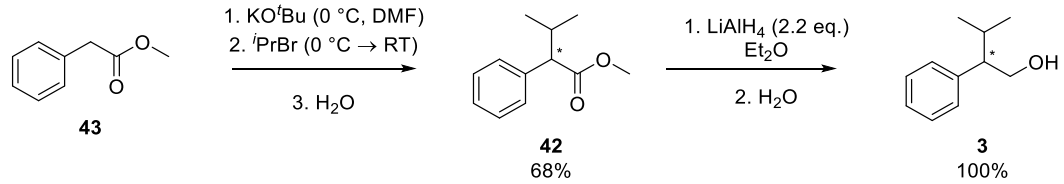
The rearrangement of epoxides in the presence of Lewis or Brønsted acids has been previously reported.[59,73-75] In particular the rearrangement of styrene oxide under Grignard conditions

has been reported, and the regiochemical outcome can be modified by changing the order of addition of reagents i.e. adding styrene oxide **44** to the Grignard reagent can give different ratios of regioisomers to adding the Grignard reagent to the styrene oxide.[70,71]

The yield of the desired alcohol **3**, after column chromatography, was 21%. While this could be scaled up to provide a reasonable amount of material from cheap starting materials, it is inefficient, and so another route was explored.

#### 2.3.1.2 Alternative route

The synthesis of alcohol **3** via a known synthetic route was carried out in accordance with previously reported procedure (Scheme 2.21).[76]




**43** | **42** 68% | **3** 100%

*Scheme 2.21 Alternative route to alcohol **3***

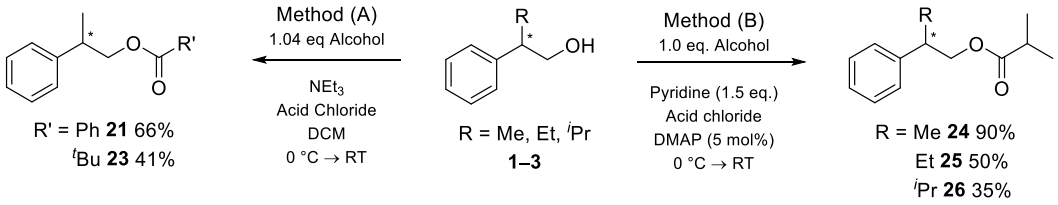
The first step involved deprotonation of the methyl 2-phenylacetate **43** by potassium *tert*-butoxide in DMF, and the subsequent alkylation using isopropyl bromide **45**. Complete removal of the DMF required extensive washing, as described in the experimental section. After column chromatography, **42** was reduced using lithium aluminum hydride, according to literature procedure, and required no purification.[76] Using this method, multigram quantities of alcohol **3** could be readily prepared.

### 2.3.2 Ester synthesis

The initial route to the esters, used in the synthesis of **21**, required the use of an excess of alcohol (1.2 equivalents) in the presence of the appropriate acid chloride. When this amount was reduced (to 1.04 eq.) the efficiency of the reaction dropped (Scheme 2.22, method A). In the synthesis of 2-phenylpropyl benzoate **21**, this resulted a slightly decreased yield from 70% to 66%.

The introduction of a nucleophilic catalyst, DMAP, increased the efficiency of the reaction, utilising only one equivalent of the alcohol and, in general, gave complete conversion to the ester (Scheme 2.22, method B).[77] The products obtained were of excellent purity, but were

subjected to column chromatography as very pure material was needed for the enzymatic reactions.




*Scheme 2.22 Ester synthesis*

## 2.4 Hydrolase-mediated kinetic resolution – analytical screens

### 2.4.1 Screening protocol – hydrolysis of esters

The hydrolysis screening reactions were carried out at approx. 50 mM scale (50 mg of ester in 4.5 mL buffer). The reaction mixture was subjected to a mini work-up, and filtered through Celite®. No drying agent was used. If conversion was detected, the crude mixture was analysed by chiral HPLC analysis. Purification was not necessary before HPLC analysis, as the reactions produce no by-products. The crude material contained only ester substrates and alcohol product. This allowed us to carry out the screening reactions on a small scale; this reduced the amount of material necessary for screening. Generally, when both the alcohol and the ester separated on a given chiral column, then other esters of the same alcohol would also separate on that column.

Transesterification reactions were carried out in neat vinyl ester (0.5 mL) using 150 mM (10 mg) of 2-phenyl-1-propanol **1**. The reaction solution was simply filtered through a plug of Celite® before analysis, and analysed like the hydrolysis reactions.

### 2.4.2 Screening – initial target 2-phenylpropyl benzoate **21**

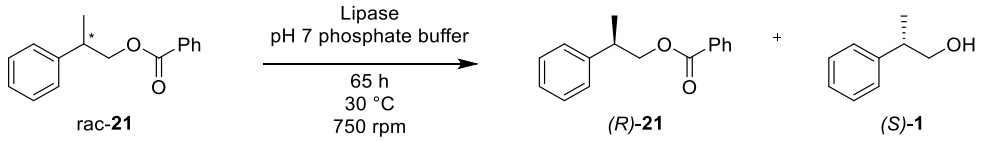
Ester **21** was initially screened against a targeted panel of lipases supplied by Almac in a previous study carried out by Gavin.[78] Of the 52 enzymes tested, 15 gave no conversion, 22 gave conversion under 10%, 4 gave 100% conversion; a selection of the results are shown in Table 2.2 (Entries 1–11). Using the lipase from *Pseudomonas cepacia*, excellent enantioselectivity toward the alcohol **1** was achieved (93% *ee*) (Entry 4). Lipases C, D, E and F from *Alcaligenes sp.* showed similar selectivities towards the product, with similar E values (Entries 7, 9, 2 and 10, respectively).

When the work was progressed in this study, a small selection of commercially available lipases was screened for activity towards the chosen substrate (Table 2.2, Entries 12–16) and subsequently used for other substrates. The commercially available lipases used were: Hog pancreas lipase, CAL-B (immob), *Pseudomonas fluorescens* (immob), Amano PS Lipase, and Lipase from *Candida cylindracea.*

During this study the lipase from *Candida cylindracea (*Table 2.2*,* entry *15)* gave a good conversion but without selectivity. This hydrolysis was subjected to a small solvent screen, including 1-octanol (Table 2.2, entry 16), which furnished us with $ee_p$ = 27%, $ee_s$ = 20%, c =

42%. While this is poorly selective overall, it does show a drastic increase in selectivity compared to the absence of the organic cosolvent (Entry 15). While the use of *Pseudomonas fluorescens* (Entry 3) gave 30% conversion with moderate enantioselectivity, the use of the immobilised lipase (Entry 13) gave <5% conversion.

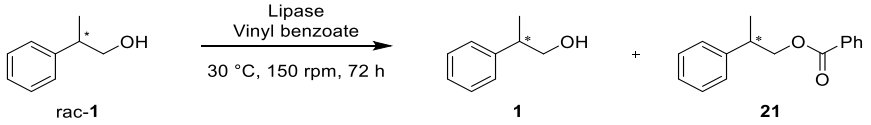
*Table 2.2 Hydrolysis of 2-Phenyl-1-propyl Benzoate **21***




| Entry | Lipase | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | Lipase A from *Burkholderia cepacia* | 47 | 48 | 66 | 71 | 11 |
| 2 | Lipase E from *Alcaligenes sp* | 30 | 33 | 23 | 46 | 3.4 |
| 3 | Lipase from *Pseudomonas fluorescens* | 29 | 29 | 28 | 69 | 7.1 |
| 4 | Lipase from *Pseudomonas cepacia* | 23 | 32 | 44 | 93 | 42 |
| 5 | Lipase from *Aspergilus niger* | 22 | 24 | 8 | 26 | 1.8 |
| 6 | Porcine *Pancrease* type II | 17 | 19 | 7 | 30 | 2 |
| 7 | Lipase C from *Alcaligenes sp* | 15 | 32 | 18 | 39 | 2.7 |
| 8 | *Candida antartica* lipase B (immob) | 15 | 19 | 21 | 87 | 17 |
| 9 | Lipase D from *Alcaligenes sp.* | 14 | 11 | 8 | 64 | 4.9 |
| 10 | Lipase F from *Alcaligenes sp.* | 11 | 7 | 4 | 57 | 3.8 |
| 11 | Lipase from *Candida antarctica* | 10 | 18 | 20 | 89 | 20 |
| 12 | Hog pancreas lipase | 4 | -[b] | -[c] | -[c] | -[b] |
| 13 | *Pseudomonas fluorescens* (immob) | 2 | -[b] | -[c] | -[c] | -[b] |
| 14 | Amano PS lipase | 9 | -[b] | -[c] | -[c] | -[b] |
| 15 | Lipase from *Candida cylindracea* | 48 | -[d] | 0 | 0 | -[d] |
| 16 | Lipase from *Candida cylindracea* with 17% *v/v* 1-octanol | -[e] | 42 | 27 *(R)* | 20 | 2 |

Entries 1 to 11 were carried out by D. Gavin. [a]Enantiomeric excess values determined by chiral HPLC analysis; [b]$E_{calc}$ and E values were not determined, as *ee* values were not measured when conversion <10%; [c]enantiomeric excess values were not determined when coversion was <10%; [d]$E_{calc}$ and E values were not determined, as enantioselectivity was <1% *ee;* [e]conversion was not determined by $^1$H NMR due to the presence of 1-octanol which was not effectively removed by rotary evaporation because of high b.p. (195°C).

The resolution was also attempted using the transesterification reaction (Scheme 2.23), as the selectivity can be changed by changing the mode of reaction.[79] Unfortunately, the transesterification reaction with vinyl benzoate gave no conversion when Hog Pancreas lipase, CAL-B (immob), *Pseudomonas fluorescens* (immob), or Amano PS Lipase were used; Lipase from *Candida cylindracea* gave 100% conversion after 72 hours.




*Scheme 2.23 Transesterification of **1** using vinyl benzoate*

Lipase from *Candida cylindracea* does not give selectivity for our compounds. It has been used for transesterification of secondary alcohols to esters having alkyl chains on the acyl moiety and for transesterification of secondary amines.[80,81]

### 2.4.3 Screening – small acyl group: acetate

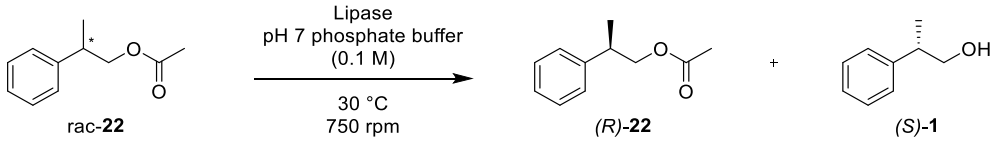
To explore the steric demands of the acyl group, it was decided to test the commercial enzymes against a much smaller ester group, the acetate group. Resolution by hydrolysis has previously been reported but was poorly selective (E = 4) for 2-phenyl-1-propyl acetate **22**.[82] More common however, is the resolution of the acetate **22** by transesterification.[25,44,83,84]

Resolution of 2-phenyl-1-propanol **1** was attempted using both hydrolysis and transesterification reactions, and the reaction times were adjusted to ensure the optimum kinetic resolution conversion ~50%. Five lipases tested gave >50% hydrolysis product after 65 hours (Table 2.3 and Table 2.4, additional timepoints are shown in **Appendix III**).

The presence or absence of molecular sieves had little effect on the transesterification reactions, with molecular sieves increasing the conversion, but having very little effect on the E value.[21]

Following these experiments, and with reference to the literature,[17,25,44,82,83,85] it was clear that the acetate group was not bulky enough to give good enantioselectivity at 30°C. One option would be to carry out the reaction at a lower temperature, which is known to aid selectivity. Looking at the time screens, where lower reaction times did not necessarily furnish better selectivity, it was decided to change the ester to a bulkier group.

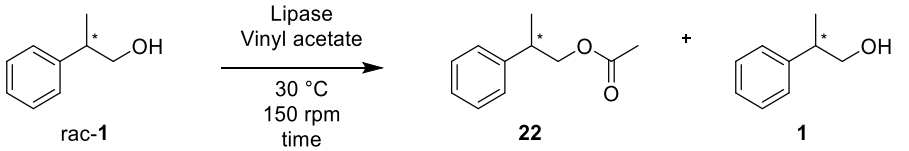
*Table 2.3 Hydrolysis of 2-phenylpropyl acetate **22***




| Entry | Lipase | Time (h) | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|---|
| | | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | Hog pancreas lipase | 65 | 61 | 55 | 65 | 54 *(S)* | 6 |
| | | 48 | 56 | 56 | 71 | 55 *(S)* | 7 |
| 2 | *Candida antarctica* lipase B (immob) | 65 | 87 | 62 | 26 | 16 *(S)* | 2 |
| | | 24 | 55 | 54 | 72 | 61 *(S)* | 9 |
| | | 18 | 56 | 57 | 73 | 55 *(S)* | 7 |
| 3 | *Pseudomonas fluorescens* lipase (immob) | 65 | 82 | 62 | 10 | 6 *(S)* | 1 |
| | | 24 | 52 | 53 | 17 | 15 *(S)* | 2 |
| 4 | Amano PS lipase | 65 | 100 | - | - | - | - |
| | | 6 | 57 | 57 | 25 | 19 *(S)* | 2 |
| 5 | Lipase from *Candida cylindracea* | 65 | 87 | 60 | 3 | 2 *(R)* | 1 |
| | | 48 | 56 | 47 | 4 | 3 *(R)* | 1 |
| | | 6 | 42 | 38 | 3 | 5 *(R)* | 1 |

In the absence of an enzyme, the reaction gave no product after 48 hours. [a]Enantiomeric excess values were determined by chiral HPLC analysis.

*Table 2.4 Transesterification of 2-phenyl-1-propanol **1** using vinyl acetate as acyl source*



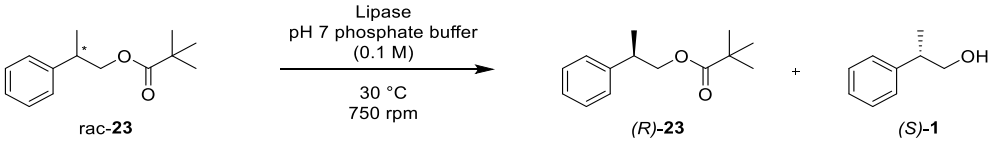

| Entry | Lipase | Time (h) | Conversion (%) | | *ee*[a] *(%)* | | E |
|---|---|---|---|---|---|---|---|
| | | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | Hog pancreas lipase | 24 | 78 | 79 | 99 *(R)* | 27 | 7 |
| | | 24[b] | 86 | 91 | 99 *(R)* | 10 | 4 |
| | | 18 | 58 | 57 | 85 *(R)* | 63 | 11 |
| 2 | *Candida antarctica* lipase B (immob) | 24 | 100 | -[c] | -[c] | -[c] | -[c] |
| | | 24[b] | 100 | -[c] | -[c] | -[c] | -[c] |
| | | 2 | 52 | 67 | 2 *(R)* | 1 | 1 |
| 3 | *Pseudomonas fluorescens* lipase (immob) | 24 | 99 | -[c] | -[c] | -[c] | -[c] |
| | | 24[b] | 100 | -[c] | -[c] | -[c] | -[c] |
| | | 2 | 46 | 43 | 4 *(R)* | 5 | 1 |
| 4 | Amano PS lipase | 24 | 79 | 80 | 45 *(R)* | 11 | 2 |
| | | 24[b] | 95 | 95 | 70 *(R)* | 4 | 2 |
| | | 6 | 44 | 45 | 16 *(R)* | 20 | 2 |
| 5 | Lipase from *Candida cylindracea* | 24 | 100 | -[c] | -[c] | -[c] | -[c] |
| | | 24[b] | 11 | 9 | 1 | 10 | 1 |

Lipase from *Candida cylindracea* gave conversion > 80% even after only 2 hours (E = 1). Reactions carried out in the absence of a lipase, gave no product after 24 hours. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]carried out in the presence of 4Å molecular sieves; [c]when conversion was 100%, enantiomeric excess was not measured, and $E_{calc}$ and E were not determined.

## 2.4.4 Screening – larger acyl group: pivalate

The pivalate ester, 2-phenylpropyl pivalate **23**, was synthesised and screened against the lipases for hydrolysis and transesterification. The hydrolysis reactions gave excellent enantioselectivity towards the alcohol **1**, when Amano PS Lipase was used, albeit coupled with poor conversion (Table 2.5, Entry 4). Other lipases tested gave little to no conversion; those which gave conversion showed poor enantioselectivity for the product **23**.

*Table 2.5 Hydrolysis of 2-Phenylpropyl pivalate **23***




rac-**23** | *(R)*-**23** | *(S)*-**1**

| Entry | Lipase | Conversion (%) | | *ee*[a] *(%)* | | E |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| **1** | Hog Pancreas lipase | 3 | 29 | 4 | 10 *(S)* | 1 |
| **2** | *Candida antarctica* lipase B (immob) | 0 | -[b] | -[c] | -[c] | -[b] |
| **3** | *Pseudomonas fluorescens* lipase (immob) | 0 | -[b] | -[c] | -[c] | -[b] |
| **4** | Amano PS lipase | 15 | 13 | 14 | 90 *(S)* | 22 |
| **5** | Lipase from *Candida cylindracea* | 9 | 11 | 0 | 8 *(R)* | 1 |

Reaction carried out using no enzyme gave no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]$E_{calc}$ conversion and E were not determined as this requires *ee* > 1; [c]enantiomeric excess values were not determined as conversion was 0%.

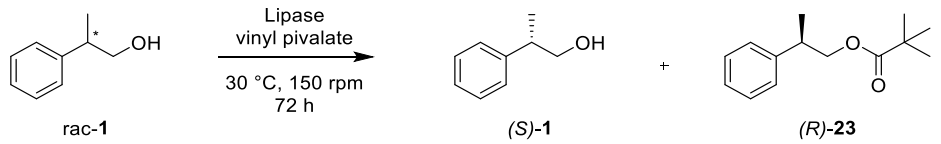
Transesterification reactions (Table 2.6) were poorly selective, giving up to 62% enantiomeric excess of the ester **23**. *Candida antarctica* lipase B (immobilised) gave complete conversion in the reaction time (72 h).

The hydrolytic reaction catalysed by Amano PS Lipase was subjected to a solvent screen (Table 2.7). The temperature was increased to 50 °C, as the lipase is stable to this temperature, and the conversion in the absence of a cosolvent was low; it was hoped that the conversion would increase with increased temperature.

The increased temperature gave a slight decrease in the enantioselectivity towards the alcohol **1** (Table 2.7, Entry 1 vs. Table 2.5, Entry 4). A moderate increase in conversion was observed in the absence of a cosolvent at the higher temperature. The addition of cosolvents gave excellent enantioselectivity, albeit with very poor conversion. It should be noted that in some cases the conversion could not be calculated, as this requires the % *ee* to be >1%; similarly, the E value could not be determined when using methyl *tert*-butyl ether, *n*-heptane and acetonitrile as solvents.

At this stage, the reaction time could have been extended but this process would not be synthetically useful, as the extended time would make the process unattractive. It was decided to try a different substrate.

*Table 2.6 Transesterification of **1** using vinyl pivalate*

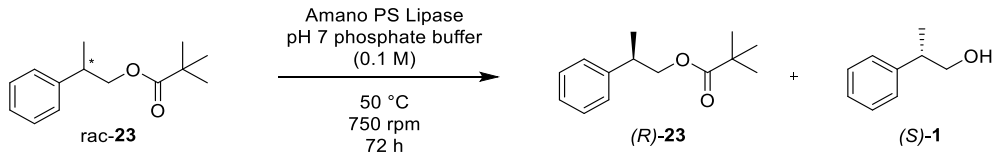



rac-**1** (*S*)-**1** (*R*)-**23**

| Entry | Lipase | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| **1** | Hog Pancreas lipase | 0 | -[b] | -[c] | -[c] | -[b] |
| **2** | *Candida antarctica* lipase B (immob) | 100 | -[b] | -[d] | -[d] | -[b] |
| **3** | *Pseudomonas fluorescens* lipase (immob) | 37 | 33 | 31 *(R)* | 62 | 6 |
| **4** | Amano PS lipase | 8 | 39 | 3 *(R)* | 5 | 1 |
| **5** | Lipase from *Candida cylindracea* | 18 | 13 | 4 *(S)* | 28 | 2 |

Reaction carried out using no enzyme gave no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]$E_{calc}$ conversion and E were not determined as this requires *ee* > 1; [c]enantiomeric excess values were not determined as conversion was 0%; [d]enantiomeric excess values were not determined as full conversion was observed.

*Table 2.7 Hydrolysis of 2-Phenylpropyl pivalate **23** -Introduction of solvents and increasing temperature*




rac-**23** (*R*)-**23** (*S*)-**1**

| Entry | Cosolvent | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| **1** | -[b] | 21 | 19 | 20 | 88 *(S)* | 20 |
| **2** | Methyl *tert*-Butyl Ether | <1 | -[c] | 0 | 81 *(S)* | -[c] |
| **3** | 2-Propanol | -[d] | 4 | 4 | 91 *(S)* | 22 |
| **4** | *n*-Heptane | <1 | -[c] | 0 | >99 *(S)* | -[c] |
| **5** | Acetonitrile | 2 | -[c] | 0 | >99 *(S)* | -[c] |

Initial reactions were carried out at 30°C using the following enzymes, which gave conversion <10%: Hog pancreas lipase, Lipase from *Candida cylindracea*. The following enzymes gave no conversion: *Pseudomonas fluorescens* (immobilised), *Candida antarctica* lipase B (immobilised). [a]Enantiomeric excess values were determined by chiral HPLC analysis; [b]carried out at 30°C, all other reactions carried out at 50°C; [c]$E_{calc}$ conversion and E were not determined as this requires *ee* > 1; [d]conversion by NMR could not be determined because of the presence of the solvent peak

### 2.4.5 Screening – isobutyrate

As a result of the high selectivity but poor conversion using the pivalate ester **23** the isobutyrate ester **24** was selected, as a compromise between the small acetate ester group,

which gave good conversion and poor selectivity, and the bulky pivalate ester group, which gave poor conversion but excellent selectivity (Figure 2.12).

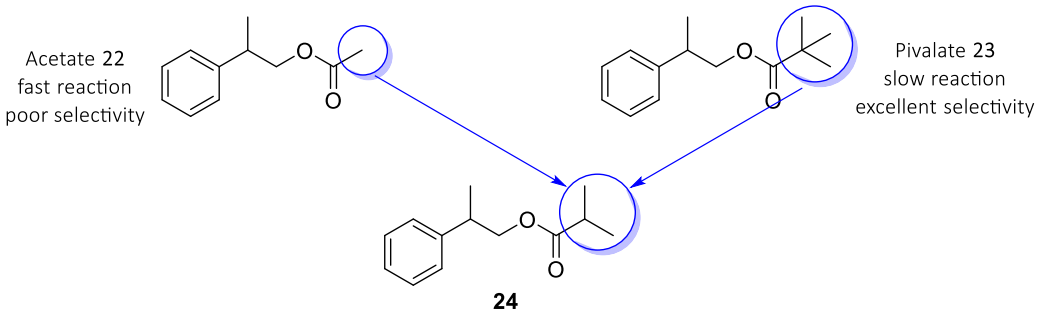
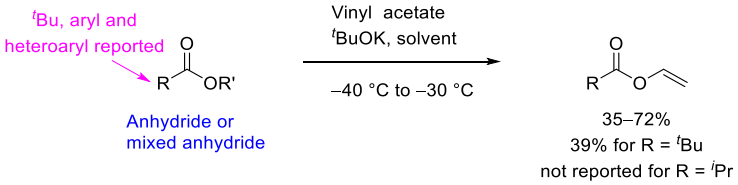



*Figure 2.12 2-Phenylpropyl Isobutyrate **24**: A compromise between ester groups*

Transesterification reactions were not carried out, partly because the vinyl ester was not commercially available. Possible synthetic routes are either low yielding, use complex or gaseous reagents or require high pressure, which would not be sufficient to access the amount of reagent required for screening reactions.[86] Scheme 2.24 shows a recently reported synthetic route to various aromatic vinyl esters through transvinylation of anhydrides or mixed anhydrides as reagents.[87]

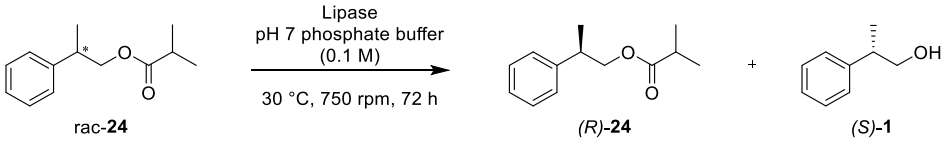



*Scheme 2.24 Synthetic route to enol ethers*

*Candida antarctica* Lipase B (immobilised) showed excellent enantioselectivity in hydrolysis leading to both the ester **24** and the alcohol **1** in good enantiopurity (

Table 2.8, Entry 2); the reaction was repeated several times and shows the reproducibility of the reaction. Amano PS Lipase also gave excellent enantioselectivity for the recovered ester, coupled with conversion >50% (Entry 4).

*Table 2.8 Hydrolysis of 2-phenylpropyl isobutyrate **24***




rac-**24** *(R)*-**24** *(S)*-**1**

| Entry | Lipase | Conversion (%) | | *ee*[a] *(%)* | | E[88] |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | Hog Pancreas lipase | 17 | 15 | 11 | 60 *(S)* | 4 |
| 2 | *Candida antarctica* lipase B (immob) | 51 | 53 | 91 | 82 *(S)* | 32 |
| | | -[b] | 45 | 73 | 90 *(S)* | 41 |
| | | -[b] | 47 | 79 | 90 *(S)* | 48 |
| | | 46 | 46 | 76 | 90 *(S)* | 44 |
| 3 | *Pseudomonas fluorescens* lipase (immob) | 9 | 8 | 4 | 51 *(S)* | 3 |
| 4 | Amano PS lipase | 64 | 67 | 81 | 40 *(S)* | 5 |
| | | -[b] | 63 | 81 | 47 *(S)* | 6 |
| | | -[b] | 51 | 61 | 58 *(S)* | 7 |
| 5 | Lipase from *Candida cylindracea* | 100 | - | - | - | - |

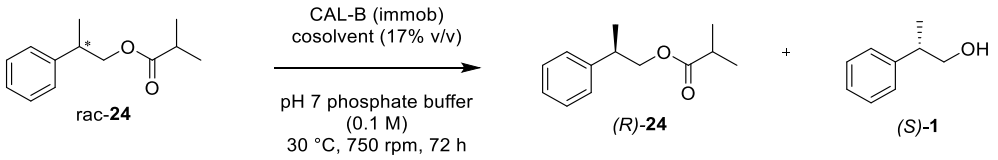
Reaction carried out with no enzyme present resulted in no conversion. [a]Enantiomeric excess values were determined by chiral HPLC analysis using Chiralcel OBH, 0.5 ml/min, 99:1 hexane:IPA; [b]conversion was not determined by $^1$H NMR.

The biocatalysed hydrolysis was subjected to an organic solvent screen (Table 2.9). E-values of up to 66 were achieved, with up to 95% *ee*. Water miscible and immiscible solvents were chosen and used as a 17% v/v mixture with the buffer. Only moderate conversions were attained using water miscible 2-propanol, acetone, and acetonitrile (Entries 3-5). The hydrocarbons pentane, hexane and heptane (entries 14, 8 and 6, respectively) gave similar conversions and enantioselectivities; interestingly, cyclohexane (entry 13) had much higher conversion than the acyclic *n*-hexane (Entry 8) (33% vs 12% conversion). Methyl *tert*-butyl ether (MTBE) and diisopropyl ether (Entries 7 and 9), two ether solvents having the same molecular formula, gave similar enantioselectivities (94% *ee* and 92% *ee*) but MTBE gave higher conversion (34% vs. 8%). Enantioselectivity towards the alcohol **1** was consistently high (87–95%).

Although *tert*-butanol is miscible with water, an interface was observed on addition of 17% *tert*-butanol to the reaction mixture; presumably, the buffer solution was sufficient to change the solubility of the solvent in the aqueous medium.

Recent literature has suggested that less polar solvents have a positive effect on the performance of *Candida antarctica* Lipase B, but no correlation was observed here; it should be noted, however, that the concentrations of solvents used were different in the literature report.[89]

*Table 2.9 Solvent screen of **24** with CAL-B (immob.)*

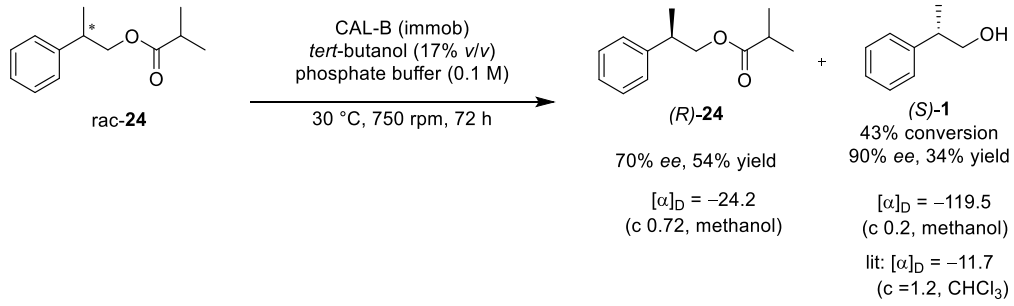



| Entry | Cosolvent | Conversion[a] (%) | *ee*[b] (%) | | E |
|---|---|---|---|---|---|
| | | | *ee*$_s$ *(R)* | *ee*$_p$ *(S)* | |
| **1** | - | 46 | 76 | 90 *(S)* | 44 |
| **2** | 1-Octanol | 2 | 2 | 89 *(S)* | 17 |
| **3** | 2-Propanol | 21 | 24 | 93 *(S)* | 33 |
| **4** | Acetone[c] | 33 | 47 | 95 *(S)* | 60 |
| **5** | Acetonitrile[c] | 33 | 47 | 95 *(S)* | 63 |
| **6** | *n*-Heptane[c] | 14 | 15 | 92 *(S)* | 29 |
| **7** | Methyl *tert*-butyl ether[c] | 34 | 48 | 94 *(S)* | 53 |
| **8** | Hexane[c] | 10 | 10 | 92 *(S)* | 27 |
| **9** | Diisopropyl ether | 8 | 8 | 92 *(S)* | 26 |
| **10** | *tert*-Butanol | 48 | 85 | 91 *(S)* | 59 |
| **11** | 2-Methyltetrahydrofuran | 14 | 15 | 95 *(S)* | 41 |
| **12** | Toluene | 41 | 62 | 87 *(S)* | 28 |
| **13** | Cyclohexane | 33 | 46 | 95 *(S)* | 66 |
| **14** | Pentane[c] | 12 | 12 | 93 *(S)* | 29 |

[a]$E_{calc}$ conversion; [b]enantiomeric excess values were determined by chiral HPLC analysis; [c]HPLC grade solvent used.

## 2.4.6 Preparative scale synthesis of *(S)*-2-phenyl-1-propanol *(S)*-**1** and *(R)*-2-phenylpropyl isobutyrate *(R)*-**24**

Following on from this successful resolution screen, and as a conclusion to the study, the resolution of 2-phenyl-1-propanol **1** was carried out on a preparative scale. The solvent chosen for scale up was *tert*-butanol on the basis of both efficiency and selectivity (Table 2.9, Entry 10) furnishing 2-phenyl-1-propanol **1** in 34% isolated yield, and the ester **24** in 54% yield, after column chromatography, accompanied by 90% *ee* and 70% *ee*, respectively (Scheme 2.25). The literature rotation agrees with the measured rotation.[25]




*Scheme 2.25 Preparative scale synthesis of (S)-2-phenyl-1-propanol **1** and (R)-2-phenyl-1-propyl isobutyrate **24**.*

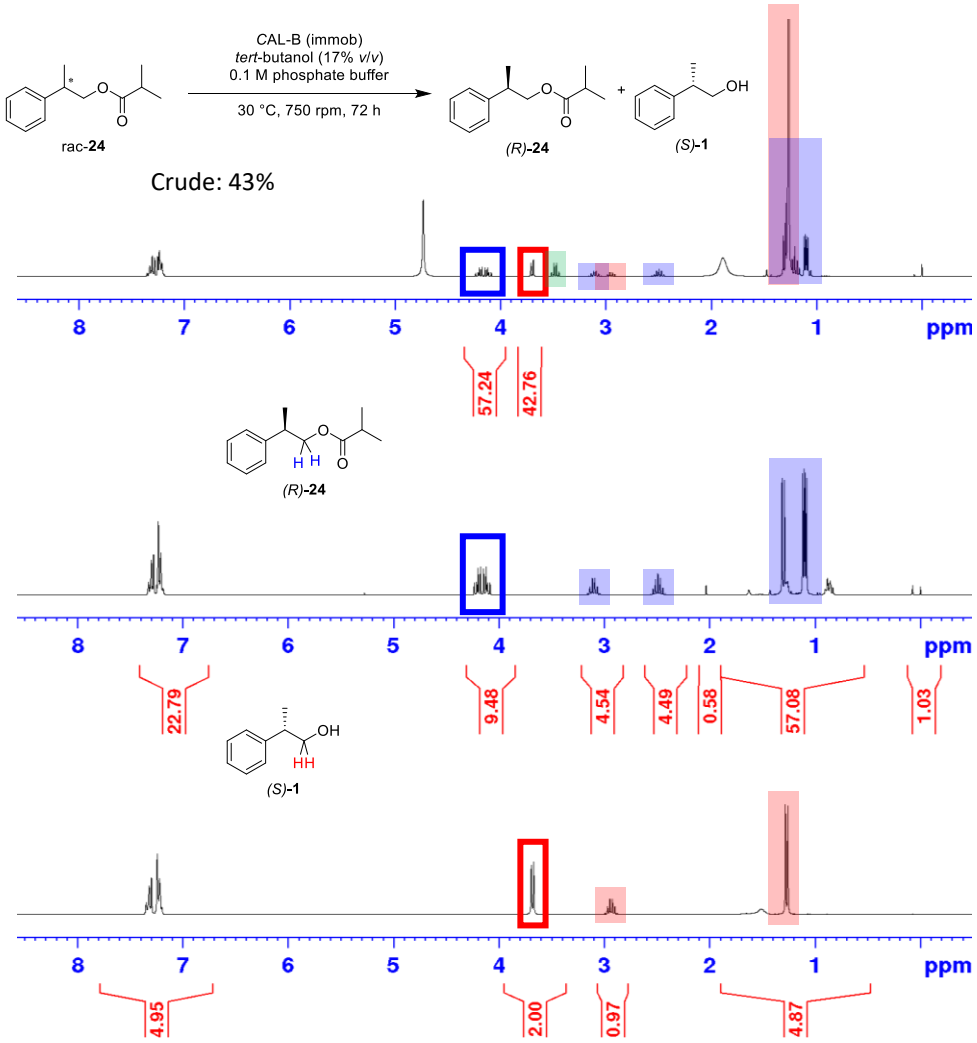



*Figure 2.13 $^{1}$H NMR spectrum of the crude preparative scale reaction (top), the purified ester **24** (middle), and the purified alcohol **1** (bottom) ($CDCl_3$, 300 MHz)*

The isobutyric acid by-product was removed in the aqueous work-up, and was not detected in the crude $^{1}$H NMR spectrum of the reaction mixture (expect singlet at 11.8 ppm, multiplet at 2.6 ppm and doublet at 1.22 ppm)[90] (Figure 2.13); only peaks corresponding to the alcohol product **1** and the ester substrate **24** were present. This is a clear advantage as this meant that only two components needed to be isolated from the crude mixture.

## 2.4.7 Determination of stereochemistry

The stereochemistry was determined by comparison to the reported optical rotation data and the HPLC trace of a sample of enantiopure (–)-*(S)*-2-phenyl-1-propanol *(S)*-**1** (Figure 2.14), purchased from Sigma Aldrich.[25] A sample of the racemic alcohol **1** was run on the HPLC, and a sample of enantiopure *(S)*-**1**. To confirm that the correct elution order had been identified a mixture of the racemic alcohol and the enantiopure alcohol was also run. The first peak was

enhanced relative to the racemic mixture, confirming that the *(S)*-enantiomer was eluted first. This was necessary, as it is known that the retention times of compounds can vary slightly, even under the same conditions.

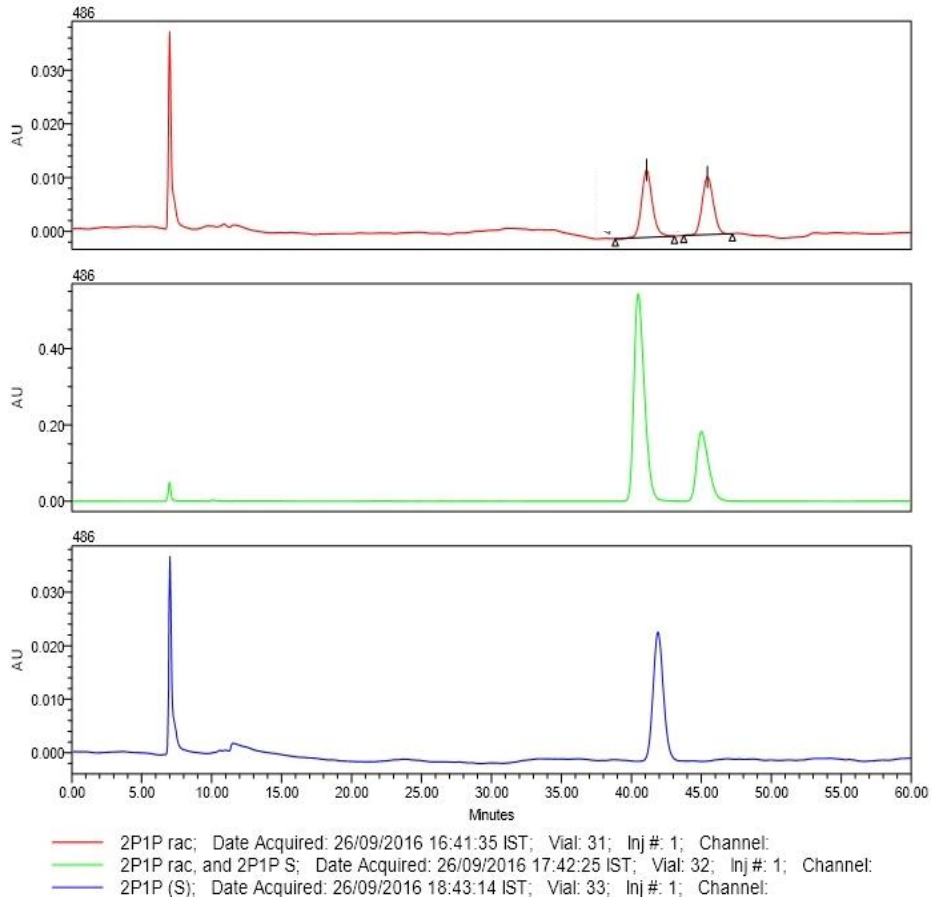


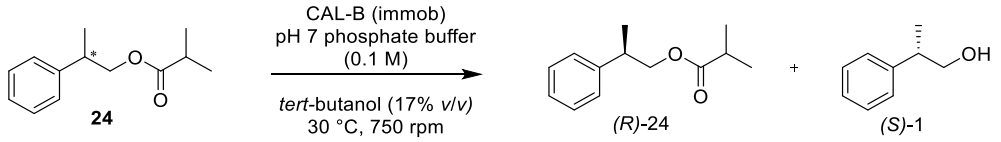

*Figure 2.14 HPLC trace showing racemic 2-phenyl-1-propanol **1** (red), (S)-**1** enriched material (green), and enantiopure (S)-**1** (blue) (Chiralcel OB-H, 0.5 mL/min, 0.5:99.5 2-propanol/hexane)*

### 2.4.7 Follow up – change lipase concentration

In order to ascertain if the reaction was a true resolution, the screen was repeated under the same conditions as the preparative scale resolution, for 24 h, 72 h and 120 h, except that the lipase concentration was doubled. The reactions attained 64% conversion, by $^{1}$H NMR, after 24h, showing that the reaction proceeded faster with higher enzyme concentration. However, the reaction continued past 50% conversion. In this case, the ester selectivity was excellent, 97–99% *ee* as expected for conversion over 50%, with the alcohol selectivity decreasing with increasing conversion (Table 2.10). This shows us that ester **24** can be prepared with excellent enantioselectivity, when using a greater concentration of enzyme, in a much shorter time. However, the higher concentration of the lipase combined with a shorter time is unsuitable

for the preparation of the enantiopure alcohol **1** as the enantioselectivity is lower due to the greater extent of conversion (Table 2.10).

*Table 2.10 Hydrolysis of 2-phenylpropyl isobutyrate **24***




**24** *(R)*-24 *(S)*-1

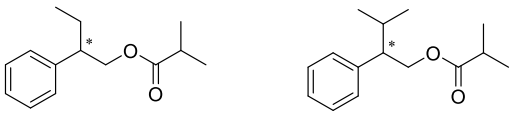
| Entry | Time | Conversion (%) | | *ee*[a] *(%)* | | E[88] |
|---|---|---|---|---|---|---|
| | | NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| **1** | 120 | 77 | 80 | 97 | 25 | 5.7 |
| **2** | 72 | 89 | 77 | >99 | 29 | 7.6 |
| **3** | 24 | 64 | 56 | >99 | 78 | 40 |

Reaction carried out with no enzyme present resulted in conversion <1% after 120 h. [a]Enantiomeric excess values were determined by chiral HPLC analysis.

Overall, these results indicate that by careful control of the reaction conditions and acyl group, the lipase-mediated hydrolysis can lead efficiently to highly enantioenriched 2-phenyl-1-propanol **1** and 2-phenylpropyl isobuyrate **24** in a synthetically useful process. The key to this was choosing the isobutyrate group as a compromise between the pivaloyl (highly selective, poorly efficient) and the acetyl (poorly selective and highly efficient) groups.

## 2.4.8 Follow up – substrate scope

Encouraged by the excellent results using 2-phenylpropyl isobutyrate **24**, the effect of increasing alkyl substituent size was briefly explored. Esters **25** and **26** (Figure 2.15) were subjected to hydrolysis using the optimised conditions for ester **24** [*Candida antarctica* lipase B (immobilised), 72 h, 750 rpm].



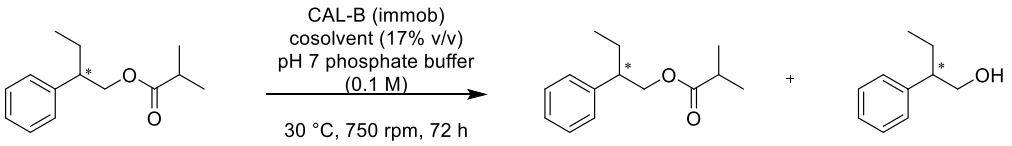
**25** **26**

*Figure 2.15 Esters **25** and **26**, substrate scope.*

Ester **25** showed encouraging results for the initial screen using *Candida antarctica* lipase B (immobilised) (Table 2.11, Entry 1) and was subjected to a small solvent screen, using the solvents which gave the highest E-values for hydrolysis of 2-phenylpropyl isobutyrate **24**. Acetonitrile (Entry 4) showed the highest product enantioselectivity (86% *ee*), which was similar to the result for **24** (Table 2.9, entry 5). Previously, the highest reported %*ee* of **2** by

lipase-catalysed resolution (transesterification) was 69% *ee,* using vinyl acetate as the acyl source.[79] The more sterically demanding ester **26** was not hydrolysed either in neat buffer or with *tert*-butanol (17% *v*/*v*). The use of the ester having a *tert*-butyl substituent at the benzylic position was not explored.

*Table 2.11 Hydrolysis of 2-phenylpropyl isobutyrate **25** with CAL-B (immob)*




rac-**25** **25** **1**

| Entry | Cosolvent | Conversion[a] (%) | *ee*[b] (%) | | E |
|---|---|---|---|---|---|
| | | | $ee_s$ | $ee_p$ | |
| **1** | - | 47 | 66 | 74 | 13 |
| **2** | *tert*-Butanol | 33 | 37 | 74 | 9.6 |
| **3** | Acetone[c] | 21 | 21 | 77 | 9.2 |
| **4** | Acetonitrile[c] | 24 | 28 | 86 | 17 |
| **5** | Methyl *tert*-butyl ether[c] | 19 | 19 | 82 | 12 |
| **6** | Cyclohexane | 18 | 17 | 82 | 12 |

[a]$E_{calc}$ conversion; [b]enantiomeric excess values were determined by chiral HPLC analysis; [c]HPLC grade solvent used.

## 2.5 Project conclusion

A series of esters of 2-phenyl-1-propanol **1** was prepared, including two novel esters, **23** and **24**, and subjected to lipase-catalysed transformations. By manipulation of the reaction conditions, as well as modification of the substrate, the resolution of 2-phenyl-1-propanol **1** was optimised.

2-Phenyl-1-propanol **1** was resolved with excellent enantioselectivity, up to 96% *ee* and up to 48% conversion, using a commercially available lipase and a small, alkyl ester group, making the transformation selective and atom economical. E values of up to 63 were obtained; while this is lower than some reported values, it uses a much smaller ester group, making it much more atom economical, and easier to purify on scale up. A preparative scale resolution was also performed, giving 43% conversion after 72 hours, coupled with 34% yield of alcohol **1** with 88% *ee* and 54% yield of ester **24**, with 68% *ee*.

It was shown that the ester can also be resolved with excellent enantioselectivity (97–99% *ee*) by increasing the lipase concentration.

The influence of the size of the alkyl substituent at the chiral centre was also investigated. The ester **25**, bearing an ethyl group at the stereocentre, was subjected to lipase-catalysed hydrolysis, giving the alcohol **2** with up to 86% *ee*, the highest reported from a lipase catalysed transformation. On further increasing the size of the alkyl substituent to $^{i}$Pr, the substrate **26** proved resistant to hydrolysis under the conditions used.

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# Chapter 3

# Resolution of 6-methylchroman-2-ol: towards the hydrolase-mediated resolution of the hemiacetal in 2-chromanols

# Contents

## Overview

Hydrolase-catalysed kinetic resolution is a powerful tool for the resolution of chiral compounds, although it suffers from a maximum theoretical yield of 50%. Combining the kinetic resolution with a racemisation step, either through use of an external catalyst or through selection of a substrate which will racemise under the reaction conditions, furnishes a dynamic system, which is synthetically more powerful.

Hydrolase-catalysed dynamic kinetic resolutions of chroman-2-ol **50** and 6-methyl chroman-2-ol **51** was effected with up to 88% conversion and 92% *ee* through the use of organic solvents. Extension to the resolution of the tolterodine precursor **52** proved more challenging. The presence of the remote phenyl substituent had a significant impact on the resolution and it was not possible to achieve high enantioselectivity together with efficient conversion from the focused panel of enzymes screened.

## 3.1 Introduction

Coumarins, or benzo-α-pyrones, are a class of molecules made up of a benzene ring fused to a pyrone ring. They are present in many naturally occurring compounds and their derivatives have been used in pharmaceuticals such as anticoagulants,[1] and anti-cancer agents.[2] The numbering system is shown in Figure 3.1.

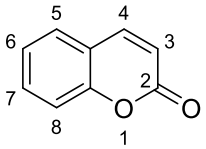
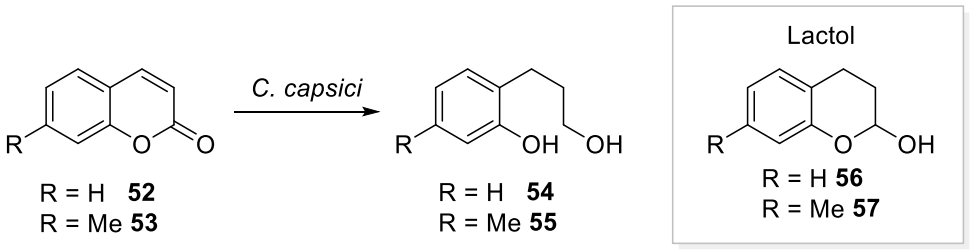
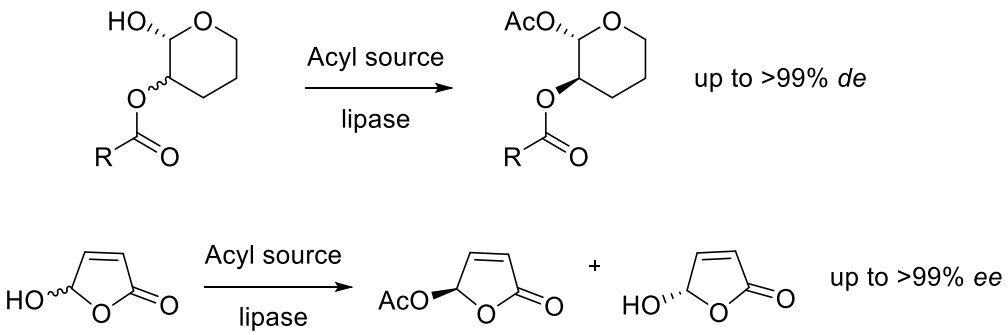



*Figure 3.1 Numbering of Coumarins*

Coumarins **52** and **53** have been used as substrates for enzymatic reduction giving the primary alcohols **54** and **55**, as a result of the reduction of the intermediate aldehyde (Scheme 3.1).[3] However, there are no reports of enzymatic resolution of the anomeric centre of the corresponding lactol compounds **56** and **57** so initially a model substrate was used in order to show that this centre could be resolved.[4] Previous reports of resolution of anomeric centres use monocyclic sugars as substrates (Scheme 3.2).[5-11]




*Scheme 3.1 Enzymatic reduction of coumarins **52** and **53** by C. capsici; corresponding lactols **56** and **57**.*




*Scheme 3.2 Examples of lipase-catalysed resolution of the anomeric centres of sugar molecules*

The lactol moiety is present in potential intermediates in the synthesis of muscarinic antagonists, tolterodine **58** and fesoterodine **59** (Figure 3.2). It is envisioned that the lactol alcohol could be dynamically resolved using lipase-catalysed transesterification, taking advantage of the dynamic ring-opening and ring-closing, which happens in solution. Once it has been shown that the dynamic resolution is possible, using the model lactol **50** and **51**, work can then follow looking at the kinetic resolution of the remote stereocentre in the Tolterodine and Fesoterodine intermediates **60** and **61**.

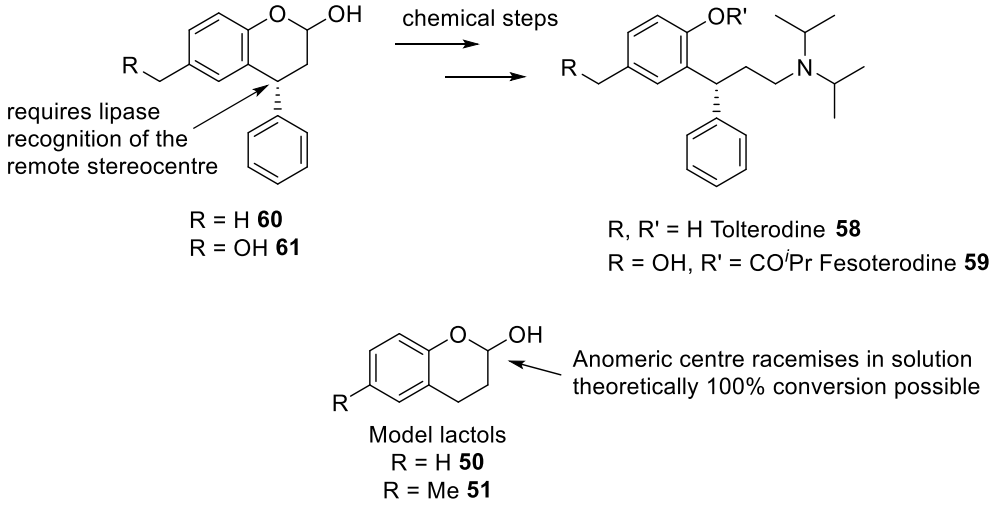
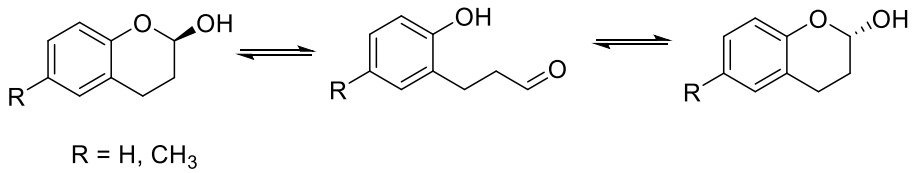



*Figure 3.2 Intermediate lactols **60** & **61**, and the model lactol, 6-methylchroman-2-ol **51***




*Scheme 3.3 Dynamic equilibrium of the lactols with the ring-opened hydroxyaldehyde*

The main goal here has been the synthesis and resolution of the title compound **51** as a preliminary step in the investigation of the resolution of intermediates in the synthesis of the tolterodine **58** and fesoterodine **59** (Figure 3.2).

The lactols exists in a dynamic equilibrium with the corresponding acyclic hydroxyaldehydes (Scheme 3.3). This is important, because it theoretically allows for complete conversion, via a dynamic kinetic resolution. The resolution of the lactol intermediates in the synthesis of tolterodine **58** and fesoterodine **59** will require, in addition, recognition of a stereocentre remote from the hemiacetal reacting site. Lipases have been shown to recognize remote stereocentres in compounds with an OH group (e.g. 3-arylalkanols).[12,13]

This preliminary research was subsequently extended within the team to the resolution of the API intermediates **60** and **61** (Figure 3.3).[4] The use of enzymes to resolve these intermediates represents a move towards a greener synthesis of these compounds.

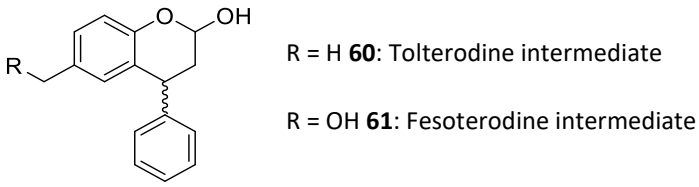
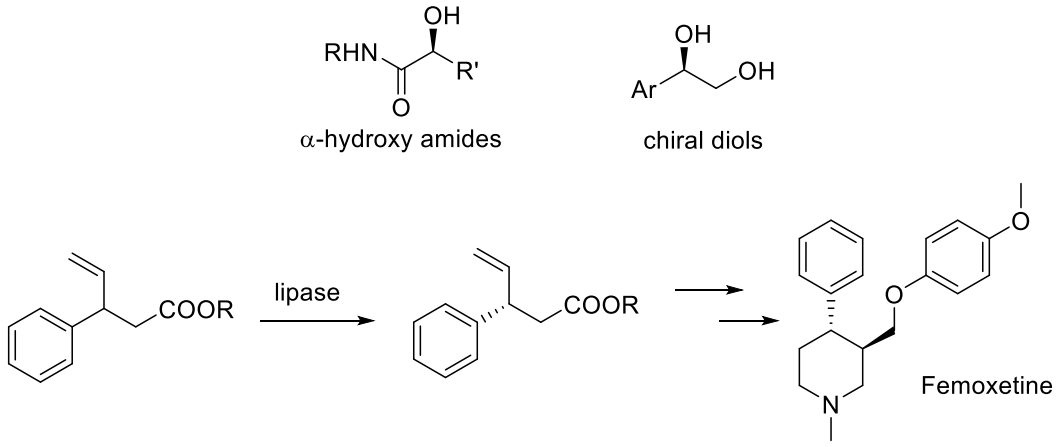



*Figure 3.3 Target compounds: intermediate in the synthesis of tolterodine **58** and fesoterodine **59***

There have been many reports of lipase mediated kinetic resolution of key synthetic intermediates, such as α-hydroxyamides,[14] chiral diols,[15,16] and other drug products and intermediates (Scheme 3.4).[17-19]

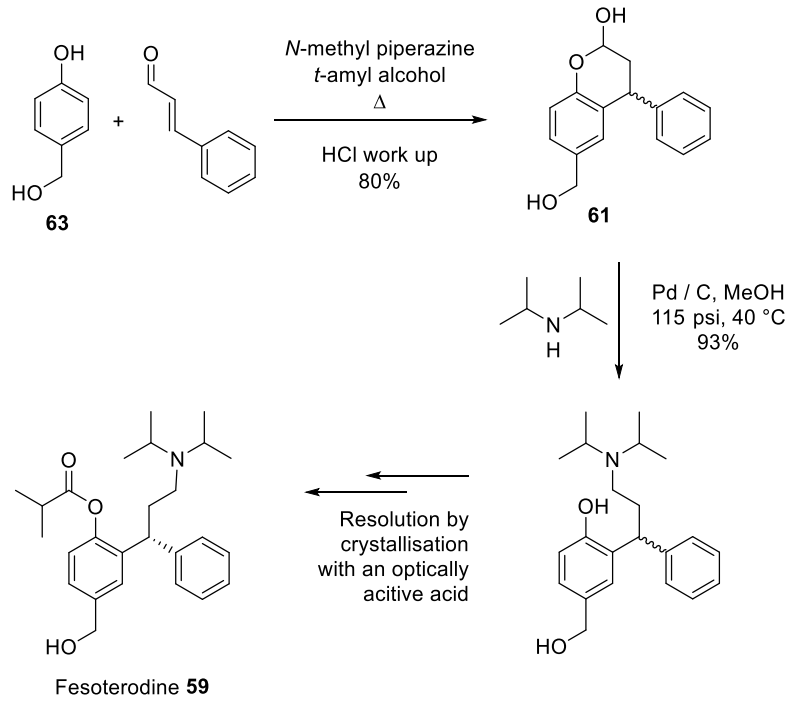



*Scheme 3.4 Some key synthetic intermediates which have been resolved by lipases*

## 3.1.1 Routes to tolterodine **58** & fesoterodine **59**

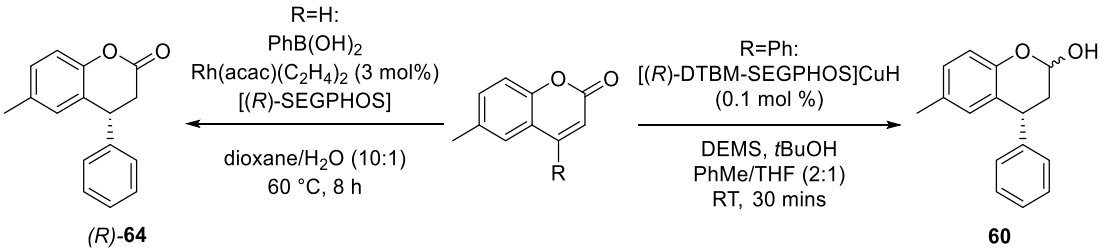
There is no shortage of routes to coumarins, dihydrocoumarins and lactols, each with advantages and disadvantages, including substrate scope and positions of substitution.[20-29]

The current route to fesoterodine **59** is by an amine-catalysed Friedel–Crafts alkylation of the substituted phenol **63** with cinnamaldehyde, followed by a reductive amination of the resulting lactol **61**, and resolution by crystallisation with an enantiopure acid in order to resolve the mixture of enantiomers (Scheme 3.5).[30] Enantiopure compounds are expensive, so the use of cheaply produced enantioenriched material potentially represents a more economical route.




*Scheme 3.5 Synthesis of fesoterodine **59***

The route to tolterodine **58** has been optimized more extensively. First patented in 1998, the original synthetic route relies on the use of a diastereomeric salt to separate the two enantiomers of tolterodine **58** and obtain the active enantiomer, (*R*)-**58**.[31,32] Early work used chiral auxiliaries, such as *N*-oxazolidinones[33] and enol ethers,[34] in conjunction with cinnamic acid **20**, and *o*-quinone methides, respectively. This allowed introduction of the stereocentre early in the synthesis and gave excellent enantioselectivity (>99%). Later, the use of SEGPHOS ligands was reported, with excellent results (Scheme 3.6). The reactions used either coumarins unsubstituted at the 4-position, with the ruthenium mediated stereoselective 1,4-addition of aryl boronic acids to give the lactone **64**, or had the substituent present already and used a copper hydride catalyst to stereoselectively reduce the double bond.[35,36] The latter route had the advantage of furnishing the lactol product **60**, eliminating the need for a separate reduction. The use of alternative ligands has also been reported for the synthesis of *(R)*-**64** including ligands where an early synthetic step in the synthesis of the ligand includes a lipase-catalysed resolution.[37]



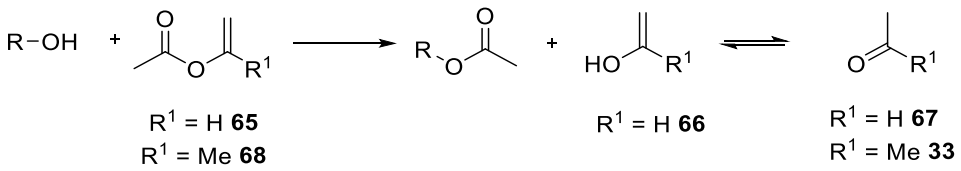

*Scheme 3.6 Enantioselective synthesis of Tolterodine intermediates **64** & **60** using Rh[35] & Cu[36] catalysis*

## 3.1.2 Enzymatic resolution

Although enzymatic resolution of the hemiacetal moiety has been reported previously,[5-11] hydrolase-mediated resolution of aromatic fused hemiacetals has not previously been reported. A model substrate (dihydrochroman-2-ol **51**) was previously synthesized and screened within our group achieving up to 95% *ee* through hydrolase-mediated acylation.[4] This will be discussed with Table 3.3.

## 3.1.3 Acylating agents

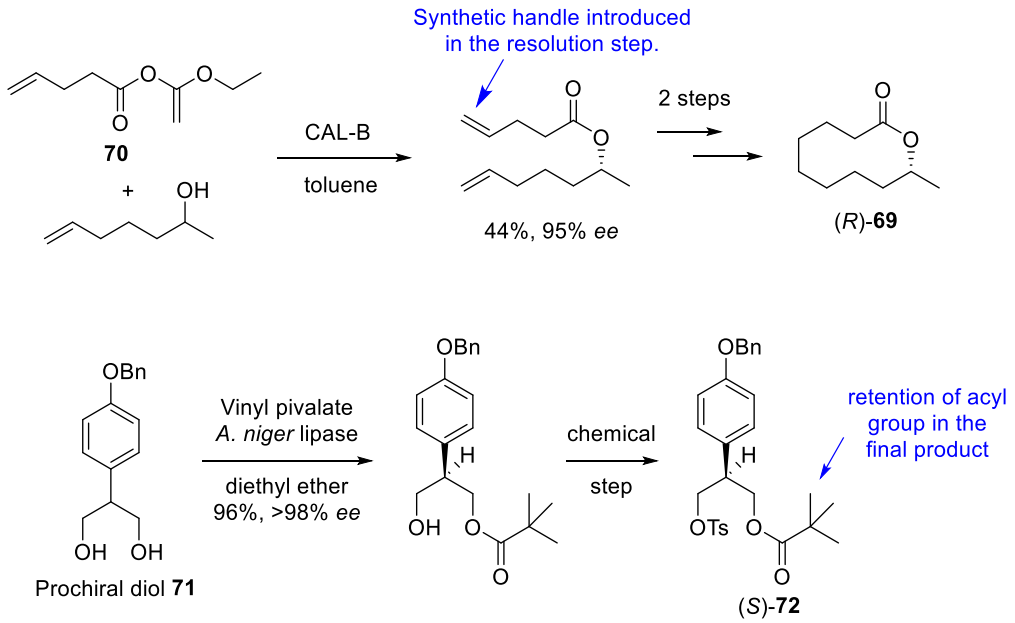
The choice of acylating agent in these reactions is important from a synthetic perspective, because one challenge with enzymatic acylation is the reversibility. The use of an acylating agent such as vinyl acetate **65** can overcome this challenge because the enol by-product **66** is unstable and tautomerises to acetaldehyde **67**, rendering the reaction irreversible (Scheme 3.7).[38] The use of vinyl acetate also has the advantage that there is only a small amount of non-selective background acylation. Isopropenyl acetate **68**, which is a stronger acylating agent than **65**, was also used during this study.[39] Much stronger acylating agents, such as acetic anhydride, would be expected to give higher conversion but lower selectivity. Succinic anhydride has been used in conjunction with lipases giving good selectivity in the resolution of alkyl alcohols.[40,41] The use of 1-ethoxyvinyl furoate has been reported for the resolution of 1,2- and 1,3- diols.[35]




*Scheme 3.7 Acetylation using **68** or **65** and generation of **33** or **67** as a side product*

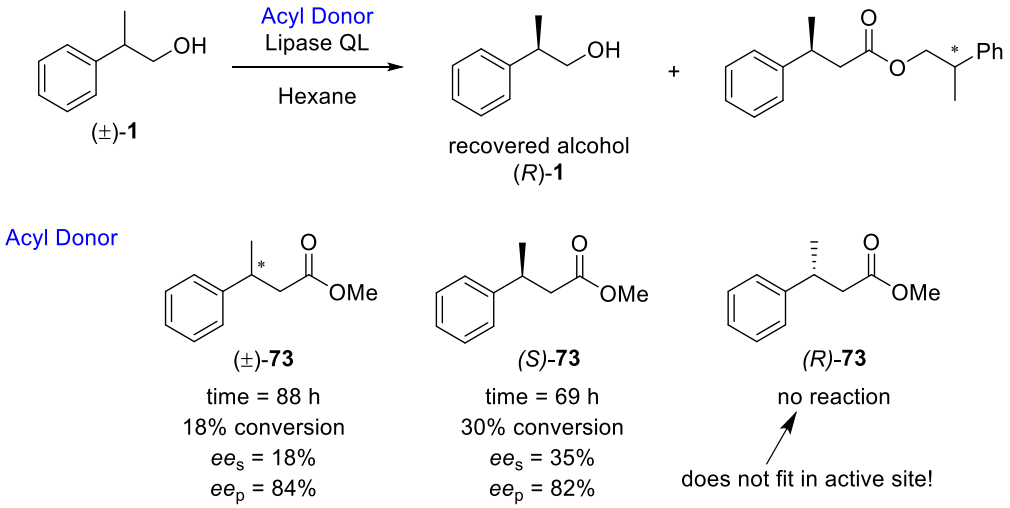
The use of longer-chain acylating agents has been reported to improve selectivity and conversion.[42-44] This is attributed to interaction of the alkyl chain with a hydrophobic channel near the binding site.[44]

Chênevert et al. investigated the resolution of 1-phenylethanol using a series of different acylating agents in order to assess the viability.[13] The ability to use different acylating agents could expand the utility of these reactions, not only allowing the resolution of the compounds but also the introduction of functional groups, which would be especially useful in the synthesis of natural products. Scheme 3.8 shows the synthesis of macrolide natural product (*R*)-phoracantholide J (*R*)-**69**, where the use of a carefully chosen acyl source **70** allowed the introduction of a synthetic handle. A prochiral diol **71**, with a remote stereocentre, was resolved using vinyl pivalate, allowing the simultaneous resolution of the compound and introduction of a pivalate group, which was retained in the final compound (*S*)-**72**.




*Scheme 3.8 Retention of acyl group used in resolution in the synthesis of protein kinase ligand (S)-**72** (bottom)*

Hirose et al. found that when a chiral acyl donor, such as **73**, was used in the resolution of 2-phenyl-1-propanol **1**, one enantiomer was inactive (Scheme 3.9).[45] When the inactive enantiomer (*R*)-**73** was screened, it furnished no acylated product. Using the active enantiomer (*S*)-**73** did not improve selectivity over the racemic mixture, but it did give greater conversion, which is attributed to less competition within the binding site.




*Scheme 3.9 Use of chiral acyl donors to resolve 2-phenyl-1-propanol **1**; one enantiomer of acyl donor **73** was inactive*

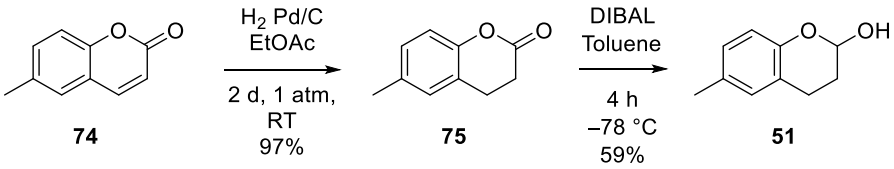
## 3.2 Objectives

The specific objectives of this project are:

- To prepare the model lactol, 6-methylchroman-2-ol **51** and prepare the acyl derivative **62** and develop analytical conditions to determine enantiomeric excess of the acyl derivatives.
- Use the model lactol **51** as a substrate for lipase-mediated transesterification screens, using a targeted panel of lipases.
- Identify lead enzymes and carry out a solvent screen.
- To conduct a preparative scale resolution of the compound and identify the enantiomer formed.

## 3.3 Synthesis of the model lactol substrate **51**

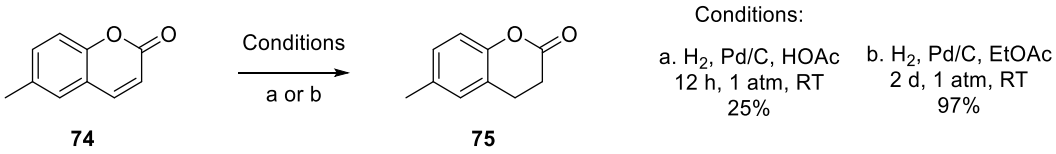
The lactol substrate **51** was synthesized using a two-step method, first reducing the alkene bond of **74** with $H_2$, Pd/C, followed by reduction of the carbonyl double bond of **75** using DIBAL.[46,47] Commercially available 6-methyl coumarin **74** was used as starting material (Scheme 3.10).




*Scheme 3.10 Synthesis of 6-methyl lactol **51***

### 3.3.1 Reduction of the alkene moiety

The first attempt at reduction of the alkene used acetic acid as a solvent and was complete in 12 h (Scheme 3.11). Unfortunately the yield was much lower than expected (25% vs >95% reported).[46] The lower yield may be caused by base-catalysed hydrolysis of the lactone, which would then remain in the aqueous layer.



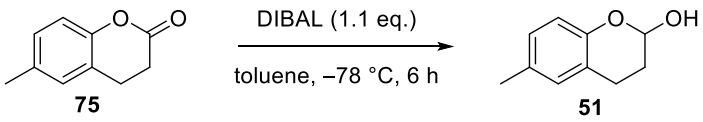

*Scheme 3.11 Reduction of the alkene*

The reduction of the alkene using ethyl acetate as the solvent, proceeded with 97% yield, gave a very clean product after drying and required no purification, albeit 48 h was required for complete consumption of the starting material.[48,47] Despite the longer reaction time, this method was found to be high yielding and gave a pure product **75** (Scheme 3.11). The same reaction was reported using a Parr hydrogenator at 57 psi, giving the product after only 3 h but it was not attempted in this investigation.[49]

### 3.3.2 Reduction of the lactone **75**

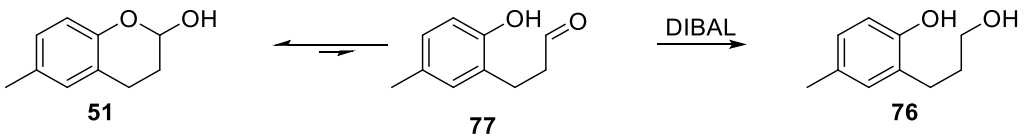
The reduction of the lactone **75** was performed under anhydrous conditions, at low temperature, with 1.1 eq. of DIBAL, as a 1M solution in toluene, using freshly distilled toluene

as solvent (Scheme 3.12). Several different sets of conditions were used to optimize the yield (Table 3.1).




*Scheme 3.12 Reduction of lactone **75** to lactol **51***

For the first attempt, TLC showed that, even after 4 h, starting material still remained. In this case, an extra 0.55 equivalents of DIBAL were added and stirred at low temperature until the starting material had disappeared. In this case, however, two products were recovered after column chromatography in a ratio of ~2:1. The major product was the desired lactol product **51**, and the minor was identified as the over-reduction product, diol **76** (Scheme 3.13).




*Scheme 3.13 Reduction of the lactol **51** to the diol **76** via the aldehyde intermediate*

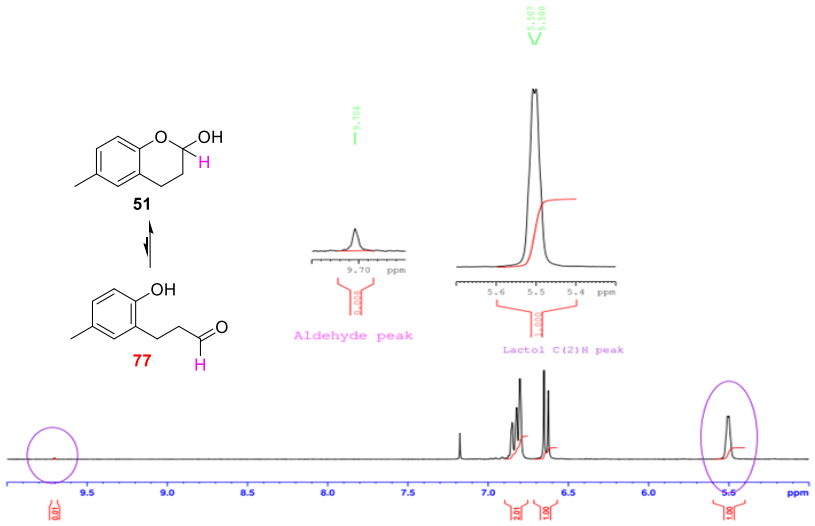



*Figure 3.4 $^{1}H$ NMR showing aldehyde peak and lactol $^{1}H$ peak, expansions are not to scale ($CDCl_3$, 300 MHz)*

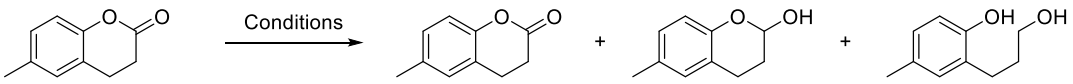
The lactol product exists in an equilibrium with the ring opened aldehyde **77**. The aldehyde is present in a very small amount (<1%, in $CDCl_3$) and appears in the $^{1}H$ NMR at 9.8 ppm (Figure 3.4). In the case above, the aldehyde form of the lactol **51** is further reduced to the corresponding alcohol **76** (Table 3.1, Entry 1). The data obtained for the diol **51** matched previously reported data for the compound.[3]

Further experiments required up to 6 h for the reaction to occur, but it was decided that after this time, that the reaction would be worked up, allowing the recovery of starting material which could be reused, along with the product, due to the challenge of maintaining the temperature for extended times overnight. The overall conversion after 6 h was calculated from the $^{1}$H NMR spectrum and was ~80% (Table 3.1, Entry 2).

Purification of **51** was generally difficult, requiring several columns to effectively separate the starting material **75** and product **51**. The unreacted dihydrocoumarin **75** eluted off the column first, then fractions containing a mixture of the unreacted dihydrocoumarin **75** and the product **51** (generally composed of 70–80% product), followed by the product. Immediately after concentration, the product was analysed and dried under vacuum overnight.

The addition of molecular sieves, to remove water during the reaction, gave much better conversion, resulting in no unreacted starting material **75**, after 6 h reaction (Table 3.1, Entry 3). The material obtained from this reaction was obtained in high purity, but chromatography was undertaken prior to enzymatic reactions as the material was required in very high purity. The synthesis of lactol **51** has been previously reported.[49]

*Table 3.1 Synthesis of lactol **51***



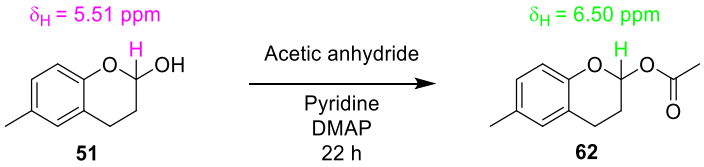

**75** **75** **51** **76**

| Entry | Conditions | % 75[a] | % 51[a] | % 76[a] |
|---|---|---|---|---|
| **1** | DIBAL (1.1 eq.), toluene, −78°C, 4 h<br>DIBAL (0.55 eq.), 2 h | 0 | 57 | 43 |
| **2** | DIBAL (1.1 eq.), toluene, −78°C, 6 h | 20 | 80 | 0 |
| **3** | DIBAL (1,1 eq.), Toluene, −78°C, 6 h<br>4Å molecular sieves | 0 | 100 | 0 |

[a] % composition determined by $^{1}$H NMR of crude reaction mixture.

### 3.3.3 Acylated lactol reference standard

In order to develop analytical methods for the reactions, it was necessary to prepare the acylated lactol **62** (Scheme 3.14). The acylation of the lactol **51** was carried out using a general acylation method previously used in our group employing acetic anhydride as the acylating agent, distilled DCM as solvent, *N*,*N*-dimethylaminopyridine (DMAP) as nucleophilic catalyst (5 mol %), and pyridine as a base (4.5 eq.).[50] The reaction proceeded at room temperature, and gave full conversion on stirring overnight. This was evident by the disappearance of the 1H signal at 5.58 ppm, and the appearance of a 1H signal at 6.50 ppm. Aqueous copper sulfate solution was employed during the work-up to remove pyridine, and any excess, which was not removed by the work-up, was removed by azeotrope with heptane. Although the $^{1}$H NMR spectrum showed complete acylation to the pure acetate **62**, the product was subjected to column chromatography as it was required in very high analytical purity. This compound is novel and was fully characterized in the course of this investigation.

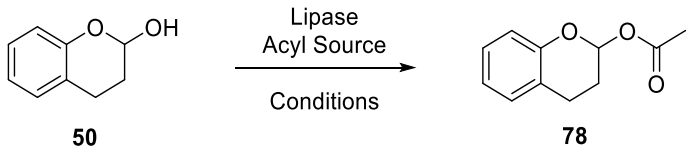



*Scheme 3.14 Preparation of acylated lactol **62***

# 3.4 Resolution of lactol **51**

## 3.4.1 Enzymatic screen of lactol substrate – background on related substrate

Previously, up to 95% *ee* was achieved for the unsubstituted lactol **50**, using neat vinyl acetate **65** (100 eq.) (Scheme 3.15).[4] However, it was observed that >70% *ee* was always coupled with low conversion, and that there was reduced enantioselectivity when the conversions were higher. Most enzymes which were screened furnished no acetylated product **78**. An investigation into the effect of organic solvents on the conversion and selectivity had also been conducted on this compound in which up to 91% *ee* was obtained, albeit with a low conversion (13%). Within this study, conversions of up to 81% were achieved, coupled with 56% *ee*. In general, it was observed that use of non-polar solvents gave better conversion and enantioselectivity. It should be noted that only 4.2 equivalents of the acylating agent were used in the solvent screen for this compound, but a higher loading of vinyl acetate was used in the screens using 6-methylchromanol **51** as substrate.




**50** **78**

*Scheme 3.15 Resolution of unsubstituted lactol **50***

## 3.4.2 Analysis of reactions

The reaction completion (conversion) was determined by $^{1}$H NMR. The peaks for the C(2) protons of the starting material **51** and the desired (acylated) product **62** were sufficiently separated on the $^{1}$H NMR spectrum (Figure 3.5) and were not overlapping with any other peaks. The enantiomeric excess was determined by chiral HPLC and the HPLC conditions are listed in **Appendix I**. The reactions were worked up by simply filtering the reaction solution through Celite® and removing the solvent by rotary evaporation. The $^{1}$H NMR analysis was carried out on the crude material, which only contained the unreacted lactol **51** and the acylated product **62**.

The results of the initial screens are shown in Table 3.2, and the results of the solvent screen are shown in Table 3.5.

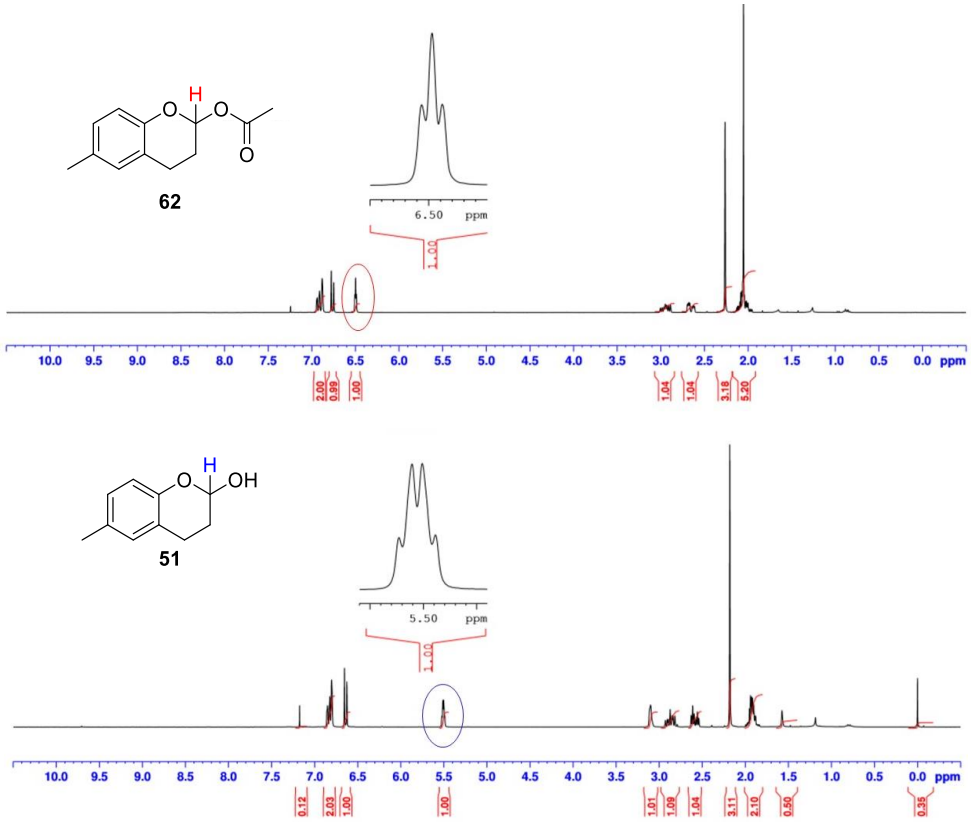



*Figure 3.5 $^{1}H$ NMR spectra for the lactol **51** and acetate **62** ($CDCl_3$, 300 MHz)*

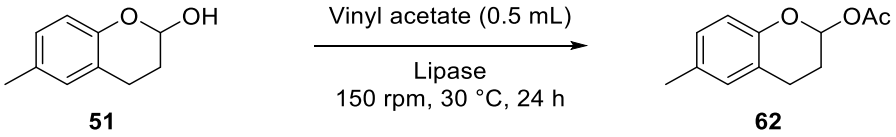
### 3.4.3 Initial 24-hour screen

The initial 24 h screens were carried out using vinyl acetate as solvent and acylating agent (Table 3.1). The lactol substrate **51** was dried prior to the reactions, and the vinyl acetate, which was stored in the fridge, was allowed to warm to room temperature before opening to minimize the ingress of water. The presence of water in these reactions could lead to the hydrolysis of the product, although strictly anhydrous conditions were not used. Control reactions were carried out, and, in the absence of an enzyme, there was no background acylation evident.

Several lead enzymes were identified for the solvent screen. Enzymes were chosen mainly on the basis of activity, but those with poor selectivity were disregarded, as high selectivity is important in this investigation. Lipase E from *Alcaligenes sp.* gave the highest conversion, coupled with moderate enantioselectivity (Table 3.1, Entry 1). Lipase from *Candida cylindracea*

and lipases A and B from *Candida rugosa*[1] performed similarly, with a slightly lower conversion, with moderate selectivity. The lipase from *Thermomyces lanuginosus* gave excellent selectivity (97% *ee*), albeit coupled with lower conversion than the other lead enzymes (Entry 7).

*Table 3.2 Initial lactol screening results using vinyl acetate as acylating agent*




**51** **62**

| Entry | Hydrolase Source | Conversion (%)[a] | *ee* (%)[b] |
|---|---|---|---|
| 1 | Lipase E from *Alcaligenes sp.* | 30 | 70 |
| 2 | Lipase from *Candida cylindracea* | 28 | 65 |
| 3 | Lipase A from *Candida rugosa* | 26 | 64 |
| 4 | *Candida antarctica* Lipase A | 26 | 8 |
| 5 | Lipase B from *Candida rugosa*[c] | 20 | 59 |
| 6 | Lipase from *Pseudomonas stutzeri* | 19 | 33 |
| 7 | Lipase from *Thermomyces lanuginosus* | 12 | 97 |
| 8 | Lipase B from *Candida rugosa*[c] | 7 | 79 |
| 9 | Lipase D from *Alcaligenes sp.* | 6 | 66 |
| 10 | Lipase F from *Alcaligenes sp.* | 5 | 39 |
| 11 | Lipase B from *Burkholderia cepacia* | 5 | 74 |
| 12 | Lipase from *Pseudomonas fluorescens* (immob) | 5 | 83 |
| 13 | Lipase from *Candida antarctica* | 4 | 93 |
| 14 | Lipase from *Pseudomonas cepacia* | 3 | 80 |
| 15 | Lipase C from *Alcaligenes sp.* | 3 | 61 |
| 16 | Lipase B from *Alcaligenes sp.* | 3 | 71 |
| 17 | CAL-B (immob) | 3 | 94 |
| 18 | *Candida antarctica* Lipase B (liq.) | 0 | -[d] |

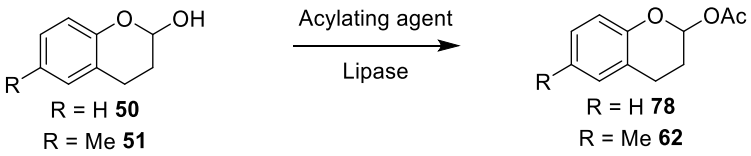
The following lipases gave conversion <2% and were not subjected to HPLC analysis: Hog pancreas lipase, Acid protease A, Protease from *Aspergillus niger*, Lipase from *Aspergillus niger*, Lipase A from *Alcaligenes sp*, Neutral Protease A, Lipase from *Rhizomucor miehei.* The following enzymes gave no conversion: Lipase from fungal source, Protease A from *Bacillus subtilis*, Phytase, Alkaline protease A, Alkaline Lipase A, Lipase from *Bromeliaceae sp,* Lipase from *Carica papaya*, Protease A from *Aspergillus oryzae*, Protease B from *Bacillus subtilis*, Acylase from *Aspergillus sp*, Lipase from *Rhizopus niveus*, Protease from *Bacillus stearothermophilus*, Lipase from *Penicillum roquefort*, Protease from *Aspergillus melleus*, Lipase from *Penicillum camembertii*, Protease B from *Aspergillus oryzae*, Protease A from *Bacillus sp*, Protease B from *Bacillus sp*, Lipase A from *Rhizomucor miehei*, Lipase Porcine Pancrease type II, Lipase from *Penicillum roquefort*, Protease C from *Bacillus subtilis*, Alkaline protease B, Proteax, Lipase from *Pseudomonas fluorescens*, Lipase A from *Burkholderia cepacia.* [a]Conversion was determined by $^1$H NMR comparing the signals for the C(2) proton in each compound; [b]enantiomeric excess was determined by chiral HPLC and is the excess of the second enantiomer detected in all cases which has retention time of approx. 6.4 mins; [c]two different samples of *Candida rugosa* lipase B were tested; [d]compounds with conversion <2% were not subjected to chiral HPLC analysis.

[1]*Candida rugosa* has been renamed to *Candida cylindracea* but will be referred to as *Candida rugosa* in this work, as two different samples of lipases were used.

*Candida antarctica* lipase B (CAL-B) (immobilised) furnished excellent selectivity (94% *ee*, Entry 17) albeit with very low conversion. The non-immobilised *Candida antarctica* lipase B (Entry 18) gave no conversion, but the mixture of lipases from this organism (Entry 13) performed similarly to the immobilised CAL-B, in terms of both selectivity (93% *ee*) and conversion. Interestingly, Lipase A from *Candida antarctica* (Entry 4), which is present in the mixture of lipases from this organism, gave good conversion but with poor selectivity. A follow-up investigation was carried out, where the acylating agent was varied, in order to see if this would affect the selectivity and conversion.

Interestingly, for the enzymes shown in Table 3.3, the enantioselectivities are very similar across the two compounds **50** and **51**, but the conversions are lower for the substituted compound **51**, showing that the methyl group may not be easily accommodated in the active site.

*Table 3.3 Comparison of acylation data for the unsubstituted lactol **50**, and the 6 methyl lactol **51***




R = H **50**
R = Me **51**

R = H **78**
R = Me **62**

| Entry | Hydrolase | 50[a] | | 51 | |
|---|---|---|---|---|---|
| | | Conv. (%)[b] | *ee* (%)[c] | Conv. (%)[b] | *ee* (%)[c] |
| 1 | CAL-B (immob) | 20 | 95 | 3 | 97 |
| 2 | Lipase E from *Alcaligenes sp.* | 47 | 77 | 30 | 70 |
| 3 | Lipase A from *Candida rugosa* | 25 | 60 | 26 | 64 |
| 4 | *Lipase from Thermomyces lanuginosus* | -[d] | -[d] | 12 | 97 |
| 5 | Lipase B from *Burkholderia cepacia* | 44 | 78 | 5 | 74 |
| 6 | Lipase from *Pseudomonas cepacia* | 19 | 65 | 3 | 80 |

[a]Reported by Gavin et al.;[4] [b]conversion was determined by $^1$H NMR; [c]enantiomeric excess was determined by chiral HPLC analysis; [d]this enzyme was not tested for activity against **50**.

## 3.4.4 *Candida Antarctica* lipase experiments

Disappointingly CAL-B (immob) gave poor conversion, but showed excellent enantioselectivity (Table 3.1, Entry 17). As a result of the poor conversion, when using the *Candida antarctica* lipases, it was decided to repeat the 24 h screens using different acylating agents, shown in Figure 3.6. The acylating agents used were vinyl acetate **65**, ethyl acetate **79**, isopropyl acetate **80**, and isopropenyl acetate **68**.

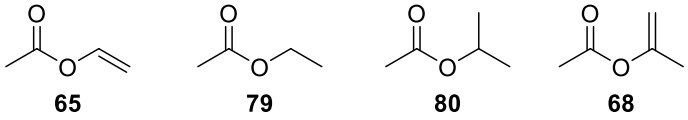



*Figure 3.6 The structures of acylating agents used in this study*

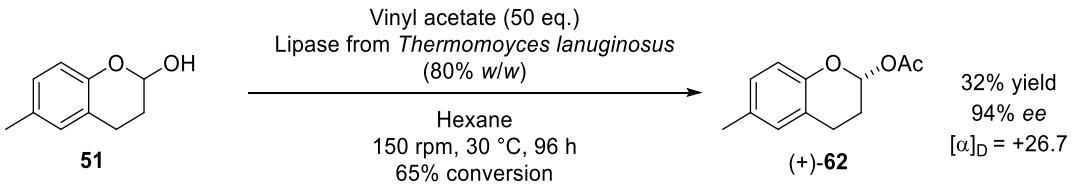
Changing the acylating agent showed a slight improvement in conversion values in some cases but no reaction in other cases (Table 3.4). Overall the different acylating agents showed little to no enhancement of the reaction over the use of vinyl acetate **33**. Acetaldehyde **67** is known to inactivate some lipases through formation of a Schiff base with the lysine residues of the protein.[51]

### 3.4.5 Solvent screen

Following the initial screens, enzymes which showed good conversion and enantioselectivity were subjected to further screens, using a range of organic solvents. Organic solvents are known to increase lipase selectivity by interfacial activation.

Six solvents were screened: hexane (HPLC grade), heptane (HPLC grade), diisopropyl ether, *tert*-butyl methyl ether (HPLC grade), and toluene (dry, distilled). Four lead enzymes were selected for screening on the basis of activity. Enzymes which had poor selectivity were disregarded. The solvent screens were carried out at 30°C and 150 rpm. The results and conditions are shown in Table 3.5.

The lipase from *Thermomyces lanuginosus* showed excellent selectivity (>92%) in the solvent screen, especially with the use of hexane and toluene (Entry 3). A preparative scale synthesis was carried out, using hexane as solvent (Scheme 3.16). After 96 h, the $^{1}$H NMR spectrum showed 65% conversion to the product, and, after chromatography, the acetate **62** was isolated in 32% yield with 94% *ee*.



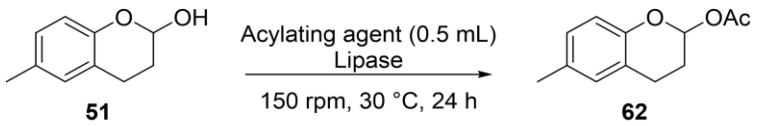

*Scheme 3.16 Preparative scale resolution of **51***

In the case of the unsubstituted compound **50**, the use of toluene and hexane gave the best results in the solvent screen, which was also observed here. With the exception of

*Thermomyces lanuginosus* the presence of the polar solvents, TBME and DIPE, decreased the conversion relative to the control reaction (vinyl acetate only), albeit, giving the highest enantioselectivities for these lipases. Previous reports of transesterification using vinyl acetate have shown that non-polar solvents, especially hexane, give superior results compared to polar solvents.[52]

*Thermomyces lanuginosus* has previously used in the production of biodiesel from soybean oil,[53] evolved for the use in the synthesis of pregabalin,[54] and has been reviewed in the context of use in an industrial setting.[55]

*Table 3.4 Investigation of the effect of the acylating agent on the resolution of **51***

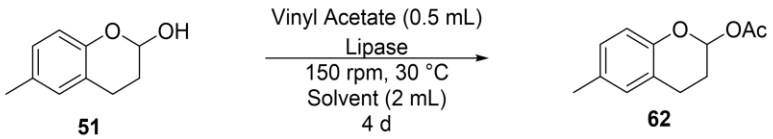



**51** **62**

| Entry | Enzyme | Vinyl acetate | | Ethyl acetate[a] | | Isopropyl acetate | | Isopropenyl acetate | |
|---|---|---|---|---|---|---|---|---|---|
| | | Conv. (%)[b] | *ee* (%)[c] | Conv. (%)[b] | *ee* (%)[c] | Conv. (%)[b] | *ee* (%)[c] | Conv. (%)[b] | *ee* (%)[c] |
| 1 | CAL-B (immob) | 3 | 94 | 1 | -[d] | 4 | 94 | 1 | 96 |
| 2 | Lipase from *Candida antarctica* | 4 | 93 | 1 | -[d] | 3 | 93 | 3 | 95 |
| 3 | *Candida antarctica* Lipase A | 26 | 8 | 1 | -[d] | - | -[d] | 2 | 15 |
| 4 | *Candida antarctica* Lipase B (liq.) | 0 | -[d] | 0 | -[d] | 0 | -[d] | 0 | -[d] |

In the absence of any lipase there was no conversion to the product. [a]The ethyl acetate reaction time was 72 h; [b]conversion was determined by $^1$H NMR comparing the signals for the C(2) proton in each compound. [c]Enantiomeric excess was determined by chiral HPLC and the second enantiomer eluted is the major enantiomer detected in all cases which has retention time of approx. 6.4 mins; [d]compounds with conversion <2% were not subjected to chiral HPLC analysis

*Table 3.5 Solvent screen results for 6-methyl lactol **51***




**51** **62**

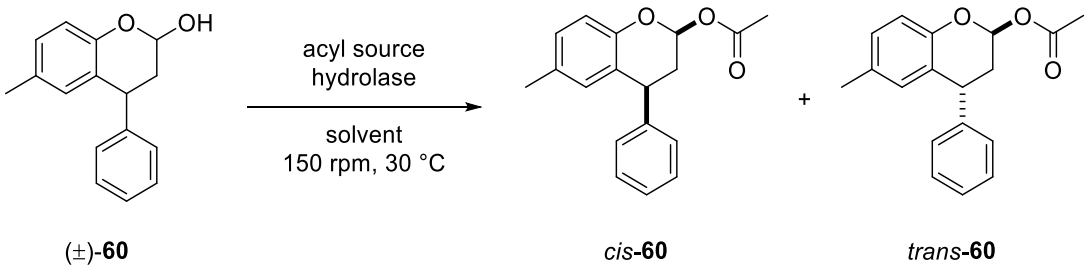
| Entry | Enzyme | no solvent[a] | | Hexane | | Heptane | | Toluene | | DIPE | | TBME | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conv (%)[b] | *ee* (%)[c] | Conv (%)[b] | *ee* (%)[c] | Conv (%)[b] | *ee* (%)[c] | Conv (%)[b] | *ee* (%)[c] | Conv (%)[b] | *ee* (%)[c] | Conv (%)[b] | *ee* (%)[c] |
| 1 | Lipase E from *Alcaligenes sp* | 57 | 67 | >95 | 44 | 58 | 58 | 53 | 62 | 6 | 67 | 21 | 67 |
| 2 | Lipase A from *Candida rugosa* | 38 | 60 | 48 | 67 | 18 | 59 | 26 | 69 | 13 | 64 | 13 | 79 |
| 3 | Lipase from *Thermomyces lanuginosus* | 13 | 94 | 86 | 92 | 38 | 95 | 55 | 94 | 27 | 96 | 51 | 94 |
| 4 | Lipase from *Candida cylindracea* | 29 | 66 | 63 | 66 | 41 | 63 | 30 | 73 | 18 | 68 | 5 | 83 |

Reactions carried out in the absence of an enzyme gave no conversion to product. [a]Reaction carried out using 0.5 mL vinyl acetate as acylating agent and solvent; [b]conversion was determined by $^1$H NMR; [c]enantiomeric excess was determined by chiral HPLC anaylsis.

## 3.5 Conclusion

We have demonstrated lipase-mediated dynamic kinetic resolution of a fused lactol system.

Extension to this work was carried out, wherein the tolterodine lactol **60**, containing a remote stereocentre, was treated with hydrolases, which proved more challenging in terms of efficiency, while retaining high enantioselectivity (up to >98% *ee*).[4]




(±)-**60** *cis*-**60** *trans*-**60**

*Scheme 3.17 Resolution of the Tolterodine intermediate lactol **60***

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# Chapter 4

# Towards dynamic kinetic resolution in the intramolecular nitroaldol (Henry) reaction through lipase catalysis

# Contents

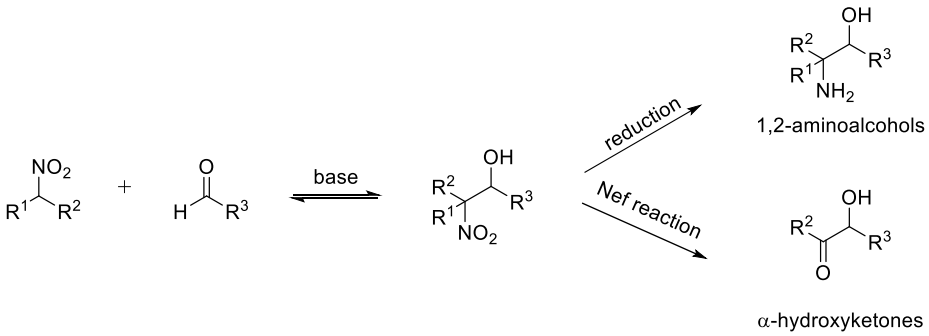
# Overview

The work described in this chapter builds on the work carried out by Deasy during her PhD, and subsequent work carried out by Milner and Gavin.[1,2] The introduction will consist of an overview of the work completed by Deasy, Milner and Gavin predating the work in this chapter, which, when combined, led to a publication.[2] This chapter will outline the work completed and will be similar to the publication; synthesis of substrates will be discussed in more detail, and the material which had been moved to the supplementary material for publication will be discussed in more detail.

Dynamic kinetic resolution (DKR) in the intramolecular nitroaldol reaction through coupling with lipase-mediated acetylation is described herein. Significant challenges in effecting the combination of the base-mediated reversible cyclisation of 6-nitroheptanal **81** with the selective enzyme-mediated transesterification were encountered. Ultimately, *trans*-2-methyl-2-nitrocyclohexyl acetate **82b** was isolated in excellent enantiopurity (>98% *ee*) *via* a sequential DKR sequence where the enzymatic resolution and base-mediated interconversion of the 2-methyl-2-nitrocyclohexanol **83** were effected alternately, demonstrating the feasibility of this approach for the first time. Further work showed, for the first time, evidence that a DKR-type system is possible: reaction engineering allowed design of a sequential one-pot reaction system which furnished the products with excellent enantioselectivity, and good diastereoselectivity.

## 4.1 Introduction

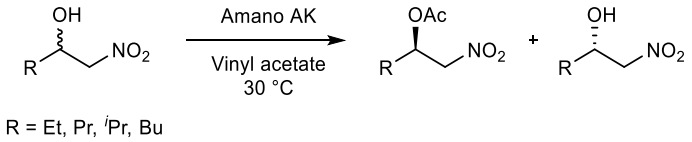
The Henry reaction is an important base-mediated transformation in organic chemistry leading to vicinal nitro alcohols which can be converted to a wide variety of synthetic intermediates, such as 1,2-aminoalcohols and α-hydroxycarbonyl compounds (Scheme 4.1).[3-5] Although the reaction is known for over a century, stereo- and diastereoselective approaches leading to enantiopure nitroalcohols are still challenging. Principal approaches to the catalytic asymmetric nitroaldol reaction, including transition metal and organo-catalysed methods, have been reviewed in detail.[5-7] The use of biocatalytic protocols to resolve the products of the Henry reaction has also been reviewed.[8]




*Scheme 4.1 Henry reaction and synthesis of 1,2-aminoalcohols & α-hydroxyalcohols*

There are two distinct biocatalytic methods: direct enzyme-catalysed asymmetric Henry reaction using hydroxynitrile lyases, or initial formation of the β-nitroalcohol product, followed by enzymatic kinetic resolution of the resulting stereoisomers.[9] The latter kinetic resolution suffers the limitation of a maximum attainable yield of 50% of the desired product.

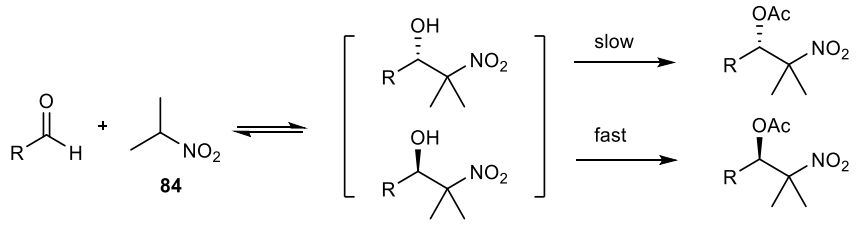
Okamoto et al. reported the first bioresolution of the products of a Henry reaction (Scheme 4.2). The use of various solvents was explored, and isopropyl ether was found to give good results.[10]




*Scheme 4.2 Resolution of Henry reaction products.*

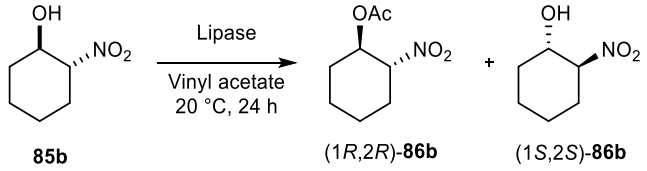
Ramström et al. developed a procedure for the one-pot intermolecular dynamic kinetic resolution of β-nitroalcohols; this was limited by the need to use a large excess of the

nitroalkane **84** in order to shift the equilibrium towards the formation of the product (Scheme 4.3).[11,12]



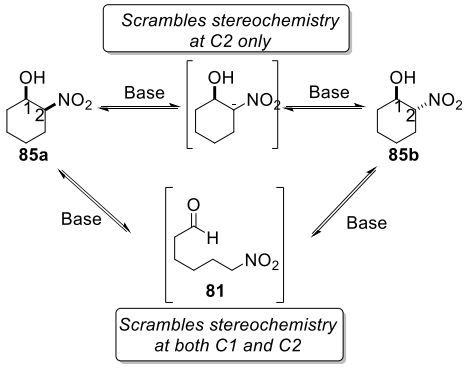

*Scheme 4.3 One-pot intermolecular Henry reaction and subsequent lipase catalysed resolution*

Previous work in the Maguire group had the added challenge of a second stereocentre, leading to potential complications in stereocontrol and diastereoselectivity (Scheme 4.4). We have previously reported efficient kinetic bioresolution for both the *cis*- and *trans*-2-nitrocyclohexanols **85a** and **85b** via enzyme-mediated transesterification.[13]




*Scheme 4.4 Resolution of 2-nitrocyclohexanol **85b***

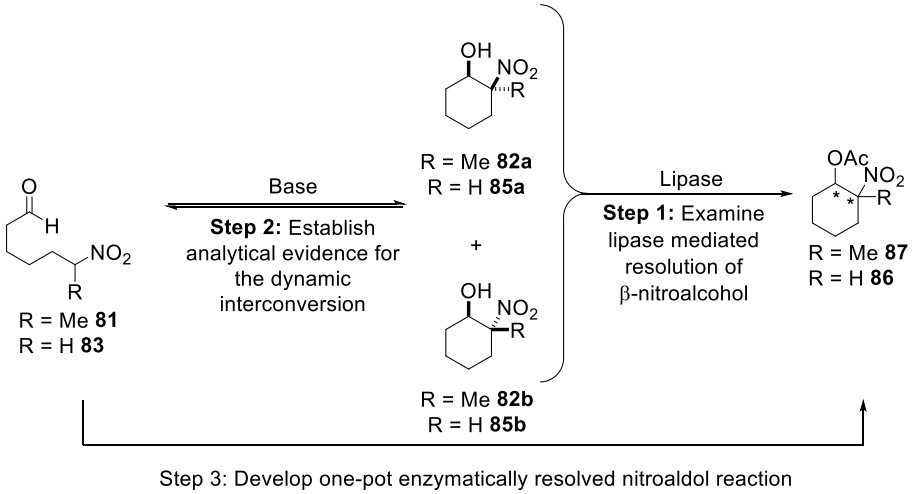
Initially, Milner reported the lipase-mediated resolution of nitrocyclohexanol **85a** and **85b**.[13] When the base-mediated interconversion was attempted using this substrate, stereochemical scrambling was only observed at one stereocentre. This was rationalised as base-catalysed epimerisation (Scheme 4.5). To overcome this problem, a methyl group was installed at the acidic site to block the epimerisation pathway. As a result, the base-catalysed interconversion through ring opening and closing was the only available reaction pathway, allowing the possibility of combining the two processes.




*Scheme 4.5 Epimerisation pathway versus the reversible nitroaldol reactions*

Crucial to the success of the lipase-mediated DKR protocol is selection of the correct base. The fundamental requirements of the selected base are firstly to mediate effective ring closure of 6-nitroheptanal **81** and then catalyse the dynamic interconversion process between the diastereomeric alcohols **82a** and **82b.** It was then envisaged that combination with the diastereoselective lipase-mediated transesterification (as discussed above) would lead to a one-pot DKR of the intramolecular nitroaldol reaction through lipase catalysis.

Deasy initially screened the substrate **83** against a variety of bases to identify the best base for the interconversion using vinyl acetate as the solvent; ultimately, combination of the two systems would require the use of the base and the acylating agent concurrently (Scheme 4.6).[1]




*Scheme 4.6 Investigation of the intramolecular nitroaldol reaction in a stepwise manner.*

Deasy showed that the use of piperidine (2 eq.) and aqueous sodium hydroxide (1M) gave no ring closure of the aldehyde **83**. Diethylamine (DEA) and Hünig's base gave <20% conversion to the alcohols **82a** and **82b**. Triethylamine and DABCO both catalysed the ring closing reaction but did not catalyse the reversible interconversion of **82a** and **82b**.

The use of DBU (0.1 eq.) gave complete cyclisation of the aldehyde in 24 hours, albeit producing a large amount of the acetates **87a** and **87b**, racemically. When the loading was reduced to 0.05 eq. and the reaction time extended from 24 hours to 48 hours, 50% of aldehyde 1 remained, but only 3% of the mixture was acetates **87a** and **87b**.

Tetramethylguanidine (TMG) was also used in the cyclisation, using 0.1 eq., resulting in a sluggish reaction, giving only 31% conversion to the alcohols **82a** and **82b**, after 24 hours; an

additional 2 equivalents of TMG gave better conversion, with only 6% of the aldehyde remaining after 48 hours, albeit with 5% of acetate **87a**.

Deasy also showed experimentally, that interconversion of the alcohol **82a** to **82b** and vice versa was taking place in the presence of 0.1 eq. DBU; this was carried out in $CDCl_3$ so that the interconversion could be monitored by $^1H$ NMR. After 24 hours, the alcohols **82a** and **82b** were in the ratio 33:67, regardless of which diastereomer was added initially. The interconversion was also carried out using TMG (2.0 eq.), however, it took 72 hours to get to the same ratio.

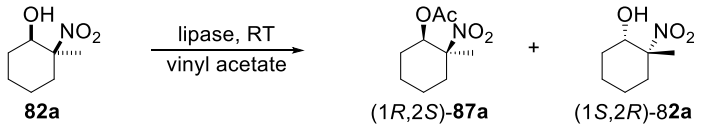
The bioresolution of an intramolecular nitroaldol reaction of 6-nitroheptanal **81** was investigated in a stepwise manner (Scheme 4.6). The first step in this study involved independent examination of the lipase-mediated kinetic resolution of the racemic *cis*- and *trans*-2-methyl-2-nitrocyclohexanols **82a** and **82b** establishing the most efficient lipase to perform this biotransformation diastereoselectively. Ideally, for an efficient dynamic process, one enantiomer of either **82a** or **82b** would be efficiently and selectively acetylated. The optimum reaction conditions reported for the kinetic resolution of *cis*- and *trans*-2-nitrocyclohexanol **85a** and **85b** employing vinyl acetate as both solvent and acyl donor were applied in this study for the lipase-mediated transesterification of **82**.[13]

Deasy examined the resolution of each alcohol diastereomer separately using a variety of lipases. A selection of the results is shown in Table 4.1 (*cis*-**82a**)[2] and Table 4.2 (*trans*-**82b**).

*P. fluorescens* furnished the product acetate *cis*-**87a** with good conversion (40%) with high enantiopurity (96% *ee*), compared to the *trans*-**87b** product, which had conversion <10%, under the same conditions (Table 4.1, Entry 3 and Table 4.2, Entry 3, respectively). The diastereoselectivity here was good, but the extended reaction time (113.5 h) was too long to be useful. *P. stutzeri*, after 48 hours, furnished *trans*-**87b** with 50% conversion, and excellent enantioselectivity (<98% *ee*), however, after the same period of time, its use gave the acetate *cis*-**87b** with 78% conversion, but with poor enantiopurity (27% *ee*), which was accompanied by excellent enantiopurity of the alcohol *cis*-**87a** (>98%) (Table 4.1, entry 2 and Table 4.2, entry 2).

[2]Throughout this work "*cis*" and "*trans*" are used referring to "seq-cis" and "seq-trans" i.e. the assignment of stereochemistry is based on the sequence rule (Cahn-Ingold-Prelog rules).

*Table 4.1 Lipase-mediated transesterification of cis-2-methyl-2-nitrocyclohexanol **82a** in vinyl acetate*

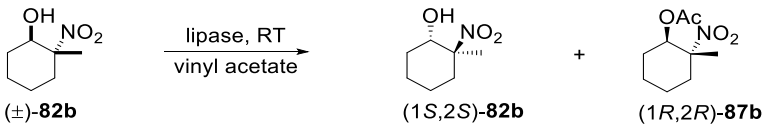



**82a** (1*R*,2*S*)-**87a** (1*S*,2*R*)-**82a**

| Entry | Enzyme Source | Time (h) | Conversion (%) | *ee* (%)[a] Alcohol *cis*-**82a** (1*S*,2*R*) | *ee* (%)[a] Acetate *cis*-**87a** (1*R*,2*S*) | E[b] |
|---|---|---|---|---|---|---|
| 1 | CAL-B (immob) | 72 | 33[b] | 49 | >98 | 159 |
| 2 | *Pseudomonas stutzeri* | 48 | 78[b] | >98 | 27 | 6.4 |
| 3 | *Pseudomonas fluorescens* | 113.5 | 40[b] | 64 | 96 | 95 |

Adapted from Deasy.[1] [a]Enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [b]conversion and the enantiomeric ratio (E value) were calculated from the enantiomeric excess of substrate alcohol **82a** ($ee_s$) and product acetate **87a** ($ee_p$);[15]

*Table 4.2 Lipase-mediated transesterification of* trans*-2-methyl-2-nitrocyclohexanol **82b** in vinyl acetate*




(±)-**82b** (1*S*,2*S*)-**82b** (1*R*,2*R*)-**87b**

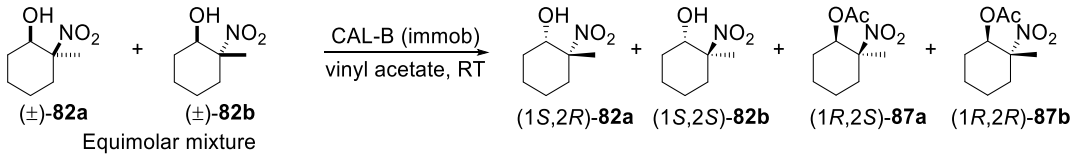
| Entry | Enzyme Source | Reaction Time (h) | Conversion (%) | *ee* (%)[a] Alcohol *trans*-**82b** (1*S*,2*S*) | *ee* (%)[a] Acetate *trans*-**87b** (1*R*,2*R*) | E[b] |
|---|---|---|---|---|---|---|
| 1 | CAL-B (immob) | 72 | 49[b] | 96 | >98 | >200 |
| 2 | *Pseudomonas stutzeri* | 48 | 50[b] | 97 | >98 | >200 |
| 3 | *Pseudomonas fluorescens* | 113.5 | <10[c] | – | – | – |

Adapted from Deasy.[1] [a]Enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [b]conversion and the enantiomeric ratio (E value) were calculated from the enantiomeric excess of substrate alcohol **82b** ($ee_s$) and product acetate **87b** ($ee_p$);[14] [c]conversion was determined by $^1$H NMR spectroscopy of the crude products to be <10%, chiral HPLC analysis was not conducted.

*Candida antarctica* lipase B (CAL-B) (immob) showed only moderate diastereoselectivity, furnishing *trans*-**87b** with 49% conversion, and *cis*-**87a** with 33% conversion, both with >98% *ee*, after 72 hours (Table 4.1, entry 1 and Table 4.2, entry 1). Further reaction using an equimolar mixture of both alcohols *cis*-**82a** and *trans*-**82b** showed that the diastereoselectivity was much better with short reaction times than after longer reaction times, albeit with lower conversion than longer reaction times (Table 4.3). This shows that one diastereomer, *trans*-**82b**, can be acylated preferentially, with excellent enantioselectivity. In the case where this is combined with an effective base-mediated interconversion, the interconversion between the

two diastereomers, constantly regenerating the trans isomer, *trans*-**82b**, should effectively overcome decreased diastereoselectivity after extended reaction time.

*Table 4.3 Diastereoselective CAL-B (immob) mediated transesterification of* cis-***2a*** *and* trans-***2b*** *in vinyl acetate*




(±)-**82a** (±)-**82b** Equimolar mixture

(1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

| Enzyme Source | Reaction Time (h) | Alcohol 82 | | Acetate 87 | |
|---|---|---|---|---|---|
| | | *cis*-**82a** (%)[a] | *trans*-**82b** (%)[a] | *cis*-**87a** (%)[a] | *trans*-**87b** (%)[a] |
| CAL-B (immob) | 12 | 44 | 38 | 3 | 15 |
| | 18.5 | 43 | 36 | 4 | 17 |
| | 40.5 | 41 | 30 | 6 | 23 |
| | 62.5 | 27 | 30 | 11 | 32 |
| | | (15% *ee*)[b,c] | (74% *ee*)[b,d] | (>98% *ee*)[b,e] | (>98% *ee*)[b,f] |

Adapted from Deasy[1] [a]Conversions were estimated by $^1$H NMR spectroscopy and were derived from integration of the $^1$H NMR spectrum of the mixture of the crude material, not mass recovery. Starting material was an equimolar mixture of *cis*-**82a** and *trans*-**82b**; [b]enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [c]the principal enantiomer was (1*S*,2*R*)-**82a**; [d]the principal enantiomer was (1*S*,2*S*)-**82b**; [e]the principal enantiomer was (1*R*,2*S*)-**87a**; [f]the principal enantiomer was (1*R*,2*R*)-**87b**.

Having established a diastereoselective enzymatic resolution, attention next focused on the aldehyde cyclisation and the interconversion. Deasy tested a variety of bases, summarised in Table 4.4. Piperidine, aqueous sodium hydroxide, diethylamine (DEA), and *N*,*N*-diisopropylethylamine (Hünig's base) showed little efficiency in the cyclisation of **81**, even at 2.0 eq. loading (Table 4.4, Entries 1–5), and their use in the base-mediated dynamic interconversion process was not further pursued.

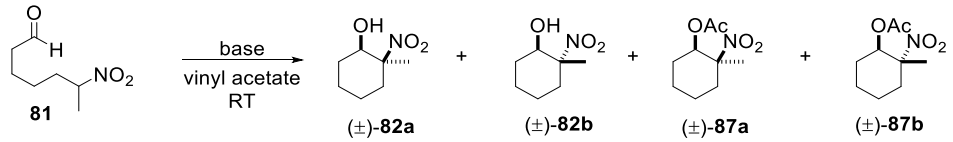
Both triethylamine and DABCO (Entries 5 and 6) successfully catalysed the nitroaldol reaction, without any competing chemical acylation. When used in the base-catalysed interconversion of *cis*-**82a** and *trans*-**82b** there was no evidence of interconversion.

The TMG-mediated dynamic interconversion process was also explored. On exposure of the more stable *trans*-**82b** diastereomer to TMG (2.0 eq.) the *cis*-**82a** diastereomer was observed within 1 h of the initial addition of the TMG and a thermodynamic ratio (*cis*-**82a**:*trans*-**82b** 26:74) was attained after 5 h (Table 4.5).

Using DBU as base successfully catalysed the cyclisation of **81** at low loadings (Table 4.5, Entries 7–9), albeit with a large extent of chemical acylation evident. Although there was a large extent of chemical acylation evident, it was hoped that on combination of the lipase-

catalysed resolution and the base-catalysed interconversion, that the resolution would effectively compete with the chemical acylation.

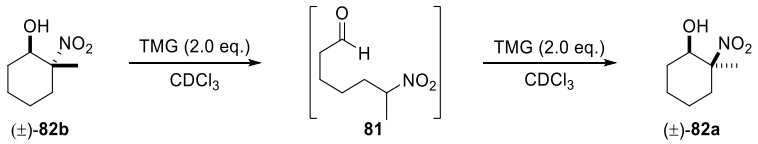
*Table 4.4 Base mediated cyclisation of aldehyde **81***




| Entry | Base | Eq. | Time (h) | Aldehyde 81 (%)[a] | Alcohol 82 | | Acetate 87 | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *cis*-82a (%)[a] | *trans*-82b (%)[a] | *cis*-87a (%)[a] | *trans*-87b (%)[a] |
| 1 | Piperidine | 2.0 | 48 | 100 | – | – | – | – |
| 2 | NaOH (1M) | – | 48 | 100 | – | – | – | – |
| 3 | DEA | 2.0 | 48 | 82 | 8 | 10 | – | – |
| 4 | Hünig's Base | 2.0 | 48 | 85 | 5 | 10 | – | – |
| 5 | $NEt_3$[b] | 2.0 | 72 | 9 | 24 | 67 | – | – |
| 6 | DABCO | 2.0 | 48 | – | 40 | 60 | – | – |
| 7 | DBU | 0.1 | 24 | – | 11 | 52 | 22 | 15 |
| 8 | DBU | 0.1 | 48 | – | – | 30 | 37 | 33 |
| 9 | DBU | 0.05 | 48 | 50 | 16 | 31 | 2 | 1 |
| 10 | TMG | 0.1 | 24 | 69 | 10 | 21 | – | – |
| 11 | TMG | 0.1–2.1[c] | 48 | 6 | 30 | 57 | 5 | – |

Adapted from Deasy.[1] [a]Conversions were determined by $^1$H NMR spectroscopy and were derived from integration of the $^1$H NMR spectrum of the mixture of the crude material not mass recovery; [b]carried out at 40°C; [c]At 24 h after analysis by $^1$H NMR spectroscopy an additional 2.0 eq. of TMG were added to the reaction vessel and stirred at room temperature for a further 24 h.

*Table 4.5 Evidence for dynamic interconversion –* trans*-2-methyl-2-nitrocyclohexanol **82b** and TMG (2.0 eq.) in $CDCl_3$*



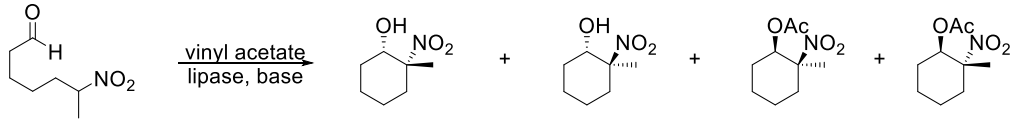

| Reaction Time (h) | 82a (%)[a] | 82b (%)[a] |
|---|---|---|
| 0 | - | 100 |
| 0.8 | 14 | 88 |
| 5.1 | 26 | 74 |
| 72 | 28 | 72 |

Adapted from Deasy.[1] [a]6-Nitroheptanal **81** was not detected in the $^1$H NMR spectra.

When DBU (0.1 eq.) was combined with CAL-B (immob), the intramolecular cyclisation did not proceed, returning only starting material after 72 h (Table 4.6, Entry 1). The use of *P. stutzeri* with this base effectively mediated the ring-closing, however, poor enantioselectivity in the resolution meant this was not a viable one-pot system (Entries 2 and 3). When the base was

changed to TMG, the ring-closing proceeded sluggishly, with aldehyde **81** (15%) still evident after 48 hours; and the low extent of conversion to the products *cis*-**87a** and *trans*-**87b**, was disappointing (Entry 4).

*Table 4.6 Combination of the lipase-catalysed resolution and the base-catalysed ring closure/interconversion*




**81** (1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

| Entry | Base (eq.) | Lipase Source | Reaction Time (h) | Aldehyde 81 (%)[a] | Alcohol 82 | | Acetate 87 | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *cis*-**82a** (%)[a] [*ee* (%)][b,c] | *trans*-**82b** (%)[a] [*ee* (%)][b,d] | *cis*-**87a** (%)[a] [*ee* (%)][b,e] | *trans*-**87b** (%)[a] [*ee* (%)][b,f] |
| 1 | DBU (0.1) | CAL-B (immob) | 72 | 100 | – | – | – | – |
| 2 | DBU (0.5) | *P. stutzeri* | 48 | – | 6 [>98][g] | 4 [>98][g] | 37 [1] | 53 [1] |
| 3 | DBU (1.0) | *P. stutzeri* | 48 | – | 6 [>98][g] | 3 [>98][g] | 53 [1] | 38 [0] |
| 4 | TMG (2.0) | *P. stutzeri* | 48 | 15 | 22 [35] | 50 [6] | 9 [80] | 4 [75] |

Adapted from Foley et al. (supporting information).[2] [a]Conversions were determined by $^1$H NMR spectroscopy and were derived from integration of the $^1$H NMR spectrum of the mixture of the crude material, not mass recovery; [b]enantiomeric excess [*ee* (%)] was determined by chiral HPLC analysis; [c]the principal enantiomer was (1*S*,2*R*)-**82a**; [d]the principal enantiomer was (1*S*,2*S*)-**82b**; [e]the principal enantiomer was (1*R*,2*S*)-**87a**; [f]the principal enantiomer was (1*R*,2*R*)-**87b**; [g]while chiral HPLC indicates high enantiomeric excess for the recovered alcohols (1*S*,2*R*)-**82a** and (1*S*,2*S*)-**82b**, the very low levels present mean the enantiopurity should be interpreted with caution.

Both the reversible ring opening/closing and the resolution worked separately, but when the two reaction were combined, neither reaction worked effectively. The use of the base and the lipase concurrently was not viable; therefore, it was decided to carry out the interconversion and the resolution consecutively, in a two-pot reaction. This overcame the base-lipase inhibition by separating the reagents and allowed the use of neat vinyl acetate as the solvent for the resolution (Figure 4.7). The problems of chemical acylation, and base-lipase inhibition were overcome by physically separating the two reactions. This ultimately returned the preferred product *trans*-**87b** with excellent enantiopurity (>98% *ee*) which made up 57% of the reaction mixture, after three cycles.

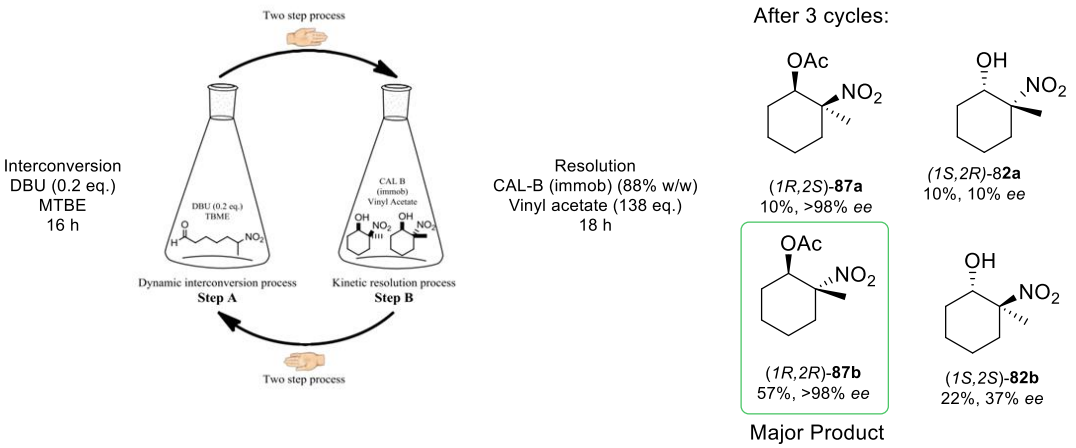



*Figure 4.7 Two-pot system developed by Deasy, % composition determined by $^{1}$H NMR.[1,2]*

## 4.2 Objectives

Deasy made significant progress in the combination of the base-mediated interconversion of nitroalcohols **82** and the lipase-catalysed resolution. Several limitations were identified and will need to be overcome before the effective combination of the base-catalysed interconversion and the lipase mediated resolution.

The use of DBU is only feasible if the problem of competing chemical acylation could be overcome, such as the use of an alternative acylating agent, or introduction of a solvent to decrease the effective concentration of the acylating agent. Potentially, reduction of the acylating agent loading could also solve this problem.

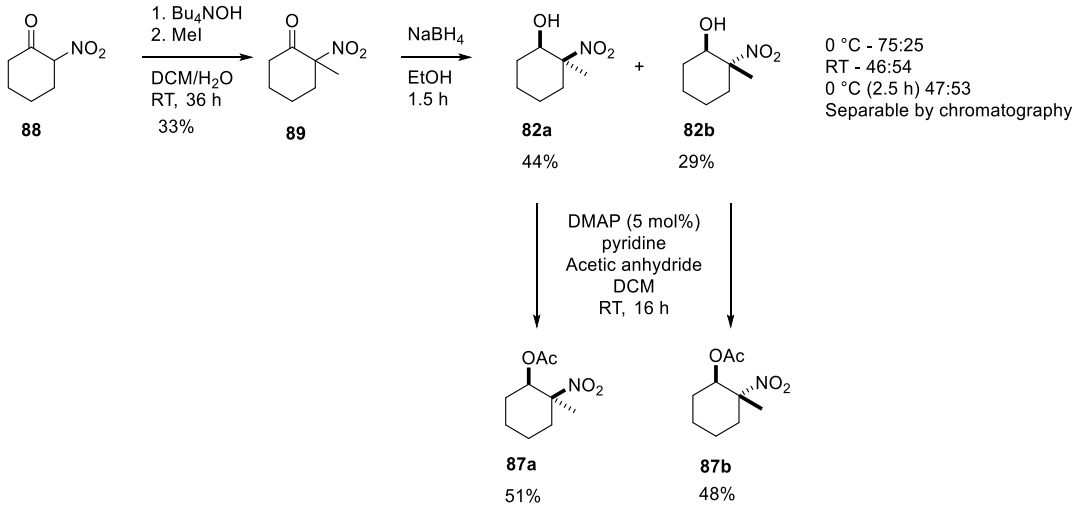
Base-lipase inhibition was a significant limitation during Deasy's work. The simultaneous introduction of the base and the lipase meant that the efficiency of both was reduced. A potential solution is the use of immobilised reagents, or physical separation of the catalysts. CAL-B, an immobilised lipase, gave excellent selectivity in this reaction; the use of an immobilised base could also be beneficial, both for physical separation of the catalyst and potentially for subsequent separation from the reaction mixture.

Further work was warranted to improve the system. As a result, the objectives of this project are:

- Synthesis of the substrate alcohols **82** and the acetates **87**, as reference standards.
- Exploration of a wider range of bases for the interconversion step
- Explore the use of solvents, ideally to find a solvent which works well for both the interconversion step and the resolution step
- Vary the acylating agent, as well as acyl agent loading, as the use of milder acylating agents could overcome the potential problem of chemical acylation
- Vary the reaction conditions
    - Temperature
    - Time
    - Addition time of the reagents
- Explore the use of a flow-like system to physically separate the catalysts and ultimately lead to the high diastereo- and enantioselectivity.

## 4.3 Synthesis of substrates

The synthesis of the substrates **82a** and **82b** was carried out using a two-step procedure (Scheme 4.7), followed by separation and acylation of the product alcohols to make acetates **87a** and **87b** which are used as standards for HPLC method development.




*Scheme 4.7 Synthesis of substrates*

The first step was the alkylation of the α-carbon of compound **88** using methyl iodide and tetra-*n*-butyl ammonium hydroxide. This reaction was low yielding, producing two by-products **90** and **91** which were separated by chromatography (Figure 4.8). These products were previously reported by Deasy and were present in roughly the same ratios as previously reported.

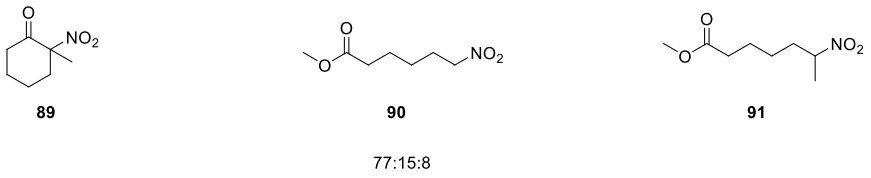
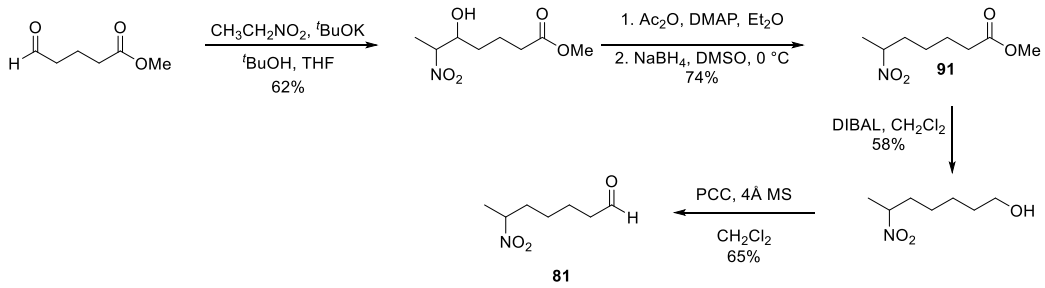



*Figure 4.8 Major products from the alkylation of **88***

The reduction of the ketone **89** was carried out using sodium borohydride as the reducing agent. Increasing the reaction time from 1.5 h to 2.5 h at 0°C gave a similar result to conducting the reaction at room temperature for 1.5 h, giving almost identical product ratios (Scheme 4.7). Separation of diastereomers **82** by chromatography was achieved using 40/60 diethyl ether/hexane as eluent. If 20/80 ethyl acetate/hexane was used as eluent, then the diastereomers did not separate effectively. The alcohol diastereomers were separated and

acylated using DMAP and acetic anhydride, giving the acetates **87a** and **87b** in moderate yields without requiring chromatographic purification.

Deasy previously synthesised aldehyde **81** by a four-step procedure (Scheme 4.8), which furnished compound **91** after the second step. This product was a by-product in the synthesis of ketone **89** and was easily purified by column chromatography and therefore can potentially be utilised in the synthesis of the aldehyde, if required, reducing the waste in the synthesis of **89** and reducing the amount of reactions required in the synthesis of aldehyde **81**.




*Scheme 4.8 Synthesis of methyl-6-nitroheptanal **81***

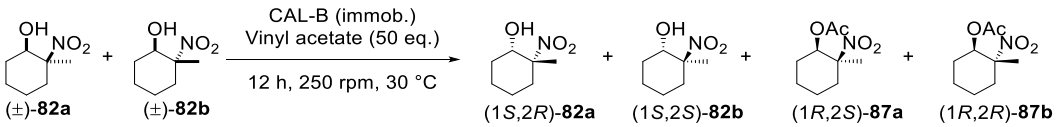
## 4.4 Initial screens – base, lipase, solvent and acylating agent

Deasy developed a two-pot cycling system, where the reagents were repeatedly exposed to the alternating reaction conditions. Work completed here built on the data collected by Deasy as well as looking at the two reactions individually. A number of experiments were carried out to quantify the effects of different components of the reaction. Considering that one of the best bases for the interconversion was DBU, immobilised DBU was explored as it would enable facile separation from the reaction mixture. Additional bases were explored but with poor results. Similarly, CAL-B (immob) was chosen as the lipase, allowing facile separation.

### 4.4.1 Solvent screen – towards a one-pot reaction

In order to combine the two separate systems, careful selection of solvent is critical to ensure compatibility with both the base-mediated interconversion, and the lipase-catalysed resolution; it is clear that the use of vinyl acetate as solvent is not feasible due to competing chemical acylation.

*Table 4.7 Solvent screen for resolution*



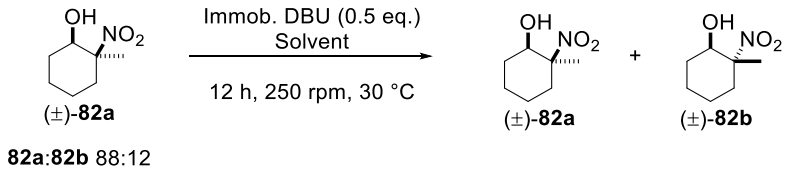

| Entry | Solvent | Acetate 87[a,b] | |
|---|---|---|---|
| | | *cis*-87a | *trans*-87b |
| 1 | Toluene | 4 (>98) | 22 (>98) |
| 2 | Hexane | 9 (>98) | 38 (>98) |
| 3 | MTBE | 10 (>98) | 33 (>98) |
| 4 | IPA | -[c] | 4 (>98) |
| 5 | 1-Octanol | 2 (>98) | 11 (33) |
| 6 | Ethyl Acetate | 2 (>98) | 23 (>98) |

Entries 1 & 2 were previously carried out by Gavin, and repeated in this work, the results shown are from this work. [a]Relative conversions, determined from *ee* values of the substrate alcohols ($ee_s$) and product acetates ($ee_p$), as the presence of some solvents made interpretation of the $^1$H NMR spectrum difficult; [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [c]no *cis*-**87a** was detected by $^1$H NMR.

In the solvent screen, we found that the resolution, mediated by CAL-B (immob), performed well in non-polar solvents (toluene and hexane) (Table 4.7, entries 1 and 2) while the interconversion is also conveniently favoured by these non-polar solvents (Table 4.8). Sampling was more reliable using toluene as solvent than using hexane, in which the substrate

was poorly soluble. Interestingly, while the interconversion catalysed by DBU (immob) was sluggish in ethyl acetate (Table 4.8, Entry 6), this solvent provides us with promising diastereomeric discrimination in the resolution step (Table 4.7, Entry 6).

*Table 4.8 Solvent screen for the interconversion catalysed by immobilised DBU*




(±)-**82a** **82a**:**82b** 88:12 (±)-**82a** (±)-**82b**

| Entry | Solvent | Alcohol 82 (%)[a] | |
|---|---|---|---|
| | | *cis*-**82a** | *trans*-**82b** |
| **1** | Toluene | 68 | 32 |
| **2** | Hexane | 87 | 13 |
| **3** | MTBE | 87 | 13 |
| **4** | IPA | 70 | 30 |
| **5** | 1-Octanol | -[b] | -[b] |
| **6** | Ethyl Acetate | 84 | 16 |

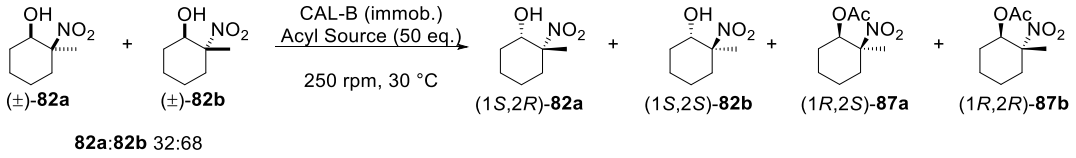
Entries 1 & 2 were previously carried out by Gavin, and repeated in this work, the results shown are from this work. [a]Values determined by $^1$H NMR; [b]values could not be determined by $^1$H NMR due to the presence of the solvent.

## 4.4.2 Variation of acyl source

We briefly explored the use of acylating agents other than vinyl acetate in conjunction with CAL-B (immob). Ramström et al. reported success in a related one-pot reaction using phenyl acetate.[12] Various acylating agents, including ethyl acetate, which could potentially be used as the solvent and the acylating agent, were explored. For these screens, 50 eq. of the acylating agent was used as the acyl source and the solvent; this is less than was used by Deasy in previous work.[1] All acylating agents provides the acetate products *cis*-**87a** and *trans*-**87b** with excellent enantioselectivity, albeit, some with very limited conversion (Table 4.9). Isopropenyl acetate **68**, and isopropyl acetate **80**, both gave similar results, but with conversion <5% for *cis*-**82a** and <15% for *trans*-**82b**, after 12 hours. Ethyl acetate, similarly, showed a low extent of acylation of *cis*-**82a**, but showed high diastereoselectivity (1:8, favouring *trans*-**87b**) when used as the acyl source.

While vinyl acetate and phenyl acetate performed equally well, including over longer reaction times (48 h), use of the higher boiling phenyl acetate did not offer any synthetic advantages over vinyl acetate but was difficult to separate from the product on an analytical scale.

*Table 4.9 Variation of acyl source*



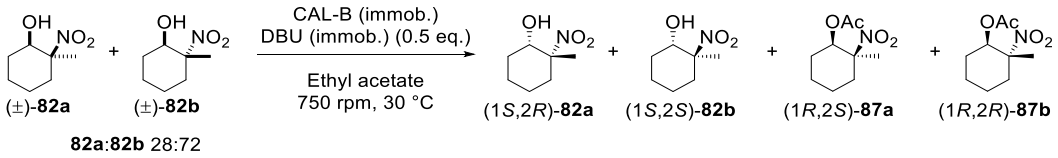

| Entry | Acyl Source | Reaction time (h) | Acetate 87 (%)[a,b] | |
|---|---|---|---|---|
| | | | *cis*-87a | *trans*-87b |
| 1 | Vinyl Acetate | 12 | 5 (*>98*) | 35 (*>98*) |
| 2 | Vinyl Acetate | 48 | 12 (*>98*) | 46 (*>98*) |
| 3 | Isopropenyl Acetate | 12 | 3 (*>98*) | 14 (*>98*) |
| 4 | Isopropyl Acetate | 12 | 3 (*>98*) | 13 (*>98*) |
| 5 | Phenyl Acetate | 12 | 6 (*>98*) | 34 (*>98*) |
| 6 | Phenyl Acetate | 48 | 14 (*>98*) | 48 (*>98*) |
| 7 | Ethyl Acetate | 12 | 2 (*>98*) | 16 (*>98*) |

[a]Relative conversions, determined from %*ee* values of the substrate alcohols ($ee_s$) and product acetates ($ee_p$); [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

The reaction was carried out again, this time with ethyl acetate as acylating agent, as it is not sufficiently reactive to effect chemical acylation. The reaction time was extended to 120 hours and the agitation was increased to 750 rpm, and extra lipase was added after 48 hours. The acylation proceeded very slowly, giving <10% of the enantiopure acetate *trans*-87b even after extended reaction times (Table 4.10, Entry 1). When using ethyl acetate as solvent the interconversion did not proceed (Table 4.8, Entry 6 and Table 4.10, Entry 2), and when the interconversion and resolution catalysts were added together (Table 4.10, Entry 3), the resolution proceeded enantioselectively. Although the material recovered here was enantioenriched, the extent of both the resolution and the interconversion was not sufficient to be synthetically useful, even after extended reaction times.

These experiments showed that the one-pot reaction was not feasible using ethyl acetate as solvent and DBU (immob) as base, as both the resolution and the interconversion were very inefficient. Although the material recovered here was enantioenriched, the extent of conversion was too low to be useful, even after 5 days, as the interconversion catalysed by DBU (immobilised) was sluggish in the polar solvent. The use of alternative bases in ethyl acetate as solvent was next explored as the diastereoselectivity in the resolution step was promising (Table 4.7, Entry 6).

*Table 4.10 One-pot system using ethyl acetate as solvent and acylating agent*




(±)-**82a** (±)-**82b** **82a**:**82b** 28:72 (1*S*,2*R*)-**82a** (1*S*,2*S*)-**82b** (1*R*,2*S*)-**87a** (1*R*,2*R*)-**87b**

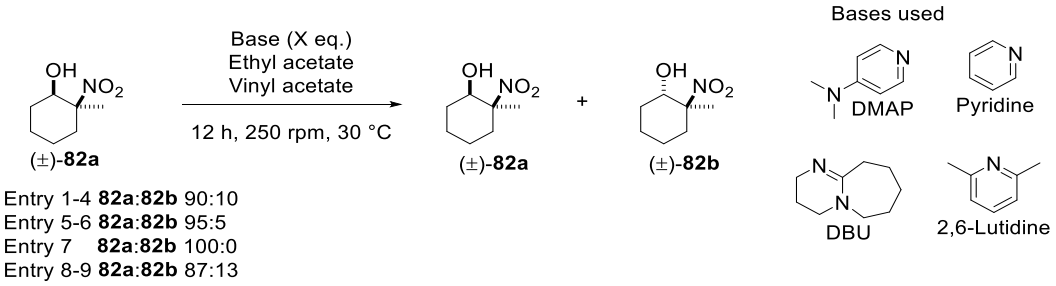
| Entry | Reaction Conditions[a] | Time (h) | Alcohol 82 | | Acetate 87 | |
|---|---|---|---|---|---|---|
| | | | *cis*-**82a**[b] | *trans*-**82b**[b] | *cis*-**87a**[b] | *trans*-**87b**[b] |
| 1 | Lipase, no base[c] | 24 | 29 (3) | 65 (12) | 0 (>99) | 6 (>99) |
| | | 120 | 30 (4) | 61 (17) | 2 (>99) | 7 (>99) |
| 2 | Base, no lipase | 24 | 23 (2) | 75 (0) | <1 (10) | 2 (1) |
| | | 120 | 25 (-) | 64 (-) | 3 (2) | 8 (1) |
| 3 | Base & Lipase[c] | 24 | 33 (2) | 67 (8) | 0 (-) | 0 (-) |
| | | 120 | 30 (6) | 57 (13) | 5 (>99) | 9 (>99) |

[a]Ethyl acetate added to give [substrate] = 10 mg/mL; reaction run in the absence of base or lipase returned *cis*-**82a**:*trans*-**82b** 28:72 after 120 h i.e. unchanged; [b]% composition of the reaction mixture, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis;[14] [c]extra lipase was added after 48 h.

## 4.4.3 Exploration of alternative bases using ethyl acetate as solvent

Based on a recent report of the use of immobilised DMAP for the Henry reaction,[15] investigation of DMAP was next explored, as well as structurally related bases (Table 4.11). Ethyl acetate was selected as solvent due to the promising diastereoselectivity in the lipase-mediated resolution (Table 4.7, Entry 6). DMAP performed poorly when only 1 eq. was used, but when the amount of DMAP was increased, up to 10 eq., the interconversion was very successful, outperforming DBU (immob) in this solvent (Table 4.11, Entry 3, 4, 1 and 2, respectively). Most importantly here, with 10 eq. of DMAP, there was no chemical acylation evident even when using 50 eq. of vinyl acetate in ethyl acetate as solvent (Entry 4). The DMAP was removed from the reaction mixture by passing through a short column of silica gel; this was necessary both for clear $^{1}$H NMR analysis, and to remove DMAP before chiral HPLC analysis. Use of DMAP (immob) proved to be poorly effective for the interconversion and led to significant background acylation in the presence of vinyl acetate, as well as decreased efficiency of the interconversion (Entry 6). In the presence of CAL-B and vinyl acetate i.e. a one-pot reaction (Entry 7), the acylation proceeded, albeit with very poor enantioselectivity. Interconversion of the diastereomers was not seen when using related bases, lutidine and pyridine (2 eq.), while chemical acylation was evident with lutidine under these conditions (Entry 8 and 9).

*Table 4.11 Investigation of different bases using ethyl acetate as solvent*




Entry 1-4 **82a**:**82b** 90:10
Entry 5-6 **82a**:**82b** 95:5
Entry 7 **82a**:**82b** 100:0
Entry 8-9 **82a**:**82b** 87:13

| Entry | Base | Eq. Base | Eq. Vinyl Acetate | Alcohol 82 | | Acetate 87 | |
|---|---|---|---|---|---|---|---|
| | | | | *cis*-**82a**[a] | *trans*-**82b**[a] | *cis*-**87a**[a] | *trans*-**87b**[a] |
| 1 | DBU (immob) | 1 | 0 | 86 | 14 | - | - |
| | | | 50 | 80 | 13 | 6 | 1 |
| 2 | | 5 | 0 | 74 | 16 | - | - |
| | | | 50 | 54 | 22 | 20 | 4 |
| 3 | DMAP | 1 | 0 | 89 | 11 | - | - |
| | | | 50 | 89 | 11 | 0 | 0 |
| 4 | | 10 | 0 | 0 | 100 | - | - |
| | | | 50 | 46 | 54 | 0 | 0 |
| 5 | DMAP (immob) | 1 | 0 | 77 | 23 | - | - |
| | | | 50 | 89 | 11 | 0 | 0 |
| 6 | | 10 | 0 | 36 | 64 | - | - |
| | | | 50 | 81 | 10 | 7 | 1 |
| 7 | DMAP & CAL-B (immob) | 10 | 50 | 71 (2) | ND | 22 (7) | 6 (4) |
| 8 | Pyridine | 2 | 0 | 87 | 13 | - | - |
| | | 2 | 50 | 85 | 15 | - | - |
| 9 | Lutidine | 2 | 0 | 87 | 13 | - | - |
| | | 2 | 50 | 79 | 18 | 2 | 1 |

Ethyl acetate added to make [substrate] = 10 mg/mL. [a]Numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

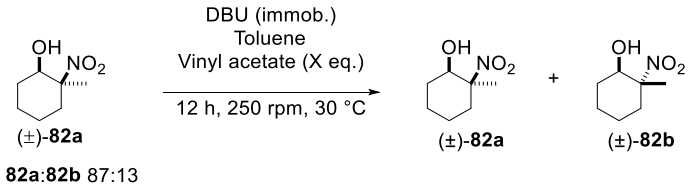
## 4.4.4 Vinyl acetate loading

As the combination of DBU (immob) and vinyl acetate led to background chemical acylation, this approach was not feasible as it led to reduced enantiopurity of the final product. Predictably, decreasing the concentration of vinyl acetate, as a solution in toluene, significantly reduced the problem of background chemical acylation (5 eq. of vinyl acetate gave <5% chemical acylation after 12 hours) (Table 4.12).

Fortunately, when the loading of vinyl acetate was decreased from 50 eq. to 3 eq. the resolution was equally effective (Scheme 4.9 and Table 4.7, Entry 1 and Table 4.9, Entry 1). This allowed the reduction of the vinyl acetate loading, reducing the extent of competing chemical acylation. It is important to note here that, even though the majority of the starting material used was the *cis* isomer, *cis*-**82a**, the conversion of this to the product was low. The

*trans* material, *trans*-**82b**, was processed preferentially by the lipase, despite making up only a minority of the material.

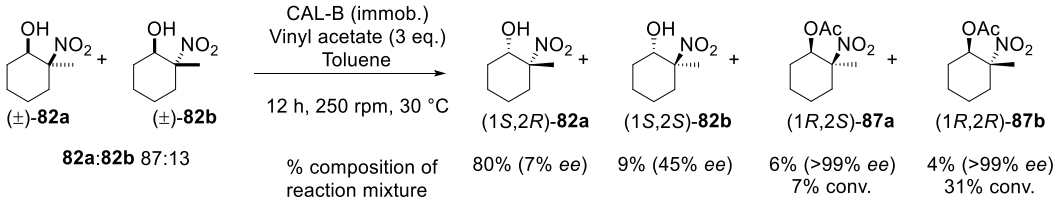
*Table 4.12 Investigation of the effect of vinyl acetate loading on interconversion and chemical acylation*




**82a**:**82b** 87:13

| Entry | Eq. Vinyl Acetate | Alcohol 82 (%)[a] | | Acetate 87 (%)[a] | |
|---|---|---|---|---|---|
| | | *cis*-**82a** | *trans*-**82b** | *cis*-**87a** | *trans*-**87b** |
| 1 | 0 | 68 | 32 | - | - |
| 2 | 5 | 68 | 28 | 3 | 1 |
| 3 | 10 | 66 | 28 | 5 | 1 |
| 4 | 20 | 69 | 24 | 6 | 1 |
| 5 | 30 | 67 | 22 | 10 | 2 |
| 6 | 40 | 69 | 20 | 9 | 2 |
| 7 | 50 | 69 | 20 | 9 | 2 |

[a]% determined by $^1$H NMR

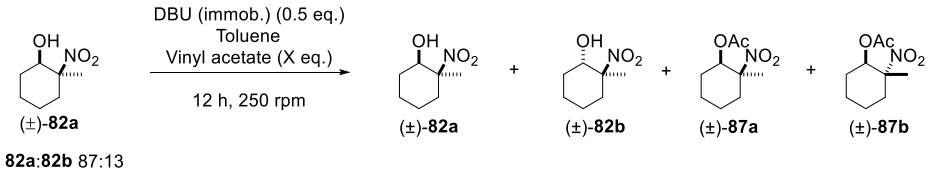



*Scheme 4.9 Resolution of 2-methyl-2-nitrocyclohexanol **82***

## 4.4.5 Temperature

The next factor explored was the impact of reaction temperature. Both the interconversion and the resolution were carried out separately with varying amounts of vinyl acetate in toluene, at three different temperatures: 30°C, 40°C and 50°C (Table 4.13 and Table 4.14, respectively). The interconversion was much faster at the higher temperatures (Table 4.13). As shown in Table 4.14, the enantioselectivity of the lipase-mediated transesterification of *cis*-**82a** and *trans*-**82b** was unchanged when the temperature was increased from 30°C to 50°C. However, there was a decrease in diastereoselectivity at the higher temperature with a higher proportion of *cis*-**87a** formed at 50°C than at 30°C (Table 4.14).

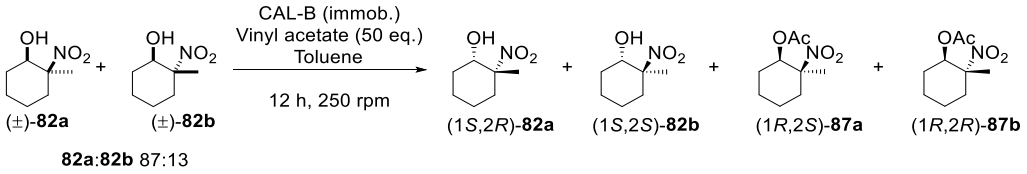
*Table 4.13 Temperature effect on the interconversion process*




| Entry | Eq. VA | 30°C[a,b] | | | | 40°C[a] | | | | 50°C[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *cis*-82a | *trans*-82b | *cis*-87a | *trans*-87b | *cis*-82a | *trans*-82b | *cis*-87a | *trans*-82b | *cis*-82a | *trans*-82b | *cis*-87a | *trans*-87b |
| 1 | 0 | 68 | 32 | - | - | 66 | 34 | - | - | 52 | 48 | - | - |
| 2 | 5 | 68 | 28 | 3 | 1 | 70 | 28 | 2 | 1 | 59 | 37 | 3 | 1 |
| 3 | 10 | 66 | 28 | 5 | 1 | 71 | 25 | 3 | 1 | 49 | 42 | 7 | 2 |
| 4 | 20 | 69 | 24 | 6 | 1 | 69 | 24 | 6 | 2 | 55 | 33 | 9 | 3 |

[a]% of mixture, determined by $^1$H NMR by comparison of the areas of the integrals for to relevant alcohols and acetates; [b]data is the same as Table 4.12, Entries 1–4.

*Table 4.14 The effect of temperature on resolution*



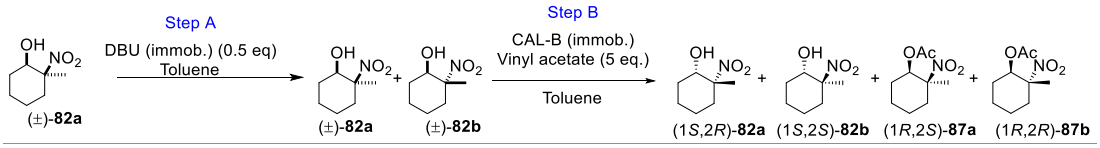

| Entry | Eq. Vinyl Acetate | 30°C[a,b] | | 40°C[a,b] | | 50°C[a,b] | |
|---|---|---|---|---|---|---|---|
| | | *cis*-87a | *trans*-87b | *cis*-87a | *trans*-87b | *cis*-87a | *trans*-87b |
| 1 | 5 | 7 (*>98*) | 34 (*>98*) | 9 (*>98*) | 38 (*>98*) | 16 (*>98*) | 43 (*>98*) |
| 2 | 10 | 9 (*>98*) | 38 (*>98*) | 14 (*>98*) | 43 (*>98*) | 16 (*>98*) | 40 (*>98*) |
| 3 | 20 | 6 (*>98*) | 27 (*>98*) | 10 (*>98*) | 37 (*>98*) | 15 (*>98*) | 35 (*>98*) |

[a]Relative conversions (%), determined by $^1$H NMR by comparison of the areas of the integrals for to relevant alcohol and acetate; [b]numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.[14]

## 4.5 Towards a one-pot dynamic kinetic resolution

Following this, a series of one-pot and "two-pot" reactions were attempted with the same solvent conditions for both the interconversion and the resolution (Table 4.15).

*Table 4.15 A selection of one-pot reactions*




| Entry | Step | Time (h) | Starting Material 82a:82b | Temp (°C) | Alcohol 82[a] | | Acetate 87[a] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *cis*-82a | *trans*-82b | *cis*-87a | *trans*-87b |
| 1 | A<br>B | 12<br>12 | 87:13 | 50 | 52<br>33 *(41)* | 48<br>18 *(87)* | -<br>17 *(>98)* | -<br>32 *(>98)* |
| 2[b] | A<br>B | 12<br>12 | 87:13 | 50 | 52<br>41 *(26)* | 48<br>26 *(80)* | -<br>11 *(>98)* | -<br>22 *(>98)* |
| 3 | A & B | 12 | 87:13 | 50 | 64 *(29)* | 9 *(81)* | 22 *(>98)* | 6 *(>98)* |
| 4 | A & B | 12 | 4:96 | 50 | 5 *(28)* | 46 *(87)* | 2 *(>98)* | 46 *(>98)* |
| 5 | A & B | 12 | 4:96 | 30 | 8 *(3)* | 79 *(13)* | 1 (ND) | 12 *(12)* |
| 6 | A & B | 12 | 87:13 | 30 | 72 *(16)* | 9 *(73)* | 13 *(>98)* | 6 *(>98)* |
| 7 | A & B | 12 | 70:30 | 30 | 63 *(10)* | 18 *(56)* | 8 *(>98)* | 11 *(>98)* |
| 8 | A & B | 12 | 87:13 | 30 | 72 *(15)* | 9 *(77)* | 13 *(>98)* | 6 *(>98)* |
| 9[c] | A & B | 12 | 87:13 | 30 | 37 *(88)* | 8 *(52)* | 48 *(>98)* | 7 *(>98)* |

[a]Given as % composition of the reaction mixture, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [b]entry 2 included a filtration to remove the DBU (immob) before addition of the lipase; [c]entry 9 included molecular sieves.

Entry 1 shows evidence of a dynamic system. The DBU (immob) was not removed prior to CAL-B (immob) addition, and the amount of *trans*-**82b** is less than the amount of *trans*-**87b**. This indicated that the reaction continued past the kinetic limit, also suggesting that the interconversion reaction continued after the addition of the lipase. In the case of Entry 2, the DBU (immob) was removed by filtration before the addition of the CAL-B (immob). If the base

was inactivated by the lipase when they were both present together, then the results for both Entry 1 and Entry 2 should be similar. These experiments showed, for the first time, evidence of a dynamic system when both the base and the lipase were present together, albeit this required a time-delayed addition, key to this system. It also showed that the base and lipase could be present in the reaction mixture at the same time.

The reaction was also carried out where both catalysts were introduced at the same time (Table 4.15). Entries 3 and 4 are identical except for the ratio of starting material. Using predominantly *trans*-**82b** starting material (Entry 4) we saw a much greater conversion, with the conversion essentially at the kinetic limit with some evidence of a dynamic process, as there are equal amounts of *trans*-**82b** and *trans*-**87b** but the % *ee* of *trans*-**82b** is not >98%, as would be expected from a non-dynamic system.

The one-pot procedure, where both the catalysts were added in at the same time, was repeated at lower temperature, 30°C, using *trans*-**82b** and *cis*-**82a** enriched material (Entries 5-7). The conversions in this case were poorer compared to the higher temperature but still retained high stereoselectivity, as expected.

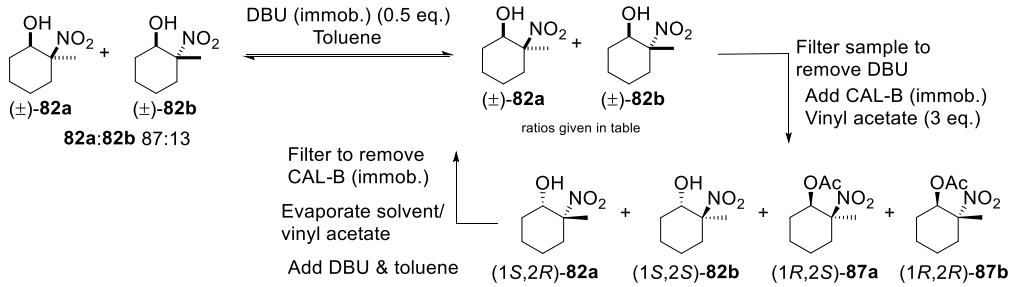
The effect of water was also investigated at this temperature (Entries 8 and 9). The reaction was run with (Entry 9) or without (Entry 8) molecular sieves, again with both the catalysts introduced at the same time. Interestingly, the presence of molecular sieves reversed the diastereoselectivity of the reaction, with the *cis*-**87a** diastereomer being preferred here, but no change in the interconversion efficiency, showing that the presence of small amounts of water is not affecting the DBU (immob) efficiency.

As the time delayed addition of the catalysts was shown to be effective, the cycling of the material through the two different reaction conditions consecutively was investigated.

In the first reaction cycle (Table 4.16, Entry 1), the material was cycled through the two steps, removing one catalyst prior to addition of the next by a simple filtration through a plug of Celite® and magnesium sulfate, and removal of the vinyl acetate by rotary evaporation at the end of the resolution step, before addition of toluene and DBU (immob) to start the cycle again. Here, the equivalents of vinyl acetate were reduced to 3, to minimise the amount of chemical acylation. For the initial cycles, the first procedure (Entry 1) performed both the interconversion and the resolution at 50°C. While this system reached the kinetic limit after

two cycles, (*trans*-**82b**:*trans*-**87b** = 1:1), there was evidence of a dynamic system i.e. that *trans*-**82b** was being interconverted albeit slowly (76% *ee*, >98% *ee*, respectively). The diastereoselectivity was poor, giving equal amounts of the acetates *cis*-**87a** and *trans*-**87b** at the end of the third cycle, with, overall, a poor conversion for a dynamic system (acetate **87** making up just over 50% of the mixture). It was assumed that the elevated temperature was the major cause of the reduced diastereoselectivity, as observed previously (Table 4.14).

*Table 4.16 One-pot reaction – removal of each catalyst before addition of the next*




| Entry | Step | Temperature (°C) | Alcohol 82[a] | | Acetate 87[a] | |
|---|---|---|---|---|---|---|
| | | | *cis*-**82a** | *trans*-**82b** | *cis*-**87a** | *trans*-**87b** |
| 1 | 1A | All steps carried out at 50°C | 56 (-) | 44 (-) | 0 (-) | 0 (-) |
| | 1B | | 56 (23) | 17 (58) | 13 (>98) | 14 (>98) |
| | 2A | | 35 (18) | 34 (22) | 15 (>98) | 16 (>98) |
| | 2B | | 30 (42) | 25 (76) | 20 (>98) | 25 (>98) |
| | 3A | | 23 (30) | 25 (40) | 23 (>98) | 29 (>98) |
| | 3B | | 21 (79) | 23 (>98) | 26 (>98) | 30 (>98) |
| 2 | 1A | 50 | 34 (4) | 66 (5) | 0 (-) | 0 (-) |
| | 1B | 30 | 38 (10) | 42 (31) | 4 (>98) | 16 (>98) |
| | 2A | 50 | 28 (4) | 41 (16) | 6 (>98) | 25 (>98) |
| | 2B | 30 | 25 (14) | 27 (70) | 8 (>98) | 40 (>98) |
| | 3A | 50 | 20 (8) | 24 (2) | 10 (>98) | 47 (>98) |
| | 3B | 30 | 28 (14) | 13 (66) | 11 (>98) | 47 (>98) |
| 3[b] | 1A | 50 | 36 (1) | 64 (3) | 0 (-) | 0 (-) |
| | 1B | 30 | 36 (9) | 39 (46) | 4 (>98) | 20 (>98) |
| | 2A | 50 | 27 (6) | 32 (33) | 8 (>98) | 33 (97) |
| | 2B | 30 | 25 (20) | 25 (78) | 10 (>98) | 40 (>98) |
| | 3A | 50 | 25 (21) | 17 (70) | 13 (>98) | 45 (96) |
| | 3B | 30 | 25 (24) | 19 (78) | 12 (>98) | 43 (97) |

Step A, interconversion: DBU (immob) (0.5 eq.), toluene (10mg/mL substrate). Step B, resolution: CAL-B (immob), vinyl acetate (3 eq.), toluene (1mL/10mg). Each step was carried out for 12 hours, except step 1A for Entry 2 & Entry 3 which were 18 h. [a]Expressed as a % determined by integration of the relevant $^1$H NMR signals, numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis; [b]evaporation step was omitted

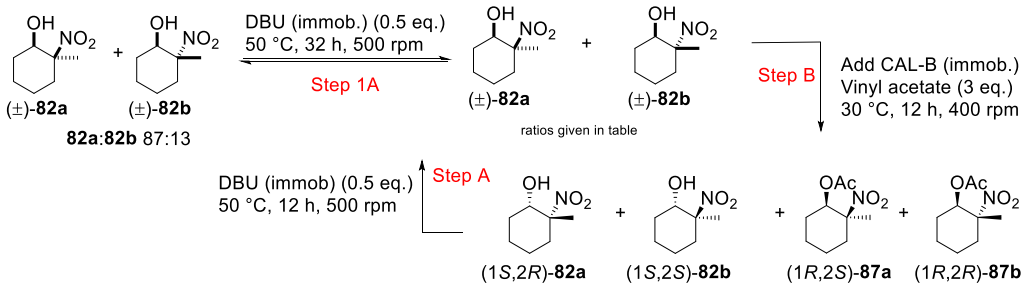
Following this, the same cycling system was maintained, this time with the resolution being carried out at the lower temperature of 30°C (Table 4.16, Entry 2), and the initial interconversion step being extended from 12 hours to 18 hours, which gave a greater

proportion of *trans*-**82b** present for the first resolution step (34:66, compared to 56:44 after 12 h). Again, before the addition of each catalyst, the previous catalyst was removed by filtration, and the vinyl acetate was removed by rotary evaporation at the end of the cycle. Throughout three cycles the enantioselectivity remained consistently high, with enantiopure *cis*-**87a** and *trans*-**87b** observed by HPLC analysis. In this case, acetate **87** again made up over 50% of the reaction mixture, but the product *trans*-**87b** was strongly preferred (47% *trans*-**87b**, 11% *cis*-**87a**, >98% *ee* for both). Here, there was no evidence that the reaction was complete (alcohol **82b** << 98% *ee*), but when it was continued through another cycle the product started to hydrolyse. The presence of a removable impurity made the spectra more difficult to interpret; it was shown that the impurity could be removed by chromatography on silica gel, and that it arose from the immobilised lipase. Here, it was shown that the resolution proceeded better at the lower temperature, and thus, it was decided to continue under these conditions.

As a result of the sustained excellent enantioselectivity, the reaction was carried out again, this time removing the evaporation step, resulting in the DBU (immob) being exposed to vinyl acetate but giving an overall simpler process from a work-up point of view (Table 4.16, Entry 3). Only a modest decrease in enantiopurity was observed, suggesting that the removal of the evaporation step was well tolerated. At the end of 3 cycles the $^{1}$H NMR and HPLC showed 43% of the reaction mixture made up of the acetate *trans*-**87b** (97% *ee*), and 12% acetate *cis*-**87a** (>98% *ee*). While this is lower than when the evaporation step is included, it is not significantly lower.

Encouraged by the success of the “two-pot” procedures, as well as previous evidence that the interconversion continued after the addition of CAL-B (immob), we decided to attempt a series of sequential one-pot reactions, where both catalysts were present at the same time, albeit after a staggered addition. In these reactions, the DBU (immob) was added, reacted for 32 hours, at 50°C, followed by a decrease in temperature, and the addition of CAL-B (immob) and vinyl acetate.

*Table 4.17 One-pot reactions*




| Entry | Step | Notes | Alcohol 82[a] | | Acetate 87[a] | |
|---|---|---|---|---|---|---|
| | | | *cis*-82a | *trans*-82b | *cis*-87a | *trans*-87b |
| 1 | 1A | Filter to remove catalyst after step B, no evaporation step. | 36 *(4)* | 64 *(0)* | 0 *(-)* | 0 *(-)* |
| | 1B | | 20 *(28)* | 26 *(96)* | 14 *(>98)* | 41 *(>98)* |
| | 2A | | 16 *(13)* | 26 *(32)* | 15 *(94)* | 42 *(>98)* |
| | 2B | | 18 *(28)* | 22 *(85)* | 15 *(96)* | 46 *(97)* |
| | 3A | | 18 *(24)* | 14 *(62)* | 20 *(96)* | 48 *(96)* |
| | 3B | | 19 *(39)* | 19 *(95)* | 19 *(95)* | 42 *(90)* |
| 2 | 1A | Filter & evaporation after step B | 36 *(4)* | 64 *(0)* | 0 *(-)* | 0 *(-)* |
| | 1B | | 18 *(39)* | 27 *(>98)* | 14 *(>98)* | 41 *(>98)* |
| | 2A | | 16 *(16)* | 27 *(7)* | 17 *(92)* | 40 *(97)* |
| | 2B | | 17 *(16)* | 25 *(62)* | 16 *(97)* | 43 *(98)* |
| | 3A | | 9 *(7)* | 21 *(13)* | 19 *(94)* | 51 *(97)* |
| | 3B | | 11 *(55)* | 17 *(87)* | 18 *(97)* | 53 *(96)* |
| 3 | 1A | No filtration or evaporation step carried out, no additional catalyst added | 33 *(7)* | 67 *(0)* | 0 *(-)* | 0 *(-)* |
| | 1B | | 20 *(41)* | 25 *(93)* | 16 *(94)* | 40 *(>98)* |
| | 2A | | 17 *(89)* | 30 *(>98)* | 19 *(98)* | 34 *(98)* |
| | 2B | | 26 *(80)* | 34 *(55)* | 17 *(98)* | 23 *(97)* |
| | 3A | | 23 *(57)* | 44 *(37)* | 15 *(96)* | 17 *(96)* |
| | 3B | | 26 *(52)* | 45 *(31)* | 15 *(96)* | 15 *(95)* |
| 4 | 1A | No filtration or evaporation step. | 33 *(4)* | 67 *(1)* | 0 *(-)* | 0 *(-)* |
| | 1B | | 19 *(35)* | 26 *(>98)* | 14 *(>98)* | 41 *(>98)* |
| | 2A | | 19 *(71)* | 43 *(36)* | 18 *(98)* | 20 *(>98)* |
| | 2B | | 20 *(64)* | 41 *(38)* | 17 *(98)* | 22 *(>98)* |
| | 3A | | 28 *(32)* | 53 *(11)* | 13 *(95)* | 6 *(85)* |
| | 3B | | 28 *(31)* | 48 *(17)* | 13 *(94)* | 11 *(63)* |

Step A, interconversion: DBU (immob) (0.5 eq.), toluene (1mL/10mg substrate), 50°C, 500 rpm. Step B, resolution: CAL-B (immob), vinyl acetate (3 eq.), toluene (1mL/10mg), 30°C, 400 rpm. Each step was carried out for 12 hours, except step 1A for which was 32 h. [a]Expressed as a % determined by integrating of the relevant $^1$H NMR signals. Numbers in parentheses are % *ee* values, which were determined by chiral HPLC analysis.

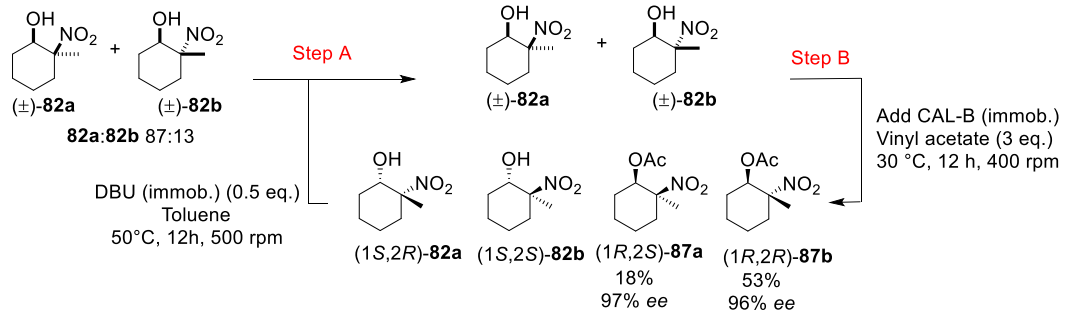
When no evaporation step was carried out (Table 4.17, Entry 1) the overall enantiopurity of the products *cis*-**87a** and *trans*-**87b** were reduced (95% *ee*, 90% *ee*, respectively), as well as reduced conversion overall. When both the filtration and the evaporation step were removed, the overall amount of acetate **87** after 3 cycles was reduced (Table 4.17, Entry 4). In this case, extra catalyst was added to the reaction mixture after each step, but without the removal of the previous catalysts, it gave a similar result to when no additional catalyst was added (Entry 3). This suggests that the presence of the old catalyst is detrimental to the reaction, resulting in hydrolysis of the product acetates *cis*-**87a** and *trans*-**87b** during each racemisation step, as well as evidence of chemical acylation, for both Entry 3 and 4. Although the presence of the catalysts is tolerated through one reaction cycle, removal of the old catalysts is essential before starting the cycle again.

Table 4.17, entry 2 shows the optimised one-pot procedure. It was found that the addition of the catalysts needed to be staggered, as DBU (immob) is less effective in the presence of CAL-B (immob). The DBU (immob) was added first, in this case interconversion was carried out for 32 h at 50°C for step 1A. Although it was subsequently shown that 32 hours was not required and that the same *cis*-**82a**:*trans*-**82b** ratio (~36:64) was reached after only 18 hours. Critically, due to staggered addition of reagents, it was possible to conduct the interconversion at 50°C and then cool the reaction mixture to 30°C for the lipase-mediated resolution, carried out in the same reaction vessel, with the vinyl acetate and the CAL-B (immob) added after the interconversion and cooling to 30°C.

The starting material used was a mixture of *cis*-**82a** and *trans*-**82b** (87:13) instead of aldehyde **81**. In the presence of DBU only, aldehyde **81** will cyclise, and in this case, the mixture of nitroalcohols **82** or aldehyde **81** would be expected to give the same outcome under the conditions for step 1A.

These results are promising, showing, for the first time, a one-pot, dynamic system for the resolution of alcohol **82**. While this is not the desired one-pot reaction, it represents a significant move towards a one-pot procedure (Scheme 4.10). In order to achieve this, several parameters were examined, and their role evaluated. Filtration (after step B) is necessary to remove the immobilised catalysts but does not need to be carried out after each step (A and B), only the end of each cycle (after step B). Sequential addition of the catalysts was found to be key to this one-pot procedure, where the immobilised base was added to the reaction mixture first, to allow interconversion to be carried out, followed by the introduction of the

immobilised lipase, to carry out the resolution. While the evaporation step (after B) was shown to give better enantiopurity after three cycles as well as more product (~60% vs ~50%) than without the evaporation step, it could be removed if necessary to simplify the procedure.




*Scheme 4.10 Optimised conditions for the cycling system*

The final ratio of the products and substrates show, that, although not a "traditional" one-pot reaction, a dynamic system has been engineered, through extensive optimisation of the individual steps.

## 4.6 Conclusion

Significant progress has been made in the individual elements of the dynamic resolution process for *trans*-**87b**. Efficient kinetic resolution has been effected for both the *trans*-2-methyl-2-nitrocyclohexanol **82b** and *cis*-2-methyl-2-nitrocyclohexanol **82a**. Significantly, CAL-B (immob) displayed high diastereoselectivity, selectively acetylating *trans*-**82b** efficiently and with excellent enantioselectivity.

The conditions were further explored, focusing on conditions which work for the individual parts. Conditions were developed which furnished a dynamic system albeit with poor efficiency. Key to this was the timing of the reagent addition, by first adding the base, to carry out the interconversion, then the lipase for the resolution, we successfully demonstrated a dynamic system was possible.

In conclusion, achieving DKR in the intramolecular nitroaldol reaction is extremely challenging due to the number of competing processes arising. By careful exploration of the process conditions including variation of biocatalyst, base and solvent, as well as reducing the loading of the acylating agent, we have demonstrated for the first time the feasibility of this process. Key to this system was the time delayed addition of the catalysts, which not only avoided base-lipase inhibition, but also allowed the reaction halves to be carried out at different temperatures.

Although extensive work has been carried out to demonstrate the utility of this dynamic kinetic resolution, further work, to extend the substrate scope was not carried out, as the synthetic utility of this protocol was not sufficient to justify such research.

## 4.7 References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# Chapter 5

# Novel transaminases – exploration of substrate scope and resolution of pharmaceutical intermediates

# Contents

# Overview

This chapter explores the substrate scope of a novel transaminase, TA3, identified through genome mining using genetic material collected by BIOMERIT. It pertains to a collaboration between the Maguire group and the group of Prof. Fergal O'Gara, UCC.

It is hoped that the material contained in this chapter, and the preceding work in the team, will form part of the supporting information for a patent application pertaining to the novel function of TA3, and for a publication.

TA3, and a similar transaminase (89% homology) TA-AD2, were identified through genome mining and their function, in comparison to the well characterised transaminase from *Chromobacterium violaceum* (CV-TA) as well as their activity compared to each other, will be explored in this work.

The first part of this chapter will focus on the substrate scope, where various compounds previously used to gauge transaminase activity in the cases where transaminases have been modified by directed evolution will be examined.

The second part of this chapter will cover initial work on the transaminase-mediated reductive amination reaction.

The final part of this chapter will explore API intermediates **92**–**94**, related to sertraline **95**, as substrates to investigate the effects of the removal of halogens, reduction of the ring size etc.

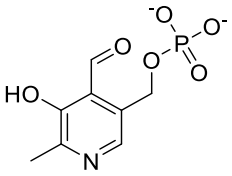
## 5.1 Introduction

The marine environment has provided chemists with a large number of bioactive molecules.[1] Natural products from the marine environment have shown novel activity, as well as containing uncommon motifs and functionality.[2] As well as this, some natural products have proven to be difficult or impossible (thus far) to synthesise in the lab. In addition to being a source of novel and interesting compounds, it follows that the organisms that produce these novel molecules should also produce enzymes with novel functionality, or differing substrate promiscuity to their terrestrial counterparts and organisms from other sources.

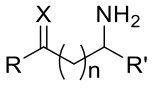
The team in BIOMERIT, led by Prof. Fergal O'Gara, collected genetic material from a marine source off the west of Ireland, and from Lough Hyne, in West Cork. Metagenomic analysis carried out on genetic material collected from a marine sponge from Lough Hyne revealed a novel ω-transaminase (TA3) from *Pseudovibrio* sp., showing, at highest, an identity of 74% with other transaminases. The transaminase showed no unusual properties in terms of pH or temperature tolerance. Due to its novelty it was thought that TA3 could have unusual or novel function. Another transaminase (AD2), which shared 87% sequence identity with TA3 was also identified and investigated in this work. As a control, a well-characterised transaminase from *Chromobacterium violaceum* (CV-TA) was used in this work; CV-TA shares 53% sequence identity with TA3.

### 5.1.1 Transaminases

Transaminases (EC 2.6.1.X) are PLP-dependant transferases that catalyse the transfer of an amine group from an amine donor to an amine acceptor, resulting in a primary amine and a carbonyl compound. There are four sub-groups of transaminases, I to IV, of which three require the substrate to contain a carbonyl group α- to the reacting site. ω-Transaminases (or ω-amino acid transferases, group II) are the exception to this, not requiring a carbonyl group at the α-position; this means that the ω-transaminases can potentially have a much broader substrate scope (Figure 5.).[3]



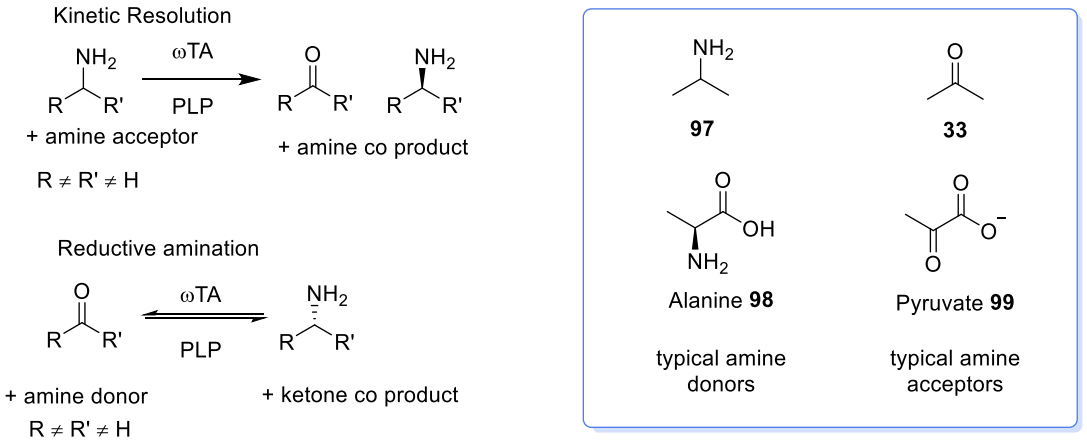

**PLP 96**




Transaminase substrates
Fold I, II, IV n = 0, X = O
Fold type III n ≥ 0, X = O or any other group
R' typically Me

*Figure 5.1 Structure of PLP, and "typical amine substrates"*

The two resulting transformations are reductive amination of a ketone to give a chiral amine, or the oxidative deamination of a racemic amine to give the ketone, and enantiopure amine (Scheme 5.). Synthetically, the reductive amination is more useful as the reaction can give up to 100% yield but is thermodynamically disfavoured and can require the use of a second or third enzyme, or excess of an amine donor (typically isopropylamine **97** or alanine **98**) to shift the equilibrium, in order to be synthetically useful.[4-7] Although the oxidative deamination is a kinetic resolution and is thus limited to a theoretical yield of 50%, the transformation is more thermodynamically favoured than the reductive amination. Potentially the limited yield could be increased by substrate recycling through racemisation of the unreacted starting material.




*Scheme 5.1 Reactions of transaminases and some typical amine donors 97 and 98 and acceptor 99*

### 5.1.1.1 Transaminase structure

The active site of transaminases contains a large pocket and a small pocket; generally wild-type transaminases are limited in their synthetic utility because of this, as the small pocket can usually only accommodate a methyl or pseudo methyl group, such as a -$CH_2$- within the framework of a ring structure (Figure 5.2). Techniques, such as directed evolution and (semi)-

rational design have been successfully used to increase the synthetic utility of wild type-transaminases, an excellent example of this is discussed in Chapter 1 (Sitagliptin).[8]

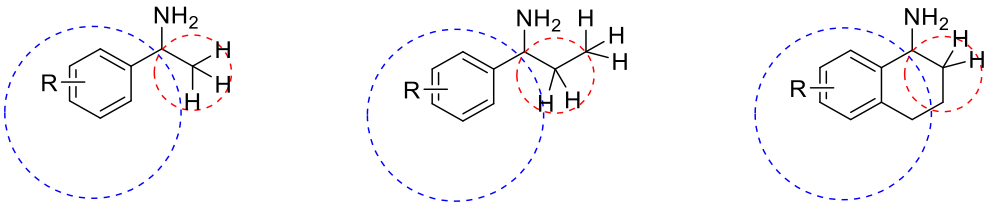
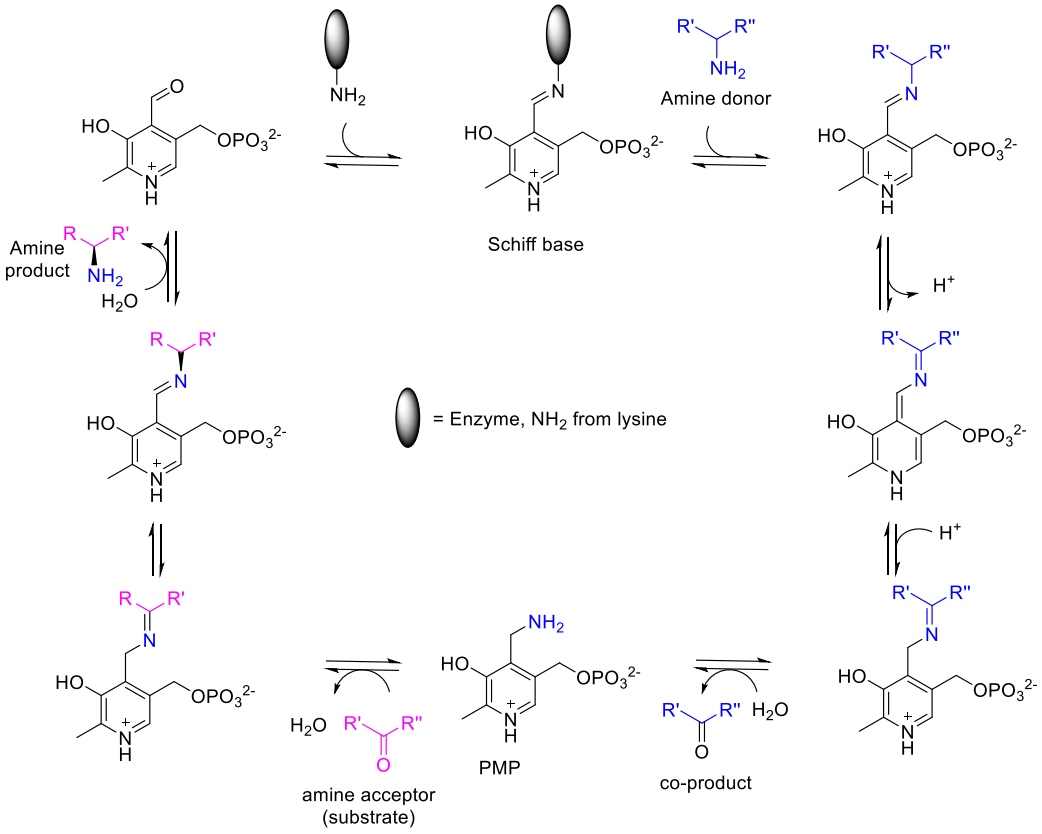


*Figure 5.2 Representation of the typical transaminase active site and substrates*

Transaminases use the cofactor PLP **96** (pyroxidal 5′-phosphate) which is the active form of vitamin B6. The initial step in the reaction is the covalent binding of **96** to a lysine residue in the transaminase active site forming a Schiff base (Scheme 5.2). The next step is the formation of a Schiff base by reaction of the amine donor with the cofactor. The donor is released from the cofactor as the ketone by-product, and the amine is left attached to the cofactor, now present as PMP (pyroxamine 5′-phosphate). The amino group is then transferred to the amine acceptor, a ketone or an aldehyde, via another Schiff base intermediate and the product amine is released from the cofactor.[9,10]




*Scheme 5.2 Mechanism of transaminase reactions, adapted from Ward et al.[10]*

## 5.1.2 Chiral amines as important synthons

Chiral amines, and functional groups derived from amines, are found in many pharmaceuticals and fine chemicals.[11] The stereogenic centres are often embedded within complex structures, and the API structures often contain at least one other stereocentre, examples include sertraline **95**, tametraline **100** and indatraline **101** (Figure 5.3). Often difficulty arises in the resolution of these additional stereocentres. One example of the use of transaminases in the resolution of pharmaceutical intermediates is the preparation of Levofloxacin **102** (Scheme 5.3).[12] Amines can also feature in ligands for asymmetric catalysis, such as bisoxazolines, which are of interest to our group, and the precursors can potentially be synthesised by transaminases; an example is shown in Scheme 5.4.[13]

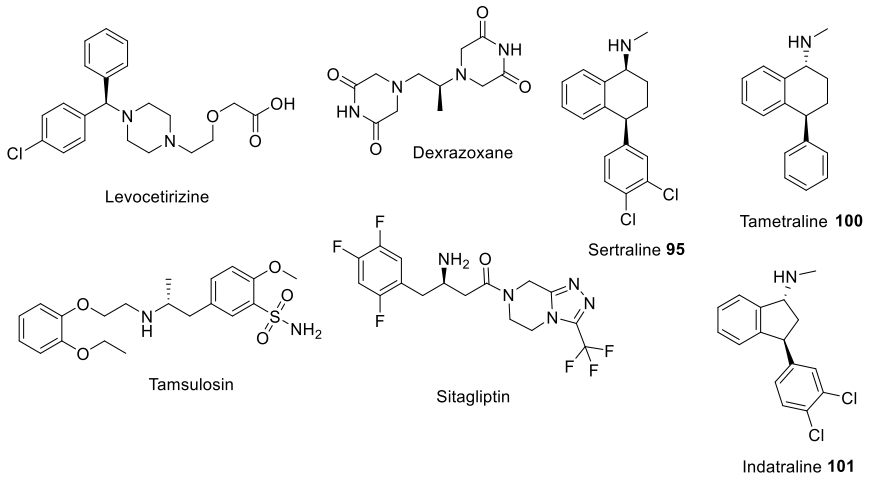
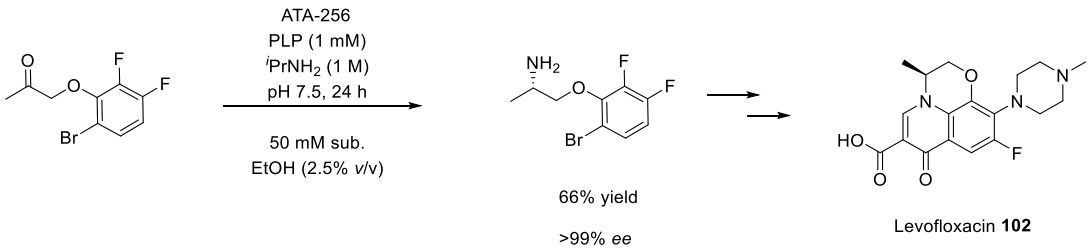
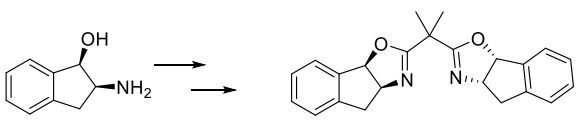



*Figure 5.3 Amine-containing pharmaceuticals*



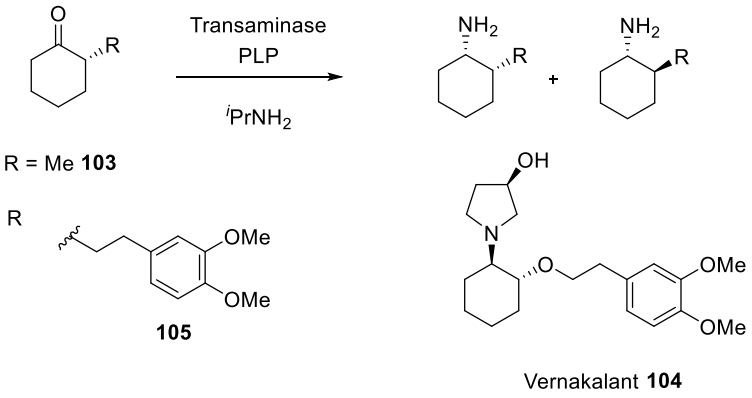

*Scheme 5.3 Use of transaminases in the enzymatic synthesis of a pharmaceutical intermediate*



*Scheme 5.4 Synthesis of bisoxazolines from enantiopure amino alcohols*

### 5.1.2.1 Remote stereoselectivity in transaminases

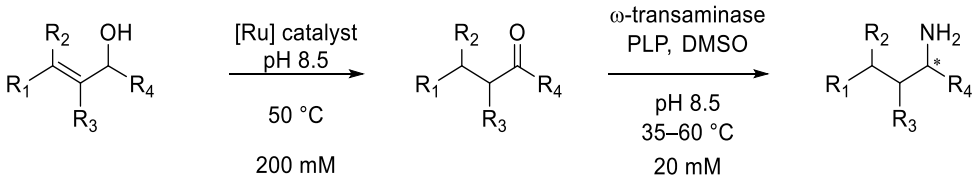
Transaminase-mediated resolution of stereocentres removed from the active site (i.e. the reacting carbonyl or amine) are uncommon and are limited to compounds with the stereocentre at the α-position. Hailes et al. studied cyclohexanes, including methyl cyclohexane **103**, and found that by varying the biocatalyst, diastereoselectivity of up to 96:4 was obtained (Scheme 5.5).[14] An intermediate in the synthesis of vernakalant **104** is cyclohexane **105**, containing an α-stereocentre which was resolved by transaminase-mediated oxidative deamination.[15]




*Scheme 5.5 Resolution of a stereocentre remote from the reactive site*

### 5.1.2.2 Transaminases in combination with other catalysts

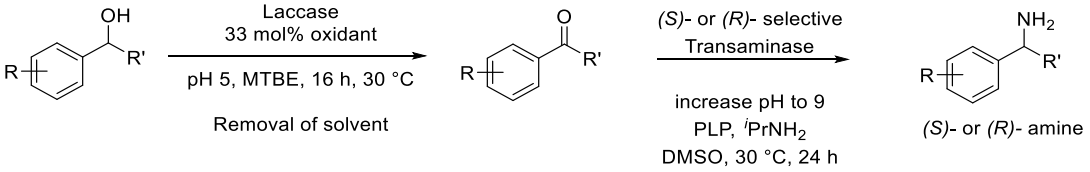
The one-pot ruthenium catalysed transformation of allylic alcohols to ketones, followed by asymmetric, ω-transaminase catalysed amination (Scheme 5.6) has also been reported.[16] The ruthenium-catalysed isomerisation was carried out first, followed by dilution of the reaction mixture and introduction of the biocatalyst, inactivating the metal catalyst by dilution of the reaction mixture, to a concentration to suit the biocatalytic reaction.




*Scheme 5.6 One pot stereoselective synthesis of chiral amines*

Recently, a one-pot, two-step process has been reported combining a transaminase with an oxidoreductase (Scheme 5.7).[17] The initial reaction non-selectively oxidises an alcohol to the corresponding ketone, and the ketone is stereoselectively reduced to an enantiopure amine.

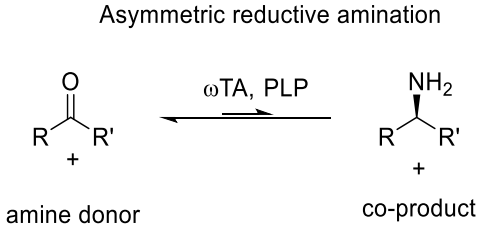
This is an example of a multi-enzyme cascade, similar to the reaction above, albeit using an enzymatic oxidant. Both halves of the reaction were optimised separately and then combined to effect the transformation. The enzymes work at different pH values, and once the initial oxidation is complete, the addition of buffer can change the pH. This deactivates the first enzyme, obviating the need to remove it before proceeding.




*Scheme 5.7 Combination of oxidase and transaminase*

## 5.1.3 Reductive amination

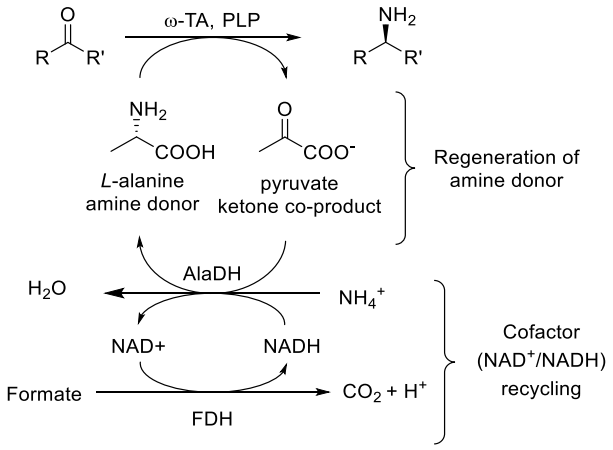
The reductive amination reaction (Scheme 5.8) is a more useful reaction than the oxidative deamination (kinetic resolution) as the reaction can theoretically yield 100% of the target compound. The stereoselective reduction should ideally give the target compound with complete selectivity for one enantiomer, however, in practice, this may not be the case. The reductive amination is less favoured than the oxidative deamination, and thus may require the addition of a second or third enzyme, or reaction engineering, in order to be synthetically useful.



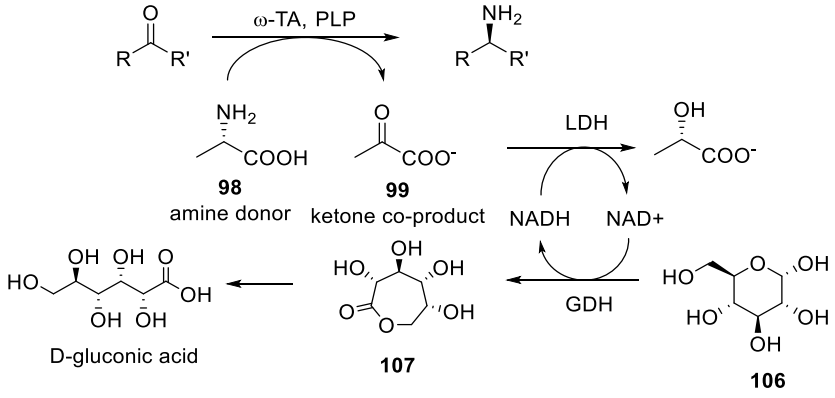

*Scheme 5.8 Asymmetric reductive amination*

There are several reported approaches to improving the conversion of the ketone to the amines.[18,19] The major challenge in this reaction is that the conversion of the ketone to the amine is thermodynamically disfavoured, meaning, like many enzymatic reactions, that the reaction is reversible. For this reason, auxiliary enzyme systems can be used, to pull the reaction forward, by acting as a thermodynamic sink; either by regenerating the amine donor or by removing the co-product. To regenerate the amine donor (alanine **98**) alanine dehydrogenase is used, converting the pyruvate by-product **99** back to **98** (Scheme 5.9).

Alanine dehydrogenase (AlaDH) requires NADH as a cofactor; to avoid the use of a large excess of the cofactor, the reduction of pyruvate is coupled with the oxidation of ammonium formate using formate dehydrogenase (FDH) which uses the reduced $NAD^+$, regenerating NADH. Similarly, the second approach removes the co-product **99** by reduction using lactate dehydrogenase (LDH) (Scheme 5.10). This reduction also uses NADH as cofactor, but in this case the redox partner is glucose dehydrogenase (GDH), which oxidises β-D-glucose **106** to D-gluconolactone **107**.




*Scheme 5.9 Regeneration of amine donor using AlaDH*

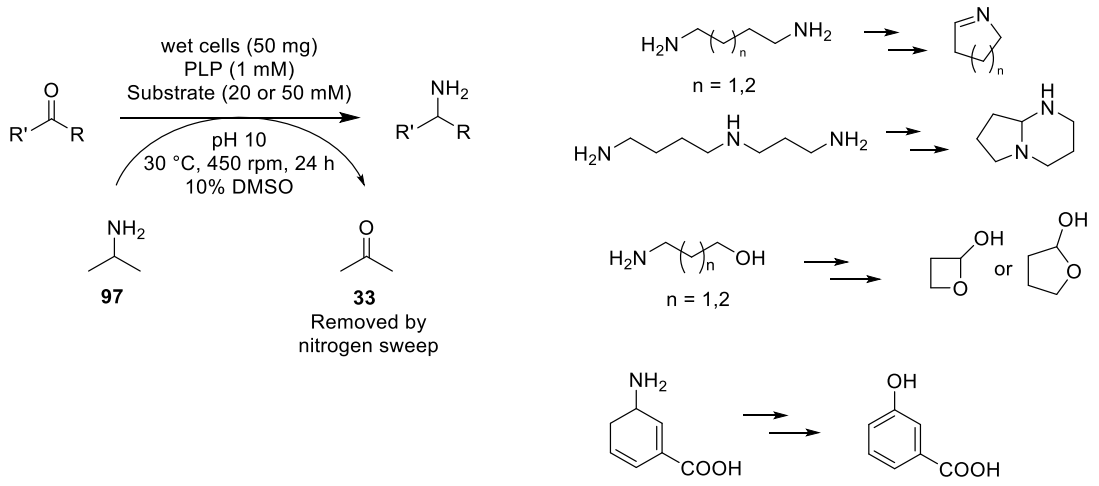



*Scheme 5.10 Removal of by-product by reduction*

In both cases, an NADH dependant enzyme uses the pyruvate by-product **99** as a substrate; these enzymes are coupled with $NAD^+$ dependant enzymes/reactions to regenerate the expensive NADH cofactor, to avoid using a large excess of the cofactor. In both cases the auxiliary enzyme systems produce water soluble by-products.[19]

Another approach is the use of amine donors which give insoluble, volatile or very stable products.[20,21] The most common example of this is the use of large excesses of isopropyl amine

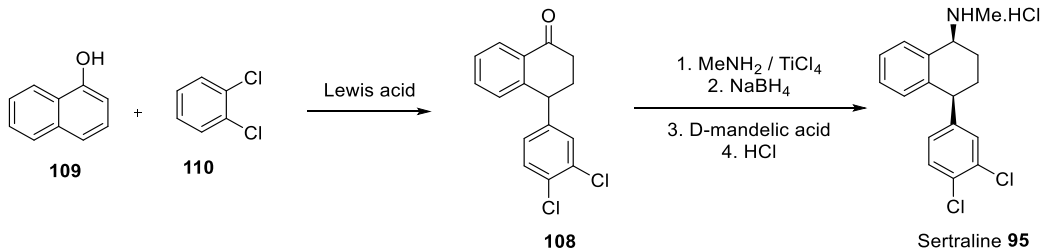
97; industrially the by-product, acetone, is continuously removed by nitrogen sweep (Scheme 5.11).[22] On a screening scale this can be difficult to carry out. The use of amine donors which polymerise after reaction or which further react with each other can be an alternative approach (Scheme 5.11).[20,21,23-25] A similar approach has been used for alcohol dehydrogenases, where diols are used as the cosubstrate in the reduction of ketones to chiral alcohols, giving hydroxy aldehyde coproducts, which cyclise to give lactols.




*Scheme 5.11 The use of alternative amine donors*

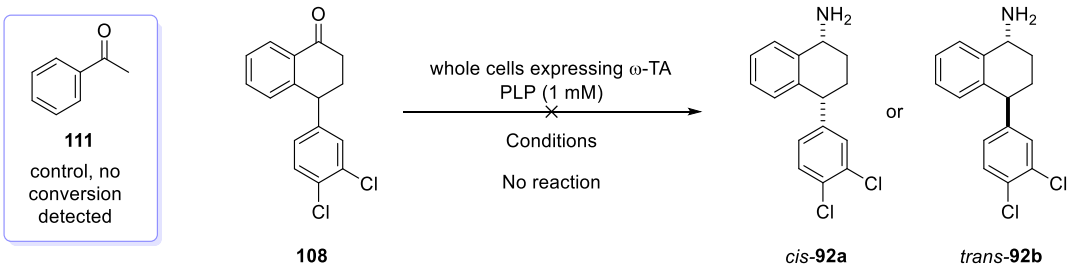
### 5.1.3.1 API intermediates: targets

Sertraline is a compound of interest within our group. The key intermediate in the synthesis of Sertraline **95**, tetralone **108**, can be synthesised by reaction of 1-naphthol **109** and 1,2-dichlorobenzene **110** in the presence of a strong Lewis acid (Scheme 5.12).[26] Sertraline **95** is resolved by diastereomeric salt formation with D-mandelic acid, followed by conversion to the HCl salt. Resolution by diastereomeric salt formation is disadvantageous as it inherently means that half the material is wasted; recycling of the undesired enantiomer can reduce the waste in this method. More attractive, however, is the asymmetric reduction of the intermediate tetralone **108** either as the enantiopure ketone or with remote stereoselectivity.




*Scheme 5.12 Synthesis of sertraline **95***

During the course of his work, Gavin attempted the reductive amination of tetralone **108** using both TA3 and CV-TA (Scheme 5.13). All attempts to reduce the tetralone **108** using transaminases were unsuccessful. The conditions used are similar to those shown in Scheme 5.9, Scheme 5.10, and Scheme 5.11. A control reaction, using acetophenone **111** as the substrate was also carried out, but this reaction furnished no product, warranting further investigation into the reductive amination, as the reduction of acetophenone should work under these conditions.

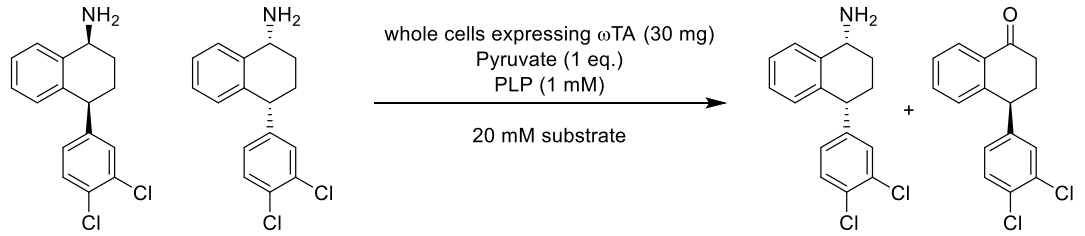



*Scheme 5.13 Attempts at reductive amination*

The amines *cis*-**92a** and *trans*-**92b** were subjected to the oxidative deamination step, showing up to 33% conversion, and up to 92% *ee* of the ketone. The initial trials were carried out without any lysis of the cells. The results show excellent selectivity and the use of sonication improved the conversion of the transaminases (Table 5.1, Table 5.2). A lysis buffer was also used to lyse the cells, but sonication was shown to be the most practical and efficient method of cell lysis.

The enantioselectivity using CV-TA was excellent, although, the diastereoselectivity was poor. However, the novel transaminase, TA3, showed excellent diastereoselectivity, furnishing product only when the *cis*-**92a** substrate was used. This is the first example, to our knowledge, of the transaminase-catalysed resolution of an amine containing a second stereocentre which is not adjacent to the reacting site.

*Table 5.1 The use of sonication for cell lysis, experiments carried out by Gavin.*

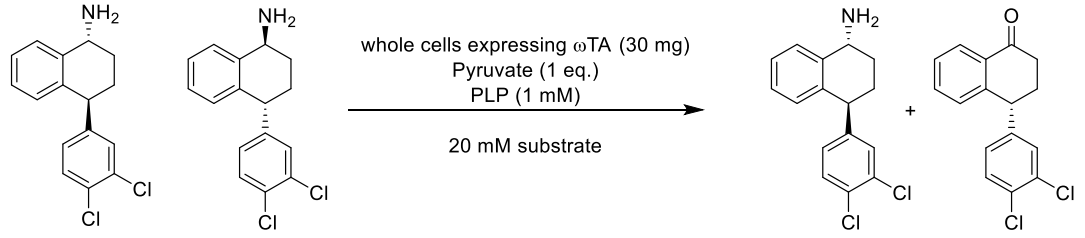



cis-**92a** *(1R,4R)*-**92a** *(4S)*-**108**

| Entry | TAm | Lysis | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|---|
| | | | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | CV-TA | - | 33 | 44 | 72 | 92 | 52 |
| 2 | CV-TA | Sonication[b] | 47 | 44 | 77 | 92 | 53 |
| 3 | TA3 | - | 22 | 25 | 29 | 88 | 21 |
| 4 | TA3 | Sonication[b] | 51 | 50 | 77 | 92 | 53 |

Carried out by Gavin. [a]Enantiomeric excess determined by chiral HPLC analysis, conditions are given in Appendix I; [b]sonication (with probe): in sodium phosphate buffer, pH 8.6, sonicate for 10s, put in ice for 30s (x5).

*Table 5.2 Different lysis methods, experiments carried out by Gavin*

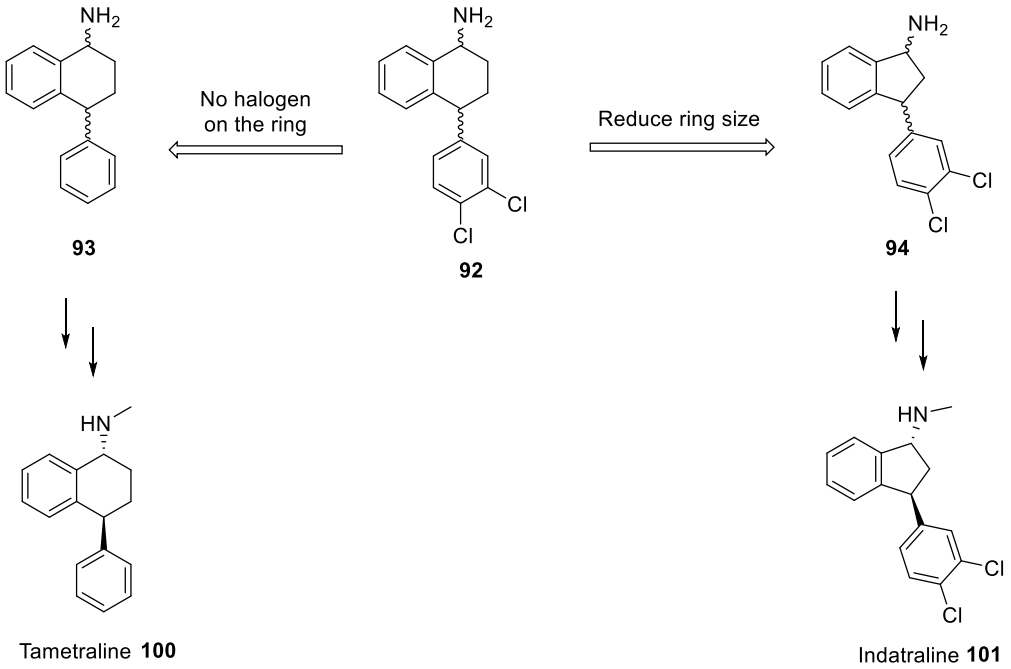



trans-**92b** *(1S,4S)*-**92b** *(4R)*-**108**

| Entry | TAm | Lysis | Conversion (%) | | *ee*[a] (%) | | E |
|---|---|---|---|---|---|---|---|
| | | | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | CV-TA | Sonication[b] | 33 | 31 | 42 | 93 (4R) | 43 |
| 2 | CV-TA | Lysis buffer[c] | 25 | 30 | 40 | 92 (4R) | 36 |
| 3 | TA3 | Sonication[b] | 0 | - | - | - | - |
| 4 | TA3 | Lysis buffer[c] | 0 | - | - | - | - |

Carried out by Gavin. [a]Enantiomeric excess determined by chiral HPLC analysis conditions are given in Appendix I; [b]sonication (with probe): in sodium phosphate buffer, pH 8.6, sonicate for 10s, put in ice for 30s (x5) [c]Lysis buffer: Tris HCl pH 8 with detergents.

As a result of this work, two related amines **93** and **94**, intermediates in the synthesis of tametraline **100** and indatraline **101**, were chosen as additional substrates, for synthesis and screening during this work; Gavin's conditions were used for the screening reactions. These amines are similar in structure to the sertraline intermediate **92**, being the des-chloro-analogue **93**, and the indane analogue **94**, respectively (Scheme 5.14)




*Scheme 5.14 Related substrates*

## 5.2 Objectives

The specific objectives of this project are:

- Design and compile a range of carefully selected amines, both commercially available and synthesised, to enable investigation of substrate scope of the novel transaminases.
- Screen this collection of substrates against two novel transaminases (TA3 & AD2).
- Compare the results to those obtained using a control transaminase, which has previously shown good activity (CV-TA).
- Prepare API intermediates, for screening against these transaminases – investigate the effect of structural changes on the substrates on the outcome of the biotransformation reactions.
- Demonstrate asymmetric synthesis through reductive amination mediated by transaminases on selected target ketone substrates, specifically investigating the effect of both conjugated and non-conjugated ketones and varying the amine source, to assess the effect on the reaction

## 5.3 Synthesis of substrates

The substrates selected for investigation are shown in Figure 5.4. The first substrates tested were methylbenzylamine *(±)*-**112**, *(S)*-methylbenzylamine *(S)*-**112** and *(R)*-methylbenzylamine *(R)*-**112** which were designed to check selectivity of the novel transaminases using a typical transaminase substrate, as well as to confirm that the transaminases are *(S)*-selective. TA3 can accommodate larger substrates than wild type transaminases, as shown by Gavin in his work on sertraline intermediates *cis*-**92a** and *trans*-**92b**; however, these substrates had larger groups which were "tied back" i.e. $CH_2$ in a ring. The next group was designed to assess the tolerance of the transaminases towards larger substituents; substrates **113**–**118** have previously been used to test the selectivity of engineered enzymes with larger active sites.[27-29] A phenyl-aryl compound **118** would be an interesting compound if it were resolved, as the phenyl group and the *p*-chloro phenyl groups of **118** would be very difficult to differentiate chemically, and this moiety is present in Levocetirizine **126** (Figure 5.5). The next group tested had the methyl group fixed and varied the large substituent **119**–**122**, looking at alkyl groups, benzyl groups, and groups with substituted phenyl rings, to determine the effect on resolution. The last group of amines studies were the cyclic aminotetralins and aminoindanes **123**–**125** enabling investigation of the effect of the conformational constraint on the cyclic system relative to the freely rotating alkyl substituents (e.g. **113**); altering the ring size from five to six carbons potentially leads to insight into the nature of the active site pocket. Investigating the 1- and 2-aminotetralins and the 1- and 2-aminoindanes provides insight not only into the impact of the position of the amino substituent, but also the impact of changing from a benzylic amine to a simple alkyl amine. These compounds are common pharmacophores found in pharmaceutically active compounds such as norsertraline (*1S,2S*)-**92a**, sertraline **95**, tametraline **100**, indatraline **101**, rasagiline **127**, ladostigil **128**, irindalone **129**,[30] and related derivatives (Figure 5.5).[31]

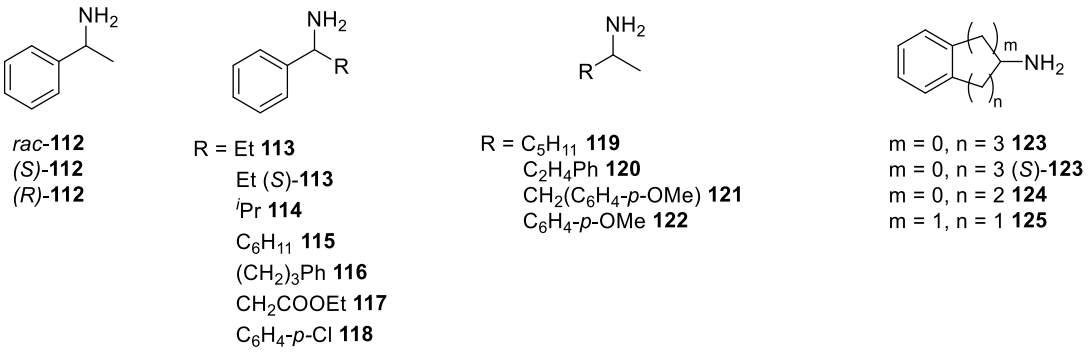



*Figure 5.4 Substrates selected*

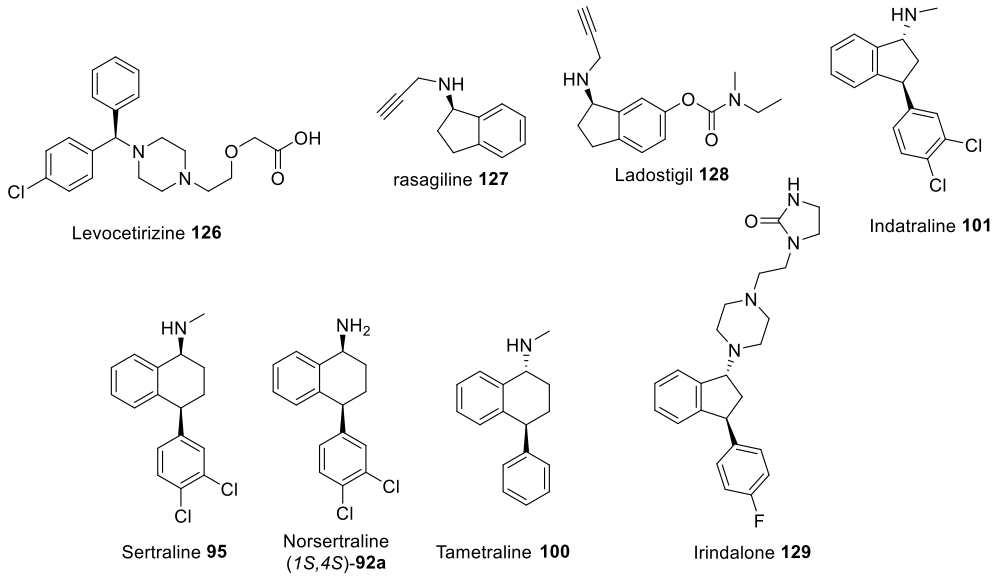
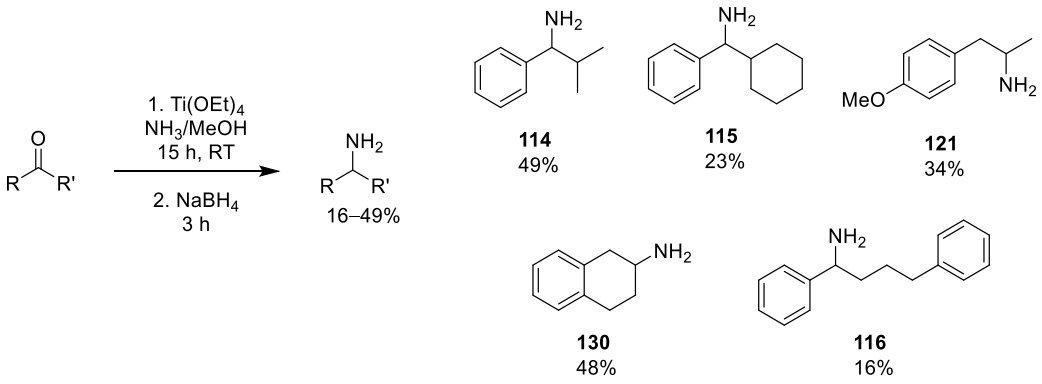



*Figure 5.5 APIs containing chiral amines*

## 5.3.1 Synthesis of model substrates

There are many reported methods for the reductive amination of ketones to the corresponding amines, using various amines sources and varying the reductant.[32,33] In this case, the substrate ketones were stirred overnight under nitrogen with methanolic ammonia and titanium ethoxide, followed by addition of sodium borohydride as reductant, and stirring for 3 hours.[34] Amines were produced in low to moderate yields, but in excellent purity and no purification was required (Scheme 5.15). The lowest yielding substrates were **115** and **116**. It is assumed that the larger molecular weight, and the non-polar groups, made the hydrochloride salts of these compounds more lipophilic, meaning that the acid-base wash used to separate the amines from the neutral side products would have been less effective. While this reaction could been optimised, in practice, sufficient material was obtained for the substrate study so no further effort was invested to optimise the yields.




*Scheme 5.15 Reductive amination*

It was also postulated that the reversible formation of the intermediate imine was responsible for the low yields, and that the addition of sodium borohydride was reducing unreacted starting material (ketone), resulting in the formation of the alcohol (Scheme 5.16). Acid-base work-up separated the amine from neutral components; this work-up would also separate any ketone, or potential alcohol by-product from the reaction mixture leaving a pure amine. During the synthesis of 2-aminotetralin **130**, the organic layer containing the neutral by-products was evaporated and analysed by $^{1}$H NMR; the spectrum is shown in Figure 5.6. Ketone **131** was present in the organic layer (green boxes), indicating that the reaction did not go to completion. Amine **130** was not present in the organic layer, as indicated by the absence of the multiplet (ttt) signal at 1.50–1.60 ppm (red box). Similarly the absence of the multiplets at 1.70–1.90 ppm and 4.00–4.30 ppm indicated that alcohol **132** was not present. This showed that sodium borohydride reduction of the ketone was not responsible for the low yield. The absence of alcohol **132** indicated that it is probably the incomplete reduction of the imine which is responsible for the low yield, and the imine is hydrolysed to the ketone during work-up.

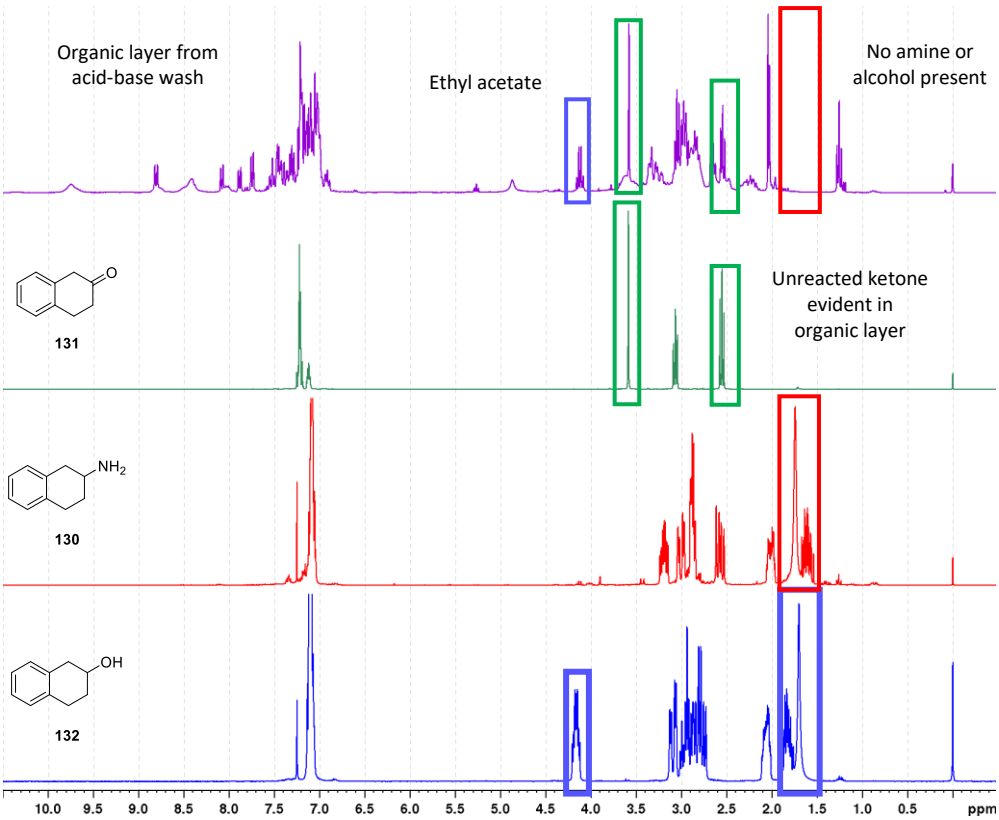
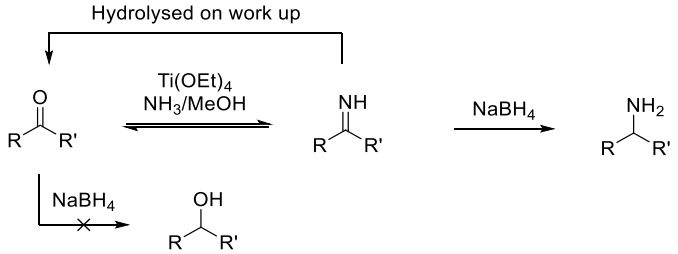
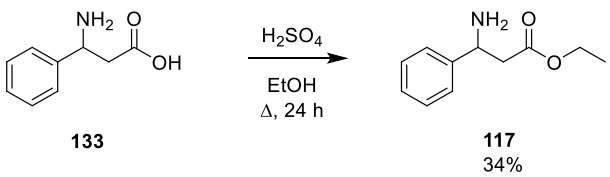



*Figure 5.6 Comparison of organic layer and potential (by)-products*




*Scheme 5.16 Alcohol by-product formation*

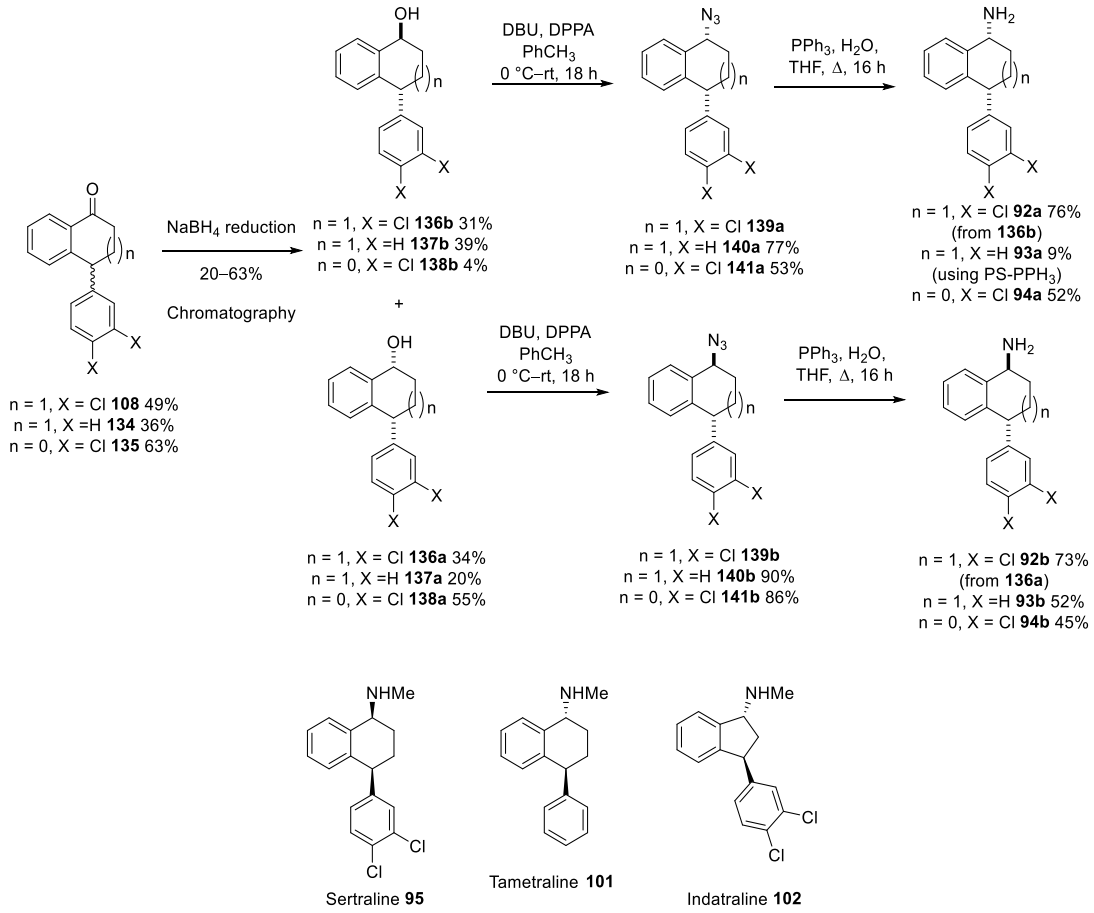
Compounds **114**–**116**, **118**, **121** and **130** were synthesised by the method above. All other amines, except amino acid ester **117**, were commercially sourced. Amino acid ester **117** was prepared by Fisher esterification of the commercially available β-amino acid **133** (Scheme 5.17). All compounds were known compounds except **116**, which was fully characterised during this work.[35-39]




*Scheme 5.17 Fisher esterification*

## 5.3.2 Synthesis of substrates related to API intermediates

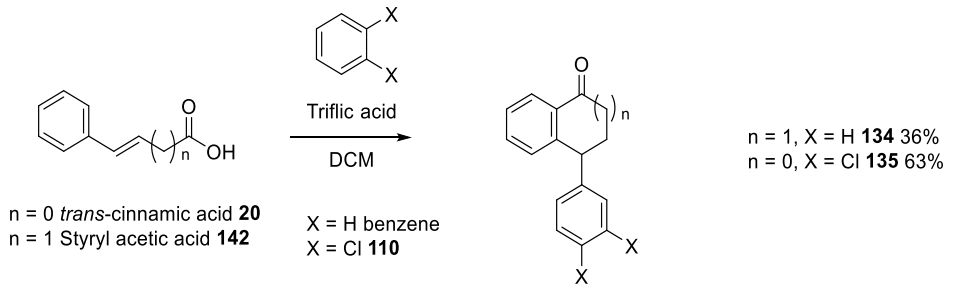
Sertraline intermediates **92a** and **92b** were previously synthesised by Gavin, and the route is outlined in Scheme 5.18; the ketone **108** was synthesised by the method shown in Scheme 5.12, using aluminium trichloride as the Lewis acid. A similar pathway was used in this work in the synthesis of tametraline amines *cis*-**93a** and *trans*-**93b** and indatraline amines *cis*-**94a** and *trans*-**94b**.




*Scheme 5.18 Synthesis of pharmaceutical intermediates*

### 5.3.2.1 Ketone synthesis

Following similar literature procedures, ketones **134** and **135** were synthesised from commercially available starting materials, styryl acetic acid **142** and cinnamic acid **20** by reaction with the appropriate arenes in the presence of a strong Lewis acid, in this case triflic acid.[40-42]




*Scheme 5.19 General scheme for the synthesis of ketones*

The tametraline ketone **134** was purified by column chromatography. The reaction was relatively low yielding, with multiple products evident by TLC, but was easily synthesised in sufficient amounts for this investigation. The product ketone **134** is reported as a solid so recrystallisation was attempted using DCM/hexane but was unsuccessful. Column chromatography was more effective, albeit resulting in a low yield (36%).[40]

The synthesis of the indatraline ketone **135** required more forcing conditions, i.e. longer reaction time, increased temperature and higher loading of arene **110**. The acid was removed by aqueous work-up, dichloromethane was removed by rotary evaporation and the dichlorobenzene by vacuum distillation. After removing the solvent, the remaining solid was easily recrystallised using DCM/hexane to give the ketone **135** in good yield (63%) without the need for chromatography.

### 5.3.2.2 Ketone reduction

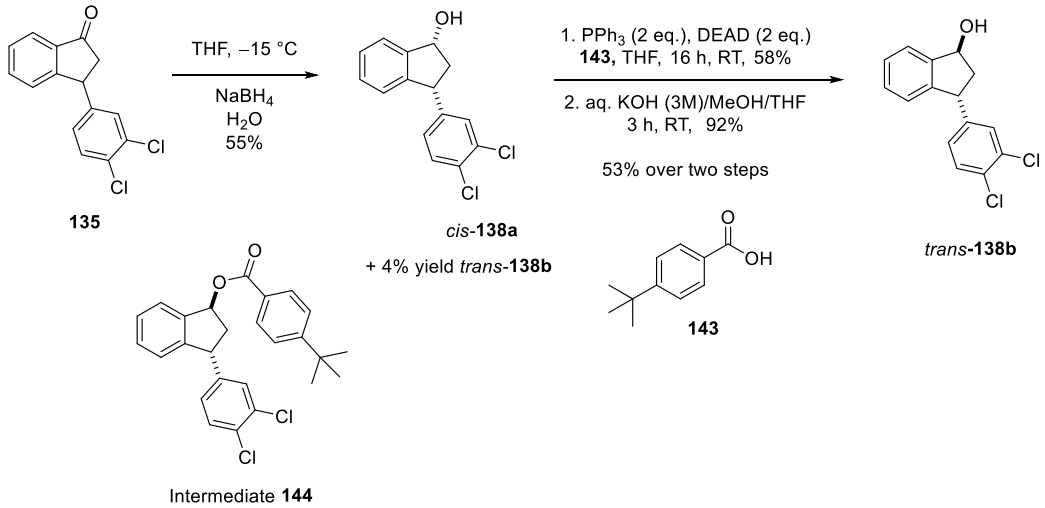
Ketones **134** and **135** were reduced according to literature procedures using sodium borohydride in methanol, or a mixture of THF and water as solvent (**134** and **135** respectively).[43-45] The reaction mixtures were stirred overnight in both cases to ensure complete conversion of ketones to alcohols.

Initially tametraline alcohols *cis*-**137a** and *trans*-**137b** (46:54) were subjected to column chromatography using ethyl acetate as the polar component of the eluent system, however, the diastereomers did not separate using this solvent. The fractions collected from the column were divided into groups of approximately ten test tubes and concentrated; different diastereomeric ratios were present in each mixture. Recrystallisation of the fractions using diethyl ether/hexane gave exclusively the *trans*-**137b** isomer, even when an almost 50/50 mixture was used. The material which was present in the mother liquor (24/76 trans/cis) was subjected to further column chromatography; the use of diethyl ether as the polar component

of the eluent gave more effective separation than using ethyl acetate. The less polar isomer, which was isolated as a viscous oil by column chromatography was identified as *cis*-**137a**, as this closely aligned with the literature data.[46] The *trans*-isomer *trans*-**137b** was isolated by recrystallisation; it was previously only reported in a mixture of diastereomers.[46,47]

The reduction of indanone **135**, was carried out in a mixture of THF and water, resulted in a mixture of diastereomers (91:9, *cis*-**138a**:*trans*-**138b**) which were purified by recrystallisation using conditions reported in the literature.[43-45] An unknown impurity was present in the recovered material, therefore the mother liquor and the isolated material were recombined and subjected to column chromatography to give pure samples of both *cis*-**138a** and *trans*-**138b**.

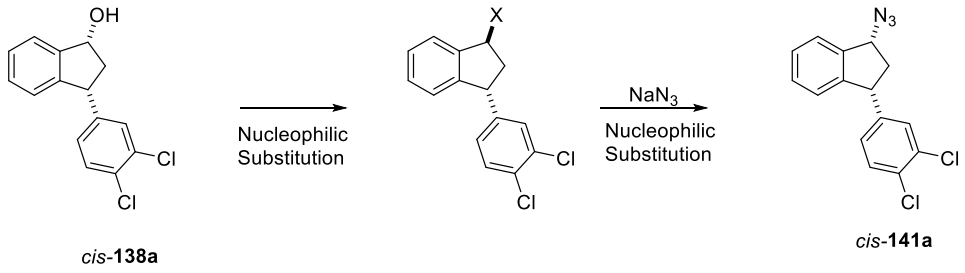
Although the minor product *trans*-**138b** was successfully isolated from the mixture, there was insufficient material for the investigation. An alternative route was identified, using the major diastereomer *cis*-**138a** (Scheme 5.20). A report by Madras et al. subsequently used a Mitsunobu reaction with benzoic acid to invert the stereochemistry at the alcohol position followed by hydrolysis of the product ester to furnish the alcohol **138b** with the required relative stereochemistry.[48] 4-*tert*-Butyl-benzoic acid **143** was used in place of benzoic acid as it was available to us. The reaction by-products were removed by column chromatography using 5% ethyl acetate in hexane, however, this resulted in the product present as a mixture of benzoic acid **143** and ester **144**.




*Scheme 5.20 Reduction of **135** and synthesis of alcohols* cis-***138a*** *&* trans-***139b***

Although it was assumed that the benzoic acid **143** would be removed in the next step by the KOH, it was desirable to remove it first, to reduce the amount of base needed, as well as to acquire an analytically pure sample of the novel ester **144** for characterisation. Several approaches were taken to remove the by-product. A hot extraction, using toluene as organic solvent, was unsuccessful, removing none of the benzoic acid. Neither stirring with saturated sodium bicarbonate nor washing with dilute sodium hydroxide solution (0.2M) removed benzoic acid **143**. The use of basic alumina was more successful, with the benzoic acid **143** retained strongly by the alumina, allowing facile recovery of the ester after washing with ethyl acetate/hexane (20/80). The acid **143** did not elute from the alumina even when the residue was washed with ethyl acetate. Because of the various work-ups, the product **144** was obtained in moderate yield (58%), which was lower than that reported for the benzoate ester (73%).[48]

The ester was hydrolysed using KOH in methanol/THF, and after work-up, the benzoic acid **143** by-product was evident in the crude $^{1}$H NMR spectrum. The benzoic acid was removed by resuspending the mixture in aqueous potassium hydroxide and stirring for 3 hours. The alcohol *trans*-**138b** remained suspended in the aqueous medium, however, the particles were too fine to be isolated by filtration. The product *trans*-**138b** was recovered by extraction into ethyl acetate in high yield (92%). Another possibility for the inversion of the stereochemistry, which would be more atom economical, is the double inversion of the stereochemistry to directly access the azide *cis*-**141a** (Scheme 5.21).




*cis*-**138a** *cis*-**141a**

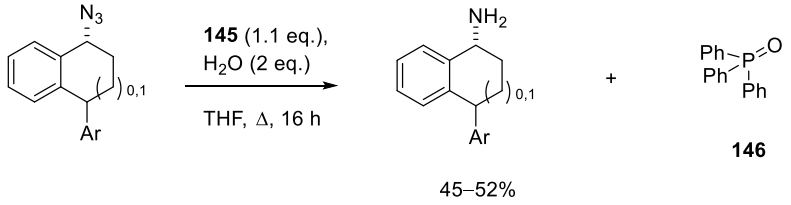
*Scheme 5.21 Potential alternative route to azide* cis-***141a***

### 5.3.2.3 Synthesis of azides—inversion of stereochemistry

At this stage synthetically useful amounts of alcohols **137** and **138** were in hand and fully characterised. A suitable literature procedure was identified, which describes the transformation of benzylic alcohols to the corresponding amines via a Mitsunobu-type reaction, inverting the stereochemistry at the benzylic position, followed by a Staudinger

reduction (Scheme 5.18).[49] The transformations of the alcohols to the azides were carried out at room temperature; the literature procedure describes reduction of azide without purification.[49] However, as it was desirable to avoid column chromatography on the amines, and the azides used in this investigation were stable to column chromatography, they were purified before reduction. On one occasion, during the synthesis of *cis* indatraline azide *cis*-**141a**, excess diphenylphosphoryl azide (DPPA) was added in error to the reaction mixture, resulting in co-elution of the DPPA with the azide *cis*-**141a** when using the usual eluent for these transformations, hexane/ethyl acetate (95/5). The presence of a phosphorus containing species was confirmed by $^{31}$P NMR spectroscopy, giving a signal at −10.4 ppm, which matches reported data for DPPA.[50] Replacing ethyl acetate with diethyl ether facilitated purification of the product without coelution of the reagent.

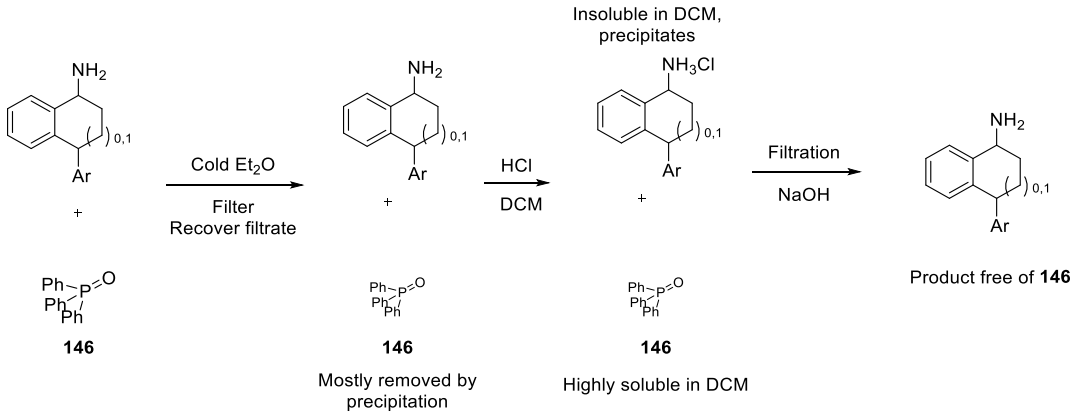
### 5.3.2.4 Staudinger reduction




*Scheme 5.22 Staudinger reduction and associated by-product **146***

A Staudinger reduction, using triphenylphosphine **145**, effectively reduced the azides to the amines with retention of stereochemistry (Scheme 5.22).[49] A challenge with this approach is the generation of stoichiometric triphenylphosphine oxide **146** as a by-product. Column chromatography to remove the by-product is undesirable as this usually results in lower yields of the amines. There are several reagents which have been used to precipitate **146** but they are generally Lewis acid-type reagents, which are incompatible with amines.[51,52] The use of scavenger resins such as the Merrifield resin, polymer-bound triphenylphosphine or the use of alternative phosphine based reagents have also been reported to overcome the need to remove **146**.[53-56] In this work, a method developed by Gavin was used to purify the product. The by-product **146** was removed by first precipitating the majority of it from cold diethyl ether, and removing it by filtration, followed by converting the amines to the HCl salts, which are insoluble in dichloromethane. The salts were then isolated by filtration and washed with dichloromethane, in which the **146** is soluble; a porosity-4 sintered glass funnel was used as the precipitate was very fine (Scheme 5.23). The hydrochloride salts were converted to the free amines for ease of analysis as the salts are not soluble in deuterated chloroform. The

absence of the triphenylphosphine oxide was confirmed by $^{31}$P NMR spectroscopy, as in low concentrations it is hard to distinguish the $^{1}$H NMR peaks from the aromatic peaks of the amine product. Since this work was carried out, the use of catalytic triphenylphosphine **145** in conjunction with poly(hydroxymethylsilane) as a green reducing agent has been reported.[57]




*Scheme 5.23 Removal of by-product **146** by precipitation and salt formation*

The use of polymer bound triphenylphosphine reportedly gives a high yield without the need for removal of the triphenylphosphine oxide **146** by-product.[54] However, when the polymer bound reagent was used, extended reaction time was required. The reaction was carried out using azide *cis*-**140a** and after 24 h azide *cis*-**140a** was still present by $^{1}$H NMR. After 5 days the reaction mixture was heated to reflux for 2 days, as there was still azide evident. After 7 days total reaction time the reaction was worked up. The azide *cis*-**140a** and the amine *cis*-**93a** were separated by acid-base work-up. As a result of the extended reaction time, the overall yield was poor (9%), and material recovered from the acid-base wash suggested decomposition products as well as the azide *cis*-**140a**.

The reduction of *trans*-**141b** was attempted using a $CeCl_3$/NaI mediated reduction, a recently-described method for the reduction of azides under neutral conditions.[58,59] After refluxing in acetonitrile for 24 hours, the reaction mixture was mainly comprised of unreacted azide; however, there was some amine *trans*-**94b** present, but only in very small amounts (30%, not pure). The presence of the amine indicates that this approach may be useful for a phosphine free reduction, but optimisation would be required.

Ultimately each of the amines were obtained diastereomerically pure, in useful amounts. The stereochemistry of each product was assigned on the basis of the expected inversion of stereochemistry; no evidence of stereochemical scrambling was observed. The amines were

stored in the freezer in nitrogen flushed vessels and there was no evidence of impurity formation in the course of this work. The azides **140a** and **140b** and amines **93a**–**94b** are novel and have been fully characterised.

The *cis*- and *trans*- compounds had characteristic patterns in the $^{1}$H NMR spectra (indatraline series shown in Figure 5.7), which were consistent for the tametraline and indatraline intermediates. The most shielded proton is each case was the C(1)H, which became more shielded as the substituent on C(1) changed from hydroxy to azide to amino. For the *cis*- compounds, the difference in the methylene [C(2)$H_2$] protons is more pronounced, as one is much more shielded than the other, due to the electron rich ring and heteroatom substituents being on the same side of the indane ring.

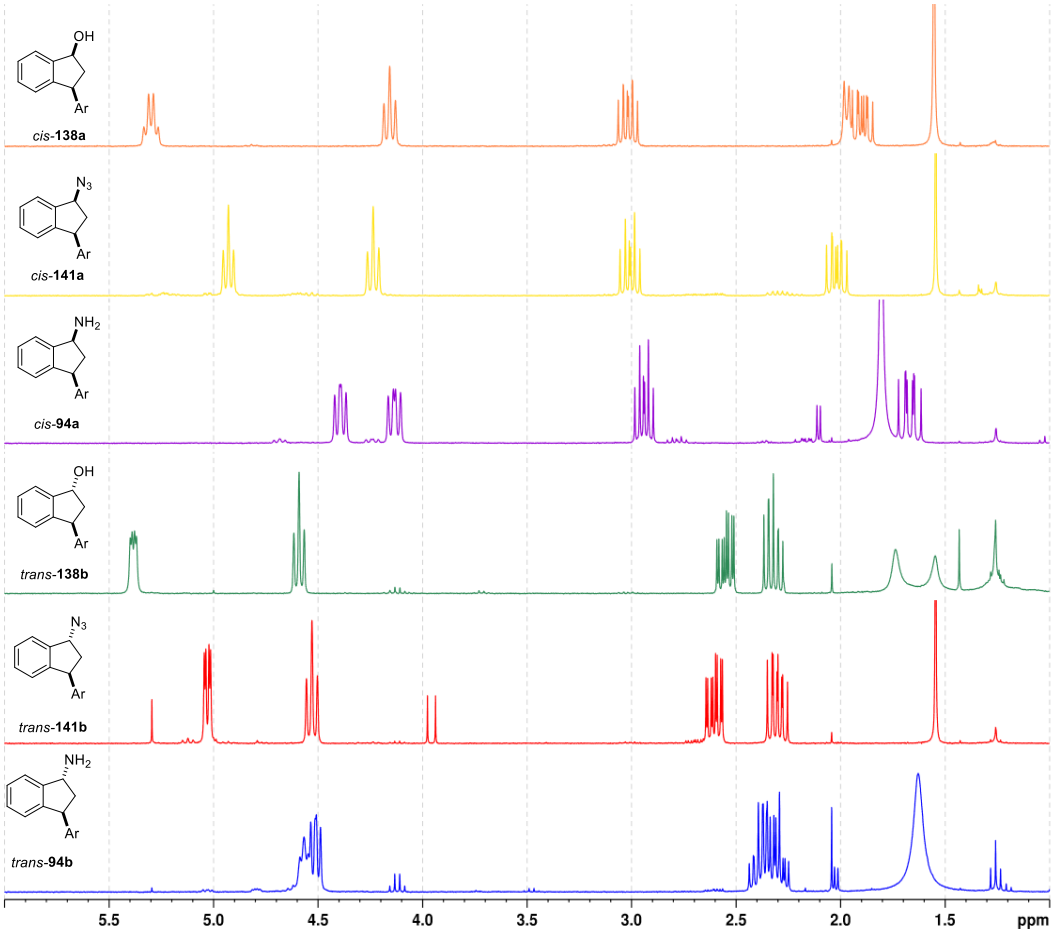



*Figure 5.7 $^{1}$H NMR spectra for indatraline-based molecules showing the range 6.0–1.0 ppm ($CDCl_3$, 300 MHz)*

## 5.4 Transaminase-mediated reactions

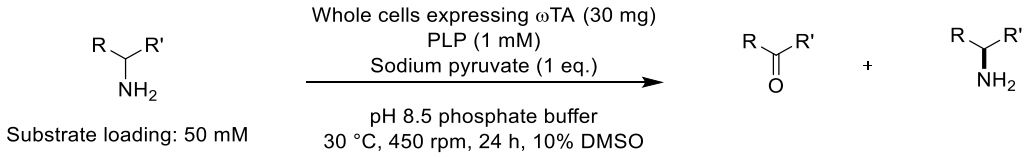
### 5.4.1 Analysis of reactions

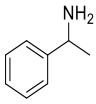
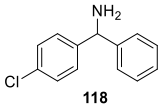
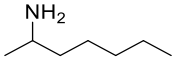
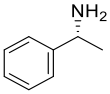
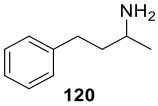
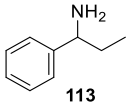
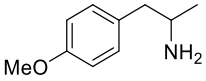
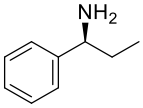
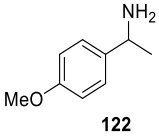
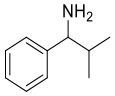
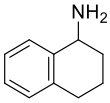
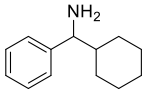
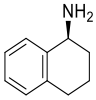
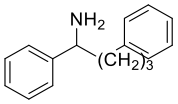
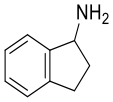
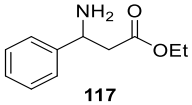
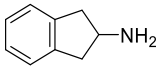
The screening reactions were carried out on a 1 mL scale for the compounds chosen, using 30 mg of whole cells, which had been stored at −80 °C and a 50 mM substrate loading, for the model substrates, this corresponded to approx. 10 mg of substrate. For the pharmaceutical intermediates **92a**–**94b** the loading was 20 mM, which was approx. 5 mg of compound. Reactions were carried out in sodium phosphate buffer, at pH 8.5, and incubated at 30 °C for 24 hours (conditions given in the Experimental section, and in the relevant tables). Due to the wide variety of amines chosen for screening, the substrates were initially subjected to the oxidative deamination as it is easier to undertake. Conversions were estimated by $^{1}$H NMR spectroscopy of the crude reaction mixtures. Efficient chiral phase HPLC analytical conditions were established for substrates which gave positive results in the initial screen (shown in Appendix I). Typically, enantiomeric excess values were measured at two wavelengths (usually approx. 230 nm and 275 nm) and if the values for enantioselectivity did not agree, then this is indicative of the presence of a coeluting impurity. In these cases a different column or different conditions were used to determine the enantiopurity. The enantiopurities of compounds **112** (MBA) and **113** (EBA) were not measured. The enantiopurity of **120** (Entry 12) was determined by derivatisation of the amine.

### 5.4.2 Oxidative deamination (kinetic resolution)

Initial screens were carried out on a range of substrates to assess the activity of the two novel transaminases (TA3 and AD2), and a known transaminase from *Chromobacterium violaceum* (CV-TA) (Table 5.3). All three transaminases were active against (±)-methylbenzylamine **112** and only showed minimal conversion of the *(R)*-enantiomer (<1%), despite giving conversion >50% for the racemic mixture using CV-TA (Entries 1–3), confirming that all three transaminases are *(S)*-selective. For this reason, it is assumed that across the series, the unreacted amines are the *(R)*-enantiomers although this was not confirmed. The high conversion could indicate that the amine is being removed during solvent evaporation despite measures taken to avoid this. For the more volatile substrates, diethyl ether was used as extraction solvent, and the samples were evaporated without external heating and were not evaporated to dryness.

*Table 5.3 Initial screens using model compounds*




| Entry | Substrate | TAm | Conv. (%)[a] | Entry | Substrate | TAm | Conv. (%)[a] [*ee* (%)][b] |
|---|---|---|---|---|---|---|---|
| 1[c] |  **112** | CV | 84 | 10 |  **118** | CV | 0 |
| | | AD2 | 34 | | | AD2 | 0 |
| | | TA3 | 18 | | | TA3 | 0 |
| 2[c] |  *(S)*-**112** | CV | 59 | 11[c] |  **119** | CV | -[e] |
| | | AD2 | 26 | | | AD2 | -[e] |
| | | TA3 | 15 | | | TA3 | -[e] |
| 3[c] |  *(R)*-**112** | CV | 1 | 12 |  **120** | CV | 20 [18][f] |
| | | AD2 | 1 | | | AD2 | 38 [26][f] |
| | | TA3 | 1 | | | TA3 | 5 [6][f] |
| 4[c] |  **113** | CV | 15 | 13 |  **121** | CV | 25 |
| | | AD2 | 6 | | | AD2 | 22 |
| | | TA3 | 2 | | | TA3 | 22 |
| 5[c] |  *(S)*-**113** | CV | 19 | 14[c] |  **122** | CV | 41 |
| | | AD2 | 4 | | | AD2 | 10 |
| | | TA3 | 5 | | | TA3 | 10 |
| 6 |  **114** | CV | 0 | 15 |  **123** | CV | 3 [2] |
| | | AD2 | 0 | | | AD2 | 6 [2] |
| | | TA3 | 0 | | | TA3 | 1 [3] |
| 7 |  **115** | CV | 0 | 16 |  *(S)*-**123** | CV | 54 |
| | | AD2 | 0 | | | AD2 | 17 |
| | | TA3 | 0 | | | TA3 | 7 |
| 8[d] |  **116** | CV | 0 | 17[c] |  **124** | CV | 19 [12] |
| | | AD2 | 0 | | | AD2 | 11 [8] |
| | | TA3 | 0 | | | TA3 | 4 [4] |
| 9 |  **117** | CV | 0 | 18[c] |  **125** | CV | 4 |
| | | AD2 | 0 | | | AD2 | 0 |
| | | TA3 | 0 | | | TA3 | <1 |

Reaction conditions: 30 mg whole cells expressing transaminases, PLP (1 mM), sodium pyruvate (50 mM), 10% DMSO, total volume 1 mL, reaction shaken for 24 h at 450 rpm, no lysis of the cells was carried out; [a]Conversion was estimated by $^1$H NMR spectroscopy; [b]Enantioselectivity of the unreacted amine was determined by chiral HPLC analysis, conditions are given in Appendix I; [c]Entries 1–5, 11, 17, 18 worked up using diethyl ether, others worked up using ethyl acetate [d]Entry 8 was carried out on 20 mM scale, 20 mM pyruvate was used; [e]No material was extracted from the aqueous layer when using diethyl ether, $^1$H NMR spectrum contained solvent only, when the procedure was repeated using heptane, no material was recovered; [f]enantioselectivity was determined by conversion to the Boc derivative.

When the size of the alkyl group[3] was increased to ethyl (**113**), there was some conversion and once again *(S)*-enantioselectivity was evident (Table 5.3, Entry 4 and 5). However, when the steric demand was further increased, no conversion was detected (Entry 6–10), indicative of the impact of increasing the steric demand in the small pocket of the transaminases. When using 2-aminoheptane **119** as substrate (Entry 11), no material was recovered from the aqueous layer during work-up with diethyl ether. The work-up was repeated, using heptane to extract the amine **119**, but this was also unsuccessful. To explore the isolation of 2-aminoheptane **119**, the substrate was suspended in buffer, and either 1% or 10% DMSO, and shaken for 24 hours; no substrate was recovered on work-up confirming that the outcome of the biotransformation is due to problems with recovery rather than reactivity. Further work is warranted on this substrate, such as direct derivatisation from the reaction mixture to decrease the aqueous solubility.

Interestingly, the aromatic ring can be substituted (Entry 14) or bridging methylene units can be inserted (Entry 12 and 13) while still retaining activity in the transaminase screen. This observation is in line with literature reports that transaminases are more tolerant to change on the larger substituent.[60]

The transaminases displayed activity against the cyclic substrates **123**–**125**, albeit to a limited extent. 1-Aminoindane **124** was transformed to a greater degree than 2-aminoindane **125** (Entry 17 & 18). This shows that the benzylic amine is more active than the non-benzylic substrate. Interestingly, for 1-aminotetralin **123**, the reaction conversions were poor when using the racemic substrate but much better conversion was observed when only the *(S)*-enantiomer was present (Entry 16 and 17); this suggests that the *(R)*-enantiomer may be inhibiting the transaminases. The 1-aminotetralin intermediate for sertraline **92a** was earlier shown to be processed by the transaminases (Table 5.1 & Table 5.2).

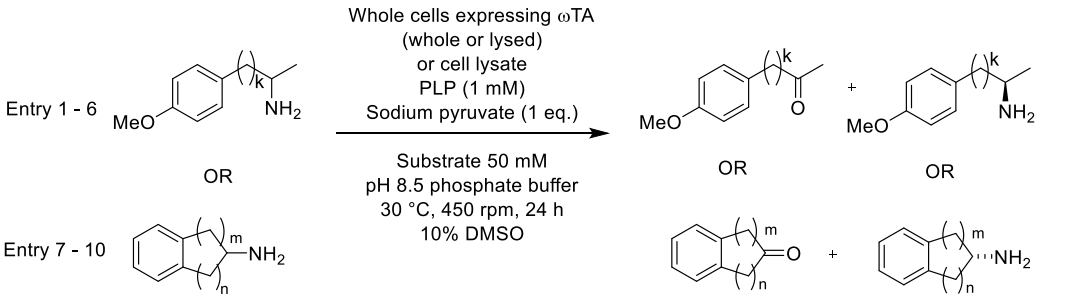
#### 5.4.2.1 Sonication of cells, and the use of cell lysate

Lysis of cells can be carried out in numerous ways. Based on work carried out by Gavin using the sertraline substrate **92** (Table 5.1 & Table 5.2), it was decided to lyse the cells by sonication. The cells were sonicated before addition of the substrates and cofactors and centrifuged after the reaction was stopped, or cells were sonicated and centrifuged, and the

[3]Although it was not carried out in this work, the size of the alkyl group could be quantified using Taft or Charlton parameters.

supernatant (cell lysate) was added to the reaction mixture. It was assumed that the use of the cell lysate would produce cleaner reactions, with lower risk of biological contamination of the reaction mixture. It also made the reaction work-ups easier to carry out as there was no biological matter which needed to be removed by filtration.

*Table 5.4 Variation of the cell preparation*




| Entry | k | m,n | Compound | Cells | Conversions (%) [*ee* (%)][a] | | |
|---|---|---|---|---|---|---|---|
| | | | | | CV-TA | AD2 | TA3 |
| **1** | 0 | - | **122** | Whole[b,c] | 41 [38] | 10 [10] | 10 [15] |
| **2** | 0 | - | **122** | Lysed[b,c] | 39 [29] | 12 [8] | 11 [9] |
| **3** | 0 | - | **122** | Supernatant[b,c] | 65 [86] | 8 [2] | 43 [35] |
| **4** | 1 | - | **121** | Whole[b,c] | 75 [97] | 59 [89] | 60 [97] |
| **5** | 1 | - | **121** | Lysed[b,c] | 67 [>98] | 43 [65] | 50 [68] |
| **6** | 1 | - | **121** | Supernatant[b,c] | 77 [>98] | 73 [>98] | 44 [18] |
| **7** | - | 0, 2 | **124** | Whole[b,c] | 3 [-][d] | 3 [-][d] | 4 [-][d] |
| **8** | - | 0, 2 | **124** | Supernatant[b,c] | 1 [-][d] | 0 [-][d] | 0 [-][d] |
| **9** | - | 1, 1 | **125** | Supernatant[b,c] | 0 [-][d] | 0 [-][d] | 0 [-][d] |
| **10** | - | 1, 2 | **130** | Supernatant[b,c] | 0 [-][d,e] | 0 [-][d,e] | 0 [-][d,e] |

Reaction conditions: 30 mg cells expressing transaminases was used per reaction, PLP (1 mM), sodium pyruvate (50 mM), 10% DMSO, total volume 1 mL, reaction shaken for 24 h at 450 rpm. [a]Enantiomeric excess of the amine substrate was determined using chiral HPLC analysis, conditions are given in Appendix I; [b]whole cells (30 mg) were not subject to lysis; lysed cells (30 mg) were lysed by sonication and added to the reaction mixture; supernatant refers to the cell lysate, with the equivalent of 30 mg cells added per reaction; [c]brine was added to the aqueous layer to aid extraction; [d]enantioselectivity not measured for conversion <5%; [e]calculation of conversion is difficult, but the absence of the ketone singlet at 2.58 ppm in the $^1$H NMR spectrum indicated that there is no product present.

The substrates chosen for the initial experiments were **121** and **122**. In addition, the cyclic compounds **124**, **125** and **130** were subjected to the same conditions (Table 5.4). Changing the reaction conditions had little apparent effect on the AD2 when using **122** (Table 5.4, Entries 1–3), with poor conversion regardless of the method used. The conversion increased for this substrate when using the supernatant of both CV-TA and TA3, showing that this mode of cell preparation was beneficial. For **121** (Entry 4–6) the conversion was >50% when using CV-TA, with excellent enantioselectivity throughout. These reactions could be further explored through variation of the concentration of amine acceptor (pyruvate) but this work was not completed in the course of this project. The cyclic compounds furnished no product regardless of whether the whole cell or cell lysate was used. It is important to note that the

concentration of protein in the cells can be variable, so the conversions can vary slightly from batch to batch, but generally similar conversions were noted. The reactions which furnished products gave enantioenriched or enantiopure compounds, indicating that the reactions are enantioselective; the challenge is in getting conversion.

### 5.4.3 Reductive amination

During his work, Gavin also attempted the asymmetric synthesis of amines *cis*-**92a** and *trans*-**92b** from tetralone **108**, using various methods. Unsurprisingly, these experiments were unsuccessful, showing no conversion of the tetralone **108** to products *cis*-**92a** or *trans*-**92b**. There is literature precedent for the resistance of these conjugated tetralone compounds towards reaction with transaminases.[61,62] The control for these experiments was the conversion of acetophenone **111** to α-methylbenzylamine **112**. Under the conditions used the control, acetophenone **111**, was not converted either, suggesting that further work was warranted.

Phenylacetone **147** was initially proposed as the new control, but due to the scheduled status of **147**, 4′-methoxyphenylacetone **148** was used instead. 4′-Methoxyphenylacetone **148** has been previously used in the CV-TA mediated reductive amination giving excellent activity and selectivity using both the LDH and ADH systems (94% conversion, >99% *ee*).[63] As a comparison, the analogous acetophenone derivative **149** and corresponding amine **122** were also selected. The use of ketones which differ by the position of the C=O were also considered. 2-Indanone **150** and 1-indanone **151** were included, as well as the corresponding tetralones **131** and **152**. It has previously been shown that transaminases can convert 2-tetralones to 2-aminotetralins, but the same transformation on 1-tetralones is more difficult.[61] The substrates are shown in Figure 5.8.

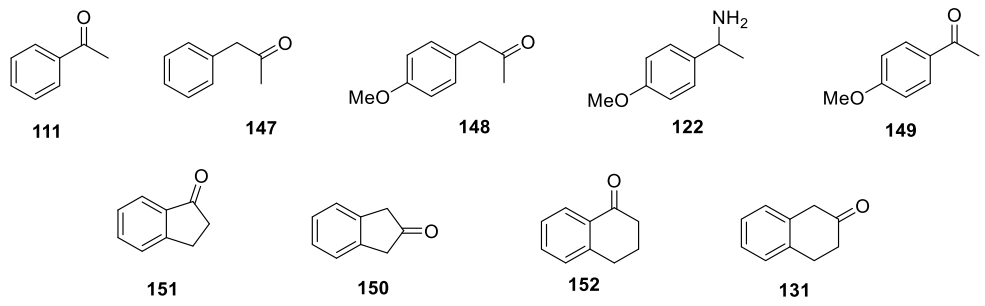
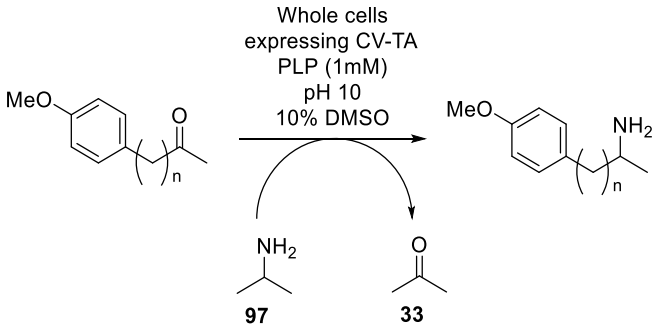



*Figure 5.8 Substrates for reductive amination*

Initially the asymmetric reactions were carried out using isopropylamine **97** as the amine source. For these reactions, a 1M solution of isopropylamine **97** was prepared and the pH adjusted to 10 using 5M HCl, as the transaminase works well at this pH. This solution was used in place of a buffer. These reaction conditions gave no conversion of the ketones **148** and **149** to the corresponding amines **121** and **122**. When using either 20 or 50 equivalents of the amine donor, no amine product was detected (Table 5.5). It is thought that this is because the reaction is carried out under standard conditions of atmospheric pressure, and ambient temperature. If further investigations using isopropylamine **97** as amine donor are carried out, elevated temperatures could be investigated. Alternatively using reaction vessels without caps on and without the cover on the shaker, could potentially allowing evaporation of the coproduct acetone **33**.

*Table 5.5 Reductive amination using isopropylamine as amine donor*



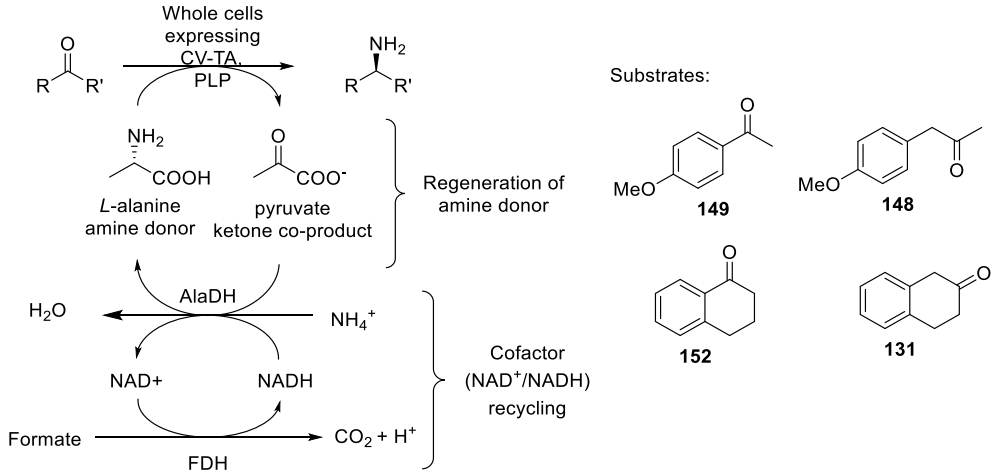

| Entry | n | Substrate | Expected product | [Substrate] (mM) | 97 (eq.) | Conversion (%)[a] |
|---|---|---|---|---|---|---|
| 1 | 0 | **149** | **122** | 50 | 20 | 0 |
| 2 | 0 | **149** | **122** | 20 | 50 | 0 |
| 3 | 1 | **148** | **121** | 50 | 20 | 0 |
| 4 | 1 | **148** | **121** | 20 | 50 | 0 |

Reaction conditions: 30 mg whole cells expressing CV-TA, PLP (1 mM), 10% DMSO, total volume 1 mL, reaction shaken for 24 h at 450 rpm, no lysis of the cells was carried out, 1M isopropylamine prepared and adjusted to pH 10 using 5M HCl solution. When [substrate]=20 mM, this is a 50 fold excess of amine, and when [substrate] = 50 mM this is a 20 fold excess of amine. [a]Conversion was estimated by $^1$H NMR spectroscopy.

Following this, two sets of conditions were used for the conversion of ketones to amines, using the AlaDH or LDH systems (Scheme 5.9 and Scheme 5.10) as auxiliary enzymes in an attempt to push the equilibrium towards the amine product. Problems were encountered in the work-up of these reactions, with some $^1$H NMR spectra showing neither ketone nor amine was present. Brine was added, and the samples were worked up again, but product was still not recovered.

Using the AlaDH system, only one of the reaction mixtures was successfully extracted. 4′-Methoxyacetophenone **149** was converted to amine **122**, with 10% conversion, and excellent enantioselectivity (91% *ee*) (Table 5.6, Entry 1). The other compounds tested were 4′-methoxyphenylacetone **148**, 1-tetralone **152** and 2-tetralone **131**. While the transaminase reactions are usually carried out at pH 8.5, these reactions were carried out at pH 7.5, as this is a suitable pH for the auxiliary enzymes. Another factor which can be explored in the future is the use of a lower substrate loading, (e.g. 20 mM), as the higher loading of substrate may inhibit the biocatalyst. Practically 50 mM is convenient for these substrates, as it provides enough material to enable a good quality $^1$H NMR spectrum to be obtained.

*Table 5.6 Reductive amination using AlaDH system*



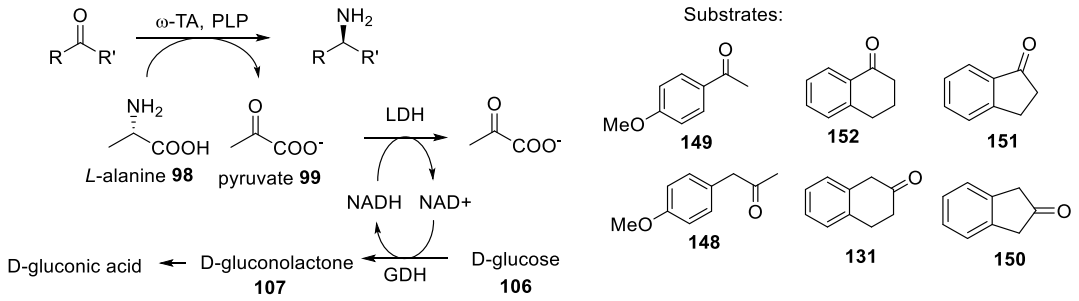

| Entry | Substrate | Conversion (%)[a] | $ee_p$ (%)[b] |
|---|---|---|---|
| 1 | **149** | 10 | 91 |
| 2 | **148** | ND[c] | -[d] |
| 3 | **152** | ND[c] | -[d] |
| 4 | **131** | ND[c] | -[d] |

Reaction conditions: [Substrate] = 50 mM; 50 mg wet cells expressing CV-TA; no sonication; 10% DMSO as cosolvent; 24 h, 30 °C, 450 rpm, pH = 7.5, total reaction volume 1 mL, NADH (1 mM), L-alanine (250 mM, 5 eq.), AlaDH (12U), ammonium formate (150 mM), FDH (10U). [a]Conversion estimated by $^1$H NMR spectroscopy; [b]enantiomeric excess was determined by chiral HPLC analysis, conditions given in appendix I; [c]neither starting material nor product were detected after work-up of the reaction mixture; [d]enantiomeric excess was not determined as no product was detected by $^1$H NMR analysis.

For the LDH system, a broader range of substrates was screened. 4′-Methoxyacetophenone **149** and 4′-methoxyphenylacetone **148** gave similar values for conversion (~20%) but the product from 4′-methoxyacetophenone **148** was produced with much lower enantioselectivity than that from **149** (~40% *ee* vs 97% *ee,* Table 5.7). 1-Tetralone **152** was extracted from the reaction mixture with no amine **123** evident in the $^1$H NMR spectrum. When the amount of DMSO was increased from 10% to 20% to enhance the substrate solubility, there was still no amine recovered. Unfortunately, when 2-tetralone **131** was

screened, using both 10% and 20% DMSO to enhance substrate solubility, neither starting material nor product were recovered from the reaction mixture.

*Table 5.7 Reductive amination using LDH system*




| Entry | Substrate | [DMSO] (%) | Conversion (%)[a] | *ee* (%)[b] |
|---|---|---|---|---|
| 1 | **149** | 10 | 20 | 97 |
| 2 | **148** | 10 | 21 | ~40%[y] |
| 3 | **152** | 10 | 0 | -[d] |
| 4 | **131** | 10 | ND[e] | -[d] |
| 5 | **151** | 10 | 0 | -[d] |
| 6 | **150** | 10 | 0 | -[d] |
| 7 | **152** | 20 | 0 | -[d] |
| 8 | **131** | 20 | ND[e] | -[d] |

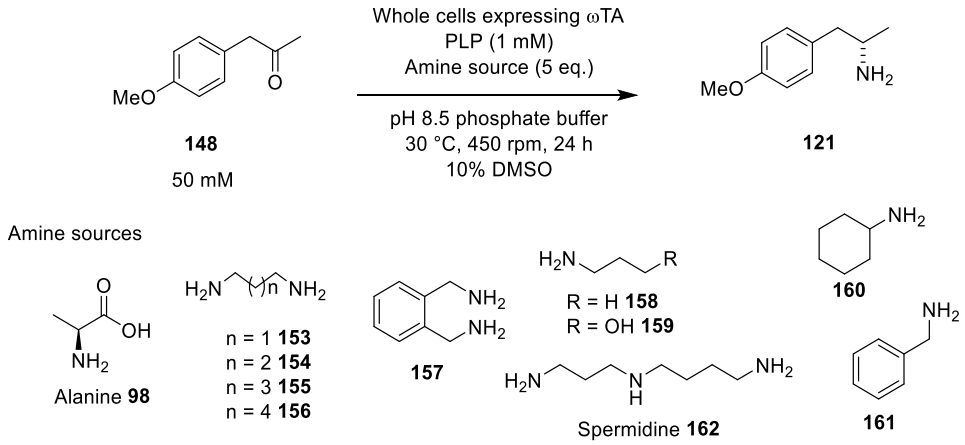
Reaction conditions: [susbtrate] = 50 mM, PLP (1 mM), NADH (1 mM), L-alanine (250 mM, 5eq.), LDH (90U), D-glucose (150 mM), GDH (15U). [a]Conversion estimated using $^1$H NMR spectroscopy; [b]enantioselectivity was determined by chiral HPLC analysis, conditions are given in Appendix I; [c]the presence of a coeluting impurity made determination of enantioselectivity difficult, baseline separation was not achieved as analysis indicated low selectivity (approx. 40% *ee)*; [d]enantioselectivity was not determined when no conversion is detected; [e]ND indicates that neither starting material nor product were recovered from the reaction mixture.

### 5.4.3.1 Alternative amine sources

The use of amines other than isopropylamine **97** in the asymmetric synthesis/reductive amination has been previously reported.[23,20] In these cases, the use of diamine co-substrates such as **153**–**157**, in theory, should help push the reaction towards the amine product, as the co-products can dimerise, cyclise or evaporate (Scheme 5.11). A similar approach was used by Hollmann et al. in the bioreduction of alcohols using ADH (Scheme 2.13).[64] In this case a diol cosubstrate is used, which can react twice, meaning that only half the amount should be required. As well as this, the co-product can cyclise to a thermodynamically more stable lactone **35**, essentially rendering the reaction irreversible. The use of an amine donor which produces a tautomerisible ketone by-product, such as those reported by Berglund et al. is also a viable alternative (Scheme 5.11).[25] More recently, *n*-butylamine has been reported as an amine donor. In this work, a transaminase was used in conjunction with a well-characterised aldehyde reductase YqhD from *E. coli*, as well as cofactor recycling.[24] In our work, a number of amine sources were tested, using 5 equivalents of the amines and diamines. In addition to

this, L-alanine **98**, short chain amines and an amino alcohol were also used as amine sources (Table 5.8).

*Table 5.8 Use of alternative amine sources*




| Entry | Amine source | Conversion (%)[a] [*ee* (%)][b] | | |
|---|---|---|---|---|
| | | CV-TA | AD2 | TA3 |
| 1 | Alanine **98** | 22 [~45%][c] | 0 | 0 |
| 2 | 1,3-diaminopropane **153** | 0 | 0 | 0 |
| 3 | 1,4-diaminobutane **154** | 0 | 0 | 0 |
| 4 | 1,5-diaminopentane **155** | 0 | 0 | 0 |
| 5 | 1,6-diaminohexane **156** | 0 | 0 | 0 |
| 6 | *o*-diaminoxylene **157** | 0 | 0 | 0 |
| 7 | 1-propylamine **158** | 0 | 0 | 0 |
| 8 | 1-aminopropanol **159** | 0 | 0 | 0 |
| 9 | Cyclohexylamine **160** | 0 | 0 | 0 |
| 10 | Benzylamine **161** | 0 | 0 | 0 |
| 11 | Spermidine **162** | 0 | 0 | 0 |
| 12 | Alanine **98** | No cells, control, 1% DMSO: 0% conversion | | |
| 13 | Alanine **98** | No cells, control, 10% DMSO: 0% conversion | | |

Reaction conditions: [substrate] = 50 mM, 50 mg whole cells expressing transaminases, PLP (1 mM), amine source (250 mM, 5 eq.), 10% DMSO, total volume 1 mL, reaction shaken for 24 h at 450 rpm, no lysis of the cells was carried out. [a]Conversion was estimated by $^1$H NMR spectroscopy; [b]enantioselectivity was determined by chiral HPLC analysis and conditions are given in Appendix I; [c]the presence of a coeluting impurity made determination of enantioselectivity difficult, baseline separation was not pursued as analysis indicated low selectivity (approx. 45% *ee).*

Overall, the results were poor. Using alanine **98** as the amine source resulted in 22% conversion and furnished ~45% *ee* (Table 5.8, Entry 1). The presence of a coeluting impurity made HPLC analysis difficult. When the sample was reanalysed the peaks were separated, albeit not with baseline resolution. The HPLC showed that the enantioselectivity was not high, and further development to get baseline separation was not carried out. Comparing this result to the results obtained when using the LDH system (Table 5.7, Entry 2) we see very similar conversion of substrate **148** and enantioselectivity in both reactions. This indicates that the auxiliary enzyme system (LDH) is not working for this substrate, and that the conversion of **148** may be as a result of the presence of L-alanine **98**, as the reaction conditions are almost

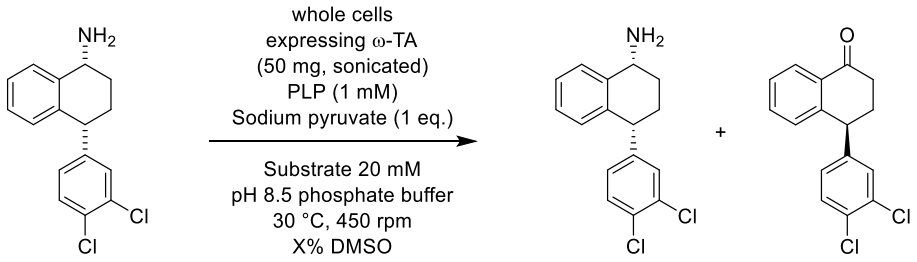
identical, except for the presence of the auxiliary enzymes and the pH. More work is required on the auxiliary enzyme systems to validate these preliminary results. To determine if the conversion of **148** was due to the presence of the biocatalyst, the substrate **148** was mixed with alanine in the buffer, shaken for 24 hours and extracted (Table 5.8, entry 12 and 13). In this case, the ketone **148** was recovered, with no amine **121** present; indicating that the conversion observed for this substrate in the presence of **98** was due to the biocatalyst. Nonetheless, the conversion of 4′-methoxyacetophenone **149** to the amine using both auxiliary enzyme systems indicates that these methods of reductive amination are working (Table 5.6 and Table 5.7, entry 1 in both cases). The remaining amine sources tested, **153**–**162**, furnished no product.

### 5.4.4 Resolution of pharmaceutical intermediates

Previous work carried out by Gavin showed that CV-TA showed excellent enantioselectivity in the oxidative deamination of *cis*-**92a** and *trans*-**92b**. This transaminase however, lacked diastereo-discrimination, converting both amines to a similar degree. The novel transaminase, TA3, displayed excellent diastereoselectivity in the kinetic resolution of amine *cis*-**92a**. Gavin initially used either *cis*-**92a** or *trans*-**92b** as substrate for the reaction, giving only conversion of the *cis*-**92a** diastereomer by TA3. When the two diastereomers were mixed together, the reaction was still diastereoselective, although the enantiopurity of the amine substrates could not be easily measured for the mixture, the tetralone enantiopurity was still high (92 % *ee*). The results for this work are shown in Table 5.1 and Table 5.2. In Gavin's initial screens 30 mg of cells were used. In this work 50 mg of whole cells was used. In both cases, cells were sonicated. Gavin used freshly prepared cells, but in this work cells which had been stored at −80 °C for up to three months were used.

In this work sertraline amines *cis*-**92a** and *trans*-**92b** were screened for activity using the novel transaminase TAad2 (AD2). The results for *cis*-**92a** and *trans*-**92b** are shown in Table 5.9 and Table 5.10, respectively. The effect of altered cosolvent concentration was assessed for all three transaminases, to show that the diastereo-discrimination showed by TA3 was not due to differing solubilities of the diastereomeric pairs. While the degree of conversion is sensitive to the DMSO concentration, the excellent diastereoselectivity was maintained using TA3 (Table 5.9 and Table 5.10, Entry 4–6 for both). Comparing Table 5.9 Entries 4 and 5 to Entries 8 and 9, the conversion ($E_{calc}$) suggests that AD2 is more tolerant to the increased cosolvent concentration than TA3.

*Table 5.9 Kinetic resolution of* cis-***92a***

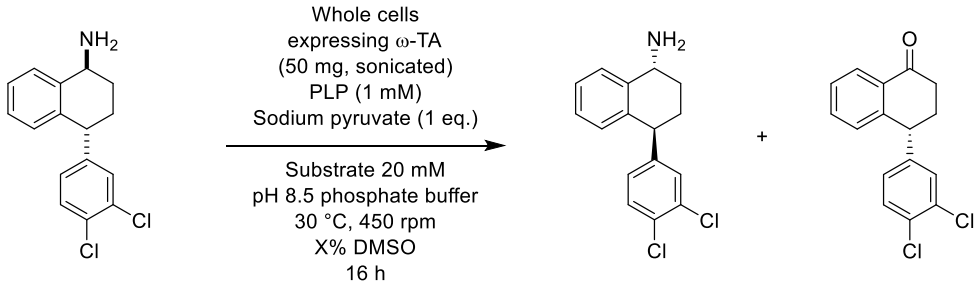



*cis*-**92a** | *(1R,4R)*-**92a** | *(4S)*-**108**

| Entry | TAm | Conditions | | Conversion (%) | | *ee* (%)[a] | | E |
|---|---|---|---|---|---|---|---|---|
| | | % DMSO | Sonication | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | CV-TA | 10% | N | 43 | 46 | 76 | 91 | 49 |
| 2 | CV-TA | 10% | Y | 45 | 47 | 77 | 87 | 33 |
| 3 | CV-TA | 20% | Y | 48 | 54 | 96 | 83 | 42 |
| 4 | TA3 | 10% | N | 16 | 17 | 18 | 86 | 16 |
| 5 | TA3 | 10% | Y | 21 | 46 | 58 | 68 | 9 |
| 6 | TA3 | 20% | Y | 25 | 35 | 36 | 66 | 7 |
| 7 | AD2 | 10% | N | 16 | 22 | 12 | 42 | 3 |
| 8 | AD2 | 10% | Y | 35 | 37 | 54 | 90 | 33 |
| 9 | AD2 | 20% | Y | 52 | 51 | 95 | 92 | 89 |

Reaction conditions: 50 mg whole cells expressing transaminases, PLP (1 mM), sodium pyruvate (50 mM, 1 eq.), 10% or 20% DMSO, total volume 1 mL, reaction shaken for 16 h at 450 rpm, cells were lysed by sonication as indicated. [a]Enantioselectivity was determined by chiral HPLC analysis and conditions are given in Appendix I.

*Table 5.10 Kinetic resolution of* trans-***92a***



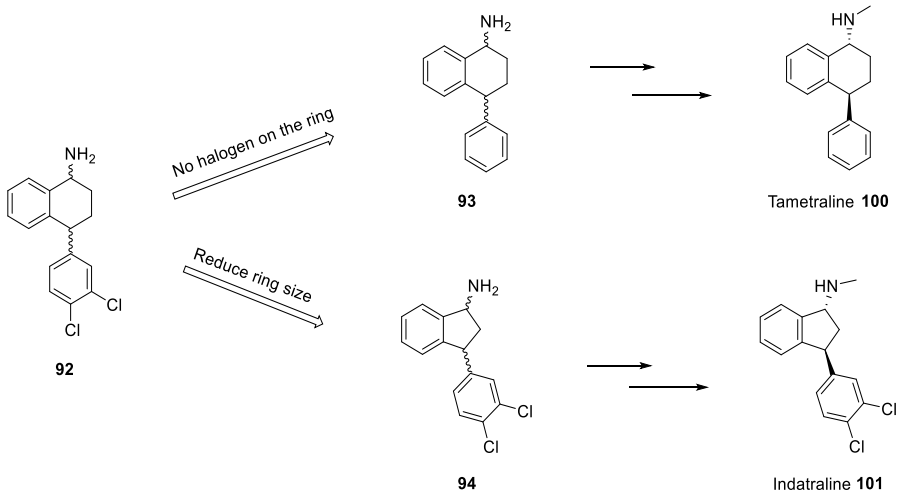

*trans*-**92b** | *(1R,4S)*-**92b** | *(4R)*-**108**

| Entry | TAm | Conditions | | Conversion (%) | | *ee* (%)[a] | | E |
|---|---|---|---|---|---|---|---|---|
| | | % DMSO | Sonication | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | CV-TA | 10% | N | 21 | 34 | 36 | 69 | 8 |
| 2 | CV-TA | 10% | Y | 37 | 46 | 67 | 78 | 16 |
| 3 | CV-TA | 20% | Y | 45 | 52 | 92 | 84 | 37 |
| 4 | TA3 | 10% | N | 0 | -[b] | -[b] | -[b] | -[b] |
| 5 | TA3 | 10% | Y | 0 | -[b] | -[b] | -[b] | -[b] |
| 6 | TA3 | 20% | Y | 0 | -[b] | -[b] | -[b] | -[b] |
| 7 | AD2 | 10% | N | 3 | -[b] | -[b] | -[b] | -[b] |
| 8 | AD2 | 10% | Y | 5 | 12 | 6 | 44 | 12 |
| 9 | AD2 | 20% | Y | 6 | 32 | 19 | 40 | 32 |

Reaction conditions: 50 mg whole cells expressing transaminases, PLP (1 mM), sodium pyruvate (50 mM, 1 eq.), 10% or 20% DMSO, total volume 1 mL, reaction shaken for 16 h at 450 rpm, cells were lysed by sonication as indicated. [a]Enantioselectivity was determined by chiral HPLC analysis and conditions are given in Appendix I; [b]enantioselectivity was not determined as not product was detected, E and $E_{calc}$ were not determined.

For these substrates (*cis*-92a and *trans*-92b), TA3 showed good enantioselectivity and excellent diastereoselectivity, with no conversion of *trans*-92b to product, even with higher cosolvent loading. AD2 had excellent enantioselectivity towards *cis*-92a (Table 5.9, Entry 8 and 9, however, the diastereoselectivity was poorer than TA3.

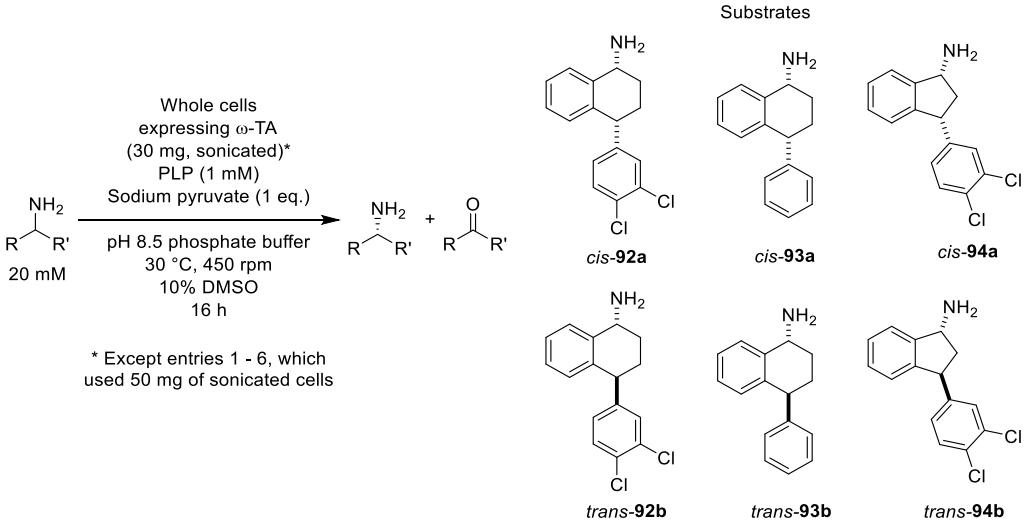
The previous experiments carried out during this work using the model substrates in Table 5.3 showed no advantage of the novel biocatalysts when compared with CV-TA; the difference arises in the use of the pharmaceutical intermediates *cis*-92a and *trans*-92b as substrates. To further assess the activity of the transaminases towards pharmaceutical intermediates other substrates were prepared; one with a reduced ring size, which is an intermediate for indatraline 101, and one where there are no halogens on the ring, an intermediate for tametraline 100 (Scheme 5.24).




*Scheme 5.24 Related substrates*

The substrates 93a–94b were synthesised as described on p. 171; the sertraline intermediates *cis*-92a and *trans*-92b used were prepared by Gavin. For these reactions, the biocatalyst loading was decreased to 30 mg of whole cells, and sonication was carried out to lyse the cells before adding them to the reaction mixture. The results differed slightly to those for experiments carried out by Gavin as freshly prepared cells were used in the earlier experiments. Here, whole cells were prepared and stored at –80°C for up to three months before use. The results are shown in Table 5.11. For the pharmaceutical intermediates 92a–94b the substrate loading used was 1 mL at 20 mM, as this is equivalent to approx. 5 mg of substrate.

*Table 5.11 Kinetic resolution of related substrates*




| Entry | Substrate | Transaminase | Conversion (%) | | *ee* (%)[a] | | E |
|---|---|---|---|---|---|---|---|
| | | | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| 1 | *cis*-**92a** | CV-TA[b] | 45 | 47 | 77 | 87 | 33 |
| 2 | *cis*-**92a** | TA3[b] | 21 | 46 | 58 | 68 | 9 |
| 3 | *cis*-**92a** | AD2[b] | 35 | 37 | 54 | 90 | 33 |
| 4 | *cis*-**92a** | CV-TA[b] | 58 | 52 | 92 | 86 | 43 |
| 5 | *cis*-**92a** | TA3[b] | 5 | 5 | 4 | 70 | 6 |
| 6 | *cis*-**92a** | AD2[b] | 55 | 50 | 96 | 95 | 154 |
| 7 | *trans*-**92b** | CV-TA | 37 | 46 | 67 | 78 | 16 |
| 8 | *trans*-**92b** | TA3 | 0 | -[c] | -[c] | -[c] | -[c] |
| 9 | *trans*-**92b** | AD2 | 5 | 12 | 6 | 44 | 12 |
| 10 | *trans*-**92b** | CV-TA | 47 | 50 | 88 | 88 | 45 |
| 11 | *trans*-**92b** | TA3 | 0 | -[c] | -[c] | -[c] | -[c] |
| 12 | *trans*-**92b** | AD2 | 4 | 12 | 12 | 86 | 15 |
| 13 | *cis*-**93a** | CV-TA | 53 | 53 | 65 | 57 | 7 |
| 14 | *cis*-**93a** | TA3 | 11 | 17 | 5 | 24 | 2 |
| 15 | *cis*-**93a** | AD2 | 50 | 48 | 74 | 80 | 16 |
| 16 | *trans*-**93b** | CV-TA | 55 | 14 | 10 | 62 | 5 |
| 17 | *trans*-**93b** | TA3 | 0 | -[c] | -[c] | -[c] | -[c] |
| 18 | *trans*-**93b** | AD2 | 3 | 23 | 25 | 85 | 16 |
| 19 | *cis*-**94a** | CV-TA | 34 | 35 | 44 | 82 | 16 |
| 20 | *cis*-**94a** | TA3 | 0 | -[c] | -[c] | -[c] | -[c] |
| 21 | *cis*-**94a** | AD2 | 13 | 26 | 26 | 74 | 9 |
| 22 | *trans*-**94b** | CV-TA | 0 | -[c] | -[c] | -[c] | -[c] |
| 23 | *trans*-**94b** | TA3 | 0 | -[c] | -[c] | -[c] | -[c] |
| 24 | *trans*-**94b** | AD2 | 4 | -[d] | 0 | ND[d] | -[d] |

Reaction conditions: [substrate] = 20 mM, PLP (1 mM), sodium pyruvate (50 mM, 1 eq.), 10% DMSO, total volume 1 mL, reaction shaken for 16 h at 450 rpm, cells were lysed by sonication; entries 1–3 and 7–9 are identical to Table 5.9 Entry 2, 5, 8 and Table 5.10 entry 2, 5, and 8. [a]Enantioselectivity was measured using the HPLC conditions which are given in Appendix I; [b]for entry 1–3 and 7–9, 50 mg of sonicated cells was used, for all other entries, 30 mg was used, all other reaction conditions were identical; [c]enantioselectivity was not determined as no product was detected by $^1$H NMR, E and $E_{calc}$ were not determined as this required % *ee* >0; [d]conversion was detected by $^1$H NMR but no ketone was detected by chiral HPLC analysis, the amine enantioselectivity was measured as 0% *ee*, E and $E_{calc}$ were not calculated as % *ee* was not measured.

Using CV-TA the enantiopurity of the product was the same using both 50 mg of cells and 30 mg of cells with *cis*-**92a** as substrate (Table 5.11, Entries 1–12). The higher loading of cells furnished a product with lower enantiopurity when using *trans*-**92b** as substrate (Table 5.11, Entry 1). TA3 showed excellent diastereoselectivity whether using the higher or lower loading of cells (Table 5.11, Entry 5 and 8 vs. Entry 14 and 17). The enantioselectivities towards the product tetralone **108** were comparable at both cell loadings (68% $ee_p$ & 70% $ee_p$, Table 5.11, Entry 2 and 5). For AD2, the enantiopurity of the product was high for the substrate *cis*-**92a** in both cases (90% $ee_p$ & 95% $ee_p$, Table 5.11 entry 3 and Entry 6, respectively). For substrate *trans*-**92b** the product enantiopurity was much poorer when using the higher cell loading (44% $ee_p$ vs 86% $ee_p$, Table 5.11, Entry 9 and Entry 12, respectively), albeit with similar values measured for conversion.

CV-TA shows no diastereoselectivity for the tetralone substrates *cis*-**92a**, *trans*-**92b**, *cis*-**93a** and *trans*-**93b** (Table 5.11, entries 4, 10, 13, 16); indane *cis*-**94a** is converted to the indanone **135** (Entry 19) but *trans*-**94b** furnished no conversion (Entry 22). The substrate *trans*-**94b** is resistant to deamination by the transaminases tested (Entries 22–24). Substrate *cis*-**94a** was converted to the indanone **135** by both CV-TA and AD2, with good enantioselectivity (82% $ee_p$ & 74% $ee_p$, Entry 19 and 21, respectively). TA3 shows no activity towards either *cis*-**94a** or *trans*-**94b** (Entry 20 and 23).

## 5.5 Conclusions

A series of amines were synthesised to assess the activity and substrate scope of novel transaminases. The novel transaminases (TA3 and AD2) explored showed excellent diastereoselectivity in the oxidative deamination reactions compared to the control transaminase (CV-TA) in the resolution of pharmaceutical intermediates **92**–**94**. While the novel transaminases showed good (AD2) to excellent (TA3) diastereoselectivity in the resolution of the pharmaceutical intermediates, CV-TA showed little diastereoselectivity. The transaminases also showed some activity against the model substrates, albeit, with a lower degree of conversion than when using the control.

Initial work on the reductive amination using the auxiliary enzymes systems in conjunction with CV-TA indicated that both 4′-methoxyacetophenone **149** and 4′-methoxyphenylacetone **148** were reduced to the corresponding amines **121** and **122**; however, further work showed that the conversion of **149** was due to the presence of L-alanine **98** as the amine donor.

## 5.6 Future work

It is anticipated that this preliminary work will be expanded to include additional substrates in the future, including variation of the substituents on the fused ring and monocyclic analogues (Figure 5.9).

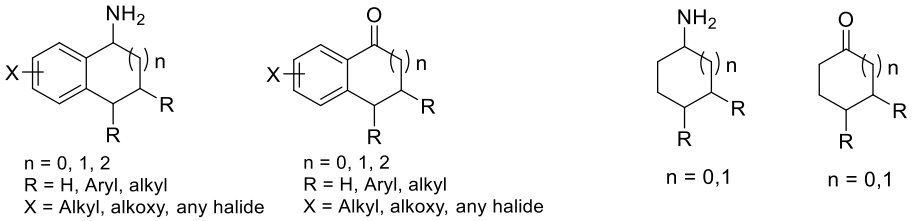
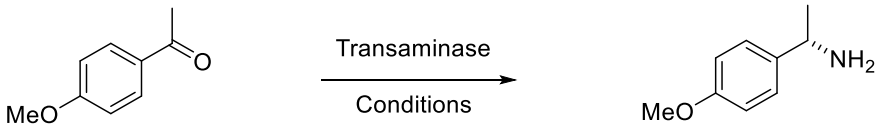



*Figure 5.9 Large molecule substrate scope for transaminases*

Preliminary work has suggested that the reductive amination transformation is working for the model substrate 4′-methoxyacetophenone **149** (Scheme 5.25). Further optimisation to this is envisaged, initially using CV-TA, and then AD2 and TA3, including the effect of substrate loading, reaction time, temperature and pH. The use of isopropylamine **97** as amine donor will be revisited, modifying some of the reaction conditions. Carrying out the reactions at elevated temperatures or using reaction vessels without caps on and without the cover on the shaker, potentially allowing the fumehood extract to help evaporate the coproduct.




*Scheme 5.25 Reductive amination*

Additionally, once the reaction conditions are optimised it is hoped to transfer these conditions to a continuous flow system. To facilitate this the immobilisation of the proteins will be investigated. This could be applied to both the reductive amination and the oxidative deamination reactions; which could potentially be coupled with a substrate recycling step.

To better control the reaction conversion, some of the earlier experiments (Table 5.4) will be carried out using shorter incubation times; in addition to this the use of substoichiometric amounts of the amine acceptor (pyruvate) in the kinetic resolution may allow for finer control of the substrate conversion, avoiding conversion >50%.

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# Experimental

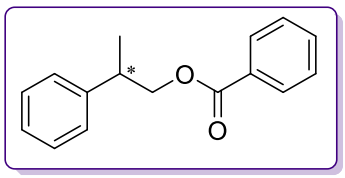
# Contents

## 6.1 General procedures

Solvents were distilled prior to use as follows: dichloromethane (DCM) was distilled from phosphorus pentoxide, ethyl acetate was distilled from potassium carbonate, THF and toluene were distilled from sodium and benzophenone. Hexane was distilled prior to use. Molecular sieves were activated by heating at 150 °C overnight. Organic phases were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate. Infrared spectra were recorded neat using a Perkin Elmer FTIR UATR2 spectrometer. $^{1}H$ (300 MHz) and $^{13}C$ (75.5 MHz) NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer, and all spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard. Chemical shifts ($\delta_H$ & $\delta_C$) are reported in parts per million (ppm) and coupling constants are expressed in Hertz (Hz). Low resolution mass spectra were recorded on a Waters Quattro Micro triple quadrupole spectrometer in electrospray ionisation (ESI) mode using 50% water/acetonitrile containing 0.1% formic acid as eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionisation mode (ESI) using 50% water/acetonitrile containing 0.1% formic acid as eluent. 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 PF254). Visualisation was achieved by UV (254 nm) light detection and $KMnO_4$ staining. Optical rotation was measured on a Perkin-Elmer 141 polarimeter at 589 nm in a 1 cm cell; concentrations (c) are expressed in g/100 mL. $[\alpha]_D^{20}$ is the specific rotation and is expressed in units of $10^{-1}$ deg $cm^2$ $g^{-1}$. Hydrolases were from Almac Sciences except Amano PS Lipase, CAL-B (on ImmoBead 150), Hog pancreas lipase, *Pseudomonas fluorescens* (immobilised on Sol-gel), and Lipase from *Candida cylindracea* which were purchased from Sigma Aldrich chemical company. Transaminases were obtained as whole cell preparations from BIOMERIT in UCC. All biotransformations were performed on a VWR Incubating Mini Shaker 4450. All reagents are analytical grade and purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, Fluorochem or TCI. HPLC analysis was performed on a Waters alliance 2690 separations module with a PDA detector. Enantiomeric excess values were measured determined using Chiralcel® chiral columns (5 × 250 mm) purchased from Daicel Chemical Industries, Japan or Phenomenex Cellulose and Amylose columns (5 × 250 mm), purchased from Phenomenex Inc., UK. Mobile phase and flow rate, detector wavelength and column temperature are included where appropriate.

## 6.2 Towards the lipase-mediated resolution of 2-phenyl-1-propanol **1**

### 2-Phenylpropyl benzoate **21**[1]

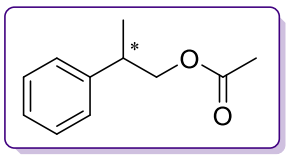


Benzoyl chloride (3.960 g, 28.2 mmol) in DCM (20 mL) was added slowly to a stirring solution of 2-phenyl-1-propanol **1** (4.0 mL, 3.98 g, 29.2 mmol) and triethylamine (4.67 mL, 3.40 g, 33.6 mmol) in DCM (20 mL) at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with DCM (90 mL). The solution was washed with water (2 × 100 mL), aq. HCl (10%, 2 × 100 mL), brine (150 mL), dried, filtered and concentrated to give the crude product **21** as a pale yellow oil. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent, giving the pure ester **21** (4.434 g, 66%) as a colourless oil.

$\nu_{max}$ (ATR) 2969 (C–H), 1716 (C=O), 1268, 1110 (C–O), 698 (C–H Ar) $cm^{-1}$; $\delta_H$ (300 MHz) 1.40 (d, 3H, *J* = 7.0, $CH_3$), 3.17–3.33 (sym m, 1H, CH), 4.33–4.48 (sym m, 2H, C(1)$H_2$), 7.19–7.46 (m, 7H, ArH), 7.49–7.57 (m, 1H, ArH), 7.94–8.04 (m, 2H, ArH) ppm; $\delta_C$ (75 MHz) 18.1 ($CH_3$), 39.1 (CH), 69.9 ($CH_2$), 126.7, 127.4, 128.3, 128.5, 129.6 (5 × aromatic CH), 130.4 (aromatic C), 132.9 (aromatic CH), 143.2 (aromatic C), 166.5 (C=O) ppm; HRMS ($ES^+$): $[M+H]^+$ 241.1229 (calculated: 241.1223); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL $min^{-1}$], $R_t$ = 10.0 min *(R)*-**21**, 12.4 min *(S)*-**21**.

All data are in agreement with previously reported data except signal for C(1)$H_2$ is reported as a doublet, and the signal here is a symmetrical multiplet.

### 2-Phenylpropyl acetate **22**[2]



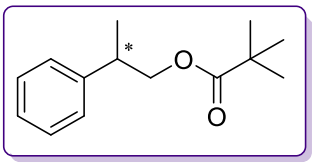
Anhydrous pyridine (2.7 mL, 2.61 g, 33.0 mmol, 1.5 eq.) and acetyl chloride (1.6 mL, 1.73 g, 22.0 mmol, 1.0 eq.) were sequentially added to a stirring solution of 2-phenyl-1-propanol **1** (3.0 g, 22.0 mmol, 1.0 eq.) and DMAP (134 mg, 1.1 mmol, 5 mol%) in DCM (75 mL) at 0 °C. The solution was removed from ice bath and was stirred for 24 h at room temperature. The reaction was quenched with sat. aq. $NaHCO_3$ (40 mL) and was stirred until no more gas was evolved (approx. 40 mins). The layers were separated, and the organic layer was washed with sat. aq. $CuSO_4$ (3 × 40 mL). The combined aqueous layers were extracted

with DCM (50 mL). The organic layers were combined and washed with water (2 × 100 mL), sat. aq. $NaHCO_3$ (100 mL), brine (100 mL), dried, filtered and concentrated to give the crude product as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **22** (2.16 g, 55%) as a colourless oil.

$\nu_{max}$ (ATR) 2968 (C–H), 1737 (C=O), 1373, 1227 (C–O), 699 (C–H Ar) $cm^{-1}$; $\delta_H$ (300 MHz) 1.30 (d, 3H, *J* = 7.1, $CH_3$), 2.00 (s, 3H, $COCH_3$), 3.00–3.19 (sym m, 1H, CH), 4.05–4.27 (sym m, 2H, $CH_2$), 7.11–7.41 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 18.1 ($CH_3$), 20.9 ($CO\mathbf{C}H_3$), 38.9 (CH), 69.4 ($CH_2$), 126.7, 128.5, 127.3 (3 × aromatic CH), 143.2 (aromatic C), 171.0 (C=O) ppm; enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99.5/0.5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 30.1 min *(S)*-**22**, 34.5 min *(R)*-**22**.

Data is in agreement with previous reports.[3]

## 2-Phenylpropyl pivalate **23**

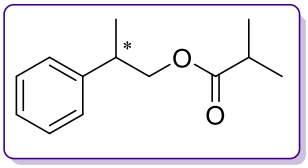


Prepared following the procedure for **22** using anhydrous pyridine (2.7 mL, 2.61 g, 33.0 mmol, 1.5 eq.), pivaloyl chloride (2.7 mL, 22.0 mmol, 1.0 eq.), 2-phenyl-1-propanol **1** (3.04 g, 22.0 mmol, 1.0 eq.) and DMAP (134 mg, 1.1 mmol, 5 mol%) in DCM (75 mL) to give the crude product **23** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **23** (3.273 g, 68%) as a colourless oil.

$\nu_{max}$ (ATR) 2971 (C–H), 1727 (C=O), 1282, 1150 (C–O), 699 (C–H) $cm^{-1}$; $\delta_H$ (300 MHz) 1.13 [9H, s, $C(CH_3)_3$], 1.31 (d, 3H, *J* = 6.9, $CH_3$), 3.02–3.29 (sym m, 1H, CH), 4.15 (qd, 2H, *J* = 10.8, 6.9, $CH_2$), 7.15–7.37 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 16.9 ($CH_3$), 26.1 [$C(\mathbf{C}H_3)_3$], 37.7 [$\mathbf{C}(CH_3)_3$], 38.0 (CH), 68.2 ($CH_2$), 125.6, 126.3, 127.4 (3 × aromatic CH), 142.3 (aromatic C), 177.3 (C=O) ppm; HRMS ($ES^+$): $[M+H]^+$ 221.1541 (calculated: 221.1542); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 9.5 min *(S)*-**23**, 13.1 min *(R)*-**23**.

This compound is novel and has been fully characterized in this investigation.

## 2-Phenylpropyl isobutyrate **24**



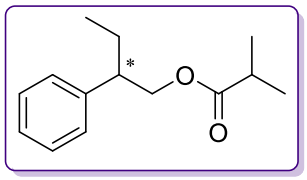
Prepared following the procedure for **22** using anhydrous pyridine (1.05 mL, 0.98 g, 15.0 mmol, 1.5 eq.), isobutyryl chloride (1.2 mL, 10.0 mmol, 1.0 eq.), 2-phenyl-1-propanol **1** (1.4 mL, 10

mmol, 1.0 eq.) and DMAP (61 mg, 0.5 mmol, 5 mol%) in DCM (35 mL) to give the crude product **24** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (90/10) as eluent gave pure ester **24** (1.862 g, 90%) as a colourless oil.

$\nu_{max}$ (ATR) 2972 (C–H), 1732 (C=O), 1190, 1153 (C–O), 699 (C–H Ar) $cm^{-1}$; $\delta_H$ (300 MHz) 1.09 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.11 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.31 [d, 3H, *J* = 7.0, C(4)**H**$_3$], 2.50 (sept, 1H, *J* = 7.0, COCH), 3.00–3.22 (sym m, 1H, C**H**CH$_2$), 4.00–4.32 (sym m, 2H, C(1)**H**$_2$), 7.10–7.43 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 18.0 [C(4)H$_3$], 18.9 [CH(**C**H$_3$)$_2$], 34.0 [**C**H(CH$_3$)$_2$], 39.0 (**C**HCH$_2$), 69.1 (CH$_2$), 126.6, 127.3, 128.4 (3 × aromatic CH), 143.3 (aromatic C), 177.0 (C=O) ppm; HRMS ($ES^+$): $[M+H]^+$ 207.1381 (calculated: 207.1380); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL $min^{-1}$], $R_t$ = 6.6 min *(R)*-**24**, 11.1 min *(S)*-**24**.

This compound is novel and has been fully characterized in this investigation.

## 2-Phenylbutyl isobutyrate **25**

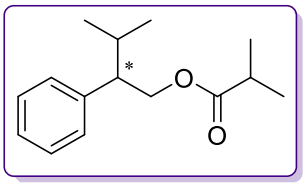



Prepared following the procedure for **22** using anhydrous pyridine (0.49 mL, 0.48 g, 6.1 mmol, 1.5 eq.), isobutyryl chloride (0.42 mL, 4.0 mmol, 1.0 eq.) 2-phenyl-1-butanol **3** (0.6 mL, 3.9 mmol, 1.0 eq.), and DMAP (27 mg, 0.2 mmol, 5 mol%) in DCM (22 mL) to give the crude product **25** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **25** (0.428 g, 50%) as a colourless oil.

$\nu_{max}$ (ATR) 2969 (C–H), 1732 (C=O), 1190, 1153 (C–O), 699 (C–H Ar) $cm^{-1}$; $\delta_H$ (300 MHz) 0.83 (t, 3H, *J* = 7.4, CH$_2$C**H**$_3$), 1.06 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.09 [d, 3H, *J* = 7.0, CH(C**H**$_3$)$_2$], 1.52–1.71 [m, 2H, C**H**$_2$CH$_3$], 2.47 [sept, 1H, *J* = 7.0, C**H**(CH$_3$)$_2$], 2.75–2.92 (sym m, 1H, C**H**CH$_2$O), 4.21 (d, 2H, *J* = 7.0, C**H**$_2$O), 7.00–7.48 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 11.8 [C(4)H$_3$], 18.9 [CH(**C**H$_3$)$_2$], 25.3 [C(3)H$_2$], 34.0 [**C**H(CH$_3$)$_2$], 46.8 [C(2)H], 68.0 (CH$_2$O), 126.6, 128.0, 128.4 (3 × aromatic CH), 141.9 (aromatic C), 177.1 (C=O) ppm; HRMS ($ES^+$): $[M+H]^+$ 221.1537 (calculated: 221.1542); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98/2, flow rate = 1 mL $min^{-1}$], $R_t$ = 6.1 min, 8.3 min.

This compound is novel and has been fully characterized in this investigation.

## 3-Methyl-2-phenylbutyl isobutyrate **26**

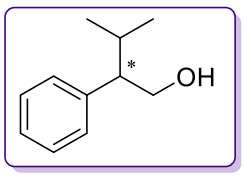


Prepared following the procedure for **22** using anhydrous pyridine (0.31 mL, 0.304 g, 3.8 mmol, 1.5 eq.), isobutyryl chloride (0.27 mL, 0.28 g, 2.6 mmol, 1.0 eq.), 3-methyl-2-phenyl-1-butanol **3** (0.413 g, 2.5 mmol, 1.0 eq.) and DMAP (16 mg, 0.13 mmol, 5 mol%) in DCM (10 mL) to give the crude product **26** as a colourless oil. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure ester **26** (0.212 g, 35%) as a colourless oil.

$\nu_{max}$ (ATR) 2969 (C–H), 1732 (C=O), 1190, 1154 (C–O), 700 (C–H Ar) $cm^{-1}$; $\delta_H$ (300 MHz) 0.76 [d, 3H, *J* = 6.7, C(4)$H_3$], 0.89–1.10 [m, 9H, C(4)$H_3$ & CH(C**H**$_3$)$_2$], 1.88–2.10 [m, 1H, $CH_2$C**H**$(CH_3)_2$], 2.41 (sept, 1H, *J* = 7.0, COCH), 2.57–2.79 (sym m, 1H, C**H**$CH_2$), 4.35 (d, 2H, *J* = 6.8, $CH_2$), 7.00–7.48 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 18.8, 20.7, 20.9 (3 × $CH_3$ signals for 4 × $CH_3$), 30.4 [C(3)H], 34.0 (CO**C**H), 51.8 [C(2)H], 66.2 ($CH_2$), 126.4, 128.1, 128.5 (3 × aromatic CH), 141.6 (aromatic C), 177.1 (C=O) ppm; HRMS ($ES^+$): $[M+H]^+$ 235.1687 (calculated: 235.1698); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL $min^{-1}$], $R_t$ = 6.0 min, 8.4 min.

This compound is novel and has been fully characterized in this investigation.

## 3-Methyl-2-phenylbutan-1-ol **3**[4]



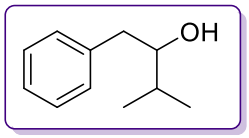
Grignard reaction:[5] In a flame dried 2-neck flask equipped with a reflux condenser, magnesium (1.505 g, 61.9 mmol) and a crystal iodine were vigorously stirred in diethyl ether (5 mL) for 1.5 h under a blanket of nitrogen. To this mixture isopropylbromide **45** (5.15 mL, 6.747 g, 54.9 mmol) in diethyl ether (15 mL) was added slowly to maintain a gentle reflux. On completion of the addition the solution was heated to reflux. After 40 mins, the solution was cooled to 0 °C using an ice bath, and a solution of styrene oxide **44** (5.72 mL, 6.006 g, 50.0 mmol) in diethyl ether (15 mL) was added over 25 mins and the solution was allowed to warm to room temperature and stirred for 40 mins, after which TLC showed the disappearance of styrene oxide **44**. The reaction solution was poured onto ice-water mixture (35 mL) and acidified to pH = 1 using conc. $H_2SO_4$. The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 20 mL). The organic layers were combined and washed with sat. aq. $Na_2CO_3$ (2 × 20 mL), water (3 × 20 mL), brine (40 mL), dried, filtered and concentrated to give the crude product as a yellow oil (5.911 g). Purification by column chromatography on silica

gel using hexane/ethyl acetate (90/10) gave the product **3** as a yellow oil (1.70 g, 21%, $R_f$ = 0.1).

$\nu_{max}$ (ATR) 3368 (OH), 2957 (C–H), 1494, 1453, 1366, 1056, 1031, 699 (C–H) $cm^{-1}$; $\delta_H$ (300 MHz) 0.74 [d, 3H, *J* = 6.9, C(4)$H_3$], 0.99 [d, 3H, *J* = 6.9, C(4)$H_3$], 1.82–2.03 [m, 1H, C(3)H], 2.51 [td, 1H, *J* = 8.7, 5.0, C(2)$H_2$], 3.72–4.02 (m, 2H, $CH_2$), 7.16–7.37 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 21.0 (2 × $CH_3$), 30.1 [C(3)H], 55.8 [C(2)H], 65.2 [C(1)$H_2$], 126.7, 128.5, 128.7 (3 × aromatic CH), 141.7 (aromatic C) ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 99/1, flow rate = 1 mL $min^{-1}$], $R_t$ = 21.9 min, 28.2 min.

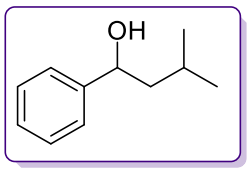
All data is in agreement with previously reported data.[4]

Another product was also isolated from the column (1.687 g, 21%, $R_f$ = 0.2) and was identified as the Meinwald rearrangement product, 3-methyl-1-phenylbutan-2-ol **47**, and agrees with previously reported data for this compound.[6]




$\nu_{max}$ (ATR) 3408 (OH), 2956 (C–H), 1495, 1453, 1367, 1075, 1031, 698 (C–H) $cm^{-1}$; $\delta_H$ (300 MHz) 1.00 [d, 6H, *J* = 6.8, CH(C**H**$_3$)$_2$], 1.69–1.85 [sym m, 1H, C**H**$(CH_3)_2$], 2.59 (dd, 1H, *J* = 13.6, 9.5, one of $CH_2$Ph), 2.85 (dd, 1H, *J* = 13.6, 3.0, one of $CH_2$Ph), 3.48–3.68 (m, 1H, C**H**OH), 7.03–7.50 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 17.7, 19.4 (2 × $CH_3$), 33.1 (**C**H$CH_3$), 40.9 (**C**$H_2$Ph), 77.6 (**C**HOH), 125.9, 127.3, 128.6, (3 × aromatic CH), 143.6 (aromatic C) ppm.

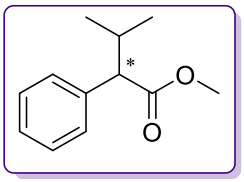
## 3-Methyl-1-phenylbutan-1-ol **46**




Magnesium (1.46 g, 60.0 mmol) and iodine (1 crystal) were stirred at high speed with small amount of diethyl ether (1.5 mL) for approx. 90 mins. 1-Bromo-2-methylpropane **48** (6.00 mL, 7.53 g, 55.0 mmol) was added dropwise as solution in diethyl ether (15 mL) and was stirred at reflux for 40 mins upon completion of the addition. The reaction mixture was cooled to 0 °C and benzaldehyde (5.0 mL, 5.20 g, 49.0 mmol) in ether (15 mL) was added slowly, over 15 mins. The reaction was warmed to room temperature and stirred for 90 mins. The mixture was poured onto ice and water and quenched by adding dilute HCl dropwise. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 25 mL). The organic layers were combined and washed with water (3 × 20 mL), brine (75 mL), dried, filtered and concentrated. Purification by column chromatography on silica gel using hexane/ethyl acetate (95/5) as eluent gave pure alcohol **46** as a yellow oil (0.47 g, 5%).

$\nu_{max}$ (ATR) 3344, 3086, 3029, 2955, 2925 (C–H), 1494, 1453 (C=C) $cm^{-1}$; $\delta_H$ (300 MHz) 0.95 [dd, 6H, *J* = 6.3, 1.4, CH(C**H**$_3$)$_2$], 1.40–1.61 [m, 1H, C**H**(CH$_3$)$_2$], 1.62–1.90 (m, 2H, C**H$_2$**CH), 4.73 (d, 1H, *J* = 8.1, C**H**OH), 4.75 (d, 1H, *J*= 8.1, CHO**H**), 7.20–7.40 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 22.3 (2 × CH$_3$), 24.8 [CH(**C**H$_3$)$_2$], 48.3 [**C**H(CH$_3$)$_2$], 72.8 (CHOH), 127.5, 128.5, 125.9 (3 × aromatic CH), 145.2 (aromatic C) ppm.

## Methyl 3-methyl-2-phenylbutanoate **42**[4]

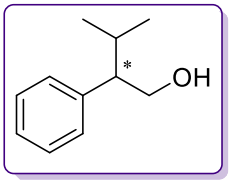


Potassium *tert*-butoxide (8.705 g, 77.7 mmol) was suspended in dry DMF (100 mL) at 0 °C under a nitrogen atmosphere and methyl phenylacetate **43** (9.4 mL, 10 g, 66.6 mmol) was added at once, followed by isopropyl bromide (6.30 mL, 8.25 g, 67.1 mmol) after 2 min. The ice bath was removed and the reaction allowed warm to room temperature (ca. 20 °C) and stirring was continued for 1 h. The reaction was quenched with water (50 mL) and diluted with DCM (40 mL), and stirred overnight. The solution was extracted with DCM (2 × 40 mL). The organic layer was washed with sat. aq. $NH_4Cl$ solution (40 mL) and water (40 mL), dried, filtered and concentrated to give crude **42** as a pale yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether (90/10) as eluent gave pure ester **42** (8.358 g, 68%) as a colorless oil.

$\delta_H$ (300 MHz) 0.70 [d, 3H, *J* = 6.6, one of C(4)H$_3$], 1.03 [d, 3H, *J* = 6.6, one of C(4)H$_3$], 2.25–2.45 [m, 1H, C(3)H], 3.15 [d, 1H, *J* = 10.5, C(2)H], 3.65 (s, 3H, OCH$_3$), 7.18–7.41 (m, 5H, ArH) ppm; $\delta_C$ (75 MHz) 20.2 [one of C(4)H$_3$], 21.5 [one of C(4)H$_3$], 28.0 [C(2)H], 51.7 (OCH$_3$), 60.0 [C(1)], 127.2, 128.4, 128.5 (3 × aromatic CH), 138.4 (aromatic C), 174.4 (C=O) ppm.

All data is in agreement with previously reported data.[4]

## 3-Methyl-2-phenylbutan-1-ol **3**[4]



Methyl 3-methyl-2-phenylbutanoate **42** (8.0 g, 41.6 mmol) in diethyl ether (40 mL) was added slowly to a stirring suspension of $LiAlH_4$ (3.30 g, 87.0 mmol) in diethyl ether (80 mL). The reaction was monitored by TLC and after complete consumption of the ester (6 h), the reaction was quenched with ethyl acetate (40 mL). The solution was stirred, water (40 mL) was added and the volatiles removed. The layers were separated, and the aqueous layer was extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried, filtered and concentrated to give the alcohol **3** (6.83 g, 100%) as a colourless oil, which was used without purification.

The spectroscopic characteristics were identical to the ones listed above.

## Lipase-catalysed reactions

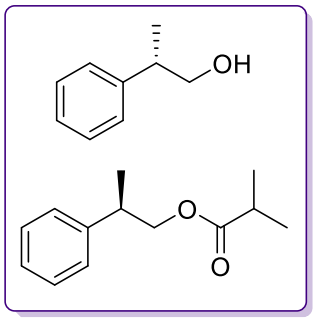
### Lipase-catalysed hydrolysis reactions

In a typical experiment 8 mg of powdered lipase, or 18 mg of immobilised lipase, was added to the ester substrates (50 mg) in 0.1 M phosphate buffer, pH 7 (4.5 mL). Cosolvents (HPLC grade, obtained from Sigma Aldrich) were added (0.77 mL, 17% *v*/*v*) as indicated. The small test tubes were sealed and agitated at 750 rpm at 30 °C for 65 h, unless otherwise stated. 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 conversion was determined by $^{1}$H NMR; the sample was reconcentrated and dissolved in *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity was determined by chiral HPLC analysis.

### Lipase-catalysed transesterification reactions

In a typical experiment 8 mg of powdered lipase, or 18 mg of immobilised lipase, was added to the substrate alcohol (10 mg) in the appropriate vinyl ester (0.5 mL), with crushed 4Å molecular sieves, as indicated. A solvent (HPLC grade, Sigma Aldrich) was added if required and the test tubes were sealed and agitated at 150 rpm for at 30 °C for the times specified in the relevant tables. The reaction mixture was diluted with ether (~5–10 mL), filtered through Celite® and concentrated under reduced pressure. The conversion was determined by $^{1}$H NMR; the sample was reconcentrated and dissolved in *n*-hexane/*i*PrOH (90/10, HPLC grade) to a concentration of ~1 mg/mL and enantioselectivity was determined by chiral HPLC analysis as indicated.

### Preparative scale lipase-mediated hydrolysis of **24**




*Candida antarctica* Lipase B (immobilised) (52 mg, 25% *w*/*w*) was added to a solution of 2-phenylpropyl isobutyrate **24** (200 mg, 1.0 mmol) in phosphate buffer (0.1M, pH 7, 10 mL) and *tert*-butanol (1.7 mL, 17% *v*/*v*). The solution was sealed in a conical flask and shaken at 750 rpm for 72 h. The reaction mixture was filtered through Celite® to remove the lipase and the filter cake was washed with diethyl ether (5 × 10 mL). The resulting solution was separated, and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The organic layers were combined and washed with brine (50 mL) and concentrated to give a crude mixture of alcohol *(S)*-**1** and ester *(R)*-**24** (43% conversion, $ee_s$ = 65%, $ee_p$ = 86%). The mixture

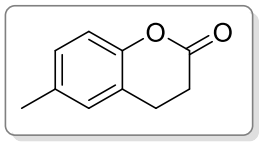
was purified by column chromatography on silica gel using hexane/ethyl acetate as eluent (95/5) to give the pure ester *(R)*-**24** (106 mg, 53%) as a colourless oil $[\alpha]_D^{20}$ −24.2 (c 0.72 in methanol), 70% *ee* and the pure alcohol *(S)*-**1** (45 mg, 34%) as a colourless oil $[\alpha]_D^{20}$ −119.5 (c 0.2 in methanol), 90% *ee*.

$^1$H NMR data for the enantioenriched *(R)*-**24** were identical to that of the racemic sample previously described. Data for *(S)*-**1**:

$\delta_H$ (300 MHz) 1.25 (d, 3H, *J* = 7.1, $CH_3$), 1.73 (broad s, 1H, OH), 2.75–3.05 (sym m, 1H, $CH_3$), 3.65 (d, 2H, *J* = 7.1, C**H**$_2$OH), 7.10–7.43 (m, 5H, ArH) ppm.

## 6.3 Synthesis & resolution of 6-methylchroman-2-ol **51**

### 6-Methylchromanone **75**



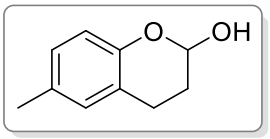
Method 1:[7] Pd/C (10 wt%, 0.072 g) was added to a stirring solution of 6-methylcoumarin **74** (1.030 g, 6.4 mmol) in acetic acid (18.5 mL) and the mixture was stirred under an atmosphere of hydrogen overnight. Reaction completion was shown by disappearance of starting material by TLC (12 h). The crude reaction mixture was filtered through Celite® to remove the catalyst, diluted with water (20 mL) and neutralized by slow addition of solid sodium carbonate. The mixture was extracted with DCM (3 × 25 mL) and the combined organic layers were washed with water (30 mL), brine (30 mL), dried, filtered and concentrated to give the product as a white solid (0.258 g, 25%) which required no further purification (m.p. 78–79 °C, lit. 78–79 °C).[8]

Method 2:[9] Pd/C (10 wt%, 0.556 g, 5 mol%) was added to a stirring solution of 6-methyl coumarin **74** (2.77 g, 16.7 mmol) in ethyl acetate (17.5 mL) and the mixture was stirred under an atmosphere of hydrogen until TLC showed disappearance of starting material (48 h). The suspension was filtered through a bed of Celite® using ethyl acetate (10 mL) and concentrated to give a yellow oil, which solidified on cooling. The solid residue was dissolved in ethyl acetate (10 mL), filtered through Celite®, and concentrated to give a white solid (2.704 g, 97%) which was used without further purification (m.p. 77–78 °C, lit. 78–79 °C).[8]

$\nu_{max}$ (ATR) 1740 (C=O), 1500 (C–C), 1209 (C–O), 810 $cm^{-1}$; $\delta_H$ (300 MHz) 2.31 (s, 3H, $CH_3$), 2.72–2.81 (m, 2H, $CH_2$), 2.90–3.00 (m, 2H, $CH_2$), 6.90–7.10 (m, 3H, ArH); $\delta_C$ (75 MHz) 20.7, 23.8, 29.4, 116.7, 122.3, 128.5, 128.7,134.0, 150.0 (6 × aromatic C), 168.8 (C=O) ppm.

Data for this compound matches previously reported data.[10]

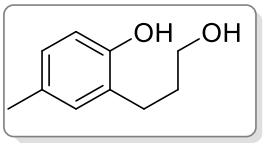
## 6-Methylchroman-2-ol **51**[11]



Method 1: Diisobutylaluminium hydride (1 M in toluene, 5.8 mL, 5.8 mmol) was added dropwise to a solution of 6-methylchromanone **75** (0.84 g, 5.2 mmol) in distilled toluene (18 mL) at −78 °C under an atmosphere of nitrogen. The mixture was stirred at −78 °C for 3 h. TLC showed that the starting material was still present and an additional portion of DIBAL (3 mL, 3.0 mmol) was added, and the reaction mixture stirred for a further 2 h. The reaction was quenched with water (7 mL) and allowed to warm to room temperature. The mixture was filtered through Celite®, and the filter cake washed with diethyl ether (30 mL) and the filtrate layers were separated. The aqueous layer was extracted with diethyl ether (20 mL) and the organic layers were combined. The organic layer was washed with water (50 mL), brine (2 × 50 mL), dried, filtered, and concentrated to give the product as a yellow oil (0.427 g, 50%). A portion of the product (0.297 g) was purified by column chromatography using hexane/ethyl acetate as an eluent (1/1) giving the pure product **51** (0.213 g) as a yellow oil. The other portion of the product was acylated directly without purification.

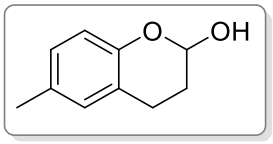
$\nu_{max}$ (ATR) 3401 (OH), 2939 (C–H), 1498, 1206 (C–O), 950, 811 $cm^{-1}$; $\delta_H$ (300 MHz) 1.85–2.11 (m, 2H, $CH_2$), 2.25 (s, 3H, $CH_3$), 2.66 (dt, *J* = 16.4, 5.3, 1H, $CH_2$), 2.82–3.04 (m, 1H, $CH_2$), 3.25 (br s, 1H, OH), 5.58 (br s, 1H, C**H**OH), 6.71 (d, *J* = 8.1, 1H, ArH), 6.78–7.00 (m, 2H, ArH) ppm; $\delta_C$ (75 MHz) 20.3, 20.5, 27.1, 92.1, 116.6, 121.7, 128.0, 129.6, 130.1, 149.7 ppm; HRMS ($ES^+$): $[M-H_2O]^+$ 147.0808 (calculated: 147.0810).

Data for this compound matches previously reported data.[10] The signal at 5.58 ppm sometimes appears as an unresolved quartet (J = 3.0)#.



A minor product (0.109g) was also recovered from the column, $R_f$ = 0.4. It was identified as the diol **76**, corresponding to the reduction of the aldehyde, which exists in equilibrium with the lactol product **51** above. It is believed to have been a result of the extra DIBAL added, and the $^1H$ NMR data matches previously reported data for this compound.[12]

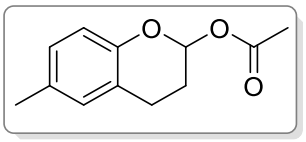
$\delta_H$ (300 MHz) 1.80–1.92 (m, 2H, C**H**$_2$$CH_2$OH), 2.25 (s, 3H, Ar$CH_3$), 2.74 (t, 2H, *J* = 6.9, Ar$CH_2$), 3.63 (t, 2H, *J* = 5.8, C**H**$_2$OH), 6.64–6.80 (m, 1.7 H, 1 × ArH & ArOH), 6.84–6.97 (m, 2H, ArH) ppm; $\delta_C$ (75.5 MHz), 20.5, 25.1, 32.3, 60.8, 115.9, 129.9, 128.0, 126.9, 131.1, 152.2 ppm.



Method 2: Diisobutylaluminium hydride (1 M in toluene, 11.2 mL, 11.2 mmol) was added dropwise to a stirring solution of 6-methylchromanone **75** (1.56 g, 9.6 mmol) and powdered 3Å molecular sieves in distilled toluene (30 mL) at −78 °C under an atmosphere of nitrogen. The mixture was stirred for 6 h. The reaction was quenched with water (5 mL) and allowed to warm to room temperature. The solution was filtered through Celite®, the filter cake washed with diethyl ether (50 mL) and the filtrate layers were separated. The aqueous layer was extracted with diethyl ether (50 mL). The combined organic layer was washed with water (2 × 50 mL), brine (100 mL), dried, filtered, and concentrated to give the product **51** as a colourless oil (0.93 g, 59%).

The spectroscopic data were identical to those reported above.

## 6-Methylchroman-2-yl acetate **62**



Acetic anhydride (0.6 mL, 0.648 g, 6.4 mmol, 6.7 eq.), DMAP (1.1 mg, 0.01 mmol) and pyridine (0.34 mL, 0.334 g, 4.2 mmol, 4.5 eq.) were added to a solution of lactol **51** (154.9 mg, 0.944 mmol) in DCM (3.5 mL). The resulting solution was stirred for 22 h. Sat. aq. $NaHCO_3$ (10 mL) was added to the solution and stirred vigorously until effervescence ceased (approx. 30 mins). The layers were separated, and the aqueous layer was extracted with DCM (10 mL). The combined organic layer was washed with sat. aq. copper sulfate solution (20 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), brine (20 mL), dried, filtered and concentrated to give an orange/yellow oil (0.291 g). Excess pyridine was removed by azetropic distillation with heptane (3 × 10 mL) to give the crude product **62** (0.161 g, 83%) as a dark yellow oil. The product was purified by chromatography on silica gel using hexane/ethyl acetate (5/1) as an eluent, which gave the pure product **62** as a pale yellow oil (0.085 g, 44%).

$\nu_{max}$ (ATR) 2933 (C–H), 1748, (C=O), 1498, 1199, 1179 $cm^{-1}$; $\delta_H$ (300 MHz) 1.90–2.18 (m, 5H, $CH_2$ & $CH_3$), 2.26 (s, 3H, $CH_3$), 2.57–2.74 (m, 1H, $CH_2$), 2.86–3.03 (m, 1H, $CH_2$), 6.50 (t, 1H, *J* = 2.6, 1H, C**H**$OCH_3$), 6.76 (d, 1H, *J* = 8.2, ArH), 6.84–6.98 (m, 2H, ArH) ppm; $\delta_C$ (75.5 MHz) 19.7, 20.5, 21.2, 25.2, 90.3, 116.8, 121.3, 128.2, 129.6, 130.7, 149.3, 169.9 ppm; HRMS ($ES^+$): $[M-OAc]^+$ 147.0816 (calculated: 147.0816); enantiomers separated using Phenomenex Cellulose 4 [conditions: *n*-hexane/*i*PrOH = 95/5, flow rate = 1 mL $min^{-1}$], (−)-**62** $R_t$ = 6.0, (+)-**62**, $R_t$ = 6.5 min.

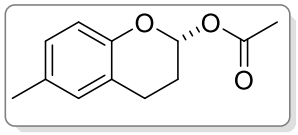
This compound is novel and has been fully characterized in this investigation.

## Enzymatic resolutions

### General procedure for enzymatic transesterification screens

The substrate **51** (10 mg) was added to a small test tube. The acyl source (0.5 mL) and solvent (2 mL, if applicable) were then added, along with a spatula tip of enzyme (~8mg powdered, or 18 mg immobilised). The reaction was sealed and incubated in a mini-shaker at 30 °C, at 150 rpm. When the stipulated time period had elapsed, the solution was passed through a Pasteur pipette containing a layer each of Celite® and $MgSO_4$, using diethyl ether as eluent. The solvent was removed under reduced pressure and the resulting crude mixture was analysed by $^1H$ NMR spectroscopy for conversion data and chiral HPLC for enantioselectivity.

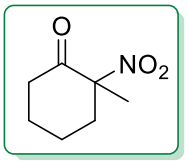
### Preparative scale resolution of (+)-6-methylchroman-2-yl acetate (+)-62



6-Methylchromanol **51** (50 mg) was added to a small test tube. Vinyl acetate (50 eq.) and hexane (4 mL) were added to a test tube with lipase from *Thermomyces lanuginosus* (40 mg) as the biocatalyst. The test tube was sealed, and the reaction was incubated at 30 °C for 4 days. The crude reaction mixture was passed through a Pasteur pipette, as above, and the solvent was removed in under reduced pressure. $^1H$ NMR analysis of the crude mixture indicated a 65% conversion. The crude mixture was purified using column chromatography with hexane/ethyl acetate (5/1) as eluent to give pure **62** in 32% yield and 94% *ee*. $[\alpha]_D^{20}$= +26.7 (c 0.2, $CHCl_3$).

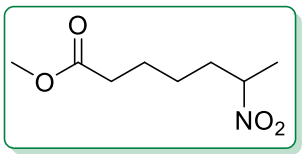
# 6.4 Synthesis & resolution of 2-methyl-2-nitrocyclohexanol **85a** & **85b**

## 2-Methyl-2-nitrocyclohexanone **89**[13]



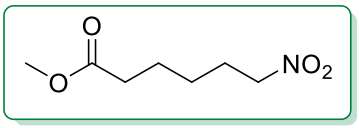

2-Nitrocyclohexanone **88** (2.09 g, 14.6 mmol) in DCM (19 mL) was added in one portion to a vigorously stirring solution of tetrabutylammonium hydroxide (11.33 g, of a 40% aq. solution, 17.5 mmol) in water (17 mL) under nitrogen. The reaction mixture was stirred for 10 min, and then methyl iodide (4.4 mL, 10.03 g, 70.7 mmol) was added in one portion. The reaction mixture was stirred vigorously for 48 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 DCM (2 × 30 mL) and the combined organic fractions were dried, filtered and concentrated under reduced pressure at room temperature. Diethyl ether (50 mL) was added to precipitate the tetrabutylammonium iodide salt, the supernatant was filtered and concentrated under reduced pressure at room temperature to give a crude mixture (2.08 g) of 2-methyl-2-nitrocyclohexanone **89** and ring cleavage products, methyl 6-nitrohexanoate **90** and methyl 6-nitroheptanoate **91** (77:15:8 respectively) as a viscous brown oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure α-nitro ketone **89** (0.754 g, 33%) as a colourless oil.

$\nu_{max}$ (ATR) 2946, 2872, 1726 (C=O), 1543 ($NO_2$), 1386 ($NO_2$) $cm^{-1}$; $\delta_H$ (300 MHz) 1.67 [s, 3H, C(2)$CH_3$], 1.69–1.85 (m, 4H, ring protons), 1.98–2.10 (m, 1H, ring protons), 2.55–2.64 (m, 2H, ring protons), 2.86–2.90 (1H, m, ring protons); $\delta_C$ (75.5 MHz) 21.5, 22.4, 26.6, 38.4, 39.2, 93.9, 200.7 ppm.




The second fraction isolated from the column was methyl 6-nitroheptanoate **91** as a pale yellow oil (269 mg).

$\nu_{max}$ (ATR) 2951, 1733 (C=O), 1546 ($NO_2$), 1390 ($NO_2$) $cm^{-1}$; $\delta_H$ (300 MHz) 1.30–1.45 (m, 1H, $CH_2$), 1.53 (d, 3H, $J$ = 6.6, $CH_3$), 1.57–1.83 [m, 3H, C(5)H & C(3)$H_2$], 1.91–2.13 [m, 1H, C(5)H], 2.32 [t, 2H, $J$ = 7.4, C(2)$H_2$], 3.67 (s, 3H, $OCH_3$), 4.47–4.67 [sym m, 1H, C(6)H] ppm; $\delta_C$ (75.5 MHz) 19.2, 24.2, 25.2, 33.6, 34.7, 51.5, 83.3, 173.6 ppm.

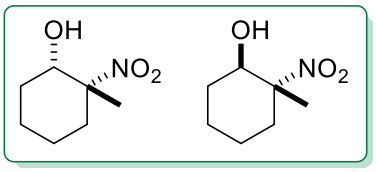


The third fraction isolated from the column was methyl 6-nitrohexanoate **90** as a yellow oil (197 mg).

$\nu_{max}$ (ATR) 2951, 1733 (C=O), 1548 ($NO_2$), 1382 ($NO_2$) $cm^{-1}$; $\delta_H$ (300 MHz) 1.33–2.19 (m, 5H, alkyl CH), 2.34 [t, 2H, *J* = 7.4, C(5)$H_2$], 3.68 (s, 3H, $OCH_3$), 4.39 [t, 2H, *J* = 7.0, C(2)$H_2$] ppm; $\delta_C$ (75.5 MHz) 24.2, 25.7, 27.0, 33.5, 51.6, 75.4, 173.6 ppm.

Data is consistent with literature values.[14]

## 2-Methyl-2-nitrocyclohexanol **82a** & **82b**[15]



A solution of 2-methyl-2-nitrocyclohexanone **89** (1.01 g, 6.4 mmol) in ethanol (11 mL) was added dropwise over 10 min to a stirred suspension of $NaBH_4$ (0.267 g, 7.1 mmol) in ethanol (11 mL) at 0 °C under nitrogen and stirring was continued for 1.5 h at 0 °C. The ice bath was then removed and aq. HCl (10%) was added to adjust to pH 1. The solution was concentrated under reduced pressure and the resulting residue was partitioned between water (20 mL) and DCM (20 mL). The aqueous phase was extracted with DCM (3 × 20 mL) and the combined organic extracts were washed with brine (30 mL), dried, filtered and concentrated under reduced pressure to give a crude mixture (1.00 g) of nitroalcohols *cis*-**82a** and *trans*-**82b** (3:1 respectively) as a yellow oil. Purification by column chromatography on silica gel using hexane/diethyl ether 60/40 as eluent gave *cis*-2-methyl-2-nitrocyclohexanol **82a** (0.450 g, 44%) as a viscous light yellow oil.

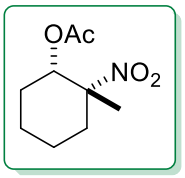
$\nu_{max}$ (ATR) 3429 (OH), 2944 (C–H), 1533 ($NO_2$), 1357 ($NO_2$) $cm^{-1}$; $\delta_H$ (300 MHz) 1.36–1.57 [m, 3H, one of C(5)$H_2$ & C(4)$H_2$], 1.63 [s, 3H, C(2)$CH_3$], 1.66–1.94 [m, 4H, one of C(5)$H_2$, one of C(3)$H_2$ and C(6)$H_2$], 2.46 [dt, 1H, *J* = 13.9, 6.0, one of C(3)$H_2$], 2.79 [d, 1H, *J* = 8.3, OH], 3.91 [td, 1H, *J* = 8.0, 3.6, C(1)H] ppm; $\delta_C$ (75.5 MHz) 21.6 [$CH_2$, C(5)], 21.9[$CH_2$, C(4)], 24.2[$CH_3$, C(2)**C**$H_3$], 30.6 [$CH_2$, C(6)], 33.0 [$CH_2$, C(3)], 73.1[CH, C(1)], 91.4 [qC, C(2)] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 40.1 min, 44.1 min.

A fraction containing both *cis*-**82a** and *trans*-**82b** (70:30) was also isolated, this was used for enzymatic screens, as it is approximately equal to the thermodynamic ratio which is achieved when subjecting equimolar mixture of *cis*-**82a** and *trans*-**82b** to the interconversion conditions.

*trans*-2-Methyl-2-nitrocyclohexanol **82b** (81 mg, 8%) was isolated as a colourless oil.

$\nu_{max}$ (ATR) 3429 (OH), 2944 C–H), 1537 ($NO_2$), 1357 ($NO_2$) $cm^{-1}$; $\delta_H$ (300 MHz) 1.29–1.53 [m, 3H, one of C(4)$H_2$, one of C(5)$H_2$ and one of C(6)$H_2$], 1.61 [s, 3H, C(2)$CH_3$], 1.66–2.05 [m, 4H, one of C(4)$H_2$ & one of C(5)$H_2$ & one of C(3)$H_2$ & one of C(6)$H_2$], 2.05–2.23 [m, 1H, one of C(3)$H_2$], 2.77 [br s, 1H, OH], 4.21–4.34 [m, 1H, C(1)H] ppm; $\delta_C$ (75.5 MHz) 16.3 [$CH_3$, br, C(2)*C*$H_3$], 22.1 [$CH_2$, C(4)$H_2$], 23.5 [$CH_2$, C(5)$H_2$], 30.6 [$CH_2$, C(6)$H_2$], 35.9 [$CH_2$, br, C(3)$H_2$], 72.4 [CH, C(1)H], 93.0 [qC, C(2)] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 47.1 min, 53.5 min.

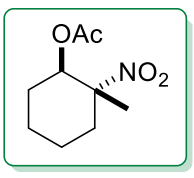
## *cis*-2-Methyl-2-nitrocyclohexyl acetate **87a**




DMAP (1 mg) was added to a stirring solution of *cis*-2-methyl-2-nitrocyclohexanol **82a** (41 mg, 0.3 mmol), acetic anhydride (0.15 mL, 1.6 mmol) and pyridine (0.1 mL, 1.2 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 21 h. Saturated aq. $NaHCO_3$ (3 mL) was added and the mixture stirred for 30 min. The solution was transferred to a separating funnel and washed with sat. aq. $CuSO_4$ (6 mL), water (6 mL), sat. aq. $NaHCO_3$ (6 mL) and brine (6 mL). The organic extract was dried, filtered and concentrated under reduced pressure to give crude acetate **87a** (27 mg, 51%) as a colourless oil which was sufficiently pure to use without further purification.

$\delta_H$ (300 MHz) 1.33–1.56 [m, 3H, C(5)$H_2$, and one of C(4)$H_2$], 1.65 [s, 3H, C(2)$CH_3$], 1.59–1.90 [m, 2H, one of C(4)$H_2$ and one of C(6)$H_2$], 2.03 [s, 3H, $COCH_3$], 1.91–2.22 [m, 3H, one of C(6)$H_2$ and C(3)$H_2$], 5.53 [dd, 1H, *J* = 9.9, 4.5, C(1)H] ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 23.5 min, 26.8 min.

## *trans*-2-Methyl-2-nitrocyclohexyl acetate **87b**




This was prepared following the procedure for **82a**, from DMAP (1 mg), *trans*-2-methyl-2-nitrocyclohexanol **82b** (43 mg, 0.3 mmol), acetic anhydride (0.15 mL, 1.6 mmol) and pyridine (0.1 mL, 1.2 mmol) in DCM (2 mL) to give the crude acetate **87b** (26 mg, 48%) as a light yellow oil which was sufficiently pure to use without further purification.

$\delta_H$ (300 MHz) 1.34–1.57 [m, 3H, one of C(4)$H_2$, one of C(5)$H_2$ and one of C(6)$H_2$], 1.61 (s, 3H, $CH_3$), 1.62–1.93 [m, 2H, one of C(5)$H_2$ and one of C(4)$H_2$], 2.02 (s, 3H, $OCH_3$), 1.95–2.14 [m, 3H, C(3)$H_2$ and one of C(6)$H_2$], 5.29–5.40 [m, 1H, C(1)H]; enantiomers separated using

Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH = 98.5/1.5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 17.8 min, 19.1 min.

## Lipase-mediated kinetic resolution

*General procedure for the development of one-pot procedures: lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol 82a or 82b with vinyl acetate as acetylating agent and dynamic interconversion process (analytical scale)*

In a typical experiment, the following were added as appropriate to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** (typically 15 mg) in the appropriate solvent (10 mg/mL): DBU (immob) (0.5 eq.) (18 mg) or vinyl acetate, and CAL-B (immob) (approx. 15 mg). The small test tube was sealed and agitated as appropriate for the specified length of time (generally 12 h). The solutions were filtered through Celite® and concentrated under reduced pressure. The sample was analysed by $^1$H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

*General procedure for the development of two-pot procedures: dynamic interconversion process and lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol cis-82a and trans-82b with vinyl acetate 65 as acetylating agent (analytical scale)*

In a typical experiment, DBU (immob) (0.5 eq., ~90 mg) was added to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** in toluene (5 mL, 10 mg/mL) and the test tube was sealed and shaken at 500 rpm for the required amount of time at the specified temperature. The reaction solution was filtered through Celite®, concentrated under reduced pressure and redissolved in the appropriate amount of solvent, if applicable. CAL-B (immob) and vinyl acetate were added and the solution shaken at the appropriate speed and temperature for the required amount of time in a sealed test tube. The solution was filtered through a plug of Celite® and concentrated under reduced pressure if applicable. The samples were taken before each filtration and filtered through Celite®, concentrated under reduced pressure. The samples were analysed by $^1$H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

*General procedure for the development of one-pot procedures: dynamic interconversion process and lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol cis-82a and trans-82b with vinyl acetate as acetylating agent (analytical scale)–cycling procedure*

In a typical experiment, immobilised DBU (0.5 eq., ~90 mg) was added to a solution of nitroalcohols *cis*-**82a** and *trans*-**82b** in toluene (5 mL, 10 mg/mL) and the test tube was sealed and shaken at 50 °C, 500 rpm for the required amount of time. CAL-B (immob) (approx. 100 %

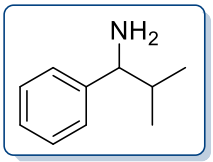
w/w) and vinyl acetate **65** (3.0 eq.) was added and the test tube was sealed, shaken at 400 rpm for 12 h at 30 °C. At the end of the cycle the solution was filtered through a plug of Celite® if appropriate and the filtrate was recycled through the process again. Before the addition of each reagent an aliquot was taken, filtered through Celite®, concentrated under reduced pressure. The samples were analysed by $^1$H NMR spectroscopy, reconcentrated and dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

*Diastereoselective lipase-mediated transesterification of 2-methyl-2-nitrocyclohexanol 82*

CAL-B (immob) (88.6 mg) was added to an equimolar mixture of *cis*- and *trans*-2-methyl-2-nitrocyclohexanol (±)-**82a** and (±)-**82b** (105.4 mg, 0.7 mmol) dissolved in vinyl acetate (5 mL). The reaction mixture was shaken at 750 rpm at room temperature. Reaction monitoring was conducted as follows: an aliquot (0.5 mL) of reaction mixture was isolated and filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure and the sample was analysed by $^1$H NMR spectroscopy. The final extraction following $^1$H NMR spectroscopy was dissolved in a mixture of *n*-hexane/*i*PrOH (90/10, HPLC grade) and enantioselectivity determined by chiral HPLC analysis.

## 6.5 Synthesis of transaminase substrates

### 2-Methyl-1-phenylpropan-1-amine **114**

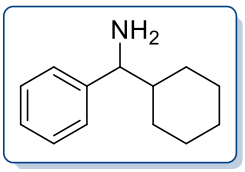



A mixture of 2-methylpropiophenone (0.740 g, 5.0 mmol), titanium(IV) ethoxide (2.1 mL, 2.28 g, 10.0 mmol) and ammonia (2 M in methanol, 12.5 mL, 25.0 mmol) was stirred under nitrogen, at ambient temperature for 15 h. Sodium borohydride (0.288 g, 7.6 mmol) was added and the resulting mixture was stirred at room temperature for an additional 3 h. The reaction was quenched by pouring onto ammonium hydroxide (2 M, 12.5 mL), the resulting inorganic precipitate was removed by filtration, and washed with ethyl acetate (2 × 15 mL). The aqueous solution was extracted with ethyl acetate (2 × 15 mL). The combined organic solution was extracted with aq. HCl (10%, 15 mL). The acidic aqueous extracts were washed with ethyl acetate (25 mL), then treated with aq. NaOH (2 M) to pH 10–12 and extracted with ethyl acetate (3 × 25mL). The combined organic extracts were washed with brine (25 mL), dried ($Na_2SO_4$), filtered and concentrated to give the primary amine **114** as a colourless oil (0.368 g, 49%), which required no further purification.

$\nu_{max}$ (ATR) 2957, 2870, 1465, 1451, 701 $cm^{-1}$; $\delta_H$ (300 MHz) 0.77 (d, 3H, *J* = 6.7, $CH_3$), 0.98 (d, *J* = 6.7, 3H, $CH_3$),1.50 (br s, 2H, $NH_2$), 1.75–1.96 (sym m, 1H, C**H**$(CH_3)_2$), 3.60 (d, 1H, *J* = 7.3, C**H**$NH_2$), 7.06–7.53 (m, 5H, ArH) ppm; $\delta_C$ (75.5 MHz) 18.9 ($CH_3$), 19.8, 35.5, 62.5, 126.8, 127.0, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm.

All data is in agreement with previously reported data.[16]

## Cyclohexyl(phenyl)methanamine **115**



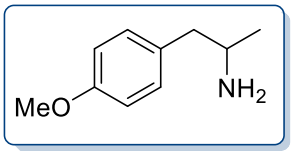

Prepared following the procedure for **114** using cyclohexyl(phenyl)ketone (1.878 g, 10.0 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.567 g, 15.0 mmol) to give the product amine **115** as a white solid (0.437 g, 23%), which required no further purification, (m.p. 99–102 °C).

$\nu_{max}$ (ATR) 3421, 2922, 2849, 1552, 1453, 1357, 704, 528 $cm^{-1}$; $\delta_H$ (300 MHz) 0.73–1.71 (m, 12H, ring CH & $NH_2$), 1.71–1.83 (m, 1H, ring CH), 1.86–2.04 (m, 1H, ring CH), 3.60 (d, 1H, *J* = 7.5, C**H**$NH_2$), 7.10–7.48 (m, 5H, ArH) ppm; $\delta_C$ (75.5 MHz) 26.2, 26.4, 29.5, 30.1 (4 × $CH_2$), 45.2, 61.7 (2 × CH), 126.8, 127.1, 128.2 (3 × aromatic CH), 145.5 (aromatic C) ppm.

$^{13}C$ signal at 145.5 ppm is very weak and was confirmed by HMBC correlation experiments. Carbon assignments were aided by DEPT experiments.

All data is in agreement with previously reported data.[17] Melting point was not previously reported.

## 1-(4-Methoxyphenyl)propan-2-amine **121**[18]



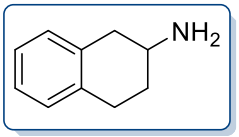

Prepared following the procedure for **114** using 4′-methoxyphenylacetone **148** (1.640 g, 10.0 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.575 g, 15.2 mmol) to give the product amine **121** as a pale yellow oil (0.559 g, 34%), which required no further purification.

$\nu_{max}$ (ATR) 2957, 2835, 1611, 1510, 1242, 1176, 1033, 802 $cm^{-1}$; $\delta_H$ (300 MHz) 1.10 (d, 3H, *J* = 6.3, $CH_3$), 2.46 (dd, 1H, *J* = 13.4, 8.0, one of $CH_2$), 2.65 (dd, 1H, *J* = 13.4, 5.3, one of $CH_2$), 3.04–3.21 (m, 1H, C**H**$NH_2$), 3.79 (s, 3H, $OCH_3$), 6.70–6.94 (m, 2H, ArH), 7.02–7.33 (m, 2H, ArH) ppm; $\delta_C$ (75.5 MHz) 23.5, 45.8, 48.6, 55.3, 113.8, 130.2, 131.8, 158.1 ppm; enantiomers separated

using Amylose 1 column [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.4 mL $min^{-1}$], $R_t$ = 16.0, $R_t$ = 16.8 min, $R_t$ (ketone) = 14.4 min.

All data is in agreement with previously reported data.[19]

## 1,2,3,4-Tetrahydronaphthalen-2-amine **130**

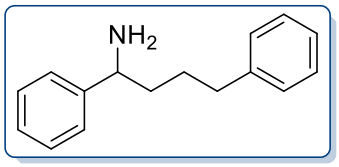



Prepared following the procedure for **114** using 2-tetralone **131** (1.472 g, 10.1 mmol), titanium(IV) ethoxide (4.2 mL, 4.56 g, 20.0 mmol), ammonia (2 M in methanol, 25 mL, 50.0 mmol) and sodium borohydride (0.584 g, 15.4 mmol) to give the product amine **130** as a dark green oil (0.713 g, 48%), which was used without further purification.

$\nu_{max}$ (ATR) 3354, 2918, 2841, 741 $cm^{-1}$; $\delta_H$ (300 MHz) 1.51–1.74 [m, 3H, one of C(3)$H_2$ and $NH_2$ (1.66, br s)], 1.91–2.10 [m, 1H, one of C(3)$H_2$], 2.56 [dd, 1H, *J* = 16.1, 9.4, one of C(1)$H_2$], 2.77–2.94 [m, 2H, C(4)$H_2$], 3.00 [ddd, 1H, *J* = 16.2, 5.0, 1.3, one of C(1)$H_2$], 3.18 (tdd, 1H, *J* = 9.5, 5.0, 3.1, C**H**$NH_2$), 6.87–7.22 (m, 4H, ArH) ppm; $\delta_C$ (75.5 MHz) 28.1, 33.0, 39.5 (3 × $CH_2$), 47.3 (CH), 125.7, 125.8, 128.7, 129.3 (4 × aromatic CH), 135.3, 135.9 (2 × aromatic C) ppm.

All data is in agreement with previously reported data.[20] Proton and carbon assignments were aided by 2D NMR and DEPT experiments.

## 1,4-Diphenylbutan-1-amine **116**

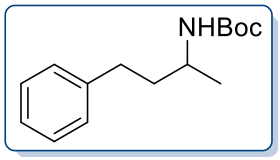



Prepared following the procedure for **114** using 1,4-diphenylbutanone (0.451 g, 2.0 mmol), titanium(IV) ethoxide (0.84 mL, 0.91 g, 4.0 mmol), ammonia (2 M in methanol, 5 mL, 10.0 mmol) and sodium borohydride (0.122 g, 3.2 mmol) to give the product amine **116** as a colourless oil (0.074 g, 16%), which required no further purification.

$\nu_{max}$ (ATR): 2934, 1265, 732, 699 $cm^{-1}$; $\delta_H$ (300 MHz) 1.38–1.98 [m, 6H, 2 × $CH_2$ & $NH_2$ (1.69, s)], 2.48–2.71 (m, 2H, $CH_2$), 3.77–3.97 (m, 1H, C**H**$NH_2$), 6.91–7.51 (m, 10H, ArH) ppm; $\delta_C$ (75.5 MHz), 28.4, 35.8, 39.1 (3 × $CH_2$), 56.3 (CH), 125.7, 126.4, 127.0, 128.3, 128.4, 128.5 (6 × aromatic CH), 142.3, 146.3 (2 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 226.1589 (calculated: 226.1596).

This compound has been fully characterized in this investigation.

## *tert*-Butyl-(4-phenylbutan-2-yl)carbamate **158**[21]

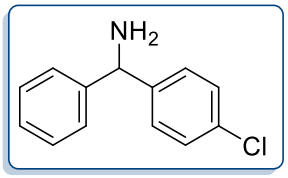



A solution of 4-phenyl-2-aminobutane **120** (16.18 µL, 0.1 mmol) in DCM (2 mL) was added to a solution of di-*tert*-butyl-dicarbonate (21.83 mg, 0.1 mmol, 1.0 eq.) in DCM (2 mL). The reaction solution was stirred for 3 h at room temperature. The solvent was evaporated to give the crude N-Boc-amine **158** as a colourless oil which was used without further purification for chiral HPLC method development.

$\delta_H$ (300 MHz) 1.16 [d, 3H, *J* = 6.2, C(1)$H_3$], 1.45 [s, 9H, C($CH_3$)$_3$], 1.62–1.82 (m, 2H, $CH_2$), 2.55–2.80 (m, 2H, $CH_2$), 3.72 (br s, 1H, C**H**$NH_2$), 4.37 (br s, 1H, $NH_2$), 7.07–7.37 (m, 5H, ArH) ppm.

All data is in agreement with previous reports.[22]

## (4′-Chlorophenyl)(phenyl)methanamine **118**

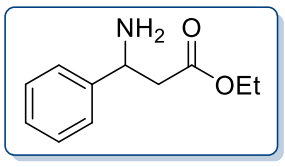



Prepared by Gavin following the procedure for **114** using 4′-chlorobenzophenone (1.08 g, 5.0 mmol), titanium(IV) ethoxide (2.1 mL, 2.28 g, 10.0 mmol), ammonia (2 M in methanol, 12.5 mL, 25.0 mmol) and sodium borohydride (0.3 g, 8.0 mmol) to give the product **118** as a colourless oil.

$\delta_H$ (300 MHz) 1.73 (v br s, 2H, $NH_2$), 5.12 (br s, 1H, C**H**$NH_2$), 7.49–7.09 (m, 9H, ArH) ppm;

All data is in agreement with previously reported data.[23]

## Ethyl 3-amino-3-phenylpropanoate **117**



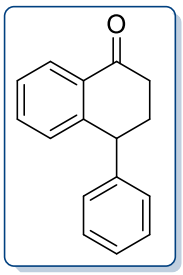

Sulfuric acid (conc., 2.4 mL, 4.42 g, 45.0 mmol) was added to a solution of 3-amino-3-phenylpropanoic acid **133** (2.48 g, 15.0 mmol) in absolute ethanol (50 mL) and heated under reflux for 24 h. Excess ethanol was evaporated under reduced pressure and the crude product was dissolved in DCM (50 mL) and washed with water (2 × 50 mL), sat. aq. $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried, filtered and concentrated to give the ester **117** as a colourless oil (0.999 g, 34%) which was used without further purification.

$\nu_{max}$ (ATR) 3381, 1726 (C=O), 1178, 1031, 699, 539 $cm^{-1}$; $\delta_H$ (300 MHz) 1.23 (t, 3H, *J* = 7.1, $CH_2$C**H**$_3$), 1.77 (br s, 2H, $NH_2$), 2.66 (d, 2H, *J* = 6.9, $COCH_2$), 4.14 (q, 2H, *J* = 7.1, OC**H**$_2$$CH_3$), 4.42

(dd appears as a t, 1H, *J* = 6.8, C**H**$NH_2$), 7.14–7.48 (m, 5H, ArH) ppm; $\delta_C$ (75.5 MHz) 14.2, 44.2, 52.6, 60.6, 126.2, 127.4, 128.6, 144.7 (4 × aromatic C), 172.1 (C=O) ppm.

All data is in agreement with previously reported data.[24]

## 4-Phenyl-3,4-dihydronaphthalen-1(*2H*)-one **134**[25]

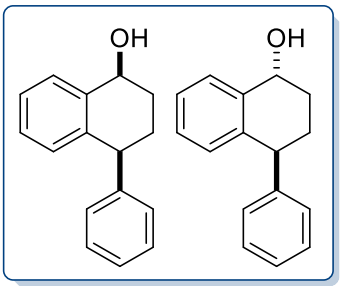


Triflic acid (50 g, 333.0 mmol) was added slowly to a stirring solution of *trans*-styrylacetic acid **142** (11.35 g, 70.0 mmol) and benzene (6.3 mL, 5.47 g, 70.0 mmol) in DCM (50 mL), at 0 °C. The reaction solution was allowed to warm to room temperature and stirred for 24 h. The reaction solution was quenched by pouring onto a mixture of water and ice (50 mL) and the layers separated. The solution was extracted with DCM (3 × 50 mL), and the combined extracts were dried, filtered, and concentrated to give the crude product (14.808 g) which was purified by column chromatography using hexane/ethyl acetate (90/10) as eluent to give the pure product **134** (5.53 g, 36%) as a white solid (m.p.: 69–72 °C, lit.: 70–72 °C).[26]

$\nu_{max}$ (ATR) 2917 (C-H), 1682 (C=O), 762, 702 $cm^{-1}$; $\delta_H$ (300 MHz) 2.20–2.38 [m, 1H, one of C(3)$H_2$], 2.38–2.54 [m, 1H, one of C(3)$H_2$], 2.54–2.81 [sym m, 2H, C(2)$H_2$], 4.30 [dd, 1H, *J* = 8.0, 4.6, 1H, C(4)H], 6.98 (d, 1H, *J* = 7.7, 1H, ArH), 7.06–7.16 (m, 2H, ArH), 7.19–7.49 (m, 5H, ArH), 8.12 (dd, 1H, *J* = 7.6, 1.1, ArH) ppm; $\delta_C$ (75.5 MHz) 31.9 [C(3)$H_2$], 36.8 [C(2)$H_2$], 45.3 [C(4)H], 126.8, 127.06, 127.13, 128.6, 128.7, 129.6 (6 × aromatic CH), 132.9 (aromatic C), 133.6 (aromatic CH), 143.7, 146.3 (2 × aromatic C), 198.1 (C=O) ppm; enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 18.5 min; 31.9 min.

All data is in agreement with previously reported data. [26,27]

## *cis*-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-ol *cis*-**137a** & *trans*-4-phenyl-1,2,3,4-tetrahydronaphthalen-1-ol *trans*-**137b**[28]



Sodium borohydride (0.697 g, 18.4 mmol) was added to a stirring solution of ketone **134** (3.823 g, 16.7 mmol) in methanol (50 mL) at 0 °C. The solution was allowed to slowly warm to room temperature and was stirred overnight. The reaction mixture was acidified to pH = 5 using aq. HCl (10%), and the volatiles were removed by rotary evaporation. The

solution was extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with brine (50 mL), dried, filtered and concentrated to give the crude material as a viscous yellow oil (4.533 g) containing a mixture of *cis*-**137a** and *trans*-**137b** diastereomers (46:54). The product *trans*-**137b** (1.46 g, 39%) was obtained as a white solid (m.p.: 121–122 °C, lit.: 122–123 °C)[29] by recrystallisation of the crude mixture from diethyl ether and hexane. The *cis*-enriched material (24:76) which remained in the mother liquor was subjected to column chromatography using diethyl ether/hexane (15/85) as eluent to give the pure product *cis*-**137a** as a colourless oil (0.754 g, 20%).

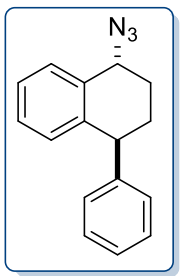
*cis*-**137a**: $\nu_{max}$ (ATR) 3307, 760, 731, 699 $cm^{-1}$; $\delta_H$ (300 MHz) 1.81 (br s, 1H, OH), 1.89–2.25 [m, 4H, C(2)$H_2$ & C(3)$H_2$], 4.02 [dd, 1H, *J* = 8.3, 5.6, C(4)H], 4.87 [br s, 1H, C(1)H], 6.85 (d, 1H, *J* = 7.7, ArH), 7.07–7.38 (m, 7H, ArH), 7.46 (m, 1H, ArH) ppm; $\delta_C$ (75.5 MHz) 28.3 ($CH_2$), 30.3 ($CH_2$), 45.8 [C(4)H], 68.2 [C(1)H], 126.3, 126.6, 127.9, 128.4, 128.8, 128.9, 130.0 (7 × aromatic CH), 139.0, 139.8, 146.6 (3 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 225.1279 (calculated: 225.1274).

$^1$H NMR data matches previously reported data.[30] $^{13}$C NMR data was not previously reported and assignments were aided by DEPT and 2D experiments.

*trans*-**137b**: $\nu_{max}$ (ATR) 3230 (OH), 745, 696 $cm^{-1}$; $\delta_H$ (300 MHz) 1.66–1.96 [m, 3H, one of C(2)$H_2$ and one of C(3)$H_2$ and OH (1.73, d, *J* = 6.0)], 2.08–2.25 [m, 1H, one of C(2)$H_2$], 2.27–2.43 [m, 1H, one of C(3)$H_2$], 4.13–4.22 [m, 1H, C(4)H], 4.85–4.96 [m, 1H, C(1)H], 6.88 (d, 1H, *J* = 7.9, ArH), 6.99–7.06 (m, 2H, ArH), 7.09–7.37 (m, 5H, ArH), 7.55 (d, 1H, *J* = 7.6, ArH) ppm; $\delta_C$ (75.5 MHz) 29.2 [C(3)$H_2$], 30.3 [C(2)$H_2$], 45.3 [C(4)H], 68.5 [C(1)H], 126.1, 126.7, 127.7, 127.9, 128.3, 128.7, 130.2 (7 × aromatic CH), 139.1, 139.7, 146.5 (3 × aromatic C) ppm; elemental analysis (Found C 85.44; H 7.17. $C_{16}H_{16}O$ requires C 85.68, H 7.19%).

$^1$H NMR data matches previously reported data.[30] $^{13}$C NMR data was not previously reported and assignments were aided by DEPT spectra and 2D experiments.

## *trans* 1-Azido-4-phenyl-1,2,3,4-tetrahydronaphthalene *cis*-**140b**[31]



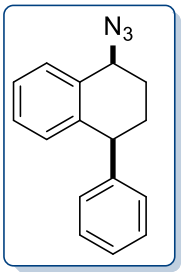

Diphenylphosphoryl azide (1.05 mL, 1.34 g, 4.9 mmol, 1.2 eq.) was added to a solution of *cis*-**137a** (0.914 g, 4.1 mmol) in dry toluene (10 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.73 mL, 0.74 g, 4.9 mmol) was added dropwise to the stirring solution. The mixture was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in

DCM/hexane (1/2) and filtered through a short silica gel pad (approx. 100 mL solvent used), and the solvent was removed in vacuo to give the crude product as a yellow oil (1.065 g). The product was purified by column chromatography using hexane/ethyl acetate (95/5) as eluent to give the pure product *trans*-**140b** (902 mg, 90%) as a colourless oil.

$\nu_{max}$ (ATR) 2939, 2091, 1491, 1239, 749, 700 $cm^{-1}$; $\delta_H$ (300 MHz) 1.80–1.96 [2H, m, one of C(2)$H_2$ and one of C(3)$H_2$], 2.05–2.23 [1H, m, one of C(2)$H_2$], 2.30–2.49 [1H, m, one of C(3)$H_2$], 4.22 [t, 1H, *J* = 5.8, C(4)H], 4.67 [t, 1H, *J* = 5.4, C(1)$HN_3$], 6.94 (d, 1H, *J* = 7.8, ArH), 6.96–7.03 (m, 2H, ArH), 7.15–7.32 (m, 5H, ArH), 7.38–7.45 (m, 1H, ArH) ppm; $\delta_C$ (75.5 MHz) 26.1 ($CH_2$), 28.9 ($CH_2$), 44.5 [C(4)H], 59.7 [C(1)$HN_3$], 126.2, 126.7, 128.32, 128.34, 128.63, 128.64, 130.7 (7 × aromatic CH), 134.6, 139.3, 146.4 (3 × aromatic C) ppm; HRMS ($ES^+$): $[M-N_3]^+$ 207.1168 (calculated: 207.1170).

This compound is novel and has been fully characterized in this investigation. NMR assignments were aided by DEPT and 2D experiments.

## *cis*-1-Azido-4-phenyl-1,2,3,4-tetrahydronaphthalene *cis*-**140a**[31]

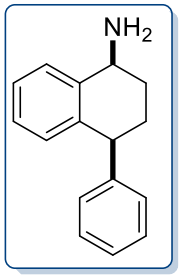



Diphenylphosphoryl azide (1.30 mL, 1.66 g, 6.0 mmol, 1.2 eq.) was added to a solution of *trans*-**137b** (1.131 g, 5.0 mmol) in dry toluene (12.5 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.90 mL, 0.918 g, 6.0 mmol, 1.2 eq.) was added dropwise to the stirring solution. The solution was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed in vacuo and the residue was dissolved in DCM/hexane (1/1) and filtered through a short silica gel pad (approx. 150 mL solvent used), and the solvent was removed in vacuo to give the crude product as a brown oil (4.01 g). The product was purified by column chromatography using hexane/ethyl acetate (95/5) as eluent to give the pure product *cis*-**140a** (966 mg, 77%) as a yellow solid (m.p.: 80–82 °C).

$\nu_{max}$ (ATR) 2943 (CH), 2091 ($N_3$), 1486, 755, 699 $cm^{-1}$; $\delta_H$ (300 MHz) 1.85–2.32 [m, 4H, C(2)$H_2$ and C(3)$H_2$], 4.04 [t, 1H, *J* = 6.7, C(4)H], 4.64 [t, 1H, *J* = 4.7, C(1)H], 6.89 (d, 1H, *J* = 7.7, ArH), 7.03–7.42 (m, 8H, ArH) ppm; $\delta_C$ (75.5 MHz) 28.1 ($CH_2$), 28.9 ($CH_2$), 45.6 [C(4)H], 59.7 [C(1)H], 126.4, 126.5, 128.5, 128.8, 129.3, 130.4 (6 × signals for 7 × aromatic CH), 133.9, 140.2, 146.3 (3 × aromatic C) ppm; elemental analysis: (Found C 77.06; H 6.13; N 16.55. $C_{16}H_{15}N_3$ requires C 77.08; H 6.06; N 16.85%).

This compound is novel and has been fully characterized in this investigation.

## *cis*-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-amine *cis*-**93a**[32]

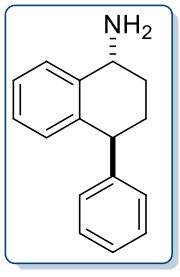



Polystyrene-bound triphenyl phosphine [4.296 g (1.4–2.0 mmol/g loading), ~7.3 mmol, 2.0 eq.] was added to a solution of *cis*-**130a** (0.854 g, 3.4 mmol) in dry THF (50 mL) and stirred for 16 h. Water (10 mL) was added and the reaction solution stirred for an additional 4 h. The reaction mixture was filtered and extracted with DCM (3 × 50 mL). The combined organic layers were dried ($Na_2SO_4$) and concentrated under reduced pressure to give a mixture of the azide *cis*-**130a** and the amine *cis*-**93a**. The reaction product and the polymer-bound catalyst were resuspended in THF (50 mL) and water (5 mL) and stirred for 4d, after which *cis*-**130a** was still evident by IR. The reaction solution was heated to reflux for 2d and worked up as previously described to give the crude amine *cis*-**93a** and unreacted azide *cis*-**130a** as an orange oil. The mixture was dissolved in DCM and aq. HCl (5M) was added until pH 1. The mixture was extracted with DCM and the residue evaporated to give the azide *cis*-**130a**. The aqueous layer was adjusted to pH > 9, extracted with DCM (3 × 10 mL) and evaporated to give the *cis*-amine *cis*-**93a** as a brown oil (0.070 g, 9%).

$\nu_{max}$ (ATR) 3279, 2928, 1489, 1447, 759, 726, 700 $cm^{-1}$; $\delta_H$ (300 MHz) 1.66 (br s, 2H, $NH_2$), 1.75–1.88 [m, 1H, one of C(2)$H_2$], 1.92–2.22 [m, 3H, one of C(2)$H_2$ and C(3)$H_2$], 3.99–4.11 [m, 2H, C(1)H and C(4)H], 6.84 (d, 1H, *J* = 7.7, ArH), 7.00–7.35 (m, 7H, ArH), 7.43 (d, 1H, *J* = 7.0, ArH) ppm; $\delta_C$ (75.5 MHz) 28.9 ($CH_2$), 30.9 ($CH_2$), 45.8 [C(4)H], 49.4 [C(1)H], 126.1, 126.5, 126.8, 128.2, 128.3, 128.9, 130.0 (7 × aromatic CH), 139.0, 141.6, 146.9 (3 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 224.1434 (calculated 224.1443); enantiomers separated using Chiralcel AS-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 10.7, $R_t$ = 11.5 min, $R_t$ (ketone **134**, no separation) = 14.6 min.

This compound is novel and has been fully characterized in this investigation.

## *trans*-4-Phenyl-1,2,3,4-tetrahydronaphthalen-1-amine *trans*-**93b**



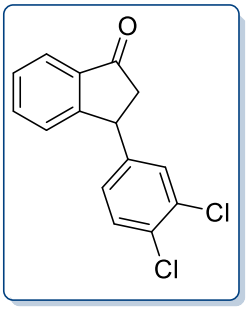

Triphenylphosphine **145** (555 mg, 2.1 mmol, 1.1 eq.) and water (68.4 µL, 2.0 eq.) were added to a stirring solution of *trans*-**140b** (479 mg, 1.9 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled, and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *trans*-**93b** and triphenylphosphine oxide **146** (1.253 g). The residue was suspended in diethyl ether (20 mL) and the insoluble triphenylphosphine oxide by-product

was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM (10 mL) and aq. HCl (5 M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL), the pH was adjusted to 14 with aq. NaOH (5M), and the solution stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried ($Na_2SO_4$), filtered and concentrated to give the product *trans*-**93b** as a yellow oil (313 mg, 52%).

$\nu_{max}$ (ATR) 3357, 2925, 1491, 1450, 749, 700 $cm^{-1}$; $\delta_H$ (300 MHz) 1.57–1.73 (m, 1H, one of C(2)$H_2$), 1.79–1.95 [m, 1H, one of C(3)$H_2$], 2.09–2.20 [m, 1H, one of C(2)$H_2$], 2.24–2.37 [m, 1H, one of C(3)$H_2$], 4.11 [t, 1H, *J* = 6.1, C(1)H], 4.15 [t, 1H, *J* = 6.8, C(4)H], 6.85 (d, 1H, *J* = 7.8, ArH), 7.05 (d, 2H, *J* = 7.1, ArH), 7.10 (d, 1H, *J* = 7.3, ArH), 7.15–7.35 (m, 4H, ArH), 7.51 (d, 1H, *J* = 7.7, ArH) ppm; $\delta_C$ (75.5 MHz) 30.1 [C(3)$H_2$], 31.8 [C(2)$H_2$], 45.7 [C(4)H], 49.8 [C(1)H], 126.1, 126.5, 126.7, 127.5, 128.3, 128.7, 130.2 (7 × aromatic CH), 139.0, 147.0 (3 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 224.1435 (calculated 224.1434); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 17.7, $R_t$ = 23.1 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments and by comparison to the related compounds ketone **134**, alcohol *trans*-**137b**, and azide *trans*-**140b**.

## 3-(3,4-Dichlorophenyl)-2,3-dihydro-*1H*-inden-1-one **135**[33]



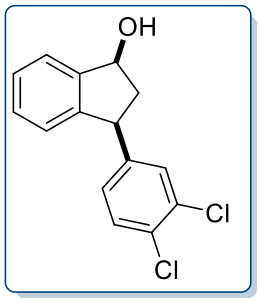

Triflic acid (50 g, 333.0 mmol) was added slowly to a stirring solution of *trans*-cinnamic acid **20** (7.60 g, 51.0 mmol) and dichlorobenzene **110** (35 mL, 45.5 g, 310.0 mmol) at 0 °C. The reaction solution was heated to reflux and stirred for 3d. The reaction solution was cooled and poured onto a mixture of water and ice (70 mL) to quench, stirred for approx. 1 h, and the layers separated. The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 50 mL), water (100 mL) and concentrated to give the crude product **135** as a solution in dichlorobenzene. The dichlorobenzene was removed by vacuum distillation (b.p. 106–108 °C, at unknown pressure), giving the crude product as an orange solid which was recrystallized

from DCM/hexane to give the pure product **135** (8.908 g, 63%) as a white solid m.p. 110–112 °C, lit.: 113–115 °C.[34]

$\nu_{max}$ (ATR) 2919, 1698 (C=O), 762 $cm^{-1}$; $\delta_H$ (300 MHz) 2.62 [dd, 1H, *J* = 19.2, 3.9, one of C(2)$H_2$, 3.23 [dd, 1H, *J* = 19.2, 8.2, one of C(2)$H_2$,], 4.55 [dd, 1H, *J* = 8.2, 3.9, C(3)H], 6.95 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.20–7.29 (m, 2H, ArH), 7.38 (d, 1H, *J* = 8.3, ArH), 7.41–7.53 (m, 1H, ArH), 7.61 (td,1H, *J* = 7.5, 1.3, ArH), 7.77–7.88 (m, 1H, ArH) ppm; $\delta_C$ (75.5 MHz) 43.6 [C(3)H], 46.5 [C(2)$H_2$], 123.7, 126.7, 127.0, 128.4, 129.7, 130.9 (6 × aromatic CH), 131.2, 133.0 (2 × aromatic C), 135.4 (aromatic CH), 136.8, 144.0, 156.5 (3 × aromatic C), 204.9 (C=O) ppm; enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 34.9, $R_t$ = 37.8 min and using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 90/10, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 35.3, $R_t$ = 38.4 min.

All data is in agreement with previously reported data.[35,36]

## *cis*-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *cis*-**138a**[28,34,35]

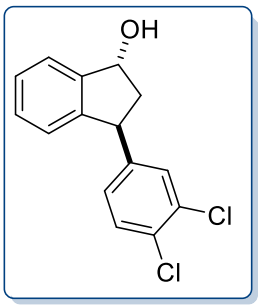



Ketone **135** (8.908 g, 32.1 mmol) in THF (100 mL) was cooled to −15 °C using a salt/ice bath. A solution of sodium borohydride (2.450 g, 64.8 mmol, 2.0 eq.) in water (10 mL) was slowly added to the stirring solution, maintaining the temperature below 0 °C. When the addition was complete the reaction solution was allowed warm to room temperature and stirred for 3 h. The solution was diluted with ice-water (50 mL) and stirred for 1 h. The THF was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water (2 × 50 mL) and brine (1 × 75 mL) and concentrated to give the crude product **138** as a mixture of diastereomers (91:9). The product was purified by column chromatography using diethyl ether/hexane (25/75) as eluent which gave the pure product *cis*-**138a** as a colourless oil (4.975 g, 55%) and the minor diastereomer *trans*-**138b** as a colourless oil (0.358 g, 4%)

*cis*-**138a:** $\nu_{max}$ (ATR) 3306 (OH), 1468, 1030, 756, 742 $cm^{-1}$; $\delta_H$ (300 MHz) 1.89 [ddd, 1H, *J* = 13.1, 8.9, 7.3, one of C(2)$H_2$], 1.99 (d, 1H, *J* = 7.0, OH), 3.01 [ddd, 1H, *J* = 13.1, 7.6, 7.0, one of C(2)$H_2$], 4.15 [dd appears as t, 1H, *J* = 8.2, C(3)H], 5.30 [m, 1H, C(1)H], 6.94 (d, 1H, *J* = 7.7, ArH), 7.07 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.22–7.36 (m, 3H, ArH), 7.38 (d, 1H, *J* = 8.3, ArH), 7.48 (d, 1H, *J* =7.4, ArH) ppm; $\delta_C$ (75.5 MHz) 46.7 [C(2)$H_2$], 47.6 [C(3)H], 74.9 [C(1)H], 123.9, 124.9, 127.67,

127.68, 128.7, 130.2, 130.55 (7 × aromatic CH) 130.59, 132.6, 144.4, 144.7, 145.2 (5 × aromatic C) ppm.

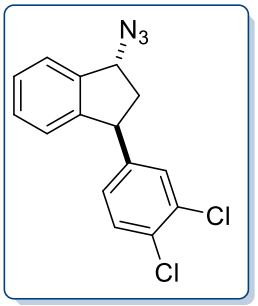
All data is in agreement with previously reported data.[33]




*trans*-**138b:** $\nu_{max}$ (ATR) 3306 (OH), 1469, 1032, 755 $cm^{-1}$; $\delta_H$ (300 MHz) 2.24–2.40 [1H, m, one of C(2)$H_2$], 2.45–2.63 [1H, m, 7.7, 2.9, one of C(2)$H_2$], 4.59 [1H, t, *J* = 7.4, C(3)H], 5.38 [1H, dd, *J* = 6.2, 2.7, C(1)H], 6.96 (1H, dd, *J* = 8.3, 2.1, ArH), 6.98–7.05 (1H, m, ArH), 7.21 (1H, d, *J* = 2.1, ArH), 7.27–7.39 (3H, m, ArH), 7.44–7.53 (1H, m, ArH) ppm; $\delta_C$ (75.5 MHz) 46.3 [C(2)$H_2$], 48.2 (CH), 75.1 (CH), 124.6, 125.3, 127.4, 127.8, 129.3, 129.8 (6 × aromatic CH), 130.5 (aromatic C), 130.6 (aromatic CH), 132.6, 144.9, 145.1, 145.6 (4 × aromatic C) ppm.

All data is in agreement with previously reported data.[33]

## *trans*-1-Azido-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-indene *trans*-**141b**

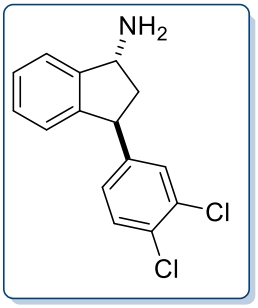



Diphenylphosphoryl azide (0.92 mL, 1.178 g, 4.3 mmol, 1.2 eq.) was added to a stirring solution of *cis*-**138a** (0.99 g, 3.5 mmol) in dry toluene (4 mL) under a nitrogen flow. The mixture was cooled to 0 °C, stirred for 10 mins, and DBU (0.64 mL, 4.3 mmol, 1.2 eq.) was added slowly. The mixture was stirred for 2 h at 0 °C and 16 h at room temperature. The solvent was removed, and the residue passed through a column of silica gel using hexane/ethyl acetate (95/5) as eluent to give the pure product *trans*-**141b** as an orange oil (0.928 g, 86%).

$\nu_{max}$ (ATR) 2935, 2092 ($N_3$), 1474, 1237, 757 $cm^{-1}$; $\delta_H$ (300 MHz) 2.30 [ddd, 1H, *J* = 13.7, 8.3, 6.8, one of C(2)$H_2$], 2.61 [ddd, 1H, *J* = 13.7, 7.5, 2.4, one of C(2)$H_2$], 4.53 [t, 1H, *J* = 7.9, C(3)H], 5.03 [dd, 1H, *J* = 6.8, 2.3, C(1)H], 6.94–7.05 (m, 2H, ArH), 7.23 (d, 1H, *J* = 2.1, ArH), 7.28–7.42 (m, 3H, ArH), 7.42–7.52 (m, 1H, ArH) ppm; $\delta_C$ (75.5 MHz) 43.4 [C(2)$H_2$], 48.5 (CH), 64.7 (CH), 124.8, 125.4, 127.4, 127.8, 129.7, 129.9, 130.7 (7 × aromatic CH), 130.8, 132.7, 140.7, 144.1, 145.8 (5 × aromatic C) ppm.

All data is in agreement with previously reported data.[32]

## *trans*-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-amine *trans*-**94b**




Method 1: Sodium iodide (1.504 g, 10.0 mmol, 12.5 eq.) and cerium chloride heptahydrate (453 mg, 1.2 mmol, 1.5 eq.) were added to a solution of azide *trans*-**141b** (246 mg, 0.8 mmol) in acetonitrile (10 mL). The suspension was stirred at reflux for 24 h. The reaction mixture was cooled, diluted with diethyl ether (25 mL) and washed with water (3 × 25 mL), sat. aq. sodium bicarbonate (2 × 25 mL), sat. aq. sodium thiosulfate (25 mL, decolourised solution), brine (30 mL) and dried ($Na_2SO_4$) to give the crude mixture, comprising of the unreacted azide *trans*-**141b** and the amine product *trans*-**94b** (242 mg). The residue was treated with aq. HCl (10%, 25 mL), washed with DCM (3 × 25 mL) and treated with aq. NaOH (5M) until pH > 9. The aqueous solution was extracted with DCM (3x20 mL) and evaporated to give the impure amine *trans*-**94b** as a green oil (67 mg, 30%).

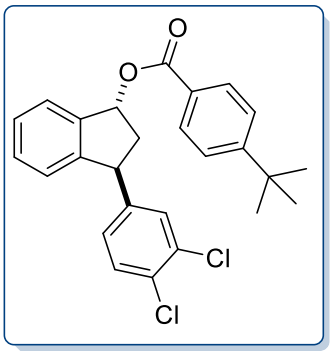
Method 2: Triphenylphosphine **145** (617 mg, 2.45 mmol, 1.1 eq.) and water (77.0 µL, 2.0 eq.) were added to a stirring solution of azide *trans*-**141b** (650 mg, 2.1 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *trans*-**94b** and triphenylphosphine oxide **146**. The residue was suspended in diethyl ether (20 mL) and the insoluble triphenylphosphine oxide by-product **146** was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM and aq. HCl (5M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL) and the pH was adjusted to 14 with aq. NaOH (5M), and the suspension stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried ($Na_2SO_4$), filtered and concentrated to give the product *trans*-**94b** as a yellow oil (265 mg, 45%).

$\nu_{max}$ (ATR) 3364, 3007, 1469, 1264, 732, 703 $cm^{-1}$; $\delta_H$ (300 MHz) 2.22–2.46 [m, 2H, C(2)$H_2$], 4.51 [dd, 1H, *J* = 8.0, 6.0, C(3)H], 4.57 [dd appears as t, 1H, *J* = 6.0, C(1)H], 6.93 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.02 (d, 1H, *J* = 7.5, ArH), 7.18 (d, 1H, *J* = 2.1, ArH), 7.20–7.37 (m, 3H, ArH), 7.40 (d, 1H, *J* = 7.3, ArH) ppm; $\delta_C$ (75.5 MHz) 46.9 [C(2)$H_2$], 48.2 [C(3)H], 55.8 [C(1)H], 123.9, 125.2, 127.2, 127.7, 128.2, 129.7 (6 × aromatic CH), 130.3 (aromatic C), 130.4 (aromatic CH), 132.5, 144.5, 145.6, 147.4 (4 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 278.0498 (calculated

278.0498); enantiomers separated using Chiralcel OJ-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 95/5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 23.1, $R_t$ = 27.1 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments.

## *trans*-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-yl 4-(*tert*-butyl)benzoate *trans*-**144**[37]

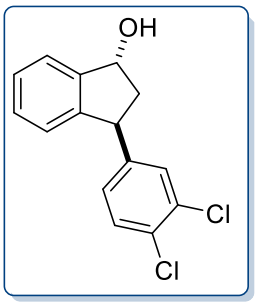



A solution of *cis*-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *cis*-**141a** (1.413 g, 5.1 mmol), triphenylphosphine **145** (2.593 g, 9.9 mmol, ~2.0 eq.), diethyl azodicarboxylate (1.780 g, 10.2 mmol, 2.0 eq.) and 4-*tert*-butylbenzoic acid **143** (1.82 g, 10.2 mmol, 2.0 eq.) in dry THF was stirred overnight at room temperature. The organic solvent was removed to give the crude product as a viscous orange oil. Purification by chromatography on silica gel using diethyl ether/ hexane (4/96) furnished a mixture of the product *trans*-**144** and the benzoic acid **143** as an orange oil. The mixture was dissolved in hexane, stirred with basic alumina for 5 mins, and filtered through a pad of Celite®. The residue was washed with hexane (100 mL), hexane/diethyl ether (99/1) (100 mL) and ethyl acetate (50 mL). All three fractions were free of benzoic acid **143** and were combined to give the product *trans*-**144** as a viscous orange oil (1.295g, 58%).

$\nu_{max}$ (ATR) 2964, 1713 (C=O), 1268, 731, 694 $cm^{-1}$; $\delta_H$ (300 MHz) 1.31 [s, 9H, $C(CH_3)_3$], 2.36–2.54 [m, 1H, one of $C(2)H_2$], 2.76 [ddd, 1H, *J* = 14.3, 7.6, 2.3, one of $C(2)H_2$], 4.64 [dd appears as t, 1H, *J* = 7.7, C(3)H], 6.54 [dd, 1H, *J* = 6.5, 2.2, C(1)H], 6.92–7.07 (m, 2H, ArH), 7.24–7.38 (m, 4H, ArH), 7.38–7.48 (m, 2H, *m*-ArH of acid moiety), 7.54–7.66 (m, 1H, ArH), 7.91–8.02 (m, 2H, *o*-ArH of acid moiety) ppm; $\delta_C$ (75.5 MHz) 31.2 (3 × $CH_3$), 35.2 [qC, $C(CH_3)_3$], 43.3 [$C(2)H_2$], 48.7 [C(3)H], 77.5 [C(1)H], 125.1, 125.4, 126.4, 127.5 (4 × aromatic CH), 127.6 (aromatic C), 127.8, 129.8, 130.0 (3 × aromatic CH), 130.7, 132.7 (2 × aromatic C), 133.7, 133.9 (2 × aromatic CH), 141.5, 144.7, 146.9 (3 × aromatic C), 156.8 [aromatic C, **C**$C(CH_3)_3$], 166.5 (C=O) ppm; HRMS ($ES^+$): $[M+Na]^+$ 461.1040 (calculated 461.1046).

This compound is novel and has been fully characterized in this investigation, data closely aligns with the data for the unsubstituted benzoic acid ester.[37]

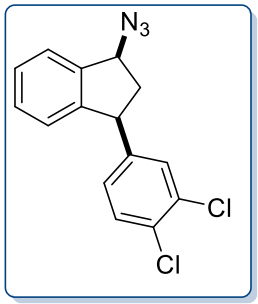
## *trans*-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-ol *trans*-**143b**[37]




Aqueous potassium hydroxide (3M, 8 mL) was added to a stirring solution of *trans*-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-inden-1-yl 4-(*tert*-butyl)benzoate *trans*-**144** (0.430 g, 1.0 mmol) in methanol (16 mL) and THF (16 mL). The reaction mixture was stirred for 3 h. The pH was adjusted to 3 using conc. HCl, followed by addition of water (20 mL). The aqueous layer was extracted with diethyl ether (3 × 20 mL) and the combined organic layers evaporated to give the crude reaction product, a mixture of the benzoic acid **143** and the alcohol *trans*-**138b**. The mixture was suspended in aq. KOH (3M, 10 mL) and stirred for 3 h, the suspension was extracted with diethyl ether (3 × 10 mL), dried, filtered and concentrated to give the product *trans*-**143b** as an orange oil (0.250 g, 92%).

Spectroscopic data were identical to those reported above.

## *cis*-1-Azido-3-(3,4-dichlorophenyl)-2,3-dihydro-1*H*-indene *cis*-**141a**




Diphenylphosphoryl azide (2.22 mL, 2.84 g, 10.4 mmol, 2.4 eq.)[4] was added to a solution of *trans*-**143b** (1.19 g, 4.3 mmol) in dry toluene (12 mL) under nitrogen. The reaction mixture was cooled to 0 °C and stirred for 10 mins. DBU (0.77 mL, 0.78 g, 5.1 mmol, 1.2 eq.) was added dropwise to the stirring solution. The solution was stirred at 0 °C for 2 h, and at room temperature for 20 h. The solvent was removed *in vacuo*. The residue was passed through a short column of silica gel using hexane/diethyl ether (95/5) as eluent. The solvent was removed *in vacuo* to give the pure product *cis*-**141a** (691 mg, 53%) as a dark orange oil.

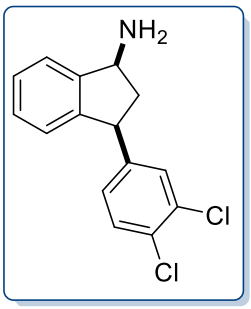
$\nu_{max}$ (ATR) 2929, 2090 (C–$N_3$), 1468, 1254, 758, 747 $cm^{-1}$; $\delta_H$ (300 MHz) 2.02 [ddd, 1H, *J* = 13.3, 8.4, 7.7, one of C(2)$H_2$], 3.01 [ddd appears as dt, 1H, *J* = 13.3, 7.7, one of C(2)$H_2$], 4.24 [dd appears as t, 1H, *J* = 8.2, C(3)H], 4.93 [dd appears as t, 1H, *J* = 7.3, C(1)H], 6.97 (d, 1H, *J* = 7.3, ArH), 7.05 (dd, 1H, *J* = 8.2, 2.1, ArH), 7.26–7.52 (m, 5H, ArH) ppm; $\delta_C$ (75.5 MHz) 42.6 [C(2)$H_2$], 48.1 [C(3)H], 64.3 [C(1)H], 124.3, 125.2, 127.6, 127.9, 129.2, 130.2, 130.7 (7 × aromatic CH),

[4] Due to error, twice the required amount of DPPA was added during this reaction. The initial purification using ethyl acetate and hexane (95/5) as eluent resulted in coelution with the DPPA. Switching to a less polar solvent system (as above) was more effective. This is discussed in the relevant section in chapter 5.

130.9, 132.7, 141.2, 144.2, 144.6 (5 × aromatic C) ppm; HRMS ($ES^+$): $[M-N_3]^+$ 261.0237 (calculated 261.0232).

This compound is novel and has been fully characterized in this investigation.

## *cis*-3-(3,4-Dichlorophenyl)-2,3-dihydro-1*H*-inden-1-amine *cis*-94a




Triphenylphosphine **145** (658 mg, 2.5 mmol, 1.2 eq.) and water (81.8 µL, 2.0 eq.) were added to a stirring solution of azide *cis*-**141b** (691 mg, 2.3 mmol) in THF (20 mL, HPLC grade). The solution was heated to reflux and stirred for 16 h. The reaction mixture was cooled and the solvent removed by rotary evaporation to give the crude reaction mixture containing the amine *cis*-**94a** and triphenylphosphine oxide **146**. The residue was suspended in diethyl ether (30 mL) and the insoluble triphenylphosphine oxide by-product **146** was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in DCM and aq. HCl (5M) was added dropwise. The solution was stirred for approx. 5 mins and the resulting precipitate was isolated by vacuum filtration using a porosity 4 sintered glass funnel and washed with DCM. The precipitate was suspended in water (10 mL) and the pH was adjusted to 14 with aq. NaOH (5M), and the mixture stirred for 30 mins. The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried ($Na_2SO_4$), filtered and concentrated to give the product *cis*-**94a** as a yellow oil (313 mg, 52%).

$\nu_{max}$ (ATR) 3372, 2957, 1468, 762, 731 $cm^{-1}$; $\delta_H$ (300 MHz) 1.67 [ddd, 1H, *J* = 12.4, 10.6, 9.5, one of C(2)$H_2$], 2.94 [ddd appears as dt, 1H, *J* = 12.4, 7.0, one of C(2)$H_2$], 4.14 [dd, 1H, *J* = 10.6, 7.3, C(3)H], 4.37 [dd, 1H, *J* = 9.0, 7.2, C(1)H], 6.88 (d, 1H, *J* = 7.4, ArH), 7.07 (dd, 1H, *J* = 8.3, 2.1, ArH), 7.16–7.50 (m, 5H, ArH) ppm; $\delta_C$ (75.5 MHz) 48.0 [C(3)H], 48.7 [C(2)$H_2$], 55.9 [C(1)H], 123.2, 124.6 (2 × aromatic CH), 127.3 (aromatic C), 127.4, 127.7, 127.8 (3 × aromatic CH), 128.6 (aromatic C), 130.3, 130.5 (2 × aromatic CH), 132.0, 132.5, 144.6 (3 × aromatic C) ppm; HRMS ($ES^+$): $[M+H]^+$ 278.0504 (calculated 278.0498); enantiomers separated using Chiralcel OB-H [conditions: *n*-hexane/*i*PrOH (containing 1% DEA) = 95/5, flow rate = 0.5 mL $min^{-1}$], $R_t$ = 16.5, $R_t$ = 19.2 min.

This compound is novel and has been fully characterized in this investigation. The carbon assignments were aided by DEPT and 2D experiments.

## Procedures for biotransformations

### *General procedure for oxidative deamination reactions*

*E. Coli* cells containing overexpressed transaminase (30 mg or 50 mg, as indicated in the appropriate tables) were suspended in 50 mM sodium phosphate buffer (pH 8.5, 400 µL) in a 15 mL centrifuge tube, the suspension was sonicated if required using a probe for 10 s, followed by 30 s on ice, this was repeated five times to lyse the cells. PLP solution (100 µL buffer, overall conc. 1mM) and sodium pyruvate (in 100 µL buffer, overall 1 eq.) solutions were added, followed by the amine substrate (20 mM or 50 mM, as indicated, in 100 µL DMSO), any additional buffer or DMSO was added as required to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 16 h or 24 h as indicated in the corresponding tables. The reaction was stopped through the addition of aqueous NaOH solution (5M, 400 µL). Ethyl acetate (≈ 4 mL) was added and the tubes were centrifuged to pellet the cells. The organic phase was passed through a plug of $Na_2SO_4$ and the solvent was removed *in vacuo*. The crude products were analysed by $^1$H NMR and chiral HPLC, except when amine **120** was used as substrate (Table 5.3, Entry 12), in this case the reaction mixture containing ketone **159** and amine **120** was derivatized (procedure for preparation of **158**) before chiral HPLC analysis (conditions in Appendix I).

Results are shown in Table 5.3, Table 5.4, Table 5.9, Table 5.10, Table 5.11.

### *General procedure for reductive amination reactions*

*Using isopropylamine **97** as amine donor:* *E. Coli* cells containing overexpressed CV-TA (30 mg) were suspended in 1M isopropylamine solution (pH adjusted to 10 using conc. HCl), in a 15 mL centrifuge tube. PLP was solution (in 100 µL of $^i$PrNH$_2$ solution, overall conc. 1 mM), substrate (in 100 µL DMSO, overall conc. 20 mM or 50 mM) solutions were added, additional buffer was added to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

The results are shown in Table 5.5.

*Using AlaDH system:*[38] *E. Coli* cells containing overexpressed transaminase (50 mg) were suspended in 50 mM sodium phosphate buffer (pH 7.5, 400 µL) in a 15 mL centrifuge tube. PLP solution (100 µL buffer, overall conc. 1 mM), L-alanine (in 100 µL buffer, overall 5.0 eq.), $NAD^+$ (in 100 µL buffer, overall 1 mM), ammonium formate (in 100 µL buffer, overall 150 mM), substrate (in 100 µL DMSO, overall conc. 50 mM) solutions were added, followed by the auxiliary enzymes formate dehydrogenase (11 U), alanine dehydrogenase (12 U), additional

buffer was added to total reaction volume 1 mL. The solution was shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

Results are shown in Table 5.6.

*Using the LDH system:*[38] *E. Coli* cells containing overexpressed transaminase (50 mg) were suspended in 50 mM sodium phosphate buffer (pH 7.5, 400 µL) in a 15 mL centrifuge tube. PLP solution (100 µL buffer, overall conc. 1 mM), L-alanine (in 100 µL buffer, overall 5.0 eq.), $NAD^+$ (in 100 µL buffer, overall 1 mM), glucose (in 100 µL buffer, overall 150 mM), substrate (in 100 µL DMSO, overall conc. 50 mM) solutions were added, followed by the auxiliary enzymes lactate dehydrogenase (90 U), glucose dehydrogenase (30 U). The reaction mixture as shaken at 30 °C, 400 rpm for 24 h. The reaction was stopped, worked up and analysed as above.

Results are shown in Table 5.7.

*Use of alternative amine sources*:[39] *E. Coli* cells containing overexpressed CV-TA (30 mg) were suspended in sodium phosphate buffer (pH 8.5, 50 mM), in a 15 mL centrifuge tube. PLP was added (in 100 µL of buffer, overall conc. 1 mM), ketone **148** (in 100 µL DMSO, overall conc. 50 mM) and amine donor (added neat or in 100 µL buffer, overall conc 250 mM) solutions were added. The solution was shaken at 30 °C, 400 rpm for 24 h. The reactions were stopped, worked up and analysed as above.

Results are shown in Table 5.8.

## 6.6 References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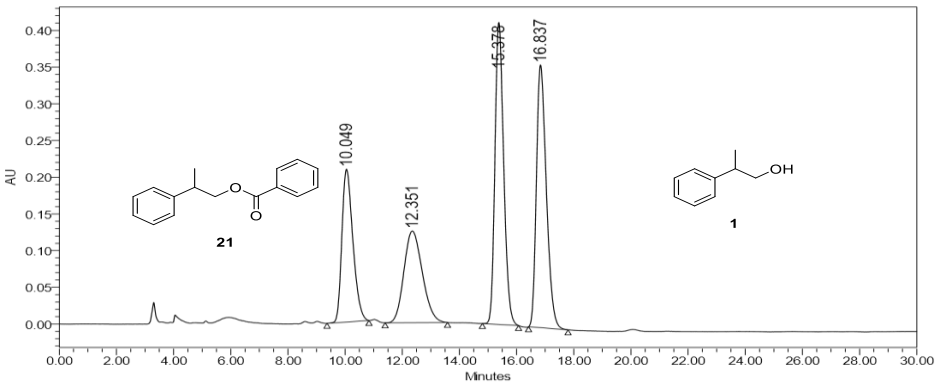
# Appendices

# Appendix I: Chiral HPLC Conditions

## Chapter 2

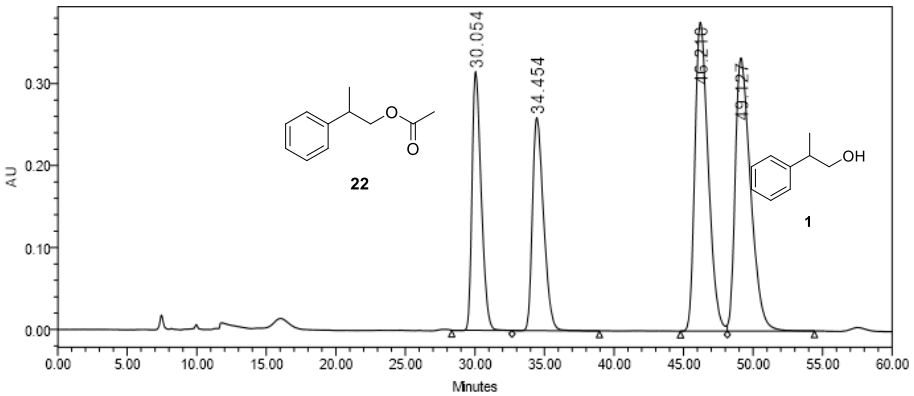
*Table A1*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 1 | 209.8 | 99/1 | 25 | *(R)*-**21** | 10.0 |
| | | | | | *(S)*-**21** | 12.4 |
| | | | | | *(S)*-**1** | 15.4 |
| | | | | | *(R)*-**1** | 16.8 |

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

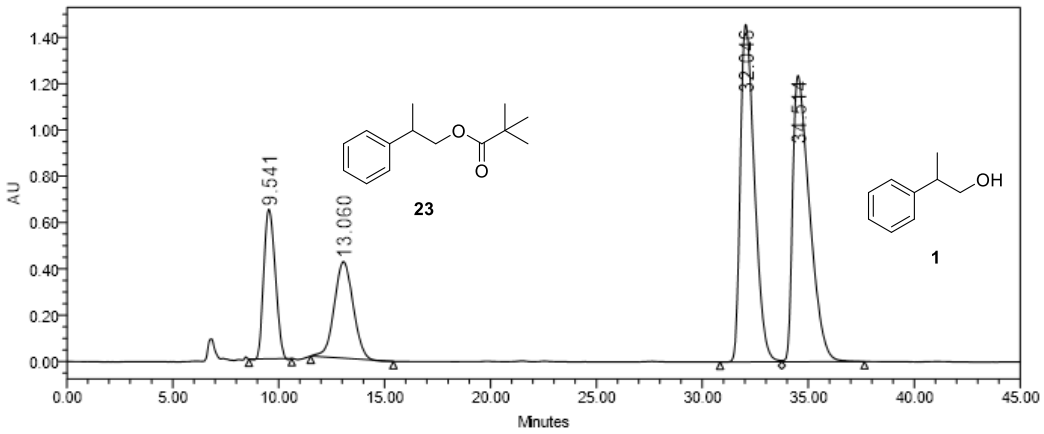
*Table A2*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 209.8 | 99.5/0.5 | 25 | *(R)*-**22** | 30.1 |
| | | | | | *(S)*-**22** | 34.5 |
| | | | | | *(S)*-**1** | 46.2 |
| | | | | | *(R)*-**1** | 49.1 |

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

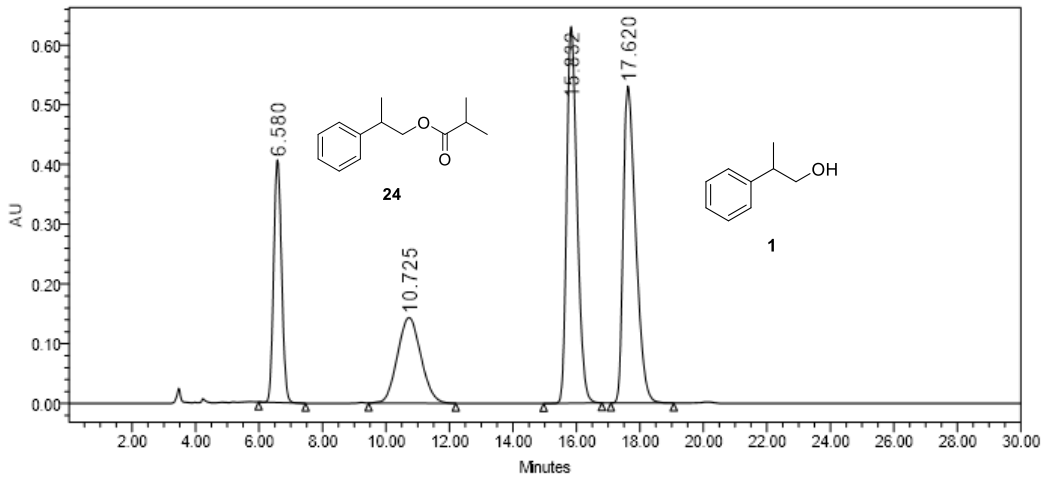
*Table A3*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 209.8 | 99/1 | 25 | *(S)*-**23** | 9.5 |
| | | | | | *(R)*-**23** | 13.1 |
| | | | | | *(S)*-**1** | 32.0 |
| | | | | | *(R)*-**1** | 34.5 |

The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

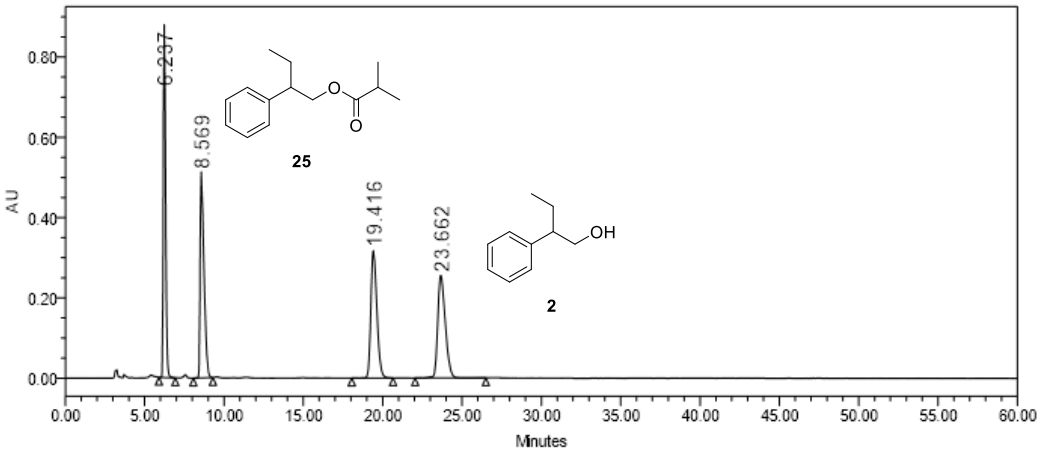
*Table A4*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 209.8 | 99/1 | 25 | *(R)*-**24** | 6.6 |
| | | | | | *(S)*-**24** | 10.7 |
| | | | | | *(S)*-**1** | 15.8 |
| | | | | | *(R)*-**1** | 17.6 |

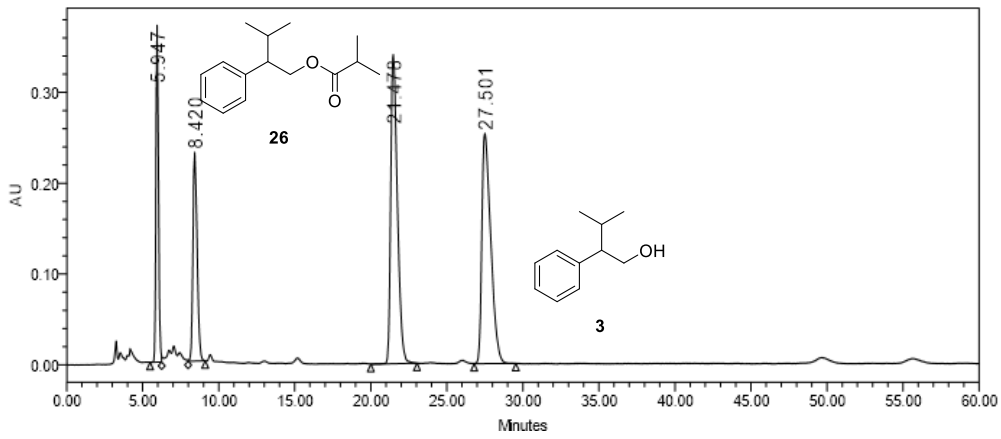
The absolute stereochemistry was assigned by comparison to the enantiopure *(S)*-**1**, which was obtained from Sigma Aldrich.

*Table A5*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 1 | 209.8 | 98/2 | 25 | **25** | 6.2 |
| | | | | | **25** | 8.6 |
| | | | | | **2** | 19.4 |
| | | | | | **2** | 23.7 |

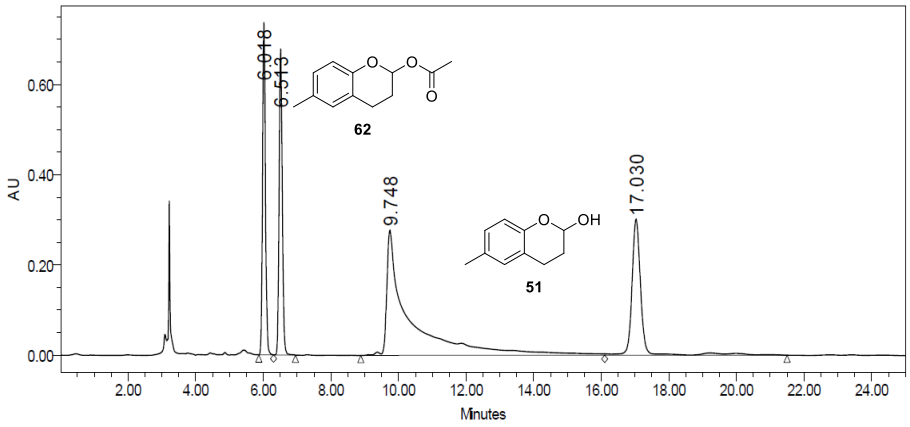
*Table A6*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 1 | 209.8 | 99/1 | 25 | **26** | 5.9 |
| | | | | | **26** | 8.4 |
| | | | | | **3** | 21.5 |
| | | | | | **3** | 27.5 |

## Chapter 3

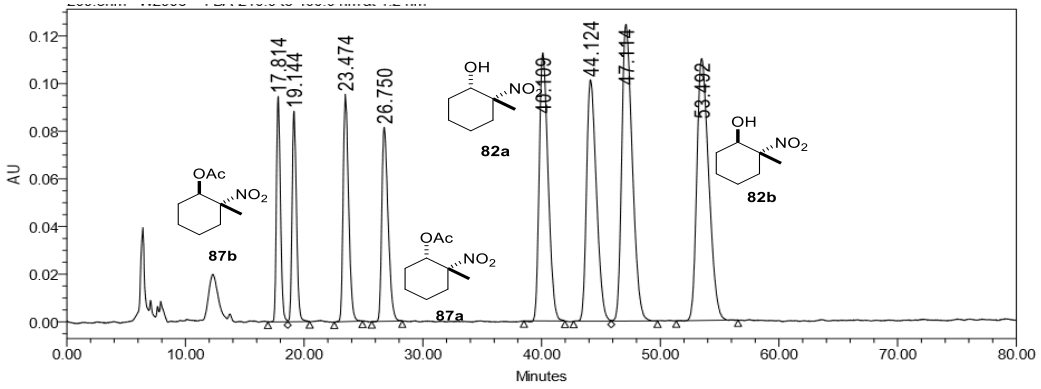
*Table A7*




| Column | Flow (mL/ min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time (min) |
|---|---|---|---|---|---|---|
| Lux Cellulose 4 | 1 | 209.8 | 95/5 | 25 | (–)-**62** | 6.0 |
| | | | | | (+)-**62** | 6.5 |
| | | | | | **51** | 9.7 |
| | | | | | **51** | 17.0 |

## Chapter 4

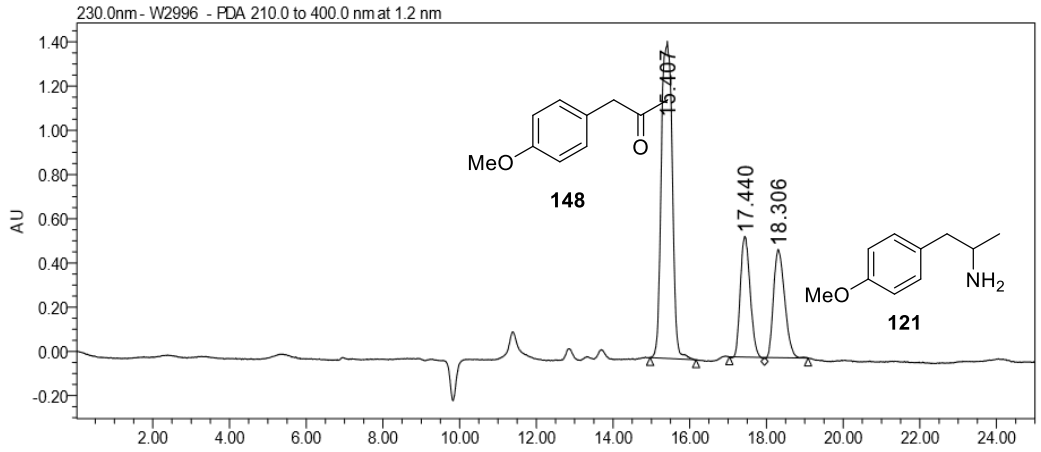
*Table A8*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 210 | 98.5/1.5 | 25 | **87b** | 17.8<br>19.1 |
| | | | | | **87a** | 23.5<br>26.8 |
| | | | | | **82a** | 40.1<br>44.1 |
| | | | | | **82b** | 47.1<br>53.4 |

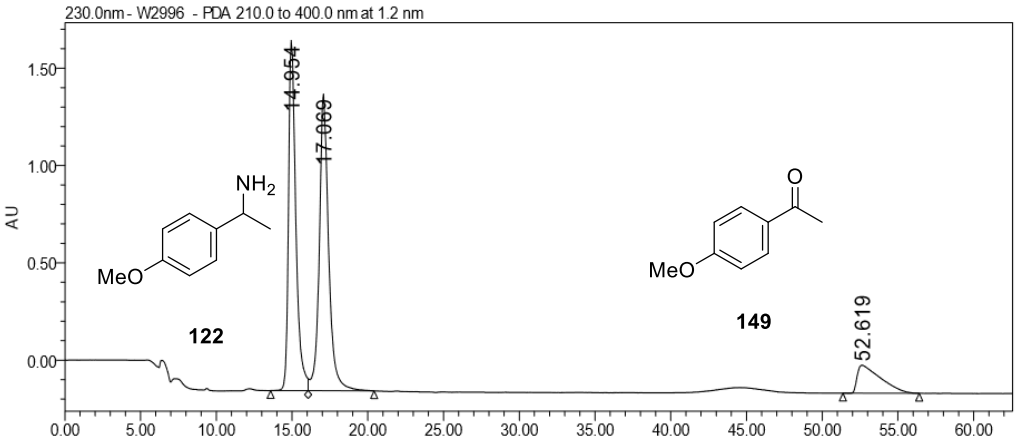
## Chapter 5

*Table A9*



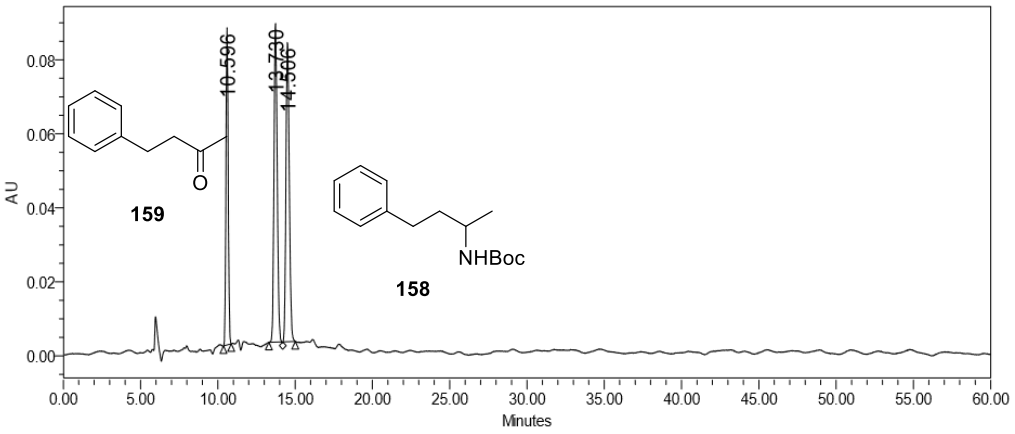

| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Lux Amylose 1 | 0.4 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **148** | 15.4 |
| | | | | | **121** | 17.4 |
| | | | | | **121** | 18.3 |

*Table A10*



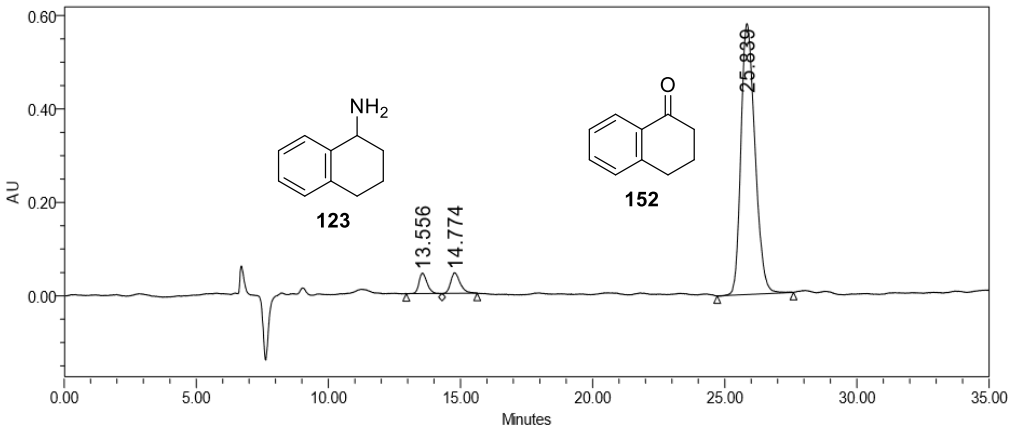

| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **122** | 15.0 |
| | | | | | **122** | 17.1 |
| | | | | | **149** | 52.6 |

*Table A11*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Lux Amylose 1 | 0.5 | 260 | 95/5* (*contains 1% v/v DEA) | 25 | **159** | 10.6 |
| | | | | | **158** | 13.7 |
| | | | | | **158** | 14.5 |

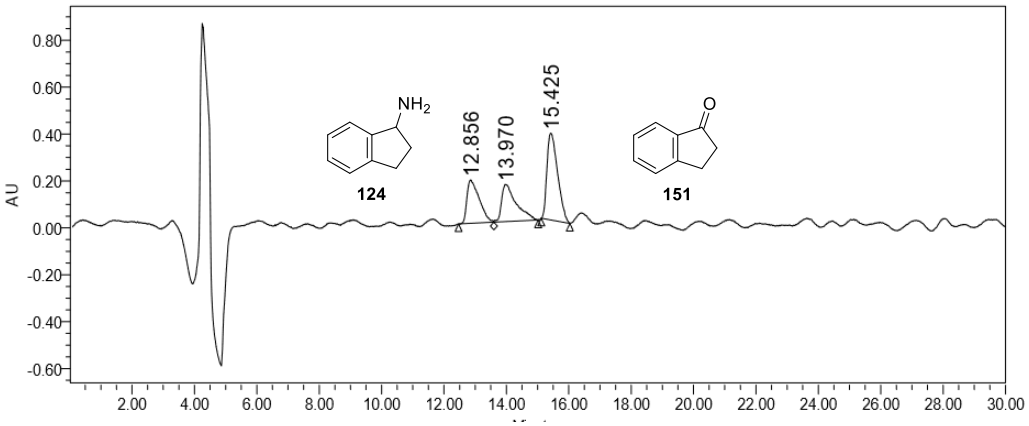
*Table A12*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 230 | 95/5* (*contains 1% v/v DEA) | 25 | *(R)*-**123** | 13.6 |
| | | | | | *(S)*-**123** | 14.8 |
| | | | | | **152** | 25.8 |

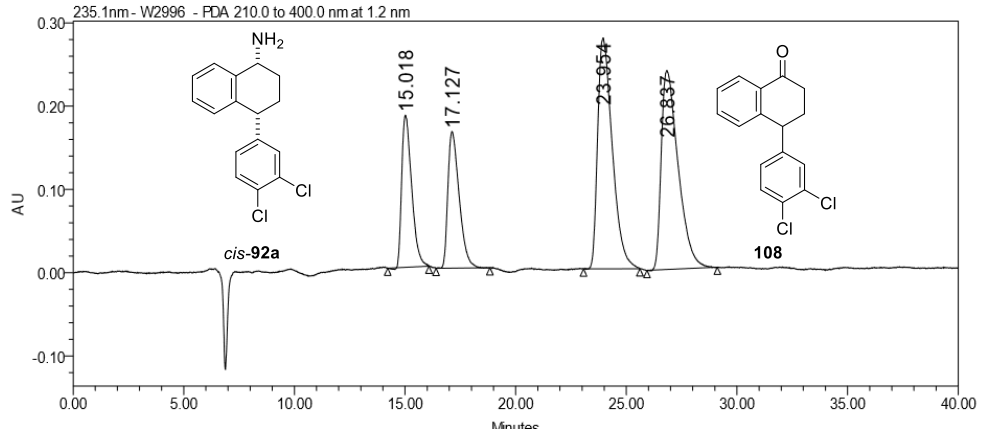
The absolute stereochemistry was assigned by comparison to an enantiopure sample of *(S)*-**123** obtained from Sigma Aldrich

*Table A13*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 1 | 215 | 99/1* (*contains 1% v/v DEA) | 25 | **124** | 12.9 |
| | | | | | **124** | 14.0 |
| | | | | | **151** | 15.4 |

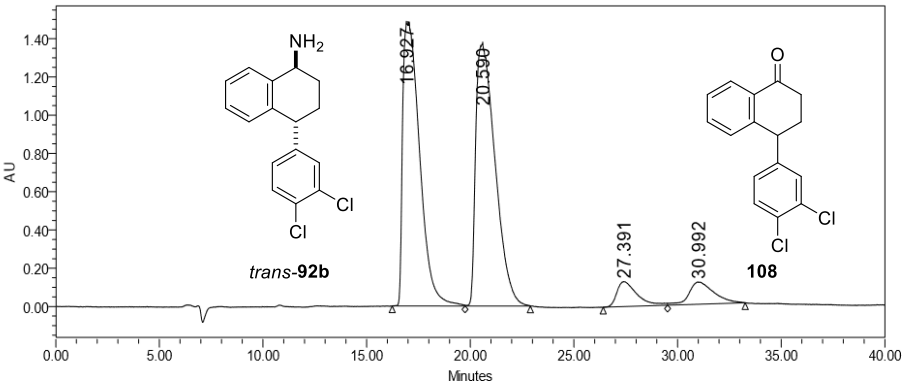
*Table A14*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 235 | 90/10* (*contains 1% v/v DEA) | 25 | *(1S,4S)*-**92a** | 15.0 |
| | | | | | *(1R,4R)*-**92a** | 17.1 |
| | | | | | *(4R)*-**108** | 24.0 |
| | | | | | *(4S)*-**108** | 26.8 |

The absolute stereochemistry was assigned by comparison to the enantiopure ketone (*4S*)-**108**, which was donated by Pfizer.

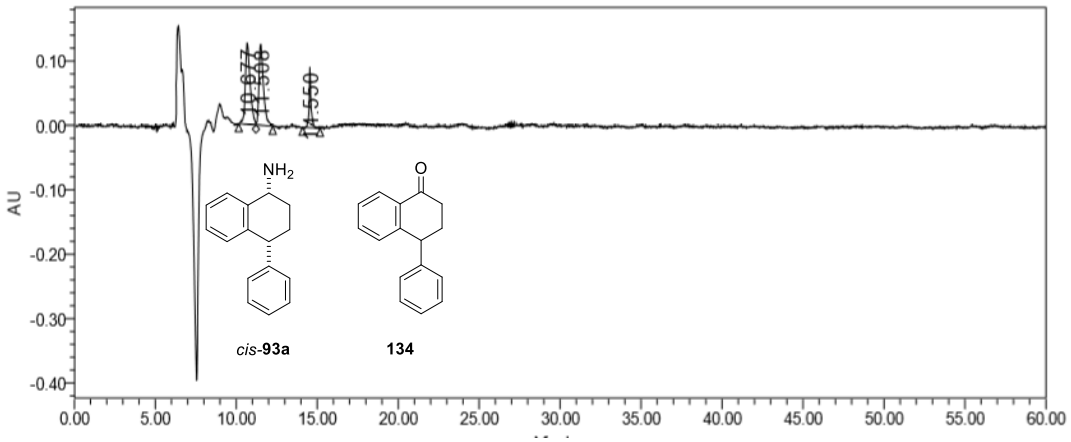
*Table A15*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | *(1S,4R)*-**92b** | 16.9 |
| | | | | | *(1R,4S)*-**92b** | 20.6 |
| | | | | | *(4R)*-**108** | 27.4 |
| | | | | | *(4S)*-**108** | 31.0 |

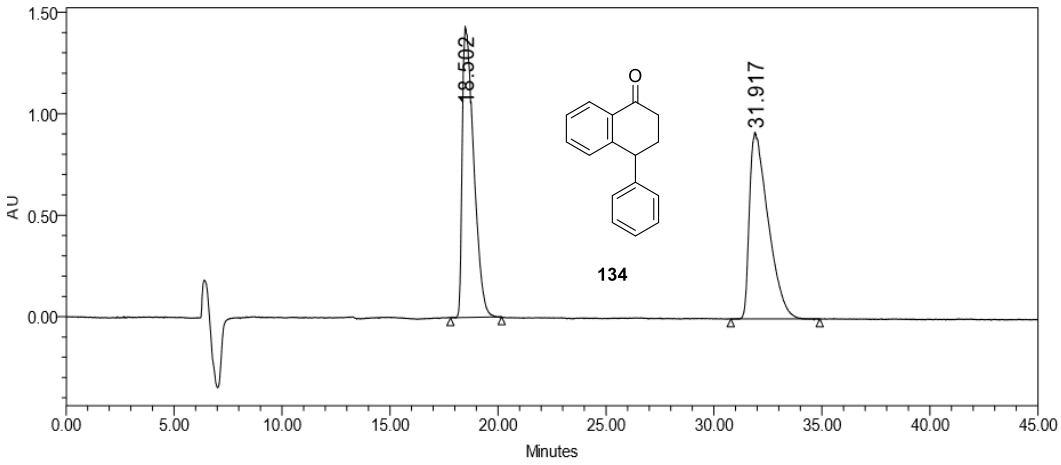
The absolute stereochemistry was assigned by comparison to the enantiopure ketone (*4S*)-**108**, which was donated by Pfizer.

*Table A16*



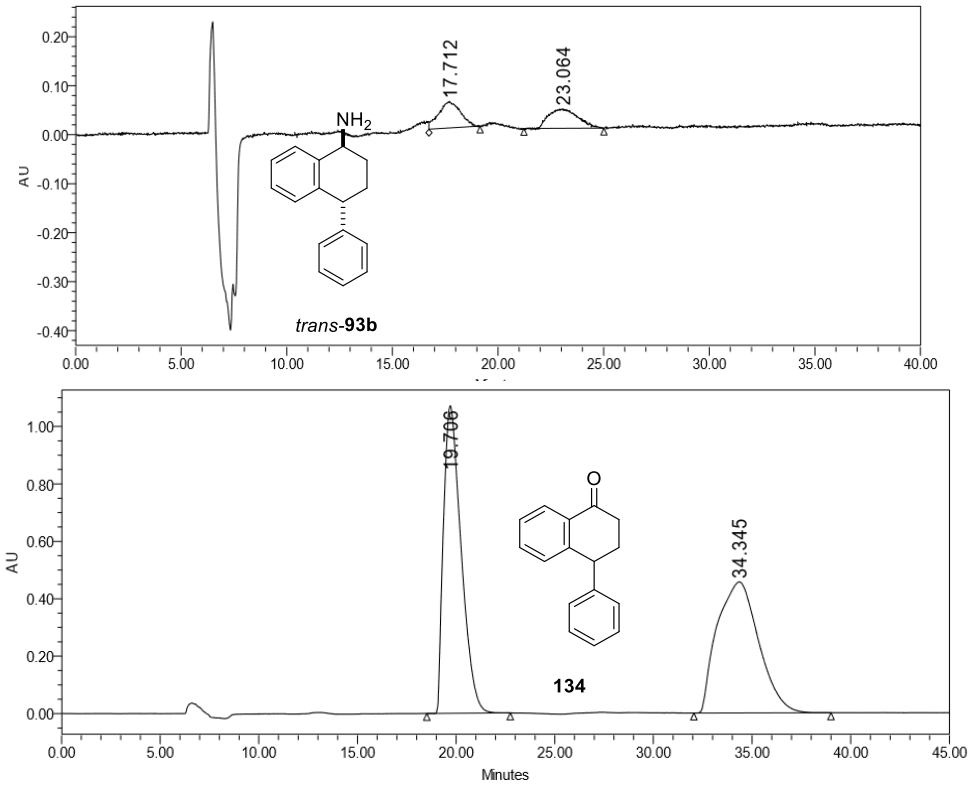

| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel AS-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **93a** | 10.7 |
| | | | | | **93a** | 11.5 |
| | | | | | **134** | 14.6 |

*Table A17*



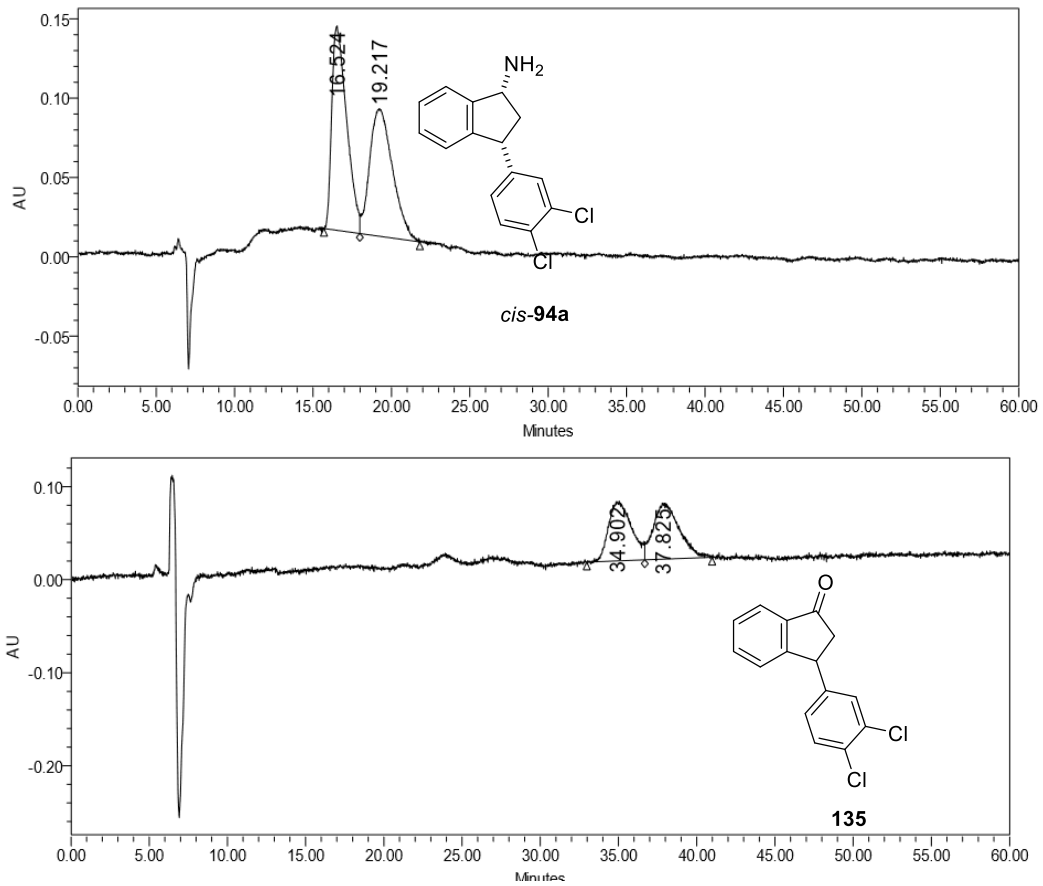

| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **134** | 18.5 |
| | | | | | **134** | 31.9 |

*Table A18*



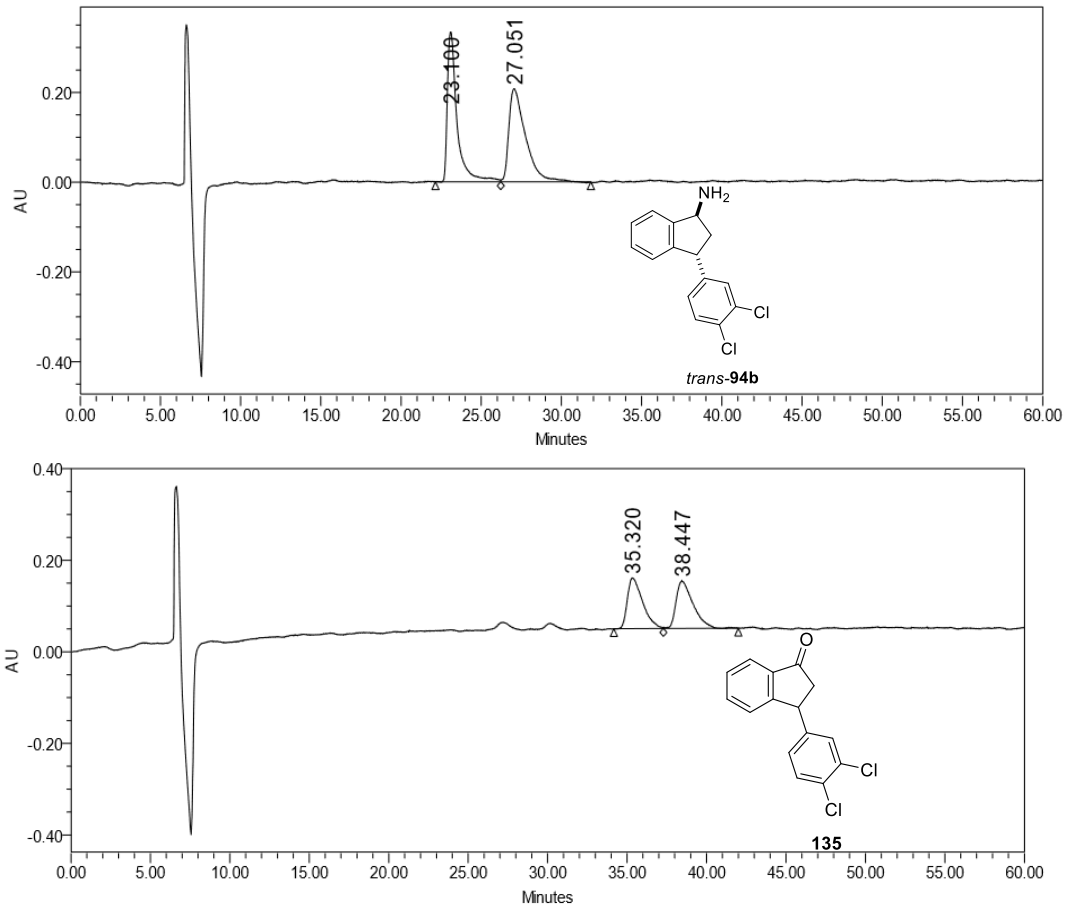

| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **93b** | 17.7 |
| | | | | | **134** | 19.7 |
| | | | | | **93b** | 23.1 |
| | | | | | **134** | 34.3 |

*Table A19*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/$^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OB-H | 0.5 | 230 | 95/5* (*contains 1% v/v DEA) | 25 | **94a** | 16.5 |
| | | | | | **94a** | 19.2 |
| | | | | | **135** | 34.9 |
| | | | | | **135** | 37.8 |

*Table A20*




| Column | Flow (mL/min) | λ (nm) | Mobile Phase (*n*-hexane/ $^{i}$PrOH) | Temp (°C) | Compound | Retention Time |
|---|---|---|---|---|---|---|
| Chiralcel OJ-H | 0.5 | 230 | 90/10* (*contains 1% v/v DEA) | 25 | **94b** | 23.1 |
| | | | | | **94b** | 27.1 |
| | | | | | **135** | 35.3 |
| | | | | | **135** | 38.4 |

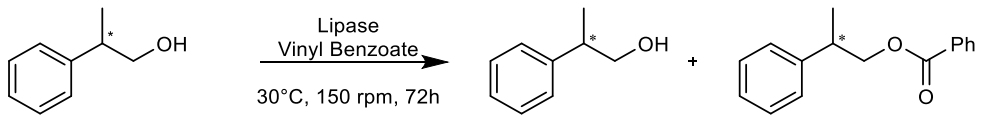
# Appendix II: List of Abbreviations

| | |
|---|---|
| $Ac_2O$ | acetic anhydride |
| ADH | alcohol dehydrogenase |
| AlaDH | alanine dehydrogenase |
| API | active pharmaceutical ingredient |
| approx. | approximately |
| aq. | aqueous |
| Ar | aryl |
| ATA | amine transaminase |
| atm | atmospheres |
| b.p. | boiling point |
| br s | broad singlet |
| CAL-B | *Candida antarctica* lipase B (immobilised) |
| conv. | Conversion |
| CV | *Chromobacterium violaceum* |
| d | doublet |
| DABCO | 1,4-diazabicyclo[2.2.2]octane |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| DCM | dichloromethane |
| dd | doublet of doublets |
| ddd | doublet of doublet of doublets |
| *de* | diastereomeric excess |
| DEA | diethylamine |
| DEAD | diethyl azodicarboxylate |
| DEPT | distortionless enhancement of polarisation transfer |
| DIP-Cl | B-Chlorodiisopinocamphenylborane |
| DKR | dynamic kinetic resolution |
| DMAP | *N*,*N*-dimethylaminopyridine |
| DMF | dimethyl formamide |
| DMSO | dimethylsulfoxide |
| DNA | deoxyribonucleic acid |
| DPPA | diphenylphosphoryl azide |
| dt | doublet of triplets |
| *E. coli* | *Escherichia coli* |
| *ee* | enantiomeric excess |
| eq. | equivalents |
| ERED | ene-reductase |
| Et | ethyl |
| $Et_2O$ | diethyl ether |
| EtOAc | ethyl acetate |
| EtOH | ethanol |
| EtONa | sodium ethoxide |
| FDH | formate dehydrogenase |
| g | gram |
| GDH | glucose dehydrogenase |
| Gl | glycine |
| h | hour |
| HHDH | halohydrin dehalogenase |
| His | histidine |
| HMBC | heteronuclear multiple bond correlation |
| HNL | hydroxynitrile lyase |
| HPLC | high performance liquid chromatography |
| HRMS | High resolution mass spectrometry |
| Hz | Hertz |
| Immob. | Immobilised |
| IPA | isopropanol |
| $^{i}PrNH_2$ | isopropylamine |

| | |
|---|---|
| $^{i}$PrOH | isopropanol |
| $^{i}$Pr | isopropyl |
| IRED | imine reductase |
| KOH | potassium hydroxide |
| KR | kinetic resolution |
| KRED | ketoreductase |
| LDH | lactate dehydrogenase |
| m | multiplet |
| M | molar |
| Me | methyl |
| MeOH | methanol |
| mg | milligram |
| min | minute |
| mL | millilitre |
| mmol | millimole |
| mol | mole |
| m.p. | melting point |
| MTBE | methyl *tert*-butyl ether |
| $NaBH_4$ | sodium borohydride |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NAD(P)H | nicotinamide adenine dinucleotide (phosphate) |
| NaOH | sodium hydroxide |
| $NEt_3$ | triethylamine |
| NMR | nuclear magnetic resonance |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| PCC | pyridinium chlorochromate |
| PDA | photodiode array |
| Ph | phenyl |
| PLP | pyroxidal 5′-phosphate |
| PMP | pyroxamine 5′-phosphate |
| PPL | porcine pancreatic lipase |
| ppm | parts per million |
| psi | pounds per square inch |
| q | quartet |
| qd | quartet of doublets |
| $R_f$ | retention factor |
| rpm | revolutions per minute |
| RT | room temperature |
| s | singlet |
| sat. | saturated |
| sept | septet |
| sym m | symmetrical multiplet |
| t | triplet |
| ω-TA | ω-transaminase |
| TA(m) | transaminase |
| $^{t}$Bu | *tert*-butyl |
| td | triplet of doublets |
| tdd | triplet of doublet of doublets |
| TEMPO | (2,2,6,6-tetramethyl-1-piperidin-1-yl)oxyl |
| THF | tetrahydrofuran |
| Thr | threonine |
| TLC | thin layer chromatography |
| TMG | 1,1,3,3-tetramethylguanidine |
| TMS | tetramethylsilane |
| TPPO | triphenylphosphine oxide |
| % v/v | volume per volume |
| % w/w | weight per weight |

# Appendix III: Additional tables for chapter 2

*Table 21 Supplementary data for the transesterification of **1** using vinyl benzoate*



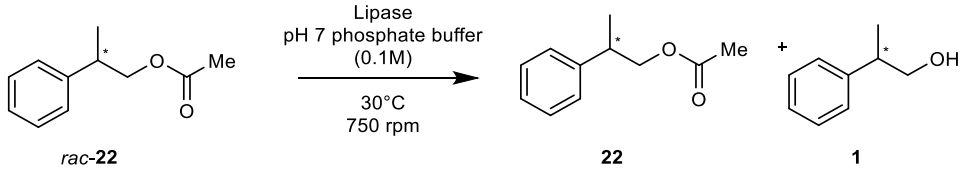

*rac*-**1** **1** **21**

| Enzyme Source | Conversion (%) | | *ee* (%) | | E |
|---|---|---|---|---|---|
| | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| No enzyme | 0 | -[a] | -[a] | -[a] | -[a] |
| Hog Pancreas Lipase | 0 | -[a] | -[a] | -[a] | -[a] |
| *Candida antarctica* Lipase B (immobilised) | 0 | -[a] | -[a] | -[a] | -[a] |
| *Pseudomonas fluorescens* (immobilised) | 0 | -[a] | -[a] | -[a] | -[a] |
| Amano PS Lipase | 0 | -[a] | -[a] | -[a] | -[a] |
| Lipase from *Candida cylindracea* | 100 | -[a] | -[a] | -[a] | -[a] |

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, $E_{calc}$, and E were not determined.

Shown below is the data from the time screen for hydrolysis of **22**, supplement to the data in **Table 2.3**, entries marked in blue are also shown in Table 2.3.

*Table 22*



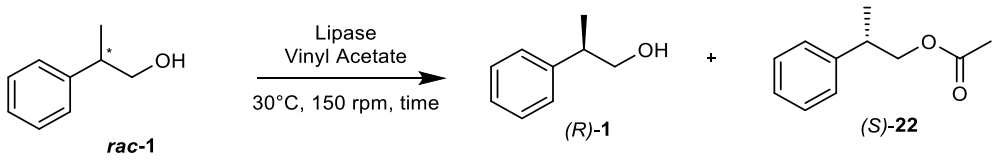

*rac*-**22** **22** **1**

| Lipase Source | Time (h) | Conversion (%) | | *ee* (%) | | E |
|---|---|---|---|---|---|---|
| | | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| Blank | 65 | 4 | -[a] | -[a] | -[a] | -[a] |
| | 48 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 24 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 0 | -[a] | -[a] | -[a] | -[a] |
| Hog pancreas Lipase | 65 | 60 | 55 | 54 | 65 | 6 |
| | 48 | 56 | 56 | 55 | 71 | 7 |
| | 24 | 72 | 72 | 35 | 92 | 6 |
| | 18 | 62 | 63 | 46 | 79 | 6 |
| | 6 | 19 | 17 | 53 | 11 | 4 |
| *Candida antarctica* Lipase B (immobilised) | 65 | 87 | 62 | 16 | 26 | 2 |
| | 48 | 69 | 70 | 38 | 87 | 6 |
| | 24 | 55 | 54 | 61 | 72 | 9 |
| | 18 | 56 | 47 | 55 | 73 | 7 |
| | 6 | 21 | 21 | 70 | 18 | 7 |
| *Pseudomonas fluorescens* (immobilised) | 65 | 87 | 62 | 6 | 10 | 1 |
| | 48 | 57 | 59 | 13 | 19 | 2 |
| | 24 | 52 | 53 | 15 | 17 | 2 |
| Amano PS Lipase | 65 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 48 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 24 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 57 | 57 | 19 | 25 | 2 |
| Lipase from *Candida cylindracea* (gives (R)-1 as product) | 65 | 87 | 60 | 2 | 3 | 1 |
| | 48 | 56 | 47 | 4 | 4 | 1 |
| | 24 | 62 | 58 | 5 | 7 | 1 |
| | 18 | 66 | 64 | 4 | 7 | 1 |
| | 6 | 42 | 38 | 5 | 3 | 1 |

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, $E_{calc}$, and E were not determined.

Shown below is the data from the time screen for transesterification of **1** with vinyl acetate, supplement to the data in **Table 2.4,** entries marked in blue are also shown in Table 2.4.

*Table 23 Supplemental data for the transesterification of **1***

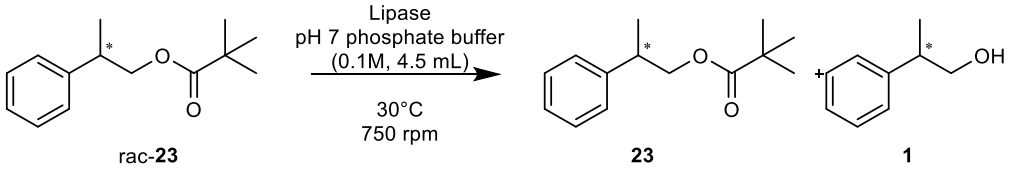



***rac*-1** *(R)*-**1** *(S)*-**22**

| Lipase Source | Time (h) | Conversion (%) | | *ee* (%) | | E |
|---|---|---|---|---|---|---|
| | | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| Blank | 24 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 8 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 4 | 0 | -[a] | -[a] | -[a] | -[a] |
| | 2 | 0 | -[a] | -[a] | -[a] | -[a] |
| Hog pancreas lipase | 24 | 78 | 79 | 99 | 27 | 7 |
| | 18 | 58 | 47 | 85 | 63 | 11 |
| | 8 | 38 | 37 | 45 | 75 | 11 |
| | 6 | 41 | 40 | 48 | 73 | 10 |
| *Candida antarctica* Lipase B (immobilised) | 24 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 93 | -[b] | 3 | 0 | -[b] |
| | 4 | 92 | -[b] | 7 | 0 | -[b] |
| | 2 | 52 | 67 | 2 | 1 | 1 |
| *Pseudomonas fluorescens* (immobilised) | 24 | 99 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 80 | 81 | 14 | 3 | 1 |
| | 4 | 72 | 72 | 12 | 4 | 1 |
| | 2 | 46 | 43 | 4 | 5 | 1 |
| Amano PS Lipase | 24 | 79 | 80 | 45 | 11 | 2 |
| | 18 | 65 | 67 | 31 | 15 | 2 |
| | 8 | 28 | 23 | 7 | 24 | 2 |
| | 6 | 44 | 45 | 16 | 20 | 2 |
| | 4 | -[a] | -[a] | -[a] | -[a] | -[a] |
| | 2 | -[a] | -[a] | -[a] | -[a] | -[a] |
| Lipase from *Candida cylindracea (gives (R)-1 as product)* | 24 | 100 | -[a] | -[a] | -[a] | -[a] |
| | 18 | 99 | -[a] | -[a] | -[a] | -[a] |
| | 6 | 98 | -[a] | -[a] | -[a] | -[a] |
| | 4 | 96 | 96 | 45 | 2 | 1 |
| | 2 | 58 | 89 | 31 | 4 | 1 |

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, $E_{calc}$, and E were not determined; [b]$E_{calc}$ and E were not determined as this required *ee* >0

*Table 24 Supplemental data for the hydrolysis of **23***




| Enzyme Source | Conversion (%) | | *ee* (%) | | E |
|---|---|---|---|---|---|
| | $^1$H NMR | $E_{calc}$ | $ee_s$ | $ee_p$ | |
| No Lipase | 0 | -[a] | -[a] | -[a] | -[a] |
| Hog pancreas Lipase | 3 | 29 | 4 | 10 (R) | 1 |
| *Candida antarctica* Lipase B (immobilised) | 0 | -[a] | -[a] | -[a] | -[a] |
| *Pseudomonas fluorescens* (immobilised) | 0 | -[a] | -[a] | -[a] | -[a] |
| Amano PS Lipase | 15 | 13 | 14 | 90 (S) | 22 |
| Lipase from *candida cylindracea* | 9 | 11 | 0 | 8 (R) | 1 |

[a]When conversion was 100% or 0% enantioselectivity values were not measured, and as a result, $E_{calc}$, and E were not determined.

# Appendix IV: Publications

Impact of variation of the acyl group on the efficiency and selectivity of the lipase-mediated resolution of 2-phenylalkanols

Foley, Aoife M.; Gavin, Declan P.; Joniec, Ilona; Maguire, Anita R., *Tetrahedron: Asymmetry*, 2017, *28*, 1144 – 1153.

Hydrolase-mediated resolution of the hemiacetal in 2-chromanols: The impact of remote substitution

Gavin, Declan P.; Foley, Aoife; Moody, Thomas S.; Rao Khandavilli, U. B.; Lawrence, Simon E.; O'Neill, Pat; Maguire, Anita R., *Tetrahedron: Asymmetry*, 2017, *28*, 577 – 585.

Dynamic kinetic resolution of 2-methyl-2-nitrocyclohexanol: Combining the intramolecular nitroaldol (Henry) reaction & lipase-catalysed resolution

Foley, A. M.; Gavin, D. P.; Deasy, R. E.; Milner, S. E.; Moody, T. S.; Eccles, K. S.; Lawrence, S. E.; Maguire, A. R., *Tetrahedron*, 2018, *74*, 1435 – 1443.

Manuscripts in preparation, not included in this appendix:

Patent pending: "A new functional ω-transaminase enzyme" European patent application number: 18186426.5

Isolation and characterization of a novel remote-stereospecific marine transaminase with a unique challenging substrate profile

Abreu-Castilla, I.; Gavin, D. P.; Reen, F. J.; Foley, A. M.; Rocha-Martin, J.; Maguire, A. R.; O'Gara, F., *Manuscript in preparation.*

Identification of a novel esterase isolated from a marine environment which displays an unusual substrate scope and its characterisation as an enantioselective biocatalyst

Declan P. Gavin, Edel J. Murphy, Aoife M. Foley, Ignacio Abreu-Castilla, F. Jerry Reen, Stuart G. Collins, Fergal O'Gara, Anita R. Maguire, *Manuscript in preparation*.