Enzymatic routes to generic building blocks leading to chiral tertiary alcohols

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Abstract

Tertiary alcohols are a common functional group in many natural products, pharmaceuticals and agrochemicals. The ability to produce highly enantiomerically pure tertiary alcohols is therefore an important goal in synthetic chemistry.

The synthesis of chiral tertiary alcohol precursors has been achieved via enzymatic desymmetrisation with the lipase Amano L, AK to generate \((S)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate 50}\) in 42\% isolated yield and 97\% enantiomeric excess. The reaction was also attempted with an immobilised lipase from \(R.\ miehei\) yielding the product in 82-90\% yield and up to 89\% ee, Figure 1.

The enantioselective ring-opening of the epoxide using different amines has been developed in high yields generating enantiomerically pure \(\beta\)-amino tertiary alcohol products. However, an undesired intramolecular migration of the acetyl group was observed during the epoxide opening with aliphatic primary amines resulting in prochiral triol products. To avoid such an intramolecular migration a TBS-protected derivative 99 has been used to prepare the tertiary alcohol products with primary amines in good yields (43\%-83\%), without any loss of enantiomeric excess of the formed ‘pseudo’-enantiomer 100a-c.

\((S)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate 50}\) has the potential to generate a large diversity of compounds; this reagent was also used as the starting material to generate azetidines 114a-j in high yields.

Figure 1: Overview.
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To Juanchi who always believed in me.
**ABREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>Selectivity of the reaction</td>
</tr>
<tr>
<td>$\Delta G$</td>
<td>Free energy change</td>
</tr>
<tr>
<td>$\Delta \Delta G$</td>
<td>Activation energy</td>
</tr>
<tr>
<td>Ac$_2$O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>AllylOAc</td>
<td>Allyl acetate</td>
</tr>
<tr>
<td>Amano L, AK</td>
<td><em>Pseudomonas fluorescens</em> lipase</td>
</tr>
<tr>
<td>Amano L, PS</td>
<td><em>Pseudomonas cepacia</em> lipase</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>CAL-A</td>
<td>Lipase A from <em>Candida antarctica</em></td>
</tr>
<tr>
<td>CAL-B</td>
<td>Lipase B from <em>Candida antarctica</em></td>
</tr>
<tr>
<td>CLEA</td>
<td>Cross-linked enzyme aggregate</td>
</tr>
<tr>
<td>$m$-CPBA</td>
<td><em>meta</em>-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>CRL</td>
<td><em>Candida rugosa</em> lipase</td>
</tr>
<tr>
<td>ddH$_2$O</td>
<td>Distilled/deionised water</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DKR</td>
<td>Dynamic kinetic resolution</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>E</td>
<td>Enantiomeric ratio</td>
</tr>
<tr>
<td>ee</td>
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<td>G</td>
<td>Glycine</td>
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<td>GC</td>
<td>Gas chromatography</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>-----------</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>k</td>
<td>First-order rate constant</td>
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<td>LAH</td>
<td>Lithium aluminium hydride</td>
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<td>Lithium perchlorate in Et₂O</td>
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<td>MW</td>
<td>Microwave</td>
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<tr>
<td>NMO</td>
<td>Methylmorpholine-N-oxide</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>PCC</td>
<td>Pyridinium chlorochromate</td>
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<td>Porcine pancreas lipase</td>
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<td>Pyridine</td>
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<td>Sodium metasilicate</td>
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<td><em>tert</em>-Butyldimethylsilylchloride</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TPAP</td>
<td>Tetrapropylammonium perruthenate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>TsCl</td>
<td>4-Toluenesulfonyl chloride</td>
</tr>
<tr>
<td>VA</td>
<td>Vinyl acetate</td>
</tr>
<tr>
<td>W</td>
<td>Watt</td>
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Chapter 1:
INTRODUCTION
NATURAL PRODUCTS AND THEIR CHIRALITY

During the past 25 years the search for biologically active natural products has been critical for the pharmaceutical and agrochemical industries. Huge efforts have been made to develop synthetic routes to these pharmacologically important compounds.\(^1\)

Many pharmaceutical compounds and medicines are isolated and purified from natural sources due to the complexity of their syntheses on an industrial scale.\(^2\)

Today's efforts tend to focus on developing new synthetic or semi-synthetic routes to drugs as well as utilising biosynthetic intermediates to generate natural products.\(^3\)

Therefore, it is important to develop new synthetic methods to generate these compounds using chemical or biochemical tools and thus improve the diversity of resources.

![Chemical structures of Taxol and Streptomycin](image)

Scheme 1: Examples of pharmaceutical compounds isolated from nature.

Scheme 1 shows two examples of pharmacologically active compounds isolated from natural sources. Taxol 1 is present in the plant kingdom, isolated from the yew tree and possesses anticancer activity,\(^4\) another example is the aminoglycosides such as streptomycin 2, present in the microbial world,\(^5\) this compound is active against bacteria and itself is isolated from the bacteria, *Legionella pneumophila*.\(^6\)

Pharmacognosy is the science of extracting medicinal compounds from natural sources. This science has the tools to identify, treat and isolate a target compound from a natural resource for pharmacological use, although this process is very slow,
expensive and inefficient, and the active ingredient is present in low concentrations. However, there is still a need to investigate and develop new efficient synthetic routes to generate not only natural products, but also their analogues and other nature-inspired compounds for potential use as medicines.

1. CHIRAL TERTIARY ALCOHOLS AND THEIR DERIVATIVES IN NATURAL PRODUCTS AND PHARMACOLOGICALLY ACTIVE COMPOUNDS

One of the common functional groups present in the natural products shown in Scheme 1 is a chiral tertiary alcohol; a moiety present in many natural products and pharmacologically active compounds.

Scheme 2 shows seven more examples of natural products or pharmaceutical compounds containing a chiral tertiary alcohol or derivative which have been isolated either from nature or synthesised in the laboratory for industrial applications.

In numerous cases, drugs and other targets containing a chiral tertiary alcohol or derivative display activity whereby one enantiomer is more active than the other or that only one shows activity altogether; this is the case with citalopram. Most of the bioactivity of this drug resides in the (S)-(+) enantiomer. (S)-(+) citalopram is a selective inhibitor of serotonin re-uptake and has proven to be an efficient antidepressant. This pharmaceutical compound is produced synthetically and in one particular route, the stereogenic centre on the tertiary alcohol is generated via enzymatic methods. Similar key enzymatic steps are used for the syntheses of voriconazole, an antifungal agent, frontalin, an insect pheromone derived from a chiral tertiary alcohol, and the essential oil linalool. Enzymatic syntheses of citalopram and frontalin will be discussed in detail in Section 3.2.
Scheme 2: Natural products and pharmaceutical drugs containing a chiral tertiary alcohol or functionality which is prepared from a chiral tertiary alcohol.

Pumiliotoxin 251D 8 is one of the toxins present in poison arrow frogs, used for chemical self-defence against predators. This natural product has been chemically synthesised using a chiral pool approach, from L-proline.

(S)-Oxybutynin 10 is an anticholinergic used to treat urinary and bladder-control problems. The (S)-enantiomer possesses anticholinergic activity and several approaches have been developed to generate the hydroxy-bearing quaternary stereocentre. Grignard addition into α-ketoesters derived from chiral cis-
aminoindanols, aldol reactions of dioxolones derived from chiral mandelic acid, Sharpless asymmetric dihydroxylation of α-cyclohexylstirene, using gadolinium complexes catalytic enantioselective cyanosilylation of cycloalkyl phenyl ketones or kinetic resolution of tertiary α-hydroxynitriles with *Pseudomonas cepacia* (PSL-C) have all been reported to synthesise the key α-hydroxy acid precursor to (S)-oxybutynin.

Camptothecin 11 was isolated from *Camptotheca acuminata decene*, a tree native in China, in 1966 by M. E. Wall and M. C. Wani. This compound showed excellent anticancer activity. The (R)-isomer is inactive and several chemical and enzymatic routes have been published in the past four decades describing methods to generate the (S)-enantiomer and its derivatives.

Some of these pharmacologically active compounds possessing a chiral tertiary alcohol require the tertiary alcohol for biological activity, or the activity increases in the presence of this functional group. For example, Chaetomugilin A 12 is an antitumour compound isolated from the marine fish *Mugil cephalus* (Figure 2), and it has been demonstrated that the analogue containing a chiral tertiary alcohol exhibits a higher cytotoxic activity than the methoxy analogue 13 (Chaetomugilin B) 33, 34

![Figure 2: Chaetomugilins A 12 and B 13, two antitumor agents.](image)

Linezolid 14 is an important antibacterial agent and the first member of the oxazolidinone family known to possess such activity. Whereas linezolid is usually a well tolerated drug, long treatments with this compound are sometimes complicated due to a reversible myelosuppression concluding with the suspension of this treatment. However, the tertiary alcohol-containing analogue 15 presents similar
antibacterial activity and could be better tolerated for prolonged treatments (Figure 3).

![Linezolid 14 and hydroxy-piperidine analog 15](image)

Figure 3: Linezolid 14 and a 4-hydroxy-piperidine analogue, 15.

Quinic acid 16 is isolated from the woody plant *Uncaria* and possesses anti-inflammatory activity (Figure 4), and it is another example of a compound with higher biological activity due to the presence of chiral secondary and tertiary alcohols. Acetylation of the hydroxyl groups giving derivative 17 reduces the anti-inflammatory activity.36

![Quinic acid 16 and less active tetra-acetylated derivative 17](image)

Figure 4: Quinic acid 16 and its tetra-acetylated derivative 17.

Due to the importance of generating compounds in a single enantiomeric form, the next section will introduce general methods used to accomplish this.
2. METHODS TO GENERATE ENANTIOPURE PRODUCTS

Many classic reactions in organic chemistry do not have the ability to produce selectively one enantiomeric form of a compound. However the need to produce pharmaceutical products as single enantiomers led to the development of many different synthetic methods and resolution techniques to achieve this (Scheme 3).\textsuperscript{37,38}

![Scheme 3: Current methods to generate enantiopure products.](image)

There are three general ways to generate chiral products, the first one is using the chiral pool approach, the second is resolution of a racemic mixture and the third is to use prochiral substrates. These three routes are described in more detail in the next sections.

2.1 Chiral pool

The chiral pool is a limited number of commercially available enantiopure reagents, such as carbohydrates, amino acids, terpenes and steroids, which are isolated from natural sources. These reagents are used as precursors or intermediates in a synthetic route to allow the introduction of the desired stereogenic centre in the final compound. The chiral pool approach is an efficient method to produce chiral compounds at low cost and 100\% enantiomeric excess, so long as the synthetic route avoids racemisation of the products. However, there are a limited number of
commercially available chiral compounds, therefore, it may be difficult to find a suitable reagent with the necessary structure and stereochemistry for a synthesis.

Lactose is a disaccharide which is isolated from the sweet or sour whey of milk. This natural product is used as a chiral pool precursor for the synthesis of the anthracycline antibiotics, daunorubicin and doxorubicin (Scheme 4). These compounds are useful drugs for the treatment of a range of human cancers and can be synthesised from the readily available precursor α-D-isosaccharino-1,4-lactone, obtained on large scale from lactose.

Scheme 4: Synthesis of daunorubicin and doxorubicin from lactose.

2.2 Kinetic resolution

The development of single enantiomers can also be achieved by the kinetic resolution of a racemic mixture. Kinetic resolution is a process in which both enantiomers of a racemic mixture are transformed into two products at different rates (Scheme 5). When the kinetic resolution is efficient, only one enantiomer of the racemic mixture is transformed into the desired product. This process displays the limitation of this method, in that a maximum yield of only 50% is achievable, i.e. the undesired enantiomer is also isolated in 50% yield, which requires time-consuming and costly removal. Kinetic resolution resolves the racemic mixture because one enantiomer reacts faster than the other.
Scheme 5: Kinetic resolution of a racemate. \( S_R \) and \( S_S \) are racemic substrates; \( P \) and \( Q \) are the enantiomeric products. If \( k_1 \gg k_2 \), product \( P \) will dominate with a high ee.

Scheme 5 represents the kinetic resolution of a racemate \( (S_R+S_S) \) to produce two enantiomeric products \( (P \) and \( Q) \) at different reaction rates. The apparent first-order rate constants \( (k_1 \) and \( k_2) \) determine the reaction rate, in the case where \( k_1 \gg k_2 \), product \( P \) will dominate with high ee. The selectivity of the reaction \( (\alpha) \) is only a function of the apparent first-order rate constants, determined by the ratio \( k_1/k_2 \). This parameter is not dependent on the conversion of the reaction and therefore will be constant over the process. Consequently, the enantiomeric excess of the product \( (\text{ee}) \) depends only on the selectivity of the reaction. When the enzyme is selective for a substrate, one rate constant will be higher than the other and thus the optical purity will be higher.

A quantitative analysis of the enantioselectivity of a kinetic resolution is the enantiomeric ratio \( (E) \). The equation is a relationship between the enantiomeric excess of the product \( (\text{ee}_P) \) and the degree of conversion \( (c) \).

\[
E = \ln[(1-c)(1-\text{ee}_P)] / \ln[(1+c)(1-\text{ee}_P)]
\]

This parameter is independent of the time of reaction and substrate concentrations. When the enzyme is totally selective, 50% of enantiopure product will be obtained but when the enzyme is less selective, the biotransformation gives lower enantioselectivity, however higher enantiomeric purity can be achieved with a higher degree of conversion. Therefore, the relationship between enantiomeric excess and conversion is important for making useful predictions and this information is generated with the enantiomeric ratio.
T. Loh and co-workers have reported a kinetic resolution of different bis-homoallylic alcohols with high enantioselectivity (Scheme 6). An oxonium-ene cyclisation catalysed by In(OTf)$_3$ resulted in remarkable 1,4-stereocomunication. Only the (S)-isomer reacts with the catalyst and the (R)-isomer is recovered with high enantiomeric purity. Finally, they applied this method to the resolution of the natural product (R)-sulcatol 27 with >98% ee; this compound is biologically active as an insect pest control agent.

Scheme 6: Kinetic resolution of bishomoallylic alcohols.

2.2.1 Dynamic kinetic resolution

Dynamic kinetic resolution (DKR) combines the classic procedure of kinetic resolution (Section 2.2) with an additional feature; an in situ racemisation step of the unreacted substrate (Scheme 7). The resolution is performed using conditions under which both enantiomers of the substrate are in a fast equilibrium or racemising, therefore, all the substrate is reacted leading to an efficient use of all starting material. Depending on the substrate stability and the rate of the equilibrium, racemisation may occur spontaneously or with the help of a catalyst. This catalyst can be either chemical or enzymatic. In dynamic kinetic resolution all the substrate can, under optimal conditions, be converted to a single enantiomer in 100% yield and enantiomeric excess.
Chemoenzymatic DKR is a very attractive process which combines traditional chemical synthesis with high regio- and enantioselectivity with the use of enzymatic catalysis.\textsuperscript{49,51,52} Unfortunately, in the majority of cases, racemisation and resolution of a racemic compound require different reaction conditions; consequently, the reaction conditions of each process can be incompatible. Finding the fine balance between both processes can give rise to a dynamic kinetic resolution.\textsuperscript{53}

\begin{center}
\includegraphics[width=0.5\textwidth]{scheme7.png}
\end{center}

Scheme 7: Kinetic dynamic resolution showing production of the (R)-product in excellent yield and ee.

J. Bäckvall and co-workers studied a chemoenzymatic DKR of secondary alcohols \textsuperscript{30} via racemisation with a Ru-catalyst combined with lipase B from \textit{Candida antarctica}, which resulted in a dynamic kinetic resolution to generate chiral secondary alcohols in high yields and >99\% ee (Scheme 8).\textsuperscript{49,50} This DKR is performed \textit{via} hydrogen transfer catalysed by the Ru-complex and the acetylation of the secondary alcohol is catalysed by lipase B from \textit{Candida antarctica} with \textit{p}-chlorophenyl acetate.
2.3 Asymmetric catalysis

Another technique for the development of enantiomerically enriched compounds is asymmetric catalysis. In this case, the chirality is present in the catalyst used in the reaction. Only one chiral molecule is needed to introduce chirality in a million molecules of the reaction product. The catalyst could be an enzyme,\textsuperscript{54-56} or a metal-based catalyst.\textsuperscript{57,58} This process can be very efficient, both on an industrial scale and synthetically in the laboratory.

The asymmetric catalysis of \textit{meso} or prochiral compounds by enzymes is a powerful strategy in organic synthesis due to the ability to reveal and/or generate multiple stereogenic centers in one step.\textsuperscript{54,59} The prochiral substrate needs to be recognised by the enzyme in order to catalyse the desymmetrisation. The desymmetrisation of \textit{meso} and prochiral compounds has been investigated with different functional groups such as epoxides,\textsuperscript{60,61} anhydrides,\textsuperscript{62} diols,\textsuperscript{63,64} dienes\textsuperscript{65} and ketones\textsuperscript{66} and applied to the synthesis of many natural products.\textsuperscript{67-69}
Scheme 9 shows a general example of enzymatic asymmetric catalysis with a lipase to transform a prochiral diol to a chiral product by selectively modify one primary alcohol function to an acetate.

As discussed, there are three general methods to produce chiral compounds, chiral pool, kinetic resolution or asymmetric catalysis, and these can be performed chemically or enzymatically. The next sections will introduce the applicability of these methods to generate enantiomerically pure chiral tertiary alcohols using enzyme catalysis.

3 ENZYMATIC CATALYSIS LEADING TO ENANTIOMERICALLY PURE TERTIARY ALCOHOLS.

Enantiomerically pure chiral tertiary alcohols are not easy to synthesise both chemically and enzymatically due to their bulkiness and as a consequence, difficult accessibility. Due to the importance of this moiety in nature, the next sections will endeavor to explain some common methods for making tertiary alcohols, using enzymatic kinetic resolution or enzymatic desymmetrisation.

Enzymes are the catalysts of biological systems, accelerating the rate of reaction by factors of a million or more compared to the corresponding non-catalysed reaction. Enzymatic catalysis has a number of advantages over non-enzymatic catalysis, such as the chemo-, regio-, stereo-, and enantioselectivity under mild conditions. Enzymes can typically catalyse a broad range of reactions, including ester hydrolysis and formation, oxidation and reduction, addition and elimination, halogenation and dehalogenation. The enzymes which catalyse the chemical reactions are formed from
chiral components ie. amino acids. These enzymes are able to differentiate both enantiomeric forms of their substrate, and therefore give the product as a single enantiomer.

Figure 5 shows the energy differences between a catalysed ($\Delta G^\#_{ES}$) and uncatalyzed reaction ($\Delta G^\#$). The enzyme accelerates the reaction rate by decreasing the free energy ($\Delta G^\#$) of the transition state. The enzyme stabilises the transition state of the reaction but is not capable of modifying the equilibrium of the process. The activation energy required to perform the catalysed reaction with an enzyme ($\Delta G^\#_{ES}$-$\Delta G_s$) is much smaller than the same reaction without catalyst ($\Delta G^\#-$-$\Delta G_s$).

![Figure 5: Free energy profile for an uncatalysed reaction (----) and enzyme catalysed reaction (---).](image)

For instance, the catalysed reaction is faster than the uncatalysed one due to the energy reduction of the enzyme-substrate transition state. While Figure 5 compares catalysed and uncatalysed reactions, Figure 6 compares the energy difference between two enantiomers and therefore the reason why one enantiomer reacts faster than the other in a catalytic reaction.

In an enzymatic reaction, both enantiomeric substrates, A and B, are competing for access to the active site of the enzyme. A selective enzyme with a specific substrate
can distinguish between both enantiomers due to the chiral environment of the active site. The catalytically active functional groups of the enzyme act as a nucleophile or electrophile with the groups of the substrate differentiating A and B; therefore, generating different free energies (ΔG) of the transition states complexes, [EA]⁺ and [EB]⁺. The result is a difference in the activation energy (ΔΔG⁺) meaning that one enantiomer, B, reacts faster than the other, A.⁴⁴

![Free energy diagram for an enzyme catalysed enantioselective reaction.](image)

Figure 6: Free energy diagram for an enzyme catalysed enantioselective reaction. E = enzyme, A and B = enantiomeric substrates, P and Q = enantiomeric products, [EA]⁺ and [EB]⁺ = transition states of diastereomeric enzyme substrate complexes, ΔΔG⁺ = differences between free energies of the transition states of [EA] and [EB].⁴⁴

### 3.1 Hydrolases and their catalytic activity

Hydrolases catalyse hydrolysis, esterification or transesterification reactions.⁴³ Scheme 10 shows a general example of a transesterification process where the enzyme reacts with the ester (acyl donor) to give an enzyme-substrate intermediate. The process is followed by a nucleophilic attack by an alcohol to produce a new ester. If the enzyme is selective only one enantiomeric form of the product will be generated.⁴⁶

Their natural function is to hydrolyse triglycerides into fatty acids and glycerol. Lipases are the most frequently used class of hydrolases in organic synthesis, playing an important role in industrial manufacture.⁴⁴
The mechanism for the hydrolysis reaction follows four general steps (Scheme 11), and is catalysed by a group of three amino acids: histidine (His), serine (Ser) and aspartic acid (Asp).

1) There is a reorganisation of the hydrogen atoms allowing the substrate to react with the active site serine residue, generating a tetrahedral intermediate which is stabilised by the histidine and aspartic acid.

2) The alcohol is eliminated and there is a covalent bond between the acyl group and the serine residue.

3) Nucleophilic attack takes place on the acyl-serine intermediate by a molecule of water (in the case of hydrolysis) or an alcohol (in the case of the transesterification), generating a tetrahedral complex stabilised by the other two amino acids.

4) Finally, elimination of the acid (or ester) regenerates the enzyme to catalyse another cycle.
Lipase-catalysed reactions can be used to generate enantiopure products or intermediates leading to chiral tertiary alcohols. A racemic tertiary alcohol can react selectively with the enzyme to transform one of the enantiomers into its corresponding acetate leaving the other enantiomer optically pure (see examples in section 3.2).

### 3.2 Enzymatic kinetic resolution leading to enantiomerically pure chiral tertiary alcohols

Only a small number of lipases are able to accept tertiary alcohols into their active site due to their bulky and spherical shape, however when they do, the enzymes cannot distinguish easily between the enantiomers.\(^{29,70-78}\) The kinetic resolution of tertiary alcohols directly is difficult, only a few hydrolyses show activity, which typically give low to modest ee’s, and are limited to small substituents.

One example is the hydrolytic resolution of tertiary acetate esters with a lipase from *Candida cylindracea* (Scheme 12).\(^{79}\) Four different tertiary acetates were tested (35-
and the most successful result was the hydrolysis of 1,1,1-trifluoro-2-phenylbut-3-yn-2-yl acetate 38, when the reaction was taken to 40% conversion, resulting in 87% and 75% enantiomeric excess of the chiral alcohol and acetate respectively.

Scheme 12: Kinetic resolution of tertiary acetate esters with a lipase from Candida cylindracea.

Further work showed that a double mutant of Bacillus subtilis esterase displayed activity against the same tertiary trifluoromethyl acetate 38 with completely opposite selectivity, 93% enantiomeric excess after 42% conversion.\(^{80}\) This particular esterase was identified as GGG(A)X-hydrolase (A = alanine, G = glycine and X = any amino acid), an amino acid sequence shown to be involved in the stabilisation of the oxyanion hole present in the active site which is able to accommodate more sterically demanding tertiary alcohols due to a larger, more open active site.\(^{81}\)

An enantioselective esterification process of tertiary alcohol 40 was also attempted, and achieved with lipase A from Candida antarctica and vinyl acetate in 97% enantiomeric excess albeit in low conversion (Scheme 13). Initially, eight different lipases and esterases were investigated for the kinetic resolution of 2-phenylbut-3-yn-2-ol 40 with vinyl acetate in hexane. Four of these enzymes, those containing the GGGX-motif at the active site, showed activity and selectivity in the enzymatic esterification.\(^{77}\)

Therefore, the amino acid motif GGGX located in the oxyanion binding pocket of esterases and lipases tend to show activity in the kinetic resolution of bulky tertiary acetate esters and alcohols.\(^{78}\)
Another enzymatic route to generate chiral tertiary alcohols is an indirect lipase-catalysed kinetic resolution. Lipase catalysis is performed on a primary or secondary alcohol near the stereogenic centre resulting in a resolution of the racemic mixture. The indirect resolution of tertiary alcohols via the hydrolysis or esterification of a proximal primary or secondary alcohol is a useful tool in enzymatic kinetic resolution.  \(^\text{16}\)

Scheme 13: Enantioselective esterification of 2-phenylbut-3-yn-2-ol, \(\text{40}\).

Scheme 14: Kinetic resolution of 4-[(4-dimethylamino)-1-(4’-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)-benzonitrile \(\text{3}\).
One example of such an approach is the enzymatic kinetic resolution of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)benzonitrile 3, a useful intermediate in the synthesis of citalopram 4 (Scheme 14). Good yields and high enantioselectivities can be achieved by an appropriate selection of the reaction parameters. Gotor et al. reported the kinetic resolution of benzonitrile 3 with lipase B from *Candida antarctica* and vinyl acetate in acetonitrile to generate the desired configuration for the synthesis of (S)-(−)-citalopram 5. A reaction performed due to the enzyme-catalysed acetylation of the distal primary alcohol.

Due to the simplicity and facile scale-up of lipase catalysed reactions, this method could be applied to the industrial preparation of the antidepressant (S)-(−)-citalopram 4.

As shown, this method can be used to achieve the enzyme catalysed resolution of a quaternary stereogenic centre by performing a transesterification or hydrolysis reaction in organic solvent. It is remarkable, that the enantioselective recognition can be achieved by the enzyme at a primary hydroxyl group four bonds removed from the stereogenic centre!

The kinetic resolution utilised a primary alcohol handle to resolve the (S)-(−)-citalopram intermediate 3, however, this method is not necessary suitable for all tertiary alcohols as the compounds must possess suitably positioned primary or secondary alcohol functionality near to the stereogenic centre for enzyme recognition. As such, there is an unmet need to develop a single, general and flexible route to access chiral tertiary alcohols.

One particular method for the synthesis of chiral tertiary alcohols can be achieved via the kinetic resolution of chiral epoxides followed by ring-opening, whereby the enzyme is able to resolve the epoxide by modification of a pendant primary alcohol, as seen with (±)-2,3-epoxy-2-(4-pentenyl)-propanol 41 (Scheme 15). Santaniello and coworkers have reported such an enantioselective esterification reaction with a lipase from *Pseudomonas fluorescens* and vinyl acetate in organic solvents to resolve the racemic epoxyalcohol 41 in 98% enantiomeric excess and 38% isolated yield; a chiral intermediate for the synthesis of both enantiomers of frontalin 7. 82
Another example of a kinetic resolution of an epoxide to generate a chiral tertiary alcohol was also reported by Santaniello the following year. In this example, itaconic acid 44 was used as the starting material for the synthesis of useful building blocks. It was shown that a methyl substituted chiral tertiary alcohol 45 could be formed by using a lipase catalysed resolution of racemic epoxide 42 to generate the desired enantiopure epoxide in 90% ee and 30% yield. Exhaustive reduction with LiAlH₄ finally achieved the desired product 45 (Scheme 16).

![Scheme 15: Kinetic resolution of (±)-2,3-epoxy-2-(4-pentenyl)-propanol 41.](image)

![Scheme 16: Synthesis of a chiral tertiary alcohol 45 via the kinetic resolution of ethyl 2,2-(hydroxymethyl)oxiran-2-yl acetate 42.](image)
Three different kinetic resolution methods to generate chiral tertiary alcohols with high enantiomeric excesses have been discussed. The principal inconvenience of using a kinetic resolution method is that only 50% yield of the desired enantiomer can be achieved with optimum conditions.

### 3.2.1 Enantioselective transformation of prochiral substrates to produce chiral tertiary alcohols in high yields

Recently, numerous isolated enzymes have been shown to catalyse asymmetric reactions with perfect stereoselectivity. Due to the availability of numerous enzymes such as esterases, lipases and amylases, etc., biocatalysts can play an important role in asymmetric synthesis.\(^4\)\(^3\),\(^4\)\(^6\) The principle limitation of this method is the high specificity of enzymes, leading to long process times to discover and develop an efficient biocatalyst for a wide range of substrates.\(^4\)\(^4\) The use of lipases to carry out ester hydrolyses and transesterification reactions have been used successfully for the desymmetrisation of different meso and prochiral alcohols. This enzymatic process could potentially generate single enantiomers in up to 100% enantiomeric excesses and yields.\(^3\)\(^7\),\(^5\)\(^4\),\(^8\)\(^4\)

For example, Sugai and co-workers reported a lipase catalysed desymmetrisation of (±)-2-hydroxy-2-(prop-2-ynyl)cyclohexane-1,3-diyl diacetate \(^4\)\(^6\), an intermediate for the synthesis of aquayamycin, \(^4\)\(^8\) (Scheme 17).\(^8\)\(^5\) The diacetate \(^4\)\(^6\) was successfully hydrolysed in high yields and enantioselectivities with a lipase from *Candida antarctica* to give the desired (R)-enantiomer, \(^4\)\(^7\). The biotransformation on a 10 g scale employed 1.8 g of enzyme to generate diol (+)-\(^4\)\(^7\) in 94% yield and >99% ee, Scheme 17.
Scheme 17: Enzymatic desymmetrisation of (±)-2-hydroxy-2-(prop-2-ynyl)cyclohexane-1,3-diy diacetate 46, an intermediate for the synthesis of aquayamycin 48.

As discussed in section 3.2, the kinetic resolution of an epoxide followed by an epoxide ring-opening is an attractive approach to generate chiral tertiary alcohols. A similar process can also be applied to the enzymatic desymmetrisation of a prochiral epoxide, where in this case, a single enantiomer, in up to 100% yield and ee, can be accessed.

Both approaches have been used for the synthesis of frontalin 7. The first, described in Section 3.2, used a kinetic resolution of racemic epoxyalcohol 41 which gave rise to enantiomerically pure (S)-41 (Scheme 15). Similarly, a desymmetrisation of prochiral epoxide 49 has also been reported (Scheme 18).
Scheme 18: Enzymatic desymmetrisation of oxirane-2,2-diylbis(methylene) diacetate 49 towards the synthesis of (1S,2R)-frontalin 7.

The optically active epoxide 50 is a versatile building block for the synthesis of tertiary alcohols. Initially, Seu and Kho reported a simple preparation of the chiral methyl substituted tertiary carbinol 51 via the asymmetric hydrolysis of oxirane-2,2-diylbis(methylene) diacetate 49 with porcine pancreas lipase (PPL) in 92% ee and 77% yield (Scheme 18) to obtain (R)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50.\textsuperscript{86} The results were further optimised with a lipase from Pseudomonas sp. to achieve the same isomer in 98.7% ee and 95% yield.\textsuperscript{87}

The ring-opening reaction of enantiopure epoxides is an attractive and powerful tool in asymmetric synthesis, and a high number of products can be synthesised by employing nucleophiles such as thiols, Grignard reagents, reducing agents, amines and alcohols, amongst others.

Another example of a chiral epoxide as a useful building block is the synthesis of glycidyl sulfide 58 as an intermediate for the synthesis of chiral tertiary alcohols, reported by T. Itoh.\textsuperscript{88,89} They studied three pathways to prepare (R)-2-(phenylthiomethyl)pent-4-ene-1,2-diol 57 (Scheme 19). Path A is a kinetic resolution of tertiary alcohol 52, whereas paths B and C employed enzymatic desymmetrisation. Unfortunately there was no enzyme capable of hydrolysing the acetate of compound 52 and path B needs a tertiary hydroxyl protection with the expensive 2-(trimethylsilyl)ethoxymethyl (SEM). The best route to generate the chiral tertiary
alcohol 57 was following the asymmetric catalysis of the epoxide 55 with porcine pancreas lipase to generate the epoxy monoacetate 56 with 96% ee and 89% yield, path C.

A few routes to generate enantiopure tertiary alcohols have been introduced, paying particular attention to the enzymatic desymmetrisation of prochiral epoxides followed by nucleophilic ring-opening. This synthetic route is of interest due to its flexibility of generating chiral tertiary alcohols with various functionalities. The enzymatic ester hydrolysis or transesterification of a prochiral epoxide with hydrolases have been investigated to generate enantiopure products. This process is followed by the ring-opening to achieve the final chiral tertiary alcohols. There are numerous synthetic methods to generate tertiary alcohols by epoxide ring-opening.

Scheme 19: Three pathways for the synthesis of 2-allyl-2-(phenylthiomethyl)oxirane 58.
with various functionalities. The following sections will introduce the synthesis of chiral β-amino tertiary alcohols in particular due to the importance of these compounds in medicinal chemistry and natural products.\textsuperscript{88-98}

\section*{4 SYNTHESIS OF CHIRAL β-AMINO TERTIARY ALCOHOLS}

Chiral β-amino tertiary alcohols are useful building blocks for the synthesis of biologically active compounds, and outlined in Scheme 20 are a number of interesting structures containing such a motif. β-Amino tertiary alcohols have been investigated for use as intermediates in the management of cardiovascular disorders,\textsuperscript{90} including hypertension\textsuperscript{91,92} and other conditions related to the sympathetic nervous system.\textsuperscript{93-97} This moiety is present in natural products and pharmacologically active compounds such as (+)-setoclavine\textsuperscript{59} which has immunomodulatory activity.\textsuperscript{98} Hydroxy-azetidines\textsuperscript{60} are another example of such compounds, and Renslo and co-workers demonstrated these linezolid analogues exhibited antibacterial activity.\textsuperscript{99} Paraherquamide A\textsuperscript{61}, which was first isolated from cultures of \textit{Penicillium paraherquei} by Yamazaki and co-workers in 1981, displays potent anthelmintic activity and antinematodal properties.\textsuperscript{100,101} Similarly, the poison arrow frog toxin pumiliotoxin A\textsuperscript{62} was isolated from the skin of the frog from the dendrobates species, and the anticancer compound vincristine\textsuperscript{63}, isolated from \textit{Catharanthus roseus}, is a mitotic inhibitor used in cancer chemotherapy also possess a β-amino tertiary alcohol.\textsuperscript{102,103} Bacilosarcin A\textsuperscript{64} was isolated from the marine bacterium \textit{Bacillus subtilis} and exhibits antibacterial, cytotoxic and antiulcer activities.\textsuperscript{104-106} Finally, Sadeé and co-workers investigated the naloxone analogue\textsuperscript{65} as a possible treatment for opioid overdose.\textsuperscript{107-109}

The nucleophilic ring-opening of epoxides by amines is one of the most common reactions in synthetic organic chemistry. The results of such a reaction are a variety of β-amino alcohols, which represent a large number of intermediates present in biologically active natural products and synthetic chemistry building blocks.

The classical approach for the synthesis of β-amino alcohols involves the treatment of epoxides with amines under thermal conditions, usually this procedure requires an excess of the amine and elevated temperatures.\textsuperscript{110} However, when poorly
nucleophilic and sterically hindered amines are used, yields of the corresponding β-amino alcohols are usually poor and often necessitate the use of catalysts or promoters. The next sections describe some examples of generating β-amino tertiary alcohols using ring-opening reactions of epoxides.

Scheme 20: Structures of biologically active compounds or natural products containing a β-amino tertiary alcohols.
4.1 Epoxide ring-opening with amine nucleophiles using catalyst/promoters

Various protocols have been developed to activate epoxides and make them more susceptible to nucleophilic ring-opening reactions.\textsuperscript{111-121} One such protocol utilises the surface of solids, like alumina or silica gel, to promote the reaction. An example was reported by Chakraborti and co-workers; they studied the ring-opening of the symmetrical epoxide cyclohexene oxide with different amines using chromatographic silica gel to promote the process (Scheme 21).\textsuperscript{122} The reaction was complete in 3 hours at room temperature, in the absence of solvent, using aromatic amines with different electronic properties such as aniline (90% yield), 4-methylaniline (87% yield) and 4-chloroaniline (92% yield) to give the trans-2-arylaminocyclohexanol products. The reaction also worked well with aliphatic amines such as pyrrolidine to give the trans-2-alkylaminocyclohexanol in 90% yield.\textsuperscript{122}

![Scheme 21: Reaction of cyclohexene oxide with different amines in the presence of silica gel.\textsuperscript{122}](image_url)

Shivani and co-workers reported on such protocols for the synthesis of this moiety using metal perchlorates, such as the commercially available zinc(II) perchlorate hexahydrate [Zn(ClO\textsubscript{4})\textsubscript{2}-6H\textsubscript{2}O], to promote an epoxide ring-opening using various amines (Scheme 22).\textsuperscript{123} This new and highly active catalyst was found to catalyses epoxide ring-opening with a number of aromatic and aliphatic amines, obtaining \(\beta\)-amino alcohols in high yields, under solvent-free conditions, at room temperature with high regioselectivities.\textsuperscript{123}
The reaction was highly regioselective, in all cases to perform on a large scale.

Scheme 22: Epoxide ring-opening with amines in the presence of metal perchlorates.\textsuperscript{123}

To prevent the limitations of the classical procedure, such as elevated temperatures, long reaction times and low yields with poorly nucleophilic or sterically bulky amines, several activators/promoters, such as Lewis acids and lithium salts, have been investigated.\textsuperscript{124,125} Lithium perchlorate in Et$_2$O (LPDE) catalyses the ring opening of epoxides using amines.\textsuperscript{126} Heydari and co-workers investigated the use of this catalyst to open different epoxides with various aromatic and aliphatic amines in high yields.\textsuperscript{127} One example is the epoxide ring-opening of 2,2-dimethyloxirane 72 with aniline 67a to generate the symmetrical alcohol 73 in 98\% yield (Scheme 23).\textsuperscript{127} The reaction was highly regioselective, in all cases only one product was detected. However, the use of perchlorates is dangerous and the work-up with Lewis acids is often complicated by the emulsions formed and this procedure is normally unsuitable to perform on a large scale.\textsuperscript{112}

Scheme 23: Epoxide ring-opening with amines catalysed by LPDE.

Epoxide ring-opening can also be catalysed by metal catalysts such (S)-(\textemdash)BINOL-Ti complexes.\textsuperscript{128-130} One example of this process was reported by Kureshy and co-
workers in the asymmetric epoxide ring-opening of \textit{meso}-stilbene oxide with aniline at room temperature (Scheme 24).\textsuperscript{131} The reaction was catalysed by (S)-(\textdagger)-BINOL-Ti(O\textsubscript{3}Pr)\textsubscript{4} \textit{76} to give \(\beta\)-amino alcohol \textit{75} in high yield (90\%) and enantiomeric excess (78\% ee) when triphenylphosphine was used as an additive. The enantioselectivity of the product was improved to 98\% after a single recrystallisation step. The catalyst can be recovered after the use and recycled four times with retention of activity, making the process favourable for an industrial use.

\[ \text{O} \begin{array}{c} \text{Ph} \\ \text{Ph} \end{array} \begin{array}{c} \text{NH}_{2} \\ \text{Ph} \\ \text{Ph} \end{array} \xrightarrow{\text{Ti(O\textsubscript{3}Pr)\textsubscript{4}} (10 \text{ mol-\%})} \xrightarrow{\text{S-Binol (10 mol-\%)} \text{ toluene \text{- room temperature}}} \begin{array}{c} \text{H} \\ \text{Ph} \end{array} \begin{array}{c} \text{N} \\ \text{Ph} \\ \text{Ph} \end{array} \text{75} \]

\textbf{Scheme 24: Asymmetric catalytic ring-opening of \textit{meso}-stilbene oxide with (S)-(\textdagger)-BINOL-Ti complex \textit{76}.}\textsuperscript{131}

Fagnou and co-workers investigated a possible catalyst for epoxide ring-opening with aromatic amines, [Rh(CO)\textsubscript{2}Cl]\textsubscript{2}.\textsuperscript{132} High yields, diastereo- and regioselectivities are obtained during the ring-opening of vinyl epoxides with aromatic amines in the presence of this catalyst to give \textit{trans}-1,2-amino alcohols at room temperature under neutral conditions with selectivities exceeding 20:1. One example is the epoxide ring-opening of 7-oxabicyclo[4.1.0]hept-2-ene \textit{77} with \textit{N}-methylaniline in 91\% yield (Scheme 25). Aliphatic amines failed to react under these conditions since the amine strongly binds to the rhodium metal destroying the catalyst. Aromatic amines are less basic than aliphatic amines, and consequently the bonding would be reduced.
4.2 Epoxide ring-opening with amine nucleophiles in water

The preparation of β-amino alcohols in water is desirable, since water reduces the environmental impact of the reaction compared to conventional organic solvents.\textsuperscript{133} However, purification of the reaction products on a large scale is sometimes difficult and certainly not always applicable on an industrial scale. Ring-opening of epoxides with a variety of amines, in water at room temperature have however been investigated.\textsuperscript{134-139} Two examples will be described involving such a process. The first example, a regioselective addition of amines in water, was reported by Azizi and co-workers.\textsuperscript{135} The reaction was performed by adding 1.2 equivalents of amine \textit{80} to an epoxide \textit{79}, in water at room temperature, to give the β-amino alcohol product \textit{81} in 97% yields.\textsuperscript{135} In some cases, the β-amino alcohols precipitate from solution and are easily separated by filtration, but usually, extraction with organic solvent is required. The reaction rate of aminolysis in water depends on the structure of the epoxide and the nucleophilicity or bulky nature of the amine (Scheme 26).\textsuperscript{135}

Scheme 26: Aminolysis of 2-(phenoxy)methyl)oxirane 79 with morpholine 80 in water.\textsuperscript{135}
The second example is the catalytic asymmetric ring-opening of epoxide 76 with aromatic amines in water, reported by Azoulay and co-workers. The reactions were performed in the presence of 1 mol% of Sc(OSO$_3$C$_{12}$H$_{25}$)$_3$ and 1.2 mol% of a chiral bipyridyl ligand to produce the β-amino alcohols in high yields and enantioselectivities. The best result was obtained when the reaction was performed with N-methylaniline 67g in the presence of the catalyst (Scheme 27).

![Scheme 27](image)

**Scheme 27: Catalytic asymmetric ring-opening of (2R,3S)-2,3-diphenyloxirane 76 with N-methylaniline 67g.**

4.3 **Epoxide ring-opening with amine nucleophiles using microwave irradiation**

Another technique that could be applied to asymmetric epoxide ring-opening is microwave irradiation. Microwave irradiation has revolutionised organic synthesis; a large number of compounds can now be constructed in a much reduced reaction time compared to that required by classical thermal conditions. This technique has been accepted rapidly as an important tool for the discovery of new drugs and the development of chemical processes.

Epoxide ring-opening by amines can also be conducted under microwave irradiation without the need of catalysts and under mild conditions. One example of this reaction was reported by Robin and co-workers. The microwave-assisted reaction can be performed cleanly with 1.5 equivalents of amine in ethanol in only 4 minutes at 140 °C (80 W). They investigated the synthesis of a large number of β-amino
alcohols using a variety of primary and secondary amines under short reaction times and in high yields. When the epoxide ring-opening of 2-(benzyloxymethyl)oxirane 83 was performed with diethylamine 84 under microwave irradiation the corresponding β-amino alcohol 85 was obtained in 96% yield (Scheme 28).

![Scheme 28: Epoxide ring-opening of 2-(benzyloxymethyl)oxirane 83 with diethylamine 84 under microwave irradiation.](image)

Different routes have been introduced for epoxide ring-opening with amines in high yields. In general, the use of catalysts increase the activity and selectivity of the reaction, microwave irradiation accelerates the reaction generating products in only a few minutes and the reaction can also be performed in water for a more environmentally friendly approach.

5 AIMS

Tertiary alcohols are a common functional group in many natural products, pharmaceuticals and agrochemicals (Figure 7). The ability to produce high enantiomerically pure tertiary alcohols is therefore an important goal in synthetic chemistry; especially important is the need to develop a general and flexible route to generate these compounds.

![Figure 7: A chiral tertiary alcohol.](image)
Only a few examples for generating enantiopure tertiary alcohols have been reported\textsuperscript{16,70,77-81,83} due to the unfavorable steric interactions resulting from the bulky substituents on the stereogenic centre, making them more difficult to access for biotransformations and chemical modifications; only a few hydrolases show activity towards tertiary alcohols.\textsuperscript{77,78,80,81} Moreover, a general and flexible route to generate chiral tertiary alcohols is not currently known.

Our flexible synthetic route to generate chiral tertiary alcohols is \textit{via} the enzymatic desymmetrisation of symmetrical epoxide 87 and it is outlined in scheme 29. The process starts with the epoxidation of commercially available 2-methylenepropane-1,3-diol 86 to generate the prochiral epoxy diol 87. A subsequent biotransformation is performed to obtain the chiral epoxy monoacetate 50, following which, the epoxide may be opened with different nucleophiles (C, N, O, S, etc.) to generate chiral tertiary alcohols with different functionalities \textit{92-97} (Scheme 32) from a common intermediate (Scheme 29).

![Scheme 29: Proposed general synthetic route to generate chiral tertiary alcohols via enzymatic desymmetrisation of an epoxide.](Image)

The enzymatic desymmetrisation of prochiral compounds potentially can generate a single enantiomer in up to 100 % enantiomeric excess and yield.\textsuperscript{43,54,64,147} The biotransformation of prochiral diol 87 with the appropriate lipase, acyl donor and organic solvent would therefore achieve this goal (Scheme 29).\textsuperscript{148} Our aim is to
discover the optimum conditions to perform this biotransformation and thus maximise the yield and enantiomeric excess of the products (Scheme 30), before opening the epoxide with various nucleophiles to generate enantiomerically pure tertiary alcohols.

Scheme 30: Enzymatic desymmetrisation of prochiral epoxy diol 87.

The enzymatic hydrolysis of oxirane-2,2-diylbis(methylene) diacetate 49 is known (Scheme 31). In 1992, Seu and Kho reported the hydrolysis of the epoxy diacetate 49 with porcine pancreatic lipase (PPL) in acetone and phosphate buffer (pH 6.5) at 0 °C for 7 hours to obtain the epoxy monoacetate (R)-50 in 77% yield and 92% ee. The enantiomerically pure epoxy monoacetate (R)-50, has been shown to be a useful building block for the synthesis of natural products, such as frontalin 7, bicyclomycin 8 and α-tocopherol.

Scheme 31: Enzymatic hydrolysis of oxirane-2,2-diylbis(methylene) diacetate 49.

We aimed to develop an enzymatic route to generate enantiopure (2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50 in organic solvents thus enabling subsequent reactions to be performed in “one pot” avoiding further purifications thereby minimising waste and reducing costs. Numerous organic products are not stable in aqueous solution and water is a poor solvent for industrial
Thus, the possibility of performing the biotransformation in organic solvents would ease both product recovery and minimise side reactions.

There are a number of nucleophiles that generate different chiral tertiary alcohols via opening enantiopure epoxides (Scheme 32). It is anticipated that the nucleophilic epoxide ring-opening process will maintain enantiomeric purity and not give rise to racemic tertiary alcohols through racemisation processes (such as acyl transfer).

Scheme 32 shows some examples of epoxide ring-opening with different nucleophiles such as sulfides, amines, allyl indium species, nitriles, alcohols and Grignard reagents.

Amine nucleophiles were chosen to screen the epoxide ring-opening process to generate chiral β-amino tertiary alcohols. These products constitute an important class of well-known organic compounds that are important in natural and non-natural products. The epoxide ring-opening of (±)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate with different amines was investigated initially to generate β-amino tertiary alcohols via this process.

Scheme 32: Epoxide ring-opening with different nucleophiles.
The ability to produce high enantiomerically pure compounds is critical to the pharmaceutical industry, since the resolution of racemic products is difficult and expensive. For this reason, it is important to synthesise enantiomerically pure intermediates.

It is important to use these compounds as intermediates for the synthesis of natural products and pharmaceutical compounds on an industrial scale since production of the unwanted enantiomer is kept to a minimum. If the yield and enantioselectivity is high, the reactions (desymmetrisation and epoxide ring-opening) could be carried out in “one pot” precluding the need for purification at the intermediates stages. This is an important consideration in industry where purification is expensive.
Chapter 2:

RESULTS AND DISCUSSION
6 SYNTHESIS OF TARGETS

6.1 Synthesis of oxirane-2,2-diyldimethanol 87

The epoxidation of 2-methylene propane-1,3-diol 86 with 1.1 equivalents of \textit{m}-chloroperoxybenzoic acid (\textit{m}-CPBA) was achieved in 98\% yield (Scheme 33). The product was purified using column chromatography, however traces of \textit{m}-CPBA or \textit{m}-CBA were detected using \textsuperscript{1}H-NMR when the reaction was performed on a larger scale. After further experimentation it was observed that the traces of \textit{m}-CPBA/\textit{m}-CBA could promote the decomposition of epoxy diol 87 upon storage. With this in mind the reaction was performed always on small scale and repeated more often to obtain fresh and pure material for use in the biotransformation process. Epoxy diol 87 was used in the enzymatic transesterification in organic solvents.

\[
\text{HO}_{\text{86}} \xrightarrow{\text{m-CPBA}} \text{HO}_{\text{87}} \text{DCM, RT 98\% yield}
\]

Scheme 33: Synthesis of epoxy diol 87.

6.2 Synthesis of oxirane-2,2-diylbis(methylene) diacetate 49

The synthesis of oxirane-2,2-diylbis(methylene) diacetate 49 was achieved via three different routes, each generating the product in moderate yield but with good purity (Scheme 34). The synthesis of diacetate 49 was developed to perform the hydrolysis and obtain a sample of enantiopure epoxy monoacetate (\textit{R})-50 in order to optimise the GC conditions required to assign the (\textit{R})-isomer, reported in the literature.\textsuperscript{86}

The first synthetic route to epoxy diacetate 49 was the double acetylation of 3-chloro-2-(chloromethyl)prop-1-ene 95 with sodium acetate in DMF followed by the epoxidation of the product with \textit{m}-CPBA (Scheme 34, reaction A). 3-Chloro-2-(chloromethyl)prop-1-ene 95 was studied due its low cost however only a 42\% yield was obtained over two steps.
The second and preferred route developed, started with the epoxidation of 2-methylene propane-1,3-diol 86 with \( m \)-CPBA followed by double acetylation with 2 equivalents of acetyl chloride, triethylamine (TEA) and 4-dimethylaminopyridine (DMAP) to obtain the epoxy diacetate 49 in an overall yield of 73\% (Scheme 34, reaction B).

![Scheme 34: Three methods for the synthesis of epoxy diacetate 49.](image)

In an attempt to improve the yield further the last method attempted for the synthesis of epoxy diacetate 49 proceeded via a diacetylation of 2-methylene propane-1,3-diol 86 with acetyl chloride, triethylamine and 4-dimethylaminopyridine in 100\% yield, followed by epoxidation of the product with \( m \)-CPBA in only 55\% yield (Scheme 19, reaction C). In this case the diacetylation is performed in quantitative yield but the epoxidation step proved more difficult, presumably due to the bulky acetates lowering accessibility and deactivating the double bond.

We decided to use route B to generate the epoxy diacetate 49, due to the higher yield of the overall process, for all subsequent reactions.
6.3 Synthesis of \((\pm)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate 50}\)

The synthesis of \((\pm)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate (}\pm\text{-}50\) was performed after monoacetylation of 2-methylenepropane-1,3-diol 86 using acetyl chloride, triethylamine and 4-dimethylaminopyridine, followed by epoxidation of the product with \(m\)-CPBA in 70% yield overall. The crude epoxidated product was difficult to purify by column chromatography since the epoxy monoacetate \((\pm)\text{-}50\) appeared to be unstable under acidic media. The best method found to purify the epoxy monoacetate \((\pm)\text{-}50\) from \(m\)-CPBA and its by-product was by treating the reaction with \(\text{K}_2\text{CO}_3\) (Scheme 35).

Two equivalents of 2-methylenepropane-1,3-diol 86 were used to perform the monoacetylation and an increased yield from 68% (1 equivalent of 86) to 77% (2 equivalent 86) was seen. The excess 2-methylenepropane-1,3-diol 86 was isolated and recycled.

Racemic monoacetate \((\pm)\text{-}50\) was used to optimise the GC analysis conditions and generate racemic standards.

\[
\begin{align*}
\text{HO} & \text{OH} & & \text{1. CH}_3\text{COCl, TEA, DMAP, RT} & \rightarrow \text{HO} & \text{O} & \text{OAc} \\
86 & & & & \rightarrow & (\pm)-50
\end{align*}
\]

Scheme 35: Synthesis of epoxy monoacetate \((\pm)\text{-}50\).

7  ENZYME CATALYSIS TO GENERATE CHIRAL EPOXIDES

7.1 Enzymatic desymmetrisation in buffer

The biotranformation of oxirane-2,2-diylbis(methylene) diacetate 49 under buffered conditions to give \((R)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate (}\text{R}\text{-}50\) is known (Scheme 36).\textsuperscript{86,87} Reported by Seu and co-workers, the hydrolysis of diacetate 49 in buffer solution with porcine pancreas lipase type II (PPL) generated acetate \((R)-50\) in 92% ee and 77% yield.\textsuperscript{86}
There are examples in the literature reporting that lipases can catalyse the reaction in both directions, i.e. perform ester hydrolysis and the transesterification process. Initially, we wanted to develop the process in both directions, perform the reaction in water and organic solvents and therefore increase the flexibility for further chemical modifications. Most of the reactions for further modifications are suitable in organic solvents. When biotransformations are performed in water provides the advantage of minimising solvent waste and environmental impact during the reaction but, in this case the purification process is more difficult. The product needs to be extracted and purify for the next step making the process more unlikely for industrial scale. When the process is performed in organic solvents the purification is easier and the process can be performed in “one pot” making the process more likely to develop on industrial scale, minimising solvent waste and time.

The synthesis of chiral tertiary alcohols was attempted initially by following the literature procedure. The hydrolysis of epoxide 49 using porcine pancreas lipase type II (PPL) in pH 6.5 buffer giving epoxy monoacetae (R)-50 in 80% yield and 76% enantiomeric excess. However this result could not be repeated reliably and thus we decided focus on the use of organic solvents.

7.2 Enzymatic desymmetrisation in organic solvents

The next sections detail a screening programme for the enzymatic desymmetrisation of oxirane-2,2-diylidimethanol 87 with a range of hydrolases and acyl donors in different organic solvents to give enantiopure (S)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50 (Scheme 37). Then, a few immobilised enzymes were tested to improve further the scope of the reaction.
Scheme 37: Enzymatic desymmetrisation of oxirane-2,2-diyldimethanol, 87 in organic solvents.

7.2.1 Screening conditions

To our knowledge, the enzymatic desymmetrisation of epoxy diol 87 in organic solvents was unknown. Therefore, it was necessary to screen the reaction with different enzymes, acyl donors and organic solvents at various temperatures in an attempt to obtain high enantiomeric excess and yield.

Due to the large number of possibilities, initially, we attempted the reaction on a small scale and estimated the conversion by TLC to establish enzymes activity. The best results, with respect to conversion, were repeated on a larger scale, purified and the enantiomeric excess was determined by GC of isolated material (Table 1).

The enzymatic desymmetrisation of epoxy diol 87 in organic solvents produced the (S)-isomer as expected due to the symmetry between the hydrolysis and transesterification reactions. The epoxy monoacetate 50 generated during the enzymatic desymmetrisation in organic solvents was analysed by GC and the results compared with those obtained from the hydrolysis of diacetate 49.

Scheme 38: Enzymatic desymmetrisation of 87 in organic solvents.

The enzymatic desymmetrisation of 87 with lipase B from Candida antarctica (CAL-B) gave low conversions and selectivities (Table 1, entries 1-6). The best
result of 20% ee in 12% yield was obtained when the reaction was performed with acetic anhydride in DCM at room temperature (Table 1, entry 5).

Porcine pancreas lipase type II (PPL type II) was also examined in organic solvents and enantiomeric excesses were very low under all conditions (Table 1, entry 7-9). It appears, this enzyme is efficient for performing the hydrolytic process, (77% yield and 92% ee) (Scheme 31), but when performed in organic solvents, gave epoxy monoacetate 50 in only 21% yield and 19% ee (Table 1, entry 8). There are several documented cases, where enzyme selectivity (enantio-, regio- and chemoselectivity) changes with the solvent used. The enantioselectivity of an enzyme with the same substrate can be modified or even inverted going from water to organic solvents. The role of water in an enzymatic reaction is to generate a specific activity, specificity and flexibility. The aqueous media generates specific interactions with the substrate and these are modified in the absence of water.

Further attempts to improve the reaction yields and ee’s resulted in the reaction being carried out with the enzyme Amano L, PS from Burkholderia cepacia (Pseudomonas cepacia) and enantiomeric excesses were significantly higher, but still too low to be synthetically useful (Table 1, entries 12-19). The best result was 66% ee when monoacetate (S)-50 was isolated in 62% yield. The reaction with this enzyme also gave the diacetylated product, oxirane-2,2-diylbis(methylene) diacetate, 49 in 40% yield (Table 1, entry 16).

The final enzyme screened was Amano L, AK from Pseudomonas fluorescens (Table 1, entries 20-30). This enzyme gave the best results with respect to enantiomeric excess; a 95% ee was obtained when the reaction was carried out with one equivalent of acetic anhydride, two weight equivalents of enzyme and DCM at 37 ºC for 0.5 hour (Table 1, entry 30). Although, the reaction with Amano L, AK gave good enantiomeric excesses, the yield of monoacetylated product was very low (11%). After further experimentation it was discovered that the starting material had decomposed upon storage, which affected the process and therefore isolated yields. Thus, the reaction was optimised with pure starting material.
Table 1: Results of the enzymatic desymmetrisation of 87 in organic solvents.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme</th>
<th>Time (h)</th>
<th>Temp. (°C)</th>
<th>Acyl donor\textsuperscript{b}</th>
<th>Solvent</th>
<th>% 50\textsuperscript{c}</th>
<th>% ee\textsuperscript{d}</th>
<th>% 49\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1</td>
<td>25</td>
<td>VA</td>
<td>CHCl\textsubscript{3}</td>
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<td>6</td>
<td>14</td>
</tr>
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<td></td>
<td>1</td>
<td>25</td>
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<td>MTBE</td>
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<td>36</td>
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<td>56</td>
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<td>10</td>
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<td>21</td>
<td>19</td>
<td>5</td>
</tr>
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<td>9\textsuperscript{e}</td>
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<td>DCM</td>
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<td>0</td>
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<td>-</td>
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<td>40</td>
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<td>24</td>
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<td>DCM</td>
<td>2</td>
<td>20</td>
<td>0</td>
</tr>
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<td>22</td>
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<td>DCM</td>
<td>39</td>
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<td>7</td>
</tr>
<tr>
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<td>25</td>
<td>VA</td>
<td>DCM</td>
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<td>47</td>
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<tr>
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<td>18</td>
<td>25</td>
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<td>12</td>
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<td>21</td>
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<td>48</td>
<td>25</td>
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<td>-</td>
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<tr>
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<td>2</td>
</tr>
<tr>
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<td></td>
<td>4</td>
<td>25</td>
<td>Ac\textsubscript{2}O</td>
<td>DCM</td>
<td>31</td>
<td>71</td>
<td>9</td>
</tr>
<tr>
<td>24\textsuperscript{e}</td>
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<td>Ac\textsubscript{2}O</td>
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<td>67</td>
<td>8</td>
</tr>
<tr>
<td>25\textsuperscript{e}</td>
<td><em>Pseudomonas fluorescens</em></td>
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<td>30</td>
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</tr>
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<td>DCM</td>
<td>22</td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td>27\textsuperscript{e}</td>
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<td>Ac\textsubscript{2}O</td>
<td>DCM</td>
<td>9</td>
<td>68</td>
<td>10</td>
</tr>
</tbody>
</table>
7.2.2 Enzymatic desymmetrisation of 90 with Amano L, AK

The biotransformation process with Amano L, AK was repeated with fresh and pure diol 87, at different temperatures and weight equivalents of enzyme in DCM (Table 2). Using pure starting material led to an increase in the yield but unfortunately this was accompanied by a decrease in the enantiomeric excess.

### Table 2: Enzymatic biotransformation of 87 with Amano L, AK in DCM

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Equivalent enzyme</th>
<th>% 50(^b)</th>
<th>% ee(^c)</th>
<th>% 49(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>22</td>
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<td>51</td>
<td>62</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>0.5</td>
<td>5</td>
<td>56</td>
<td>66</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>4.5</td>
<td>0.5</td>
<td>49</td>
<td>73</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>1.5</td>
<td>2</td>
<td>62</td>
<td>74</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^a\) Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride (1 molar equivalent) and Amano L, AK (2 weight equivalent).  
\(^b\) Isolated yields.  
\(^c\) Results from GC of the crude reaction.

Comparing the best results of pure and impure material, the biotransformation process employing 2 weight equivalents of Amano L, AK at 37 °C, the yield increased from 11% to 62% whilst the enantiomeric excess decreased from 95% to 74% (Table 1, entry 11 and Table 2, entry 4).
Initially, it was believed that these differences could be due to impurities present in the starting material. When the reaction was carried out with impure material the proposed polymer impurity could potentially stabilise the enzyme, and thus we postulated that the use of additives would increase the enantiomeric excess whilst maintaining high yields and the results of these studies are displayed in the next section.

### 7.2.3 Use of additives

Certain additives are able to increase the performance of enzymatic hydrolysis in organic solvents.\textsuperscript{43,177-181} As such, a range of additives, for example: sodium chloride (NaCl), triethylamine (TEA), polyethyleneglycol (PEG) (Table 3) were screened in attempts to improve the selectivity of the enzyme.

Table 3: Enzymatic biotransformation of 87 with Amano L, AK in DCM using various additives.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (h)</th>
<th>Additive</th>
<th>Equivalent additive</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{c}</th>
<th>% 49\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>NaCl</td>
<td>0.5</td>
<td>51</td>
<td>78</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>TEA</td>
<td>0.5</td>
<td>45</td>
<td>81</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>PEG</td>
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<td>51</td>
<td>67</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>NaCl</td>
<td>2</td>
<td>63</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>TEA</td>
<td>2</td>
<td>50</td>
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<td>6</td>
<td>1.5</td>
<td>PEG</td>
<td>2</td>
<td>47</td>
<td>67</td>
<td>15</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride (1 molar equivalent), Amano L, AK (2 weight equivalent) and the additive. \textsuperscript{b} Isolated yields. \textsuperscript{c} Results from GC of the crude reaction mixture.

Table 3 shows that the yields of epoxy monoacetate 50 are similar to the results with pure epoxy diol 87 in the absence of additives (Table 2). However, an 81% ee and 45% yield was obtained when the reaction was carried out with 0.5 equivalents of
triethylamine (Table 3, entry 2). The ee values decreased when 2 equivalents of triethylamine were used (Table 3, entry 5).

Based on these results, we believe that these differences were not due to the proposed polymer impurities present in the starting material but due to the biotransformation following a two-step process, Section 7.2.4.

7.2.4 Two-step process

Another potential reason for obtaining lower enantiomeric excesses (ee’s) with pure material could be that using the impure epoxy diol 87 leaves an excess of acyl donor.

Failing to repeat the high enantiomeric excesses when performing the biotransformation with impure starting material (Table 1) we focussed our attention to varying the amount of acyl donor added to the reaction. When the reaction was carried out with impure starting material the acetic anhydride (acyl donor) was, unknown at the time, in excess, and therefore not completely consumed in the reaction.

The enzymatic desymmetrisation of 87 using Amano L, AK gives the epoxy monoacetate 50 and epoxy diacetate 49 as a consequence of a two-step process. The first step is an enantioselective biotransformation followed by a second step, a kinetic resolution of the newly formed chiral material (Scheme 39).

The enzymatic biotransformation of epoxy diol 87 gives both enantiomers of the desymmetrised material. The (S)-isomer is formed faster than the (R)-isomer. At the same time, a kinetic resolution of these enantiomers is performed by the enzyme, reacting faster with the (R)-isomer (Scheme 39). This two-step process increases the enantiomeric excess of the (S)-enantiomer considerably by removing the undesired (R)-enantiomer as a function of time, but with an associated decrease in the yield. It was therefore necessary to find a balance between high ee together with a synthetically useful yield. By varying the molar equivalents of acyl donor and the reaction time it is possible to increase each parameter independently, but unfortunately, when one increases the other decreases.55
Scheme 39: Enzymatic desymmetrisation of epoxy diol 87 with Amano L, AK and an excess of acyl donor.

### 7.2.5 Kinetic resolution

To confirm the selectivity of the second step of the two-step process and to better understand the biotransformation, a kinetic resolution of (±)-50 was performed (Scheme 40).

Scheme 40: Kinetic resolution of (±)-50 with 2 weight equivalents of Amano L, AK and Ac₂O in DCM at 37 °C.

To compare this result with the enzymatic desymmetrisation of 87, the kinetic resolution of (±)-50 was performed under the same conditions (see section 7.2.6), two weight equivalents of Amano L, AK and 1.8 molar equivalents of acetic anhydride in DCM at 37 °C. The reaction was very fast, in only 30 minutes the process was complete giving (S)-50 in 99% ee and 20% yield, as well as epoxy diacetate 49 in 32% yield which will decrease in the amount of (S)-50 obtained in the process. Amano L, AK gives good selectivity for the kinetic resolution of (±)-50, the
second part of the two-step process; by removing the \((R)\)-isomer it is possible to generate the \((S)\)-enantiomer in very high ee.

### 7.2.6 Equivalents of the acyl donor

To optimise the two-step process, the enzymatic desymmetrisation with Amano L, AK was performed varying the equivalents of acetic anhydride (Figure 8). Increasing the amount of acetic anhydride in the biotransformation will provide enough acyl donor to remove the undesired \((R)\)-isomer during the kinetic resolution of \((\pm)\)-50. The biotranformation process using 1.8 equivalents of acetic anhydride gave 44% yield of \((S)\)-50 in 96% enantiomeric excess (Appendix, Table 1).

The enzymatic biotransformation with Amano L, AK gave a high enantiomeric excess with a moderate reaction rate. Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), different equivalents of acetic anhydride and 2 weight equivalents of Amano L, AK. Yields of \((S)\)-50 were isolated and the ee’s were determined by GC (Appendix, Table 1 and 2).

![Figure 8: Graphical representation of the enzymatic desymmetrisation with different equivalents of acyl donor. Reactions were performed in DCM and Ac₂O at 37 °C for one hour. See Appendix, Table 1 and 2 for raw data.](image)

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As can be seen in Figure 8, the enantiomeric excess increases markedly when the amount of acetic anhydride increases from 1.2 to 1.5 equivalents (Figure 8, red line). As expected, the opposite trend is obtained with the yield of monoacetate \((S)-\text{50}\) (Figure 8, blue line) along with an increase in the yield of epoxy diacetate \text{49} as enantiomer \((R)-\text{50}\) is consumed. The enzymatic biotransformation of epoxide \text{87} can proceed with a high enantiomeric excess, but with a decreasing yield of epoxy monoacetate \((S)-\text{50}\).\textsuperscript{55}

In summary, the kinetic resolution of \((\pm)-\text{50}\) generates the desired epoxide \((S)-\text{50}\) in 99% ee and 20% yield whilst the enzymatic desymmetrisation generates the same enantiomer in 44% yield and 96% ee using, in both cases, 1.8 equivalents of \(\text{Ac}_2\text{O}\). The process was performed with the same commercially available starting material diol \text{86}. Monoacetate \((\pm)-\text{50}\) was synthesised in two steps in 70% yield and one step for prochiral diol \text{87} in 98% yield (Scheme 41). It is concluded that the enzymatic desymmetrisation of prochiral diol \text{87} to generate \((S)-\text{50}\) is the most efficient route from diol \text{86}.

![Scheme 41: Kinetic resolution vs enzymatic desymmetrisation.](image)

**7.2.7 Enzymatic desymmetrisation as a function of time**

For a better understanding of the enzymatic process, the reaction was studied over different reaction times using 1.5 and 1.8 equivalents of the acyl donor. The results of the GC analysis of the crude reaction mixture are described below.
The enzymatic desymmetrisation of epoxy diol 87 with 1.5 equivalents of acetic anhydride was very fast. In 40 minutes the enantiomeric excess was 99% and the monoacetate (S)-50 was isolated in 42% (Appendix, Table 3).

To ascertain if we could maximise the yield under these conditions, we studied the reaction over the course of 1 hour.

Figure 9: Graphical representation of the enzymatic desymmetrisation of prochiral diol 87 (10 mg) with 1.5 equivalents of Ac₂O over 1 hour. Reactions were performed in DCM (0.1 M) at 37 °C. Raw data in Appendix, Table 3.

The enzymatic desymmetrisation with Amano L, AK with 1.5 equivalents of acetic anhydride, over 1 hour, shows an increasing enantiomeric excess of (S)-50 until it reaches a maximum after about 40 minutes (Figure 9, red line). The conversion of epoxy monoacetate (S)-50 increases very fast during the first 10 minutes to 55%. However, the conversion of epoxy monoacetate (S)-50 starts to decrease as the kinetic resolution of the enantiomers begins to occur (Figure 9, blue line). For the same reason, after the first 10 minutes of the reaction, the conversion of epoxy diacetate 49 increases very fast as a consequence of the kinetic resolution of (R)-50.
The epoxy diol 87 is consumed within 30 minutes (Figure 9, violet line).

The enzymatic desymmetrisation was also studied with 1.8 equivalents of acetic anhydride and as expected, it was observed to be faster than the biotransformation with 1.5 equivalents. Unfortunately, the yields of (S)-50 were lower along with concomitant higher enantiomeric excesses (compare Figures 9 and 10).

Figure 10 depicts the same trends as Figure 9, but using 1.8 molar equivalents of acetic anhydride the enantiomeric excess increases faster than with 1.5 molar equivalents, as would be expected. The consumption of epoxy diol 87 using 1.8 equivalents of acetic anhydride takes only 30 minutes, whereby the epoxy monoacetate 50 is achieved in 40% yield and 98% ee.

Figure 10: Graphical representation of the enzymatic desymmetrisation of prochiral diol 87 (10 mg) with 1.8 equivalents of Ac₂O over 1 hour. Reactions were performed in DCM (0.1 M) at 37 °C. Raw data in Appendix, Table 4.

In conclusion, the enzymatic desymmetrisation of 87 has been achieved with Amano L, AK, acetic anhydride in DCM at 37 °C. The biotransformation occurs via a two step process whereby the enzymatic desymmetrisation of the epoxy diol 87 is
followed by a kinetic resolution of epoxy monoacetate 50. After optimisation of the reaction over an average of four repeat reactions, a 46% isolated yield and 97% enantiomeric excess was obtained when using 2 equivalents of acetic anhydride in DCM at 37 °C in one hour.

7.3 Enzymatic desymmetrisation with immobilised enzymes

The enzymatic desymmetrisation of prochiral epoxy diol 87 could potentially generate a single enantiomer in 100 % enantiomeric excess and yield.182 If such a result could be achieved, it would minimise waste and post-reaction processing, therefore creating a process more appropriate for industry for the synthesis of enantiopure tertiary alcohols. In order to develop an enzymatic method to achieve this we focussed our attention on immobilised enzymes, where the enzyme is attached to a solid support in the hope of improving the desymmetrisation process. The immobilisation of enzymes is known to improve purification and recycling, since many enzymes are soluble in many solvents. The use of enzyme immobilisation makes separation from the reaction mixture very simple leading to the ability to scale-up the biotransformation making it applicable on an industrial scale. The selectivity and stability of enzymes may also be improved upon immobilisation. Many enzymes are not stable under operational conditions in a biotransformation process and can quickly lose their catalytic activity during the reaction. Usually, enzymes suffer from denaturation by organic solvents, however, the increase in stability due to immobilisation can overcome this.43,183-189

Attempts to improve our results of the enzymatic desymmetrisation of epoxy diol 87, led us to employ a kit of immobilised enzymes which were screened using different acyl donors (Scheme 42). The biotransformation was attempted with 9 lipases and 6 proteases using 10 different acyl donors. The first screening was performed at 37 °C in dichloromethane and the reactions were left until no further changes were observed by TLC. The conditions selected were consistent with the biotransformation with non-immobilised Amano L, AK (Section 7.2).
Scheme 42: Enzymatic desymmetrisation of oxirane-2,2-diyldimethanol 87 with immobilised enzymes.

The kit of enzymes used was provided by Codexis and the acyl donors used were: acetic anhydride, vinyl acetate, iso-propylacetate, allyl acetate, pentyl acetate, ethyl acetate, 2-ethoxyethyl acetate, lauryl acetate, 2-methoxyphenyl acetate, and 2-methoxyethyl acetate. The biotransformations were carried out on 10 mg of oxirane-2,2-diyldimethanol 87 and the crude reaction mixture was injected directly into the GC to determine the enantiomeric excess and conversion of the product. The results of the enzymatic desymmetrisation of epoxy diol 87 with immobilised enzymes are shown in Table 4.

Lipase A from Candida antarctica (NZL-101) presents low activity with the substrate (Table 4, entries 1-10). The enzyme is active with Ac₂O generating acetate (S)-50 in 74% yield but low enantioselectivity, only 7% ee was obtained (Table 4, entry 1). Similar results were obtained with lipase B from Candida antarctica (NZL-102), presenting moderate activity and low selectivity (Table 4, entries 11-20).

Rhizomucor miehei (NZL-103) displayed higher activity and enantioselectivity with epoxy diol 87 (Table 4, entries 21-30). The reaction temperature, weight equivalents of enzyme, molar equivalents of acyl donor and the use of additives were all screened to optimise the biotransformation process with this enzyme vide infra. The enzyme displayed the highest activity and selectivity with acetic anhydride and vinyl acetate. A high enantiomeric excess (94%) was obtained using acetic anhydride with 69% conversion to the desired product (S)-50 (Table 4, entry 21). In this case, 31% epoxy diacetate 49 was obtained due to the kinetic resolution described in Scheme 39. Optimisation and scaling-up of the process using acetic anhydride would be more difficult due to the need to remove the undesired diacetate by-product. In contrast, only 3% conversion to the epoxy diacetate 49 was observed when using vinyl acetate with this enzyme, the desired product obtained in 79% enantiomeric excess and 79% conversion (Table 4, entry 22). R. miehei appeared to be more
selective in the first step of the biotransformation using vinyl acetate due to the observed higher conversion and high enantiomeric excess (the kinetic resolution of the undesired enantiomer is kept to a minimum) than the process performed with Amano L, AK and Ac₂O (the kinetic resolution remove the undesired enantiomer increasing the enantiomeric excess and decreasing the conversion of product). Therefore, the use of vinyl acetate would appear to be more suited for further study for this system.

Table 4: Results of the enzymatic desymmetrisation of epoxy diol 87 with immobilised enzymes.

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<tr>
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<td>Acyl donor(^c)</td>
<td>Time (^{d})</td>
<td>% (^{50})</td>
<td>% ee (^{d})</td>
<td>% (^{49})</td>
<td>% (^{87})</td>
</tr>
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<td>9</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td>82</td>
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<td>VA</td>
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<td>22</td>
<td>5</td>
</tr>
<tr>
<td>83</td>
<td></td>
<td>(^{t})PrOAc</td>
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<td>18</td>
<td>1</td>
<td>64</td>
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<td>0</td>
<td>83</td>
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<tr>
<td>85</td>
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<td>0</td>
<td>_</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>86</td>
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<td>0</td>
<td>100</td>
</tr>
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<td>87</td>
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<td>EthoxyethylOAc</td>
<td>48</td>
<td>0</td>
<td>_</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>88</td>
<td></td>
<td>LaurylOAc</td>
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<td>0</td>
<td>_</td>
<td>0</td>
<td>100</td>
</tr>
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<td>89</td>
<td></td>
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<td>8</td>
<td>12</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>MethoxyethylOAc</td>
<td>24</td>
<td>0</td>
<td>_</td>
<td>0</td>
<td>100</td>
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</tbody>
</table>
Biotransformations were carried out in dichloromethane (0.1 M) at 37 °C using prochiral diol (10 mg), acyl donor (1 equivalent) and immobilised enzyme (1 weight equivalent) (biotransformations were followed by TLC until no further changes were seen). Enzyme provided by Codexis. Abbreviations: acetic anhydride (Ac₂O), vinyl acetate (VA), iso-propyl acetate (PrOAc), allyl acetate (AllylOAc), pentyl acetate (PentylOAc), ethyl acetate (EtOAc), 2-ethoxyethyl acetate (EthoxyethylOAc), lauryl acetate (LaurylOAc), 2-methoxyphenyl acetate (MethoxyPhOAc), 2-methoxyethyl acetate (MethoxyethylOAc). Results from GC of the crude reaction mixture.

Another enzyme tested was a lipase from Thermomyces lanuginosus (NZL-104) which resulted in poor activity and selectivity with the substrate (Table 4, entries 31-40). Codexis provided two mutants of this enzyme (Table 4, entries 41-60), the first (NZL-105) gave the product with high ee, 79% when Ac₂O was used but in 20% yield (Table 4, entry 41). The second mutant (NZL-106) generated the product in 46% ee and 29% yield (Table 4, entry 51).

Finally three more lipases from a fungus were tested (NZL-107, NZL-108 and NZL-109) with little success (Table 4, entries 61-90). In all cases the enzyme generated the product in low yields and poor enantiopurities.

Five immobilised proteases were also screened using the same acyl donor under the same reaction conditions however, none of them gave successful results (see data in Appendix, Table 5).

### 7.3.1 Equivalents of R. miehei used

Attempts to improve the yield and enantiomeric excess of the desymmetrised product (S)-50 using the lipase from R. miehei with vinyl acetate were undertaken with varying molar equivalents of the enzyme.

Reducing the amount of enzyme only served to slow down the process and did not improve either the enantiomeric excess or yield (Table 5). Interestingly, increasing the equivalents of enzyme in the reaction gave higher enantiomeric excesses, such that using 2 weight equivalents of enzyme resulted in 99% ee but with only 24%
conversion obtained (Table 5, entry 4). Whilst using 1 weight equivalent of enzyme a 81% ee was achieved but accompanied by a substantial increase in product conversion (90%) in 8 hours (Table 5, entry 3). Gratifyingly, the conversion of epoxy diacetate 49 in the process with 1 weight equivalent of enzyme was very low (Table 5, entry 3). These results are consistent with a second kinetic resolution of desymmetrised product (see scheme 39) and a simultaneous increase in the amount of the epoxy diacetate 49 to 76% when using 2 weight equivalents of the enzyme (Table 5, entry 4).

Table 5: Enzymatic biotransformation of 87 using different equivalents of R. miehei.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Weight equivalent enzyme</th>
<th>Time (h)</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
<th>% 87\textsuperscript{b}</th>
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</thead>
<tbody>
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<td>1</td>
<td>0.2</td>
<td>24</td>
<td>22</td>
<td>41</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>24</td>
<td>36</td>
<td>64</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>8</td>
<td>90</td>
<td>81</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>24</td>
<td>99</td>
<td>76</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Biotransformations were carried out in dichloromethane (0.1 M) using prochiral diol 87 (10 mg), vinyl acetate (1 equivalent) and the correspondent weight equivalent of the immobilised R. miehei at 37 °C. \textsuperscript{b} Results from GC of the crude reaction mixture.

The enzymatic desymmetrisation of epoxy diol 87 was further optimised with 1 weight equivalent of enzyme (see below).
7.3.2 Temperature of the reaction

Table 6: Enzymatic biotransformation of compound 87 using different reaction temperatures.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (°C)</th>
<th>% 50b</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
<th>% 87\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>88</td>
<td>85</td>
<td>11</td>
<td>1</td>
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<tr>
<td>2</td>
<td>25</td>
<td>82</td>
<td>89</td>
<td>18</td>
<td>0</td>
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<tr>
<td>3</td>
<td>37</td>
<td>90</td>
<td>81</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Biotransformations were carried out in dichloromethane (0.1 M) using prochiral-diol 87 (10 mg), vinyl acetate (1 equivalent) and immobilised \textit{R. miehei} (1 weight equivalent) for 8 hours. \textsuperscript{b} Results from GC of the crude reaction mixture.

It was found that the temperature of the reaction plays a small role in the process. At 25 °C the enzyme displayed optimum selectivity, but, due to the two-step biotransformation process (Scheme 39), when the enantiomeric excess increases the conversion of the epoxy monoacetate 50 decreases (Table 6).

7.3.3 Equivalents of acyl donor

The process was also studied by varying the amount of acyl donor (1.1 to 1.5 molar equivalents) at different temperatures (Table 7).

A big difference was observed with respect to enantiomeric excess and yields upon increasing the amount of vinyl acetate over all temperatures. The enantiomeric excess increases rapidly with a concomitant decrease in the conversion of the epoxy monoacetate (S)-50 at all temperatures. Simultaneously, a higher proportion of epoxy diacetate 49 was obtained as expected, concluding that 1 equivalent of vinyl acetate is optimal for future optimisation.
Table 7: Enzymatic biotransformation of diol 87 using different equivalents of vinyl acetate at different reaction temperatures.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (ºC)</th>
<th>Equivalents acyl donor</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
</tr>
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<td>1</td>
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<td>1.1</td>
<td>12</td>
<td>99</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>1.3</td>
<td>9</td>
<td>99</td>
<td>91</td>
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<tr>
<td>3</td>
<td>37</td>
<td>1.5</td>
<td>8</td>
<td>99</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>1.1</td>
<td>36</td>
<td>98</td>
<td>64</td>
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<tr>
<td>6</td>
<td>25</td>
<td>1.5</td>
<td>21</td>
<td>99</td>
<td>79</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>1.1</td>
<td>21</td>
<td>99</td>
<td>79</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>1.3</td>
<td>25</td>
<td>99</td>
<td>75</td>
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<tr>
<td>9</td>
<td>4</td>
<td>1.5</td>
<td>20</td>
<td>99</td>
<td>80</td>
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</tbody>
</table>

\textsuperscript{a} Biotransformations were carried out in dichloromethane (0.1 M) using prochiral diol 87 (10 mg), vinyl acetate (see table) and immobilised \textit{R. miehei} (1 weight equivalent) for 8 hours at different reaction temperatures. \textsuperscript{b} Results from GC of the crude reaction mixture.

7.3.4 Enzymatic desymmetrisation over time

The enzymatic desymmetrisation of diol 87 over time was studied using 1 equivalent of vinyl acetate in dichloromethane at 37 ºC. The results were comparable with Amano L, AK (compare Figure 11 with Figures 9 and 10), but over a longer reaction period.

The enzymatic desymmetrisation of 87 with \textit{R. miehei} underwent the same two-step process as when the biotransformations were performed with Amano L, AK (Scheme 39). However, in this case, the enzyme is more selective in the first step due to the
higher conversion to (S)-50, and consequently a smaller amount of the (R)-50 for removal in the kinetic resolution step.

Figure 11: Graphical representation of the enzymatic desymmetrisation of diol 87 with R. miehei and vinyl acetate at 37 °C in dichloromethane over 8 hours. Raw data in Appendix, Table 6.

7.3.5 Use of additives

Immobilised enzymes are more stable in organic solvents and all reactions described herein were run with molecular sieves (4Å) to minimise water in the reaction. However, the use of water as an additive can stabilise the enzyme and increase selectivity.43

In order to test this hypothesis the biotransformation was performed with 0.1 to 0.5 molar equivalents of water. The results show that the ee decreased significantly from 94% to 58% on addition of 0.1 equivalents of water (Table 8, entries 1 and 2). The decrease in enantiomeric excess when the reaction was performed in the presence of water is presumably due to the non-selective hydrolysis of epoxy diacetate 49 produced during the reaction which lowers the overall ee.
Table 8: Results of the enzymatic biotransformation of 87 and the corresponding additive.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additive</th>
<th>Equivalents additive</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
<th>% 87\textsuperscript{b}</th>
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<td>94</td>
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<td>48</td>
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<td>8</td>
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<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Biotransformations were carried out in dichloromethane (0.1 M) using prochiral diol 87 (10 mg), vinyl acetate (1.1 equivalents), immobilised \textit{R. miehei} (1 weight equivalent) and the corresponding additive (see table) for 5 hours at 37 °C. \textsuperscript{b} Results from GC of the crude reaction mixture.

Triethylamine is a common additive in biotransformations and an increase in selectivity was obtained when Amano L, AK was used with Ac\textsubscript{2}O (Section 7.2.3, Table 3, entry 2).\textsuperscript{43,190} Unfortunately, when \textit{R. miehei} was used with 0.1 mol% of triethylamine, the ee decreased from 94% to 82% but surprisingly, the product yield increased from 34% to 86% when the biotransformation was incubated for 5 hours (Table 8, entries 1 and 5). However, 90% epoxy monoacetate (S)-50 with 81% ee was produced during the biotransformation without additives over 8 hours (Table 6, entry 3).
7.3.6 Organic solvents.

Enzymatic regio- and enantioselectivity is remarkably dependent on the nature of the organic solvent.\textsuperscript{153} As such, in our studies, the lipase-catalysed transesterification of epoxy diol \textbf{87} was investigated employing immobilised \textit{R. miehei} and vinyl acetate in different organic solvents (Table 9).

The biotransformation worked best in dichloromethane with respect to the conversion of product and enantiomeric excess (Table 9, entry 1). Chloroform and dioxane improved the selectivity of the enzyme slightly but in both cases the conversion of product decreased drastically (Table 9, entries 3 and 9).

Table 9: Results of the enzymatic biotransformation of \textbf{87} with immobilised \textit{R. miehei} in different organic solvents.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Solvent</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
<th>% 87\textsuperscript{b}</th>
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</tr>
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<td>11</td>
<td>24</td>
<td>Et\textsubscript{2}O</td>
<td>0</td>
<td>_</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>Pentane</td>
<td>15</td>
<td>4</td>
<td>85</td>
<td>0</td>
</tr>
</tbody>
</table>
Biotransformations were carried out in the corresponding organic solvent (0.1 M) using prochiral diol 87 (10 mg), vinyl acetate (1 equivalent), immobilised \textit{R. miehei} (1 weight equivalent) at 37 °C (biotransformations were monitored by TLC until no further changes were detected). \(^b\) Results from GC of the crude reaction mixture.

7.3.7 \textit{Rhizomucor miehei} immobilised as a CLEA

Due to the improved results obtained using immobilised enzymes, an alternative enzyme immobilisation technique was examined. In this case, \textit{R. miehei} was used as a cross-linked enzyme aggregate (CLEA). CLEA methodology involves the precipitation of the enzyme and the additional chemical cross-linking of the protein aggregate.\(^{191-195}\)

The enzymatic desymmetrisation of diol 87 was performed with 1 weight equivalent of \textit{R. miehei} CLEA in DCM, using vinyl acetate at 37 °C. The biotransformation was incubated for 5 hours and the crude reaction mixture was analysed by GC. The reaction gave the desired product with 13% conversion and 90% ee. Unfortunately, CLEA immobilisation did not improve the results as desired although the use of different immobilisation methods can potentially modify the enantio- and regioselectivity of enzymes in a biotransformation.\(^{44}\)

7.3.8 Non-immobilised enzyme

So far, a different enzyme had been used for the enzyme immobilisation work compared to previous work without immobilisation (Amano L, AK from \textit{P. fluorescens}), therefore we deemed it necessary to compare the results more directly by using non-immobilised \textit{R. miehei}.

The reaction was carried out with a non-immobilised lipase from \textit{R. miehei} in dichloromethane (0.1 M), using vinyl acetate (1 equivalent) at 37 °C. The
biotransformation was incubated for 8 hours and the crude reaction mixture was analysed by GC which gave the same product, (S)-(++)-50, in 75% conversion (81% ee), demonstrating the success of immobilisation on this enzymatic desymmetrisation (90% conversion and 82% ee). The enzymatic desymmetrisation of diol 87 has been optimised from non-immobilised *Pseudomonas fluorescens* (46% yield of (S)-50 (97% ee)) to immobilised *Rhizomucor miehei* (90% conversion of (S)-50 (81% ee)).

7.4 Silica encapsulation of Amano L, AK.

Due to the improved yield observed using immobilised *Rhizomucor miehei* on a solid support from Codexis, and comparing this result with the same reaction performed with free enzyme under the same conditions, we wanted to apply the reaction to immobilised Amano L, AK.

We tried initially a commercially available immobilised enzyme. Lipase immobilised on Sol-Gel-AK from *Pseudomonas fluorescens* was tested using 1 equivalent of Ac₂O in dichloromethane and the biotransformation was incubated at 37 ºC for 3 hours to generate (S)-50 in 50% yield and 74% ee. Sol-Gel is a chemical synthesis to produce gels, glasses, and ceramic powders. The Sol-Gel process generally involves the use of metal alkoxides, which undergo via hydrolysis and condensation to give polymerization reactions and synthesise gels.

The biotransformation was performed with Ac₂O to be consistent with the acyl donor used with non-immobilised Amano L, AK. Comparing this result with the non-immobilised Amano L, AK under the same conditions, the selectivity maintains roughly constant but the conversion to products decreases when immobilised *Pseudomonas fluorescens* was used. The contact of an enzyme with a support may modify its activity due to the interactions between enzyme and carrier. With this in mind, it was considered that not all the solid supports would increase the activity of the enzyme for our biotransformation. However, no information available from Codexis was on the support used to immobilise their enzyme. For this reason, the immobilisation of Amano L, AK encapsulated on silica was employed; this work was performed in collaboration with the University of Strathclyde (Scheme 43).
Scheme 43: Enzymatic desymmetrisation of 87 with silica-immobilised Amano L, AK.

The immobilisation technique used to encapsulate the enzyme was via an entrapment into inorganic matrix such as silica gel. This entrapment is a mild immobilisation method where the biocatalyst is physically encaged in a macroscopic matrix. This immobilisation constitutes an inorganic matrix with a highly stable porous matrix that grows around the enzyme in a three-dimensional space. Sol-gel aggregates exhibit higher stability towards changes in temperature, pH and mechanical stability than other biological matrices such as agar gels or alginate gels.

Many studies have been reported with lipase immobilisation in silica using different techniques such as cross-linking, covalent coupling and entrapment, increasing the activity, stability, selectivity and recycling of the enzyme.

Amano L, AK was immobilised in silica with sodium metasilicate (Si-Na), pentaethylenehexamine (PEHA), a catalyst for silica formation, distilled/deionised water (ddH₂O) and the enzyme required for the entrapment.

The biotransformation with immobilised Amano L, AK on silica was performed on a 10 mg scale and the crude reaction mixture was injected into the GC to determine the yield and optical purity of 50. The immobilisation of the enzyme on silica provided us with four different samples and their supernatants, two blanks at different pHs (sample 1 at pH 3 and sample 2 at pH 6.5) and their corresponding supernatants (sup. 1 and sup. 2 respectively), two samples with immobilised of Amano L, AK at different pHs (sample 3 at pH 8 and sample 4 at pH 7) and their corresponding supernatants (sup. 3 and sup. 4 respectively). Initially, the biotransformations were performed with 2 weight equivalents of immobilised Amano L, AK on silica and 1.5 molar equivalents of Ac₂O in DCM and the samples were incubated at 37 ºC. Ac₂O
was used as acyl donor due to the higher enzyme selectivity exhibited in previous work with non-immobilised Amano L, AK (Section 7.2).

The reaction with blanks and their corresponding supernatants were performed under the described conditions and the reaction yielding up to 4% product \((S)-50\) (Table 10, entries 1 and 2; table 11, entries 1 and 2; table 12, entry1).

The biotransformation was performed with the encapsulated enzyme and surprisingly the activity increased whilst maintaining its high selectivity. Amano L, AK immobilised on silica at pH 8 carried out the biotransformation generating \((S)-50\) in 76% yield and 95% ee (Table 10, entry 3). The biotransformation with non-immobilised Amano L, AK, performed under the same conditions, gave \((S)-50\) 42% yield and 97% ee (Appendix, Table 1) concluding that the enzyme has a better activity when immobilised on silica.

The activity of this immobilised enzyme increases but the supernatants of the corresponding samples also exhibit activity and selectivity for the substrate 90. The supernatant of sample 4 catalysed the biotransformation achieving the desired enantiomer in 74% yield and 93% ee (Table 10, entry 9)! Supernatants contains enzyme that was not immobilised. When the encapsulation is efficient, the concentration of enzyme in the supernatants should be zero and therefore inactive with the substrate. Since it is difficult to estimate the amount of enzyme encapsulated on silica we used the following assay: the supernatants were collected and the mass of enzyme present in the supernatants was determined \((a\ \text{mg})\) by a standard enzyme assay or a protein assay (e.g. Bradford).\(^{197}\) From this estimation and with the mass of enzyme present initially (45 mg in 30 mL batch), the difference \((b = 45 - a)\) will be the mass of enzyme present on silica.

Enzyme encapsulation efficiency \((\%) = \frac{b \times 100}{45} .\)

In this case, we obtained 64.9 mg for sup.3 and 69.5 mg for sup.4 giving negative enzyme encapsulation efficiency. The weight of supernatant after freeze-drying was higher than initially used for the encapsulation. The immobilisation method should be repeated due to the unusual results obtained.
Table 10: Results of enzymatic desymmetrisation of epoxy diol 87 with 2 weight equivalents of immobilised Amano L, AK on silica. 

<table>
<thead>
<tr>
<th>Entry</th>
<th>Name</th>
<th>Description</th>
<th>Time (h)</th>
<th>% 50°</th>
<th>% ee°</th>
<th>% 49°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>‘blank’ (pH 3)</td>
<td>1</td>
<td>4</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>‘blank’ (pH 6.5)</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Silica-enzyme (pH 8)</td>
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<td>76</td>
<td>95</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Silica-enzyme (pH 7)</td>
<td>1</td>
<td>67</td>
<td>89</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Sup. 1</td>
<td>Supernatant sample 1</td>
<td>1</td>
<td>3</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Sup. 2</td>
<td>Supernatant sample 2</td>
<td>1</td>
<td>4</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Sup. 3</td>
<td>Supernatant sample 3</td>
<td>1</td>
<td>33</td>
<td>78</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Sup. 4</td>
<td>Supernatant sample 4</td>
<td>1</td>
<td>74</td>
<td>93</td>
<td>23</td>
</tr>
</tbody>
</table>

a Biotransformations were carried out in dichloromethane (0.1 M) at 37 °C using prochiral diol 87 (10 mg), Ac₂O (1.5 molar equivalent) and immobilised Amano L, AK (2 weight equivalent) for 1 hour. bResults from GC of the crude reaction mixture.

As the enzyme encapsulation on silica was performed on a small scale the biotransformations were performed under the same conditions but using only 1 weight equivalent of enzyme to investigate if an excess of catalyst was required. The same biotransformations were performed without drastic modification; in this case the reactions with the blank only gave 1% yield of product due to the reduction of silica in the reaction mixture (Table 11, entry 1 and 2).

As commented before, again there is active enzyme in the supernatant solution; in this case the solid dried from sup. 3 gave (S)-50 in 70% yield and 98% ee (Table 11, entry 10). More studies of the immobilisation and biotransformations are required to understand these unusual results.

With these interesting results it was decided to scale-up the immobilisation of Amano L, AK and screen more reaction conditions.
Table 11: Results of enzymatic desymmetrisation of epoxy diol 87 with 1 weight equivalent of immobilised Amano L, AK on silica.\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Name</th>
<th>Description</th>
<th>Time (h)</th>
<th>% 50(^b)</th>
<th>% ee(^b)</th>
<th>% 49(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>‘blank’ (pH 3)</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>‘blank’ (pH 6.5)</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>0</td>
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<td>3</td>
<td>3</td>
<td>Silica-enzyme (pH 8)</td>
<td>1</td>
<td>75</td>
<td>95</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Silica-enzyme (pH 7)</td>
<td>1</td>
<td>73</td>
<td>91</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Sup. 1</td>
<td>Supernatant sample 1</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Sup. 1</td>
<td>Supernatant sample 1</td>
<td>5</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Sup. 2</td>
<td>Supernatant sample 2</td>
<td>1</td>
<td>2</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Sup. 2</td>
<td>Supernatant sample 2</td>
<td>5</td>
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<tr>
<td>9</td>
<td>Sup. 3</td>
<td>Supernatant sample 3</td>
<td>1</td>
<td>29</td>
<td>78</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Sup. 3</td>
<td>Supernatant sample 3</td>
<td>5</td>
<td>70</td>
<td>98</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>Sup. 4</td>
<td>Supernatant sample 4</td>
<td>1</td>
<td>19</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Sup. 4</td>
<td>Supernatant sample 4</td>
<td>5</td>
<td>54</td>
<td>66</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\) Biotransformations were carried out in dichloromethane (0.1 M) at 37 °C using prochiral diol 87 (10 mg), Ac\(_2\)O (1.5 molar equivalent) and immobilised Amano L, AK (1 weight equivalent) for 1 hour.\(^b\)Results from GC of the crude reaction mixture.

The encapsulation of Amano L, AK on a larger scale, 180 mg of free enzyme, produced 212.3 mg of enzyme encapsulated on silica and 88 mg of freeze-dried supernatant with an encapsulation efficiency of 51%. In this case, the immobilisation was only performed at pH 7 generating two samples (sample 1: blank; sample 2: Amano L, AK on silica; sup. 2: supernatants of sample 2).
Table 12: Results of enzymatic desymmetrisation of epoxy diol 87 with 1 weight equivalent of immobilised Amano L, AK on silica.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Name</th>
<th>Description</th>
<th>Time (h)</th>
<th>% 50\textsuperscript{b}</th>
<th>% ee\textsuperscript{b}</th>
<th>% 49\textsuperscript{b}</th>
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<td>‘blank’ (pH 6.5)</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Silica-enzyme (pH 7)</td>
<td>1</td>
<td>66</td>
<td>84</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Silica-enzyme (pH 7)</td>
<td>5</td>
<td>59</td>
<td>&gt;99</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>Sup. 2</td>
<td>Supernatant sample 3</td>
<td>1</td>
<td>7</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Sup. 2</td>
<td>Supernatant sample 3</td>
<td>5</td>
<td>26</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Biotransformations were carried out in dichloromethane (0.1 M) at 37 \degree C using prochiral diol 87 (10 mg), Ac\textsubscript{2}O (1.8 molar equivalent) and immobilised Amano L, AK (1 weight equivalent) for 1 hour.\textsuperscript{b}Results from GC of the crude reaction mixture.

As we wanted to screen more reaction conditions with the immobilised enzyme, the biotransformations were performed using 1.8 molar equivalents of Ac\textsubscript{2}O. Following the two step process, the expected results were an increase in the optical purity with a concomitant decrease in the yield. Surprisingly, optical purity and yield decreased when the biotransformations were performed with 1.8 equivalents of Ac\textsubscript{2}O (compare results from tables 11 and 12). The biotransformation performed with sample 2 generated (S)-50 with 66% yield and 84% ee in 1 hour (Table 12, entry 2) but when the reaction was incubated longer, (S)-50 was obtained in 59% yield and >99% ee (Table 12, entry 3). In this case, the optical purity of (S)-50 was excellent but the yield of reaction decreased as expected. The enzyme isolated from the supernatant (sup. 2) performed the reaction in very low yield presumably due to an encapsulation efficiency of 50%.

To determine if the immobilisation of the enzyme on a larger scale provides the same activity and selectivity as on small scale, the enzymatic desymmetrisation of diol 87 was performed using 1 weight equivalent of immobilised Amano L, AK, 1.5 molar equivalents of Ac\textsubscript{2}O in DCM and the reaction was incubated for 1 hour. The results gave (S)-50 in 71% yield and 84% ee, with a decreasing on yield from 73% to 71%
and the enantiomeric excess from 91% to 84%. The encapsulation efficiency and the biotransformation results changed when the process was scaled-up due to unknown factors. Unfortunately, there was no more time to continue developing the immobilisation process, however the initial results of the biotransformation shows that the immobilised of Amano L, AK on silica improved the activity of the enzyme.

8 EPOXIDE OPENING TO GENERATE CHIRAL TERTIARY ALCOHOLS

8.1 Epoxide ring-opening of epoxy monoacetate 50 with nitrogen nucleophiles

Chiral β-amino tertiary alcohols are present in natural products and pharmaceuticals including pumiliotoxin A 62,98 and alkaloids from the poison dart frogs of the dendrobates genus, setoclavine 59,98 paraherquamide A 61,101 as well as the anticancer compound vincristine 63 (Scheme 20).103 Likewise, numerous biologically active non-natural compounds possess this moiety.35,172-174 With our asymmetric synthesis of (S)-50, we chose several amine nucleophiles to open the epoxide thus generating enantiomerically pure β-amino tertiary alcohols (Scheme 44).

![Scheme 44: Our synthesis of enantiopure β-amino tertiary alcohols.](image)

Numerous protocols have been developed for activating epoxides towards nucleophilic ring-opening by amines.123,135,136,160,161,166-171 Some of these methods
were investigated and are discussed in detail below (Scheme 45). The reactions were performed initially with (±)-50 to save costly material.

![Scheme 45: Proposed scheme for the epoxide ring-opening of (±)-50 using amines.](image)

Since the enzymatic desymmetrisation was carried out in dichloromethane, it allows the possibility of performing the epoxide ring-opening reaction in “one pot”. Performing the reaction in “one pot” would be advantageous due to a reduction in synthetic steps and lengthy purification process, therefore saving time and resources.

Initial approaches to develop the reaction utilised 100 mg of (±)-50 with 1.1 equivalents of the amine in DCM. Benzylamine and allylamine were tested and the reactions were left for three days at room temperature. Unfortunately, no product was detected in either reaction.

### 8.1.1 Microwave irradiation

The ring-opening of epoxides by amines promoted by microwave irradiation is known.\(^{161}\) Normally, the reactions are faster than the corresponding thermally controlled reaction and thus it is not necessary to use a catalyst or promoter.

Reactions were carried out on a 50 mg scale of (±)-50, using 1.1 equivalents of amine in dichloromethane. The reactions were left from between 5 to 30 minutes at 100 °C to 140 °C (200W). The amines screened were benzylamine, allylamine, morpholine and piperidine.

Unfortunately, it was not possible to isolate the desired ring-opened product. The reactions were not clean and gave a complex mixture of products which prevented
purity of any desired product. As such, it was necessary to find another protocol to prepare the β-amino tertiary alcohols from (±)-50.

8.1.2 Use of promoters/activators.

Scheme 46: General scheme for the ring-opening of (±)-50 with amines, promoted by Ti(OiPr)₄, in DCM at room temperature.

There are many catalytic promoters to activate the ring-opening of epoxides using amines,¹⁶⁰,¹⁶⁹ as such, the ring-opening of (±)-50 was studied using the Lewis acid Ti(OiPr)₄ as a catalyst. The reactions were performed on a 100 mg scale, using 0.1 equivalent of Ti(OiPr)₄, 1.1 equivalents of the amine in dichloromethane at room temperature (Scheme 46). When the reaction was carried out with piperidine, the tertiary alcohol 99b was obtained, but in a disappointing 25% yield after purification. Benzylamine was also used but, in this case, no product was obtained, and only starting material was recovered. The ring-opening reaction was also attempted with 2-(piperidin-4-yl)ethanol, but this too was unsuccessful. After these disappointing results, other methods were examined to open the epoxide.

8.1.3 Classical conditions.

Epoxide ring-opening was attempted in dichloromethane using classical conditions, an excess of amine (benzylamine, allylamine and piperidine) and heating to reflux. However, under such conditions, there was no product formed, even after 48 hours heating the reaction to reflux.
Better results were obtained when the reaction was performed with an excess of amine refluxing in EtOH.\textsuperscript{170,171} In this case, the epoxide ring-opening was carried out on a 200 mg scale of (±)-50, three equivalents of the amine at 78 °C over 1 to 4 hours in EtOH (Scheme 47). After this time the products were purified and characterised as β-amino tertiary alcohols (Table 13).

\[
\text{HO-} \xrightarrow{R_1R_2NH} \xrightarrow{\text{EtOH, reflux}} \xrightarrow{1-4 \text{ hours}} \text{HO-} \quad (±)-50 \rightarrow \quad \text{HO-} \quad \text{96}
\]

Scheme 47: General reaction for the ring-opening of (±)-50 with an excess of amine refluxing in EtOH.

When aliphatic secondary amines were used, the reaction produced the desired β-amino tertiary alcohols (Table 13, entries 1 to 5, 10, 12 and 14). Using piperidine as a nucleophile gave a modest yield (55\%) of 96b (Table 13, entry 2). However, the other secondary amines morpholine, 2,6-dimethylmorpholine and 2-(piperidin-4-yl)ethanol gave yields of 76, 84 and 77\% respectively (Table 13, entries 3-5). Interestingly, the tertiary alcohol was not obtained with pyrrolidine (Table 13, entry 1); the un-repeatable reaction product obtained was tentatively assigned as N-acetyl pyrrolidine, presumably formed \textit{via} an acyl exchange reaction with the acetate starting material (±)-50.

Table 13: Results of the ring-opening of (±)-50 with three equivalents of amine refluxing in EtOH.\textsuperscript{a}
\[
\text{HO-}OAc \quad \xrightarrow{\text{R}_1\text{R}_2\text{NH}} \quad \text{EtOH, reflux} \quad \text{HO-}OAc \\
(\pm)-50 \quad \xrightarrow{\text{R}_1\text{R}_2\text{NH}} \quad \text{HO-}OAc \quad \xrightarrow{\text{NR}_1}\text{R}_2 \\
(\pm)-96 \quad \text{secondary aliphatic amines} \\
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Nucleophile</th>
<th>Product type</th>
<th>Yield(^b) (%)</th>
<th>Yield(^d) (%)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td><img src="Image1" alt="Nucleophile" /></td>
<td>96a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><img src="Image2" alt="Nucleophile" /></td>
<td>96b</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><img src="Image3" alt="Nucleophile" /></td>
<td>96c</td>
<td>76</td>
<td>81</td>
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<tr>
<td>4</td>
<td><img src="Image4" alt="Nucleophile" /></td>
<td>96d</td>
<td>84</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td><img src="Image5" alt="Nucleophile" /></td>
<td>96e</td>
<td>77</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><img src="Image6" alt="Nucleophile" /></td>
<td>97a</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td><img src="Image7" alt="Nucleophile" /></td>
<td>97b</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td><img src="Image8" alt="Nucleophile" /></td>
<td>97c</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td><img src="Image9" alt="Nucleophile" /></td>
<td>97d</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Structure</td>
<td>Isolated Yield</td>
<td>Trace Product</td>
<td>NMR Analysis</td>
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<td>---------------</td>
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</tr>
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<td>-</td>
<td>80</td>
</tr>
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<td>-</td>
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<td>-</td>
<td>55</td>
</tr>
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<td>74</td>
<td>-</td>
</tr>
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<td>14</td>
<td><img src="https://example.com/structure14.png" alt="Structure" /></td>
<td>96h</td>
<td>63</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td><img src="https://example.com/structure15.png" alt="Structure" /></td>
<td>97g</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td><img src="https://example.com/structure16.png" alt="Structure" /></td>
<td>96i</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>17</td>
<td><img src="https://example.com/structure17.png" alt="Structure" /></td>
<td>96j</td>
<td>93</td>
<td>96</td>
</tr>
</tbody>
</table>

*Reagents and conditions*: Epoxide (±)-50 in ethanol (0.1 M), amine (3 equivalents) and reflux for 1 or 4 hours. Isolated yields. Trace product was observed after 1 h at reflux. The reaction was repeated and left at reflux for 4 hours. The reaction was purified by the removal of excess amine under high vacuum.

Aliphatic primary amines were also screened in the reaction and interestingly different results were obtained than using secondary aliphatic amines under the same conditions (See Scheme 47 and Table 13, entries 6 to 9, 11, 13 and 15). NMR analysis of products together with X-ray crystallography of the crystalline products derived from propargylamine and allylamine (Figures 12 and 13 respectively) revealed an intramolecular migration of the acetyl group had occurred during the reaction onto the newly, mldk formed secondary amine to give acetamides 97d and 97e.
The chiral centre in products 97a-g is lost due to the intramolecular O to N-migration of the acetyl group, resulting in a prochiral triol, when primary amines 4-(2-aminoethyl)morpholine, iso-propylamine, cyclopentylamine, propargylamine,
allylamine, benzylamine and phenylethylamine were used (Table 13, entries 6-9, 11, 13 and 15).

To confirm that migration of the acetyl group takes place when using primary amines, analogous secondary amines were used. It was thought that a lack of a replaceable hydrogen atom in the products would prevent acyl migration. Three secondary N-methylated amines were reacted alongside their analogous primary amines, N-methylprop-2-yn-1-amine, N-methylprop-2-en-1-amine and N-methyl-1-phenylmethanamine (Table 13, entries 9 and 10; 11 and 12; 13 and 14). Indeed it was found that the reactions of the N-methylated compounds (Table 13, entries 10, 12 and 14) gave the desired products 96f, 96g and 96h exclusively in good yields without the concomitant acyl migration seen in the cases of the primary amines (Table 13, entries 9, 11 and 13).

The N-methylated amines produce the desired compounds albeit in lower yields than the corresponding primary amines (Table 13, entries 10, 12 and 14), presumably due to the increased steric hindrance in these cases.

In conclusion, it appears as though using primary aliphatic amines in the reaction gives an intramolecular $\text{O to N}$-migration of the acetyl group onto the newly formed secondary amine resulting in a prochiral triol, whilst secondary aliphatic amines give the desired products exclusively.

Primary aromatic amines, such as 4-fluoroaniline and $o$-anisidine were used in the reaction with epoxide (±)-50, and interestingly, tertiary alcohols 96i and 96j were obtained in both cases, with no migration of the acetyl group, in high yields (Table 13, entries 16 and 17 respectively). It would appear as though primary aromatic amines are not nucleophilic enough to attack the acetyl group and initiate intramolecular $\text{O to N}$-migration.\(^{135,136,154,160}\) Confirmation of the structure of 96i was proved by X-ray crystallography (Figure 14).
Figure 14: X-ray structure of tertiary alcohol 96i; obtained when 4-fluoroaniline was reacted with epoxide (±)-50.

A number of differences between diol 96 and triol 97 could be seen by $^1$H and $^{13}$C NMR. For example, with 96g and 97e, $^1$H NMR of the pure products from the reaction of (±)-50 with allylamine and N-methylprop-2-en-1-amine respectively show a difference in the signal splitting patterns. Allylamine derived product 97e is achivable as a result of acyl migration, thus there is only one signal integrating to four protons for the two equivalent -CH$_2$OH groups (Figures 16 and 17). However, N-methylprop-2-en-1-amine derived product 96g is chiral, and thus the two methylene protons appear as diastereotopic AB systems (Figures 15 and 19).

Figure 15: Diastereotopic protons are seen by $^1$H-NMR in the asymmetric tertiary 96.

Figure 16: Symmetrical protons are seen by $^1$H-NMR with the prochiral triol product 97.
The difference in structure in all examples, is also evident from $^{13}$C NMR, since the ester carbonyl of the tertiary alcohols appears at 171 ppm and the amide carbonyl of the prochiral triol appears at 174 ppm.

Figure 17: $^1$H NMR of allylamine derived product 97e in CDCl$_3$.

Figure 19: $^1$H NMR of N-methylprop-2-en-1-amine derived product 96g in CDCl$_3$. 
8.1.4 Epoxide ring-opening of enantiopure epoxide (S)-50 with o-anisidine and 4-fluoroaniline.

The reaction was attempted with enantiomerically pure epoxide (S)-50 to determine if these conditions can allow us to develop an asymmetric synthesis of enantiomerically pure β-amino tertiary alcohols. The enantiomers of racemic 96i and 96j were separated by chiral HPLC, thus o-anisidine and 4-fluoroaniline were selected to open the epoxide of enantiomerically pure material. To avoid purification of intermediates, the reaction was performed in “one pot” (Scheme 48).

![Scheme 48: Enzymatic desymmetrisation and epoxide ring-opening in “one pot”](image)

The reaction was carried out on a 100 mg of prochiral diol 87, 1.8 equivalents of Ac₂O and 2 weight equivalents of Amano L, AK in DCM at 37 °C for 1 hour (97% ee). After this time, the enzyme was filtered and the solvent evaporated in vacuo. Ethanol was added to the reaction mixture along with 3 equivalents of the amine, and the reaction was heated at reflux for 4 hours. After this time the solvent was removed and the mixture purified by column chromatography.

Using this “one pot” procedure, the chiral tertiary alcohols were prepared with no loss of enantiomeric excess; in both cases the enantiomeric excess was 97% (Table 15).
Table 15: Results obtained in the enzymatic desymmetrisation and epoxide ring-opening in “one pot”.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Nucleophile</th>
<th>Product type</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F—NH₂</td>
<td>96i</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>NH₂—OMe</td>
<td>96j</td>
<td>97</td>
</tr>
</tbody>
</table>

These reactions were performed in “one-pot” as follows: To a solution of 87 (100 mg, 0.96 mmol), Amano L, AK (200 mg, 2 weight equivalents) and molecular sieves (4 Å) in dichloromethane (10 ml, 0.1 M) was added acetic anhydride (1.8 equivalents) and the reaction left for 1 hour at 37 ºC. The mixture was filtered through Celite ® and the filtrate was reduced in vacuo. Ethanol (10 ml, 0.1M) and the corresponding amine (see Table 15) was added and the mixture was heated at reflux for 4 hours. The mixture was reduced in vacuo and purified by column chromatography (SiO₂; 20% EtOH in EtOAc). The enantiomeric purity was determined by HPLC; Agilent 1100 Series. Chiralpack AD, 1 ml/min, 80/20 iso-hexene in EtOH at 25 ºC.

8.2 Preventing the O to N-migration of the acetyl group.

To avoid the intramolecular migration of the acetyl group when using primary aliphatic amines and thus avoid the conversion of chiral products 96 into prochiral triols 100, an alternative synthetic route was developed introducing a more stable protecting group (Scheme 49). It was found that, as expected, a silyl protecting group does not migrate during the epoxide opening reaction therefore allowing the use of aliphatic primary amines to make chiral β-amino tertiary alcohols, however, the opposite enantiomer of the β-amino tertiary alcohols was generated.
Scheme 49: Synthetic route to avoid the intramolecular migration of the acetate group using primary aliphatic amines.

The new synthetic route began with the protection of racemic (2-(hydroxymethyl)oxiran-2-yl)methyl acetate (±)-50 using tert-butylidemethylsilyl chloride, triethylamine and 4-dimethylaminopyridine in dichloromethane. The product was purified by column chromatography and the resulting yield was 82%. The process was followed by the removal of the acetyl group with potassium carbonate in methanol, giving a 91% yield after aqueous work-up. The protected epoxide, (±)-(2-((tert-butylidemethylsilyloxy)methyl)oxiran-2-yl)methanol (±)-99 was reacted with three primary amines, propargylamine, allylamine and benzylamine respectively. No migration of the tert-butylidemethylsilyl protecting group was detected, yields are summarised in Table 16. The epoxide was reacted with three equivalents of the amine in ethanol and the mixture was refluxed for 1 hour to obtain the racemic β-amino tertiary alcohols, 100a-c.

This route was also performed with enantiopure epoxide (S)-50 to determinate if these conditions enable us to develop an asymmetric synthesis of β-amino tertiary alcohols using aliphatic primary amines. When the enantiomerically enriched epoxide (R)-99 (97% ee) was reacted with benzylamine, no loss of enantiomeric excess was observed, confirming that the method is suitable for the synthesis of chiral β-amino tertiary alcohols with aliphatic primary amines which were previously unobtainable due to acyl migration. The “pseudo”-enantiomer was obtained when the chiral tertiary alcohols were synthesised with this alternative route. However, simple
protecting group manipulations could allow them to be converted to the other (R)-enantiomer (Scheme 50).\textsuperscript{199,200}

Table 16: Reaction of (±)-99 with primary amines.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product type</th>
<th>Yield (%)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\text{NH}_2)</td>
<td>100\textsuperscript{a}</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>(\text{NH}_2)</td>
<td>100\textsuperscript{b}</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>(\text{NH}_2)</td>
<td>100\textsuperscript{c}</td>
<td>83</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reagents and conditions: Epoxide (±)-99 was dissolved in ethanol (0.1 M), the amine added (3 equivalents) and the reaction heated to reflux for 4 hours. \textsuperscript{b} Isolated yields.

Scheme 50: Absolute configuration of the chiral tertiary alcohols.

8.3 Synthesis of β-amino methyl alcohols, 101.

β-Amino methyl alcohols are a common functional group in many natural products and non-natural products.\textsuperscript{106,201,202} Some examples are: pumilitoxin A, B and 251D\textsuperscript{203} initially isolated from dendrobatid frogs; setoclavine\textsuperscript{98} which has immunomodulatory activity, paraherquamide A\textsuperscript{100,101} displays potent anthelmintic activity and antinematodal properties, bacilosarcin A\textsuperscript{105,106} exhibits antibacterial, cytotoxicity and antiulcer activities (see structures in Schemes 2 and 20).
With such a plethora of tertiary alcohols we focussed our efforts on developing a new building block which would lead to chiral $\beta$-amino methyl alcohols (Figure 21). In the next sections we try to find a synthetic route to achieve this potentially useful building block.

![Figure 21: $\beta$-amino tertiary methyl alcohols 101.](image)

8.3.1 Synthesis of $\beta$-amino methyl alcohols 101 via oxidation followed by reductive amination.

The first route proposed to generate enantiomerically enriched alcohols of type 101 ($R_1=\text{OH}$) was to oxidise the primary alcohol of (±)-50 followed by a reductive amination (Scheme 51). The oxidation of the primary alcohol was performed using different reagents (Table 17). The oxidation reaction was optimised to obtain aldehyde 102 in 33% - 66% yields using tetra-propylammonium perruthenate (TPAP) and $N$-methylmorpholine-$N$-oxide (NMO) in dichloromethane at room temperature. Other oxidants were unsuccessful. Experimentally, the starting material was disappearing but no product was observed (TLC) during the reaction probably due to the decomposition of the aldehyde product 102.

![Scheme 51: Proposed synthetic route for the synthesis of chiral $\beta$-amino methyl alcohols.](image)
Table 17: Reagents used to perform the oxidation of (±)-50.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidising reagents</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;212&lt;/sup&gt;</td>
<td>NMO/TPAP in anhydrous DCM at RT</td>
<td>33-66</td>
</tr>
<tr>
<td>2&lt;sup&gt;205&lt;/sup&gt;</td>
<td>KMnO&lt;sub&gt;4&lt;/sub&gt; / MnO&lt;sub&gt;2&lt;/sub&gt; in DCM at RT</td>
<td>-</td>
</tr>
<tr>
<td>3&lt;sup&gt;209&lt;/sup&gt;</td>
<td>CrO&lt;sub&gt;3&lt;/sub&gt; / H&lt;sub&gt;5&lt;/sub&gt;IO&lt;sub&gt;6&lt;/sub&gt; in MeCN at 0ºC</td>
<td>-</td>
</tr>
<tr>
<td>4&lt;sup&gt;206&lt;/sup&gt;</td>
<td>PCC / H&lt;sub&gt;5&lt;/sub&gt;IO&lt;sub&gt;6&lt;/sub&gt; in MeCN at 0ºC</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>PCC in DMF</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>PCC in DCM</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>PCC in DCM with Celite&lt;sup&gt;1M&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>8&lt;sup&gt;213,214&lt;/sup&gt;</td>
<td>Swern oxidation</td>
<td>-</td>
</tr>
<tr>
<td>9&lt;sup&gt;215&lt;/sup&gt;</td>
<td>Parikh-Doering oxidation</td>
<td>-</td>
</tr>
<tr>
<td>10&lt;sup&gt;210&lt;/sup&gt;</td>
<td>IBX (o-iodoxibenzoic acid) and β-cyclodextrin</td>
<td>-</td>
</tr>
<tr>
<td>11&lt;sup&gt;211&lt;/sup&gt;</td>
<td>NaOCl&lt;sub&gt;2&lt;/sub&gt; / TEMPO</td>
<td>-</td>
</tr>
</tbody>
</table>

The reductive amination of 102 was performed using piperidine in anhydrous dichloromethane, but a mixture of products was obtained without the isolation of the desired tertiary alcohol. Initially, the process was performed in two steps; starting with the amination of the aldehyde with piperidine in the presence of 4Å molecular sieves or magnesium sulphate. This was followed by an exhaustive reduction with lithium aluminium hydride. The conversion of the reaction by TLC was poor and as such, the process was repeated in one step to prevent problems associated with the instability of imine 103.<sup>216,217</sup> Using this approach, the amine and reducing agent were added at the same time; theoretically the reduction step would force the equilibrium over to the side of the products and it would not be necessary to use a
drying reagent to remove the water. When the process was performed a mixture of products were obtained, none of which was the desired tertiary alcohol.

The low yield of the oxidation reaction and the poor results of reductive amination did not prove to be synthetically useful. Thus, we decided to develop an alternative route to make the chiral β-amino tertiary methyl alcohols.

8.3.2 Synthesis of β-amino methyl alcohol 101 via the tosylation of primary alcohol 96i followed by an exhaustive reduction

Another route to make the desired compound is shown in Scheme 52 and consists of the tosylation of the previously synthesised alcohol 96i to give 104a, followed by exhaustive reduction with lithium aluminium hydride to give the desired tertiary alcohol 105a (Scheme 52).

\[\text{Scheme 52: Possible synthetic route to make the β-amino methyl alcohols.}\]

The tosylation reaction was performed with an excess of 4-toluenesulfonyl chloride (1.5 equivalents) in pyridine at room temperature. After purification and characterisation of the final product (after reduction) it was observed that the secondary amine was tosylated, making a very stable bond which is very difficult to cleave, as well as the desired tosylation of the less hindered primary alcohol and its subsequent reduction with lithium aluminium hydride (Figure 22). β-Amino methyl alcohols were made using this synthetic route but the tosylated amine is too stable and difficult to deprotect, as such, the final product is not useful for further modifications leading to pharmaceuticals or natural products; the nitrogen-sulphur...
bond is too stable and the reactivity is too low for additional alterations. Therefore, this route, with primary amines, was not repeated further.

Figure 22: Undesired product obtained by performing the tosylation of 96i with an excess of 4-toluenesulfonyl chloride and subsequent reduction.

A similar synthetic route was attempted with secondary amines to avoid this undesired $N$-tosylation, as outlined in scheme 53. The tosylation of the primary alcohol was performed following the same procedure above, but surprisingly, no reaction occurred. After 24 hours at room temperature only starting material was recovered, and the crude NMR showed decomposition of compound 96h.

Scheme 53: Proposed synthetic route to make the $\beta$-amino methyl alcohols.
8.3.3 Synthesis of β-amino methyl alcohol 101 via tert-butyldimethylsilyl chloride protection.

One explanation for the decomposition of the starting material observed in the above reaction could be the sensitivity of the acetate group. Thus a modification of the protecting group strategy was introduced, a more robust tert-butyldimethylsilyl maybe beneficial. (Scheme 54).

![Scheme 54: Possible synthetic route to make the β-amino tertiary methyl alcohols.]

The alternative route follows the selective protection of the primary alcohol of 2,3-dihydroxy-2-(morpholinomethyl)propyl acetate 96c with tert-butyldimethylsilyl chloride, triethylamine and 4-dimethylaminopyridine in dichloromethane. Protection of this primary alcohol, before the hydrolysis of the acetate group, is essential to maintain the stereogenic centre of the tertiary alcohol when the process performed with enantiopure material (the diol would be prochiral). After the hydrolysis of the acetate group with potassium carbonate in MeOH, the newly formed primary alcohol
can be tosylated followed by a subsequent reduction with lithium aluminium hydride to reveal the desired compound 109 (Scheme 54).

To attempt the new synthetic route, primary alcohol 96c was used due its stability as demonstrated in previous work (Section 8.1.3). Unfortunately, protection of primary alcohol 96c with tert-butyldimethylsilyl chloride was met with no success. The reaction was left at reflux for 8 hours and no product was seen by TLC.

### 8.3.4 Synthesis of β-amino methyl alcohol 101 via tert-butyldimethylsilyl chloride protection of epoxide (±)-50.

Due to the adjacent quaternary centre in 96c, the accessibility to the primary alcohol is difficult due to the sterically encumbering functional groups attached to the stereogenic centre. Therefore, we decided to protect epoxy monoacetate and open the epoxide after the protection of the primary alcohol (Scheme 55). The protection was performed with tert-butyldimethylsilyl chloride, triethylamine and 4-dimethylaminopyridine in dichloromethane in 82% yield. The epoxide was subsequently reacted with morpholine to arriving at the chiral tertiary alcohol 107 in a gratifying 95% yield (Scheme 55). Hydrolysis of the acetate group was performed with potassium carbonate in methanol in 98% yield generating diol 100d. However, tosylation of primary alcohol 100d using 4-toluenesulfonyl chloride in pyridine met with no success.
8.3.5 Synthesis of β-amino methyl alcohol 101 using (±)-50.

Since modification of the tertiary alcohols was problematic presumably due to the increased steric bulk, the reaction of tosyl epoxide 110 towards amines was investigated. This would give the desired alcohols 108 with the required tosylate functionality (Scheme 56). Derivatisation of the primary alcohol (±)-99 with 4-toluenesulfonyl chloride, triethylamine and 4-dimethylaminopyridine in dichloromethane was achieved in 90% yield to obtain the desired epoxide (2-((tert-butyl(dimethyl)silyloxy)methyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate 110 (Scheme 56).
The ring-opening of epoxide 110 was performed using four different amines, 4-fluoroaniline, allylamine, propargylamine and benzylamine. The reaction with 4-fluoroaniline gave very low conversion by TLC after 48 hours at reflux, presumably due to the low nucleophilicity of the aromatic amine (especially being electron deficient) and the product was not isolated. When the epoxide ring-opening was performed with propargylamine and benzylamine the tosyl group was lost during the process. However, when allylamine was used the desired compound 108 was obtained. Reduction of alcohol 108 with lithium aluminium hydride gave a product without the expected methyl group!
Scheme 57: Proposed products for the epoxide ring-opening with aliphatic primary amines.

Scheme 57 shows the tentatively assigned products for the epoxide ring-opening with aliphatic primary amines. Product 108 was obtained when the reaction was performed with allylamine in EtOH at reflux for 1 hour. In this case, the signals from the tosyl group were observed by ^1^H-NMR of the purified material. The mass spectrum in conjunction with ^1^H-NMR data of the products obtained when propargylamine and benzylamine were used as a nucleophiles discount products 108, 112 and 113. Finally, azetidines can be differentiated from structure 111 by ^13^C-NMR due to the symmetry of azetidines 114. After analysing all possibilities we concluded that the products made from the epoxide ring-opening using propargylamine and benzylamine in EtOH at reflux for 1 hour were azetidines 114.

Confirmation of the azetidine structures for products as 114a and 114b (R = allyl and propargyl respectively) generated by the epoxide ring-opening of 110, was obtained using X-ray crystallography (Figure 23 and 24).
Figure 23: X-ray structure of azetidine 114a; obtained when allylamine was used to open epoxide 110.

Figure 24: X-ray structure of azetidine 114b; obtained when propargylamine was used to open epoxide 110.
In an effort to generate β-amino methyl alcohols 101 and avoid azetidine formation the reactions were repeated at room temperature. Allylamine, N-methylallylamine and benzylamine were used as nucleophiles for the epoxide ring-opening of tosylate epoxide 110; the reactions were performed in ethanol at room temperature for 24 hours. In the cases of allylamine and benzylamine, azetidines 114a and 114c were obtained in 31% and 51% yields respectively. Interestingly, the reaction with allylamine gave azetidine 114a at room temperature in 24 hours, but tertiary alcohol 108b when left at reflux for 1 hour. In both cases the yields were low. Unfortunately, it was not possible to isolate again 108b and the compound was only characterised by 1H-NMR. However, 1H-NMR data of the product obtained after reduction of tertiary alcohol 108b with lithium aluminium hydride was the same azetidine 114a as the one produced via epoxide ring-opening with allylamine at room temperature (Scheme 58). In the first reaction, lithium aluminium hydride acts as a base to deprotonate the NH proton of amine 108b and the resulting anion perform a 4-exo-tet cyclisation to displace the tosylate. Possible mechanisms of these reactions will be discussed in detail in the next section.

Scheme 58: Epoxide ring-opening with primary and secondary aliphatic amines.
The epoxide ring-opening of tosylate epoxide 110, when performed with N-methylallylamine generated a symmetrical tertiary alcohol 115a. The reaction was performed with an excess of the amine and a second equivalent should attack a presumed second epoxide intermediate generating the symmetric tertiary alcohol (see later section).

We wanted to study the possibility that compound 108b is an intermediate towards azetidine 114a and if the reaction is refluxed longer the yield of the azetidine may increase. Therefore, the reaction was performed with an excess of different amines under identical conditions, refluxing for 8 hours in EtOH in the hope that all intermediates and reaction pathways would be forced through to the azetidine products.

In both cases, when using either primary or secondary amines, symmetrical compounds are produced and the chiral centre is lost during the reaction. Even though the final product loses its chirality, the biotransformation with R. miehei (see section 7.3) generates the epoxy monoacetate in higher yield than the reaction performed chemically with acetyl chloride and thus could still be a viable synthetic protocol. However, it is pleasing to discover a new route to azetidines in the process which we decided to pursue as outlined in the section below.

8.4 Proposed mechanism for the epoxide ring-opening of 110.

As shown in section 8.3.5, we developed a synthetic route to produce azetidines via ring-opening of epoxide 110 using different amines. We propose two possible mechanisms for the formation of the azetidines from (2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate 110 and an excess of primary amine in EtOH (Schemes 59 and 60).

The first mechanism begins with an amine induced epoxide ring-opening (Scheme 59); the amine attacks the epoxide generating alkoxide intermediate 116. An intramolecular ring-closure follows causing the displacement of the tosylate leaving group producing a new epoxide 111. The secondary amine generated in this process, attacks the newly formed epoxide, opening the ring, in a 4-endo-tet cyclisation forming the four membered nitrogen heterocycle 114. In this case, it is
assumed that the alkoxide attacks faster than a proton transfer in intermediate 116 (see the alternative mechanism (Scheme 60)).

![Scheme 59: Proposed mechanism for the synthesis of azetidines.](image)

During this process, the chiral centre is lost by the formation of the azetidine. Following Baldwin’s rules of epoxide ring-opening, the second intramolecular reaction is a 4-endo-tet cyclisation and therefore a disfavoured heterocyclic formation (Figure 24).\(^{220}\) Baldwin suggests that this type of three-membered ring generally prefers exo-modes, thus following his rules, the proposed mechanism is not favoured for the synthesis of azetidines using epoxide 110.\(^{220}\)

![Figure 24: Baldwin’s rules for opening three-membered rings to form cyclic structures.](image)
The second mechanism proceeds via the tertiary alcohol (Scheme 60). The reaction begins with the same epoxide ring-opening as before with the amine attacking the epoxide generating a quaternary ammonium ion and an alkoxide intermediate 116, however, immediately it undergoes a proton transfer generating the tertiary alcohol 108. Finally, the formation of the azetidine is via an intramolecular attack of the amine via an S\textsubscript{N}2 reaction onto the neopentyl carbon with the better leaving group, and a consequent elimination of the tosylate. Normally, direct S\textsubscript{N}2 to a neopentyl centre are very rare, the neopentyl centre is too hindered to react via S\textsubscript{N}2, although when the reaction is intramolecular S\textsubscript{N}2 substitutions can proceed.\textsuperscript{221}

![Scheme 60: Alternative proposed mechanism for the synthesis of azetidines using primary amines.]

Similarly, following Baldwin’s rules, the intramolecular cyclisation in this case is 4-exo-tet and therefore a favoured reaction, making this mechanism more likely.\textsuperscript{220}

The reaction with secondary amines produced a symmetrical tertiary alcohol. The first mechanism proposed agrees with the product produced via double epoxide ring-opening. The reactions were performed with an excess of the amine and there was enough to open the second epoxide to generate the tertiary alcohol (Scheme 61).
Scheme 61: Proposed mechanism for the synthesis of tertiary alcohol 115 using a secondary amine.

Considering the second mechanism proposed, when reactions were performed with secondary amines, the second equivalent of the amine attacks intermolecularly to a neopentyl centre and this reaction is very slow and normally unfavoured (Scheme 62, mechanism via 1). Having in mind that intramolecular S_n2 reactions at neopentyl centres can proceed via rearrangement of the atoms, it is more likely that the reaction progresses through an azetidinium ion 117,221,222 rather than direct attack at the neopentyl centre (Scheme 62, mechanism via 2) followed by the azetidinium ion ring-opening to produce the final product 115. Azetidinium ions are stable enough to be isolated and store for months without significant decomposition and the heterocycle can be easily opened due to the strong activation of the amonium group.223 There are some examples of azetidine formation from epoxides via azetidinium ions making this mechanism more suitable for the synthesis of tertiary alcohol 115.224-227
Scheme 62: Alternative proposed mechanism for the synthesis of tertiary alcohol 115 using secondary amines.

Considering only the epoxide ring-opening of 110 with primary amines to generate azetidine 114, the second mechanism proposed would be the more likely following Baldwin’s rules. The reaction was attempted with one equivalent of allylamine (see section 8.4.5) without isolation of the expected β-amino epoxide 101, and when the reaction was attempted for the first time with an excess of allylamine refluxing for 1 hour, the chiral tertiary alcohol 108 was isolated. Unfortunately, it was not possible to isolate this intermediate again.

On the other hand, reactions with secondary amines rarely go via S_N2 substitution at neopentyl centres and it is more likely that these go via azetidinium ion formation.

Even though the second mechanism is more likely to generate both products, more experiments needs to be performed.
8.4.1 Synthesis of azetidines with primary amines.

Recently, it has been demonstrated that azetidines find wide application as an important functional group in many pharmacologically active compounds making them an interesting synthetic topic. However, there is a remarkable lack of general methods for the synthesis of azetidines.

The epoxide ring-opening of (2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate 110 was performed with different primary aliphatic and primary aromatic amines to generate a range of azetidines (Table 18). The reactions were performed in ethanol, with an excess of the amines (3 equivalents) and refluxed for 8 hours to produce different amino substituted azetidines in high yields (43% - 92%).

When the reaction was performed with 4-fluoroaniline the yield of azetidine 114d was low, presumably due to the lower nucleophility of the electron-deficient aromatic amine. The only product isolated from the reaction was the azetidine; even with the low nucleophility of the aromatic amines, none of the proposed intermediates from Schemes 59 and 60 could be isolated to provide evidence as to which reaction mechanism was taking place.
Table 18: Results of the ring-opening of epoxide 110 with 3 equivalents of primary amines in EtOH at 78 °C.\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product type</th>
<th>Yield (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H(_2)N(\equiv)</td>
<td>114a</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>H(_2)N(\equiv)</td>
<td>114b</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>H(_2)N(\equiv)</td>
<td>114c</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>F(\equiv)NH(_2)</td>
<td>114d</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>OMe(\equiv)NH(_2)</td>
<td>114e</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>NH(_2)</td>
<td>114f</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>NH(_2)</td>
<td>114g</td>
<td>92</td>
</tr>
</tbody>
</table>

\(^a\) Reagents and conditions: Epoxide 110 was dissolved in ethanol (0.1 M), the amine added (3 equivalents) and the reaction heated at reflux for 8 hours. \(^b\) Isolated yields.

8.4.2 Synthesis of azetidines with chiral aliphatic primary amines.

The ring-opening of epoxide 110 was performed with a small number of chiral amines in high yields (Scheme 64). Though the stereogenic centre in the epoxide 110 is lost during the azetidine formation, the final azetidine is chiral due to the chirality of the amine.
Scheme 64: Epoxide ring-opening with chiral amines to produce chiral azetidines.

Table 19: Results of the ring-opening of \textbf{110} with 3 equivalents of chiral amine in EtOH at 78 °C to produce chiral azetidines.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product type</th>
<th>Yield (%)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textsuperscript{a}NH\textsubscript{2}</td>
<td>\textbf{114h}</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>\textsuperscript{b}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>\textsuperscript{c}NH\textsubscript{2}</td>
<td>\textbf{114i}</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>\textsuperscript{d}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>\textsuperscript{e}NH\textsubscript{2}</td>
<td>\textbf{114j}</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>\textsuperscript{f}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reagents and conditions: Epoxide \textbf{110} was dissolved in ethanol (0.1 M), the amine added (3 equivalents) and the reaction heated at reflux for 8 hours. \textsuperscript{b} Isolated yields.

Table 19 summarises the results obtained in these reactions. Chiral amines, such as: (\textit{R})-(\textit{+})-\textit{α}-methylbenzylamine (entry 1), (\textit{R})-(\textit{+})-\textit{α}-ethylbenzylamine (entry 2) and (\textit{R})-(\textit{+})-1-(2-naphthyl)ethylamine (entry 3) were reacted with epoxide \textbf{110} and the corresponding chiral azetidine was produced in high yield in each case.
8.4.3 Epoxide ring-opening with secondary amines.

The experiment was performed with secondary amines to gain a better understanding of the process. Three secondary amines, \( N \)-benzylmethylamine, \( N \)-methylprogargylamine and \( N \)-allylmethylamine, were reacted with epoxide 11 (Scheme 65). As previously observed, the reaction with secondary amines gave prochiral product 115.

![Scheme 65: Epoxide ring-opening with secondary amines.](image)

The epoxide ring-opening of \((2-(\text{tert-butyl}d\text{imethyl}silyloxy})\text{methyl)}\text{oxiran-2-yl})\text{methyl-4-methylbenzenesulfonate 110 with an excess of secondary amines agrees with both mechanisms proposed in Schemes 61 an 62. The products were obtained in high yields and are summarised in Table 20.}
Table 20: Results of the ring-opening of epoxide 110 with 3 equivalents of secondary amines in EtOH at 78 °C.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Product type</th>
<th>Yield (%)\textsuperscript{b}</th>
</tr>
</thead>
</table>
| 1     | \begin{tikzpicture}[scale=0.5]
        \draw[thick] (0,0) -- (1,0);
        \draw[thick] (0,0) -- (0,1);
        \node at (0.5,0.5) {NH};
    \end{tikzpicture} | 115\textsuperscript{a} | 81 |
| 2     | \begin{tikzpicture}[scale=0.5]
        \draw[thick] (-0.5,0) -- (0.5,0);
        \draw[thick] (0,0) -- (0,1);
        \node at (0,0.5) {NH};
    \end{tikzpicture} | 115\textsuperscript{b} | 78 |
| 3     | \begin{tikzpicture}[scale=0.5]
        \draw[thick] (-0.5,0) -- (0.5,0);
        \draw[thick] (0,0) -- (0,1);
        \node at (0,0.5) {NH};
        \draw[thick] (0,1) -- (0,2);
        \draw[thick] (0,2) -- (1,2);
    \end{tikzpicture} | 115\textsuperscript{c} | 83 |

\textsuperscript{a}Reagents and conditions: Epoxide 110 was dissolved in ethanol (0.1 M), the amine added (3 equivalents) and the reaction heated to reflux for 8 hours.\textsuperscript{b} Isolated yields.

8.4.4 Epoxide ring-opening with two different amines.

Our interest in developing a synthetic route to chiral tertiary alcohols using this process focused our attention on ascertaining the outcome of the reaction depicted in Scheme 65. Could chiral, non-symmetrical products be made by a judicious choice of two amines? To avoid the intramolecular cyclisation and therefore the generation of azetidines, two secondary amines with different reactivities were used in the reaction. Performing the reaction with a mixture of two amines with different chemical properties in the hope that the reaction may be more selective for the less bulky amine to attack the epoxide, leaving the more bulky amine for the second (epoxide or azetidinium ion) ring-opening step to generate a product without loss of the stereogenic centre. A mixture of \textit{N}-methylpropargylamine and \textit{N}-benzylmethylamine were tested to attempt such a formation of a chiral tertiary alcohol (Scheme 66). One equivalent of each amine was refluxed for 8 hours in ethanol. Unfortunately, after this time, a mixture of products was observed by TLC and the crude reaction mixture was not able to be purified.
8.4.5 Epoxide ring-opening with 1 equivalent of amine.

In order to prove that the synthesis of azetidines follow the first mechanism proposed (Scheme 67), via epoxide formation, we believe that it would be useful to attempt to isolate the proposed epoxide intermediate 111 (R=Me and Bn). This epoxide, if isolable, could also be a building block to generate chiral tertiary alcohols. The reaction was performed using only one equivalent of a secondary amine to avoid the second step of the process. A secondary amine, N-benzylmethylamine, was used to avoid the proposed intramolecular cyclisation which would give the undesired azetidine. Unfortunately, the starting material did not react with only one equivalent of the amine and no product was seen by TLC.
9 CONCLUSION.

The synthesis of enantiomerically pure (S)-2-acetoxymethylglycidol 50 has been achieved via enzymatic desymmetrisation of epoxy diol 87 with lipase Amano L, AK in 42% isolated yield and 97% enantiomeric excess. An improved on yield was observed when the enzyme was immobilised on silica (70% yield and 98% ee). Commercially immobilised lipase from R. miehei was also used to achieve the same isomer in 82-90% yield and up to 89% ee.

The ring-opening of the biotransformed epoxide using different amines has been developed in high yields generating some enantiomerically pure β-amino tertiary alcohol products. The intramolecular migration, when primary amines were used to open the epoxide, was avoided using a TBS-protected compound 99 with good yields (43%-83%), without any loss of enantiomeric excess of the formed ‘pseudo’-enantiomer.

(S)-2-acetoxymethylglycidol 50 was also used as a starting material to generate azetidines in high yields. Tertiary alcohol-azetidines can be generated via enzymatic desymmetrisation with R. miehei to generate the overall process in higher yield.
10 FUTURE WORK

The immobilisation of Amano L, AK on silica was promising and there is a need to spend more time in the future developing this encapsulation method.

Another way to increase activity and selectivity of the enzyme could be by directed mutagenesis. Modifying the appropriate amino acids in the active site, the interactions between solvent-enzyme-acyl donor-substrate can be affected thus influencing the activity and specificity of the enzyme.

\((S)-(2-(\text{hydroxymethyl})\text{oxiran-2-yl})\text{methyl acetate}\) 50 is a useful building block to generate chiral tertiary alcohols, we focussed our attention towards synthesising \(\beta\)-amino tertiary alcohols, however there are a large number of nucleophiles that could open the epoxide and produce chiral tertiary alcohols (Scheme 32). We could extend the diversity of nucleophiles to react with the epoxide 50 and produce a large number of functionalised tertiary alcohols. This could expand the synthesis and applicability of this compound leading to pharmaceutical and agrochemical products.

We were trying to develop a synthetic route to generate \(\beta\)-amino methyl alcohols without success. In Scheme 68 and 69 there are two new routes for the synthesis of this target. The first one starts with \(\beta\)-amino tertiary alcohol 96 and a protection of the diol, followed by the hydrolysis of the acetate group and tosylation of the newly generated primary alcohol. Finally, an exhaustive reduction with lithium aluminium hydride should produce \(\beta\)-amino methyl alcohol.
Scheme 68: Proposed synthetic route to generate β-amino methyl alcohols.

The second possible route to generate β-amino tertiary methyl alcohols is via an exhaustive reduction of epoxide 98 with lithium aluminium hydride to produce a tertiary methyl alcohol intermediate followed by the tosylation of the primary alcohol. Treating compound 123 with a base to generate the tertiary alkoxide and posterior intramolecular $S_N2$ attacks displacing the tosylate would generate a new epoxide 124. This epoxide would be a new building block that could be reacted with different nucleophiles to achieve a diversity of β-substituted tertiary methyl alcohols.

Scheme 69: Proposed synthetic route to generate β-amino tertiary methyl alcohols.
An unexpected and interesting synthesis of tertiary hydroxyl-azetidines has been achieved using \((S)\)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50 in high yields. However, more experiments are required to determine the mechanism of this reaction. If the reaction proceeds via an azetidinium ion and, if stable, the isolation of it would be of great interest. This azetidinium species could be reacted with different reagents such as heteronucleophiles\(^{234}\), hydrides\(^{235}\) and carbon nucleophiles\(^{236}\) and therefore the synthesis of chiral tertiary alcohols (Scheme 70).

Scheme 70: Ring-opening of azetidinium ions.

Throughout this thesis we have related the importance of chiral tertiary alcohols and chiral \(\beta\)-amino tertiary alcohols in natural and non-natural products. We hope that these intermediates will find wide applicability in pharmaceutical and agrochemical industries.
Chapter 3:

EXPERIMENTAL SECTION
11 GENERAL

Commercially available reagents were used as received without purification. Analytical thin layer chromatography was performed with Keiselgel 60 F254, in a variety of solvents on aluminium-backed plates. The plates were visualised by UV light (254 nm), p-anisaldehyde and KMnO₄. Flash column chromatography was conducted with Merck silica gel 60H (40-60 µm, 230-400 mesh) under bellows pressure. Nominal mass spectra were recorded on a Waters LCT mass spectrometer connected to a Waters Alliance 1100 LC autosampler and controlled by Waters Masslynx 4.1 and OpenAccess software using electrospray (ES) ionisation. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 300 (300 MHz) or a Bruker DPX 400 (400 MHz) spectrometer. All chemical shifts (δ) are quoted in parts per million (ppm) relative to a calibration reference of the residual protic solvent; CD₃OD (δH 5.84, s) was used as the internal standard in ¹H NMR spectra, and ¹³C NMR shifts were referenced using CD₃OD (δC 49.05, hept) with broad band decoupling and CDCl₃ (δH 7.26, s) was used as the internal standard in ¹H NMR spectra, and ¹³C NMR shifts were referenced using CDCl₃ (δC 77.0, t) with broad band decoupling. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; hept, heptet; m, multiplet. Petroleum ether refers to the fraction that boils between 40-60 °C.

11.1 Synthesis of (2-hydroxymethyl-oxiranyl)-methanol, 87.

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{O} & \quad \text{OH}
\end{align*}
\]

To a solution of 2-methylene-1,3-propanediol 86 (2.47 g, 28.0 mmol) in dichloromethane (280 ml, 0.1 M) was added m-CPBA (7.6 g, 30.8 mmol). The solution was stirred at room temperature for 4 hours. The mixture was reduced in vacuo and the crude material was purified by column chromatography (SiO₂; 100% EtOAc) to yield the title compound 87 as a colourless oil (2.8 g, 96 %). ¹H NMR (300 MHz, CDCl₃) δH 2.12 (2H, dd, J = 5.4, 7.4, CH₂O), 2.88 (2H, s, OH), 3.72 (2H, dd, J = 7.4, 12.4, CH₂OH), 3.89 (2H, dd, J = 5.4, 12.4, CH₂OH); ¹³C NMR (75
MHz) 49.1 (CH$_2$OC), 60.1 (C(CH$_2$)$_3$O), 62.6 (CH$_2$OH); MS ES (+ve) found m/z 231 ([2M+23Na]$^+$, 100%).

11.2 Synthesis of (±)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate, 50.

![Structure of the compound](image)

To a solution of 2-methylene-1,3-propanediol 86 (3.0 g, 34.0 mmol) in dichloromethane (340 ml, 0.1 M) was added 4-dimethylaminopyridine (208 mg, 1.70 mmol), triethylamine (3.6 ml, 25.5 mmol) and acetyl chloride (1.2 ml, 17.0 mmol). The solution was stirred at room temperature for 20 hours. After this time potassium carbonate (9.4 g, 68.0 mmol) was added to the solution and the slurry was stirred for 15 minutes. The mixture was filtered and concentrated in vacuo to yield the title compound 50 as colourless oil (551 mg, 98% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 2.08 (3H, s, CH$_3$CO), 4.13 (2H, s, CH$_2$OH), 4.63 (2H, s, CH$_2$OAc), 5.17 (1H, s, CH$_3$H$_5$C(CH$_2$)$_2$), 5.23 (1H, s, CH$_3$H$_5$C(CH$_2$)$_2$); $^{13}$C NMR (100 MHz) 21.3 (CH$_3$CO), 64.1 (CH$_2$OH), 65.2 (CH$_2$OAc), 114.9 (CH$_2$C(CH$_2$)$_2$), 143.8 (C(CH$_2$)$_3$), 171.4 (CO); MS ES (+ve) found m/z 131 ([M+H]$^+$, 100%). To a solution of the above oil (500 mg, 3.85 mmol) in dichloromethane (0.1 M) was added m-CPBA (1.42 g, 5.77 mmol). The solution was stirred at room temperature for 20 hours. After this time potassium carbonate (9.4 g, 68.0 mmol) was added to the solution and the slurry was stirred for 15 minutes. The mixture was filtered and concentrated in vacuo to yield the title compound 50 as colourless oil (551 mg, 98% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H 2.09 (3H, s, CH$_3$CO), 2.80 (1H, d, $J = 4.6$, CH$_3$H$_5$OC), 2.92 (1H, d, $J = 4.6$, CH$_3$H$_5$OC), 3.68-3.83 (2H, m, CH$_2$OH), 4.14 (1H, d, $J = 12.2$, CH$_3$H$_5$OAc), 4.32 (1H, d, 12.2, CH$_3$H$_5$OAc); $^{13}$C NMR (75 MHz) 20.9 (CH$_3$CO), 49.4 (CH$_2$OC), 57.8 (C(CH$_2$)$_3$), 61.7 (CH$_2$OH), 64.4 (CH$_2$OAc), 171.0 (CO); MS ES (+ve) found m/z 147 ([M+H]$^+$,
11.3  **Synthesis of acetic 2-acetoxymethyl-oxiranylmethyl ester, 49.**

![Chemical structure of the title compound](image)

To a solution of (2-hydroxymethyl-oxiranyl)-methanol 87 (252.0 mg, 2.42 mmol) in dichloromethane (24 ml, 0.1 M) was added 4-dimethylaminopyridine (29 mg, 0.1 mmol), triethylamine (1.01 ml, 7.26 mmol) and acetyl chloride (0.43 ml, 6.1 mmol). The solution was stirred at room temperature for 1.5 hours. The mixture was extracted with diethyl ether and the organic extract was washed with brine (20 ml, three times). The organic extract was dried (MgSO$_4$) and concentrated in vacuo to yield the title compound 49 as a colourless oil (348.0 mg, 76% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 2.08 (6H, s, 2xC$_3$H$_2$CO), 2.84 (2H, s, C$_2$H$_2$OC), 4.13 (2H, d, $J$ = 12.0, C$_2$H$_3$H$_2$OAc), 4.27 (2H, d, $J$ = 12.0, CH$_2$HOAc), $^{13}$C NMR (100 MHz) 20.7 (C$_3$H$_2$CO), 49.7 (C$_2$H$_2$OC), 63.8 (CH$_2$OAc), 55.3 (C(CH$_2$)$_2$O), 170.4 (CO); MS ES (+ve) found m/z 189 ([M+H]$^+$, 40%), 147 ([M-Ac+2H]$^+$, 60%), 129 ([M-OAc]$^+$, 80%).

11.4  **Enzymes.**

The enzymes used were sourced as follows:

- **PPL:** Lipase from porcine pancreas type II., Sigma-Aldrich, L3126.
- **Amano L, AK:** Amano L, AK from *Pseudomonas fluorescens*, Sigma-Aldrich, 534730.
- **Amano L, PS:** Amano L, PS from *Burkholderia cepacia* (*Pseudomonas cepacia*), Sigma-Aldrich, 534641.
- **CRL:** Lipase from *Candida rugosa*, Fluka, 90860.
- **CAL-B:** Novozyme 435, Novozymes.

A kit of immobilized enzymes was used from CODEXIS. The enzyme codes are as follows:

- **NZL-101 Lipase A** from *Candida antarctica*.
- **NZL-102 Lipase B** from *Candida antarctica*.  

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NZL-103 Lipase from *Rhizomucor miehei*.

NZL-104 Lipase from *Thermomyces lanuginosus*.

NZL-105 Lipase from *Thermomyces lanuginosus*, mutant.

NZL-106 Lipase from *Thermomyces lanuginosus*, mutant, broad range.

NZL-107 Lipase from fungal mutant, accepting bulky substrates.

NZL-108 Lipase from fungal, broad range.

NZL-109 Lipase from fungal mutant, broad range.

### 11.5 General enzymatic reactions.

To a mixture of (2-hydroxymethyl-oxiranyl)-methanol 87 (100 mg, 0.96 mmol), enzyme (200 mg, 2 wt. eq.) and molecular sieves (4 Å) in the corresponding solvent (10 ml, 0.1 M) was added the acyl donor (see Tables 1-3 in Results and Discussion section). The solution was shaken vigorously in an incubator set at the required temperature for the specified times (see Tables 1-3 in Results and Discussion section). The mixture was filtered through Celite® and the filtrate was reduced *in vacuo* to yield an oil which was purified by column chromatography (SiO₂; 30% EtOAc in petroleum ether). The enantiomeric purity was determined by chiral gas chromatography; CP-Chirasil-Dex CB; 25 m × 0.25 mm × 0.39 mm; 130 °C; split 50:1, He flow 1.0 ml/min; 1 µl injection using FID detection.

### 11.6 General immobilised enzyme reactions.

To a mixture of (2-hydroxymethyl-oxiranyl)-methanol 87 (10 mg, 0.1 mmol), the appropriate lipase (10 mg, 1 wt. eq.) and molecular sieves (4 Å, 10 mg) in dichloromethane (1 ml, 0.1 M) was added the acyl donor (see Tables 4-12 in Results and Discussion section). The solution was shaken vigorously (250 rpm) in an incubator set at the required temperature for the specified times (see Tables 4-12 in Results and Discussion section). The mixture was filtered through 0.3 μm filters and the filtrate was analysed by chiral gas chromatography; CP-Chirasil-Dex CB; 25 m × 0.25 mm × 0.39 mm; 130 °C; split 50:1, He flow 1.0 ml/min; 1 µl injection using FID detection.
11.7 General synthesis of tertiary alcohols 96a-j and 97a-g.

To a solution of acetic (±)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate, 50 (200 mg, 1.37 mmol) in ethanol (14 ml, 0.1 M) was added the corresponding amine (3 eq., see Table 13 in Results and Discussion). The solution was heated at reflux for 4 hours with stirring. The mixture was reduced in vacuo and the crude material was purified by column chromatography (SiO$_2$; MeOH/EtOAc).

11.7.1 (±)-2,3-Dihydroxy-2-(piperidin-1-ylmethyl)propyl acetate, 96b.

![Chemical structure of 96b](image)

Yield 55%, colourless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 1.56 (6H, m, 3xCH$_2$CH$_2$N), 2.07 (3H, s, CH$_3$CO), 2.51 (6H, m, 3xCH$_2$N), 3.53 (1H, d, $J$ = 11.6 CH$_a$H$_b$OH), 3.58 (1H, d, $J$=11.6 CH$_a$H$_b$OH), 3.96 (1H, d, $J$ = 11.2 CH$_a$H$_b$OAc), 3.99 (1H, d, $J$=11.2 CH$_a$H$_b$OAc). $^{13}$C NMR (100 MHz) 20.8 (CH$_3$CO), 23.5 (CH$_2$CH$_2$CH$_2$N), 26.3 (CH$_2$CH$_2$N), 56.7 (CH$_2$CH$_2$N), 62.11 (CCH$_2$N), 66.3 (CH$_2$OH), 66.8 (CH$_2$OAc), 71.4 (C(CH$_2$)$_3$OH), 171.1 (CO); MS ES (+ve) found $m/z$ 232.1 ([M+H]$^+$, 100%). HRMS FAB [M+H]$^+$ 232.1542 C$_{11}$H$_{21}$O$_4$N+H$^+$ requires 232.1549. IR (cm$^{-1}$) 3416, 1737.

11.7.2 (±)-2,3-Dihydroxy-2-(morpholinomethyl)propyl acetate, 96c.

![Chemical structure of 96c](image)

Yield 81%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 2.10 (3H, s, CH$_3$CO), 2.55 (2H, d, $J$=4.0, CCH$_2$N), 2.59 (2H, m, OCH$_2$CH$_2$N), 2.72 (2H, m, OCH$_2$CH$_2$N), 3.54 (1H, d, $J$ = 11.4 CH$_a$H$_b$OH), 3.58 (1H, d, $J$=11.4 CH$_a$H$_b$OH), 3.70 (4H, t, $J$ = 4.8, 2xOCH$_2$CH$_2$N), 4.00 (1H, d, $J$ = 11.6 CH$_a$H$_b$OAc), 4.03 (1H, d, $J$=11.6 CH$_a$H$_b$OAc). $^{13}$C NMR (100 MHz) 20.9 (CH$_3$CO), 55.6 (OCH$_2$CH$_2$N), 61.7 (CCH$_2$N), 65.9
(CH₂OH), 66.6 (OCH₂CH₂N), 67.2 (CH₂OAc), 72.2 (C(CH₂)₂OH), 171.2 (CO); MS ES (+ve) found m/z 234.1 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 234.1336 C₁₀H₁₀O₅N⁺H⁺ requires 234.1341. IR (cm⁻¹) 3420, 1735, 1113.

(±)-3-(2,6-Dimethylmorpholino)-2-hydroxy-2-(hydroxymethyl)propyl acetate, 96d.

Yield 84%, colorless oil. ¹H NMR (400 MHz, CDCl₃) δH 1.14 (6H, d, J=5.6, CH₃CHO), 2.02 (1H, d, J=10.0), 2.07 (1H, d, J=10.0), 2.12 (3H, s, CH₃CO), 2.48 (1H, d, J= 9.2), 2.53 (1H, d, J= 6.4), 2.72 (1H, d, J= 11.2), 2.93 (1H, d, J= 11.2), 3.56 (1H, d, J= 12.4 C(CH₂H₂N), 3.61 (1H, d, J= 12.4 C(CH₂H₂N), 3.64-3.70 (2H, m, CH₂OH), 4.00-4.06 (2H, m, CH₂OAc). ¹³C NMR (100 MHz) 19.0 (CH₃CH), 20.9 (CH₃CO), 71.9 (NCH₂CH), 61.2 (NCH₂CH), 61.4 (CCH₂N), 66.1 (CH₂OH), 66.8 (CH₂OAc), 72.0 (C(CH₂)₂OH), 171.1 (CO); MS ES (+ve) found m/z 262.2 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 262.1648 C₁₂H₂₃O₅N⁺H⁺ requires 262.1654. IR (cm⁻¹) 3435, 1736.

11.7.3 (±)-2,3-Dihydroxy-2-((4-(2-hydroxyethyl)piperidin-1-yl)methyl)propyl acetate, 96e.

Yield 77%, colorless oil. ¹H NMR (400 MHz, CDCl₃) δH 1.27-1.58 (5H, m, (CH₂)₂CHCH₂CH₂OH), 1.71-1.76 (2H, m, CCH₂N), 2.13 (3H, s, CH₃CO), 2.29-2.62 (4H, m, N(CH₂CH₂)₂CH), 2.88 (1H, m, CH₃H₂O), 3.09 (1H, m, CH₃H₂O), 3.60-3.64 (2H, m, CH₂CH₂OH), 3.71-3.76 (2H, m, CH₂CH₂OH), 4.03-4.07 (2H, m, CH₂OAc). ¹³C NMR (100 MHz) 20.9 (CH₃CO), 31.6 (CHCH₂CH₂OH), 32.7(CHCH₂CH₂OH), 32.9 (N(CH₂CH₂)₂CH), 39.2 (N(CH₂CH₂)₂CH, 56.1
(CCH₂N), 60.5 (CH₂CH₂OH), 66.4 (CCH₂OH), 66.91 (CH₂OAc), 71.6 (C(CH₃)₃OH), 171.1 (CO); MS ES (+ve) found m/z 276.2 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 276.1814 C₁₃H₂₅O₅N⁺H⁺ requires 276.1811. IR (cm⁻¹) 3376, 1735.

11.7.4 (±)-2,3-Dihydroxy-2-((methyl(prop-2-ynyl)amino)methyl)propyl acetate, 96f.

Yield 80%, yellow oil. ¹H NMR (400 MHz, CDCl₃) δH 2.10 (3H, s, CH₃CO), 2.25 (1H, t, J = 2.4, CHC), 2.46 (3H, s, CH₃N), 2.62 (1H, d, J = 14.0, CCH₃H₅N), 2.67 (1H, d, J = 14.0 CCH₃H₅N), 3.41 (2H, d, J = 2.4, CH₂CCH), 3.53 (1H, d, J = 11.2, CH₃H₅OH), 3.57 (1H, d, J=11.2, CH₃H₅OH), 4.03 (2H, s, CH₃OAc). ¹³C NMR (100 MHz) 20.9 (CH₃CO), 44.5 (CH₃N), 47.9 (CH₂CCH), 58.7 (CCH₂N), 65.7 (CH₂OH), 66.4 (CH₂OAc), 72.3 (C(CH₃)₃OH), 73.3 (CCH), 78.6 (CCH), 171.0 (CO); MS ES (+ve) found m/z 216.1 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 216.1232 C₁₀H₁₇O₄N⁺H⁺ requires 216.1236. IR (cm⁻¹) 3419, 1729.

11.7.5 (±)-3-(Allyl(methyl)amino)-2-hydroxy-2-(hydroxymethyl)propyl acetate, 96g.

Yield 55%, yellow oil. ¹H NMR (400 MHz, CDCl₃) δH 2.09 (3H, s, CH₃CO), 2.39 (3H, s, CH₃N), 2.52 (1H, d, J = 13.8 CCH₃H₅N), 2.64 (1H, d, J = 13.8 CCH₃H₅N), 3.06 (1H, dd, J = 7.2, 14, NCH₃H₅CH), 3.19 (1H, dd, J = 6.4, 14, NCH₃H₅CH), 3.53 (1H, d, J = 11.4 CCH₃H₅OH), 3.57 (1H, d, J = 11.4 CH₂H₅OH), 3.99 (1H, d, J = 11.8 CH₃H₅OAc), 4.02 (1H, d, J = 11.8 CH₃H₅OAc), 5.17 (1H, s, CHCH₃H₅), 5.20 (1H,
d, $J = 3.6, CHCH_2H_b$, 5.85 (1H, m, $CHCH_2$). $^{13}$C NMR (100 MHz) 20.8 ($CH_3CO$), 44.7 ($CH_3$), 59.8 ($NCH_2CH$), 62.6 ($CCH_3$), 66.1 ($CH_2OH$), 66.6 ($CH_2OAc$), 72.2 ($C(CH_3)_2OH$), 118.5 ($CH_2CHCH_2N$), 134.6 ($CH_2CHCCH_3N$), 171.0 (CO); MS ES (+ve) found $m/z$ 218.1 ([M+H]$^+$, 100%). HRMS FAB [M+H]$^+$ 218.1384 $C_{10}H_{19}O_4N$ requires 218.1392. IR (cm$^{-1}$) 3384, 1736.

11.7.6 (±)-3-(Benzyl(methyl)amino)-2-hydroxy-2-(hydroxymethyl)propyl acetate, 96h.

![Molecule Image]

Yield 63%, yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 2.06 (3H, s, $CH_3CO$), 2.38 (3H, s, $CH_3$N), 2.58 (1H, d, $J = 13.6$ $CH_2H_bN$), 2.69 (1H, d, $J = 13.6$ $CH_2H_bN$), 3.46 (1H, d, $J = 11.4$, $CH_2H_bOH$), 3.49 (1H, d, $J = 11.4$, $CH_2H_bOH$), 3.54 (1H, d, $J = 12.8$, $CH_2H_bOAc$), 3.72 (1H, d, $J = 12.8$, $CH_2H_bOH$), 3.97 (2H, s, $CH_2Ar$), 7.31-7.34 (5H, m, Ar). $^{13}$C NMR (100 MHz) 20.9 ($CH_3CO$), 45.0 ($CH_3$N), 60.2 ($CH_2Ar$), 64.3 ($CH_2OH$), 66.1 ($CH_2OAc$), 66.7 ($CCH_3N$), 72.1 ($C(CH_2)_2OH$), 127.6, 128.6, 129.2 (CH, Ar), 138.1 (C, Ar), 171.1 (CO); MS ES (+ve) found $m/z$ 268.2 ([M+H]$^+$, 100%). HRMS FAB [M+H]$^+$ 268.1542 $C_{13}H_{21}O_4N$ requires 268.1549. IR (cm$^{-1}$) 3386, 1735.

11.7.7 (R)-(+)3-(4-Fluorophenylamino)-2-hydroxy-2-(hydroxymethyl)propyl acetate, 96i.

![Molecule Image]

Yield 94%, green solid. M. p. 76-79 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 2.13 (3H, s, $CH_3CO$), 3.17 (1H, d, $J = 12.8$, $CH_2H_bN$), 3.21 (1H, d, $J = 12.8$, $CH_2H_bN$), 3.61 (2H, s, $CH_2OH$), 4.17 (1H, d, $J = 11.6$, $CH_2H_bOAc$), 4.20 (1H, d, $J = 11.6$, $CH_2H_bOAc$), 6.61-6.65 (2H, m, Ar), 6.87-6.92 (2H, m, Ar). $^{13}$C NMR (100 MHz) 20.9 ($CH_3CO$),
48.1 (CH₂N), 64.4 (CH₂OH), 65.2 (CH₂OAc), 73.3 (C(CH₂)₃OH), 114.6 (J(C-F) = 29, CHCF), 115.7 (J(C-F) = 88) (CHCN), 144.5 (CHCN), 168.1 (CHCF), 171.7 (CO); MS ES (+ve) found m/z 258.0 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 258.1135 C₁₂H₁₆O₄NF+H⁺ requires 258.1141. IR (cm⁻¹) 3372, 1720. [α₀] (EtOH, c=0.9) +5.6.

11.7.8 (R)-(+)-2,3-Dihydroxy-2-((2-methoxyphenylamino)methyl)propyl acetate, 96j.

\[
\text{HO} \quad \text{N} \quad \text{O} \\
\text{O} \quad \text{H} \quad \text{O}
\]

Yield 96%, oil. ¹H NMR (400 MHz, CDCl₃) δH 2.14 (3H, s, CH₃CO), 3.25 (1H, d, J = 13.2, CH₃H₂N), 3.32 (1H, d, J = 13.2, CH₃H₂N), 3.63 (2H, s, CH₂OH), 3.85 (3H, s, CH₂OAr), 4.20 (2H, s, CH₂OAc), 6.70-6.74 (2H, m, Ar), 6.79 (1H, dd, J = 1.4, 8.0, Ar), 6.85-6.89 (1H, m, Ar). ¹³C NMR (100 MHz) 20.9 (CH₃CO), 47.2 (CH₃N), 55.5 (CH₃O), 64.4 (CH₂OH), 66.0 (CH₂OAc), 73.4 (C(CH₂)₃OH), 109.6, 110.7, 117.6, 121.2 (CH, Ar), 138.1 (CN), 147.3 (CHCO), 171.5 (CO); MS ES (+ve) found m/z 270.1 ([M+H]⁺, 100%). HRMS FAB [M+H]⁺ 270.1329 C₁₃H₁₉O₄N+H⁺ requires 270.1341. IR (cm⁻¹) 3401, 1720. [α₀] (EtOH, c=0.6) +11.2.

11.7.9 N-(2,3-dihydroxy-2-(hydroxymethyl)propyl)-N-(2-morpholinoethyl) acetamide, 97a.

\[
\text{HO} \quad \text{N} \quad \text{O} \\
\text{OH} \quad \text{O} \quad \text{O}
\]

Yield 89%, colourless oil. ¹H NMR (400 MHz, CDCl₃) δH 2.13 (3H, s, CH₃CO), 2.46 (4H, t, J= 4.4, 2xNCH₂CH₂O), 2.61 (2H, t, J= 5.6, NCH₂CH₂N), 3.41 (4H, s, 2xNCH₂CH₂O), 3.50 (2H, t, J= 5.6, NCH₂CH₂N), 3.54 (2H, s, CCH₂N), 3.65 (4H, t, J = 4.4, CH₂OH). ¹³C NMR (100 MHz) 21.8 (CH₃CO), 48.6 (NCH₂CH₂N), 52.3
(NCH$_2$CH$_2$N), 54.0 (CCH$_2$N), 58.9 (NCH$_2$CH$_2$O), 65.3 (NCH$_2$CH$_2$O), 66.4 (CH$_2$OH), 74.6 (C(\text{CH}_2)_3\text{OH}), 174.1 (\text{CO}); MS ES (+ve) found \( m/z \) 277.2 ([M+H]$^+$, 100%). HRMS FAB [M+H]$^+$ 277.1773 C$_{12}$H$_{24}$O$_3$N$_2$H$^+$ requires 277.1763. IR (cm$^{-1}$) 3376, 1612, 1113.

11.7.10 \( N\)-(2,3-dihydroxy-2-(hydroxymethyl)propyl)-\(N\)-isopropylacetamide, 97b.

![Structural diagram]

Yield 18\%, colourless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 1.24 (6H, d, \( J=6.8\), (\text{CH}_3)$_2$CH), 2.20 (3H, s, CH$_3$CO), 3.43 (2H, s, CH$_2$N), 3.48 (4H, m, 2xCH$_2$OH), 4.03 (1H, m, CH$_3$CH). $^{13}$C NMR (100 MHz) 21.2 (CH$_3$CO), 22.6 (CH$_3$CH), 46.9 (CH$_2$N), 64.5 (CH$_2$OAc), 51.2 (CHCH$_3$), 74.3 (C(\text{CH}_2)$_3$OH), 174.6 (\text{CO}); MS ES (+ve) found \( m/z \) 206.1 ([M+H]$^+$, 100\%). HRMS FAB [M+H]$^+$ 206.1393 C$_9$H$_{19}$O$_4$N$^+$H$^+$ requires 206.1392. IR (cm$^{-1}$) 3242, 1613.

11.7.11 \( N\)-cyclopentyl-\(N\)-(2,3-dihydroxy-2-(hydroxymethyl)propyl) acetamide, 97c.

![Structural diagram]

Yield 31\%, colourless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 1.56 (4H, m, 2xCH$_2$CH$_2$CH), 2.69-2.80 (4H, m, 2xCH$_2$CH$_2$CH), 2.15 (3H, s, CH$_3$CO), 3.37 (2H, s, CH$_2$N), 3.41 (4H, t, \( J=3.2\), CH$_2$OH), 3.98 (1H, m, NCH). $^{13}$C NMR (100 MHz) 22.3 (CH$_2$CH$_2$CH), 22.3 (CH$_3$CO), 29.7 (CH$_2$CH$_2$CH), 47.4 (CH$_2$N), 61.2 (CHN), 64.2 (CH$_2$OH), 74.2 (C(\text{CH}_2)$_3$OH), 174.8 (\text{CO}); MS ES (+ve) found \( m/z \) 232.2 ([M+H]$^+$, 100\%). HRMS FAB [M+H]$^+$ 232.1546 C$_{11}$H$_{21}$O$_4$N$^+$H$^+$ requires 232.1549. IR (cm$^{-1}$) 3363, 1601.

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11.7.12 \(N\)-(2,3-dihydroxy-2-(hydroxymethyl)propyl)-\(N\)-(prop-2-ynyl) acetamide, 97d.

\[
\begin{align*}
\text{Yield 93\%, white solid. M. p. 76-77 °C.} & \quad ^1H \text{NMR (400 MHz, CDCl}_3) \delta_H 2.27 \ (3H, s, CH}_3\text{CO}), \ 2.35 \ (1H, t, J=2.4, CHC), \ 3.49 \ (6H, m, CH}_2\text{C(CH}_2\text{OH)}_2), \ 4.21 \ (2H, d, J=2.4, CH}_2\text{CHC).} \\
& \quad ^13C \text{NMR (100 MHz) 22.1 (CH}_3\text{CO), 41.3 (NCH}_2\text{CCH), 49.9 (CCH}_2\text{N), 64.5 (CH}_2\text{OH), 73.7 (CHCCH}_2), 76.0 (C(CH}_2\text{)}_3\text{OH), 78.7 (CHCCH}_2),} \\
& \quad 174.6 (CO); \text{MS ES (+ve) found } m/z 202.2 ([M+H]^+, 100\%). \text{HRMS FAB [M+H]^+ 202.1085 C}_9\text{H}_{15}\text{O}_4\text{N+H}^+ \text{requires 202.1079. IR (cm}^{-1}) 3232, 1729.}
\end{align*}
\]

11.7.13 \(N\)-allyl-\(N\)-(2,3-dihydroxy-2-(hydroxymethyl)propyl)acetamide, 97e.

\[
\begin{align*}
\text{Yield 96\%, yellow solid. M. p. 84-86 °C.} & \quad ^1H \text{NMR (400 MHz, CDCl}_3) \delta_H 2.14 \ (3H, s, CH}_3\text{CO),} \ 2.44 \ (6H, m, CH}_2\text{C(CH}_2\text{OH)}_2), \ 4.07 \ (2H, d, J=4.0, NCH}_2\text{CHCH}_2), \ 5.12 \ (1H, d, J=17.6, NCH}_2\text{CHCH}_3\text{H}_b), \ 5.25 \ (1H, d, J=12.0, NCH}_2\text{CHCH}_3\text{H}_b), \ 5.79 \ (1H, ddt, J=4.0, 12.0, 17.6, NCH}_2\text{CHCH}_2). \\
& \quad ^13C \text{NMR (100 MHz) 21.3 (CH}_3\text{CO), 48.8 (NCH}_2\text{CHCH}_2), 53.0 (CCH}_2\text{N), 63.7 (CH}_2\text{OH), 75.4 (C(CH}_2\text{)}_3\text{OH), 116.8 (NCH}_2\text{CHCH}_2), 131.9 (NCH}_2\text{CHCH}_2), 174.3 (CO); MS ES (+ve) found } m/z 204.1 ([M+H]^+, 100\%). \text{HRMS FAB [M+H]^+ 204.1246 C}_9\text{H}_{17}\text{O}_4\text{N+H}^+ \text{requires 204.1236. IR (cm}^{-1}) 3216, 1611.}
\end{align*}
\]

11.7.14 \(N\)-benzyl-\(N\)-(2,3-dihydroxy-2-(hydroxymethyl)propyl)acetamide, 97f.
Yield 74%, yellow oil. \( ^1H \text{NMR} \ (400 \text{ MHz, CDCl}_3) \delta_H \ 2.11 \ (3H, s, CH_3CO), \ 3.43 \ (6H, m, CH_2C(CH_2OH)_2), \ 4.68 \ (2H, s, CH_2Ar), \ 7.07 \ (2H, d, J = 6.8, Ar), \ 7.30 \ (1H, m, Ar). \ 1^3C \text{NMR} \ (100 \text{ MHz}) \ 21.6 \ (CH_3CO), \ 49.0 \ (CH_2Ar), \ 54.0 \ (CCH_2N), \ 64.0 \ (CH_2OH), \ 75.8 \ (C(CH_2)_2OH), \ 126.1, \ 127.8, \ 129.1 \ (CH, Ar), \ 136.1 \ (NCH_2C), \ 174.1 \ (CO); \ MS \ ES \ (+ve) \ found \ m/z \ 254.2 ([M+H]^+, 100%). \ HRMS \ FAB \ [M+H]^+ 254.1380 \ C_{15}H_{19}O_4N+H^+ \ requires \ 254.1348. \ IR \ (cm^{-1}) \ 3295, \ 1605.

11.7.15 N-(2,3-dihydroxy-2-(hydroxymethyl)propyl)-N-phenethylacetamide, 97g.

Yield 54%, oil. \( ^1H \text{NMR} \ (400 \text{ MHz, CDCl}_3) \delta_H \ 1.97 \ (3H, s, CH_3CO), \ 2.88 \ (2H, t, J = 7.6, NCH_2CH_2Ar), \ 3.38-3.49 \ (6H, m, CH_2C(CH_2OH)_2), \ 3.66 \ (2H, t, J = 7.6, NCH_2CH_2Ar), \ 7.15 – 7.33 \ (5H, m, Ar). \ 1^3C \text{NMR} \ (100 \text{ MHz}) \ 21.08 \ (CH_3CO), \ 34.6 \ (NCH_2CH_2Ar), \ 48.2 \ (NCH_2CH_2Ar), \ 52.2 \ (CCH_2N), \ 64.0 \ (CH_2OH), \ 75.6 \ (C(CH_2)_2OH), \ 128.7, \ 128.8, \ 128.9 \ (CH, Ar), \ 137.7 \ (C, Ar), \ 174.0 \ (CO); \ MS \ ES \ (+ve) \ found \ m/z \ 268.2 ([M+H]^+, 100%). \ HRMS \ FAB \ [M+H]^+ 268.1541 \ C_{14}H_{21}O_4N+H^+ \ requires \ 268.1504. \ IR \ (cm^{-1}) \ 3272, \ 1602.

11.8 General synthesis of chiral tertiary alcohols 96i-j.

To a solution of 87 (100 mg, 0.96 mmol), Amano L, AK (200 mg, 2 wt. eq.) and molecular sieves (4 Å) in dichloromethane (10 ml, 0.1 M) was added acetic anhydride (1.8 equivalents) and the reaction was left for one hour with stirring at 37 °C. The mixture was filtered through Celite® and the filtrate was reduced in vacuo. Ethanol (10 ml, 0.1 M) and the corresponding amine (3 equivalents, see Table 15 in Results and Discussion section) was added and the mixture heated at reflux for 4 hours. The mixture was reduced in vacuo and purified by column chromatography (SiO2). The enantiomeric purity was determined by HPLC; Agilent 1100 Series. Chiralpack AD, 1 ml/min, 80/20 isohexene in EtOH at 25°C.
11.9 Synthesis of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl acetate, 98.

To a solution of (±)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50 (150 mg, 1.03 mmol) in dichloromethane (10 ml, 0.1 M) was added 4-dimethylaminopyridine (13 mg, 0.10 mmol), triethylamine (430.7 μl, 3.09 mmol) and tert-butyldimethylsilyl chloride (170 mg, 1.13 mmol). The solution was stirred and heated at reflux for 5 hours. The mixture was reduced in vacuo and the crude material was purified by column chromatography (SiO2; 5% ethyl acetate in petroleum ether) to yield the title compound as a colorless oil (220 mg, 82% yield). 1H NMR (400 MHz, CDCl3) δH 0.00 (3H, s, SiCH3), 0.08 (3H, s, SiCH3), 0.89 (9H, s, Si′Bu), 2.09 (3H, s, CH3CO), 2.70 (1H, d, J = 5.2, CHdHbOC), 2.81 (1H, d, J = 5.2, CH2HbOC), 3.71 (1H, d, J = 11.6, CH2HbOAc), 3.80 (1H, d, J = 11.6, CH3HbOAc), 4.11 (1H, d, J = 12.0, CHdHbOSi), 4.32 (1H, d, J = 12.0, CHdHbOSi); 13C NMR (100 MHz) -5.5 (SiCH3), 18.3 (SiC(CH3)), 20.8 ((CH3)CO), 25.8 (SiC(CH3)), 49.3 (CH2OC), 57.6 (COCH2), 63.2 (CH2OAc), 63.8 (CH2OSi), 170.6 (C(CH2)3O); MS ES (+ve) found m/z 261.2 ([M+H]+, 30%), 201.1 (100%); HRMS FAB [M+H]+ 261.1530, C12H24O4Si+H+ requires 261.1522; νmax/cm⁻¹ 3417, 1746.

11.10 Synthesis of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methanol, 99.

To a solution of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl acetate 98 (220 mg, 0.84 mmol) in methanol (8 ml, 0.1 M) was added potassium carbonate (350 mg, 2.53 mmol). The solution was stirred at room temperature for 1 hour. The mixture was evaporated in vacuo and the crude material was filtered through Celite® to yield the title compound as a colorless oil (199 mg, 91% yield). 1H NMR (400 MHz, CDCl3) δH 0.00 (3H, s, SiCH3), 0.09 (3H, s, SiCH3), 0.89 (9H,
s, Si‘Bu), 2.76 (1H, d, J = 4.8, CH$_2$H$_6$OC), 2.87 (1H, d, J = 4.8, CH$_2$H$_6$OC), 3.74 (1H, dd, J = 7.2, 12.2, CH$_{3}$H$_8$OSi), 3.80 (2H, s, CH$_2$OH), 3.89 (1H, dd, J = 3.6, 12.2, CH$_{3}$H$_8$OSi); $^{13}$C NMR (100 MHz) -5.5 (SiCH$_3$), 18.3 (SiC(CH$_3$)), 25.8 (SiC(CH$_3$)), 49.0 (CH$_2$OC), 56.5 (CO), 62.2 (CH$_2$OCO), 64.4 (CH$_2$OSi); MS ES (+ve) found m/z 260.0 ([M+H+MeCN]$^+$, 100%); HRMS FAB [M+H+MeCN]$^+$ 260.1687, C$_{10}$H$_{22}$O$_3$Si+H$^+$+MeCN requires 260.1682; $\nu_{max}$/cm$^{-1}$ 3427.

11.11 Synthesis of tertiary alcohols using primary aliphatic amines.

To a solution of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methanol 99 (100 mg, 0.46 mmol) in ethanol (4.6 ml, 0.1 M) was added the corresponding amine (3 equivalents, see Table 16 in results and discussion). The solution was heated with stirring at reflux for 1 hour then allowed to cool slowly to room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO$_2$; methanol/ ethyl acetate).

11.11.1 (±)-3-(Tert-butyldimethylsilyloxy)-2-((prop-2-ynylamino)methyl)propane-1,2-diol, 100a.

Yield 70%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 0.07 (6H, s, SiCH$_3$), 0.89 (9H, s, Si‘Bu), 2.23 (1H, t, J = 2.4, CHC), 2.75 (1H, d, J = 12.0, HOCCCH$_3$H$_8$NH), 2.90 (1H, d, J = 12.0, HOCCCH$_3$H$_8$NH), 3.40 (1H, dd, J = 2.4, 16.8, CHCCH$_3$H$_8$NH), 3.48 (1H, dd, J = 2.4, 16.8, CHCCH$_3$H$_8$NH), 3.53 (1H, d, J = 9.6, CH$_2$H$_8$OH), 3.56 (1H, d, J = 9.6, CH$_2$H$_8$OH), 3.59 (1H, d, J = 11.6, CH$_2$H$_8$OSi), 3.63 (1H, d, J = 11.6, CH$_2$H$_8$OSi); $^{13}$C NMR (100 MHz) -5.5 (SiCH$_3$), 18.2 (SiC(CH$_3$)), 25.8 (SiC(CH$_3$)), 38.7 (CHCCH$_2$NH), 52.6 (HOCCCH$_3$NH), 66.8 (CH$_2$OH), 66.9 (CH$_2$OSi), 71.9 (CHC), 72.9 (C(CH$_3$)$_2$OH), 81.4 (CHC); MS ES (+ve) found m/z 274.1 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 274.1845, C$_{13}$H$_{25}$NO$_3$Si+H$^+$ requires 274.1838; $\nu_{max}$/cm$^{-1}$ 3416, 1638.
11.11.2 (±)-3-(Allylamino)-2-((tert-butyldimethylsilyloxy)methyl)propane-1,2-diol, 100b.

Yield 53%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.06 (6H, s, SiCH$_3$), 0.89 (9H, s, Si$i^t$Bu), 2.72 (1H, d, $J = 12.0$, HOCH$_2$H$_9$NH), 2.79 (1H, dd, $J = 0.8$, 12.0, HOCH$_2$H$_9$NH), 3.26 (2H, m, CH$_2$CHCH$_2$NH), 3.51 (1H, d, $J = 10.0$, CH$_2$H$_9$OH), 3.55 (1H, d, $J = 10.0$, CH$_2$H$_9$OH), 3.60 (1H, dd, $J = 0.8$, 11.2, CH$_2$H$_9$OSi), 3.64 (1H, dd, $J = 11.2$, CH$_2$H$_9$OSi), 5.11 (1H, ddd, $J = 1.4$, 3.2, 10.2, CH$_2$H$_9$CH), 5.22 (1H, ddd, $J = 1.4$, 3.2, 17.2, CH$_2$H$_9$CH), 5.86 (1H, ddt, $J = 6.2$, 10.2, 17.2, CH$_2$CH); $^{13}$C NMR (100 MHz) -5.6 (SiCH$_3$), 18.2 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$), 52.7 (HOCH$_2$NH), 53.1 (CH$_2$CHCH$_2$NH), 66.9 (CH$_2$OH), 67.4 (CH$_2$OSi), 72.6 (C(CH$_2$)$_3$OH), 116.4 (CH$_2$CH), 136.2 (CH$_2$CH); MS ES (+ve) found m/z 276.2 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 276.1993, C$_{13}$H$_{29}$NO$_3$Si+H$^+$ requires 276.1995; $\nu_{\text{max}}$/cm$^{-1}$ 3399, 1639.

11.11.3 (±)-3-(Benzylamino)-2-((tert-butyldimethylsilyloxy)methyl)propane-1,2-diol 100c.

Yield 83%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.04 (6H, s, SiCH$_3$), 0.87 (9H, s, Si$i^t$Bu), 2.74 (1H, d, $J = 11.6$, HOCH$_2$H$_9$NH), 2.81 (1H, d, $J = 11.6$, HOCH$_2$H$_9$NH), 3.49 (1H, d, $J = 10.4$, CH$_2$H$_9$OH), 3.52 (1H, d, $J = 10.4$, CH$_2$H$_9$OH), 3.59 (1H, d, $J = 11.2$, CH$_2$H$_9$OSi), 3.63 (1H, d, $J = 11.2$, CH$_2$H$_9$OSi), 3.78 (1H, d, $J = 12.8$, CH$_2$Ar), 3.82 (1H, d, $J = 12.8$, CH$_2$Ar), 7.30 (5H, m, Ar); $^{13}$C NMR (100 MHz) -5.6 (SiCH$_3$), 18.2 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$), 53.2 (HOCH$_2$NH), 54.3 (CH$_2$OH), 66.8 (CH$_2$OSi), 67.4 (CH$_2$Ar), 72.8 (C(CH$_2$)$_3$OH), 127.2, 128.1, 128.5 (CH, Ar), 139.4 (C, Ar); MS ES (+ve) found m/z 326.1 ([M+H]$^+$,
100%); HRMS FAB [M+H]$^+$ 326.2149, $^{13}$C$_{17}$H$_{31}$NO$_3$Si+H$^+$ requires 326.2151; $\nu_{\text{max}}$/cm$^{-1}$ 3395, 1638.

### 11.12 Synthesis of (S)-(−)-3-(benzylamino)-2-((tert-butyldimethylsilyloxy)methyl)propane-1,2-diol 100c.

![Chemical Structure]

To a solution of 90 (100 mg, 0.96 mmol), immobilised *R. miehei* (100 mg, 1 wt. eq.) and molecular sieves (4 Å, 100 mg) in dichloromethane (10 ml, 0.1 M) was added vinyl acetate (1 eq.) and the reaction was left for 8 hours with shaking at 37 ºC. The mixture was filtered through Celite® and the filtrate was evaporated *in vacuo*. Dichloromethane (10 ml, 0.1 M), 4-dimethylaminopyridine (11 mg, 0.09 mmol), triethylamine (401.4 µl, 2.88 mmol) and tert-butyldimethylsilyl chloride (145 mg, 0.96 mmol) were added to the colourless oil. The solution was heated at reflux for 5 hours. The mixture was evaporated *in vacuo* and the crude material was purified by column chromatography (SiO$_2$; 5% ethyl acetate in petroleum ether). To the pure oil was added methanol (5 ml, 0.1 M) and potassium carbonate (199 mg, 1.44 mmol). The solution was stirred at room temperature for 1 hour. The mixture was reduced *in vacuo* and the crude material was filtered through Celite®. To a solution of the resulting oil (97 mg, 0.44 mmol) in ethanol (4.4 ml, 0.1 M) was added benzylamine (144 µl, 1.32 mmol). The solution was heated at reflux for 1 hour and allowed to cool slowly to room temperature. The mixture was evaporated *in vacuo* and the crude material was purified by column chromatography (SiO$_2$; methanol/ethyl acetate).

The enantiomeric excess was determined by HPLC; Agilent 1100 Series. Chiralpack AS-H, 1 ml/min, 80/20 iso-hexene in EtOH at 25 ºC. [α]$_D$ (H$_2$O, c=0.16) +8.75. $^1$NMR obtained was identical that of racemic 100c.
11.13 Synthesis of (2-formyloxiran-2-yl)methyl acetate, 102.

To a solution of (2-(hydroxymethyl)oxiran-2-yl)methyl acetate 50 (100 mg, 0.685 mmol) in anhydrous dichloromethane (7 mL, 0.1 M) at room temperature, were added tetrapropylammonium perruthenate (TPAP) (12.04 mg, 0.034 mmol) and N-methylmorpholine-N-oxide (NMO) (120.36 mg, 1.027 mmol) under an atmosphere of nitrogen. After 3 hours the mixture was evaporated in vacuo and the crude material was filtered through silica (2.5 g, column diameter = 1 cm, 100 ml EtOAc). The resulting fraction was evaporated in vacuo and the crude material was purified by column chromatography (SiO$_2$; 50 % petroleum ether in ethyl acetate) to yield the title compound as a colorless oil (65.7 mg, 66% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 3.07 (3H, s, CH$_3$CO), 3.15 (1H, d, J = 4.8, CH$_a$H$_b$OC)), 3.19 (1H, d, J = 4.8, CH$_a$H$_b$OC)), 4.27 (1H, d, J = 12.4, CH$_a$H$_b$OAc), 4.65 (1H, d, J = 12.4, CH$_a$H$_b$OAc), 8.97 (1H, s, CHO).

11.14 Synthesis of (±)-N-(2,3-dihydroxy-2-methylpropyl)-N-(4-fluorophenyl)-4-methylbenzenesulfonamide, 106.

To a solution of 3-(4-fluorophenylamino)-2-hydroxy-2-(hydroxymethyl)propyl acetate 96i (100 mg, 0.39 mmol) in pyridine (3.7 mL) at 0 ºC was added p-toluene-sulfonyl chloride (223.06 mg, 1.17 mmol) and the reaction was stirred overnight at room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO$_2$; 50 % petroleum ether in ethyl acetate) to yield the title compound as a colorless oil (65.7 mg, 66% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 3.07 (3H, s, CH$_3$CO), 3.15 (1H, d, J = 4.8, CH$_a$H$_b$OC)), 3.19 (1H, d, J = 4.8, CH$_a$H$_b$OC)), 4.27 (1H, d, J = 12.4, CH$_a$H$_b$OAc), 4.65 (1H, d, J = 12.4, CH$_a$H$_b$OAc), 8.97 (1H, s, CHO).
evaporated in vacuo to obtain a colorless oil. The oil was dissolved in anhydrous diethyl ether (4 mL), under an atmosphere of nitrogen at 0 °C, and LiAlH₄ (74.0 mg, 1.96 mmol) was added to the cold solution and the reaction was allowed to warm slowly to room temperature. After 1 hour the crude mixture was cooled down to 0 °C and 74 µL of water were added slowly. NaOH (74 µL of 15 % aqueous solution) and water (222 µL) were added and the mixture was allowed to warm at room temperature. After an hour the white solid was diluted with diethyl ether and filtered. The solution was dried (MgSO₄) and the resulting filtrate was evaporated in vacuo to yield the title compound as a colorless oil (50 mg, 36% yield).

$^{1}$H NMR (400 MHz, CDCl₃) δH 0.96 (3H, s, C(CH₂)₂C₃H₇OH), 2.43 (3H, s, C₃H₇Ts), 3.43 (1H, d, J = 12.8, NCH₃H₅b), 3.45 (1H, d, J = 12.8, NCH₃H₅b), 3.67 (1H, d, J = 13.2, C₄H₅aH₅bOH), 3.78 (1H, d, J = 13.2, CH₅aH₅bOH), 7.02 (4H, m, NCC₃H₅), 7.26 (2H, m, FCC₃H₅), 7.40 (2H, m, ArTs); $^{13}$C NMR (100 MHz) 21.6 (C(CH₂)₂C₃H₇OH), 22.4 (C₃H₇Ts), 57.2 (NCH₃), 66.7 (C₃H₇OH), 73.3 (C(CH₂)₂C₃H₇OH), 121.7 (J(C-F) = 80, NC₃H₅), 130.0 (J(C-F) = 40, FC₃H), 133.8 (NC₃), 137.0 (SC), 144.3 (CH₅C₃H₅), 160.6 (CF); MS ES (+ve) found m/z 376.1 ([M+H+Na]+, 100%); HRMS FAB [M+H+Na]+ 376.0994, C₁₇H₂₀FNO₄S+H⁺+Na requires 376.0995; νmax/cm⁻¹ 3387, 1232.


To a solution of (±)-(2-(((tert-butylmethyldisilyloxy)methyl)oxiran-2-yl)methyl acetate 98 (273.7 mg, 1.05 mmol) in ethanol (10.0 ml, 0.1 M) was added morpholine (272.4 µL, 3.15 mmol). The solution was heated at reflux for 1 hour and allowed to cool slowly to room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO₂; 100% ethyl acetate) to obtain the title compound as colorless oil (345.7 mg, 95% yield). $^{1}$H NMR (400 MHz, CDCl₃) δH 0.05 (6H, s, SiCH₃), 0.88 (9H, s, Si'Bu), 2.08 (3H, s, CH₃OCO),
2.36 (1H, d, J = 13.8, CCH₂H₃N), 2.56 (1H, d, J = 13.8, CCH₂H₃N), 2.60 (4H, m, (2xNCH₂CH₂O)), 3.45 (1H, d, J = 10.0, CH₃H₃OAc), 3.58 (1H, d, J = 10.0, CH₃H₃OAc), 3.69 (4H, t, J = 4.4 (2xNCH₂CH₂O)), 3.99 (1H, d, J = 11.2, CH₃H₃OSi), 4.07 (1H, d, J = 11.2, CH₃H₃OSi); ¹³C NMR (100 MHz) -5.6 (SiC(°)), 14.22 (SiCH₃), 18.2 (SiC(CH₃)₃), 20.9 (CH₃OAc), 25.8 (SiC(CH₃)₃), 55.6 (NCH₂CH₂O), 59.9 (CCH₂N), 64.6 (NCH₂CH₂O), 66.1 (CH₂OSi), 67.2 (CH₂OAc), 72.7 (C(CH₂)₃OH), 170.9 (CO); MS ES (+ve) found m/z 348.1 ([M+H]⁺, 100%); HRMS FAB [M+H]⁺ 348.2201, C₁₆H₃₅NO₅Si+H⁺ requires 348.2206; νmax/cm⁻¹ 3351, 1730.

11.16 Synthesis of (±)-3-(tert-butyldimethylsilyloxy)-2-(morpholinomethyl)propane-1,2-diol, 100d.

To a solution of 3-(tert-butyldimethylsilyloxy)-2-hydroxy-2-(morpholinomethyl)propyl acetate 107 (100 mg, 0.29 mmol) in methanol (3 ml, 0.1 M) was added potassium carbonate (118.8 mg, 0.86 mmol). The solution was stirred at room temperature for 1 hour. After that time water (10 ml) was added to the mixture and the product extracted with DCM (10 ml x 3). The organic layer was dried (MgSO₄), filtered and the solution was evaporated in vacuo to yield the title compound as colourless oil (86.4 mg, 98% yield). ¹H NMR (400 MHz, CDCl₃) δH 0.06 (6H, s, SiCH₃), 0.89 (9H, s, Si°Bu), 2.55 (4H, m, (2xNCH₂CH₂O)), 2.75 (2H, m, CCH₂N), 2.46 (1H, d, J = 9.6, CH₃H₃OSi), 3.49 (1H, d, J = 9.6, CH₃H₃OSi), 3.59 (1H, d, J = 11.4, CH₃H₃OH), 3.65 (1H, d, J = 11.4, CH₃H₃OH), 3.69 (4H, t, J = 4.4, (2xNCH₂CH₂O)); ¹³C NMR (100 MHz) -5.5 (SiCH₃), 18.2 (SiC(CH₃)₃), 25.8 (SiC(CH₃)₃), 55.6 (NCH₂CH₂O), 62.6 (CCH₂N), 66.5 (CH₂OSi), 67.2 (CH₂OAc), 67.3 (NCH₂CH₂O), 72.9 (C(CH₂)₃OH); MS ES (+ve) found m/z 307.4 ([M+2H]⁺, 100%); HRMS FAB [M+H]⁺ 306.2097, C₁₄H₃₅NO₃Si+H⁺ requires 306.2100; νmax/cm⁻¹ 3298.
11.17 Synthesis of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl-4-methylbenzenesulfonate, 110.

[Chemical structure image]

To a solution of (±)-(2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methanol 99 (1.09 g, 5.01 mmol) in dichloromethane (50 ml, 0.1 M) was added 4-dimethylaminopyridine (61.2 mg, 0.50 mmol), triethylamine (2.09 ml, 15.03 mmol) and 4-toluenesulfonyl chloride (1.05 g, 5.51 mmol). The solution was heated at reflux for 5 hours. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO2; 10% ethyl acetate in petroleum ether) to yield the title compound as a colorless oil (1.59 g, 90% yield). 1H NMR (400 MHz, CDCl3) δH 0.01 (3H, s, SiCH3), 0.02 (3H, s, SiCH3), 0.84 (9H, s, SiBu), 2.45 (3H, s, CH3Ts), 2.72 (1H, d, J = 4.8, CHaHbOC), 2.75 (1H, d, J = 4.8, CHaHbOC), 3.65 (1H, d, J = 11.6, CHaHbOTs), 3.73 (1H, d, J = 11.6, CHaHbOTs), 4.10 (1H, d, J = 10.4, CHaHbOSi), 4.18 (1H, d, J = 10.4, CHaHbOSi), 7.34 (2H, d, J = 8.4, CH, Ar), 7.79 (2H, d, J = 8.4, CH, Ar); 13C NMR (100 MHz) -5.4 (SiCH3), 18.3 (SiC(CH3)3), 25.6 (CH3CCH), 25.8 (SiC(CH3)3), 63.3 (CH2OC), 63.4 (CH2OSi), 68.0 (CH2OTs), 70.0 (C(CH3)2O), 128.3, 128.4 (CH, Ar), 139.4 (CH2CCH), 146.4 (CS); MS ES (+ve) found m/z 395.1 ([M+H+Na]+, 100%); HRMS FAB [M+H+Na]+ 395.1315, C17H28O5SSi+Na+H requires 395.1324, IR 1196.

11.18 Synthesis of 3-(allylamino)-2-((tert-butyldimethylsilyloxy)methyl)-2-hydroxypropyl 4-methylbenzenesulfonate, 108.

[Chemical structure image]
To a solution of (2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate 110 (100 mg, 0.27 mmol) in ethanol (2.7 ml, 0.1 M) was added allylamine (3 equivalents, 46.2 µl). The solution was heated at reflux for 1 hours and allowed to cool slowly to room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO₂; 50% ethyl acetate in petroleum ether). Yield 21%, yellow oil. ¹H NMR (400 MHz, CDCl₃) δH 0.12 (6H, s, SiC₃H₃), 0.93 (9H, s, Si'tBu), 2.37 (3H, s, CH₃CCH), 3.43 (1H, s, CH₂H₂), 3.44 (1H, s, CH₂H₂), 3.65 (2H, s, NC₃H₂CH), 3.76 (4H, m, CH₂O), 5.35 (2H, m, CH₂CH), 5.80 (1H, m, CH₂C₃H), 7.21 (2H, d, J = 7.8, CH, Ar), 7.81 (2H, d, J = 7.8, CH, Ar).

11.19 Synthesis of azetidines.

To a solution of (2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl 4-methylbenzenesulfonate 110 (100 mg, 0.27 mmol) in ethanol (2.7 ml, 0.1 M) was added the corresponding amine (3 equivalents, see Tables 18 and 19 in Results and Discussion section). The solution was heated at reflux for 8 hours and allowed to cool slowly to room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO₂; ethyl acetate in petroleum ether).

11.19.1 1-Allyl-3-((tert-butyldimethylsilyloxy)methyl)azetidin-3-ol, 114a.

Yield 50%, yellow solid. M. p. 65-67 °C. ¹H NMR (400 MHz, CDCl₃) δH 0.09 (6H, s, SiC₃H₃), 0.90 (9H, s, Si'Bu), 2.96 (1H, d, J = 1.6, CH₃H₆CH), 2.98 (1H, d, J = 1.6, CH₃H₆CH), 3.12 (1H, t, J = 1.2, (CCH₃H₆N), 3.13 (1H, t, J = 1.2, CCH₃H₆N), 3.36 (1H, d, J = 1.8, CCH₃H₆N), 3.38 (1H, d, J = 1.8, CCH₃H₆N), 3.79 (2H, s, CH₂OSi), 5.10 (1H, ddt, J = 1.2, 1.6, 10.4, CHCH₃H₆b), 5.17 (1H, ddt, J = 1.6, 3.2, 17.2, CHCH₃H₆b), 5.76 (1H, ddt, J = 6.2, 10.4, 17.2, CHCH₂); ¹³C NMR (100 MHz) -
5.4 (SiCH₃), 18.3 (SiC(CH₃)₃), 25.8 (SiC(CH₃)₃), 61.9 (CCH₂N), 63.3 (NCH₂CH), 67.9 (CH₂OSi), 69.9 (CCH₂N), 117.4 (CH₂CH), 134.3 (CH₂CH); MS ES (+ve) found m/z 258.0 ([M+H]+, 100%); HRMS FAB [M+H]+ 258.1897, C₁₃H₂₅NO₂Si+H⁺ requires 258.1811; νₚₓ/ cm⁻¹ 3395.

11.19.2 3-((Tert-butyldimethylsilyloxy)methyl)-1-(prop-2-ynyl)azetidin-3-ol, 114b.

Yield 88%, yellow solid. M. p. 70-73 °C. ¹H NMR (400 MHz, CDCl₃) δH 0.09 (6H, s, SiCH₃), 0.90 (9H, s, Si'Bu), 2.27 (1H, t, J = 2.0, CHC), 3.19 (1H, d, J = 2.0, CH₃H₂OSi), 3.21 (1H, d, J = 2.0, CH₃H₂OSi), 3.33 (4H, m, (2xCHC₂N)), 3.81 (2H, s, CH₂CCH); ¹³C NMR (100 MHz) -5.4 (SiCH₃), 18.3 (SiC(CH₃)₃), 25.8 (SiC(CH₃)₃), 45.3 (CH₂CCH), 61.4 (CCH₂N), 68.0 (CH₂OSi), 69.5 (C(CH₂)₃OH), 73.1 (CHC), 78.6 (CHC); MS ES (+ve) found m/z 256.1 ([M+H]+, 100%); HRMS FAB [M+H]+ 256.1733, C₁₃H₂₅NO₂Si+H⁺ requires 256.1733; νₚₓ/ cm⁻¹ 3391.

11.19.3 1-Benzyl-3-((tert-butyldimethylsilyloxy)methyl)azetidin-3-ol, 114c.

Yield 84%, oil. ¹H NMR (400 MHz, CDCl₃) δH 0.09 (6H, s, SiCH₃), 0.90 (9H, s, Si'Bu), 3.01 (2H, d, J = 2.0, (2xCHC₂H₃N), 3.35 (2H, d, J = 2.0, (2xCHC₂H₃N), 3.67 (2H, s, CH₂OSi), 3.80 (2H, s, CH₂Ar), 7.28 (5H, m, Ar); ¹³C NMR (100 MHz) -5.4 (SiCH₃), 18.3 (SiC(CH₃)₃), 25.8 (SiC(CH₃)₃), 63.5(CCH₂N), 63.5 (CH₂Ar) 68.1 (CH₂OSi), 70.0 (C(CH₂)₃OH), 127.0, 128.3, 128.4 (CH, Ar), 138.1 (C, Ar); MS ES
(+ve) found \( m/z \) 308.1 ([M+H]\(^+\), 100%); HRMS FAB [M+H]\(^+\) 308.2053, \( \text{C}_{17}\text{H}_{29}\text{NO}_2\text{Si}+\text{H}^+ \) requires 308.2046; \( \nu_{\text{max}}/\text{cm}^{-1} \) 3388.

11.19.4 3-((Tert-butyldimethylsilyloxy)methyl)-1-(4-fluorophenyl)azetidin-3-ol, 114d.

Yield 43%, yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta_H \) 0.12 (6H, s, SiCH\(_3\)), 0.92 (9H, s, Si^Bu), 3.65 (2H, d, \( J = 8.0 \), CCH\(_3\)H\(_2\)N), 3.81 (2H, d, \( J = 8.0 \), CCH\(_3\)H\(_2\)N), 3.86 (2H, s, CH\(_2\)OSi), 6.41 (2H, m, Ar), 6.92 (2H, m, Ar); \(^{13}\)C NMR (100 MHz) -5.3 (SiCH\(_3\)), 18.3 (SiC(CH\(_3\))\(_3\)), 25.9 (SiC(CH\(_3\))\(_3\)), 61.9 (CCH\(_2\)N), 68.2 (CH\(_2\)OSi), 70.1 (C(CH\(_2\))\(_3\)OH), 112.8 (J(C-F) = 40, CH, Ar), 115.4 (J(C-F) = 80, CH, Ar), 148.2 (CN), 157.3 (CF); MS ES (+ve) found \( m/z \) 312.1 ([M+H]\(^+\), 100%); HRMS FAB [M+H]\(^+\) 312.1565, \( \text{C}_{16}\text{H}_{26}\text{FNO}_2\text{Si}+\text{H}^+ \) requires 312.1750; \( \nu_{\text{max}}/\text{cm}^{-1} \) 3388.

11.19.5 3-((Tert-butyldimethylsilyloxy)methyl)-1-cyclopentylazetidin-3-ol, 114f.

Yield 80%, white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta_H \) 0.09 (6H, s, SiCH\(_3\)), 0.90 (9H, s, Si^Bu), 1.48 (4H, m, 2xCHCH\(_2\)CH\(_2\)), 1.71 (4H, m, 2xCHCH\(_2\)CH\(_2\)), 2.88 (1H, m, CH), 3.16 (2H, d, \( J = 8.0 \), 2xCH\(_2\)H\(_2\)N), 3.45 (2H, d, \( J = 8.0 \), 2xCH\(_2\)H\(_2\)N), 3.73 (2H, s, CH\(_2\)OSi); \(^{13}\)C NMR (100 MHz) -5.4 (SiCH\(_3\)), 18.2 (SiC(CH\(_3\))\(_3\)), 24.4 (CHCH\(_2\)CH\(_2\)), 25.8 (SiC(CH\(_3\))\(_3\)), 30.0 (CHCH\(_2\)CH\(_2\)), 62.3 (CCH\(_2\)N), 67.4 (CH\(_2\)OSi), 69.1 (CHCH\(_2\)CH\(_2\)), 69.5 (C(CH\(_2\))\(_3\)OH); MS ES (+ve) found \( m/z \) 287.4 ([M+2H]\(^+\), 100%); HRMS FAB [M+H]\(^+\) 286.2205, \( \text{C}_{15}\text{H}_{31}\text{NO}_2\text{Si}+\text{H}^+ \) requires 286.2202; \( \nu_{\text{max}}/\text{cm}^{-1} \) 3355.
11.19.6 3-((Tert-butyldimethylsilyloxy)methyl)-1-isopropylazetidin-3-ol, 114g.

Yield 92%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.07 (6H, s, SiCH$_3$), 0.88 (9H, s, Si'Bu), 0.94 (6H, d, $J$ = 6.4, CH(CH$_3$)$_2$), 2.38 (1H, hept, $J$ = 6.4, CHCH$_3$), 2.99 (2H, d, $J$ = 9.6, 2xCH$_2$H$_b$N), 3.36 (2H, d, $J$ = 9.6, 2xCH$_2$H$_b$N)), 3.72 (2H, s, CH$_2$OSi); $^{13}$C NMR (100 MHz) -5.4 (Si, N), 62.5 (C, b), 3.32 ($\nu$Si=O); 3.87 (1H, d, $J$ = 7.6, CHCH$_3$), 3.42 (1H, d, $J$ = 7.6, CCH$_a$H$_b$N), 3.79 (1H, d, $J$ = 6.4, CCH$_b$N), 3.84 (2H, s, CH$_2$OSi), 68.6 (C, Ar)); MS ES (+ve) found m/z 260.1 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 260.2043, C$_{13}$H$_{29}$NO$_2$Si+H$^+$ requires 260.2046; $\nu_{max}$/cm$^{-1}$ 3371.

11.19.7 (S)-3-((Tert-butyldimethylsilyloxy)methyl)-1-(1-phenylethyl)azetidin-3-ol, 114h.

Yield 88%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.08 (3H, s, SiCH$_3$), 0.10 (3H, s, SiCH$_3$), 0.90 (9H, s, Si'Bu), 1.21 (3H, d, $J$ = 6.8, CHCH$_3$), 2.84 (1H, d, $J$ = 7.8, CCH$_a$H$_b$N)), 2.99 (1H, d, $J$ = 7.8, CCH$_a$H$_b$N), 3.09 (1H, d, $J$ = 7.6, CCH$_a$H$_b$N), 3.34 (1H, q, $J$ = 6.8, CHCH$_3$), 3.42 (1H, d, $J$ = 7.6, CCH$_a$H$_b$N), 3.79 (1H, d, $J$ = 10.0, SiOCH$_2$H$_b$), 3.81 (1H, d, $J$ = 10.0, SiOCH$_2$H$_b$), 7.27 (5H, m, CH, Ar); $^{13}$C NMR (100 MHz) -5.3 (SiCH$_3$), 18.3 (SiC(CH$_3$)$_3$), 21.5 (CHCH$_3$), 25.9 (SiC(CH$_3$)$_3$), 62.4 (CCH$_2$N), 62.5 (CCH$_2$N), 68.3 (CH$_2$OSi), 68.7 (CHCH$_3$), 69.0 (C(CH$_2$)$_3$OH), 127.0, 127.1, 128.3 (CH, Ar), 143.4 (C, Ar); MS ES (+ve) found m/z 322.1 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 322.2204, C$_{18}$H$_{31}$NO$_2$Si+H$^+$ requires 322.2202; $\nu_{max}$/cm$^{-1}$ 3397. [\alpha]$_D$ (EtOH, c=0.034) –29.4.
11.19.8 (S)-3-((Tert-butyldimethylsilyloxy)methyl)-1-(1-phenylpropyl)azetidin-3-ol, 114i

Yield 78%, white solid. M. p. 59-61 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 0.06 (3H, s, SiCH$_3$), 0.08 (3H, s, SiCH$_3$), 0.66 (3H, t, $J=7.6$, CH$_2$CH$_3$), 0.88 (9H, s, Si$^t$Bu), 1.45 (1H, dqd, $J=13.4$, 9.6, 7.6 (CH$_3$H$_2$CH$_3$), 1.69 (1H, dqd, $J=13.4$, 7.6, 3.6 CH$_3$H$_2$CH$_3$), 2.82 (1H, d, $J=8.0$, CCH$_3$H$_2$N), 2.95 (1H, d, $J=8.0$, CCH$_3$H$_2$N), 3.03 (1H, dd, $J=7.8$, 2.0, 0.8, CCH$_3$H$_2$N), 3.07 (1H, dd, $J=3.6$, 9.6, CHCH$_2$), 3.40 (1H, ddd, $J=7.8$, 2.0, 0.8, CCH$_3$H$_2$N), 3.73 (1H, d, $J=10.4$, CCH$_3$H$_2$OSi), 3.77 (1H, d, $J=10.4$, CCH$_3$H$_2$OSi), 7.24 (5H, m, Ar); $^{13}$C NMR (100 MHz) -5.4 (SiCH$_3$), 10.0 (CH$_3$CH$_2$), 18.3 (SiC(CH$_3$_3), 25.8 (SiC(CH$_3$_3), 27.3 (CH$_3$CH$_2$), 62.3 (CCH$_3$), 62.5 (CCH$_2$N), 68.2 (CH$_2$OSi), 69.3 (C(CH$_3$_3)OH), 75.5 (CHCH$_2$), 127.0, 128.0, 128.1 (CH, Ar), 141.3 (C, Ar); MS ES (+ve) found $m/z$ 336.1 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 336.2356, C$_{19}$H$_{33}$NO$_2$Si+H$^+$ requires 336.2359; $\nu_{\text{max}}$/cm$^{-1}$ 3375. [$\alpha_D$] (EtOH, c=0.30) +40.3.

7.20.9 (S)-3-((Tert-butyldimethylsilyloxy)methyl)-1-(1-(naphthalen-2-yl)ethyl)azetidin-3-ol, 114j.

Yield 97%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 0.11 (3H, s, SiCH$_3$), 0.12 (3H, s, SiCH$_3$), 0.92 (9H, s, Si$^t$Bu), 1.31 (3H, d, $J=6.4$, CH$_3$CHN), 2.96 (1H, d, $J=8.0$, CCH$_3$H$_2$N), 3.06 (1H, d, $J=8.0$, CCH$_3$H$_2$N), 3.16 (1H, d, $J=8.4$, CCH$_3$H$_2$N), 3.49 (1H, d, $J=8.4$, CCH$_3$H$_2$N), 3.54 (1H, q, $J=6.4$, CH$_3$CHN), 3.80 (1H, d, $J=10.0$, SiOCH$_3$H$_2$), 3.84 (1H, d, $J=10.0$, SiOCH$_3$H$_2$), 7.46 (3H, m, (CH, Ar)), 7.79 (4H, m, (CH, Ar)); $^{13}$C NMR (100 MHz) -5.4 (SiCH$_3$), 18.3 (SiC(CH$_3$_3), 21.3
(CHCH₃), 25.9 (SiC(CH₃)₃), 62.5 (CCH₂N), 62.6 (CCH₂N), 68.1 (CH₂OSi), 68.8 (CHCH₃), 69.0 (C(CH₂)₃OH), 125.4, 125.5, 125.7, 125.8, 127.6, 127.7, 128.0 (CH, Ar), 132.8, 133.3, 141.0 (C, Ar); MS ES (+ve) found m/z 372.2 ([M+H]+, 100%); HRMS FAB [M+H]+ 372.2280, C₂₂H₃₃NO₂Si+H+ requires 372.2314; νₘₚₓ/cm⁻¹ 3348.

11.20 Synthesis of tertiary alcohols using secondary amines from epoxide 110.

To a solution of (2-((tert-butyldimethylsilyloxy)methyl)oxiran-2-yl)methyl-4-methylbenzenesulfonate 110 (100 mg, 0.27 mmol) in ethanol (2.7 ml, 0.1 M) was added the corresponding amine (3 equivalents, Table 20 in Results and Discussion section). The solution was heated at reflux for 8 hours and allowed to cool slowly to room temperature. The mixture was evaporated in vacuo and the crude material was purified by column chromatography (SiO₂; methanol in ethyl acetate).

11.20.1 1,3-bis(Allyl(methyl)amino)-2-((tert-butyldimethylsilyloxy)methyl) propan-2-ol, 115a.

![Chemical structure](image)

Yield 81%, yellow oil. ¹H NMR (400 MHz, CDCl₃) δH 0.03 (6H, s, 2xSiCH₃), 0.87 (9H, s, Si’Bu), 2.31 (6H, s, 2xCH₂N), 2.37 (2H, d, J = 13.6, 2xCH₂H₅N), 2.48 (2H, d, J = 13.6, 2xCH₂H₅N), 3.05 (2H, dd, J = 6.4, 14.0, 2xNCH₃H₅CH), 3.14 (2H, dd, J = 6.4, 14.0, 2xNCH₂H₅CH), 3.40 (2H, s, CH₂OSi), 5.11 (4H, m, 2xCH₂CH), 5.83 (2H, m, 2xCH₂CH); ¹³C NMR (100 MHz) -5.5 (SiCH₃), 18.1 (SiC(CH₃)₃), 25.8 (SiC(CH₃)₃), 44.4 (CH₂N), 59.8 (NCH₂CH), 62.6 (CH₂OSi), 65.2 (CCH₂N), 73.8 (C(CH₂)₃OH), 117.0 (CH₂CH), 136.2 (CH₂CH); MS ES (+ve) found m/z 343.2 ([M+H]+, 100%); HRMS FAB [M+H]+ 343.2770, C₁₈H₃₈N₂O₂Si+H+ requires 343.2736; νₘₚₓ/cm⁻¹ 3295.
11.20.2 1-(Tert-butylidemethylsilyloxy)-3-(methyl(prop-2-ynyl)amino)-2-((methyl(prop-2-ynyl)amino)methyl)propan-2-ol, 115b.

![Chemical Structure Image]

Yield 78%, colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.05 (6H, s, 2xSiCH$_3$), 0.88 (9H, s, Si'Bu), 2.20 (2H, t, $J = 2.0$, 2xCHC), 2.39 (2H, d, $J = 15.6$, 2xCCH$_2$H$_3$N), 2.40 (6H, s, 2xNCH$_3$), 2.56 (2H, d, $J = 15.6$, 2xCCH$_2$H$_3$N), 3.41 (4H, d, $J = 2.0$, 2xCCH$_2$CH), 3.43 (2H, s, CH$_2$OSi); $^{13}$C NMR (100 MHz) -5.5 (SiCH$_3$), 18.1 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$), 44.2 (CH$_3$N), 47.6 (CCH$_2$N), 58.4 (NCH$_2$CH), 65.0 (CH$_2$OSi), 72.5 (CHC), 73.8 (C(CH$_2$)$_3$OH), 79.6 (CHC); MS ES (+ve) found m/z 339.2 ([M+H]$^+$, 100%); HRMS FAB [M+H]$^+$ 339.2420, C$_{18}$H$_{34}$N$_2$O$_2$Si+H$^+$ requires 339.2423; $\nu$$_{max}$/cm$^{-1}$3371.

11.20.3 1,3-bis(Benzyl(methyl)amino)-2-((tert-butyldimethylsilyloxy)methyl)propan-2-ol, 115c.

![Chemical Structure Image]

Yield 83%, yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 0.04 (6H, s, 2xSiCH$_3$), 0.80 (9H, s, Si'Bu), 2.22 (6H, s, 2xCH$_3$N), 2.52 (4H, s, 2xCCH$_2$N), 3.44 (2H, s, CH$_2$OSi), 3.54 (2H, d, $J = 13.6$, CH$_3$H$_3$Ar), 2.65 (2H, d, $J = 13.6$, CH$_3$H$_3$Ar), 7.23 (10H, m, Ar); $^{13}$C NMR (100 MHz) -5.6 (SiCH$_3$), 18.0 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$), 44.4 (CH$_3$N), 60.2 (NCH$_2$CH), 63.9 (CH$_2$OSi) 65.6 (NCH$_2$CH), 74.2 (C(CH$_2$)$_3$OH), 126.9, 128.2, 128.8 (CH, Ar), 139.6 (C, Ar); MS ES (+ve) found m/z 443.3 ([M+H]$^+$,
100%); HRMS FAB [M+H]$^+$ 443.3091, C$_{26}$H$_{42}$N$_2$O$_2$Si+H$^+$ requires 443.3094; $v_{\text{max}}$/cm$^{-1}$ 3329.
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Table 1: Results of the enzymatic desymmetrisation of 87 with Amano L, AK in organic solvents.

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<th>Entry</th>
<th>Eq acyl donor</th>
<th>% 50&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% ee&lt;sup&gt;c&lt;/sup&gt;</th>
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<sup>a</sup> Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride and Amano L, AK (2 weight equivalents) at 37 ºC for 1 hour. <sup>b</sup> Isolated yields. <sup>c</sup> Results from GC of the crude reaction mixture. <sup>d</sup> Average of two reactions. <sup>e</sup> Average of three reaction. <sup>f</sup> Average of four reaction. (More results in table 2).
Table 2: Results of the enzymatic desymmetrisation of 87 with Amano L, AK in organic solvents.

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<sup>a</sup> Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride (1 equivalent) and Amano L, AK (2 weight equivalents) at 37
°C for 1 hour. \textsuperscript{b} Isolated yields. \textsuperscript{c} Results from GC of the crude reaction mixture. Results in red were excluded from the average of table 1.

Table 3: Results of the enzymatic desymmetrisation of 87 with Amano L, AK in organic solvents.

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\textsuperscript{a} Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride (1.5 equivalents) and Amano L, AK (2 weight equivalent) at 37 °C. \textsuperscript{b} Results from GC of the crude reaction mixture.
Table 4: Results of the enzymatic desymmetrisation of 87 with Amano L, AK in organic solvents.

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<sup>a</sup> Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (100 mg), acetic anhydride (1.8 equivalents) and Amano L, AK (2 weight equivalents) at 37 °C. <sup>b</sup>Results from GC of the crude reaction mixture.
Table 5: Results of the enzymatic desymmetrisation of 87 with different proteases in organic solvents.

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<tr>
<th>Entry</th>
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<th>% ee^d</th>
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*a* Biotransformations were carried out in dichloromethane (0.1 M) at 37 °C using prochiral diol 87 (10 mg), acyl donor (1 equivalent) and 1 weight equivalent of the immobilised enzyme for the corresponding time (biotransformations were followed by TLC until no changes were seen). *b* Names of enzyme provide from Codexis. *c* Abbreviations: acetic anhydride (Ac$_2$O), vinyl acetate (VA), *iso*-propylacetate ('PrOAc), pentyl acetate (PentylOAc), ethyl acetate (EtOAc). *d* Results from GC of the crude reaction mixture.
Table 6: Results of the enzymatic desymmetrisation of 87 with *R. miehei* in organic solvents.

<table>
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<th>Entry(^a)</th>
<th>Time (min)</th>
<th>% 50(^b)</th>
<th>% ee(^c)</th>
<th>% 49(^b)</th>
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\(^a\) Biotransformations were carried out in DCM (0.1 M) using prochiral diol 87 (10 mg), 1 equivalent of vinyl acetate and 1 weight equivalent of NZL-103 from *R. miehei* at 37ºC. \(^b\) Results from GC of the crude reaction.