Lipase-catalysed Biotransformations in Organic Synthesis.

A thesis presented in part fulfilment of the requirement for the Degree of Doctor of Philosophy

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Quaeque ipse miserrima vidi,
Et quorum pars magna fui.

Virgil.

"Among 'hot' chemistry papers, the top ten (as measured by citations received in September-October last year) include only three *not* on fullerenes. The 'hottest' of these was on the use of enzymes in organic solvents."

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Abbreviations

The following abbreviations are used in this text.

ANL	:*Aspergillus niger* lipase.
BOC	:*t*-Butoxycarbonyl.
BOC-ON	:2-(*t*-Butoxycarbonyloxyimino)-2-phenylacetonitrile.
CDI	:1,1'-Carbonyldiimidazole.
CRL	:*Candida rugosa* lipase.
CVL	:*Chromobacterium viscosum* lipase.
DBU	:1,8-Diazabicyclo-[5.4.0]-undec-7-ene.
DCC	:*N,N'*-Dicyclohexylcarbodiimide.
DCM	:Dichloromethane.
DCU	:*N,N'*-Dicyclohexylurea.
DEPT	:Distortionless Enhancement by Polarisation Transfer.
DIPE	:Diisopropyl ether.
DMAP	:4-Dimethylaminopyridine.
DMF	:*N,N*-Dimethylformamide.
DMSO	:Dimethylsulphoxide.
EDC-HCl	:1-Ethyl-3-(3-dimethylpropyl)carbodiimide hydrochloride.
GC	:Gas Chromatography.
HPLC	:High Performance Liquid Chromatography.
LAH	:Lithium Aluminium Hydride.
LDA	:Lithium *N,N*-Diisopropylamide.
LICA	:Lithium *N*-isopropylcyclohexylamide.
NBS	:*N*-Bromosuccinimide.
PCL	:*Pseudomonas cepacia* lipase.
PFPA	:Pentafluorophenyl acetate.
PLE	:Pig Liver Esterase.

PPL	:Porcine Pancreatic Lipase.
PSL	:*Pseudomonas* species Lipase.
SAT	:Sodium Ammonium Tartrate.
TBDMS	:*t*-Butyldimethylsilyl.
THF	:Tetrahydrofuran.
TLC	:Thin Layer Chromatography.
TMS	:Tetramethylsilane.
TMSCl	:Trimethylsilyl chloride.

Table of Contents

Summary

In this thesis are described our investigations into the use of lipases as asymmetric catalysts in organic synthesis. The primary objective of this work was to achieve the resolution of pyrrolidine and piperidine alcohols *via* porcine pancreatic lipase catalysed transacetylations, using ethyl acetate as both the acyl donor and the solvent. Various synthetic routes directed towards the synthesis of these amino alcohols are described, along with our attempts at synthesising aziridine alcohols for use as enzyme substrates. Several experimental procedures were developed for the porcine pancreatic lipase mediated resolution of these amino alcohols, and acetylated products were obtained in enantiomeric excesses of between 0 and 57%. Also described in this thesis are our attempts to extend this work to the lipase-mediated resolution of α- and β- amino acid derivatives. Herein is described the preparation and lipase-mediated alcoholysis of several 5(4H)-oxazolones ('azlactones') and 4,5-dihydro-1,3-oxazin-6-ones ('dihydrooxazinones'), using both porcine pancreatic lipase and *Mucor miehei* lipase. Low enantioselectivities were observed in these reactions. Finally, our investigation into the effect of varying the nature of the acyl donor in the lipase-catalysed acylation of (±)-2-octanol is described. No esterified products were obtained in the attempted coupling of simple carboxylic acids to the alcohol. The preparation of a number of 2,2,2-trichloroethyl esters of a number of simple alkyl, alkenyl and aromatic acids is described, and our investigation of the lipase catalysed reaction of these irreversible acyl donors with (±)-2-octanol is described. The monitoring of the progress of these reactions by gas chromatography is described. Results at variance with published results were obtained, and the implications of the results obtained are discussed.

Chapter One

Introduction

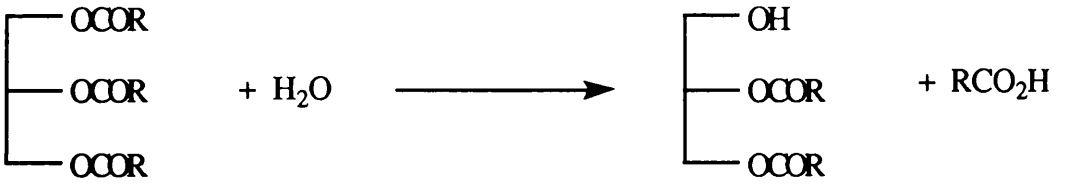
1.1. Lipases

The production of optically active compounds is of great importance to chemistry today, both in research and industrial applications. The biological activities of the two enantiomers of a compound are seldom equal, and on occasion the beneficial aspects of one enantiomer are more than outweighed by the detrimental effects of the other (Thalidomide being a prime example). Therefore, it is clearly of tremendous importance to the pharmaceutical industry to have easy access to both enantiomers of a test compound. This can be achieved by both resolution and asymmetric synthesis. Resolution techniques are often wasteful, resulting in a large amount of material being discarded (although this disadvantage can be partially overcome by recycling the unresolved material). Asymmetric synthesis, although seemingly more attractive, often requires the use of expensive chiral auxiliaries and reaction conditions which are less than ideal for industrial scale processes. In addition, the chiral auxiliary itself must be obtainable in optically active form, and for 'unnatural' compounds this ultimately means that some form of resolution must occur. Clearly then, there is scope for the development of new, economical, environmentally friendly techniques for the obtention of optically active compounds. Therefore, the discovery that some enzymes catalysed asymmetric transformations in organic solvents has created much interest in the use of these compounds for the resolution of many types of compounds. Foremost among the

classes of enzymes which have been used in organic solvents have been lipases, and it is the reactions of these enzymes which have been investigated in this work.

1.2 Definition of Lipases

The exact definition of a lipase (E.C. 3.1.1.3) has, in the past, been confused. Lipases are ester hydrolases, but collectively they form a sub-group clearly distinct from other carboxylesterases. The definition of a lipase as an enzyme which hydrolyses triacylglycerols[1] according to Scheme 1.1 has proved to be inadequate, not least because of the ability of lipases to hydrolyse triacylglycerols to monoacylglycerols and



Scheme 1.1

even to glycerol itself.[2] Sarda and Desnuelle[3] have proposed that the most useful differentiation between lipases and carboxylesterases lies in the physical state of the substrate. Lipases show a marked preference for emulsified[3] or micellar[4] substrates, whereas esterases prefer water-soluble substrates. Thus, lipases are unusual in that they operate *in vivo* in a low-water environment. This fact must surely be a significant factor in the unique stability and catalytic activity of lipases in organic solvents.

1.3. Occurrence of Lipases

Lipases are ubiquitous in nature, being found in both plant and animal kingdoms. This reflects the widespread usage of triacylglycerols as the main energy storage device *in vivo*.[5] An especially rich source of lipases in plants is in germinating seeds,[6] when the organism needs to convert the store of triacylglycerols rapidly into energy for growth. Bacterial lipases are generally exocellular enzymes,[6] excreted by the organism into the surrounding medium.

The most widely studied of animal lipases are of mammalian origin. In 1849, Claude Bernard realised that the pancreas had a crucial role in the mammalian digestion of fat through the pancreatic hydrolysis of long-chain triglycerides.[7] This observation led to the study of pancreatic juices from a range of species, culminating in the isolation of lipases from pig,[8], rat,[9] cow,[10] sheep[11] and human[12] pancreas. The primary sequences of these enzymes, where known, show a high degree of similarity.[13]

In addition to the well studied pancreatic lipases, lipolytic activity has been reported in various mammalian tissues and organs, including heart, liver, testicular and adipose tissues.[6] Lipases have also been isolated from a number of non-mammalian animal sources, including chickens,[14] honey bees.[15] and dogfish.[16] However, none of these sources has been subjected to the same degree of study as the pancreatic lipases.

1.4 Advantages of Lipases as Asymmetric Catalysts

This topic is treated in greater detail in Chapter 4, and so will only be discussed briefly here. A wide range of lipases are currently commercially available from both animal and microbial sources. The ready availability of a large number of lipases allows the chemist to screen several enzymes to find the one best suited to a particular substrate and reaction. In addition, lipases as a whole show a remarkable ability to accommodate a diverse range of substrate structures. It is for a combination of these two facts that the use of lipases has become increasingly popular as a method for obtaining optically active compounds.

Lipases, being proteins, are the ultimate in bio-degradable reagents, an important consideration in these environmentally conscious times. The use of isolated lipases can be advantageous over whole-cell processes, since unwanted side reactions catalysed by other enzymes can be eliminated. In addition, isolated enzyme reactions do not have to be carried out under the carefully controlled conditions of a fermentation vessel. It is also true that the high stability of lipases in organic solvents makes the technique applicable to a wide range of substrates which cannot survive in the aqueous environment of a fermentation process.

Lipase-catalysed reactions can proceed with extremely high enantioselectivities and/or regioselectivities, forming products which are essentially optically pure. Where the substrate molecule is prochiral, lipases can create an optically pure molecule in high chemical and optical yields. Moreover, when lipase-catalysed reactions proceed with unacceptably low levels of enantioselection, the reaction conditions can be altered to maximise the efficiency of the process. Temperature, solvent, agitation rate, the nature

of the lipase, and the type of acyl donor or acceptor molecules have all been shown to affect the enantioselectivity of an enzyme-catalysed reaction.

Lipases have been, and continue to be, important reagents for the production of optically pure compounds of many kinds. The use of such reagents under mild conditions (typically between 0-40°C) in organic solvents has opened up the field of biotransformations to a wide number of applications impossible in an aqueous environment. In the following chapters is recounted our use of lipases in the enantioselective resolutions of a variety of compounds of amino acid or amino alcohol origin. Chapter Two reviews the current knowledge about the structure of lipases, concentrating on the main enzyme used in this work, Porcine Pancreatic Lipase (PPL). This chapter also reviews what is known about the mechanism of lipase-catalysed triacylglycerol hydrolysis, and a likely reaction scheme is proposed. Methods of obtaining optically active amino acids and amino acid derivatives are reviewed in Chapter Three, with particular reference to resolution techniques and asymmetric synthesis. In Chapter Four is discussed the use of lipases in organic synthesis, concentrating on three main areas; the conversion of prochiral substrates into optically active products, the regiospecific reactions of lipases with sugars, and the lipase-mediated resolution of amino alcohols and amino alcohol precursors. This chapter also contains a discussion of the various methods used to increase the efficiency and selectivity of lipase-catalysed processes. Chapter Five tells of our work using cyclic amino alcohols as lipase substrates. In this chapter is described the various routes developed to synthesise both racemic and optically pure 2-pyrrolidinemethanols ('prolinols') and 2-piperidinemethanols ('homoprolinols'). The synthesis of the corresponding *N*-methyl amino alcohols is also described, as are our abortive attempts at preparing 2-aziridinemethanols. The latter part of Chapter Five describes the various

procedures we used to obtain optically active amino alcohols and amino alcohol derivatives *via* lipase-catalysed transacylations in organic solvents.

In Chapter Six, we describe our work aimed at resolving amino acid derivatives by the action of lipases in organic solvents. The preparation of several racemic oxazol-5(4H)-ones from α-amino acids is described, along with our attempts at effecting enantioselective alcoholysis of these compounds with lipase. Chapter Six also contains details of the many methods employed to prepare 4,5-dihydro-1,3-oxazin-6-ones ('dihydrooxazinones') from the corresponding β-amino acids, and our investigations into the lipase-catalysed alcoholysis of these compounds. Finally, Chapter Seven details our investigations into the effect of varying substrate structures on lipase-catalysed transesterifications. The preparation of a wide range of 2,2,2-trichloroethyl esters is described, along with details of the screening of these compounds as achiral acyl donors in lipase-catalysed transesterifications, using (±)-2-octanol as a model substrate. The effect of varying reaction parameters such as water content and shaking rate is also investigated here, obtaining results of significance to our previous work.

Chapter Two

Structure and Mechanism of Lipases

2.1 Lipase Structure

The most widely studied of the lipases has been Porcine Pancreatic Lipase (PPL), and so this is the enzyme which will be discussed in greatest detail, and will be referred to as "lipase".

To date, no X-ray crystal structure analysis of PPL has been carried out. Therefore, current knowledge of the structure and mode of action of PPL is based on chemical methods of probing the active site of the enzyme.

The first purified preparation of Porcine Pancreatic Lipase was obtained in 1955 by Herr and Sumner.[17] With improving isolation techniques, PPL was separated into two isoenzymes of very similar amino acid compositions, in a relative proportion of 1:4.[18] In addition, the two isoenzymes, labelled L_A and L_B, were found to have similar activities towards the hydrolysis of both long- and short-chain triglycerides. Both isoenzymes were found to have molecular weights in the range 45 000 - 50 000 daltons. An improved large-scale purification procedure for PPL developed by Rovery and co-workers[19] led them to conclude that the isoenzymes differed from each other only in the composition of a glycan chain covalently bound to the polypeptide.

The complete primary structure of PPL was reported in 1981.[20] The enzyme was found to be a single-chain polypeptide containing 449 amino acid residues, with a calculated molecular weight for the polypeptide of 49 859. The enzyme contains two thiol groups on cysteine residues[21] and six disulphide bridges.[22] The positions of 5 of these bridges have been unambiguously identified as being between Cys-4 and Cys-10, Cys-238 and Cys-261, Cys-285 and Cys-296, Cys-299 and Cys-304, and Cys-433 and Cys-449. The position of the final disulphide linkage has not been determined, although it is known to involve the cysteine residue at position 90 along with one of two cysteines at positions 101 and 103. It has been suggested that two isomeric forms of lipase exist, differing only in the location of this cystine residue.[22] In any case, the disulphide bridges form small loops in the enzyme structure, imparting a degree of flexibility to the molecule[13] which may go a long way to explaining the extraordinarily wide range of substrate molecules accepted by the enzyme at the hands of organic chemists. PPL has little α-helix structure, but contains about 75% β-structure.[23]

The purified PPL enzyme proved to be totally inactive as a catalyst for the hydrolysis of emulsified olive oil in the presence of bile salts. However, it was found that activity could be restored by the addition of a second protein, present in the exocrine secretion of the pancreas.[24] This proteinaceous cofactor of lipase, christened colipase, was isolated in 1971 by Maylie *et al.*[25] Erlanson and Borgstrom characterised colipase as a single chain polypeptide of approximately 95 amino acid residues, with isoleucine as the *N*-terminal amino acid residue.[26] Charles *et al.* isolated two candidates for colipase, termed colipase I and II.[27] Colipase I corresponds to the protein of Erlanson and Borgstrom, while colipase II contains 84 amino acid residues. It now appears that the various forms of colipase isolated result from partial enzymic degradation of a common precursor, either *in vivo* or during purification.[28] The amino acid sequence of colipase II has been determined,[27] as has the location of 3 out of 5 cystine residues.[29] From this

evidence, colipase II appears to be organised into a heavily crosslinked central core and two loosely bound tails. The function of the colipase will be discussed in a later section.

The third gross structural feature of Porcine Pancreatic Lipase is the glycan chain,[30] which is covalently bound to the enzyme *via* an asparagine residue at position 166.[31] The composition of this sugar moiety was reported for lipases L_A and L_B respectively (Table 2.1).[30]

Table 2.1[30]

Sugar	Lipase L_A	Lipase L_B
Fucose	0.83	0.92
Galactose	1.05	0.53
Glucose	0.48	0.16
Mannose	4.12	4.81
Glucosamine	3.05	2.98
Sialic Acid	0.31	0.04

Sugar Composition of glycan chains in Lipases L_A & L_B (mol sugar/mol lipase)

The structure of the glycan chain was described as a mixture of several structures.

2.2 Mechanism of Lipase Action

Despite intensive study in this area, the mechanism by which lipase effects hydrolysis has still not been ascertained. This is due in part to the unusual nature of the enzyme, preferring as it does water insoluble substrates. This means that the mechanism of catalysis must be divided into three separate processes:[32]

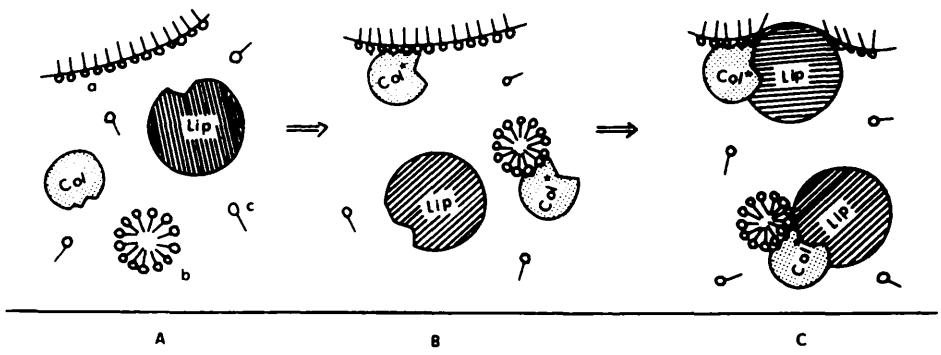
1. interfacial adsorption;
2. interfacial activation; and
3. catalysis proper.

Therefore, it is not sufficient to determine the residues involved in the process: the role which they play must also be revealed.

2.2.1 Mode of action of Colipase

The addition of bile salts such as sodium taurodeoxycholate (TDC) strongly inhibits the PPL - catalysed hydrolysis of emulsified substrates.[24] Full catalytic activity can be restored by adding colipase to the reaction system. Addition of colipase to the lipase - emulsified substrate system has no effect on the catalytic behaviour of the enzyme in the absence of bile salts. The role of colipase was investigated by Chapus *et al.*[33] using siliconised glass beads as a model hydrophobic interface (glass beads increase the activity of lipase on dissolved substrates 1000 fold).[34] They found that bile salts prevented the adsorption of enzyme at the interface, and explained this as being due to the competition between lipase and bile salts for possession of the interface.

Colipase itself was not adsorbed onto this model interface, nor did adding colipase affect the adsorption of lipase in the absence of bile salts. Furthermore, a large excess of bile salts inhibits lipase activity even with colipase present. Finally, colipase binds to micelles but pure colipase does not bind to pure lipase. These results have been explained by Chapus *et al.* in terms of colipase acting as an anchor, binding lipase to the interface (Scheme 2.1).



Tentative scheme illustrating the role of colipase for the fixation of lipase in the presence of bile salts. (a) Interface coated with detergent molecules. (b) Detergent micelle. (c) Detergent monomer. (A) When alone lipase recognizes neither the micelle nor the interface. (B) Colipase can recognize both creating a binding site for lipase. (C) Binding of lipase mediated by colipase.

Scheme 2.1: Schematic Representation of lipase - colipase - amphiphile interactions (From ref 33)

Thus, lipase itself does not recognise either micelle or amphiphile-coated interface. Colipase recognises both, and the binding of colipase onto the surface causes a conformational change in the colipase molecule which reveals a binding site for lipase. A large excess of amphiphile inhibits lipolysis due to the increasing amount of micelles present.

The molecular basis of lipase - colipase - interface interactions is poorly understood at present. Due to the proposed mode of action of colipase, it might be expected to possess two distinct regions. A hydrophobic site would be necessary for interfacial adsorption, whereas a hydrophilic site would be suited towards lipase - colipase binding.[13]

Porcine colipase contains two strongly hydrophobic domains.[27] The first resides in the *N*-terminal chain and comprises three adjacent isoleucine residues. The second is located in the tightly bound central core of the molecule, in the sequence Leu-Tyr-Gly-Val-Tyr-Tyr. Colipases from a wide range of sources show

a high degree of homology in the latter sequence.[13] Colipase activity is not seriously affected by several proteolytic splittings in the tails of the molecule,[28] indicating that the isoleucine sequence is not essential for activity. In addition, spectroscopic data suggest that it is the hydrophobic sequence in the core of the molecule which is involved in interfacial binding. It has been shown that complex formation between horse colipase and taurodeoxycholate micelles results in significant perturbation of a tryptophan residue at position 52 along with two out of three tyrosine residues in positions 55, 58 and 59.[35] In porcine colipase, Trp-52 is replaced by a phenylalanine residue, maintaining the aromatic nature of the side chain. Thus, the available evidence suggests the involvement of three aromatic residues at positions 52, 55 and 58 in the micellar binding site.

Erlanson *et al.* studied the effects of various chemical modifications of colipase on its activity.[36] This group found that nitration of a tyrosine residue in colipase caused a small decrease in activity. In addition, acetylation of the three tyrosines located in the hydrophobic region of colipase using a 120 fold excess of acetylimidazole reduced colipase activity by up to 40%. This they interpreted as evidence that the tyrosines do not play an integral role in colipase binding. They proposed instead that colipase binding to a triglyceride interface proceeds *via* ionic interactions between lysine residues of colipase and the ester carbonyl groups of the triglyceride. However, this explanation has two main faults. Firstly, it would be highly unusual for Lewis acid behaviour to be seen between an ester and an amine. Secondly, one would expect that the conditions used to acetylate the tyrosine residues would also acetylate the lysine side-chains, thus lowering the basicity if the nitrogen atoms. Therefore the weight of evidence still seems to favour the involvement of tyrosine in colipase-interface binding.

In a separate experiment, Erlanson's group also amidated four free carboxylate groups in porcine colipase using glycine methyl ester.[36] Two of the

modified carboxylate groups were located in the *N*-terminal tail of the molecule, and two were in the *C*-terminal chain. The resulting protein displayed no colipase activity, although it did bind to tributyrin in the presence of bile salt. Thus the modified carboxylate groups are believed to be involved in the colipase - lipase interaction.

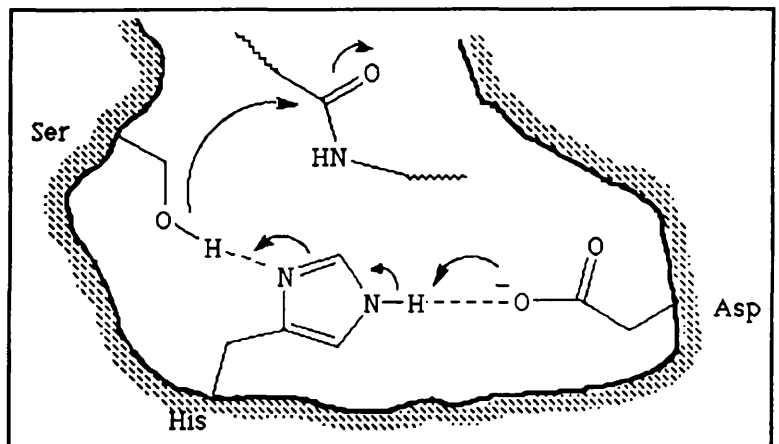
Finally, Erlanson's group acylated lysine residues of colipase using ethyl thiofluoroacetate. The modified colipase was unable to bind to a substrate interface in the presence of bile salts. Thus the lysine residues appear to be important in the adsorption of colipase to a substrate interface, possibly aiding in the displacement of amphiphile from the interface.

2.2.2 Mode of action of Lipase

Although the nature of most of the active residues in lipase has been identified, the locations and the roles which they play has not in all cases been determined. However, from the large amount of work devoted to this enzyme certain conclusions have emerged.

Early claims that lipase was a sulphydryl enzyme were proved to be erroneous, as substituting the two thiol groups with phenylmercuric radicals had little effect on lipase activity.[21] It was found that incubating pure lipase with diethyl *p*-nitrophenyl phosphate (labelled E_{600}) solution in the presence of bile salts inactivated the enzyme, releasing a stoichiometric equivalent of *p*-nitrophenol.[37] The residue reacting with the phosphate ester has been shown to be Ser-152.[38] In addition, lipase activity was found to be dependent on two residues with pK_a values of 5.6 and 9.3, the first of which was assumed to be a histidine residue.[39]

Photooxidative inactivation of the enzyme, correlating to the loss of a histidine residue, has also been observed.[6] Hence, it was assumed that pancreatic lipase was a serine - histidine enzyme, operating *via* a charge-relay mechanism involving an aspartate residue in much the same way as serine proteases such as α-Chymotrypsin[40] (Scheme 2.2).



Scheme 2.2

However, this explanation of lipase activity is not now held to be correct. It has been shown by X-ray crystal structure analysis that human pancreatic lipase (which has conserved 86% of the amino acid sequence of PPL) does contain a Ser-152 - His263 - Asp-176 'catalytic triad'.[41] Such a triad also exists in a more structurally dissimilar lipase, from *Mucor miehei*.[42] Experimental evidence, though, suggests that Ser-152 is intimately involved in interfacial binding, rather than catalysis proper. As stated previously, lipase suffers complete inactivation when incubated with E_{600} in the presence of bile salts, where the organophosphate forms mixed micelles with the amphiphiles. Lipase is also inhibited in the same manner by emulsified E_{600}, but no inhibition is observed when lipase is incubated with E_{600} solution in the absence of bile salts.[37] Furthermore, lipase modified with diethyl *p*-nitrophenyl phosphate (DP-lipase) retains appreciable activity towards dissolved *p*-nitrophenyl acetate.[43] Finally, DP-lipase is unable to bind to

siliconised glass beads. Thus Ser-152 seems to be involved in either adsorption to an interface or in interfacial binding of substrate molecules prior to catalysis.

Garner[44] investigated the reaction of lipase with alkyl and aryl boronic acids. These are believed to react with nucleophilic residues to form tetrahedral boronate adducts analogous to the tetrahedral intermediates involved in ester hydrolysis. Lipase reacts with boronic acids *via* an unidentified serine residue, the resulting modified enzyme being completely inhibited towards emulsified substrates. The modified enzyme could still adsorb onto a model interface, but would not react with E_{600}. In addition, lipase treated with boronic acids retained full activity towards water-soluble substrates such as *p*-nitrophenyl acetate. Garner interpreted these results as evidence of serine involvement in the catalytic site. However, in light of the results of other groups, it appears that the active serine (presumably Ser-152) is involved in the interfacial binding of substrate molecules once the enzyme is adsorbed onto the substrate - water interface.

Dufour *et al.*[45] found that modifying five free carboxyl groups of lipase with carbodiimide and norleucine methyl ester completely suppressed lipase activity towards emulsified substrates. Kinetic data suggest that only one of these carboxyls is essential for activity. However, the modified enzyme still possessed an active site titratable with E_{600}, indicating that the modified carboxyls were not intimately involved in catalysis proper. It is believed that the role of this carboxyl is structural, stabilising a super-active form of the enzyme formed by conformational changes occurring when the enzyme is adsorbed onto an interface. If this explanation is correct, the native enzyme contains a catalytic site which is only weakly active (witness the much reduced reactivity of lipase towards water-soluble substrates), but which is transformed by adsorption onto an interface into the fully active form. This would also explain the high reactivity of the carboxyl in the native enzyme, since it would lack stabilising hydrogen bonds in the native form.[45]

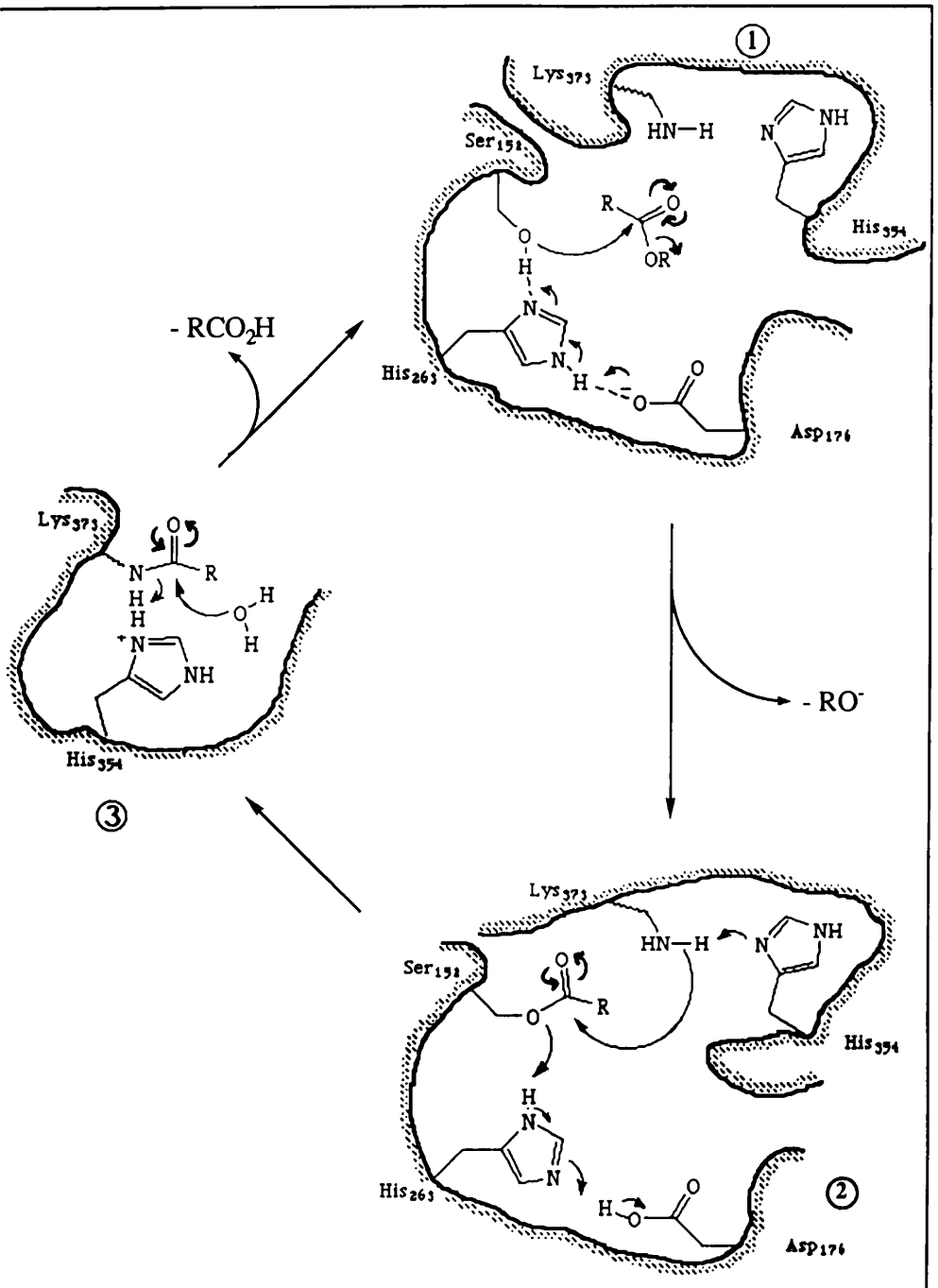
Recently published X-ray crystal structure analyses of lipases from human pancreas[41] and *Mucor miehei*[42] seem to lend weight to this theory. In both of these structurally dissimilar lipases, a Ser-His-Asp 'catalytic triad' is located under a hydrophobic α-helical surface loop which acts as a 'lid', rendering the catalytic triad inaccessible to substrate molecules. For lipolytic activity, this lid would have to be reoriented away from the active site, and it is entirely conceivable that the free carboxylates would stabilise a new conformation of the molecule which would do just that.

This leaves two fundamental questions to be answered. Firstly, by what method does lipase anchor itself to the substrate - water interface? And secondly, if the classical Ser - His - Asp 'catalytic triad' does not constitute the active site proper, then where does this El Dorado of the lipase world lie within the molecule?

The method of fixation of lipase to colipase is implied by the work of Erlanson *et al.*[36] to be essentially ionic. Certainly the involvement of free carboxylate groups from colipase suggests interaction with basic residues of lipase. Sikk *et al.*[46] found that succinylating an amino group not involved directly in the hydrolysis of *p*-nitrophenyl acetate resulted in an enzyme which could not bind to colipase in the presence of bile salts. This they interpreted as evidence that the involvement of the *N*-terminal amino group was essential for lipase-colipase binding.

If we accept the premise that Ser-152 is involved in interfacial binding of substrate, then the nature of the true catalytic site has still to be determined. Semeriva and Desnuelle[32] suggest that a histidine residue is involved in the catalytic site. However, while an imidazole ring of a histidine can act as a nucleophilic catalyst in enzymic processes, it is more common for such residues to act as acid-

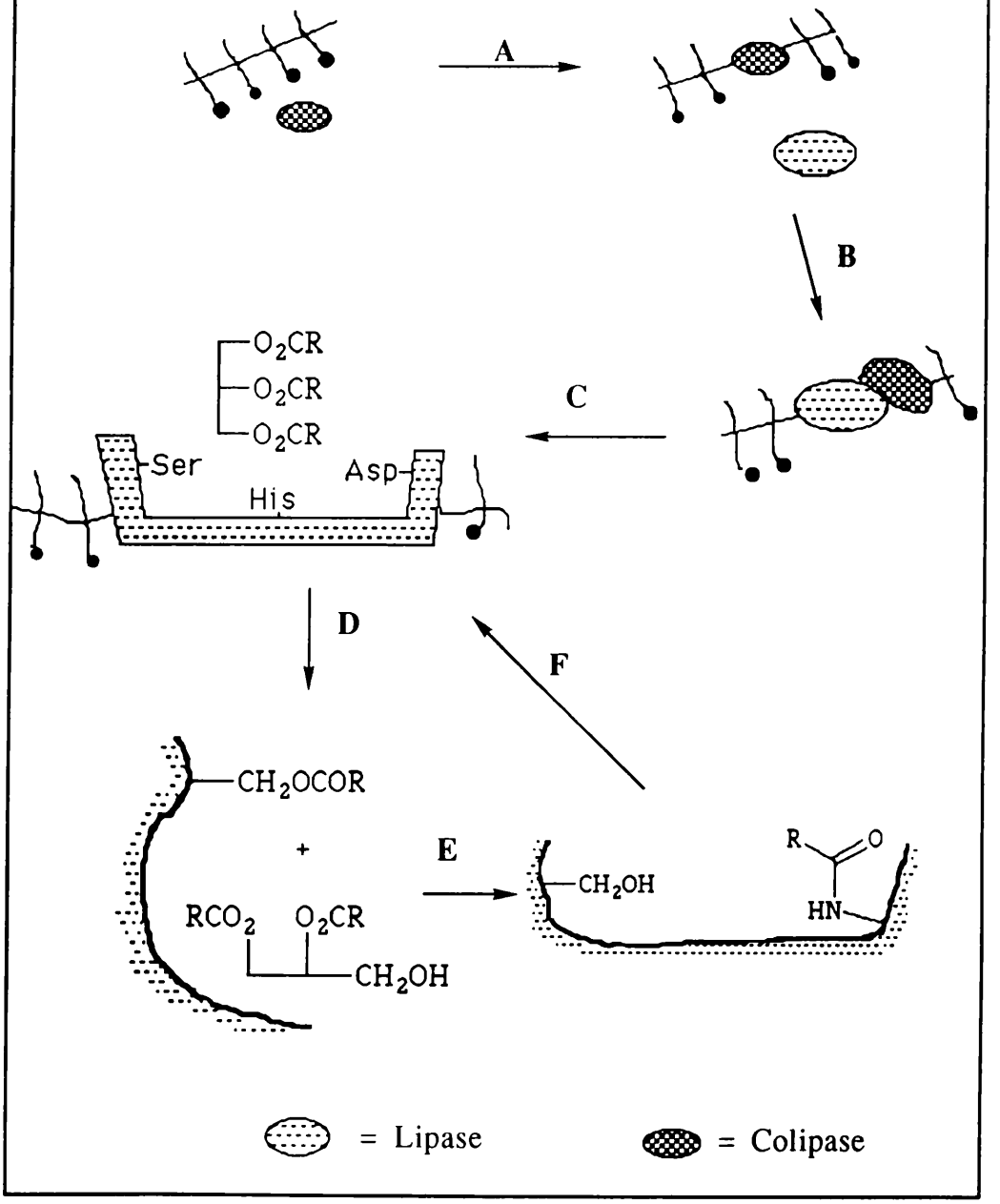
base catalysts.[47] In addition, the identification by Momsen and Brockman of two ionisable groups with pK_a values of 5.6 and 9.3 controlling lipase activity is suggestive of histidine and lysine residues being involved. Kaimal and Saroja found that subjecting lipase to a reductive alkylation procedure produced an enzyme with greater activity than the native form.[48] Such a procedure is selective for lysine residues.[48] In addition, limited α-chymotrypsin proteolysis of lipase produces a fragment peptide (residues 336-449) which is inactive towards natural (emulsified) substrates but which retains full activity towards water-soluble *p*-nitrophenyl acetate.[49] de Caro *et al.* found that subjecting both lipase and the lipase fragment to *p*-nitrophenyl acetate solution resulted in stoichiometric acetylation of Lys-373 residue. Treatment of both lipase and the lipase fragment with diethyl pyrocarbonate caused ethoxyformylation of His-354. The modified enzyme and enzyme fragment were both inactivated towards *p*-nitrophenyl acetate hydrolysis.[50] These results are certainly consistent with Lys-373 being at the active site proper of the enzyme, with the key step in lipolysis being an acyl transfer between Ser-152 and Lys-373, aided by His-354 acting as a base catalyst (Scheme 2.3). Certainly, the X-ray crystal analysis of human pancreatic lipase shows that Lys-373 is proximal to His-354.[41] Furthermore, in the solid state the Lys-373 - His-354 system is in an easily accessible area of the molecule, and thus available as a weakly catalytic site even when the enzyme has not been activated by adsorption onto an interface.



Scheme 2.3: Proposed acyl-transfer mechanism for Lipases.

Thus a proposed reaction scheme for the lipase-colipase-substrate interface system which is consistent with the available data can be summarised in Scheme 2.4. The key steps indicated are as follows:

Scheme 2.4



(A) Adsorption of colipase onto an amphiphile-coated water - lipid interface.

(B) Adsorption of lipase onto interface, aided by ionic interactions with colipase.

(C) Conformational change on adsorption removes 'lid', revealing the Ser-His-Asp binding site.

(D) Interfacial binding of substrate to Ser-152.

(E) Acyl-transfer from Ser-152 to Lys-373.

(F) Hydrolysis of acyl-enzyme complex to regenerate active enzyme.

It is interesting to note that if the acyl transfer step is blocked (either by ethoxyformylation of His-354 or dialkylation of the crucial lysine residue), lipase will still hydrolyse emulsified substrates but not soluble ones. Kaimal and Saroja found that dimethyl-lysyl lipase had no esterase activity in organic solvents, but would hydrolyse peptides in a two-phase system.[48] Thus it appears that blocking the acyl transfer step in lipolysis converts a lipase into a serine protease type enzyme.

No biological function has been found for the glycan chain of lipases. Indeed, not all lipases isolated are glycoproteins. Canioni *et al.*[11] have noted that pancreatic lipases from non-ruminants (such as humans and pigs) are glycoproteins, whereas those from ruminants (sheep, cows, horses) are not. However, as they themselves admit, there are insufficient examples available to afford this observation anything more than curiosity status at the current time.

Chapter Three

Production of Optically Active Amino Acids: Resolution and Asymmetric Synthesis

3.1 Introduction

The production of uncommon amino acids is an ever-expanding area of chemistry. Such compounds are of interest as constituents of many naturally-occurring substances which display interesting pharmaceutical properties. The incorporation of so-called 'unnatural' amino acids into peptides can provide information on the peptide's conformation,[51] alter the biological activity of the peptide,[52] and produce hydrolysis-resistant peptides.[53] In addition, amino acids have attracted considerable interest as chiral auxiliaries in a wide range of synthetic applications.[54-56] Clearly, for both the medicinal chemist and the synthetic chemist, it is important that novel amino acids can be prepared in optically pure form. In this chapter are reviewed various procedures for obtaining optically active amino acids, covering both resolution of racemates and asymmetric synthesis.

3.2 Resolution of Amino Acids and Derivatives

The first recorded isolation of an amino acid from a natural source was in 1806, when the juice of asparagus plants yielded what is now called asparagine.[57] However, it was to be nearly fifty years before Louis Pasteur recognised that both asparagine and the derived aspartic acid from this source were optically active.[58] For the fledgeling science of synthetic organic chemistry, this observation highlighted a problem which was to remain of central importance throughout its history and which is, if anything, of even greater importance today. For when Pasteur examined aspartic acid synthesised by Dessaignes from ammonia and fumaric acid, he found that the synthetic material was optically inactive.[59] For a chemist to emulate nature truly in the laboratory, it would be necessary to separate the two enantiomers of racemic compounds. This was achieved in 1886 by Schulze and Bosshard.[60] They found that when the mould *Penicillium glaucum* was grown in a medium containing either racemic leucine or racemic glutamic acid, the amino acids subsequently isolated from the medium were not only optically active, but had the opposite direction of specific rotation to the naturally occurring compounds. By being the first to separate the two enantiomers of a racemic amino acid, Schulze and Bosshard were also the first to access the 'unnatural' enantiomer of an amino acid.

In modern times, the situation has of course improved. The 'natural' enantiomers of the common α-amino acids are readily available at reasonable cost, and in most cases the 'unnatural' enantiomer is also not prohibitively expensive. In addition, various methods have been developed for the asymmetric synthesis of unusual α-amino acids, and this is a field of study which shows every sign of continuing to be fruitful in the future. However, the growing interest in the properties

of 'unnatural' α- and β-amino acids continues to make the development of convenient and economic resolution methods an attractive proposition.

3.3 Methods of Resolution

It is indicative of the genius of Louis Pasteur that virtually all the methods of enantiomer separation in use today stem, in one way or another, from procedures first described by him. They can be summarised thus:

1 Spontaneous Resolution
2 Diastereoisomeric Salt Formation
3 Diastereoisomeric Derivatisation
4 Chromatographic Methods
5 Biological methods.

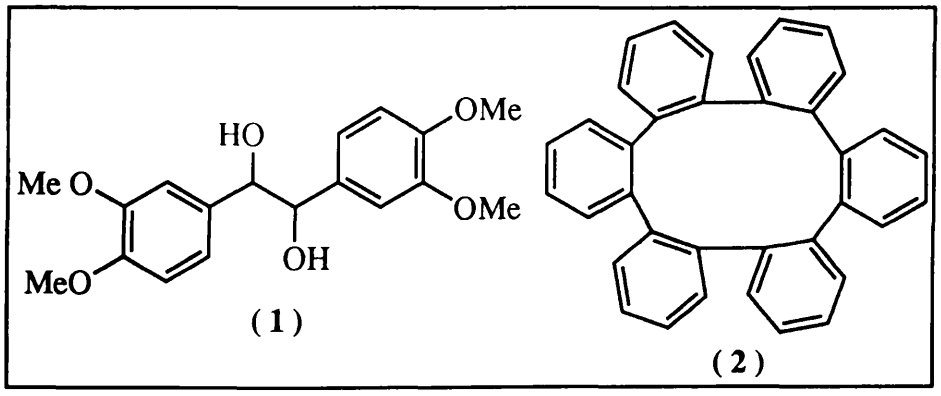
An in-depth discussion of any one of these methods would fill a book. This review is therefore by necessity rather selective, and is intended merely to provide an overview of the topics.

3.3.1 Spontaneous Resolution[61,62]

Crystalline chiral compounds can be classified into two main types, racemic crystals and conglomerates.[61] A conglomerate can be defined as a mechanical mixture of crystals, wherein each crystal contains only one enantiomeric form. It is immediately apparent that if such a situation pertains then the resolution of the

compound is potentially much easier than if one were handling a true racemate. A limitation of the method is the comparative rarity of conglomerates (by 1980, only about 250 were known). However, if one so desires, it is often possible to find a derivative of the molecule of interest which can be resolved spontaneously. For example, of the naturally occurring amino acids only cysteine, arginine and tyrosine have not succumbed to this method of resolution. The method can be used in large-scale processes: between 1963 and 1973, 13 000 tons of L-glutamic acid were prepared industrially by the spontaneous resolution of the racemate, which was in turn prepared from acrylonitrile.[61]

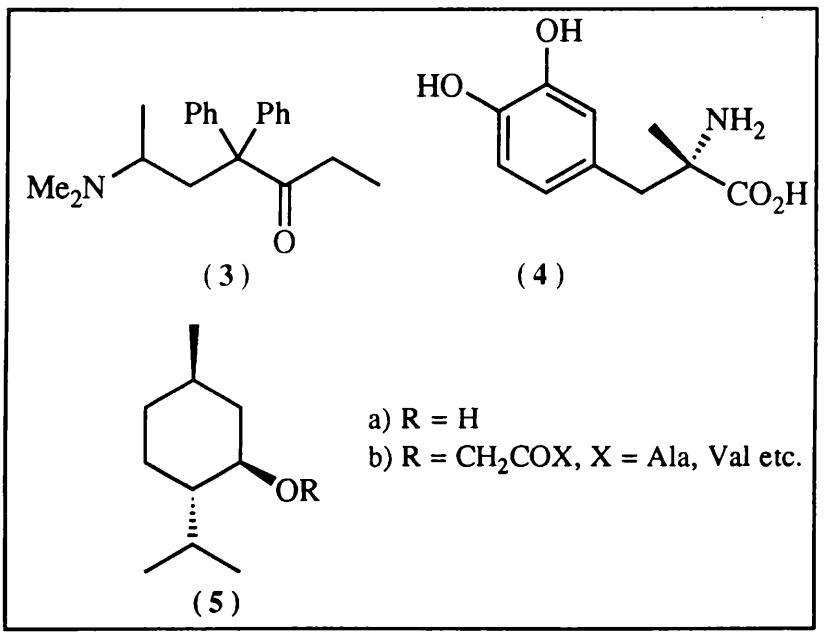
The classical study of spontaneous resolution was that of Pasteur in 1848.[63,64] He observed that racemic sodium ammonium tartrate (SAT) formed two crystal types, whose shapes were enantiomeric. Resolution was achieved by the mechanical separation of the two crystal types. Such a method was extremely laborious, and suffered from the additional drawback that only well-defined crystals could be utilised. For these reasons, the method is not of any practical importance. However, the observation of such spontaneous resolution was decisive in the distinguishing of the *meso*- and DL- forms of hydroveratroin (**1**).[65] Spontaneous resolution was also used by Wittig and Rumpler to obtain optically pure *o*-hexaphenylene (**2**).[66]



(**1**)

(**2**)

The industrially important method of spontaneous resolution is resolution by entrainment.[61] The genesis of this procedure came when Gernez (a student of Pasteur) observed that a supersaturated solution of (*l*)-SAT would not form crystals when seeded with (*d*)-SAT, and *vice-versa*.[67] However, a supersaturated solution of racemic SAT seeded with an enantiomerically pure crystal would grow only crystals of the same rotatory sense. Using a similar procedure, Zaugg obtained 13 grams of both enantiomers of the analgesic methadone (**3**) from 50 grams of racemate.[68] Dolling *et al.*[69] used the method to resolve up to 13 kg lots of 2-deuterio-3-fluoro-DL-alanine as its benzenesulphonate salt. A variation of the method, favoured by industry, involves a circulating solution of racemate passing over alternating beds of each enantiomer.[61] Such a system has been used in the commercial resolution of L-glutamic acid,[61] L-α-methyl DOPA (**4**),[70] and (-)-menthol (**5a**)[71] (as its benzoate ester).



(3) (4)

(5)

3.3.2 Diastereoisomeric Salt Formation[62]

The principle of diastereoisomeric salt formation for the resolution of a racemate was first evinced by Pasteur when he resolved (±)-tartaric acid using alkaloid bases.[72] The procedure involves utilising the different physical properties of two diastereoisomeric salts to effect separation, normally by crystallisation of the less soluble diastereoisomer. Due to the amphoteric nature of amino acids, salt formation can be effected with either optically active acids or bases. Once separation is achieved, the corresponding optically active amino acid can be generated by a metathetical reaction between the diastereoisomeric salt and a stronger acid or base than the corresponding resolving agent.

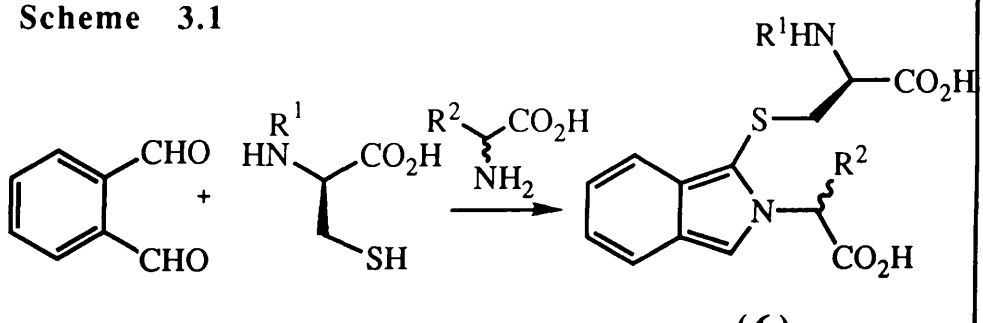
This is a method which has found wide utility in the resolution of the common amino acids. However, the method does involve several recrystallisations of the diastereoisomeric salts, which is both time consuming and frequently involves a concomitant loss of material which can be unacceptably high. In addition, the use of strong acids or bases to decompose the salts could prevent the method being extended to uncommon amino acids with acid- or base-labile functionality. Finally, the search for a suitable resolving agent is often long and not always successful (although Wilen *et al.*[73] have described a systematic procedure for the selection of a resolving agent). The resolution of amino acids by diastereoisomeric salt formation is still of great practical value today, but the drawbacks mentioned above make it at times an unattractive proposition.

3.3.3 Diastereoisomeric Derivatisation

A distinction is drawn here between diastereoisomeric salt formation, in which the interactions between racemate and chiral auxiliary are ionic, and diastereoisomeric derivatisation, where the chiral auxiliary is linked to the racemate by a covalent bond.

When discussing amino acids, the most commonly applicable diastereoisomeric derivatives are esters or amides, formed from homochiral alcohols, amines, or acid derivatives. An early example of this technique is the resolution by Holmes and Adams of racemic alanine, valine and α-phenylglycine by crystallisation of the appropriate (-)-menthoxyacetamide derivatives (**5b**).[74] More recently, Buck and Krummen[75] resolved 21 amino acids by high performance liquid chromatography (HPLC) separation of the fluorescent diastereoisomeric derivatives (**6**) formed by the amino acid in the presence of *o*-phthalyldialdehyde and *N*-butoxycarbonyl- (*N*-BOC) or *N*-acetyl-L-cysteine (Scheme 3.1).

Scheme 3.1



(**6**)

Manning and Moore[76] reported a method of separation which has proved popular. They formed diastereoisomeric dipeptides by reacting the racemic amino acid with the *N*-carboxyanhydride derivative of an optically pure amino acid. The

separation of the resultant dipeptide has been performed by ion-exchange,[76] paper[77] and Sephadex chromatography.[78]

Diastereoisomeric derivatisation has been a reasonably popular and successful method of resolution. The chiral auxiliaries necessary are readily accessible from cheap starting materials, and the procedure normally requires less steps than would a crystallisation of diastereoisomeric salts. The drawback of such a method is the need for cleavage of a covalent bond to separate the amino acid and chiral auxiliary at the end of the resolution. This normally involves the hydrolysis of an amide bond, requiring reaction conditions which could conceivably induce racemisation in susceptible molecules. However, the method is of widespread application and, with care, such problems are usually avoidable.

3.3.4 Chromatographic Methods[79]

The possibility of resolving racemates by chromatography on a chiral stationary phase has been recognised for many years. Initial attempts, using animal tissue proteins such as wool, failed to produce any significant resolution.[72] A more successful approach has involved the resolution of DL-amino acids by thin layer chromatography (TLC) on cellulose.[80] Racemic amino acids have also been resolved by TLC on silica gel impregnated with (-)-brucine.[80] DL-Tryptophan has been resolved by column chromatography, using powdered cellulose as the stationary phase.[81]

HPLC has also proved very efficacious for resolving racemic amino acids. Two protocols have been developed, one involving a chiral stationary phase and the other a chiral mobile phase. Cyclodextrin-bonded stationary phases have been used

successfully in the resolution of several *N*-dansyl amino acids.[82] The use of chiral additives in the mobile phase is a more attractive proposition, due to the ease of preparation of the chiral phase and the comparative cheapness of the chiral auxiliaries used. Chiral copper(II) complexes of optically pure amino acids have been popular as mobile-phase chiral additives. Gil-Av *et al.* found that a dilute aqueous solution of L-proline and copper(II) acetate facilitated the chromatographic resolution of racemic amino acids by reversed-phase liquid chromatography.[83] Chiral copper(II) complexes of both *N*-(*p*-toluenesulphonyl)-D-phenylglycine[79] and also *N,N*-di-*n*-propyl-L-alanine[84] have also been used as chiral mobile phase additives in a similar manner.

Chromatographic methods can offer rapid and quantitative resolution of racemic amino acids. However, the equipment necessary for modern techniques is often expensive, as are some of the chiral materials employed. Therefore a simple and less costly resolution method is often more attractive than chromatography.

3.3.5 Biological Methods

When discussing biological methods of producing optically active amino acids from racemates, an important distinction should be drawn between a *separation* (which produces only one enantiomer) and a true *resolution* (where both enantiomers become available). Therefore, although the use of whole organisms to access optically pure amino acids from racemates is often called a resolution, it is strictly speaking a separation of a racemate. While one enantiomer is obtained in high optical purity, its antipode is metabolised by the organism and is thus inaccessible. However, to place this field in the correct historical perspective, one must briefly discuss such methods.

3.3.5.1. Use of Whole Organisms

The first demonstration of a biological separation of enantiomers was, once again, due to Pasteur.[86] In 1860, he described how *Penicillium glaucum*, when grown in a medium containing racemic tartaric acid, consumed only (+)-tartaric acid and so enabled optically pure (-)-tartaric acid to be obtained from the broth. Ehrlich further demonstrated the wide applicability of this method, using it to obtain a range of D-amino acids from their racemates.[72]

The use of whole organisms for the production of optically active amino acids is a common industrial process today. However, such processes tend to involve the synthesis of only one enantiomer from prochiral precursors in the reaction broth, rather than a resolution. As such, these important processes lie outwith the scope of this review, and the interested reader is referred to recent publications.[87]

Piutti's observation[88] in 1886 that L-asparagine had a bitter taste while its antipode tasted sweet was a graphic illustration that the capacity to recognise enantiomers was not confined to lower organisms. Wohlgemuth[89] demonstrated that racemic amino acids could be separated by injecting the racemate into rabbits and collecting the D-form from the animals' urine. However, as one might imagine, the use of whole animals for such resolution is far from satisfactory. Quite apart from the ethical problems, the yields from such processes are low and the optical purity of the product is often not of the highest order. Hence the use of whole animals for resolutions (as opposed to separations) is today of little importance.

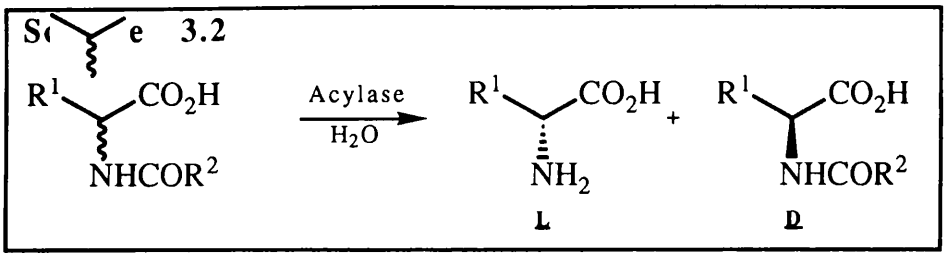
3.3.5.2. Use of Isolated Enzymes

With the advent of commercially available purified enzyme preparations, the use of enzymes as resolving agents for amino acids has mushroomed. The reasons for this are easy to see - enzymes offer rapid reactions under mild conditions, giving good yields and a high degree of enantioselectivity. Using isolated enzymes, the problem of unwanted side reactions is eliminated, both enantiomers can be accessed, and separation of reaction products is usually extremely simple. Although the number of enzymes which can be used for resolving amino acids is large, several have become popular for reasons of both economy and their proven efficacy. These are all hydrolases, catalysing the hydrolysis of either amide of ester bonds, and the most important of these is Acylase I.

a) Acylase I

In 1881, Schmiedeberg[72] noted that an aqueous extract of kidneys catalysed the hydrolysis of hippuric acid into benzoic acid and glycine. The enzyme responsible (Acylase) was subsequently identified and purified. Acylases are available from a range of sources, the most prominent being pig kidneys and *Aspergillus* species. They catalyse the hydrolysis of amide bonds with a high degree of enantioselectivity, according to Scheme 3.2. With few exceptions, these enzymes show a marked preference for L-amino acid derivatives.

Scheme 3.2



Birnbaum and co-workers[90] were the first to report a systematic study of Renal Acylase I. Among their observations were the facts that the enzyme would accept a broad range of *N*-acyl-L-amino acids as substrates, in all cases leaving the *N*-acyl-D-amino acids largely untouched. With the cheapness and ready availability of Acylase I (see Table 3.1), and the great desirability of obtaining optically pure amino acids, the use of this enzyme for amino acid resolutions has grown dramatically. Whitesides and co-workers[91] investigated the substrate specificity of Acylase I from both pig kidney and *Aspergillus* species in a wide-ranging study covering over 50 *N*-acyl-α- and β-amino acids, including α-methyl substituted α-amino acids. They found that both enzymes exhibited a remarkably high tolerance for structural modifications in α-amino acid substrates, always preferentially hydrolysing the L-isomer and generally giving products with high enantiomeric excesses (>90%). However, β-amino acids were found not to be substrates for these enzymes, a result in agreement with earlier studies.

Table 3.1

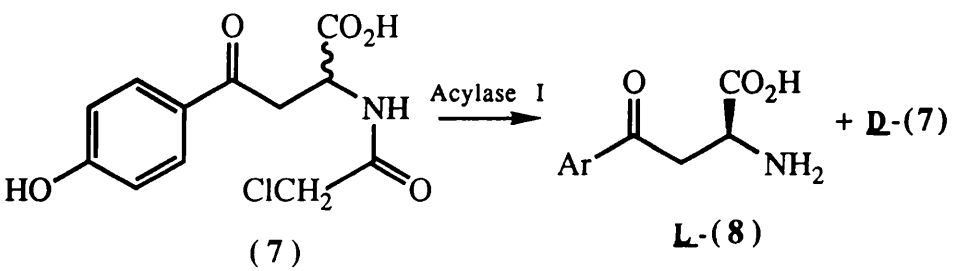
Enzyme	Cost per gram (£)‡
Acylase I (pig kidney)	20.50
(Aspergillus)	7.50*
α-Chymotrypsin	9.20
Papain (Crude Powder)	4.70
Carboxypeptidase A	270.00
Subtilisin Carlsberg	81.80

‡ Prices quoted in Sigma Chemical Company 1990 catalogue for cheapest enzyme preparation available. Purified enzymes are significantly more expensive.

* Cost for 5g

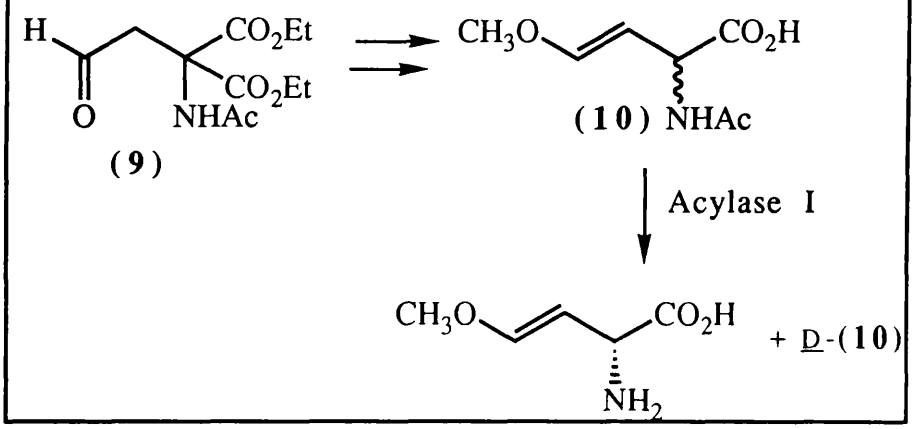
There are numerous reports of the use of Acylase I for the resolution of racemic reaction products. DL-4-Oxo-homotyrosine (**8**), an intermediate in the synthesis of an unusual naturally-occurring amino acid, was resolved by the action of Acylase I on the *N*-chloroacetamide derivative (**7**), which was obtained *via* the condensation of diethyl acetamidomalonate with 4-methoxyphenacyl bromide (Scheme 3.3).[92]

Scheme 3.3



Keith *et al.*[93] used pig kidney Acylase I to resolve the racemic amino acid (**10**), a natural product synthesised in five steps from the aldehyde (**9**) (Scheme 3.4).

Scheme 3.4

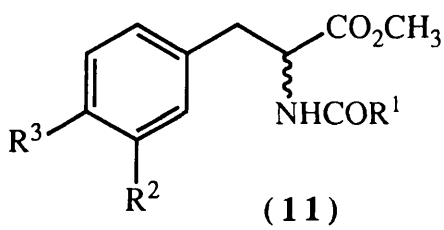


The usefulness of Acylase I has been further extended by the use of polymer supports, making the isolation of reaction products even easier and enabling the enzyme to be used in continuous-flow processes.[81] Barth and Maskova[94] have described just such a continuous resolution, using pig kidney Acylase I supported on DEAE-cellulose to resolve *N*-acetyl-DL-methionine. A continuous resolution procedure is especially valuable in cases such as this, when the liberated L-amino acid inhibits the enzyme and must thus be removed from the reaction medium as it is formed.

b) Other Hydrolytic Enzymes

Due to its cheapness and wide substrate specificity, Acylase I is by far the most commonly used enzyme for the resolution of α-amino acids. However, this review would not be complete without mention of the other main hydrolytic enzymes used for such resolutions.

α-Chymotrypsin is an enzyme of narrow substrate specificity, acting only on substrates with large hydrophobic groups, especially favouring a proximal aromatic ring.[95] However, within this well-defined area it has proved to be an extremely efficacious resolving agent. Matta *et al.*[96] have used the enzyme to resolve DOPA analogues **(11)** by standard methods.



$R^1 = CH_3$, Ph
$R^2 = H$, CH_3O
$R^3 = OH$, CH_3O
$R^2, R^3 = OCH_2O$

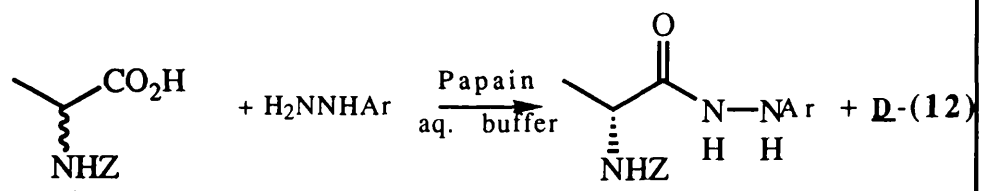
(11)

Anantharamaiah and Roeshe[97] found α-Chymotrypsin to be more tolerant of α-methyltryptophan and phenylalanine derivatives than Acylase I, as witnessed by the relative rates of the substituted and unsubstituted amino acids for both enzymes. However, they found best resolution was achieved by allowing α-Chymotrypsin to hydrolyse the methyl esters of these amino acids, rather than the amides. This they attributed to an incorrect bond alignment in the enzyme-substrate complex for α-methyl derivatives preventing effective hydrolysis of the amide bond. Similarly, Tong *et al.*[98] resolved a series of ring-substituted phenylalanine derivatives by the action of α-Chymotrypsin on their ethyl esters.

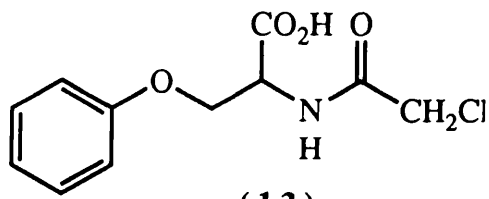
Thus it can be seen that α-Chymotrypsin is a good resolving agent, given that the substrate possesses a suitably located hydrophobic residue. However, this rather narrow substrate specificity means that the enzyme cannot be considered as a generally applicable resolving agent.

The vast majority of amino acid resolutions have used enzymes in a hydrolytic mode. However, Abernethy *et al.*[99] used papain (a proteolytic enzyme of broad structural specificity) to resolve *N*-carbobenzoxy-DL-alanine (**12**) *via* the coupling of the compound with various substituted phenylhydrazines (Scheme 3.5). It is generally assumed that the large amount of water present in such systems will suppress any such condensation reactions, but this example shows that this is not always the case.

Scheme 3.5



Two other enzymes which should be mentioned are subtilisin Carlsberg and Carboxypeptidase A. The subtilisins are bacterial proteases of wide substrate specificity,[100] subtilisin Carlsberg being the most commonly used one. This enzyme has been used in the resolution of amino acids by ester hydrolysis.[101] The substrate specificity of Carboxypeptidase A has been summarised by Hartsuck and Lipscomb.[102] The most critical requirement is a free carboxyl group adjacent to the amide bond being cleaved. Thus the enzyme has been used to resolve *N*-trifluoroacetyl-α-methyl amino acids[103] and the *N*-chloroacetyl amino acid (**13**).[104] However, the high cost of this enzyme (Table 3.1) makes it a less than ideal choice of resolving agent.

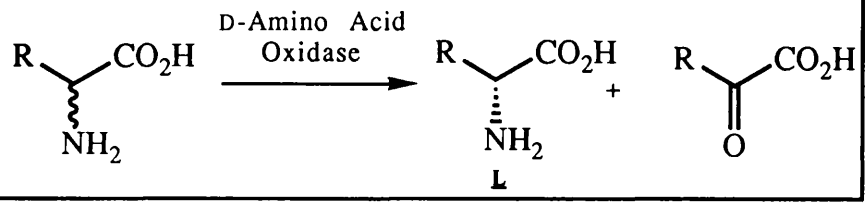


(**13**)

3.3.5.3. Amino Acid Oxidases

The use of D- or L-amino acid oxidases for the obtention of optically pure amino acids by the reaction shown in Scheme 3.6 has been in use for some years.[81,105] However, as was the case for using whole organisms, this method destroys one enantiomer and thus cannot be considered a resolution in the true sense. Such reactions are thus outwith the scope of this review.

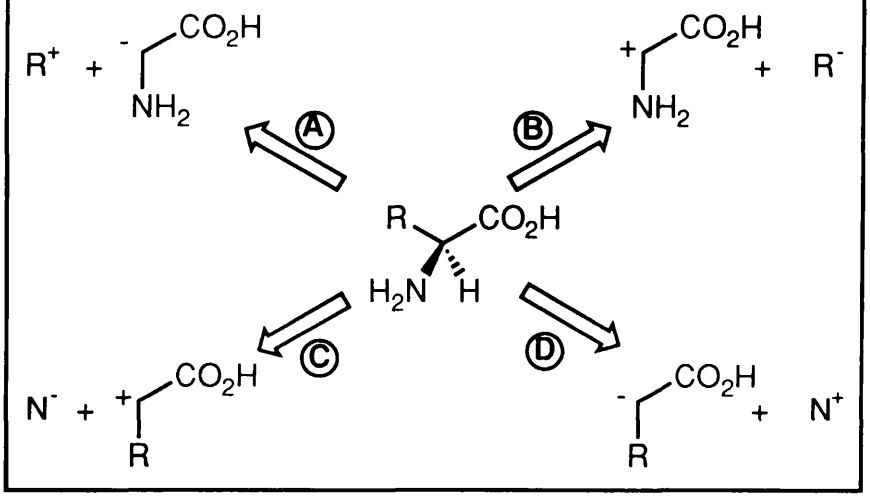
Scheme 3.6



3.4 Asymmetric Synthesis of α-Amino Acids[106-108]

The challenge of devising new methodologies for the asymmetric synthesis of amino acids has proved irresistible to a large number of organic chemists, and much research has been carried out in this area. The vast majority of such asymmetric syntheses fall into one of four main categories - glycine enolates (A), glycine cations (B), and nucleophilic (C) or electrophilic (D) aminations (Scheme 3.7).[109]

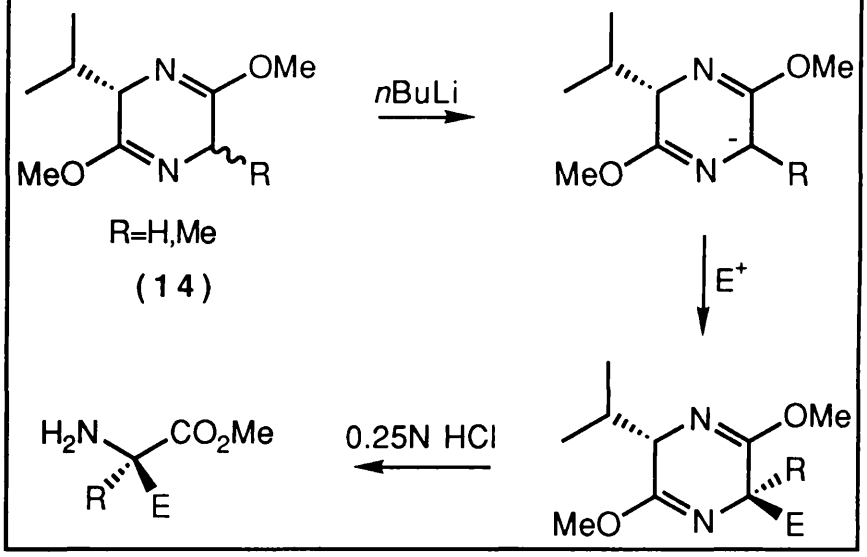
Scheme 3.7



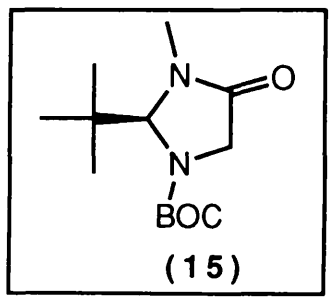
3.4.1 Glycine Enolates

Asymmetric glycine enolate equivalents have proved by far the most popular method of synthesising optically active amino acids. In general, chirality in this process is derived from an asymmetric centre within the enolate anion, and the various methods developed differ only in the nature of the chiral auxiliary used. One highly successful example of this method has been Schollkopf's use of bislactim ethers such as **(14)** (Scheme 3.8).[110-112] Deprotonation of this moiety with strong base generates an anion, which is attacked by an electrophile approaching from the less hindered face. Hydrolysis of the bislactim ether with aqueous acid then generates the amino acid.

Scheme 3.8



Seebach's group have also studied the asymmetric alkylation of glycine enolates, developing a number of heterocyclic chiral templates for this purpose.[113-115] Representative of these is *t*-butyl 2-(*t*-butyl)-3-methyl-4-oxo-1-imidazolidinecarboxylate (BOC-BMI,(**15**)), which is commercially available in both enantiomeric forms. Seebach and co-workers have used this glycine equivalent to prepare optically active examples of cyclic, heterocyclic, α-dialkyl substituted and α-deuterioamino acids.[113]

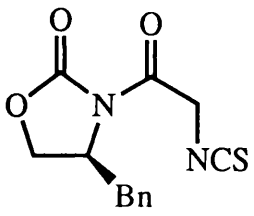


(**15**)

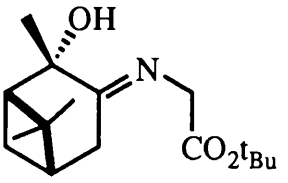
Clearly, such methodology can be extended virtually *ad infinitum*, simply by altering the chiral enolate source. Thus the literature contains reports of asymmetric

amino acid syntheses using Evans' enolate chemistry (**16**) and various glycine-derived imines (**17**) - (**20**) (Scheme 3.9), all following the same general methodology.

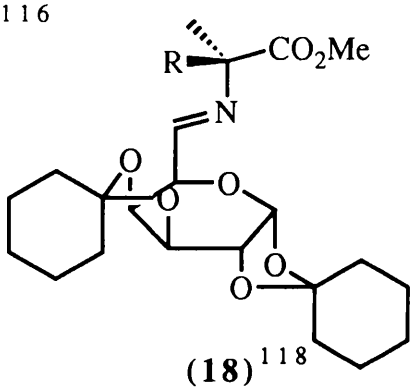
Scheme 3.9



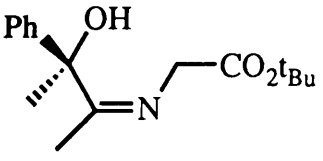
(**16**)[116]



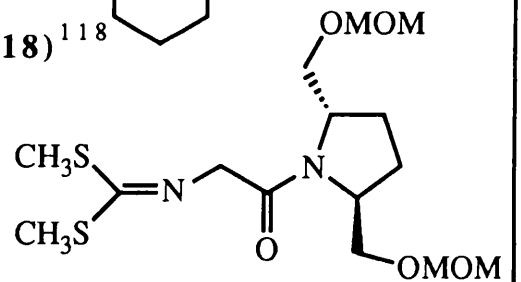
(**17**)[117]



(**18**)[118]



(**19**)[119]



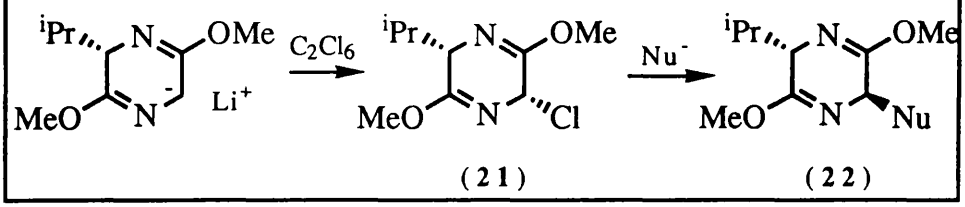
(**20**)[120]

3.4.2 Glycine Cation Equivalents

The use of glycine cation equivalents is obviously complementary to glycine enolates, and there exists more than a passing similarity between the chiral templates used in both cases. Thus Schollkopf's bislactim ether has been adapted to form the

glycine cation synthetic equivalent (**21**).[121] This reacts well with 'soft' nucleophiles to give the α-amino acid precursor (**22**) in good yield (Scheme 3.10).

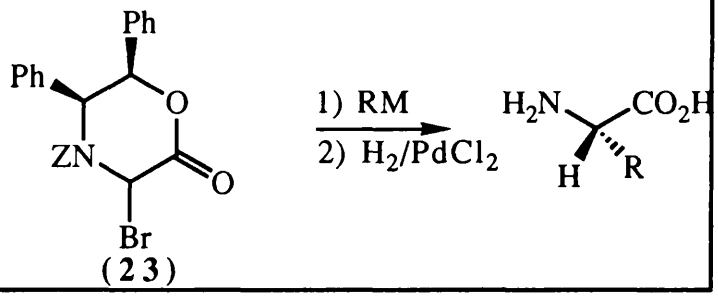
Scheme 3.10



(21) (22)

Sinclair *et al.*[122] have used the amino-alcohol derived heterocycle (**23**) as a chiral glycine template, reacting it with a variety of organometallic reagents to form α-alkyl amino acids in good optical yield (Scheme 3.11).

Scheme 3.11

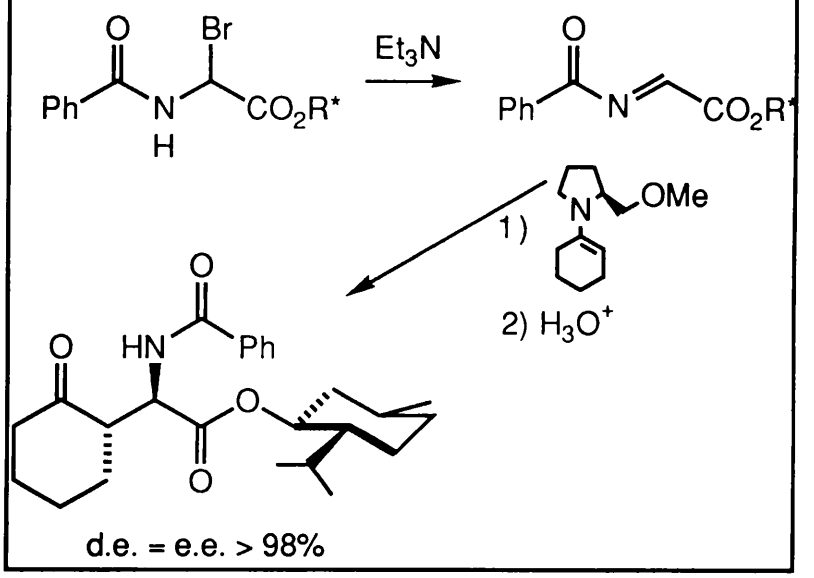


(23)

This template has also been used to prepare α-deuterioglycine in up to 82% enantiomeric excess.[124]

Steglich and co-workers[123] have used the (+)-menthyl ester of *N*-benzoyl-α-bromoglycine as a cation equivalent, generating the imine and reacting this with a variety of enamines. The resulting alkylated amino acid derivatives were obtained in essentially optically pure form (Scheme 3.12).

Scheme 3.12



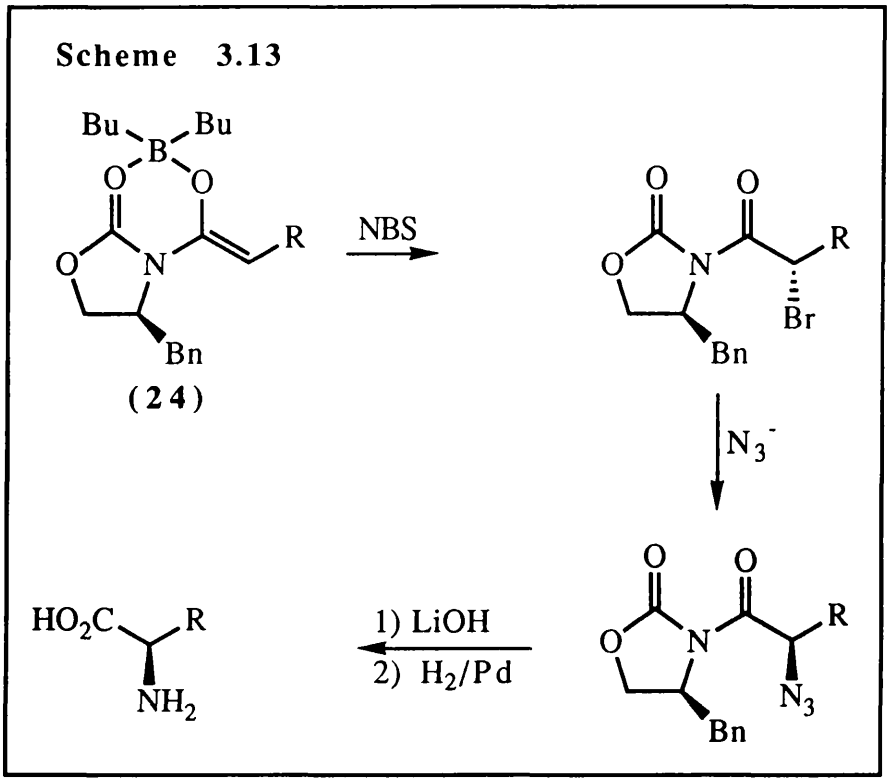
3.4.3 Nucleophilic and Electrophilic Aminations

The amination of acid derivatives has not found nearly as much use as glycine derivatives in the synthesis of amino acids. The main reason for this is undoubtedly the ready availability of glycine itself. Such a cheap starting material is the obvious progenitor of more complex amino acids. Nevertheless, the amination of carbonyl compounds has been successfully used to prepare optically active amino acids.

Nucleophilic amination invariably involves azide displacement of a halogen, with subsequent reduction of the azide to form the amino compound. Thus Evans *et al.*[125] stereoselectively brominated the boron enolate (**24**) with NBS, azide attack inverting the stereochemistry at that centre to give the azido acid (Scheme 3.13).

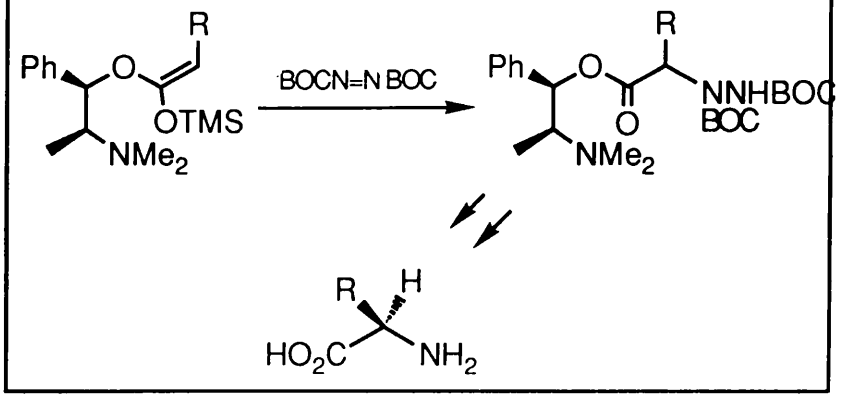
Similarly, Oppolzer *et al.*[126] have used isobornyl esters of α-bromocarboxylic acids to obtain α-alkyl amino acids.

Scheme 3.13



Electrophilic aminations have usually been carried out using dialkylazodicarboxylates, which are reacted with chiral enolate equivalents to give hydrazine derivatives. These can then be converted into the amino acids. A typical example of this is the procedure of Gennari *et al.*[127] (Scheme 3.14). Similar procedures have been carried out using Evans' enolate chemistry,[128,129] again giving α-amino acids in good optical yields.

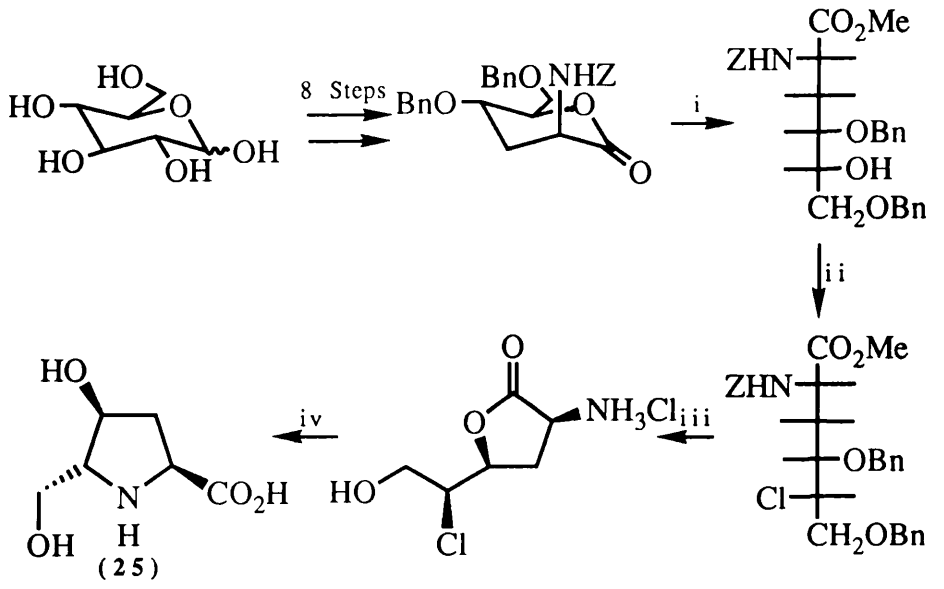
Scheme 3.14



3.4.4 Miscellaneous Methods

The asymmetric synthesis of amino acids has not been exclusively confined to the areas of methodology outlined in Scheme 3.7. For example, the use of carbohydrates as starting materials for the synthesis of more complex amino acids has proved fruitful, especially when a number of contiguous chiral centres are present in the target molecule. Thus Shiba and co-workers have prepared the proline analogue bulgecinine **(25)** from D-glucose (Scheme 3.15).[130]

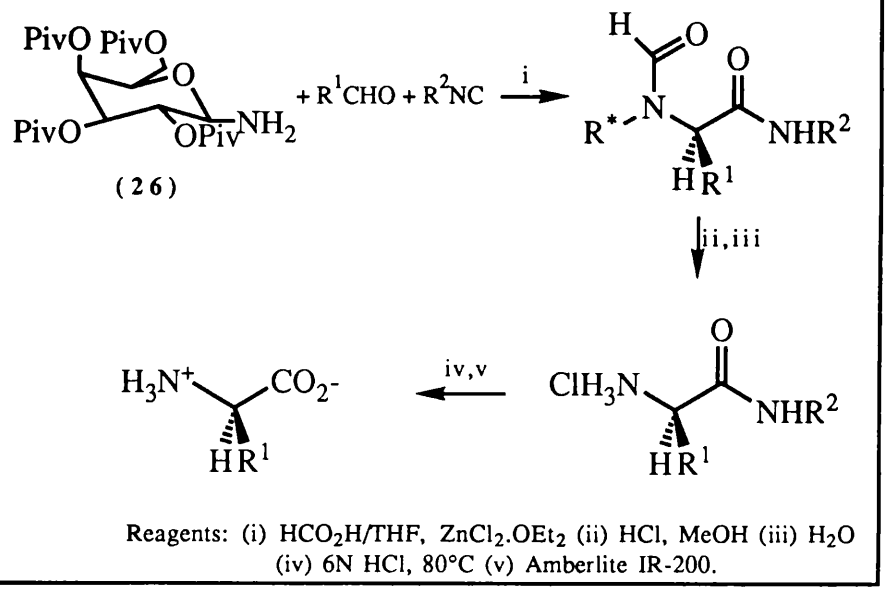
Scheme 3.15



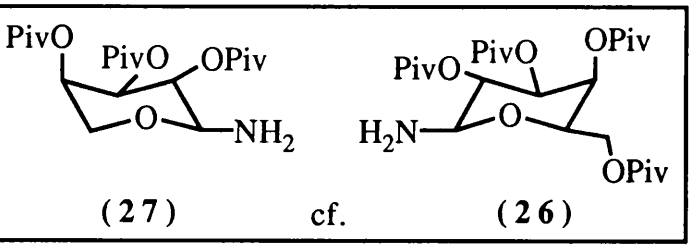
Reagents: (i) MeOH, Δ (ii) Ph_3P, CCl_4 (iii) H_2, Pd, MeOH, HCl (iv) sat. $Ba(OH)_2$, pH9.

While such an approach is elegant, its applicability is clearly limited, and a separate synthetic scheme must be created for each such compound to be synthesised. Of more general interest is the use of sugars as chiral auxiliaries. Thus Kunz and Pfrengle[131] prepared (R)-α-alkyl amino acids *via* an asymmetric zinc chloride-catalysed Ugi condensation, using 2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosylamine (R^*NH_2, **(26)**) as the source of chirality (Scheme 3.16). This route was also used to prepare the corresponding (S)-amino acids by substituting the near-enantiomeric 2,3,4-tri-*O*-pivaloyl-α-D-arabinopyranosylamine **(27)** for **(26)** in Scheme 3.16.[132]

Scheme 3.16



Reagents: (i) HCO_2H/THF, $ZnCl_2.OEt_2$ (ii) HCl, MeOH (iii) H_2O (iv) 6N HCl, 80°C (v) Amberlite IR-200.

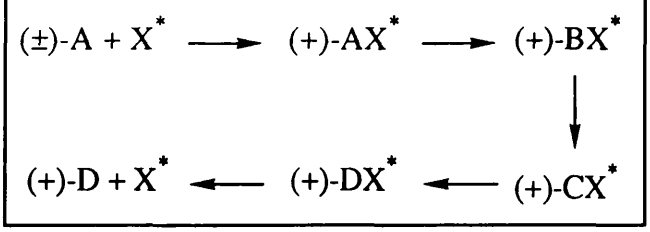


3.5 Conclusion

The most popular methods employed currently for amino acid resolution are probably the traditional one of diastereoisomeric salt formation and the more recent techniques involving isolated enzymes. While chromatographic methods (especially GC and HPLC) are potentially very powerful, their widespread use in this field for preparative purposes still lies some way off. The fundamental reason for this must lie in the high cost of the necessary equipment, rendering the technique beyond the reach of many research workers.

Spontaneous resolution seems destined never to achieve popularity as a resolution technique, due largely to the somewhat serendipitous nature of such a resolution. Diastereoisomeric derivatisation can be a useful method of resolution, although breakdown of the intermediate to reform the resolving agent and the amino acid can be problematical. An interesting strategy might be to use such a homochiral molecule as both a resolving agent and a protecting group, and so carry the group through a reaction scheme before removing it later on in a synthesis (Scheme 3.17). This additional chiral element might even be used to influence the steric outcome of a reaction in the synthesis. In such a case, the one homochiral moiety would be serving as a resolving agent, protecting group *and* a chiral auxiliary - a most economical use of resources!

Scheme 3.17



Diastereoisomeric salt formation has been, and will continue to be, a hugely popular method for resolving amino acids. The method is applicable to amino acids of widely differing structures, unlike many enzymes which tend to react with α-substituted or β-amino acids much more slowly than with common amino acids. As Wilen *et al.*[68] have pointed out, the selection of a suitable resolving agent does not have to rely on good fortune nearly as much as is often assumed. The major drawback with this method is the loss of material which is usually experienced with repeated crystallisations. If the racemic amino acid is cheaply obtained and easily accessible,

such material handling losses may be acceptable. However, in cases where the racemic amino acid is the product of a long synthesis, or is itself scarce, repeated crystallisations of diastereoisomeric salts can be a luxury the chemist cannot afford.

The use of isolated enzymes (and especially acylases) in the kinetic resolution of amino acids has proved a very useful tool indeed. The method allows access to unnatural amino acids in high optical purity, using simple methods and achieving good yields of both enantiomers. However, the necessity to use these enzymes in aqueous environments prevents the method being used for the resolution of amino acids containing water-sensitive functionality. In addition, the methods are almost exclusively limited to α-amino acids. Hence, although it is a very powerful resolution technique for amino acids, the use of isolated enzymes is by no means a universally applicable method.

The asymmetric synthesis of amino acids continues to generate tremendous interest, and new procedures are constantly being developed. However, we have not yet arrived at a point where unnatural amino acids can be routinely synthesised in optically active form on a large scale. In addition, it is often easier and more convenient to resolve the product of a racemic process than to attempt the asymmetric synthesis. Thus, although apparently more wasteful of materials, resolution techniques are still of great importance in the production of optically active amino acids.

Chapter Four

The Use of Lipases in Organic Synthesis

4.1 Introduction

An enzyme is a catalyst which has been subjected to the incessant demands of evolutionary pressure, and hence can promote a desired reaction with an efficiency and selectivity unequalled by any other chemical process. It has even been suggested that enzymes have achieved the maximum possible catalytic activity.[133] And yet, until comparatively recently, organic chemists virtually ignored such catalysts in their quest for ever milder and more selective reagents. The reasons for the small usage of enzymes in organic chemistry were simple: everyone knew that enzymes would only function in water, and water was far from an ideal solvent for organic compounds. The vast majority of organic compounds display at best low solubility in water, and many functional groups are highly unstable in the presence of water. In addition, the extremely high concentration of water molecules in aqueous media results in hydrolytic reactions overriding any other nucleophilic processes. Therefore, the use of enzymes in organic chemistry remained in the domain of a relatively small number of chemists, working mainly in the amino acids field.

This situation was to change with the discovery by Klibanov and Cambou[134] that some enzymes (particularly proteases and lipases) retained their catalytic activity when used as heterogeneous catalysts in organic solvents. This

discovery greatly increased the potential of enzyme-catalysed transformations, allowing a much wider range of compounds to be resolved by enzyme action. In addition, the range of reactions which these enzymes could catalyse was now extended to include esterification, amide formation and transacylation reactions as well as the conventional hydrolyses.

The three type of enzymes which are most commonly used to resolve organic compounds are esterases (e.g. Pig Liver Esterase, PLE), proteases (e.g. α-Chymotrypsin, Subtilisin) and lipases. Of these, lipases have two unique properties which make them extremely attractive to organic chemists.[135] They are stable in non-polar organic solvents, probably reflecting their *in vivo* working environment. Moreover, they show a conformational flexibility within the active site which allows lipases to accept a wide range of substrate structures and functionalities. In addition, a wide range of lipases are commercially available from both mammalian and microbial sources, allowing the screening of various lipases to select the best one to catalyse a given transformation.

In this chapter is given a brief review of the use of lipases in organic chemistry. In addition, various strategies aimed at increasing the efficiency of lipase-catalysed resolutions are discussed.

4.2 Lipase-Catalysed Reactions in Organic Synthesis

The use of lipases as catalysts in organic chemistry has increased dramatically in recent years, and lipase substrates include such diverse compounds as steroids,[136] ferrocene-alcohols,[137] chromium-benzyl alcohol complexes,[138] and diacids and diols in the synthesis of macrocyclic lactones.[139] Consequently, this review will merely highlight several important classes of compound which have

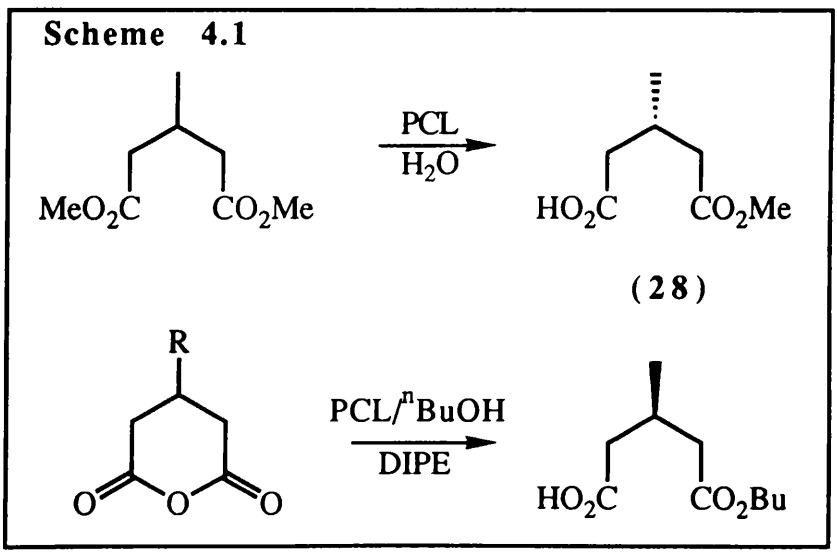
been resolved using lipases. For further examples, the interested reader is referred to several recent reviews covering this area in greater depth.[135,140-143]

4.2.1 Stereoselective Conversion of *meso* Compounds into Chiral Compounds.

The conversion of an achiral molecule to an optically pure chiral molecule is an extremely attractive proposition. The advantage of this technique is that it is theoretically possible to convert all of the starting material into the desired enantiomer of the product molecule, whereas conventional resolution techniques result in half the product being discarded. Therefore, the use of *meso* substrates for lipase-catalysed reactions has attracted considerable attention.

Perhaps the most popular procedure in use is the mono-hydrolysis of *meso* diesters of substituted malonic and glutaric acids. Thus Oda *et al.*[144] produced (S)-(**28**) by the hydrolysis of the corresponding diester, catalysed by lipase from *Pseudomonas cepacia*[145] (PCL) (Scheme 4.1).

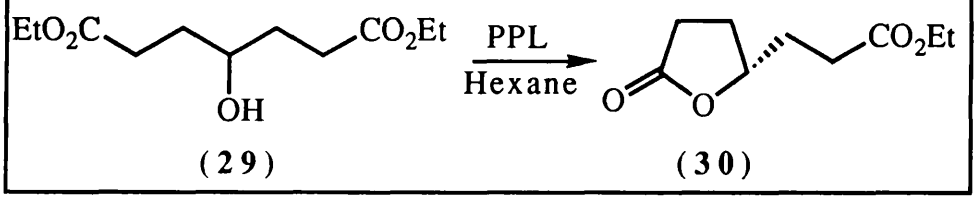
Scheme 4.1



In a variation of this method, Oda *et al.*[144,146] have described the PCL-catalysed butanolysis of cyclic anhydrides to give optically active monoesters (Scheme 4.1). Using this procedure, 3-methylglutaric anhydride reacts with butanol to give (R)-(**28**), making the procedure complementary to lipase-catalysed hydrolysis of the diester.

In a similar vein, Gutman and Bravdo[147] have used PPL to produce the optically active lactone (**30**) *via* intra-molecular transesterification of the appropriate hydroxydiester (**29**) (Scheme 4.2).

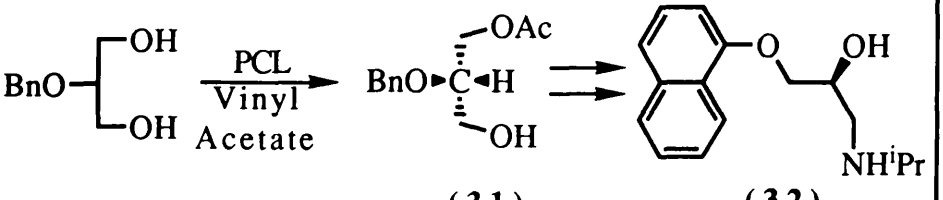
Scheme 4.2



(29) (30)

Lipases have also been used to produce optically active alcohols from *meso* starting materials. Thus Achiwa *et al.*[148] have produced the optically active glycerol derivative (**31**) in up to 94% enantiomeric excess (e.e.) *via* PPL-catalysed esterification of 2-*O*-benzylglycerol. This was then converted chemically into (S)-propanolol (**32**) (Scheme 4.3).

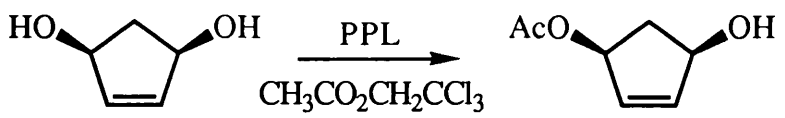
Scheme 4.3



(31) (32)

Jommi *et al.*[149] prepared 3-acetoxy-5-hydroxycyclopent-1-ene **(34)** by PPL-catalysed transesterification of *cis*-3,5-dihydroxycyclopent-1-ene **(33)** and 2,2,2-trichloroethyl acetate in anhydrous pyridine (Scheme 4.4). This compound is a versatile synthetic unit for natural product syntheses.

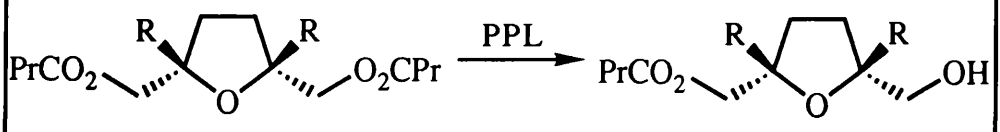
Scheme 4.4



(33) **(35)**

Substituted tetrahydrofuran groups occur in many natural polyether antibiotics. Prasad and co-workers[150] have reported the formation of optically active asymmetrised bis(hydroxymethyl)-tetrahydrofurans *via* PPL-catalysed hydrolysis of the *meso* dibutanoate esters **(35)** (Scheme 4.5).

Scheme 4.5

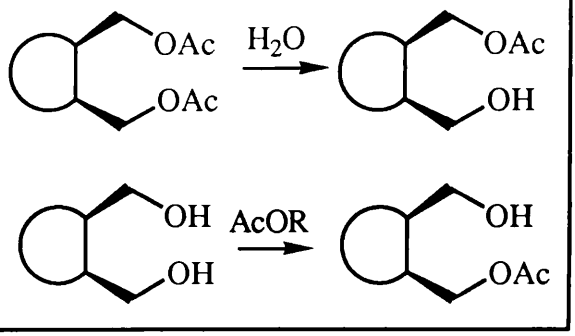


R=Me, H

(35)

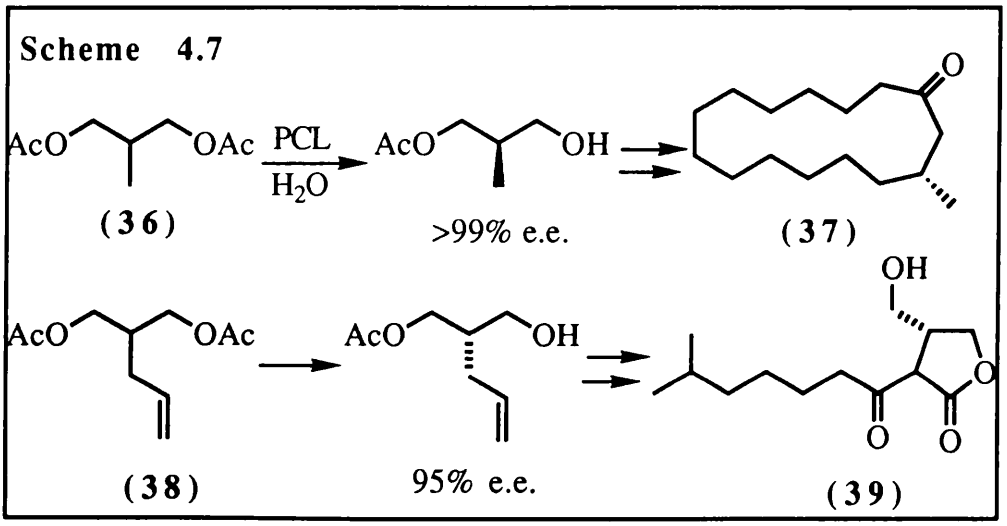
Schneider *et al.*[151] have reported that lipase catalyses the formation of various asymmetrised cycloalkanedimethanols by both hydrolysis and esterification reactions (Scheme 4.6).

Scheme 4.6



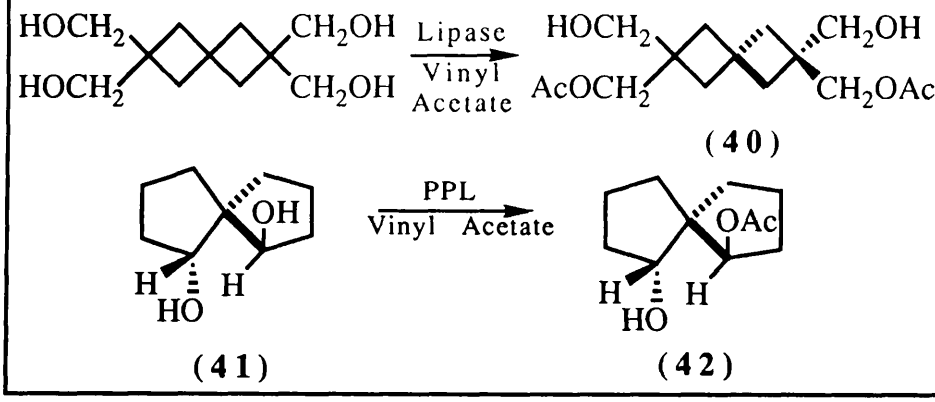
The lipase-mediated conversion of *meso* compounds into optically active ones has been used in the asymmetric synthesis of several compounds of interest industrially. The preparation of (S)-propanolol by Achiwa *et al.* *via* PCL-catalysed acylation of 2-*O*-benzylglycerol has already been mentioned.[148] Xie *et al.*[152] produced (R)-(-)-muscone (**37**) by a route involving the PCL-catalysed monohydrolysis of the *meso* diester (**36**), while Wang and Sih[153] have prepared (-)--A-factor (**39**), which induces streptomycin biosynthesis in inactive mutants of *Streptomyces griseus*, *via* PPL-catalysed hydrolysis of another *meso* diester (**38**) (Scheme 4.7).

Scheme 4.7



Finally, lipases have been shown to be able to convert *meso* substrates into chiral compounds which possess a chiral axis rather than a chiral centre. Thus Naemura and Furutani[154] obtained the chiral spiro[3.3]heptanetetraol diacetate (**40**) in 60% yield and 59% e.e. *via* lipase Amano-P catalysed transesterification with vinyl acetate. Similarly, the spiro[5.5]nonane (**41**) was selectively acetylated by PPL to give (**42**) (Scheme 4.8).

Scheme 4.8

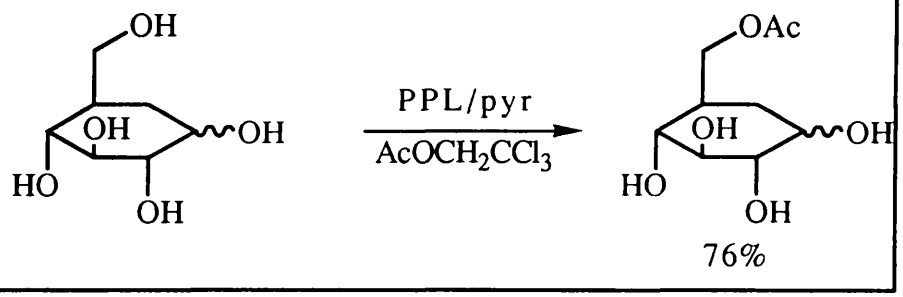


4.2.2 Regioselective Acylation of Sugars[155]

The regioselective functionalisation of the various hydroxyl groups in sugars has long been an important aim of organic chemists, since such protected compounds form a large chiral pool of great utility to the synthetic organic chemist.[156] For this reason, many procedures have been developed to functionalise sugars at specific positions. These methods involve multi-step transformations which, although often high-yielding, are time consuming and tedious. The use of lipases has allowed the masking or unmasking of hydroxyl groups in sugars to be performed in a highly selective, predictable and convenient manner. For example, Therisod and Klibanov[157] reported that PPL catalysed the acylation of the primary hydroxyl group of several unprotected monosaccharides such as glucose and

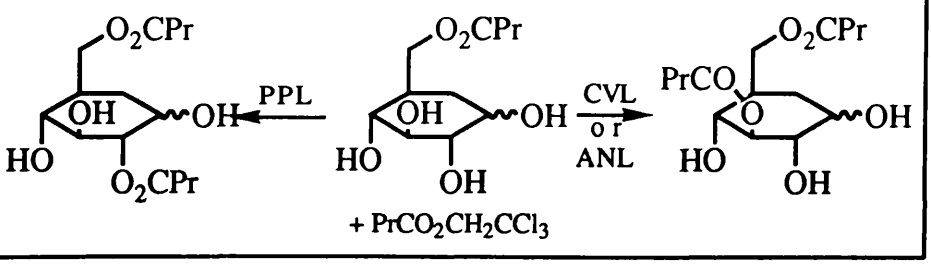
mannose (Scheme 4.9). These workers found that disaccharides such as sucrose, lactose and maltose were very poor substrates for PPL, a fact they attributed to steric hindrance.

Scheme 4.9



The same workers found that the 6-*O*-acylated monosaccharides so produced could also act as substrates for lipases, and that different lipases exhibited different regiochemical preferences.[158] Thus, PPL selectively acylates 6-*O*-butanoyl-D-glucose at the 2-position, whereas lipases from *Chromobacterium viscosum* (CVL) and *Aspergillus niger* (ANL) showed a strong preference for the 3-position of the same substrate (Scheme 4.10).

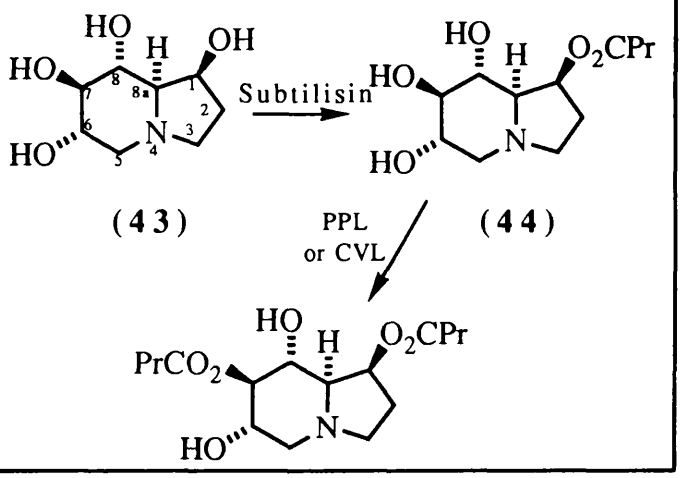
Scheme 4.10



Lipases have also been shown to catalyse the selective hydrolysis of polyacylated sugar derivatives, again showing a strong preference for reaction at the primary hydroxyl group.[159]

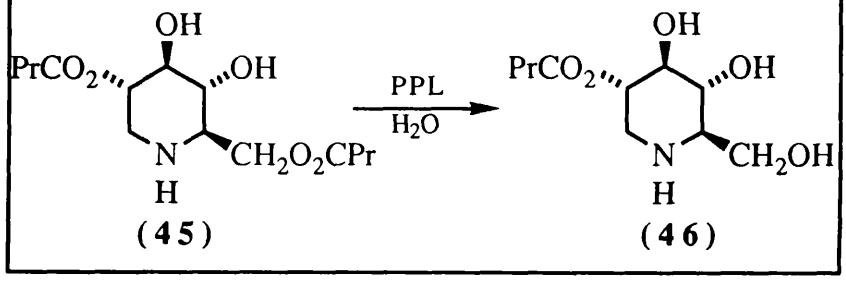
The regioselectivity displayed by lipases has also been used to good effect in the functionalisation of azasugar derivatives. For example, Margolin *et al.*[160] have used PPL and CVL to obtain diacylcastanospermine derivatives (Scheme 4.11). Such compounds have been shown to be potent inhibitors of Human Immunodeficiency Virus replication, thus attracting interest as possible anti-AIDS drugs. PPL and CVL were used to acylate 1-*O*-butanoylcastanospermine (**44**) preferentially at the 7-position, although some 1,6-diacylated product was also formed in both cases. 1-*O*-Butanoylcastanospermine was obtained *via* Subtilisin-catalysed esterification of castanospermine (**43**).

Scheme 4.11



Delinck and Margolin[161] have used the hydrolytic action of PPL to obtain 2-*O*-butanoyl-1-deoxynojirimycin (**46**) from the 2,6-diester (**45**), which was in turn prepared by an enzymic acylation (Scheme 4.12).

Scheme 4.12



(45) (46)

4.2.3 Resolution of Amino Alcohols and Amino Alcohol Precursors

Amino alcohols are important chemicals, being useful synthetic intermediates, important chiral auxiliaries, and often displaying interesting biological or pharmaceutical properties (see Chapter 5). Consequently, new methods for obtaining such compounds in optically active form are constantly being sought, and the use of lipases to effect such resolutions has often proved successful.

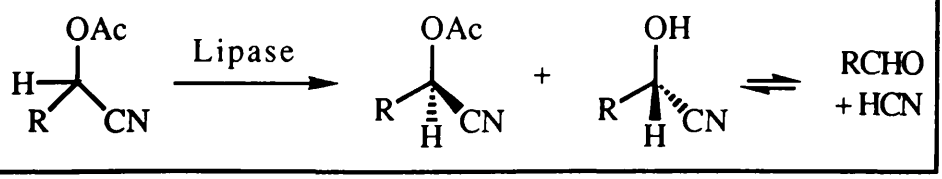
There have been conflicting reports about whether amino alcohols themselves are good substrates for lipase-catalysed acylations. Francalanci *et al.*[162] reported that only *N*-protected amino alcohols could be resolved by PCL, whereas Gotor *et al.*[163] have obtained acylated derivatives of (±)-1-amino-2-propanol and (±)-2-amino-1-butanol in over 95% enantiomeric excess *via* PPL-catalysed transacylation in ethyl acetate. Bevinakatti and Newadkar[164] found that a large amount of crude lipase powder had to be used in order to repeat the work of Gotor *et al.*, and implied that this was due to the free amine deactivating the lipase in some way. Bevinakatti and Newadkar circumvented this problem by performing a PPL-catalysed transesterification between (±)-2-acetamido-1-butyl acetate and 1-butanol, the (R)-enantiomer of the ester reacting faster to give (R)-2-acetamido-1-butanol in good optical purity.

When unprotected amino alcohols are subjected to enzymic acylation, the nitrogen generally reacts much faster than the oxygen, and so one gets the amidoalcohol formed before the amidoester. However, Chinsky *et al.*[165] have reported that the relative reactivities of the hydroxyl and amino groups depends upon the acyl donor used. Thus, using *Aspergillus niger* lipase (ANL) as the biocatalyst and 6-amino-1-hexanol as a model substrate, acylation occurred on the oxygen when 2-chloroethyl butanoate was used as the acyl donor, and on the nitrogen when the acyl donor was 2-chloroethyl *N*-acetyl-L-phenylalaninate. This phenomenon was also observed using PPL or PCL in place of ANL.

In view of the confusion about amino alcohols as lipase substrates, it is perhaps not surprising that many workers have preferred to concentrate on the resolution of amino alcohol precursors. Typically, these compounds are alcohols containing another functional group which can be readily transformed into an amine. The commonest examples include azido alcohols, nitro alcohols, cyano alcohols and cyanohydrins.

Schneider and co-workers[166] have obtained a large number of structurally dissimilar cyanohydrin acetates in high optical purity *via* lipase-catalysed hydrolyses. The cyanohydrins formed in this reaction were not obtained in optically pure form, due to their breakdown into aldehyde and hydrogen cyanide (Scheme 4.13).

Scheme 4.13



Wang *et al.*[167] have found that cyanohydrins can be acylated by lipase in dichloromethane, using vinyl acetate as the acyl donor. Under such conditions, the cyanohydrins are quite stable, and so both acetate and alcohol can be obtained in high enantiomeric excess.

Honig *et al.*[168] have studied the enzyme-catalysed hydrolysis of racemic butanoate esters of cyclic *trans*-2-azido, *trans*-2-nitro, and *trans*-2-cyano cycloalcohols. Lipases from *Candida rugosa*[169] and *Pseudomonas* species (PSL) proved to be excellent catalysts for the formation of the derived optically active alcohols, although a significant decrease in the rate of hydrolysis by PSL was observed as the ring size of the substrate was increased. The choice of nitrogen functionality (azide, nitro or nitrile) proved to be unimportant in terms of the enantioselectivity of ester hydrolysis. Honig's group have extended this work to acyclic amino alcohol precursors,[170] obtaining optically pure 2-azidoalcohols *via* CRL or PCL-catalysed hydrolysis of the corresponding butanoate esters.

Clearly, good results have been achieved in the resolution of amino alcohol precursors. However, the position regarding amino alcohols themselves is less clear, with contradictory reports appearing in the literature. It would obviously be useful if amino alcohols themselves could be resolved quickly and easily by the action of lipases, and so the search for a generally applicable method of effecting such resolutions goes on.

4.3 Strategies Used to Increase the Efficiency of Lipase Resolutions

Many different experimental procedures have been developed to improve the enantioselectivity of lipase-catalysed reactions. In this section, the main techniques employed to improve lipase catalysed processes in organic solvents are discussed.

4.3.1 Irreversible Acyl Donors

In a transesterification reaction, an alcohol reacts with an ester to form a new ester and release a second alcohol (Scheme 4.14). Unless some factor interferes, it is possible for the liberated alcohol (R^3OH) to participate in another transesterification

Scheme 4.14

$$R^1OH + R^2CO_2R^3 \rightleftharpoons R^2CO_2R^1 + R^3OH$$

reaction, and so the process is reversible. This is clearly undesirable, as the yield of ester $R^2CO_2R^1$ is decreased. More importantly, such reversible reactions serve to lower the enantiomeric excess of the final product. Therefore, several irreversible acyl donors have been developed to suppress the reverse reaction, mainly by reducing or removing the nucleophilic character of R^3OH.

The simplest method to achieve this would be to use esters of sterically hindered alcohols such as *t*-butanol as acyl donors. However, such bulky acyl donors prove to be poor substrates for lipases,[171] and so this procedure is not useful.

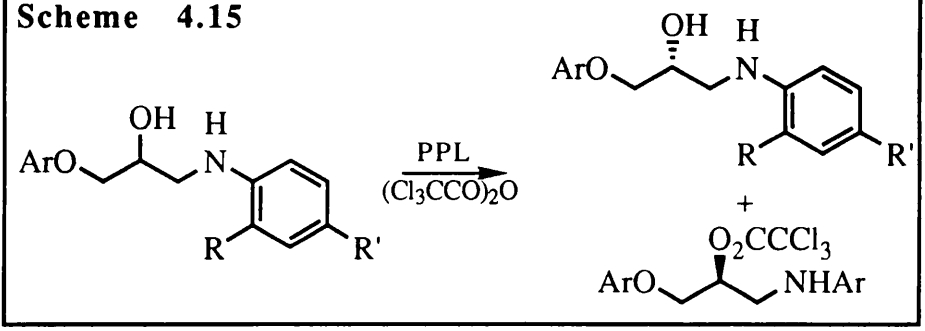
A common approach uses electonegative substituents on the α-carbon of the alcohol R^3OH, which reduces the nucleophilicity of the hydroxyl by an inductive effect. The most commonly used examples of this are the 2,2,2-trichloroethyl esters, although 2,2,2-trifluoroethyl esters have been shown to give faster reactions.[171] Another method of reducing the nucleophilicity of the liberated hydroxyl group involves the use of oxime esters as acyl donors.[172] The by-product of this reaction is an oxime, the hydroxyl group of which is significantly less nucleophilic than normal.

An alternative strategy used to prevent reversible reactions occurring involves the removal of the troublesome hydroxyl group. For example, when an enol ester is used as the acyl donor, the liberated enol quickly tautomerises to the corresponding carbonyl compound and so will not participate in the reverse reaction.[173] The enol esters most commonly used are vinyl acetate and isopropenyl acetate, both of which are commercially available. However, such compounds can undergo polymerisation, and in the case of vinyl acetate the acetaldehyde produced can, in sufficient quantities, deactivate the enzyme.

The use of acid anhydrides as irreversible acyl donors has also attracted some attention. Bianchi *et al.*[174] acylated a number of racemic alcohols asymmetrically using anhydrides of short-chain carboxylic acids and PCL adsorbed on Celite. Kamal and Rao[175] enantioselectively acylated the hydroxyl group of chiral 2-propanolamines using trichloroacetic anhydride in dioxane (Scheme 4.15). When acetic anhydride was used as the acyl donor, the main product was diacylated material. Other acyl donors such as ethyl acetate, *O*-acetylcyclohexanone oxime and vinyl acetate failed to give satisfactory results. Using anhydrides rather than esters as acyl donors is said to give much faster reactions. In addition, the liberated carboxylic acid does not participate in a reverse reaction. However, Berger *et al.*[176] have reported that when various lipases and acid anhydrides were used to acylate

tetrachlorobicyclo[2.2.1]hept-5-en-2-ols, the enzyme's enantioselectivity dropped dramatically if the liberated carboxylic acid was not removed. Addition of a weak base to the reaction mixture, or the adsorption of the lipase onto Celite proved equally effective at counteracting this problem.

Scheme 4.15



4.3.2 Effect of Solvent and Water Content

The choice of solvent has been shown to be an important consideration in optimising the enantioselectivity of lipase-catalysed transacylations. The stability of lipases decreases as the polarity of the solvent increases.[135] This has been interpreted as being due to a combination of polar solvents penetrating the hydrophobic core of the protein, thus denaturing the enzyme, and also hydrophilic solvents stripping away essential water from the protein chain.[177] Either way, the effect is to reduce the catalytic efficiency of lipases in polar solvents, although successful resolutions have been performed in polar solvents such as pyridine[149] and acetone.[178]

The logarithm of the partition coefficient of a solvent between octanol and water (log P) has been used as a quantitative measure of solvent polarity.[179] Chen and Sih[135] have tabulated log P values for some common organic solvents, and have stated the general rule that the catalytic activity of lipases is highest in solvents

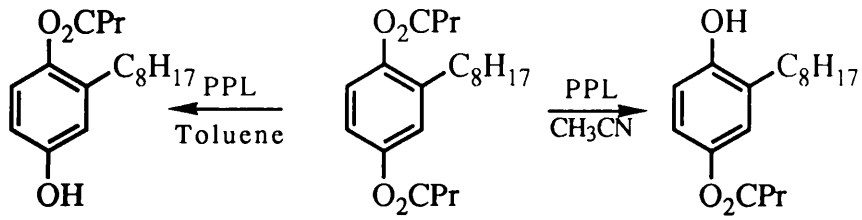
having log P > 4. However, the instability of lipases in polar solvents can be partially counteracted by adsorbing the enzyme on a polymer support.[135]

Nakamura *et al.*[180] have studied the effect of cyclic and acylic solvents on the lipase-catalysed transesterification of (±)-3-nitro-2-propanol, using vinyl acetate as the acyl donor. They found that, using cyclic solvents, the enantioselectivity of the reaction was highest for THF (log P = 0.5), and generally was higher for cyclic solvents than for acylic ones of similar log P values. However, it remains to be seen if these results are of general applicability.

The water content of solvents is also an important parameter in lipase-catalysed transacylations. In the CRL-catalysed coupling of α-bromopropanoic acid and 1-butanol in hexane, Kitaguchi *et al.*[181] found that the initial rate of the reaction was increased significantly when a little water (< 0.125%) was added to the reaction mixture. In addition, the enantioselectivity of the process increased as the water content was increased. These results were thought to be due to the enhanced conformational flexibility of the enzyme. Similar results were observed using water-mimicking co-solvents such as formamide or ethylene glycol, which are capable of forming multiple hydrogen bonds to the enzyme.

Choice of solvent has also been demonstrated to affect the regioselectivity of lipase action. Thus Rubio *et al.*[182] have reversed the regioselectivity of the PCL-catalysed transesterification reaction between butanol and 2-octylhydroquinone dibutanoate (**47**), merely by altering the solvent (Scheme 4.16). This result was explained in terms of a hydrophobic cleft in the enzyme, which accommodated the octyl group when in polar solvents and so oriented the molecule such that the more sterically hindered ester was placed at the enzyme's active site.

Scheme 4.16



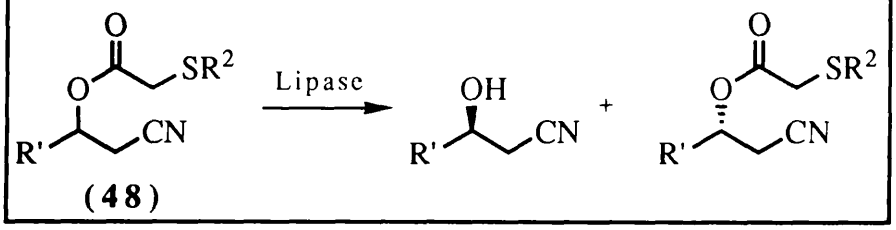
(47)

4.3.3 Modification of Substrate Structure

It is sometimes possible to alter the structure of a substrate molecule to achieve better results in lipase-catalysed reactions. This substrate modification may be nothing more dramatic than the addition of a protecting group to some part of the molecule. Thus, Francalanci *et al.*[162] have stated that the effective resolution of amino alcohols by lipases can only be achieved after the amino group has been masked as an amide or carbamate.

A more common approach has been the modification of an achiral component in the reaction. A simple method which has proved effective in the resolution of racemic esters is to vary the size of the ester acyl group.[183,184] In general, butanoate esters are hydrolysed by lipases more rapidly than propanoate esters, which in turn are hydrolysed more rapidly than acetate esters. More exotic modifications of the acyl group have also proved useful. For example, Itoh and Takagi[185] have found that 3-phenylthioacetoxy- ((**48**), R^2=Ph) and 3-methylthioacetoxynitriles ((**48**), R^2=Me) were hydrolysed to the corresponding β-hydroxynitriles in high enantiomeric excess by PCL (Scheme 4.17). Under identical reaction conditions, the PCL-catalysed hydrolysis of 3-valeroyloxybutyronitrile proceeded with a significantly lower enantioselectivity.

Scheme 4.17

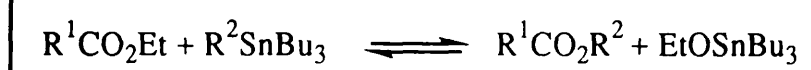


(48)

Scilimati *et al.*[186] have found that to achieve high enantioselectivities in the lipase-catalysed hydrolysis of racemic acyloxycarboxylic esters, the carboxyl terminal must be protected with a non-hydrolysable ester such as a *t*-butyl ester.

Therisod[187] has reported that *O*-stannyl ethers of alcohols can be used in place of the alcohol in lipase-catalysed transesterifications (Scheme 4.18). In this case, the more nucleophilic tributylstannyl ether reacts up to three times faster than the free alcohol. However, no details were reported about the stereochemical consequences of the replacement of alcohols with stannyl ethers, and the procedure has not been widely adopted.

Scheme 4.18



4.3.4 Miscellaneous Methods

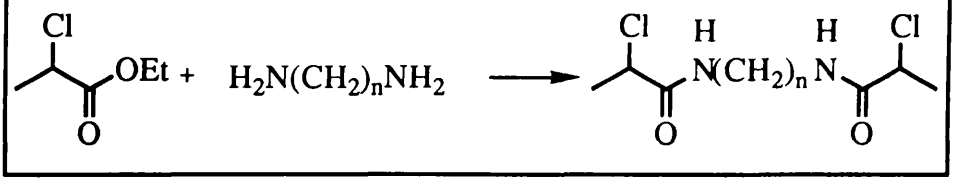
Sih and co-workers have reported that treating *Candida rugosa* lipase (CRL) with sodium deoxycholate in organic solvents caused the enzyme to unfold and refold to give a more stable conformer.[188] This new form of CRL was found to display much higher enantioselectivity than the native enzyme towards the esterification of (±)-arylpropanoic acid and (±)-phenoxypropanoic acid. However,

their method involved a lengthy purification procedure, and has not been used to any great extent.

A second procedure aimed at increasing the enantioselectivity of lipase-catalysed reactions invokes the concept of enantioselective inhibition. Guo and Sih[189] found that various chiral amines, most notably dextromethorphan (DM) and levomethorphan (LM) increased 20-fold the enantioselectivity of the CRL-catalysed hydrolysis of methyl (±)-2-(2,4-dichlorophenoxy)propanoate (DCCP). Nor was the phenomenon limited to this substrate: DM or LM increased the enantioselectivity of CRL-catalysed hydrolyses of several other (±)-aryloxypropanoate and (±)-arylpropanoate esters. Dextromethorphan appears to act as a non-competitive inhibitor for (S)-DCCP and as a partial non-competitive inhibitor for (R)-DCCP. Once again, though, this technique has not found widespread use in lipase-catalysed resolutions.

Gotor *et al.*[190] have used the lipase-catalysed acylation of achiral diamines with chiral esters to obtain chiral diamides (Scheme 4.19). To form the diamide, the monoamide must react with an acyl-enzyme complex. Since the monoamide is

Scheme 4.19



itself chiral, it was hoped that the enzyme would show a double enantioselection, discriminating between the enantiomers of both ester and monoamide. However, only CRL showed complete diastereoselectivity in the reaction, with the (S,S)-

diamide obtained in 88% e.e. over the (R,R)-enantiomer. With all other lipases tested, a significant amount of (R,S)-diastereoisomer was formed.

4.4 Conclusion

In this chapter, an impression of the tremendous utility of lipases in organic chemistry has been given. These enzymes have demonstrated an ability to accept an incredibly large variety of substrate molecules. With such a wide range of lipase-mediated reactions in the literature, the examples cited here have been selected to give a representative sample of the major areas of research. It seems from the vast wealth of literature that, given sufficient time and patience, reaction conditions can be found which will allow the lipase-mediated reaction of virtually any conceivable substrate. It seems likely that the use of lipases as biocatalysts in organic solvents will increase even more in future.

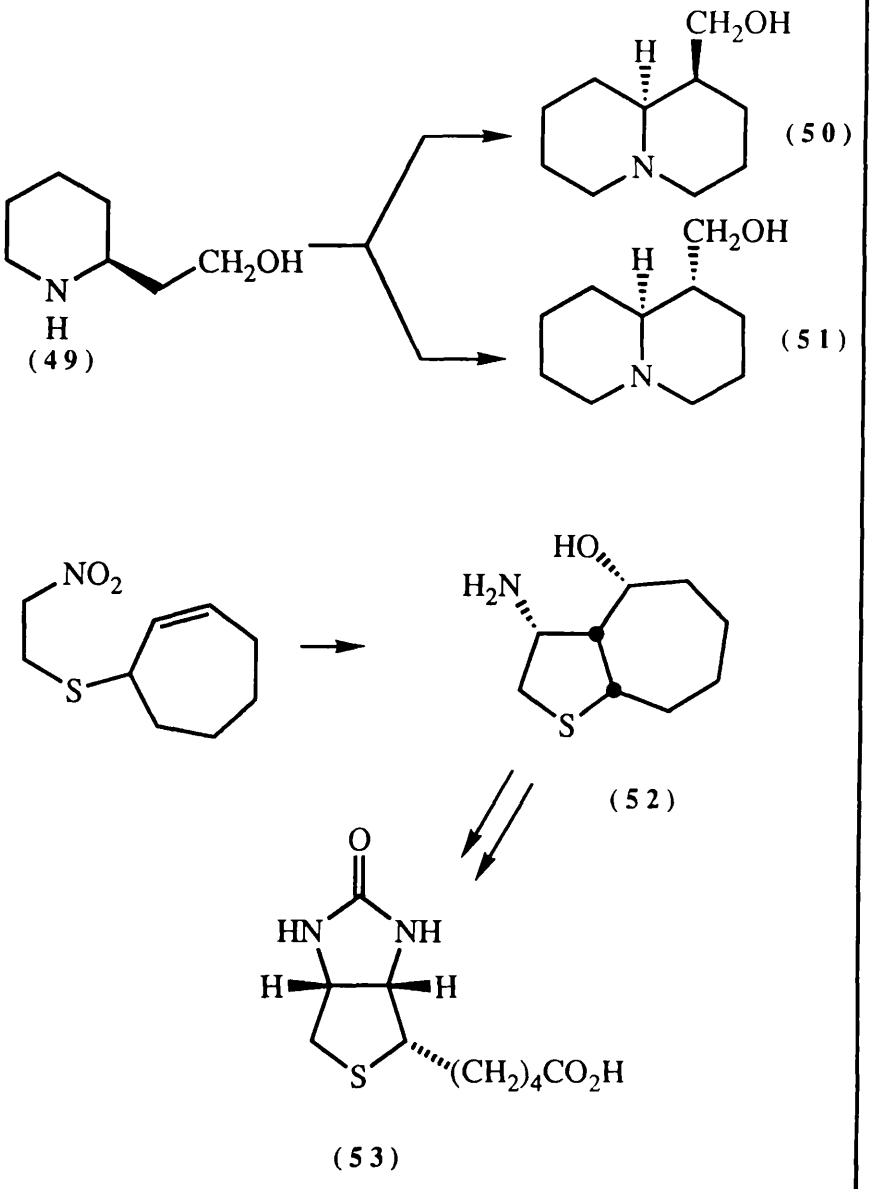
Chapter Five

Synthesis and Lipase-Catalysed Resolution of Cyclic Amino Alcohols

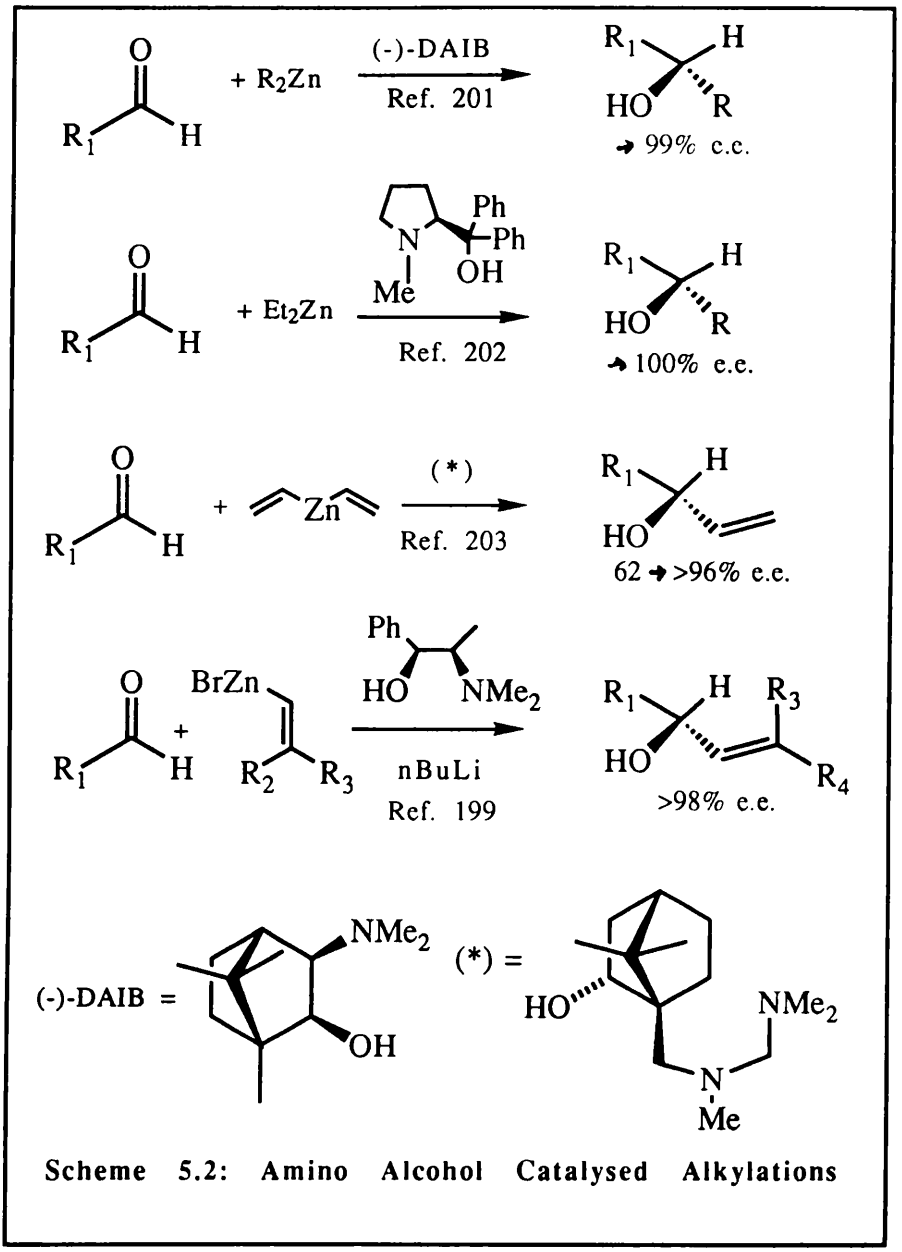
5.1 Introduction

1,2- and 1,3-amino alcohols are compounds of considerable interest, due to both their synthetic utility and their biological importance.[191-194] They have been used as starting materials and intermediates in the synthesis of natural products,[195,196] and have also attracted much interest as chiral auxiliaries and catalysts.[197,198] Their utility as reagents is perhaps limited only by the chemists ingenuity, due to the vast number of reactions which can be performed at each functional group present. For example, Knight and co-workers[195] prepared the quinolizidine alkaloids (+)-lupinine (**50**) and (-)-epilupinine (**51**) from (S)-2-piperidineethanol (**49**), while Confalone *et al.*[196] used the amino alcohol (**52**) as a key intermediate in their synthesis of (±)-biotin (**53**) (Scheme 5.1).

Amino alcohols have been found to catalyse alkylation of carbonyl compounds by dialkyl and dialkenyl zinc compounds.[197] When optically active amino alcohols have been used, good to excellent enantioselection has been observed in the alkylation of aldehydes (Scheme 5.2).[199,200]

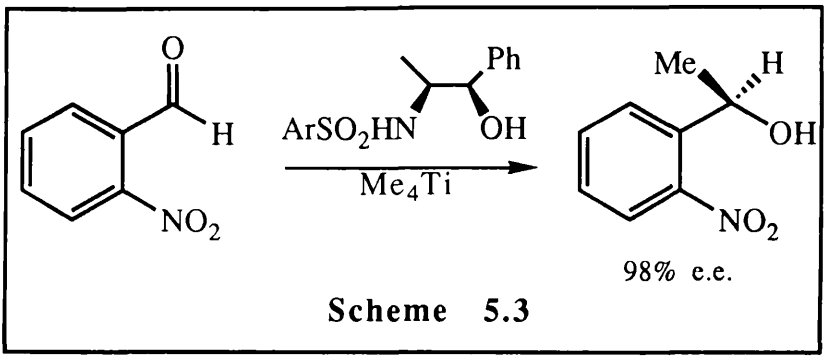


Scheme 5.1 Amino Alcohols in Synthesis



Scheme 5.2: Amino Alcohol Catalysed Alkylations

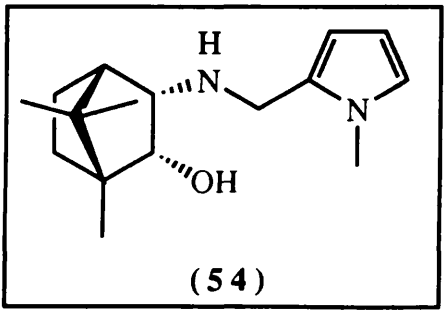
The utility of amino alcohols as chiral ligands is not confined to zinc compounds. High enantioselectivity has been achieved in the tetramethyltitanium (IV) alkylation of aldehydes (Scheme 5.3).[204]



Scheme 5.3

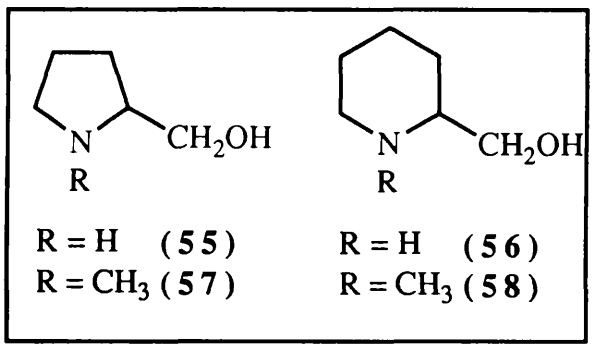
Optically active amino alcohols form complexes with borane which have been used to catalyse asymmetric Diels-Alder reactions.[205] Such complexes have also been used to effect asymmetric reductions of carbonyl compounds.[206-208] In addition, amino alcohol derivatives such as (S)-1-amino-2-methoxymethylpyrrolidine (SAMP) have proved useful chiral auxiliaries for α-alkylation of carbonyl compounds.[209,210]

It should be noted that in many of the above examples, amino alcohols not only serve as chiral auxiliaries, but also catalyse the reactions. Moreover, with dialkyl zinc reactions a phenomenon dubbed *Chiral Amplification*[197] has been observed. For example, a reaction catalysed by optically impure (-)-3-*exo*-dimethylaminoisoborneol (DAIB) (ca.10% e.e) leads to alkylation products with enantiomeric excesses very close to those obtained using enantiomerically pure auxiliary.[211] Chiral amplification has also been observed in the conjugate addition of chiral alkoxydimethylcuprates to an enone system, using amino alcohol **(54)** as a chiral additive.[212]



(54)

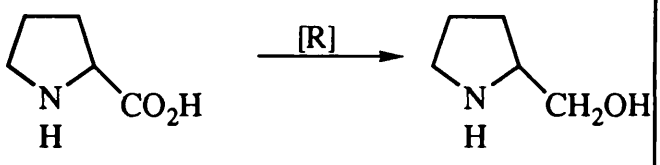
Due to the importance of amino alcohols, many methods have been found to prepare them.[213-217] Many of these routes result in racemic products, and it would be of obvious benefit if a simple, efficient and general method of resolution was available for amino alcohols. In this chapter is described the study of lipase-catalysed transacetylations as a resolution technique for the cyclic amino alcohols 2-pyrrolidinemethanol (prolinol (**55**)), the homologous 2-piperidinemethanol (homoprolinol (**56**)), and the *N*-methyl derivatives (**57**) and (**58**). The search for synthetic routes to these compounds is also discussed.



5.2 Synthesis of (±)-2-Pyrrolidinemethanol

The obvious method of preparing prolinol is by the reduction of proline (Scheme 5.4), and this was indeed the course pursued.

Scheme 5.4



(55)

It was expected that this reduction would proceed readily with BH_3.THF. However, the procedure used (subjecting the reaction mixture to prolonged heating) proved to be inappropriate, giving a complex mixture of products with the desired prolinol only a minor constituent if it was present at all. Therefore, our attention turned to other possible methods of reduction.

Kostyanovsky *et al.*[218] have reported a procedure for an LAH-mediated reduction of L-proline. The fact that this literature procedure involved heating the reaction mixture at reflux for 6 days indicated that this was a troublesome reaction to perform, and the published optical rotation for the product ($[\alpha]_D$ +3.38°) differed substantially from the accepted value ($[\alpha]_D$ -31°).[219] However, as we were only interested in obtaining racemic material in this case, the reported reaction was attempted. Once again though, the reaction mixture consisted of a complex mixture of products, with the desired prolinol being only a minor constituent.

Giannis and Sandhoff[220] have reported a procedure for reducing amino acids with a mixture of a metal borohydride (MBH_4) and trimethylsilyl chloride (TMS-Cl) in refluxing THF. As the metal borohydride itself will not effect such a reduction, it is apparent that a second species, generated *in situ*, must be the active reductant in this case. Giannis and Sandhoff propose that borane is the active reductant, formed *in situ* according to Scheme 5.5.

$$(CH_3)_3SiCl + MBH_4 \rightleftharpoons (CH_3)_3Si.BH_4 + MCl$$

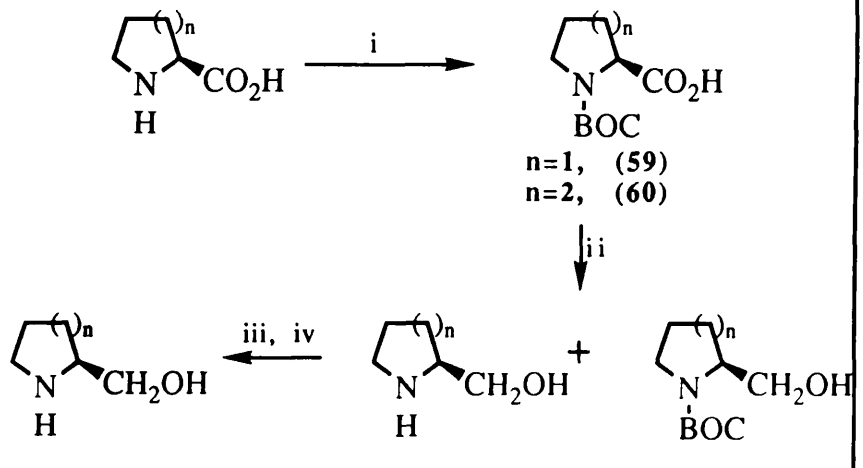
$$(CH_3)_3Si.BH_4 \rightleftharpoons (CH_3)_3SiH + BH_3$$

Scheme 5.5

However, in our hands neither $LiBH_4$ nor $NaBH_4$ produced more than a minimal amount of amino alcohol, even after varying both the reaction times and the relative amounts of MBH_4 and TMS-Cl used.

It was surprising that no success had been achieved with the attempted borane reduction of proline, and it was suspected that the low solubility of proline in THF might have been an important factor in this. This view seemed to be confirmed when Pettit *et al.* reported the facile reduction of THF-soluble *N*-*t*-butoxycarbonyl-L-proline (**59**) (*N*-BOC-L-proline) as a step in their synthesis of Dolastatin X.[221] Indeed, borane reduction of *N*-BOC-L-proline and *N*-BOC-2-piperidineacetic acid (**60**) procedeed in good yield to give a mixture of *N*-protected and unprotected amino alcohol (Scheme 5.6). However, the attempted hydrolysis of the BOC group gave a complex mixture of reaction products. In addition, an unacceptable loss of material was experienced in the deprotection step, almost certainly due to the appreciable water solubility of the amino alcohols.

Scheme 5.6



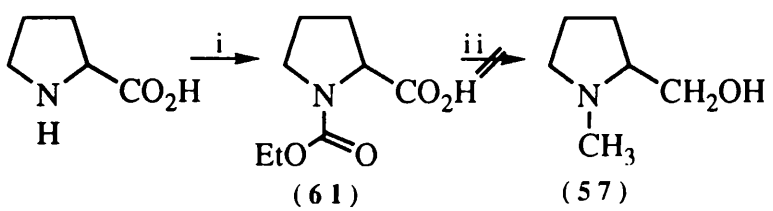
Reagents: (i) BOC-ON, Et_3N, H_2O. dioxan; (ii) BH_3.THF, Δ; (iii) 3M HCl, EtOAc; (iv) $NaHCO_3$.

Fortunately, these problems became of little consequence, for at this point a literature survey revealed that the procedure used for reducing an amino acid with borane bore little resemblance to the original method proposed by Brown *et al.*[222] Whereas the reaction had been subjected to prolonged heating, Brown and co-workers recommended stirring at room temperature for one hour. In addition, the work-up procedure used (extraction from basic solution) failed to recognise that a β-amino alcohol such as prolinol will form a strong chelate complex with borane, which requires to be hydrolysed with dilute acid before the amino alcohol can be obtained Thus, following the procedure of Brown *et al.* gave access to prolinol in low to good yield, and with no racemisation when optically pure proline was used.

5.3 Synthesis of *N*-Methylprolinol and *N*-Methylhomoprolinol

Once again, the corresponding amino acid was chosen as the starting material for the synthesis. The initial attempt to synthesise *N*-methylprolinol (**57**) involved formation of the ethyl carbamate of proline (**61**), and then reduction of both the carbamate and carboxylic acid functionalities to give the desired product (Scheme 5.7).

Scheme 5.7



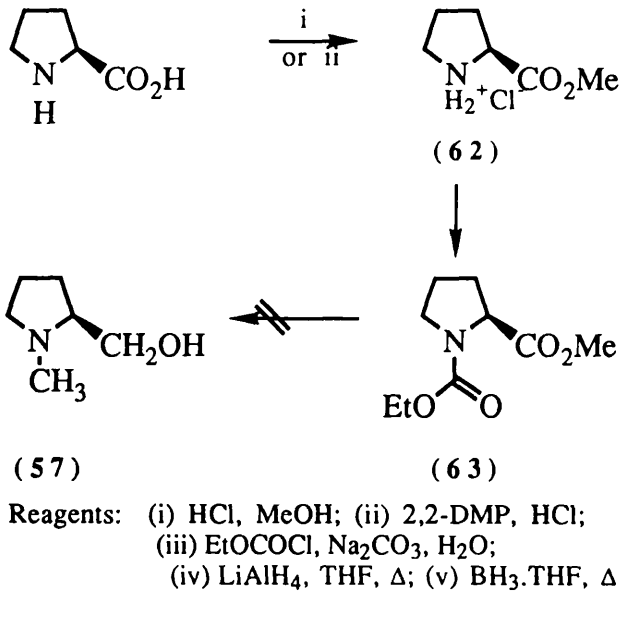
(61) (57)

Reagents: (i) EtOCOCl, Na_2CO_3, H_2O, 0°C→RT; (ii) BH_3.THF, Δ, 16h.

Formation of the carbamate from L-proline and ethyl chloroformate was effected by a standard procedure,[223] although the product could only be obtained as

an oil. Borane reduction of this compound proved to be troublesome, and this was attributed either to impurities in the amino acid derivative or to the low solubility of the oil in THF. Therefore, it was decided to prepare the methyl ester of *N*-ethoxycarbonyl-L-proline (**63**) *via* the corresponding methyl ester hydrochloride salt (**62**), and to attempt to reduce this (Scheme 5.8).

Scheme 5.8



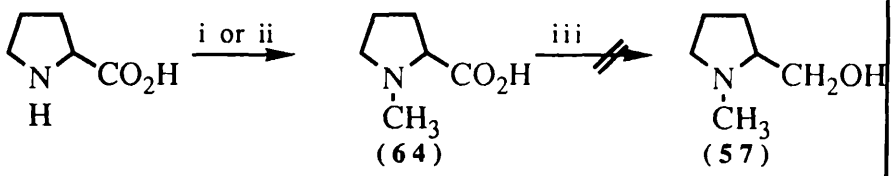
Reagents: (i) HCl, MeOH; (ii) 2,2-DMP, HCl; (iii) $EtOCOCl$, Na_2CO_3, H_2O; (iv) $LiAlH_4$, THF, Δ; (v) BH_3.THF, Δ

The formation of L-proline methyl ester hydrochloride (**62**) was first attempted by the standard method of bubbling HCl gas through a solution of the amino acid in anhydrous methanol.[224] However, the yield obtained by this procedure was disappointing, and the product could only be obtained as a coloured oil. Therefore, an alternative method of forming this compound was attempted, reacting the amino acid with 2,2-dimethoxypropane in the presence of conc. HCl.[225] Not only was the yield of methyl ester hydrochloride significantly greater using this method, but it also had the added advantage of avoiding the inconvenient use of gaseous HCl. Formation of the ethyl carbamate methyl ester (**63**) was

performed as before. Once again, purification of these amino acid derivatives proved to be problematical, all being obtained as oils. Attempted reduction of **(63)** using LAH failed to produce any of the desired product, and so this approach to the *N*-methyl amino alcohols was abandoned.

As the reduction step was the most troublesome in these routes, an alternative strategy was devised whereby the methyl group was introduced by a reductive methylation, followed by reduction of the carboxylic acid (Scheme 5.9).

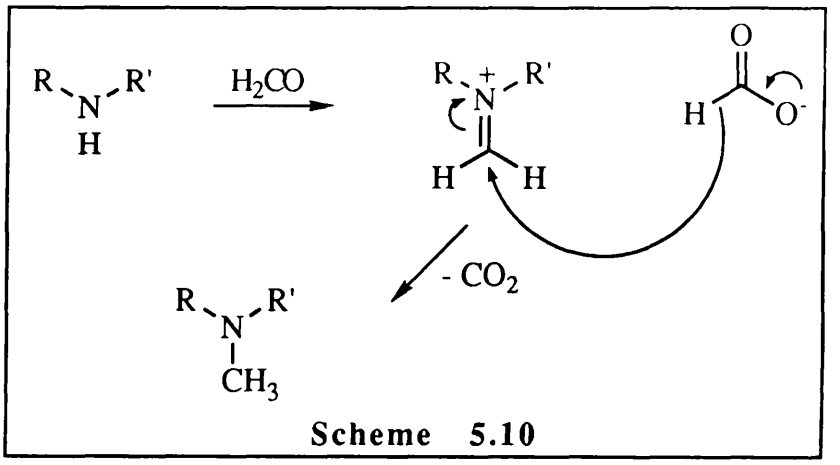
Scheme 5.9



(64) **(57)**

Reagents: (i) H_2CO, $NaHCO_3$, HCO_2H, Δ; (ii) H_2CO, PtO_2, AcOH, H_2O, H_2; (iii) BH_3.THF, Δ, 3 days.

The initial attempt at methylation involved a standard Eschweiler-Clarke procedure, in which the amino group is condensed with formaldehyde to form an iminium ion which is then reduced by hydride transfer from a formate anion (Scheme 5.10).[226]

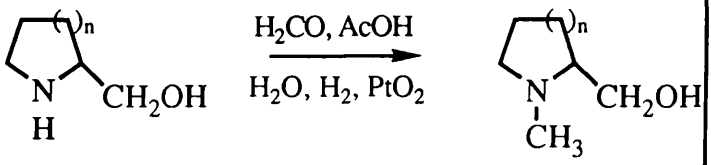


Scheme 5.10

However, when the reaction was attempted only a polymeric material was obtained, possibly originating with formaldehyde. A similar strategy for *N*-methylating amino acids had been reported by Japanese workers, but they reduced the intermediate iminium ion by catalytic hydrogenation.[227] This was claimed to form *N*-methyl-L-proline (hygric acid) (**64**) in optically pure form. When the reaction was performed, hygric acid was obtained in low yield (22%) and it was partially racemised. The low yield was due mainly to losses incurred in crystallising the crude product, and so it was decided to attempt the reduction of the carboxylic acid group using the crude product from the hydrogenation step. However, once again borane reduction of this crude reaction mixture led only to a complex mixture of products.

Until this juncture, the reduction step to give an alcohol had proved the difficult one. However, the relative success of the reductive methylation procedure had demonstrated that the methyl group could be introduced successfully by this procedure, albeit in low yield. In addition, a procedure had by this time been developed for preparing prolinol or homoprolinol in acceptable yields from the amino acids. Therefore, the obvious approach was to attempt to *N*-methylate the amino alcohols by the reductive methylation procedure (Scheme 5.11).

Scheme 5.11



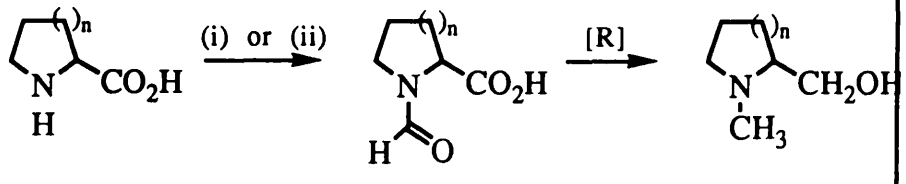
n=1, (55)
n=2, (56)

(57)
(58)

This was first attempted using (±)-2-piperidinemethanol, obtained from Aldrich Chemical Company. The reaction proceeded smoothly to give (±)-*N*-methyl-2-piperidinemethanol (**58**) in good yield. Thus it seemed that we had at last found a method of synthesising the *N*-methyl amino alcohols in reasonable quantities. However, when the procedure was repeated using L-prolinol, the product obtained proved to have been almost totally racemised during the reaction. Therefore, although this route could provide racemic compounds for use in enzyme studies, another procedure was required for the synthesis of optically pure compounds which would serve as reference standards.

Our last attempt at introducing the *N*-methyl group was *via* the reduction of the corresponding formamide (Scheme 5.12).

Scheme 5.12



n=1, (65)
n=2, (66)

Reagents: (i) AcOH, HCO_2H, 0°C→R.T.; (ii) Ac_2O, HCO_2H, Δ.

The *N*-formyl amino acids (**65**) and (**66**) were prepared in moderate to good yield by a standard method.[228] Once again, however, the reduction of these proved extremely troublesome, with a variety of reagents and reaction conditions proving ineffective (Table 5.1).

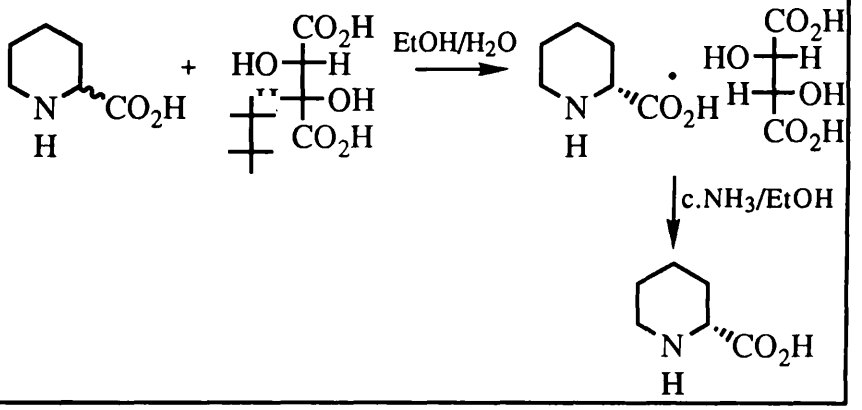
Table 5.1

Reaction Conditions	Result
LAH, THF, Δ	No amino alcohol formed
$NaBH_4$/TMSCl (9.1:4.8), 16 h	No amino alcohol formed
$NaBH_4$/TMSCl (9.1:5.2), 41 h	Minor product only
BH_3.THF, Δ65 h	Minor product only

However, as was the case in the previous section, it was discovered that the procedure used for attempting a borane reduction had been far more savage than necessary. Carrying out the reaction with optically pure *N*-formyl-L-proline using Brown's milder method[229] gave the corresponding *N*-methyl amino alcohol (**57**) in low to good yields and without racemisation.

Having established these synthetic routes to the various amino alcohols, it was hoped to use them to prepare optically pure forms of the various piperidine alcohols to serve as reference standards. For this aim to be achieved, (±)-2-piperidinecarboxylic acid (only available economically in racemic form) had to be resolved chemically. This was achieved by a tartrate resolution (Scheme 5.13), the optically pure amino acid being freed by a metathetical reaction with ammonia.[230,231]

Scheme 5.13



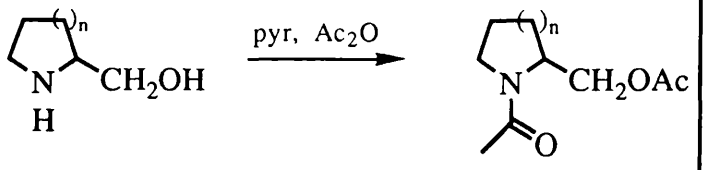
The resolution was initially performed on a small scale, and L-(-)-2-piperidinecarboxylic acid was obtained in optically pure form. The resolution was then attempted on a larger scale to obtain sufficient quantities for synthetic work. However, the starting material used for this larger scale resolution proved to contain impurities which were not removed by repeated crystallisations, and so the resolution was not completed.

5.4 Synthesis of Acyl- and Carbamoyl-protected Amino Alcohols

Having obtained optically pure samples of the amino alcohols, it was then necessary to prepare reference standards of the various acylated products expected from the enzyme reactions. The purpose of this was twofold; firstly to establish unequivocably the identity of the reaction products, and secondly to facilitate the determination of the optical purity of the enzyme reaction products.

The diacetates of prolinol and homoprolinol ((**67**) and (**68**) respectively) were both obtained in good yield by standard methods (Scheme 5.14).

Scheme 5.14



n=1, (**67**)
n=2, (**68**)

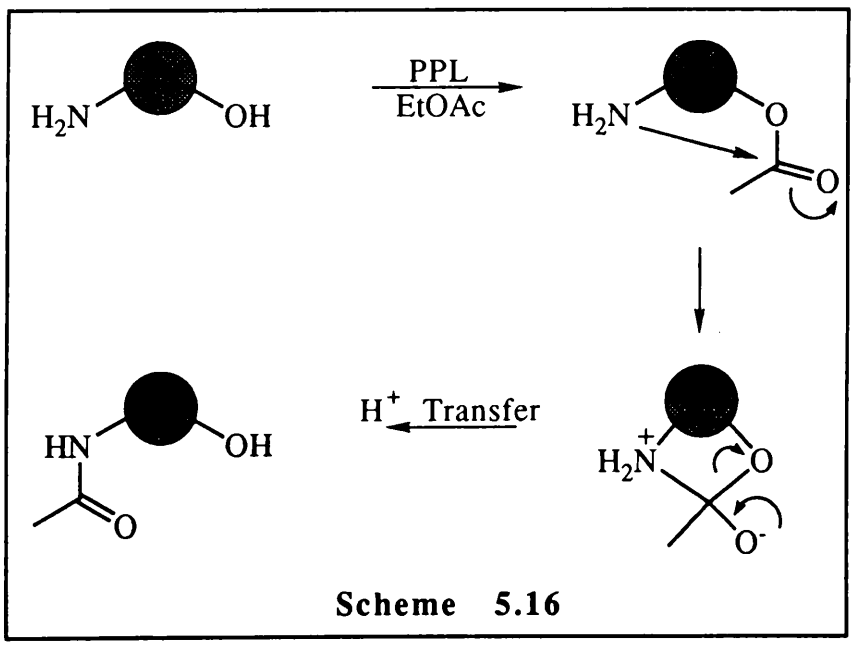
N-Acetylprolinol (**69**) was obtained by the method of Kisfaludy *et al.*,[232] involving the reaction of the amino alcohol with pentafluorophenyl acetate (PFPA) (**70**) in DMF (Scheme 5.15).

Scheme 5.15

(**69**)

Presumably this furnishes only the amido alcohol as the effect of the halogenated aromatic ring is to decrease the susceptibility of the carbonyl group towards nucleophilic attack to such an extent that a hydroxyl group will not react to form the ester. Of course, a more usual way of forming the amido alcohol would be to acylate exhaustively (with, for example, acetic anhydride) and then to hydrolyse selectively the ester using methanolic K_2CO_3. With the benefit of hindsight, it must be said that the acylation/hydrolysis procedure is far more satisfactory, due to the toxicity of fluorinated aromatic rings, the necessity of forming the pentafluorophenyl acetate, and also the difficulty in removing DMF from small, water-soluble amido alcohols such as those handled in this project.

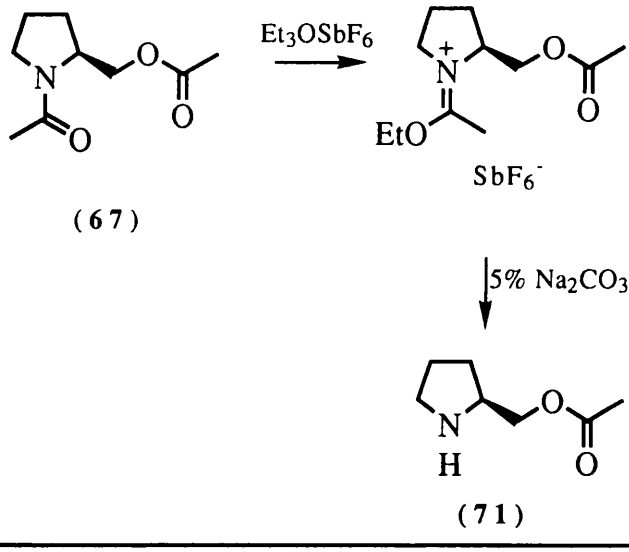
Although it is normally extremely difficult, if not impossible, to acylate selectively an alcohol in the presence of a 1° or 2° amine, it was felt that for completeness an attempt should be made at synthesising *O*-acetylprolinol (**71**). There were two reasons for this. First of all, it would establish if such a compound was a product of the enzyme reactions. However, even if such a compound was not detectable in the reaction mixture, its synthesis would still be valuable. It is possible that when confronted with an amino alcohol, PPL acylates not the amine but the alcohol. A rapid inter- or intra- molecular acyl transfer can then occur to form the observed *N*-acyl alcohol product (Scheme 5.16). Clearly, if the amino ester was stable enough to be isolated, its behaviour in solution would provide an indication of the likelihood of such a process occurring.



Scheme 5.16

To prepare the amino ester, a method developed by Hanessian was used (Scheme 5.17).[233] This involved the selective alkylation of an amide bond in the presence of an ester, using a trialkyloxonium salt. The intermediate alkylacetimidium ion would then be hydrolysed by aqueous base to give the amino ester.

Scheme 5.17

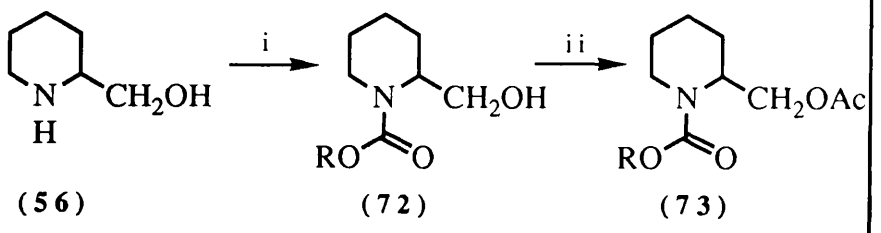


(67) (71)

However, when the reaction was attempted a mixture of four compounds was obtained. Three of these were identified by TLC as being prolinol, *N*-acetylprolinol and *N,O*-diacetylprolinol. The fourth compound present was not identified, but as it was the least polar compound in the mixture it seems unlikely that it was the amino ester. The preponderance of de-acetylated compounds in the reaction mixture was probably due to too long an exposure to aqueous base. However, no attempt was made to repeat the reaction.

Finally, Francalanci *et al*, reported that no resolution of amino alcohols was possible unless the amine group was masked as a carbamate.[162] Therefore, (±)-*N*-ethoxycarbonylhomoprolinol (**72**) and the corresponding acetate ester (**73**) were synthesised by standard procedures for use in further enzyme studies (Scheme 5.18).

Scheme 5.18



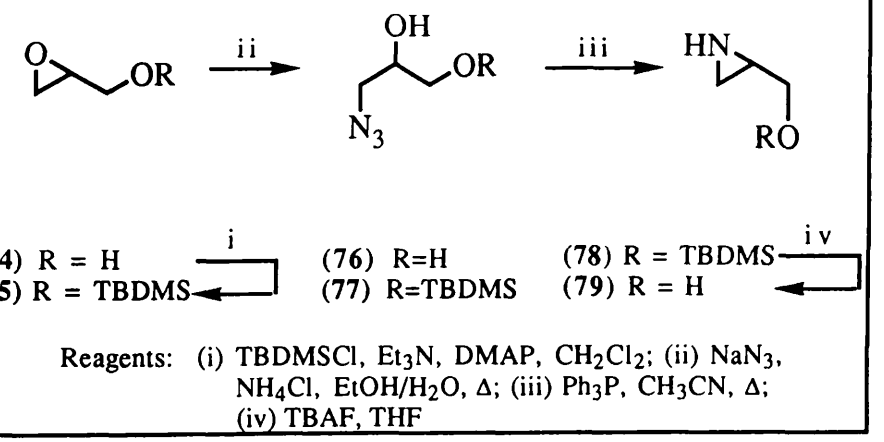
(56) (72) (73)

Reagents: (i) ROCOCl, Et_3N, Me_2CO; (ii) CH_3COCl, pyr, CH_2Cl_2

5.5 Attempted Synthesis of (±)-2-Aziridinemethanols

In an attempt to evaluate the effect of differing ring size on the rate of enzyme-catalysed acetylation of cyclic amino alcohols, it was decided to prepare (±)-2-aziridinemethanol (**79**) and (±)-*N*-benzoyl-2-aziridinemethanol (**84**).

Scheme 5.19



(74) R = H
(75) R = TBDMS
(76) R=H
(77) R=TBDMS
(78) R = TBDMS
(79) R = H

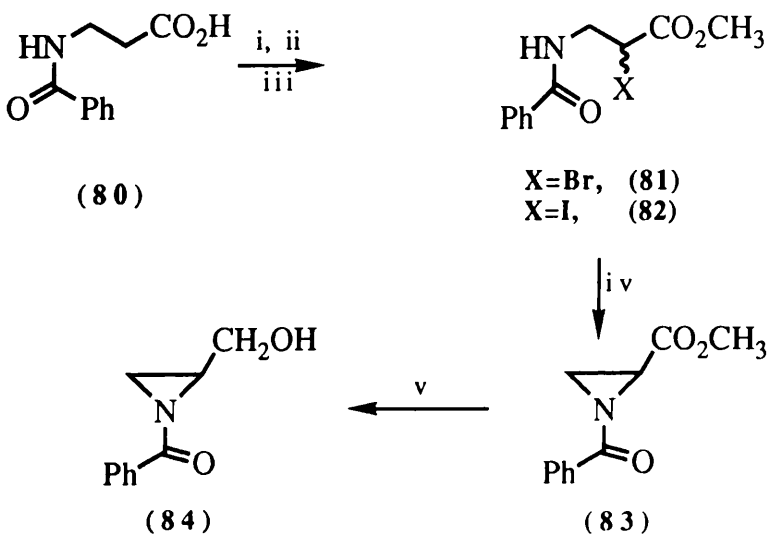
Reagents: (i) TBDMSCl, Et_3N, DMAP, CH_2Cl_2; (ii) NaN_3, NH_4Cl, $EtOH/H_2O$, Δ; (iii) Ph_3P, CH_3CN, Δ; (iv) TBAF, THF

The synthesis of (±)-2-aziridinemethanol (**79**) was attempted from (±)-glycidol (**74**) *via* the azido diol (**76**) (Scheme 5.19). Cyclisation was effected using triphenylphosphine, by a method reported by Legters *et al.*[234] However, the yield of azido diol obtained was low, and the product of the cyclisation appeared to be a polymeric or oligomeric species derived from the desired (±)-2-aziridinemethanol. To prevent such self-condensation, and also to increase the

yield of the first step, the reaction sequence was repeated using the *t*-butyldimethylsilyl (TBDMS) protected glycidol (**75**). Although this did indeed increase the yield obtained in the first step (presumably due to the reduced water-solubility of the protected azido diol), the ring-closure reaction failed completely. Due to time constraints, it was not possible to repeat this reaction or to attempt an alternative route to (**79**).

(±)-2-Aziridinemethanol was expected to be a material which would prove difficult to handle, as evidenced by the ready polymerisation reaction it seemed to undergo. Therefore, the synthesis of (±)-*N*-benzoyl-2-aziridinemethanol (**84**) was attempted from *N*-benzoyl-β-alanine (**80**), rather than attempting to acylate the amino alcohol (Scheme 5.20).

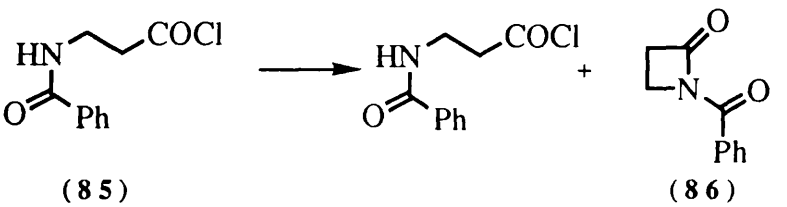
Scheme 5.20



Reagents: (i) $SOCl_2$, CCl_4, Δ; (ii) NBS, CCl_4, 48% HBr, Δ; (iii) MeOH; (iv) NaOEt, EtOH; (v) DIBAL

This approach relied upon the base-promoted cyclisation of the α-halo-β-amino acid esters (**81,82**),[235] followed by the reduction of the cyclised ester

(**83**). Unfortunately, our attempts at generating an α-halo-β-alanine derivative proved fruitless. Bromination of *N*-benzoyl-β-alanine was attempted by a variation of the Hell-Vollhard-Zelinsky reaction described by Haipp *et al.*,[236] but only *N*-benzoyl-β-alanine methyl ester was isolated from the reaction mixture. It is possible that the intermediate acid chloride (**85**) cyclised spontaneously under the reaction conditions to give a mixture of acid chloride and β-lactam (**86**) (Scheme 5.21). Certainly, ^{1}H N.M.R. spectroscopic analysis of the reaction mixture after treatment with thionyl chloride revealed twice the expected number of signals, none of which represented the starting material.

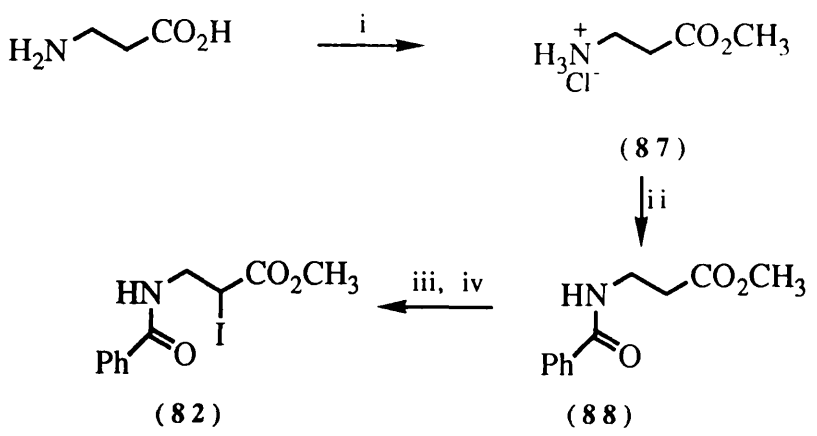


(**85**) (**86**)

Scheme 5.21

As the bromination had failed, attention turned to the generation of the α-iodo-β-alanine derivative (**82**) (Scheme 5.22). The iodination was attempted according to a literature procedure reported by Rathke and Lindert,[237] generating the enolate anion of (**88**) using lithium *N*-isopropylcyclohexylamine (LICA), and allowing this to react with iodine. However, this attempt at generating an α-halo species was also a failure, and due to pressure of time the quest for the 2-aziridinemethanols had to be abandoned.

Scheme 5.22



Reagents: (i) CH_3OH, HCl; (ii) PhCOCl, 10% NaOH;
(iii) LICA, THF, -78°C; (iv) I_2, THF, -78°C.

5.6 PPL-Catalysed Acetylation of Cyclic Amino Alcohols

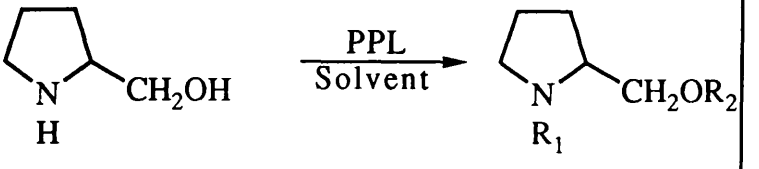
While synthetic routes towards the various amino alcohols were being developed, enzyme studies were confined to commercially available substrates. Both enantiomers of prolinol (**55**) are available from Aldrich Chemical Company, but the racemate is not sold. The opposite situation holds for homoprolinol (**56**), which is only commercially available in racemic form. As it was highly desirable to have access to optically pure reference standards of the various acylated amino alcohols, prolinol was chosen for testing as an enzyme substrate.

5.6.1 Enzymic Acetylation of (+)-, (-)-, and (±)-Prolinol

When lipase reacts with a racemic substrate to give optically active products, it is an example of a kinetic resolution *i.e.* the resolution depends upon the differing rates of reaction of the two enantiomers rather than on differing thermodynamic

stabilities of the two products. Therefore, to ascertain if such a resolution was feasible for (±)-prolinol, the PPL-catalysed acetylation of the separate enantiomers under identical conditions was studied (Scheme 5.23).

Scheme 5.23



(55)

$R_1 = H$, $R_2 = H$ (55)
$R_1 = Ac$, $R_2 = H$ (69)
$R_1 = Ac$, $R_2 = Ac$ (67)

Initial results were not promising. When the reaction was carried out in ethyl acetate at 28 °C, no difference in the rates of formation of either *N*-acetylprolinol (**69**) or *N,O*-diacetylprolinol (**67**) was observed by TLC. However, repeating the reaction at 20 °C resulted in the (R)-isomer reacting markedly faster than the (S)-isomer (see Table 5.2). Therefore, reducing the overall rate of the reaction resulted in the enzyme showing enantiodifferentiation. Unfortunately, our facilities did not allow the running of reactions for prolonged periods below ambient temperature. Therefore, to attempt to slow down the reaction still further, and hopefully to increase the difference in reaction rates between the two enantiomers, the reaction was performed using hexane as solvent and three equivalents of ethyl acetate. However, under these conditions no reaction was observed. Therefore it was decided to attempt the resolution of (±)-prolinol under the optimum conditions discovered above.

Table 5.2

Reaction Conditions	Appearance Times			
	N-Acetylprolinol		*N,O*-Diacetylprolinol	
	(R)	(S)	(R)	(S)
EtOAc, 28°C	20 min	20 min	2 h	2 h
EtOAc, 20°C	5 h	26 h	60 h	> 12 d
EtOAc/hexane 17°C	*No reaction after 48 h*			

Since a satisfactory method of preparing (±)-prolinol had not at this stage been found, racemic prolinol was prepared by the simple expedient of mixing equal amounts of the two enantiomers. This was clearly not a completely satisfactory method, as the procedure introduced an avoidable source of error in the data collected. However, in the circumstances no alternative was available.

The reaction was first studied over short reaction times, when only *N*-acetylation of prolinol was observed. Separation of the amido alcohol and the unreacted amino alcohol was attempted by column chromatography. The amido alcohol was isolated in good yield by this method, but satisfactory recovery of the amino alcohol from the chromatography column was never achieved. The high polarity of the amino alcohol was undoubtedly the reason for its reluctance to chromatograph, and even methanol/ammonia solvent mixtures proved ineffective at recovering the amino alcohol. The optical rotations of the isolated *N*-acetylprolinol varied erratically (e.e. 6 - 26%), but always indicated an excess of (S)-(-)-*N*-acetylprolinol (Table 5.3). This was surprising, as the previous work on the separated enantiomers lead us to expect the enzyme to prefer the (R)-isomer. As for the unreacted amino alcohol, the small amount of material that was obtained always displayed no optical activity. It may well be true that the prolinol was racemising due to prolonged exposure to the chromatography surface, or even due to the polar solvents used to elute it. It is unlikely that the preponderance of (S)-*N*-acetylprolinol in the reaction mixture was due to inaccurate preparation of the initial

racemate. Such an explanation certainly does not account for the reproducibility of the results.

Table 5.3

50 mg prolinol, 120 mg PPL, 30 ml EtOAc, 25°C

Reaction Time (h)	*N*-Acetylprolinol		Prolinol	
	Yield mg (%)	$[\alpha]_D$	Yield mg (%)	$[\alpha]_D$
4	28 (40)	-3.6	24 (48)	0
7	18 (25)	-15.0	37 (74)	0
7.40	24 (33)	-5.6	26 (51)	0

(Optical Rotations were measured as $CHCl_3$ solns, c 0.4 - 0.8 g/100 ml)

Leaving the reaction on for longer gave mixtures of prolinol, *N*-acetylprolinol and *N,O*-diacetylprolinol. Once again, after column chromatography prolinol was obtained in racemic form and in poor yield. *N*-Acetylprolinol was again obtained with a small enantiomeric excess (e.e. 0 - 33%), but this time with the (R)-isomer in excess. The observed enantiomeric excess of *N,O*-diacetylprolinol proved remarkably constant no matter how long the reaction was run (e.e. 44 - 57%), with the (S)-isomer always in excess (Table 5.4). It was extremely difficult to make sense of these results, and it was felt that the large number of possible reactions such a bifunctional system might undergo was precluding any sensible results from being obtained. Therefore, our attention turned to the *N*-methyl amino alcohols, in which only one acylation site is available.

Table 5.4

Reactions Conditions as Table 5.3

Reaction Time (h)	*N*-Acetylprolinol		*N,O*-Diacetylprolinol	
	Yield mg (%)	$[\alpha]_D$	Yield mg (%)	$[\alpha]_D$
48	60 (42)	+4.5	17 (9)	-29.4
93	66 (46)	+3.2	18 (10)	-29.2
145.30	25 (17)	+3.1	26 (14)	-35.8
174	31 (22)	0.0	60 (32)	-34.4
357	43 (30)	+19.4	71 (38)	-30.0
425.30	27 (19)	+2.4	98 (54)	-27.8

5.6.2 Enzymic Acetylation of (±)-*N*-Methylhomoprolinol and (±)-Homoprolinol

N-Methylhomoprolinol **(58)** was used as the substrate at this point due to necessity rather than choice, as this was the only *N*-methyl amino alcohol which we had successfully synthesised at the time.

Contrary to what had been hoped, the results obtained using *N*-methylhomoprolinol did not form a simple pattern. The acetate ester produced was usually obtained with a small negative optical rotation, but the size of this rotation varied erratically with reaction time (Table 5.5). Increasing both the substrate and the enzyme concentrations did not alter the seemingly random variance of these rotations (Tables 5.6, 5.7, 5.8). In addition, the increased basicity of the tertiary amine caused even more problems in eluting unreacted amino alcohol from basic or neutral alumina, and no satisfactory recovery of amino alcohol was ever achieved.

Table 5.5

50 mg amino alcohol, 120 mg PPL, 30 ml EtOAc, 25°C

Reaction Time (h)	*N*-Methylhomoprolinyl acetate	
	Yield mg (%)	$[\alpha]_D$
9.30	14 (21)	0.0
28.30	15 (22)	-8.1
33.30	32 (47)	0.0
39	21 (31)	-8.3
75	55 (81)	0.0

(Optical rotations were measured as $CHCl_3$ solns, c 0.3 - 1.2 g/100 ml).

Table 5.6

100 mg homoprolinol, 120 mg PPL, 30 ml EtOAc, 25°C

Reaction Time (h)	*N*-Methylhomoprolinyl acetate	
	Yield mg (%)	$[\alpha]_D$
14	25 (18)	-4.1

Table 5.7

100 mg homoprolinol, 150 g PPL, 30 ml EtOAc, 25°

Reaction Time (h)	*N*-Methylhomoprolinyl acetate	
	Yield mg (%)	$[\alpha]_D$
24	63 (47)	-6.3
48	71 (53)	-52.6

Table 5.8

100 mg homoprolinol, 610 mg PPL, 20 ml EtOAc, 27°C

Reaction Time (h)	*N*-Methylhomoprolinyl acetate	
	Yield mg (%)	$[\alpha]_D$
6	34 (26)	-6.7
24	76 (58)	-7.4

Because of the recovery problems we had been experiencing, we next attempted to resolve *N*-ethoxycarbonylhomoprolinol (**72**) *via* lipase catalysed transacetylation. Our reasons for choosing this substrate were twofold. Firstly it was expected that the reaction products would undergo column chromatography better than free amines, and secondly, Francalanci *et al.* had experienced some success in lipase-catalysed resolutions of simple amino alcohols protected as the ethyl carbamate.[162] The products of the reactions were indeed much easier to separate, but unfortunately the enzyme showed no enantioselectivity. In an attempt to demonstrate some enantioselection, the reaction was repeated several times using vinyl acetate rather than ethyl acetate as the acyl donor. However, under these conditions the vinyl acetate rapidly polymerised, and no solution was found to this problem.

With these failures at achieving understandable results, our interest in the amino alcohols as substrates waned. However, a recent paper by Asensio *et al.*[238] reported the successful resolution of (±)-homoprolinol with PPL under reaction conditions almost identical to those used in our work. In fact, the sole difference upon which success hinged was the use of magnetic stirring in the published work. It now appears that the likeliest cause of the erratic results noted in our work was varying rates of shaking in the water bath. Clearly, in such a heterogeneous system it is important that the enzyme is given the maximum opportunity to react with the dissolved substrate *i.e.* it must be stirred well. However, in our initial work we felt that an enzyme would be denatured by the vigorous stirring of a magnetic stirrer, and so concentrated our efforts on shaking reactions. Previous work within our research group had suggested that there was substantial degradation of the enzyme during prolonged stirring. In addition, advice from Prof. Peter Halling of the Department of Bioscience and Biotechnology at the University of Strathclyde (Glasgow) had supported our approach in preferring to use more gentle agitation for enzyme reactions. Later work using 2-octanol as a substrate showed this belief

to be wrong (see Chapter 7), but due to pressure of time no attempt was made to repeat these reactions.

The PPL-catalysed acetylation of (±)-homoprolinol was attempted using magnetic stirring, and *N*-acetylhomoprolinol was obtained in similar yield and enantiomeric excess to the published report. However, satisfactory recovery of the unreacted amino alcohol once again proved elusive.

It can only be said that it is unfortunate that the solution to the problems encountered in this work proved to be as frustratingly simple as the method of stirring the enzyme-catalysed reaction.

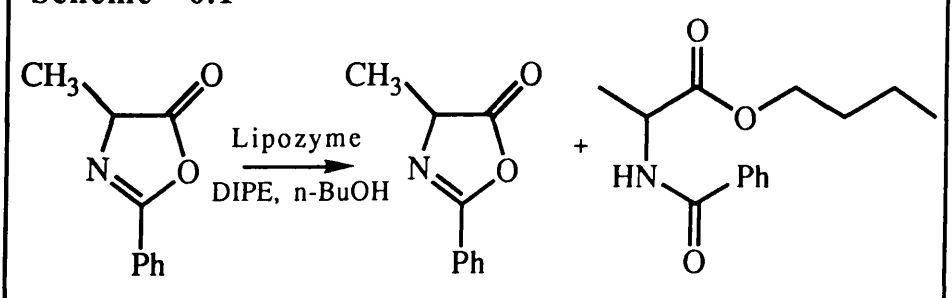
Chapter Six

Resolution of α- and β-Amino Acids via Lipase-Catalysed Alcoholyis of Azlactones and Dihydrooxazinones

6.1 Introduction

The failure to resolve fully amino alcohols using lipase caused us to search for other suitable substrates. Our interest was aroused by a report of Bevinakatti *et al.*,[239] who obtained butyl (R)-*N*-benzoylalaninate (**90**) in optically enriched form (e.e. 57%) *via* Lipozyme-catalysed butanolysis of the oxazol-5(4H)-one ('azlactone') (**89**) (Scheme 6.1).

Scheme 6.1

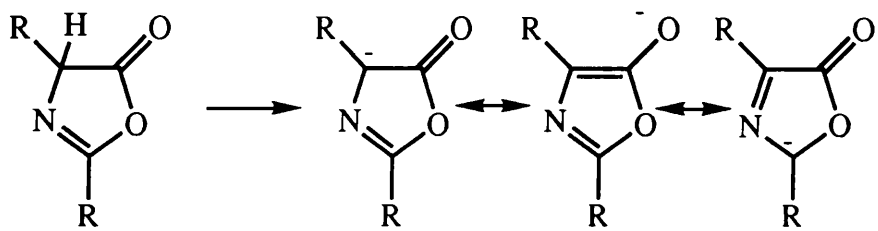


(89) (90)

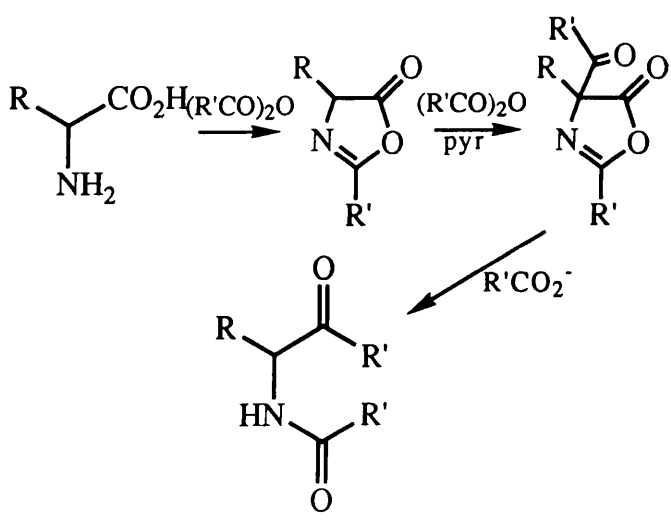
In itself, this result was hardly of great significance: the amino acid is, after all, available commercially in optically pure forms. However, it was felt that if the enantiomeric excess in such a resolution could be increased, then this system allowed great scope for some interesting chemistry. For example, the chemistry of the azlactones is dominated by the remarkable reactivity of the lactone group

towards nucleophiles, and also the enhanced acidity of the α-hydrogen.[240] Indeed, deprotonation of an azlactone to form an extensively delocalised anion (Scheme 6.2) is an important mechanism of racemisation in peptide synthesis.[241] The ease of generating such an enolate has been recognised for a long time in synthetic chemistry. For example, in the Dakin-West reaction[242,243] an amino acid is reacted with an acid anhydride in the presence of pyridine to form an amido ketone *via* the azlactone (Scheme 6.3).[244,245]

Scheme 6.2

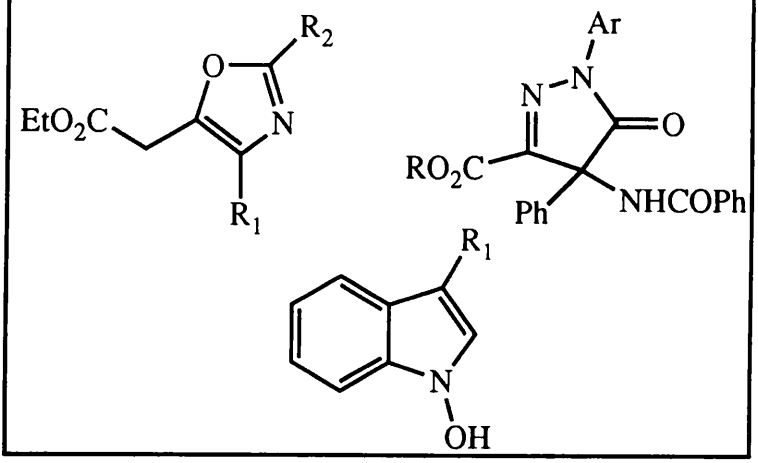


Scheme 6.3



In addition, there have been several reports in the recent literature of the use of azlactone enolates in the synthesis of heterocyclic compounds of the types shown in Scheme 6.4.[246-249]

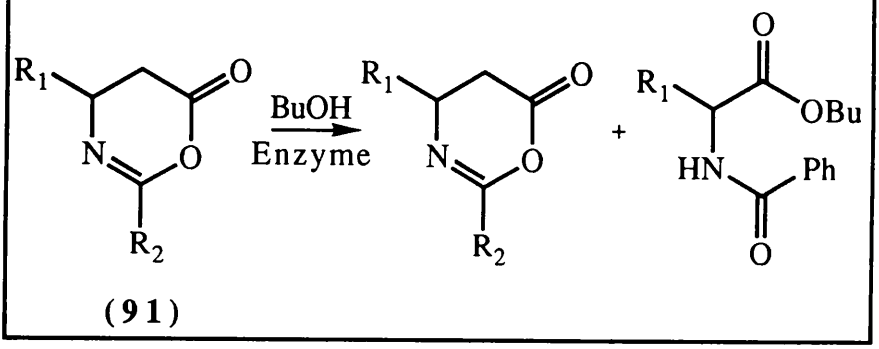
Scheme 6.4



As the azlactone enolate is extremely easy to generate, such a system can provide entry into a wide range of uncommon α-disubstituted amino acids of interest.[250] If the azlactone product of such a reaction could be resolved enzymatically, then this method would become extremely attractive.

However, this was not our main interest in this system. Many methods have been developed for the asymmetric synthesis of non-proteinogenic amino acids, and many of these give both excellent yields and enantioselectivities.[107,110,111,113,114,116,251-254] In general, however, these methods are specific to α-amino acid synthesis and cannot easily be extended to the synthesis of β-amino acids. We wondered if 4,5-dihydro-1,3-oxazin-6-ones ('dihydrooxazinones') (**91**) could be resolved enzymatically in an extension of Bevinakatti's groups work (Scheme 6.5).

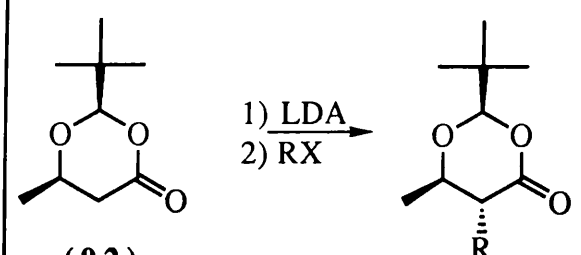
Scheme 6.5



(91)

If such a resolution succeeded, another interesting possibility arose. Could the chirality of the β-carbon be used to induce chirality at the α-carbon? There is some precedent for such a result in the work of Seebach *et al.* with β-hydroxybutyric acid derivatives (**92**) (Scheme 6.6).[255] Thus, the potential of this area was great indeed if the enzymic resolution could be effected.

Scheme 6.6



(92)

In this chapter is described the preparation and attempted resolution of several azlactones and dihydrooxazinones. Azlactone derivatives of (±)-alanine, (±)-phenylalanine and (±)-valine were prepared to ascertain the generality of Bevinakatti's groups results, and resolutions were attempted under various conditions. Dihydrooxazinone derivatives of (±)-3-aminobutanoic acid and (±)-3-amino-3-phenylpropanoic acid were also prepared, and resolution was attempted in the same manner as for the azlactones. Finally, in an attempt to discover the effect

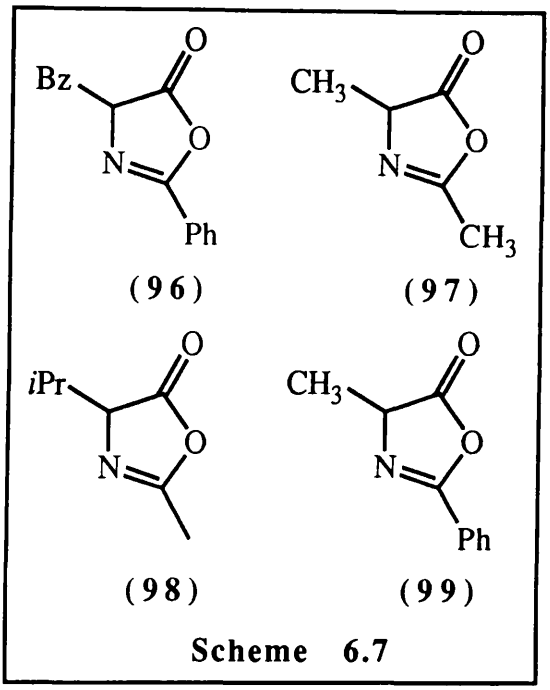
of alkyl substitution on the basic dihydrooxazinone framework, a range of β-alanine derived dihydrooxazinones was prepared for enzyme testing.

6.2 Synthesis and Enzymatic Resolution of Azlactones

6.2.1 Synthesis of Azlactones[256,257]

Azlactones can be considered the internal anhydrides of *N*-acyl α-amino acids,[257] and as such the obvious method of synthesis is dehydration of the amido acid. The *N*-benzoyl derivatives of (±)-alanine, (±)-phenylalanine and (±)-valine were prepared by a standard Schotten-Baumann procedure.[258] Racemic *N*-acetylalanine was obtained from the Sigma Chemical Company. Attempted formation of other *N*-acetyl amino acids using acetic anhydride gave impure oils as products, which on cyclisation gave extremely low yields of the desired products.

The cyclisation reagent chosen initially was *N,N'*-dicyclohexylcarbodiimide (DCC), which formed the cyclised product in quantitative yield. Unfortunately, removal of the last traces of *N,N'*-dicyclohexylurea (DCU) from the product proved problematical, especially with non-crystalline low molecular weight azlactones. Warming the *N*-acyl amino acids in acetic anhydride (a traditional method of azlactone formation)[259] proved equally unsuitable for the synthesis of the non-crystalline azlactones. The most satisfactory method found for azlactone synthesis was use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl),[260] as with this reagent removal of the urea by-product was effected by a simple aqueous wash. This method proved to be suitable for the synthesis of both solid and liquid azlactones. The azlactones prepared in this manner are shown in Scheme 6.7.

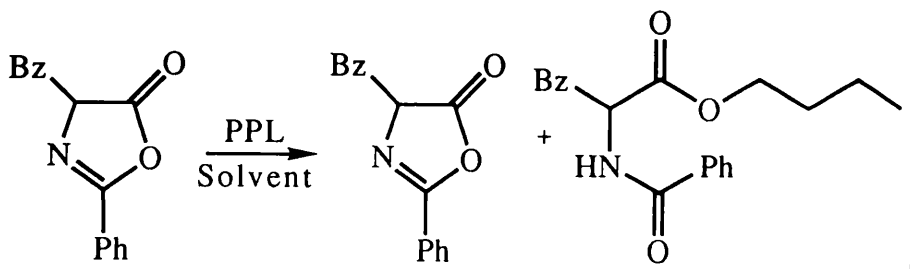


Scheme 6.7

6.2.2 Enzymic Resolution of Racemic Azlactones

Bevinakatti's group had reported the partial enzymic resolution of (±)-2-phenyl-4-methyloxazol-5(4H)-one (**89**) by Lipozyme-catalysed butanolysis in diisopropylether (DIPE) (Scheme 6.8).[239] Although we were not primarily interested in azlactones as enzyme substrates, it seemed sensible for us to study this system initially, and attempt to increase the enantioselectivity of the process. However, at this stage only the azlactone derived from *N*-benzoylphenylalanine (**96**) could be obtained free from DCU. Therefore, this was the substrate on which our efforts were initially concentrated. Towards the end of the project, sufficiently pure samples of the other azlactones were obtained and subsequently tested as enzyme substrates.

Scheme 6.8



(96) (100)

6.2.2.1 Enzymic Resolution of (±)-2-Phenyl-4-benzyloxazol-5(4H)-one (96)

The reaction conditions used by Bevinakatti *et al.*[239] were unsatisfactory from our perspective, for the following reasons. The actual enzymic resolution achieved by Bevinakatti and co-workers was low (57% e.e.), and the solvent used (diisopropyl ether) was unsuitable for safety reasons. In addition, the enzyme preparation used by Bevinakatti *et al.* (Lipozyme, lipase from *Mucor miehei* on a polymer support) was not commercially available. Therefore, we had to find suitable reaction conditions for the resolution using a different enzyme, a different substrate, and a different solvent.

Porcine Pancreatic Lipase (PPL) was the enzyme used in this work. Bevinakatti *et al.* also reported the use of PPL in their work, but achieved lower enantioselectivities with PPL than with using Lipozyme.[239] It was hoped that if some enantioselection could be achieved with PPL, better results might be obtainable under the same reaction conditions but using Lipozyme if and when any became available to us.

Initially, we studied the effect of changing solvent on the reaction shown in Scheme 6.8. It is a well established fact that the choice of solvent has a dramatic and unpredictable effect on the course of an enzyme resolution.[180,261] Therefore, we hoped that a suitable choice of solvent would result in an acceptable degree of resolution.

The first solvent we tried was hexane, and after 72 h shaking in a water bath the butyl ester **(100)** was obtained with a small specific rotation ($[\alpha]_D$ -1.8°). However, over the course of the reaction the azlactone crystallised from the hexane, and so another solvent was sought. It is possible that we were a little hasty in rejecting hexane merely because it did not dissolve the substrate effectively. Kuhl *et al.* have reported a successful lipase-catalysed resolution in which care was taken to ensure the substrates remained insoluble.[262] When working with enzymes in organic solvents, it seems that chemical common-sense is not necessarily an advantage!

However, the search for a suitable solvent continued. Repeating the reaction using THF in place of hexane resulted in a slower reaction, and after 72 h only 46% of the azlactone had been converted into the ester (cf. 58% in hexane). Neither the butyl ester nor the azlactone recovered from the reaction after five days showed any optical activity. In addition, due to the prolonged reaction times some hydrolysis of the azlactone was occurring.

To ensure that the products obtained had been the result of a non-selective enzyme-catalysed butanolysis rather than a simple chemical reaction, a 'blank' experiment was performed in THF with no enzyme present. Under these conditions, no butyl ester was formed after five days, although some hydrolsis of the azlactone did occur. Therefore, we could be sure that the enzyme had been catalysing the butanolysis, albeit in a non-selective manner.

At this stage, pure (±)-2-phenyl-4-methyloxazol-5(4H)-one (**99**) became available, and attention was switched to this compound. Performing the butanolysis of this in diethyl ether with PPL resulted in the butyl ester (**101**) being obtained in 3% enantiomeric excess, with the (R)-isomer predominating. Repeating the reaction using Lipozyme (kindly donated by Novo Industri A/S.) gave the ester (**101**) in 6% e.e., this time with the (S)-isomer in excess. This reversal of enantioselectivity between PPL and Lipozyme was also noted by Bevinakatti's group.[239]

It was felt at this stage that too much time had been spent on the azlactone reaction, when the system of real interest to us involved the dihydrooxazinones. In addition, our previous experience suggested that results obtained using azlactones as substrates might not be applicable when using dihydrooxazinones. Therefore, we reluctantly turned our attention away from the azlactones and focussed instead on the dihydrooxazinones.

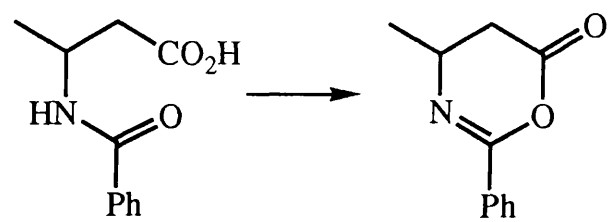
6.3 Synthesis and Enzyme Studies of Racemic Dihydrooxazinones

In contrast to the azlactones, whose chemistry has been studied for many years, there is a paucity of literature references to 4,5-dihydro-1,3-oxazin-6-ones ('dihydrooxazinones'). It had been hoped that a simple DCC cyclisation of the corresponding *N*-acyl-β-amino acid would be effective in the formation of these compounds. However, once again removal of the DCU by-product proved impossible without severely reducing the yield of desired product. Therefore, our primary task was to discover an efficient method of synthesising dihydrooxazinones.

6.3.1 Synthesis of (±)-2-Phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one (103)

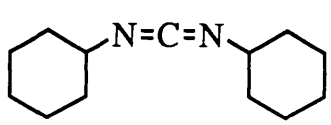
The synthesis of the dihydrooxazinone (**103**) was attempted *via* cyclisation of the *N*-benzoyl-β-amino acid (**102**) (Scheme 6.9), in direct analogy with the synthesis of azlactones described previously. Preparation of the *N*-benzoyl-β-amino acids was again performed by the Schotten-Baumann procedure. Cyclisation of (±)-3-benzamidobutyric acid (**102**) was then attempted by various methods, summarised in Scheme 6.10.

Scheme 6.9

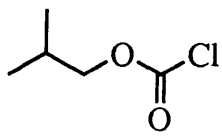


(**102**) (**103**)

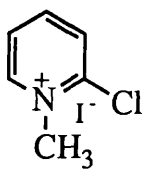
As stated above, the first reagent used to attempt cyclisation was DCC (**104**). A variety of solvents (DCM, CCl_4, EtOAc, Et_2O) were used, but in each case the cyclised product (detected by 90 MHz ^1H N.M.R. spectroscopy) could only be obtained with DCU as a major contaminant. For this reason, an alternative procedure for effecting the cyclisation was sought. As before with the azlactones, acetic anhydride proved completely ineffective as a cyclising reagent. Drey and Mtetwa[263] reported the preparation of (**103**) using isobutyl chloroformate (**105**) to cyclise the *N*-benzoylamino acid. When we repeated their procedure, the reaction yielded the dehydrooxazinone heavily contaminated with isobutanol, and purification procedures resulted in loss of the cyclised product.



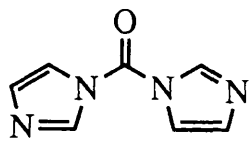
(104)



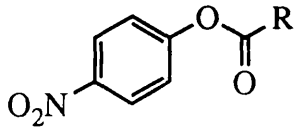
(105)



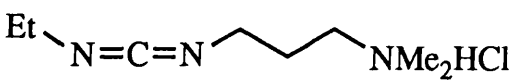
(106)



(107)

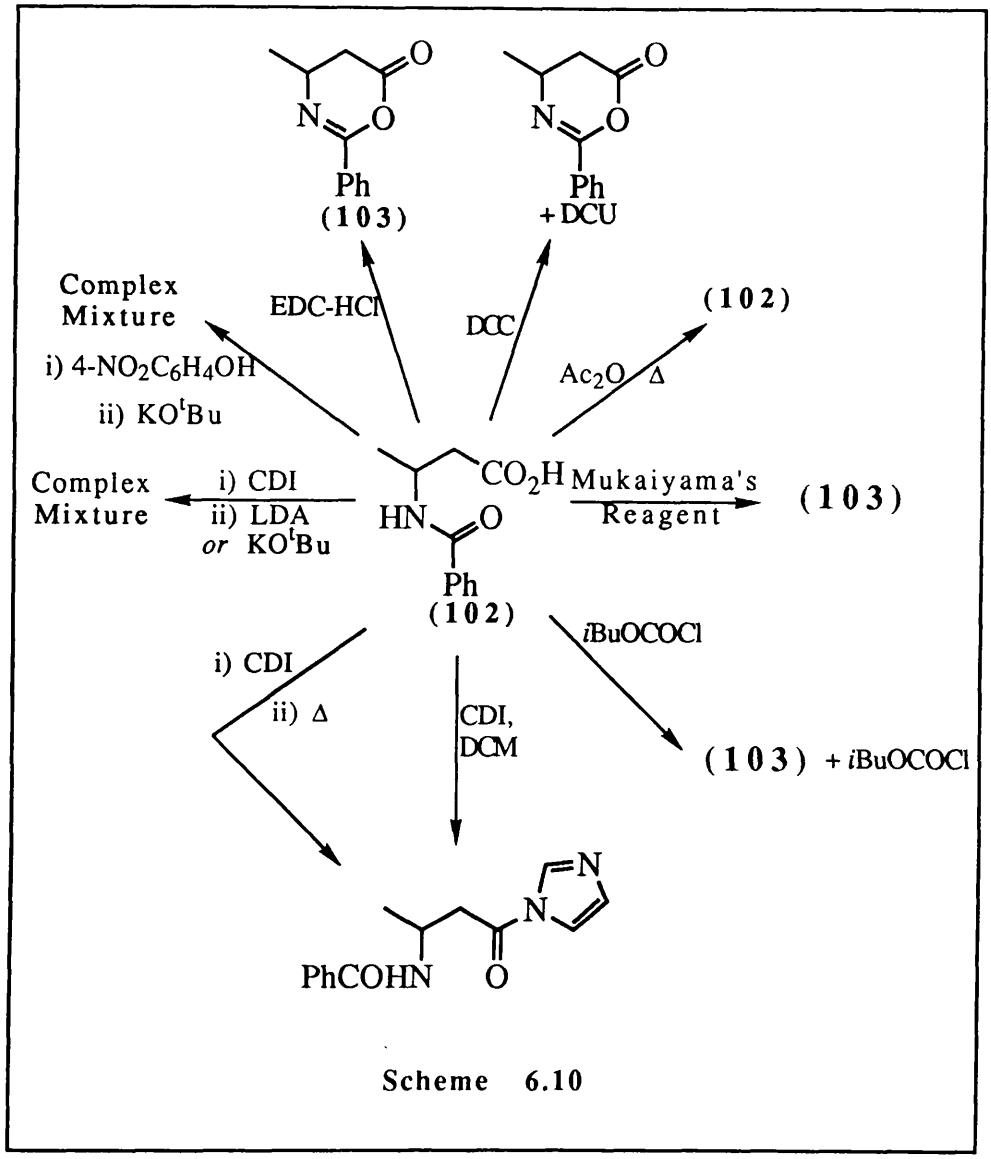


(108)



(109)

Coupling Reagents used to Cyclise 3-Benzamidobutanoic Acid.



Scheme 6.10

The next attempt at forming the desired compound involved the use of Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide (**106**)).[264] In small scale reactions, this gave the desired product as a yellow oil in excellent (>90%) yield. However, repeating the reaction on a larger scale gave drastically reduced yields, making the reaction unsuitable for producing sufficient quantities of material. Using chlorosulphonylisocyanate (**107**) to activate the carboxylic acid[265] gave no cyclised product, while 1,1'-carbonyldiimidazole[266] (CDI (**108**)) formed

only the imidazolide after stirring overnight with the acid at room temperature. However, as the imidazolide was formed quantitatively, attention turned to seeking methods of cyclising this intermediate. In all the following reactions, the imidazolide was pre-formed from benzamidobutyric acid and CDI in an appropriate solvent at room temperature. Heating to reflux a dichloromethane (DCM) solution (b.p. 40 °C) of the imidazolide gave no cyclised product, and this was also the case when acetonitrile (b.p. 82 °C) was used as the solvent. As heat did not offer sufficient incentive for the compound to cyclise, base-promoted cyclisations were next attempted.

As imidazole was already present in the reaction mixture due to the breakdown of CDI, it was assumed that using tertiary amine bases such as triethylamine or pyridine would not prove fruitful. Therefore, attention focussed instead on the use of alkoxide and amide bases. With such strong bases, two sites of deprotonation in the imidazolide are possible; at the amide nitrogen to generate an amide anion, and at the α-carbon, to generate an enolate anion. Of these, it it is reasonable to assume that the more acidic amide proton would be abstracted first. It was then hoped that the amide anion generated would prove more nucleophilic than the protonated form, and hence that efficient cyclisation could be achieved.

The first attempt at realising this strategy was to use lithium diisopropylamide (LDA) as the base. This reaction gave a complex mixture of products resulting from the degradation of the starting material, with no evidence of the cyclised compound being formed. On reflection, the use of a lithium base for such a reaction was not the ideal choice, as the lithium ion will associate closely with the oxygen atom of the amide anion and so reduce the nucleophilicity of the oxygen atom.

To overcome this, the generation of the amide anion was attempted using potassium *t*-butoxide as the base. The affinity of K^+ for anionic oxygen in organic solvents is much reduced compared to Li^+, and so it was hoped that this time the cyclisation would proceed smoothly. Unfortunately, once again the reaction yielded only a complex mixture of products, with no cyclised compound detectable.

There are several possible explanations why this reaction failed. It is unlikely that the base would generate an enolate anion in preference to an amide anion. However, if the rate of cyclisation of the amide anion is slow (as it appears to be since no evidence of cyclised products was ever detected), then it is eminently possible that an enolate anion could be generated by the amide anion abstracting an α-proton in an intermolecular process. Once such an enolate is generated in the reaction mixture, various condensation processes become possible and it would not then be surprising if a number of unwanted products was obtained.

In an attempt to increase the rate of cyclisation of the intermediate anion, the *p*-nitrophenyl ester (**110**) was prepared by a Mukaiyama coupling of acid and alcohol. This was then treated with a solution of KO^tBu in THF at -10 °C to effect cyclisation, but once again a complex and uncharacterisable mixture of products was obtained.

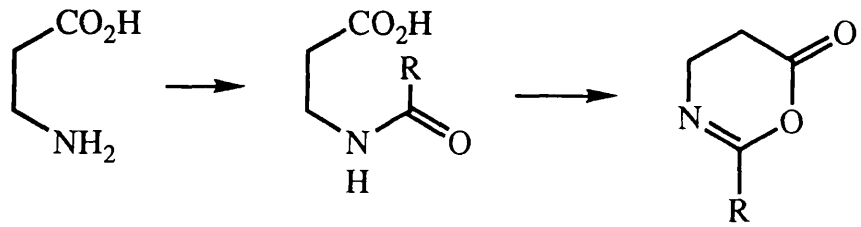
The most effective method of cyclising (±)-3-benzamidobutyric acid had been with DCC, but this procedure had proved useless due to the heavy contamination of product with DCU. However, this did indicate that a carbodiimide which formed a water-soluble urea on hydration would be an excellent reagent for forming the dihydrooxazinones. Such a reagent is 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) (**109**). Using this reagent, the desired (±)-2-phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one (**103**) was indeed obtained as a clear oil in excellent yield. This method proved quite

general for the synthesis of dihydrooxazinones, and was also used in the preparation of the 2,4-diphenyl substituted compound (**112**).

6.3.2 Synthesis of β-Alanine derived Dihydrooxazinones

In an attempt to evaluate the effect of alkyl substitution on the enzyme-catalysed alcoholysis of dihydrooxazinones, it was decided to synthesise a range of 2-substituted dihydrooxazinones (**119**) - (**124**) (Scheme 6.11).

Scheme 6.11

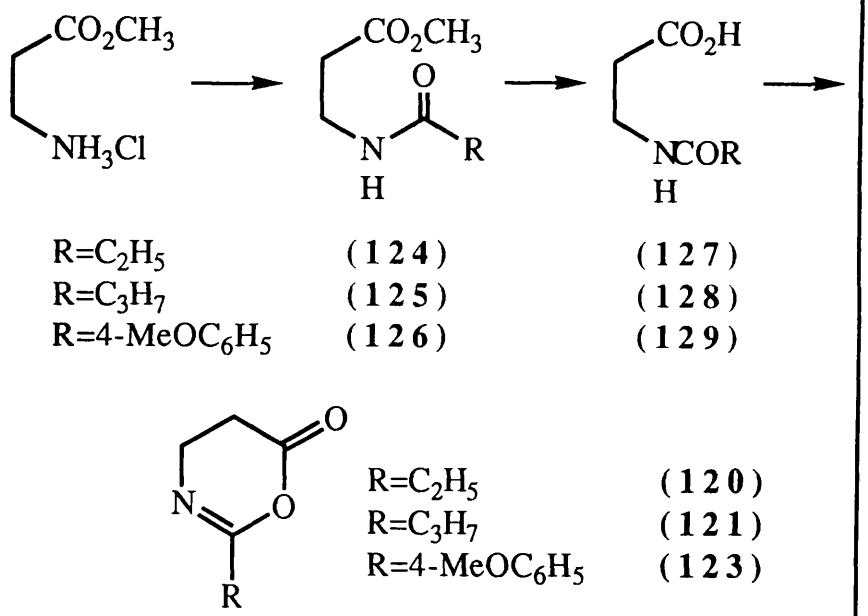


R=H	(**113**)	(**118**)
R=CH_3	(**114**)	(**119**)
R=C_2H_5	(**115**)	(**120**)
R=C_3H_9	(**116**)	(**121**)
R=Ph	(**80**)	(**122**)
R=4-$MeOC_6H_5$	(**117**)	(**123**)

N-Formyl-β-alanine (**113**) was prepared by the mixed anhydride method used previously in the synthesis of *N*-formyl proline. *N*-Acetyl-β-alanine (**114**) was prepared from acetic anhydride in excellent yield, and *N*-benzoyl-β-alanine (**80**) was formed from benzoyl chloride by a Schotten-Baumann procedure. Unfortunately, Schotten-Baumann conditions failed to give any *N*-propanoyl- or *N*-butanoyl-β-alanine ((**115**) and (**116**) respectively), presumably due to hydrolysis of the acid chloride. Attempting to form these using the appropriate acid anhydrides also proved unsatisfactory, due to the difficulty experienced in removing the non-

volatile acid anhydrides. Therefore, we attempted to prepare the *N*-propanoyl-, *N*-butanoyl- and *N*-(*p*-anisoyl)-β-alanines ((**115**), (**116**) and (**117**) respectively) from β-alanine methyl ester hydrochloride (**87**) according to Scheme 6.12.

Scheme 6.12



The amido esters (**124**), (**125**) and (**126**) were prepared in low to good yields. However, all attempts at hydrolysing the amido esters to the corresponding amido acids gave only unreacted starting materials. Therefore, the 2-ethyl, 2-propyl and 2-(*p*-anisoyl)-dihydrooxazinones were not prepared.

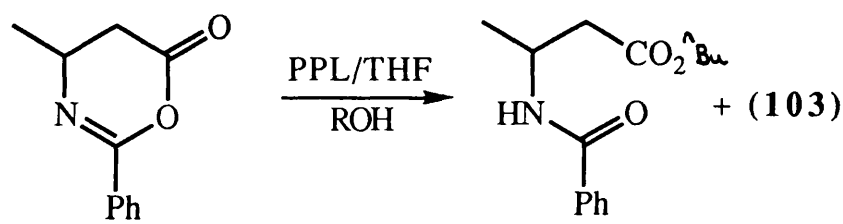
Cyclisation of the *N*-formyl-, *N*-acetyl- and *N*-benzoyl-β-alanines was attempted using EDC-HCl as before. However, no cyclised product was obtained from the *N*-formyl and *N*-acetyl derivatives. It may be the case that such 2-alkyl dihydrooxazinones are extremely prone to hydrolysis, and thus difficult to obtain. 2-Benzoyl-4,5-dihydro-1,3-oxazin-6-one (**122**) was obtained in good yield, but this too proved to be too prone to hydrolysis for use as an enzyme substrates. The increased stability of 2-aryl substituted dihydrooxazinones compared to the 2-alkyl

compounds parallels a phenomenon noted in the azlactones, where a 2-aryl substituent greatly enhances the stability of the azlactone ring.[257] However, it seems that in the 6-membered ring, a second substituent in the ring is required to hinder hydrolysis of the dihydrooxazinone.

6.3.3 P.P.L.-Catalysed Alcoholysis of Dihydrooxazinones

The asymmetric alcoholysis of racemic 2-phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one (**103**) was first attempted using PPL and 1-butanol, in direct analogy with Bevinakatti's work with the azlactones.[239] (Scheme 6.13, R=C_4H_9).

Scheme 6.13



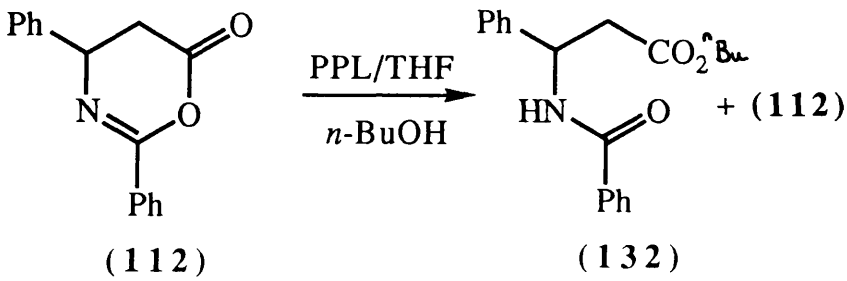
(**103**) + (**103**) (**130**)

This compound did prove to be a substrate for the enzyme, and after 24 h the butyl ester (**130**) was isolated from the reaction mixture in 42% yield. Unfortunately, both the butyl ester and the unreacted dihydrooxazinone proved to be optically inactive.

The reaction was repeated using methanol as the nucleophile (Scheme 6.13, R=CH_3). It was hoped that using a smaller nucleophile would increase the reaction rate, and perhaps also encourage enantioselective catalysis. However, after 24 h only methyl 3-benzamidobutanoate (**131**) was present in the reaction mixture.

Attention then turned to the enzyme-catalysed butanolysis of (±)-2,4-diphenyl-4,5-dihydro-1,3-oxazin-6-one (**112**) (Scheme 6.14).

Scheme 6.14



(112) (132)

Using a somewhat simplistic approach, we felt that the larger substituent at the 4-position of the dihydrooxazinone ring would enhance the enantioselectivity in the P.P.L. catalysed hydrolysis. The resolution was attempted using P.P.L. and 1-butanol in the same manner as before. After 24 h, the butyl ester (**132**) was isolated from the reaction mixture in 28% yield, but in racemic form. Unreacted dihydrooxazinone was recovered in 18% yield, again in racemic form. In addition, 3-benzamido-3-phenylpropanoic acid was recovered in 10% yield from the reaction mixture, presumably arising from non-enzymatic hydrolysis. This was a little surprising, as we thought that the 4-phenyl substituent might help prevent hydrolysis, both by a steric effect and also because of the hydrophobic nature of the aromatic ring. However, this proved not to be the case, and at this point our attempts at resolving such cyclic derivatives of amino acids were abandoned.

Chapter Seven

Studies of Enzymatic Acylation Using (±)-2-Octanol as a Model Enzyme Substrate

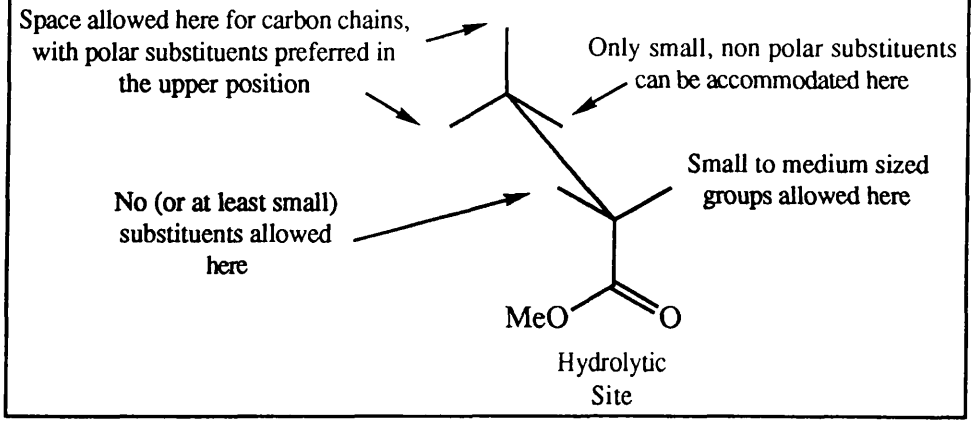
7.1 Introduction

In the previous two chapters are described our attempts at lipase-catalysed resolutions of amino alcohols (Chapter 5) and amino acid derivatives (Chapter 6). In the course of this work, we attempted to improve the observed enantioselectivities of the processes under study by altering various reaction conditions for each substrate in turn. However, these attempts proved fruitless, and a survey of the literature revealed a plethora of reaction conditions with no guidance available as to which would prove the most suitable for our purposes. It occurred to us that what was needed was a systematic survey of reaction conditions which would be applicable to a wide range of substrate structures. This would serve two purposes, the most important being establishing the optimum reaction conditions for lipase catalysed resolutions. It was also possible that a systematic screening of substrates would enable us to construct a model of the enzyme active site, which could prove useful for predicting the stereochemical outcome of lipase-mediated reactions. It was with these hopes that we began the course of work detailed in this chapter. However, in order to select a suitable system for study, we first surveyed the previous work done in this field.

7.1.2 Previous Studies

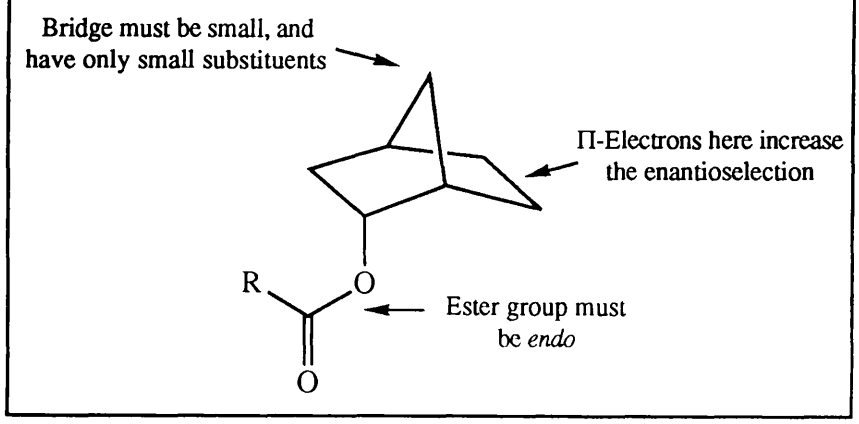
Enzyme substrate selectivity patterns have been used for several years to construct predictive models of the catalytic site, with some success. Such studies can give rise to two types of models, substrate models and active site models.[267] A substrate model proposes an ideal structure for an enzyme substrate, and for best results the substrate to be resolved should conform broadly to the idealised structure. For example, Mohr *et al.*[268] have proposed a substrate model for pig liver esterase (PLE) catalysed mono-hydrolyses of symmetrical diesters (Scheme 7.1).

Scheme 7.1

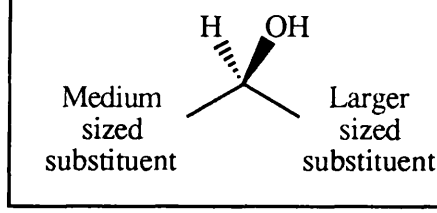


Similarly, Oberhauser *et al.*[267] have proposed a substrate model for the hydrolysis of esters of bicyclic secondary alcohols, catalysed by *Candida rugosa* lipase (CRL) (Scheme 7.2). Finally, Kazlavskas *et al.*[269] have proposed a model to predict which enantiomer of a secondary alkyl ester will react faster in hydrolytic reactions catalysed by bovine cholesterol esterase, *Pseudomonas cepacia* lipase (PCL) and CRL. (Scheme 7.3). This model is based on the size of the substituents around the chiral centre, and the authors claim a predictive accuracy of greater than 93%.

Scheme 7.2

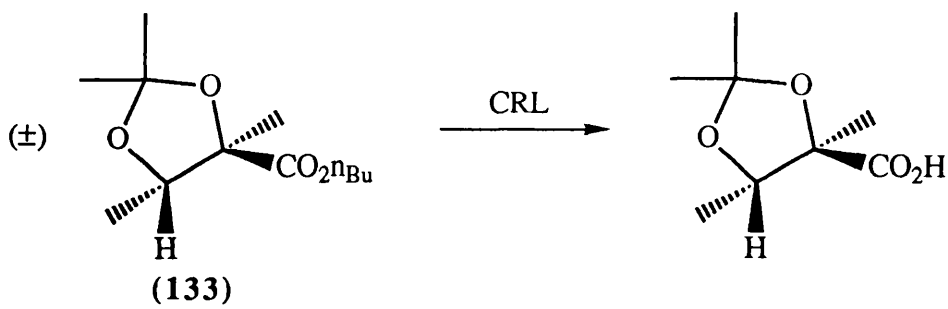


Scheme 7.3



The main disadvantage of such substrate-based model systems is that they are only relevant to compounds with similar structures to those used in the construction of the model. Thus, the CRL-based substrate model of Oberhauser *et al.* would be of little use in predicting the efficient stereoselective hydrolysis of **(133)** by CRL reported by Pottie *et al.*[270] (Scheme 7.4).

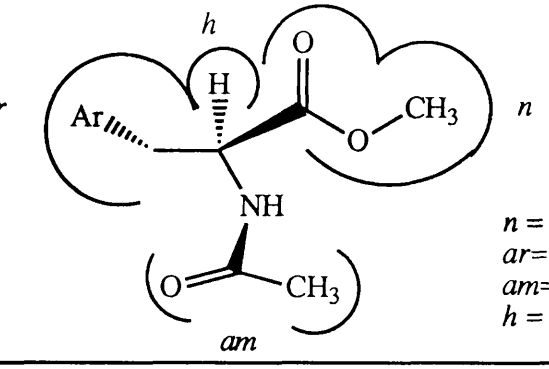
Scheme 7.4



(133)

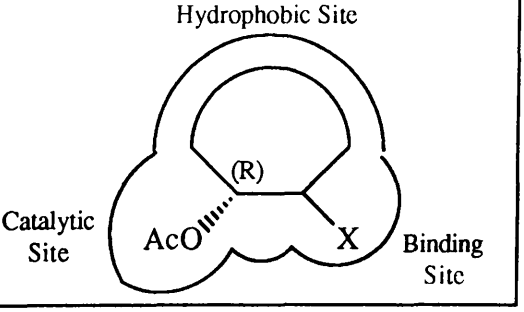
Clearly, such a disadvantage is inherent in the substrate model concept. More popular has been the construction of active site models, again based on observed substrate selectivity patterns. An enzyme particularly suited to such an approach is α-chymotrypsin, due to the high degree of conformational rigidity about the active site. Thus Cohen[271] proposed a model (Scheme 7.5) for the active site of α-chymotrypsin which has since been verified by X-ray crystal structure studies.[272]

Scheme 7.5



Xie *et al.*[273] have proposed a simple three-site model for the active site of PCL, based on the results of cycloalkyl acetate hydrolyses (Scheme 7.6).

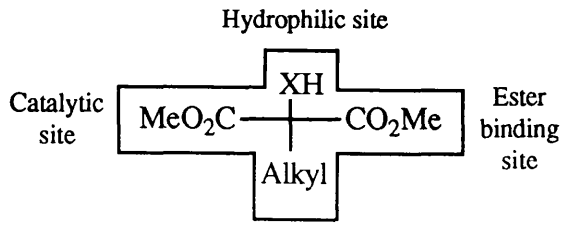
Scheme 7.6



PLE is an enzyme which has attracted much attention in this field. Zemlicka *et al.*[274] proposed a binding site model for this enzyme (Scheme 7.7) which involved four distinct sites in the enzyme. More recently, Toone *et al.*[275] published a more sophisticated model of the PLE active site based on cubic space descriptors

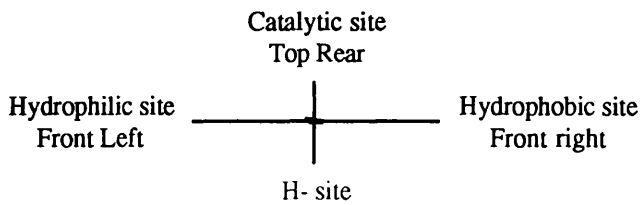
which is consistent with previous studies and is, they claim, of predictive value for new substrate structures.

Scheme 7.7



When attention is turned to PPL, however, surprisingly little work in this area has been reported. The only attempt at an active-site model has come from Seebach's group,[276] who surveyed the hydrolyses of cyclic diesters published by various other workers[277-289] to produce their model (Scheme 7.8).

Scheme 7.8



Although such active site models have achieved predictive success with some enzymes, they must be considered of limited value for lipases due to the unusual mode of action of these enzymes. As was discussed in Chapter 2, the current theory of lipase action suggests that large conformational changes must occur before a substrate can bind to the active site of the enzyme, and thus the enzyme has a wide degree of conformational flexibility. This flexibility is beneficial to organic chemists, as it allows the enzyme to accept a wide range of substrate structures. However, the disadvantage is that the active site cannot be as

conformationally rigid, and hence well-defined, as in (for example) α-chymotrypsin. Herein may well lie the reason for the dearth of active site models for PPL. Indeed, Kavlaskas has stated somewhat pessimistically that *"it may never be possible to define an exact size and shape for the substrate binding region of lipases, because this region may change for each substrate"*.[269] Clearly, this was a problem which had to be surmounted if we were to obtain any meaningful results.

7.1.3 Choice of Reaction System for Study

Having surveyed the literature, we had to choose a reaction system to study. All the model systems described previously have been based on hydrolytic reactions performed in aqueous media. However, most organic chemistry is carried out in non-aqueous conditions, and with good reason. Organic compounds are on the whole fairly insoluble in water. Many compounds are extremely unstable in the presence of water, and reactions performed in aqueous conditions often suffer from extremely unfavourable equilibria. For these reasons, the use of enzymes in organic solvents with no or very little water present is extremely common, and it is by no means certain that a model of an active site in an aqueous environment would accurately reflect the enzyme conformation in organic solvent. Therefore, our first requirement was that the enzyme should be studied under non-aqueous conditions. Our second requirement was to overcome the problem of the enzyme's flexibility. One possible solution was to use a polymer-supported enzyme such as Lipozyme. If one assumes that the process of adsorbing the enzyme onto the polymer support is akin to the adsorption of lipase onto a water-lipid interface, then it seems likely that the gross conformational changes of the enzyme will already have occurred before any substrate binding takes place. However, some deformation of the active site is still likely when the substrate binds to the lipase. Therefore, it seemed to us that the best method to obtain results applicable to a wide range of lipase-catalysed

reactions was to vary not the chiral component, as was the case with all previous studies, but an *achiral* component. For instance, Kirchner *et al.*[290] have studied the lipase-catalysed resolutions of a variety of chiral alcohols using a transesterification reaction (Scheme 7.9), varying the nature of the chiral alcohol (R^*OH). We wondered if results of more general interest could be obtained by keeping the chiral alcohol the same and varying the nature of the achiral acyl donor. Kanerva *et al.*[291]

Scheme 7.9

$$R^*OH + PrCO_2CH_2CCl_3 \xrightarrow{\text{Lipase}} (R)\text{-}PrCO_2R^* + (S)\text{-}R^*OH$$

have carried out a similar study on the PPL-catalysed transesterification between (±)-2-octanol and various haloalkyl butanoate esters (Scheme 7.10). In this work, Kanerva *et al.* varied the haloalkyl moiety and found that increasing the polarity of this group resulted in faster reactions and, in general, better enantiomeric excesses (Table 7.1). They also noted a steric effect in operation, and indeed attributed the difference in reaction rates of 2,2,2-trifluoroethyl and 2,2,2-trichloroethyl esters as being mainly steric in origin.

Scheme 7.10

$$R^*OH + PrCO_2CH_2CCl_3 \xrightarrow{\text{Lipase}} (R)\text{-}PrCO_2R^* + (S)\text{-}R^*OH$$

Table 7.1

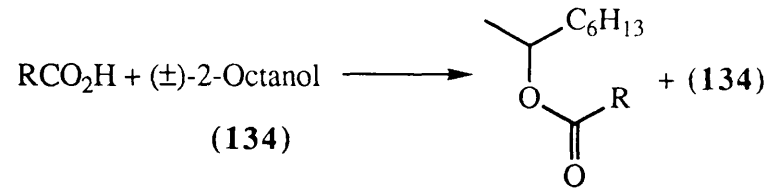
R	Initial Rate (μmol $min^{-1}g^{-1}$)	Reaction Time (h)	Degree of Conversion (%)	Enantiomeric Excess (%)
CF_3	13	70	46	100
CCl_3	3.9	134	43	97
$CHCl_2$	2.4	340	45	90
CH_2Cl	0.23	1750	36	87
C_2H_5	0.07	1750	26	93
^tBu	-	-	-	-

Therefore, we decided to study the transesterification reaction between various 2,2,2-trichloroethyl esters and a chiral alcohol. The use of 2,2,2-trichloroethyl esters rather than 2,2,2-trifluoroethyl esters was simply a matter of which chemicals were available to us at the time. The chiral alcohol used was (±)-2-octanol, for two reasons. Firstly, it allowed us to begin our study by repeating work in the literature before sailing into uncharted waters. Secondly, 2-octanol has been frequently used as a *de facto* standard for the screening of lipase activity.[292,293] We thus embarked on our study of lipase-catalysed transesterifications involving (±)-2-octanol.

7.2 Attempted Coupling of (±)-2-Octanol and Carboxylic Acids

There have been a few reports of the lipase-mediated direct coupling of a carboxylic acid and an alcohol,[148] but such an obvious reaction has not been a popular choice for enzymic resolutions. Therefore, the first system we investigated was the P.P.L.-catalysed reaction of (±)-2-octanol with simple carboxylic acids (Scheme 7.11).

Scheme 7.11



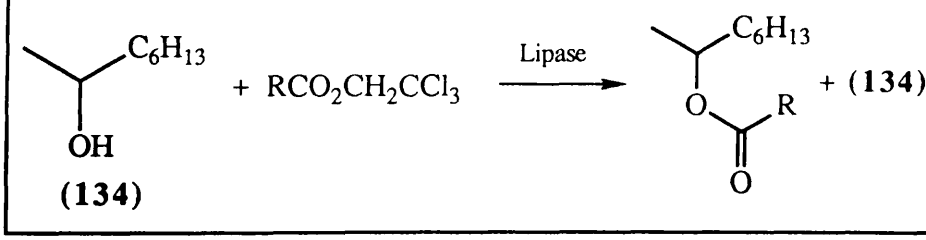
However, none of these reactions formed any acylated octanol, and so this reaction was not investigated any further.

7.3 Lipase-Catalysed Reactions of (±)-2-Octanol and 2,2,2-Trichloroethyl Esters

7.3.1 Preparation of 2,2,2-Trichloroethyl Esters

Following the work of Kirchner *et al.*,[290] we chose to study the transacylation of (±)-2-octanol and various 2,2,2-trichloroethyl esters (Scheme 7.12).

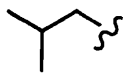
Scheme 7.12



Twelve different 2,2,2-trichloroethyl esters were prepared by the reaction of the appropriate acid chloride and 2,2,2-trichloroethanol (Scheme 7.13). In the cases of the 4-methylvalerate **(138)**, phenylacetate **(140)**, 3,3-dimethylacrylate **(143)**, sorbate **(144)** and diphenylacetate **(146)** esters, the acid chloride was first prepared by the action of thionyl chloride on the corresponding acid.

Scheme 7.13

$$RCOCl + Cl_3CCH_2OH \xrightarrow{Et_3N} RCO_2CH_2CCl_3$$

R		R	
CH_3	**(135)**	$PhCH_2$	**(140)**
C_2H_5	**(136)**	$H_2C{=}CH$	**(141)**
C_3H_7	**(137)**	$MeCH{=}CH$	**(142)**
		$Me_2C{=}CH$	**(143)**
	**(138)**	$MeCH{=}CHCH{=}CHCH$	**(144)**
		$PhCH{=}CH$	**(145)**
		Ph_2CH	**(146)**
C_6H_5	**(139)**		

The preparation of the acetate and propanoate derivatives proved uneventful. However, when 2,2,2-trichloroethyl butanoate (**137**) was prepared in this manner, a second compound was isolated from the reaction mixture. The ^1H N.M.R. spectrum of this compound consisted solely of an AB system located at δ 4.55 and δ 4.69, while the ^{13}C N.M.R. DEPT spectrum showed two signals, a triplet at δ 72.42 (indicative of a methylene group with electron withdrawing substituents attached) and a singlet at δ 94.43 (typical of a CCl_3 moiety). The infra-red spectrum showed no carbonyl absorption, but did show strong absorptions in the region ν_{max} 1 100 - 1 000 cm^{-1}, typical of C-O bonds. In addition, the mass spectrum of this compound showed isotopic peaks M, M+2 and M+4 with intensities in the ratio of 3:3:1. The calculated ratio of intensities for a compound possessing 3 chlorine atoms is 3.2:3.1:1, strongly suggesting the presence of a CCl_3 group in the molecule. However, we could not describe a structure for this compound which was consistent with all the physical data collected.

A similar AB system was seen in the ^1H N.M.R. spectrum of the 4-methylvalerate ester (**138**), but no further purification of this compound was carried out.

Most of the other 2,2,2-trichloroethyl esters were prepared without difficulty. However, the sorbate ester (**144**) proved unstable, polymerising slowly at room temperature. In addition, the crotonate (**142**) and 3,3-dimethylacrylate (**143**) decomposed slowly on storage. Consequently, no enzyme reactions were attempted using these three compounds.

7.3.2 Preparation of 2-Octyl Esters

For identification purposes, it was deemed highly desirable that the expected ester products of the enzyme-catalysed reactions be synthesised by more conventional chemical means. However, this proved more troublesome than at first expected, and several methods of esterification were attempted.

Standard DCC coupling of 2-octanol with the appropriate carboxylic acid furnished the 2-octyl ester in low yield, and with DCU present as a major contaminant. In an attempt to obtain pure ester, CDI was used as the coupling reagent.[266] However, no ester product was formed in the reaction. This surprising result was almost certainly due to the long alkyl chain of 2-octanol reducing the nucleophilicity of the hydroxyl group to such an extent that no reaction occurred. Clearly, this did not augur well for the use of other coupling reagents to form the ester, as almost without exception such reagents act by activating the carboxyl group of the acid towards nucleophilic attack.

We therefore attempted to prepare 2-octyl acetate (**147**), 2-octyl propanoate (**148**), and 2-octyl butanoate (**149**) by reacting the alcohol with the appropriate acid anhydride. However, G.C. analysis of the crude products showed at best less than 10% acylation had occurred. Reaction of the alcohol with an acid chloride also failed to furnish acceptable yields of ester. In any case, using acid chlorides or anhydrides would not have been the most convenient method of forming the 2-octyl esters, due to the need to preform most of the anhydrides or chlorides from the parent acids.

It was hoped that EDC-HCl would, once again, allow access to the pure esters free from urea side-products. However, in this case EDC-HCl failed to form any 2-octyl ester. Our attention was drawn to a report in the literature of a method for the formation of *t*-butyl esters using CDI and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU).[294] This reaction was reported to give good yields of hindered esters.

However, when this reaction was applied to 2-octanol, once again no ester product was formed. At this stage, time was becoming scarce and so esters (**147**) - (**151**) were prepared by the DCC/DMAP method.[295] The yields obtained by this method were variable, and once again the products tended to contain DCU as a contaminant However, the procedure did allow the preparation of sufficient quantities of 2-octyl esters to serve as reference standards for gas chromatography.

7.3.3 P.P.L.-Catalysed Esterification of (±)-2-Octanol using 2,2,2-Trichloroethyl Esters.

We first studied the P.P.L.-catalysed transesterification of (±)-2-octanol using the acetate (**135**), propanoate (**136**) and butanoate (**137**) esters of 2,2,2-trichloroethanol (Scheme 7.12). Kirchner *et al.*[290] had reported the P.P.L.-

mediated resolution of (±)-2-octanol with 2,2,2-trichloroethyl butanoate, while Kanerva *et al.*[291] used the same reaction conditions for their study of the effect of varying the nature of the haloalkyl moiety in the reaction of various haloalkyl butanoate esters. Therefore, the procedure seemed quite well established for a variety of substrates. However, when we attempted the P.P.L.-catalysed transacetylation of (±)-2-octanol with 2,2,2-trichloroethyl acetate (**135**) using this procedure, the enzyme quickly turned into a paste and no transesterification was detected. Thinking that perhaps the enzyme was proving unstable in the presence of water, we attempted the same reaction using freshly dried ether (the amount of water present in lipase-catalysed reactions has been shown in several cases to be a crucial factor in achieving good resolutions).[181,296,297] The progress of the reaction was monitored by gas chromatography (G.C.), aliquots being withdrawn from the reaction mixture at various time intervals. The extent of reaction was determined from the heights of the appropriate peaks in the G.C. trace. The results obtained are shown in Table 7.2, and also graphically in Fig. 7.1.

Table 7.2

Reaction Time (h)	% Acylation	Reaction Time (h)	% Acylation
1	1.0	18	16.0
2	2.0	24	19.8
3	3.8	48	31.2
4	4.6	77	37.8
6	6.7	96	40.7
8	9.0	120	46.7

P.P.L.-catalysed transesterification of 2,2,2-Trichloroethyl acetate & (±)-2-Octanol (Et_2O/mol.sieves).

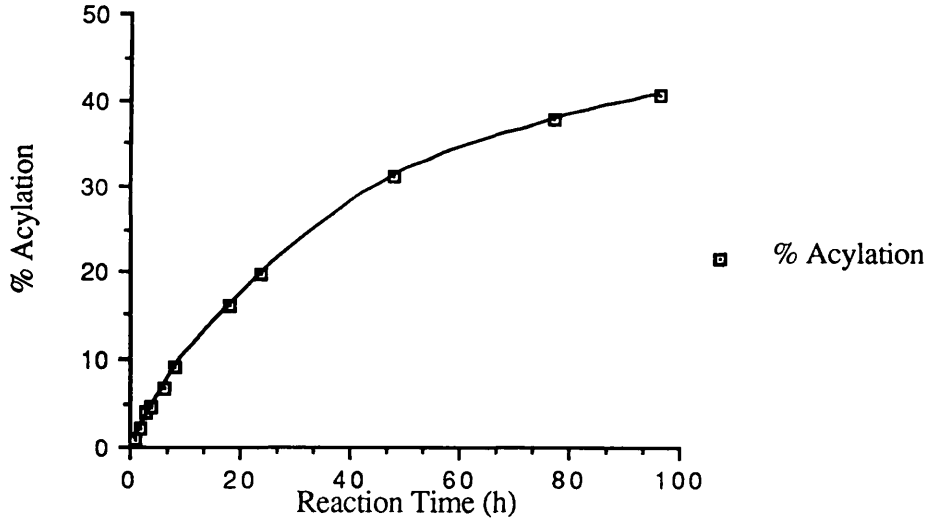


Fig. 7.1

These results were highly encouraging, the data fitting a smooth curve and acetylation stopping at 40 - 50% conversion. However, it was not clear if the reaction was slowing down due to a high degree of enantioselection being displayed or whether it was due to deactivation of the enzyme. The reaction was therefore repeated using 2,2,2-trichloroethyl propanoate (**136**) as the acyl donor. Conventional wisdom has it that the initial rate of acylation increases as the alkanoate moiety of the ester increases in chain length from C_2 - C_4,[6,291] and so we expected the propanoate ester to prove a better substrate for the enzyme than the acetate. However, when the reaction was performed, the reaction displayed a more complex behaviour than expected (Table 7.3).

Table 7.3

Reaction Time (h)	% Acylation	Reaction Time (h)	% Acylation
2	0.9	12	6.0
3	1.5	18.25	10.8
4	2.1	24	16.1
5	2.2	53	31.5
6	3.0	74	39.2
8	4.3	98	41.4
10	4.7	119	45.4

P.P.L.-catalysed transesterification of 2,2,2-Trichloroethyl propanoate & (±)-2-Octanol

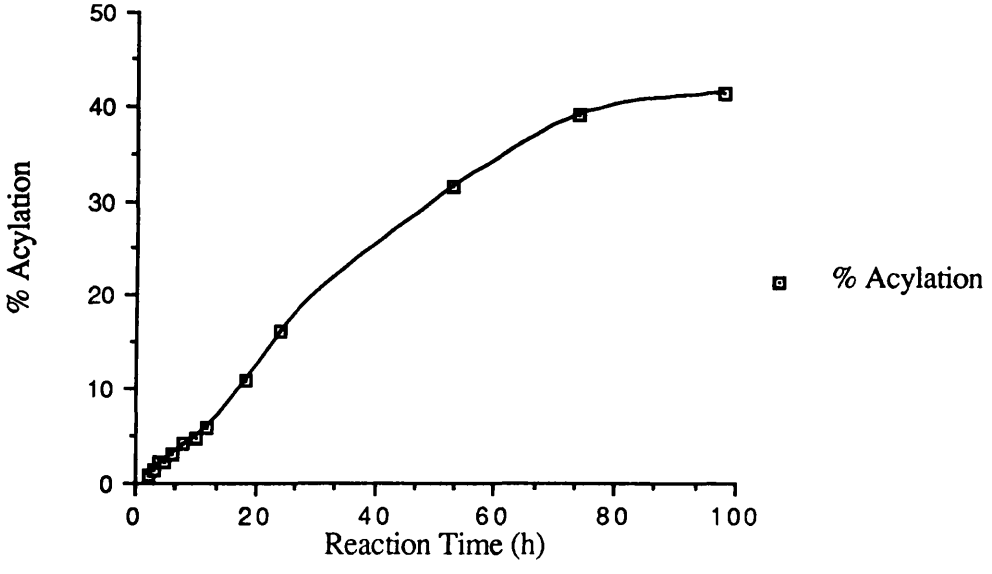


Fig. 7.2

When a graph of percentage acylation *versus* time was plotted (Fig. 7.2), the resulting curve was not smooth as expected. Such a phenomenon has not been reported before for such systems, and the reaction was repeated to obtain a second set of data (Table 7.4, Fig. 7.3). This second reaction showed exactly the same type of behaviour as before.

Table 7.4

Reaction Time (h)	% Acylation	Reaction Time (h)	% Acylation
2.5	<1.0	10	5.0
3	1.2	11.5	5.4
4	1.7	24	15.7
5	2.2	48	32.8
6	2.8	77	41.9
8	3.8	103	44.9

P.P.L.-catalysed transesterification of 2,2,2-Trichloroethyl propanoate & (±)-2-Octanol.

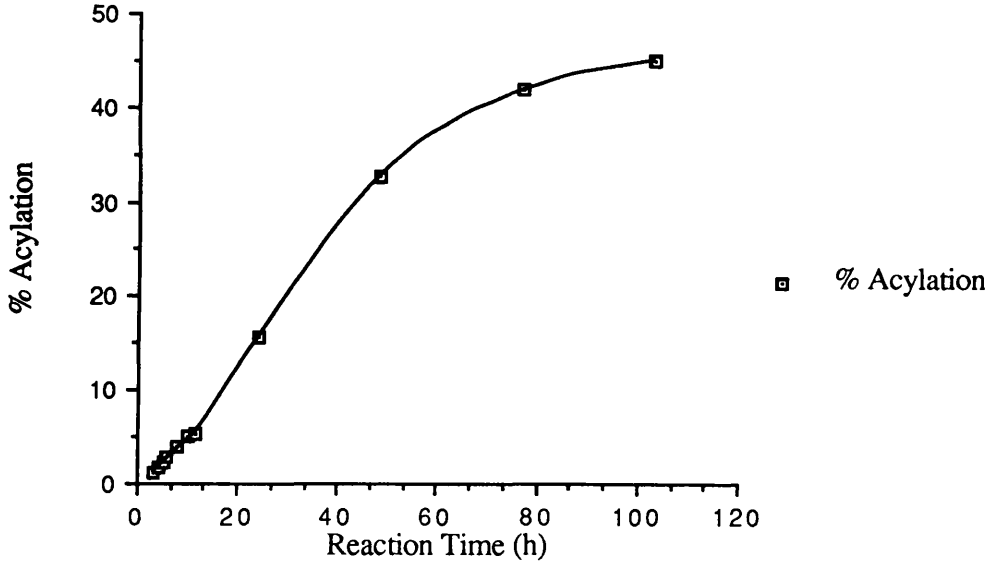


Fig. 7.3

It is hard to explain such a result using the simple mechanistic description of lipase catalysis outlined in Chapter 2. The displayed behaviour would be consistent with an induction period during which the enzyme adopts a more active conformation, possibly due to the adsorption of water from the surroundings or the solvent.

As the propanoate ester failed to react as expected, it was with great interest that we repeated the reaction using 2,2,2-trichloroethyl butanoate (**137**). This had

been expected to be the best substrate for the enzyme, and so it was not surprising when the reaction failed to furnish more than a trace of 2-octyl butanoate (**149**).

In addition to their study of the structural effects of the alkyl group of alkyl butanoates on the P.P.L.-catalysed resolution of 2-octanol in Et_2O, Kanerva *et al.* have also reported the results of a study of solvent effects in the P.P.L.-catalysed transesterification of 2,2,2-trifluoroethyl butanoate and (±)-2-octanol.[171] They found that the choice of solvent exerted a slight effect on the course of the reaction, but this effect was minimal. Therefore, at this stage we decided to use THF as the reaction solvent, rather than diethyl ether. The reason for this change was purely a matter of operational convenience. However, to ensure that THF was not drastically affecting the reaction, we repeated the P.P.L.-mediated transacylation between (±)-2-octanol and 2,2,2-trichloroethyl butanoate using THF as solvent and with magnetic stirring (Table 7.5, Fig. 7.4).

Table 7.5

Reaction Time (h)	% Acylation	Reaction Time (h)	% Acylation
1	-	24	5
2	-	48	12
3	-	97	22
4	-	120	22

P.P.L.-Catalysed Transesterification of 2,2,2-Trichloroethyl acetate & (±)-2-Octanol in THF

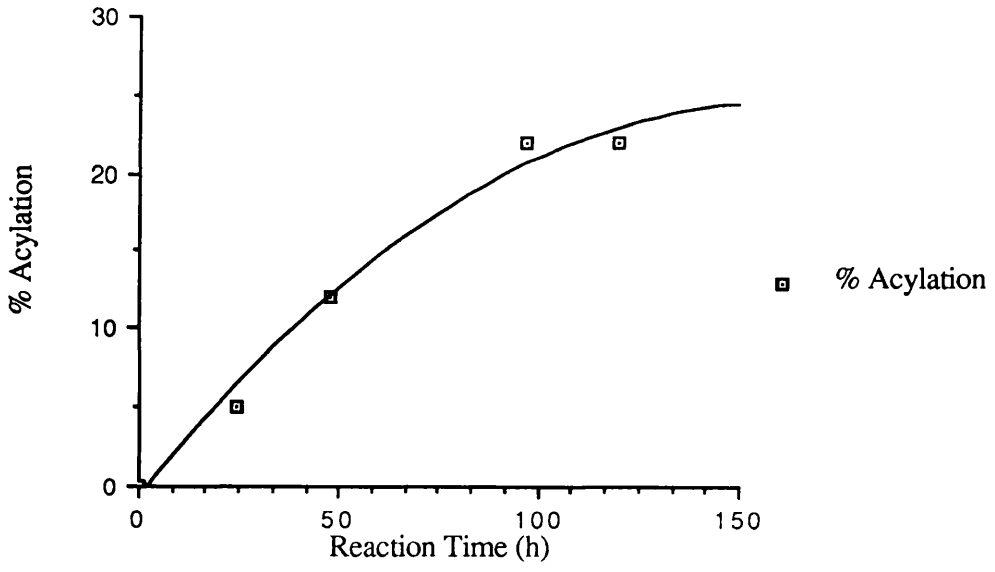


Fig.7.4

Clearly, the reaction in THF was slower than that in Et_2O, and the reaction stopped at a lower degree of acylation than before. This we interpreted as being due to enzyme degradation through the grinding action of the stirring bar over five days reaction. Therefore, the reaction was repeated using the more gentle agitation of a water-bath shaker. However, G.C. analysis of the reaction mixture after 14 days revealed no 2-octyl acetate to be present in the reaction. Clearly, reducing the level of agitation had slowed the reaction down to a negligible rate. This result had immediate repercussions for our other work, as much of the work we had carried out previously with amino alcohols (Chapter 5) used gentle shaking rather than magnetic stirring. This implied that we had not used reaction conditions which gave the enzyme the best chance of reacting with the substrates, and so opened the possibility of repeating much of this work using more efficient agitation of the reaction mixture.

7.3.4 Lipozyme-Catalysed Esterification of (±)-2-Octanol with 2,2,2-Trichloroethyl Esters.

At this stage in our work, a sample of Lipozyme (*Mucor meihei* lipase on a polymer support) became available to us. The enhanced stability of such a supported enzyme suggested that this preparation would be better suited to withstand the rigours of long periods of magnetic stirring. In addition, the structure of this enzyme has been determined recently by X-ray crystal structure analysis.[42] This led us to the intriguing prospect of relating the substrate preference of the enzyme to the crystal structure of the proposed active site.

However, as a small digression we first investigated whether Lipozyme would catalyse the transesterification of 2,2,2-trichloroethyl acetate and (±)-2-octanol in refluxing THF. To the best of our knowledge, no reports have been made of refluxing enzyme reactions, although some workers have used oven-dried P.P.L. at elevated temperatures.[290] We wondered if a supported enzyme would exhibit sufficient thermal stability to operate under such conditions. However, when the reaction was attempted, no 2-octyl acetate was formed and no further investigation of this method were carried out.

We decided to investigate the substrate-structure preferences of Lipozyme for the same transesterification reactions as before (Scheme 7.12). The reactions were monitored by G.C. as before. Unfortunately, and somewhat surprisingly, none of the trichloroethyl esters were particularly good substrates for the enzyme. The reactions between 2-octanol and 2,2,2-trichloroethyl propanoate (**136**) or 2,2,2-trichloroethyl butanoate (**137**) formed very small amounts of the respective 2-octyl esters (**148**) and (**149**) after several days reaction. In all other cases, no 2-octyl esters could be detected by G.C. Having obtained these results, we wished to repeat the reactions using 2,2,2-trifluoroethyl esters as acyl donors, as these have

been reported as being more reactive than the corresponding trichloroethyl esters. However, no time was available for this, and so our work with (±)-2-octanol came to an end.

Chapter Eight

Experimental

8.1 General

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with an Optical Activity Ltd. AA 10 Polarimeter. Infra red spectra were obtained on a Perkin Elmer 580 spectrophotometer. Nuclear magnetic resonance (N.M.R.) spectra were recorded with a Perkin Elmer R32 spectrophotometer operating at 90 MHz (δ_H), a Varian EM 390 spectrophotometer operating at 90 MHz (δ_H), or a Bruker WP200-SY spectrophotometer operating at 200 MHz (δ_H) or 50.3 MHz (δ_C). The multiplicities of the ^{13}C resonances were determined using DEPT spectra with pulse angles of $\theta = 90°$ and $\theta = 135°$. Unless otherwise stated, spectra were recorded for solutions in deuteriochloroform, with $CHCl_3$ as internal standard. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers. Elemental analyses were performed using a Carlo-Erba 1106 elemental analyser.

Thin Layer Chromatography (TLC) was carried out on Merck Kieselgel G (silica) plates of 0.25 mm thickness, and visualised with iodine unless otherwise stated. Boiling points refer to the oven temperature using a Kugelrohr apparatus.

Tetrahydrofuran (THF) and diethyl ether were dried by distillation from sodium-benzophenone under nitrogen prior to use. Where necessary, THF was pre-distilled from CuCl to remove peroxides prior to drying. Dichloromethane (DCM) was distilled from phosphorous pentoxide and stored over 4Å molecular sieves. Other solvents and reagents were purified by standard techniques. Benzoyl chloride was distilled and stored over 4Å molecular sieves. Pyridine and triethylamine were distilled from, and stored over, potassium hydroxide. *N*-Bromosuccinimide was recrystallised from water. 4-*N*,*N*-dimethylaminopyridine (DMAP) was recrystallised from hexanes.

8.2 *Experimental to Chapter Five*

8.2.1 Synthesis of Prolinol and Homoprolinol

Attempted LAH Reduction of Proline[218]

To a suspension of lithium aluminium hydride (4.0 g) in dry THF (80 ml), cooled to 0-5°C, was added L-proline (4.0 g). The resulting mixture was heated at reflux under nitrogen for 6 d, and then allowed to cool to room temperature. A saturated aqueous solution of Na_2SO_4 (13 ml) was carefully added by syringe, and the reaction mixture was stirred at room temperature for 3 h. A further 15 ml of saturated Na_2SO_4 solution was added, and the reaction mixture was filtered through Celite, the residue being washed with several portions of THF. The combined filtrate and washings were concentrated *in vacuo*, and the residue was dried by azeotropic distillation with benzene *in vacuo*. The residue was taken up in $CHCl_3$, and insolubles were removed by filtration. The filtrate was again concentrated *in vacuo*, to give no product.

Attempted Borane Reduction of Proline

A suspension of L-proline (5.059 g, 44 mmol) in dry THF (50 ml) was heated to reflux in an atmosphere of nitrogen. To this was added carefully a 1.0M BH_3.THF solution (50 ml, 50 mmol), and the reaction mixture was heated at reflux overnight. After cooling to room temperature, excess borane was destroyed by the careful addition of water (100 ml) by syringe. The aqueous layer was saturated with K_2CO_3, chloroform (10 ml) was added, and the mixture was filtered through Celite. The mixture was then extracted with $CHCl_3$ (4 x 30 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. Concentration *in vacuo* gave a brown oil (1.943 g), which was examined by TLC (*i*PrOH/NH_3, 5:3). The complex mixture of reaction products contained only a trace amount of the desired product, and so the reaction was abandoned.

The reaction was repeated with L-proline (2.00 g, 17.4 mmol) and 1.0M BH_3.THF solution (18 ml, 18 mmol), heated at reflux for 7 d. After work-up, no prolinol was present in the crude product. Repeating the procedure, but heating at reflux for 36 h, again yielded no prolinol.

Attempted $LiBH_4$/TMS-Cl Reduction of L-Proline[220]

To a solution of trimethylsilyl chloride (1.887 g, 17.4 mmol, 4 equiv) in dry THF (10 ml) in an atmosphere of nitrogen was added gradually a suspension of lithium borohydride (0.195 g, 9.0 mmol, 2 equiv) in dry THF (10 ml). On addition of the borohydride, some effervescence was observed and a precipitate formed. L-Proline (0.500 g, 4.3 mmol, 1 equiv) was then added portionwise to the reaction

vessel over a period of five min. The reaction mixture was stirred at room temperature under nitrogen for 24 h. Methanol (10 ml) was then carefully added to the reaction mixture. More effervescence was observed, and the precipitate dissolved. The reaction mixture was stirred for 10 min at room temperature, and then concentrated *in vacuo*. The residue was taken up in 20% KOH solution (20 ml) and extracted with dichloromethane (4 x 20 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a brown oil (18 mg). The crude product was examined by TLC (with either *i*PrOH/NH_3, 5:3 or $CHCl_3$/CH_3OH/NH_3, 85:14:1 solvent systems), using authentic L-2-pyrrolidinemethanol as a reference standard. The reaction product was found to be a mixture of compounds, with prolinol a minor component. Due to the very low yield, it was decided not to attempt any further purification.

<u>*N*-*t*-Butoxycarbonyl-L-proline</u> (59)[298]

To a stirred mixture of L-proline (0.576 g, 5 mmol) in triethylamine (2.0 ml, 15 mmol) was added a mixture of 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON) (1.36 g, 5.5 mmol) in dioxane (4 ml) and water (4 ml). The reaction mixture quickly became homogeneous, and stirring was continued at room temperature for 7 h. To the reaction mixture was added water (7 ml) and ethyl acetate (10 ml). The aqueous layer was removed and washed with ethyl acetate (2 x 10 ml). The aqueous layer was acidified with 5% citric acid solution, and extracted with ethyl acetate (5 x 10 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, and volatiles were removed *in vacuo* to give a clear oil which solidified on standing to give a white solid (0.801 g). Of this, 0.689 g was crystallised from toluene to give *N*-*t*-butoxycarbonyl-L-proline as a white crystalline solid (0.616 g, 67%), m.p. 138-140°C (lit,[299] 136-137 °C); $[\alpha]_D$ -85° (c 0.7, $CHCl_3$) (lit,[299] $[\alpha]_D$ -

60.2° (c 2, AcOH)); ν_{max}/cm^{-1} ($CHCl_3$) 3 560 - 2 400, 2 990, 1 760, 1 720, 1 690, 1 405, 1 210 and 1 160; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.5 (9H, s, *t*Bu), 1.8 - 2.4 (4H, m, 3-H_2 and 4-H_2), 3.3 - 3.7 (2H, m, 5-H_2), 4.1 - 4.4 (1H, m, 2-H), 11.8 (1H, br s, CO_2H); *m/z* 170 (M^+-CO_2H, 5.5%), 114 (81.4), 70 (100.0), 57 (93.6) and 41 (70.6) (Found: C, 55.8%; H, 7.8; N, 6.5; M^+-CO_2H, 170.1179. $C_{10}H_{17}NO_4$ requires C, 55.8; H, 8.0; N, 6.5%; $C_{10}H_{17}NO_4$ - CO_2H requires M, 170.1181).

Repeating this reaction on a 20 mmol scale, stirring the reaction mixture for 2 h, gave *N*-BOC-L-proline in only 12.5% yield after recrystallisation.

(±)-*N*-(*t*-Butoxycarbonyl)-2-piperidinecarboxylic acid (60)

(±)-2-Piperidinecarboxylic acid (0.658 g, 5.1 mmol) and BOC-ON (1.38 g, 5.6 mmol) were reacted as described above to give (±)-*N*-(*t*-butoxycarbonyl)-2-piperidinecarboxylic acid as a white solid (0.949 g, 81%), shown by ^{13}C N.M.R. spectroscopy to exist in two rotameric forms; m.p. 140-142°C (toluene); ν_{max}/cm^{-1} (KBr disc) 3 600 - 2 500, 1 750, 1 630, 1 435, 1 160 and 1 140; δ_H (200 MHz) 1.01 - 1.74 (15H, unres. m), 2.05 - 2.28 (1H, unres. m), 2.72 - 3.03 (1H, unres. m), 3.75 - 4.05 (1H, unres. m), 4.62 - 4.96 (1H, unres, m), 11.69 (1H, br s); δ_C (50 MHz) 20.56 (br t), 24.36 (t), 24.53 (t), 26.42 (t), 27.70 (t), 28.10 (q), 40.86 (t), 41.88 (t), 53.39 (d), 54.49 (d), 79.76 (s), 80.16 (s), 155.47 (s), 155.96 (s), 177.23 (br s); *m/z* 184 (M^+- CO_2H, 4.4%), 128 (88.3), 84 (100.0) and 57 (92.1) (Found: C, 57.6%; H, 8.3; N, 6.0; M^+, 229.1324. $C_{11}H_{19}NO_4$ requires C, 57.6; H, 8.4; N, 6.1%; M^+, 229.1314).

Borane Reduction of *N*-BOC-L-proline

A stirred solution of *N*-BOC-L-proline (0.500 g, 2.33 mmol) in dry THF (30 ml) was heated to reflux under N_2. To the refluxing solution was added a 1.0M BH_3.THF solution (6 ml, 6 mmol). The reaction mixture was heated to reflux under N_2 overnight. After cooling to room temperature, water (15 ml) was carefully added. The aqueous layer was saturated with K_2CO_3 and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow-green oil (0.431 g). TLC analysis of this ($CHCl_3$/MeOH/NH_3, 85:14:1) showed a mixture of compounds to be present. The oil was taken up in ethyl acetate (3 ml), and to it was added 3M HCl (4 ml). The resulting mixture was stirred at room temperature for 30 min, and volatiles were removed *in vacuo* to give a clear oil. This was taken up in 5% Na_2CO_3 (5 ml), and to it was added ethyl acetate (3 ml). The mixture was stirred at room temperature for 1 h, and then saturated with K_2CO_3. The mixture was extracted with ethyl acetate (3 x 5 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. Volatiles were removed *in vacuo* to give a brown oil (0.061 g). TLC analysis of this (iPrOH/NH_3, 5:3) showed a complex mixture of compounds to be present.

Borane Reduction of (±)-*N*-BOC-2-piperidinecarboxylic Acid

(±)-*N*-(*t*-Butoxycarbonyl)-2-piperidinecarboxylic acid (0.500 g, 2.2 mmol) and a 1.0M BH_3.THF solution (3.0 ml, 3 mmol) were reacted as described above to give a creamy oil (0.414 g, >88%). TLC analysis of this revealed the crude product to be a mixture of unprotected and *N*-protected amino alcohols. The product was taken up in ethyl acetate (5 ml), and to this was added 3M HCl (5 ml), and the biphasic mixture was stirred at room temperature for 35 min. Solvent was removed *in vacuo* to give a

white solid (0.280 g), which was crystallised from ethanol to give (±)-2-piperidinemethanol hydrochloride as a white solid (0.089 g, 31%), m.p. 131-133°C (lit.; 130-132°C, mixed melting point with authentic sample 130-133°C). The solid was dissolved in 1.0M NaOH (5 ml), and the basic solution was extracted with ethyl acetate (3 x 8 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give no product.

(±)-2-Pyrrolidinemethanol (55)[222]

To a mixture of DL-proline (2.303 g, 20 mmol) in dry THF (100 ml), cooled to 0-5°C in an N_2 atmosphere, was added a 1.0M BH_3.THF solution (50 ml, 50 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. To the resulting clear solution was added carefully 6M HCl (30 ml), and THF was removed by distillation at atmospheric pressure. The residue was saturated with NaOH and extracted with Et_2O (4 x 30 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a brown oil. This was subjected to Kugelrohr bulb-to-bulb distillation under reduced pressure to give (±)-2-pyrrolidinemethanol as a clear, air-sensitive oil (1.031 g, 51%), b.p. 85-87°C (4 mm); ν_{max}/cm^{-1} (neat) 3 700 - 2 400, 2 940, 2 880, 1 445 and 1 060; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.5 - 1.9 (4H, m, 3-H_2 and 4-H_2), 2.8 - 3.0 (2H, m, 5-H_2), 3.1 - 3.7 (3H, m, CH_2OH and 2-H), 4.0 (2H, br s, OH and NH); *m/z* 101 (M^+, 1.4%), 84 (0.8), 83 (0.8) and 70 (100.0). This compound displayed identical spectroscopic and chromatographic behaviour to an authentic sample of (S)-(+)-2-pyrrolidinemethanol.

8.2.2 Synthesis of *N*-Methylprolinol and *N*-Methylhomoprolinol

N-Ethoxycarbonyl-L-proline (61)[223]

To a solution of L-proline (0.690 g, 6 mmol) and sodium carbonate (0.640 g, 6 mmol) in water (4 ml), cooled in an ice-bath to 5 °C, was added dropwise ethyl chloroformate (0.58 ml, 6 mmol). The reaction mixture was stirred at room temperature for two hours, and then acidified by the addition of 5% HCl. The reaction mixture was then extracted with chloroform (3 x 5 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, and volatiles were removed *in vacuo* to give 1-ethoxycarbonyl-L-proline as a clear viscous oil (0.524 g, 47%); ν_{max}/cm^{-1} (neat) 3 600 - 2 700, 2 980, 1 720, 1 690 and 1 430; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.0 - 1.5 (3H, unres. t, CH_3), 1.8 - 2.5 (4H, m, 3-H_2 and 4-H_2), 3.4 - 3.8 (2H, unres. m, 5-H_2), 4.1 - 4.6 (3H, m + q, 2-H and OCH_2CH_3), 10.6 (1H, s, CO_2H); *m/z* 187 (M^+, 1.1%), 142 (57.7), 114 (47.6) and 70 (100.0).

Attempted Reduction of N-Ethoxycarbonyl-L-proline

A solution of *N*-ethoxycarbonyl-L-proline (0.268 g, 1.43 mmol) in dry THF (20 ml) was heated to reflux under N_2. To the refluxing solution was carefully added by syringe a 1.0M BH_3.THF solution (3.0 ml, 3 mmol), and heating was continued for 18 h. After cooling to room temperature, water (20 ml) was carefully added. The aqueous layer was saturated with K_2CO_3 and extracted with Et_2O (4 x 20 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow oil (0.104 g). TLC analysis of this product (*i*PrOH/NH_3, 5:3) revealed no 1-methyl-2-pyrrolidinemethanol to be present, and so the reaction was abandoned.

L-Proline Methyl Ester Hydrochloride (62)

[HCl method][224]

L-Proline (11.500 g, 0.1 mol) was dissolved in freshly prepared anhydrous methanol (125 ml). The solution was cooled in an ice-salt bath, and dry gaseous HCl was passed into the solution until saturation was achieved. Volatiles were removed *in vacuo* at 35 °C, and the saturation and distillation procedures were repeated. The resulting oil was dried overnight in a vacuum desiccator to give L-proline methyl ester hydrochloride as a yellow oil (9.440 g, 57%). Despite repeated trituration with ether, no further purification was achieved; $[\alpha]_D$ -31.9° (c 0.80, H_2O) (lit,[300] $[\alpha]_D$ -40.1° (c 0.5, H_2O)); ν_{max}/cm^{-1} (neat) 3 600 - 3 200, 2 960, 1 740, 1 440 and 1 240; δ_H (90 MHz, D_2O, ref. HOD) 1.9 - 2.2 (4H, m, 3-H_2 and 4-H_2), 3.2 - 3.5 (2H, m, 5-H_2), 3.7 (3H, s, CO_2CH_3); *m/z* 129 (M^+- HCl, 1.5%), 115 (0.3), 70 (100.0) and 69 (4.6) (Found: M^+- HCl, 129.0790. $C_6H_{11}NO_2$ requires M^+- HCl, 129.0790).

L-Proline Methyl Ester Hydrochloride (62)

[2,2-dimethoxypropane method][225]

To a suspension of L-proline (5.00 g, 43 mmol) in freshly distilled 2,2-dimethoxypropane (80 ml) was added conc. HCl (44 ml, 51 mmol). The reaction mixture quickly became homogeneous, with spontaneous cooling. The stoppered flask was allowed to stand in the dark for 40 h, with occasional shaking. The reaction mixture was then concentrated *in vacuo* to give a dark red oil. Further purification was achieved by repeated trituration with diethyl ether to give L-proline methyl ester hydrochloride as a slightly yellow oil (5.43 g, 77%), $[\alpha]_D$ -42.0° (c 2.24, water). In

all other aspects, the product was identical to samples prepared by the HCl/methanol procedure.

***N*-Ethoxycarbonyl-L-proline Methyl Ester (63)**

To a solution of L-proline methyl ester hydrochloride (8.298 g, 50 mmol) and sodium carbonate (6.36 g, 60 mmol) in water (40 ml), cooled in an ice-bath to 5 °C, was added dropwise ethyl chloroformate (4.8 ml, 50 mmol). The reaction mixture was allowed to warm to room temperature and stirred for seven hours. The reaction mixture was then extracted with chloroform (3 x 20 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, and volatiles were removed *in vacuo* to give *N*-ethoxycarbonyl-L-proline methyl ester as a clear oil (4.670 g, 46%); $[\alpha]_D$ -62.6° (c 3.82, $CHCl_3$); ν_{max}/cm^{-1} (neat) 2 980, 2 960, 2 880, 1 750, 1 705, 1 420, 1 380, 1 350, 1 200 and 1 175; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.2 (3H, br m, $CH_2\underline{CH_3}$), 2.0 (4H, br m, 2-H_3 & 3-H_4), 3.5 (2H, br m, 2-H_5), 3.7 (3H, s, $CO_2\underline{CH_3}$), 3.9 - 4.4 (3H, m, 1-H_2 & $O\underline{CH_2}CH_3$); *m/z* 201 (M^+, 6.2%), 142 (100.0), 128 (9.4) and 70 (79.1) [Found: M^+, 201.0998. $C_9H_{15}NO_4$ requires M^+, 201.1001].

Attempted Reduction of *N*-Ethoxycarbonyl-L-proline Methyl Ester

To a stirred suspension of lithium aluminium hydride (1.51 g, 0.04 mol) in dry THF (20 ml), cooled in an ice bath under N_2, was added a solution of *N*-ethoxycarbonyl-L-proline methyl ester (1.00 g, 5 mmol) in dry THF (20 ml). The reaction mixture was refluxed in an atmosphere of nitrogen overnight. After cooling to room temperature, water (15 ml) was added dropwise by syringe, and the mixture was stirred overnight. The reaction mixture was filtered through Celite, and the filtrate was

concentrated *in vacuo*. Proton N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) revealed only starting material to be present, and the reaction was abandoned.

Attempted Synthesis of *N*-Methyl-L-proline (64)

A mixture of L-proline (1.00 g, 8.68 mmol), 37% formaldehyde solution (1.435 g, 16 mmol) and sodium bicarbonate (0.603 g, 7.2 mmol) in formic acid (1.8 ml) was refluxed for 18 h. The resulting clear solution was evaporated to dryness *in vacuo* to give a clear oil which slowly solidified on standing (0.725 g). Proton N.M.R. spectroscopy (90 MHz, D_2O, ref. HOD) showed no *N*-methyl signal, the product appearing to be a polymeric material.

<u>*N*-Methyl-L-proline</u> (64)[227]

A mixture of L-proline (1.000 g, 8.7 mmol), 37% formaldehyde solution (0.869 g, 10.7 mmol), Pt(IV)O_2 (26 mg), acetic acid (9.8 ml) and water (5.4 ml) was hydrogenated for 5 h at atmospheric pressure and room temperature. After filtration through Celite, volatiles were removed *in vacuo* to give a brown solid (1.302 g). The crude product was dissolved in water (15 ml) and heated at reflux for 15 min with activated charcoal. The mixture was then filtered and concentrated *in vacuo* to give a creamy oil (1.291 g). Of this, 0.232 g was crystallised from EtOH to give *N*-methyl-L-proline as fine white needles (0.044 g, 22%), m.p. 153-155 °C (lit,[301] 116-117 °C (L-form), 169-170 °C (DL-form)); $[\alpha]_D$ -69.3° (c 1.35, H_2O) (lit,[301] $[\alpha]_D$ -80.1° (H_2O)); ν_{max}/cm^{-1} (KBr disc) 3 700 -2 400, 1 630 and 1 390; δ_H (90 MHz, D_2O, ref. HOD) 1.6 - 2.5 (4H, m, 3-H_2 and 4-H_2), 2.8 (3H, s, N-CH_3), 2.9 - 3.2 (1H, m, 2-H), 3.4 - 3.9 (2H, m, 5-H_2); *m/z* 129 (M^+, 2.0%), 85 (9.1), 84 (100.0) and 70 (7.5) (Found: M^+, 129.0787. $C_6H_{11}NO_2$ requires M^+, 129.0790).

Attempted Borane Reduction of Crude *N*-Methyl-L-proline

A stirred suspension of crude *N*-methyl-L-proline (0.746 g) in dry THF (25 ml) was heated to reflux under nitrogen. To this was carefully added a 1.0M BH_3-THF solution (18 ml, 18 mmol). The reaction mixture was heated at reflux for 3 d, then allowed to cool to room temperature. Water (5 ml) was carefully added, and the precipitate which formed was removed by filtration. The reaction mixture was extracted with $CHCl_3$ (3 x 10 ml), and the combined extracts were dried over anhydrous Na_2SO_4. Volatiles were removed *in vacuo* to give a brown oil (0.134 g). TLC analysis of this product ($CHCl_3$/MeOH/NH_3, 85:14:1 or iPrOH/NH_3, 5:3) revealed a complex mixture of compounds.

Reductive Methylation of (S)-(-)-2-Pyrrolidinemethanol

A mixture of (S)-(-)-2-pyrrolidinemethanol (0.200 g, 2.0 mmol), 37% formaldehyde solution (0.198 g, 2.4 mmol), Pt(IV)O_2 (11 mg), glacial acetic acid (2.75 ml) and water (1.5 ml) was hydrogenated overnight at atmospheric pressure and room temperature. After filtration, the reaction mixture was concentrated *in vacuo* to give a brown oil (0.643 g), identified as 1-methyl-2-pyrrolidinemethanol (**57**) by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) and comparison with an authentic sample; $[\alpha]_D$ -7.6° (c 0.8, MeOH).

(±)-1-Methyl-2-piperidinemethanol (58)

A mixture of (±)-2-piperidinemethanol (0.500 g, 4.3 mmol), 37% formaldehyde solution (0.434 g, 5.3 mmol), Pt(IV)O_2 (13 mg), acetic acid (4.9 ml)

and water (2.7 ml) was hydrogenated for 28 h at atmospheric pressure and room temperature. After filtration through Celite, volatiles were removed *in vacuo* to give a yellow oil (0.541 g). This was purified by Kugelrohr bulb-to-bulb distillation under reduced pressure to give (±)-*N*-methyl-2-piperidinemethanol as an air sensitive clear oil (0.351 g, 63%), b.p. 120-123 °C (20 mm) (lit,[219] 79-80 °C, 7 mm); ν_{max}/cm^{-1} ($CHCl_3$) 3 700 - 3 300, 3 010, 2 970 and 1 215; δ_H (200 MHz) 1.03 - 1.73 (6H, m), 1.74 - 1.91 (1H, m), 1.91 - 2.09 (1H, m), 2.18 (3H, s), 2.68 - 2.81 (1H, m), 3.32 - 3.73 (3H, m and br s); δ_C (50 MHz) 23.93 (t), 25.34 (t), 28.35 (t), 42.81 (q), 56.74 (t), 63.17 (t), 64.51 (d); *m/z* 128 (M^+-H, 0.6%), 98 (100.0) and 84 (14.6) (Found: M^+, 129.1160. $C_7H_{15}NO$ requires M^+, 129.1154).

<u>*N*-Formyl-L-proline</u> (65)[228]

A mixture of 98% formic acid (7.6 ml, 0.20 mol, 20 equiv.) and acetic anhydride (9.4 ml, 0.10 mol, 10 equiv.) was heated on an oil bath at 55-60 °C for two hours. The mixture was allowed to cool to ambient temperature, and to it was added a solution of L-proline (1.151 g, 0.01 mol) in formic acid (5 ml). The flask was equipped with a silica gel guard tube, and the reaction mixture was stirred overnight. Ice-cold water (20 ml) was added to the solution, which was then concentrated *in vacuo* to give a yellow oil. This was taken up in ethyl acetate and dried over anhydrous Na_2SO_4. Volatiles were again removed *in vacuo* to give a yellow oil which was crystallised from ethyl acetate. Two crops of crystals were obtained, washed with petroleum ether (b.p. 60-80°C) and dried in a vacuum desiccator to give *N*-formyl-L-proline as a white crystalline solid (0.954 g, 67%), m.p. 90-91 °C (lit,[302] 88-91 °C); $[\alpha]_D$ -145.7° (c 2.36, $CHCl_3$) (lit,[302] -125° (c 1, EtOH)); ν_{max}/cm^{-1} (KBr disc) 3 700 - 2 200, 1 730, 1 625, 1 380, 1 215 and 1 180; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.8 - 2.5 (4H, m, 3-H_2 and 4-H_2), 3.4 - 3.8 (2H, m, 5-H_2), 4.3 - 4.6 (1H,

m, 2-H), 8.3 (1H, s, COH), 10.3 (1H, s, CO_2H); *m/z* 143 (M^+, 6.3%), 115 (1.9), 99 (41.2), 98 (55.0), 71 (33.4), 70 (100.0), 68 (25.7) and 28 (88.8) [Found: C, 49.9%; H, 6.6; N, 9.7; M^+, 143.0568. $C_6H_9NO_3$ requires C, 50.4; H, 6.3; N, 9.8%; M^+, 143.1411].

N-Formyl-D-proline

N-Formyl-D-proline was prepared from formic acid (13.2 ml, 0.347 mol), acetic anhydride (16.4 ml, 0.174 mol) and D-proline (2.000 g, 17.4 mmol) as described above, and was obtained as a white crystalline solid (2.229 g, 90%), m.p. 98-100 °C (EtOAc); $[\alpha]_D$ +145.9° (c 4.8, $CHCl_3$); [Found: C, 50.2%; H, 6.3; N, 9.7. $C_6H_9NO_3$ requires C, 50.4; H, 6.3; N, 9.8%]. All other data for this compound were identical to the L-enantiomer.

N-Formyl-DL-proline

N-Formyl-DL-proline was prepared from formic acid (13.2 ml, 0.347 mol), acetic anhydride (16.4 ml, 0.174 mol) and DL-proline (2.000 g, 17.4 mmol) as described previously. After crystallisation from ethyl acetate, *N*-formyl-DL-proline was obtained as a white solid (2.147 g, 86%), m.p. 125-126 °C. Satisfactory analytical data could not be obtained for this compound, as it had apparently crystallised in a hydrated form.

(±)-N-Formyl-2-piperidinecarboxylic acid (66)

(±)-*N*-Formyl-2-piperidinecarboxylic acid was prepared from formic acid (3.5 ml, 92 mmol), acetic anhydride (13.1 ml, 140 mmol) and (±)-2-

piperidinecarboxylic acid (2.000 g, 15.5 mmol) as described previously. On drying the crude product as an ethyl acetate solution over anhydrous Na_2SO_4, the solution turned black. After concentration *in vacuo*, the crude product was crystallised from ethyl acetate to give a black solid (2.031 g). This was taken up in ethyl acetate and warmed for 15 min with activated charcoal. The mixture was filtered and concentrated *in vacuo* to give a yellow oil, which was crystallised from ethyl acetate to give (±)-*N*-formyl-2-piperidinecarboxylic acid as a white solid, which was shown by ^1H and ^{13}C N.M.R. spectroscopy to exist in two rotameric forms (1.371 g, 56%); ν_{max}/cm^{-1} ($CHCl_3$) 3 600 - 2 200, 1 720 (br), 1 620 and 1 590 (sh); δ_H (200 MHz) 1.30 - 1.90 (4.9H, unres. m), 2.25 - 2.31 (1.0H, unres. m), 2.75 - 2.95 (0.3H, m), 3.25 - 3.66 (1.4H, m), 4.22 - 4.52 (0.6H, unres. m), 5.13 - 5.16 (0.7H, br d), 8.06 (0.3H, s), 8.10 (0.7H, s), 11.97 (1H, br s); δ_C (50 MHz) [Major Isomer] 21.32 (t, C-4), 25.28 (t, C-3 or C-5), 26.26 (t, C-5 or C-3), 44.51 (t, C-6), 50.81 (d, C-2), 163.04 (d, C-7), 173.76 (s, C-8); [Minor Isomer] 21.22 (t, C-4), 24.06 (t, C-3 or C-5), 27.26 (t, C-5 or C-3), 38.46 (t, C-6), 57.13 (d, C-2), 163.51 (d, C-7), 173.62 (s, C-8); *m/z* 157 (M^+, 10.8%), 113 (17.5), 112 (100.0) and 84 (8.1) (Found: C, 53.5%; H, 7.2; N, 8.9. $C_7H_{11}NO_3$ requires C, 53.5; H, 7.1; N, 8.9%).

Attempted TMS-Cl/$NaBH_4$ Reduction of *N*-Formylproline[220] (Procedure A)

To a solution of trimethylsilyl chloride (3.451 g, 31.8 mmol, 9.1 equiv) in dry THF (6 ml), in a nitrogen atmosphere, was added sodium borohydride (0.633 g, 16.7 mmol, 4.8 equiv). The resulting mixture was heated at reflux under nitrogen for 2.5 h. A solution of *N*-formyl-L-proline (0.500 g, 3.5 mmol, 1 equiv) in dry THF (5 ml) was added by syringe over a period of 10 min to the refluxing reaction mixture. The reaction mixture was heated at reflux overnight, then allowed to cool to ambient

temperature. Methanol (13 ml) was added carefully by syringe. The reaction mixture was filtered through celite, and the filtrand was washed with several portions of methanol. Volatiles were removed *in vacuo*, and the residue was taken up in 5% HCl (5 ml). The acidic solution was washed with diethyl ether (3 x 3 ml), then basified with dilute sodium hydroxide solution. The basic solution was then repeatedly extracted with dichloromethane (7 x 5 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and then reduced *in vacuo* to give 26 mg of a yellow oil. The basic washings were concentrated to half volume *in vacuo*, and again repeatedly extracted with dichloromethane. Concentration of the organic extracts *in vacuo* revealed no product had been extracted. The basic layer was saturated with NaCl and repeatedly extracted with ethyl acetate. Again, concentrating the organic extracts *in vacuo* yielded no further product. At this stage, the reaction was abandoned.

Attempted TMS-Cl/$NaBH_4$ Reduction of *N*-Formylproline (Procedure B)

To a solution of trimethylsilyl chloride (3.451 g, 32 mmol, 9.1 equiv.) in dry THF (20 ml) was added sodium borohydride (0.692 g, 18.3 mmol, 5.2 equiv.). The resulting mixture was heated at reflux under nitrogen for 3 h. A solution of *N*-formylproline (0.500 g, 3.5 mmol, 1 equiv.) in dry THF (20 ml) was added dropwise by syringe. The reaction mixture was heated at reflux for a further 41 h, by which time no *N*-formylproline was detectable by TLC analysis ($CHCl_3$/MeOH/NH_3, 85:14:1, visualised with bromocresol green spray). To the reaction mixture was carefully added methanol (30 ml). Volatiles were removed *in vacuo*, and the residue was taken up in 20% KOH (10 ml). This was extracted with CH_2Cl_2 (6 x 50 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. After removal of solvent *in vacuo*, the crude product was examined by TLC (iPrOH/NH_3, 5:3). The reaction mixture consisted of a complex mixture of products, with 1-methyl-2-

pyrrolidinemethanol as a minor component only. Due to the low yield, the reaction was abandoned at this stage.

(S)-(-)-1-Methyl-2-pyrrolidinemethanol (57)[229]

To a solution of *N*-formyl-L-proline (0.500 g, 3.5 mmol) in dry THF (25 ml), cooled to 0-5°C under N_2, was added a 1.0M BH_3.THF solution (10 ml, 10 mmol). The reaction mixture was heated to reflux for one hour, and then cooled to room temperature. To the reaction mixture was carefully added 6M HCl (2 ml). THF was removed by distillation at atmospheric pressure, a further 10 ml of water being added during the distillation. The residue was saturated with NaOH and extracted with Et_2O (5 x 20 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a green oil. This was subjected to Kugelrohr bulb-to-bulb distillation under reduced pressure to give (S)-(-)-1-methyl-2-pyrrolidinemethanol as a clear oil which quickly turned brown on exposure to air (0.093 g, 23%); $[\alpha]_D$ -44.9° (c 3.5, MeOH) (lit,[219] $[\alpha]_D$ -49.5° (c 5, MeOH)); ν_{max}/cm^{-1} ($CHCl_3$) 3 700 - 2 400, 2 970, 2 680, 2 795, 1 450, 1 400, 1 240, 1 080 and 1 020; δ_H (200 MHz) 1.55 -1.94 (4H, m, 3-H_2 and 4-H_2), 2.11 - 2.39 (m) and 2.28 (s) (4H), 2.94 - 3.17 (1H, m), 3.27 - 3.45 (2H, m), 3.46 - 3.63 (2H, m); δ_C (50 MHz) 23.01 (t), 27.34 (t), 40.67 (q), 57.42 (t), 62.14 (t), 66.27 (d); *m/z* 115 (M^+, 1.3%), 100 (1.2), 98 (58.2), 84 (100.0) and 82 (31.0).

(±)-1-Methyl-2-pyrrolidinemethanol

N-Formyl-DL-proline (2.571 g, 18.0 mmol) and a 1.0M BH_3.THF solution (50 ml, 50 mmol) were reacted as described above to give (±)-1-methyl-2-pyrrolidinemethanol as an air-sensitive, clear oil (1.375 g, 66%), identical in all

respects (other than optical activity) to previously obtained (S)-(-)-1-methyl-2-pyrrolidinemethanol.

(±)-1-Methyl-2-piperidinemethanol (58)

(±)-*N*-Formyl-2-piperidinecarboxylic acid (2.478 g, 15.8 mmol) and a 1.0M BH_3.THF solution (55 ml, 55 mmol) were reacted together as described above to give (±)-1-methyl-2-piperidinemethanol as a clear oil (1.596 g, 78%), identical in all respects with an authentic sample obtained from Aldrich Chemical Company.

Tartrate Resolution of (±)-2-Piperidinecarboxylic Acid[230,231]

(±)-2-Piperidinecarboxylic acid (2.000 g, 15.5 mmol) was dissolved with heating in 95% EtOH/H_2O (50 ml). To this was added a solution of L-(+)-tartaric acid (3.10 g, 20.7 mmol) in ethanol. The resulting mixture was stored at 0°C, and scratched occasionally until crystallisation had started. The mixture was kept in the freezer for 24 h, and the precipitated white solid was collected by filtration. The solid was dissolved in a little hot water, ethanol was added and the reaction mixture was again left to crystallise at 0°C. The crystallisation procedure was repeated a further four times, until the measured specific optical rotation of the isolated tartrate salt had reached a maximum value; $[\alpha]_D$ -20.8° (c 3.6, H_2O) {lit.,[231] $[\alpha]_D$ -20.7° (c 6.8, H_2O)}. This was dissolved in methanol (5 ml) and cooled in an ice bath. To this was added conc. NH_3 (1.125 ml). The reaction mixture was stirred in an ice bath for 30 min, and ammonium tartrate was removed by filtration. The filtrate was concentrated *in vacuo* at 40°C to give L-(-)-2-piperidinecarboxylic acid as a white powder (0.435 g, 44%), m.p. 271-273°C (lit.,[219] 272°C); $[\alpha]_D$ -26.8° (c 4.22, H_2O) {lit.,[219] $[\alpha]_D$ -26.4° (c 1, H_2O)}; ν_{max}/cm^{-1} (KBr disc) 3 700 - 2 300, 1 630 and 1 400; δ_H (200 MHz, D_2O,

ref. dioxan) 1.28 - 1.80 (5H, m), 1.85 - 2.10 (1H, m), 2.66 - 2.89 (1H, m), 3.11 - 3.42 (2H, m); δ_C (50 MHz, D_2O, ref. dioxan) 22.34 (t), 22.66 (t), 27.34 (t), 44.47 (t), 59.81 (d), 175.35 (s); *m/z* 129 (M^+, 1.0%) and 84 (100.0) (Found: C, 55.2%; H, 8.6; N, 10.8. $C_6H_{11}NO_2$ requires C, 55.8; H, 8.6; N, 10.8%).

8.2.3 Synthesis of Acyl- and Carbamoyl-protected Prolinols and Homoprolinols

(S)-*N*-Acetyl-2-pyrrolidinemethyl Acetate (67)

A mixture of (S)-(+)-2-pyrrolidinemethanol (0.203 g, 2 mmol), pyridine (1 ml) and acetic anhydride (4 ml) was stirred overnight at room temperature. Volatiles were removed by azeotropic distillation with toluene *in vacuo* to give a brown solid. This was crystallised from ethyl acetate/pentane to give (S)-*N*-acetyl-2-pyrrolidinemethyl acetate as a white solid (0.352 g, 95%), shown by ^{13}C N.M.R. spectroscopy to exist in two rotameric forms; m.p. 50-51°C; ν_{max}/cm^{-1} ($CHCl_3$) 3 000, 1 740, 1 630, 1 420 and 1 240; δ_H (200 MHz) 1.64 - 1.98 (9.3H, m), 2.52 - 2.62 (0.6H, m), 3.18 - 3.47 (2.2H, m), 3.85 - 4.07 (2.1H, m), 4.09 - 4.12 (0.7H, m); δ_C (50 MHz) 20.55 (q, minor), 20.58 (q, major), 21.56 (t, minor), 21.73 (q, minor), 22.46 (q, major), 23.67 (t, major), 27.14 (t, major), 28.32 (t, minor), 45.21 (t, minor), 47.58 (t, major), 54.90 (d, major), 56.18 (d, minor), 63.48 (t, major), 64.21 (t, minor), 169.41 (s, minor), 169.44 (s, major), 170.30 (s, minor), 170.48 (s, major); *m/z* 126 (M^+-AcO, 1.3%), 125 (8.9), 112 (17.7) and 70 (100.0).

(±)-*N*-Acetyl-2-piperidinemethyl Acetate (68)

To a solution of (±)-2-piperidinemethanol (1.000 g, 8.7 mmol) and triethylamine (2.4 ml, 17.4 mmol) in acetone (15 ml), cooled to 0-5°C, was added dropwise acetyl chloride (1.23 ml, 17.4 mmol). The reaction mixture was allowed to warm to room temperature, and stirred overnight. The reaction mixture was filtered and concentrated *in vacuo* to give a brown semi-solid. This was subjected to flash column chromatography (21 g SiO_2, 230-400 mesh), eluting with $CHCl_3$ to give (±)-*N*-acetyl-2-piperidinemethyl acetate as a yellow oil (1.633 g, 94%), which was shown by ^1H and ^{13}C N.M.R. spectroscopy to exist in two rotameric forms; ν_{max}/cm^{-1} ($CHCl_3$) 3 010, 2 950, 2 870, 1 740, 1 630, 1 430 and 1 230; δ_H (200 MHz) 1.10 - 1.75 (6H, unres. m), 1.93, 1.94, 1.98 and 2.02 (6H, 4 x s), 2.47 - 2.62 (0.8H, br t), 2.95 - 3.22 (0.7H, br t), 3.40 - 3.65 (0.6H, unres. m), 3.85 - 4.26 (2.1H, m), 4.26 - 4.57 (2.1H, m), 4.80 - 5.00 (0.5H, unres. m); δ_C (50 MHz) 19.12 (t), 19.18 (t), 20.54 (q), 20.63 (q), 21.30 (q), 21.68 (q), 24.79 (t), 24.95 (t), 25.50 (t), 26.01 (t), 36.33 (t), 42.28 (t), 46.14 (d), 51.75 (d), 61.44 (t), 61.64 (t), 169.55 (s), 169.69 (s), 170.38 (s), 170.70 (s); *m/z* 199 (M^+, 0,8%), 156 (1.0), 139 (4.4), 126 (33.7) and 84 (100.0) (Found: M^+, 199.1197. $C_{10}H_{17}NO_3$ requires M^+, 199.1208).

Pentafluorophenyl Acetate (70)[232]

To a round-bottomed flask charged with pentafluorophenol (10.000 g, 54 mmol) was added acetyl bromide (5.0 ml, 68 mmol). The reaction mixture was stirred at room temperature for 2 h. Dry nitrogen gas was then bubbled through the reaction mixture for 1.5 h to remove HBr, and the mixture was then distilled from glass wool under vacuum (water-pump), the fraction distilling at 80-82°C being collected to give pentafluorophenyl acetate as a clear oil (10.888 g, 89%); ν_{max}/cm^1 (neat) 1 790,

1 520, 1 380, 1 180 and 1 010; δ_H (90 MHz, $CDCl_3$, ref. TMS) 2.43; *m/z* 226 (M^+, 2.2%), 184 (14.9), 183 (7.6), 155 (12.5) and 43 (100.0) (Found: C, 42.3%; H, 1.1. $C_8H_3F_5O_2$ requires C, 42.5; H, 1.3%).

(S)-(-)-*N*-Acetyl-2-pyrrolidinemethanol (69)[232]

To a round-bottomed flask charged with (S)-(+)-2-pyrrolidinemethanol (0.253 g, 2.5 mmol) was added a solution of pentafluorophenyl acetate (**70**) (1.676 g, 7.4 mmol) in dried DMF. The flask was fitted with a silica gel guard tube, and the reaction mixture was allowed to stir at room temperature until TLC (*i*PrOH/NH_3, 5:3) indicated all the amino alcohol had been consumed (2 d). Solvent was removed by azeotropic distillation with toluene *in vacuo* at 40 °C to give a brown oil. This was subjected to flash column chromatography (15 g SiO_2, 230-400 mesh), eluting with CH_2Cl_2 (40 ml), then EtOAc/CH_2Cl_2 (10%, 20%, 40%, 80% and 100% EtOAc) and finally MeOH/EtOAc (10%, 20%, 30%, 70% and 100% MeOH). The product started appearing in the fraction eluted with 40% EtOAc/CH_2Cl_2 and continued to elute with all further solvent mixtures. Fractions containing product were combined and concentrated *in vacuo* to give (S)-(-)-*N*-acetyl-2-pyrrolidinemethanol as an air-sensitive oil (0.302 g, 84%); $[\alpha]_D$ -58.0° (c 1.0, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3 700 - 2 600, 3 000, 1 620, 1 520 and 1 450; δ_H (200 MHz) 1.51 - 1.97 (7H, m and s, CH_3CO, 3-H_2 and 4-H_2), 3.32 - 3.47 (4H, m, 5-H_2 and CH_2OH), 3.89 - 4.18 (1H, m, 2-H), 4.20 - 4.67 (1H, br s, OH); *m/z* 143 (M^+, 0.6%), 125 (4.9), 112 (22.3), 84 (1.2) and 70 (100.0) (Found: M^+, 143.0929. $C_7H_{13}NO_2$ requires M^+, 143.0946).

Attempted Synthesis of (S)-2-Pyrrolidinemethyl Acetate (71)[233]

To a stirred solution of (S)-1-acetyl-2-pyrrolidinemethyl acetate (**67**) (0.052 g, 0.28 mmol) in dried CH_2Cl_2 (8 ml) under N_2 was added a solution of triethyloxonium hexachloroantimonate (0.123 g, 0.20 mmol) in dried CH_2Cl_2 (2 ml). The reaction mixture was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The residue was taken up in $CHCl_3$ (3 ml), and treated with 5% Na_2CO_3 (1.5 ml). The mixture was stirred at room temperature for 2 h and then extracted with $CHCl_3$ (4 x 3 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a brown oil (0.042 g), which was shown by TLC ($CHCl_3$/MeOH/NH_3, 85:14:1) to be a mixture of 4 compounds, 3 of which were identified by TLC as prolinol, *N*-acetylprolinol and *N,O*-diacetylprolinol.

(±)-*N*-Ethoxycarbonyl-2-piperidinemethanol (72)

To a stirred solution of (±)-2-piperidinemethanol (0.241 g, 2.1 mmol) and triethylamine (0.3 ml, 1.0 mmol) in acetone (10 ml), cooled to ~10°C, was added dropwise ethyl chloroformate (0.9 ml, 8.7 mmol). The reaction mixture was allowed to warm to room temperature and monitored by TLC (*i*PrOH/NH_3, 5:3) until all the amino alcohol had been consumed (30 min). The reaction mixture was then filtered and concentrated *in vacuo* to give a yellow oil. This was subjected to flash column chromatography (SiO_2, 230-400 mesh), eluting with ethyl acetate, to give (±)-*N*-ethoxycarbonyl-2-piperidinemethanol as a clear oil (0.344 g, 88%), which was shown by ^{13}C N.M.R. spectroscopy to exist in two rotameric forms; ν_{max}/cm^{-1} ($CHCl_3$) 3 700 - 3 240, 3 005, 2 970, 1 670, 1 430, 1 215 and 1 100; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.3 (3H, t), 1.4 - 1.8 (6H, m), 2.6 - 3.1 (2H, m and br s), 3.4 - 4.5 (6H, m); δ_C (50 MHz) 14.16 (q, minor rotamer), 14.53 (q, major rotamer), 18.61 (t,

minor), 19.15 (t, major), 19.52 (t, major) 24.89 (t, major), 25.06 (t, minor) 25.12 (t, minor), 39.65 (t, major), 39.91 (t, minor), 48.73 (d, major), 48.80 (d, minor), 61.27 (t, major), 64.01 (t, minor), 64.91 (t, major), 65.22 (t, minor), 154.98 (s, minor), 155.78 (s, major); *m/z* 187 (M^+, 0.7%), 157 (9.2), 156 (100.0), 128 (37.1), 112 (14.7) and 84 (87.3) (Found: M^+- CH_3O, 156.1028. $C_9H_{17}NO_3$ - CH_3O requires M^+, 156.1025).

(±)-*N*-Ethoxycarbonyl-2-piperidinemethyl Acetate (73)

A solution of (±)-*N*-ethoxycarbonyl-2-piperidinemethanol (**72**) (0.200 g, 1.1 mmol) and pyridine (0.1 ml, 1.2 mmol) in $CHCl_3$ (2 ml) was cooled in an ice-bath to 0-5°C. To this was added dropwise acetyl chloride (0.09 ml, 1.2 mmol). The reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. The reaction mixture was washed with water (2 x 2 ml), 5% $NaHCO_3$ (2 x 2 ml), 5% HCl (2 x 2 ml), and again water (2 x 2 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. This was subjected to flash column chromatography (SiO_2, 230-400 mesh), eluting with Et_2O, to give (±)-*N*-ethoxycarbonyl-2-piperidinemethyl acetate as a clear oil (0.081 g, 33%); ν_{max}/cm^{-1} ($CHCl_3$) 3 005, 2 975, 2 935, 1 740, 1 690, 1 430, 1 260 and 1 250; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.3 (3H, t), 1.5 - 1.9 (6H, unres. m), 2.1 (3H, s), 2.7 - 3.1 (1H, unres. m), 4.0 - 4.7 (6H, unres. m); *m/z* 169 (M^+-CH_3CO_2H, 4.3%), 156 (100.0), 128 (36.1), 112 (14.8) and 84 (89.2).

8.2.4 Synthesis of 2-Aziridinemethanol

(±)-3-Azido-1,2-propanediol (76)[303]

2,3-Epoxy-1-propanol (3.3 ml, 50 mmol, 1 equiv.) and ammonium chloride (3.477 g, 65 mmol, 1.3 equiv.) were dissolved in an 80% ethanol/water mixture (85 ml). To this solution was added sodium azide (4.226 g, 65 mmol, 1.3 equiv.), and the reaction mixture was heated to 70-75 °C for 24 h. The reaction mixture was allowed to cool to room temperature, poured into water (85 ml) and extracted with diethyl ether (3 x 75 ml). The combined organic layers were washed with water (3 x 30 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give (±)-3-azido-1,2-propanediol as a clear oil (1.845 g, 32%); ν_{max}/cm^{-1} (neat) 3 800 - 2 700, 2 120, and 1 640; δ_H (200 MHz, d_6-DMSO, ref. d_5-DMSO) 3.10 - 3.41 (4H, m, 1-H_2 and 3-H_2), 3.55 - 3.65 (1H, br m, 2-H), 4.85 (1H, br t, primary OH), 5.22 (1H, br d, secondary OH); δ_C (50 MHz, d_6-DMSO, ref. d_6-DMSO) 53.72 (t, C-3), 63.39 (t, C-1), 71.28 (s, C-2).

(±)-2-Aziridinemethanol (79)[234]

A suspension of (±)-3-azido-1,2-propanediol **(76)** (1.171 g, 10 mmol, 1 equiv.) and triphenylphosphine (2.623 g, 10 mmol, 1 equiv.) in dry acetonitrile (30 ml) was stirred at room temperature for 30 min, by which time the reaction mixture had become homogeneous. The solution was then heated to reflux for 5 h. The reaction mixture was concentrated *in vacuo*, and the residue was taken up in dichloromethane (20 ml) and extracted with water (3 x 20 ml). The combined aqueous layers were concentrated *in vacuo* and dried in a vacuum desiccator overnight to yield a brown oil which quickly solidified. This was characterised as either a

polymer or oligomer of 2-aziridinemethanol (0.340 g, 47%); ν_{max}/cm^{-1} (neat) 3 700 - 2 200, 2 910, 2 870, 1 600, 1 460, 1 080, and 1 035; δ_H (200 MHz, d_6-DMSO, ref. d_5-DMSO) 2.3 - 2.7 (br m, CH_2N), 3.2 - 3.4 (br m, 2 x CH_2O); δ_C (50 MHz, d_6-DMSO, ref. d_5-DMSO) 44.73 (br t), 63.95 (br t), 77.43 (br d); *m/z* 262 (0.2%), 208 (0.4), 187 (1.0), 178 (3.1), 166 (2.8), 134 (14.5), 116 (10.0), 104 (89.3), 72 (11.3), 56 (16.9), 44 (100.0).

(±)-1-*t*-Butyldimethylsilyloxy-2,3-epoxypropane (75)

A solution of 2,3-epoxy-1-propanol (**74**) (0.665 ml, 10 mmol, 1 equiv.), triethylamine (2.1 ml, 15 mmol, 1.5 equiv.) and DMAP (20 mg, catalytic) in dry dichloromethane (20 ml) was cooled in an ice bath. To this solution was added *t*-butyldimethylsilyl chloride (1.507 g, 10 mmol, 1 equiv.). The reaction mixture was allowed to warm gradually to room temperature, and stirring was continued overnight. Volatiles were removed *in vacuo*, and the residues were taken up in diethyl ether and insoluble triethylamine hydrochloride was removed by suction filtration. Volatiles were again removed *in vacuo* to give (±)-1-*t*-butyldimethylsilyloxy-2,3-epoxypropane as a clear oil (1.676 g, 89%); ν_{max}/cm^{-1} (neat) 2 960, 2 930, 2 860, 1 420, 1 255, 1 100, 920, 840, and 780; δ_H (200 MHz) 0.016 and 0.024 (6H, 2 x s, 2 x CH_3Si), 0.84 (9H, s, t-Bu), 2.55 - 2.73 (2H, m, 1-H_2), 2.99 - 3.06 (1H, m, 2-H), 3.55 - 3.84 (2H, m, 3-H_2); δ_C (50 MHz) -5.48 (q, CH_3Si), -5.44 (q, CH_3Si), 18.50 (s, $(\underline{CH_3})_3C$), 25.76 (q, $(\underline{CH_3})_3C$), 44.27 (t, C-3), 52.28 (d, C-2), 63.62 (t, C-1); *m/z* 189 ($[M+H]^+$, 0.3%), 131 (M^+-*t*-Bu, 23.9), 101 (100.0); (Found: $[M+H]^+$, 189.1137. $[C_9H_{20}O_2Si+H]^+$ requires 189.1311).

(±)-3-Azido-1-*t*-butyldimethylsilyloxy-2-propanol (77)

(±)-1-*t*-Butyldimethylsilyloxy-2,3-epoxypropane (**75**) (1.301 g, 6.9 mmol, 1 equiv.) and ammonium chloride (0.481 g, 9.1 mmol, 1.3 equiv.) were dissolved in an 80% ethanol/water mixture (10 ml). To this was added sodium azide (0.584 g, 9.1 mmol, 1.3 equiv.), and the resulting mixture was heated to reflux for 24 h. After cooling to room temperature, the reaction mixture was poured into water (10 ml) and extracted with diethyl ether (4 x 10 ml). The combined organic layers were washed with water (2 x 10 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (±)-3-azido-1-*t*-butyldimethylsilyloxy-2-propanol as a golden oil (1.013 g, 63%); ν_{max}/cm^{-1} (neat) 3 600 - 3 040, 2 950, 2 930, 2 860, 2 100, 1 260, 1 120 - 1 060 (br), and 840; δ_H (200 MHz) 0.05 (6H, s, 2 x $\underline{CH_3}Si$), 0.87 (9H, s, *t*-Bu), 2.68 (1H, br s, OH), 3.31 - 3.33 (2H, m, 1-H_2), 3.51 - 3.70 (2H, m, 3-H_2), 3.72 - 3.89 (1H, m, 2-H); δ_C (50 MHz) -5.55 (q, $\underline{CH_3}Si$), -4.88 (q, $\underline{CH_3}Si$), 18.18 (s, $(CH_3)_3\underline{C}$), 25.64 (q, $(\underline{CH_3})_3C$), 53.08 (t, C-1), 64.03 (t, C-3), 70.74 (d, C-2).

(±)-2-(*t*-Butyldimethylsilyloxymethyl)-aziridine (78)

A suspension of (±)-3-azido-1-*t*-butyldimethylsilyloxy-2-propanol (**77**) (0.694 g, 3 mmol, 1 equiv.) and triphenylphospine (0.787 g, 3 mmol, 1 equiv.) in dry acetonitrile (5 ml) was stirred under nitrogen at room temperature for 30 min. The resulting solution was then heated to reflux for 3 h, after which volatiles were removed *in vacuo*. Work-up was attempted by the same method as before (extraction of product into water from a dichloromethane solution), without success. The dichloromethane layer was instead concentrated *in vacuo*, and subjected to flash column chromatography on basic alumina, eluting first with DCM/EtOAc solvents mixtures, and then finally with methanol. No aziridine was recovered from the column.

N-Benzoyl-β-alanine (80)

To a solution of β-alanine (4.516 g, 51 mmol, 1 equiv.) in 4M NaOH (50 ml) was added benzoyl chloride (6.5 ml, 56 mmol, 1.1 equiv.). The reaction mixture was stirred at room temperature until homogeneous. The solution was then acidified to Congo Red indicator paper with conc. HCl, and the precipitated product was collected by suction filtration. The resulting white solid was recrystallised from ethyl acetate and dried in a vacuum desiccator to give *N*-benzoyl-β-alanine as a white crystalline solid (7.598 g, 77%), m.p. 133-135 °C; ν_{max}/cm^{-1} (KBr disc) 3 640 - 2 400, 3 320, 1 705, 1 620, 1 555 and 1 260; *m/z* 193 (M^+, 18.1 %), 176 (6.8), 148 (4.0), and 105 (100.0); (Found: C, 62.0%; H, 5.7; N, 7.4; M^+, 193.0741. $C_{10}H_{11}NO_3$ requires C, 62.2; H, 5.7; N, 7.3%; M^+, 193.0739).

Attempted Formation of Methyl (±)-2-Bromo-3-benzamidopropionate (81)[236]

To a stirred suspension of *N*-benzoyl-β-alanine (**80**) (3.091 g 16 mmol, 1 equiv.) in carbon tetrachloride (4 ml) was added thionyl chloride (4.7 ml, 64 mmol, 4 equiv.). The flask was equipped with a reflux condenser and silica gel guard tube, and heated to 60-65 °C for 30 min. The reaction mixture was allowed to cool to room temperature. At this stage, a sample of the reaction mixture was examined by ^1H N.M.R. spectroscopy [δ_H (90 MHz, $CDCl_3$, ref. TMS) 3.2 (t), 3.4 (t), 3.65 (t), 4.0 (t), 7.3 - 8.1 (m)].

A suspension of *N*-bromosuccinimide (3.417 g, 19.2 mmol, 1.2 equiv.) in carbon tetrachloride (8 ml) was added to the reaction mixture, followed by two drops of

48% aqueous HBr solution. The reaction mixture was heated at 70 °C for 10 min. The temperature was then raised to 80 °C, and maintained there until the reaction mixture became light yellow in colour (approx. 4.5 h). The reaction mixture was cooled in an ice bath, and to it was slowly added methanol (20 ml). The reaction mixture was allowed to warm to room temperature, volatiles were removed *in vacuo*, the residue was filtered and the filtrand was extracted with CCl_4. The combined organic extracts were again concentrated *in vacuo* to give a brown semi-solid. This was then subjected to flash column chromatography (30 g SiO_2, 230-400 mesh), eluting with pentane/ethyl acetate solvent mixtures. Incomplete separation was obtained, and so all fractions containing product were combined and subjected to positive pressure column chromatography (60 g SiO_2, 230-400 mesh). The product was eluted with ethyl acetate to give a brown solid which was characterised as methyl *N*-benzoyl-β-alaninate (0.810 g, 24%); ν_{max}/cm^{-1} (KBr disc) 3 280, 1 730, 1 630, 1 550, 1 200, and 1 180; δ_H (200 MHz) 2.64 (2H, t, J 6Hz, 2-H_2), 3.70 (q, J 6Hz) and 3.69 (s) (5H, 3-H_2 and ester CH_3), 6.95 (1H, br s, NH), 7.39 - 7.44 (3H, m, Ph), 7.72 - 7.77 (2H, m, Ph); δ_C (50 MHz) 33.64 (t, C-2), 35.24 (t, C-3), 126.88 (d, Ph), 128.49 (d, Ph), 131.47 (d, Ph), 134.24 (s, Ph), 167.34 (s, C=O amide), 173.31 (s, C=O ester); *m/z* 207 (M^+, 4.3%), 176 (3.9), 147 (2.9), and 105 (100.0); (Found: M^+, 207.0890. $C_{11}H_{13}NO_3$ requires M^+, 207.0895).

Methyl β-Alaninate Hydrochloride (87)[224]

Into a 500 ml three-necked, round bottomed flask equipped with $CaCl_2$ drying tube, was placed β-alanine (10.691 g, 0.12 mol) and anhydrous methanol (175 ml). The resulting suspension was cooled in an ice-bath, and a stream of dry HCl gas was passed through the reaction mixture until saturation was achieved. The reaction mixture was allowed to warm gradually to room temperature, with stirring, and then stirred a

further hour at ambient temperature. The suspension quickly became homogeneous on stirring. Volatiles were then removed *in vacuo* to give a white solid which was taken up in methanol and precipitated with diethyl ether. The resulting white solid was dried overnight in a vacuum desiccator to give methyl β-alaninate hydrochloride as a white, hygroscopic solid (14.145 g, 84%), m.p. 35-60 °C; ν_{max}/cm^{-1} (KBr disc) 3 800 - 2 100, 1 730, and 1 230; δ_H (200 MHz, D_2O, ref. HOD) 2.69 (2H, t, J 6.5 Hz), 3.16 (2H, t, J 6.5 Hz), 3.60 (3H, s); δ_C (50 MHz, D_2O, external ref. dioxan at 67.4 ppm) 32.26 (t), 36.30 (t), 53.84 (q), 174.28 (s); *m/z* 104 (M^+- Cl, 1.0%), 103 (3.5), 88 (1.5), 72 (6.8), 70 (10.5), and 30 (100.0).

Methyl *N*-benzoyl-β-alaninate (88)

To a stirred suspension of methyl β-alaninate hydrochloride (**87**) (4.885 g, 35 mmol, 1 equiv.) and triethylamine (9.2 ml, 70 mmol, 2 equiv.) in dry dichloromethane (75 ml), cooled in an ice-bath, was added dropwise benzoyl chloride (4.1 ml, 35 mmol, 1 equiv.). The reaction mixture was allowed to warm to room temperature, and stirring was continued at ambient temperature for 1 h. Volatiles were removed *in vacuo*, the residue was taken up in water (50 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a white solid which was crystallised from diethyl ether to give methyl *N*-benzoyl-β-alaninate as a white solid (5.323 g, 74%), m.p. 59-62 °C; ν_{max}/cm^1 ($CHCl_3$) 3 460, 3 020, 2 960, 1 730, 1 660, 1 605, 1 585, 1 520, 1 490, 1 440, 1 370, 1 200 and 1 180; δ_H (200 MHz) 2.61 (2H, t, J 6.0 Hz), 3.66 (3H, s), 3.68 (2H, q, J 6.1 Hz), 7.08 (br s), 7.29 - 7.51 (3H, m), 7.68 - 7.79 (2H, m); δ_C (50 MHz) 33.57 (t), 35.24 (t), 51.72 (q), 126.84 (d), 128.39 (d), 131.39 (d), 134.13 (s), 167.40 (s), 173.13 (s); *m/z* 207 (M^+, 8.9%), 192 (2.2), 176 (7.0), 148 (1.8), 105 (100.0), and 77 (54.0); (Found: M^+, 207.0878. $C_{11}H_{13}NO_3$ requires M^+, 207.0895).

Attempted Synthesis of Methyl 2-Iodo-3-benzamidopropanoate (82)[237]

To a stirred solution of *N*-isopropylcyclohexylamine (4.1 ml, 25 mmol, 2 equiv.) in dry THF (25 ml), cooled in an N_2 atmosphere to -78 °C, was carefully added by syringe a 1.67M butyl lithium-hexanes solution (15 ml, 25 mmol, 2 equiv.). The resulting solution was stirred at -78 °C for 5 min, then a solution of methyl 3-benzamidopropionate (2.591 g, 12.5 mmol, 1 equiv.) in dry THF (10 ml) was added by syringe. The reaction mixture was stirred for 30 min at -78 °C, then allowed to warm to ambient temperature. A yellow solid precipitated. The resulting suspension was transferred by syringe into a flask charged with a solution of iodine (7.614 g, 30 mmol, 2 equiv.) in dry THF (15 ml) at -78 °C. The last traces of the suspension were washed into the iodine solution with THF (15 ml). Concentrated HCl (5 ml) was injected, the solution was allowed to warm to room temperature and stirring was maintained overnight. The reaction mixture was washed with 1M sodium thiosulphate solution (4 x 100 ml), the organic layer was dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give a tan-coloured semi-solid (1.605 g). TLC analysis (eluted with pet. ether, visualised with 254 nm UV light) revealed a complex mixture of reaction products, the main component of which was identified as methyl 3-benzamidopropanoate by TLC.

8.2.5 Enzyme-catalysed Reactions of Amino Alcohols and Derivatives

8.2.5.1 P.P.L.-Catalysed Acetylation of (+)-Prolinol and (-)-Prolinol

A mixture of (R)-(-)-2-pyrrolidinemethanol (0.026 g, 0.26 mmol) and P.P.L. (0.130 g) in ethyl acetate (20 ml) was shaken in a water bath at 28 °C. A second reaction was run simultaneously using (S)-(+)-2-pyrrolidinemethanol (0.026 g). The progress of the reactions was monitored by TLC ($CHCl_3$/MeOH/NH_3; 85:14:1) and the time taken for *N*-acetylprolinol and *N,O*-diacetylprolinol to be detected in the reaction mixture was noted.

The two reactions were repeated using a water bath temperature of 20 °C. Finally, another two reactions were carried out, using (R)- or (S)-2-pyrrolidinemethanol (0.120 g, 1.15 mmol) and P.P.L. (0.500 g) in a mixture of ethyl acetate (0.35 ml, 3.6 mmol) and hexane (20 ml), shaken in a water bath at 17 °C. The observed appearance times of *N*-acetyl- and *N,O*-diacetylprolinol are shown in Table 5.2 (See Chapter 5).

8.2.5.2 P.P.L.-Catalysed Acetylation of (±)-2-Pyrrolidinemethanol

Procedure 1: A mixture of (R)-(-)-prolinol (25 mg), (S)-(+)-prolinol (25 mg) and P.P.L. (120 mg) in ethyl acetate was shaken in a water bath at 25 °C. After the required reaction time had elapsed, the enzyme was removed by filtration and the filtrate was concentrated *in vacuo* to give a brown oil. This was subjected to column chromatography on basic alumina. *N*-Acetylprolinol was eluted with 2%

MeOH/CH_2Cl_2, and prolinol was eluted with 1% NH_3/MeOH. Products were characterised by ^1H N.M.R. (90 MHz, $CDCl_3$, ref. TMS), mass spectrum and TLC, using chemically synthesised material as reference standards. No *N,O*-diacetylprolinol was formed in the duration of these enzyme reactions. The results obtained are shown in Table 5.3 (See Chapter 5).

Procedure 2: A mixture of (R)-(-)-prolinol (50 mg), (S)-(+)-prolinol (50 mg), and P.P.L. (150 mg) in ethyl acetate (30 ml) was shaken in a water bath at 25 °C. After the required reaction time, the reactions were treated as described in Procedure 1 to give a brown oil, which was subjected to column chromatography on basic alumina. *N,O*-Diacetylprolinol was eluted with 20% EtOAc/CH_2Cl_2 and *N*-acetylprolinol was eluted with 20% MeOH/EtOAc. Satisfactory recovery of prolinol from the column could not be obtained, even when eluting with 10% NH_3/MeOH. Products were characterised as described in Procedure 1, and the results obtained are shown in Table 5.4 (See Chapter 5).

8.2.5.3 P.P.L.-catalysed Acetylation of *N*-Methyl-2-piperidinemethanol

Procedure 1: A mixture of (±)-2-*N*-methyl-2-piperidinemethanol (50 mg) and P.P.L. (120 mg) in EtOAc (30 ml) was shaken in a water bath at 25 °C. After the required reaction time had elapsed, the enzyme was removed by filtration and the filtrate was concentrated *in vacuo* to give a brown oil. This was then subjected to column chromatography on basic alumina. *N*-Methyl-2-piperidinemethyl acetate was eluted with 30% EtOAc/CH_2Cl_2. *N*-Methyl-2-piperidinemethanol could not be satisfactorily recovered from the column. The reaction product was identified by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS), mass spectrum and TLC, using

chemically-prepared material as a reference standard. The results obtained are shown in Table 5.5 (See Chapter 5).

Procedure 2: A mixture of (±)-*N*-methyl-2-piperidinemethanol (100 mg) and P.P.L. (120 mg) in EtOAc (30 ml) was shaken in a water bath at 25 °C. After 14 h, the enzyme was removed by filtration and the filtrate was concentrated *in vacuo* to give a brown oil. This was subjected to column chromatography on neutral alumina, eluting with 15% CH_2Cl_2/hexane to give *N*-methyl-2-piperidinemethyl acetate, identified by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS), mass spectrum and TLC, by comparison with a chemically prepared reference standard. The result obtained is shown in Table 5.6 (See Chapter 5).

Procedure 3: A mixture of (±)-*N*-methyl-2-piperidinemethanol (100 mg) and P.P.L. (150 mg) in EtOAc (30 ml) was shaken in a water bath at 25 °C. After the required reaction time had elapsed, the reaction mixture was subjected to the work-up procedure described previously to yield a brown oil. this was subjected to column chromatography on basic alumina. *N*-Methyl-2-piperidinemethyl acetate was eluted with 30% EtOAc/CH_2Cl_2, and was characterised by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS), mass spectrum and TLC, by comparison with a chemically prepared reference sample. The results obtained are shown in Table 5.7 (See Chapter 5).

Procedure 4: A mixture of (±)-*N*-methyl-2-piperidinemethanol (100 mg) and P.P.L. (610 mg) in EtOAc (20 ml) was shaken in a water bath at 27 °C. The reaction mixture was subjected to the work-up procedure described previously, to give a brown oil. This was subjected to flash column chromatography on silica (230-400 mesh), eluting with a freshly prepared solution of $CHCl_3$/MeOH/NH_3, 85;14;1. Once again,

satisfactory recovery of *N*-methyl-2-piperidinemethanol could not be achieved. The results obtained are shown in Table 5.8 (See Chapter 5).

8.2.5.4 P.P.L.-Catalysed Acetylation of (±)-*N*-Ethoxycarbonyl-2-piperidinemethanol

Procedure 1: A mixture of (±)-*N*-ethoxycarbonyl-2-piperidinemethanol (0.150 g, 0.8 mmol) and P.P.L. (0.634 g) in ethyl acetate (20 ml) was shaken in a water bath at 20°C. After 48 h, the enzyme was removed by filtration, and washed with EtOAc. The combined washings and filtrate were concentrated *in vacuo* to give a mixture containing both ester and alcohol. This was subjected to flash column chromatography (17 g SiO_2, 230-400 mesh), eluting with EtOAc. Insufficient carbamoyl-ester (<4 mg) was obtained for characterisation. Unreacted carbamoyl-alcohol was obtained as a clear oil (0.138 g, 92%), identified by comparison with a previously obtained sample; $[\alpha]_D$ 0° (c 1.9, MeOH).

Procedure 2: A mixture of (±)-*N*-ethoxycarbonyl-2-piperidinemethanol (0.187 g, 1 mmol), vinyl acetate (0.300 ml, 3.2 mmol) and P.P.L. (0.610 g) in hexane (5 ml) was stirred magnetically at room temperature. After 15 h, the reaction mixture had aquired an orange colour and had become very viscous. No *N*-ethoxycarbonyl-2-piperidinemethyl acetate was detectable in the reaction mixture by TLC.

A similar reaction was performed using DCM (5 ml) as solvent. Once again, polymerisation occurred and no ester was detectable by TLC. The reaction was repeated in a flask wrapped in aluminium foil to exclude light, with similar results. At this stage, the reaction was abandoned.

8.2.5.5 P.P.L.-Catalysed Acetylation of (±)-2-Piperidinemethanol (after Asensio *et al.*)[238]

A mixture of (±)-2-piperidinemethanol (0.461 g, 4 mmol) and P.P.L. (1.000 g) in ethyl acetate (20 ml) was stirred magnetically at room temperature for 4 h. The enzyme was removed by filtration, the filtrate was evaporated *in vacuo,* and the residue was subjected to positive pressure column chromatography (SiO_2, 230 - 400 mesh). *N*-Acetyl-2-piperidinemethanol was eluted with EtOAc/MeOH (10:1) as a thick yellow, air sensitive oil (0.183 g, 29%); $[\alpha]_D$ -31.6° (c 0.5, $CHCl_3$) (e.e. 69%); ν_{max}/cm^{-1} ($CHCl_3$) 3 600 - 2 700, 3 010, 2 940, 2 860, 1 620 and 1 440; *m/z* 157 (M^+, 0.9%), 139 (1.4), 126 (24.0) and 84 (100.0).

Some unreacted 2-piperidinemethanol was eluted with EtOAc/MeOH/Et_2O (100:20:1) as a yellow solid (0.161 g, 35%); $[\alpha]_D$ 0.0 °; ν_{max}/cm^{-1} ($CHCl_3$) 3 600 - 2 300, 2 940, 1 450, 1 225 and 1 050; *m/z* 115 (M^+, 0.7%), 98 (6.4) and 84 (100.0).

8.3 *Experimental for Chapter Six*

8.3.1 General Procedures

General Procedure for Synthesis of *N*-Benzoyl Amino Acids[258]

To a solution of the amino acid (1 equiv.) in 10% NaOH solution was added, in several portions, benzoyl chloride. After each addition of acid chloride, the flask was subjected to vigorous shaking until all the benzoyl chloride had reacted. Once reaction

was complete, conc. HCl was added to the solution until the reaction mixture was acidic to Congo Red indicator paper. Crude product was isolated by filtration, and final purification was achieved by crystallisation from ethyl acetate.

General Procedures for the Cyclisation of *N*-Acyl Amino Acids

Procedure A (DCC method)

To a stirred suspension of the *N*-acyl amino acid (1 equiv.) in Et_2O was added DCC (1 equiv.) as an ethereal solution. The reaction mixture was stirred at room temperature for 4-24 h. Precipitated DCU was removed by filtration, and the filtrate was concentrated *in vacuo*. The crude product was crystallised from Et_2O/pet.ether to give the azlactone.

Procedure B (EDC-HCl method)[260]

To a stirred suspension of the *N*-acyl amino acid (1 equiv.) in dried DCM was added EDC-HCl (1 equiv.). The reaction mixture was stirred at room temperature until homogeneous (30 - 60 min), and then washed with water (3 x 5 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the azlactone.

8.3.2 Synthesis of Azlactones

N-Benzoyl-DL-phenylalanine (93)

DL-Phenylalanine (3.304 g, 20 mmol) and benzoyl chloride (2.5 ml, 22 mmol) were reacted as described above to obtain *N*-benzoyl-DL-phenylalanine as a white solid (3.929 g, 73%), m.p. 189-191 °C (lit,[302] 187-188 °C); ν_{max}/cm^{-1} (KBr

disc) 3 700 - 2 200, 3 320, 1 720, 1 610, 1 570, 1 540, 1 270 and 1 230; δ_H (200 MHz, d_6-DMSO, ref. d_5-DMSO) 3.02 - 3.31 (2H, m, 3-H_2), 4.57 - 4.69 (1H, m, 2-H), 7.10 - 7.83 (10H, m, 2 x Ph), 8.57 (1H, br d, NH); δ_C (50 MHz, d_6-DMSO, ref. DMSO) 36.68 (t, C-3), 54.68 (d, C-2), 126.28 (d, Ph), 127.34 (d, Ph), 128.17 (d, Ph), 128.32 (d, Ph), 128.48 (d, Ph), 129.23 (d, Ph), 131.32 (d, Ph), 134.30 (s, Ph), 138.66 (s, Ph), 166.27 (s, C=O amide), 173.78 (s, C=O acid); *m/z* 269 (M^+, 1.7%), 225 (6.0), 148 (39.2), 105 (100.0) and 91 (29.2) (Found: C, 71.3%; H, 5.7; N, 5.0; M^+, 269.1047. $C_{16}H_{15}NO_3$ requires C, 71.4; H, 5.6; N, 5.2%; M^+, 269.1052).

<u>*N*-Benzoyl-DL-valine</u> (94)

DL-Valine (0.586 g, 5 mmol) and benzoyl chloride (0.64 ml, 5.5 mmol) were reacted as described above to obtain *N*-benzoyl-DL-valine as a white solid (0.659 g, 60%), m.p. 131-133 °C (EtOAc) (lit.,[302] 132.5 °C); ν_{max}/cm^{-1} (KBr disc) 3 600 - 2 300, 3 360, 1 730, 1 630, 1 575 and 1 535; δ_H (200 MHz, D_2O + NaOD, ref. HOD at 4.63 ppm) 0.73 - 0.79 (6H, m), 1.87 - 2.12 (1H, m), 4.05 (1H, d, J 6 Hz), 7.23 - 7.57 (5H, m); δ_C (50 MHz, D_2O + NaOD, ref. dioxan at 67.4 ppm) 18.67 (q), 20.08 (q), 31.90 (d), 62.58 (d), 128.28 (d), 129.86 (d), 133.23 (d), 134.90 (s), 170.20 (s), 180.00 (s); *m/z* 221 (M^+, 1.7%), 222 (0.3), 176 (10.9), 161 (9.0) and 105 (100.0) (Found: C, 65.2%; H, 6.7; N, 6.2; M^+, 221.1058. $C_{11}H_{15}NO_3$ requires C, 65.1; H, 6.8; N. 6.3; M^+, 221.1052).

<u>*N*-Benzoyl-DL-alanine</u> (95)

DL-Alanine (8.909 g, 0.1 mol) and benzoyl chloride (12.8 ml, 0.11 mol) were reacted as described above to obtain *N*-benzoyl-DL-alanine as a white solid

(14.788 g, 77%), m.p. 174-176 °C (EtOAc) (lit.,[302] 165-166 °C); ν_{max}/cm^{-1} (KBr disc) 3 600 - 2 200, 3 370, 1 710, 1 630, 1 580, 1 540 and 1 210; δ_H (200 MHz, D_2O + NaOD, ref. dioxan) 1.25 - 1.29 (3H, d, 3-H_3), 4.06 - 4.24 (1H, q, 2-H), 7.26 - 7.58 (5H, m, Ph); δ_C (50 MHz, D_2O + NaOD, ref. dioxan) 18.35 (q, C-3), 52.49 (d, C-2), 128.00 (d, Ph), 129.56 (d, Ph), 132.95 (d, Ph), 134.21 (s, Ph), 170.67 (s, C=O amide), 181.14 (s, C=O acid); *m/z* 193 (M^+, 3.8%), 148 (34.6) and 105 (100.0) (Found: M^+, 193.0734. $C_{10}H_{11}NO_3$ requires M^+, 193.0739).

(±)-2-Phenyl-4-benzyl-1,3-oxazol-5(4H)-one (96)

(±)-*N*-Benzoylphenylalanine (1.000 g, 3.7 mmol) was reacted with DCC (0.766 g, 3.7 mmol) in Et_2O (30 ml) according to method A. The reaction mixture was stirred for 24 h, and then purified as described previously to give (±)-2-phenyl-4-benzyl-1,3-oxazol-5(4H)-one as a white solid (0.213 g, 23%), m.p. 72-73 °C (lit.,[256] 69-71 °C); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 1 820, 1 660, 1 490, 1 445, 1 210 and 1 050; δ_H (200 MHz) 3.12 - 3.43 (2H, m, Ph**C**H_2), 4.64 - 4.72 (1H, m, 4-H), 7.24 - 7.93 (10H, m, 2 x Ph); δ_C (50 MHz) 37.24 (t, Ph**C**H_2), 66.46 (d, C-4), 125.69 (s, Ph), 127.13 (d, Ph), 127.80 (d, Ph), 128.35 (d, Ph), 128.67 (d, Ph), 129.52 (d, Ph), 132.65 (d, Ph), 135.20 (s, Ph), 161.62 (s, C-2), 172.53 (s, C-5); *m/z* 251 (M^+, 17.6%), 105 (28.9) and 91 (100.0) (Found: M^+, 251.0940. $C_{16}H_{15}NO_2$ requires 251.0946).

(±)-2,4-Dimethyl-1,3-oxazol-5(4H)-one (97)

(±)-*N*-Acetylalanine (0.656 g, 5 mmol) was reacted with EDC-HCl (0.958 g. 5 mmol) in dried CH_2Cl_2 (10 ml) according to method B, to give (±)-2,4-dimethyl-1,3-oxazol-5(4H)-one as a clear oil (0.370 g, 65%); ν_{max}/cm^{-1} ($CHCl_3$) 3 020,

2 990, 2 930, 1 820, 1 680, 1 500, 1 385, 1 230, 1 110 and 1 005; δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.4 (3H, d, J 7.6 Hz), 2.2 (3H, d, J 2.0 Hz), 4.0 - 4.2 (1H, m); δ_C (50 MHz) 14.84 (q), 16.14 (q), 60.06 (d), 162.46 (s), 178.98 (s); *m/z* 113 (M^+, 1.4%), 114 (0.2), 85 (8.4), 69 (63.4) and 43 (100.0).

(Product decomposed before an accurate mass could be obtained).

(±)-2-Phenyl-4-isopropyl-1,3-oxazol-5(4H)-one (98)

(±)-*N*-Benzoylvaline (0.443 g, 2 mmol) was reacted with EDC-HCl (0.383 g, 2 mmol) in CH_2Cl_2 (10 ml) according to method B, to give (±)-2-phenyl-4-isopropyl-1,3-oxazol-5(4H)-one as a clear oil (0.352 g, 87%); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 960, 2 860, 1 810, 1 650, 1 580 and 1 450; δ_H (200 MHz) 0.93 (3H, d, J 6.9 Hz, CH_3), 1.05 (3H, d, J 6.9 Hz, CH_3), 2.17 - 2.37 (1H, m, **CH**Me_2), 4.20 (1H, d, J 3.5 Hz, 4-H), 7.33 - 7.47 (3H, m, Ph), 7.89 - 7.94 (2H, m, Ph); δ_C (50 MHz) 17.20 (q, CH_3), 18.41 (q, CH_3), 30.88 (d, **CH**Me_2), 70.19 (d, C-5), 125.61 (s, Ph), 127.55 (d, Ph), 128.44 (d, Ph), 132.33 (d, Ph), 161.34 (s, C-2), 177.22 (s, C-5); *m/z* 203 (M^+, 2.5%), 204 (0.4), 161 (30.0), 144 (10.5), 133 (22.1), 105 (69.3), 77 (97.0) and 29 (100.0).

(Sample decomposed before an accurate mass could be obtained).

(±)-2-Phenyl-4-methyl-1,3-oxazol-5(4H)-one (99)

N-Benzoyl alanine (0.966 g, 5 mmol) was reacted with EDC-HCl (0.958 g, 5 mmol) in dried CH_2Cl_2 (10 ml) according to method B, to give (±)-2-phenyl-4-methyl-1,3-oxazol-5(4H)-one as a white solid.(0.664 g, 76%), m.p. 38-40 °C; ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 1 830, 1 665, 1 460, 1 330, 1 300, 1 220 and 1 170; δ_H (200 MHz) 1.48 (3H, d, J 6.5 Hz), 4.32 (3H, q, J 6.5 Hz), 7.26 - 8.01 (5H, m);

m/z 175 (M^+, 1.1%), 147 (5.3), 131 (47.6), and 105 (100.0) (Found: M^+, 175.0642. $C_{10}H_9NO_2$ requires M^+, 175.0640).

1-Butyl (±)-*N*-benzoylalaninate (101)

To a suspension of (±)-*N*-benzoylalanine (0.966 g, 5 mmol) in dried CH_2Cl_2 (15 ml) was slowly added 1,1'-carbonyldiimidazole (0.811 g, 5 mmol). When effervescence had ceased, 1-butanol (0.500 ml, 5.5 mmol) was added to the reaction mixture. The resulting solution was stirred at room temperature for 30 min, and then washed with 1M HCl (2 x 10 ml), 2% Na_2CO_3 (10 ml), and water (10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 1-butyl (±)-*N*-benzoylalaninate as a clear oil which solidified on standing (0.852 g, 68%), m.p. 58-60 °C; ν_{max}/cm^{-1} (KBr disc) 3 290, 2 960, 1 740, 1 635, 1 540 and 1 270; δ_H (200 MHz) 0.88 - 0.96 (3H, t), 1.14 - 1.90 (7H, m and d), 4.12 - 4.25 (2H, t), 4.67 - 4.89 (1H, m), 6.75 - 6.97 (1H, br d, NH), 7.34 - 7.58 (3H, m, Ph), 7.74 - 7.87 (2H, m, Ph); δ_C (50 MHz) 13.61 (q), 18.68 (q), 18.98 (t), 30.48 (t), 48.52 (d), 65.42 (t), 126.97 (d), 128.50 (d), 131.62 (d), 133.94 (s), 166.72 (s), 173.29 (s); *m/z* 249 (M^+, 3.1%), 148 (48.9) and 105 (100.0) (Found: C, 67.3%; H, 7.4; N, 5.7; M^+, 249.1371. $C_{14}H_{19}NO_3$ requires C, 67.5; H, 7.6; N, 5.6%; M^+, 249.1365).

8.3.3 Enzyme-Catalysed Alcoholysis of Azlactones

PPL-Catalysed Butanolysis of (±)-2-Phenyl-4-benzyl-1,3-oxazol-5(4H)-one (96)

To a solution of the azlactone (0.251 g, 1 mmol) in hexane (10 ml) was added PPL (0.600 g) and 1-butanol (0.185 ml, 2 mmol). The resulting mixture was shaken in a water bath at 40 °C for 72 h. The enzyme was then removed by filtration, and washed with acetone. Solvent was removed *in vacuo* to give a thick oil (0.219 g), which was shown by ^{1}H N.M.R. spectroscopy to contain a 58:42 mixture of ester and azlactone. This was subjected to flash column chromatography (30 g SiO_2, 230-400 mesh), eluting with DCM. Butyl *N*-benzoylphenylalaninate (**100**) was obtained as a viscous oil (0.132 g, 41%), $[\alpha]_D$ -1.8° (c 0.7, acetone); ν_{max}/cm^{-1} ($CHCl_3$) 3 440, 3 020, 2 960, 2 940, 1 735, 1 670, 1 605, 1 580, 1 520 and 1 220; δ_H (90 MHz, $CDCl_3$, ref. TMS) 0.8 - 1.1 (3H, unres. t), 1.1 - 2.0 (4H, unres. m), 3.3 (2H, d), 4.1 (2H, t), 5.0 - 5.2 (1H, m), 6.8 (1H, br d), 7.1 - 7.6 (8H, m), 7.6 - 7.9 (2H, m); *m/z* 325 (M^+, 0.9%), 234 (0.3), 224 (5.0), 148 (20.4) and 105 (100.0).

Unreacted (±)-2-Phenyl-4-benzyl-1,3-oxazol-5(4H)-one was obtained as a white solid (0.048 g, 19%), $[\alpha]_D$ 0° (c 1.1, acetone), identified by comparison with an authentic sample.

The above reaction was repeated using anhydrous THF (5 ml) as solvent. After 72 h, the reaction mixture was examined by ^{1}H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref TMS), and found to contain a 54:46 mixture of azlactone and ester. After 96 h, the reaction was worked up as before, and the resulting oil was subjected to flash column chromatography (20 g SiO_2, 230-400 mesh), eluting with DCM. Butyl *N*-

benzoylphenylalaninate was obtained as a white solid (0.054 g, 17%), which gave identical physical data to the previously obtained sample ($[\alpha]_D$ 0° (c 1.4, acetone)). Unreacted azlactone was recovered as a white solid (0.062 g, 25%), which gave identical physical data to previously obtained material ($[\alpha]_D$ 0° (c 1.2, acetone)).

Non-Enzymatic Butanolysis of (±)-2-Phenyl-4-benzyl-1,3-oxazol-5(4H)-one

A solution of azlactone (0.251 g, 1 mmol) and 1-butanol (0.370 ml, 4 mmol) in anhydrous THF (5 ml) was shaken in a water bath at 40 °C, and the reaction was monitored by TLC. After 5 days, no ester was detectable in the reaction mixture.

8.3.4 Synthesis of 4,5-Dihydro-1,3-oxazin-6-ones

8.3.4.1 Synthesis of *N*-Acyl β-Amino Acids

(±)-3-Benzamidobutanoic Acid (102)

(±)-3-Aminobutanoic acid (2.005 g, 19.4 mmol) and benzoyl chloride (3 ml, 25.9 mmol) were reacted as described above to give (±)-3-benzamidobutanoic acid as a white crystalline solid (3.667 g, 91%), m.p. 160-161 °C (EtOAc); ν_{max}/cm^{-1} (KBr disc) 3 600 - 2 400, 3 320, 1 700, 1 630, and 1 555; δ_H (200 MHz, $CDCl_3$ + d_6-DMSO, ref. TMS) 1.32 (3H, d, 4-H_3), 2.47 - 2.71 (2H, m, 2-H_2), 4.42 - 4.61 (1H, m, 3-H), 7.36 - 7.83 (5H, m, Ph); δ_C (50 MHz, $CDCl_3$ + d_6-DMSO, ref. $CDCl_3$) 19.42 (q, C-4), 39.45 (t, C-2), 41.84 (d, C-3), 126.40 (d, Ph), 127.52 (d, Ph), 130.44 (d, Ph), 133.97 (s, Ph), 165.86 (s, C=O amide), 172.87 (s, C=O acid); *m/z*

207 (M^+, 8.4%), 148 (3.9), 105 (100), and 77 (58.3); (Found: C, 63.9%; H, 6.4; N, 6.8; M^+, 207.0891. $C_{11}H_{13}NO_3$ requires C, 63.8; H, 6.3; N, 6.8%; M^+, 207.0896).

(±)-3-Benzamido-3-phenylpropanoic Acid (111)

(±)-3-Amino-3-phenylpropanoic acid (2.005 g, 12.1 mmol) and benzoyl chloride (1.6 ml, 13.8 mmol) were reacted as described above to give (±)-3-benzamido-3-phenylpropanoic acid as a white crystalline solid (2.894 g, 89%), m.p. 211-213 °C (EtOAc); ν_{max}/cm^{-1} (KBr disc) 3 700 - 2 400, 3 370, 1 710, 1 640, and 1 520; δ_H (200 MHz, D_2O + NaOD, ref. replacement dioxan in D_2O at 4.63 ppm) 2.35 (2H, d, J 8 Hz), 4.95 (1H, t, J 8 Hz), 6.84 - 7.12 (8H, m), 7.17 - 7.26 (2H, m); δ_C (50 MHz, D_2O + NaOD, external ref. dioxan at 67.4 ppm) 44.91 (t), 53.40 (d), 127.31 (d), 128.20 (d), 128.67 (d), 129.80 (d), 129.91 (d), 133.18 (d), 134.72 (s), 142.75 (s), 171.31 (s), 180.05 (s); *m/z* 269 (M^+, 1.7%), 164 (44.5), 105 (71.3), and 77 (100.0); (Found: M^+, 269.1051. $C_{16}H_{15}NO_3$ requires M^+, 269.1052).

8.3.4.2 Cyclisation of *N*-Acyl β-Amino Acids

a) DCC Cyclisation of (±)-3-Benzamidobutanoic Acid

To a stirred suspension of (±)-3-benzamidobutanoic acid **(102)** (0.518 g, 2.5 mmol, 1 equiv) in dry diethyl ether (15 ml) was added a solution of *N,N'*-dicyclohexylcarbodiimide (0.516 g, 2.5 mmol, 1 equiv) in dry diethyl ether (5 ml). The resulting mixture was stirred at room temperature for 1 h. The mixture was filtered, and volatiles were removed *in vacuo* to give a white solid, shown by 90 MHz ^1H N.M.R. spectroscopy to be highly contaminated with *N,N'*-dicyclohexylurea. The residue was taken up in diethyl ether, urea was removed by filtration and volatiles were

removed *in vacuo*. This procedure was repeated several times, with no noticeable improvement in the purity of the cyclised product.

The reaction was repeated using other solvents (ethyl acetate, dichloromethane, carbon tetrachloride). In all cases, it proved impossible to remove *N,N'*-dicyclohexylurea completely from the reaction product.

b) Attempted Acetic Anhydride Cyclisation of (±)-3-Benzamidobutanoic Acid

A suspension of (±)-3-benzamidobutanoic acid (0.207 g, 1 mmol, 1 equiv) in acetic anhydride (10 ml) was heated to 110-115 °C for 15 min, by which time the reaction mixture had become homogeneous. The reaction mixture was allowed to cool to room temperature, and volatiles were removed by lyophilisation *in vacuo*. The last traces of acetic anhydride were removed by azeotropic distillation *in vacuo* with toluene to give a white solid, identified by ^{1}H N.M.R. spectroscopy (90 MHz, $CDCl_3$ + d_6-DMSO, ref. TMS) and by melting point as starting material.

c) Attempted Cyclisation of (±)-3-Benzamidobutanoic Acid Using Isobutyl chloroformate[263]

A solution of (±)-3-benzamidobutanoic acid (0.500 g, 2.24 mmol, 1 equiv) and triethylamine (0.42 ml, 2.9 mmol, 1.2 equiv) in dry DCM (5 ml) was cooled in a nitrogen atmosphere to -15 °C. To this was added, carefully by syringe, isobutyl chloroformate (0.38 ml, 2.9 mmol, 1.2 equiv). During the addition, the temperature of the ice-bath rose to -12 °C. The reaction mixture was stirred at between -10 °C and -15 °C for 15 min, then at 0 °C for 10 min, and then finally at room temperature for 15 min.

Volatiles were removed *in vacuo*, the residue was taken up in pet. ether, filtered, and concentrated *in vacuo* to give (±)-2-phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one as a clear oil, heavily contaminated with isobutyl chloroformate (0.456 g). Final purification was attempted by a variety of methods (flash column chromatography, trituration, reduced pressure distillation) to no avail; ν_{max}/cm^{-1} (neat) 2 970, 2 940, 2 880, 1 795 and 1 620; δ_C (50 MHz) 18.18 (q), 21.08 (q), 27.23 (d), 34.82 (t), 48.65 (t), 75.30 (t), 127.26 (d), 128.04 (d), 131.31 (d), 130.08 (s), 148.32 (s), 151.96 (s), 165.74 (s).

d) Cyclisation of (±)-3-Benzamidobutanoic Acid using Mukaiyama's Reagent[264]

To a stirred solution of (±)-3-benzamidobutanoic acid (0.500 g, 2.42 mmol, 1 equiv) and triethylamine (0.81 ml, 5.8 mmol, 2.4 equiv) in dry DCM (10 ml), in a N_2 atmosphere, was added 2-chloro-1-methylpyridinium iodide (0.742 g, 2.9 mmol, 1.2 equiv). The resulting mixture was heated to reflux under nitrogen for 3 h. Volatiles were removed *in vacuo*, the residue was taken up in acetone and insoluble salts were removed by filtration. Volatiles were again removed *in vacuo*, and the residue was purified by flash column chromatography (SiO_2, 230-400 mesh), eluting with ethyl acetate to give (±)-2-phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one as a yellow oil (0.412 g, 90%); ν_{max}/cm^{-1} (neat) 1 790, 1 670, 1 230, 1 140, 1 040, 1 020 and 695; δ_H (200 MHz) 1.33 (3H, d, CH_3), 2.26 - 2.79 (2H, m, 5-H_2), 3.83 - 4.03 (1H, m, 4-H), 7.31 - 7.49 (3H, m, Ph), 7.92 - 8.02 (2H, m, Ph); δ_C (50 MHz) 21.27 (q, CH_3), 35.03 (t, C-5), 49.79 (d, C-4), 127.42 (d, Ph), 128.21 (d, Ph), 130.24 (s, Ph), 131.50 (d, Ph), 152.17 (s, C-2), 165.94 (s, C-6); *m/z* 189 (M^+, 14.8%), 148 (4.4), 105 (100.0); (Found: M^+, 189.0786. $C_{11}H_{11}NO_2$ requires M^+, 189.0790).

e) Cyclisation of (±)-3-Benzamidobutanoic Acid Using Mukaiyama's Reagent (Large Scale)

(±)-3-Benzamidobutanoic acid (1.892 g, 9 mmol, 1 equiv.), triethylamine (3.4 ml, 24 mmol, 2.7 equiv.), and 2-chloro-1-methylpyridinium iodide (3.066 g, 12 mmol, 1.3 equiv.) were reacted as before to give (±)-2-phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one as a yellow oil, identical in all respects to the previously obtained sample (0.705 g, 41%).

f) Attempted CDI Cyclisation of (±)-3-Benzamidobutanoic Acid[266]

A suspension of (±)-3-benzamidobutanoic acid (0.385 g, 1.857 mmol, 1 equiv.) in dry DCM (8 ml) was stirred in an atmosphere of nitrogen. To this was added *N,N'*-carbonyldiimidazole (0.301 g, 1.857 mmol, 1 equiv.). The reaction mixture quickly became homogeneous. An aliquot was withdrawn and examined by 90 MHz ^{1}H N.M.R. spectroscopy. The doublet at δ 2.4 ppm due to starting material had disappeared, to be replaced by a multiplet centred at δ 3.2 ppm, indicating complete conversion into the acylimidazole intermediate. Stirring was continued overnight at room temperature. A further aliquot was withdrawn and studied by ^{1}H N.M.R. spectroscopy as before. No cyclised product was observed. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (SiO_2, 230-400 mesh), eluting with acetone to give *N*-[(±)-3-benzamidobutanoyl]-imidazole as a white, moisture-sensitive solid (0.381 g, 80%), which rapidly decomposed before physical data could be obtained.

g) Attempted Cyclisation of *N*-[(±)-3-Benzamidobutanoyl]-imidazole by Heating at Reflux

A suspension of (±)-3-benzamidobutanoic acid (0.385 g, 1.857 mmol, 1 equiv.) in dry DCM (8 ml) was stirred at room temperature in an atmosphere of nitrogen. To this was added *N,N'*-carbonyldiimidazole (0.301 g, 1.857 mmol, 1 equiv.). The resulting mixture was stirred at room temperature until homogeneous, then heated to reflux for 3 h. An aliquot was withdrawn and examined by 90 MHz ^{1}H N.M.R. spectroscopy. No cyclised product was present. Heating was continued overnight, and the reaction mixture was concentrated *in vacuo* to give a white solid, identified as *N*-[(±)-3-benzamidobutanoyl]-imidazole by 90MHz ^{1}H N.M.R. spectroscopy and TLC analysis.

This procedure was repeated using distilled acetonitrile (8 ml) as solvent. Again, after heating at reflux overnight no cyclised product was identified.

h) Attempted Base-promoted Cyclisation of *N*-[(±)-3-Benzamidobutanoyl]-imidazole Using LDA

A solution of distilled diisopropylamine (0.112 ml, 0.8 mmol, 1 equiv) in dry THF (4 ml) was cooled to -78 °C in an atmosphere of nitrogen. To this was added a 1M solution of butyl lithium in hexanes (0.8 ml, 0.8 mmol, 1 equiv). The resulting solution was stirred for 15 min at -78 °C. To this was added by syringe a solution of *N*-[(±)-3-benzamidobutanoyl]-imidazole (0.200 g, 0.8 mmol, 1 equiv.) in dry THF (2 ml). The resulting solution was stirred at -78 °C for 15 min, and was then allowed to warm to room temperature while stirring was continued for 3 h. Volatiles were removed *in vacuo*, the residue was taken up in ethyl acetate (5 ml), washed with 1M

HCl (3 ml), saturated $NaHCO_3$ solution (3 ml), then water (3 ml). The organic layer was dried over anhydrous Na_2SO_4 and volatiles were removed *in vacuo* to give a yellow oil (15 mg), with a complex 90 MHz ^1H N.M.R. spectrum, and it was shown to contain several compounds by TLC.

i) Attempted Base-promoted Cyclisation of *N*-[(±)-3-Benzamidobutanoyl]-imidazole using Potassium *t*-Butoxide

A suspension of (±)-3-benzamidobutanoic acid (0.385 g, 1.857 mmol, 1 equiv.) in dry DCM (8 ml) was stirred at room temperature in an atmosphere of nitrogen. To this was added *N,N'*-carbonyldiimidazole (0.301 g, 1.857 mmol, 1 equiv.). Stirring was continued for 1 h, after which time the solution was cooled in an ice/methanol bath to -10 °C. A solution of potassium *t*-butoxide (0.208 g, 1.857 mmol, 1 equiv.) in dry THF (2 ml) was added by syringe, the reaction mixture was allowed to warm to room temperature, and stirring was continued for 2 h. The reaction mixture was then washed with 3M HCl (2 x 8 ml), saturated $NaHCO_3$ solution (8 ml), and finally water (8 ml). The organic layer was dried over anhydrous Na_2SO_4. Volatiles were removed *in vacuo* to give 26 mg of solid, shown by TLC to contain a complex mixture of compounds.

j) Attempted Cyclisation of (±)-3-Benzamidobutanoic Acid *via p*-Nitrophenyl Ester

i) Formation of *p*-Nitrophenyl (±)-3-Benzamidobutanoate (110)

To a solution of *p*-nitrophenol (0.335 g, 2.4 mmol), (±)-3-benzamidobutanoic acid (0.500 g, 2.4 mmol) and triethylamine (0.8 ml, 5.8 mmol) in dried DCM

(25 ml) was added. under N_2, 2-chloro-1-methylpyridinium iodide (0.738 g, 2.9 mmol). The reaction mixture was heated at reflux for 3 h, and then allowed to cool to room temperature. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a yellow oil. This was subjected to flash column chromatography (20 g SiO_2, 230-400 mesh), eluting with EtOAc to give the product as a yellow solid. This was recrystallised from EtOAc to give *p*-nitrophenyl (±)-3-benzamidobutanoate as a white solid (0.413 g, 52%); δ_H (200 MHz) 1.22 - 1.42 (3H, m), 2.58 - 3.04 (2H, m), 4.63 - 4.76 (1H, m), 6.83 (1H, d, J 8 Hz), 7.22 - 7.49 (5H, m), 7.73 - 7.76 (2H, m), 8.17 - 8.21 (2H, m); δ_C (50 MHz) 20.17 (q), 40.48 (t), 42.54 (d), 122.39 (d), 125.05 (d), 126.80 (d), 128.45 (d), 131.51 (d), 134.07 (s), 145.22 (s), 154.97 (s), 166.84 (s), 169.19 (s); (Found: C, 62.3%; H, 4.9; N, 8.5. $C_{17}H_{16}N_2O_5$ requires C, 61.5; H, 4.9; N, 9.6%).

ii) Attempted Cyclisation of *p*-Nitrophenyl (±)-3-Benzamidobutanoate

To a stirred solution of *p*-nitrophenyl (±)-3-benzamidobutanoate (0.100 g, 0.3 mmol) in dried THF (2 ml), under a nitrogeneous atmosphere, was carefully added a solution of potassium *t*-butoxide (0.034 g, 0.3 mmol) in dry THF (2 ml). The reaction mixture stirred at room temperature for 6 h. Methanol (1 ml) was cautiously added, and the reaction mixture was filtered and concentrated *in vacuo*. T.L.C. analysis of the reaction mixture showed no dihydrooxazinone to be present in a complex mixture of products, and so the reaction was abandoned.

k) EDC-HCl Cyclisation of *N*-Acyl β-Amino Acids

(±)-3-Benzamidobutanoic acid (0.385 g, 1.857 mmol) and EDC-HCl (0.356 g, 1.857 mmol) were reacted in dried DCM (20 ml) as described previously to give (±)-2-

phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one as a clear oil, identical in all respects to previously obtained material (0.305 g, 87%).

(±)-2,4-Diphenyl-4,5-dihydro-1,3-oxazin-6-one (112)

(±)-3-Benzamido-3-phenylpropanoic acid (0.537 g, 2 mmol) and EDC-HCl (0.383 g, 2 mmol) were reacted in dried CH_2Cl_2 (10 ml) as described previously to give (±)-2,4-diphenyl-4,5-dihydro-1,3-oxazin-6-one as a yellow solid, which proved to be remarkably prone to hydrolysis (0.466 g, 93%); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 1 785, 1 670, 1 510 and 1 210; δ_H (200 MHz, $CDCl_3$ + d_6-DMSO, ref. $CHCl_3$) 3.14 - 3.43 (2H, m, 5-H_2), 4.67 - 4.73 (1H, m, 4-H), 7.21 - 7.31 (6H, m, Ph), 7.39 - 7.54 (4H, m, Ph), 7.91 - 7.96 (2H, m, Ph) [contains some free acid from hydrolysis of product]; δ_C (50 MHz, $CDCl_3$ + d_6-DMSO, ref. $CDCl_3$) 37.05 (t, C-5), 66.22 (d, C-4), 125.51 (s, Ph), 127.01 (d, Ph), 128.22 (d, Ph), 128.56 (d, Ph), 129.41 (d, Ph), 132.55 (d, Ph), 135.09 (d, Ph), 161.56 (s, C=N), 177.26 (s, C=O); *m/z* 250 (M^+-H, 7.6%), 223 (6.4), 105 (82.3) and 77 (100.0).

8.3.4.2 Formation of β-Alanine-derived Dihydrooxazinones

N-Formyl-β-alanine (113)

N-Formyl β-alanine was prepared from β-alanine (0.356 g, 4 mmol), acetic anhydride (3.8 ml, 40 mmol) and formic acid (3.0 ml, 80 mmol) by the method described previously for the formation of *N*-formyl-L-proline. Crystallisation of the crude product from ethyl acetate gave *N*-formyl-β-alanine as a white crystalline solid (0.288 g, 62%), m.p. 72-75 °C (EtOAc) (lit.,[304] 74-75 °C); ν_{max}/cm^{-1} (KBr disc) 3 700 - 2 200, 3 320, 1 710, 1 640, 1 530, and 1 215; δ_H (200 MHz) 2.41 (2H, t, J 6.5

Hz), 3.28 (2H, t, J 6.5 Hz), 7.81 (1H, s); δ_C (50 MHz) 34.50 (t), 34.80 (t), 165.36 (d), 177.14 (s); *m/z* 117 (M^+, 1.5%), 100 (7.6), 99 (26.2), 89 (59.9), 71 (43.9), 58 (32.1), and 30 (100.0); (Found: C, 40.8%; H, 5.7; N, 11.7; M^+, 117.0423. $C_4H_7NO_3$ requires C, 41.0; H, 6.0; N, 12.0%; M^+, 117.0426).

N-Acetyl-β-alanine (114)

A suspension of β-alanine (3.000 g, 33.7 mmol) in acetic anhydride (40 ml) was stirred at room temperature overnight. The reaction mixture became homogeneous after 30 min. Water (80 ml) was added to the reaction mixture, which was then stirred at room temperature a further 30 min. Volatiles were removed *in vacuo* to give a clear oil which was crystallised from ethyl acetate to give *N*-acetyl-β-alanine as a white crystalline solid (4.027 g, 91%), m.p. 82-83 °C; ν_{max}/cm^{-1} (KBr disc) 3 600 - 2 100, 3340, 1 710, 1 620, 1 560, 1 225, and 1 205; δ_H (200 MHz, D_2O, ref. HOD) 1.77 (3H, s, CH_3), 2.40 (2H, t, J 6.5 Hz, 3-H_2), 3.23 (2H, t, J 6.5 Hz, 2-H_2); δ_C (50 MHz, D_2O, ref. replacement dioxan in D_2O at 67.4 ppm) 22.58 (q, CH_3), 34.21 (t, C-3), 35.94 (t, C-2), 174.94 (s, C=O amide), 176.97 (s, C=O acid); *m/z* 131 (M^+, 17.4%), 116 (0.5), 113 (5.8), 88 (69.5), 85 (23.0), and 43 (100.0); (Found: C, 45.9%; H, 6.7; N, 10.7. $C_5H_9NO_3$ requires C, 45.8; H, 6.7; N, 10.7%).

N-Propanoyl-β-alanine Methyl Ester (124) ***via acid chloride***

A stirred solution of β-alanine methyl ester hydrochloride (0.698 g, 5 mmol, 1 equiv.) and triethylamine (1.4 ml, 10 mmol, 2 equiv.) in dry DCM (20 ml) was cooled in an ice-bath. To this was added dropwise propanoyl chloride (0.43 ml, 5 mmol, 1 equiv.). The reaction mixture was allowed to warm gradually to room temperature, and stirred overnight. The reaction mixture was washed with water (2 x 10 ml), and dried

over anhydrous Na_2SO_4. Volatiles were removed *in vacuo* to give crude product as a brown oil (0.285 g). Due to the low yield, this oil was combined with *N*-propanoyl-β-alanine methyl ester prepared *via* CDI coupling for final purification.

<u>*N*-Propanoyl-β-alanine Methyl Ester</u> (124) - *via CDI coupling*

To a stirred solution of propanoic acid (0.375 ml, 5 mmol, 1 equiv.) in dry DCM (20 ml) was slowly added *N,N'*-carbonyldiimidazole (0.811 g, 5 mmol, 1 equiv.). The reaction mixture was stirred at room temperature until effervescence had ceased, and to it was then added β-alanine methyl ester hydrochloride (0.841 g, 6 mmol, 1.2 equiv.). The reaction mixture was stirred at room temperature for 1 h. The solution was washed with 1M HCl (1 x 15 ml) and water (1 x 15 ml). The organic layer was dried over anhydrous Na_2SO_4 and volatiles were removed *in vacuo* to give a brown oil (0.306 g). This was combined with the crude product obtained previously and subjected to flash column chromatography (SiO_2, 230-400 mesh), eluting with diethyl ether/ethyl acetate solvent mixtures to give *N*-propanoyl-β-alanine methyl ester as a yellow oil (0.427 g, 27% combined yield); ν_{max}/cm^{-1} ($CHCl_3$) 3 470, 3 020, 2 960, 1 730, 1 620, 1 520, 1 440, 1 370, 1 110 and 1 095; δ_H (200 MHz) 1.08 (3H, t, J 7.6 Hz), 2.14 (2H, q, J 7.6 Hz), 2.49 (2H, t, J 6.0 Hz), 3.46 (2H, q, J 6.0 Hz), 3.65 (3H, s), 6.18 (1H, br s); δ_C (50 MHz) 9.68 (q), 29.54 (t), 33.76 (t), 34.65 (t), 51.67 (q), 173.08 (s), 173.75 (s); *m/z* 159 (M^+, 2.2%), 145 (0.2), 128 (4.4), 102 (22.8), 98 (13.8), 57 (27.6), and 28 (100.0); (Found: M^+, 159.0891. $C_7H_{13}NO_3$ requires M^+, 159.0895).

N-Butanoyl-β-alanine Methyl Ester (125)

A DCM solution (30 ml) of butanoic acid (1.37 ml, 15 mmol, 1 equiv.), *N,N'*-carbonyldiimidazole (2.433 g, 15 mmol, 1 equiv.) and β-alanine methyl ester hydrochloride (2.513 g, 18 mmol, 1.2 equiv.) were reacted together as described previously. The reaction mixture was then washed with 1M HCl (2 x 10 ml) and water (1 x 10 ml), then dried over anhydrous Na_2SO_4. Volatiles were removed *in vacuo* to give *N*-butanoyl-β-alanine methyl ester as a clear oil (1.430 g, 55%); ν_{max}/cm^{-1} (neat) 3 700 - 2 600, 3 080, 2 960, 2 880, 1 740, 1 640, 1 550, 1 440, 1 365, 1 200, and 1 175; δ_H (200 MHz) 0.85 (3H, t, J 7.3 Hz), 1.57 (2H, sextet, J 8.1 Hz), 2.07 (2H, t, J 7.5 Hz), 2.48 (2H, t, J 6.1 Hz), 3.44 (2H, q, J 6.1 Hz), 3.62 (3H, s), 6.27 (1H, br s); δ_C (50 MHz) 13.53 (q), 18.96 (t), 33.73 (t), 34.63 (t), 38.43 (t), 51.64 (q), 173.01 (s), 173.06 (s); *m/z* 173 (M^+, 0.5%), 158 (0.2), 142 (3.1), 102 (15.5), 71 (15.7), and 28 (100.0); (Found: M^+, 173.1047. $C_8H_{15}NO_3$ requires M^+, 173.1052).

N-(*p*-Anisoyl)-β-alanine Methyl Ester (126)

A solution of *p*-anisic acid (4-methoxybenzoic acid) (0.761 g, 5 mmol, 1 equiv), *N,N'*-carbonyldiimidazole (0.811 g, 5 mmol, 1 equiv.) and β-alanine methyl ester hydrochloride (0.698 g, 5 mmol, 1 equiv.) in dry DCM (20 ml) was reacted together as described previously. The reaction mixture was washed with water (2 x 10 ml), 1M HCl (1 x 10 ml), and then again with water (1 x 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and volatiles removed *in vacuo* to give 0.697 g of a white solid. Further purification was achieved by flash column chromatography (28 g SiO_2, 230-400 mesh), eluting with ethyl acetate to give *N*-(*p*-anisoyl)-β-alanine methyl ester as a white solid (0.633 g, 53%), m.p. 68-72 °C; ν_{max}/cm^{-1} ($CHCl_3$) 3 460, 3 020, 1 730, 1 660, 1 610, 1 500, 1 260, 1 110 and 1 095; δ_H (200 MHz) 2.64 (2H, t,

J 5.9 Hz), 3.70 (5H, s and q, J 6.0 Hz), 3.83 (s), 6.78 (br s), 6.88 - 6.92 (2H, m,), 7.69 - 7.74 (2H, m); δ_C (50 MHz) 33.72 (t), 35.14 (t), 51.77 (q), 55.33 (q), 113.66 (d), 126.55 (s), 128.66 (d), 162.13 (s), 166.78 (s), 173.38 (s); *m/z* 237 (M^+, 10.5%), 222 (1.7), 206 (3.2), 135 (100.0), and 107 (10.5); (Found: M^+, 237.1002. $C_{12}H_{15}NO_4$ requires M^+, 237.1001).

Attempted Ester Hydrolysis of Amido β-Alanine Methyl Esters

Procedure A:

The amido β-alanine methyl ester was stirred overnight at room temperature in a saturated methanolic solution of potassium carbonate. The solution was then carefully acidified by the addition of conc. HCl, and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4 and volatiles were removed *in vacuo* to give only starting material, identified by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS).

Procedure B:

A mixture of *N*-benzoyl-β-alanine methyl ester (0.518 g, 2.5 mmol, 1 equiv) and $Ba(OH)_2.8H_2O$ (3.155 g, 10 mmol, 4 equiv) in 5:1 water/ethanol was heated at reflux for 4 h. After cooling to room temperature, dry ice was added to the reaction mixture and insoluble barium carbonate was removed by filtration. The precipitation/filtration procedure was repeated until no more barium carbonate was precipitated. The filtrate was then concentrated *in vacuo* to give a white solid, identified by both ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) and melting point as being starting material only.

2-Methyl-4,5-dihydro-1,3-oxazin-6-one (119)

N-Acetyl-β-alanine (0.656 g, 5 mmol) and EDC-HCl (0.958 g, 5 mmol) were reacted in dried CH_2Cl_2 (10 ml) as described previously. After washing the reaction mixture with water and drying over anhydrous Na_2SO_4, volatiles were removed *in vacuo* to give no product.

2-Phenyl-4,5-dihydro-1,3-oxazin-6-one (122)

N-Benzoyl-β-alanine (0.966 g, 5 mmol) and EDC-HCl (0.958 g, 5 mmol) were reacted in dried CH_2Cl_2 (10 ml) as described previously to give 2-phenyl-4,5-dihydro-1,3-oxazin-6-one as a white solid (0.702 g, 80%), which proved very susceptible to hydrolysis; ν_{max}/cm^{-1} (KBr disc) 3 310, 3 060, 1 800, 1 740, 1 675, 1 630, 1 540, 1 100 and 1 080; δ_H (90 MHz, $CDCl_3$, ref. TMS) 2.5 - 2.7 (2H, m, 4-H_2), 3.7 - 3.9 (2H, m, 5-H_2), 7.3 - 7.6 (3H, m, Ph), 7.9 - 8.1 (2H, m, Ph); *m/z* 175 (M^+, 14.9%), 176 (2.0), 105 (97.0) and 77 (100.0).

(Compound had decomposed before satisfactory ^{13}C N.M.R. data and accurate mass data could be obtained).

8.3.5 PPL-Catalysed Alcoholysis of (±)-4,5-Dihydro-1,3-oxazin-6-ones

PPL-Catalysed Methanolysis of (±)-2-Phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one (103)

To a stirred suspension of racemic dihydrooxazinone (0.189 g, 1 mmol) and PPL (0.600 g) in anhydrous THF (3 ml) was added methanol (0.080 ml, 2 mmol). The resulting mixture was shaken in a water bath at 38 °C for 24 h. The enzyme was removed by filtration, and volatiles were removed *in vacuo* to give a yellow oil (0.182 g, 82%), shown by TLC to be one compound and identified by ^{1}H N.M.R. to be methyl 3-benzamidobutanoate (**131**); δ_H (90 MHz, $CDCl_3$, ref. TMS) 1.2 - 1.5 (m), 2.6 - 2.8 (d), 3.8 (s), 4.3 - 4.7 (m), 7.2 - 7.6 (m), 7.7 - 7.9 (m).

PPL-Catalysed Butanolysis of (±)-2-Phenyl-4-methyl-4,5-dihydro-1,3-oxazin-6-one (103)

To a stirred suspension of racemic dihydrooxazinone (0.189 g, 1 mmol) and PPL (0.600 g) in anhydrous THF (3 ml) was added 1-butanol (0.185 ml, 2 mmol). The resulting mixture was shaken in a water bath at 40 °C for 24 h. The enzyme was removed by filtration, and volatiles were removed *in vacuo* to give a yellow oil. This was subjected to flash column chromatography (20 g SiO_2, 230 - 400 mesh), eluting with DCM/EtOAc (1:1). 1-Butyl 3-benzamidobutanoate (**130**) was obtained as a clear oil (0.110 g, 42%); $[\alpha]_D$ 0° (c 0.5, acetone); ν_{max}/cm^{-1} ($CHCl_3$) 3 440, 3 020, 2 960, 2 940, 1 720, 1 660, 1 610, 1 580, 1 520, 1 490 and 1 210; *m/z* 263 (M^+, 2.7%), 190 (4.7), 158 (21.3), 148 (9.1) and 105 (100.0).

Unreacted dihydrooxazinone was obtained as a pale yellow oil (0.038 g, 20%); $[\alpha]_D$ 0° (c 0.8, acetone). This compound gave identical physical data to previously obtained samples.

PPL-Catalysed Butanolysis of (±)-2,4-Diphenyl-4,5-dihydro-1,3-oxazin-6-one (112)

A mixture of racemic dihydrooxazinone (0.251 g, 1 mmol), 1-butanol (0.185 ml, 2 mmol) and PPL (0.600 g) in dried THF (5 ml) was stirred magnetically at room temperature for 24 h. The enzyme was removed by filtration, and washed exhaustively with acetone. The combined filtrate and washings were concentrated *in vacuo* and subjected to flash column chromatography (30 g SiO_2, 230 - 400 mesh). Fractions were concentrated *in vacuo* and examined by TLC (developed with DCM, visualised with U.V. light at 254 nm or by hydroxylamine/ferric chloride spray). Unreacted dihydrooxazinone was eluted with DCM (45 mg, 18%); $[\alpha]_D$ 0.0° (c 1.2, acetone); δ_H (90 MHz, $CDCl_3$, ref. TMS) 2.5 - 3.2 (2H, ABX, 5-H_2), 4.9 - 5.1 (1H, ABX, 4-H), 7.3 - 7.7 (8H, m, Ph), 8.1 - 8.3 (2H, m, Ph). This compound gave identical physical data to previously obtained samples.

1-Butyl 3-benzamido-3-phenylpropanoate **(132)** was eluted with 5% EtOAc/DCM (90 mg, 28%); $[\alpha]_D$ 0.0° (c 1.0, acetone); ν_{max}/cm^1 ($CHCl_3$) 3 440, 3 040, 2 980, 1 720, 1 670, 1 520, 1 490 and 1 220; δ_H (90 MHz, $CDCl_3$, ref. TMS) 0.7 - 1.0 (3H, br t), 1.0 - 1.7 (4H, m), 2.9 - 3.1 (2H, m), 3.9 - 4.2 (2H, m), 5.4 - 5.7 (1H, m), 7.2 - 7.6 (8H, m), 7.6 - 7.9 (2H, m); *m/z* 325 (M^+, 1.0%), 220 (39.8), 164 (8.4), 105 (80.9) and 77 (100.0) (Found: M^+, 325.1687. $C_{20}H_{23}NO_3$ requires M^+, 325.1678).

A little 3-benzamido-3-phenylpropanoic acid was eluted with ethyl acetate (28 mg, 10%). This compound gave identical physical data to previously obtained samples.

8.4 Experimental for Chapter Seven

8.4.1 General Procedures for the Synthesis of 2,2,2-Trichloroethyl Esters

Method A: To a cooled solution of 2,2,2-trichloroethanol (1 equiv.) in either diethyl ether or DCM was added dropwise triethylamine (1 equiv.). To the resultant solution was added, dropwise with stirring, the appropriate acid chloride (1 equiv.). The reaction mixture was allowed to warm to room temperature, and stirring was continued until the reaction was complete. The product was then purified as described for individual compounds.

Method B: To a stirred solution or suspension of the appropriate acid (10 mmol) in dried DCM (10 ml) was added carefully thionyl chloride (0.8 ml, 10 mmol). The reaction mixture was heated at reflux for 2-5 h, and then concentrated *in vacuo*. The crude acid chloride was added as an ethereal solution (3 ml) to a pre-cooled solution of 2,2,2-trichloroethanol (10 mmol) and triethylamine (10 mmol) in diethyl ether (10 ml). The resulting mixture was then stirred overnight, and purified as described for individual compounds.

Method C: To a round-bottomed flask charged with the appropriate carboxylic acid (10 mmol) was added, at room temperature, thionyl chloride (10 ml). The resulting

solution was heated to reflux for 5 h, then allowed to cool to room temperature. Volatiles were removed *in vacuo*, and the crude acid chloride was taken up in dried DCM (3 ml). The solution was carefully added to an ice-cold solution of 2,2,2-trichloroethanol (0.96 ml, 10 mmol) and triethylamine (1.4 ml, 10 mmol) in dried DCM (10 ml). The reaction mixture was allowed to warm gradually to room temperature and stirred overnight. Volatiles were removed *in vacuo*, the residue was taken up in diethyl ether and insoluble triethylamine hydrochloride was removed by filtration. The solution was again concentrated *in vacuo*, and final purification was achieved by Kugelrohr bulb-to-bulb distillation at reduced pressure.

2,2,2-Trichloroethyl Acetate (135)

2,2,2-Trichloroethyl acetate (**135**) was prepared from 2,2,2-trichloroethanol (9.6 ml, 0.1 mol) and acetyl chloride (9.2 ml, 0.1 mol) in DCM (50 ml) by method A. After stirring in an ice bath for 15 min, the reaction mixture was washed with water (2 x 25 ml), dried over anhydrous Na_2SO_4 and finally concentrated *in vacuo*. Proton N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) showed a substantial amount of triethylamine hydrochloride was still present. Extraction of the salt into water was attempted once more, this time using acetone as the organic solvent. Once again, a substantial quantity of triethylamine hydrochloride was present in the organic layer. The crude ester product was divided into seven equal portions, and DCM solutions of these portions were washed with water. The combined organic extracts were then dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 2,2,2-trichloroethyl acetate as a golden oil (12.567 g, 66%); ν_{max}/cm^{-1} (neat) 1 770, 1 440, 1 380, 1 215, 1 090, 1 050, 800 and 720; δ_H (90 MHz, $CDCl_3$, ref. TMS) 2.2 (3H, s, CH_3), 4.7 (2H, s, CH_2); *m/z* 155 (M^+-HCl, 7.9%), 159 (0.1), 157 (1.0), 95 (10.0), and 73 (100.0) (Found: M^+, 189.9360. $C_4H_5{}^{35}Cl_3O_2$ requires M^+, 189.9355).

2,2,2-Trichloroethyl Propanoate (136)

2,2,2-Trichloroethyl propanoate (**136**) was prepared from 2,2,2-trichloroethanol (4.8 ml, 0.05 mol) and propionyl chloride (4.4 ml, 0.05 mol) in DCM (15 ml) by method A. After stirring in an ice bath for 15 min, the reaction mixture was concentrated *in vacuo*. The residue was taken up in acetone and insoluble triethylamine hydrochloride was removed by filtration. Volatiles were again removed *in vacuo*, and the resulting oil was filtered through glass wool. The filtrate was then subjected to distillation under oil-pump vacuum, the fraction boiling at 44-46 °C being collected to give 2,2,2-trichloroethyl propanoate as a clear oil (6.810 g, 66%); ν_{max}/cm^{-1} (neat) 2 990, 2 950, 1 760, 1 150 and 1 095; δ_H (200 MHz) 1.14 (3H, t, J 7.5 Hz), 2.42 (2H, q, J 7.5 Hz), 4.68 (2H, s); δ_C (50 MHz) 8.81 (q), 27.13 (t), 73.68 (t), 94.96 (s), 172.50 (s); *m/z* 169 (M^+-HCl, 2.6%), 173 (0.3), 171 (1.7), 133 (2.7), 131 (2.8), 95 (7.4), 87 (14.3) and 57 (100.0); (Found: C, 29.3%; H, 3.6; $[C_5H_7{}^{37}Cl^{35}ClO_2]^+$, 170.9824. $C_5H_7Cl_3O_2$ requires C, 29.2; H, 3.4; $[C_5H_7{}^{37}Cl^{35}ClO_2]^+$, 170.9794).

2,2,2-Trichloroethyl Butanoate (137)

2,2,2-Trichloroethyl butanoate (**137**) was prepared from 2,2,2-trichloroethanol (2.9 ml, 30 mmol) and butyryl chloride (3.5 ml, 30 mmol) in DCM (20 ml) by method A. The reaction mixture was stirred at room temperature for 30 min, and then concentrated *in vacuo*. The residue was taken up in diethyl ether, filtered, and again concentrated *in vacuo* to give a brown oil. Proton N.M.R. spectroscopy of this crude product (90 MHz, $CDCl_3$, ref. TMS) showed an extra signal at δ 4.7 ppm. The crude reaction mixture was distilled twice under oil pump vacuum to give 2,2,2-trichloroethyl

butanoate as a clear oil (2.088g, 32%); ν_{max}/cm^{-1} (neat) 2 970, 2 940, 2 880, 1 760, 1 155, 815 and 725; δ_H (200 MHz) 0.92 (3H, t, J 7.4 Hz, CH_3), 1.65 (2H, m, $CH_2\underline{CH_2}CH_3$), 2.36 (2H, t, J 7.3 Hz, $O\underline{CH_2}Et$), 4.67 (2H, s, $\underline{CH_2}CCl_3$); δ_C (50 MHz) 13.40 (q, CH_3), 18.07 (t, $OCH_2\underline{CH_2}CH_3$), 35.53 (t, $O\underline{CH_2}Et$), 73.55 (t, $O\underline{CH_2}CCl_3$), 94.96 (s, CCl_3), 171.46 (s, C=O); *m/z* 217 (M^+-H, 0.1%), 183 (3.6), 101 (2.8), 43 (85.1) and 71 (100.0). The distillation residue was subjected to flash cloumn chromatography (SiO_2, 230-400 mesh), eluting with DCM to give 0.242 g of a brown oil. The identity of this by-product has not been ascertained; ν_{max}/cm^{-1} ($CHCl_3$) 3 040, 2 960, 1 450, 1 230, 1 080, 1 050, 1 020, 990 and 830; δ_H (200 MHz) 4.55 and 4.69 (AB, J 11.7 Hz); δ_C (50 MHz) 72.42 (t), 94.43 (s); *m/z* 229 (1.1%), 227 (3.3), 225 (3.3), 199 (3.4), 197 (9.7), 195 (9.9), 135 (30.2), 133 (94.9), 131 (100.0), 121 (5.2), 119 (16.7), 117 (17.5), 99 (4.3), 97 (20.7), 95 (30.8), 61 (25.2), and 31 (25.9).

2,2,2-Trichloroethyl 4-Methylvalerate (138)

2,2,2-Trichloroethyl 4-methylvalerate (**138**) was prepared from 4-methylvaleric acid (1.162 g, 10 mmol), thionyl chloride (2.2 ml, 30 mmol) and 2,2,2-trichloroethanol (0.96 ml, 10 mmol) by method B. After stirring overnight at room temperature, insoluble triethylamine hydrochloride was removed by filtration. The filtrate was concentrated *in vacuo* to give a brown oil, which was purified by Kugelrohr bulb-to-bulb distillation under oil-pump vacuum to give 2,2,2-trichloroethyl 4-methylvalerate as a clear oil (1.374 g, 56%), b.p. 198-200 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 960, 2 930, 1 745, 1 150 and 1 105; δ_H (200 MHz) 0.90 (6H, d, 2 x CH_3), 1.55 - 1.61 (3H, m, $CHCH_2$), 2.30 - 2.49 (2H, m, CH_2CO), 4.72 (2H, s, CH_2CCl_3)‡; δ_C (50 MHz) 22.15 (q, 2 x CH_3), 27.55 (d, CH), 31.98 (t, CH_2), 33.45 (t, CH_2CO), 73.84 (t, CH_2CCl_3), 95.02 (s, CCl_3), 172.35 (s, C=O); *m/z*

247 ([M+1]$^+$, 0.2%), 211 (26.2), 129 (4.3), 116 (7.4), 99 (98.7), 71 (12.8), 57 (55.3) and 43 (100.0) (Found: M$^+$+2, 247.9873. $C_8H_{13}{}^{35}Cl_2{}^{37}ClO_2$ requires M$^+$, 247.9952).

‡ In addition, a small AB system was located under the singlet at δ 4.72, similar to the signal observed in the 2,2,2-trichloroethyl butanoate reaction.

2,2,2-Trichloroethyl Benzoate (139)

2,2,2-Trichloroethyl benzoate **(139)** was prepared from 2,2,2-trichloroethanol (2.9 ml, 30 mmol) and benzoyl chloride (3.83 ml, 33 mmol) in DCM (30 ml) by method A. After stirring in an ice bath for 15 min, volatiles were removed *in vacuo*. The residue was taken up in acetone, and insoluble triethylamine hydrochloride was removed by filtration. Volatiles were again removed *in vacuo*, and the crude product was subjected to flash column chromatography (35 g SiO_2, 230 - 400 mesh), eluting with DCM. The resulting oil was still contaminated with benzoyl chloride. Final purification was achieved by vacuum distillation to give 2,2,2-trichloroethyl benzoate as a clear oil (4.638 g, 61%), b.p. 104-106 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 950, 1 730, 1 600, 1 450, 1 370, 1 260, 1 170 and 1 115; δ_H (200 MHz) 4.97 (2H, s, $\underline{CH_2}CCl_3$), 7.45 - 7.62 (3H, m, Ph), 8.11 - 8.16 (2H, m, Ph); δ_C (50 MHz) 74.39 (t, $\underline{CH_2}CCl_3$), 95.02 (s, CCl_3), 128.58 (d, Ph), 128.65 (d, Ph), 133.78 (d, Ph), 164.85 (s, C=O); *m/z* 252 (M$^+$, 1.0%), 257 (0.1), 256 (0.2), 255 (1.1), 254 (1.0), 253 (2.9), 217 (1.6), 135 (3.1), 122 (1.0), 105 (100.0) and 77 (40.2) (Found: M$^+$, 251.9521. $C_9H_7{}^{35}Cl_3O_2$ requires M$^+$, 251.9512).

2,2,2-Trichloroethyl Phenylacetate (140)

2,2,2-Trichloroethyl phenylacetate (**140**) was prepared from phenylacetic acid (1.362 g, 10 mmol) and 2,2,2-trichloroethanol (0.96 ml, 10 mmol) by method C. The crude reaction product was purified by Kugelrohr bulb-to-bulb distillation under oil-pump vacuum to give 2,2,2-trichloroethyl phenylacetate as a clear oil (1.357 g, 51 %), b.p. 248-250 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 975, 1 750, 1 490, 1 450, 1 370, 1 220 and 1 130; δ_H (200 MHz) 3.77 (2H, s, CH_2Ph), 4.75 (2H, s, CH_2CCl_3), 7.28 - 7.45 (5H, m, Ph); δ_C (50 MHz) 40.89 (t, CH_2Ph), 74.16 (t, $\underline{CH_2}CCl_3$), 94.80 (s, CCl_3), 127.41 (d, Ph), 128.65 (d, Ph), 129.38 (d, Ph), 132.90 (s, Ph), 169.93 (s, C=O); *m/z* 266 (M^+, 3.9%), 270 (1.2), 268 (3.7), 231 (0.5), 149 (0.3), 135 (1.3), 119 (12.0) and 91 (100.0) (Found: M^+, 265.9680. $C_{10}H_9{}^{35}Cl_3O_2$ requires M^+, 265.9668).

2,2,2-Trichloroethyl Acrylate (141)

2,2,2-Trichloroethyl acrylate (**141**) was prepared from 2,2,2-trichloroethanol (0.96 ml, 10 mmol) and acryloyl chloride (0.81 ml, 10 mmol) in diethyl ether (15 ml) by method A. The reaction mixture was stirred at room temperature for 2 h, and triethylamine hydrochloride was removed by filtration. Volatiles were removed *in vacuo* to give a clear oil. Flash column chromatography (SiO_2, 230-400 mesh), eluting with diethyl ether, failed to effect separation of the ester from unreacted alcohol. Consequently, the crude product was subjected to Kugelrohr bulb-to-bulb distillation under oil-pump vacuum to yield 2,2,2-trichloroethyl acrylate as a clear oil (0.852 g, 42%), b.p. 55-57 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 1 735, 1 635, 1 405, 1 290 and 1 160; δ_H (200 MHz) 4.81 (3H, s), 5.95 - 6.01 (1H, m), 6.15 - 6.29 (1H, m), 6.52 - 6.61 (1H, m); δ_C (50 MHz) 74.02 (t, $\underline{CH_2}CCl_3$), 95.00 (s,

CCl_3), 127.02 (d, $\underline{CH}=CH_2$), 133.05 (t, $\underline{CH_2}=CHCO$), 164.33 (s, C=O); *m/z* 167 (M^+- Cl, 4.6%), 132 (1.2), 97 (4.0), 85 (30.2), 55 (100.0) and 27 (64.7) (Found: M^+-Cl, 166.9643. $C_5H_5{}^{35}Cl_3O_2$ - ^{35}Cl requires M^+, 166.9667).

2,2,2-Trichloroethyl Crotonate (142)

2,2,2-Trichloroethyl crotonate (**142**) was prepared from 2,2,2-trichloroethanol (0.96 ml, 10 mmol) and crotonoyl chloride (1.06 ml, 10 mmol) in diethyl ether (20 ml) by method A. The reaction mixture was stirred at room temperature for one hour, and triethylamine hydrochloride was removed by filtration. The reaction mixture was concentrated *in vacuo* to give a yellow, sweet-smelling oil. This was subjected to Kugelrohr distillation under oil-pump vacuum to give 2,2,2-trichloroethyl crotonate as a clear oil (1.789 g, 82%), b.p. 232-235 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 940, 1 730, 1 655, 1 440 and 1 160; δ_H (200 MHz) 0.92 (3H, dd, J 6.9 Hz and 1.7 Hz, CH_3), 4.77 (2H, s, $\underline{CH_2}CCl_3$), 5.90 - 5.98 (1H, m, Me$\underline{CH}$=CH), 7.08 - 7.19 (1H, m, $\underline{CH}CO$); δ_C (50 MHz) 18.23 (q, CH_3), 73.80 (t, $\underline{CH_2}CCl_3$), 95.11 (s, CCl_3), 121.19 (d, $\underline{CH}CH_3$), 147.49 (d, $\underline{CH}CO$), 164.54 (s, C=O); *m/z* 216 (M^+, 0.4%), 220 (0.1), 219 (0.1), 218 (0.4), 217 (0.1), 181 (3.7), 99 (12.5) and 69 (100.0) (Found: M^+, 215.9518. $C_6H_7{}^{35}Cl_3O_2$ requires M^+, 215.9512).

2,2,2-Trichloroethyl 3,3-Dimethylacrylate (143)

2,2,2-Trichloroethyl 3,3-dimethylacrylate (**143**) was prepared from 3,3-dimethylacrylic acid (1.001 g, 10 mmol), thionyl chloride (2.2 ml, 30 mmol) and 2,2,2-trichloroethanol (0.96 ml, 10 mmol) by method B. After stirring the mixture overnight, insoluble triethylamine hydrochloride was removed by filtration. The filtrate was concentrated *in vacuo* to give a brown oil which was purified by Kugelrohr bulb-

to-bulb distillation to give 2,2,2-trichloroethyl 3,3-dimethylacrylate as a clear oil (1.305 g, 56%), b.p. 165-167 °C (oil-pump vacuum); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 990, 2 970, 2 950, 1 745, 1 650 and 1 130; *m/z* 230 (M^+, 10.4%), 235 (0.2), 234 (3.0), 233 (0.8), 232 (9.7), 231 (1.0), 195 (5.5), 113 (2.3), 100 (40.4), 83 (100.0), 55 (98.5) and 43 (29.3) (Found: M^+, 229.9674. $C_7H_9{}^{35}Cl_3O_2$ requires M^+, 229.9668).

2,2,2-Trichloroethyl Sorbate (144)

2,2,2-Trichloroethyl sorbate (**144**) was prepared from sorbic acid (hexa-2,4-dienoic acid) (1.121 g, 10 mmol), thionyl chloride (1.4 ml, 20 mmol) and 2,2,2-trichloroethanol (0.96 ml, 10 mmol) by method B. After stirring overnight at room temperature, insoluble triethylamine hydrochloride was removed by filtration. The filtrate was then concentrated *in vacuo* to give a yellow oil. Proton N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) showed that approx. 75% of the alcohol had been converted into ester. The crude product was purified by Kugelrohr bulb-to-bulb distillation under oil-pump vacuum to give 2,2,2-trichloroethyl sorbate as a clear oil (1.763 g, 72%), b.p. 177-180 °C (oil pump vacuum). This ester was found to be relatively unstable, an appreciable amount of polymerisation occurring at room temperature overnight; ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 990, 2 975, 1 735, 1 640, 1 370, 1 235 and 1 150; δ_H (90 MHz, $CDCl_3$, ref. TMS)‡ 1.8 (3H, d, Me), 4.8 (2H, s, CH_2CCl_3), 5.8 - 6.3 (3H, m), 7.2 - 7.5 (1H, m); *m/z* 242 (M^+, 5.2%), 248 (0.1), 247 (0.2), 246 (1.6), 245 (1.1), 244 (5.1), 243 (1.7), 209 (2.6), 207 (4.1), 112 (13.6), 95 (87.7), 67 (100.0), 41 (51.7) and 28 (24.5) (Found: M^+, 241.9674. $C_8H_9{}^{35}Cl_3O_2$ requires M^+, 241.9668).

‡ Sample had polymerised before 200 MHz ^1H and 50 MHz ^{13}C N.M.R. spectra could be obtained.

2,2,2-Trichloroethyl Cinnamate (145)

2,2,2-Trichloroethyl cinnamate (**145**) was prepared from 2,2,2-trichloroethanol (0.96 ml, 10 mmol) and cinnamoyl chloride (1.666 g, 10 mmol) in diethyl ether (25 ml) by method A. The reaction mixture was stirred at room temperature for 90 min, and triethylamine hydrochloride was removed by filtration. Volatiles were removed *in vacuo* to give a yellow oil which slowly solidified on standing. This was crystallised from hexane to give 2,2,2-trichloroethyl cinnamate as a pale yellow solid (2.122 g, 76%), m.p. 46-48 °C; ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 1 720, 1 635, 1 575, 1 490, 1 445, 1 305 and 1 150; δ_H (200 MHz) 4.87 (2H, s, CH_2CCl_3), 6.55 and 7.80 (2H, AB, J 16 Hz), 7.37 - 7.42 (3H, m, Ph), 7.54 - 7.61 (2H, m, Ph); δ_C (50 MHz) 74.07 (t, $\underline{CH_2}CCl_3$), 95.10 (s, CCl_3), 116.26 (d, CH olefinic), 128.35 (d, Ph), 128.96 (d, Ph), 130.84 (d, Ph), 133.91 (s, Ph), 147.06 (d, CH olefinic), 165.18 (s, C=O); *m/z* 279 (M^++1, 1.3%), 284 (0.2), 283 (0.2), 282 (1.7), 281 (0.9), 280 (5.6), 243 (2.7), 147 (7.2), 131 (100.0), 103 (49.8) and 77 (35.7) (Found: C, 48.2%; H, 3.3; M^+, 277.9654. $C_{11}H_9Cl_3O_2$ requires C, 47.3; H, 3.2%; M^+, 277.9668).

2,2,2-Trichloroethyl Diphenylacetate (146)

2,2,2-Trichloroethyl diphenylacetate (**146**) was prepared from 2,2,2-trichloroethanol (0.96 ml, 10 mmol), diphenylacetic acid (2.122 g, 10 mmol) and thionyl chloride (10 ml) by method C. The crude reaction mixture was purified by Kugelrohr bulb-to-bulb distillation under reduced pressure. The fraction distilling at 260-263 °C was collected to give 2,2,2-trichloroethyl diphenylacetate as a viscous oil which slowly solidified on standing (2.891 g, 84%), m.p. 53-55 °C; ν_{max}/cm^{-1}

($CHCl_3$) 3 090, 3 060, 1 745, 1 600, 1 490, 1 450, 1 370 and 1 130; δ_H (200 MHz) 4.82 (2H, s, CH_2CCl_3), 5.20 (1H, s, CH), 7.26 - 7.47 (10H, m, 2 x Ph); δ_C (50 MHz) 56.73 (d, CH), 74.30 (t, CH_2CCl_3), 94.66 (s, CCl_3), 127.52 (d, Ph), 128.63 (d, Ph), 128.70 (d, Ph), 137.66 (s, Ph), 170.87 (s, C=O); *m/z* 342 (M^+, 2.3%), 346 (0.7), 344 (2.3), 307 (0.3), 212 (0.3), 195 (0.4), 167 (100.0), 115 (6.7), 89 (5.0) and 77 (3.3) (Found: M^+, 341.9973. $C_{16}H_{13}{}^{35}Cl_3O_2$ requires M^+, 341.9981).

8.4.2 Enzyme-Catalysed Acylation of (±)-2-Octanol

Attempted PPL-catalysed Coupling of (±)-2-Octanol With Carboxylic Acids

A mixture of acetic acid (0.227 g, 3.7 mmol), (±)-2-octanol (0.50 ml, 3.1 mmol) and PPL (0.314 g) in dried heptane (2.2 ml) was stirred magnetically at room temperature. After 48 h, the enzyme was removed by filtration and washed thoroughly with hexane. The combined filtrate and washings were evaporated *in vacuo* to give an oil, which was shown by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) to contain no ester.

The reaction was repeated using propanoic acid (0.280 g, 3.8 mmol). Once again, no acylated alcohol was detectable in the reaction mixture after 48 h. The reaction was attempted once more, this time using butanoic acid (0.332 g, 3.8 mmol). Yet again, no acylated product was detectable in the reaction mixture after 48 h.

General Procedures for the Lipase-catalysed Transesterification of (±)-2-Octanol and 2,2,2-Trichloroethyl Esters.

Gas Chromatography

Gas Chromatographic (G.C.) analysis of reaction mixtures was performed with a Hewlett-Packard 5880A gas chromatograph, equipped with a CP Sil 19CB fused-silica capillary column (25 m x 0.32 mm I.D. x 0.18 μm). Aliquots (~5μl) were periodically withdrawn from the reaction mixtures, and diluted with an appropriate solvent. Samples (~1 μg/μl) were injected into a Grob-type injector, operated in split mode (50:1). Helium carrier and make-up gas flow rates were 3 ml/min and 25 ml/min respectively. The column was operated under the following programmes, and the instrument employed a flame ionisation detector.

The identity of the 2-octyl ester peaks in the G.C. trace was confirmed where possible by comparison with an authentic, chemically synthesised sample.

Programme 1: The column temperature was maintained at 80 °C for 2 min, then raised at 5 °C/min to a final temperature of 180 °C.

Programme 2: The column temperature was maintained at 80 °C for 2 min, raised at 5 °C/min to 100 °C, and maintained at 100 °C for 1 min.

Programme 3: The column temperature was maintained at 80 °C for 2 min, raised at 5 °C/min to 150 °C, and maintained at 150 °C for 1 min.

Programme 4: The column temperature was maintained at 80 °C for 2 min, raised at 10 °C/min to 250 °C, and maintained at 250 °C for 1 min.

Programme 5: The column temperature was maintained at 80 °C for 2 min, raised at 10 °C/min to 240 °C, and maintained at 240 °C for 1 min.

Programme 6: The column temperature was maintained at 80 °C for 2 min, raised at 15 °C/min to 240 °C, and maintained at 240 °C for 1 min.

Programme 7: The column temperature was maintained at 80 °C for 2 min, raised at 30 °C/min to 200 °C, and maintained at 200 °C for 1 min. The column temperature was then raised at 5 °C/min to 260 °C, and maintained at 260 °C for 10 min.

Programme 8: The column temperature was maintained at 80 °C for 2 min, raised at 5 °C/min to 240 °C, and maintained at 240 °C for 1 min.

General Procedures for the Enzyme-catalysed Transesterification of (±)-2-Octanol and 2,2,2-Trichloroethyl Esters.

Method A: To a mixture of (±)-2-octanol (0.635 ml, 4 mmol), a 2,2,2-trichloroethyl ester (4.8 mmol) and water (0.135 ml) in freshly dried diethyl ether (5 ml) was added PPL (0.400 g). The reaction mixture was stirred magnetically at room temperature. 5ml Aliquots were withdrawn periodically from the reaction mixture. Each aliquot was filtered through glass wool with ether (1 ml), and examined by G.C. using programme 1.

Method B: To a mixture of (±)-2-octanol (0.635 ml, 4 mmol) and a 2,2,2-trichloroethyl ester (4.8 mmol) in freshly dried diethyl ether (5 ml) was added PPL (0.400 g). 3 Å Molecular sieves were added, and the reaction mixture was stirred magnetically at room temperature. 5 µl Aliquots were withdrawn periodically from the reaction mixture. Each aliquot was filtered through glass wool with diethyl ether (1 ml), evaporated to dryness under nitrogen, and taken up in ethyl acetate (1 ml). The ethyl acetate solutions were then analysed by G.C. using programme 1.

Method C: To a mixture of (±)-2-octanol (0.635 ml, 4 mmol) and a 2,2,2-trichloroethyl ester (4.8 mmol) in freshly dried THF was added PPL (0.400 g). The reaction mixture was stirred magnetically at room temperature, and 2 µl aliquots were withdrawn periodically from the reaction mixture. Each aliquot was filtered through glass wool with diethyl ether (1 ml), evaporated to dryness under nitrogen and taken up in ethyl acetate (1 ml). The ethyl acetate solutions were then analysed by G.C. using programme 1.

Method D: To a mixture of (±)-2-octanol (0.635 ml, 4 mmol) and a 2,2,2-trichloroethyl ester (4.8 mmol) in freshly dried THF (5 ml) was added PPL (0.400 g). The reaction mixture was shaken in an enzyme bath at 25 °C. 5 µl Aliquots were withdrawn periodically from the reaction mixture, filtered through glass wool with THF (1 ml), and analysed by G.C. using programme 1.

Method E: To a solution of (±)-2-octanol (0.635 ml, 4 mmol) and a 2,2,2-trichloroethyl ester (4.8 ml) in freshly dried THF (5 ml) was added Lipozyme (50 mg). The reaction mixture was then refluxed with magnetic stirring in an atmosphere of nitrogen for 7 h. After filtration, the reaction mixture was concentrated *in vacuo* and examined by ^{1}H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS).

Method F: To a solution of (±)-2-octanol (0.320 ml, 2 mmol) and a 2,2,2-trichloroethyl ester (2.4 mmol) in Analar diethyl ether (3 ml) was added Lipozyme (0.200 g). The reaction mixture was stirred magnetically at room temperature. 5 µl Aliquots were withdrawn periodically from the reaction mixture, filtered through glass wool with diethyl ether (1 ml) and analysed by G.C. as described for individual compounds.

Reaction of 2,2,2-Trichloroethyl Esters and (±)-2-Octanol

(1) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (P.P.L./Et_2O/H_2O)

The reaction of 2,2,2-trichloroethyl acetate (0.917 g, 4.8 mmol) and (±)-2-octanol was monitored over 4 days under the conditions described by method A. After 30 min, the powdered enzyme had turned into a paste on the bottom of the flask. The aliquot withdrawn after four days was analysed by G.C. as described previously. No 2-octyl acetate was detectable in the reaction mixture by G.C.

(2) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (P.P.L./Et_2O/mol. sieves)

The lipase-catalysed reaction of 2,2,2-trichloroethyl acetate (0.917 g, 4.8 mmol) and (±)-2-octanol was monitored over 5 days under the conditions described by method B. The results obtained are summarised in Table 7.1 (See Chapter 7).

(3) Transesterification of 2,2,2-Trichloroethyl Propanoate and (±)-2-Octanol (P.P.L./Et_2O/mol. sieves)

The P.P.L.-catalysed reaction of 2,2,2-trichloroethyl propanoate (0.986 g, 4.8 mmol) and (±)-2-octanol was monitored over 5 days under the conditions described by method B. The results obtained are summarised in Table 7.2 (See Chapter 7).

The reaction was repeated under similar conditions to obtain a second set of data, summarised in Table 7.3 (See Chapter 7).

(4) Transesterification of 2,2,2-Trichloroethyl Butanoate and (±)-2-Octanol (P.P.L./Et_2O/mol. sieves)

The P.P.L.-catalysed reaction of 2,2,2-trichloroethyl butanoate (1.054 g, 4.8 mmol) and (±)-2-octanol was monitored over 3 days under the conditions described by method B. After 79 h reaction, only a very small amount (<1%) amount of 2-octyl butanoate was detected in the reaction mixture by G.C.

(5) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (P.P.L./THF/Magnetic Stirring)

The P.P.L.-catalysed reaction of 2,2,2-trichloroethyl acetate (0.917 g, 4.8 mmol) and (±)-2-octanol was monitored under the conditions described in method C. Under these conditions, the retention times of 2-octanol and 2-octyl acetate were

2.34 min and 3.96 min respectively. The results obtained are summarised in Table 7.4 (See Chapter 7).

(6) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (P.P.L./THF/Shaking)

The P.P.L.-catalysed reaction of 2,2,2-trichloroethyl acetate (0.917 g, 4.8 mmol) and (±)-2-octanol was monitored under the conditions described by method D. After 25 h, no 2-octyl acetate was detectable in the reaction mixture by G.C. The reaction mixture was shaken in an enzyme bath for 14 d. The enzyme was then removed by filtration, and volatiles were removed *in vacuo* to give a yellow oil. Proton N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS) showed no 2-octyl acetate to be present in the reaction mixture, as evidenced by the lack of a multiplet at δ 4.8 ppm due to the ester methine proton.

(7) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (Lipozyme/THF/reflux)

The reaction of 2,2,2-trichloroethyl acetate (0.917 g, 4.8 mmol) and (±)-2-octanol was monitored under the conditions described by method E. After cooling to room temperature, the reaction mixture was filtered to remove the supported enzyme. The filtrate was concentrated *in vacuo* to give a yellow oil, which was examined by ^1H N.M.R. spectroscopy (90 MHz, $CDCl_3$, ref. TMS). No 2-octyl acetate was present in the reaction mixture, as evidenced by the lack of a multiplet at δ 4.8 ppm due to the ester methine proton.

(8) Transesterification of 2,2,2-Trichloroethyl Acetate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl acetate (0.459 g, 2.4 mmol) and (±)-2-octanol was monitored over 3 days under the conditions described by method F. Aliquots were analysed by G.C. using programme 2. Under these conditions, authentic 2-octyl acetate and 2-octanol had retention times of 5.2 min and 3.5 min respectively. Samples withdrawn from the reaction mixture after 6 h, 19 h and 47 h showed no octyl acetate present by G.C.

(9) Transesterification of 2,2,2-Trichloroethyl Propanoate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl propanoate (0.493 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 3. Under these conditions, authentic 2-octyl propanoate and 2-octanol had retention times of 7.0 min and 3.5 min respectively. A sample withdrawn from the reaction mixture after 70 h showed ~5% acylation had occurred, while after 30 h only a trace of 2-octyl propanoate could be detected by G.C.

(10) Transesterification of 2,2,2-Trichloroethyl Butanoate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl butanoate (0.527 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 2. Under these conditions,

authentic 2-octyl butanoate and 2-octanol had retention times of 9.0 min and 3.5 min respectively. After 48 h, ~1% acylation had occurred.

(11) Transesterification of 2,2,2-Trichloroethyl Benzoate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl benzoate (0.507 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 4. Under these conditions, authentic 2-octyl benzoate and 2-octanol had retention times of 12.2 min and 3.4 min respectively. Samples withdrawn from the reaction mixture after 1 h, 2 h, 3 h, 6 h, 9 h, 12 h, 15 h, 24 h, 29 h and 36 h showed two peaks present in the G.C. trace, with retention times of 3.4 min and 11.0 min. The relative proportions of the two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction. The sample withdrawn after 36 h was subjected to high vacuum to remove 2-octanol, and then examined by solid probe M.S.; *m/z* 252 (4.1%), 254 (3.9), 256 (1.3) and 258 (0.2).

(12) Transesterification of 2,2,2-Trichloroethyl 4-Methylvalerate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl 4-methylvalerate (0.495 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 5. Samples withdrawn from the reaction mixture after 9 h and 49.5 h showed 2 peaks in the G.C. trace, with retention times of 3.4 min and 7.6 min. The relative proportions of the

two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction.

(13) Transesterification of 2,2,2-Trichloroethyl Phenylacetate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl phenylacetate (0.535 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 6. Under these conditions, authentic 2-octyl phenylacetate and 2-octanol had retention times of 10.2 min and 3.3 min respectively. Samples withdrawn from the reaction mixture after 2 h, 12 h and 48 h showed 2 peaks present in the G.C. trace, with retention times of 3.3 min and 9.6 min. The relative proportions of the two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction.

(14) Transesterification of 2,2,2-Trichloroethyl Cinnamate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl cinnamate (0.671 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 6. Samples withdrawn from the reaction mixture after 1 h, 6 h and 24 h showed two peaks present in the G.C. trace, with retention times of 3.3 min and 9.6 min. The relative proportions of the two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction.

(15) Transesterification of 2,2,2-Trichloroethyl Diphenylacetate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl diphenylacetate (0.823 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 7. Samples withdrawn from the reaction mixture after 1 h, 6 h and 24 h showed two peaks in the G.C. trace, with retention times of 3.1 min and 12.1 min. The relative proportions of the two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction.

(16) Transesterification of 2,2,2-Trichloroethyl Acrylate and (±)-2-Octanol (Lipozyme/Et_2O)

The Lipozyme-catalysed reaction of 2,2,2-trichloroethyl acrylate (0.488 g, 2.4 mmol) and (±)-2-octanol was monitored under the conditions described by method F. Aliquots were analysed by G.C. using programme 8. Samples withdrawn from the reaction mixture after 2 h, 9 h and 24 h showed two peaks present in the G.C. trace, with retention times of 3.5 min and 4.3 min. The relative proportions of the two compounds in the reaction mixture remained essentially invariant throughout the course of the reaction.

8.4.3 Preparation of 2-octyl ester standards

Method A (DCC method)

To a solution of 2-octanol (1 equiv) and the appropriate acid (1 equiv) in diethyl ether (10 ml) was added a solution of *N,N'*-dicyclohexylcarbodiimide (1 equiv) in diethyl ether (5 ml). The reaction mixture was stirred at room temperature until esterification was complete, and then filtered. The filtrate was concentrated *in vacuo*, taken up in diethyl ether, and again filtered. This filtration/concentration procedure was continued until no *N,N'*-dicyclohexylurea was present, to give the product.

Method B (CDI method)

To a solution of the appropriate carboxylic acid (1 equiv) in dried DCM (10 ml) was slowly added 1,1'-carbonyldiimidazole (1 equiv). Once effervescence had ceased, 2-octanol (1 equiv) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 30 min, and was then washed with water (2 x 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the product.

Method C (Anhydride Method)

To a mixture of the appropriate acid anhydride (4 equiv) and pyridine (2 equiv), cooled to 0-5 °C, was slowly added 2-octanol (1 equiv). The reaction mixture was stirred at 0-5 °C for 30 min, and then allowed to warm to room temperature. Stirring was continued for a further 30 min, and the reaction mixture was then concentrated *in vacuo*. Further purification was attempted as detailed for individual compounds.

Method D (Acid Chloride method)

To a mixture of 2-octanol (1 equiv) and pyridine (1.2 equiv) in dried DCM (2 ml), cooled to 0 °C, was added dropwise the appropriate acid chloride (1.2 equiv). The reaction mixture was stirred at 0 °C for 5 min, and then allowed to warm to room temperature. Stirring was continued for 30 min. The reaction mixture was then washed with water (1 ml), 2M Na_2CO_3 (2 x 1 ml), 5% HCl (1 ml) and water again (1 ml). The organic layer was dried over anhydrous Na_2SO_4 and volatiles were removed *in vacuo* to give the product.

Method E (EDC-HCl Method)

To a mixture of 2-octanol (2 mmol) and the appropriate carboxylic acid (2 mmol) in dried DCM (10 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC-HCl, 1 equiv.). The reaction mixture was stirred at room temperature for 1 h, and was then washed with water (3 x 5 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the product.

Method F (CDI/DBU method)[294]

To a mixture of the appropriate carboxylic acid (1 mmol) in dried DMF (1 ml) in an atmosphere of nitrogen was added 1,1'-carbonyldiimidazole (1 mmol). The reaction mixture was warmed to 30 °C for 1 h. 2-Octanol (2 mmol) and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) (1 mmol) were added to the reaction mixture, which was then stirred under nitrogen at 40 °C overnight. Diethyl ether (3 ml) was added to the reaction mixture, and the solution was washed with 4M HCl (1 ml), water

(1 ml) and 5% Na_2CO_3 (1 ml). The organic layer was dried over anhydrous Na_2SO_4, and volatiles were removed *in vacuo* to give no product.

Method G (DCC/DMAP method)[295]

To a solution of *N,N'*-dicyclohexylcarbodiimide (1 equiv), 4-(*N,N'*-dimethylamino)-pyridine (DMAP, 0.1 equiv) and 2-octanol (1.1 equiv) in diethyl ether (1 ml) was added the appropriate carboxylic acid (1 equiv). The reaction mixture was stirred at room temperature for 1 h, and then filtered. The filtrate was concentrated *in vacuo*, taken up in diethyl ether and filtered again. This filtration/concentration procedure was repeated until no more DCU was present in the reaction mixture, to give the product.

(R)-2-Octyl Acetate (147)

Acetic acid (0.040 ml, 0.66 mmol) was reacted according to method G to give (R)-2-octyl acetate as a clear oil, slightly contaminated with DCU (0.042 g, 37%); ν_{max}/cm^{-1} ($CHCl_3$) 3 010, 2 950, 2 920, 2 850, 1 720, 1 460, 1 450, 1 375 and 1 250; δ_H (200 MHz) 0.86 (3H, unres. t), 1.15 - 1.65 (12H, m, product + DCU), 2.00 (3H, s), 4.78 - 4.97 (1H, m); δ_C (50 MHz) 14.03 (q), 19.92 (q), 21.36 (q), 22.55 (t), 25.34 (t), 29.09 (t), 31.71 (t), 35.89 (t), 71.05 (d), 170.80 (s); *m/z* 157 (M^+-CH_3, 0.3%), 143 (1.0), 112 (1.7) and 43 (100.0).

(R)-2-Octyl Propanoate (148)

Propanoic acid (0.050 ml, 0.66 mmol) was reacted according to method G to give (R)-2-octyl propanoate as a clear oil, slightly contaminated with DCU (0.050 g,

41%); ν_{max}/cm^{-1} ($CHCl_3$) 3 020, 2 920, 2 850, 1 720, 1 460, 1 380 and 1 195; δ_H (200 MHz) 0.87 (3H, unres. t), 1.08 - 2.09 (30H, m, product + DCU), 2.28 (2H, q, J 7.6 Hz), 2.46 (2H, q, J 7.5 Hz), 4.86 (1H, m); *m/z* 143 (M^+-C_3H_7, 1.1%), 126 (8.7), 120 (14.5) and 43 (100.0).

Satisfactory ^{13}C N.M.R. data could not be obtained for this compound due to lack of material.

(R)-2-Octyl Butanoate (149)

Butanoic acid (0.060 ml, 0.66 mmol) was reacted according to method G to give (R)-2-octyl butanoate as a clear oil, slightly contaminated with DCU (0.051 g, 39%); ν_{max}/cm^{-1} ($CHCl_3$) 3 010, 2 950, 2 920, 2 850, 1 715, 1 450, 1 380 and 1 190; δ_H (200 MHz) 0.80 - 1.02 (6H, unres. t), 1.02 - 1.78 (18H, unres. m), 2.23 (2H, unres. t), 4.78 - 4.97 (1H, m); δ_C (50 MHz) 13.61 (q), 14.02 (q), 18.54 (t), 19.99 (q), 22.54 (t), 25.34 (t), 29.08 (t), 31.71 (t), 35.92 (t), 36.60 (t), 70.68 (d), 173.36 (s); *m/z* 170 (M^+-C_3H_7, 1.6%), 126 (11.8), 114 (1.6), 98 (34.6), 88 (38.0) and 43 (100.0).

(R)-2-Octyl Benzoate (150)

Benzoic acid (0.081 g, 0.66 mmol) was reacted according to method G to give (R)-2-octyl benzoate as a white solid (0.017 g, 11%), slightly contaminated with DCU; ν_{max}/cm^{-1} ($CHCl_3$) 3 010, 2 920, 2 850, 1 710, 1 450, 1 280 and 1 160; δ_H (200 MHz) 0.86 (3H, unres. t), 1.05 - 1.65 (14H, unres. m), 5.05 - 5.23 (1H, m), 7.38 - 7.58 (3H, m), 8.01 - 8.06 (2H, m); δ_C (50 MHz) 14.06 (q), 20.08 (q), 22.59 (t), 25.41 (t), 29.16 (t), 31.74 (t), 33.94 (t), 36.06 (t), 71.75 (d), 128.26 (d), 129.49

(d), 132.67 (d); *m/z* 234 (M^+, 0.4%), 235 (0.1), 123 (31.9), 112 (19.6), 105 (100.0) and 77 (43.7) (Found: M^+, 234.1620. $C_{15}H_{22}O_2$ requires M^+, 234.1620).

(R)-2-Octyl Phenylacetate (151)

Phenylacetic acid (0.091 g, 0.66 mmol) was reacted according to method G to give (R)-2-octyl phenylacetate as a clear oil (0.098 g, 60%), slightly contaminated with DCU; ν_{max}/cm^{-1} ($CHCl_3$) 3 010, 2 930, 2 850, 1 710, 1 450, 1 310 and 1 280; δ_H (200 MHz) 0.86 (3H, unres. t), 0.95 - 2.10 (16H, unres. m), 3.58 (2H, s), 4.81 - 4.97 (1H, m), 7.24 - 7.32 (5H, m); δ_C (50 MHz) 14.04 (q), 19.90 (q), 22.50 (t), 25.50 (t), 29.01 (t), 31.67 (t), 35.84 (t), 41.79 (t), 71.51 (d), 126.90 (d), 128.45 (d), 129.16 (d), 134.36 (s), 171.25 (s); *m/z* 248 (M^+, 0.1%), 163 (0.3), 157 (2.8), 119 (3.7), 112 (6.6), 91 (89.4), 71 (41.7), 65 (28.7), 57 (64.7) and 43 (100.0).

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