The Utilization of Enzymes in the Synthesis and Modification of Natural and NonNatural Compounds: A Chemo-Enzymatic Approach to Enantiomerically Pure Compounds

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The Utilization of Enzymes in the Synthesis and Modification of Natural and Non-Natural Compounds: A Chemo-Enzymatic Approach to Enantiomerically Pure Compounds

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Chemistry
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... shake off all the fears of servile prejudices under which weak minds are servilely crouched. Fix reason firmly in her seat, and call to her tribunal for every fact, every opinion. Question with boldness even the existence of a god because, if there be one, he must more approve of the homage of reason than that of blindfolded fear. You will naturally examine first the religion of your own country. Read the bible then, as you would read Livy or Tacitus. The testimony of the writer weighs in their favor in one scale, and their not being against the laws of nature does not weigh against them. But those facts in the bible which contradict the laws of nature, must be examined with more care, and under a variety of faces. Here you must recur to the pretensions of the writer to inspiration from god. Examine upon what evidence his pretensions are founded, and whether that evidence is so strong as that it's [sic] falsehood [sic] would be more improbable than a change of the laws of nature in the case he relates.... Do not be frightened from this enquiry by any fear of its [sic] consequences. If it ends in a belief that there is no god, you will find incitements to virtue in the comfort and pleasantness you feel in it's [sic] exercise, and the love of others which it will procure you. If you find reason to believe there is a god, a consciousness that you are acting under his eye, and that he approves you, will be a vast additional incitement. If that there be a future state, the hope of a happy existence in that increases the appetite to deserve it; if that Jesus was also a god, you will be comforted by a belief of his aid and love. In fine, I repeat that you must lay aside all prejudice on both sides, and neither believe nor reject any thing because any other person, or description of persons have rejected or believed it. Your own reason is the only oracle given you by heaven, and you are answerable not for the rightness but uprightness of the decision....

(Thomas Jefferson, letter to his young nephew Peter Carr, August 10, 1787)
Dedication

TO MOM and DAD whose love has helped to make this dissertation a reality and for whom this dissertation means a great deal, their love has been a spring of continuous encouragement, inspiration and motivation,

TO WOLFF who is able to dissipate the misery of a day full of intractable tars with a hug and a kiss, to my best and dearest friend whose charisma, whose art, imbedded a moral feeling for life,

TO EDITH and FAMILY who have been a constant source of support whenever I felt the world stood against me. They always believed in me and for this they will always have my eternal gratitude,

TO DWIGHT my dear brother who is in Iraq fighting an unjust and malevolent war; my advice is best summarized by that given by Winston Churchill – ‘In war, Resolution; in defeat, Defiance; in victory, Magnanimity’.
Acknowledgments

We see then how far the monuments of wit and learning are more durable than the monuments of power, or of the hands. For have not the verses of Homer continued twenty-five hundred years, or more, without the loss of a syllable or letter; during which time infinite palaces, temples, castles, cities have been decayed and demolished?

Learning is the greatest adventure in life. The author wishes to express his appreciation to Dr. K.S. Bisht for his encouragement and assistance in this episode of the author’s development and for making this learning process stimulating and joyous. It is a testimonial to Dr. Bisht’s effectiveness as a professor that he is able to transmit to his students the enthusiasm with which he approaches both life and chemistry.

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<td>Synthesis of $R$-($-$)-Imperanene through the Coupling of Aldehyde 10 with Wittig reagent 6.</td>
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## List of Symbols and Abbreviations

<table>
<thead>
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<tr>
<td>δ</td>
<td>Chemical shift in parts per million units</td>
</tr>
<tr>
<td>$^1$H</td>
<td>Isotope of hydrogen with mass of 1 amu</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>Isotope of carbon with mass of 13 amu</td>
</tr>
<tr>
<td>AK</td>
<td>Lipase from <em>Pseudomonas fluorescens</em></td>
</tr>
<tr>
<td>AYS</td>
<td>Lipase from <em>Candida rugosa</em></td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>CH$_3$OD</td>
<td>Deuterated methanol</td>
</tr>
<tr>
<td>COSY</td>
<td>COrrelation SpectroscopY</td>
</tr>
<tr>
<td>DDQ</td>
<td>Dichloro Dicyano Quinone</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>DEPT-45</td>
<td>Distortionless Enhancement by Polarization Transfer at a flip angle of 45 degrees</td>
</tr>
<tr>
<td>DEPT-90</td>
<td>Distortionless Enhancement by Polarization Transfer at a flip angle of 90 degrees</td>
</tr>
<tr>
<td>DEPT-135</td>
<td>Distortionless Enhancement by Polarization Transfer at a flip angle of 135 degrees</td>
</tr>
<tr>
<td>DMSO-d$_6$</td>
<td>Deuterated Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>EAC</td>
<td>Enzyme-activated complex</td>
</tr>
<tr>
<td>e.e.</td>
<td>Enantiomeric excess</td>
</tr>
<tr>
<td>HETCOR</td>
<td>HETeronuclear Chemical Shift CORrelation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
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<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium Aluminum Hydride</td>
</tr>
<tr>
<td>MPPIM</td>
<td>Methyl N-(3-phenyl-propanoyl)-2-oxoimidazoline-4-carboxylate</td>
</tr>
<tr>
<td>MMPP</td>
<td>Magnesium monoperoxyphthalate</td>
</tr>
<tr>
<td>Novozym-435</td>
<td>Lipase from <em>Candida antarctica</em></td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PMB</td>
<td>Para-methoxy benzyl</td>
</tr>
<tr>
<td>PPL</td>
<td>Lipase from <em>Porcine pancreatic lipase</em></td>
</tr>
<tr>
<td>PS-30</td>
<td>Lipase from <em>Pseudomonas cepacia</em></td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>RAMP</td>
<td>(R)-1-amino-2-methoxy-methylpyrrolidine</td>
</tr>
<tr>
<td>SAMP</td>
<td>(S)-1-amino-2-methoxy-methylpyrrolidine</td>
</tr>
<tr>
<td>SL</td>
<td>Sophorolipid</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium floride</td>
</tr>
<tr>
<td>TBDMS</td>
<td>Tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
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The Utilization of Enzymes in the Synthesis and Modification of Natural and Non-Natural Compounds: A Chemo-Enzymatic Approach to Enantiomerically Pure Compounds

Jason A. Carr

ABSTRACT

The employment of enzymes and whole cells has been important in many industries for centuries. However, it is only in the last 30 years that the use of enzymes for the synthesis of high-value fine chemicals has enjoyed increasing popularity. In fact, esterases and lipases are used almost routinely these days to provide optically active building blocks for the construction of imaginative new routes to chiral target molecules. The major topic of this work describes the utilization of enzymes (namely lipases) in the synthesis and modification of natural and non-natural compounds.

Chapter 1 outlines the strengths and weaknesses of the most widely used enzyme systems and a description of a brief summary on the state of the art of biotransformations with special emphasis on the general applicability and reliability of various reaction types is described.

Chapter 2 describes the enzymatic resolution of various 3-acetoxy-4-aryl-substituted azetidin-2-ones. Following screening of enzymes, such as Novozym-435, PS-30, PPL and AYS the best conditions were a phosphate buffer with PS-30 as the enzyme. The resulting products were the (3S, 4R)-3-hydroxy-4-aryl-substituted azetidin-2-ones and the unreacted
(3R, 4S)-3-acetoxy-4-aryl-substituted azetidin-2-ones. Reactions generally occurred with high conversion and high selectivity.

In Chapter 3, the regioselective transesterifications and hydrolysis of peracylated sophorolipid (SL) derivatives catalyzed by lipases was investigated. It was confirmed from the detailed spectral analysis of the products that transesterification failed to furnish any free hydroxyls on the sophorose ring. Instead, transesterification took place on the methyl ester located at the carboxylic end of the 17-hydroxyoctadecenoic acid chain attached to the C-1′ position of the sophorose ring.

In Chapter 4, the chemo-enzymatic syntheses of enantiomerically pure R and S imperanene from vanillin are described. The key step entails the asymmetrization of a prochiral diol using lipase PS-30. The resulting monoacetate has enantiomeric excesses of >97%.

Biocatalysts represent a new class of chiral catalysts useful for a broad range of selective organic transformations. It is stating the obvious to say that biocatalysis is not a panacea for synthetic organic chemistry. However, advances over the past thirty years mean that it would be a serious mistake not to consider the employment of a biocatalyst, in, perhaps, the key step in a sequence of transformations that turn a cheap starting material into an expensive fine chemical.
1.1 General Introduction

The use of enzymes for the biotransformation of non-natural organic compounds is not at all new as they have been used for about a century\(^1\). What has changed, however, is the objective; that is, ‘biocatalysis’ has evolved as a trend-setting segment of organic synthesis since the mid-eighties thus providing a powerful tool to the arsenal of modern organic synthesis. Whereas most of the early studies were directed towards the elucidation of biochemical pathways and enzyme mechanisms, the enormous potential for employing enzymes to transform non-natural organic compounds was only realized relatively recently, \textit{i.e.} during the late 1980’s.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Annual publications on the biocatalytic transformation of non-natural organic compounds\textsuperscript{a}}
\end{figure}

\textsuperscript{a} Data obtained from database Faber, ~8000 entries (05/1997).
As a result of this intense research, enzymes have been secured an important place in contemporary organic synthesis. A testament to the importance of biocatalysis in synthetic organic chemistry is illustrated when an entire issue (*Tetrahedron*, Vol 60, Issue 3, 2004) was dedicated to synthetic biocatalysis.

Much of the early research was impeded by a tacitly accepted dogma which stated that *enzymes are Nature’s own catalysts developed during evolution for the regulation of metabolic pathways*. This narrow definition implied that man-made or non-natural organic compounds cannot be regarded as substrates. However, once this pedagogic problem was attacked by non-traditionalists, it was quickly shown that the substrate tolerance of enzymes is much wider than believed. An impressive number of biocatalysts have been shown to possess a wide substrate tolerance by keeping their exquisite catalytic properties with respect to chemo-, regio- and, most importantly, enantio-selectivity. For many of these enzymes, the natural substrates - if there are any - are not known. As a consequence, the frequency of use of a particular enzyme is not evenly distributed among the various types of biocatalysts but follows a pattern shown in *Fig. 1-2*. Herein, a description of a brief summary on the state of the art of biotransformations with special emphasis on the general applicability and reliability of various reaction types is described.
Fig. 1-2 Frequency of use of enzymes in biotransformations; (i) Ester formation, - aminolysis, -hydrolysis; (ii) ester hydrolysis; (iii) ester and amide hydrolysis, peptide synthesis; (iv) nitrile hydrolysis; (v) hydrolysis of epoxides, halogens, phosphates, glycosylation; (vi) reduction of aldehydes, ketones and enoates; (vii) biohydroxylation, sulfoxidation, epoxidation, Baeyer-Villiger oxidation, dihydroxylation; (viii) cyanohydrin formation, acyloin and aldol reaction; (ix) glycosyl transfer; (x) Claisen-type rearrangement, isomerization of carbohydrates, racemization and epimerization.

1.2 Hydrolases

Reactions catalyzed by various types of hydrolases are predominant among biotransformations. The lack of sensitive cofactors, which have to be recycled, makes them particularly attractive for organic synthesis. Consequently, they account for about two thirds of all reactions reported. In particular, reactions involving the cleavage (or formation) of an amide- or ester bond are most easy to perform by using lipases, esterases and proteases, respectively. Other types of hydrolysis reactions involving phosphate esters, epoxides, organo-halogenes and nitriles are still hampered by a restricted availability of enzymes, but they hold great synthetic potential.
1.2.1 Lipases

A large number of fat-cleaving enzymes - lipases - are produced on an industrial scale for applications in the food, detergent and fine chemical industry\(^\text{18}\). Due to their ready availability, they have enjoyed enormous use in the development of enantioselective hydrolysis and carboxyl esters formation\(^\text{19}\). Considering that the natural substrates for lipases - glycerides - possess a chiral alcohol moiety, it is reasonable to understand why lipases are synthetically useful for the resolution or asymmetrization of esters bearing a chiral alcohol moiety (Fig. 1-3).\(^\text{16c, 20}\)

From the literature data available, the following broad-spectrum blueprint of lipase-substrates interface can be agreed upon:

(i) optimal chiral recognition can be guaranteed if the stereogenic center is located as close as possible to the reaction site (i.e. the ester carbonyl group); (ii) The nature of both substituents \(R_1\) and \(R_2\) should differ in size and/or polarity\(^\text{2}\); (iii) The alkyl chain of the acid moiety (\(R_3\)) should preferably possess a straight-chain nature at at least three to four carbon atoms\(^\text{3}\); (iv) The hydrogen atom must not be replaced by a substituent, since esters of tertiary alcohols and \(\alpha,\alpha,\alpha\)-trisubstituted carboxylates are typically not accepted by lipases. (v) The stereochemical preference of the most commonly used lipases (e.g. from *Pseudomonas* and *Candida* spp.) for esters of secondary alcohols follows an empirical model generally referred to as 'Kazlauskas-rule'\(^\text{21,22}\).
Fig. 1-3 Substrate types for lipases, esterases or proteases

The general guidelines described above can be successfully employed in resolving almost every secondary alcohol by lipase biocatalysis. The stereochemical outcome (a 'digital' decision) can be predicted with fair precision, whereas the stereoselectivity (i.e., an 'analog' value) is largely empirical. Despite an impressive effort in molecular

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4 Usually expressed as the ratio of the relative rate constants of the enantiomers, the 'Enantiomeric Ratio' (E) (ref. 27).
modelling,\textsuperscript{23} attempts to develop reliable methods for the prediction of lipase-stereoselectivity have failed so far.

The high stability of lipases towards organic solvents makes them extremely useful for the reverse reaction, \textit{i.e.} ester formation by condensation or (more advantageous) transesterification reactions\textsuperscript{24,25}. Moreover, the use of non-natural nucleophiles in acyl-transfer reactions such as amines\textsuperscript{26}, ammonia, hydrazine, oximes, and hydrogen peroxide allows the lipase-catalyzed aminolysis, ammonolysis\textsuperscript{27}, hydrazinolysis\textsuperscript{28}, oximolysis and perhydrolysis\textsuperscript{29} of esters giving rise to carboxamides, hydrazides, hydroxamic acids and peracids, respectively.

Stereoselective variation on enzyme selectivity can be controlled by varying the solvent’s properties\textsuperscript{30}. Given the fragility of enzymes, it may be anticipated that any solvent exerts a significant influence on the catalytic properties of an enzyme, such as reaction rate. For reactions performed in water, however, this is hardly possible, because its physicochemical properties are determined by Nature and can be altered only within a very narrow margin. An organic solvent may be chosen within certain limits\textsuperscript{5} from a wide arsenal having different properties such as dipole moment, water-solubility, straight-chain or cyclic structure, flexibility and the ability to form hydrogen bonds\textsuperscript{31}. Therefore, the stereoselectivity of an enzyme-catalyzed reaction in organic solvent is still a largely empirical task.

Presently, lipases are continuously used for the generation of enantiomerically enriched primary and secondary alcohols. However, limitations with respect to the predictability of stereoselectivity will persist for some time.

\textsuperscript{5} From a number of methods to estimate the compatibility of an organic solvent ensuring sufficient enzyme activity, the log P-value has been shown to be most reliable. As a rule of thumb, an organic solvent having a log P of 1 should not deactivate a reasonably stable enzyme.
1.2.2 Esterases

The large majority of esterase-catalyzed reactions have been performed by using porcine liver esterase (PLE)\textsuperscript{32}. This enzyme has been widely used for the hydrolysis of Type II esters Fig. 1-3 with $R_3$ being preferably methyl or ethyl, whilst Type I substrates (employed as the acetate esters) have been used to a lesser extent. In comparison to lipases, the applicability of PLE is significantly restricted to reactions performed in an aqueous medium, as PLE has been shown to exhibit low activity and erratic results with respect to stereoselectivity when placed in (nearly) anhydrous organic solvents\textsuperscript{6}.

Because of both the smaller availability and narrow operational range of esterases as compared to lipases, whole microbial cells have been used instead of isolated enzyme preparations\textsuperscript{33}. Although some surprising highly selective transformations have been reported, this technique is of limited use because the nature of the active enzyme system is unknown and, as a consequence, optimization is a complicated task.

The general rules for the substrate-construction for esterases are closely related to those for lipases (Fig. 1-3). However, $R_3$ should preferably be a short-chain unit, leading to acetates (Type I). The number of commercially available esterases is small and their applicability in organic solvents is limited. Thus, the development of novel esterases from microbial sources is a worthwhile endeavour.

1.2.3 Proteases

Peptides have gained increasing attention due to their diverse biological activity. They may be used as sweeteners, antibiotics and chemotactic agents, as well as growth

\textsuperscript{6} This problem has been circumvented recently by using chemically modified PLE (ref. 35).
factors and they play an important role in hormone release by acting either as stimulators or inhibitors. The enzymatic hydrolysis of the carboxamide bond\(^{34}\) is naturally linked to amino acid and peptide chemistry and an increasing number of optically pure L-amino acids are produced on an industrial scale by an enzymatic method. The exquisite specificity of proteases for L-configured substrates turns into a drawback when the incorporation of D-amino acids is required.\(^{35}\) Attempts have been reported to ‘weaken’ the L-specificity of proteases by ‘solvent engineering’ or by enzyme-modification on the genetic level. The general rules for the substrate-construction for esterases and proteases are closely related to those for lipases (Fig. 1-3). However, \(R_3\) should preferably be a short-chain unit, leading to acetates (Type I) and methyl carboxylates (Type II), respectively.

1.2.4 Epoxide Hydrolases

Epoxides are versatile building blocks in synthetic organic chemistry. As a result of the ring strain, their susceptibility towards nucleophiles, oxidizing and reducing agents makes them versatile intermediates for the preparation of various complex molecules. Optically active epoxides have been synthesized by a variety of biotransformation reactions (Figure 1-4). They can be prepared by direct epoxidation of alkenes using monooxygenases or peroxidases, by enantioselective hydrolysis using epoxide hydrolases\(^{37b}\), or by enantioselective nucleophilic ring opening using a haloalcohol dehalogenase or lipase.\(^{38-42}\) Optically active halohydrins, which are direct precursors of epoxides, have been prepared using haloalcohol dehalogenases, haloperoxidases, lipases or by microbial reduction of haloacetophenones.
Figure 1-4. Biocatalytic preparation methods of optically active epoxides and chloroalcohols.

The asymmetric hydrolysis of an epoxide provides access to chiral 1,2-diols and (in kinetic resolutions) also to enantiomERICALLY enriched epoxides. Both of these materials are widely used as highly valuable intermediates\(^7\) for asymmetric synthesis\(^46\). In principle, the asymmetric biocatalytic hydrolysis of an epoxide may be conducted in various ways (Fig. 1-4): (i) Kinetic resolution provides the corresponding 1,2-diol and the residual substrate in 50% theoretical yield. (ii) Alternatively, enantioconvergent hydrolysis leads to the vicinal diol as the sole product in 100% theoretical yield. The latter transformation may be achieved by using (iia) two different biocatalysts possessing opposite enantio- and regio-selectivity\(^47\) or (iib) by using a bio- and chemo-catalytic step in a sequence\(^48\).

\(^7\) Vicinal diols are employed as the corresponding cyclic sulfite or sulfate esters.
1.2.5 Phosphatases and Kinases

Phosphate ester formation is of considerable importance because numerous bioactive agents display their highest activity only when they are transformed into phosphorylated analogues. Thus the phosphorylation of OH-groups at the expense of adenosine triphosphate (ATP) by using kinases has been developed to a great extent\(^49\).

1.2.6 Dehalogenases and Halohydrin Epoxidases

The replacement of halogen with hydroxyl in a formal hydrolysis reaction is catalyzed by dehalogenases\(^8,50\) (Fig 1-4). Depending on the enzyme used, the reaction usually proceeds with inversion of configuration, \textit{e.g.} L-\(\alpha\)-chloropropionic acid yields D-lactate, and \textit{vice versa}\(^51\). On the other hand, the formal elimination of hydrogen halide from a halohydrin under catalysis of a halohydrin epoxidase yields an epoxide. The bulk of research published so far has shown that these enzymes exhibit low enantio-selectivity in general\(^52\).

1.3 Novel Techniques

At present, the research goals within the area of biotransformations are undergoing a rapid change. The majority of biotransformations involving chiral molecules are performed with racemic substrates rather than prochiral or \textit{meso}-forms\(^9\) - \textit{i.e.} they constitute kinetic resolutions as opposed to asymmetrizations. The former, however, are impeded by several drawbacks such as (i) limited yield (50\% of each enantiomer), (ii) reduced enantiomeric purity of substrate and product for kinetic reasons,

\(^8\) Dehalogenases are classified depending on their substrate type; for biotransformations, haloalkane dehalogenase and \(\alpha\)-haloacid dehalogenase are most important.

\(^9\) The ratio of kinetic resolution versus asymmetrization is about 4:1.
(iii) close reaction monitoring required, because the process has to be stopped at a certain point of conversion at (or near) 50% and (iv) separation of the product formed from unreacted substrate. All of these impediments are largely circumvented by using processes which lead to the formation of a single enantiomeric product. As a consequence, the theoretical yield is now 100% with optical purities being higher, the separation step is obsolete and monitoring can be simplified because the reaction can be run to completion. Such processes are, for instance, (i) dynamic resolutions and (ii) stereo-inversions (Fig. 1-5). Any kinetic resolution of a racemic starting material ($R, S$) following a classic profile can be converted into a dynamic process by adding one additional reaction - an in-situ-racemization of the substrate. While in classic resolutions the fast reacting enantiomer (e.g. $R$) is transformed into P leaving its enantiomer S behind, in a dynamic process S is converted to P via racemization. As a consequence, all of the starting material ($R, S$) is eventually transformed into P as the sole product. The dynamic resolution processes developed so far made use of racemization through chemical catalysis, but it can be expected that two-enzyme processes involving racemases are possible. The latter shows great potential, considering the greater compatibility of biocatalysts as opposed to the combination of chemo- and biocatalysis.
A particular reaction sequence leading to the formation of a single enantiomeric product makes use of an enantioselective stereoinversion\textsuperscript{53}. Suppose that one enantiomer out of a racemic mixture is selectively inverted into its mirror-image counterpart, the final product is 100\% of one enantiomer. This type of process has been exemplified with the stereoinversion of racemic secondary alcohols through a two-step oxidation-reduction sequence using microbial whole cells\textsuperscript{54}. Although the actual nature of the enzymes and cofactors involved is not known at present, the chemical and optical yields achieved are impressive. It can be anticipated, that once the catalytic and thermodynamic details about this sequence are known, more synthetic applications will appear. The methodology concerning the application of enzymes in non-aqueous solvents with respect to enzyme activity is sufficiently understood as to be highly useful for organic chemists. Thus, the synthesis of esters, lactones, amides, peptides and peracids by using enzymes is standard.
methodology. On the other hand, an understanding of the influence of the nature of the solvent on an enzyme’s selectivity is still in its infancy and we are far away from being able to provide rules of general applicability\(^{30}\). The same is true with regard to the understanding of the factors involved in substrate-binding where assistance will be given by means of molecular modelling\(^{55}\).

1.4 Common Prejudices Against Enzymes

In the bio-transformations of non-natural organic compounds the following prejudices are often encountered:

– ‘Enzymes are sensitive’. This is certainly true for most enzymes but if certain precautions are met, enzymes are remarkably stable. Some are known to tolerate hostile environments such as temperature greater then 100\(^\circ\)C and pressures beyond several hundred bar\(^{56-58}\).

– ‘Enzymes are expensive’. Some enzymes are indeed expensive, but many are produced cheaply on a large scale. Considering their high catalytic power and the fact that they are recyclable\(^{10}\) most reasonably price crude enzyme preparations are adequate when compared to chemical catalysts.

– ‘Enzymes are only active on their natural substrates’. Although this statement is certainly true for some enzymes, for the majority of them it’s false. Biocatalysts are capable of accepting non-natural substrates of an unrelated structural type by often exhibiting the same high specificities as for the natural counterparts.

– ‘Enzymes work only in their natural environment’. Although it is generally true that enzymes display their highest catalytic activity in aqueous media, this is often not the

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\(^{10}\) The overall efficiency of a process may be better even if a rather expensive enzyme is required.
solvent of choice for organic chemists. Providing certain guidelines are followed, enzymes can function in organic solvents. Although lower activity may be expected, other advantages can be accrued\(^{59-62}\).

1.5 Advantages and Disadvantages of Biocatalysts

1.5.1 Advantages of Biocatalysts

– ‘Enzymes are very efficient catalysts’. Compared to their non-enzymatic reactions, enzyme-assisted processes are accelerated by a factor of \(10^8-10^{10}\), far out seeding those of chemical catalysts. Consequently, whereas chemical catalysts are generally employed in concentrations of mole percentage 0.1-1\%, enzymatic reactions can be performed at reasonable rates with mole percentage of 0.001-0.0001\%.

– ‘Enzymes are environmentally acceptable.’ Biocatalysts are environmentally benign reagents since they are completely degradable, unlike heavy metals.

– ‘Enzymes act under mild conditions.’ Enzymes act in a pH range of about 5-8, typically around 7, and in a temperature range of 20-40\(\text{oC}\), preferably around 30\(\text{oC}\). This minimizes undesired side-reactions\(^{11}\) which often plague traditional methodology.

– ‘Enzymes are compatible with each other\(^{63}\).’ Since enzymes normally function under similar conditions, biocatalytic reaction can be carried out in tandem making sequential reactions feasible in multienzyme synthetic methodology, particularly if isolation of unstable intermediates is not always possible.

– ‘Enzymes are not bound to their natural role.’ Enzymes can tolerate a large variety of man-made unnatural substrates. Often, aqueous medium can sometimes be replaced by an organic solvent.

\(^{11}\) Side-reactions may include decomposition, isomerization, racemization and rearrangement.
Enzymes can catalyze a broad spectrum of reactions. There’s an enzyme-catalyzed process equivalent to almost every type of organic reaction. For example, as have been previously described common examples range from:

(i) Hydrolysis-synthesis of esters, amides, lactones, lactams, ethers, acid anhydrides, epoxides and nitriles.

(ii) Oxidation-reduction of alkanes, alkenes, aromatics, alcohols, aldehydes and ketones, sulfides and sulfoxides.

(iii) Addition-elimination of water, ammonia, hydrogen cyanide.

(iv) Halogenation and dehalogenation, alkylation and dealkylation, carboxylation and decarboxylation, isomerization, acyloin and aldol reactions. Even Michael-additions, Diels-Alder and Claisen-rearrangement reactions have been reported.

Enzymes display three major types of selectivities:

– Chemoselectivity. Since enzymes tend to act on a single type of functional group, other sensitive functionalities that are sensitive to chemical catalysis do survive. Thus, reactions tend to be cleaner and laborious purification can be largely omitted.

– Regioselectivity and Diastereoselectivity. The complexity of an enzyme’s three dimensional structure affords an enzyme to distinguish between functional groups which are chemically situated in different regions of the same substrate molecule.

– Enantioselectivity. Since almost all enzymes are made from L-amino acids, they are thus chiral catalysts. Consequently, chirality present within a substrate molecule is ‘recognized’ upon enzyme-substrate complex formation. A prochiral substrate, as a consequence, may be transformed into an optically active product through

12 Enzymes can only accelerate a reaction, but they have no impact on the position of the thermodynamic equilibrium of the reaction. Thus, in principle, some enzyme-catalyzed reactions can be run in both directions.
asymmetrization and both enantiomers of a racemic substrate may react at different rates, affording a kinetic resolution. Collectively, these properties constitute the ‘specificity’ of an enzyme and represents its most important feature for selective and asymmetric exploitation.\textsuperscript{13, 66}

1.5.2 Disadvantages of Biocatalysis

There are certainly drawbacks to the use of biocatalysis:

-- ‘Enzymes are provided by Nature in only one enantiomeric form.’ Since there’s no general way of creating mirror-image enzymes, it is impossible to invert the chiral induction of a given enzymatic reaction by choosing the ‘other enantiomer’ of the biocatalyst, a strategy possible if chiral chemical catalysts are involved.

-- ‘Enzymes require narrow operation parameters.’ Working under mild reaction conditions can certainly have its drawback. For example, if a reaction has a narrow window of operation at a given pH and temperature, elevated temperature, extreme pH or even high salt concentration may deactivate the enzyme. Some enzymes, however, remain catalytically active even in ice.\textsuperscript{67}

-- ‘Enzymes display their highest catalytic activity in water.’ Enzymes display their highest catalytic activity in water, but the majority of organic compounds are only poorly soluble. Hence, shifting to an organic medium would be highly desirable, but some loss in activity is the opportunity cost in shifting the enzymatic reactions.

-- ‘Enzymes are bound to their natural cofactors.’ Although enzymes are extremely flexible in accepting non-natural substrates, they are almost exclusively bound to their natural cofactors. The majority of these ‘biological reagents’ are relatively unstable.

\textsuperscript{13} It’s remarkable that this key feature was recognized by E. Fischer as long ago as 1898!
molecules and are prohibitively expensive when used in stoichiometric amounts. Unfortunately, they cannot be replaced by more economical man-made substitute.\(^\text{14}\)

– ‘Enzymes are prone to inhibition phenomena.’ The efficiency of a reaction may be limited if the enzymatic reactions are prone to substrate or product inhibition. Whereas substrate inhibition can be circumvented easily by keeping substrate concentration low, product inhibition is a little more complicated.

– ‘Enzymes may cause allergic reactions.’ Enzymes may cause allergic reactions but careful handling may minimize reaction.

1.6 Summary and Outlook

As the field of biocatalysis undergoes still fast development, it can be expected, that some of the limitations will be overcome in the near future by using novel (genetically engineered) enzymes and through the development of novel techniques. Despite tremendous efforts in the field of genetic engineering (such as site-directed mutagenesis) aimed at the development of enzymes possessing altered specificities it is clear that at this stage, screening for novel microorganisms and enzymes is much more likely to lead to a solution of a particular problem than painstaking modification of the enzyme.

In summary, biocatalysts represent a new class of chiral catalysts useful for a broad range of selective organic transformations. Synthetic chemists capable of using this potential will have a clear advantage over those limited to non-biological methods in their ability to tackle the new generation of synthetic problems appearing at the interface between chemistry and biology.

\(^\text{14}\) Despite an impressive amount of progress, the recycling of cofactors is still not a trivial task.
1.7 References


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63. Only proteases are exception to this rule for obvious reasons.


CHAPTER TWO

Lipase-Catalyzed Resolution of 4-Aryl-Substituted β-Lactams: Effect of Substitution on the 4-Aryl Ring

2.1 General Introduction

The use of lipolytic enzymes, such as lipases, provides one of the most useful and versatile biocatalytic methods in asymmetric synthesis and resolution of organic substrates with high efficiency and selectivity.\textsuperscript{1-4} Lipases, in this regard, are excellent biocatalysts since they have the remarkable ability of assuming a variety of conformations to accommodate substrates of varying sizes and complexities.\textsuperscript{5-9} Above all lipases do not require the use of co-factors, can be recycled, and are effective under mild, environmentally benign conditions. As a result of this broad substrate specificity and their distinctive stereopreferences, lipases have been used in numerous applications ranging from enantioselective syntheses to the resolution of racemic mixtures.\textsuperscript{5-9}

Stereoselective synthesis of enantiomerically pure compounds is required not only for clinical compounds but also for fine chemical industries and agrochemicals. It is possible that an enantiomer in a racemic mixture may have detrimental side effects. Syntheses of such homochiral compounds, however, are often too difficult to obtain via conventional chemical synthetic methods. Recently we have reported the preparation of optically active substituted ε-caprolactones,\textsuperscript{10} versatile building blocks in organic synthesis, through lipase-catalyzed kinetic resolution in absence of added solvent. In another report,\textsuperscript{11} we have described solventless stereoselective polymerizations of racemic substituted caprolactones catalyzed by \textit{Candida antarctica} lipase.
The β-lactam (azetidin-2-one) is an interesting ring system found in penicillins, one of the most utilized antibacterials in clinical medicine.\textsuperscript{12,13} Moreover, β-lactams can be readily synthesized via the Staudinger reaction starting from an imine and an aldehyde (Scheme 2) and serve as chiral synthons in organic synthesis.\textsuperscript{13} For example, β-lactams have been used for assembly of the C-13 side chain in the anti-tumor drug paclitaxel,\textsuperscript{14} in the asymmetric synthesis of human leukocyte elastase inhibitors,\textsuperscript{15} and as key intermediates for penems and carbepenems.\textsuperscript{16} Recently, the stereocontrolled synthesis of monocyclic β-lactams has been an active area of chemical research owing to their utility as synthons for a variety of natural products\textsuperscript{14} and activity against MRSA bacterial strains.\textsuperscript{13} Since these compounds are interesting from chemical and pharmacological aspects, our goal was to study lipase catalyzed resolution of monocyclic β-lactams in order to obtain them in optically pure form. The previous results on enzymatic resolution of racemic β-lactams have largely concentrated on resolution of N-hydroxymethylated derivatives\textsuperscript{17-22} via lipase catalyzed asymmetric acylation of the primary hydroxyl on the N-hydroxymethyl group or by the hydrolysis of their corresponding ester derivatives.\textsuperscript{17-22} In a different approach, the β-lactam ring has been enantiospecifically hydrolyzed via enzymatic catalysis to the corresponding β-amino acid.\textsuperscript{23,24} This approach, however, results in loss of the β-lactam ring in the hydrolyzed enantiomer and would not be synthetically appealing especially if the hydrolyzed enantiomer is needed for subsequent synthetic transformations. In their quest to prepare the C-13 side chain of paclitaxel, Brieva et al.\textsuperscript{14} obtained enantiomerically enriched 3-hydroxy-4-phenyl-β-lactams derivatives \textit{via} lipase-catalyzed hydrolysis and transesterification of racemic ester and alcohols, respectively. These authors\textsuperscript{14} subjected three β-lactams with different
substituents (H-, PhCO-, and 4-CH$_3$OPh-) on the ring nitrogen and observed that lipase-catalyzed resolutions were influenced by the nature of the substituents on the lactam nitrogen.$^{14}$ For example, introduction of the $N$-benzoyl substituent, an electron withdrawing group, caused the cleavage of the β-lactam ring in tert-butyl methyl ether in the presence of CH$_3$OH as a nucleophile.$^{14}$

The goal in this study was to develop an enzyme system that invokes a resolution of the monocyclic β-lactams upon hydrolysis of its 3-acetoxy derivatives and to probe the effects of the nature of the lactam ring C-4 substituents on enzyme selectivity. In this report kinetic enzymatic resolution of the 3-acetoxy derivatives was investigated as an attractive alternative to the classical salt resolution or the resolution of the $N$-hydroxymethyl derivatives.$^{25}$ The kinetic resolution led to quantitative yields and enantiomerically pure (>98% ee) β-lactams were thus prepared. Importantly, the nature of the substituent on the C-4 position affected the enzymatic resolution to a great extent. Efforts to understand the effect of the C-4 substituent on the selectivity of the lipase catalyst are reported herein.

### 2.2 Introduction to Azetidin-2-ones (β-lactams)

Azetidin-2-ones and their derivatives, because of their diverse and interesting antibiotic activities,$^{26-30}$ have occupied a central place among medicinally important compounds. With the discovery of Penicillin in 1928, new and novel synthetic methods have been developed toward the effort of synthesizing azetidin-2-one derivatives with novel biological properties. In fact, β-lactam antibiotics constitute over 60% of the
clinically valuable antibiotics today.\textsuperscript{31} For the synthetic organic chemist, the $\beta$-lactam ring holds a particular attraction. These four-membered, chiral heterocycles are present in many natural products and many homochiral, synthetic approaches have been developed. A testament to the biological and synthetic importance of $\beta$-lactams in organic chemistry was illustrated when an entire issue of \textit{Tetrahedron} (2000, Issue 31, Vol. 56) was devoted to $\beta$-lactam chemistry. Reports attributing the therapeutic utility of azetidinones as agents to cholesterol absorption inhibitors\textsuperscript{32-35} and enzyme inhibitors\textsuperscript{36-37} are sure to give momentum to their therapeutic use other than antibiotics.

2.3 General Synthesis of Azetidin-2-ones

The Staudinger’s ketene-imine reaction (\textbf{Scheme 2-1}) is the most common method for the synthesis of $\beta$-lactams\textsuperscript{38} and has been the subject of a recent review by Palomo et al.\textsuperscript{39} The reaction utilizes the use of an acid chloride and an imine (Schiff base) in the presence of a tertiary amine (typically triethylamine). A ketene is generated in situ from the $\beta$-elimination of the base and the acid chloride followed by a [2 + 2] cycloaddition reaction between the generated ketene and the imine. This results in the formation of up to four possible racemic diastereomers.

In cases where the acid halide was too unstable or difficult to prepare, carboxylic acid activating agents were employed like trifluoroacetic anhydride\textsuperscript{40}, ethyl chloroformate\textsuperscript{41}, p-toluenesulfonyl chloride\textsuperscript{42}, phosphorous derived reagents\textsuperscript{43}, Mukaiyama reagent\textsuperscript{44}, cyanuric chloride\textsuperscript{45}, and triphosgene\textsuperscript{46}. 

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Scheme 2-1. The Staudinger Ketene-imine Reaction.

2.3.1 Synthesis of 4-Aryl-Substituted \( \beta \)-Lactams (±1 - ±23)

Herein, the racemic 3-acetoxy-1,4-diaryl-azetidin-2-ones (±1 - ±23) were synthesized in a three-step reaction scheme as described previously13 starting with the acid-catalyzed Schiff base formation from the corresponding substituted benzaldehyde and \( \text{para} \)-methoxyaniline. The Schiff base was then reacted with acetoxyacetyl chloride and triethylamine in dichloromethane to yield the \( \beta \)-lactam (Scheme 2-2). In all reactions, only one diastereoisomer possessing the C-3 acetoxy and C-4 aryl groups in the \textit{cis} configuration was produced resulting in formation of (±) racemates.

Scheme 2-2. General Synthesis of 4-Aryl-substituted \( \beta \)-lactams. 1 \( R = \text{H} \); 2 \( C_4 = (-\text{CH}=\text{CH}-\text{Ph}) \); 3 \( C_4 = (-\text{C} = \text{C}-\text{Ph}) \); 4 \( C_4 = (-\text{C}_2\text{H}_3\text{S}) \); 5 \( R = 4-\text{OCH}_3 \); 6 \( R = 4-(\text{O})\text{OCH}_3 \); 7 \( R = 4-\text{NO}_2 \); 8 \( R = 3-\text{NO}_2 \); 9 \( R = 4-\text{CN} \); 10 \( R = 4-(\text{CH}_3)_2\text{N} \); 11 \( R = 3-\text{F} \); 12 \( R = 4-\text{F} \); 13 \( R = 4-\text{Cl} \); 14 \( R = 3-\text{Cl} \); 15 \( R = 2-\text{Cl} \); 16 \( R = 2,4-\text{di-Cl} \); 17 \( R = 2-\text{Br} \); 18 \( R = 3-\text{Br} \); 19 \( R = 2-\text{I} \); 20 \( R = 4-\text{Br} \); 21 \( R = 4-\text{CF}_3 \); 22 \( R = 4-\text{C}(\text{CH}_3)_3 \); 23 \( R = 4-\text{CH}(\text{CH}_3)_2 \).
2.4 Chemical Synthesis of Optically Enriched 3-Hydroxy-4-Aryl-cis-β-lactams

As stated, 3-hydroxy-4-aryl-cis-β-lactams have become important substrates as precursors for analogues of the taxol side chain and also as a source of enantiomerically pure α-hydroxy-β-amino acids which are present in numerous biologically important compounds.\(^{47-49}\) To date, a number of enantioselective and diastereoselective syntheses have been developed in order to make available optically pure large scale quantities.

Shinkre et al\(^ {50}\) has reported the use of a chiral auxiliary derived from (-)-ephedrine (Scheme 2-3) toward the synthesis of enantiopure 3-hydroxy-4-aryl-cis-β-lactams. (-)-Ephedrine was reacted with α-ketopropionyl chloride or oxalyl chloride followed by Grignard addition to prepare a hemiketal of known stereochemistry. Subsequent alkylation with ethyl bromoacetate followed by base hydrolysis afforded the chiral acid which was reacted with triphosgene, an acid activator, and various imines in the presence of triethylamine. Refluxing the resulting compounds in THF with PTSA afforded the 3-hydroxy-4-aryl-cis-β-lactams.

In an effort to synthesize enantiomerically pure taxol C-13 side chain (N-benzoyl-(2R,3S)-3-phenylisoserine), Ojima et al. successfully applied a lithium chiral ester enolate-imine cyclocondensation resulting in the synthesis of 3-hydroxy-4-aryl-β-lactams in good yield and with enantiomeric excesses of >96%.\(^ {51}\) The reactions were carried out using a chiral lithium ester enolates, generated in situ from (silyloxy)acetates and N-(trimethylsilyl)imines to give the corresponding chiral β-lactams (Scheme 2-3).
Scheme 2-3. β-lactam Formation from Lithium Chiral Ester Enolate-imine Cyclocondensation.

Fernandez et al. focused on the [2+2] cycloaddition of chiral aldehyde hydrazone to α-benzylxoyketene (Scheme 2-4) as an approach to the synthesis of enantioenrich 4-alkyl(aryl)-3-hydroxy-β-lactams. Studies started with the reaction of proline-derived hydrazones 1a ± 5a with benzylxoyacetylchloride 6 which leads to the desired cycloadducts 7a ± 11a, respectively, in excellent yields (84 – 98%), even for compounds derived from easily enolizable substrates (Table 2-1, entries 1 – 4). Under optimized conditions (toluene, Et₃N, 80°C for primary substrates, 100°C for secondary or aromatic substrates), moderate to good 3R, 4S/3S, 4R selectivities were found, and only traces of
trans isomers were detected in some cases. Nevertheless, the collected results were satisfactorily evaluated because the diastereomers could be separated easily in all cases, thus allowing the isolation of the optically pure major cis isomers (3R, 4S)-7a – (3R, 4S)-11a in yields of 70 – 82%.

Scheme 2-4. [2+2] Cycloadditions of N,N-dialkylhydrazones to benzyloxyketene. 1: R= n-pentyl, 2: R= iBu, 3: R= PhCH₂CH₂, 4: R= iPr, 5: R= Ph. Bn= benzyl.
Table 2-1. Synthesis of 1-N,N-dialkylamino-3-benzyloxyazetidin-2-ones 7a ± 11a, 11b, and 8c.

<table>
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<tr>
<th>Entry</th>
<th>Hydration</th>
<th>$T$</th>
<th>Product</th>
<th>Yield [%][a]</th>
<th>3R,4S: cis: trans[b]</th>
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<tr>
<td>1</td>
<td>1a</td>
<td>80</td>
<td>7a</td>
<td>85 (70)</td>
<td>82:18 98:2</td>
</tr>
<tr>
<td>2</td>
<td>2a</td>
<td>80</td>
<td>8a</td>
<td>84 (73)</td>
<td>87:13 98:2</td>
</tr>
<tr>
<td>3</td>
<td>3a</td>
<td>80</td>
<td>9a</td>
<td>97 (78)</td>
<td>80:20 &gt;99:1</td>
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<tr>
<td>4</td>
<td>4a</td>
<td>100</td>
<td>10a</td>
<td>90 (82)</td>
<td>91:9  &gt;99:1</td>
</tr>
<tr>
<td>5</td>
<td>5a</td>
<td>100</td>
<td>11a</td>
<td>98 (75)</td>
<td>76:24  &gt;99:1</td>
</tr>
<tr>
<td>6</td>
<td>5b</td>
<td>100</td>
<td>11b</td>
<td>66</td>
<td>&lt;1:99  &gt;99:1</td>
</tr>
<tr>
<td>7</td>
<td>2c</td>
<td>80</td>
<td>8c</td>
<td>53</td>
<td>99:1   90:10</td>
</tr>
</tbody>
</table>

[a] Yield of isolated product. Values in parentheses correspond to the pure (98% de) major isomer. [b] Determined by means of $^{13}$C and $^1$H NMR spectroscopy.
The N-N bond cleavage, which is required to remove the auxiliaries, was successfully removed with magnesium monoperoxyphthalate (MMPP) to afford the deaminated lactams in high yields.

In yet another synthesis, Georg et al reported the first synthesis of taxol derivatives with substituted phenyl rings at the C-13 N-benzoyl-(2′R,3′S)-3′-phenylisoserine side chain of taxol. The synthesis of the novel phenylisoserine side chains was achieved through the asymmetric synthesis of 3-hydroxy-4-aryl-2-azetidinone derivatives via the ester enolate-imine cyclocondensation reaction (Scheme 2-5).

\[ R_1O\overset{\text{Ph}}{\text{OR}_2} + \overset{\text{Me}_3\text{Si}}{\text{NH}} \rightarrow \overset{\text{O}}{\text{NH}} \rightarrow \overset{\text{OH}}{\text{O}} \]

Scheme 2-5. Ester Enolate-imine Condensation of Optically Active Glycolates and N-(trimethylsilyl)imines.

The N-trimethylsilyl aldimines are readily available from aldehydes and lithium bis(trimethylsilyl)amide. The ester enolate cyclocondensation allows for the facile introduction of different substituents at the C-4 position of the β-lactam ring system. Thus, the methodology is very well suited for the synthesis of 3-phenylisoserine analogues with modified C-3 phenyl groups.

By all means, the above procedures are not an exhaustive review of the synthesis of 3-hydroxy-4-aryl-2-azetidinones. They do, however, form the basis of many common synthetic methodologies. Our method utilizes enzymes in the resolution of racemic 3-hydroxy-4-aryl-2-azetidinones as discussed below.
2.5 Lipase-Catalyzed Asymmetric Deacetylation of ±1 - ±23

Our experiences with lipase catalyzed resolution of esters and lactones in anhydrous organic solvents prompted investigation of the deacetylation of the β-lactams in anhydrous solvents. *Pseudomonas fluorescens* (AK), *Porcine pancreatic lipase* (PPL) and *Candida antarctica* (Novozym-435) lipases did not catalyze the transesterification reaction between the n-butanol and β-lactam acetoxy group in anhydrous tetrahydrofuran (THF), which we have previously found to be a solvent of choice for lipase catalyzed transesterifications. 

*Pseudomonas cepacia* (PS-30) lipase did catalyze the transesterification in anhydrous THF, however, deacetylation proceeded at an exceedingly slow rate and prolonged reaction times (> 6 days) were required for any reasonable substrate conversion. For example, hydrolysis of compound 7 in buffer (pH 7.2) led to 50% conversion within 48 hours, while deacetylation in dry THF/ n-butanol gave only 35% substrate conversion after 6 days.

The synthesis of the stereochemically pure β-lactam, therefore, was conceived via hydrolysis (*Scheme 2-6*) of the racemic acetates (±)1 – (±)23, which were exposed to a series of lipases from different sources (PS-30, Novozym-435, AK, PPL) in aqueous buffer.
Scheme 2-6. Lipase-Catalyzed Asymmetric Deacetylation of 4-Aryl-substituted β-lactams.  

1 R = H; 2 C4 = (-CH=CH-Ph); 3 C4 = (-C≡C-Ph); 4 C4 = (-C4H3S); 5 R = 4-OCH3; 6 R = 4-C(O)OCH3; 7 R = 4-NO2; 8 R = 3-NO2; 9 R = 4-CN; 10 R = 4-(CH3)2N; 11 R = 3-F; 12 R = 4-F; 13 R = 4-Cl; 14 R = 3-Cl; 15 R = 2-Cl; 16 R = 2,4-di-Cl; 17 R = 2-Br; 18 R = 3-Br; 19 R = 2-I; 20 R = 4-Br; 21 R = 4-CF3; 22 R = 4-C(CH3)3; 23 R = 4-CH(CH3)2.

Generally, microbial lipases exhibit pH optima ranging from 5.6-8.5 and at temperatures between 30-40 °C; we found that highest conversions were obtained in pH 7.2 phosphate buffer at 25 °C. Of the enzymes screened, lipases Novozym-435, AK and PPL did catalyze the hydrolysis of the β-lactam acetates in buffer solution however long reaction times (>10 days) were required. With Pseudomonas cepacia lipase (PS-30) reactions proceeded with moderate to excellent substrates conversion and hence it was identified as the most suitable biocatalyst for hydrolysis. Brieva et al also had previously described the lipase PS-30 as a suitable catalyst for enantioselective hydrolysis of the racemic 3-acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (1) in sodium phosphate buffer (pH 7.5). For optimal production of the alcohols (-) 1a – (-) 23a (Scheme 2), water insoluble racemic acetates (±) 1 - (±) 23 were dissolved in a minimal
amount of acetone and dispersed in an aqueous phosphate medium (pH 7.2) at 25 °C. The reaction was initiated by addition of predetermined amounts of lipase PS-30. The product alcohol and the unreacted acetate were extracted with ethyl acetate and separated readily by silica gel column chromatography (Table 2-2). Using a stepwise elution of ethyl acetate/petroleum ether (15:85) followed by ethyl acetate/petroleum ether (30:70), unreacted acetate and the alcohol were isolated in successive order.

The enantiopurity of the unreacted acetates were calculated from their $^1$H-NMR spectra acquired in presence of (+)-Eu(hfc)$_3$, a chiral shift reagent (Figure 2-1). The resonance signal of the acetoxy protons in the racemic mixture, a singlet in absence of the chiral shift reagent, was split into two signals of equal intensity for the two enantiomers in the presence of (+)-Eu(hfc)$_3$. The product alcohol was acetylated and enantiomeric purity was determined using (+)-Eu(hfc)$_3$ in $^1$H-NMR experiments.
Table 2-2. Lipase PS-30 Catalyzed Hydrolysis of Substituted β-lactams (±)1 – (±)23.

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<th>Substratea</th>
<th>Convb (%)</th>
<th>(3R, 4S)-acetoxy-c</th>
<th>%yieldd</th>
<th>[α]D 25 e</th>
<th>E</th>
<th>(3S, 4R)-hydroxy-c</th>
<th>%yieldd</th>
<th>[α]D 25 e</th>
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aRepresents the aryl substituents on the β-lactam ring. bDetermined from 1H-NMR of crude product mixture after 48 h. cDetermined from 1H-NMR spectra in the presence of Eu(hfc)₃, by integration of CH₃C=O. dYield 100% at 50% conversion. 
ec=0.01 (g/ml), CHCl₃. fYield 100% at 0% conversion. gc=0.01 (g/ml), CH₃OH.
Figure 2-1. a) $^1$H-NMR of Racemic 3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-β-lactam with Eu(hfc)$_3$. b) $^1$H-NMR of unreacted (+) (3R, 4S)-3-acetoxy-1-(4-methoxyphenyl)-4-phenyl-β-lactam with Eu(hfc)$_3$. c) $^1$H-NMR of acetylated (-) (3R, 4S)-3-hydroxy-1-(4-methoxyphenyl)-4-phenyl-β-lactam with Eu(hfc)$_3$. 
The activity of different lipases was greatly dependent upon its source, i.e., while lipases CALB (Novozym-435, immobilized form from *Candida antarctica*), AK (from *Pseudomonas fluorescens*) and *Porcine pancreatic lipase* (PPL) showed little activity, the lipase PS-30 (from *Pseudomonas cepacia*) was highly efficient in buffer solution. The effect of the enzyme concentration on the substrate conversion was studied using a 1:1 (w/w) and 2:1 (w/w) lactam to lipase PS-30 ratio (*Figure 2-2*). The rate of acetate hydrolysis, as expected, was higher using more lipase in the reaction, for example 50 % conversion was achieved after 11 hours using 1:1 while more than 18 hours were required using 2:1 substrate to lipase ratios. For subsequent studies, a 1:1 (w/w) substrate to lipase ratio was used (*Table 2-2*).

![Figure 2-2](image)

**Figure 2-2.** Variation in Substrate Conversion with Time using Lactam 1 to PS-30 ratio (w/w) of 1:1 (▲) and 2:1 (■).
To understand the effect of β-lactam ring substitution on hydrolytic activity of lipase PS-30, twenty three β-lactam substrates with different substituents on C-4 were synthesized and subjected to lipase-catalyzed hydrolysis in phosphate buffer (pH 7.2). The results of the *Pseudomonas cepacia* lipase-catalyzed hydrolysis after 48 hours are shown in Table 2-2. Even though lipase PS-30 accepted substituted β-lactam rings with phenyl (1), thiophen-2-yl (4), phenylethenyl (2), and phenylethynyl (3) at the C-4 position as substrates, the C-4 substituent on the β-lactam ring influenced the hydrolysis to a great extent and substrate conversions were greatly affected (Table 2-2). For all substrates, the lipase-catalyzed hydrolysis proceeded with high enantioselectivity towards the 3S enantiomer. For example, in compound 1, prolonged reaction time (>72 h, not shown) beyond 50% conversion did not result in hydrolysis of the 3R enantiomer (Figure 2) indicating that the enzymatic reaction proceeded with great efficiency as measured by enantioselectivity ($E = 1057$) and substrate conversion (~50%). The lipase enantioselectivity ($E$) values were determined from the following equation: $E = \ln[(1-c)(1-ee_s)] / \ln[(1-c)(1+ee_s)]$ where the $ee = [(S)-(R)] / [(S)+(R)] * 100\%$. The enantiomeric excesses were determined from $^1$H-NMR spectra recorded in presence of the (+)-[Eu(hfc)$_3$] (Figure 2-1).

To understand how the C-4 substituent affected lipase PS-30’s ability to hydrolyze the acetoxy group, a number of substrates that differ in the position, size, and electronic nature of substituent(s) on the C-4 phenyl ring were subjected to hydrolysis and substrate conversions were carefully monitored as a function of reaction time by $^1$H-NMR (Figure 2-3). From figure 2-3, it is important to note that:
The hydrolytic ability of the lipase PS-30, as illustrated by substrate conversion, was greatly affected by the substituent(s) on the C-4 aryl ring.

Substrates 6, 14, 13, 20 and 21 with electron withdrawing groups (EWG) on the aryl ring typically had quantitative conversions (~50 %) within 48 h, while compounds 5, 10, 22 and 23 with electron donating groups (EDG) had lower substrate conversions and more than 48 h were needed to achieve reasonable conversion (Figure 2-3 and Table 2-2).

Among the electron withdrawing groups screened, the position of the substituent(s) on the phenyl ring was of importance in realizing high conversion. In general, a group at the C-4′ position, i.e., further away form the lactam ring, on the phenyl moiety gave higher conversion. For example, with C-4′ chloro (in 13) or bromo (in 20) substituents quantitative conversions were achieved but the C-2′ chloro (in 15) and bromo (in 17) analogs gave only 32 and 27 % substrate conversions, respectively, in 13 hours (Figure 3). In the case of compound 16 having two chloro groups at C-4′ and C-2′, the substrate conversion were about 32%, i.e., much lower than that in compound 13 with a C-4′ chloro substituent.

The size of a substituent on the C-4 phenyl ring was another important factor that affected substrate conversion. For an electron withdrawing group at C-4′ the conversion increased with increasing size (12<7<21<13<20), while for group at C2′ or C-3′ the conversion decreased with increasing size of the substituent (15<17<19). For example, only 21 % substrate conversion in 48h was achieved for compound 19, with a large iodo group at C-2′, while 35 % substrate conversion was observed for compound 15 with chloro, a relatively smaller
group, at C-2'. Compounds 15, 17, and 19 with chloro, bromo and iodo groups have decreased substrate conversion, which follow the order of their size (Cl<Br<I, Figure 2-3) or electronegativity (Cl>Br>I). For compounds with electron donating groups on the C-4 phenyl ring the substrate conversion decreased as the size of the substituent increased, ie., 4-tert-butyl < 4-isopropyl < 4-N,N-dimethylamino < 4-methoxy < phenyl. The lower substrate conversion in these substrates may be attributed to their steric bulkiness, which could result in van der Waals repulsive interactions in the enzyme active site.
Figure 2-3. Effect of C-4 Substitution on Substrate Conversion in PS-30 Catalyzed Hydrolysis.
• The dependence of the substrate conversion on the size and electronic nature of the C-4 substituent suggested that steric as well as electronic characteristic of the substrates drive the lipase PS-30 catalyzed hydrolysis.

In compound 7, analysis of the $^1$H-NMR spectra (Figure 2-4) of the reaction mixture at 50 % conversion revealed that the product alcohol was a 1:1 racemic mixture of the cis and trans alcohols. The reaction mixture was subjected to flash column chromatography to obtain the recovered (3R, 4S)-acetate (50 %, $[\alpha]_D^{25} = +56^\circ$, %ee = 99), racemic-cis-alcohol (25 %, $[\alpha]_D^{25} = 0^\circ$, %ee= 0), and racemic-trans-alcohol (25 %, $[\alpha]_D^{25} = 0^\circ$, %ee= 0) (Figure 2). The formation of the cis- and trans- alcohols from a cis- acetate can only be explained via an unusual ring opening across the lactam C3-C4 bond to a benzylic carbanion (stabilized by the 4'-nitro group) that regenerates the lactam ring by attack on the planar carbonyl group resulting in formation of the racemic cis- and trans-3-hydroxy-β-lactam derivatives upon double epimerization (Scheme 2-7). The observation that the recovered β-lactam derivative, i.e., (3R)-acetoxy-(4S)-(4-nitrophenyl) was optically active ($[\alpha]_D^{25} = +112^\circ$) but the hydroxy compounds were not only racemic but a mixture of cis- and trans- stereoisomers suggested that the ring opening across the C3-C4 bond in the hydroxyl compound might involve a non enzymatic process. However, it was not obvious if the double epimerization involved an enzymatic process or was just a reaction artifact.
To uncover the role of enzyme in the epimerization process, 3-hydroxy-4-(4-nitrophenyl)-1-(4-methoxyphenyl)azetidin-2-one was sought. The compound, however, was stable to hydrolysis in phosphate buffer (pH 7.2) in the absence of the lipase, indicating that the hydrolysis observed in the presence of lipase indeed was an enzymatic process. Several attempts to obtain the cis-hydroxy compound upon chemical hydrolysis of racemic cis-7 (Scheme 2-7) using K$_2$CO$_3$/acetone and K$_2$CO$_3$/CH$_3$OH at room temperature or 0°C led to the formation of the diastereomeric racemic cis- and trans- alcohols, suggesting that the epimerization of the hydroxy lactam (under basic conditions) was a chemical process. Further, when the cis-3-hydroxy-4-(4-nitrophenyl)-1-(4-methoxyphenyl)azetidin-2-one, isolated upon column chromatography from the cis-/trans- mixture, was stirred in phosphate buffer (pH 7.2, without lipase) it rapidly led to an equilibrium mixture of the cis-/trans- hydroxy products. To elucidate the role of buffer in this racemization reaction, we removed the buffer from the enzymatic reaction.
and instead the lipase catalyzed deacetylation of 7 was carried out in dry THF/n-butanol. Though deacetylation in THF/n-butanol was slow, optically pure cis-hydroxy compound ([α]D$^{25} = -71.2^\circ$) and unreacted optically enriched (+)-cis-acetate were isolated after 6 days. The positive and negative sign of the optical rotation for the recovered acetate and the hydroxy product, respectively, established that the enantiopreference of the lipase PS remained unchanged between the buffer and the THF reaction media. The optically pure (-)-cis-hydroxy compound was subsequently stirred in phosphate buffer, pH 7.4, in absence of lipase. The isolated compound was found to be racemic mixture of the cis- and trans- hydroxyl compounds. The formation of the trans- isomer from the cis-isomer in absence of the lipase confirmed that the double epimerization was a result of a non-enzymatic reaction and that a free hydroxyl group was required for the double epimerization. In light of these observations, it was concluded that (1) racemization of the product alcohol was an artifact and not a result of the enzymatic catalysis and (2) a non-enzymatic cleavage of the C3-C4 bond followed by ring closure involving double epimerization, it is believed, resulted in formation of a racemic, diastereomeric mixture of the hydroxyl products (Scheme 2-7).
Scheme 2-7. Schematic Representation of Different Chemical and Enzymatic Processes leading to the Double Epimerization of 3-Hydroxy-4-(4-nitrophenyl)azetidin-2-one.
2.6 Determination of Absolute Configuration

The absolute configuration of the two enantiomers was determined by X-ray crystallography. Single crystals suitable for x-ray crystallographic analysis were selected following examination under a microscope. Single-crystal x-ray diffraction data for the compound were collected on a Bruker-AXS SMART APEX/CCD diffractometer. Diffraacted data were corrected for absorption using the SADABBS program. Direct methods and the structure solution solved the structures and refinement was based on $F^2$. All non-hydrogen atoms were refined with anisotropic displacement parameters whereas hydrogen atoms were placed in calculated positions and given isotropic $U$ values based on the atom to which they are bonded. All crystallographic calculations were conducted with the SHELXTL 6.1 program package.

For the crystal of (+)-(3$R$, 4$S$)-3-acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one, two molecules were found in each unit cell. The compound crystallizes in a monoclinic space group P2 (1), with cell dimensions $a = 5.7617$ (8) Å, $b = 8.1159$ (11) Å, $c = 16.889$ (2) Å. A total of 4910 unique reflection data were obtained to give a final $R$ index [$I > 2\sigma(I)]$ of 0.0466 and a flack parameter of 0.0 (9). The results of the structure determination study confirmed the cis- (3$R$, 4$S$) configuration of the unreacted substrate, (+)-(3$R$, 4$S$)-3-acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (Figure 2-5). In the crystal of (+)-(3$R$, 4$R$)-3-acetoxy-1-(4-methoxyphenyl)-4-thiophen-2-ylazetidin-2-one, two molecules were found in each unit cell. The compound crystallized in a monoclinic space group P2 (1), with cell dimensions $a = 5.3082$ (10) Å, $b = 8.4869$ (16) Å, $c = 17.005$ (3) Å. A total of 4638 unique reflection data were obtained to give a final $R$ index.
[I > 2σ(I)] of 0.0618 and flack parameter of 0.19(16). The results of the structure determination study confirmed the cis- (3R, 4R) configuration (Figure 2-5).

**Figure 2-5.** Ortep Plot for the X-ray structures (a) of (+)-(3R, 4S)-3-acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (1) and (b) (+)-(3R,4R)-3-acetoxy-1-(4-methoxyphenyl)-4-thiophen-2-ylazetidin-2-one (4).
2.7 Stereopreference of the Lipase PS-30

X-ray crystallographic data (Figure 2-5) collected using a single crystal of the unreacted enantiomer of the 4-phenyl (1) and 4-thiophen-2-yl (4) β-lactam supported the (S)-enantiopreference of the Pseudomonas cepacia lipase. The data confirmed (3R, 4S) and (3R, 4R) as the absolute configurations for the unreacted enantiomer of 1 and 4, respectively. Hence the reacted enantiomers must possess the (3S) absolute configurations.

Modeling experiments\textsuperscript{56-58} have indicated that Pseudomonas cepacia has a stereochemical preference for the (R)-substrate over the (S)-substrate but its stereoselectivity seems to be dependent on the chemical nature and/or physical state of the substrate, as has been observed for other lipases.\textsuperscript{59} The stereopreference of the lipase PS-30 for the (3S, 4R) enantiomer was rationalized using a known active site model (Figure 2-6) for the lipase as described by Zuegg et al.\textsuperscript{56} While the hydrogen at the asymmetric center of both enantiomers is directed towards the same site (referred to as \textbf{H}-alignment), the medium sized group (the carbonyl function) and the large group (the phenyl attached to the C-4 stereogenic center) trade places. This allows the medium-sized group to fit into the smaller hydrophobic pocket in the favored enantiomer whereas the larger-sized group is made to accommodate the smaller hydrophobic pocket in the disfavored enantiomer.
Table 2-6. Schematic representations of the orientation of favored (3S, 4R) and disfavored (3R, 4S) enantiomer in active site of the lipase PS-30. M = medium, L = large.
2.8 Experimental Methods and Materials

Lipases PS-30 and AYS were generous gifts from Amano Enzymes, Novozym-435 was donated by Novo Nordisk. $^1$H-NMR spectra were recorded on a Bruker 250 MHz spectrometer and a Bruker 360 MHz spectrometer (Appendix A) in CDCl$_3$ or CD$_3$OD with TMS as the internal standard. Optical rotations were measured with an AutoPol-IV (Rudolph research analytical) automatic polarimeter. Elemental analysis was performed using a NC-2100 Soil Analyzer. Thin-layer chromatography (TLC) was performed on glass plates coated with 0.25mm thickness of silica-gel. All solvents were distilled prior to use and organic solvent extracts dried over Na$_2$SO$_4$.

Single-crystal x-ray diffraction data for the compounds 1 and 4 were collected on a Bruker-AXS SMART APEX/CCD diffractometer using a Mo radiation source. Diffracted data were corrected for absorption using the SADABS program. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 191837 & 191838. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union road, Cambridge CB2 1Ez, UK [Fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

2.8.1 General Procedure for Synthesis of Racemic 3-Acetoxy-4-arylazetidin-2-ones 1-23

A solution of acetoxyacetyl chloride (7.30 mmol) in dry dichloromethane (12 ml) was added drop wise over 1 hr at room temperature to a mixture of the appropriately substituted Schiff base (8.76 mmol) and triethylamine (17.52 mmol) in dry
dichloromethane (18 ml). The reaction mixture was stirred for an additional hour at room
temperature and was then washed with water (2 x 50 ml). The organic layer was
separated and dried over sodium sulfate. Evaporation of the solvent followed by
purification by column chromatography (dichloromethane/ ether, 9:1) afforded the
desired product.

*Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (±)* – 1

With the procedure described above 1 was prepared as a white solid, mp 163 – 165°C
(lit. 158-160°C). \(^1\)H-NMR (250 MHz, CDCl\(_3\)) \(\delta\) (ppm): 1.67 (3H, s, CH\(_3\)C=O), 3.75
(3H, s, CH\(_3\)O), 5.34 (1H, d, \(J = 4.84\) Hz, 4\(H\)), 5.94 (1H, d, \(J = 4.85\) Hz, 3\(H\)), 6.78-7.34
(9H, m, Ph). \(^13\)C-NMR (62.5 MHz, CDCl\(_3\)): 19.8, 55.5, 61.4, 77.3, 114.1, 114.4, 118.8,
127.9, 128.5, 128.8, 130.3, 132.2, 156.6, 161.3, 169.3.

*Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(2-phenylethynyl)azetidin-2-one (±)* – 2

With the procedure described above 2 was prepared as a yellow solid, mp 138 – 140°C.
\(^1\)H-NMR (250 MHz, CDCl\(_3\)) \(\delta\) (ppm): 2.07 (3H, s, CH\(_3\)C=O), 3.77 (3H, s, CH\(_3\)O), 4.94
(1H, dd, \(J = 4.71, 2.98\) Hz, 3\(H\)), 5.90 (1H, d, \(J = 4.92\) Hz, 4\(H\)), 6.16 (1H, dd, \(J = 8.0, 8.04\)
Hz, 5\(H\)), 6.80 (1H, d, \(J = 17.0\) Hz, 6\(H\)), 6.83-7.43 (9H, m, Ph). \(^13\)C-NMR (62.5 MHz, CDCl\(_3\)): 20.3, 55.4, 60.1, 76.3, 114.1, 114.4, 118.7, 121.4, 126.7, 128.7, 130.6,
135.5, 137.1, 156.6, 161.1, 169.5.
Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(phenylethynyl)azetidin-2-one (±) -3

With the procedure described above 3 was prepared as a white solid, mp 94-96°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 2.21 (3H, s, CH$_3$C=O), 3.80 (3H, s, CH$_3$O), 5.12 (1H, d, $J = 4.67$ Hz, 4H), 5.88 (1H, d, $J = 4.66$ Hz, 3H), 6.90-7.54 (9H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 20.3, 51.7, 55.3, 76.7, 81.0, 114.2, 118.7, 121.5, 128.3, 129.1, 130.0, 132.0, 156.6, 161.1, 169.5. Anal calcd. for C$_{20}$H$_{17}$NO$_4$0.22H$_2$O: C, 70.78; H, 5.05; N, 4.13. Found: C, 70.92; H, 5.06; N, 3.95.

Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(thiophen-2-yl)azetidin-2-one (±) –4

With the procedure described above 4 was prepared as a white solid, mp 148-149°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.87 (3H, s, CH$_3$C=O), 3.76 (3H, s, CH$_3$O), 5.62 (1H, d, $J = 4.72$ Hz, 4H), 5.96 (1H, d, $J = 4.73$ Hz, 3H), 6.80-7.38 (7H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 20.0, 55.4, 57.6, 114.4, 118.8, 126.8, 127.4, 128.4, 130.0, 135.6, 156.7, 161.0, 169.3.

Racemic 3-Acetoxy-1, 4-bis-(4-methoxyphenyl)azetidin-2-one (±) –5

With the procedure described above 5 was prepared as a white solid, mp 144-145°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.66 (3H, s, CH$_3$C=O), 3.68 (3H, s, CH$_3$O), 3.73 (3H, s, CH$_3$O), 5.22 (1H, d, $J = 4.78$ Hz, 4H), 5.82 (1H, d, $J = 4.78$ Hz, 3H), 6.71-7.22 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.9, 55.4, 61.1, 113.6, 113.9, 114.3, 118.8, 124.0, 129.2, 130.3, 156.5, 159.9, 161.4, 169.3.
Racemic 3-Acetoxy-4-(4-methoxycarbonylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (±)
- 6

With the procedure described above 6 was prepared as a white solid, mp 151-152°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.62 (3H, s, CH$_3$C=O), 3.68 (3H, s, CH$_3$O), 3.85 (3H, s, CH$_3$O), 5.32 (1H, d, $J = 4.85$ Hz, 4H), 5.89 (1H, d, $J = 4.9$ Hz, 3H), 6.72-7.97 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.8, 52.3, 55.5, 61.1, 76.3, 114.5, 118.7, 127.9, 129.7, 130.0, 130.6, 137.6, 156.7, 160.9, 166.5, 169.2. Anal calcd. for C$_{20}$H$_{19}$NO$_6$·0.82H$_2$O: C, 62.52; H, 4.98; N, 3.64. Found: C, 62.64; H, 4.99; N, 3.48.

Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one (±) –7

With the procedure described above 7 was prepared as a yellow solid, mp 162-164°C (lit.55 165°C). $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.74 (3H, s, CH$_3$C=O), 3.76 (3H, s, CH$_3$O), 5.45 (1H, d, $J = 4.93$ Hz, 4H), 5.99 (1H, d, $J = 4.93$ Hz, 3H), 6.80-8.24 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.9, 55.5, 60.6, 114.6, 118.6, 123.7, 128.9, 129.6, 140.0, 148.2, 156.9, 160.6, 169.1.

Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(3-nitrophenyl)azetidin-2-one (±) –8

With the procedure described above 8 was prepared as a brown solid, mp 89-91°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.73 (3H, s, CH$_3$C=O), 3.76 (3H, s, CH$_3$O), 5.45 (1H, d, $J = 4.85$ Hz, 4H), 5.99 (1H, d, $J = 4.90$ Hz, 3H), 6.80-8.24 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.8, 55.3, 60.2, 76.3, 113.2, 114.3, 118.6, 122.9, 123.5, 129.6, 133.8, 135.00, 148.3, 156.8, 160.6, 169.0. Anal calcd. for C$_{18}$H$_{16}$N$_2$O$_6$·0.75H$_2$O: C, 58.46; H, 4.36; N, 7.57. Found: C, 58.79; H, 4.39; N, 7.00.
Racemic 3-Acetoxy-4-(4-cyanophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -9

With the procedure described above 9 was prepared as a yellow solid, mp 178-180°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 1.66 (3H, s, CH$_3$C=O), 3.69 (3H, s, CH$_3$O), 5.32 (1H, d, $J = 4.88$ Hz, 4H), 5.90 (1H, d, $J = 4.9$ Hz, 3H), 6.73-7.61 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.8, 55.5, 60.8, 76.4, 112.8, 114.6, 118.2, 118.6, 128.6, 129.7, 132.3, 138.0, 156.9, 160.6, 169.1. Anal calcd. for C$_{19}$H$_{16}$N$_2$O$_4$: C, 62.26; H, 4.39; N, 7.64. Found: C, 62.83; H, 4.44; N, 6.87.

Racemic 3-Acetoxy-4-(4-dimethylaminophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -10.

With the procedure described above 10 was prepared as a white solid, mp 198-199°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 1.67 (3H, s, CH$_3$C=O), 2.88 (6H, s, N(CH$_3$)$_2$), 3.67(3H, s, CH$_3$O), 5.18 (1H, d, $J = 5.11$ Hz, 4H), 5.81 (1H, d, $J = 5.11$ Hz, 3H), 6.61-7.24 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) $\delta$ (ppm): 20.0, 40.6, 55.4, 61.4, 112.9, 114.3, 118.9, 129.0, 130.5, 156.4, 161.6, 169.4. Anal calcd. for C$_{20}$H$_{22}$N$_2$O$_4$: C, 59.45; H, 5.49; N, 6.93. Found: C, 60.35; H, 5.57; N, 5.71.

Racemic 3-Acetoxy-4-(3-fluorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -11

With the procedure described above 11 was prepared as a white solid, mp 166-167°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 1.67 (3H, s, CH$_3$C=O), 3.69 (3H, s, CH$_3$O), 5.26 (1H, d, $J = 4.85$ Hz, 4H), 5.88 (1H, d, $J = 4.88$ Hz, 3H), 6.73-7.22 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.8, 55.4, 60.8, 76.2, 114.5, 114.7, 115.6, 116.0, 118.7,
Racemic 3-Acetoxy-4-(4-fluorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -12

With the procedure described above 12 was prepared as a yellow solid, mp 136 °C (lit.60 136-137°C). 1H-NMR (250 MHz, CD3OD) δ (ppm): 1.60 (3H, s, CH3C=O), 3.62 (3H, s, CH3O), 5.39 (1H, d, J = 4.79 Hz, 4H), 5.84 (1H, d, J = 4.82 Hz, 3H), 6.73-7.24 (8H, m, Ph). 13C-NMR (62.5 MHz, CD3OD): 19.7, 55.9, 62.0, 77.7, 115.2, 115.5, 116.2, 116.5, 120.1, 130.2, 131.4, 158.3, 163.3, 170.5.

Racemic 3-Acetoxy-4-(4-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -13.

With the procedure described above 13 was prepared as a white solid, mp 172-173°C. 1H-NMR (250 MHz, CDCl3) δ (ppm): 1.67 (3H, s, CH3C=O), 3.68(3H, s, CH3O), 5.25 (1H, d, J = 4.81 Hz, 4H), 5.85 (1H, d, J = 4.82 Hz, 3H), 6.72-7.28 (8H, m, Ph). 13C-NMR (62.5 MHz, CDCl3) δ (ppm): 55.4, 58.8, 84.9, 114.4, 118.6, 127.0, 128.9, 129.5, 130.3, 131.1, 133.2, 156.4, 163.6. Anal calcd. for C18H16ClNO4.1.25H2O: C, 58.71; H, 4.38; N, 3.80. Found: C, 57.50; H, 4.29; N, 3.35.

Racemic 3-Acetoxy-4-(3-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -14

With the procedure described above 14 was prepared as a yellow solid, mp 132-134°C. 1H-NMR (250 MHz, CDCl3) δ (ppm): 1.68 (3H, s, CH3C=O), 3.69 (3H, s, CH3O), 5.23 (1H, d, J = 4.88 Hz, 4H), 5.87 (1H, d, J = 4.90 Hz, 3H), 6.73-7.25 (8H, m, Ph). 13C-NMR (62.5 MHz, CDCl3): 19.8, 55.4, 60.8, 76.2, 114.2, 114.5, 118.7, 126.1, 127.9, 157.6.
129.1, 130.0, 134.6, 156.7, 161.0, 169.2. Anal calcd. for C$_{18}$H$_{16}$ClNO$_4$: C, 62.52; H, 4.66; N, 4.05. Found: C, 61.23; H, 4.56; N, 3.91.

*Racemic 3-Acetoxy-4-(2-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -15.*

With the procedure described above 15 was prepared as a yellow solid, mp 95-97°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.69 (3H, s, CH$_3$C=O), 3.69(3H, s, CH$_3$O), 5.71 (1H, d, $J = 4.98$ Hz, 4H), 6.09 (1H, d, $J = 4.98$ Hz, 3H), 6.74-7.38 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ (ppm): 19.9, 55.4, 58.2, 75.4, 114.5, 118.6, 126.8, 128.7, 129.8, 130.0, 130.2, 133.8, 156.7, 161.4, 168.7. Anal calcd. for C$_{18}$H$_{16}$ClNO$_4$·2.45H$_2$O: C, 55.45; H, 4.13; N, 3.59. Found: C, 51.82; H, 4.56; N, 3.56.

*Racemic 3-Acetoxy-4-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) –16*

With the procedure described above 16 was prepared as a white solid, mp 116-118°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.86 (3H, s, CH$_3$C=O), 3.75 (3H, s, CH$_3$O), 6.07 (1H, d, $J = 5.0$ Hz, 4H), 6.21 (1H, d, $J = 5.0$ Hz, 3H), 6.80-7.43 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ (ppm): 20.0, 55.4, 57.8, 75.4, 114.5, 118.6, 127.2, 128.9, 129.7, 134.6, 135.2, 156.8, 161.2, 168.7. Anal calcd. for C$_{18}$H$_{15}$Cl$_2$NO$_4$: C, 56.86; H, 3.98; N, 3.68. Found: C, 56.51; H, 3.96; N, 3.57.

*Racemic 3-Acetoxy-4-(2-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) –17*

With the procedure described above 17 was prepared as a white solid, mp 79-80°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.77 (3H, s, CH$_3$C=O), 3.77 (3H, s, CH$_3$O), 5.76 (1H,
d, $J = 5.0$ Hz, $4H$), 6.19 (1H, d, $J = 5.0$ Hz, $3H$), 6.81-7.64 (8H, m, Ph). $^{13}$C-NMR: 114.2, 114.8, 118.7, 123.7, 127.3, 128.9, 130.0, 131.7, 133.1, 156.7, 161.4, 168.7.

Racemic 3-Acetoxy-4-(3-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) –18

With the procedure described above 18 was prepared as a yellow solid, mp 150-152°C.

$^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.75 (3H, s, CH$_3$C=O), 3.76 (3H, s, CH$_3$O), 5.29 (1H, d, $J = 4.86$ Hz, $4H$), 5.94 (1H, d, $J = 4.86$ Hz, $3H$), 6.80-7.46 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.8, 55.4, 60.8, 76.2, 114.5, 118.7, 122.6, 126.6, 130.0, 130.8, 132.1, 134.8, 156.7, 161.0, 169.1.

Racemic 3-Acetoxy-4-(2-iodophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) -19

With the procedure described above 19 was prepared as a white solid, mp 139-142°C.

$^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.76 (3H, s, CH$_3$C=O), 3.77 (3H, s, CH$_3$O), 5.63 (1H, d, $J = 4.96$ Hz, $4H$), 6.18 (1H, d, $J = 4.99$ Hz $3H$), 6.82-7.91 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 20.0, 55.5, 65.4, 75.4, 98.8, 114.5, 118.7, 128.2, 128.6, 130.0, 130.3, 134.5, 139.8, 156.7, 161.3, 168.7. Anal calcd. for C$_{18}$H$_{16}$INO$_4$: C, 49.45; H, 3.69; N, 3.20. Found: C, 49.37; H, 3.68; N, 3.04.

Racemic 3-Acetoxy-4-(4-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one (±) –20

With the procedure described above 20 was prepared as a white solid, mp 178-180°C. $^1$H-NMR (360 MHz, CDCl$_3$) δ (ppm): 1.76 (3H, s, CH$_3$C=O), 3.78 (3H, s, CH$_3$O), 5.30 (1H, d, $J = 4.95$ Hz, $4H$), 5.92 (1H, d, $J = 5.0$ Hz, $3H$), 6.80-7.51 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 19.9, 55.4, 60.9, 76.2, 114.5, 118.7, 122.9, 129.6, 130.0, 131.5, 131.7,
Racemic 3-Acetoxy-4-(4-trifluoromethylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (+) – 21

With the procedure described above 21 was prepared as a white solid, mp 154-156°C. \( \text{\textsuperscript{1}}H\)-NMR (250 MHz, CDCl\(_3\)) \( \delta \) (ppm): 1.70 (3H, s, CH\(_3\)C=O), 3.76 (3H, s, CH\(_3\)O), 5.40 (1H, d, \( J = 5.0 \) Hz, 4H), 5.97 (1H, d, \( J = 5.0 \) Hz, 3H), 6.80-7.66 (8H, m, Ph). \( \text{\textsuperscript{13}}C\)-NMR (62.5 MHz, CDCl\(_3\)): 19.7, 55.4, 60.8, 76.3, 114.5, 118.6, 125.4, 128.3, 129.8, 130.7, 136.6, 156.7, 160.8, 169.1. Anal calcd. for C\(_{19}\)H\(_{16}\)F\(_3\)NO\(_4\): C, 60.16; H, 4.25; N, 3.69. Found: C, 60.14; H, 4.25; N, 3.74.

Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-tert-butyl phenyl)azetidin-2-one (+) – 22

With the procedure described above 22 was prepared as a white solid, mp 175 – 177°C. \( \text{\textsuperscript{1}}H\)-NMR (250 MHz, CDCl\(_3\)) \( \delta \) (ppm): 1.22 (9H, s, C(CH\(_3\))\(_3\)), 1.56 (3H, s, CH\(_3\)C=O), 3.68 (3H, s, CH\(_3\)O), 5.24 (1H, d, \( J = 4.8 \) Hz, 4H), 5.84 (1H, d, \( J = 4.8 \) Hz, 3H), 6.72-7.29 (8H, m, Ph). \( \text{\textsuperscript{13}}C\)-NMR (62.5 MHz, CDCl\(_3\)): 19.7, 31.2, 34.6, 55.4, 61.2, 114.3, 118.8, 125.3, 127.6, 129.1, 130.3, 151.9, 156.5, 161.3, 169.3. Anal calcd. for C\(_{22}\)H\(_{25}\)NO\(_4\): C, 71.91; H, 6.86; N, 3.81. Found: C, 71.85; H, 6.72; N, 3.74.

Racemic 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-isopropylphenyl)azetidin-2-one (+) – 23

With the procedure described above 23 was prepared as a white solid, mp 131 – 133°C. \( \text{\textsuperscript{1}}H\)-NMR (250 MHz, CDCl\(_3\)) \( \delta \) (ppm): 1.15 (6H, d, \( J = 7 \) Hz, CH(CH\(_3\))\(_2\)), 1.58 (3H, s,
$CH_3C=O$), 2.82 (1H, sep., $J = 6.9$ Hz, $CH(CH_3)_3$), 3.68 (3H, s, $CH_3O$), 5.24 (1H, d, $J = 4.83$ Hz, 4$H$), 5.84 (1H, d, $J = 4.83$ Hz, 3$H$), 6.72-7.24 (8H, m, $Ph$). $^{13}C$-NMR (62.5 MHz, CDCl$_3$): 19.7, 23.9, 33.8, 55.4, 61.3, 114.3, 118.8, 126.5, 127.9, 129.4, 130.3, 149.6, 156.5, 161.4, 169.2. Anal calcd. for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.30; H, 6.44; N, 3.85.

2.8.2 General Procedure for Lipase-Catalyzed Hydrolysis of the Acetoxy $\beta$-Lactam

The racemic 3-acetoxy $\beta$-lactam (1-23, 200 mg) was dissolved in acetone (5 ml) and then added to phosphate buffer (25 ml, pH 7.2) in a 50 ml round bottom flask. To this reaction mixture was added the enzyme catalyst (lipase PS-30, 100mg) and reaction flask was capped to prevent loss of co-solvent due to evaporation. The reaction mixture was stirred at 25 $^\circ$C and progress of the reaction was monitored via TLC until no further change was detected. The products were isolated by extraction of the reaction mixture with ethyl acetate. The crude product mixture was analyzed by $^1$H-NMR to determine the percent conversion. The hydrolyzed product (the secondary alcohol) and the unreacted O-acetate were separated by column chromatography using a stepwise elution of ethyl acetate: petroleum ether (15:85) followed by ethyl acetate: petroleum ether (30:70).

For monitoring substrate conversion with time, reactions were done in 20 mL scintillation vials using 1:1 ratio (w/w) of substrate to the lipase PS-30. After predetermined times, aliquot were withdrawn from the reaction, extracted with ethyl acetate, and the crude reaction product after evaporation of the solvent was analyzed by
1H-NMR spectroscopy to determine the substrate conversion. (See Appendix A for NMR spectra.)

3-Hydroxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (--)1a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 1 afforded 1a as a white solid, mp 190-192°C (lit.60 210-212°C). 1H-NMR (250 MHz, CDCl₃) δ (ppm): 3.76 (3H, s, CH₃O), 5.16 (1H, d, J = 5.0 Hz, 4H), 5.27 (1H, d, J = 4.98 Hz, 3H), 6.79-7.45 (9H, m, Ph). 13C-NMR (62.5 MHz, CDCl₃): 55.4, 62.2, 77.2, 114.1, 114.4, 118.8, 127.4, 129.0, 129.2, 130.4, 133.1, 156.4, 165.3.

3-Hydroxy-1-(4-methoxyphenyl)-4-(2-phenylethynyl)azetidin-2-one (--)2a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 2 afforded 2a as a white solid, mp 166-167°C (lit.65 157-160°C). 1H-NMR (250 MHz, CDCl₃) δ (ppm): 3.74 (3H, s, CH₃O), 4.38 (1H, bs, OH), 4.80 (1H, dd, J = 5.13, 2.73 Hz, 3H), 5.13 (1H, d, J = 4.98 Hz, 4H), 6.41 (1H, dd, J = 8.0, 8.0 Hz, 5H), 6.81-6.84 (9H, m, Ph). 13C-NMR (62.5 MHz, CDCl₃): 55.4, 61.6, 77.2, 113.6, 114.0, 114.3, 114.6, 118.8, 123.0, 126.8, 128.6, 130.8, 135.8, 136.5, 156.4, 166.3.

3-Hydroxy-1-(4-methoxyphenyl)-4-(phenylethynyl)azetidin-2-one (--)3a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 3 afforded 3a as a yellow solid, mp 170-171°C. 1H-NMR (250 MHz, CDCl₃) δ (ppm): 3.66 (3H, s, CH₃O), 4.20 (1H, d, J = 4.95 Hz, 4H), 4.93 (1H, d, J = 4.70 Hz, 3H), 5.04 (1H, bs, OH), 6.66-7.42 (9H, m, Ph). 13C-NMR (62.5 MHz, CDCl₃): 51.7, 55.3, 76.7, 81.0,
Anal calcd. for C_{18}H_{15}NO_{3}.0.47H_{2}O: C, 71.64; H, 5.01; N, 4.65. Found: C, 71.39; H, 4.99; N, 4.46.

3-Hydroxy-1-(4-methoxyphenyl)-4-(thiophen-2-yl)azetidin-2-one (−)-4a\textsuperscript{34}

With the enzymatic hydrolysis procedure described above, the reaction of racemic \textbf{4} afforded \textbf{4a} as a white solid, mp 189-191°C. \textsuperscript{\textsuperscript{1}}H-NMR (250 MHz, CDCl\textsubscript{3}) \(\delta\) (ppm): 3.76 (3H, s, \(CH_{3}\)O), 5.18 (1H, dd, \(J = 5.05\) Hz, 4\(H\)), 5.51 (1H, d, \(J = 5.05\) Hz, 3\(H\)), 6.80-7.38 (7H, m, \textit{Ph}). \textsuperscript{\textsuperscript{13}}C-NMR (62.5 MHz, CDCl\textsubscript{3}): 55.4, 58.7, 114.4, 118.9, 126.6, 127.3, 127.6, 130.2, 136.5, 156.5, 165.5.

3-Hydroxy-1,4-bis-(4-methoxyphenyl)-azetidin-2-one (−) \textbf{5a}\textsuperscript{66}

With the enzymatic hydrolysis procedure described above, the reaction of racemic \textbf{5} afforded \textbf{5a} as a white solid, mp 158-159°C. \textsuperscript{\textsuperscript{1}}H-NMR (250 MHz, CDCl\textsubscript{3}) \(\delta\) (ppm): 3.67 (3H, s, \(CH_{3}\)O), 3.72 (3H, s, \(CH_{2}\)O), 5.07 (1H, d, \(J = 5.17\) Hz, 4\(H\)), 5.13 (1H, d, \(J = 5.13\) Hz, 3\(H\)), 6.69-7.22 (8H, m, \textit{Ph}). \textsuperscript{\textsuperscript{13}}C-NMR (62.5 MHz, CDCl\textsubscript{3}): 55.3, 61.9, 114.3, 114.4, 114.6, 118.9, 124.8, 128.8, 130.5, 156.3, 159.9, 166.0.

3-Hydroxy-4-(4-methoxycarbonylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (−)-\textbf{6a}

With the enzymatic hydrolysis procedure described above, the reaction of racemic \textbf{6} afforded \textbf{6a} as a white solid, mp 180-182 °C. \textsuperscript{\textsuperscript{1}}H-NMR (250 MHz, CDCl\textsubscript{3}) \(\delta\) (ppm): 3.67 (3H, s, \(CH_{3}\)O), 3.82 (3H, s, \(CH_{2}\)O), 5.12 (1H, d, \(J = 4.53\) Hz, 4\(H\)), 5.18 (1H, d, \(J = 4.9\) Hz, 3\(H\)), 6.71-7.97 (8H, m, \textit{Ph}). \textsuperscript{\textsuperscript{13}}C-NMR (62.5 MHz, CDCl\textsubscript{3}): 52.1, 55.3, 62.3, 114.3,
Cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one (±)-7a55

With the enzymatic hydrolysis procedure described above, the reaction of racemic 7 afforded 7a as a yellow solid, mp 182-183 °C, ([α]D25 = 0°). 1H-NMR (250 MHz, CDCl3) δ (ppm): 3.65 (3H, s, CH3O), 5.22 (1H, d, J = 5.18 Hz, 4H), 5.26 (1H, d, J = 5.28 Hz, 3H), 6.64-8.16 (8H, m, Ph). 13C-NMR (62.5 MHz, CDCl3): 55.9, 63.4, 78.6, 115.2, 115.8, 119.9, 124.5, 130.0, 131.7, 143.7, 149.2, 158.1, 167.7.

Trans-3-Hydroxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one (±)-7b55

With the enzymatic hydrolysis procedure described above, the reaction of racemic 7 afforded 7b as a yellow solid, mp 98-99°C, ([α]D25 = 0°). 1H-NMR (250 MHz, CD3OD) δ (ppm): 3.60 (3H, s, CH3O), 4.52 (1H, d, J = 1.52 Hz, 4H), 4.91 (1H, d, J = 1.40 Hz, 3H), 6.69-8.13 (8H, m, Ph). 13C-NMR (62.5 MHz, CDCl3): 55.9, 66.4, 85.2, 115.2, 115.5, 120.3, 125.2, 128.4, 131.3, 145.6, 149.3, 158.2, 167.5.

3-Hydroxy-1-(4-methoxyphenyl)-4-(3-nitrophenyl)azetidin-2-one (−)-8a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 8 afforded 8a as a brown solid, mp 151-152°C. 1H-NMR (250 MHz, CDCl3) δ (ppm): 3.65 (3H, s, CH3O), 4.70 (1H, bs, OH), 5.22 (2H, d, J = 5.13 Hz, 3H, 4H), 6.69-8.15 (8H, m, Ph). 13C-NMR (62.5 MHz, CDCl3): 55.4, 61.9, 144.5, 118.8, 122.9, 123.5, 129.7, 133.9,
136.0, 148.3, 156.7, 166.2. Anal calcd. for C_{16}H_{14}N_{2}O_{5}.0.37H_{2}O: C, 59.88; H, 4.40; N, 8.73. Found: C, 59.99; H, 4.41; N, 8.09.

4-(4-Cyanophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-9a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 9 afforded 9a as a white solid, mp 85-88 °C. \(^1^H\)-NMR (250 MHz, CDCl\(_3\)) \(\delta\) (ppm): 3.69 (3H, s, CH\(_3\)O), 4.17 (1H, bs, OH), 5.20 (2H, s, 3H, 4H), 6.72-7.61 (8H, m, Ph). \(^1^3^C\)-NMR (62.5 MHz, CDCl\(_3\)): 55.5, 60.8, 77.2, 112.4, 114.5, 118.4, 118.7, 128.4, 129.8, 132.5, 139.2, 156.6, 165.8. Anal calcd. for C\(_{17}\)H\(_{14}\)N\(_2\)O\(_3\): C, 69.38; H, 4.79; N, 9.52. Found: C, 69.25; H, 4.55; N, 9.32.

3-Hydroxy-4-(4-dimethylaminophenyl)-1-(4-methoxyphenyl)azetidin-2-one (-)-10a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 10 afforded 10a as a brown solid, mp 187-190°C. \(^1^H\)-NMR (250 MHz, CDCl\(_3\)) \(\delta\) (ppm): 2.88 (6H, s, N(CH\(_3\))\(_2\)), 3.67 (3H, s, CH\(_3\)O), 5.02 (1H, d, \(J = 5.0\) Hz, 4H), 5.11 (1H, d, \(J = 5.0\) Hz, 3H), 6.67-7.25 (8H, m, Ph). \(^1^3^C\)-NMR (62.5 MHz, CDCl\(_3\)) \(\delta\) (ppm): 40.5, 55.4, 62.0, 112.9, 114.3, 118.9, 128.4, 130.7, 150.4, 156.2, 165.9. Anal calcd. for C\(_{18}\)H\(_{20}\)N\(_2\)O\(_3\): C, 69.21; H, 6.45; N, 8.97. Found: C, 68.98; H, 6.43; N, 8.80.

4-(3-Fluorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-11a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 11 afforded 11a as a white solid, mp 217-218°C. \(^1^H\)-NMR (250 MHz, CDCl\(_3\)/CD\(_3\)OD) \(\delta\) (ppm): 3.67 (3H, s, CH\(_3\)O), 5.08 (1H, d, \(J = 4.88\) Hz, 4H), 5.12 (1H, d, \(J = 5.05\) Hz, 3H),
6.71-7.21 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$/CD$_2$OD): 55.3, 61.1, 76.8, 114.3, 114.7, 115.1, 115.4, 118.7, 123.2, 130.2, 136.3, 136.4, 156.3, 166.4. Anal calcd. for C$_{19}$H$_{16}$FNO$_3$: C, 66.89; H, 4.91; N, 4.88. Found: C, 66.43; H, 4.88; N, 4.58.

4-(4-Fluorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-12a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 12 afforded 12a as a yellow solid, mp 163-166°C. $^1$H-NMR (250 MHz, CDCl$_3$/CD$_2$OD) $\delta$ (ppm): 3.69 (3H, s, C$_2$H$_5$O), 6.16 (1H, d, $J = 5.09$ Hz, 4H), 6.23 (1H, d, $J = 5.07$ Hz, 3H), 7.82-8.36 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$/CD$_2$OD): 55.3, 62.0, 76.6, 114.6, 115.3, 118.7, 129.3, 130.2, 156.3, 160.7, 164.6, 166.5. Anal calcd. for C$_{19}$H$_{16}$FNO$_3$: C, 66.89; H, 4.91; N, 4.88. Found: C, 66.43; H, 4.88; N, 4.58.

4-(4-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-13a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 13 afforded 13a as a white solid, mp 180-181°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 3.68 (3H, s, CH$_3$O), 5.13 (1H, d, $J = 5.13$ Hz, 4H), 5.16 (1H, d, $J = 5.13$ Hz, 3H), 6.71-7.31 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) $\delta$ (ppm): 55.4, 61.9, 114.4, 118.8, 129.0, 129.1, 130.1, 131.8, 134.7, 156.6.

4-(3-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-14a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 14 afforded 14a as a white solid, mp 197-198°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 3.68 (3H, s, CH$_3$O), 5.12 (2H, s, 3H, 4H), 6.71-7.28 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz,
(CDCl₃): 55.4, 62.0, 77.2, 114.2, 114.4, 118.8, 125.8, 127.7, 129.0, 130.1, 134.9, 135.6, 156.6, 165.8. Anal calcd. for C₁₆H₁₄ClNO₃·0.75H₂O: C, 60.56; H, 4.45; N, 4.41. Found: C, 60.96; H, 4.48; N, 4.09.

4-(2-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one  (-)-15a.

With the enzymatic hydrolysis procedure described above, the reaction of racemic 15 afforded 15a as a brown solid, mp 214-217°C. ¹H-NMR (250 MHz, CDCl₃/CD₃OD) δ (ppm): 3.69 (3H, s, CH₃O), 5.19 (1H, d, J = 4.98 Hz, 4H), 5.53 (1H, d, J = 4.95 Hz, 3H), 6.75-7.38 (8H, m, Ph). ¹³C-NMR (62.5 MHz, CDCl₃/CD₃OD) δ (ppm): 55.1, 60.2, 114.2, 118.5, 126.6, 128.2, 129.0, 129.3, 130.2, 131.3, 133.1, 156.2, 166.3. Anal calcd. for C₁₆H₁₄ClNO₃·1.13H₂O: C, 59.30; H, 4.36; N, 3.35. Found: C, 58.30; H, 4.28; N, 3.69.

4-(2,4-Dichlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one  (-)-16a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 16 afforded 16a as a brown solid, mp 181-183°C. ¹H-NMR (250 MHz, CDCl₃/CD₃OD) δ (ppm): 3.70 (3H, s, CH₃O), 5.19 (1H, d, J = 4.55 Hz, 4H), 5.49 (1H, d, J = 4.18 Hz, 3H), 6.75-7.41 (7H, m, Ph). ¹³C-NMR (62.5 MHz, CDCl₃/CD₃OD) δ (ppm): 55.3, 59.8, 114.4, 118.5, 127.1, 129.4, 130.1, 130.2, 134.0, 156.5. Anal calcd. for C₁₆H₁₃Cl₂NO₃: C, 56.82; H, 3.87; N, 4.14. Found: C, 56.75; H, 3.90; N, 4.05.

4-(2-Bromophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one  (-)-17a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 17 afforded 17a as a white solid, mp 205-206°C. ¹H-NMR (250 MHz, CDCl₃) δ (ppm): 3.69
(3H, s, CH$_3$O), 5.32 (1H, d, $J = 5.09$ Hz, 4H), 5.56 (1H, d, $J = 5.06$ Hz, 3H), 6.74-7.53 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 29.3, 55.1, 62.4, 114.1, 118.5, 127.1, 128.4, 129.3, 130.2, 132.5, 132.8, 156.2, 166.3. Anal calcd. for C$_{16}$H$_{14}$BrNO$_3$.0.23H$_2$O: C, 54.54; H, 4.00; N, 3.97. Found: C, 54.71; H, 4.01; N, 3.37.

4-(3-Bromophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-18a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 18 afforded 18a as a yellow solid, mp 182-183°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 3.68 (3H, s, CH$_3$O), 5.11 (2H, s, 3H, 4H), 6.71-7.44 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 55.4, 62.0, 77.2, 114.2, 114.5, 118.7, 118.9, 123.0, 126.2, 127.7, 130.1, 130.6, 130.7, 131.9, 135.9, 156.6, 165.9. Anal calcd. for C$_{16}$H$_{14}$BrNO$_3$: C, 55.15; H, 4.05; N, 4.02. Found: C, 55.10; H, 4.00; N, 4.05.

3-Hydroxy-4-(2-iodophenyl)-1-(4-methoxyphenyl)azetidin-2-one (-)-19a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 19 afforded 19a as a yellow solid, mp 78-80°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 3.69 (3H, s, CH$_3$O), 5.27 (1H, bs, 4H), 5.36 (1H, d, $J = 5.01$ Hz, 3H), 6.73-7.86 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 55.4, 66.5, 76.9, 98.2, 114.2, 114.5, 118.8, 128.4, 130.1, 130.3, 135.5, 139.7, 156.5, 165.4. Anal calcd. for C$_{16}$H$_{14}$INO$_3$: C, 48.63; H, 3.57; N, 3.54. Found: C, 43.65; H, 3.45; N, 3.50.
4-(4-Bromophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-20a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 20 afforded 20a as a pale yellow solid, mp 193-194°C. $^1$H-NMR (360 MHz, CDCl$_3$) $\delta$ (ppm): 3.68 (3H, s, CH$_3$O), 5.05 (2H, s, 3-H,4H), 6.62-7.46 (8H, dd, $J$ = 7.1, 5.98 Hz Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 55.4, 61.9, 114.5, 118.8, 122.9, 129.3, 130.2, 132.1, 132.4, 156.6, 165.6. Anal calcd. for C$_{16}$H$_{14}$BrNO$_3$: C, 55.19; H, 4.05; N, 4.02. Found: C, 55.02; H, 3.97; N, 3.99.

4-(4-Trifluoromethylphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one (-)-21a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 21 afforded 21a as a white solid, mp 220-223°C. $^1$H-NMR (250 MHz, CDCl$_3$/CH$_3$OH) $\delta$ (ppm): 3.66 (3H, s, CH$_3$O), 5.10 (1H, d, 3-H), 5.17 (1H, d, 3H), 6.70-7.55 (8H, 2dd, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 55.2, 62.1, 76.8, 114.3, 118.6, 125.3, 128.0, 130.1, 138.0, 156.4, 166.3. Anal calcd. for C$_{17}$H$_{14}$F$_3$NO$_3$: C, 60.54; H, 4.18; N, 4.15. Found: C, 60.32; H, 4.25; N, 4.10.

3-Hydroxy-4-(4-tert-butylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (-)-22a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 22 afforded 22a as a white solid, mp 180-182°C. $^1$H-NMR (250 MHz, CDCl$_3$) $\delta$ (ppm): 1.30 (9H, s, C(CH$_3$)$_3$), 3.76 (3H, s, CH$_3$O), 5.14 (1H, d, $J$ = 5.03 Hz, 4H), 5.21 (1H, d, $J$ = 5.05 Hz, 3H), 6.79-7.41 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$): 30.8, 34.2, 55.0, 62.4, 114.0, 118.6, 125.2, 127.1, 130.1, 130.3, 151.0, 156.1. Anal calcd. for C$_{20}$H$_{23}$NO$_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.74; H, 7.05; N, 4.19.
3-Hydroxy-4-(4-isopropylphenyl)-1-(4-methoxyphenyl)azetidin-2-one (-)-23a

With the enzymatic hydrolysis procedure described above, the reaction of racemic 23 afforded 23a as a white solid, mp 238-240°C. $^1$H-NMR (250 MHz, CDCl$_3$) δ (ppm): 1.17 (6H, d, $J$ = 6.83 Hz, CH(CH$_3$)$_2$), 2.50 (1H, bs, OH), 2.83 (1H, sep., $J$ = 6.85 Hz, CH(CH$_3$)$_3$), 3.68 (3H, s, CH$_3$O), 5.07 (1H, bs, 4H), 5.17 (1H, d, $J$ = 5 Hz, 3H), 6.71-7.24 (8H, m, Ph). $^{13}$C-NMR (62.5 MHz, CDCl$_3$/ CH$_3$OH): 23.3, 33.5, 54.9, 62.5, 114.0, 118.6, 126.3, 127.3, 130.3, 130.5, 148.7, 156.0, 166.5. Anal calcd. for C$_{19}$H$_{21}$NO$_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.15; H, 6.55; N, 4.40.

2.8.3 Non-enzymatic Hydrolysis of Racemic cis-7.

In a 50 mL round bottom flask, 200 mg of the racemic cis-7 was added to a suspension of potassium carbonate (100 mg) in 25 mL of methanol. The reaction mixture was stirred for 15 minutes at 0°C. The potassium carbonate was filtered off and the crude reaction mixture was isolated upon removal of methanol under reduced pressure. The crude mixture was analyzed from its $^1$H-NMR spectrum, which confirmed the formation of the diastereomeric racemic cis- and trans- alcohols 7a and 7b.

2.8.4 Enzymatic Transesterification of Racemic cis-7.

In a 50 mL round bottom flask, 100 mg of the racemic cis-7 was dissolved in dry THF (10 mL) to which 40 µL of n-butanol was added. To this reaction mixture, 100 mg PS-30 was added and the reaction was stirred at 40°C for 6 days. The reaction mixture was filtered through a bed of celite and the crude reaction mixture was analyzed by $^1$H-NMR to determine its percent conversion. The hydrolyzed product (the secondary
alcohol, ee > 98%, $[\alpha]_{D25} = -71.2^\circ$) and the enantioenriched $O$-acetate were separated by column chromatography using a stepwise elution of ethyl acetate: petroleum ether (20:80) followed by ethyl acetate: petroleum ether (50:50).

2.8.5 Determination of the Enantiomeric Excess.

The enantio-purity (the e.e. values) was determined from the $^1$H-NMR spectrum acquired on a Bruker 250-MHz NMR spectrometer in presence of (+)-[Eu(hfc)$_3$], a chiral shift reagent. The resonance signal of the acetoxy protons, a singlet in absence of the chiral shift reagent, was split into two signals of equal intensity for the two enantiomers in the racemic mixture. The ratio of the intensity of the CH$_3$C=O signal in the $^1$H-NMR spectra for the respective enantiomer was used to calculate the % enantiomeric excess. The % ee values for the hydrolyzed alcohols were also obtained using (+)-[Eu(hfc)$_3$] as a chiral shift reagent after their acetylation with Ac$_2$O/DMAP in dry THF.
2.9 References


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Chapter Three

Enzyme-catalyzed Regioselective Transesterification of Peracylated Sophorolipids

3.1 General Introduction

Sophorolipids, dimeric sophorose sugars (2'-O-β-D-glucopyranosyl-β-D-glucopyranose) linked β-glycosidically to a hydroxy-fatty acid, are amphipathic biomolecules produced by the yeast Candida bombicola (formerly Torulopsis bombicola)\(^1\) or Candida apicola\(^2\) from simple sugars and lipid substrates. Native sophorolipid is a complex mixture of up to 14 different compounds with the macrolactone and the sophorose glycoside being the major constituents of such mixtures\(^3a-c\). The fatty acid portion (17-hydroxyoctadecenoic acid) forms a 1′,4″ macrocyclic lactone ring (lactonic SLs) or has a free carboxylic end (acidic SLs) (Figure 3-1). These molecules, because of their structure, naturally act as biosurfactants (any type of compound produced by microorganisms with surface active or emulsifying properties) finding applications in the petroleum, pharmaceuticals and food processing industries where they can be used to reduce surface tension, stabilize emulsions, and promote foaming.\(^4\) Compared to their synthetic counterparts, biosurfactants offer some distinct advantages: they can be produced from renewable resources or even industrial waste, they are biodegradable and nontoxic, they are environmentally friendly and are effective under extreme conditions in small quantities and are structurally diverse\(^5a-c\). In addition, biosurfactants can enhance the emulsification of hydrocarbons and therefore have the potential to solubilize hydrocarbon contaminants and increase their availability for microbial degradation\(^6a-c\).
There has been considerable interest in the physiological properties of sophorolipids, which have shown exciting potential in the treatment of a host of disorders. SLs have been reported to have caused differentiation and protein kinase C inhibition in the HL60 leukemia cell line. Additionally, they are useful as immunomodulators for Parkinson’s disease, Alzheimer’s disease, psoriasis, AIDS treatment, as well as for antiviral immunostimulation. Consequently, there has been a great deal of interest in the synthesis of novel SL derivatives. To date, however, the primary strategy identified for the ‘tailoring’ of SL structure has been during in vivo formation by the selective-feeding of lipophilic substrates. For example, changing the co-substrate from sunflower to canola oil resulted in a large increase (50 to 73%) of the lactonic portion of SLs. Interestingly, using oleic acid (alone or with glucose) increased the fraction of non-acetylated 1’,4” sophorolipid lactone. Unsaturated C-18 fatty acids such as oleic acid may be incorporated unchanged into sophorose lipids.

![Figure 3-1](image)

**Figure 3-1.** Classes of Sophorolipid: the 1’, 4” lactonic sophorolipid (lactonic SLs, 1) and the acidic sophorolipid (acidic SLs, 2) where R₁ is the acetyl group.
A limited number of studies on selective \textit{in-vitro} enzymatic modifications of the natural SLs have been reported.\textsuperscript{10,12a-d}  Deacetylation of the 6', 6''-diacetylated lactonic SLs has been reported to result in formation of the 6'-hydroxy compound upon incubation with the enzymes acetylesterase,\textsuperscript{10} cutinase,\textsuperscript{12b} or lipases\textsuperscript{12a} (from \textit{Candida antarctica}-B, \textit{Candida rugosa}, \textit{Humicola} sp., porcine pancreas, \textit{Pseudomonas} sp., and \textit{Mucor miehei}), while keeping the lactone ring intact.\textsuperscript{12c,d} Enzymatic conversion of SLs to glucose lipids has also been reported using glycosidases, which released one glucose molecule from the disaccharide lipid.\textsuperscript{13} Highly regioselective lipase Novozym-435 (\textit{Candida antartica-Lipase B})-catalyzed acylations of the C-6' and C-6'' hydroxyl groups, in the non-lactonic SL methyl ester, have also been described for synthesis of well-defined sophorolipid analogs.\textsuperscript{14a,b} Such derivatives are essential for evaluation of their bioactivities and for preparation of glycolipid-based polymers.\textsuperscript{14a,b}

Lipase-catalyzed transformations provide a useful mean for the selective modification of sophorolipids and its derivatives since the glycosidic bonds between the glucose moieties and many acetyl groups may also be targets for hydrolysis under acidic or basic hydrolytic conditions. The utility of lipases in catalyzing selective transformations in lactonic sophorolipids has been previously demonstrated.\textsuperscript{12c} Lipases, in addition to having a large fatty acid binding site are activated at the hydrophobic hydrophilic interfaces, which are likely to be present in the sophorolipids solutions. Our interest in lipases arises from their ability to catalyze reactions in non-aqueous solvents under relatively mild conditions with high chemo-, regio- and enantio-selectivities, to accept a broad spectrum of substrates, and their biocompatibility.\textsuperscript{15a-c}
In this thesis, we describe and discuss regioselective transesterifications and hydrolysis of peracetylated sophorolipid derivatives catalyzed by lipases. This study is the first evaluation of the lipase-catalyzed reactions on the peracetylated non-lactonic SL derivatives. Four lipases, namely from Porcine pancreas (PPL, Type II), *Candida rugosa* (AYS, Type VII), *Pseudomonas cepacia* (PS-30), and *Candida antarctica* (Novozym 435, carrier fixed lipase fraction B) were used in anhydrous THF or in phosphate buffer (pH = 7.4, 0.2M). Literature reports attest to the binding of the lactonic SLs in the lipase binding pocket such that the sophorose moiety is accessible to the active site. The data presented in this thesis suggests that in peracetylated sophorolipids the absence of the 1′,4″-macrolactone ring results in its binding in the lipase active site such that the carboxyl end of the octadecenoic acid chain rather than that on the sophorose head group is preferentially accessible.

**3.2 Sophorolipid Biosynthesis**

Sophorolipids belong to a special class of biosurfactants known as glycolipids, low molecular weight compounds containing a mono- or disaccharide unit linked to a fatty acid moiety. Sophorolipids and biosurfactants in general, may either be synthesized *de novo* or may be assembled from substrates and commonly available central intermediates. For instance, Daniel et al. developed a two-step batch cultivation process where they used the yeast *C. curvatus* in the first and the yeast *C. bombicola* in the second step (*Fig. 3-2*). In the first step was a crossflow filtration for removing major solid particles. The second step was a sterile filtration into an autoclave bioreactor. When deproteinized whey was used as a substrate *C. bombicola* was not able to consume
lactose directly therefore the first cultivation step of *C. curvatus* (formerly known as *Candida Curvata* and *Apiotrichum Curvatum*) was necessary. A low yield (12g/L) of sophorolipids production was achieved using the above cultivation process. The same authors later described an improved method than that previously described. Another two-step bioprocess was used but rapeseed oil was continually fed in the second step increasing the yield of sophorolipids to an excellent final concentration of 422g/L.\textsuperscript{12a}

\begin{center}
\includegraphics[width=\textwidth]{Figure_3-2.png}
\end{center}

**Figure 3-2.** Flow Chart of the Coupled Process including Filtration.
3.3 Chemical Synthesis of Sophorolipid

Recently, the first total synthesis of sophorolipid was reported by Fürstner et al. In this approach, the 26-membered macrolide that contains the Z-alkene in the lipidic moiety spanning the sophorose backbone was created by an intramolecular ring-closure metathesis reaction of a diyne catalyzed by Mo[N(t-Bu)(Ar)]₃ (where Ar = 3,5-dimethylphenyl) activated in situ by CH₂CL₂, followed by catalytic reduction of the resulting cycloalkyne (Scheme 3-1). The β-glycosidic linkages were installed by means of glucal epoxide method followed by a modified Koenigs-Knorr reaction promoted by AgOTf/lutidine. It is of importance to note that the ring-closing alkyne metathesis constituted the first application to the carbohydrate series. Compared to the two-step, high-yielding biosynthetic method, many steps in this synthetic strategy suffer from low to moderate yields resulting in an overall low yield. However, it provides a concise preparative method to the parent sophorolactone (Fig. 3-1) in analytically pure samples.
Scheme 3-1. Synthesis of Sophorolipid Macrolactone. Legend: (a) Mo[N(t-Bu)(Ar)]₃ (Ar) 3,5-dimethylphenyl), 5 (10 mol %), CH₂Cl₂/toluene, 80 °C, 78%; (b) H₂ (1 atm), Lindlar catalyst (Pd, 5% w/w, on CaCO₃ poisoned with Pb), quinoline, CH₂Cl₂, room temperature, quantitative; [c] DDQ, CH₂Cl₂/MeOH (18/1), room temperature, 8 h, 93% (containing only trace impurities; analytically pure sample after preparative HPLC: 64%).

3.4 Lipase-catalyzed Modification of Sophorolipids

Scheme 3-2 shows how the peracylated sophorolipids were synthesized from the corresponding naturally occurring lactonic sophorolipids (1). Methanolysis of the sophorolipid macrolactone with freshly prepared 0.022 N sodium methoxide resulted in
formation of the sophorolipid methyl ester (SL-Me) (3). Structural assignment of the methyl ester (3) was previously described using $^1$H-NMR, $^{13}$C-NMR, $^1$H-$^1$H COSY and $^1$H-$^{13}$C HETCOR spectral data. Since the naturally occurring SLs are a mixture of as many as 14 different compounds, the use of the methyl ester intermediate provides a useful alternative to the otherwise cumbersome purification procedures while preserving the structural motif of the natural acidic SLs. The resulting SL-Me ester was acylated using acetic and propionic anhydride in dry THF in the presence of dimethylamino pyridine (DMAP) as the catalyst to give the peracetylated and perpropionated sophorolipid methyl esters 4 and 5, respectively. The structures of the peracetate and perpropionate 4 and 5, respectively, were confirmed from their respective $^1$H-NMR and $^{13}$C-NMR spectral data. For example, the proton NMR spectra of 4 showed multiple new singlets at ~2.0 ppm which integrated for additional 21 hydrogens compared to 2 hydrogens of C-2 H signal at 2.3 ppm (Table 2). The $^{13}$C NMR spectrum also showed additional resonance signals for the acetoxy carbonyls (-C(O)CH$_3$) between 169.2-170.6 ppm and the methyl ester carbonyl carbon (C-1) appeared at 174.2 ppm (Table 3-2). The structure of the perpropionate 5 was also established from its NMR spectral data and the assignments in the proton NMR were made using $^1$H-$^1$H COSY correlations (Table 3-3 and Appendix B). For example, in the $^1$H-$^1$H COSY NMR spectrum, the propionyl methyl (C(O) CH$_2$CH$_3$) proton resonance signals in 5 at 1.01-1.17 ppm showed cross peaks to the methylene protons (C(O) CH$_2$CH$_3$) signals at 2.21-2.39 ppm. The integral ratio of the methyl protons (1.01-1.17 ppm, 21H), in the propionyl group, to the resonance signal of the C-9H and 10 H signal (5.35 ppm, 2H) in 5 was used to confirm complete acylation of the SL-Me ester.
Scheme 3-2. Synthesis of Peracylated SL-Me Esters: (a) 0.022 N sodium methoxide, reflux, 30 minutes (b) acetic anhydride or propionic anhydride, DMAP, dry THF (4, R₁ = CH₃C(O); 5, R₁ = CH₃CH₂C(O)).

### 3.4.1 Screening of Lipases

The screening for lipases that would accept the peracylated SL-Me esters as substrates were carried out in a 50 mL round bottom flasks using 1:1 substrate to lipase ratio (w/w). The transesterification reactions were carried out in dry THF in presence of 1-butanol and 2-methylpropanol for 72 h. The hydrolysis reactions were evaluated by incubating the lipases with the substrates in phosphate buffer (pH = 7.4, 0.2M) for 72 h. Since the size of the scissile fatty acid binding pocket in lipases is known to vary considerably, four lipases were screened, namely, Novozym-435 (immobilized preparation from Candida antarctica), PS-30 (from Pseudomonas cepacia), PPL (from porcine pancreas) and AYS (from Candida rugosa) exhibited different specificity for the SL substrates (Table 3-1). While no activity was seen for PPL and PS-30 lipases in any of the tested reaction media, formation of a single product was observed (different R_f values compared to the substrate) within 72 h upon incubation of the peracylated SL-Me ester substrates (4 and 5) with the lipase from Candida antarctica (Novozym-435). The lipase AYS did not catalyze transesterifications in THF although was able to catalyze the
hydrolysis of 4 and 5 in aqueous phosphate buffer (pH = 7.4, 0.2M). Interestingly, the specificity of the lipases Novozym-435 and lipase AYS was same for the peracetylated (4) and perpropionated (5) substrate, i.e., the corresponding products formed had the same $R_f$ and same structures, confirmed from their NMR data as discussed in following sections.

**Table 3-1. Screening of Commercially Available Lipases for Peracylated SL-Me Esters Transesterification after 72h Incubation.**

<table>
<thead>
<tr>
<th>Media</th>
<th>Lipases</th>
<th>Substrate 4 (5) conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AYS</td>
<td>Novozym</td>
</tr>
<tr>
<td>THF/ $n$-butanol</td>
<td>n.r</td>
<td>83 (77)$^a$</td>
</tr>
<tr>
<td>THF/ iso-butanol</td>
<td>n.r</td>
<td>58 (55)</td>
</tr>
<tr>
<td>Buffer (pH = 7.4, 0.2M)</td>
<td>91 (54)</td>
<td>85 (90)</td>
</tr>
</tbody>
</table>

$^a$ The value in parenthesis are conversions for perpropionate 5. No reactions were observed in absence of lipase. n.r - no conversion observed.

### 3.4.2 Lipase-catalyzed Regioselective Transesterifications.

Enzyme catalyzed deacetylation in aqueous buffer of 6′,6″-diacetylated lactonic SLs has been reported to give the corresponding 6′-hydroxy lactonic SL.$^{12a}$ It has been proposed, based upon 3D models, that the lactonic SL fit well in the binding pocket of several lipases and that while the 6″-OAc is buried inside the macrolactone structure the 6′-OAc is well accessible to the active site of the lipase.$^{19}$ The lipase catalyzed deacylation reactions, however, have only been reported with lactonic sophorolipids and no investigation of the acidic SLs has been reported to date.

No report on the investigation of the lipase catalyzed transesterification/hydrolysis of peracylated acidic SLs were reported. Since the only lipase that accepted
peracylated SL-Me esters as substrates in transesterification reactions was Novozym-435, it was the only lipase used in further studies (Table 3-1). The transesterifications of the peracylated compounds 4 and 5 were conducted using excess 1-butanol and 2-methylpropanol in dry THF (Scheme 3-3). The product of the reaction was isolated by column chromatography. Upon comparison of the $R_f$ values of the starting materials with the product, which had a higher value, the deacylation of one or more ester groups on the sophorose seemed unreasonable. The deacetylated product should have had $R_f$ value lower than the starting material because of the resulting free hydroxyl group(s). It was confirmed from the detailed spectral analysis of the products that alcoholysis failed to furnish any free hydroxyls on the sophorose ring. Instead, transesterification took place on the methyl ester located at the carboxylic end of the 17-hydroxyoctadecenoic acid chain attached to the C-1' position of the sophorose ring.
Scheme 3-3. Lipase-catalyzed Hydrolysis and Transesterification Reactions of Peracylated Sophorolipid Methyl Esters.
3.4.3 NMR spectroscopy.

Detailed structural analysis of the products isolated from the transesterification reactions was undertaken using $^1$H-, $^{13}$C- and 2D- NMR spectral data (Figure 3-3, Tables 3-2 and 3-3). The $^1$H-NMR spectra of the products 6-11 when compared to the respective starting compounds 4 and 5, varied little and because of a relatively short width of the spectra (0-10 ppm) unambiguous assignments were not possible (Tables 2 and 3). For example in proton NMR spectrum of product 6, the integral value of the resonance attributed to the acetoxy methyl protons (1.98-2.08 ppm, 21H) was unchanged relative to the integral of the H-9 and H-10 vinylic protons (5.35 ppm, 2H). Although the sharp singlet for protons of the methyl ester between 3-5 ppm was absent in its proton NMR spectrum, structure of the product 6 could not be ascertained unambiguously from its $^1$H-NMR spectrum.

Bisht et al$^{14a}$ had previously carried out detailed NMR analysis of the SL-methyl ester and noted limited utility of the $^1$H NMR spectra in establishing the structure and assignment of various resonances. It was suggested that the $^{13}$C NMR spectrum because of its wider spread (0-200 ppm) was better suited for the assignment of individual carbon in SLs.$^{14a}$ The $^{13}$C-NMR assignments were used to assign resonances in the proton spectra, utilizing correlations obtained from a $^1$H-$^{13}$C HETCOR spectrum. In this paper we will restrict ourselves to spectral information concerning the identification of the position of the various acyl groups. In particular, we will focus on the position of the transesterification reaction in the peracetate 4 upon its incubation with lipase Novozym in dry THF containing 1-butanol and extend the argument, in light of data observed, to products 7-9.
In the $^{13}$C-NMR spectrum of the peracetylated sophorolipid methyl ester 4 (Figure 3-3), the methyl carbon (C(O)OCH$_3$) resonates at 51.4 ppm. Upon comparing the $^{13}$C-NMR spectra of 4 and 6, the disappearance of the methyl ester carbon (C(O)OCH$_3$) in product 6 and concomitant new resonance signals appearing at 63.8 (C(O)OCH$_2$C$_2$H$_5$), 30.5 (C(O)OCH$_2$-), 18.8 (C(O)OCH$_2$-CH$_2$-C$_2$H$_5$) and 13.4 (C(O)OCH$_2$-CH$_2$-CH$_2$-CH$_3$) ppm suggested formation of the butyl ester upon Novozym-435 catalyzed transesterification in the presence of 1-butanol. Importantly, a downfield shift was observed for the three methylene group protons in the butyl chain as compared to that of 1-butanol further suggesting formation of the butyl ester. The assignments were supported by a DEPT-135 and a $^1$H-$^{13}$C HETCOR spectra (see supporting information).

For example in the DEPT-135 spectrum of 6, negative resonance signals at 63.8, 30.5 and 18.8 ppm and a positive resonance signal at 13.4 ppm were assigned to the methylene and the methyl carbons, respectively, of the butyl group. In the $^1$H-$^{13}$C HETCOR spectrum a direct correlation was observed between the butyl carbons and corresponding protons allowing us to unambiguously assign the resonance signals of the hydrogens for this independent spin system (Tables 3-2 and 3-3). The methylene carbon at 63.8 along the $f_2$ axis of $^1$H-$^{13}$C HETCOR spectrum showed correlation with the resonance signals on the $f_1$ axis at 4.03-4.10 ppm, which integrated to four hydrogens. The –OCH$_2$- hydrogens in the butyl ester were thus assigned to the resonance multiplet at 4.03-4.10 ppm. Furthermore, the two different methylene carbons at 30.5 and 18.8 ppm showed correlations to the broad singlet (4H) at 1.32 ppm and the methyl carbon at 13.4 ppm to the triplet (3H) at 0.92 ppm.
Importantly, carbon resonances of the sophorose, the acetates groups, and the fatty acid chain were relatively unchanged (Figure 3-3). It was therefore concluded that regioselective transesterification of methyl ester took place and the acetate groups on sophorose sugar did not participate in this reaction. In a separate experiment, when the peracetate 4 was incubated with Novozym-435 in dry THF in presence of 2-methylpropanol under similar transesterification reaction conditions, disappearance of the methyl ester at 52.0 ppm was also observed with concomitant resonance signals appearing at 70.2 (C(O)OCH\(_2\)), 27.5 (C(O)OCH\(_2\)CH(CH\(_3\))\(_2\)), and 18.9 (C(O)OCH\(_2\)CH(CH\(_3\))\(_2\)) ppm suggesting the formation of the isobutyl ester at the carboxyl end of the octadecenoic acid. Again, the carbon resonances of the sophorose moiety and the acetate groups were relatively unaffected (Figure 3-3). It is important to mention that all reactions were carried out using 2:1 and 5:1 molar excess of the alcohol to the substrate and that the resulting product was the same regardless of the quantity of alcohol used. After column chromatographic purification yields were typically above 75%.

The \(^{13}\)C-NMR spectrum of the perpropionate 5 was more complex because of additional resonances arising from the propionate groups. Assignments shown in Table 3 were established using DEPT-135, \(^1\)H-\(^{13}\)C-HETCOR, and \(^1\)H-\(^1\)H-COSY spectra. The transesterification reaction catalyzed by Novozym in the presence of 1-butanol and 2-methylpropanol resulted in formation of the corresponding butyl (8) and isobutyl (9) esters, respectively at the carboxyl end of the octadecenoic acid. To reiterate, carbon resonances of the sophorose moiety and propionate groups were unchanged in products 8 and 9, compared to the starting perpropionate methyl ester, 5. It was thus established that
Figure 3-3. $^{13}$C-NMR Spectra of Peracetyl Sophorolipid Methyl Ester and its Derivatives.
Novozym catalyzed regioselective transesterification of the methyl ester in the peracetate 5 and perpropionate 6.

Hu et al\textsuperscript{12c} have recently reported lipase catalyzed deacetylation of 6',6"-diacetoxy lactonic SL in anhydrous organic solvents suggesting that the lactonic SL fit well in the binding pocket of several lipases such that 6'-OAc is well accessible to the active site. Therefore, it was somewhat intriguing that the non-lactonic (or acidic) peracylated SLs were not deacylated under the reaction condition described in this report. Bisht et al\textsuperscript{14a} have previously investigated lipase catalyzed acylation of non-lactonic SL-methyl ester (3) in anhydrous organic solvents. In absence of an acylating agent, the authors reported formation of a (17-hydroxyoctadec-9-enoic acid)-1',6"-lactonized sophorolipid. The observation made by Bisht et al.\textsuperscript{14a} and those made in this report suggest that presence of the macrolactone is necessary for proper placement of the peracylated sophorose moiety in the binding pocket of a lipase such that it is accessible to the active site of the lipase for deacylation of the sophorose esters in SLs. Herein, we suggest that the macrolactone provides a structural motif to the SLs that allow it to fit in the binding cavity of the lipase such that an acylated sophorose moiety is accessible to the active site. In absence of the lactonic structural motif, the binding of the SLs in the lipase binding pocket takes place such that the carboxyl group of the octadecenoic acid, not the acyl groups on sophorose sugar, is preferentially accessible to the active site.

The formation of 6'-acyloxy-(17-hydroxyoctadec-9-enoic acid)-1',6"-lactonized sophorolipid in two steps, reported by Bisht et al.,\textsuperscript{14a} thus can be rationalized as a two step mechanistic process. In the first step, the SL methyl ester binds such that an enzyme activated complex (EAC) is formed upon attack of the Ser-105 (in the active triad of the
lipase-B from *Candida antarctica*) on the carbonyl of the methyl ester group in the 17-hydroxyoctadec-9-enoic acid chain. In absence of another nucleophile (the reaction was done under anhydrous solvents) the 6″-hydroxyl of the sophorose attacks the EAC to yield a macrolactone. In the second step, the macrolactone reenters the binding pocket such that the sophorose unit is now accessible to the active site and the acylation proceeds to give the 6′-acyloxy- (17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipids in presence of an acylating agent. If this sequence of events were actually what was happening in the lipase active site then the presence of another nucleophile, during the first step, would not result in formation of a macrolactone. If the nucleophile were an alcohol, a transesterification of the methyl ester and the alcohol would result.

To explore this hypothesis, we performed an experiment to investigate formation of the (17-hydroxyoctadec-9-enoic acid)-1′,6″-lactonized sophorolipids as described previously by Bisht el al. but in presence of a nucleophile, i.e., 1-butanol. The SL-Me ester was incubated with Novozym in THF at 40 °C in presence of 3 mole equivalent of 1-butanol for 72 h (Scheme 3). Progress of the reaction was monitored by thin layer chromatography. The product 12 was characterized from its detailed NMR spectral analysis. The 1H-NMR spectrum of 12, had the methyl ester singlet missing, observed at 3.64 ppm (3H) in the proton NMR spectrum of 3, with concomitant appearance of a triplet at 3.96 ppm (2H), a broad singlet at 1.21 ppm (4H) and another triplet at 0.84 ppm (3H) suggesting the formation of the butyl ester. The triplets at 3.96 ppm and 0.84 ppm were assigned to the methyleneoxy (–C(O)OCH₂) and the methyl (-CH₃) groups, respectively, and the broad singlet at 1.21 ppm was assigned to the two methylene groups (–CH₂CH₂–) of the butyl ester. These assignments were supported by the 13C-NMR
spectrum in which the –OCH₃ signal at 52.0 ppm, observed in 3, disappeared with new resonance signals appearing at 65.2, 31.8, 20.2 and 14.1 ppm for the butyl carbons. The carbon resonances of the sophorose moiety were unperturbed, i.e., formation of the macrolactone was not observed. The structure of the resulting product (Scheme 3-4) was thus determined to be butyl-17-L-[(2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-cis-9-octadecenoate (12), i.e., butyl ester of sophorolipids.
Table 3-2. Assignment of $^1$H and $^{13}$C Signals of Sophorolipid-Me ester (3) and Peracetylated Sophorolipid Derivatives (4, 6, 7, and 10).

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Table 3-3. Assignment of $^1$H and $^{13}$C Signals of Perpropionyl Sophorolipid Derivatives (5, 8, 9, and 11).

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3.4.4 Lipase-Catalyzed Hydrolysis.

Otto et al.\textsuperscript{12a} have described the selective deacetylation of lactonic sophorolipid 6′, 6″-diacetate using lipase AYS (from \textit{Candida rugosa}) in a phosphate buffer medium (pH = 7.4, 0.2M). The author reported that the 6′,6″-diacetate was converted to the 6′-hydroxy lactonic sophorolipid. Choice of a reaction medium in an enzymatic reaction is very important because organic solvents may alter enzyme conformation or at worst denature the enzyme.\textsuperscript{16} Hydrolysis of the peracetate 4 and perpropionate 5 was therefore investigated upon incubation with the lipases Novozym-435 and AYS in phosphate buffer (pH = 7.4, 0.2M) at 25 °C.

For the current study, the reaction procedure needed to be modified due to poor solubilities of the compounds 4 and 5 in buffer. A common approach for improving the biocatalytic reaction rates of water insoluble substrates is the use of cosolvents.\textsuperscript{15c} Compounds 4 or 5 was therefore dissolved in minimum amount of acetone prior to their addition to the buffer solution followed by addition of the lipase. The reaction product was isolated upon its extraction in ethyl acetate followed by rotoevaporation of the solvent. In separate reactions with compound 4 and 5 only one hydrolysis product in each was isolated. In the $^1$H-NMR spectra of the compound 10 and 11, isolated from reaction of 4 and 5, respectively, the methyl ester resonance between 3.65-3.73 ppm disappeared. The $^{13}$C-NMR spectra of 10 and 11 corroborated the hydrolysis of the methyl ester. For example, in the $^{13}$C-NMR spectrum of the product 10, the resonance for O-methyl ester carbon (C(O)O\textsubscript{OCH$_3$}) at 51.4 ppm had disappeared along with an observed downfield shift in the carboxylic acid carbonyl carbon to 178.8 ppm (in 4). More significantly, the resonances for sophorose carbons and acetate groups remained
unchanged confirming that sophorose acetate esters were not hydrolyzed by the lipase under reaction conditions described. Similarly, the $^{13}$C-NMR spectrum of the product 11 (of the perpropionate 5) also had the O-methyl ester carbon ($\text{C}($O$)$OCH$_3$) resonance missing at 51.4 ppm along with a carboxyl carbonyl carbon signal shifted downfield to 179 ppm. The resonances for the sophorose carbons and the perpropionates groups were unchanged in the $^{13}$C-NMR spectrum of 11 (Scheme 3-3) reaffirming that deacylation of the propionate esters was not catalyzed by the lipase. The NMR results thus established that enzymatic hydrolysis of the peracylated SL-Me esters 4 and 5 by Novozym-435 and AYS occurred with high chemo and regioselectivity, i.e., only hydrolysis of the methyl ester took place while the peracyl esters were left untouched. These observations lend additional support to the proposition that the macrolactone ring is necessary for proper binding of the SLs in the lipase binding pocket such that the acyl ester on the sophorose moiety are accessible to the lipase active site.

\begin{center}
\includegraphics[width=\textwidth]{3-4.png}
\end{center}

**Scheme 3-4.** Formation of the Butyl Ester from Sophorolipid Methyl Ester.
3.5 Experimental Section

Chemicals and enzymes. All reagents were purchased from commercial sources and used as received. All solvents were purified and dried prior to use by known literature procedures. Prior to their use, the sophorolipids were dried over P$_2$O$_5$ in a vacuum desiccator (0.1 mmHg, 12 h, 56°C). Porcine pancreatic lipase (PPL) Type II Crude (activity = 61 units/mg protein) and Candida rugosa lipase (AYS) TypeVII (activity = 4570 u/mg protein) were purchased from the Sigma Chemical Co. The lipase PS-30 from Pseudomonas cepacia (20,000 units/g) was obtained from Amano Enzymes Co., Ltd. The carrier fixed lipase Novozym 435 (10,000 units/g from Candida antarctica, fraction B) was a gift from Novo Nordisk Inc.

Column chromatography. Column chromatographic separations were performed over silica gel 60 (Silicycle Inc.). In a typical separation, silica gel was used to pack a glass column (5cm x 50cm) in the eluent (ethyl acetate/ hexane mixture). The compounds were dissolved in a minimal volume of eluent and loaded onto the top of the silica bed in the column. Different fractions were subsequently eluted and monitored by thin-layer chromatography (TLC). Fractions containing the purified compounds were pooled together, and the solvent was evaporated to give the pure compound.

Nuclear magnetic resonance. $^1$H-NMR and $^{13}$C-NMR spectra were recorded using Bruker ARX-250 spectrometer (Appendix B). Chemical shifts in parts per millions are reported downfield from 0.00 ppm using deuterated chloroform (unless stated otherwise) with trimethylsilane (TMS) as the internal reference. Unambiguous assignments were
derived from COSY and HETCOR spectra. The following abbreviations are used to present the spectral data: s = singlet, bs = broad singlet, bm = broad multiplet, d = doublet, t = triplet.

Optical rotations were measured with an AutoPol-IV (Rudolph research analytical) automatic polarimeter. IR spectra were recorded on a Nicolet Avatar Smart Miracle 320 FT-IR spectrometer. High resolution mass analyses (HRESIMS) were obtained using a JEOL JMS-600H with Agilent 6890 Series GC System at Florida State University (Tallahassee) Mass Spectral Analysis Facilities.

Synthesis of methyl 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (SL-Me, 3).\textsuperscript{14a}

In a 100 mL round-bottomed flask equipped with a reflux condenser 10 g of dry crude sophorolipid and 10 mL 0.022 N freshly prepared sodium methoxide in methanol were added. The reaction assembly was protected from atmospheric moisture by a CaCl\textsubscript{2} guard tube. The reaction mixture was refluxed for 3 h, cooled to room temperature (25 °C), and neutralized using glacial acetic acid. The reaction mixture was concentrated by rotoevaporation and poured with stirring onto 100 g of crushed ice that resulted in the precipitation of the sophorolipid methyl ester as a white solid. The white precipitate was filtered, washed with ice-water, and dried overnight under pressure in a vacuum oven (8.77 g, yield = 95.0%).

In a 50ml round-bottomed flask was added 2g (3.06 mmol) of methyl 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (3) and dissolved in dry THF (30ml). Acetic anhydride 8 mL (0.847 mmol) and 0.15g of dimethylamino pyridine (DMAP) was added to the solution and the reaction was allowed to stir at room temperature for 6 h while protected from atmospheric moisture by a CaCl₂ guard tube. The reaction mixture was concentrated by rotoevaporation followed by extraction with ethyl acetate and washing with sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and concentrated by rotoevaporation. The viscous liquid was then dried overnight in a vacuum desiccator at 40°C to yield the peracetylated sophorolipid methyl ester (4) (2.79g, yield = 98%) as a clear viscous liquid, [α]²⁵_D = -7.3° (CHCl₃, c = 0.01); IR ν 2923, 1748, 1430, 1376, 1240, 1037, 888 cm⁻¹. HRESIMS m/z: 930.4466 [M + Na]⁺ (calcd for C₄₅H₇₀O₂₀, 930.4461).

Synthesis of methyl 17-L-([2″,3″,3′,4″,4′,6″,6′″-heptapropionyloxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (5).

A procedure similar to the one above for the peracetylated sophorolipid methyl ester (4) was used to prepare the perpropionated sophorolipid methyl ester. Sophorolipid methyl ester 3 (2.0 g, 3.06 mmol), propionic anhydride (10.0g, 0.0768 mmol) and DMAP (0.15g) were dissolved in dry THF (30ml). The reaction mixture was allowed to stir for 6 h at room temperature. After usual workup the perpropionated sophorolipid methyl ester (5) was isolated as a light amber colored viscous liquid (3.0 g, yield = 97%), [α]²⁵_D = -4.5°
(CHCl₃, c = 0.01); IR ν 2936, 1740, 1351, 1165, 1053, 1012, 813 cm⁻¹. HRESIMS m/z: 1028.5571 [M + Na]⁺ (calcd for C₅₂H₈₄O₂₀, 1028.5556).

**Screening of lipases and general procedure of lipase catalyzed transesterification reactions.** In a 50ml round-bottomed flask was added the appropriately substituted analogue (4 or 5) dissolved in dry THF (30 ml). To this solution, the appropriate enzyme (AYS, Novozym-435, PPL, PS-30) was added followed by addition of n-butanol (or iso-butanol) and the reaction mixture was stirred at 40°C for 72 h. Novozym-435 was found to be the only enzyme that catalyzed the transesterification reaction and was used in subsequent reactions. The reaction mixture was then filtered through a bed of celite and concentrated by rotoevaporation. The resulting crude product was purified by wet column chromatography using an ethyl acetate: hexane mixture as eluent.

**Synthesis of n-butyl 17-L-([2″,3′,3″,4′,4″,6′,6″-heptaacetoxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (6).**

A procedure similar to the one above was used to prepare (6). 0.8g (8.6 mmol) of 4, 0.4g of Novozym-435 and 456 µl (4.98 M mol) of 1-butanol was stirred at 40°C for 72 h. The reaction was worked up as specified and the resulting crude product was purified by wet column chromatography using a 35:65 ethyl acetate: hexane mixture as eluent to afford 6 (0.159g, yield = 83%) as a light amber viscous liquid, [α]²⁵_D = -5.9⁰ (CHCl₃, c = 0.01); IR ν 2921, 1749, 1376, 1229, 1045, 903 cm⁻¹. HRESIMS m/z: 972.4937 [M + Na]⁺ (calcd for C₄₈H₇₆O₂₀, 972.4930).
Synthesis of \( n \)-butyl 17-L-\([2''',3''',4''',4'',6''',6''''-heptapropionyloxy-2'-O-\(\beta\)-D-glucopyranosyl-\(\beta\)-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (8).

A procedure similar to the one above was used to prepare (8). 0.8g (0.79 mmol) of 5, 0.4g of Novozym-435 and 456 µl (4.98 M mol) of 1-butanol was stirred at 40°C for 72 h. The reaction mixture was worked up as specified and the resulting crude product was purified by silica gel column chromatography using a 35:65 ethyl acetate: hexane mixture as eluent to afford 8 (0.645g, yield = 77%) as an amber colored viscous liquid, \([\alpha]^{25}_{D} = -5.2^\circ\) (CHCl₃, c = 0.01); IR \(\nu\) 2942, 2871, 1739, 1450, 1352, 1169, 1057, 1031, 804 cm\(^{-1}\). HRESIMS \(m/z\): 1070.6022 [M + Na]\(^+\) (calcd for C\(_{55}\)H\(_{90}\)O\(_{20}\), 1070.6026).


A procedure similar to the one above was used to prepare (7). 0.4g (4.3 mmol) of 4, 0.4g of Novozym-435, and 456 µl (4.98 M mol) of 2 methylpropanol was stirred at 40°C for 72 h. The reaction mixture was worked up as specified and the resulting crude product was purified by silica gel column chromatography using a 35:65 ethyl acetate: hexane mixture as eluent to afford 7 (0.245g, yield = 58%) as a light amber colored viscous liquid, \([\alpha]^{25}_{D} = -6.7^\circ\) (CHCl₃, c = 0.01); IR \(\nu\) 2929, 2840, 1757, 1374, 1227, 1036, 906 cm\(^{-1}\). HRESIMS \(m/z\): 972.4931 [M + Na]\(^+\) (calcd for C\(_{48}\)H\(_{76}\)O\(_{20}\), 972.4930).
Synthesis of isobutyl 17-L-([2″,3″,3′,4′,4″,6′,6″-heptapropionyloxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate (9).

A procedure similar to the one above was used to prepare (9). 0.4g (3.95 mmol) of 5, 0.4g of Novozym-435 and 456 µl (4.98 Mmol) of 2-methylpropanol and the reaction was stirred at 40°C for 72 h. The reaction mixture was worked up as specified and the resulting crude product was purified by silica gel column chromatography using a 35:65 ethyl acetate: hexane mixture as eluent to afford 9 (0.231g, yield = 55%) as a light amber colored viscous liquid, \([\alpha]_{25}^D = -4.2^\circ (\text{CHCl}_3, c = 0.01)\); IR \(\nu 2929, 1735, 1454, 1347, 1174, 1049, 1022, 804 \text{ cm}^{-1}\). HRESIMS \(m/z\): 1070.6038 [M + Na]⁺ (calcd for C\(_{55}\)H\(_{90}\)O\(_{20}\), 1070.6026).

General procedure of lipase screening for hydrolysis reactions. To a 50 mL Erlenmeyer flask containing 20 ml phosphate buffer solution (pH = 7.4, 0.2M), the appropriately substituted sophorolipid analogue dissolved in 5mL acetone was added. To this solution, lipase (AYS or Novozym-435) was added and the flask was closed with a rubber stopper (to prevent evaporation of the co-solvent). The reaction mixture was stirred at room temperature for 72 h. The reaction mixture was extracted with ethyl acetate (3 x 30 mL), and the organic layer was dried over anhydrous sodium sulfate and concentrated by rotoevaporation. Purification was achieved using ethyl acetate : hexane as the eluent in a silica gel chromatographic column.

A procedure similar to the one above was utilized in the synthesis of 10. 0.3g (0.32 mmol) of 4, 0.15g of lipase (AYS or Novozym-435) was added to a phosphate buffer solution and stirred at room temperature for 72 h. Workup was accomplished as directed to yield 10 (Novozym-435- 150 mg, yield 85%, [α]$_{25}^{D}$ = -11.1° (CHCl$_3$, c = 0.01); AYS-150 mg, yield 91%) as a clear viscous liquid which was purified using ethyl acetate : hexane (50:50) as the chromatographic eluent; IR ν 2925, 2854, 1746, 1366, 1218, 1019 cm$^{-1}$. HRESIMS m/z: 916.4308 [M + Na]$^+$ (calcd for C$_{55}$H$_{90}$O$_{20}$, 916.4304).

Synthesis of 2″,3″,4″,6″-heptapropionyloxy-17-L-((2’-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoic acid (11).

A procedure similar to that above was used in the synthesis of 11. 0.3g (0.30 mmol) of 5, 0.15g of lipase (AYS or Novozym-435) was added to phosphate buffer solution and stirred at room temperature for 72 h. Workup was done as directed to yield 11 (Novozym-435- 150 mg, yield 90%, [α]$_{25}^{D}$ = -5.0° (CHCl$_3$, c = 0.01); AYS-150 mg, yield 54%) as a light amber viscous liquid which was purified using ethyl acetate : hexane (30:70) as the eluent in chromatographic separation; IR ν 2929, 2846, 1754, 1362, 1164, 1058, 1021 cm$^{-1}$. HRESIMS m/z: 1014.5399 [M + Na]$^+$ (calcd for C$_{51}$H$_{82}$O$_{20}$, 1014.5400).

In a 50ml round bottomed flask was added 0.1g (0.153 M mol) of 5 and dissolved in dry THF (3 ml). To this solution, 0.1g of Novozym-435 was added followed by .057g (.077 mmol) of n-butanol and the reaction was stirred at 40°C for 72 h. The reaction mixture was then filtered over a celite bed and concentrated by rotoevaporation. The resulting crude product was purified by silica gel column chromatography using a 35:65 ethyl acetate: hexane mixture as eluent to afford 12 (0.103g, yield = 95%); IR ν 3328, 3248, 2921, 2846, 1749, 1258, 1078, 1011, 813 cm⁻¹; HRESIMS m/z: 678.4195 [M + Na]⁺ (calcd for C₃₄H₆₂O₁₃, 678.4190). ¹H- NMR (250 MHz, CD₃OD) δ 0.84 (3H, t, J = 7.5 Hz, -O(CH₂)₃CH₃), 1.21 (16H, brs, H- 4-7,12-14,16), 1.21 (4H, brs, -OCH₂(CH₂)₂CH₃), 1.50 (4H, bs, H-3,15), 1.93 (4H, bs, H-8 and -11), 2.20 (2H, t, J = 7.5 Hz, H-2), 3.16 (4H, m, H-2″,4″,-4′, and -5′), 3.34 (3H, m, H-3″,H-3′,H-2’), 3.54 (2H, m, H-6′), 3.76 (3H, m, H-6″ and H-17), 3.96 (2H, t, -OCH₂(CH₂)₂CH₃), 4.35 (1H, d, J = 7.5 Hz, H-1′), 4.54 (1H, J = 7.5 Hz, H1″) and 5.24 (2H, bs, H-9 and -10); ¹³C-NMR (62.5 MHz): 14.08, 20.18, 21.93, 24.83, 26.05, 26.22, 28.09, 28.14, 30.13, 30.23, 30.40, 30.75, 30.82, 30.90, 31.81, 35.07, 37.79, 62.63, 63.01, 65.19, 68.22, 71.35, 71.68, 75.79, 77.67, 78.17, 78.26, 78.90, 81.75, 102.70, 104.57, 130.75, 130.89, 175.58.
3.6 References.


CHAPTER FOUR

An Enantioselective Synthesis of Imperanene via Lipase-Catalyzed Asymmetrization of a 1,3-Diol

4.1 General Introduction

Imperanene (1), a phenolic compound of the rare C₆-C₄-C₆ class of natural products, has been isolated from Imperata cylindrica¹ and shown to possess platelet aggregation inhibitory activity² making it a suitable candidate in the search for platelet aggregation inhibitors for the treatment of disease such as stroke. The rhizomes of I. cylindrica have been used in Chinese medicine as a diuretic and anti-inflammatory agent³-⁵, but only few studies concerning the chemical constituent of this plant have been reported⁶-⁹.

![Figure 4-1](image)

**Figure 4-1.** Structure of Imperanene, a novel phenolic compound from *I. cylindrica*.

While the Imperanene was isolated as a single (+) isomer, its absolute stereochemistry was not reported. Shattuck *et al.*¹⁰ have since established, through comparison of optical rotation data, the natural product to be the (S)-enantiomer.

4.2 Synthetic Approaches to the Synthesis of Imperanene

To date, four different synthetic approaches of imperanene have been reported. Shattuck *et al.*¹⁰ employed the Ender’s RAMP/SAMP chiral auxiliary method toward the
asymmetric synthesis of both enantiomers of imperanene (1) in an eight-step synthesis with enantiomeric excesses of 82-90%. The key step of asymmetric induction utilizes Ender’s method with (S)-1-amino-2-methoxy-methylpyrrolidine (SAMP) or (R)-1-amino-2-methoxy-methylpyrrolidine (RAMP), the chiral auxiliaries used for enantiofacial selectivities of alkylation with benzyl chloromethyl ether (Scheme 4-1). The chiral auxiliaries were made to react with an aldehyde to form a hydrazone capable of being alkylated. Benzyl chloromethyl ether, the alkylation agent used in the Ender’s method, introduced a one-carbon benzyl-protected alcohol in a single step. Subsequent oxidation with ozone, followed by Wittig coupling gave both protected enantiomers of imperanene (1), which were then deprotected to give the desired products.

Scheme 4-1 Key step in Shattuck’s synthesis of imperanene utilizing Ender’s method. Legend: (a) SAMP, 0°C to rt, 20h, 84%; (b) RAMP, 0°C to rt, 20h, 82%; (c) (i) LDA, 0°C, 5.5h; (ii) benzyl chloromethyl ether, -120°C for 20 min, then rt for 20h (77% for pathway (a), 75% for (b)).

In another reported synthesis, Eklund et al11 used a semisynthetic method for the synthesis of the (R)-(−)-enantiomer of imperanene (1) starting from hydroxymatairesinol,
a natural lignan from the Norway spruce. The semisynthetic method is very efficient but is not amenable to the synthesis of the natural enantiomer or its structural analogs. The key step in Eklund’s synthetic pathway was the synthesis of the a carboxylic acid intermediate using hydroxymatairesinol as starting material (Scheme 4.2)

\[ \text{Scheme 4-2} \text{ Key step in Eklund’s synthesis of imperanene using hydromatairesinol. (a) NaOH (0.6 M), 80°C, 2.5h.} \]

The resulting carboxylic acid was then esterified to yield the methyl ester by refluxing in acidified methanol, which was reduced with LiAlH₄ to afford (–)-imperanene with a high enantiomeric purity (ee ~ 90%) and overall yield of 60%.

Doyle et al\textsuperscript{12} reported the synthesis of \textit{S}-(+)-imperanene by use of regio- and enantioselective intramolecular carbon-hydrogen insertion reactions catalyzed by chiral dirhodium(II) carboxamidates. The key step in Doyle’s synthetic approach used \textit{Rh}₂(4\textit{S}-MPPIM)\textsubscript{4} and \textit{Rh}₂(4\textit{R}-MPPIM)\textsubscript{4} to give both enantiomeric forms of the 3-benzyl-δ-butyrrolactone products (Scheme 4.3). Other rhodium catalysts were screened, but only the \textit{Rh}₂(MPPIM)\textsubscript{4} catalysts led to enantioselection of greater than 20:1. This is due
primarily to the increased steric interactions of ligands with the reacting carbine, which restricts available conformations and direct product formation. The use of the Rh₂(4S-MPPIM)₄ catalyst gave the highest enantiomeric excess of the S-configured lactone product (93%).

Scheme 4-3. Key step in Doyle’s synthesis of imperanene using chiral dirhodium(II) carboxamidates. (a) Rh₂(4S-MPPIM)₄, CH₂Cl₂, 68%; (b) DIBAL-H, 95%; (c) aryllithium, THF, -78°C to r.t., 74%.

The final reported synthesis of imperanene (1) was reported by Davies et al. who established the efficient C-H activation of primary benzylic positions by means of rhodium carbenoid induced intermolecular C-H insertion toward the synthesis of (+)-imperanene and (−)-conidendrin (Scheme 4-4).
Scheme 4-4. Key step in Davies’ synthesis of imperanene by intermolecular C-H insertion. (a) (i) LAH, -40°C; (ii) TBAF, rt, 87%.

The total synthesis of (+)-imperanene by Davies et al. is summarized in Scheme 4-4. Rh$_2$(R-DOSP)$_4$-catalyzed decomposition of α-diazo methyl ester in the presence of the very electron-rich aromatic at 50°C generates the C–H activation product (S) in 43% yield and 91% ee. Lithium aluminum hydride reduction of (S) followed by silyl deprotection generates (+)-imperanene in 87% yield. The specific rotation was in agreement with the literature value and demonstrated that Rh$_2$(R-DOSP)$_4$ generates the S configured C–H insertion product. Interestingly, all synthetic methods described above
required the use of a chiral auxiliary or an organometallic catalyst. The semisynthetic method is very efficient but is not amenable to synthesis of the natural enantiomer or its structural analogs.

4.3 Synthesis of $S$ and $R$ imperanene by Lipase-catalyzed Asymmetrization

In our synthesis of imperanene, we envisaged a divergent-convergent synthetic strategy to imperanene starting from vanillin, which can be converted to a 1,3-diol (8) and the Wittig reagent (6) (Scheme 4-5).

![Scheme 4-5](image)

Scheme 4-5. Retrosynthetic analysis of Imperanene.

The 1,3-diol is a key intermediate as it is prochiral and can be converted to an enantiomerically pure intermediate (B) (Scheme 4-5) in high yield. Thus diverging from a commercially inexpensive starting material (vanillin), the two pieces of the carbon skeleton of Imperanene would be obtained. ($S$)-(+) Imperanene would be readily derived through a Wittig coupling reaction of the aldehyde obtained from the $S$ enantiomer of B.
with the ylide generated from A. Similarly, the (R)-(−) enantiomer would be derived from the R enantiomer of B. The highlight of this strategy is the enantiocontrol exhibited by the biocatalyst during enzymatic acetylation of the prochiral 1,3 diol. Asymmetrization of prochiral diols make very useful synthetic intermediates since the maximum feasible yield upon lipase-catalyzed transformation is not limited to 50%, as what happens when resolving racemates.14

The choice of lipases as biocatalyst for the asymmetrization of the prochiral 1, 3-diol was based upon their ability to assume a variety of conformations to accommodate substrates of varying sizes and complexities, providing one of the most useful and versatile biocatalytic methods in asymmetric synthesis and resolution of organic substrates with high efficiency and selectivity.15 Furthermore, the lipase used herein was recyclable and was reused without significant lost in activity.

Our strategy for the synthesis of imperanene began with the tosylation of vanillin (2) with tosyl chloride affording the tosylated aldehyde (3) in 95% yield (Scheme 4-6). The choice of the protecting group turned out to be quite important. Other protecting groups such as TBDMS, Bn and PMB were initially utilized, but they proved to be liabilities during the course of the subsequent reactions often resulting in low yielding or failed reactions. The benzyl group in particular was an excellent protecting group but the difficulties of its deprotection in the presence of a carbon-carbon double bond made this approach unrealistic in the overall synthetic scheme. Subsequent reduction with sodium borohydride yielded the alcohol (4) in 99% yield which was readily brominated using phosphorus tribromide to afford the bromide (5) quantitatively.16 It is important to note that the first four steps were readily amendable to large scales; high yields were achieved.
and no purifications by wet column chromatography were required. Purifications were easily accomplished by recrystallization.

\[
\begin{align*}
\text{HO} & \quad \text{TsO} \\
2 & \quad \text{H} & \quad \text{TsO} \\
\text{3} & \quad \text{H} & \quad \text{TsO} \\
\text{4} & \quad \text{OH} & \quad \text{TsO} \\
\text{6} & \quad \text{PPh}^+ & \quad \text{Br}^- \\
\text{5} & \quad \text{TsO} & \quad \text{Br} \\
\text{7} & \quad \text{TsO} & \quad \text{OEt} & \quad \text{OEt} & \quad \text{OEt} & \quad \text{OEt}
\end{align*}
\]

**Scheme 4-6.** Synthesis of Wittig Reagent starting from vanillin. Conditions: (i) tosyl chloride, K₂CO₃, acetone, reflux, 24h, 95%; (ii) NaBH₄, MeOH, 0°C, 8h, 99%; (iii) PBr₃, ether, r.t., 3h, 98%; (iv) triphenylphosphine, toluene, reflux, 24h, 97%; (v) diethylmalonate, NaH, 0°C, 8h, 88%.

The bromide (5) served as the intermediate for the divergent synthesis of the triphenylphosphonium bromide salt (6) (yield = 97%) which was synthesized by refluxing 5 with triphenylphosphine in toluene for 24 hours and for the symmetrical alkylation of diethylmalonate affording the monoalkylated diethylmalonate (7) (yield =
88%) which was easily separated from the dialkylated product (5-10% yield) by column chromatography.

Reduction of the alkylated diethylmalonate (7) to the prochiral 1,3-diol (8) was accomplished using NaBH₄:LiCl (4:4) in methanol:ether (1:3) (Scheme 4-7).¹⁷ The resulting diol was then selectively acylated using vinyl acetate as the acylating agent in the presence of lipase form *Pseudomonas cepacia* (Amano lipase PS-30). A systematic screening of a number of different lipases available in our laboratories led us to the most efficient route through use of the lipase PS-30.

![Scheme 4-7](image)

Scheme 4-7. Synthesis of *R*-aldehyde through asymmetrization of a 1,3-diol. (i) NaBH₄, LiCl, MeOH:ether (1:3), 0°C, 24h, 87%; (ii) vinyl acetate, *PS-30*, 40°C, 48h, 90%; (iii) Dess-Martin periodinane, CH₂Cl₂, r.t., 6h, 75%.

The reaction gave the *R*-(+)-monoacetate in good yield with high enantioselectivity. The enantiopurity of the product acetate was calculated from its ¹H-NMR spectra acquired in the presence of (+)-Eu(tfc)₃, a chiral shift reagent (Figure 4-2). The resonance signal of the methoxy and acetoxy protons in the racemic mixture, singlets
in absence of the chiral shift reagent, was split into a set of two signals of equal intensity for the two enantiomers in the presence of (+)-Eu(tfc)$_3$. The product monoacetate (9) obtained by lipase desymmetrization reveals a singlet for both the methoxy and acetoxy protons in the presence of (+)-Eu(tfc)$_3$. The enantiomeric excess was determined from the $^1$H-NMR spectrum recorded in the presence of the (+)-Eu(tfc)$_3$ using the following equation: ee = $[(R)-(S)]/[(R)+(S)] \times 100\%$.

The enantiomerically enriched monoacetate was oxidized to the aldehyde (10) which was particularly sensitive to the choice of oxidizing agent used. Oxidation with pyridinium chlorochromate (PCC) or with Parikh-Doering reagent (SO$_3$·pyridine) proceeded with β-elimination of the β-acetoxy aldehyde to give the α,β-unsaturated aldehyde. Dess-Martin periodinane in dry dichloromethane at 0°C gave the maximum yield of the desired product with minimal side reaction; however, it important to note that if the reaction proceeded for longer than six hours, the yield of the eliminated product increased. Due to its instability, the aldehyde was taken directly to the next step without further purification.

The final assembly of $R$-(−)-imperanene began with the coupling of aldehyde 10 with Wittig reagent 6 that was synthesized in Scheme 4-6. The trans-pertosylated imperanene (11) was the favored diastereomer of the product alkene (yield = 88%, a 9:1 diastereomeric ratio) (Scheme 4-8). The isomers were separated by wet column chromatography over silica gel. Global deprotection of the $p$-tosyl and acetyl protecting groups was achieved by refluxing isomer 11 in ethanolic KOH for 3 hr followed by chromatographic purification to yield $R$-(−)-imperanene in 60% yield. The specific
**Figure 4-2.** Determination of enantiomeric excess of $R$-$(+)$-monoacetate ($9$).

The rotation value of the product matched with that of the $R$-$(−)$-imperanene reported in the literature ($[\alpha]_{D}^{25} = -98.2^\circ$ (c = 0.011 g/ml, CHCl$_3$)).$^{20}$
Scheme 4-8. Synthesis of $R$-(-)-imperanene through the coupling of aldehyde 10 with Wittig reagent 6. Conditions: (i) 6, $n$-butyl lithium, 0°C, 24h, 88%; (ii) KOH, ethanol, reflux, 3hr, 60%.

To synthesize the $S$-(+)-imperanene, inversion of stereochemistry in the monoacetate was accomplished after the enantiopure monoacetate (9) was tosylated with tosyl chloride in dichloromethane using triethylamine: DMAP to furnish 12 (yield =93%) (Scheme 4-9). Deacetylation of 12 using $K_2$CO$_3$ in methanol gave the mono-ol (13) in 86% yield which was oxidized under conditions previously stated to give the desired aldehyde 14. The aldehyde (14) proved to be more stable that it’s acetylated counterpart (10). This stability is probably due to steric encumbrance of the β-tosyl group thus suppressing elimination of the α-proton. Similarly, the final assembly of $S$-(+)-imperanene began with the coupling of aldehyde 14 with the Wittig reagent 6 to give 15 (yield = 72%), in a diastereomeric ratio 7:1 in favor of the trans- stereoisomer. Separation of diastereomers was accomplished via wet column chromatography and removal of the
$p$-tosyl protecting groups in refluxing ethanolic KOH gave $S$-(+)-imperanene in 56% yield ($[\alpha]_{D}^{25} = +104^\circ$ (c = 0.01 g/ml, CHCl$_3$)).

**Scheme 4-9.** Synthesis of $S$-(+)-imperanene through stereochemical inversion of the enantiopure monoacetate (9). Conditions: (i) tosyl chloride, triethylamine, DMAP, dry CH$_2$Cl$_2$, 0°C, 93%; (ii) K$_2$CO$_3$, MeOH, 0°C, 87%; (iii) Dess-Martin periodane, CH$_2$Cl$_2$, r.t., 6h, 83%; (iv) 6, $n$-butyllithium, THF, 0°C, 80%; (v) KOH, ethanol, reflux, 3h, 56%.
In conclusion, enantioselective syntheses of both $S$-$(+)$-and $R$-$(–)$-imperanene have been demonstrated starting from vanillin with overall yields of 19% and 24%, respectively. In the core reaction, PS-30 served as the biocatalyst for asymmetrization of the prochiral diol (8) providing the monoacetate (9) in enantiomeric excesses of $>97\%$. The specific rotation values are in agreement with reported literature values.\textsuperscript{20}

\section*{4.4 Stereopreference of the Lipase PS-30.}

Modeling experiments\textsuperscript{21-23} have indicated that \textit{Pseudomonas cepacia} has a stereochemical preference for the $(R)$-substrate over the $(S)$-substrate but its stereoselectivity seems to be dependent on the chemical nature and/or physical state of the substrate, as has been observed for other lipases.\textsuperscript{24} The stereochemical outcome from the PS-30-catalyzed formation of the monoacetate 9 (Scheme 4-7) does supported the stereochemical preference for the $R$-substrate. The stereopreference of the lipase PS-30 $R$-enantiomer was rationalized using a known active site model (Figure 4-3) for the lipase as described by Zuegg et al.\textsuperscript{21} While the hydrogen at the asymmetric center of both enantiomers is directed towards the same site (referred to as H-alignment), the medium sized group (the acetate function) and the large group (the tosyl function) trade places. This allows the medium-sized group to fit into the smaller hydrophobic pocket in the favored enantiomer whereas the larger-sized group is made to accommodate the smaller hydrophobic pocket in the disfavored enantiomer.
Figure 4-3. Schematic Representations of the Orientation of Favored (R) and Disfavored (S) Enantiomer in Active Site of the Lipase PS-30. M = medium, L = large.
4.5 Experimental Section

Lipases PS-30 and AYS were generous gifts from Amano Enzymes and Novozym-435 was donated by Novo Nordisk. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker 250 MHz spectrometer (Appendix C) in CDCl$_3$ with TMS as the standard. Optical rotations were measured with a Rudolph Research Analytical AutoPol IV Automatic polarimeter. Thin-Layer chromatography (TLC) was performed on glass plates coated with 0.25mm thickness of silica-gel. All solvents were dried and distilled prior to use and organic solvent extracts dried over Na$_2$SO$_4$.

4-Formyl-2-methoxyphenyl 4-methylbenzenesulfonate (3). To a solution of vanillin (100 g, 657 mmol) dissolved in dry acetone (500 ml) was added anhydrous K$_2$CO$_3$ (182 g, 1314 mmol) and the mixture allowed stirring for 10 minutes at room temperature. To the solution was added tosyl chloride (251 g, 1314 mmol) and the mixture refluxed for 24 hours. The solution was cooled to room temperature, filtered, and concentrated in vacuo. To the resulting solid was added hexane, the solution was warmed, then cooled to room temperature. The solid was vacuum-filtered and the solid washed with cold hexane to afford an off-white solid (191.5 g, 657.2 mmol, 95%). $^1$H-NMR (250MHz, CDCl$_3$) δ: 2.46 (s, 3H, Ph-C$_2$H$_3$), 3.63 (s, 3H, -OCH$_3$), 7.28-7.77 (comp, 7H), 9.93 (s, 1H, C(O)H).

$^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 21.7, 55.7, 110.9, 124.3, 124.5, 128.5, 129.5, 132.7, 135.6, 142.9, 145.5, 152.5, 190.9.

4-(Hydroxymethyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4). To a solution of protected vanillin 3 (75 g, 245 mmol) in dry methanol (250 ml) cooled at 0°C was added
sodium borohydride (23 g, 612 mmol) portionwise over a 2 hr period. The reaction was brought to room temperature and allowed to stir for a further 12 hours. The mixture was concentrated under reduced pressure and to the resulting solid was added ethyl acetate (400 ml) followed by a solution of ammonium chloride (200 ml). The solution was extracted, the organic layer dried over sodium sulfate and concentrated under reduced pressure. Acetone was added to the resulting solid, cooled to 0°C and vacuum-filtered to afford a white solid (74.5 g, 245 mmol, 99%), m.p. 84-87°C. $^1$H-NMR (250MHz, CDCl$_3$) δ: 2.44 (s, 3H, Ph-CH$_3$), 3.54 (s, 3H, -OCH$_3$), 4.63 (s, 2H, -CH$_2$OH), 6.81-7.09 (comp, 3H), 7.28-7.74 (dd, 4H, $J = 8.2, 8.2$ Hz, -OTs). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 21.6, 55.4, 64.4, 110.9, 118.5, 123.7, 128.5, 129.3, 133.0, 137.3, 141.2, 145.0, 151.7.

4-(Bromomethyl)-2-methoxyphenyl 4-methylbenzenesulfonate (5). To a solution of the alcohol 4 (36 g, 117 mmol) in dry ether (250 ml) was added phosphorus tribromide (47 g, 175 mmol) dissolved in dry ether (50 ml) and the reaction allowed to stir at room temperature for 45 minutes. The reaction was cooled to 0°C and ice water (approximately 100ml) was carefully added over a 30 minute period. The mixture was extracted and the organic layer dried over sodium sulfate and concentrated under reduced pressure. Hexane (100 ml) was added to the resulting solid and vacuum-filtered to afford an off-white solid (42.5g, 117 mmol, 98%), m.p. 69-71°C. $^1$H-NMR (250MHz, CDCl$_3$) δ: 2.45 (s, 3H, Ph-CH$_3$), 3.57 (s, 3H, -OCH$_3$), 4.42 (s, 2H, -CH$_2$Br), 6.86-7.09 (comp, 3H), 7.27-7.76 (dd, 4H, $J = 8.2, 8.2$ Hz, -OTs). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 21.7, 32.7, 55.5, 113.2, 121.1, 124.0, 128.5, 129.4, 133.0, 137.7, 138.1, 145.1, 151.8.
2-Methoxy-4-(triphenylphosphinomethyl bromide)phenyl 4-methylbenzenesulfonate (6). To a solution of the bromide 5 (25 g, 67 mmol) in dry toluene was added freshly recrystallized triphenylphosphine (26 g, 101 mmol) and the solution refluxed for 24 hours. The mixture was cooled to room temperature, filtered under reduced pressure, and washed with dry ether to afford a white solid (41.5g, 67.3 mmol, 97%), 235-238°C. $^1$H-NMR (250MHz, DMSO-d$_6$) $\delta$: 2.39 (s, 3H, Ph-C$_2$H$_3$), 3.02 (s, 3H, -OC$_3$H$_3$), 5.26 (d, 2H, J = 15.6 Hz, Ph-C$_2$H$_2$), 6.56-6.99 (comp, 3H), 7.39-7.93 (comp, 19H). $^{13}$C-NMR (62.5 MHz, DMSO-d$_6$) $\delta$: 21.2, 28.3, 55.2, 115.9, 116.8, 118.2, 123.2, 123.8, 128.3, 128.6, 129.7, 130.0, 130.2, 131.6, 134.1, 134.2, 135.2, 137.2, 145.7, 150.8.

Diethyl 2-(3-methoxy-4-(tosyloxy)benzyl)malonate (7). To a solution of distilled diethyl malonate (19 g, 121 mmol) in dry THF (150 ml) at 0°C was added sodium hydride (2.9 g, 121 mmol) portionwise under a nitrogen atmosphere. The reaction was allowed to stir for 20 minutes and a solution of bromide 5 (30 g, 81 mmol) in dry THF (100 ml) was added dropwise over a 90 minute period. The solution was warmed to room temperature and allowed to stir for a further 12 hours. The solution was concentrated under reduced pressure and the viscous liquid taken up in ethyl acetate (400 ml) and extracted with aqueous sodium chloride (250 ml). The organic layer was dried over sodium sulfate and concentrated. The product was purified by silica gel chromatography using a gradient eluent of ethyl acetate: hexane (2:8) followed by ethyl acetate: hexane (3:7) to afford 7 (33g, 81 mmol, 91%) as light yellow viscous oil. $^1$H-NMR (250MHz, CDCl$_3$) $\delta$: 1.21 (t, 6H, J = 7.1 Hz, -OCH$_2$CH$_3$), 2.44 (s, 3H, Ph-CH$_3$), 3.17 (d, 2H, J = 7.8 Hz, -CH$_2$CH), 3.53 (s, 3H, -OCH$_3$), 3.60 (t, 1H, J = 7.8 Hz, -CH$_2$CH), 4.16 (q, 4H, J =...
7.1 Hz, -OCH₂CH₃), 6.69-7.04 (comp, 3H), 7.28-7.74 (dd, 4H, J = 8.2 Hz, OTs). ¹³C-NMR (62.5 MHz, CDCl₃) δ: 13.9, 21.6, 34.4, 53.5, 55.4, 61.5, 113.2, 120.8, 123.8, 128.5, 129.2, 133.1, 137.0, 138.1, 145.0, 151.5, 168.6.

**4-(3-Hydroxy-2-(hydroxymethyl)propyl)-2-methoxyphenyl-4-methylbenzene sulfonate (8).** To a solution of 7 (20 g, 44 mmol) in methanol:ether (1:3) cooled at 0°C was added lithium chloride (8 g, 178 mmol) and the reaction stirred for an additional 10 minutes. Sodium borohydride (6.7 g, 178 mmol) was then added portionwise over a 30 minute period and the reaction warmed to room temperature and allowed to stir for a further 8 hours. The solution was concentrated, the residue taken up in ethyl acetate (400 ml) and extracted with aqueous ammonium chloride solution (150 ml). The organic layer was dried over sodium sulfate and the resulting product was purified by silica gel chromatography using ethyl acetate as the eluent affording 6 as a colorless viscous liquid.

¹H-NMR (250MHz, CDCl₃) δ: 2.20 (m, 1H, -CH₂CH), 2.44 (s, 3H, Ph-CH₃), 2.54 (d, 2H, J = 7.6 Hz, -CH₂CH), 3.55 (s, 3H, -OCH₃), 3.72 (dd, 2H, J = 10 Hz and 3 Hz, -CH₂OH), 3.98 (dd, 2H, J = 10 Hz and 3 Hz, -CH₂OH), 6.61-6.68 (comp, 3H), 7.27-7.76 (dd, 4H, J = 8.2 Hz, OTs). ¹³C-NMR (62.5 MHz, CDCl₃) δ: 14.1, 21.6, 34.0, 37.8, 55.5, 60.4, 66.6, 112.9, 120.7, 123.9, 128.5, 129.3, 133.1, 136.9, 138.7, 145.0, 151.7.

**2-(Hydroxymethyl)-3-(3-methoxy-4-(tosyloxy)phenypropyl acetate (9).** To a solution of 8 (10 g, 27 mmol) in dry THF (30 ml) was added PS-30 (5g) followed by vinyl acetate (3.5 g, 41 mmol). The reaction was allowed to stir at 40°C for 48 hours and the reaction was filtered under vacuum with subsequent concentration of the filtrate. The
product was purified by wet column chromatography using ethyl acetate: hexane (2:1) to afford a clear viscous liquid (7.5 g, 27 mmol, 67%). $^1$H-NMR (250MHz, CDCl$_3$) δ: 1.85 (m, 1H, -CH$_2$CH), 2.08 (s, 3H, -C(O)CH$_3$), 2.44 (s, 3H, Ph-CH$_3$), 2.54 (dd, 2H, $J = 7.6$ Hz and 18.9 Hz, -CH$_2$CH), 3.55 (m, 5H, -OCH$_3$, -OCH$_2$OH), 4.06 (dd, 2H, $J = 11.3$ Hz and 4.8 Hz, -CH$_2$C(O)CH$_3$), 6.65-6.71 (comp, 3H), 7.27-7.76 (dd, 4H, $J = 8.2$ Hz, OTs). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 20.6, 21.3, 34.3, 38.7, 55.5, 63.5, 113.1, 120.9, 124.0, 128.5, 129.3, 133.1, 136.7, 139.2, 145.6, 151.6, 171.0.

2-Formyl-3-(3-methoxy-4-(tosyloxy)phenyl)propyl acetate (10). To a solution of 9 (1 g, 2.4 mmol) in dry dichloromethane (30 ml) at 0°C was added Dess-Martin periodinane (5ml, 15%w/v). The reaction was allowed to stir at 0°C for a further 90 minutes then allowed to warm to room temperature and stirred for an additional 2 hours. A solution of sodium thiosulfate (15ml) was added and the solution stirred for an additional 15 minutes, then vacuum-filtered over celite. An additional 30 ml of dichloromethane was added and extracted with aqueous sodium chloride (20 ml). The organic layer was dried over sodium sulfate, concentrated, and the product (amber liquid, 0.750 g, 2.4 mmol, 75%) used immediately in the next reaction without further purification. $^1$H-NMR (250MHz, CDCl$_3$) δ: 2.04 (s, 3H, -C(O)CH$_3$), 2.45 (s, 3H, Ph-CH$_3$), 2.77 (dd, 2H, $J = 7.8$ Hz and 13.6 Hz, -CH$_2$CH), 2.91 (m, 1H, -CH$_2$CH), 3.53 (s, 3H, -OCH$_3$), 4.2 (dd, 2H, $J = 11.3$ Hz and 4.8 Hz, -CH$_2$C(O)CH$_3$), 6.65-6.71 (comp, 3H), 7.27-7.76 (dd, 4H, $J = 8.2$ Hz, OTs), 9.66 (s, 1H, -C(O)H).
(E)-2-(3-methoxy-4-(tosyloxy)benzyl)-4-(3-methoxy-4-(tosyloxy)phenyl)but-3-enyl acetate (11). To a solution of the Wittig reagent (9.7 g, 15 mmol) 6 in dry THF under a nitrogen atmosphere at 0°C was added n-butyllithium (0.98 g, 15 mmol). The reaction was allowed to stir for an additional 30 minutes and a solution of the aldehyde 9 (2.5 g, 6.2 mmol) in dry THF (10 ml) was added to the sanguineous solution at 0°C. The reaction was allowed to warm to room temperature and stirred for an additional 19 hours. The solution was then concentrated, and the residue taken up in ethyl acetate (50 ml) and extracted with brine (20 ml). Purification over wet silica chromatography using ethyl acetate: hexane (2:8) provided compound 11 as a viscous, amber liquid (3.58 g, 6.15 mmol, 88%). 1H-NMR (250MHz, CDCl3) δ: 1.98 (s, 3H, C(O)CH3), 2.43 (s, 6H, Ph-CH3), 2.61 (m, 3H, Ph-CH2-CH-), 3.37 (s, 3H, -OCH3), 3.44 (s, 3H, -OCH3), 4.01 (m, 2H, -CH2OAc), 5.84 (dd, 1H, J = 7.9, 15.8 Hz), 6.27 (d, 1H, J = 15.8 Hz), 6.33-7.15 (comp, 6H), 7.19-7.70 (comp, 8H); 13C-NMR (62.5 MHz, CDCl3) δ: 14.1, 21.6, 37.8, 43.8, 55.5, 60.5, 66.2, 110.0, 113.3, 118.2, 121.0, 123.5, 124.0, 128.5, 129.3, 130.6, 131.0, 133.0, 136.3, 136.7, 137.1, 139.2, 145.0, 151.3, 151.6, 170.5.

3-(3-Methoxy-4-(tosyloxy)phenyl)-2-((tosyloxy)methyl)propyl acetate (12). To a solution of the monoacetate 9 (2.17 g, 5.31 mmol) in dry dichloromethane (30 ml) was added triethyl amine (0.81 g, 8 mmol) and DMAP (100 mg) at 0°C. Tosyl chloride (1.52 g, 8.0 mmol) was then added and allowed to stir at r.t. for 8 hours. The solution was then concentrated and ethyl ether was added and the solution filtered. The filtrate was concentrated under reduced pressure and purified by flash column chromatography using EA/hexane (4:6) to afford a viscous colorless liquid (2.79 g, 5.31 mmol, 93%). 1H-NMR
(250MHz, CDCl$_3$) δ: 1.88 (s, 3H, C(O)CH$_3$), 2.45 (m, 1H, CH), 2.38 (s, 3H, Ph-CH$_3$), 2.39 (s, 3H, Ph-CH$_3$), 2.54 (d, 2H, J = 7.4 Hz, Ph-CH$_2$), 3.45 (s, 3H, -OCH$_3$), 3.85 (m, 4H, -CH$_2$OAc, -CH$_2$OTs), 6.50-7.12 (comp, 3H, -Ph), 7.25-7.77 (m, 8H, -OTs); $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 20.6, 21.6, 33.7, 39.3, 55.5, 62.9, 68.5, 113.3, 120.8, 124.0, 127.8, 128.5, 129.3, 129.9, 132.5, 133.1, 145.0, 151.7, 170.5.

3-(3-Methoxy-4-(tosyloxy)phenyl)-2-(hydroxymethyl)-1-(tosyloxy)propane (13). To a solution of the monoacetate 12 (2.5 g, 4.4 mmol) in dry methanol (20 ml) was added anhydrous potassium carbonate (1.2 g, 8.8 mmol). The reaction was allowed to stir at room temperature for 3 hours upon which it was concentrated and purified by flash column chromatography using ethyl acetate: hexane (4:6) affording a viscous colorless liquid 13 (2.0 g, 4.4 mmol, 86%). $^1$H-NMR (250MHz, CDCl$_3$) δ: 1.98 (m, 1H, CH), 2.37 (s, 6H, Ph-CH$_3$), 2.52 (dd, 2H, J = 6.3, 5.9 Hz, Ph-CH$_2$), 3.44 (s, 3H, -OCH$_3$), 3.47 (m, 2H, -CH$_2$OH), 3.94 (m, 2H, -CH$_2$OTs), 6.53-6.94 (comp, 3H), 7.19-7.70 (comp, 8H); $^{13}$C-NMR (62.5 MHz, CDCl$_3$) δ: 21.6, 33.4, 42.4, 55.5, 69.2, 113.3, 120.9, 127.8, 128.5, 129.3, 132.6, 133.2, 136.8, 139.0, 145.0, 151.6.

3-(3-Methoxy-4-(tosyloxy)phenyl)-2-((tosyloxy)methyl)propanal (14). To a solution of 13 (1.5 g, 2.4 mmol) in dry dichloromethane (30 ml) at 0°C was added Dess-Martin periodinane (5 ml, 15%w/v). The reaction was allowed to stir at 0°C for a further 90 minutes then allowed to warm to room temperature and stirred for an additional 2 hours. A solution of sodium thiosulfate (15 ml) was added and the solution stirred for an additional 15 minutes, then vacuum-filtered over celite. An additional 30 ml of
dichloromethane was added and extracted with aqueous sodium chloride (20 ml). The organic layer was dried over sodium sulfate, concentrated, and the product 14 (amber liquid, 1.25 g, 2.88 mmol, 83%) used immediately in the next reaction without further purification. \(^1\)H-NMR (250MHz, CDCl\(_3\)) \(\delta\): 2.45 (s, 6H, Ph-CH\(_3\)), 2.77 (dd, 2H, \(J = 7.8\) Hz and \(13.6\) Hz, -CH\(_2\)CH), 2.97 (m, 1H, -CH\(_2\)CH), 3.45 (s, 3H, -OCH\(_3\)), 4.2 (dd, 2H, \(J = 11.3\) Hz and \(4.8\) Hz, -CH\(_2\)OTs), 6.47-6.91 (comp, 3H), 7.13-8.25 (m, 8H, -OTs), 9.60 (s, 1H, -C(O)H)

**Pertosylated Imperanene (15).** To a solution of the Wittig reagent (3.66 g, 5.78 mmol) 6 in dry THF under a nitrogen atmosphere at 0°C was added \(n\)-butyl lithium (0.37 g, 5.8 mmol). The reaction was allowed to stir for an additional 30 minutes and a solution of the aldehyde 14 (1 g, 2 mmol) in dry THF (10 ml) was added to the sanguineous solution at 0°C. The reaction was allowed to warm to room temperature and stirred for an additional 19 hours. The solution was then concentrated, and the residue taken up in ethyl acetate (50 ml) and extracted with brine (20 ml) to afford a viscous, amber liquid 15 (1.1 g, 1.93 mmol, 72%) which was taken onto the next step without further purification. \(^1\)H-NMR (250MHz, CDCl\(_3\)) 2.44 (s, 9H, Ph-CH\(_3\)), 2.54 (m, 3H, Ph-CH\(_2\)-CH-), 3.35 (s, 3H, -OCH\(_3\)), 3.45 (s, 3H, -OCH\(_3\)), 3.87 (m, 2H, -CH\(_2\)OTs), 5.84 (dd, 1H, \(J = 7.9\), 15.8 Hz), 6.27 (d, 1H, \(J = 15.8\) Hz), 6.49-7.01 (comp, 6H), 7.1-7.75 (m, 12H, -OTs); \(^{13}\)C-NMR (62.5 MHz, CDCl\(_3\)) \(\delta\): 21.6, 37.2, 43.8, 55.5, 71.5, 110.2, 112.6, 113.5, 118.3, 120.0, 121.1, 123.8, 124.0, 127.8, 128.5, 129.0, 129.3, 129.9, 131.7, 132.5, 133.1, 136.7, 136.8, 137.7, 138.5, 145.1, 151.5, 151.7.
Imperanene (1). To a solution of 11 or 15 dissolved in ethanol was added aqueous potassium hydroxide solution. The mixture was brought to reflux for 3 hours after which they were concentrated under vacuum. The viscous liquid was taken up in ethyl acetate then extracted with brine. The organic layer was dried over sodium sulfate and concentrated. Purification by flash column chromatography using ethyl acetate: hexane (15:85) provided the $R$ (60%) and $S$ (56%) isomers, respectively. $^1$H-NMR (250MHz, CDCl$_3$) $\delta$: 2.61 (m, 3H, Ph-CH$_2$-CH-), 3.48 (m, 2H, -CH$_2$OH), 3.74 (s, 3H, -OCH$_3$), 3.80 (s, 3H, -OCH$_3$), 5.51-5.65 (comp, 2H, Ph-OH), 5.84 (dd, 1H, $J = 7.9, 15.8$ Hz), 6.27 (d, 1H, $J = 15.8$ Hz), 6.59-6.76 (comp, 6H). $^{13}$C-NMR (62.5 MHz, CDCl$_3$) $\delta$: 37.6, 47.6, 55.8, 65.2, 108.2, 111.7, 114.1, 114.4, 119.7, 121.8, 128.3, 129.7, 131.5, 132.1, 143.8, 145.2, 146.3, 146.6.
4.6 References


18. Maximum yield upon enzymatic acetylation was 62%. The unreacted diol was recovered and acetylation was repeated with recovered enzyme to give a total yield of 90% of combined enantiopure monoacetate (9), $[\alpha]^{25}_{D} = +7.9^\circ$.

19. The diastereomeric ratio was determined via $^1$H-NMR by comparing the ratio of the alkenyl protons of the crude reaction mixture.

20. While the natural Imperanene was reported to have $[\alpha]^{25}_{D}$ value of +700 by Matsunaga, et al.¹, Doyle et al.² reported $[\alpha]^{25}_{D} = + 103$ for 93% enantioenriched sample and have explained the discrepancy in reported $[\alpha]^{25}_{D}$ for natural product and observed value for the synthetic sample.


Figure A-1. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one.
Figure A-2. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one.
Figure A-3. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-(2-phenylethynyl)azetidin-2-one.
Figure A-4. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1-(4-methoxyphenyl)-4-(2-phenylethynyl)azetidin-2-one.
Figure A-5. $^1$H-NMR Spectrum of 3-Acetoxy-1-(4-methoxyphenyl)-4-(phenylethynyl)azetidin-2-one.
Figure A-6. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1-(4-methoxyphenyl)-4-(phenylethynyl)azetidin-2-one.
Figure A-7. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-thiophen-2-ylazetidin-2-one.
Figure A-8. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1-(4-methoxyphenyl)-4-thiophen-2-ylazetidin-2-one.
**Figure A-9.** $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1, 4-bis-(4-methoxyphenyl)azetidin-2-one.
Figure A-10. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1,4-bis-(4-methoxyphenyl)-azetidin-2-one.
Figure A-11. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(4-methoxycarbonylphenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-12. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-4-(4-methoxycarbonylphenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-13. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one.
Figure A-14. $^1$H and $^{13}$C-NMR Spectra of Cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one.
Figure A-15. $^1$H and $^{13}$C-NMR Spectra of Trans-3-Hydroxy-1-(4-methoxyphenyl)-4-(4-nitrophenyl)azetidin-2-one.
Figure A-16. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-(3-nitrophenyl)azetidin-2-one.
Figure A-17. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-1-(4-methoxyphenyl)-4-(3-nitrophenyl)azetidin-2-one.
Figure A-18. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(4-cyanophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-19. $^1$H and $^{13}$C-NMR Spectra of 4-(4-Cyanophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-20. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(4-dimethylaminophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-21. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-4-(4-dimethylaminophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-22. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(3-fluorophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-23. $^1$H and $^{13}$C-NMR Spectra of 4-(3-Fluorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-24. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxyl-4-(4-fluorophenyl)-1-(4-methoxynaphthyl)azetidin-2-one.
Figure A-25. $^1$H and $^{13}$C-NMR Spectra of 4-(4-Fluorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-26. $^1$H-NMR Spectrum of 3-Acetoxy-4-(4-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-27. $^1$H and $^{13}$C-NMR Spectra of 4-(4-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-28. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(3-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-29. $^1$H and $^{13}$C-NMR Spectra of 4-(3-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-30. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(2-chlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-31. $^1$H and $^{13}$C-NMR Spectra of 4-(2-Chlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-32. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-3. $^1$H and $^{13}$C-NMR Spectra of 4-(2,4-Dichlorophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-34. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(2-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-35. $^1$H and $^{13}$C-NMR Spectra of 4-(2-Bromophenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-36. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(3-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-37. $^1$H and $^{13}$C-NMR Spectra of 4-(3-Bromophenyl)-3-hydroxy-1-(4-methoxyp phenyl)azetidin-2-one.
Figure A-38. $^1$H and $^{13}$C-NMR Spectra of 3-Acetoxy-4-(2-iodophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-39. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-4-(2-iodophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-40. $^1$H-NMR Spectrum of 3-Acetoxy-4-(4-bromophenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-41. $^1$H and $^{13}$C-NMR Spectra of 3-acetoxy-4-(4-trifluoromethylphenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-42. $^1$H and $^{13}$C-NMR Spectra of 4-(4-trifluoromethylphenyl)-3-hydroxy-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-4. $^1$H and $^{13}$C NMR spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-tert-butyl phenyl)azetidin-2-one.
Figure A-44. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-4-(4-tert-butylphenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure A-45. $^1$H and $^{13}$C NMR spectra of 3-Acetoxy-1-(4-methoxyphenyl)-4-(4-isopropylphenyl)azetidin-2-one.
Figure A-46. $^1$H and $^{13}$C-NMR Spectra of 3-Hydroxy-4-(4-isopropylphenyl)-1-(4-methoxyphenyl)azetidin-2-one.
Figure B-1. $^1$H and $^{13}$C-NMR Spectra of Methyl 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-2. $^1$H and $^{13}$C-NMR Spectra of Methyl 17-L-((2″,3′,3″,4′,4″,6″)-heptaacetoxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-3. HRMS Spectrum of Methyl 17-L-([2\”',3\″',3\′',4\″',4\′',6\″',6\″'-heptaacetoxy-2\'-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-4. $^1$H and $^{13}$C-NMR Spectra of Methyl 17-L-([2",3",3",4",4",6",6"
-heptapionyloxy-2'-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-
octadecenoate.
Figure B-5. $^1$H and $^{13}$C-NMR Spectra of $n$-Butyl 17-L-([2″,3″,4″,4″,6″,6″-heptaacetoxy-2′-O-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-6. HRMS Spectrum of *n*-Butyl 17-L-((2″,3″,4″,6″-heptaacetoxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-7. $^1$H and $^{13}$C-NMR Spectra of Isobutyl 17-L-([2",3",3",4",4",6",6"-heptaacetoxy-2'-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-8. $^1$H and $^{13}$C-NMR Spectra of Isobutyl 17-L-([2\(^{"},3\(^{"},3\(^{"},4\(^{"},4\(^{"},6\(^{"},6\(^{"}-heptaacetoxy-2\(^{`}\)-O-\(\beta\)-D-glucopyranosyl-\(\beta\)-D-glucopyranosyl]-oxy)-\(cis\)-9-octadecenoate.
Figure B-9. $^1$H and $^{13}$C-NMR Spectra of $n$-Butyl 17-L-([2$''$,3$''$,3$'$,4$'$,4$''$,6$'$,6$''$-heptapropionyloxy-2$'$-O-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-10. HRMS Spectrum of \textit{n}-Butyl 17-L-((2″,3′,3″,4′,4″,6′,6″-heptapropionyloxy-2′-\textit{O}-\textit{\beta}-D-glucopyranosyl-\textit{\beta}-D-glucopyranosyl]-oxy)-\textit{cis}-9-octadecenoate.
Figure B-11. $^1$H and $^{13}$C-NMR Spectra of Isobutyl 17-L-((2",3",3"′,4",4"′,6",6"′-heptapropionyloxy-2'-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoate.
Figure B-12. $^1$H and $^{13}$C-NMR Spectra of 17-L-([2″,3′′,3″,4′,4″,6′′,6″-Heptaacetoxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoic acid.
Figure B-13. HRMS Spectrum of 17-L-([2″,3″,4″,4′,6″-Heptaacetoxy-2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-cis-9-octadecenoic acid.
Figure B-14. $^1$H and $^{13}$C-NMR Spectra of $2",3",3",4",4",6",6"$-Heptapionyloxy-17-L-([2'-O-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranosyl]-oxy)-cis-9-octadecenoic acid.
Figure B-15. HRMS Spectrum of 2\textsuperscript{''},3\textsuperscript{''},3\textsuperscript{''},4\textsuperscript{''},4\textsuperscript{''},6\textsuperscript{''},6\textsuperscript{''}-Heptapropionyloxy-17-L-([2\textsuperscript{''}-O-\beta-D-glucopyranosyl-\beta-D-glucopyranosyl]-oxy)-\textit{cis}-9-octadecenoic acid.
Figure B-16. $^1$H and $^{13}$C-NMR Spectra of Butyl 17-L-[(2'-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-cis-9-octadecenoate.
Figure B-17. HRMS Spectrum of Butyl 17-L-[(2'-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-cis-9-octadecenoate.
APPENDIX C

Spectroscopic data for compounds of Chapter 4

Figure C-1. $^1$H and $^{13}$C-NMR Spectra of 4-Formyl-2-methoxyphenyl 4-methylbenzenesulfonate.
Figure C-2. $^1$H and $^{13}$C-NMR Spectra of 4-(Hydroxymethyl)-2-methoxyphenyl 4-methylbenzenesulfonate.
Figure C-3. $^1$H and $^{13}$C-NMR Spectra of 4-(Bromomethyl)-2-methoxyphenyl 4-methylbenzenesulfonate.
Figure C-4. $^1$H and $^{13}$C-NMR Spectra of 2-Methoxy-4-(triphenylphosphinomethyl bromide)phenyl 4-methylbenzenesulfonate.
Figure C-5. $^1$H and $^{13}$C-NMR Spectra of Diethyl 2-(3-methoxy-4- (tosyloxy)benzyl)malonate.
Figure C-6. $^1$H and $^{13}$C-NMR Spectra of 4-(3-Hydroxy-2-(hydroxymethyl)propyl)-2-methoxyphenyl 4-methylbenzenesulfonate.
Figure C-7. $^1$H and $^{13}$C-NMR Spectra of 2-(Hydroxymethyl)-3-(3-methoxy-4-(tosyloxy)phenyl)propyl acetate.
Figure C-8. $^1$H and $^{13}$C-NMR Spectra of (E)-2-(3-Methoxy-4-(tosyloxy)benzyl)-4-(3-methoxy-4-(tosyloxy)phenyl)but-3-enyl acetate.
Figure C-9. $^1$H and $^{13}$C-NMR Spectra of 3-(3-Methoxy-4-(tosyloxy)phenyl)-2-((tosyloxy)methyl)propyl acetate.
Figure C-10. $^1$H and $^{13}$C-NMR Spectra of 3-(3-Methoxy-4-(tosyloxy)phenyl)-2-(hydroxymethyl)-1-(tosyloxy)propane.
Figure C-11. $^1$H Spectrum of 3-(3-Methoxy-4-(tosyloxy)phenyl)-2-((tosyloxy)methyl)propanal.
Figure C-12. $^1$H Spectrum of Pertosylated Imperanene.
Figure C-13. $^1$H and $^{13}$C-NMR Spectra of Imperanene.
About the Author

Jason A. Carr was born in Belize, Central America to Winston and Victoria Carr. In 1995, Jason left Belize to further his education and he settled in Sarasota, FL where he received his Associate’s Degree from Manatee Community College in 1997 followed by a Bachelor’s Degree in Chemistry from New College of Florida in 1999. He then decided to pursue a doctorate at the University of South Florida.

While in the Ph.D. program at the University of South Florida, Mr. Carr was very active in research in the Department of Chemistry. He has coauthored four publications in peer reviewed journals and made several poster presentations at regional and national meetings of the American Chemical Society. He is an active member of the International Union of Pure and Applied Chemistry, the American Chemical Society Organic Chemistry Division and the American Chemical Society Younger Chemist Society.