Dynamic Kinetic Resolution:
Synthesis of Optically Active $\alpha$-Amino
Acid Derivatives

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The University of Edinburgh

A thesis submitted for the degree of Doctor of Philosophy

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Declaration

This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work is original, and has not been previously submitted, in whole or in part, for any degree at this, or any other university.
Abstract

The dynamic kinetic resolution of 2-phenyl-4-substituted-5(4H)-oxazolones 89a-g has been investigated as a method for the synthesis of optically active α-amino acid derivatives. The effects of lipase, [either Novozyme® (Candida antarctica lipase B), or Lipozyme® (Rhizomucor miehei lipase)], solvent, nucleophile, and the addition of external triethylamine to the reaction is described. When \( R' = \text{Ph} \), an 88% yield and 98% enantiomeric excess (e.e.) of α-amino acid ester 96a was obtained with Novozyme® in acetonitrile as solvent. The synthesis of novel 5(4H)-oxazolones 129a-e, which are identified as key intermediates in the synthesis of a series of matrix metalloproteinase inhibitors 94, is described. Application of the lipase catalysed dynamic kinetic resolution conditions to 129a-e, afforded high yields (96%) and diastereomeric excesses (d.e.'s), (86%) of the resulting pseudodipeptides \((2R,2'S)-127a-e\) and 130-132, by careful selection of the reaction conditions.

\[
\begin{align*}
\text{Scheme 1 Dynamic kinetic resolution of } 5(4H)-\text{oxazolones} \\
\end{align*}
\]
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<td>Ac</td>
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<tr>
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<td>benzyl alcohol</td>
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<td>tert-butoxycarbonyl</td>
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<td>d.e.</td>
<td>diastereomeric excess</td>
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<td>DIPE</td>
<td>diisopropylether</td>
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<td>DMAP</td>
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<tr>
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<td>enantiomeric excess</td>
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<td>IPA</td>
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<tr>
<td>Lip ozyme® Rhizomucor miehei lipase</td>
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<td>m</td>
<td>multiplet</td>
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<td>Me</td>
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</tr>
<tr>
<td>MMPI</td>
<td>matrix metalloproteinase inhibitor</td>
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<td>Mp</td>
<td>melting point</td>
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<td>MS</td>
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<tr>
<td>NMM</td>
<td>N-methyl morpholine</td>
</tr>
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<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
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<tr>
<td>nmr</td>
<td>nuclear magnetic resonance</td>
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<td>Candida antarctica lipase B</td>
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<td>2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
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<tr>
<td>ZBG</td>
<td>zinc binding group</td>
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1.1.1. Role of lipases in nature

In nature, lipases, or triacylglycerol hydrolases (E.C. 3.1.1.3) as they are systematically known, are classified as hydrolases that catalyse the hydrolysis of fatty acids and glycerol at the lipid/water interface. In contrast to proteases and esterases, lipases generally exhibit little or no catalytic activity at low substrate concentrations, and therefore do not follow normal Michaelis-Menten kinetics. Hydrolysis is only realised once the substrate concentration has reached its saturation limit, or critical micellar concentration (CMC) (Figure 1).

![Activity of lipases and esterases](image)

**Figure 1** Activity of lipases and esterases

In the early 1990’s when the first X-ray crystal structures of lipases began to emerge, it became evident that the mechanism of interfacial activation was due, in part, to the presence of an amphiphilic peptidic loop, or lid, covering the active site of the enzyme. The analysis of X-ray co-crystals between lipase and substrate analogues suggested that once CMC is reached, a conformational change occurs at the lipid/water interface, and the lid ‘flips’, thus allowing access to the previously buried
It should be noted however that not all lipases have a lid present in their tertiary structure, or show interfacial activation.\(^4\)

All of the lipases whose structures have been elucidated, (12 based on data up to 1998) contain a common architecture. Catalytic activity is realised by a triad consisting of a nucleophilic serine residue, a histidine residue, and an aspartate or glutamate residue (Scheme 2) similar to that found in the serine proteases. Also, in

---

**Scheme 2** *Catalytic mechanism of lipases*
over thirty cases of cloned lipases, a consensus sequence of -Gly/Ala-X-Ser-X-Glu- has been identified at the active site serine.\(^5\)

Recently there has been some degree of confusion in the literature with regards to the naming of some lipases. For example, *Candida rugosa* was previously classified as *Candida cylindracea*, and *Pseudomonas cepacia* was previously classified as *Pseudomonas fluorescens*. To avoid any confusion, the name given in the cited article is used here.

1.1.2. The introduction of organic solvent

There are several advantages to using an organic solvent as opposed to an aqueous system for enzymatic transformations, these include, (a) water insoluble substrates, and substrates and products that may be unstable in aqueous media can be investigated, (b) a broader range of nucleophiles can be used including e.g. alcohols, amines, ammonia, oximes, and hydrazines, (c) separation of reagents from the enzymes is greatly simplified as the enzymes are insoluble in organic solvents therefore only require filtration, and (d) the enzyme can be recovered and reused.

The use of organic media in enzymatic catalysis was first documented by Pottevin\(^6\) in 1906 when he used a pancreatic extract in methanol to catalyse the formation of methyl oleate. It was not until the pioneering work of Klibanov and co-workers almost eighty years later in the mid 1980's, that biocatalysis in organic solvent was implemented in the synthetic chemistry laboratory. Initial perception was that enzymes would exhibit only low catalytic activity as the concentration of organic solvent was increased, due to denaturation of the enzyme.

To circumvent this problem, Klibanov and co-workers\(^7\) encapsulated aqueous solutions of hog liver carboxylesterase and lipase from *Candida cylindracea* inside the porous supports Sepharose or Chromosorb. The resulting beads were suspended in a water-immiscible organic solvent, methyl propionate in the case of hog liver carboxylesterase, and tributyrin for lipase from *Candida cylindracea*. In both cases the solvent also served as substrate (Scheme 3a and b respectively). A number of
primary and secondary alcohols were tested as nucleophiles and both enzymes exhibited high transesterification activity and stereoselectivity.

a) 

\[
\text{OCH}_3 \quad + \quad \text{HOR}^* 
\xrightarrow{\text{hog liver carboxylesterase}} 
\text{OR}^* \quad + \quad \text{HOR}^* 
\]

\( (RS) \quad (S) \quad (R) \)

b) 

\[
\text{Tributyrin} \quad + \quad \text{HO} - \left( \text{C}_{\text{C}} \text{andida cylindracea} \right) \text{lipase} \quad \text{OR}^2 \quad \text{OR}^1 
\xrightarrow{\text{Candida cylindracea lipase}} 
\text{OR}^2 \quad \text{OR}^1 
\]

\( (RS) \quad (R) \quad (S) \)

Scheme 3

Expanding on these promising initial results, Klibanov and co-workers\(^8\) utilised lyophilised *Candida cylindracea* and porcine pancreatic lipases in hexane in the esterification and transesterification of racemic carboxylic acids and esters respectively. Klibanov *et al.*,\(^9\) were also the first to implement lipases in organic solvent for amide bond formation and peptide synthesis (see Section (1.2.2.) below).

A major problem with using lyophilised enzyme preparations in organic solvents is that the observed activity is often 10,000 fold less than that observed in aqueous systems due to denaturation.\(^{10}\) Immobilisation of enzymes onto solid supports, *via* covalent attachment, or electrostatic interactions, and more recently the introduction of cross-linked enzyme crystals,\(^{11,12}\) (CLECs, prepared by cross-linking the enzyme with glyceraldehyde), heralded a new approach to enzyme preparation and provided enzymes with increased stability and activity in organic solvents. The introduction of surfactants,\(^{13}\) hydrophobic sol-gel materials,\(^{14}\) solid state acid-base buffers\(^{15}\) and control of the hydration level of enzymes\(^{16}\) have all added to a greater understanding of the processes of enzyme activation in organic solvents. The advances in techniques for using biocatalysts in organic media have been reviewed in two informative articles.\(^{17,18}\)
1.2.0. Resolution processes

1.2.1. Kinetic resolution

The general reaction is outlined in Figure 2. The success of a kinetic resolution relies on the fact that in the presence of a chiral environment, e.g. an enzyme, the rate of reaction, \(k_{(R)}\) and \(k_{(S)}\) for each enantiomer, \((R)\) and \((S)\) of a racemic mixture differ significantly. In the ideal case, the rate of reaction of the slower reacting enantiomer would be 0, resulting in the reaction of a single enantiomer. As a result, a maximum inherent yield of 50% is obtainable in kinetic resolution processes.

\[
\begin{align*}
\text{Substrate (R)} & \quad \xrightarrow{k_{(R)}} \quad \text{Product (R)} \\
\text{Substrate (S)} & \quad \xrightarrow{k_{(S)}} \quad \text{Product (S)}
\end{align*}
\]

Figure 2

Kinetic resolution of alcohols and carboxylic acids are carried out routinely in the laboratory and are far too wide ranging to be covered in the required detail in this thesis. Kazlauskas and co-workers\(^{19-21}\) have published an empirical rule predicting the chiral preference of lipases for the resolution of secondary alcohols. In all cases, the preferred enantiomer, drawn with the hydroxyl group pointing forward out of the plane of the paper, was as depicted in Figure 3a. Kazlauskas\(^{22}\) also published a similar rule for the resolution of primary alcohols (with no oxygen substituent at the adjacent stereocentre) using lipase from *Pseudomonas cepacia*. Although the opposite enantiopreference is predicted, it is hypothesised that the substrate interacted with the lipase in a similar manner to secondary alcohols, with the substituents on the adjacent chiral carbon functioning as the large, L, and medium, M, sized groups. The addition of the methylene group allows the primary alcohol moiety the flexibility to adopt a similar conformation to that of the secondary alcohol (Figure 3b).
Similar rules have been predicted for the enantioselectivity of Candida rugosa lipase towards carboxylic acids (Figure 4a), and Aspergillus niger lipase for L-α-amino acids (Figure 4b). The predicative rules for carboxylic acids, however, are less reliable than those for secondary alcohols, and are best applied to reactions carried out with purified lipase instead of the crude commercial preparation which often contain contaminating enzymes.

A large number of excellent articles already exist in the literature documenting kinetic resolutions in both non-aqueous and aqueous media. A number of unusual cases merit closer inspection.

1.2.2. Kinetic resolution with nitrogen nucleophiles

Although there are far fewer examples documenting the use of nitrogen nucleophiles in enzymatic processes in the literature, the area is growing rapidly. As indicated above, Klibanov was the first to document the use of amines of amino acid esters, as nucleophiles for lipase mediated biotransformations in organic solvent. However, Gotor and co-workers were the first to implement the use of primary amines as nucleophiles in the aminolysis of ethyl (±)-2-halopropionate, 1 (X= Cl and Br) catalysed by Candida cylindracea lipase (Scheme 4).
Greater enantioselectivity was obtained for the Novozyme®, \textit{(Candida antarctica lipase B)}, mediated resolution of ethyl 3-substituted-3-hydroxypropionates 3 in dioxan with benzylamine as the nucleophile (Scheme 5).\textsuperscript{30,31} The resulting (R)-benzylamides 4 were obtained in >99% and 98% e.e. at 45% and 50% conversion when \( R = \) methyl and chloromethyl respectively. Also, in the case where \( R = \) chloromethyl the corresponding (S)-ethyl ester 3 was recovered in optically pure form. Both chiral \( \beta \)-hydroxy amides and esters are of interest as they serve as intermediates in the synthesis of \( \beta \)-aminoalcohols, which are themselves intermediates in the synthesis of a number of antibiotics and antidepressants.

Gotor et al.\textsuperscript{32} also introduced the transamidation reaction illustrated in Scheme 6. The activated racemic trifluoroethylamide 5 was reacted with \textit{n}-butylamine in the presence of \textit{Candida cylindracea} lipase (CCL) to give the (S)-\textit{n}-butylamide 6 in 49% yield and 78% e.e.
An excellent example of amide bond formation was achieved by Conde et al.\textsuperscript{33} Separate exposure of each enantiomer of diethyl Cbz-glutamate 7 to Novozyme\textsuperscript{®} in the presence of a variety of amines, afforded aminolysis predominantly at the \(\alpha\)-position for the L-enantiomer, and predominantly at the \(\gamma\)-position for the D-enantiomer (Scheme 7). Best results were obtained at an elevated temperature of 45-60 °C.

Greater success has been achieved in the resolution of amines, although a number of issues must be addressed before carrying out such reactions. Amines, being more nucleophilic than alcohols, are prone to react non-enzymatically with activated esters resulting in low e.e.'s. The use of enol esters must also be avoided as the aldehydes or ketones liberated in these processes undergo Schiff-base reactions with the amine substrates to form imines. The synthesis of chiral (R)-propiolamides 12, using 3-substituted-propylate methyl esters 10 as acyl donors, proceeded in good yield with e.e.'s ranging from 63-97% (Scheme 8).\textsuperscript{34,35} The best results, collected in Table 1, were achieved with 1-phenylethylamine in diisopropylether. Lower e.e.'s were obtained (63-84%) when 2-propyl and 2-hexylamine were used.
Introduction

Scheme 8

<table>
<thead>
<tr>
<th>Entry</th>
<th>10 R₁</th>
<th>11 R₂</th>
<th>12 Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂=CH</td>
<td>C₅H₁₁</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>CH₂=C(CH₃)</td>
<td>C₅H₅</td>
<td>27</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>Ph</td>
<td>83</td>
<td>&gt;95</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>Ph</td>
<td>84</td>
<td>&gt;95</td>
</tr>
<tr>
<td>5</td>
<td>Ph</td>
<td>Ph</td>
<td>79</td>
<td>&gt;95</td>
</tr>
<tr>
<td>6</td>
<td>Ph</td>
<td>Ph</td>
<td>78</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

Table 1

Gotor et al.³⁶ expanded on these results publishing the resolution of 1-(heteroaryl)ethylamines 13 with Novozyme®, to furnish the desired (R)-amides 14 with excellent e.e.’s (Scheme 9) Hedenström³⁷ also used Novozyme® for the resolution of amines. Using a variety of organic solvents and temperatures, diastereomeric 2-methyloctanoic phenylethylamide was prepared from racemic phenylethylamine and racemic ethyl 2-methyloctanoate with moderate selectivity.

Scheme 9

BASF AG (Germany) introduced the resolution of 1-phenylethylamine 15 as a commercial process utilising lipase from Burkholderia plantarii with ethyl
methoxyacetate \(16\) as acyl donor (Scheme 10).\(^{38}\) Yields in excess of 45% of pure \((S)\)-1-phenylethylamine \(15\) are reported.

\[
\begin{align*}
\text{NH}_2 & \quad \text{O} \quad \text{O}\text{CO}_\text{CH}_2\text{OH} \\
\text{Ph} & \quad \text{O} \quad \text{O}\text{CO}_\text{Me} \\
\text{NH}_2 & \quad \text{O} \quad \text{O}\text{CO}_\text{Me}
\end{align*}
\]

\(\text{rac-15} + 16 \xrightarrow{\text{Burkholderia}} \text{plantarii lipase} \xrightarrow{\text{1BuOMe}} \text{(S)-15} >99\% \text{ e.e.} \quad \text{(R)-17} 93\% \text{ e.e.}
\]

Scheme 10

The use 3-methyl-3-pentanol as solvent has proved successful in suppressing non-enzymatic amidation. In the resolution of \(1\)-(1-naphthyl)ethylamine \(18\) with cyanomethyl pent-4-enoate \(19\) as acyl donor, the use of THF, \(\text{CH}_2\text{Cl}_2\), DMF and tert-butyl alcohol as solvent resulted in a degree of non-enzymatic amidation. On switching to 3-methyl-3-pentanol, \((S)\)-amide \(20\) was isolated in 43% yield and 97% e.e. as depicted in Scheme 11.\(^{39}\)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \quad \text{O}\text{CN} \\
\text{Ph} & \quad \text{O} \quad \text{O}\text{CN}
\end{align*}
\]

\(\text{rac-18} + 19 \xrightarrow{3\text{-methyl-3-pentanol}} \text{Subtilisin Carlsberg} \xrightarrow{\text{S}} \text{(S)-20}
\]

43% yield, 97% e.e.

Scheme 11 Enantioselective acylation of \(1\)-(1-naphthyl)ethylamine

A recent advance in enzymatic amidation reactions has been the introduction of symmetrical\(^\text{40,41}\) and unsymmetrical\(^\text{39,42-44}\) carbonates as acyl donors. The reactions are irreversible as the carbamates formed are not substrates for lipases or proteases. Carbamates also have the advantage of simple removal under mild conditions to furnish the valuable chiral amine.
Gotor and co-workers\textsuperscript{44} were the first to implement unsymmetrical vinyl carbonates in the resolution of amines (Scheme 12). The best results obtained were for the resolution of 1-phenylethylamine 22 with \textit{n}-octyl vinyl carbonate in the presence of Novozyme\textsuperscript{®}. The resulting (\textit{R})-carbamate 23 was obtained in 98\% e.e. at 39\% conversion. Lower e.e.'s were observed when \textit{n}-butyl vinyl carbonate was used as acyl donor. Lower e.e.'s were also obtained in the resolution of 2-heptyl and 2-butyl amine.

\begin{equation}
\begin{array}{c}
\begin{array}{c}
\text{R}^1 \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\begin{array}{c}
\text{Ph} \\
\text{NH}_2
\end{array}
\end{array}
\xrightarrow{\text{Novozyme\textsuperscript{®} hexane}}
\begin{array}{c}
\begin{array}{c}
\text{Ph} \\
\text{NH}_2 \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\begin{array}{c}
\text{R}
\end{array}
\end{array}
\end{equation}

\(\text{R= a) \textit{n}-octyl, b) \textit{n}-butyl 21 \quad \text{rac-22} \quad (\text{R})-23\)

\textbf{Scheme 12}

Wong and co-workers\textsuperscript{45} carried out a systematic study of amine protecting groups and categorised their utility. By investigating the amount of non-enzymatic background reaction in non-reaction suppressing solvents, such as toluene, or reaction suppressing solvent (3-methyl-3-pentanol) against the IR absorption of the carbonyl groups, it is possible to obtain an accurate estimation of reactivity of similar and dissimilar esters and carbonates. This is possible because the frequency of the carbonyl stretch reflects the bond length, and therefore the reactivity of the carbonyl group. A larger wavenumber indicates a shorter C=O bond, thus a more reactive carbonyl group. The study identified novel enzymatic protecting groups, namely 24, and carbonate 25, and the known dibenzyl carbonate 26,\textsuperscript{41} which were utilised in amine resolutions with yields of 33-46\% and e.e. of 81-99\% (Table 2).

\begin{center}
\begin{tabular}{ccc}
24 & 25 & 26
\end{tabular}
\end{center}
<table>
<thead>
<tr>
<th>Entry</th>
<th>Product*</th>
<th>Acyl Donor</th>
<th>Yield</th>
<th>e.e. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Ph O" /> 24</td>
<td>43</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="HN O" /> 24</td>
<td>46</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="OH NHCbz" /> 26</td>
<td>36</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="NHCbz" /> 26</td>
<td>41</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

*a) Reaction conditions: toluene (high concentration), Novozyme®

Table 2

The use of ammonia as the nucleophile was simultaneously reported by the groups of Sheldon and Goto. Sheldon reported that by bubbling ammonia gas through a solution of the 2-chloroethyl ester of ibuprofen 27, and adding Novozyme®, the resulting (R)-amide 28 was formed (Scheme 13). At 56% conversion, the (S)-ester 27 was recovered in 93% e.e. The corresponding hydrolysis reaction in water furnished ester (S)-27 in only 58% e.e. at 63% conversion.

Similarly, in the ammoniolysis of α-methylbenzyl n-butyrate 29, α-methylbenzyl alcohol 30 was recovered at 45% conversion in 98% e.e (Scheme 14). Enzymatic ammoniolysis has also been employed in a one pot, double resolution process for the synthesis of fatty acid amides and carboxylic amides.
1.2.3. Kinetic resolution of organometallic substrates

Lipases have proved to be robust enzymes and their use in the kinetic resolution of organometallic compounds such as ferrocenes, (η⁶-arene)-chromium and (η⁴-diene)-iron tricarbonyl complexes is known. Chiral 1-hydroxymethyl-2-substituted ferrocenes, possessing planar chirality, are useful in asymmetric catalysis and therefore routes to optically pure ferrocenes are of great interest. 1-Hydroxymethyl-2-substituted ferrocenes have been resolved using a number of lipases with excellent selectivity, as depicted in Scheme 15, with a summary of the results presented in Table 3. The results lead to a preferred enantiomer model (with regard to the size of the ortho substituent to the hydroxymethyl group) similar to that proposed by Kazlauskas for the enantiopreference of lipases in the recognition of secondary alcohols.
**Introduction**

![Chemical structure](image)

**Scheme 15**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Solvent</th>
<th>31 R</th>
<th>conv.</th>
<th>32 e.e.</th>
<th>33 e.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas cepacia&lt;sup&gt;51&lt;/sup&gt;</td>
<td>benzene</td>
<td>CH₂OH</td>
<td>80</td>
<td>100&lt;sup&gt;(1S)&lt;/sup&gt;</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>CCL&lt;sup&gt;52&lt;/sup&gt;</td>
<td>tBuOMe</td>
<td>CH₂N(CH₃)₂</td>
<td>65</td>
<td>-</td>
<td>&gt;95&lt;sup&gt;(32a, 1R)&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>CCL&lt;sup&gt;52&lt;/sup&gt;</td>
<td>tBuOMe</td>
<td>CH₂N(CH₃)</td>
<td>42</td>
<td>92&lt;sup&gt;(1S)&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Novozyme&lt;sup&gt;0,53&lt;/sup&gt;</td>
<td>DIPE</td>
<td>SCH₃</td>
<td>32</td>
<td>90&lt;sup&gt;(1R)&lt;/sup&gt;</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Lipozyme&lt;sup&gt;0,54&lt;/sup&gt;</td>
<td>DIPE</td>
<td>S′Bu</td>
<td>55</td>
<td>-</td>
<td>95&lt;sup&gt;(43a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Novozyme&lt;sup&gt;0,54&lt;/sup&gt;</td>
<td>DCM</td>
<td>SPh</td>
<td>40</td>
<td>90&lt;sup&gt;(1R)&lt;/sup&gt;</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Novozyme&lt;sup&gt;0,50&lt;/sup&gt;</td>
<td>DCM</td>
<td>I</td>
<td>52</td>
<td>89</td>
<td>96</td>
</tr>
</tbody>
</table>

*Table 3*

Ferrocenes containing central chirality have also been resolved enzymatically. Boaz<sup>55</sup> resolved 1-ferrocenylethanol 34 into the (R)-acetate 35 and recovered (S)-alcohol 34 with a high degree of selectivity (Scheme 16). Similarly, Kim *et al.*<sup>56</sup> prepared the (R)-acetates 37 and recovered (S)-alcohols 36 of ferrocenylpropanol and ferrocenylbutanol (Scheme 17).

![Chemical structure](image)

**Scheme 16**

*a) Isolated yield, b) Rhizomucor miehei lipase, c) Vinyl propionate used as acyl donor, product was corresponding propionate ester.*
The (R)-(+) cyanohydrin acetate 38, was prepared in 84% e.e. from the corresponding racemic cyanohydrin.\textsuperscript{57}

Nakamura \textit{et al.}\textsuperscript{58} and Yamazaki\textsuperscript{59} independently reported the resolution of (±)-tricarbonyl (η\textsubscript{4}-2-methylbenzyl alcohol)-chromium 39 in isopropenyl acetate and vinyl palmitate in toluene respectively. Excellent selectivity was observed, furnishing both the unreacted (R)-39 and acetylated (S)-40 products in optically pure form (Scheme 18). The 2-methoxy, 3-methyl\textsuperscript{59} and 2-trimethylsily\textsuperscript{58} derivatives were also prepared with equally impressive results.

\begin{scheme}
\centering
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{(R)-38}};
\end{tikzpicture}
\caption{Resolution of (±)-tricarbonyl (η\textsubscript{4}-2-methylbenzyl alcohol)-chromium 39}
\end{scheme}
The resolution of (arene)Cr(CO)_3 complexes with central chirality has also been achieved. When 41 was exposed to lipase Toyobo A from *Pseudomonas aeruginosa*, acetate 43 and unreacted alcohol 42 were obtained in optically pure form in >40% yield (Scheme 19).^{60}

![Scheme 19](image)

The resolution of hydroxymethyl substituted (diene)Fe(CO)_3 complexes 44, has also been realised (Table 4).^{60} By careful choice of the lipase, it was also possible to obtain either enantiomer of the alcohol 44 (entries 2 and 3).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>R_1</th>
<th>R_2</th>
<th>yield/%</th>
<th>e.e./% (config.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amano PS</td>
<td>H</td>
<td>CH_3</td>
<td>22</td>
<td>97 (S)</td>
</tr>
<tr>
<td>2</td>
<td>Amano PS</td>
<td>H</td>
<td>Ph</td>
<td>36</td>
<td>96 (S)</td>
</tr>
<tr>
<td>3</td>
<td>Amano AY</td>
<td>H</td>
<td>Ph</td>
<td>18</td>
<td>99 (R)</td>
</tr>
<tr>
<td>4</td>
<td>Amano PS</td>
<td>CH_3</td>
<td>Ph</td>
<td>48</td>
<td>99 (S)</td>
</tr>
<tr>
<td>5</td>
<td>Amano PS</td>
<td>CH_3</td>
<td>&quot;Bu</td>
<td>47</td>
<td>92 (S)</td>
</tr>
</tbody>
</table>

Table 4

The resolution of a number of α-hydroxystannanes was achieved via formation of the corresponding butyl esters (Scheme 20). At 50% conversion, the butyl acetate
(S)-46 was obtained in 38% yield, 98% e.e., and the unreacted alcohol (R)-45 in 41% yield, 97% e.e.\(^6\)

\[
\begin{align*}
\text{MeSn(CH}_3\text{)}_3 & \quad \stackrel{\text{PPL, Et}_2\text{O, 25 °C}}{\rightarrow} & \quad \text{BuCO}_2\text{CH}_2\text{CF}_3 \\
45 & \quad \rightarrow & \quad \text{MeSn(CH}_3\text{)}_3
\end{align*}
\]

Scheme 20

Optically active silane\(^6\) 47 and germane\(^6\) 48 compounds have also been prepared. Both enantiomers of silane 47 were prepared by correct choice of lipase, CCL producing (+)-47, and lipase from *Chromobacterium viscosum* (-)-47.

\[
\begin{align*}
R= \text{Ph, n-octyl 47} & \quad 50-80\% \text{ yield, 70-76\% e.e.} \\
(-)-48 & \quad 57\% \text{ yield, 50\% e.e.}
\end{align*}
\]

1.2.4. The desymmetrisation of meso or prochiral substrates

Although kinetic resolution processes have proved very successful, they suffer from the limitation of a maximum yield of 50%. Close monitoring of the progress of the reaction is often required so as to maximise the e.e. of the desired compound. In general, if the product is required in optically pure form, the reaction is stopped at low conversion, 20-30%, whereas if the substrate is required, the reaction is stopped at high conversion, 70-80%. The end result in both instances is a reduction in yield to significantly less than the theoretical 50%. The use of meso or prochiral substrates is one method of overcoming this limitation (Scheme 21). As in a normal kinetic resolution, the enzyme will react with one alcohol group faster than the other, thereby creating a chiral product, in this case (R)-50 with a theoretical yield and e.e. of 100%.
Introduction

Scheme 21 Desymmetrisation of prochiral 2-substituted-1,3-diols

Through the desymmetrisation of meso or prochiral compounds a large number of acyclic and cyclic, mono and unsymmetrical diacetates and diacids have been prepared. A number of excellent reviews summarising the compounds prepared utilising meso or prochiral substrates exist in the literature.\textsuperscript{25,64-66}

Johnson has carried out an extensive programme in the enzymatic preparation of 5, 6, 7, and 8 membered ring unsaturated diols, represented in Figure 5. These chiral cyclic intermediates have been used in the synthesis of a number of biologically important compounds, such as C-glycosides, several (deoxy)norjirimycins, conduritol derivatives, 3-deoxy-D-arabino-heptulosonic acid derivatives, and the tropane alkaloid calystegine A\textsubscript{3}.\textsuperscript{67}

Figure 5

A comprehensive study on the effect of solvent, acyl donor, enzyme, temperature and concentration was carried out in the desymmetrisation of the 2-substituted-1,3-propanediol 51, an intermediate in the synthesis of a potential antifungal agent SCH51408 developed by Schering-Plough.\textsuperscript{68} Of the 205 commercial enzyme preparations tested, four furnished the desired (S)-acetate 52 with an acceptable e.e.
of >97%. Novozyme® was chosen for further examination and the optimised conditions (Scheme 22) were operated on pilot plant scale to furnish the product in 81% yield and 97% e.e.

\[
\begin{array}{c}
\text{51} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Novozyme®, CH₃CN} \\
0 °C \\
\end{array}
\]

\[
\begin{array}{c}
\text{(S)-52 97% e.e.} \\
\end{array}
\]

Scheme 22 Resolution of 2-substituted-1,3-propanediol

Chiral phosphonate derivatives have recently been prepared from prochiral phosphonates (Scheme 23).⁶⁹ Best results were obtained with Lipase PS in a range of ether solvents at 25 °C. The results were found to be in agreement with those predicted for the enantiopreference of chiral primary alcohols with lipases from Pseudomonas cepacia reported by Kazlauskas.²²

\[
\begin{array}{c}
\text{R=a) CH₂, b) CH₂CH₂, c) CH₂CF₂} \\
\end{array}
\]

83-99% yield, 91-98% e.e.

Scheme 23

The desymmetrisation of mono-substituted malonates 54 was achieved under transesterification conditions by Gutman et al.⁷⁰,⁷¹ By the correct choice of nucleophile and substrate ester, both enantiomers of the unsymmetrical diester 53 were obtained with the same lipase. Although excellent e.e.’s were obtained, the reactions were stopped at moderate conversions (48-53%) to minimise the formation of diester by-product.
1.2.5. Dynamic kinetic resolution

Another method of maximising the obtainable yield is through a dynamic kinetic resolution process (Figure 6) which involves a classical kinetic resolution coupled with \textit{in situ} substrate racemisation.\textsuperscript{72-76} Substrate racemisation enables complete conversion to the desired product, resulting in a theoretical yield and e.e. of 100%.

For a dynamic kinetic resolution to be effective, the rate of racemisation, $k_{\text{rac}}$ must be greater than (or at least equal to) the rate of enzymatic reaction $k_{(S)}$ for the faster reacting enantiomer ($S$). Also, the initial kinetic resolution must be selective in that $k_{(S)}$ is greater than $k_{(R)}$.

\[
\begin{array}{c}
\text{Substrate (R)} \xrightarrow{k_{(R)} \text{ (slow)}} \text{Product (R)} \\
| \xrightarrow{k_{(\text{rac}} \text{ (fast)}} \\
\text{Substrate (S)} \xrightarrow{k_{(S)} \text{ (fast)}} \text{Product (S)}
\end{array}
\]

\textbf{Figure 6 Dynamic kinetic resolution}

Zwanenburg and co-workers\textsuperscript{77} utilised a dynamic kinetic resolution in the synthesis of the 5-acetyloxy-2(5H)-furanones \textit{56} (Scheme 25). The stereogenic centre at C-5 of the substrate hydroxy-2(5H)-furanones \textit{55} is labile due to mutarotation. Acylation of the faster reacting ($S$) enantiomer with lipase PS-30 furnished the stable 5-acetyl-2(5H)-furanones \textit{56} in 78-86% e.e. at 100% conversion.

\textbf{Scheme 25 Dynamic resolution of 5-hydroxy-2(5H)-furanones}
In a subsequent report, the synthesis of 6-acetyloxy-2H-pyran-3(6H)-one 57 under identical conditions was achieved.\textsuperscript{78}

\[
\begin{align*}
\text{(S)}-57 & \quad 76\% \text{ e.e.}
\end{align*}
\]

Kellog and Feringa\textsuperscript{79} reported the synthesis of 5-acetyloxy-2(5H)-furanone 58, and the corresponding pyrrolinone 60, in optically pure form using Lipase R or Novozyme\textsuperscript{®} respectively (Scheme 26).

\[
\begin{align*}
\text{58} & \quad 90\% \text{ yield} \\
& >99\% \text{ e.e.} \\
\text{59} & \\
\text{60} & \quad 100\% \text{ conversion} \\
& >99\% \text{ e.e.}
\end{align*}
\]

Scheme 26

Oda and co-workers\textsuperscript{8} employed cyanohydrins in the enzymatic synthesis of optically active aryl cyanohydrin acetates 62 (Scheme 27). Cyanohydrins are ideal substrates for dynamic kinetic resolution as they readily equilibrate under basic conditions to the corresponding aldehyde and cyanide. The racemic cyanohydrins 61 were prepared \textit{in situ} through reversible transhydrocyanation with aryl aldehydes and acetone cyanohydrin as a mild source of HCN, catalysed by basic ion exchange resin. The subsequent transesterification catalysed by immobilised \textit{Pseudomonas} species lipase furnished the stable aryl cyanohydrin acetates 62 in high yields and e.e. of 80-90%.

\[
\begin{align*}
\text{rac-61} \\
\text{(S)-62}
\end{align*}
\]

Scheme 27 \textit{Synthesis of optically active cyanohydrin acetates}
Rayner and co-workers exploited reversible hemithioacetal formation and their conversion to stable thioacetates catalysed by *Pseudomonas fluorescens* lipase was achieved (Scheme 28). The racemic hemithioacetals were prepared in situ by mixing an aldehyde and thiol, with dissociation of the hemithioacetals promoted by SiO$_2$. The one pot process gave yields of ~80% and e.e.’s of 90-95% for the acylated products.

$$\text{HS} - R^2 + R^1 \text{H} \xrightleftharpoons{\text{SiO}_2}\text{O} \rightarrow R^1 \text{S} - R^2 \text{OH} \xrightarrow{\text{Pseudomonas fluorescens lipase}} R^1 \text{S} \text{OAc} \xrightarrow{\text{tBuOMe}} \text{OAc}$$

\text{rac-63} \quad \text{Pseudomonas fluorescens lipase} \quad \text{90-95% e.e.}

Thioesters have also proved fruitful substrates in dynamic resolution processes. It has been shown that the α-protons of thioesters can be removed under basic conditions that do not deprotonate the corresponding oxoesters. Most of the resolutions were carried out in aqueous media, however, in the case of the thioesters of 2,4-dichlorophenoxypropionate, it was discovered that non-enzymatic hydrolysis occurred, resulting in low e.e.’s. To overcome unwanted hydrolysis the transesterification was performed in toluene with lipase PS-30. The n-butyl ester was obtained in 75% e.e. at 98% conversion.

$$\text{Cl} \quad \text{O} \quad \text{O} \quad \text{S} \quad \text{CF}_3 \xrightarrow{\text{lipase PS-30}} \text{Et}_3\text{N, toluene}} \text{Cl} \quad \text{O} \quad \text{O} \quad \text{Bu}^n$$

\text{rac-65} \quad \text{lipase PS-30} \quad \text{98% conversion} \quad \text{(R)-66 (75% e.e.)}

Scheme 29 *Dynamic resolution of 2,4-dichlorophenoxypropionate trifluoroethyl thioester*

Another example of base catalysed substrate racemisation was demonstrated by Ogasawara and co-workers in the dynamic kinetic resolution of tricyclic acyloins. In the presence of triethylamine, the racemic acyloins formed the meso-1,2-
enediol intermediate 68. On exposure to Lipase PS, the (-)-endo-acyloin acetates 70 were obtained in yields of 75% and 67% and e.e.'s of 97 and 99% when n = 1 and 2 respectively.

\[
\begin{align*}
&\text{(CH}_2\text{)}_n \quad \text{lipase PS} \\
&\text{OH THF, Et}_3\text{N} \\
&\text{OAc}
\end{align*}
\]

\[\text{n} = 1, 2\]

\[\text{(+)-67} \quad \text{meso-68} \quad \text{(-)-69} \quad \text{(-)-70}\]

Scheme 30

5(4H)-Oxazolones 71 have also been used as substrates for dynamic kinetic resolution and will be discussed in detail in Chapter 2.

\[\text{71}\]

1.2.6. Dynamic kinetic resolution with transition metals

An interesting area that has emerged over the last few years is the use of transition metals as racemisation agents, coupled with an enzymatic resolution. For this approach to be effective, the enzyme and the product must be unreactive to the transition metal used. Williams et al.\(^6\) used a ruthenium catalyst and enzyme combination in the resolution of phenyl ethyl alcohol 72 as illustrated in Scheme 31. The role of the transition metal is to facilitate a temporary oxidation of the alcohol to the corresponding ketone, which in turn is reduced by hydrogen transfer catalysis. Coupling the racemisation reaction with an enzymatic acylation using \textit{Pseudomonas fluorescens} lipase resulted in good to excellent e.e.'s for the product acetate 73 as shown in Table 5.
Introduction

**Pseudomonas sp. lipase**

\[
\begin{align*}
\text{OH} & \quad \text{Ph} \quad \text{CH}_3 \\
\rightarrow & \quad \text{OAc} & \quad \text{PhCOMe (1 equiv.) catalyst, KOH} \\
\end{align*}
\]

\[
\text{rac-72} \quad \text{Ph} \quad \text{CH}_3 \\
\rightarrow & \quad \text{OAc} & \quad \text{PhCOMe (1 equiv.) catalyst, KOH} \\
\text{(S)-73}
\]

**Scheme 31**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Temp./ °C</th>
<th>Conv./ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 mol %, [Rh(_2)(cod)Cl]_2</td>
<td>cyclohexane</td>
<td>50</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td>2*</td>
<td>2 mol % Rh(_2)(OAc)_4</td>
<td>CH(_3)Cl</td>
<td>20</td>
<td>60</td>
<td>98</td>
</tr>
</tbody>
</table>

a) no KOH

**Table 5**

Bäckvall\[^{86,87}\] used ruthenium catalyst 76 in the synthesis of a number of optically pure acetates of secondary alcohols in the presence of the Novozyme\(^\circ\) as illustrated in Scheme 32 and Table 6. The choice of acyl donor proved crucial for reaction success. When normal enol esters were employed, the resulting aldehydes or ketones were reduced to alcohols under the reaction conditions. These alcohols then participated in the acylation process reducing the yield of the desired product. The use of \(p\)-chlorophenyl acetate prevented this from occurring as the \(p\)-chlorophenol released does not contain an \(\alpha\)-proton; therefore cannot interfere with the transition metal catalyst.

\[
\begin{align*}
\text{OH} & \quad \text{R}^1 \quad \text{R}^2 \\
\rightarrow & \quad \text{OAc} & \quad \text{R}^1 \quad \text{R}^2 \\
\end{align*}
\]

**Scheme 32**
The use of enzymatic, transition metal assisted, dynamic kinetic resolution has also been extended to the production of chiral amides by Reetz et al.\textsuperscript{88} Palladium on carbon racemised phenyl ethylamine 77 via transient oxidation to the corresponding imine. Using Novozyme\textsuperscript{®} in triethylamine, amide 78 was isolated in 64\% yield and 99\% e.e. (Scheme 33).

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Substrate 74 & Product 75 & Yield/\% & e.e./\% \\
\hline
\hspace{1cm}$\text{Ph}\text{-CH}_3$ & $\text{Ph}\text{-CH}_3$ & 80 & >99 \\
\hspace{1cm}$\text{Ph}_2\text{C}\text{-CH}_2\text{OH}$ & $\text{Ph}_2\text{C}\text{-CH}_2\text{CH}_2\text{OAc}$ & 77 & >99 \\
\hspace{1cm}$\text{Ph}_2\text{C}\text{-CH}_2\text{OH}$ & $\text{Ph}_2\text{C}\text{-CH}_2\text{CH}_3\text{OAc}$ & 79 & >99 \\
\hspace{1cm}$\text{Ph}\text{-OH}$ & $\text{Ph}\text{-CH}_2\text{CH}_3\text{OAc}$ & 80 & 98 \\
\hspace{1cm}$\text{Ph}\text{-CH}_2\text{CH}_2\text{OH}$ & $\text{Ph}\text{-CH}_2\text{CH}_2\text{CH}_3\text{OAc}$ & 63 & >99\textsuperscript{a} \\
\hline
\end{tabular}
\caption{Table 6}
\end{table}

\textsuperscript{a} R,R:meso 86:14

Scheme 33
1.3.0. The use of lipases in combinatorial chemistry

Over the past decade combinatorial chemistry has become extremely popular for generating molecular diversity to aid drug discovery. One method of generating diversity relies on elaborating a core substrate unit containing a number of functional groups with a selection of partners. However, until very recently, the derivatisation of polyfunctionalised core units without protection/deprotection protocols was problematic due to a lack of sufficiently selective chemical procedures. The inherent regioselectivity offered by enzymes proved attractive and was employed by Adamczyk and co-workers\(^89\) in the lipase catalysed solution phase synthesis of a library of 26 compounds as shown in Scheme 34. When 1,2-phenylenedioxydiacetate \(79\) was simultaneously exposed to five mono Boc protected amines in the presence of lipase from \textit{Pseudomonas cepacia} species, followed by deprotection of the resulting Boc-amides, a yield of 93% based on the average weight of the bis-amide TFA salt product was obtained. ESMS analysis identified twenty-six different products including all fifteen desired bis-amides \(80\), five mono-amide mono-esters \(81\), and five mono-amide mono-acid \(82\) products from their corresponding MH\(^+\) peak (the hydrolysis products attributed to the presence of water in the hygroscopic monoprotected amine substrates).

\begin{align*}
\text{i. BocNHR}_{1-5}, \text{toluene/Pr}_2O \\
\text{Pseudomonas cepacia lipase} \\
\text{ii. TFA}
\end{align*}

\begin{align*}
\text{79} & \xrightarrow{\text{lipase}} \text{NHR}_{1-5} \\
\text{80} & + \\
\text{81} & + \\
\text{82} & + \\
\text{83} & \\
R^1 = (\text{CH}_2)_2\text{NH}_2; R^2 = (\text{CH}_2)_3\text{NH}_2; R^3 = (\text{CH}_2)_5\text{NH}_2; R^4 = (\text{CH}_2)_6\text{NH}_2; R^5 = \text{p-CH}_2\text{C}_6\text{H}_4\text{CH}_2\text{NH}_2
\end{align*}

\text{Scheme 34}
Khmelnitsky et al. synthesized a 167 member library of regioselectively acylated derivatives of the polyhydroxylated flavanoid, bergenin 84 (Scheme 35).

A mixture of four lipases (Chirazymes L-2 and L-9, and lipases PS30 and FAP-15) was identified as the best catalyst for the initial regioselective acylation at position-11. Subtilisin was utilised in the second regioselective acylation step at position-4, followed by selective hydrolysis at position-11, again mediated by the lipase mixture. In all, twelve acyl donors were employed, resulting in a library of $N^2+2N$ compound. All but one of the expected products was identified by HPLC/MS. The unaccounted product, a diacylation product from two bulky aromatic acyl donors, was discounted due to unfavourable steric interactions in the second acylation step.

1.4.0. Closing remarks

The utility of enzymes, and their diversity of applications is increasing in the field of chemistry. As well as the areas covered in this article, the rapidly growing area of directed evolution has the potential to provide enzymes designed for specific
substrates or reactions. Industrially enzymes offer a clean, reusable, and with the advances in cloning and purification technologies, a cheap alternative to conventional approaches. Biocatalysis in non-aqueous media has grown into an independent discipline and due to the increased understanding of enzyme properties provides an invaluable tool to the synthetic chemist.
2.0.0. Results and Discussion I

2.1.0. Development of 5(4H)-oxazolone methodology

2.1.1. 5(4H)-Oxazolones

The formation of 5(4H)-oxazolones 71, also known as oxazolin-5(4H)-ones or azlactones, in peptide synthesis has been documented as the main cause of racemisation.\textsuperscript{92,93} Racemisation occurs as a result of the decrease in the pKa of the C-4 proton upon cyclisation from the activated \( \alpha \)-amino acid residue. de Jersey \textit{et al.}\textsuperscript{94} showed that the pKa of the C-4 proton was 8.9-9.5 depending on the \( R^1 \) and \( R^2 \) substituents. Deprotonation at C-4 results in the formation of the stable pseudoaromatic anion intermediate as depicted in Scheme 36. Re protonation of the pseudoaromatic intermediate can occur from either face to produce either oxazolone enantiomer.

In enzyme catalysed reactions involving serine proteases, the oxazolone can act as an acyl donor. One enantiomer, \textit{e.g.} (S) preferentially acylates the enzyme to give an acyl-enzyme intermediate which is further attacked by a nucleophile (Nu\textsuperscript{+}), \textit{i.e.} alcohol, amine, water \textit{etc.} to produce the chiral \( \alpha \)-amino acid derivative. The remaining enantiomer (R) is racemised (due to the pKa of the C-4 proton) and the enzyme again preferentially reacts with the (S) enantiomer, thus dynamic resolution occurs. For the dynamic resolution process to be effective however, the rate of racemisation (\( k_{\text{rac}} \)), must be greater than the rate of enzymatic catalysis (\( k_{\text{enz}} \)), which in turn must be much greater than the rate of chemical reaction (\( k_{\text{chem}} \)). Furthermore, for the enzymatic catalysis to be enantioselective, the rates of enzymatic catalysis for each enantiomer must differ significantly, \textit{i.e.} \( k_{\text{rac}} > (k_{\text{enz}}(S)) > k_{\text{enz}}(R)) >> k_{\text{chem}} \).\textsuperscript{95}
Bevinakatti et al.\textsuperscript{96} described 5(4\textit{H})-oxazolones as cyclic aza enol esters (Scheme 37) and hypothesised that in organic solvents they would react in a similar manner to the enol esters in lipase catalysed transesterification studies. When a nucleophile attacks the activated carbonyl of an enol ester, the enol released tautomerises to the corresponding aldehyde or ketone, thus preventing reversible transesterification which can cause problems when normal alkyl esters are used as acyl donors.

\begin{center}
\begin{tabular}{ccc}
\ce{R} & \ce{O} & \ce{N}\textsuperscript{\textit{Nu}} \\
Alkyl ester & Enol ester & Aza enol ester
\end{tabular}
\end{center}

\textbf{Scheme 37 Comparison of ester reactivity}

\subsection*{2.1.2. Biocatalytic ring opening of 5(4\textit{H})-oxazolones}

Bevinakatti\textsuperscript{96} was the first to demonstrate the lipase catalysed ring opening of oxazolones in organic solvent to yield optically active $\alpha$-amino acid derivatives, albeit with moderate enantioselectivity as illustrated in Scheme 38.
Results and Discussion

HN\textsubscript{C}O\textsubscript{2}B

\textbf{(S)-88} 34\% e.e.

Scheme 38 \textit{Reagents and conditions:} i. DIPE, "BuOH, Lipozyme"

Sih \textit{et al.}\textsuperscript{95} expanded on the work of Bevinakatti and screened a number of lipases for the corresponding hydrolysis of phenylalanine derived oxazolone \textbf{89a} using phosphate buffer at pH 7.6 as the solvent and the source of the nucleophile. Of the ten lipases tested, two were found to give opposite enantiomers in optically pure form. Porcine pancreatic lipase (PL Fermlipase) catalysed the formation of \textbf{(S)-90a} while \textit{Aspergillus niger} lipase (AN) yielded \textbf{(R)-90a} as shown in Scheme 39.

\textbf{Scheme 39 Enantioselective hydrolysis of 2-phenyl-4-benzyl-5(4H)-oxazolone 89a}\textsuperscript{95}

Sih \textit{et al.} also tested a selection of 2-phenyl-4-substituted-5(4H)-oxazolones using these two lipases (Scheme 40).\textsuperscript{95} Moderate to good enantioselectivities, (Table 7) were observed but as with the work of Bevinakatti no yields were reported.

\textbf{Scheme 40 Reagents and conditions:} i. Lipase, phosphate buffer, pH 7.6
Results and Discussion I

Table 7 Results obtained for hydrolysis of 5(4H)-oxazolones

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Lipase</th>
<th>time/ h</th>
<th>e.e. %</th>
<th>Conf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂CH₂SCH₃</td>
<td>AP</td>
<td>5</td>
<td>83</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>16</td>
<td>80</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>p-HOC₆H₄CH₃</td>
<td>AP</td>
<td>20</td>
<td>37</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>20</td>
<td>19</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>CH₂icaidine</td>
<td>AP</td>
<td>100</td>
<td>77 (34)*</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>100</td>
<td>99 (55)*</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>CH₃CH(CH₃)₂</td>
<td>AP</td>
<td>18</td>
<td>6</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>18</td>
<td>87</td>
<td>S</td>
</tr>
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<td>20</td>
<td>80</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PL</td>
<td>20</td>
<td>76</td>
<td>S</td>
</tr>
</tbody>
</table>

a) results in parenthesis were obtained with new enzyme

Table 7 Results obtained for hydrolysis of 5(4H)-oxazolones

Bevinakatti also studied the effect of solvent in the reaction with Lipozyme® and n-butanol as the nucleophile (Table 8). It was shown that by careful screening of solvents the e.e. of the product could be dramatically increased. For example, when the reaction was carried out in DIPE an e.e. of 33% was obtained, whereas the same reaction in dichloromethane produced an e.e. of 69%. As a footnote a purified general yield of >90% was quoted.

Reagents and conditions: i. Solvent Lipozyme®, nBuOH

<table>
<thead>
<tr>
<th>Solvent</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIPE</td>
<td>33</td>
<td>34</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>59</td>
<td>42</td>
<td>66</td>
<td>39</td>
</tr>
<tr>
<td>DCM</td>
<td>69</td>
<td>47</td>
<td>61</td>
<td>43</td>
</tr>
</tbody>
</table>

R= (a) CH₂Ph, (b) CH₃, (c) CH₂CH(CH₃)₂, (d) CH₂CH₂CH₃

Table 8 Results obtained for alcoholyses of 5(4H)-oxazolones in various solvents
A far more comprehensive study documenting the methanolysis of a number of 2-phenyl-5(4H)-oxazolone derivatives with *Pseudomonas cepacia* lipase (P-30) in tert-butyl methyl ether was conducted by Sih *et al.*\(^9\) (Table 9). The lipase catalysed alcoholyses were carried out in the presence and absence of five equivalents of water. In both cases the formation of the preferred \((S)\) enantiomer was observed. The rate of the reaction in the presence of water did increase but the yields of the products were generally lower due to the competing hydrolysis reaction. The e.e. of the product appeared to increase as the C-4 substituent increased in size, but also slightly decreased when the reactions were carried out in the presence of water.

\[
\text{Reagents and conditions: i. Lipase P-30, 'BuOMe, CH}_3\text{OH (5 equiv.), (H}_2\text{O (5 equiv.)}, 50 ^\circ\text{C}}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>H(_2)O</th>
<th>Time/ h</th>
<th>Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH(CH(_3))(_2)</td>
<td>yes</td>
<td>130</td>
<td>47</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>CH(_2)CH(CH(_3))(_2)</td>
<td>yes</td>
<td>72</td>
<td>85</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no</td>
<td>72</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>CH(_2)CH(_2)SCH(_3)</td>
<td>yes</td>
<td>48</td>
<td>31</td>
<td>82</td>
</tr>
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<td></td>
<td></td>
<td>no</td>
<td>114</td>
<td>56</td>
<td>71</td>
</tr>
</tbody>
</table>
| 4     | \(\text{Ph} = \begin{array}{c} \text{C} \hspace{1cm} \text{C} \\
\text{H} \hspace{1cm} \text{H} \\
\text{Ph} \end{array}\) | yes     | 91     | 83       | 49      |
|       |       | no      | 86     | 90       | 75      |
| 5     | CH\(_2\)C\(_6\)H\(_5\)\(_p\)CH\(_3\)) | yes     | 84     | 78       | 63      |
|       |       | no      | 156    | 8        | 66      |
| 6     | Ph    | yes     | 84     | 46       | 75      |
| 7     | CH\(_2\)Ph | yes     | 35     | 80       | 78      |
|       |       | no      | 22     | 93       | 69      |
| 8     | CH\(_2\)CH\(_2\)Ph | yes     | 42     | 61       | 93      |
| 9     | CH\(_2\)CH\(_2\)CH\(_2\)Ph | yes     | 72     | 76       | 95      |
|       |       | no      | 72     | 91       | 84      |

*Table 9 Results obtained for the methanolysis of 5(4H)-oxazolones*\(^9\)
Turner et al. applied the oxazolone methodology in the lipase catalysed synthesis of the non-proteinogenic α-amino acid L-(S)-tert-leucine 92g as shown in Scheme 41.

\[
\begin{align*}
\text{rac-89g} & \xrightarrow{\text{i.}} 94\%, 99.5\% \text{ e.e.} \\
(S)-91 & \xrightarrow{\text{ii-iv}} (S)-92g
\end{align*}
\]

Scheme 41 Reagents and conditions: i. Toluene, Lipozyme®, n-BuOH, Et₃N (0.25 equiv.), 37 °C, ii. Alcalase®, iii. 6 N HCl, iv. Amberlite IRA-67.

2-Phenyl-4-tert-butyl-5(4H)-oxazolone 89g was dissolved in toluene and Lipozyme®, n-butanol and a catalytic amount of triethylamine were added. The optically pure product was isolated by chromatography in 94% yield. Alcalase® mediated deprotection of the ester followed by acid hydrolysis to remove the \(N\)-benzoyl protecting group and finally ion exchange chromatography furnished the optically pure L-(S)-tert-leucine 92g.

In 1998, Fu et al. published a chemical dynamic kinetic resolution of 2-phenyl-4-substituted-5(4H)-oxazolone derivatives utilising a planar chiral DMAP based catalyst 93.

\[ (-)-93 \quad \text{R} = \text{CH}_3 \]

Toluene was identified as the best solvent and the reaction proceeded with excellent yields and moderate e.e.’s, as illustrated in Table 10. It was also discovered that the reaction rate and e.e. of the product were both increased if 10% benzoic acid was
added to the reaction mixture. The e.e. of the product in entry 1 dropped to <2% in
the absence of benzoic acid. No explanation for this effect was given.

\[
\text{Reagents and conditions: i. Toluene, 5\% (-)-93, 10\% PhCO}_2\text{H, MeOH, room temp.}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH\textsubscript{3}</td>
<td>98</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>CH\textsubscript{2}=CHCH\textsubscript{2}</td>
<td>94</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}</td>
<td>95</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>CH\textsubscript{2}CH\textsubscript{2}SCH\textsubscript{3}</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>CH(C\textsubscript{6}C\textsubscript{11})</td>
<td>93</td>
<td>54</td>
</tr>
</tbody>
</table>

Table 10 Results obtained for the chemical dynamic kinetic resolution of 5(4H)-oxazolones\textsuperscript{100}

2.2.0. Aim of project

As discussed above, Turner \textit{et al}.\textsuperscript{99} published a stereospecific high yielding route
to L-(S)-\textit{tert}-leucine \textit{92g}. However, when the optimised conditions were applied to
less sterically demanding substrates such as 2-phenyl-4-isopropyl-5(4H)-oxazolone,
there was a dramatic drop in the observed e.e. of the product from 99% for the
\textit{tert}-leucine derivative to 0% for the valine derivative. Substituting a methyl group for a
proton resulted in a complete loss of selectivity.\textsuperscript{101}

The aim of the project was to expand this 5(4H)-oxazolone methodology to gain a
more general route to optically active \textit{\alpha}-amino acid derivatives and later apply this to
the synthesis of pertinent biologically active compounds. The target chosen was the
matrix metalloproteinase inhibitors (MMPI's) of structure \textit{94}. These will be
discussed in detail in Chapter 3.
2.3.0. Preliminary studies

2.3.1. Synthesis of substrates

2-Phenyl-4-benzyl-5(4H)-oxazolone 89a was chosen as the substrate for the initial studies into the lipase catalyse dynamic kinetic resolution process. Oxazolone 89a was synthesised in two steps from the DL-phenylalanine 92a. N-Benzoylation under Schotten-Baumann conditions, followed by acetic anhydride mediated cyclodehydration proceeded in high yield to furnish the desired oxazolone as a colourless solid. Oxazolone 89a decomposed readily at room temperature over a period of a few days. The oxazolone, and all other oxazolones subsequently prepared in this study were stored in the fridge or freezer until required.

![Scheme 42](image)

**Scheme 42 Reagents and conditions:** i. 2M NaOH, PhCOCl, 0 °C, ii. 1,4-dioxane:acetic anhydride (1:1), 40 °C

2.3.2. Screening of lipases

As a starting point the Fluka Lipase Basic Kit was tested, as illustrated in Scheme 43, with the results shown in Table 11. Small scale reactions (0.04 mmol substrate and 1 mL solvent) were incubated at 37 °C with methanol as the nucleophile, and the progress of the reactions monitored by chiral HPLC. A sample of the racemic methyl ester 96a, (and all subsequent racemic derivatives), was prepared by acid catalysed alcoholyis of the corresponding oxazolone. The experimental details are given in Chapter 4.
Results and Discussion

**Scheme 43 Reagents and conditions:** i. Toluene, lipase (see Table 11), Et₃N(0.25 mmol), CH₃OH (2 equiv.), 37°C

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Conversion</th>
<th>e.e./%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aspergillus niger</em></td>
<td>+</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td><em>Candida antarctica</em></td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida cylindracea</em></td>
<td>++</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td><em>Rhizomucor miehei</em></td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas cepacia</em></td>
<td>+</td>
<td>43</td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>+++</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td><em>Rhizopus arrhizus</em></td>
<td>+</td>
<td>5*</td>
</tr>
<tr>
<td>8</td>
<td><em>Rhizopus niveus</em></td>
<td>++</td>
<td>3*</td>
</tr>
<tr>
<td>9</td>
<td><em>Hog pancreas</em></td>
<td>++</td>
<td>42</td>
</tr>
</tbody>
</table>

a) Opposite enantiomer

**Table 11 Results obtained for screening of Fluka Lipase Basic Kit**

Employing the optimised conditions found for the Lipozyme®/tert-leucine system, the immobilised lipases Lipozyme® (*Rhizomucor miehei* lipase immobilised on an anionic exchange resin) and Novozyme® (*Candida antarctica* lipase B immobilised on acrylic resin) were tested, and the results are shown in Table 12. The natural (S) stereochemistry of the product, and subsequent biotransformation products, was assigned by comparison with previous results obtained using Lipozyme® and optical rotation values in the literature.¹⁰¹

The results obtained with the Fluka Lipase Basic Kit (Table 11) were disappointing with only poor to moderate e.e. and conversions obtained. In contrast, the immobilised lipases produced interesting results. The Lipozyme® experiments, (entries 1 and 2, Table 12) were a repeat of previous experiments¹⁰¹ for reference and showed again the enhancement of e.e. with the addition of external triethylamine.
Results and Discussion

Ph\nPh
HN\nN\nCO_2Bu
Ph
89a
\rightarrow i. \rightarrow
Ph
HN\nCO_2Bu
Ph
97a

Reagents and conditions: i. Toluene, (Et_3N (0.25 equiv.)), lipase, CH_3OH (2.0 equiv.), 37 °C

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Et_3N</th>
<th>Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lipozyme®</td>
<td>no</td>
<td>59</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Lipozyme®</td>
<td>yes</td>
<td>74</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>Novozyme®</td>
<td>no</td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>Novozyme®</td>
<td>yes</td>
<td>53</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>Novozyme®</td>
<td>yes</td>
<td>81</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 12 Results obtained for screening Lipozyme® and Novozyme®

The Novozyme® results also exhibit a dependence on triethylamine addition to increase the e.e. of the product (entries 3 and 4). Although the Novozyme® mediated biotransformation resulted in an excellent e.e. of 95%, (entry 4), only a moderate yield of 51% pure product was obtained. On closer examination of the ^1H nmr (200 MHz, CDCl_3) spectrum of the crude biotransformation product it could be seen that a 1:1 ratio of desired product to hydrolysis product, i.e. N-benzoyl phenylalanine was obtained. The ratio was calculated from the integrals corresponding to the α-CH of each amino acid derivative, δ 5.06 and 4.94 for the ester and acid respectively. To minimise the formation of the undesired hydrolysis product Novozyme® was crushed in a mortar and pestle and dried to constant weight. It was hoped that the use of phosphorus pentoxide as desiccant would remove any excess water trapped in the solid support, or associated with the surface of the lipase, but would not be powerful enough to remove the water held at the catalytic triad of the lipase that is essential for catalytic activity. Indeed the resulting powder, when subjected to the biotransformation, produced an identical e.e. of 95% while the yield increased to 81% (entry 5).
2.3.3. Effect of alkyl chain length of nucleophile

As an effective, and reproducible set of biotransformation conditions had been established, it seemed logical to probe the parameters of the reaction to achieve a greater understanding of the role of each component. The first variable to be investigated was the effect of the alcohol chain length and the results are shown in Table 13, with the previous results obtained for Lipozyme® also shown.\textsuperscript{99,101}

\begin{center}
\begin{align*}
\text{Reagents and conditions: } & \text{i. Toluene, (Et}_3\text{N (0.25 equiv.)), lipase, } \text{R'OH (2.0 equiv.), 37 °C} \\
\end{align*}
\end{center}

\begin{table}
\centering
\begin{tabular}{cccccc}
\hline
\text{Compound} & \text{R'} & \text{Novozyme®} & \text{Lipozyme®} \\
 & & \text{Yield} / \% & \text{e.e.} / \% & \text{Yield} / \% & \text{e.e.} / \% \\
\hline
\text{96a} & \text{CH}_3 & 79 & 94 & 55 & 40 \\
\text{98a} & \text{CH}_2\text{CH}_2 & 82 & 97 & 53 & 83 \\
\text{99a} & \text{CH}_3\text{CH}_2\text{CH}_2 & 83 & 97 & - & - \\
\text{97a} & \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2 & 81 & 95 & 69 & 73 \\
\text{100a} & \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 & 32 & 88 & - & - \\
\hline
\end{tabular}
\caption{Effect of alkyl chain length of alcohol nucleophile for the alcoholysis of 5(4H)-oxazolone 89a}
\end{table}

The results show that there is very little effect on the resulting e.e. of the Novozyme\textsuperscript{®} mediated biotransformation product when the length of the alkyl chain of the primary alcohol nucleophile is varied. This is in direct contrast to the erratic results obtained in the Lipozyme\textsuperscript{®} mediated alcoholysis.

2.3.4. Secondary alcohols

The use of secondary alcohols as nucleophiles was also investigated. When isopropanol was used as the nucleophile, (Scheme 44), the biotransformation proceeded at a very slow rate with a reaction time of 26 days required for 100\% conversion. The isolated product yield of only 18% with an e.e. of 29\% indicates that secondary alcohols are poor nucleophiles under the conditions studied. An explanation for this
result could be that in processes where Novozyme® has been used successfully with secondary alcohol nucleophiles, it has been to resolve the alcohol using achiral acyl donors. In the above reaction, the oxazolone is a relatively bulky chiral acyl donor. The resulting acyl enzyme intermediate formed will have considerable steric constraints in comparison to simple acyl enzyme intermediates, thus the secondary alcohol nucleophile has difficulty accessing the crowded active site of the enzyme.

\[
\begin{align*}
\text{Ph} & \quad \text{HN CO}_2 \text{H} \\
\text{Ph} & \quad \text{Ph} \\
\end{align*}
\]

\[
\begin{align*}
89a & \xrightarrow{i.} 101a \\
\end{align*}
\]

Scheme 44 Reagents and conditions: i. Toluene, Novozyme®, Et,N, 'PrOH, 37°C

2.4.0. Expansion of substrate range

2.4.1. Synthesis of substrates

The next and most obvious variable to probe was the C-4 substituent (R) to examine the scope of the substrate range tolerated by Novozyme®. The substrates were all prepared in two steps as described in Section (2.3.1.) and illustrated in Scheme 45. They all proceeded smoothly in good to excellent yield with the results shown in Table 14. It was decided to carry out the biotransformations with methanol and n-propanol as the nucleophiles so that the two sets of results could be compared (see Section (2.4.2.)).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CO}_2\text{H} \\
\text{R} & \quad \text{H} \\
\end{align*}
\]

\[
\begin{align*}
92b-g & \xrightarrow{i.} 95b-g \xrightarrow{ii.} 89a-g \\
\end{align*}
\]

Scheme 45 Reagents and conditions: i. 2M NaOH, PhCOCl, 0 °C, ii. 1,4-dioxane:acetic anhydride (1:1), 40 °C
Table 14 Synthesis of 2-phenyl-4-substituted-5(4H)-oxazolones 89b-g

2.4.2. Testing of substrates

Using the optimised reaction conditions each substrate was tested, Scheme 46, and the results are shown in Table 15.

![Scheme 46](image)

The results in Table 15 again indicate that there is only a small change in the e.e. of the product when the nucleophile is changed from methanol to n-propanol. They also indicate that Novozyme® prefers flexible alkyl side chains with the iso-propyl and iso-butyl side chains giving the highest e.e.'s. The small methyl side chain gave poor chiral induction, presumably due to lack of steric interactions with the lipase. The bulky tert-butyl group gave only poor to moderate yields (after the recovery of starting oxazolone) and poor e.e.'s, perhaps due to excessive steric interactions with the lipase. As the flexibility of the C-4 side chain was increased, as in the case of the methyl thioethyl group, the e.e. began to fall to ~80%. The aromatic indolemethylene side chain also gave good e.e.'s of 80 and 90%. Although the indolemethylene group
is relatively bulky, the methylene spacer (as in the case of the benzyl side chain) allowed the substrate the flexibility to fit into the active site of the lipase.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R'</th>
<th>Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>96b</td>
<td>CH₂CH(CH₃)₂</td>
<td>CH₃</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>99b</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>89</td>
<td>98</td>
</tr>
<tr>
<td>96c</td>
<td>CH(CH₃)₂</td>
<td>CH₃</td>
<td>82</td>
<td>95</td>
</tr>
<tr>
<td>99c</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>70</td>
<td>93</td>
</tr>
<tr>
<td>96d</td>
<td>CH₂CH₂SCH₁</td>
<td>CH₃</td>
<td>69</td>
<td>80</td>
</tr>
<tr>
<td>99d</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>64</td>
<td>83</td>
</tr>
<tr>
<td>96e</td>
<td></td>
<td>CH₃</td>
<td>90</td>
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</tr>
<tr>
<td>99e</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>48</td>
<td>80</td>
</tr>
<tr>
<td>96f</td>
<td>CH₃</td>
<td>CH₃</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>98f</td>
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</tr>
<tr>
<td>99f</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>96g</td>
<td>C(CH₃)₃</td>
<td>CH₃</td>
<td>40*</td>
<td>35</td>
</tr>
<tr>
<td>99g</td>
<td></td>
<td>CH₃CH₂CH₃</td>
<td>15*</td>
<td>19b</td>
</tr>
</tbody>
</table>

a) Yield based on recovered starting material, b) not baseline separation

Table 15 Results obtained for Novozyme® mediated alcoholysis of 2-phenyl-4-substituted 5(4H)-oxazolones 89b-g

2.4.3. Studies on the effect of solvent

To gain a further understanding of the reaction parameters the solvent was varied. The methanolysis of 89a was performed in the presence and absence of a catalytic amount of triethylamine to also examine the role of the base in the reaction. A number of conclusions can be drawn from the results shown in Table 16. Firstly, if we examine the results obtained when triethylamine was present, we can see that chlorinated solvents, (entries 1 and 2) gave poorer yields and e.e.'s. while ethers, (entries 3-5) gave excellent yields and e.e.'s comparable to the results obtained in toluene. The polar solvents of tetrahydrofuran and acetonitrile both produced products with excellent e.e.'s but with only moderate yields. Lower e.e.'s were observed when the same series of reactions were carried out in the absence of triethylamine. The effect was most prominent in the ether series (entries 3-5), where
the e.e. dropped from 96% to 33% in the case of DIPE. Examining the relative rates of reaction, it is evident that in the presence of triethylamine the reactions are much faster. The ethers gave relatively fast reactions both in the presence and absence of triethylamine and in all cases were complete after being left overnight. Chlorinated solvents resulted in the largest decrease in rate.

The results obtained in tetrahydrofuran and acetonitrile in the absence of triethylamine are the most interesting. In the absence of triethylamine the e.e. remained very high and the yield increased, (doubled in the acetonitrile case).

![Chemical structure](image)

Reagents and conditions: i. Solvent, (Et$_3$N (0.25 equiv.)), Novozyme®©, R'O (2.0 equiv.), 37 °C

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time/ h</th>
<th>Et$_3$N Present</th>
<th>Time/ h</th>
<th>Et$_3$N Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>41</td>
<td>78</td>
<td>89</td>
<td>124</td>
</tr>
<tr>
<td>2</td>
<td>CHCl$_3$</td>
<td>39</td>
<td>66</td>
<td>75</td>
<td>124</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>48</td>
<td>64</td>
<td>95</td>
<td>123</td>
</tr>
<tr>
<td>4</td>
<td>Et$_2$O</td>
<td>17</td>
<td>87</td>
<td>97</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>tBuOMe</td>
<td>15</td>
<td>90</td>
<td>96</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>DIPE</td>
<td>15</td>
<td>86</td>
<td>96</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>toluene</td>
<td>26</td>
<td>82</td>
<td>94</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>acetonitrile</td>
<td>22</td>
<td>44</td>
<td>97</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 16 Solvents studies for the methanolysis of 2-phenyl-4-benzyl-5(4H)-oxazolone 89a with Novozyme®©

2.4.4. Acetonitrile as solvent

To explore if the acetonitrile solvent effect discovered above was specific to Novozyme®, or was a more general phenomenon, the reaction was repeated with Lipozyme®© as illustrated in Scheme 47.
Scheme 47 Reagents and conditions: i. Solvent, (Et3N, 0.25 equiv.), Lipozyme®, CH2OH, 37 °C

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Et3N</th>
<th>Time/ h</th>
<th>Yield/ %</th>
<th>e.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene</td>
<td>No</td>
<td>-</td>
<td>48</td>
<td>19101</td>
</tr>
<tr>
<td>2</td>
<td>toluene</td>
<td>Yes</td>
<td>-</td>
<td>55</td>
<td>40101</td>
</tr>
<tr>
<td>3</td>
<td>acetonitrile</td>
<td>No</td>
<td>23</td>
<td>94</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>acetonitrile</td>
<td>Yes</td>
<td>23</td>
<td>61</td>
<td>73</td>
</tr>
</tbody>
</table>

Table 17 Acetonitrile studies for the methanolysis of 2-phenyl-4-benzyl-5(4H)-oxazolone 89a with Lipozyme®

The results in Table 17 show that using acetonitrile as the solvent with Lipozyme® produced the same effect as observed with Novozyme®. Not only did the yield increase from 55% to 94% but the e.e. almost doubled from 40% to 73%.

2.4.5. Testing of substrates with acetonitrile as solvent

To expand on the results found with acetonitrile as solvent, the 2-phenyl-5(4H)-oxazolones tested in Section (2.4.2.) were also subjected to the acetonitrile conditions with both lipases, (Table 18).

Reagents and conditions: i. Acetonitrile, lipase, CH3OH, 37 °C
Examining the Novozyme® data first, and comparing it with the results in Section (2.4.2), Table 15, it can be clearly seen that for the C-4 alkyl side chains the results are almost identical in yield and e.e. In the case of the tert-butyl side chain the enzyme activity was entirely depressed and no desired product was isolated after several weeks. An identical result was obtained with the indolemethylene side chain (compared to a yield and e.e. of 90% when the reaction was carried out in toluene with triethylamine). An explanation for the above results could be that the use of acetonitrile as the solvent causes a conformational change in the lipase, resulting in a decrease in the accessibility of the active site of the lipase. The corresponding Lipozyme® reactions were generally high yielding, with only modest enhancement of the e.e. of the product when compared with the results obtained in toluene/triethylamine. The e.e. for the iso-propyl side chain increased from 0 to 19%. The tert-butyl side chain also gave a dramatic change in reactivity. Under the previous conditions a 47% yield and 80% e.e. was obtained. With acetonitrile as the solvent the activity was again entirely depressed. It appears that the best substrates for Novozyme® are flexible side chains i.e. iso-propyl or iso-butyl. It is also implied that the presence of a methylene spacer, as in the benzyl and methylsulfanyl-ethyl side chains can be advantageous as higher e.e. were observed with these substrates. Lipozyme® on the other hand appears to have a wider cavity at the active site and therefore is most stereoselective for sterically demanding side chains such as tert-butyl. In the absence of any steric bulk, only poor enantioselectivity was achieved. In
addition, once the lipase has been selected for the biotransformation the choice of solvent between toluene and acetonitrile can dramatically effect the enantioselectivity.

2.5.0. The role of the base

The intended role of the addition of triethylamine to the reaction system was to increase the rate of racemisation of the oxazolone substrates. However, during the course of our studies it was realised that the actual role of the triethylamine was more complex. To gain a greater understanding of the role of the base an in depth kinetic study was undertaken in our laboratory by Dr. M.-C. Parker.103

The most reliable predictor of enzyme catalytic activity in low water organic media is thermodynamic water activity (a_w). The hydration level was controlled by equilibrating the enzyme and solvent (over a period of 48-72 h) with the appropriate saturated salt solution of known a_w. For example, a low a_w system was one where the solvent was poorly hydrated, therefore the enzyme was similarly poorly hydrated. At high a_w the solvent was near water saturation and the enzyme was fully hydrated as in an aqueous system. The reaction depicted in Scheme 48 was performed in solvents with a range of known a_w.

Scheme 48 Reagents and conditions: i. Solvent, (Et_3N (0.25 equiv.)), Novozyme®, _n_BuOH (2.0 equiv.), 37 °C

The effect of the level of hydration on the initial catalytic rate and enantioselectivity found in three different solvents in the presence and absence of triethylamine is shown in Table 19. In the absence of triethylamine, even low levels of hydration present in non-polar solvents such as hexane and toluene were detrimental to the rate and e.e. When the reactions were carried out in the presence of triethylamine, the rate
did drop, but to a lesser degree, while the e.e. remained high. The conditions which resulted in the optimum rate and e.e. were when the lipase, solvent and triethylamine were rigorously dried.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Water activity $a_w$</th>
<th>No $\text{Et}_3\text{N}$</th>
<th>$\text{Et}_3\text{N}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-hexane</td>
<td>~0 (anhydrous)</td>
<td>26 (±1.5) 85 (± 3)</td>
<td>30 (± 1.5) 90 (± 3)</td>
</tr>
<tr>
<td>$n$-hexane</td>
<td>0.69</td>
<td>4 (±0.5) 55 (±2)</td>
<td>20 (±1) 87 (±3)</td>
</tr>
<tr>
<td>$n$-hexane</td>
<td>0.97</td>
<td>1.5 (±0.15) 30 (± 5)</td>
<td>18 (± 0.9) 80 (± 5)</td>
</tr>
<tr>
<td>toluene</td>
<td>~0</td>
<td>15 (± 0.8) 85 (± 4)</td>
<td>27 (±1.5) 93 (± 3)</td>
</tr>
<tr>
<td>toluene</td>
<td>0.22</td>
<td>3 (± 6) 61 (± 6)</td>
<td>17 (±1) 95 (± 2)</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>~0</td>
<td>15 (± 0.15) 30 (± 5)</td>
<td>18 (± 0.9) 80 (± 5)</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>0.1 (0.5% v/v $\text{H}_2\text{O}$)</td>
<td>no reaction</td>
<td>5 (± 0.3) 90 (±4)</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>0.4 (2% v/v $\text{H}_2\text{O}$)</td>
<td>no reaction</td>
<td>no reaction</td>
</tr>
</tbody>
</table>

Table 19 Effect of water activity on initial catalytic rate and enantioselectivity as a function of hydration, in the presence and absence of triethylamine

The effect of triethylamine addition to a reaction already proceeding with poor enantioselectivity was also examined. Triethylamine was added to the reaction in hexane ($a_w = 0.69$) after 140 min, resulting in an instantaneous increase in rate and e.e. as illustrated in Figure 7.
The addition of organic bases to increase the enantioselectivity of enzyme catalysed reactions carried out in organic solvents has been documented in the literature.\textsuperscript{104-106} One hypothesis is that the addition of external base results in the formation of an ion-pair with any acid by-product formed during the reaction. As mentioned earlier in Section (2.3.2.), the formation of \textit{N}-benzoyl amino acids such as 95a has been observed by \textsuperscript{1}H nmr in the resolution of oxazolone 89a. We have also found that addition of acid 95a to an already hydrated system results in loss of activity. On subsequent addition of triethylamine the activity was regained, presumably \textit{via} ion-pair formation. Ion-pair formation was observed in both low and high, non-hydrogen bonding dielectric solvents such as hexane and acetonitrile. The acid 95a was found to be more soluble in acetonitrile than hexane. In the absence of triethylamine, the dissolved acid remains bound to the surface of the lipase through electrostatic interactions, thus altering the protonation state, and leading to protein deactivation. In polar solvents such as acetonitrile, the acid by-product is more soluble and therefore is not bound to the surface of the enzyme and no reduction in the e.e. is observed. However, for reactions carried out with rigorously dried reagents and low $a_\nu (< 0.7)$ there was no evidence of hydrolysis over the initial rate measurements, yet the addition of triethylamine did enhance the enantioselectivity of the reaction. A role of the triethylamine in the reaction has been identified but does not give the complete answer. Investigations to further understand the activation process are currently under way.

\subsection*{2.6.0. Nitrogen nucleophiles}

\subsection*{2.6.1. Amines}

As well as testing alcohol nucleophiles, nitrogen based nucleophiles were investigated. As mentioned in Section (2.2.0.), one aim of the project was to synthesise a number of MMPI derivatives 94, all of which contain a methyl amide functionality in the position resulting from nucleophilic attack of the corresponding oxazolone. In control reactions 2-phenyl-4-benzyl-5(4H)-oxazolone 89a was subjected to the amine nucleophiles benzylamine and allylamine in the absence of any lipase (Scheme 49). Unfortunately in both cases the amide product 102 was isolated in high yield indicating that amines are too nucleophilic and would by-pass
the lipase and produce only racemic amide products. Shriner et al.\textsuperscript{107} observed amide formation in their studies involving the reactions of 2-phenyl-4-benzyl-5(4H)-oxazolone 89a and secondary amines such as piperidine, morpholine, dimethylamine, diethylamine, methylaniline and ethylaniline.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{CONHR} & \quad \text{CONHR}
\end{align*}
\]

Scheme 49 Reagents and conditions: i. (a) Toluene, PhCH\textsubscript{2}NH\textsubscript{2}, 77%, (b) THF, H\textsubscript{2}NCH\textsubscript{2}CH=CH\textsubscript{2}, 90%

The use of α-amino acid derivatives as nucleophiles was also investigated as an extension of the work carried out by Sih\textsuperscript{108,109}. Sih subjected 2-phenyl-4-benzyl-5(4H)-oxazolone 89a to nucleophilic attack by various α-amino acid derivatives in the presence of α-chymotrypsin or the cysteine protease papain. Either acetonitrile:phosphate buffer (1:1, pH 8) or DMF:phosphate buffer (1:1, pH 8.5) were used as solvent respectively, Scheme 50.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{R} & \quad \text{I} \\
\text{HN} & \quad \text{V} \\
\text{H} & \quad \text{H} \\
\text{NCONH}_2 & \quad \text{NCONH}_2 \\
\text{Ph} & \quad \text{Ph}
\end{align*}
\]

Scheme 50 Reagents and conditions: i. (a) α-chymotrypsin, acetonitrile:phosphate buffer (1:1, pH 8), (b) papain, DMF:phosphate buffer (1:1, pH 8.5)

The experimental results obtained by Sih are shown in Table 20. They indicate that when optically pure oxazolone 89a was used as the acyl donor only one diastereomeric dipeptide (L,L) was isolated, indicating that under the reaction conditions the L-oxazolone did not racemise. When racemic oxazolone was used, a mixture of (L,L) and (D,L) diastereomers of varying ratios were produced. These results suggest that the enzymes show a degree of enantioselectivity.
Results and Discussion

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxazolone configuration</th>
<th>acyl acceptor</th>
<th>Conditions</th>
<th>Time/ min</th>
<th>Yield/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>Phe-NH₂</td>
<td>a</td>
<td>30</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>Arg-NH₂</td>
<td>a</td>
<td>30</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>DL</td>
<td>Phe-NH₂</td>
<td>a</td>
<td>30</td>
<td>59 (L,L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13 (D,L)</td>
</tr>
<tr>
<td>4</td>
<td>DL</td>
<td>Arg-NH₂</td>
<td>a</td>
<td>5</td>
<td>70 (L,L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 (D,L)</td>
</tr>
<tr>
<td>5</td>
<td>L</td>
<td>Ala-NH₂</td>
<td>b</td>
<td>30</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>L</td>
<td>Glu-NH₂</td>
<td>b</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>DL</td>
<td>Ala-NH₂</td>
<td>b</td>
<td>30</td>
<td>40 (L,L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19 (D,L)</td>
</tr>
<tr>
<td>8</td>
<td>DL</td>
<td>Glu-NH₂</td>
<td>b</td>
<td>30</td>
<td>62 (L,L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18 (D,L)</td>
</tr>
</tbody>
</table>

Table 20 Results obtained by Sih for the aminolysis of 2-phenyl-4-benzyl-5(4H)-oxazolone 89a with α-chymotrypsin and papain

It is also clear that during the course of the reaction, the oxazolone partially racemised because the yield of the (L,L) diastereomer is greater than 50%. However, no control experiment where the reagents were reacted in the absence of the enzyme is documented, therefore the optically pure diastereomers obtained in entries 1, 2, 5 and 6 could have been formed by direct reaction of the amino acid amide with the oxazolone. The diastereomeric ratios of the products in entries 3, 4, 7 and 8 could also have been a result of chemical diastereoselectivity because a chiral nucleophile was used. The yields in excess of 50% could result from racemisation of the relatively less reactive D-oxazolone (for steric reasons). A closer examination of the rate of reaction could shed light on this.

To provide more information on the mechanism of the above reaction we carried out the following control experiment. 2-Phenyl-4-benzyl-5(4H)-oxazolone 89a was dissolved in toluene and freshly prepared glycine methyl ester added as shown in Scheme 51. The dipeptide product 105 precipitated almost instantly as a colourless solid, and was isolated in a yield of 80%. This is conclusive proof that these α-amino
acid derivatives are too nucleophilic for use in enzymatic oxazolone aminolysis reactions.

\[
\begin{align*}
\text{Ph} & \quad \text{H} & \quad \text{N} & \quad \text{CO}_2\text{CH}_3 \\
\text{Ph} & \quad \text{H} & \quad \text{N} & \quad \text{CO}_2\text{CH}_3 \\
\end{align*}
\]

Scheme 51 Reagents and conditions: i. Et$_2$O, NH$_3$, 0°C, ii. Toluene, room temp.

2.6.2. Amides

In an attempt to lower the nucleophilicity of the nitrogen nucleophiles the use of amides was investigated. Unfortunately, the two attempts shown in Scheme 52 failed. It is hypothesised that in the case of phenylacetamide, (R= Ph), the amide was too weak a nucleophile due to the relatively poor electron withdrawing nature of the aromatic ring. Trifluoroacetamide, (R= CF$_3$) was tested in the hope that the greater electron withdrawing affect of the trifluoromethyl group would activate the amide sufficiently. Unfortunately no reaction was observed.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{Ph} & \quad \text{Ph} \\
\end{align*}
\]

Scheme 52 Reagents and conditions: i. THF, Novozyme®, Et$_3$N, a) R= Ph, phenylacetamide, 0%, b) R= CF$_3$, trifluoroacetamide, 0%

2.6.3. Kinetic resolution with amines

Another route for preparing amides was tested. A double resolution process was envisaged as illustrated in Scheme 53. Ideally, both reactions would be carried out in the same reaction vessel. The initial dynamic kinetic resolution, upon completion, would be followed by amine addition to facilitate the enzymatic kinetic aminolysis utilising the first product as the new substrate. Not only would this provide the
desired functionality, but it could also potentially produce optically pure amide product.

\[
\begin{align*}
&\text{Scheme 53 Reagents and conditions: i. Solvent, Novozyme®, (Et}_3\text{N), R'}\text{OH, 37 °C, ii. solvent,} \\
&\text{Novozyme®, H}_2\text{NR''} \\
&\text{Reagents and conditions: i. Solvent, Novozyme®, (Et}_3\text{N), R'}\text{OH, 37 °C, ii. solvent,} \\
&\text{Novozyme®, H}_2\text{NR''}
\end{align*}
\]

Conde et al.\textsuperscript{33} (see Chapter 1, Section (1.2.2.)) have shown that when both enantiomers of diethyl Cbz-glutamate are separately exposed to Novozyme® in the presence of a variety of amines, aminolysis occurs predominantly at the α-position for the L-enantiomer, and predominantly at the γ-position for the D-enantiomer.

To test the utility of the aminolysis methodology, racemic N-benzoyl phenylalanine (R= benzyl) and N-benzoyl valine methyl esters (R= iso-propyl) were subjected to methyl and benzyl amine in various solvents as shown in Scheme 54 and Table 21. Unfortunately, no reaction was observed.

\[
\begin{align*}
&\text{Scheme 54 Reagents and conditions: i. Solvent, Novozyme®, H}_2\text{NR'} \\
&\text{Reagents and conditions: i. Solvent, Novozyme®, H}_2\text{NR''}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Temp/ °C</th>
<th>R</th>
<th>R'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene + Et_3N (0.25 equiv.)</td>
<td>37</td>
<td>CH_2Ph</td>
<td>CH_3</td>
</tr>
<tr>
<td>2</td>
<td>1,4-Dioxane</td>
<td>37</td>
<td>CH_2Ph</td>
<td>CH_3Ph</td>
</tr>
<tr>
<td>3</td>
<td>DIPE</td>
<td>37</td>
<td>CH_2Ph</td>
<td>CH_3Ph</td>
</tr>
<tr>
<td>4</td>
<td>DIPE</td>
<td>60</td>
<td>CH_2Ph</td>
<td>CH_2Ph</td>
</tr>
<tr>
<td>5</td>
<td>DIPE</td>
<td>37</td>
<td>CH(CH_3)_2</td>
<td>CH_3Ph</td>
</tr>
<tr>
<td>6</td>
<td>DIPE</td>
<td>60</td>
<td>CH(CH_3)_2</td>
<td>CH_2Ph</td>
</tr>
</tbody>
</table>

Table 21 Attempted aminolysis of α-amino acid esters in the presence of Novozyme®
In a subsequent report, Conde\textsuperscript{10} demonstrated that acyl protected amines react slower than their carbamate counterparts. It was hypothesised that the alkyl-oxygen atom of the carboxamides interacted with the hydrogen bond network of the polar residues of the active site of the lipase. As a result, the energy required to bind the substrate to the active site will be significantly smaller than that for amines. Due to the unsuccessful results obtained in attempting to introduce nitrogen nucleophiles into the biotransformations, no further research on nitrogen nucleophiles was carried out.

\subsection*{2.7.0. Conclusions}

To conclude, a reliable and reproducible assay has been developed utilising 2-phenyl-4-substituted-5(4\textit{H})-oxazolones 89\textit{a-g} as substrates in a lipase mediated dynamic kinetic resolution process in the synthesis of optically active L-\textalpha-amino acid derivatives. High enantioselectivities were achieved by the correct selection of lipase and solvent. It was found that Novozyme\textsuperscript{®} preferred relatively flexible C-4 substituents, with the incorporation of a methylene spacer at C-4 proving advantageous for achieving a high degree of enantioselectivity. Lipozyme\textsuperscript{®} on the other hand required sterically demanding C-4 substituents for high enantioselectivities to be achieved. The addition of triethylamine to the reaction proved crucial in obtaining high enantioselectivities, with the base acting as a counter ion to any hydrolysis product formed, thus removing any inhibitory effects. Acetonitrile was the solvent of choice for flexible C-4 side chains, whereas toluene and a catalytic volume of triethylamine were preferred for bulky C-4 side chains.

\subsection*{2.8.0. Future work}

As there are already viable, economical routes to the proteinogenic \textalpha-amino acids, the utility of this methodology lies in the synthesis of unnatural \textalpha-amino acid derivatives. The oxazolone skeleton can provide a template for Knoevenagel condensation with various imines. Subsequent reduction or alkylation yields novel saturated 5(4\textit{H})-oxazolones 11\textit{1} which can be utilised as the substrates in the biotransformation process as illustrated in Scheme 55.
Scheme 55 Proposed synthesis of novel 4-substituted-5(4H)-oxazolones
3.0.0. Results and Discussion II

3.1.0. Application of developed methodology: The synthesis of potential matrix metalloproteinase inhibitors

3.1.1. Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs) are a family of closely related zinc containing endopeptidases. Their role in the body is to cleave large biomolecules such as collagens, proteoglycans, and gelatins. They have also been implicated in the remodelling of the extracellular matrix. To date there are 14 known MMPs, and a high degree of sequence homology has been identified. All the MMPs have been found to contain a zinc (II) metal-ion at the active site and are inhibited by metal ion chelating agents.

The role of MMPs in nature is to cleave molecules such as collagens, (the most abundant protein in the body and the major structural component of many organs and tissues). Their activity in the body must be precisely regulated. Expression of MMPs is tightly controlled by pro- and anti-inflammatory cytokines and growth factors. On production, the MMPs are generally secreted as inactive zymogens, or pro-enzymes. To activate these zymogens the N-terminal must be modified or cleaved, which results in the freeing of the zinc (II) containing active site. This is achieved by cleaving the bond between the Cys73 residue and the Zn (II) as illustrated in Figure 8. In the inactive form, the pro-domain is folded over, allowing the Cys73 residue to interact with the Zn (II), thereby preventing access to the active site by the water molecules that are required for the cleavage mechanism. The active forms of the MMPs are also regulated by natural inhibitors in the body, the tissue inhibitors of metalloproteinases, or TIMPs, and general plasma proteinase inhibitors such as α2-macroglobulin.
3.1.2. Matrix metalloproteinases and disease

In a number of pathological conditions such as cancers and arthritis, there is an imbalance between the levels of the active MMPs and the native inhibitors. The increased levels of MMPs result in increased degradation of the extracellular matrix,
which in turn, cause irreversible damage to the body. For example, in various (but not all) cancers, abnormally high levels of MMPs cause the breakdown of the extracellular matrix of the cancer tumour, allowing proliferation and local invasion of the primary tumour. As the tumour cells travel through the blood vessels, they adhere to the blood vessel wall. Again MMP activity causes a breakdown of the cell wall allowing tumour cells to cross the vascular basement membrane, culminating in secondary tumour growth. In addition, there is evidence that MMP activity contributes to the invasive growth of new blood cells, (angiogenesis) which allows the tumour to grow. This process is illustrated in Figure 9.

![Figure 9](image.png)

**Figure 9** Potential sites of action of matrix metalloproteinases in tumour growth

### 3.1.3. Synthetic matrix metalloproteinase inhibitors

A number of pharmaceutical companies currently have active research projects directed towards finding an orally active matrix metalloproteinase inhibitor (MMPI). The strategy of treatment is the continuous administration of low toxicity therapeutics that will stabilise malignant disease and prevent further growth. To this end, the MMPI’s do not constitute a magic bullet or a preventative treatment, but are better regarded as a quality of life enhancing tool for patients. Two approaches in MMPI development have been followed, namely the screening of natural compounds and the substrate-based design of pseudo-peptide derivatives. The second approach has been by far the most successful. The starting point for structure based design lay
with the sequence around the glycine-isoleucine and glycine-leucine cleavage sites of collagen, which frees the zinc (II) containing active site as indicated in Figure 10. Of the three resulting substructures, it has been MMPI’s based on the right hand side (RHS) of the sequence that have proved the most potent.

Figure 10 Design of matrix metalloproteinase inhibitors on the basis of the cleavage site of collagen

A summary of the structure activity relationship (SAR) found for MMPI’s is given in Figure 11. The incorporation of a zinc (II) binding group, (ZBG) to chelate to the active site of the MMPs proved essential for potent inhibitory levels. Of the zinc binding groups (ZBG’s) tested, including carboxylate, aminocarboxylate, sulfhydryl,
derivatives of phosphorus acids and hydroxamic acids, it has been the hydroxamic acids that have been incorporated most frequently. From an X-ray crystal structure of a hydroxamate substrate binding to matrilysin (MMP7) it is evident that the hydroxamate acts as a bidentate ligand, with each oxygen at an optimum distance (1.9-2.3 Å) from the active Zn(II) ion. The P₁' group fits into the S₁' pocket of the enzyme and offers the greatest opportunity for selective inhibitor design. A large number of the developed inhibitors have incorporated an iso-butyl group which results in non-selective inhibition. The P₂' and P₃' groups can tolerate a variety of α-amino acid residues which shows that the P₂' side chain does not play a major role in enzyme binding. Bulky R₃ groups such as tert-butyl, as in the Roche compound Ro 31-9790, and an indolemethylene as in Glycomed’s Galardin, have shown enhanced inhibition in comparison with other P₂' alkyl groups. The use of bulky R₃ groups also prevents amide hydrolysis in vivo. It has also been discovered that the P₁'-P₂', P₂'-P₃' C=O and N-H are all involved in hydrogen bonding interactions with the enzyme.¹¹³

![Diagram](image)

Figure 11 Summary of the SAR for RHS matrix metalloproteinase inhibitors. HFC- human fibroblast collagenase¹¹³

3.2.0. Retrosynthetic analysis

Through retrosynthetic analysis, (Scheme 56), it can clearly be illustrated how the oxazolone methodology developed in the previous chapter can be utilised in the
synthesis of the MMPI's such as Galardin (R= indolemethylene) and Ro 31 9790 (R= tert-butyl). Through a number of simple functional group interconversions, the diester 112 is obtained, which can be considered as the dynamic kinetic resolution product from the corresponding oxazolone 113. Synthesis of oxazolone 113 from racemic α-amino acid methyl esters and the substituted succinate monoester 114 was envisaged. The first goal was therefore the synthesis of the substituted succinate monoester 114.

**Scheme 56 Retrosynthetic analysis of matrix metalloproteinase inhibitors**

Succinate 114 can be considered as the downstream product from the alkylation of a unsymmetrical malonate 118 with the acceptor molecule 117 (Scheme 57). The acceptor molecule 117, where X = I, Br or TfO, TsO, could be synthesised from either enantiomer of leucine.

**Scheme 57 Retrosynthetic analysis of succinate 114**
3.3.0. Synthesis of substituted succinate mono ester 114

Starting from D-leucine, as illustrated in Scheme 58, the amine group was converted to a hydroxyl group via diazotisation to form (R)-leucic acid 119 according to the procedure detailed by Mori. The literature yield was moderate for this reaction and could not be improved on. Acid catalysed benzylation furnished the α-hydroxy ester 120 in excellent yield after column chromatography; which was used to form the triflate 121 as described by Degerbeck. Evidence for the formation of the triflate was obtained from the $^1$H and $^{13}$C nmr spectra. In the $^1$H nmr the α-proton was shifted downfield from δ 4.24 for the hydroxy ester 120 to δ 5.19 due to the electron withdrawing effect of the trifluoromethyl group. In the $^{13}$C nmr spectrum of 121, a quartet at δ 118.29 with a coupling constant of 319 Hz was observed which is characteristic for the trifluoromethyl group of a triflate. The triflate was stable to chromatography but readily decomposed on standing and was therefore used immediately in the next stage of the synthesis. All the above reactions were carried out on at least a 70 mmol scale providing multigram quantities (~24 g) of pure triflate 121.

![Scheme 58](image)

The optically purity of α-hydroxy ester 120 was also determined by HPLC analysis of the acetate 122 and found to be 97% e.e. (Scheme 59). This was in agreement with the optical purity of the D-leucine (97% e.e. commercially available) used at the beginning of the synthesis.

![Scheme 59](image)
The unsymmetrical malonate 125 was synthesised in high yield from the commercially available tert-butyl ethyl malonate 123 as illustrated in Scheme 60. Base hydrolysis of 123 proceeded quantitatively yielding mono tert-butyl malonate 124. Next, the potassium salt was formed and dried overnight under vacuum before being used in the alkylation reaction with benzyl bromide in DMF, to furnish tert-butyl benzyl malonate 125 in 75% yield on an 80 mmol scale.

With the two fragments in hand, the carbon-carbon bond forming reaction was attempted. The $S_N2$ displacement of the triflate group with the enolate derived from malonate 125 proceeded in an excellent yield of 91% on a 60 mmol scale (Scheme 61). The triester product 126 was isolated as a 1:1 mixture of diastereomers, as calculated from the integrals from the $^1$H nmr of the OCC$^6$CH proton. Deprotection of the benzyl esters to furnish diacid 116 proceeded quantitatively using 10% Pd/C and hydrogen at atmospheric pressure. Diacid 116 was not purified but used directly in the subsequent decarboxylation step.

A number of conditions were screened for the decarboxylation step outlined in Scheme 62 in order to obtain the optimum yield. Due to the tert-butyl ester
functionality, acid catalysed decarboxylation was avoided. The results are summarised in Table 22.

![Diagram](image)

Scheme 62 Reagents and conditions: i. See Table 22

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Crude Yield/ %</th>
<th>Purified Yield/ %</th>
<th>Comment, ratio 116:114</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene reflux, 20 h</td>
<td>-</td>
<td>60</td>
<td>desired product</td>
</tr>
<tr>
<td>2</td>
<td>THF, reflux</td>
<td>-</td>
<td>-</td>
<td>recovered starting material</td>
</tr>
<tr>
<td>3</td>
<td>THF Cu₂O, 65 °C</td>
<td>-</td>
<td>-</td>
<td>recovered starting material</td>
</tr>
<tr>
<td>4</td>
<td>THF, Et₃N, room temp.</td>
<td>-</td>
<td>-</td>
<td>recovered starting material</td>
</tr>
<tr>
<td>5</td>
<td>THF, Et₃N, reflux 5 h</td>
<td>98</td>
<td>-</td>
<td>1:4</td>
</tr>
<tr>
<td>6</td>
<td>CH₃CN, Cu₂O, 70 °C</td>
<td>0</td>
<td>0</td>
<td>recovered starting material</td>
</tr>
<tr>
<td>7</td>
<td>CH₃CN, reflux 20 h</td>
<td>-</td>
<td>-</td>
<td>1:1</td>
</tr>
<tr>
<td>8</td>
<td>CH₃CN, Cu₂O, reflux, 1.5 h</td>
<td>78</td>
<td>-</td>
<td>2:7</td>
</tr>
<tr>
<td>9</td>
<td>CH₃CN Cu₂O, reflux, 5 h</td>
<td>92</td>
<td>67</td>
<td>desired product</td>
</tr>
<tr>
<td>10</td>
<td>CH₃CN, Cu₂O, reflux, 24 h</td>
<td>51</td>
<td>-</td>
<td>desired product</td>
</tr>
<tr>
<td>11</td>
<td>CH₃CN, Et₃N, reflux, 5 h</td>
<td>90</td>
<td>-</td>
<td>1:2</td>
</tr>
<tr>
<td>12</td>
<td>CH₃CN, Et₃N, reflux, 19 h</td>
<td>-</td>
<td>85</td>
<td>desired product</td>
</tr>
</tbody>
</table>

Table 22 Decarboxylation studies for diacid 116

Petit utilised catalytic (0.1 equiv.) copper (I) oxide in the decarboxylation of chiral diacids similar to 116 in an extension of the procedure developed by Maumy. Maumy proposed that the reaction proceeded by an ionic mechanism involving copper (I) carboxylates as shown in Scheme 63. Brunner et al. carried out a number of experiments to disprove the participation of the proposed copper (I) carboxylates and discovered that the monoanionic malonate derivatives were the reactive species undergoing decarboxylation. The effect of copper (I) was attributed to basicity influence, therefore any compound that increases the concentration of the
monoanionic species will also increase the rate of decarboxylation. Brunner applied these experimental findings and used chiral amine alkaloids such as cinchonine, and cinchonidine in catalytic quantities as base to furnish chiral ethyl 2-phenyl propionate from mono ethyl methylphenylmalonate in excellent yields, albeit with only moderate enantioselectivity (11-34% e.e.).

\[
\begin{align*}
\text{R}^1 \underset{\text{CO}_2\text{H}}{\text{C}} \text{R}^2 \\
\text{R}^1 \underset{\text{CO}_2\text{H}}{\text{C}} \text{R}^2
\end{align*}
\]

Scheme 63 Proposed catalytic cycle for Cu(I) catalysed decarboxylation of malonic acids\textsuperscript{119}

The results in Table 22 for the decarboxylation of diacid 116 show that thermal decarboxylation required high temperatures and proceeded with moderate yields of 60% in toluene, entry 1. The conditions described by Petit and Maumy were also screened. When tetrahydrofuran was employed as the solvent, only starting material was recovered, entry 3. With acetonitrile and Cu (I), 5 h under reflux was required for complete decarboxylation, with the desired product isolated in a 67% yield, entry 9. The use of catalytic triethylamine as base resulted in complete decarboxylation after 19 h, and furnished the desired succinate 114 in 85% (~10 g) yield from triester 126, entry 11.
3.4.0. Synthesis of novel 5(4H)-oxazolones for lipase catalysed dynamic kinetic resolution

To produce the required 4-substituted-5(4H)-oxazolones, the succinate 114 was coupled to racemic α-amino acids to produce a 1:1 mixture of diastereomeric amides 127 using either of two sets of peptide coupling conditions illustrated in Scheme 64. The second set of conditions, (ii.), were required because uneven ratios of the resulting diastereomers were obtained when the reaction conditions (i.) were employed with leucine, tryptophan and tert-leucine methyl esters. The results in Table 23 indicate the good to excellent yields of amides (2R,2'RS)-127a-e obtained.

![Scheme 64](image) 

**Scheme 64** Reagents and conditions: i. (i.) DCM, HOBT, EDCI, Et3N, room temp., or (ii.) DMF, HOBT, TBTU, DIPEA, room temp.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Method</th>
<th>Yield/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,2'RS)-127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>CH2Ph</td>
<td>i.</td>
<td>80</td>
</tr>
<tr>
<td>b</td>
<td>CH(CH3)2</td>
<td>i.</td>
<td>77</td>
</tr>
<tr>
<td>c</td>
<td>CH2CH(CH3)2</td>
<td>ii.</td>
<td>77</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>ii.</td>
<td>78</td>
</tr>
<tr>
<td>e</td>
<td>C(CH3)3</td>
<td>ii.</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 23 Synthesis of 1:1 mixture of diastereomeric amides (2R,2'RS)-127a-e

Succinate 114 was also coupled to L-phenylalanine methyl ester as shown in Scheme 65. The product was isolated in 95% d.e. as calculated from the 600 MHz 1H nmr spectrum by comparison of the signals corresponding to the methyl ester protons, δ 3.69 and 3.67 for the minor and major diastereomers respectively. The crystal structure of the amide 127a was also obtained, (see Appendix I for details), and is
Results and Discussion II

represented in Figure 12. The structure clearly indicates that C-5 (as numbered in Figure 12) has an (R) configuration since the C-8 configuration was fixed as (S) due to the use of L-phenylalanine methyl ester. This result confirms that the synthesis of succinate 114 proceeded with the desired stereochemical integrity, and that the carbon-carbon bond forming reaction involving malonate 125 and triflate 121 did proceed via an \( S_N2 \) inversion mechanism. As the e.e. of \( \alpha \)-hydroxy ester 120 was determined to be 97\% (Scheme 59), and amide 127a was obtained with 95\% d.e. when reacted with optically pure L-phenylalanine, it can be concluded that (R)-succinate 114 was obtained in 97\% e.e. It should be noted that samples of the C-5-(R)-C-8-(S)-pseudodipeptides for all five C-8 substituents were prepared in high yield and d.e. from coupling of succinate 114 and the corresponding L-\( \alpha \)-amino acid ester to aid \( ^{1} \)H nmr assignment. Details can be found in the experimental section.

\[
\begin{align*}
\text{Ph} & \quad \text{BuO}_2\text{C} \quad \text{CO}_2\text{H} \\
+ & \quad \text{HCl} \text{H}_{2}\text{N} \quad \text{CO}_2\text{CH}_3 \\
\rightarrow & \quad \text{83}\% \\
\text{(R)-114} & \quad \text{(S)-115a} \\
\text{83}\% & \quad \text{(2R,2'S)-127a}
\end{align*}
\]

Scheme 65 Reagents and conditions: i. (a) DCM, HOBt, EDCI, Et\(_3\)N, room temp.

Figure 12 X-ray crystal structure obtained for (2R,2'S)-127a synthesised chemically
As illustrated in Scheme 66, base catalysed hydrolysis of the diastereomeric amides \((2R,2'RS)\)-127a-e proceeded in quantitative yield; subsequent cyclisation using EDCI in acetonitrile furnished the desired oxazolone substrates 129a-e in good to excellent yield (Table 24). The characteristic carbonyl stretch at \(~1818\) \(\text{cm}^{-1}\) was observed in the infrared spectra of all oxazolone products. In the \(^1\text{H}\) nmr spectra, oxazolone formation was accompanied by the loss of the N-H signal. These oxazolones were considered to be relatively unstable and were used immediately, or stored in the freezer at \(-26\) °C. The low yield obtained for the cyclisation of the tert-leucine derived acid 128e appeared to be due to cleavage of the tert-butyl ester as a results of prolonged reaction conditions (see experimental). From the \(^1\text{H}\) and \(^13\text{C}\) nmr of the crude hydrolysis product 128e, it was evident that a mixture of four compounds (two sets of diastereomers) was obtained in an almost equal ratio. A pure sample of acid 128e was obtained by repeated trituration of the undesired diacid. Cyclisation of crude hydrolysis product 128e, followed by purification by column chromatography furnished pure oxazolone 129e.

**Scheme 66** Reagents and conditions: ii. THF:H\(_2\)O (1:1), LiOH (2.0 equiv.), room temp., iii. CH\(_3\)CN, EDCI, room temp.
3.5.0. Dynamic kinetic resolution of novel 5(4H)-oxazolones

3.5.1. Initial studies

The first substrate tested was the phenylalanine derived oxazolone 129a (Scheme 67). The compound was subjected to both sets of optimised conditions developed in Chapter 2, and to both the lipases Novozyme® and Lipozyme®. The results in Table 25 can be directly compared with those obtained in Chapter 2, where the C-2 substituent was a phenyl group.

The use of acetonitrile as solvent was detrimental to the rate of reaction. In the case of Novozyme®, entry 2, a good yield of 88% was obtained but this figure is based on the recovery of 25% starting oxazolone 129a. Under identical reaction conditions, but using Lipozyme®, entry 5, only a 47% yield was obtained with 24% recovery of starting material after a period of 48 days. These results reinforce the hypothesis from Chapter 2 that the use of acetonitrile as solvent causes a conformational change in

---

**Table 24** Synthesis of 2-alkyl-5(4H)-oxazolone substrates 129a-e

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>128 Yield/ %</th>
<th>129 Yield/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>CH₂Ph</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>b</td>
<td>CH(CH₃)₂</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td>c</td>
<td>CH₂CH(CH₃)₂</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>d</td>
<td><img src="image.png" alt="Image" /></td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>e</td>
<td>C(CH₃)₃</td>
<td>100</td>
<td>52</td>
</tr>
</tbody>
</table>

a) Product was a mixture of desired product and tert-butyl ester cleavage product (1:1)

---

Scheme 67 Reagents and conditions: i. Solvent, (Et₃N, (0.25 equiv.)), lipase, CH₃OH (2.0 equiv.), 37 °C
the lipase that results in a decrease in the size of the active site of the lipases. The introduction of a more complex C-2 substituent, which also contains an additional chiral centre, imposes greater restrictions on the fit of the substrate into the active site of the enzyme.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Et$_3$N</th>
<th>Lipase</th>
<th>Time/ days</th>
<th>Yield/ %</th>
<th>d.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene</td>
<td>yes</td>
<td>Novozyme®</td>
<td>4</td>
<td>85</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>acetonitrile</td>
<td>no</td>
<td>Novozyme®</td>
<td>10</td>
<td>88*</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>'BuOMe</td>
<td>yes</td>
<td>Novozyme®</td>
<td>2</td>
<td>90</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>toluene</td>
<td>yes</td>
<td>Lipozyme®</td>
<td>1</td>
<td>73</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>acetonitrile</td>
<td>no</td>
<td>Lipozyme®</td>
<td>48</td>
<td>47*</td>
<td>55</td>
</tr>
</tbody>
</table>

a) Yield based on recovered starting material

Table 25 Results obtained for the methanolysis of 5(4H)-oxazolone 129a with Novozyme® and Lipozyme®

The d.e. for the Novozyme® and Lipozyme® reactions were consistent under the conditions studied, within the error of analysis (±5% by nmr); with Novozyme® producing the higher d.e. of 80% compared to 55% for Lipozyme®. The use of tert-butyl methyl ester as solvent with Novozyme® did enhance the rate, as predicted from the results in Chapter 2, but had no positive effect on the d.e. of the product. By comparison of the $^1$H nmr of the methyl esters obtained in Table 25 with the $^1$H nmr of (2R,2'S)-127a synthesised chemically, (Scheme 65), it was ascertained that the products had the (S) conformation at C-8. This was proved conclusively by obtaining a X-ray crystal structure (See Appendix II for data) of the Novozyme® mediated methanolysis product shown in Figure 13.

![Figure 13 X-ray crystal structure obtained for enzymatic methanolysis product (2R,2'S)-127a](image-url)
3.5.2. The effect of alkyl chain length of the nucleophile

In a similar process as described in Chapter 2, the alcohol nucleophile chain length was varied in the biotransformation as illustrated in Scheme 68. All the reactions were carried out with Novozyme® and the results reported in Table 26. Again, as discovered in Chapter 2, the d.e. of the product varied very little in going from a methyl to n-butyl alkyl chain. A range of ± 4% was observed which is within the accuracy of the 'H nmr measurements (± 5%). The rate and high yield for each reaction also remained constant throughout the series.

![Scheme 68](image)

**Scheme 68 Reagents and conditions:** i. Toluene, Et₃N, (0.25 equiv.), Novozyme®, ROH (2.0 equiv.), 37 °C

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Time/ days</th>
<th>Yield/ %</th>
<th>d.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,2'S)-127a</td>
<td>CH₃</td>
<td>4</td>
<td>85</td>
<td>81</td>
</tr>
<tr>
<td>130</td>
<td>CH₂CH₂</td>
<td>3</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>131</td>
<td>CH₃CH₂CH₃</td>
<td>3.5</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>132c</td>
<td>CH₃CH₂CH₂CH₂</td>
<td>3.5</td>
<td>85</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 26 Effect of alkyl chain length of the nucleophile on d.e. of the product**

3.5.3. Testing of remaining substrates

The remaining 5(4H)-oxazolone substrates 129b-e were tested with both Lipozyme® and Novozyme® (Scheme 69) and the results, collected in Table 27. The results indicate the following trends. From analysis of the results obtained for the simple C-4 alkyl substituents, where R= iso-propyl and iso-butyl, entries 1-4, it can be seen that the Novozyme® mediated reactions proceeded at a faster rate than the corresponding Lipozyme® reactions. The choice of enzyme had little effect on the yield. In all four reactions, excellent yields of 87-96% were obtained.
Scheme 69 Reagents and conditions: i. Toluene, Et₃N, (0.25 equiv.), lipase, CH₃OH (2.0 equiv.), 37 °C

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Lipase</th>
<th>Time/ days</th>
<th>Yield/ %</th>
<th>d.e./ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,2'S)-127</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>CH₂CH(CH₃)₂</td>
<td>Novozyme®</td>
<td>2</td>
<td>96</td>
<td>86</td>
</tr>
<tr>
<td>b</td>
<td>CH₂CH(CH₃)₂</td>
<td>Lipozyme®</td>
<td>12</td>
<td>96</td>
<td>54</td>
</tr>
<tr>
<td>c</td>
<td>CH(CH₃)₂</td>
<td>Novozyme®</td>
<td>11</td>
<td>93</td>
<td>75</td>
</tr>
<tr>
<td>c</td>
<td>CH(CH₃)₂</td>
<td>Lipozyme®</td>
<td>29</td>
<td>87</td>
<td>27</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>Novozyme®</td>
<td>13</td>
<td>84</td>
<td>74</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>Lipozyme®</td>
<td>13</td>
<td>89</td>
<td>7</td>
</tr>
<tr>
<td>e</td>
<td>C(CH₃)₃</td>
<td>Lipozyme®</td>
<td>28</td>
<td>12(44)°</td>
<td>72</td>
</tr>
<tr>
<td>e</td>
<td>C(CH₃)₃</td>
<td>Lipozyme°</td>
<td>28</td>
<td>5°</td>
<td>-</td>
</tr>
</tbody>
</table>

a) Yields in parentheses are based on recovered 5(4R)-oxazolones, b) n-Butanol (2.0 equiv.) as nucleophile, c) yield based on conversion as calculated from 'H nmr integrals.

Table 27 Results obtained for the methanolyis of 2-alkyl-4-substituted-5(4H)-oxazolones 129b-e

On examination of the d.e. of each product, the Novozyme® mediated reactions again produced the best optical resolution product. In the case of the indolemethylene side chain, high yield and good d.e. was also obtained with Novozyme®, in contrast to Lipozyme® which produced almost racemic material (entries 5 and 6 respectively). In the case of tert-butyl side chain it was decided not to attempt the Novozyme® mediated reaction due to the extremely poor results obtained with the equivalent substrate where the C-2 substituent was a phenyl ring. Instead, the Lipozyme® reaction was investigated with methanol and n-butanol as the nucleophile. A d.e. of
72% was obtained for the reaction with methanol, but only a 12% yield (44% based on recovered starting oxazolone) was achieved after 28 days (entry 7). In the case of the n-butanol reaction, only 5% conversion was observed by examination of the crude \(^1\)H nmr (200 MHz, CDCl\(_3\)) after 28 days, therefore the reaction was abandoned. If all the d.e. results obtained in Table 27 are compared with there counterparts in Chapter 2, it can be seen that the chiral induction obtained at C-4 in reduced by 10-20% with the introduction of the chiral C-2 substituent. It is speculated that this effect is due to an increase in the steric interactions of the C-2 substituent with the active site of the lipase.

### 3.6.0. Introduction of methyl amide functionality

The desired MMPI targets 94 all contain a methylamide functionality adjacent to the stereocentre at C-5 (numbered as in Figure 12). Using the biotransformation product \((2R,2'S)-127a\) from Table 25, entry 1, and the previously employed hydrolysis and peptide coupling conditions, (method (ii.)) with methyl amine as the nucleophile, the methyl amide 134 was synthesised in 85% for the two steps. On examination of the resulting \(^1\)H nmr (600 MHz) it was discovered that the d.e. had fallen from 81% to 42%. It is hypothesised that the cause of epimerisation was the reformation of the oxazolone 129a.

![Scheme 70](image)

**Scheme 70 Reagents and conditions:** i. THF:H\(_2\)O (1:1), LiOH, room temp., ii. DMF, HOBt, TBTU, DIPEA, CH\(_3\)NH\(_2\).HCl, room temp.

In an attempt to prevent reformation of the oxazolone, the Weinreb\(^{22,23}\) amide approach was considered. Under Weinreb conditions, the ester \((2R,2'S)-127a\) is reacted with the desired amine in the presence of trimethylaluminium to furnish the diamide 134a as illustrated in Scheme 71. It has recently been demonstrated that when such conditions were employed in the synthesis of peptides where the amine is
an unprotected α-amino acid, there is a 10-20% racemisation of the chiral centre alpha to the substrate ester. The cause of racemisation is attributed to the formation of the corresponding oxazolone. Unfortunately, due to lack of time, no further attempts to synthesise the desired amide 134a were possible.

\[
\begin{align*}
\text{(2R,2'S)-127a-e} & \quad \text{+} \quad \text{H}_3\text{C}\text{AlNHCH}_3 \\
& \quad \text{i.} \quad \rightarrow \quad \text{134a-e}
\end{align*}
\]

Scheme 71 Reagents and conditions: Benzene, reflux

3.7.0. Conclusions

A high yielding synthesis of novel, chiral C-2-substituted-4-substituted-5(4H)-oxazolones 129a-e was achieved, and their application in a dynamic kinetic resolution process under lipase mediated conditions in organic solvent proved successful. Excellent yields as high as 96%, and high diastereomeric excesses up to 86% were achieved for a range of C-4 substituents with the use of Novozyme®. The best results were obtained when the C-2 substituent was either benzyl or iso-butyl, again demonstrating the apparent advantage of the incorporation of a methylene spacer into the C-4 side chain to be resolved by the lipase. The choice of solvent also proved important. The use of acetonitrile resulted in a large decrease in the rate of reaction compared to the corresponding reaction in toluene with triethylamine. It is speculated that the decreased reaction rate in acetonitrile is a result of a conformational change in the lipase, tightening the active site channel, thus limiting access to the substrate.

3.8.0. Future work

Obviously future work would involve the completion of the synthesis of the MMPI’s. The introduction of the methyl amide bond could be realised utilising an enzyme such as papain or Subtilisin. Instead of using the methyl ester as the substrate more success may be obtained utilising the corresponding acid as depicted in Scheme 72.
The synthesis of the MMPI's could then be completed using published procedures for the introduction of the hydroxamic acid moiety as illustrated in Scheme 73.125

**Scheme 72 Reagents and conditions:** i. Solvent, enzyme, CH₃NH₂

On a broader level, the novel 2-substituted-5(4H)-oxazolone 136 could be utilised in alkylation/reduction reactions to generate oxazolones 137 with increased diversity at the C-4 position as discussed in Chapter 2. Subsequent dynamic kinetic resolution would furnish potentially new MMPI’s. The approach outlined in Scheme 74 could also be used to generate a combinatorial library of potential MMPI’s on solid support. The tert-butyl ester functionality of oxazolone 136 could be used to tether a linker and resin. O-Hydroxyamines 139126 and 140127 have recently been used as linkers to solid supports; cleavage resulting in the formation of the desirable hydroxamic acids.
Scheme 74 Proposed synthesis of novel MMPI's
4.0.0. Experimental

4.1.0. General experimental

$^1$H and $^{13}$C nmr were recorded on Bruker AC 200, Varian Gemini 200, Bruker AC 250, Bruker WH 360 or Varian UNITY INOVA 600 instruments. Chemical shifts ($\delta_H$, $\delta_C$) are reported in ppm and coupling constants ($J$) are in Hertz (Hz). Chemical shifts were referenced to residual undeuterated solvent present in the deuterated sample, \textit{i.e.} CHCl$_3$ in CDCl$_3$.

Electron Impact (EI) and Chemical Ionisation (CI) mass spectrometry were carried out on a Finnegan 4500 mass spectrometer. Fast atom bombardment (FAB) was performed on a Kratos MS50TC.

Infra-red spectra were recorded on a Biorad FTS-7 or a Perkin Elmer Paragon 1000 FT-IR spectrophotometer with the frequencies (v) measured in wavenumbers (cm$^{-1}$). Samples were measured on disposable IR Cards (Type 61 3M, polyethylene, 19 mm aperture), in CHCl$_3$ or as nujol mulls.

Melting points were measured on a Gallenkamp melting point apparatus, and are quoted in °C and uncorrected.

Chiral HPLC analysis was carried out using a Waters 486 Tunable Absorbance Detector and a Waters 600E Pump and Controller. Waters Millennium Chromatography Manager software package was used to analyse the results. A Chiracel OD column was used as the stationary phase eluting with hexane:isopropanol (9:1) with a flow rate of 0.5 mL min$^{-1}$ unless otherwise stated. Retention times ($R_t$) are quoted in minutes.

Elemental analysis (CHN) was performed on a Perkin Elmer 2400 CHN Elemental Analyser.
Optical rotations were measured on an Optical Activity AA-1000 polarimeter (sodium 589 nm detection). Sample concentration was measured in g/100 mL and $\left[\alpha\right]_{D}^{20}$ are quoted in $10^{-1}$ deg cm$^2$ g$^{-1}$.

Thin layer chromatography (tlc) was carried out on 2.5 mm glass plates coated with silica gel 60 F254, with detection by UV (254 nm) fluorescence, ammonium molybdate, bromocresol, ninhydrin or potassium permanganate dips. Chromatography was carried out using silica gel 60 (Merck 9385).

All reagents were used as supplied from commercial sources unless stated. Lipozyme® and Novozyme® were received as gifts from Novo-Nordisk and the Fluka Lipase Basic Kit was a gift from Fluka. Dichloromethane was distilled from calcium hydride while tetrahydrofuran was pre dried over sodium wire and distilled from sodium benzophenone ketal. Toluene was dried over sodium wire for 24 h before use. n-Butanol was dried, and distilled from sodium metal and stored over 4 Å molecular sieves. Novozyme® was crushed with a mortar and pestle and dried to constant weight over phosphorus pentoxide.

4.2.0. Development of 5(4H)-oxazolone methodology

4.2.1. General procedure for N-benzoyl DL-amino acids 95a-g

Benzoyl chloride (1.05 equiv.) and aqueous sodium hydroxide (2M, 100 mL) were simultaneously added to a stirring solution of DL-amino acid (1.00 equiv.) in aqueous sodium hydroxide (2M, 10 mL per 1.00 g of amino acid) at 0 °C over a period of 10 min. The resulting solution was stirred at room temperature for 30 min. The reaction mixture was cooled to -10 °C and conc. hydrochloric acid added until precipitation was complete. The mixture was stirred for a further 1 h, filtered, dried, and recrystallised from ethanol:water (2:1) to furnish the desired product as a colourless solid.
4.2.2. *N*-Benzoyl-DL-phenylalanine 95a

![Chemical structure](https://example.com/structure)

The general procedure outlined above (4.2.1) with DL-phenylalanine (10.00 g, 60.0 mmol) and benzoyl chloride (7.38 mL, 63.6 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (12.1 g, 74%).

Mp 186-188 °C (EtOH:water), Lit.\textsuperscript{128} 187-189 °C; $\nu_{\text{max}}$(nujol)/cm\textsuperscript{-1} 3322 (NH), 2800-2500 (acid OH), 1717 (acid C=O), 1612, 1536 (CONH); $\delta_{\text{H}}$ ((CD\textsubscript{3})\textsubscript{2}SO; 200 MHz), 12.76 (1 H, br s, OH), 8.73 (1 H, d, $J$ 8.0, NH), 7.83-7.77 (2 H, m, CH\textsubscript{ar}), 7.71-7.41 (3 H, m, CH\textsubscript{ar}), 7.31-7.12 (5 H, m, CH\textsubscript{ar}), 4.60 (1 H, ddd, $J$ 11.0, 8.0 and 5.0, NHCH), 3.18 (1 H, dd, $J$ 16.5, 11.0, CH\textsubscript{A}H\textsubscript{B}Ph), 3.08 (1 H, dd, $J$ 16.5, 5.0, CH\textsubscript{A}H\textsubscript{B}Ph); $\delta_{\text{C}}$ ((CD\textsubscript{3})\textsubscript{2}SO; 63 MHz) 173.3, 166.6 (CO\textsubscript{2}H, CONH), 138.9, 134.1 (ipso-Ar), 131.5, 129.2, 128.4, 128.3, 127.5, 126.5 (CH\textsubscript{ar}), 54.4 (HNCH), 36.4 (CH\textsubscript{Ph}); m/z (FAB) 270 (90%, MH\textsuperscript{+}), 224 (82, M-CO\textsubscript{2}H), 105 (85, 224-NHCHCH\textsubscript{2}Ph), 77 (72, 105-CO).

4.2.3. *N*-Benzoyl-DL-leucine 95b

The general procedure outlined above (4.2.1) with DL-leucine (10.00 g, 76 mmol) and benzoyl chloride (9.29 mL, 80 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (14.07 g, 78%).

Mp 140-142 °C, (EtOH:water), Lit.\textsuperscript{129} 141-143°C; $\nu_{\text{max}}$(nujol)/cm\textsuperscript{-1} 3271 (NH), 2800-2500 (acid OH), 1720 (acid C=O), 1634, 1536 (CONH); $\delta_{\text{H}}$ ((CD\textsubscript{3})\textsubscript{2}SO; 200 MHz) 8.59 (1 H, d, $J$ 8.0, NH), 7.92-7.86 (2 H, m, CH\textsubscript{ar}), 7.58-7.42 (3 H, m, CH\textsubscript{ar}), 4.44 (1 H, m, NHCH), 1.85-1.55 (3 H, m, CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2} and CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 0.92 (3 H, d, $J$ 6.0, CH\textsubscript{3a}CHCH\textsubscript{3b}), 0.88 (3 H, d, $J$ 6.0, CH\textsubscript{3a}CHCH\textsubscript{3b}); $\delta_{\text{C}}$ ((CD\textsubscript{3})\textsubscript{2}SO; 63 MHz) 174.4, 166.6 (CO\textsubscript{2}H, CONH), 134.1 (ipso-Ar), 131.5, 128.3, 127.6 (CH\textsubscript{ar}), 51.0 (HNCH), 24.7 (CH(CH\textsubscript{3})\textsubscript{2}) 23.1, 21.3 (CH(CH\textsubscript{3})\textsubscript{2}); m/z (FAB), 236 (94%, MH\textsuperscript{+}), 190 (82, M-CO\textsubscript{2}H\textsubscript{2}), 105 (94, 190-NHCHCH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 77 (76, 105-CO.).
4.2.4. \(N\)-Benzoyl-\(DL\)-valine 95c

![Chemical Structure](attachment:image.png)

The general procedure outlined above (4.2.1) with \(DL\)-valine (10.00 g, 85 mmol) and benzoyl chloride (10.4 mL, 87 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (15.75 g, 83%).

Mp 127-129 °C, (EtOH:water), Lit.\(^{130}\) 129.5-130.5 °C; \(\nu_{\text{max}}\) (nujol)/cm\(^{-1}\) 3330 (OH), 2800-2475 (acid OH), 1727 (acid C=O), 1626, 1537 (CONH); \(\delta_{\text{H}}\) ((CD\(_3\))\(_2\)SO; 250 MHz) 12.51 (1 H, br s, OH), 8.44 (1 H, d, \(J 8.0, \text{NH}\)), 7.89 (2 H, d, \(J 8.0, \text{CH}_\text{ar}\)), 7.57-7.43 (3 H, m, \text{CH}_\text{ar}), 4.28 (1 H, dd, \(J 8.0, 7.0, \text{NHCH}\)), 2.19 (1 H, dq, \(J 7.0, \text{CH}((\text{CH}_3)_2)\), 0.97 (3 H, d, \(J 7.0, \text{CH}_5\text{CHCH}_3\)) 0.95 (3 H, d, \(J 7.0, \text{CH}_3\text{CHCH}_3\)); \(\delta_{\text{C}}\) ((CD\(_3\))\(_2\)SO; 63 MHz) 173.3, 167.1 (CO\(_2\)H, CONH), 134.3 (ipso-Ar), 131.4 128.3, 127.8 (CH\(_\text{ar}\)), 58.5 (HNCH), 29.6 (CH(CH\(_3\))\(_2\)), 19.5 18.9 (2 x CH\(_3\)); \(m/z\) (FAB) 222 (80%, MH\(^+\)), 176 (97, M-CO\(_2\)H, 97), 105 (100, 176-NHCHCHCH\(_3\)), 77 (88, 105-CO).

4.2.5. \(N\)-Benzoyl-\(DL\)-methionine 95d

![Chemical Structure](attachment:image.png)

The general procedure outlined above (4.2.1) with \(DL\)-methionine (10.00 g, 67 mmol) and benzoyl chloride (8.17 mL, 70 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (12.78 g, 75%).

Mp 146-148 °C, (EtOH:water), Lit.\(^{131}\) 146-149 °C; \(\nu_{\text{max}}\) (nujol)/cm\(^{-1}\) 3320 (NH), 2800-2500 (acid OH), 1717 (acid C=O), 1626, 1537 (CONH); \(\delta_{\text{H}}\) ((CD\(_3\))\(_2\)SO; 200 MHz) 8.63 (1 H, d, \(J 8.0, \text{NH}\)), 7.90-7.84 (2 H, m, \text{CH}_\text{ar}), 7.53-7.41 (3 H, m, \text{CH}_\text{ar}), 4.51 (1 H, ddd, \(J 8.0, 7.5, 7.0, \text{NHCH}\)), 2.59-2.47 (2 H, m partially obscured by DMSO peak, CH\(_2\)CH\(_2\)S), 2.05 (2 H, dd, \(J 7.5, \text{CH}_5\text{S}\)), 2.03 (3 H, s, SCH\(_3\)); \(\delta_{\text{C}}\) ((CD\(_3\))\(_2\)SO; 63 MHz) 173.6, 166.9 (CO\(_2\)H, CONH), 134.1 (ipso-Ar), 131.6, 128.4, 127.6 (CH\(_\text{ar}\)), 51.8 (HNCH), 30.4, 30.3 (CH\(_2\)CH\(_2\)S), 14.7 (CH\(_3\)); \(m/z\) (FAB) 254 (100%, MH\(^+\)), 206 (26, M-SCH\(_3\)), 105 (85, 206-NHCH(CH\(_2\)CH\(_2\)SCH\(_3\))CO\(_2\)H), 77 (50, 105-CO).
4.2.6. **\(N^2\)-Benzoyl-DL-tryptophan 95e**

The general procedure outlined above (4.2.1) with DL-tryptophan (7.70 g, 37.7 mmol) and benzoyl chloride (4.60 mL, 40 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (8.85 g, 76%).

Mp 192-194 °C, (EtOH:water), Lit.\(^{132}\) 192-193 °C; \(\nu_{\text{max}}\) (nujol)/cm\(^{-1}\) 3392 (indole NH), 3359 (amide NH), 2800-2500 (acid OH), 1729 (acid C=O), 1628, 1547 (CONH); \(\delta_{\text{H}}\) ((CD\(_3\))\(_2\)SO; 250 MHz); 10.85 (1 H, d, J 2.0, NH\(_{\text{indole}}\)), 8.67 (1 H, d, J 8.0, NH\(_{\text{amide}}\)), 7.86-7.80 (2 H, m, CH\(_{\text{ar}}\)), 7.62-7.59 (1 H, m, CH\(_{\text{ar}}\)), 7.56-7.31 (4 H, m, CH\(_{\text{ar}}\)), 7.22 (1 H, d, J 2.5, CH\(_{\text{ar}}\)), 7.10-6.96 (2 H, m, CH\(_{\text{ar}}\)), 4.68 (1H, ddd, J 10.0, 8.0, 5.0, NH\(_{\text{CH}}\)), 3.31 (1H, dd, J 14.5, 5.0, CH\(_{\text{A}}\)H\(_{\text{B}}\)Indole), 3.23 (1 H, dd, J 14.5, 10.0, CH\(_{\text{A}}\)H\(_{\text{B}}\)Indole); \(\delta_{\text{C}}\) ((CD\(_3\))\(_2\)SO; 63 MHz) 173.8, 166.6 (CO\(_2\)H, CONH), 136.3, 134.1 (ipso-Ar), 131.4, 128.4, 127.5 (CH\(_{\text{ar}}\)), 127.3 (ipso-Ar), 123.7, 121.1, 118.5, 118.3, 111.6 (CH\(_{\text{ar}}\)), 110.6 (ipso-Ar), 53.9 (HNCH), 26.8 (CH\(_2\)) ; m/z (FAB) 331 (100%,[M+Na]\(^{+}\)), 309 (57, MH\(^{+}\)), 263 (51, M-CO\(_2\)H), 105 (62, 263-NHCHCH\(_2\)Indole), 77 (54, 105-CO).

4.2.7. **N-Benzoyl-DL-alanine 95f**

The general procedure outlined above (4.2.1) with DL-alanine (10.00 g, 112 mmol) and benzoyl chloride (13.7 mL, 118 mmol, 1.05 equiv.) was followed and gave the title compound as a colourless solid (15.28 g, 70%).

Mp 164-165 °C, (EtOH:water), 165-166 °C\(^{133}\); \(\nu_{\text{max}}\) (nujol)/cm\(^{-1}\) 3367 (NH), 2800-2500 (acid OH), 1723 (acid C=O), 1662, 1546 (CONH); \(\delta_{\text{H}}\) ((CD\(_3\))\(_2\)SO; 200 MHz) 8.65 (1 H, d, J 7.5, NH), 7.89-7.84 (2 H, m, CH\(_{\text{ar}}\)), 7.53-7.40 (3H, m, CH\(_{\text{ar}}\)), 4.43 (1 H, dq, J 7.5, NHCH), 1.37 (3 H, d, J 7.5 CH\(_3\)); \(\delta_{\text{C}}\) ((CD\(_3\))\(_2\)SO; 63 MHz) 174.4, 166.4 (CO\(_2\)H, CONH), 134.1 (ipso-Ar), 131.6, 128.4, 127.6 (CH\(_{\text{ar}}\)), 48.3 (HNCH), 14.7 (
Experimental

4.2.8. N-Benzoyl-DL-tert-leucine 95g

\[
\text{Ph} \quad \begin{array}{c}
\text{O} \\
\text{N} \\
\text{CO}_2\text{H}
\end{array} 
\]

The general procedure outlined above (4.2.1) with DL-tert-leucine (1.00 g, 7.6 mmol) and benzoyl chloride (1.43 mL, 8.4 mmol, 1.10 equiv.) was followed with recrystallisation from ethanol:water (1:1) and furnished the title compound as a colourless solid (1.46 g, 81%).

\[\text{mp} \ 165-166 \ ^\circ\text{C}, \ (\text{EtOH:water}), \ \text{Lit.}^{134} 165-166 \ ^\circ\text{C}; \ \nu_{\text{max}}(\text{nujol})/\text{cm}^{-1} 3362(\text{NH}), 1724 (\text{acid } \text{C}=\text{O}), 1625, 1540 (\text{CONH}); \ \delta_{\text{H}} ((\text{CD}_3)_2\text{SO}; 200 \text{ MHz}) 8.13 (1 \text{ H, d, } J 8.0, \text{ NH}), 7.86-7.81 (2 \text{ H, m, CH_AR}), 7.56-7.39 (3 \text{ H, m, CH_AR}), 4.34 (1 \text{ H, d, } J 8.0, \text{ NHCH}), 1.02 (9 \text{ H, s, CH}_3); \ \delta_c ((\text{CD}_3)_2\text{SO}; 63 \text{ MHz}) 172.6, 167.2 (\text{CO}_2\text{H, CONH}), 134.4 (\text{ipso-Ar}), 131.4, 128.3, 127.8 (\text{CH_AR}), 61.1 (\text{HNCH}), 33.7 (\text{C(CH}_3)_3), 27.0 (\text{C(CH}_3)_3); \ m/z (\text{FAB}) 236 (100\%, \text{ MH}^+), 190 (64, \text{ M-CO}_2\text{H}), 105 (34, 190-\text{NHCHC(CH}_3)_3), 77 (11, 105-CO).

4.2.9. General procedure for (SR)-2-phenyl-4-substituted-5(4H)-oxazolones 89a-g

A solution of N-benzoyl DL-amino acid in 1,4-dioxane:acetic anhydride (1:1, 10 mL per 1.00 g of N-benzoyl amino acid) was stirred at 22-60 °C until a clear solution was obtained. On cooling, the solution was filtered and concentrated under reduced pressure. The excess acetic anhydride was co-evaporated with toluene to give the crude product which was purified by recrystallisation or column chromatography as described.
4.2.10. (SR)-2-Phenyl-4-benzyl-5(4H)-oxazolone 89a

The general procedure outlined above (4.2.9.) with N-benzoyl-DL-phenylalanine (5.17 g, 19.2 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (4.30 g, 89%).

Rf (Hexane:EtOAc, 4:1) 0.62; Mp 67-69 ºC, Lit.135 68-70 ºC; v_max(CHCl3)/cm^-1 1819 (C=0), 1652 (C=N); δ_H (CDCl3; 250 MHz) 7.94 (2 H, m, CH_ar), 7.58-7.40 (3 H, m, CH_ar), 7.29-7.20 (5 H, m, CH_ar), 4.68 (1 H, dd, J 6.5, 5.0, CHCH2), 3.32 (1 H, dd, J 14.0, 5.0, CH_A H_B Ph), 3.25 (1 H, dd, J 14.0, 6.5, CH_A H_B Ph); δ_C (CDCl3; 63 MHz) 177.3 (C=O), 161.7 (C=N), 135.0 (ipso-Ar), 132.7, 129.4, 128.6, 128.3, 127.8, 127.1 (CH_ar), 125.5 (ipso-Ar), 66.3 (CH), 37.1 (CH2Ph); m/z (FAB) 252 (100%, MH^+), 224 (49, MH^+-CO).

4.2.11. (SR)-2-Phenyl-4-iso-butyl-5(4H)-oxazolone 89b

The general procedure outlined above (4.2.9.) with N-benzoyl-DL-leucine (2.10 g, 8.9 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (1.58 g, 81%).

Rf (Hexane:EtOAc, 8:1) 0.66; Mp 54-55 ºC, Lit.135 54-56 ºC; v_max(nujol)/cm^-1 1812 (C=O), 1653 (C=N); δ_H (CDCl3; 200 MHz) 8.02-7.97 (2 H, m, CH_ar), 7.61-7.43 (3 H, m, CH_ar), 4.41 (1 H, dd, J 9.0, 5.5, NCH), 2.06 (1 H, ddq, J 7.0, 6.5, 6.0, CH(CH3)_2), 1.84 (1 H, ddd, J 13.5, 7.0, 5.5, CHCH_A H_B), 1.69 (1 H, ddd, J 13.5, 9.0, 6.5, CHCH_A H_B), 1.03 (3 H, d, J 6.5, CH_A CHCH_B), 1.00 (3 H, d, J 6.5, CH_CHCH_B); δ_C (CDCl3; 63 MHz) 178.9 (C=O), 161.2 (C=N), 132.5, 128.6, 127.7, (CH_ar), 125.9 (ipso-Ar) 63.8 (NCH), 40.7 (CHCH2), 25.1 (CH(CH3)_2), 22.6, 21.9 (2x CH3); m/z (FAB) 218 (12%, MH^+).
Experimental

4.2.12. (SR)-2-Phenyl-4-iso-propyl-5(4H)-oxazolone 89c

The general procedure outlined above (4.2.9.) with N-benzoyl-DL-valine (2.00 g, 9.0 mmol) was followed with recrystallisation from hexane:diethyl ether (1:1) to give the title compound as a colourless solid (1.74 g, 95%).

R$_f$ (Hexane:EtOAc, 4:1) 0.61; Mp 48-51 °C, Lit.$^{135}$ 48-51 °C; $\nu_{max}$(CHCl$_3$/cm$^{-1}$) 1821 (C=O), 1652 (C=N); $\delta_H$ (CDCl$_3$; 200 MHz) 8.05-7.99 (2 H, m, CH$_{aryl}$), 7.61-7.44 (3 H, m, CH$_{aryl}$), 4.29 (1 H, d, J 4.5, NCH), 2.39 (1 H, dqq, J 7.0, 4.5, CH(CH$_3$)$_2$), 1.15 (3 H, d, J 7.0 CH$_{3A}$CHCH$_3B$), 1.12 (3 H, d, J 7.0 CH$_{3A}$CHCH$_3B$); $\delta_C$ (CDCl$_3$; 63 MHz) 177.7 (C=O), 161.5 (C=N), 132.5, 128.6, 127.7, (CH$_{aryl}$), 125.8 (ipso-Ar) 70.6 (NCH), 31.1 (NCH$_2$CH), 18.6, 17.4 (2 x CH$_3$); m/z (FAB) 204 (74%, MH$^+$), 176 (47, MH$^+$.CO).

4.2.13. (SR)-2-Phenyl-4-(2-methylsulfanyl-ethyl)-5(4H)-oxazolone 89d

The general procedure outlined above (4.2.9.) with N-benzoyl-DL-methionine (2.50 g, 9.9 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:diethyl ether (7:1) to give the title compound as a colourless oil (1.31 g, 56%). Spectroscopic data was in agreement with previously reported results.$^{136}$

R$_f$ (Hexane:EtOAc, 1:1) 0.40; $\nu_{max}$(CHCl$_3$/cm$^{-1}$) 1825 (C=O), 1653 (C=N); $\delta_H$ (CDCl$_3$; 200 MHz) 8.02-7.99 (2 H, m, CH$_{aryl}$), 7.98-7.43 (3H, m, CH$_{aryl}$), 4.69 (1 H, dd, J 7.5, 6.0, NCH), 2.74 (2 H, t, J 7.0, CH$_2$S), 2.29 (1 H, ddd, J 14.5, 7.0, 6.0, CHCH$_{A}$H$_{B}$CH$_2$), 2.22 (1H, ddd, J 14.5, 7.5, 7.0, CHCH$_{A}$H$_{B}$CH$_2$), 2.11 (3 H, s, SCH$_3$); $\delta_C$ (CDCl$_3$; 63 MHz) 178.26 (C=O), 161.8 (C=N), 132.6, 128.6, 127.7, (CH$_{aryl}$), 125.6 (ipso-Ar) 63.5 (NCH), 30.2, 29.9 (CH$_2$CH$_2$S), 14.9 (CH$_3$); m/z (FAB) 236 (42%, MH$^+$), 208 (32, MH$^+$.CO).
4.2.14. (SR)-2-Phenyl-4-(1H-indol-3-ylmethyl)-5(4H)-oxazolone 89e

The general procedure outlined above (4.2.9.) with \( N\)-benzoyl-DL-tryptophan (2.00 g, 6.5 mmol) was followed with recrystallisation from ethyl acetate to give the title compound as a colourless solid (669 mg, 36%).

\[ \text{Rf (Hexane:EtOAc, 1:1) 0.75; Mp139-142 °C, Lit.}^{137} 142 °C; \nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1} 3477 (\text{NH}), 3018 (\text{CH}_\text{ar}), 1817 (\text{C}=\text{O}), 1651 (\text{C}=\text{N}); \delta_h (\text{CDCl}_3; 200 \text{ MHz}) 8.05 (1 \text{ H, br s, NH}\text {Indole}), 7.89 (2 \text{ H, d, J 7.0, CH}^\text{Ph}), 7.74-7.70 (1 \text{ H, m, CH}^\text{Indole}), 7.56-7.37 (3 \text{ H, m, CH}^\text{Ph}), 7.3-7.25 (2 \text{ H, m, CH}^\text{Indole}), 7.18-7.08 (2 \text{ H, m, CH}^\text{Indole}), 4.76 (1 \text{ H, dd, J 6.0, 5.5, NCH}), 3.51 (1 \text{ H, dd, J 15.0, 5.5, CHCH}_A H_B), 3.42 (1 \text{ H, dd, J 15.0, 6.0, CHCH}_A H_B); \delta_c (\text{CDCl}_3; 63 \text{ MHz}) 177.9 (\text{C}=\text{O}), 161.6 (\text{C}=\text{N}), 135.7 (ipso-Ar), 132.4, 128.5, 127.65 (\text{CH}_\text{ar}), 127.2, 125.6 (ipso-Ar), 123.3, 121.8, 119.3, 118.9, 110.9 (\text{CH}_\text{ar}), 66.4 (\text{NCH}), 27.1 (\text{CH}^3); m/z \text{(FAB) 291 (67%, MH})^+, 263 (33, MH-CO), 130 (100, CH}_2\text{Indole), 105 (51 (PhCO).}

4.2.15. (SR)-2-Phenyl-4-methyl-5(4H)-oxazolone 89f

The general procedure outlined above (4.2.9.) with \( N\)-benzoyl-DL-alanine (2.00 g, 10.3 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:ethyl acetate (4:1) to give the title compound as a colourless solid (1.79 g, 99%).

\[ \text{Rf (Hexane:EtOAc, 4:1) 0.52; Mp 36 °C, Lit.}^{135} 37-38 °C; \nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1} 1825 (\text{C}=\text{O}), 1653 (\text{C}=\text{N}); \delta_h (\text{CDCl}_3; 200 \text{ MHz}) 8.02-7.96 (2 \text{ H, m CH}_\text{ar}), 7.61-7.43 (3 \text{ H, m, CH}_\text{ar}), 4.44 (1 \text{ H, q, J 7.5, NCH}), 1.58 (3 \text{ H, d, J 7.5, CH}_3); \delta_c (\text{CDCl}_3; 63 \text{ MHz}) 178.6 (\text{C}=\text{O}), 161.3 (\text{C}=\text{N}), 132.5, 128.5, 127.6 (\text{CH}_\text{ar}), 125.4 (ipso-Ar), 60.7 (\text{CH}). \]
16.6 (CH₃); m/z (FAB) 176 (31%, MH⁺), 148 (11, MH⁺-CO), 105 (100, 148-NHCHCH₃).

4.2.16. (SR)-2-Phenyl-4-tert-butyl-5(4H)-oxazolone 89g

The general procedure outlined above (4.2.9.) with N-benzoyl-DL-tert-leucine (1.25 g, 5.3 mmol) was followed and the crude product purified by column chromatography, eluting with hexane:diethyl ether (14:1) to give the title compound as a colourless solid (0.94 g, 82%).

R₁ (Hexane:EtOAc, 6:1) 0.70; Mp 72-73 °C, Lit.¹³ 73-74 °C; u max(CHCl₃)/cm⁻¹; 1820 (C=O), 1656 (C=N) δ H (CDCl₃; 200 MHz) 8.01 (2 H, d, J 7.0, CH₆), 7.60-7.43 (3 H, m, CH₆), 4.07 (1 H, s, NCH), 1.13 (9 H, s, CH₃); δ C (CDCl₃; 63 MHz) 176.8 (C=O), 161.1 (C=N), 132.5, 128.6, 127.8 (CH₆), 125.9 (ipso-Ar), 73.9 (NCH), 35.8 (C(CH₃)₃), 26.1 (C(CH₃)₃); m/z (FAB) 218 (56%, MH⁺), 190 (24, MH⁺-CO), 105 (100, 190-NHCHC(CH₃)₃).

4.2.17. General procedure for N-benzoyl-DL-amino acid esters

To a solution of (RS)-2-phenyl-4-substituted-5(4H)-oxazolones 89a-g (100 mg) in alcohol (5 mL) was added a catalytic volume of conc. hydrochloric acid (30 μL). The solution was heated under reflux overnight, cooled and the solvent removed under reduced pressure. The crude product was purified by column chromatography as described, furnishing the desired ester as a colourless solid unless stated.

4.2.18. N-Benzoyl-DL-phenylalanine methyl ester 96a
The general procedure outlined above (4.2.17.) with oxazolone 89a in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (4:1), (96 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.12; R, 19.57 and 25.72; Mp 85-87 °C, Lit.138 86.5-87.5 °C; ν_max(CHCl_3)/cm^{-1} 3410 (NH), 3029 (CH_ar), 1740 ester C=O), 1644, 1526 (CONH); δ_H (CDCl_3; 250 MHz) 7.74-7.70 (2 H, m, CH_ar), 7.53-7.28 (3 H, m, CH_ar), 7.27-7.21 (3 H, m, CH_ar), 7.15-7.11 (2 H, m, CH_ar), 6.63 (1 H, d, J 7.0, NH), 5.09 (1 H, ddd, J 7.0, 6.0, 5.5, NHCH), 3.75 (3 H, s, OCH_3), 3.29 (1 H, dd, J 14.0, 6.0, CHCH_A.CH_B), 3.23 (1 H, dd, J 14.0, 5.5, CHCH_A.CH_B); δ_C (CDCl_3; 63 MHz) 171.9, 166.7 (CO_2CH_3, CONH), 135.7 133.7 (ipso-Ar), 131.6, 129.1, 128.4, 127.0, 126.8 (CH_ar), 53.4 (HNCH), 52.3 (OCH_3), 37.7 (CH_2Ph); m/z (FAB) 284 (68%, MH^+), 268 (13, M-Me), 224 (73, 268-CO_2), 105 (100, 224-NHCHCH_2Ph), 77 (37, 105-CO).

4.2.19. N-Benzoyl-DL-phenylalanine ethyl ester 98a

The general procedure outlined above (4.2.17.) with oxazolone 89a in ethanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (4:1), (96 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.18; R, 12.13 and 16.78; Mp 90-92 °C, Lit.139 94-95 °C; ν_max(CHCl_3)/cm^{-1} 3321 (NH), 3029 (CH_ar), 1740 (ester C=O), 1641, 1534 (CONH); δ_H (CDCl_3; 250 MHz) 7.75-7.70 (2 H, m, CH_ar), 7.53-7.37 (3 H, m, CH_ar) 7.32-7.20 (3 H, m, CH_ar), 7.16-7.12 (2 H, m, CH_ar), 6.65 (1 H, d, J 7.0, NH), 5.06 (1 H, ddd, J 6.0, 5.5, NHCH), 4.22 (2 H, q, J 7.5, OCH_2), 3.28 (1 H, dd, J 14.0, 6.0, CH_A.CH_B.PH), 3.24 (1 H, dd, J 14.0, 5.5, CH_A.CH_B.PH); 1.28 (3 H, t, J 7.0, CH_2CH_3); δ_C (CDCl_3; 63 MHz) 171.4, 166.6 (CO_2CH_2, CONH), 135.7 133.8 (ipso-Ar), 131.6, 129.2, 128.4, 128.0, 126.8 (CH_ar), 61.5 (OCH_3), 53.4 (HNCH), 37.7 (CH_2Ph), 14.0 (CH_2CH_3); m/z (FAB) 298 (49%, MH^+), 252 (30, M-OEt), 224 (73, 252-CO), 105 (100, 224-NHCHCH_2Ph), 77 (45, 105-CO).
4.2.20. *N*-Benzoyl-**DL**-phenylalanine propyl ester 99a

\[
\text{Ph}^\text{O} \quad \text{Ph} \quad \text{N} \quad \text{CO}_2\text{Pr}^\text{O}
\]

The general procedure outlined above (4.2.17.) with oxazolone 89a in *n*-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (109 mg, 88%).

R<sub>f</sub> (Hexane:EtOAc, 8:1) 0.18; R<sub>r</sub> 11.67 and 17.47; Mp 69-69 °C; ν<sub>max</sub>(CHCl<sub>3</sub>)cm<sup>-1</sup> 3332 (NH), 3030 (CH<sub>ar</sub>), 1736 (ester C=O), 1647, 1522 (CONH); δ<sub>H</sub> (CDCl<sub>3</sub>; 200 MHz) 7.75 (2 H, m, CH<sub>ar</sub>), 7.55-7.40 (3 H, m, CH<sub>ar</sub>), 7.38-7.23 (3 H, m, CH<sub>ar</sub>), 7.21-7.12 (2 H, m, CH<sub>ar</sub>), 6.6 (1 H, d, J 7.0, NH), 5.08 (1 H, ddd, J 7.0, 6.0, 5.5, NHCH), 4.11 (1 H, dt, J 10.5, 6.5, OCH<sub>H</sub>B<sub>Ph</sub>), 4.10 (1 H, dt, J 10.5, 6.5, OCH<sub>H</sub>B<sub>Ph</sub>), 3.28 (1 H, dd, J 13.5, 6.0, CH<sub>H</sub>B<sub>Ph</sub>), 3.24 (1 H, dd, J 13.5, 5.5, CH<sub>H</sub>B<sub>Ph</sub>), 1.66 (2 H, tq, J 7.5, OCH<sub>2</sub>CH<sub>2</sub>), 0.93 (3 H, t, J 7.5, CH<sub>3</sub>); δ<sub>C</sub> (CDCl<sub>3</sub>; 63 MHz) 171.5, 166.7 (CO<sub>2</sub>CH<sub>3</sub>, CONH), 135.7 133.8 (ipso-Ar), 131.5, 129.2, 128.4, 126.9, 126.8 (CH<sub>ar</sub>), 67.0 (OCH<sub>2</sub>), 53.4 (HNCH), 37.8 (CH<sub>2</sub>Ph), 21.7 (OCH<sub>2</sub>CH<sub>2</sub>), 10.2 (CH<sub>2</sub>CH<sub>3</sub>); m/z (FAB) 312 (81%, MH<sup>+</sup>), 252 (30, M-OPr), 224 (76, 252-CO), 105 (100, 224-NHCHCH<sub>2</sub>Ph), 77 (46, 105-CO), Found (FAB) 312.1592, C<sub>19</sub>H<sub>22</sub>NO<sub>3</sub> requires 312.1600.

4.2.21. *N*-Benzoyl-**DL**-phenylalanine butyl ester 97a

\[
\text{Ph}^\text{O} \quad \text{Ph} \quad \text{N} \quad \text{CO}_2\text{Bu}^\text{O}
\]

The general procedure outlined above (4.2.17.) with oxazolone 89a in *n*-butanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (9:1), (121 mg, 93%).

R<sub>f</sub> (Hexane:EtOAc, 9:1) 0.29; R<sub>r</sub> 13.70 and 17.12; ν<sub>max</sub>(CHCl<sub>3</sub>)cm<sup>-1</sup> 3325 (NH), 3030 (CH<sub>ar</sub>), 1736 ester C=O), 1654, 1517 (CONH); δ<sub>H</sub> (CDCl<sub>3</sub>; 200 MHz) 7.74-7.70 (2 H, m, CH<sub>ar</sub>), 7.51-7.40 (3 H, m, CH<sub>ar</sub>), 7.38-7.20 (3 H, m, CH<sub>ar</sub>), 7.19-7.11 (2 H, m, CH<sub>ar</sub>), 6.64 (1 H, d, J 7.5, NH), 5.07 (1 H, ddd, J 7.5, 6.0, 5.5, NHCH), 4.14 (1 H, dt, J 11.0, 6.5, OCH<sub>H</sub>B<sub>Ph</sub>), 4.11 (1 H, dt, J 11.0, 6.5, OCH<sub>H</sub>B<sub>Ph</sub>), 3.26 (1 H, dd, J 14.0, 6.0, CH<sub>H</sub>B<sub>Ph</sub>), 3.24 (1 H, dd, J 14.0, 5.5, CH<sub>H</sub>B<sub>Ph</sub>), 1.68-1.54 (2 H, m,
Experimental

OCH₂CH₃), 1.34 (2 H, septet, J 7.5, CH₂CH₃), 0.92 (3 H, t, J 7.5, CH₃); δC (CDCl₃; 63 MHz) 171.5, 166.6 (CO₂CH₂, CONH), 135.8, 133.8 (ipso-Ar), 131.5, 129.2, 128.4, 128.4, 126.9, 126.8 (CH₆), 65.3 (OCH₂), 53.4 (HNCH), 37.8 (CH₂Ph), 30.3, 18.9 (OCH₂CH₂CH₂), 10.2 (CH₂CH₃); m/z (FAB) 326 (94%, MH⁺), 252 (25, M-OBu), 224 (53, 252-CO), 105 (100, 224-NHCHCH₂Ph), 77 (35, 105-CO), Found (FAB) 326.1579, C₂₀H₂₄NO₃ requires 326.1756.

4.2.22. N-Benzoyl-DL-phenylalanine pentyl ester 100a

\[ \text{Ph} \quad \text{O} \quad \text{N} \quad \text{CO}_2\text{CH} \]

\( n \)-Pentanol (87 µl, 0.80 mmol, 2.0 equiv.) was added to a solution of oxazolone 89a (100 mg, 0.40 mmol) in toluene (8 mL). Conc. hydrochloric acid (30 µL) was added, and the solution heated under reflux for 6 h. On cooling, the solvent was removed under reduced pressure and the crude product purified by column chromatography, eluting with hexane:ethyl acetate (10:1), to furnish the desired product as a colourless wax (129 mg, 95%).

Rₓ (Hexane:EtOAc, 8:1) 0.48; Rₛ 11.20 and 13.08; \( ν_{\text{max}}\) (CHCl₃)/cm⁻¹ 3432 (NH), 3030 (CH=O), 1735 (ester C=O), 1658, 1515 (CONH); δH (CDCl₃; 200 MHz) 7.77-7.70 (2 H, m CH₆), 7.62-7.37 (3 H, m, CH₆), 7.34-7.21 (3 H, m, CH₆), 7.19-7.11 (2 H, m CH₆), 6.62 (1 H, d, J 7.5, NH), 5.08 (1 H, ddd, J 7.5, 6.0, 5.5, NHCH), 4.14 (1 H, dt, J, 10.5, 6.5, OCH₆CH₆CH₃), 4.12 (1 H, dt, J, 10.5, 6.5, OCH₆CH₆CH₃), 3.26 (1 H, dd, J 14.0, 6.0, CH₆H₆Ph), 3.24 (1 H, dd, J, 14.0, 5.5, CH₆H₆Ph), 1.63 (2 H, m, OCH₂CH₂), 1.32 (4 H, m, CH₂(CH₂)₂CH₃), 0.90 (3 H, t, J 6.5, CH₃); δC (CDCl₃; 63 MHz) 171.5, 166.6 (CO₂CH₂, CONH), 135.8, 133.8 (ipso-Ar), 131.6, 129.2, 128.4, 128.4, 126.9, 126.8 (CH₆), 65.6 (OCH₃), 53.4 (HNCH), 37.8 (CH₃Ph), 28.0, 27.8, 22.1 (OCH₂CH₂CH₂CH₂), 13.8 (CH₃CH₂); m/z (FAB) 340 (93%, MH⁺), 252 (53, M-OPen), 224 (77, 252-CO), 120 (100, 224-CHCH₂Ph), 105 (68, 120-NH), 77 (21, 105-CO), Found (FAB) 340.1903, C₂₁H₂₆NO₃ requires 340.1913.
4.2.23. N-Benzoyl-DL-phenylalanine iso-propyl ester 101a

![Chemical structure of N-Benzoyl-DL-phenylalanine iso-propyl ester 101a]

The general procedure outlined above (4.2.17.) with oxazolone 89a in iso-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (114 mg, 92%).

\[ \text{R}_f \ (\text{Hexane:EtOAc, 4:1}) \ 0.50; \ \text{R}_s \ 13.00 \text{ and} \ 17.00; \ \text{M}p \ 97-99 \degree C; \ \nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1} \ 3332 \ (\text{NH}), \ 3030 \ (\text{CH}_\text{ar}), \ 1737 \ (\text{ester C}=\text{O}), \ 1644, \ 1533 \ (\text{CONH}); \ \delta_{\text{H}} \ (\text{CDCl}_3; \ 200 \ \text{MHz}) \ 7.75-7.70 \ (2 \ \text{H}, \ \text{m, CH}_\text{ar}), \ 7.61-7.37 \ (3 \ \text{H}, \ \text{m, CH}_\text{ar}), \ 7.33-7.20 \ (3 \ \text{H}, \ \text{m, CH}_\text{ar}), \ 7.18-7.13 \ (2 \ \text{H}, \ \text{m, CH}_\text{ar}), \ 6.62 \ (1 \ \text{H, d, J} \ 7.5, \ \text{NH}), \ 5.11-4.95 \ (2 \ \text{H, m, NHCH and CH(CH}_3)_2), \ 3.26 \ (1 \ \text{H, dd, J} \ 6.0, \ 13.5, \ \text{CH}_3 \text{H}_3\text{Ph}), \ 3.23 \ (1 \ \text{H, dd, J} \ 13.5, \ 6.5, \ \text{CH}_3\text{H}_3\text{Ph}), \ 1.25 \ (3 \ \text{H, d, J} \ 6.5, \ \text{CHCH}_3), \ 1.24 \ (3 \ \text{H, d, J} \ 6.5, \ \text{CHCH}_3); \ \delta_{\text{C}} \ (\text{CDCl}_3; \ 63 \ \text{MHz}) \ 171.0, \ 166.6 \ (\text{CO}_2\text{CH, CONH}), \ 135.8, \ 133.9 \ (\text{ipso-Ar}), \ 131.5, \ 129.3, \ 128.4, \ 128.4, \ 126.9, \ 126.8 \ (\text{CH}_\text{ar}), \ 69.4 \ (\text{OCH}), \ 53.5 \ (\text{HNCH}), \ 37.8 \ (\text{CH}_2\text{Ph}), \ 21.6, \ 21.5 \ (\text{CH(CH}_3)_2); \ m/z \ (\text{FAB}) \ 312 \ (63\%, \text{MH}^+), \ 252 \ (25, \text{M-OPr}), \ 224 \ (80, \text{252-CO}), \ 120 \ (88, \text{224-CHCH}_2\text{Ph}), \ 105 \ (100, \text{120-NH}), \ 77 \ (69, \text{105-CO}), \ \text{Found (FAB)} \ 312.1594, \ C_{19}H_{22}NO_3 \text{ requires 312.1600.}

4.2.24. N-Benzoyl-DL-leucine methyl ester 96b

The general procedure outlined above (4.2.17.) with oxazolone 89b in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (92 mg, 80%).

\[ \text{R}_f \ (\text{Hexane:EtOAc, 8:1}) \ 0.14; \ \text{R}_s \ 9.63 \text{ and} \ 13.28; \ \text{M}p \ 94-96 \degree C, \ \text{Lit.}^{138} \ 95-96 \degree C; \ \nu_{\text{max}}(\text{Card})/\text{cm}^{-1} \ 3317 \ (\text{NH}), \ 3030 \ (\text{CH}_\text{ar}), \ 1747 \ (\text{ester C}=\text{O}), \ 1641, \ 1534 \ (\text{CONH}); \ \delta_{\text{H}} \ (\text{CDCl}_3; \ 200 \ \text{MHz}) \ 7.82-7.76 \ (2 \ \text{H, m, CH}_\text{ar}), \ 7.54-7.37 \ (3 \ \text{H, m, CH}_\text{ar}), \ 6.59 \ (1 \ \text{H, d, J} \ 8.5, \ \text{NH}), \ 4.55 \ (1\text{H, ddd, 8.5, 5.5, NHCH}), \ 3.74 \ (3 \ \text{H, s, OCH}_3), \ 1.69 \ (3 \ \text{H, m, CH}_2\text{CH and CH}_2\text{CH}), \ 0.97 \ (3 \ \text{H, d, J} \ 6.5, \ \text{CH}_3\text{ACHCH}_3\text{B}), \ 0.96 \ (3 \ \text{H, d, J} \ 6.5, \ \text{CH}_3\text{ACHCH}_3\text{B}); \ \delta_{\text{C}} \ (\text{CDCl}_3; \ 63 \ \text{MHz}) \ 173.5, \ 167.0 \ (\text{CO}_2\text{CH}_3, \ \text{CONH}), \ 133.8 \ (\text{ipso-}...
Experimental

Ar), 131.5, 128.4 126.90 (CH₆), 52.1 (OCH₃), 51.0 (HNCH), 41.6 (CH₂CH), 24.8 (CH(CH₃)₂), 22.6, 21.8 (CH(CH₃)₂), m/z (FAB) 250 (100%, MH⁺), 234 (11, M-CH₃), 190 (67, 234-CO₂), 105 (53, 190-NHCHCH₂CH(CH₃)₂), 77 (37, 105-CO).

4.2.25. N-Benzoyl-DL-leucine propyl ester 99b

\[
\begin{align*}
\text{Ph} & \quad \text{N} \\
& \quad \text{CO₂Pr} \\
\end{align*}
\]

The general procedure outlined above (4.2.17.) with oxazolone 89b in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (114 mg, 93%).

Rₜ (Hexane:EtOAc, 8:1) 0.32; Rₜ 8.22 and 16.02; Mp 62-63 °C; νₓ max(Card)/cm⁻¹; 3329 (NH), 3029 (CH₆), 1743 ester (CO), 1642, 1535, (CONH); δₓ (CDCl₃; 200 MHz) 7.82-7.76 (2 H, m, CH₆), 7.54-7.37 (3 H, m, CH₆), 6.58, (1 H, d, J 7.5, NH), 4.84 (1H, ddd, 8.5, 7.5, 5.5, NHCH), 4.11 (2 H, t, J 6.5 OCH₂), 1.79-1.59 (5 H, m, CHCH₂CH, CHCH₂CH, and CH₂CH₃), 0.99 (3 H, d, J 6.0, CH₃CHCH₂), 0.97 (3 H, d, J 6.0, CH₃CHCH₂); 0.95 (3 H, t, J 7.5, CH₂CH₃); δₓ (CDCl₃; 63 MHz) 173.2, 166.9 (CO₂CH₂, CONH), 133.9 (ipso-Ar), 131.5, 128.4, 126.9 (CH₆), 66.8 (OCH₂), 51.1 (HNCH), 41.8 (CHCH₂), 24.9 (CH(CH₃)₂), 22.6, 22.0 (CH(CH₃)₂), 21.8 (CH₂CH₃), 10.2 (CH₂CH₃); m/z (FAB) 278 (81%, MH⁺), 218 (16, M-OPr), 190 (66, 218-CO), 105 (100, 190-NHCHCH₂CH(CH₃)₂), 77 (12, 105-CO), Found (FAB) 278.1744, C₁₁H₂₃NO₃ requires 278.1756.

4.2.26. N-Benzoyl-DL-valine methyl ester 96c

\[
\begin{align*}
\text{Ph} & \quad \text{N} \\
& \quad \text{CO₂CH₃} \\
\end{align*}
\]

The general procedure outlined above (4.2.17.) with oxazolone 89c in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (87 mg, 75%).

Rₜ (Hexane:EtOAc, 4:1) 0.23; Rₜ 9.55 and 11.38; Mp 84-86 °C, Lit.¹⁴⁰ 86 °C; νₓ max(nujol)/cm⁻¹ 3436 9NH), 3018 (CH), 1734 (ester C=O), 1663, 1517 (CONH); δₓ
Experimental

(CDCl₃; 200 MHz) 7.84 (2 H, m, CH₆), 7.55-7.39 (3 H, m, CH₆), 6.63 (1 H, d, J 7.0, NH), 4.76 (1 H, dd, J 7.0, 8.5, NHCH), 3.76 (3 H, s, OCH₃), 2.26 (1 H, dqq, J 7.0, 8.5 CHCH₃), 1.00 (3 H, d, J 7.0 CHCH₃), 0.98 (3 H, d, J 7.0 CHCH₃); δ c (CDCl₃; 63 MHz) 172.5, 167.1 (CO₂CH₃, CONH), 134.0 (ipso-Ar), 131.6, 128.5, 126.9 (CH₆), 57.3, (NHCH), 52.1 (OCH₃), 31.5 (CH(CH₃)₂), 18.9, 17.8 (CH(CH₃)₂); m/z (FAB) 236 (81%, MH⁺), 204 (3, M-OMe), 176 (30, 204-CO), 105 (100, 176-NHCHCH(CH₃)₂), 77 (25, 105-CO).

4.2.27. N-Benzoyl-DL-valine propyl ester 99c

\[
\text{Ph} \quad \text{NCO₂Pr}
\]

The general procedure outlined above (4.2.17.) with oxazolone 89c in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (10:1), (105 mg, 81%).

R f (Hexane:EtOAc, 10:1) 0.18; R f 6.43 and 8.35; Mp 53-54 ºC; νmax(nujol)/cm⁻¹ 3432 (NH), 3017 (CH), 1730 (ester C=O), 1663, 1517 (CONH); δ H (CDCl₃; 200 MHz) 7.78 (2 H, M, CH₆), 7.50-7.33 (3 H, m, CH₆), 6.59 (1 H, d, J 9.0, NH), 4.72 (1 H, dd, J 9.0, 7.0, NHCH), 4.10 (1 H, dt, J 12.1, 6.5, OCH₃H₆B), 4.04 (1 H, dt, J 12.1, 6.5, OCH₃H₆B) 2.21 (1 H, dqq, J 7.0, 6.5, CH(CH₃)₂) 1.63 (2 H, tq, J 7.0, OCH₂CH₂), 0.96 (3 H, d, J 7.0, CH₃CHCH₃B), 0.92 (3 H, d, J 7.0, CH₃CHCH₃B), 0.90 (3 H, t, J 7.5, CH₂CH₃); δ c (CDCl₃; 63 MHz) 172.1, 167.1 (CO₂CH₃, CONH), 134.1 (ipso-Ar), 131.6, 128.5, 126.9, (CH₆), 66.9 ((OCH₂), 57.2 (NHCH), 31.6 (CH(CH₃)₂), 21.8 (OCH₂CH₃), 18.9, 17.8 (CH(CH₃)₂), 10.3 (CH₂CH₃); m/z (FAB) 264 (71%, MH⁺), 204 (39, M-O₂Pr) 176 (78, 204-CO), 105 (100, 176-NHCHCH(CH₃)₂), 77 (46, 105-CO), Found (FAB) 264.1599, C₁₅H₂₂NO₃ requires 264.1600.

4.2.28. N-Benzoyl-DL-methionine methyl ester 96d

\[
\text{Ph} \quad \text{NHCO₂CH₃}
\]
The general procedure outlined above (4.2.17.) with oxazolone 89d in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (98 mg, 83%).

R_f (Hexane:EtOAc, 8:1) 0.10; R_f 15.08 and 20.70; Mp 85-87 °C, Lit. 87.5-88 °C; v_max (Card/cm^-1) 3322 (NH), 3030 (CH_ar), 1734 (ester C=O), 1642, 1534 (CONH); δ_H (CDCl_3; 200 MHz) 7.84-7.77 (2 H, m, CH_ar), 7.55-7.38 (3 H, m, CH_ar), 6.99 (1 H, d, J 6.5, NH), 4.92 (1 H, ddd, J 7.5, 7.0, 6.5, NHCH), 3.78 (3 H, s, OCH_3), 2.57 (2 H, dd, J 8.0, 7.5, CH_2S), 2.36-2.21 (2 H, m, CH_AH_B CH_2S and CH_AH_B CH_2S), 2.09 (3 H, s, SCH_3); δ_C (CDCl_3; 63 MHz) 172.4, 166.9 (CO_2CH_3, CONH), 133.5 (ipso-Ar), 131.6, 128.4, 126.9 (CH_ar), 52.4 (OCH_3), 51.9 (HNCH), 31.4, 29.9 (CH_2CH_3S), 15.3 (SCH_3); m/z (FAB) 268 (96%, MH^+), 236 (19, M-OMe), 208 (51, 236-CO), 105 (83, 208-NHCHCH_2CH_2SCH_3), 77 (50, 105-CO).

4.2.29. N-Benzoyl-DL-methionine propyl ester 99d

The general procedure outlined above (4.2.17.) with oxazolone 89d in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (107 mg, 86%).

R_f (Hexane:EtOAc, 8:1) 0.29; R_f 11.57 and 20.03; Mp 76-78 °C; v_max (Card/cm^-1) 3326 (NH), 3029 (CH_ar), 1740 (ester C=O), 1643, 1534 (CONH); δ_H (CDCl_3; 200 MHz) 7.82-7.77 (2 H, m, CH_ar), 7.54-7.38 (3 H, m, CH_ar), 6.98 (1 H, d, J 7.5, NH), 4.91 (1 H, ddd, J 7.5, 7.0, 5.0, NHCH), 4.13 (2 H, t, J 7.0, OCH_2), 2.58 (2 H, m, CH_2S), 2.34-2.04 (2 H, m, CHCH_AH_B CH_2 and CHCH_AH_B CH_2), 2.09 (3 H, s, SCH_3), 1.69 (2 H, t, J 7.0, 7.5, OCH_2CH_2), 0.95 (3 H, t, J 7.5, CH_3CH_3); δ_C (CDCl_3; 63 MHz) 172.0, 166.9 (CO_2CH_3, CONH), 133.6 (ipso-Ar), 131.6, 128.4, 126.9 (CH_ar), 67.1 (OCH_3), 52.0 (HNCH), 31.6, 29.9 (CH_2CH_3S and CH_2CH_3S), 21.7 (OCH_2CH_2), 15.3 (SCH_3), 10.2 (CH_2CH_3); m/z (FAB) 296 (100%, MH^+), 280 (7, M-CH_3), 248 (63, 280-S), 236 (17, M-OPr), 208 (33, 236-CO), 105 (31, 208-NHCHCH_2CH_2SCH_3), 77 (30, 105-CO). Found (FAB) 296.1328, C_{15}H_{22}NO_3S requires 296.1320.
4.2.30. Nα-Benzoyl-dl-tryptophan methyl ester 96e

The general procedure outlined above (4.2.17.) with oxazolone 89e (60 mg, 0.21 mmol) in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (2:1), (65 mg, 98%).

Rf (Hexane:EtOAc, 1:1) 0.46; Rf (Hexane:EtOH, 9:1) 50.10 and 53.92 (not baseline separation); Mp 105-107 °C; Lit.141 109-110 °C; $\nu_{\max}$(CHCl$_3$/cm$^{-1}$; 3475 (indole NH), 3424 (amide NH), 3017 (CH$_x$), 1738 (ester C=O), 1656, 1519 (CONH); $\delta_H$ (CDCl$_3$; 200 MHz) 8.27 (1 H, br s, NH$_{(\text{Indole})}$), 7.62-7.57 (2 H, m, CH$_{ar}$), 7.49-7.24 (5 H, m, CH$_{ar}$), 7.17-6.95 (2 H, m, CH$_{ar}$), 6.90 (1 H, d, J 2.0, CH$_{ar}$), 6.64 (1 H, d, J 7.5, NH), 5.07 (1 H, ddd, J 7.5, 5.5, 5.0, NHCH), 3.63 (3 H, S, OCH$_3$), 3.45 (1 H, dd, J 15.0, 5.5, CH$_A$CH$_B$Indole) 3.43 (1 H, dd, J 15.0, 5.0 CH$_A$CH$_B$Indole); $\delta_C$ (CDCl$_3$; 63 MHz) 172.3, 167.0 (CO$_2$CH$_3$, CONH), 136.0, 133.5 (ipso-Ar), 131.6, 128.4 (CH$_{ar}$), 127.4 (ipso-Ar), 126.9, 122.8, 122.0, 119.4, 118.3, 111.3, (CH$_{ar}$) 109.5 (ipso-Ar), 53.4 (HNCH), 52.3 (OCH$_3$), 27.4 (CHCH$_2$); m/z (FAB) 323 (28%, MH$^+$), 291 (2, M-OH), 263 (13, 291-CO), 130 (100, CH$_2$Indole), 105 (69, 263-NHCHCH$_2$Indole), 77 (15, 105-CO).

4.2.31. Nα-Benzoyl-dl-tryptophan propyl ester 99e

The general procedure outlined above (4.2.17.) with oxazolone 89e (60 mg, 0.21 mmol) in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (1:1), (65 mg, 89%).

Rf (Hexane:Et$_2$O, 1:1) 0.16; Rf (Hexane:EtOH, 9:1) 38.75 and 41.73 (not baseline separation); Mp 104-106 °C; $\nu_{\max}$(CHCl$_3$/cm$^{-1}$; 3476 (indole NH), 3421 (amine NH),
Experimental

3017 (CH<sub>3</sub>), 1736 (ester C=O), 1656, 1519; δ<sub>H</sub> (CDCl<sub>3</sub>; 200 MHz) 8.46 (1 H, br s, NH<sub>(Indole)</sub>), 7.70-7.66 (2 H, m, CH<sub>ar</sub>), 7.59-7.21 (5 H, m, CH<sub>ar</sub>), 6.98 (1 H, d, J 2.0, NHCH<sub>ar</sub>), 6.74 (1 H, d, J 7.5 NH), 5.15 (1 H, ddd, J 7.5, 5.5, NHCH), 4.07 (1 H, dt, J 10.5, 6.5, OCH<sub>α</sub>CH<sub>β</sub>CH<sub>2</sub>), 4.04 (1 H, dt, J 10.5, 6.5, OCH<sub>α</sub>CH<sub>β</sub>CH<sub>2</sub>), 3.45 (1 H, dd, J 15.5, 5.5, CH<sub>α</sub>CH<sub>β</sub>Indole) 3.43 (1 H, dd, J 15.5, 5.5, CH<sub>α</sub>CH<sub>β</sub>Indole); 1.63 (2 H, tq, J 7.5, 7.0, OCH<sub>2</sub>CH<sub>2</sub>), 0.89 (3 H, t, J 7.5, CH<sub>2</sub>CH<sub>3</sub>); δ<sub>C</sub> (CDCl<sub>3</sub>; 63 MHz) 171.9, 166.9 (CO<sub>2</sub>CH<sub>3</sub>, CONH), 136.0, 133.7 (ipso-Ar), 131.5, 128.4 (CH<sub>ar</sub>), 127.5 (ipso-Ar), 126.9, 122.7, 122.0, 119.4, 118.4, 111.2, (CH<sub>ar</sub>), 109.7 (ipso-Ar), 53.4 (NHCH), 27.6, 21.7 (OCH<sub>2</sub>CH<sub>2</sub> and CHCH<sub>2</sub>), 10.1 (CH<sub>3</sub>); m/z (FAB) 351 (15%, MH<sup>+</sup>), 291 (3, M-OPr), 263 (15, 291-CO), 130 (100, CH<sub>2</sub>Indole), 105 (62, 263-NHCHCH<sub>2</sub>Indole), 77 (13, 105-CO), Found (FAB) 351.1705, C<sub>21</sub>H<sub>23</sub>N<sub>2</sub>O<sub>3</sub> requires 351.1709.

4.2.32. N-Benzoyl-DL-alanine methyl ester 96f

![Structure](attachment:structure1.png)

The general procedure outlined above (4.2.17.) with oxazolone 89f in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (97 mg, 82%).

R<sub>f</sub> (Hexane:EtOAc, 4:1) 0.13; R<sub>f</sub> 10.61 and 14.21; Mp 80-82 °C, Lit. 80-82 °C; ν<sub>max</sub>(nujol)/cm<sup>-1</sup> 3431 (NH), 3017 (CH), 1750 (ester C=O), 1653, 1517 (CONH); δ<sub>H</sub> (CDCl<sub>3</sub>; 200 MHz) 7.78 (2 H, m, CH<sub>α</sub>), 7.52-7.37 (3 H, m, CH<sub>α</sub>), 6.81 (1 H, b d, J 5.5, NH), 4.63 (1 H, dq, J 7.0, NHCH), 3.76 (3 H, s, OCH<sub>3</sub>), 1.50 (3 H, d, J 7.0, CHCH<sub>3</sub>); δ<sub>C</sub> (CDCl<sub>3</sub>; 63 MHz) 173.5, 160.7 (CO<sub>2</sub>C, CONH), 133.7 (ipso-Ar), 131.6, 128.4, 126.9 (CH<sub>ar</sub>), 52.4 (OCH<sub>3</sub>), 48.3 (CH), 18.4 (CHCH<sub>3</sub>); m/z (FAB) 208 (100%, MH<sup>+</sup>), 176 (6, M-OMe), 148 (13, 176-CO), 105 (55, 148-NHCHCH<sub>3</sub>).

4.2.33. N-Benzoyl-DL-alanine ethyl ester 98f

![Structure](attachment:structure2.png)
The general procedure outlined above (4.2.17.) with oxazolone 89f in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (107 mg, 85%).

R_f (Hexane:EtOAc, 6:1) 0.15; R_t 10.97 and 14.07; Mp 75 \degree C; \nu_{\text{max}}(\text{nujol})/\text{cm}^{-1} 3431 (NH), 3018 (CH), 1734 (ester C=O), 1653, 1517 (CONH); \delta_H (CDCl_3; 200 MHz) 7.83 (2 H, m, CH_ar), 7.54-7.38 (3 H, m, CH_ar), 6.77 (1 H, d, J 6.0, NH), 4.75 (1 H, dq, J 7.0, NHCH), 4.23 (2 H, q, J 7.0, OCH_2), 1.51, (3 H, d, J 7.0, CHCH_3), 1.30, (3 H, t, J 7.0, CH_2CH_3); \delta_C (CDCl_3; 63 MHz) 173.1, 166.6 (CO_2CH_2, CONH), 133.8 (ipso-Ar), 131.5, 128.4, 126.9 (CH_ar), 61.5 (OCH_2), 48.4 (CH), 18.6 (CHCH_3), 14.01 (CH_2CH_3); m/z (FAB) 222 (100%, MH^+), 176 (92, M-OEt), 148 (99, 176-CO), 105 (48, 148-NHCHCH_3), 77 (37, 105-CO), Found 222.1136, C_{12}H_{16}NO_3 requires 222.1130.

4.2.34. N-Benzoyl-DL-alanine propyl ester 99f

The general procedure outlined above (4.2.17.) with 89f in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), furnished the desired product (95 mg, 71%).

R_f (Hexane:EtOAc, 6:1) 0.18; R_t 10.43 and 13.90; Mp 60-61 \degree C; \nu_{\text{max}}(\text{nujol})/\text{cm}^{-1} 3429 (NH), 3016 (CH), 1731 (ester C=O), 1655, 1518 (CONH); \delta_H (CDCl_3; 200 MHz) 7.83-7.77 (2 H, m, CH_ar), 7.54-7.38 (3 H, m, CH_ar), 6.78 (1 H, d, J 6.0, NH), 4.79 (1 H, dq, J 7.0, NHCH), 4.17 (1 H, dt, J 11.5, 6.5, OCH_2H_9CH_2), 4.10 (1 H, dt, J 11.5, 6.5, OCH_2H_9CH_2), 1.78-1.60 (2 H, m, OCH_2CH_2), 1.52 (3 H, d, J 7.0, CHCH_3), 0.95 (3 H, t, J 7.5, CH_2CH_3); \delta_C (CDCl_3; 63 MHz) 173.2, 166.7 (CO_2CH_2, CONH), 133.8 (ipso-Ar), 131.5, 128.4, 126.9 (CH_ar), 67.0 (OCH_2), 48.4 (NHCH), 21.8 (OCH_2CH_2), 18.6 (CHCH_3), 10.2 (CH_2CH_3); m/z (FAB) 236 (100%, MH^+), 176 (78, M-OEt), 148 (81, 176-CO), 105 (39, 148-NHCHCH_3), 77 (29, 105-CO), Found 236.1290, C_{12}H_{18}NO_3 requires 236.1286.
4.2.35. N-Benzoyl-DL-tert-leucine methyl ester 96g

The general procedure outlined above (4.2.17.) with oxazolone 89g in methanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (6:1), (96 mg, 84%).

Rf (Hexane:EtOAc, 4:1) 0.28; Rf (Heptane:IPA, 98:2, not baseline separation) 48.13 and 51.50; Mp 65-66 °C, Lit.143 66 °C; νmax(CHCl3)/cm⁻¹; 3437 (NH), 3018 (CHα), 1736 (ester C=O), 1664, 1518 (CONH); δH (CDCl3; 200 MHz) 7.80-7.76 (2 H, m, CHα), 7.50-7.37 (3 H, m, CHα), 6.67 (1 H, d, J 9.0 NH), 4.76 (1 H, d, J 9.0, NHCH), 3.73 (3 H, s, OCH3), 1.04 (9 H, s, C(CH3)3); δc (CDCl3; 63 MHz) 172.0, 166.9 (CO2CH3, CONH), 134.0 (ipso-Ar), 131.5, 128.4, 126.8 (CHα), 60.0 (NHCH), 51.7 (OCH3), 34.9 (C(CH3)3), 26.4 (C(CH3)3); m/z (FAB) 250 (100%, MH⁺), 105 (36, M-NHCH(C(CH3)3)CO2CH3), 77 (5, 105-CO).

4.2.36. N-Benzoyl-DL-tert-leucine propyl ester 99g

The general procedure outlined above (4.2.17.) with 89g in n-propanol was followed. Product purified by column chromatography, eluting with hexane:ethyl acetate (8:1), (95 mg, 74%).

Rf (Hexane:EtOAc, 6:1) 0.41; Rf (Heptane:IPA, 98:2) 40.35 and 43.52; Mp 60-62 °C; νmax(CHCl3)/cm⁻¹ 3435 (NH), 3018 (CHα), 1725 (ester C=O), 1663, 1517 (CONH); δH (CDCl3; 200 MHz) 7.81-7.77 (2 H, m, CHα), 7.54-7.39 (3 H, m, CHα), 6.69 (1 H, d, J 9.0, NH), 4.69 (1 H, d, J 9.0, NHCH), 4.10 (2 H, t, J 7.0, OCH2), 1.69 (2 H, tq, J 7.5, 7.0, OCH2CH3), 1.04 (9 H, s, C(CH3)3), 0.95 (3 H, t, J 7.5, CH2CH3); δc (CDCl3; 63 MHZ) 171.7, 166.9 (CO2CH2, CONH), 134.1 (ipso-Ar), 131.5, 128.4, 126.8 (CHα), 66.6 (OCH3), 60.1 (NHCH), 35.1 (C(CH3)3), 26.5 (C(CH3)3), 21.7 (CH2CH3), 10.3 (CH2CH3); m/z (FAB) 278 (100%, MH⁺), 105 (67, M-NHCH(C(CH3)3)CO2Pr), Found (FAB) 278.1764, C16H24NO3 requires 278.1756.
4.2.37. General procedure for the lipase catalysed ring opening of (RS)-2-phenyl-4-substituted-5(4H)-oxazolones 89a-g

i. In the presence of triethylamine

Catalytic triethylamine (0.25 equiv.), lipase (crushed and dried Novozyme®, 100 mg pre-dried weight, or Lipozyme®, 100 mg) and alcohol (2.0 equiv.) were added to a solution of (RS)-2-phenyl-DL-4-substituted-5(4H)-oxazolone 89a-g (100 mg) in solvent (8 mL). The flask was stoppered and placed in an orbital incubator at 37 °C and 200 rpm. The reactions were monitored by tlc and on complete consumption of the starting 5(4H)-oxazolone the lipase was filtered, washed with solvent (2x 10 mL) and the combined organic fractions concentrated under reduced pressure. Purification by column chromatography, as described for the corresponding racemate, furnished the desired product as a colourless solid unless otherwise stated. Spectroscopic data for all purified products were in agreement with the corresponding racemic sample.

ii. In the absence of triethylamine

As above with the elimination of triethylamine.

4.2.38. N-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, methanol, triethylamine, and Novozyme®. Reaction time 26 h, (93 mg, 82%).

R, 25.72, e.e. 94%; [α]D20 +96.4 (c 1.00, CHCl₃); Mp 86-88 °C.

4.2.39. N-Benzoyl-L-phenylalanine ethyl ester 98a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, ethanol, triethylamine and Novozyme®. Reaction time 26 h, (94 mg, 79%).

R, 16.78, e.e. 97%; [α]D20 +92.0 (c 1.00, CHCl₃); Mp 95-97 °C.
4.2.40. N-Benzoyl-L-phenylalanine propyl ester 99a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, with n-propanol, triethylamine and Novozyme®. Reaction time 26 h, (103 mg, 83%).

R₁ 17.47, e.e. 97%; [α]$_D^{20}$ +85.8 (c 1.00, CHCl₃); Mp 78-79 °C.

4.2.41. N-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, n-butanol, triethylamine and Novozyme® (100 mg as supplied). Reaction time 2 days, (69 mg, 53%).

R₁ 17.12, e.e. 95%; [α]$_D^{20}$ +78.6 (c 1.00, CHCl₃).

4.2.42. N-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89a, toluene, n-butanol, and Novozyme® (100 mg as supplied). Reaction time 6 days, (51 mg, 40%).

R₁ 17.12, e.e. 64%; [α]$_D^{20}$ +54.0 (c 1.00, CHCl₃).

4.2.43. N-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, with n-butanol, triethylamine, and Novozyme®. Reaction time 2 days, (104 mg, 81%).

R₁ 17.12, e.e. 95%; [α]$_D^{20}$ +78.6 (c 1.00, CHCl₃); Mp 68-70 °C.

4.2.44. N-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, n-butanol, triethylamine, and Lipozyme®. Reaction time 2 days, (96 mg, 74%).

R₁ 17.12, e.e. 69%; [α]$_D^{20}$ +52.7 (c 1.00, CHCl₃).
4.2.45. N-Benzoyl-L-phenylalanine butyl ester 97a

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89a, toluene, n-butanol, and Lipozyme®. Reaction time 3 days, (76 mg, 59%).

R, 17.12, e.e. 55%.

4.2.46. N-Benzoyl-L-phenylalanine pentyl ester 100a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, n-pentanol, triethylamine, and Novozyme®. Reaction time 26 h, (43 mg, 32%).

R, 13.08, e.e. 88%; [α]$_D^{20}$ +75.4 (c 1.00, CHCl$_3$); Mp 55-58 °C.

4.2.47. N-Benzoyl-L-phenylalanine iso-propyl ester 101a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, toluene, iso-propanol, triethylamine, and Novozyme®. Reaction time 26 days, (22 mg, 18%).

R, 17.00, e.e. 29%; Mp 95-97 °C.

4.2.48. N-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89b, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days, (110 mg, 96%).

R, 13.28, e.e. 97%; [α]$_D^{20}$ -22.1 (c 0.98, CH$_3$OH), Lit.$^{144}$ [α]$_D^{25}$ -22.8 (c 0.80, CH$_3$OH); Mp 101-103 °C.

4.2.49. N-Benzoyl-L-leucine propyl ester 99b

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89b, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days. Product obtained as a colourless oil (114 mg, 89%).

R, 16.02, e.e. 98%; [α]$_D^{20}$ -21.4 (c 1.20, CH$_3$OH).
4.2.50. N-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89c, toluene, methanol, triethylamine, and Novozyme®. Reaction time 6 days, (95 mg, 82%).
R, 11.38, e.e. 95%; \([\alpha]_D^{20} +40.2\) (c 1.02, CHCl₃); Lit. [145] \([\alpha]_D^{20} +35\) (c 0.4, CHCl₃); Mp 109-110 °C.

4.2.51. N-Benzoyl-L-valine propyl ester 99c

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89c, toluene, n-propanol, triethylamine, and Novozyme®. Reaction time 6 days, (91 mg, 70%).
R, 8.35, e.e. 93%; \([\alpha]_D^{20} +36.4\) (c 0.52, CHCl₃); Mp 59-60 °C.

4.2.52. N-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89d, toluene, methanol, triethylamine, Novozyme®. Reaction time 26 h, (79 mg, 69%).
R, 20.70, e.e. 80%; \([\alpha]_D^{20} +27.9\) (c 1.12, CHCl₃); Mp 79-81 °C.

4.2.53. N-Benzoyl-L-methionine propyl ester 99d

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89d, toluene, n-propanol, triethylamine, and Novozyme®. Reaction time 26 h, (80 mg, 64%).
R, 20.03, e.e. 83%; \([\alpha]_D^{20} +27.0\) (c 1.18, CHCl₃); Mp 51-53 °C.

4.2.54. N⁶-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89e, toluene, methanol, triethylamine, and Novozyme®. Reaction time 2 days, (100 mg, 90%).
R, 50.10, 90%; \([\alpha]^D_{20}\) -39.7 (c 0.70, CH$_3$OH), Lit.$^{146}$ \([\alpha]^D_{20}\) - 45 (c 0.175, CH$_3$OH); Mp 102-104 °C.

4.2.55. N°-Benzoyl-L-tryptophan propyl ester 99e

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89e, toluene, n-propanol, triethylamine, and Novozyme®. Reaction time 7 days, (58 mg, 48%).
R, 38.73, e.e. 80%; \([\alpha]^D_{20}\) -30.9 (c 0.80, CH$_3$OH); Mp 107-109 °C.

4.2.56. N-Benzoyl-L-alanine ethyl ester 98f

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89f, toluene, ethanol, triethylamine, and Novozyme®. Reaction time 6 days, (76 mg, 60%).
R, 14.07, e.e. 14%; \([\alpha]^D_{20}\) -1.7 (c 0.24, CHCl$_3$); Mp 74-76 °C.

4.2.57. N-Benzoyl-L-alanine propyl ester 99f

The general procedure outlined above (4.2.38.i) was followed using oxazolone 89f, toluene, n-propanol, triethylamine, and Novozyme®. Reaction time 6 days, (97 mg, 72%).
R, 13.90, e.e. 14%; \([\alpha]^D_{20}\) +0.4 (c 0.54, CHCl$_3$); Mp 45-46 °C.

4.2.58. N-Benzoyl-L-tert-leucine methyl ester 96g

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89g, toluene, methanol, triethylamine, and Novozyme®. After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl acetate (6:1) furnished recovered starting oxazolone, (22 mg, 22%) and desired product, (36 mg, 31%, or 40% based on recovered oxazolone. 
R, (Heptane:IPA 98:2, not baseline separation) 51.50, e.e. 35%.
4.2.59. *N*-Benzoyl-\(L\)-\(\text{tert}\)-leucine propyl ester 99g

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89g, toluene, \(n\)-propanol, triethylamine, and Novozyme\(^\circledR\). After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl acetate (8:1) furnished recovered starting oxazolone (42 mg, 42%, and desired product, (11 mg, 11%, or 15% based on recovered oxazolone). R\(_s\) (Heptane:IPA, 98:2), 43.52, e.e. 19%; Mp 57-58 °C.

4.2.60. Solvent studies: *N*-benzoyl-\(L\)-phenylalanine methyl ester 96a in the presence of triethylamine

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a in (a) dichloromethane, (b) chloroform, (c) tetrahydrofuran, (d) diethyl ether, (e) \(\text{tert}\)-butyl methyl ether (f), diisopropylether, and (g) acetonitrile, with methanol, triethylamine, and Novozyme\(^\circledR\). Reaction times are quoted after the yield. Products were purified by column chromatography, eluting with hexane:ethyl acetate (8:1). R\(_s\), 25.72; (a) (88 mg, 78%, e.e. 89%), 41 h, (b) (74 mg, 66%, e.e. 75%), 39 h, (c) (72 mg, 64%, e.e. 95%), 48 h, (d) (98 mg, 87%, e.e. 97%), 17 h (e) (102 mg, 90%, e.e. 96%) 15 h, (f) (97 mg, 86%, e.e. 96%), 15 h, (g) (49 mg, 44%, e.e. 97%), 22 h.

4.2.61. Solvent studies: *N*-benzoyl-\(L\)-phenylalanine methyl ester 96a in the absence of triethylamine

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89a in (a) dichloromethane, (b) chloroform, (c) tetrahydrofuran, (d) diethyl ether, (e) \(\text{tert}\)-butyl methyl ether (f), diisopropylether, and (g) acetonitrile, with methanol and Novozyme\(^\circledR\). Reaction times are quoted after the yield. Products were purified by column chromatography, eluting with hexane:ethyl acetate (8:1). R\(_s\), 25.72; (a) (73 mg, 65%, e.e. 75%), 124 h, (b) (71 mg, 63%, e.e. 83%), 124 h, (c) (80 mg, 71%, e.e. 97%) 123 h, (d) (102 mg, 90%, e.e. 58%), 18 h, (e) (103 mg 91%, e.e. 34%), 18 h, (f) (102 mg, 90%, e.e. 33%), 18 h, (g) (99 mg, 88%, e.e. 98%), 51 h.
4.2.62. N-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above (4.2.37.i.) was followed using oxazolone 89a, acetonitrile, triethylamine, methanol, and Lipozyme®. Reaction time 23 h, (69 mg, 61%).
R, 25.72, e.e. 73%

4.2.63. N-Benzoyl-L-phenylalanine methyl ester 96a

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89a, acetonitrile, methanol, and Lipozyme®. Reaction time 23 h, (106 mg, 94%).
R, 25.72, e.e. 73%

4.2.64. N-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89b, acetonitrile, methanol, and Novozyme®. Reaction time 19 h, (110 mg, 96%).
R, 13.28, e.e. 97%

4.2.65. N-Benzoyl-L-leucine methyl ester 96b

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89b, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (102 mg, 89%).
R, 13.28, e.e. 62%

4.2.66. N-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89c, acetonitrile, methanol, Novozyme®. Reaction time 4 days, (96 mg, 83%).
R, 11.38, e.e. 97%; $[\alpha]_D^{20}$ + 1.63 (c 1.66, CHCl₃).

4.2.67. N-Benzoyl-L-valine methyl ester 96c

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89c, acetonitrile, methanol, and Lipozyme®. After 21 days, the incomplete reaction was worked up. Purification by column chromatography, eluting with hexane:ethyl
Experimental acetate (4:1), afforded starting oxazolone, (34 mg, 34%) and desired product (52 mg, 45%, or 68%, yield based on recovered starting oxazolone).

R, 11.38, e.e. 19%

4.2.68. N-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89d, acetonitrile, with methanol, and Novozyme®. Reaction time 16 h, (90 mg, 79%).

R, 20.70, e.e. 73%

4.2.69. N-Benzoyl-L-methionine methyl ester 96d

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89d, acetonitrile, methanol, and Lipozyme®. Reaction time 2 days, (94 mg, 83%).

R, 20.70, e.e. 59%

4.2.70. N-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89e, acetonitrile, methanol, and Novozyme®. Reaction time 16 days. Column chromatography, eluting with hexane:ethyl acetate (2:1), (0 mg, 0%).

4.2.71. N-Benzoyl-L-tryptophan methyl ester 96e

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89e, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (109 mg, 98%).

R, 50.10, e.e. 46%

4.2.72. N-Benzoyl-L-alanine methyl ester 96f

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89f, acetonitrile, methanol, and Novozyme®. Reaction time 19 h, (111 mg, 94%).

R, 14.21, e.e. 10%
4.2.73. *N*-Benzoyl-L-alanine methyl ester 96f

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89f, acetonitrile, methanol, and Lipozyme®. Reaction time 4 days, (94 mg, 79%). Rf 14.21, e.e. 39%; Mp 79-81 °C.

4.2.74. *N*-Benzoyl-L-tert-leucine methyl ester 96g

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89g, acetonitrile, methanol, and Novozyme®. Reaction time 22 days. No product formation was observed by tlc, hexane:ethyl acetate (6:1), or ¹H nmr (200 MHz, CDCl₃). (0 mg, 0%).

4.2.75. *N*-Benzoyl-L-tert-leucine methyl ester 96g

The general procedure outlined above (4.2.37.ii.) was followed using oxazolone 89g, acetonitrile, methanol, and Lipozyme®. Reaction time 22 days. No product formation was observed by tlc, hexane:ethyl acetate (6:1), or ¹H nmr (200 MHz, CDCl₃). (0 mg, 0%).

4.2.76. Nitrogen based nucleophiles

4.2.77. Amine nucleophiles

4.2.78. N-Benzyl-DL-phenylalanine benzylamide 102a

\[
\text{Ph} \quad \text{N} \quad \text{Ph} \\
\text{O} \quad \text{Ph} \quad \text{N} \quad \text{Ph} \\
\text{O}
\]

To a solution of oxazolone 89a (50 mg, 0.20 mmol) in toluene (5 mL) was added triethylamine (8 µL, 0.25 equiv.) and benzylamine (44 µL, 0.4 mmol, 2.0 equiv.). The solution was stirred at room temperature and almost instantly a white precipitate formed. The solid was filtered, washed with toluene (2 x 10 mL) and dried under vacuum overnight to give the product (55 mg, 77%).

Rf (Hexane:EtOAc, 8:1) 0.31; Mp 210-211 °C Lit.¹⁴⁴ 211-212 °C; νₘₐₓ(IR Card)/cm⁻¹ 3239 (NH), 1664, 1633, 1570, 1537 (CONH); δ₂H ((CD₃)₂SO; 200 MHz) 8.64 (1 H, d, J 8.0, NHCH), 8.62 (1H, d, 5.5, NHCH₂), 7.81 (2 H, m, CH₃), 7.72-7.16 (13 H,
Experimental

CH(Ph), 4.75 (1 H, ddd, 10.0, 8.0, 5.0, NHCH), 4.32 (2 H, d, J 5.5, NHCH2), 3.13 (1 H, dd, J 13.5, 5.0, CHA Hb Ph), 3.03 (1 H, dd, J 13.5, 10.0, CHA Hb Ph); \( \delta_c \) ((CD3)2SO; 63 MHz) 171.51, 166.47 (CONH), 139.41, 138.51, 134.17 (ipso-Ar), 131.42, 129.31, 128.37, 128.29, 128.23, 127.58, 127.20, 126.85, 126.39 (CHar), 55.25 (NHCH), 42.25 (NHCH2), 37.41 (CH2Ph); m/z (FAB) 359 (75%, MH+), 252 (45, M-NHBn), 224 (100, 252-CO), 105 (224-NHCHCH2Ph).

4.2.79. N-Benzoyl-DL-phenylalanine allylamide 102b

\[
\begin{align*}
\text{Ph} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{NH} \\
\text{N} & \quad \text{H} \\
\text{Ph} & \quad \text{N} \\
\end{align*}
\]

To a solution of oxazolone 89a (100 mg, 0.40 mmol) in tetrahydrofuran (3 mL) was added allylamine (30 \( \mu \)L, 0.4 mmol, 1.0 equiv.) and the solution stirred at room temperature. The solvent was evaporated under reduced pressure and the resulting colourless solid purified by column chromatography, eluting with hexane:ethyl acetate (1:1) to furnish the product (110 mg, 90%).

R\(_f\) (Hexane:EtOAc, 1:1) 0.50; Mp 160-161°C, Lit.\(^{129}\) 164-166 °C; \( \nu_{\max} (\text{CHCl}_3)/\text{cm}^{-1} \) 3435 (NH), 3018 (CH), 1653, 1646, 1517 (CONH); \( \delta_H \) (CDCl3; 200 MHz); 7.83 (2 H, m, CHa), 7.65-7.40 (3 H, m, CHar), 7.39-7.25 (5 H, m, CHar) 5.85 (1 H, ddt J 17.0, 10.0, 5.5, CH=CH\(_2\)), 5.19 (1 H, ddt, J 17.0, 2.0, CH=CHH(trans)), 5.14 (1 H, ddt, J 10.0, 1.5, C=CHH(cis)), 4.91 (1 H, dd, J, 8.5, 6.5, NHCH), 3.87 (2 H, ddd, J 5.5, 2.0, 1.5, OCHCH), 3.30 (1 H, dd, J 13.5, 6.5, CHA Hb Ph), 3.16 (1 H, dd, J 13.5, 8.5, CHA Hb Ph); \( \delta_c \) (CDCl3; 63 MHz) 171.58, 168.17 (CONH), 136.64, 133.36 (ipso-Ar), 133.20, 120.90, 128.46, 127.57, 126.53 (CHar), 125.89 (CH=CH\(_2\)), 114.37 (CH=CH\(_2\)), 54.94 (NHCH), 40.80 and 37.16 (CH\(_2\)Ph and CH\(_2\)CH=CH\(_2\)); m/z (FAB) 309 (55%, MH+), 252 (100, M-CH\(_2\)CH=CH\(_2\)), 224 (48, 252-CO), 105 (87, 224-NHCHCH\(_2\)Ph), 77 (15, 105-CO).
Experimental

**4.2.80. Amino acid ester nucleophiles**

**4.2.81. N-benzoyl-DL-phenylalanine glycine methyl ester 104**

![Chemical Structure]

Glycine methyl ester was prepared following the procedure outlined by Frankel.\(^{147}\) Glycine methyl ester hydrochloride (2.70 g, 21.5 mmol) was suspended in ether (25 ml) and ammonia gas bubbled through the vigorously stirred mixture for a period of 1 h. Nitrogen gas was bubbled through the resulting suspension to remove any excess ammonia and the precipitated ammonium chloride was filtered. The solvent was removed by distillation at 40 °C and the crude product was distilled, collecting the fraction boiling at 62 °C, 15 mm Hg to give the desired product as a colourless oil (0.99 g, 51%). H nmr (200 MHz, CDCl\(_3\)) indicated the desired product. The product was not characterised further but used directly in the next step of the synthesis.

To a solution of oxazolone 89a (50 mg, 0.20 mmol) in toluene (3 mL) was added freshly prepared glycine methyl ester (20 mg, 0.22 mmol, 1.1 equiv.) and the solution stirred at room temperature. A colourless precipitate formed almost immediately which was filtered, washed with ether (2 x 5 mL) and dried under vacuum to give the product (54 mg, 80%).

R\(_f\) (EtOAc:Hexane, 2:1) 0.37; Mp 155-157 °C; \(\nu_{\text{max}}\)(CHCl\(_3\))/cm\(^{-1}\) 3317 (NH), 3018 (CH), 1750 (ester C=O), 1651, 1627, 1577, 1551 (CONH); \(\delta_H\) (CDCl\(_3\); 200 MHz) 7.69 (2 H, m CH\(_{\text{ar}}\)), 7.52-7.31 (3 H, m, CH\(_{\text{ar}}\)), 7.25 (5 H, s, CH\(_{\text{ar}}\)), 7.06 (1 H, d, 7.5 NHCH), 6.88 (1 H, brt, NHCH\(_2\)), 4.98 (1 H, ddd, \(J 7.5, \text{NHCH}\)), 3.99 (1 H, dd, \(J 18.5, 6.0, \text{CH}_A\text{H}_B\text{Ph}\)), 3.95 (1 H, dd, \(J 18.5, 6.0, \text{CH}_A\text{H}_B\text{Ph}\)), 3.68 (3 H, s, OCH\(_3\)), 3.19 (2 H, d, \(J 7.0, \text{NHCH}_2\)); \(\delta_C\) (CDCl\(_3\); 63 MHz) 171.26, 169.65 (CONH), 167.27 (CO\(_2\)C), 136.40, 133.53 (ipso-Ar), 131.70, 129.20, 128.51, 128.43, 126.98, 126.88 (CH\(_{\text{ar}}\)), 54.49 (CHCONH), 52.19 (OCH\(_3\)), 41.09 and 38.07 (CH\(_2\)CO\(_2\) and CH\(_2\)Ph); m/z (FAB) 341 (100%, MH\(^+\)), 252 (30, M-NHCH\(_2\)CO\(_2\)CH\(_3\)), 224 (32, 252-CO), 105 (44, 252-NHCHCH\(_2\)Ph).
4.3.0. Application of 5(4H)-oxazolone methodology: Synthesis of matrix metalloproteinase inhibitors

4.3.1. (R)-2-Hydroxy-4-methyl-hexanoic acid 119

The title compound was prepared by the method outlined by Mori.\textsuperscript{116} To a solution of D-leucine (50.0 g, 0.38 mol) in aqueous sulfuric acid (0.5M, 250 mL) at 0 °C was added a solution of aqueous sodium nitrite (41.98 g, 0.61 mol, 1.6 equiv.) in water (150 mL) over a period of 3 h. The resulting suspension was stirred at 0 °C for a further 2 h, and overnight at room temperature. The resulting clear aqueous solution was extracted with ether (3 x 200 mL), and the combined organic phase dried (Na\textsubscript{2}SO\textsubscript{4}), filtered and evaporated under reduced pressure to give a pale yellow solid. Recrystallisation three times from petroleum ether (40:60):diethyl ether (1:1) furnished the desired product as a colourless solid (25.70 g, 51%).

Mp 78-79 °C, Lit.\textsuperscript{116} 80-81 °C; [\alpha]\textsubscript{D}\textsuperscript{20} +26.2 (c 1.0 in 1 N NaOH), Lit.\textsuperscript{116} [\alpha]\textsubscript{D}\textsuperscript{20} +26.5 (c 1.52, 1 N NaOH); \upsilon_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1} 3420 (OH), 3200-2400 (acid OH), 1718 (acid C=O); \delta_H (CDCl\textsubscript{3}; 250 MHz) 6.99 (1 H, br s, OH), 4.28 (1 H, dd, J 7.5 7.0, HOCH), 1.89 (1 H, ddq, J 6.5, CH(CH\textsubscript{3})\textsubscript{2}), 1.63, (1 H, ddd, J 14.0, 7.0, 6.5, CHCH\textsubscript{3A}H\textsubscript{8}CH), 1.61 (1 H, ddd, J 14.0, 7.5, 6.5, CHCH\textsubscript{3A}H\textsubscript{8}CH) 0.95 (6 H, d, J 6.5, 4.0 7.5, CH\textsubscript{3A}CHCH\textsubscript{3B} and CH\textsubscript{3A}CHCH\textsubscript{3B}); \delta_C (CDCl\textsubscript{3}; 63 MHz) 188.2 (CO\textsubscript{2}H), 68.8 (HOCH), 43.0 (CH\textsubscript{2}CH), 24.3 (CH(CH\textsubscript{3})\textsubscript{2}), 23.1, 21.3 (CH(CH\textsubscript{3})\textsubscript{2}; m/z (FAB) 113 (91%, MH\textsuperscript{+}), 115 (42, M- OH), 87 (69, 115-CO).

4.3.2. (R)-Benzyl 2-hydroxy-4-methyl pentanoate 120

D-Leucic acid (10.00 g, 75.67 mmol) was suspended in toluene (100 mL). Benzyl alcohol (11.75 mL, 113.50 mmol) and p-toluenesulfonic acid (720 mg, 3.78 mmol, 0.05 equiv.) were added and the mixture heated under reflux using Dean-Stark conditions for 2.5 h. The reaction was quenched with saturated aqueous sodium
hydrogen carbonate (100 mL) and the aqueous layer extracted with ethyl acetate (2 x 70 mL). The combined organic extracts were washed with brine (2 x 50 mL), dried (Na₂SO₄), filtered, and evaporation under reduced pressure produced a yellow oil. Purification by column chromatography, eluting with hexane:ethyl acetate (10:1) furnished the title compound as a colourless oil (16.02 g, 95%).

R₉ (Hexane:EtOAc, 4:1) 0.50; [α]²⁰ D +16.1 (c 1.0, CHCl₃), Lit.¹¹⁷ [α]²⁰ D +14.4 (c 2.98, CHCl₃); ν_max(neat)/cm⁻¹ 3470 (OH), 2955 (saturated CH), 1738 (ester C=O); δH (CDCl₃; 250 MHz) 7.36 (5 H, s, CH₉), 5.20 (2 H, s, CH₂Ph), 4.24 (1 H, ddd, J 7.5, 7.0, 6.0, HOCH), 2.79 (1 H, d, J 6.0 OH), 1.87 (1H, ddq, J 6.5, CH(CH₃)₂), 1.60 (1 H, ddd, J 14.0, 7.5, 6.5, CHCH₃CH₃CH), 1.51 (1 H, ddd, J 14.0, 7.5, 6.5, CHCH₃CH₃CH), 0.93 (3 H, d, J 6.6 CH₃A CH₃B) 0.92 (3 H, d, J 6.6 CH₃A CH₃B); δC (CDCl₃; 63 MHz) 175.6 (CO₂CH₂), 135.1 (ipso-Ar), 128.5, 128.4, 128.2 (CH₉), 69.0 (HOCH), 67.1 (CH₂Ph), 43.2 (CH₂CH), 24.2 (CH(CH₃)₂), 23.1, 21.4 (CH(CH₃)₂); m/z (FAB) 223 (42%, MH⁺), 91 (100, CH₂Ph), Found (FAB) MH⁺ 233.1342, C₁₃H₁₀O₃ requires 233.1334.  

4.3.3. (R)-Benzyl 2-trifluoromethanesulfonyl-4-methyl pentanoate 121

The title compound was prepared according to the procedure outlined by Degerbeck.¹¹⁷ To a solution of α-hydroxy ester 120 (15.75 g, 70.86 mmol) in DCM (160 mL) and 2,6-lutidine (10.73 mL, 92.11 mmol, 1.3 equiv.) cooled to -78 °C was added trifluoromethanesulfonic anhydride (13.71 mL, 81.48 mmol, 1.1 equiv.) over a period of 30 minutes. The solution was stirred at -78 °C for a further 1 h, followed by warming to -10 °C over 40 minutes, and 40 minutes stirring at room temperature. The reaction was quenched with water (100 mL) and the aqueous layer extracted with DCM (2 x 100 mL). The combined organic fractions were dried (Na₂SO₄), filtered and evaporated under reduced pressure. The crude brown product was filtered through a plug of silica, eluting with hexane:DCM (3:1) to furnish the title compound as a colourless oil (24.62 g, 98%).
**Experimental**

$R_f$ (Hexane:DCM, 1:1) 0.76; $[\alpha]^{20}_D +42.8$ (c 1.8, CH$_2$Cl$_2$), Lit.$^{117}$ $[\alpha]^{20}_D +43.8$ (c 1.0, CH$_2$Cl$_2$); $\nu_{\text{max}}$(neat)/cm$^{-1}$ 2963 (CH), 1763 (ester C=O), 1419 (SO$_2$-O), 1208 (C-F), 1043 (S=O); $\delta_{\text{H}}$ (CDCl$_3$; 250 MHz) 7.37 (5 H, s, CH$_{\text{ar}}$), 5.25 (2 H, s, CH$_2$Ph), 5.19 (1 H, dd, J 9.0, 3.5, OCH), 1.94 (1 H, obscured ddq, CH(CH$_3$)$_2$), 1.85-1.69 (2 H, m, CHCH$_A$H$_B$CH and CHCH$_A$H$_B$CH), 0.96 (3 H, d, J 6.0, CH$_3$A,CHCH$_3$B), 0.95 (3 H, d, J 6.0, CH$_3$A,CHCH$_3$B); $\delta_{\text{C}}$ (CDCl$_3$; 63 MHz) 167.4 (CO$_2$CH$_2$); 134.2 (ipso-Ar), 128.7, 128.6, 128.4 (CH$_{\text{ar}}$); 118.3 (1 C, q, J 319, F,$^3$C), 82.2 (HOCH), 68.1 (CH$_2$Ph), 40.5 (CH$_2$CH), 23.9 (CH(CH$_3$)$_2$), 22.7, 20.9 (CH(CH$_3$)$_2$).

4.3.4. *Mono tert*-butyl malonate 124

\[ \text{tBuO}_2\text{C} = \text{CO}_2\text{H} \]

*tert*-Butyl ethyl malonate (15.10 g, 80.23 mmol) was suspended in a solution of THF:water (1:1, 350 mL) and lithium hydroxide monohydrate (3.70 g, 88.25 mmol, 1.1 equiv.) added. The suspension was stirred at room temperature for 20 minutes until a clear solution was obtained. The solution was acidified to pH= 3 with aqueous citric acid (1M, 260 mL) and extracted with ethyl acetate (3 x 250 mL). The combined organic extracts were washed with brine (2 x 150 mL), dried (Na$_2$SO$_4$), filtered, and evaporation under reduced pressure gave the desired product as a colourless oil (12.85 g, 100%).

$\nu_{\text{max}}$(neat)/cm$^{-1}$ 3204-2400 (acid OH), 1734 (ester C=O and acid C=O); $\delta_{\text{H}}$ (CDCl$_3$; 250 MHz) 10.42 (1 H, br s, OH), 3.34 (2 H, s, CH$_2$), 1.48 (9 H, s, C(CH$_3$)$_3$); $\delta_{\text{C}}$ (CDCl$_3$; 63 MHz) 171.3, 166.3 (CO$_2$H, CO$_2$C), 82.9 (C(CH$_3$)$_3$), 41.7 (CH$_2$), 27.7 (C(CH$_3$)$_3$); m/z (FAB) 161 (52%, MH$^+$), 57 (100, 'Bu), Found (FAB) 161.0815, C$_7$H$_{13}$O$_4$ requires 161.0813.

4.3.5. *tert*-Butyl benzyl malonate 125

\[ \text{tBuO}_2\text{C} = \text{CO}_2\text{Bn} \]

Mono acid 124 (12.85 g, 80.23 mmol) was suspended in THF:water (1:1, 200 mL) and potassium hydroxide (4.95 g, 88.24 mmol, 1.1 equiv.) added. Once complete dissolution had occurred the solvent was removed under reduced pressure to give a colourless solid which was dried under vacuum overnight. The solid was suspended
in DMF (300 mL); benzyl bromide (10.50 mL, 88.25 mmol, 1.1 equiv.) was added and the suspension stirred at room temperature for 7 h. The resulting mixture was quenched with saturated aqueous ammonium chloride (200 mL) and the aqueous solution extracted with ethyl acetate (4 x 200 mL). The combined organic extracts were washed with saturated aqueous ammonium chloride (2 x 200 mL), brine (2 x 150 mL), dried (Na₂SO₄), filtered, and evaporated under reduced pressure to give a yellow oil. Purification by column chromatography, eluting with hexane:diethyl ether (20:1) furnished the title compound as a colourless oil (15.06 g, 75%).

R_f (Hexane:EtOAc, 4:1) 0.65; \( \nu_{\text{max}} \) (neat)/cm\(^{-1} \) 2978 (saturated CH), 1729 (ester C=O); \( \delta_H \) (CDCl\(_3\); 250 MHz) 7.34 (5 H, s, CH₂), 5.16 (2 H, s, CH₂Ph), 3.32 (2 H, s, CH₂CO₂), 1.42 (9 H, s, C(CH₃)₃); \( \delta_C \) (CDCl₃; 63 MHz) 166.6, 165.3 (2 x CO₂C), 135.2 (ipso-Ar), 128.3, 128.2, 128.1, (CH₂), 81.8 (C(CH₃)₃), 66.8 (CH₂Ph), 42.7 (C(CH₂)C), 27.6 (C(CH₃)₃); \( m/z \) (Cl) 251 (56%, MH⁺), Found (El) 250.1202, C\(_{14}\)H\(_{18}\)O₄ requires 250.1205.

4.3.6. (2RS,3R)-4-Benzyl 1-tert-butyl 2-benzyloxycarbonyl-3-iso-butyl-succinate ester 126

Malonate 125 was dissolved in THF (150 mL), cooled to -78 °C and added to a suspension of sodium hydride (1.45 g, 60.17 mmol, 1.0 equiv.) in THF (150 mL) at -78 °C via cannulation over 15 minutes. The suspension was stirred for 40 minutes and a solution of triflate 121 (22.39 g, 63.18 mmol, 1.05 equiv.) was added over a period of 15 minutes. The reaction mixture was stirred at -78 °C for 1 h followed by warming to -10 °C and stirring for a further 1 h until a clear solution was obtained. The solution was allowed to warm to room temperature for a further 1 h and quenched with saturated aqueous ammonium chloride (100 mL). The aqueous layer was extracted with ethyl acetate (3 x 200 mL), washed with brine (2 x 100 mL), water (2 x 100 mL), dried (Na₂SO₄), filtered, and evaporated to give a colourless oil. Purification by column chromatography, eluting with hexane:DCM (1:3) produced
the title product as a colourless oil. $^1$H nmr indicated a 1:1 mix of diastereomers (24.99 g, 91%).

$R_f$ (Hexane:EtOAc, 4:1) 0.68; $[\alpha]^{20}_{D} +22.8$ (c 1.0, CHCl$_3$); $v_{max}$(neat)/cm$^{-1}$ 2959 (saturated CH), 1733 (ester C=O); $\delta_H$ (CDCl$_3$; 360 MHz) 7.34 (2.5 H, s, CH$_{ar}$), 7.33 (5 H, s, CH$_{ar}$), 7.32 (2.5 H, s, CH$_{ar}$), 5.20, 5.17, 5.15, 5.13, 5.11, 5.09, 5.05 and 4.99 (total of 2 H, 8 x d, $J$ 12.4, 12.2 and 12.0, 2 x CH$_A$H$_B$Ph and CH$_A$H$_B$Ph of each diastereomer), 3.69 (0.5 H, d, $J$ 10.2, CH$_A$CHCO$_2$), 3.66 (0.5 H, d, $J$ 10.4, CH$_B$CHCO$_2$), 3.18 and 3.17 (total of 1 H, 2 x m, CHCHCO$_2$, of each diastereomer), 1.7201.52 (total of 2 H, 2 x m, CHCH$_A$H$_B$CH and CHCH$_A$H$_B$CH of each diastereomer), 1.34, 1.33 (total of 9 H, 2 x s, C(CH$_3$)$_3$ of each diastereomer), 1.26, 1.16 (total of 1 H, 2 x m, CH(CH$_3$)$_2$ of each diastereomer), 0.90, 0.85, 0.83, 0.78 (total of 6 H, 4 x d, $J$ 6.4, CH$_{3a}$CHCH$_{3b}$ and CH$_{3a}$CHCH$_{3b}$ of each diastereomer); $\delta_C$ (CDCl$_3$; 90 MHz) 173.6, 173.4, 167.9, 167.8, 167.5, 167.3, (CO$_2$C), 135.5, 135.1 (ipso-Ar), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0 (CH$_{ar}$), 82.3, 82.2, (C(CH$_3$)$_3$), 66.9, 66.4, 66.3 (CH$_2$Ph), 55.6, 55.4 (O$_2$CCH$_2$CO$_2$), 42.7, (CHCHCH$_2$), 39.3, 39.2 (CH$_2$CH(CH$_3$)$_2$), 27.5 (C(CH$_3$)$_3$), 25.5 (CH(CH$_3$)$_2$), 23.4, 23.2, 21.1, 21.0 (CH(C$_{H_2}$)$_2$); m/z (FAB) 455 (9%, MH$^+$), 399 (78, MH$^+$-tBu), 91 (100, CH$_2$Ph), 57 (23, tBu), Found (FAB) 455.2424, C$_{27}$H$_{35}$O$_6$ requires 455.2433.

4.3.7. (2RS,3R)-4-tert-Butyl 2-carboxy-3-iso-butyl-succinic acid 116

!BuO$_2$C

\[
\begin{align*}
  &\text{HO}_2\text{C} \\
  &\text{CO}_2\text{H}
\end{align*}
\]

10% Palladium on carbon (2.50 g, 10% w) was added to a solution of triester 126 (24.99 g, 54.98 mmol) in THF (400 mL) and the reaction vessel placed under a atmosphere of hydrogen. The mixture was stirred at room temperature for 2 h until the required volume of hydrogen had adsorbed (~2.5 L). The catalyst was filtered through celite and washed with THF (2 x 100 mL). The combined organic fractions were evaporated under reduced pressure to give a colourless solid which was not further purified but carried directly to the next step.
4.3.8. (R)-2-iso-butyl-succinic acid 4-tert-butyl ester 114

\[
\text{\textsuperscript{1}BuO\textsubscript{2}C} \quad \text{CO}_2\text{H}
\]

Triethylamine (756 L, 5.43 mmol, 0.1 equiv.) was added to a suspension of crude diacid 116 (14.88 g, 54.25 mmol expected yield) in acetonitrile (250 mL) and the mixture heated under reflux for 14 h under and atmosphere of nitrogen. On cooling, the clear solution was filtered through a plug of silica, eluting with acetonitrile. The solvent was removed under reduced pressure to give the title compound as a pale yellow oil (10.76 g, 85% from triester 126).

\[
[n]_D^{20} +14.0 \text{ (c 2.20, CHCl}_3); \nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1} 3400-2500 \text{ (acid OH), 1733 (ester C=O), 1710 (acid C=O); } \delta_H (\text{CDCl}_3; 360 \text{ MHz}) 1.16 (1 \text{ H, br s, OH}), 2.83 (1 \text{ H, m, CHCO}_2\text{H}), 2.56 (1 \text{ H, dd, J 16.4, 9.2, O}_2\text{CCCH}_3\text{H}_2\text{CH}) 2.36 (1 \text{ H, dd, J 16.4, 6.4, O}_2\text{CCCH}_3\text{H}_2\text{CH}) 1.65-1.54 (2 \text{ H, 2 x m, CHCH}_3\text{H}_2\text{CH and CHCH}_3\text{H}_2\text{CH}), 1.41 (9 \text{ H, s, C(CH}_3)_3, 1.26 (1 \text{ H, m, CH(CH}_3)_2, 0.92 (3 \text{ H, d, J 6.4, CH}_3\text{CHCH}_3\text{H}), 0.88 (3 \text{ H, d, J 6.4, CH}_3\text{CHCH}_3\text{H}); \delta_C (\text{CDCl}_3; 63 \text{ MHz}) 181.7, 170.9 (\text{CO}_2\text{H, CO}_2\text{C}), 80.8 (\text{C(CH}_3)_3), 40.7 (\text{O}_2\text{CCCH}_3), 39.5 (\text{CHCO}_2\text{H}), 37.5 (\text{CHCH}_2\text{CH}), 27.8 (\text{C(CH}_3)_3), 25.6 (\text{CH(CH}_3)_2), 22.4, 22.1 (\text{CH(CH}_3)_2); m/z (FAB) 231 (82%, MH\textsuperscript{+}), 175 (100, MH\textsuperscript{+}-\textsuperscript{t}Bu), 57 (71, \textsuperscript{t}Bu), Found (FAB) 231.1592, C\textsubscript{12}H\textsubscript{23}O\textsubscript{4} requires 231.1596.

4.3.9. General procedure for the coupling of \(\alpha\)-amino acid esters to (R)-2-iso-butyl-succinic acid 4-tert-butyl ester 114

Method (i)

EDCI (1.2 equiv.), HOBt (1.2 equiv.) and triethylamine (2.4 equiv.) were added to a solution of succinate 114 (1.0 equiv.) in DCM (40 mL). Once complete dissolution occurred \(\alpha\)-amino acid ester hydrochloride (1.2 equiv.) was added and the solution stirred at room temperature for 6 h. The reaction mixture was diluted with DCM (50 mL), washed with saturated aqueous ammonium chloride (2 x 50 mL), water (50 mL), saturated aqueous sodium hydrogen carbonate (2 x 50 mL), water (50 mL), dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and evaporated under reduced pressure to give the crude product. Purification by column chromatography furnished the desired product.
Experimental

Method (ii)

TBTU (1.2 equiv.), HOBt (1.2 equiv.) and DIPEA (3.0 equiv.) were added to a solution of succinate 114 (1.0 equiv.) in DMF (40 mL). α-Amino acid ester hydrochloride (1.2 equiv.) was added and the resulting solution stirred at room temperature under an atmosphere of nitrogen for 1.5 h. After quenching with saturated aqueous ammonium chloride (50 mL), the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with saturated aqueous ammonium chloride (50 mL), saturated aqueous sodium hydrogen carbonate (2 x 50 mL), brine (50 mL), dried (Na₂SO₄), filtered, and evaporated under reduced pressure to give the crude product. Purification by column chromatography furnished the desired product.

4.3.10. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.9.i.) was followed using EDCI (68 mg, 0.36 mmol, 1.2 equiv.), HOBt (48 mg, 0.36 mmol, 1.2 equiv.), triethylamine (99 μL, 0.72 mmol, 2.4 equiv.), succinate 114 (70 mg, 0.30 mmol, 1.0 equiv.) and L-phenylalanine methyl ester hydrochloride (78 mg, 0.36 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (98 mg, 83%). ¹H nmr indicated a d.e. of 95%. The spectroscopic data given is for the diastereomer whose absolute stereochemistry was determined from the crystal structure (see appendix 1).

Rₜ (Hexane:EtOAc, 6:1) 0.27; d.e. 95%; Mp 97-99 °C; (Found: C, 67.58; H, 8.50; N, 3.75; C₂₂H₃₃NO₅ requires C, 67.49; H, 8.49; N 3.58); [α]°D +62.3 (c 0.82, CHCl₃); νₚₚₚ.max(CHCl₃)/cm⁻¹ 3430 (amide NH), 3018 (saturated CH), 1726 (ester CO), 1673, 1510 (CONH); δH (CDCl₃; 600 MHz) 7.27 (2 H, tt, J 7.3, CH₃-meta), 7.22 (1 H, tt, J 7.3, CH₃-ortho), 7.16 (2 H, dt, J 7.3, CH₃-ortho), 6.81 (1 H, d, J 7.9 NH), 4.85 (1 H, ddd, J 7.9, 6.0, 5.9, NHCH), 3.67 (3 H, s, OCH₃), 3.09 (1 H, dd, J 14.3, 5.9, CH₃-H₅-Ph)
3.08 (1 H, dd, J 14.3, 6.0, CH$_2$A-H$_B$Ph), 2.61 (1 H, m, CHCONH), 2.52 (1 H, dd, J 16.6, 8.9, O$_2$CCH$_A$H$_B$CHCO), 2.27 (1 H, dd, 16.6, 4.9, O$_2$CCH$_A$H$_B$CHCO), 1.55 (1 H, ddd, J 13.6, 8.9, 5.9, CHCH$_A$H$_B$CH), 1.53 (1 H, m, CH(CH$_3$)$_2$), 1.42 (9 H, s, C(CH$_3$)$_3$), 1.15 (1 H, ddd, J 13.6, 7.6, 5.6, CH$_{A4}$H$_B$CH(CH$_3$)$_2$), 0.85 (3 H, d, J 6.4, CH$_{3A}$CHCH$_{3B}$), 0.84 (3 H, d, J 6.4, CH$_{3A}$CHCH$_{3B}$); δ$_C$ (CDCl$_3$; 63 MHz) 174.3, 171.8, 171.5 (CONH, CO$_2$R); 135.8, (ipso-Ar); 129.2 128.4, 126.9 (CH$_n$); 80 6 (C(CH$_3$)$_3$); 53.0 (NHCH); 52.0 (CO$_2$CH$_3$); 41.2 (O$_2$CCH$_2$); 40.7 (CHCONH); 38.0, 37.9 (CH$_2$Ph and CH$_2$CH); 27.9 (C(CH$_3$)$_3$), 25.4 (CH(CH$_3$)$_2$); 22.8, 22.0 (CH(CH$_3$)$_2$); m/z (FAB) 392 (34%, MH$^+$), 91 (100, CH$_2$Ph), 57 (35, 'Bu), Found (FAB) 392.2419, C$_{22}$H$_{34}$NO$_5$ requires 392.2437.

4.3.11. (2R,2'S)-4-(tert-butyl)-2-iso-butyl-succinyl-valine methyl ester 127b

The procedure outlined in Section (4.3.9.i.) was followed using EDCI (70 mg, 0.36 mmol, 1.2 equiv.), HOBt (49 mg, 0.36 mmol, 1.2 equiv.), triethylamine (102 µL, 0.73 mmol, 2.4 equiv.), succinate 114 (70 mg, 0.30 mmol, 1.0 equiv.) and L-valine methyl ester hydrochloride (61 mg, 0.36 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (74 mg, 71%).

R$_f$ (Hexane:EtOAc, 6:1) 0.20; d.e. 86%; Mp 75-76 °C; (Found: C, 63.05; H, 9.60; N, 3.98; C$_{18}$H$_{34}$NO$_5$ requires C, 62.95; H, 9.68; N 4.08); [α]$_D^{20}$ +12.7 (c 1.26, CHCl$_3$); ν$_{max}$(CHCl$_3$)/cm$^{-1}$ 3428 (amide NH), 3018 (saturated CH), 1734 (ester C=O), 1675, 1512 (CONH); δ$_H$ (CDCl$_3$; 600 MHz) 6.21 (1 H, d, J 8.9, NH), 4.53 (1 H, dd, J 8.9, 4.9, NHCH), 2.68 (1 H, m, CHCONH), 2.58 (1 H, dd, J 17.0, 9.6, O$_2$CCH$_2$CH), 2.29 (1 H, dd, J 17.0 4.0, O$_2$CCH$_2$CH), 2.14 (1 H, dqq, J 6.9, 4.9, CH$_2$CH(CH$_3$)$_2$), 1.60 (1 H, ddd, J 14.6, 9.0, 5.6, CH$_2$CH$_2$CH), 1.55 (1 H, m, CH$_2$CH(CH$_3$)$_2$), 1.40 (9 H, s, C(CH$_3$)$_3$), 1.15 (1 H, ddd, J 14.6, 8.0, 5.6, CHCH$_A$H$_B$CH), 0.90 (6 H, d, J 6.8, CH(CH$_3$)$_2$CHCH$_3$) and CH(CH$_3$)$_2$CHCH$_3$), 0.89, (3 H, d, J 6.8, CH$_2$(CH$_3$)$_2$CHCH$_3$), 0.86 (3 H, d, J 6.8, CH$_2$(CH$_3$)$_2$CHCH$_3$); δ$_C$ (CDCl$_3$; 63 MHz)
174.56, 172.3, 171.7 (CONH, CO₂C), 80.6 (C(CH₃)₂), 56.7 (NHCH), 51.9 (OCH₃),
41.2 (OCCH₂CH), 40.7 (CHCONH), 38.3 (CH₂CH(CH₃)₂), 31.2 (CHCH(CH₃)₂), 27.9
(C(CH₃)₂), 25.5 (CH₂CH(CH₃)₂), 22.8, 22.0 (CH₂CH(CH₃)₂), 18.8, 17.5
(CHCH(CH₃)₂); m/z (FAB), 344 (70%, MH⁺), 288 (100, MH₂⁻Bu), 270 (11, 288-
H₂O), 132 (43), 72 (57) Found (FAB), 358.2595 C₁₉H₃₆N₀₅ requires 3518.2594.

4.3.12. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (117 mg,
0.36 mmol, 1.2 equiv.), HOBT (49 mg, 0.36 mmol, 1.2 equiv.),
diisopropylethylamine (159 µL, 0.91 mmol, 3.0 equiv.), succinate 114 (70 mg, 0.30
mmol, 1.0 equiv.) and L-leucine methyl ester hydrochloride (66 mg, 0.36 mmol, 1.2
equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished
the title compound as a colourless solid (80 mg, 74%).

Rᵣ (Hexane:EtOAc, 6:1) 0.55; d.e. 90%; Mp 106-108 °C; (Found: C, 63.25; H, 9.74;
N, 3.79; C₁₉H₃₄N₀₅ requires C, 63.48; H, 9.87; N 3.94); [α]₂₀⁰ +3.2 (c 1.44, CHCl₃);
νₓₒₓ (CHCl₃)/cm⁻¹ 3431 (amide NH), 3018 (saturated CH), 1736 (ester C=O), 1673,
1513 (CONH); δₓ (CDCl₃; 600 MHz) 6.08 (1 H, d, J 8.3 NH), 4.9 (1 H, ddd, J 13.6, 8.3,
5.0, NHCH), 3.69 (3 H, s, OCH₃), 2.64 (1 H, m, OCCH₂CH), 2.57 (1 H, dd, J 16.9,
9.6, OCCH₃H₅CH), 2.28 (1 H, ddd, J 16.9, 4.0, OCCH₃H₅CH), 1.68-1.49 (total
of 5 H, m, CH₂CHCH₃H₅CH, CH₂CHCH₂CH, NHCHCH₃H₅CH, NHCHCH₃H₅CH,
and NHCHCH₂CH), 1.40 (9 H, s, C(CH₃)₃), 1.13 (1 H, ddd, J 13.3, 7.9, 5.6
OCCH₂CHCH₃H₅CH), 0.90 (3 H, 2 d, J 6.3, CH₃CHCH₃H₅(Leucine)), 0.89 (3 H, 2 d, J 6.6,
CH₃ACHCH₃H₅(Leucine)), 0.88 (3 H, d, J 6.3, CH₃ACHCH₃H₅(Leucine)), 0.86 (3 H, d, J 6.6,
CH₃ACHCH₃H₅(Leucinate)); δᵧ (CDCl₃; 63 MHz) 174.6, 173.4, 171.7 (CONH, CO₂C), 80.6
(C(CH₃)₂), 52.1 (OCH₃), 50.4 (NHCH), 41.6, 41.3 (OCCH₂CH and NHCHCH₃H₅CH), 40.6
(CHCONH), 38.3 (OCCH₂CHCH₃H₅), 28.0 (C(CH₃)₃), 25.5, 24.5 (CH(CH₃)₂), 23.0,
22.8 (CH(CH₃)₂(Leucinate)), 21.8, 21.7 (CH(CH₃)₂(Leucinate)); m/z (FAB) 357 (24%, MH⁺),
Experimental

302 (100, MH$_2$-t-Bu), 284 (7, 302-H$_2$O), 146 (20), 86 (38), 57 (54, t-Bu), Found (FAB) 344.2439, C$_{18}$H$_{36}$NO$_5$ requires 344.2437.

4.3.13. (2R,2'S)-[4-(t-tert-butyl)-2-iso-butyl-succinyl]-tryptophan methyl ester 127d

\[
\begin{align*}
\text{H} & \quad \text{CO}_2\text{CH}_3 \\
\text{N} & \quad \text{CO}_2\text{CH}_3 \\
\text{CH} & \quad \text{CH} \\
\end{align*}
\]

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (119 mg, 0.37 mmol, 1.2 equiv.), HOBt (50 mg, 0.37 mmol, 1.2 equiv.), diisopropylethylamine (161 uL, 0.92 mmol, 3.0 equiv.), succinate 114 (71 mg, 0.31 mmol, 1.0 equiv.) and L-tryptophan methyl ester hydrochloride (94 mg, 0.37 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:diethyl ether (1:1) furnished the title compound as a colourless glass (109 mg, 82%).

R$_f$ (Hexane:Et$_2$O, 1:1) 0.20; d.e. 90%; Mp 96-97 °C; (Found: C, 66.45; H, 7.93; N, 6.33; C$_{24}$H$_{34}$N$_2$O$_5$ requires C, 66.95; H, 7.96; N 6.51); [\(\alpha\)]$_D^{20}$ +64.9 (c 1.96, CHCl$_3$);

\(\nu_{\text{max}}\) (CHCl$_3$/cm$^{-1}$ 3480 (indole NH), 3428 (amide NH), 3020 (saturated CH), 1726 (ester C=O), 1668, 1513 (CONH); \(\delta_H\) (CDCl$_3$; 600 MHz) 8.29 (1 H, brs, NH$_{\text{indole}}$), 7.56 (1 H, d, \(J=7.6\), CH$_{\text{ar}}$), 7.32 (1 H, d, \(J=8.0\), CH$_{\text{ar}}$), 7.15 (1 H, m, CH$_{\text{ar}}$), 7.13 (1 H, d, \(J=2.4\), CH$_{\text{NH}}$), 7.09 (total of 1 H, m, CH$_{\text{ar}}$), 6.27 (1 H, d, \(J=7.8\), NH$_H$), 4.92 (1 H, ddd, \(J=7.8\), 5.6, 5.4, NHCH), 3.64 (3 H, s, OCH$_3$), 3.34 (1 H, dd, \(J=14.8\), 5.4, CH$_{\text{CH}_A\text{H}_B\text{Indole}}$), 3.24 (1 H, dd, \(J=14.8\), 5.6, CH$_{\text{CH}_A\text{H}_B\text{Indole}}$), 2.61 (1 H, m, CHCONH), 2.58 (1 H, dd, \(J=15.8\), 9.0, OCCH$_A$H$_B$CH), 2.29 (1 H, dd, \(J=15.8\), 4.0, OCCH$_A$H$_B$CH), 1.58-1.50 (2 H, m, CH$_{\text{CH}_3}$; and CH$_2$CHCH$_A$H$_B$CH), 1.40 (9 H, s, C(CH$_3$)$_3$), 1.17 (1 H, ddd, \(J=15.4\), 9.8, 5.4 CH$_2$CHCH$_A$H$_B$CH), 0.84 (3 H, d, \(J=6.4\), CH$_{\text{3A}CH\text{CH}_3}\)), 0.82 (3 H, d, \(J=6.4\), CH$_{\text{3A}CH\text{CH}_3}\)); \(\delta_C\) (CDCl$_3$; 63 MHz) 174.4, 172.2, 171.6 (CONH, CO$_2$C), 136.0, 127.4 (ipso-Ar), 123.1, 121.9, 119.4, 118.4, 111.1 (CH$_{\text{ar}}$), 109.6 (ipso-Ar), 80.5 (C(CH$_3$)$_3$), 52.5 (NHCH), 52.1 (OCH$_3$), 41.3 (OCCH$_2$CH), 40.7 (CHCONH), 37.9 (CH$_2$CH$_2$CH), 27.9 (C(CH$_3$)$_3$), 27.6
Experimental

(CH₂Indole), 25.4 (CH(CH₃)₂), 22.7, 22.0 (CH(CH₃)₂); m/z (FAB) 431 (1%, MH⁺), 375 (12, MH⁺-tBu), 202 (24), 130 (100, CH₂Indole), 57 (41, tBu), Found (FAB) 431.2550, C₂₄H₃₅N₂O₅ requires 431.2546.

4.3.14. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tert-leucine methyl ester

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (119 mg, 0.37 mmol, 1.2 equiv.), HOBt (50 mg, 0.37 mmol, 1.2 equiv.), diisopropylethylamine (161 µL, 0.92 mmol, 3.0 equiv.), succinate 114 (71 mg, 0.32 mmol, 1.0 equiv.) and L-tert-leucine methyl ester hydrochloride (67 mg, 0.37 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (84 mg, 77%).

Rf (Hexane:EtOAc, 6:1) 0.38; d.e. 86%; Mp 104-106 °C; (Found: C, 63.82; H, 9.67; N, 3.91; C₁₉H₃₅NO₅ requires C, 63.84; H, 9.87; N 3.92); [α]₂⁰D +5.3 (c 1.48, CHCl₃);
νmax (CHCl₃)/cm⁻¹ 3433 (amide NH), 3018 (saturated CH), 1729 (ester C=O), 1675, 1512 (CONH); δh (CDCl₃; 600 MHz) 6.29 (1 H, d, J 9.3, NH), 4.42 (1 H, d, J 9.3, NHCH), 3.69 (3 H, s, OCH₃), 2.67 (1 H, m, CHCONH), 2.58 (1 H, dd, J 17.0, 9.6, OCCH₃H₆CH), 2.29 (1 H, dd, J 17.0, 4.0, OCCH₃H₈CH), 1.59 (1 H, ddd, J 13.6, 8.9, 5.9, CHCH₃H₈CH), 1.51 (1 H, m, CH(CH₃)₂), 1.14 (1 H, ddd, J 13.6, 7.9, 5.9, CHCH₃H₈CH), 0.88 (3 H, d, J 6.6, CH₃₃CHCH₃β), 0.84 (3 H, d, J 6.6, CH₃₃CHCH₃β); δc (CDCl₃; 63 MHz) 174.3, 171.9, 171.7 (CONH, CO₂C), 80.6 (OC(CH₃)₂), 59.7 (NHCH), 51.6 (OCH₃), 41.1 (OCCH₂CH) 40.7 (CHCONH), 38.1 (CHCH₂CH), 34.7 (CHCH(CH₃)₂), 27.9 (OC(CH₃)₂), 26.4 (CHC(CH₃)₂), 25.5 (CH(CH₃)₂), 22.8, 22.1 (CH(CH₃)₂); m/z (FAB) 358 (24%, MH⁺), 302 (100, MH₂⁺-tBu), 242 (5), 146 (12), 86 (44), 57 (Bu), Found (FAB) 358.2609, CHNO₅ requires 358.2593.
4.3.15. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester and (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.9.i.) was followed using EDCI (1.05 g, 5.47 mmol, 1.2 equiv.), HOBt (739 mg, 5.47 mmol, 1.2 equiv.), triethylamine (1.53 mL, 10.94 mmol, 2.4 equiv.), succinate 114 (1.05 g, 4.56 mmol, 1.0 equiv.) and DL-phenylalanine methyl ester hydrochloride (1.18 g, 5.47 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless solid (1.42 g, 80%). 'H nmr indicated a (1:1) ratio of diastereomers.

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27, 0.23; Mp 69-72 °C (Found: C, 67.52; H, 8.49; N, 3.49; C<sub>22</sub>H<sub>33</sub>N<sub>3</sub>O<sub>5</sub> requires C, 67.49; H, 8.49; N 3.58); [α]<sub>D</sub><sup>20</sup> +7.9 (c 1.34, CHCl<sub>3</sub>); ν<sub>max</sub>(CHCl<sub>3</sub>) cm<sup>-1</sup> 3429 (amide NH), 3019 (saturated CH), 1737, 1732 (ester C=O), 1668, 1510 (CONH); δ<sub>H</sub> (CDCl<sub>3</sub>; 600 MHz) 7.25 (1 H, tt, <em>J</em> 7.3, CH<sub>ar</sub> (meta)), 7.23 (1 H, tt, <em>J</em> 7.3, CH<sub>ar</sub> (meta)), 7.21-7.17 (total of 1 H, m, CH<sub>ar</sub>(para) of each diastereomer), 7.13 (1 H, brt, <em>J</em> 7.3, CH<sub>ar</sub> (ortho)), 7.08 (1 H, brt, <em>J</em> 7.3, CH<sub>ar</sub> (ortho)), 6.18 (total of 1 H, br d, NH of each diastereomer), 4.84 (total of 1 H, 2 x m, NHCH<sub>2</sub> of each diastereomer), 3.66 (1.5 H, s, OCH<sub>3</sub>), 3.64 (1.5 H, s, OCH<sub>3</sub>B), 3.11 (0.5 H, dd, <em>J</em> 14.0, 5.9, CH<sub>A</sub>H<sub>8</sub>Ph) 3.06 (0.5 H, dd, <em>J</em> 14.3, 6.0, CH<sub>A</sub>H<sub>6</sub>Ph) 3.05 (0.5 H, dd, <em>J</em> 14.3, 5.9 CH<sub>A</sub>H<sub>8</sub>Ph), 3.00 (0.5 H, dd, <em>J</em> 14.0, 6.9, CH<sub>B</sub>H<sub>8</sub>Ph), 2.57 (total of 2 H, m, CHCONH of each diastereomer), 2.50 (0.5 H, dd, <em>J</em> 16.3, 9.0, OCC<sub>A</sub>H<sub>8</sub>CH), 2.49 (0.5 H, dd, <em>J</em> 16.6, 8.9, OCC<sub>A</sub>H<sub>6</sub>CH), 2.23 (0.5 H, dd, <em>J</em> 16.6, 4.9, OCC<sub>A</sub>H<sub>6</sub>CH) 2.22 (0.5 H, dd, <em>J</em> 16.3, 5.0, OCC<sub>A</sub>H<sub>8</sub>CH), 1.55-1.47 (total of 1.5 H, m, CHCH<sub>A</sub>H<sub>8</sub>CH, CH<sub>A</sub>(CH<sub>3</sub>)<sub>2</sub> and CHCH<sub>B</sub>H<sub>8</sub>CH) 1.39 (4.5 H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.36 (4.5 H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.32 (0.5 H, m, CH<sub>B</sub>(CH<sub>3</sub>)<sub>2</sub>), 1.13 (0.5 H, ddd, <em>J</em> 13.3, 7.6, 5.6,
CHCH$_2$CH$_3$, 1.06 (0.5 H, ddd, $J$ 13.6, 8.6, 5.3, CHCH$_2$CH$_3$), 0.83 (1.5 H, d, $J$ 6.6, CH$_3$CHCH$_3$, 0.81 (1.5 H, d, $J$ 6.6, CH$_3$CHCH$_3$), 0.79 (1.5 H, d $J$ 6.6 CH$_3$CHCH$_3$), 0.79 (1.5 H, d $J$ 6.6 CH$_3$CHCH$_3$); $\delta_c$ (CDCl$_3$; 63 MHz) 174.3, 174.2, 171.8, 171.5, 171.3 (CONH, CO$_2$R), 135.8 (ipso-Ar), 129.1, 129.0, 128.3, 126.9, (CH$_3$), 80.6, (C(H$_3$)$_2$), 52.9, 52.8 (NHCH), 52.0 (CO$_2$CH$_3$), 41.2, 41.0 (O$_2$CCCH$_2$), 40.6, 40.5 (CHCONH), 38.2, 37.9, 37.8 (CH$_2$CH(CH$_3$)$_2$ and CH$_2$Ph), 27.9, 27.8 (C(H$_3$)$_2$), 25.4 (CH(CH$_3$)$_2$), 22.9, 22.7, 22.0, 21.9 (CH(CH$_3$)$_2$); m/z (FAB) 392 (62%, MH$^+$), 336 (100, MH$^+$.t Bu), 258 (13), 180 (52), 120 (30), 91 (18, CH$_2$Ph), 57 (25, t Bu), Found (FAB), 392.2412, C$_{22}$H$_{34}$N$_2$O$_5$ requires 392.2437.

4.3.16. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine methyl ester and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine methyl ester 127b

The procedure outlined in Section (4.3.9.i.) was followed using EDCI (1.04 g, 5.42 mmol, 1.2 equiv.), HOBt (732 mg, 5.42 mmol, 1.2 equiv.), triethylamine (1.51 mL, 10.84 mmol, 2.4 equiv.), succinate 114 (1.04 g, 4.52 mmol, 1.0 equiv.) and DL-valine methyl ester hydrochloride (1.18 g, 5.47 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless wax (1.20 g, 77%). $^1$H nmr indicated a (1:1.5) ratio of diastereomers.

$R_f$ (Hexane:EtOAc, 6:1) 0.20, 0.18; (Found: C, 62.97; H, 9.98; N, 4.01; C$_{18}$H$_{33}$NO$_5$ requires C, 62.95; H, 9.68; N 4.08); [$\alpha$]$^D_{20}$ + 4.74 (c 1.16, CHCl$_3$); $\nu_{max}$(CHCl$_3$)/cm$^{-1}$ 3428 (amide NH), 1730, 1738 (ester C=O), 1671, 1507 (CONH); $\delta_h$ (CDCl$_3$; 600 MHz) 6.30 (0.6 H, d, $J$ 8.3, NH$^+$), 6.22 (0.4 H, d, $J$ 8.9, NH$^+$), 4.53 (0.4 H, dd, $J$ 8.9, 4.9, NHCH$^+$), 4.48 (0.6 H, dd, $J$ 8.3, 4.9 NHCH$^+$, 3.70 (1.8 H, s, OCH$^+$), 3.68 (1.2 H, s, OCH$_3^+$), 2.86 (total of 1 H, m, CHCONH of each diastereomer), 2.57 (0.4 H, dd, $J$ 17.0, 9.6, OCCH$_A$H$_B$CH), 2.54 (0.6 H, dd, $J$ 16.9, 8.6, OCCH$_A$H$_B$CH), 2.29
Experimental

(0.4 H, dd, J 17.0, 4.0, OCCH\textsubscript{A}H\textsubscript{B}CH), 2.28 (0.6 H, dd, J 16.9, 5.0, OCCH\textsubscript{A}H\textsubscript{B}CH), 2.12 (total of 1 H, m, CHCH(CH\textsubscript{3})\textsubscript{2} of each diastereomer), 1.64 (0.6 H, ddd, J 15.0, 9.6, 5.6, CHCH\textsubscript{A}H\textsubscript{B}CH), 1.60 (0.4 H, ddd, J 14.6, 9.0, 5.6, CHCH\textsubscript{A}H\textsubscript{B}CH), 1.54 (total of 1 H, m, CHCH\textsubscript{A}H\textsubscript{B}CH of each diastereomer), 1.40 (3.6 H, s, C(CH\textsubscript{3})\textsubscript{3}), 1.39 (2.4 H, C(CH\textsubscript{3})\textsubscript{3}), 0.89 (0.6 H, ddd, J 15.0, 9.6, 5.6 CHCH\textsubscript{A}H\textsubscript{B}CH), 1.13 (0.4 H, ddd, J 14.6, 8.0, 5.6 CHCH\textsubscript{A}H\textsubscript{B}CH), 0.90 (1.8 H, d, J 6.6, CH\textsubscript{2}(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B}), 0.89 (3 H, d, J 6.9, CH(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B} of each diastereomer), 0.88 (3 H, d, J 6.9, CH(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B} of each diastereomer), 0.88 (1.2 H, d, J 6.6, CH\textsubscript{2}(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B}), 0.87 (1.8 H, d, J 6.6 CH\textsubscript{2}(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B}), 0.85 (1.2 H, d, J 6.6, CH\textsubscript{2}(CH\textsubscript{3})\textsubscript{3}CHCH\textsubscript{3B}), \delta\textsubscript{C} (CDCl\textsubscript{3}; 63 MHz) 174.7, 174.6, 172.3, 172.1, 171.7, 171.6 (CONH, CO\textsubscript{2}R), 80.7, 80.6 (C(CH\textsubscript{3})\textsubscript{3}), 56.9, 56.7 (NHCH), 51.9 (CO\textsubscript{2}CH\textsubscript{3}), 41.2, 41.0 (O\textsubscript{2}CCH\textsubscript{2}), 40.7, 40.6 (CHCONH), 38.4, 38.2 (CHCH\textsubscript{2}CH), 31.1, 30.9 (CHCH(CH\textsubscript{3})\textsubscript{2}), 27.9 (C(CH\textsubscript{3})\textsubscript{3}), 25.6, 25.5 (CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 23.0, 22.8, 22.0, 21.8 (CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 18.8, 18.8, 17.7, 17.5 (CHCH(CH\textsubscript{3})\textsubscript{2}); m/z (FAB) 344 (49%, MH\textsuperscript{+}), 288 (100, MH\textsuperscript{+} t-Bu), 210 (17), 157 (12), 132 (59), 72 (100), 57 (55, t-Bu), Found (FAB), 344.2432, C\textsubscript{18}H\textsubscript{34}N\textsubscript{2}O\textsubscript{5} requires 344.2437.

4.3.17. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine methyl ester and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (1.74 g, 5.42 mmol, 1.2 equiv.), HOBT (732 mg, 5.42 mmol, 1.2 equiv.), diisopropylethylamine (2.36 mL, 13.55 mmol, 3.0 equiv.), succinate 114 (1.04 g, 4.52 mmol, 1.0 equiv.) and DL-leucine methyl ester hydrochloride (984 mg, 5.42 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1)
furnished the title compound as a colourless solid (1.25 g, 77%). $^1$H nmr indicated a (1:1) mixture of diastereomers.

R$_f$ (Hexane:EtOAc, 6:1) 0.55, 0.46; Mp 63-66°C, (Found: C, 64.15; H, 10.02; N, 3.68; C$_{19}$H$_{35}$NO$_5$ requires C, 63.84; H, 9.87; N 3.94); $[\alpha]^2_{D}$ + 6.08 (c 1.20, CHCl$_3$); $\nu_{\text{max}}$(CHCl$_3$)/cm$^{-1}$; 3434 (amide NH), 1740, 1734 (ester C=O), 1675, 1672, 1517, 1512 (CONH); $\delta_H$ (CDCl$_3$; 600 MHz) 6.21 (0.5 H, d, J 8.0, NH$^A$), 6.10 (0.5 H, d, J 8.3 NH$^B$), 4.60 (0.5 H, ddd, J 13.6, 8.3, 5.0, NHCH$^B$), 4.54 (0.5 H, ddd, J 13.6, 8.0, 5.0, NHCH$^A$), 3.69 (1.5 H, s, OCH$_3^B$), 3.67 (1.5 H, s, OCH$_3^A$), 2.65 (total of 1 H, m, CHCONH of each diastereomer), 2.56 (0.5 H, dd, J 16.9, 9.6, OCCH$_3^B$H$_n$CH), 2.53 (0.5 H, dd, J 16.9, 8.6, OCCH$_3^A$H$_n$CH) 2.28 (0.5 H, dd, J 16.9, 5.3, OCCH$_3^A$H$_n$CH), 2.27 (0.5 H, dd, J 16.9, 4.0, OCCH$_3^A$H$_n$CH), 1.66-1.48 (total of 5 H, m, CH$_2$CHCH$_3$H$_n$CH, CH$_2$CHCH$_2$CH, NHCHCH$_3$H$_n$CH, NHCHCH$_2$H$_n$CH, and NHCHCH$_2$CH$_n$ of each diastereomer), 1.40 (4.5 H, s, C(CH$_3$)$_3$), 1.39 (4.5 H, s, C(CH$_3$)$_3$), 1.14 (0.5 H, ddd, J 13.6, 8.3, 5.6, CH$_2$CHCH$_3$H$_n$CH), 1.13 (0.5 H, ddd, J 13.6, 8.3, 5.6, CH$_2$CHCH$_3$H$_n$CH), 0.91 (1.5 H, d, J 6.3, CH$_3$A CHCH$_3$B(leucine)), 0.90 (1.5 H, d, J 6.3, CH$_3$A CHCH$_3$B(leucine)), 0.89 (1.5 H, d, J 6.3, CH$_3$A CHCH$_3$B(leucine)), 0.88 (1.5 H, d, J 6.6, CH$_3$A CHCH$_3$B(succinate)), 0.87 (1.5 H, d, J 6.3, CH$_3$A CHCH$_3$B(succinate)), 0.87 (1.5 H, d, J 6.6, CH$_3$A CHCH$_3$B(succinate)), 0.86 (1.5 H, d, J 6.6, CH$_3$A CHCH$_3$B(succinate)), 0.85 (1.5 H, d, J 6.6, CH$_3$A CHCH$_3$B(succinate)); $\delta_C$ (CDCl$_3$; 63 MHz) 174.6, 174.5 (CONH), 173.3, 173.2, 171.7, 171.6 (CO$_2$R), 80.7, 80.6 (C(CH$_3$)$_3$), 52.0 (CO$_2$CH$_3$), 50.6, 50.3 (NHCH), 41.5, 41.3, 41.2, 40.9, (O$_2$CCCH$_2$ and CH$_2$CH(CH$_3$)$_2$(leucine)), 40.5, 40.4 (CHCONH), 38.3, 38.2 (CH$_2$CH(CH$_3$)$_2$(succinate)), 27.9 (C(CH$_3$)$_3$), 25.6, 25.4, 24.7, 24.4 (2 x CH(CH$_3$)$_2$), 22.9, 22.8, 22.7, 22.6, 22.0, 21.9, 21.7, 21.6 (2 x CH(CH$_3$)$_2$); m/z (FAB) 358 (55%, MH$^+$), 302 (100, MH$_2$-"Bu), 284 (26, 284-H$_2$O), 154 (26), 146 (44), 86 (52), 57 (38, "Bu), Found (FAB), 358.2588, C$_{19}$H$_{36}$NO$_5$ requires 358.2593.
4.3.18. (2R,2'R)-(4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan methyl ester and
(2R,2'S)-(4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan methyl ester 127d

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (1.82 g, 5.68 mmol, 1.2 equiv.), HOBt (767 mg, 5.68 mmol, 1.2 equiv.), diisopropylethylamine (2.47 mL, 14.20 mmol, 3.0 equiv.), succinate 114 (1.09 g, 4.73 mmol, 1.0 equiv.) and DL-tryptophan methyl ester hydrochloride (1.45 g, 5.68 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (2:1) furnished the title compound as a colourless solid (1.55 g, 78%). 1H nmr indicated a (1:1) ratio of diastereomers.

\( \text{R} \text{f} \) (Hexane:EtOAc, 1:1) 0.76, 0.76; mp 51-53 °C, (Found: C, 66.93; H, 8.09; N, 6.48; C\(_{24}\)H\(_{34}\)N\(_2\)O\(_5\) requires C, 66.95; H, 7.95; N 6.51); \([\alpha]\)\(_D\)^{20} + 21.47 (c 1.36, CHCl\(_3\)); \(\nu_{\text{max}}\) (CHCl\(_3\))/cm\(^{-1}\); 3476 (indole NH), 3433 (amide NH), 3017 (saturated CH), 1734, 1725 (ester C=O), 1675, 1669, 1512, 1507 (CONH); \(\delta\)\(_H\) (CDCl\(_3\); 600 MHz) 8.29 (total of 1 H, brs, NH\(_{\text{indole}}\) of each diastereomer) 7.56 (total of 1 H, m, CH\(_{\text{ar}}\) of each diastereomer), 7.32 (total of 1 H, m, CH\(_{\text{ar}}\) of each diastereomer), 7.15 (total of 1 H, m, CH\(_{\text{ar}}\) of each diastereomer), 7.13 (total of 1 H, m, CH\(_{\text{ar}}\) of each diastereomer), 7.09 (total of 1 H, m, CH\(_{\text{ar}}\) of each diastereomer), 6.27 (0.5 H, d, J 7.8, NH\(_B\)), 6.25 (0.5 H, d, J 7.8, NH\(_A\)), 4.92 (0.5 H, ddd, J 7.8, 5.6, 5.4, NHCH\(_B\)), 4.91 (0.5 H, ddd, J 7.8, 6.0, 5.8, NHCH\(_A\)), 3.64 (1.5 H, s, OCH\(_A\)), 3.61 (.5 H, s, OCH\(_B\)), 3.34 (0.5 H, dd, J 14.8, 5.4, CHCH\(_A\)H\(_B\)Indole), 3.28 (0.5 H, dd, J 5.8, CHCH\(_A\)H\(_B\)Indole), 3.27 (0.5 H, dd, J 5.8, CHCH\(_A\)H\(_B\)Indole), 3.24 (0.5 H, dd, J 14.8, 5.6, CHCH\(_A\)H\(_B\)Indole), 2.63-2.56 (1 H, m, CHCONH of each diastereomer), 2.58 (0.5 H, dd, J 15.8, 9.0, OCCH\(_A\)H\(_B\)CH), 2.53 (0.5 H, dd, J 16.2, 5.6, OCCH\(_A\)H\(_B\)CH), 2.29 (0.5 H, dd, J 15.8, 4.0, OCCH\(_A\)H\(_B\)CH), 2.26 (0.5 H, dd, J 16.2, 5.2, OCCH\(_A\)H\(_B\)CH), 1.58-1.48 (total of 1.5 H, m, CH\(_A\)(CH\(_3\))\(_2\), CH\(_B\)(CH\(_3\))\(_2\), 123
Experimental

CH₃CHCH₃H₂CH and CH₂CHCH₃H₂CH), 1.40 (4.5 H, s, C(CH₃)₃), 1.38 (4.5 H, s, C(CH₃)₃), 1.37 (0.5 H, m, obscured by 'Bu peaks, CH₃(CH₃)₂), 1.17 (0.5 H, ddd, J 15.4, 9.8, 8.4), 5.4 CH₂CHCH₃H₂CH), 1.11 (0.5 H, ddd, J 13.8, 8.4, 5.8 CH₂CHCH₃H₂CH), 0.84 (1.5 H, d, J 6.4, CH₃CHCH₃CH₃), 0.82 (1.5 H, d, J 6.4, CH₃CHCH₃CH₃), 0.76 (1.5 H, d, J 6.4, CH₃CHCH₃CH₃), 0.75 (1.5 H, d, J 6.4, CH₃CHCH₃CH₃), δ(CDC1₃; 63 MHz) 174.5, 174.4, 172.2, 171.6, 171.4 (CONH, CO₂R), 136.0, 136.2, 127.4 (ipso-Ar), 123.1, 122.7, 122.0, 121.9, 119.4, 119.3, 118.4, 118.3, 111.1, 111.0 (C₆H₅), 109.8, 109.6 (ipso-Ar), 80.6, 80.6 (C(CH₃)₃), 52.6, 52.5 (NH), 52.0 (O₂CCH₃), 41.3, 40.9 (O₂CCH₂), 40.8, 40.5 (CHCONH), 38.2, 37.9, (CH₂CH(CH₃)₂), 27.9, 27.8 (C(CH₃)₂), 27.6 (CH₁Indole), 25.4, 25.3 (CH(CH₃))₂, 22.7, 22.6, 22.0, 21.9 (CH(CH₃)₂); m/z (FAB) 431 (50, MH⁺), 375 (88, MH₂⁺'Bu), 201(83), 159 (42), 130 (100, CH₂Indole), 57 (58, 'Bu), Found (FAB), 431.2533, C₂₄H₃₅N₂O₅ requires 431.2546.

4.3.19. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tert-leucine methyl ester and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tert-leucine methyl ester 127e

The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (1.52 g, 4.73 mmol, 1.2 equiv.), HOBt (639 mg, 4.73 mmol, 1.2 equiv.), diisopropylethylamine (2.06 mL, 11.83 mmol, 3.0 equiv.), succinate 114 (908 mg, 3.94 mmol, 1.0 equiv.) and DL-tert-leucine methyl ester hydrochloride (788 g, 4.34 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless wax (873 g, 62%). ¹H nmr indicated a (1:1). ratio of diastereomers.

Rₜ (Hexane:EtOAc, 6:1) 0.38, 0.32; [α]²⁰ + 5.78 (c 1.16, CHCl₃); vₘₐₓ(CHCl₃)/cm⁻¹ 3432 (amide NH), 3010 (saturated CH), 1731 (ester C=O), 1673, 1513 (CONH); δₜ (CDCl₃; 600 MHz) 6.35 (0.5 H, d, J 9.3, NH₃⁺), 6.29 (0.5 H, d, J 9.3, NH₃⁺), 4.42 (0.5
Experimental

H, d, J 9.3, NHCH\textsuperscript{B}), 4.38 (0.5 H, d, J 9.3, NHCH\textsuperscript{A}), 3.69 (1.5 H, s, OCH\textsubscript{3}\textsuperscript{B}), 3.67 (1.5 H, s, OCH\textsubscript{3}\textsuperscript{A}), 2.68 (total of 1 H, m, CHCONH of each diastereomer), 2.58 (0.5 H, dd, J 17.0, 9.6, OCCH\textsubscript{A}H\textsubscript{B}CH), 2.51 (0.5 H, dd, J 16.9, 9.0, OCCH\textsubscript{A}H\textsubscript{B}CH), 2.29 (0.5 H, dd, J 17.0, 4.0, OCCH\textsubscript{A}H\textsubscript{B}CH), 2.28 (0.5 H, dd, J 16.9, 5.0, OCCH\textsubscript{A}H\textsubscript{B}CH), 1.65 (0.5 H, ddd, J 13.6, 9.3, 5.6, CHCH\textsubscript{A}H\textsubscript{B}CH), 1.59 (0.5 H, ddd, J 13.6, 8.9, 5.9, CHCH\textsubscript{A}H\textsubscript{B}CH), 1.58-142 (total of 1 H, m, CH(CH\textsubscript{3})\textsubscript{2} of each diastereomer), 1.14 (0.5 H, ddd, J 13.6, 7.9, 5.9, CHCH\textsubscript{A}H\textsubscript{B}CH), 1.13 (0.5 H, ddd, J 13.6, 8.3, 5.6, CHCH\textsubscript{A}H\textsubscript{B}CH), 0.90 (1.5 H, d, J 6.6, CH\textsubscript{A}CHCH\textsubscript{B}), 0.88 (1.5 H, d, J 6.6, CH\textsubscript{A}CHCH\textsubscript{B}), 0.87 (1.5 H, d, J 6.6, CH\textsubscript{A}CHCH\textsubscript{B}), 0.84 (1.5 H, d, J 6.6, CH\textsubscript{A}CHCH\textsubscript{B}); \text{\textdelta}_{C} (CDCl\textsubscript{3}; 63 MHz) 174.6, 174.3, 171.9, 171.7, 171.6 (CONH, CO\textsubscript{2}C), 80.7, 80.6 (OC(CH\textsubscript{3})\textsubscript{3}), 60.0, 59.7 (NHCH), 51.5, 51.5 (OCH\textsubscript{3}), 41.1, 41.0 (O\textsubscript{2}CCH\textsubscript{2}), 40.7 (CHCONH), 38.4, 38.2 (CHCH\textsubscript{2}CH), 34.7, 34.3 (CH(C(CH\textsubscript{3}))\textsubscript{2}), 27.9, 27.9 (OCC(CH\textsubscript{3})\textsubscript{3}), 26.5, 26.4 (CH(C(CH\textsubscript{3})\textsubscript{3})), 25.7, 25.5 (CH(CH\textsubscript{3})\textsubscript{2}), 23.0, 22.8, 22.1, 21.8 (CH(C(CH\textsubscript{3})\textsubscript{2}); m/z (FAB), 358 (24%, MH\textsuperscript{+}), 302 (100, MH\textsuperscript{+}+Bu), 284 (16, 302-H\textsubscript{2}O), 242 (12), 86 (30), 57 (30, 'Bu), Found (FAB) 358.2593, C\textsubscript{19}H\textsubscript{36}N\textsubscript{0} requires 358.2594.

4.3.20. General procedure for the hydrolysis of (2R,2'RS)-[4-(tert-butyl)-2-iso-butyl-succinyl]-\textalpha-aminomethyl esters 127a-e

To amide 127a-e suspended in THF:water (1:1, 20 mL), was added lithium hydroxide monohydrate (2.0 equiv.) and the resulting suspension stirred for 30 minutes at room temperature until a clear solution was obtained. The reaction mixture was acidified to pH 3 with aqueous citric acid (1M), and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with brine (30 mL), dried (Na\textsubscript{2}SO\textsubscript{4}), filtered, and evaporated under reduced pressure to give the desired product.
4.3.21. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine and (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine 128a

The general procedure outlined in Section (4.3.20.) was followed with DL-phenylalanine derived amide 127a (1.03 g, 2.63 mmol) and lithium hydroxide monohydrate (221 mg, 5.26 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (0.99 g, quantitative). \( \alpha \) H nmr indicated a (1:1) ratio of diastereomers. \( \alpha \) 20 + 11.02 (c 1.18, CHCl 3 ); \( \nu _{\text{max}} \) (CHCl 3 )/cm'; 3425 (amide NH), 3200-2400 (acid OH), 3019 (saturated CH), 1723 (ester C=O, and acid C=O), 1671, 1516 (CONH); O (CDC 13 ; 600 MHz) 175.6, 175.2, 174.5, 174.3, 172.2, 171.8 (CONH, CO 2 H, CO 2 C), 135.9, 135.8, (ipso-Ar), 129.3, 129.1, 128.5, 126.97 (CH ary), 81.8, 81.0 (C(CH 3 ) 3 ), 53.2, 53.01 (NHCH), 41.0 (O,C,C(CH 3 ) 2 ), 40.7, 40.5 (CHCONH), 38.1, 38.0 (CH 2 CH(CH 3 ) 2 ), 37.3, 37.2 (CH 2 Ph), 27.9, 27.9 (C(CH 3 ) 3 ), 25.5, 25.4 (CH(CH 3 ) 2 ), 22.9, 22.7, 22.0, 21.9 (CH(CH 3 ) 2 ); m/z (FAB) 378 (41%, MH + ), 322 (100, MH 2 + 'Bu), 258 (8), 166 (38), 126.
120 (67), 91 (34, CH₂Ph), 57 (62, 'Bu), Found (FAB), 378.2263, C₂₁H₃₂N₀₅ requires 378.2280.

4.3.22. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine 128b

The general procedure outlined in Section (4.3.20.) was followed with DL-valine derived amide 127b (1.02 g, 2.97 mmol) and lithium hydroxide monohydrate (249 mg, 5.94 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (0.98 g, quantitative). ¹H nmr indicated a (1.5:1) ratio of diastereomers.

\[ \alpha \left[^{2}D \right] + 6.67 \text{ (c 1.08, CHCl₃); } \nu_{\text{max}}(\text{CHCl₃})/\text{cm}^{-1}; 3496 \text{ (amide NH), 3400-2400 (acid OH), 3020 (saturated CH), 1752 (ester C=O) 1718 (acid C=O), 1654, 1519 (CONH); } \delta_{H}(\text{CDCl₃}; 600 \text{ MHz}) 9.88 \text{ (total of 1 H, br s, OH of each diastereomer), 6.62 (0.6 H, d, J 8.6, NH²), 6.50 (0.4 H, d, J 8.9, NH³), 4.56 (0.4 H, dd, J 8.9, 4.6, NHCH²), 4.51 (0.6 H, dd, J 8.6, 4.6, NHCH³), 2.73 (total of 1 H, m, CHCONH of each diastereomer), 2.58 (0.4 H, dd, J 17.3, 9.6, OCH² HbCH), 2.35 (0.6 H, dd, J 17.3, 4.3, OCH³ HbCH), 2.30 (0.6 H, dd, J 16.6, 8.6, OCH³ HbCH), 2.31 (0.4 H, dd, J 17.3, 4.3, OCH³ HbCH), 2.30 (0.6 H, dd, J 16.6, 8.6, OCH³ HbCH), 2.21 (total of 1 H, m, CHCH(CH₃)₂ of each diastereomer), 1.64 (0.6 H, ddd, J 13.3, 9.6, 5.3, CHCH² HbCH), 1.60 (0.4 H, ddd, J 13.3, 9.0, 5.9, CHCH³ HbCH), 1.56-1.49 (total of 1 H, 2 x m, CH₂CH(CH₃)₂ of each diastereomer), 1.39 (3.6 H, s, C(CH₃)₃), 1.38 (5.4 H, s, C(CH₃)₃), 1.1 (0.6 H, ddd, J 13.3, 8.6, 5.3, CHCH² HbCH), 1.13 (0.4 H, ddd, J 13.3, 8.0, 5.9, CHCH³ HbCH), 0.95 and 0.92 (total of 6 H, 2 x d, J 6.6, CH(CH₃)₃CHCH₃b and CH(CH₃)₃CHCH₃b of each diastereomer), 0.89, 0.87, 0.86, 0.83 (total of 6 H, 4 x d, J 6.6, CH₂(CH₃)₃CHCH₃b and CH₂(CH₃)₃CHCH₃b of each diastereomer); \delta_C(\text{CDCl₃}; 63 MHz) 175.5, 175.3, 175.2, 172.1, 171.9 (CONH, CO₂H, CO₂C), 81.1, 80.9 (C(CH₃)₃), 57.0, 56.8 (NHCH), 41.0, 40.9 (O₂CC₃H₇), 40.7, 40.6 (CHCONH), 38.3, 38.2 (CH₂CH(CH₃)₂), 30.9, 30.6 (CH(CH₃)₂(valine)), 27.9, 27.8 (C(CH₃)₃), 25.6, 25.5 (CH(CH₃)₂(succinate)), 22.9,
22.8, 22.1, 22.0, 21.8, (CH(CH₃)₂(succinate)), 18.9, 18.8, 17.5, 17.3 (CH(CH₃)₂(valine)); m/z (FAB), 330 (25%, MH⁺), 274 (100, MH₂⁺-Bu), 256 (16, 274-H₂O), 210 (7), 157 (9), 118 (37), 72 (46), 57 (30, -Bu), Found (FAB), 330.2283, C₁₇H₃₂N₀₅ requires 330.2280.

4.3.23. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine 128c

The general procedure outlined in Section (4.3.20.) was followed with DL-leucine derived amide 127c (1.12 g, 3.13 mmol) and lithium hydroxide monohydrate (263 mg, 6.26 mmol, 2.0 equiv.) to furnish the title compound as a colourless gum (1.08 g, quantitative). ¹H nmr indicated a (1:1) ratio of diastereomers. [α]D²⁰ + 8.56 (c 1.32, CHCl₃); υ max(CHCl₃)/cm⁻¹; 3442 (amide NH), 3200-2450 (acid OH), 3019 (saturated CH), 1720 (ester C=O and acid C=O), 1655, 1522 (CONH); δH (CDCl₃; 600 MHz) 8.16 (total of 1 H, br s, OH of each diastereomer), 6.49 (0.5 H, d, J 8.0, NH₄⁺), 6.35 (0.5 H, d, J 8.0, NH₃⁺), 4.58 (0.5 H, ddd, J 9.6, 8.0, 4.9, NHCH₃⁺), 4.53 (0.5 H, ddd, J 9.3, 8.0, 4.6, NHCH₃⁺), 2.62 (total of 1 H, m, CHCONH), 2.58 (0.5 H, dd, J 16.9 10.0, OCCCH₃H₃CH₂), 2.56 (0.5 H, dd, J 16.9, 9.0, OCCCH₃H₃CH₂) 2.31 (0.5 H, dd, J 16.9 5.1, OCCCH₃H₃CH₂), 2.30 (16.9, 4.3, OCCCH₃H₃CH₂), 1.71-1.51 (total of 5 H, m, CH₂CHCH₂CH₂, CH₂CHCH₂CH₂, NHCHCH₂CH₂, and NHCHCH₂CH₂CH₂CH₂, of each diastereomer), 1.40 (total of 9 H, s, C(CH₃)₃ of each diastereomer), 1.15 (0.5 H, ddd, J 13.6, 8.3, 5.6, CH₂CHCH₃CH₂CH₃CH₂CH₃), 1.14 (0.5 H, ddd, J 13.6, 7.9, 6.0, CH₂CHCH₃CH₂CH₂CH₂CH₃), 0.94, 0.92, 0.91, 0.90, 0.89(2), 0.87, 0.85 (total of 12 H, 8 x d, J 6.6, CH₃CHCH₃ and CH₃CHCH₃ of each iso-butyl group of each diastereomer); δC (CDCl₃; 63 MHz) 176.5, 176.2, 175.8, 175.3, 172.1, 171.9 (CONH, CO₂H, CO₂C), 81.2, 80.9 (C(CH₃)₃), 50.9, 50.8 (NHCH), 41.1 (O₂CC₂H), 40.9, 40.7 (CH₂CH(CH₃)₂(leucine)), 40.6, 40.5 (CHCONH), 38.3, 38.1 (CH₂CH(CH₃)₂(succinate)), 27.9 (C(CH₃)₃), 25.7, 25.4 (CH(CH₃)₂(succinate)), 24.8,
24.5 (CH(CH₃)₂(Leucine)), 22.9, 22.8, 22.7, 22.6, 22.0, 21.9, 21.6 (2 x CH(CH₃)₂); m/z (FAB), 344 (23%, MH⁺). 288 (100, MH₂⁺-Bu), 270 (18, 288-H₂O), 229 (30), 157 (17), 132 (53), 86 (81), 57 (72, 'Bu). Found (FAB), 344.2445, C₁₈H₃₄NO₅ requires 344.2437.

4.3.24. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan 128d

![Chemical Structure]

The general procedure outlined in Section (4.3.20.) was followed with DL-tryptophan derived amide 127d (1.35 g, 3.14 mmol) and lithium hydroxide monohydrate (263 mg, 6.27 mmol, 2.0 equiv.) to furnish the title compound as a colourless foam (1.31 g, quantitative). ¹H nmr indicated a (1:1) ratio of diastereomers.

Mp 77-79°C; [α]D + 22.82 (c 1.10, CHCl₃); νmax(CHCl₃)/cm⁻¹; 3476 (amide NH and indole NH), 3400-2400 (acid OH), 3019 (saturated CH), 1720 (ester C=O and acid C=O), 1669, 1519 (CONH); δH (CDCl₃; 600 MHz) 8.46 (total of 1 H, br s, NH(indole) of each diastereomer), 7.63 and 7.55 (total of 1 H, 2 x d, J 7.9, CH_ar of each diastereomer), 7.32 and 7.31 (total of 1 H, 2 x d, J 7.3, CH_ar of each diastereomer), 7.16 (total of 1 H, m, CH_ar of each diastereomer), 7.08 (total of 1.0 H, m, CH_ar of each diastereomer) 7.06 (0.5 H, d, J 2.3, CH_ar), 6.97 (0.5 H, d, J 2.3, CH_ar), 6.45 and 6.42 (total of 1 H, 2 x d, J 7.6, NH(amide) of each diastereomer), 4.92 and 4.90 (total of 1 H, 2 ddd, J 7.6, 5.6, and 7.6, 5.8, NHCH of each diastereomer), 3.33 (0.5 H, dd, J, 15.0, 6.6, CHCH_BH₄Indole), 3.30 (total of 1.0 H, m, CHCH_AH₄Indole and CHCH_AH₄Indole), 3.26 (0.5 dd, J 15.0, 6.6, NHCHCH_AH₄B), 2.61 (0.5 H, m, CH_BCONH), 2.52 (total of 1.5 H, m, CHCONH, OCCH_BH₄CH and OCCH_AH₄CH), 2.28 (0.5 H, dd, J 16.6, 5.3, OCCH_AH₄B CH) 2.24 (0.5 H, dd, J 20.2, 8.6,
OCCH\textsubscript{3}H\textsubscript{4}\textsuperscript{+}), 1.49 (total of 1 H, m, CH(CH\textsubscript{3})\textsubscript{2} of each diastereomer), 1.43 (0.5 H, m, CHCH\textsubscript{3}H\textsubscript{3}), 1.39 (4.5 H, s, C(CH\textsubscript{3})\textsubscript{3}), 1.35 (4.5 H, s, C(CH\textsubscript{3})\textsubscript{3}), 1.25 (0.5 H, m, CHCH\textsubscript{3}H\textsubscript{3}), 1.14 (0.5 H, ddd, J 13.0, 9.6, 5.6, CHCH\textsubscript{3}H\textsubscript{3}), 1.07 (0.5 H, ddd, J 13.6, 8.3, 5.3, CHCH\textsubscript{3}H\textsubscript{3}), 0.77, 0.76 and 0.70, 0.68 (total of 6 H, 4 x d, J 6.3, CH\textsubscript{3}A CHCH\textsubscript{3}B and CH\textsubscript{3}A CHCH\textsubscript{3}B of each diastereomer); \(\delta\) (CDCl\textsubscript{3}; 63 MHz) 175.7, 175.4, 174.9, 174.7, 172.1, 171.9 (CON\textsubscript{2}, 0\textsubscript{2} H, 0\textsubscript{2} C), 136.1, 136.0, 127.5, 127.5 (ipso-Ar), 123.4, 123.2, 122.0, 121.9, 119.5, 119.4, 118.4, 118.3, 111.2, (CH\textsubscript{a}), 109.4 (ipso-Ar), 81.3, 80.9 (C(CH\textsubscript{3})\textsubscript{3}), 53.0 (NHCH), 41.1, 40.9 (O\textsubscript{2} CCH\textsubscript{2}), 40.7, 40.5 (CHCONH), 38.1, 37.9 (CH\textsubscript{2}CH(CH\textsubscript{3})\textsubscript{2}), 27.9, 27.8 (C(CH\textsubscript{3})\textsubscript{3}), 27.2, 26.9 (CH\textsubscript{2}Indole), 25.4 (C(CH(CH\textsubscript{3})\textsubscript{2}), 22.6, 22.0, 21.9 (CH(CH\textsubscript{3})\textsubscript{2}); \(m/z\) (FAB), 417 (3%, MH\textsuperscript{+}), 361 (21, MH\textsuperscript{2+}-tBu), 288 (10), 229 (23), 188 (19), 130 (30, CH\textsubscript{2}Indole), 88 (19), 57 (100, tBu), Found (FAB), 417.2372, C\textsubscript{23}H\textsubscript{33}N\textsubscript{2}O\textsubscript{5} requires 417.2389.

4.3.25. (2R,2'R)-(4-(t tert-butyl)-2-iso-butyl-succinyl]-tert-leucine and (2R,2'S)-(4-(t tert-butyl)-2-iso-butyl-succinyl]-tert-leucine 128e

The general procedure outlined in Section (4.3.20.) was followed with DL-tert-leucine derived amide 127e (843 mg, 3.36 mmol) and lithium hydroxide monohydrate (396 mg, 9.43 mmol, 4.0 equiv.). The reaction mixture was stirred for 2 days. The product was obtained as a colourless gum (810 mg, 100%). \(^1\)H nmr (200 MHz) indicated a mixture of two products, the desired acid and the corresponding diacid amide resulting from concomitant cleavage of the tert-butyl ester in an almost 1:1 ratio. The bulk crude product was not further purified but was used immediately in the next step (see Section (4.3.28.)). A portion of the crude product was purified by repeated trituration, (Et\textsubscript{2}O:hexane) to remove the diacid amide. The mother liquor was evaporated under reduced pressure to furnish a pure sample of the desired product. \(^1\)H nmr indicated a (1:1) ratio of diastereomers. 

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[α]_D^0 +6.04 (c 1.44, CHCl_3); ν_\text{max}(\text{CHCl}_3)/\text{cm}^{-1}; 3428 (amide NH), 3200-2400 (acid OH), 1719 (ester C=O and acid C=O), 1676, 1515 (CONH); δ_H (CDCl_3; 600 MHz) 10.19 (total of 1 H, br s, OH), 6.69 (0.5 H, d, J 9.0, NH\text{A}), 6.57 (0.5 H, d, J 9.3, NH\text{B}), 4.44 (0.5 H, d, J 9.3, NHCH\text{B}), 4.39 (0.5 H, d, J 9.0, NHCH\text{A}), 2.75 (total of 1 H, m, CHCONH of each diastereomer), 2.58 (0.5 H, dd, J 17.0, 9.6, CHCH\text{A}H\text{B}CH), 2.53 (0.5 H, dd, J 16.9, 8.6, CHCH\text{A}H\text{B}CH), 2.31 (0.5 H, dd, J 17.1 4.2, CHCH\text{A}H\text{B}CH), 2.30 (0.5 H, dd, J 16.9, 5.0, CHCH\text{A}H\text{B}CH), 1.64 (0.5 H, m, CH(CH_3)_2 of one diastereomer), 1.61-1.49 (total of 1 1.5 H, m, CHCH\text{B}H\text{B}CH, CH(CH_3)_2 of each diastereomer), 1.39 (OC(CH_3)_3), 1.38 (4.5 H, s, OC(CH_3)_3), 1.15 (total of 1 H, m, CHCH\text{A}H\text{B}CH of each diastereomer), 0.99 (4.5 H, s, CH(CH_3)_3), 0.92 (4.5 H, s, CH(CH_3)_3), 0.89 (1.5 H, d, J 6.6, CH\text{A}CHCH\text{B}), 0.87 (1.5 H, d, J 6.6, CH\text{A}CHCH\text{B}), 0.86 (1.5 H, d, J 6.6, CH\text{A}CHCH\text{B}), 0.83 (1.5 H, d, J 6.6, CH\text{A}CHCH\text{B}), δ_C (CDCl_3; 63 MHz) 175.3, 175.2, 175.1, 175.0, 172.1, 172.0 (CONH, CO_2H, CO_2C), 81.2, 80.9 (OC(CH_3)_3), 60.2, 59.9 (NHCH), 41.0, 40.8 (O_2CCH_2), 40.6, 40.5 (CHCONH), 38.2, 38.0 (CHCH_2CH), 34.6, 34.1 (CHC(CH_3)_3), 27.9, 27.8 (OCC(CH_3)_3), 26.5, 26.4 (CHC(CH_3)_3), 25.6, 25.4 (CH(CH_3)_3), 22.9, 22.8, 22.0, 21.8 (CH(CH_3)_3); m/z (FAB), 344 (26%, MH\text{A}), 288 (100, MH_2-\text{Bu}), 270 (39, 288-H_2O), 242 (9, 270-CO), 132 (20), 86 (32), 57 (40, \text{tBu}), (FAB) 344.2436, C_{18}H_{34}NO_5 requires 344.2437.

4.3.26. General procedure for (3R,4'RS)-2'-substituted-4'-substituted-5'(4'H)-oxazolones 129a-e

EDCI (1.1 equiv.) was added to a solution of acid (1.0 equiv.) in acetonitrile (30 mL) and the solution stirred at room temperature under an atmosphere of nitrogen for 90 minutes. The reaction was quenched with saturated aqueous ammonium chloride (20 mL) and the aqueous layer extracted with diethyl ether (3 x 30 mL). The combined organic extracts were washed with brine (2 x 20 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure with a water bath temperature of no greater than 30 °C. Purification by column chromatography yielded the desired product as a colourless oil.
4.3.27. (3R,4'SS)-5-Methyl-3-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid tert-butyl ester 129a

The procedure outlined in Section (4.3.26.) was followed using DL-phenylalanine derived acid 128a (1.09 g, 2.89 mmol) and EDCI (1.09 g, 3.18 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (896 mg, 86%). 'H nmr indicated a (1:1) ratio of diastereomers.

$R_f$ (Hexane:EtOAc, 6:1) 0.27; $\left[\alpha\right]_D^{20}$ -1.03 (c 2.34, CHCl$_3$); $\nu_{\text{max}}$(CHCl$_3$/cm$^{-1}$) 3019 (saturated CH), 1817 (oxazolone C=O), 1724 (ester C=O), 1671 (C=N); $\delta_H$ (CDCl$_3$; 600 MHz) 7.26-7.18 (total of 3 H, m, CH$_{\text{ar}}$ of each diastereomer), 7.17-7.15 (total of 2 H, m, CH$_{\text{ar}}$ of each diastereomer), 4.42 (total of 1 H, m, NCH of each diastereomer), 3.22 (0.5 H, dd, $J$ 13.6, 5.2, CH$_A$H$_B$Ph of one diastereomer), 3.21 (0.5 H, dd, $J$ 13.6, 5.2, CH$_A$H$_B$Ph of one diastereomer), 3.13 (0.5 H, dd, $J$ 13.6, 5.4, CH$_A$H$_B$Ph of one diastereomer), 3.11 (0.5 H, dd, $J$ 13.6, 5.8, CH$_A$H$_B$Ph of one diastereomer), 2.93 (0.5 H, m, CHC=NH of one diastereomer), 2.86 (0.5 H, m, CHC=NH of one diastereomer), 2.45 (0.5 H, dd, $J$ 16.0, 7.8, OCCH$_A$H$_B$CH of one diastereomer), 2.42 (0.5 H, dd, $J$ 16.6, 8.0, OCCH$_A$H$_B$CH of one diastereomer), 2.29 (0.5 H, dd, $J$ 16.6, 6.4, OCCH$_A$H$_B$CH of one diastereomer), 2.26 (0.5 H, dd, $J$ 16.6, 6.4, OCCH$_A$H$_B$CH of one diastereomer), 1.40 (total of 9 H, s, C(CH$_3$)$_3$ of each diastereomer), 1.37 (0.5 H, ddd, obscured by 'Butyl peak, CHCH$_A$HBCH of one diastereomer), 1.30 (0.5 H, ddd, $J$ 13.2, 8.6, 6.0, CHCH$_A$HBCH of one diastereomer), 1.25 (0.5 H, m, CH(CH$_3$)$_2$ of one diastereomer), 1.12 (0.5 H, ddd, $J$ 12.8, 8.8, 5.8, CHCH$_A$H$_B$CH of one diastereomer), 1.10 (0.5 H, ddd, $J$ 13.2, 8.0, 6.0, CHCH$_A$H$_B$CH of one diastereomer), 0.93 (0.5 H, m, CH(CH$_3$)$_2$ of one diastereomer), 0.80, 0.77, 0.77, 0.74 (total of 6 H, 4 x d, $J$ 6.6, CH$_{3A}$CH$_{3B}$ and CH$_{3A}$CH$_{3B}$ of each diastereomer); $\delta_C$ (CDCl$_3$; 63 MHz) 177.8, 177.7 (NCH$_2$CO$_2$C), 170.2, 170.1 (CO$_2$C), 167.8, 167.1 (C=N), 135.0, 134.7 (ips-Ar), 129.6, 129.5, 128.3, 128.1, 127.0 (CH$_{\text{ar}}$), 80.8, 80.7 (C(CH$_3$)$_3$), 65.5, 65.4 (NCHCO), 40.5, 40.1 (O$_2$CCH$_2$), 37.6,
37.4 (CH2CH(CH3)2), 36.6, (CH2Ph), 34.8, 34.1 (CH2CHCN), 27.9 (C(CH3)3), 25.2, 25.0 (CH(CH3)2), 22.7, 22.6, 21.8, 21.7 (CH(CH3)3); m/z (FAB) 360 (69%, MH+), 304 (100, MH+-'Bu), 286 (29, 304-H2O), 258 (96, 286-CO), 212 (10), 154 (33), 120 (77), 91 (51, CH2Ph), 57 (74, 'Bu), Found (FAB) 360. 2174, C21H30NO4 requires 360.2174.

4.3.28. (3R,4'SR)-4-Methyl-2-(4'-iso-propyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid tert-butyl ester 129b

The procedure outlined in Section (4.3.26.) was followed using DL-valine derived acid 128b (587 mg, 1.81 mmol) and EDCI (382 mg, 1.99 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (486 mg, 81%). 'H nmr indicated a (1:1) ratio of diastereomers.

Rf (Hexane:EtOAc, 6:1) 0.54; [α]D20 -7.5 (c 1.16, CHCl3); υmax(CHCl3)/cm-1 1819 (oxazolone C=O), 1725 (ester C=O), 1673 (C=N); δH (CDCl3; 600 MHz) 4.01 (0.5 H, d, J 5.4, NCH of one diastereomer), 4.00 (0.5 H, d, J 5.0, NCH of one diastereomer), 3.06 (total of 1 H, m, CHC=N of each diastereomer), 2.64 (0.5 H, dd, J 16.2, 9.2, OCCH4CH6CH of one diastereomer), 2.61 (0.5 H, dd, J 16.0, 8.2, OCCH4CH6H of one diastereomer), 2.44 (0.5 H, dd, J 16.0, 6.0, OCCH4CH6CH of one diastereomer), 2.42 ((0.5 H, dd, J 16.2, 5.6, OCCH4CH6H of one diastereomer), 2.25 (0.5 H, m, CHCH(CH2)3 of one diastereomer), 2.24 (0.5 H, m, CHCH(CH2)3 of one diastereomer), 1.66- 1.58, (total of 2 H, m, CH2CH(CH3)2 and CHCH4H9CH of each diastereomer), 1.42 (4.5 H, s, C(CH3)3 of one diastereomer), 1.41 (4.5 H, s, C(CH3)3 of one diastereomer), 1.34 and 1.33 (total of 1 H, m, CHCH4H9CH of each diastereomer), 1.05, 1.04, 0.93(2), 0.92(2), 0.90, 0.89 (total of 12 H, 8 x d, J 6.6, CH3ACHCH3B and CH3ACHCH3B of the iso-buty1 and iso-propyl groups of each diastereomer); δC (CDCl3; 63 MHz) 177.9 (NCHCO2C), 170.4, 170.2 (CO2C), 167.7, 167.5 (C=N), 80.9, 80.8 (C(CH3)3), 69.8, 69.7 (HCHCO), 40.6 (O2CCH2), 37.5, 37.3

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(CHCH₂CH), 34.6, 34.5 (CH₂CHN), 30.6, 30.5 (CHCH(CH₃)₂), 27.9, 27.8 (C(CH₃)₃), 25.6, 25.5 (CH₂CH(CH₃)₂), 22.6, 22.50, 22.1, 22.0 (CH₂CH(CH₃)₂), 18.6, 18.5, 17.3, 17.2 (CHCH(CH₃)₂); m/z (FAB) 312 (49%, MH⁺), 256 (100, MH⁺⁻-tBu), 210 (74, 256-H₂O-CO), 157 (9), 111 (7), 83 (9), 72 (39), 57 (33, 'Bu), Found (FAB) 312.2171, C₁₇H₂₂NO₄ requires 312.2174.

4.3.29. (3R,4'R'S)-5-Methyl-3-(4'-iso-butyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-hexanoic acid tert-butyl ester 129c

![Chemical Structure](image)

The procedure outlined in Section (4.3.26.) was followed using DL-leucine derived acid 128c (753 mg, 2.19 mmol) and EDCI (462 mg, 2.41 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title product (533 mg, 75%). ¹H nmr indicated a (1:1) ratio of diastereomers. Rₐ (Hexane:EtOAc, 6:1) 0.45; [α]²⁰D -4.1 (c 1.22, CHCl₃); νₘₐₓ(CHCl₃)/cm⁻¹ 1822 (oxazolone C=O), 1723 (ester C=O), 1670 (C=N); δₜ (CDCl₃; 600 MHz) 4.13 (0.5 H, dd, J 8.6, 5.8 HCH of one diastereomer), 4.12 (0.5 H, dd, J 9.0, 5.8 HCH of one diastereomer), 3.03 (total of 1 H, m, CHC=N of each diastereomer), 2.64 (0.5 H, dd, J 16.0, 9.0, CH₂CHCH₃H₆CH of one diastereomer), 2.60 (0.5 H, dd, J 15.8, 8.4, CH₂CHCH₃H₆CH of one diastereomer), 2.44 (0.5 H, dd, J 15.8, 6.2, CH₂CHCH₃H₆CH of one diastereomer), 2.42 (0.5 H, dd, J 16.0, 5.6, CH₂CHCH₃H₆CH of one diastereomer), 1.96-1.90 (total of 1 H, m, CH₂CHCH₂CH(CH₃)₂ of each diastereomer), 1.69 (0.5 H, ddd, J 13.4, 7.4, 5.6, CHCHHCH of one diastereomer), 1.68 (0.5 H, ddd, J 13.4, 7.6, 5.8, CHCHHCH of one diastereomer), 1.65-1.58 (total of 2 H, NCHCH₂CH(CH₃)₂ of one diastereomer, and 3 x CHCHHCH of either diastereomer), 1.52 (0.5 H, ddd, J 13.6, 8.8, 6.4, CHCHHCH of one diastereomer), 1.49 (0.5 H, ddd, J 13.6, 9.0, 6.4, CHCHHCH of one diastereomer), 1.41 (4.5 H, s, C(CH₃)₃ of one diastereomer), 1.40 (4.5 H, s,
C(CH₃)₃ of one diastereomer), 1.37-1.32 (total of 1 H, m, NCH₂CH(CH₃)₂ of one diastereomer and CHCHCH of either diastereomer), 0.96, 0.95, 0.93, 0.92, 0.90 0.89 (total of 12 H, 8 x d, J 6.6, CH₃A(CH₂)₃B and CH₃(CH₂)₃B of each iso-butyl group of each diastereomer); δC (CDCl₃; 63 MHz) 179.2 (NCHCO₂C), 170.2, 170.0 (CO₂C), 167.3, 167.0 (C=N), 80.8 (C(CH₃)₂), 63.0, 62.9 (HCHCO), 40.6, 40.4 40.3, 40.2 (O₂CCH₂ and NCH₂CH₂), 37.5, 37.3 (O₂CCH₂CH₂CH₂), 36.6, 34.4 (CH₂CHN), 27.9 (C(CH₃)₂), 25.6, 25.5, 24.9 (2 x CH(CH₃)₂), 22.5, 22.1, 22.0, 21.9, 21.9 (2 x CH(CH₃)₂); m/z (FAB) 326 (80%, MH⁺), 270 (86, MH₂⁺-'Bu), 252 (27, 270-H₂O), 224 (68, 252-CO), 182 (29), 154 (96), 136 (59), 91 (23, CH₂Ph), 57 (43, 'Bu), Found (FAB) 326.2345, C₁₈H₃₂N₀₄ requires 326.2331.

4.3.30. (3R,4'SS)-4-Methyl-2-[4'-{(1H-indol-3-ylmethyl)-5'-oxo-4',5'-dihydro-oxazol-2'-yl]-hexanoic acid tert-butyl ester 129d

![Chemical structure]

The procedure outlined in Section (4.3.26.) was followed using DL-tryptophan derived acid 128d (914 mg, 2.19 mmol) and EDCI (463 mg, 2.41 mmol, 1.1 equiv.). Column chromatography, eluting with hexane:ethyl acetate (3:1) furnished the title product (900 mg, 91%). ¹H nmr indicated a (1:1) ratio of diastereomers. Rf (Hexane:EtOAc, 2:1) 0.60, 0.53; [α]D²⁰ +14.9 (c 1.20, CHCl₃); νmax(CHCl₃)/cm⁻¹ 3479 (indole NH), 1815 (oxazolone C=O), 1724 (ester C=O), 1672 (C=N); δH (CDCl₃; 600 MHz) 8.15 (total of 1 H, s, NHindole of each diastereomer), 7.64 (0.5 H, d, J 7.2, CHar of one diastereomer), 7.63 (0.5 H, d, J 7.0, CHar of one diastereomer), 7.29 (total of 1 H, m, CHar of each diastereomer), 7.15 (total of 1 H, m, CHar of each diastereomer), 7.10 (total of 1 H, m, CHar of each diastereomer), 7.03 (total of 1 H, m, CHar of each diastereomer), 4.51 (0.5 H, dd, J 5.0 NCH on one diastereomer), 4.49 (0.5 H, dd, J 5.0 NCH on one diastereomer), 3.45 (0.5 H dd, J 15.0, 5.0,
Experimental

CH₃A,H₈Indole of one diastereomer), 3.44 (0.5 H dd, J 15.0, 5.0, CH₃A,H₈Indole of one diastereomer), 3.30 (0.5 H dd, J 15.0, 5.0, CH₃A,H₈Indole of one diastereomer), 3.29 (0.5 H dd, J 15.0, 5.0, CH₃A,H₈Indole of one diastereomer), 2.87 (0.5 H, m, CHC=N of one diastereomer), 2.79 (0.5 H, m, CHC=N of one diastereomer), 2.32 (0.5 H, dd J 16.0, 8.0, OCCH₃A,H₈CH of one diastereomer), 2.26 (0.5 H, dd J 16.2, 7.8, OCCH₃A,H₈CH of one diastereomer), 2.16 (0.5 H, dd J 16.2, 6.4, OCCH₃A,H₈CH of one diastereomer), 2.14 (0.5 H, dd J 16.0, 6.6, OCCH₃A,H₈CH of one diastereomer), 1.40 (4.5 H, s, C(CH₃)₃ of one diastereomer), 1.39 (4.5 H, s, C(CH₃)₃ of one diastereomer), 1.25 (0.5 H, ddd, J 13.6, 9.0, 5.8, CHCH₃A,H₈CH of one diastereomer), 1.14 (0.5 H, m, CH(CH₃)₂ of one diastereomer), 1.03 (0.5 H, ddd, J 14.2, 8.4, 6.0, CHCH₃A,H₈CH of one diastereomer), 1.01 (0.5 H, ddd, J 13.6, 8.4, 6.4, CHCH₃A,H₈CH of one diastereomer), 0.95 (0.5 H, m, CH(CH₃)₂ of one diastereomer), 0.82 (0.5 H, ddd, J 14.2, 8.0, 6.4, CHCH₃A,H₈CH of one diastereomer), 0.70, 0.66, 0.64, 0.63 (total of 6 H, 4 x d, J 6.6, CH₃A,CHCH₃B and CH₃A,CHCH₃B of each diastereomer); δ_C (CDCl₃; 63 MHz) 178.6, 178.5 (NCHCO₂C), 170.4, 170.2 (CO₂C), 167.7, 167.1 (C=N), 137.7, 127.5, 127.4 (ipso-Ar), 123.2, 123.1, 122.0, 121.9, 119.5, 119.3, 119.2, 110.9 (CH₃), 109.3, 109.1 (ipso-Ar), 80.8, 80.7 (C(CH₃)₃), 66.1, 66.0 (HCHCO), 40.0, 39.9 (O₂CCCH₂), 37.4, 37.0 (CH₂CH(CH₃)₂), 34.6, 33.9 (CH₂CHN), 27.9, 27.8 (C(CH₃)₃), 26.6, 26.5 (CH₂Indole), 25.1, 24.9 (CH₂CH(CH₃)₂), 22.5, 22.3, 21.9, 21.5 (CH₂CH(CH₃)₂); m/z (FAB) 399 (36%, MH⁺), 361 (71), 343 (17, (MH₂⁺·Bu), 297 (6, 343·H₂O-CO), 277 (16), 205 (12), 188 (48), 159 (82), 130 (100, CH₂Indole), 57 (36, 'Bu), Found (FAB) 399.2266, C₂₃H₃₁N₂O₄ requires 399.2283.

4.3.31. (3R, 4'RS)-4-Methyl-2-(4'-tert-butyl-5'-oxo-4',5'-dihydro-oxazol-2-yl)-hexanoic acid tert-butyl ester 129e

![Chemical structure](image-url)
The procedure outlined in Section (4.3.26.) was followed using crude DL-tert-leucine derived acid 128e (500 mg, 1.60 mmol) and EDCI (367 mg, 1.91 mmol, 1.2 equiv.). Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the title compound as a colourless oil in a diastereomeric ratio of 2:1 as calculated from the integrals of the $^1$H nmr (244 mg, 52%). $^1$H nmr indicated a (1:1) ratio of diastereomers.

R$_f$ (Hexane:EtOAc, 6:1) 0.60; $[\alpha]^20_D$ -17.3 (c 0.74, CHCl$_3$); $\nu_{max}$(CHCl$_3$)/cm$^{-1}$; 3018 (saturated CH), 1821 (oxazolone C=O), 1729 (ester C=O), 1675 (C=N); $\delta_H$ (CDCl$_3$; 600 MHz) 3.80 (0.5 H, s, NCH of one diastereomer), 3.79 (0.5 H, s, NCH of one diastereomer), 3.06 (total of 1 H, m, CHC=N of each diastereomer), 2.65 (0.5 H, dd, J 16.3 9.0, OCCH$_A$H$_B$CH of one diastereomer), 2.60 (0.5 H, dd, J 16.3 8.0, OCCH$_A$H$_B$CH of one diastereomer) 2.44 (0.5 H, dd, J 16.3 6.0, OCCH$_A$H$_B$CH of one diastereomer) 2.42 (0.5 H, dd, J 16.3 5.6, OCCH$_A$H$_B$CH of one diastereomer), 1.66-1.52 (total of 2 H, m, CHCH$_A$CH$_B$CH of each diastereomer, CHCH$_A$CH$_B$CH of one diastereomer, and CH$_2$(CH$_3$)$_2$ of one diastereomer), 1.42 (4.5 H, s, OC(CH$_3$)$_3$ of one diastereomer), 1.41 (4.5 H, s, OC(CH$_3$)$_3$ of one diastereomer), 1.33 (total of 1 H, m, CHCH$_A$H$_B$CH of one diastereomer, and CH$_2$(CH$_3$)$_2$ of one diastereomer), 1.04 (4.5 H, s, CHC(CH$_3$)$_3$ of one diastereomer), 1.03 (4.5 H, s, CHC(CH$_3$)$_3$ of one diastereomer), 0.93, 0.92, 0.90, 0.89 (total of 6 H, 4 x d, J 6.4, CH$_3$A CHCH$_3$B and CH$_3$A CHCH$_3$B of each diastereomer); $\delta_C$ (CDCl$_3$; 63 MHz) 177.1 (NCHCO$_2$C), 170.4, 170.2 (CO$_2$C), 167.3, 167.0 (C=N), 80.8 (OC(CH$_3$)$_3$), 73.1 (NCHCO), 40.6, 40.5 (OCCH$_2$), 37.5, 37.1 (CHCH$_2$CH), 35.2 (CHC(CH$_3$)$_3$), 34.5, 34.3 (CH$_2$CHCN), 28.0 and 27.9 (OC(CH$_3$)$_3$ and CHC(CH$_3$)$_3$), 26.0 (CHC(CH$_3$)$_3$), 25.6, 25.5 (CH(CH$_3$)$_3$) 22.5, 22.4, 22.1, 22.0 (CH(CH$_3$)$_3$); m/z (FAB) 326 (100%, MH$^+$), 270 (81, MH$_2$-t-Bu) 252 (11, 270-H$_2$O), 224 (30, 252-CO), 137 (10), 86 (26), 57 (50, t-Bu), Found (FAB) 326.2328, C$_{18}$H$_{32}$N$_{2}$O$_4$ requires 326.2331.

4.3.32. General procedure for the lipase catalysed ring opening of (4RS)-2-substituted-4-substituted-5(4H)-oxazolones 129a-e

i. In the presence of triethylamine

Triethylamine (0.25 equiv.), lipase (crushed dried Novozyme®, 100 mg pre-dried weight, or dried Lipozyme®, 100 mg pre-dried weight) and alcohol (2.0 equiv.) were
added to a solution of oxazolone 129a-e (100 mg) dissolved in solvent (8 mL). The flask was stoppered and placed in an orbital incubator at 37 °C at 200 rpm. The reactions were monitored by tlc and on complete consumption of the starting 5(4H)-oxazolone the lipase was filtered, washed with solvent (2x 10 mL), and the combined organic fractions concentrated under reduced pressure. Purification by column chromatography as described for the corresponding 1:1 mix of diastereomers afforded the desired product as a colourless solid.

**ii. In the absence of triethylamine**

As above with the elimination of triethylamine.

### 4.3.33. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129a, triethylamine, Novozyme®, methanol, and toluene. Reaction time 4 days, (93 mg, 85%).

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27; d.e. 81%; Mp 94-96 °C; [α]<sub>D</sub> +54.7 (c 1.90, CHCl<sub>3</sub>).

### 4.3.34. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.32.ii.) was followed using oxazolone 129a, Novozyme®, methanol, and acetonitrile. Reaction stopped after 10 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (25 mg, 25%), and the desired product as a colourless solid (72 mg, 66%, 88% based on recovered starting material).

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27; d.e. 78%; Mp 92-95 °C; [α]<sub>D</sub> +60.2 (c 1.38, CHCl<sub>3</sub>).

### 4.3.35. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129a, triethylamine, Novozyme®, methanol, and tert-butyl methyl ether. Reaction time 2 days, (98 mg, 90%).
Experimental

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27; d.e. 79%; Mp 94-96 °C; [α]<sup>20</sup> + 52.6 (c 1.80, CHCl<sub>3</sub>).

4.3.36. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129a, triethylamine, Lipozyme<sup>®</sup>, methanol, and toluene. Reaction time 1 day, (80 mg, 73%).

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27; d.e. 58%; Mp 89-91 °C; [α]<sup>20</sup> + 42.6 (c 1.52, CHCl<sub>3</sub>).

4.3.37. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a

The procedure outlined in Section (4.3.32.ii.) was followed using oxazolone 129a, Lipozyme<sup>®</sup>, methanol, and acetonitrile. Reaction stopped after 48 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (24 mg, 24%) and desired product, (39 mg, 36%, 47% based on recovered oxazolone).

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.27; d.e. 55%; Mp 88-90 °C; [α]<sup>20</sup> + 37.76 (c 0.76, CHCl<sub>3</sub>).

4.3.38. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine ethyl ester 130

![Chemical Structure](image)

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129a, triethylamine, Novozyme<sup>®</sup>, ethanol, and toluene. Reaction time 3 days. Column chromatography eluting, with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (85 mg, 76%). Spectroscopic data given is for the major product diastereomer.

R<sub>f</sub> (Hexane:Et<sub>2</sub>O, 2:1) 0.38 (major), 0.29; d.e. 84%; Mp 65-67 °C; (Found: C, 68.09; H, 9.00; N, 3.30; C<sub>23</sub>H<sub>33</sub>N0<sub>5</sub> requires C, 68.12; H, 8.70; N, 3.45); [α]<sup>20</sup> + 49.6 (c 1.39...
Experimental

1.00, CHCl₃); υₘₐₓ(CHCl₃)/cm⁻¹ 3435 (amide NH), 3018 (saturated CH), 1732 (ester C=O), 1682, 1511 (CONH); δₗ (CDCl₃; 600 MHz) 7.20 (2 H, d, J 7.0, CH₉), 7.15 (1 H, d, J 7.0, CH₉), 7.11 (2 H, d, J 7.0 CH₉), 6.14 (1 H, d, J 7.9, NH), 4.78 (1 H, ddd, J 7.9, 6.0, NHCH), 4.03 (2 H, q, J 7.0, OCH₃), 3.04 (1 H, dd, J 13.6, 5.9, CH₉H₆Ph), 3.01 (1 H, dd, 13.6, 6.0, CH₉H₆Ph), 2.55 (1 H, m, CHCONH), 2.47 (1 H, dd, J 16.6, 8.9, OCCH₉H₆CH), 2.21 (1 H, dd, J 16.6, 4.9, OCCH₉H₆CH), 1.50 (1 H, ddd, J 13.6, 8.9, 5.9, CHCH₉H₆CH), 1.40 (1 H, m, CH(CH₃)₂), 1.36 (9 H, s, C(CH₃)₃), 1.13 (3 H, t, J 7.3, CH₂CH₃), 1.09 (1 H, ddd, obscured by CH₃ ethyl ester peak, CH(CH₃)₂), 0.80 (3 H, d, J 6.6, CH₃A CHCH₃B), 0.78 (3 H, d, J 6.5, CH₃A CHCH₃B); δC (CDCl₃; 63 MHz) 174.2, 171.5, 171.3 (CONH, CO₂C), 135.9 (ipso-Ar), 129.3, 128.3, 126.8 (CH₉), 80.5 (C(CH₃)₂), 60.2 (OCH₂CH₃), 53.0 (NHCH), 41.2 (O₂CC₂H₂), 40.7 (CHCONH), 38.0 (CH₂CH(CH₃)₂ and CH₂Ph), 27.9 (C(CH₃)₃), 25.4 (CH(CH₃)₂), 22.8, 22.0 (CH(CH₃)₂), 13.9 (CH₃CH₃); m/z (FAB) 406 (27%, MH⁺), 378 (38, MH⁺-Et) 350 (74, MH⁺⁻⁻Bu), 332 (15, 350-H₂O), 304 (12, 332-CO), 258 (25), 222 (14), 194 (35), 157 (12), 120 (100), 91 (14, CH₂Ph), 57 (30, 'Bu), Found (FAB), 406.2577, C₂₃H₃₆NO₅ requires 406.2593.

4.3.39. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine n-propyl ester 131

![Chemical structure](image)

The procedure outlined in Section (4.3.32.1.) was followed using 129a, triethylamine, Novozyme®, n-propanol, and toluene. Reaction time 3.5 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (98 mg, 84%). Spectroscopic data given is for the major product diastereomer.

Rₜ (Hexane:EtoAc, 2:1) 0.52; d.e. 84%; Mp 50-53 °C; (Found: C, 68.46; H, 9.11; N, 3.21; C₂₄H₃₇NO₅ requires C, 68.68; H, 8.89; N, 3.38); [α]²₀ D +47.3 (c 1.76, CHCl₃). υₘₐₓ(CHCl₃)/cm⁻¹ 3429 (amide NH), 3017 (saturated CH), 1728 (ester C=O), 1669, 1513 (CONH); δₗ (CDCl₃; 600 MHz) 7.21 (2 H, d, 7.0, CH₉), 7.16 (1 H, d, 7.0,
4.3.40. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine n-butyl ester 132

The procedure outlined in Section (4.3.32.i.) was followed using 129a, triethylamine, Novozyme®, n-butanol, and toluene. Reaction time 3.5 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished the desired product as a colourless solid (103 mg, 85%). Spectroscopic data given is for the major product diastereomer.

Rf (Hexane:EtOAc, 2:1) 0.55; d.e. 80%; Mp 53-56 °C; (Found: C, 68.84; H, 9.14; N, 3.14; C_{26}H_{37}NO_{5} requires C, 69.21; H, 8.99; N 3.23); [α]_{D}^{20} +45.2 (c 1.76, CHCl_{3}).

ν_{max} (CHCl_{3})/cm^{-1} 3427 (amide NH), 3018 (saturated CH), 1726 (ester C=O), 1675, 1507 (CONH); δ_{H} (CDCl_{3}; 600 MHz) 7.20 (2 H, d, J 7.0, CH_{ar}), 7.16 (1 H, d, J 7.0, CH_{ar}), 7.11 (2 H, d, J 7.0, CH_{ar}), 6.14 (1 H, d, J 7.9, NH), 4.79 (1 H, ddd, J 7.9, 6.0, NHCH), 4.00 (1 H, dt, J 10.6, 6.6, OCH_{A}H_{B}CH_{2}), 3.99 (1 H, dt, J 10.6, 6.6,

\[ \text{BuO}_2\text{C} \underset{\text{N}}{\overset{\text{CO}_2\text{Bu}}{\text{Ph}}} \]
OCH$_3$, 3.04 (1 H, dd, $J$ 13.9, 5.6, CH$_3$H$_8$Ph), 3.01 (1 H, dd, $J$ 13.9, 6.3, CH$_3$H$_8$Ph), 2.55 (1 H, m, CHCONH), 2.48 (1 H, dd, $J$ 16.6, 9.0, OCCH$_3$H$_8$CH), 2.22 (1 H, dd, $J$ 16.6, 4.9, OCCH$_3$H$_8$CH), 1.53-1.44 (total of 4 H, m, CHCH$_3$H$_8$CH, CH(CH$_3$)$_2$, and CH$_2$CH$_2$CH$_3$), 1.36 (9 H, s, C(CH$_3$)$_3$), 1.23 (2 H, q, $J$ 7.3, CH$_2$CH$_3$), 1.10 (1 H, ddd, $J$ 13.6, 7.6, 5.9, CHCH$_3$H$_8$Ph), 0.83 (3 H, t, $J$ 7.3, CH$_2$CH$_3$), 0.80 (3 H, d, $J$ 6.4, CH$_3$A CHCH$_3$B), 0.78 (3 H, d, $J$ 6.4, CH$_3$A CHCH$_3$B); $\delta$C (CDCl$_3$; 63 MHz) 174.3, 171.7, 171.6 (CONH, COO$_2$C), 136.0 (ipso-Ar), 129.4, 128.4 127.0 (CH$_3$Ar), 80.7 (C(CH$_3$)$_3$), 65.2 (OCCH$_2$CH$_3$), 53.1 (NHCH), 41.3 (O$_2$CCH$_2$), 40.8 (CCHCONH), 38.2, 38.1 (CH$_2$CH(CH$_3$)$_2$ and CH$_2$Ph), 30.4 (OCH$_2$CH$_2$), 28.1, (C(CH$_3$)$_3$), 25.6 (CH(CH$_3$)$_2$), 22.9, 22.2 (CH(CH$_3$)$_2$), 19.0 (CH$_2$CH$_3$), 10.3 (CH$_2$CH$_3$); m/z (FAB) 434 (32%, MH$^+$), 378 (100, MH$_2$-Bu), 360 (20, 378-H$_2$O), 304 (9), 258 (17), 222 (24), 157 (6), 120 (48), 91 (5, CH$_2$Ph), 57 (1, 'Bu), Found (FAB), 434.2896, C$_{25}$H$_{40}$N$_2$O$_5$ requires 434.2906.

4.3.41. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine methyl ester 127b

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129b, triethylamine, Novozyme®, methanol, and toluene. Reaction time 11 days, (103 mg, 93%).

R$_f$ (Hexane:EtOAc, 6:1) 0.20; d.e. 75%;Mp 70-73 °C; $[\alpha]_D^{20}$ +11.36 (c 1.98, CHCl$_3$)

4.3.42. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-valine methyl ester 127b

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129b, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 29 days, (96 mg, 87%).

R$_f$ (Hexane:EtOAc, 6:1) 0.20; d.e. 27%; Mp 58-59 °C; $[\alpha]_D^{20}$ +7.14 (c 1.68, CHCl$_3$)

4.3.43. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129c, triethylamine, Novozyme, methanol, and toluene. Reaction time 2 days, (105 mg, 96%).
Experimental

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.55; d.e. 86%; Mp 102-103 °C; \([\alpha]^{20}_D +2.48\) (c 2.06, CHCl₃)

4.3.44. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-leucine methyl ester 127c

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129c, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 12 days, (105 mg, 96%).

R<sub>f</sub> (Hexane:EtOAc, 6:1) 0.55; d.e. 54%; Mp 94-96 °C; \([\alpha]^{21}_D +1.44\) (c 1.88, CHCl₃)

4.3.45. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan methyl ester 127d

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129d, triethylamine, Novozyme®, methanol, and toluene. Reaction stopped after 13 days, (91 mg, 84%).

R<sub>f</sub> (Hexane:Et₂O, 1:1) 0.20; d.e. 74%; Mp 71-73 °C; \([\alpha]^{20}_D +60.1\) (c 1.76, CHCl₃)

4.3.46. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tryptophan methyl ester 127d

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129d, triethylamine, Lipozyme®, methanol, and toluene. Reaction stopped after 13 days, (96 mg, 89%).

R<sub>f</sub> (Hexane:Et₂O, 1:1) 0.20; d.e. 7%; Mp 54-56 °C; \([\alpha]^{20}_D +15.72\) (c 1.80, CHCl₃)

4.3.47. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tert-leucine methyl ester 127e

The procedure outlined in Section (4.3.32.i.) was followed using oxazolone 129e, triethylamine, Lipozyme®, methanol, and toluene. Reaction time 28 days. Column chromatography, eluting with hexane:ethyl acetate (6:1) furnished starting oxazolone (73 mg, 73%) and the desired product as a colourless solid (13 mg, 12%, 44% based on recovered starting material).
R_f (Hexane:EtOAc, 6:1) 0.38; d.e. 72%; Mp 100-102 °C; \([\alpha]_D^{20} +3.75\) (c 0.24, CHCl_3)

4.3.48. (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-tert-leucine n-butyl ester 132e

The procedure outlined in Section (4.3.32.1.) was followed using oxazolone 129e, triethylamine, Lipozyme®, n-butanol, and toluene. Reaction time 28 days. \(^1\)H nmr (200 MHz, CDCl_3) of the crude product indicated mainly starting oxazolone. The title compound appeared to be present in only 5% calculated by comparison of the integrals at \(\delta_H\) 4.45 (NHCH) of product and \(\delta_H\) 3.81 (NCH) of each diastereomer of starting oxazolone. Due to the low conversion no further purification was attempted. R_f (Hexane:EtOAc, 6:1) 0.48.

4.3.49. (2R,2'R)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine-N-methylamide and (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine-N-methylamide 134a

The general procedure outlined in Section (4.3.20.) was followed with the biotransformation product obtained in Section (4.3.33.) (88 mg, 0.22 mmol) and lithium hydroxide monohydrate (19 mg, 0.45 mmol, 2.0 equiv.) to furnish the hydrolysis product as a colourless gum (85 mg, quantitative). The product was not characterised but used directly in the next step of the synthesis.
The procedure outlined in Section (4.3.9.ii.) was followed using TBTU (87 mg, 0.27 mmol, 1.2 equiv.), HOBt (37 mg, 0.27 mmol, 1.2 equiv.), diisopropylethylamine (118 µL, 0.67 mmol, 3.0 equiv.), the acid obtained above (85 mg, 0.22 mmol, 1.0 equiv.) and methylvamine hydrochloride (18 mg, 0.26 mmol, 1.1 equiv.). Column chromatography, eluting with ethyl acetate:hexane (2:1) furnished the title compound as a colourless solid (78 mg, 88% from starting methyl ester). 'H nmr indicated a (1:2.75) ratio of diastereomers.

R<sub>f</sub> (EtOAc, 100%) 0.50; d.e. 42%; Mp 168-171 °C; (Found: C, 67.35; H, 9.05; N, 7.00; C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O<sub>4</sub> requires C, 67.66; H, 8.78; N, 7.17); [α]<sub>D</sub><sup>20</sup> +46.1 (c 1.56, CHCl<sub>3</sub>); v<sub>max</sub>(CHCl<sub>3</sub>)<sup>-1</sup> 3384 (amide NH), 3019 (saturated CH), 1712 (ester C=O), 1664, 1542 (CONH); δ<sub>H</sub> (CDCl<sub>3</sub>; 600 MHz)7.27-7.25 (total of 2 H, m, CH<sub>ar</sub> of each diastereomer), 7.22-7.18 (total of 3 H, m, CH<sub>ar</sub> of each diastereomer), 6.37 (0.71 H, br q NH<sup>B</sup>CH<sub>3</sub>), 6.13 (0.29 H, br q, NH<sup>B</sup>CH), 5.83 (total of 1 H, d, J 8.9, NHCH of each diastereomer), 4.84 (0.71 H, ddd, J 8.9, 4.9, NH<sup>A</sup>CH), 4.54 (0.29 H, ddd, NHCH<sup>B</sup>), 3.30 (0.71 H, dd, J 14.3, 5.0, CH<sup>A</sup>CH<sub>B</sub>Ph); 3.10 (0.29 H, dd, J 13.6, 6.6, CH<sup>A</sup>CH<sub>B</sub>Ph), 3.06 (0.29 H, dd, J 13.6, 7.6, CH<sub>B</sub>CH<sup>B</sup>Ph), 3.05 (0.29 H, dd, J 14.3, 8.6, CH<sub>B</sub>CH<sup>B</sup>Ph), 2.74 (2.13 H, d, J 4.6, NHCH<sup>B</sup>), 2.72 (0.71 H, dd, J 17.7, 11.3, OCH<sup>A</sup>CH<sub>B</sub>CH), 2.68 (0.87 H, d, J 4.9, NHCH<sup>B</sup>), 2.57 (0.29 H, m, CH<sub>2</sub>CH<sup>B</sup>CONH), 2.45 (0.29 H, dd, J 16.3, 8.9, OCH<sup>B</sup>CH<sub>B</sub>CH), 2.44 (0.29 H, dd, J 16.3, 4.9, OCH<sub>B</sub>CH<sub>B</sub>CH). 2.32 (0.71 H, m, CH<sub>2</sub>CH<sup>B</sup>CONH), 2.18 (0.71 H, dd, J 17.6, 3.3, OCCH<sub>A</sub>H<sub>B</sub>CH), 1.45 (0.29 H, ddd, J 14.6, 8.6, 6.0, CHCH<sup>B</sup>AH<sub>B</sub>CH), 1.40 (2.61 H, s, C(CH<sub>B</sub>)<sub>3</sub>), 1.39 (0.71 H, ddd, obscured by 'Bu ester peaks, CHCH<sup>A</sup>AH<sub>B</sub>CH), 1.38 (6.39 H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.15 (0.29 H, m, CH<sup>B</sup>(CH<sub>3</sub>)<sub>2</sub>), 0.98 (0.71 H, m, CH<sup>A</sup>(CH<sub>3</sub>)<sub>2</sub>), 0.93 (0.71 H, ddd, 13.6, 9.6, 4.6, CHCH<sub>A</sub>H<sub>B</sub>CH), 0.92 (0.29 H, ddd, J 14.6, 9.6, 4.9, CHCH<sub>A</sub>H<sub>B</sub>CH), 0.84 (0.87, d, J 6.3, CH<sub>3</sub>A CHCH<sub>3</sub>B), 0.80 (0.87, d, J 6.3, CH<sub>3</sub>A CHCH<sub>3</sub>B), 0.70 (2.13, d, J 6.3, CH<sub>3</sub>A CHCH<sub>3</sub>B), 0.68 (2.13, d, J 6.3, CH<sub>3</sub>A CHCH<sub>3</sub>B); δ<sub>CH</sub> (CDCl<sub>3</sub>; 63 MHz) 174.8, 174.6, 173.1, 171.6, 171.3, 171.1 (CO<sub>2</sub>C and CONH), 136.9 (Ipso-Ar), 129.1, 129.0, 128.5, 128.4, 126.7 (CH<sub>ar</sub>), 81.3, 80.9 (C(CH<sub>3</sub>)<sub>3</sub>), 54.7, 53.6 (NHCH), 41.5, (O<sub>2</sub>CCH<sub>2</sub>), 41.0, 40.9 (CHCONH), 38.3, 38.0,
37.8, 37.1 (CH$_2$Ph and CHCH$_2$CH), 27.9 (C(CH$_3$)$_3$), 26.1, 26.0 (NHCH$_3$), 25.5, 24.9 (CH(CH$_3$)$_2$), 23.1, 22.6, 22.2, 21.7 (CH(CH$_3$)$_2$); m/z (FAB) 391 (52%, MH$^+$), 335 (69, MH$_2$$^+$-Bu), 304 (335-NH$_2$CH$_3$), 279 (33), 179 (27), 91 (40, CH$_2$Ph), 57 (54, 'Bu), Found (FAB) 391.2595, C$_{22}$H$_{35}$N$_2$O$_4$ requires 391.2597.
5.0.0. Bibliography


73 S. Caddick and K. Faber, *Synthesis*, 1996, **447**.
H. S. Bevinakatti, A. A. Banerji, R. V. Newadkar, and A. A. Mokashi,

J. Z. Crich, R. Brieva, P. Marquart, R.-L. Gu, S. Flemming, and C. J. Sih,

N. J. Turner, J. R. Winterman, R. McCague, J. S. Parratt, and S. J. C. Taylor,


J. R. Winterman, *Thesis: Chemo-Enzymic Methods for The Synthesis of
Optically Active α-Amino Acids*, Ph. D, University of Exeter, 1996.

The Fluka Lipase Basic Kit was a gift from Fluka.

M.-C. Parker, S. A. Brown, L. Robertson, and N. J. Turner,

B. Berger, C. G. Rabiller, K. Königsberger, K. Faber, and H. Griengel,

T. Maugard, M. Remaud-Simeon, D. Petre, and P. Monsan,

N. W. Boaz and R. L. Zimmerman,

D. K. Barnes, E. Campagne, and R. L. Shriner,

B. K. Hwang, Q.-M. Gu, and C. J. Sih,

B. K. Hwang, Q.-M. Gu, and C. J. Sih,

S. Conde, P. Lopez-Serrano, M. Fierros, M. I. Biezama, A. Martinez, and M. I. Rodriguez-Franco,

N. R. A. Beeley, P. R. J. Ansell, and A. J. P. Docherty,

A. H. Davidson, A. H. Drummond, W. A. Galloway, and M. Whittaker,

R. P. Beckett, A. H. Davidson, A. H. Drummond, P. Huxley, and M. Whittaker,
*Drug Discovery Today*, 1996, 1, 16.

A. Zask, J. I. Levin, L. M. Killar, and J. S. Skotnicki,

Bibliography

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X-ray crystal structure for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically
Appendices

Crystal data and structure refinement for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically

A. Crystal data

Empirical formula \( \text{C}_{22}\text{H}_{33}\text{NO}_{5} \)
Formula weight 391.51
Wavelength 1.54180 Å
Temperature 150 K
Crystal system Monoclinic
Space group P21
Unit cell dimensions
\( a = 12.401(5) \text{ Å} \) \( \alpha = 90^\circ \)
\( b = 6.238(3) \text{ Å} \) \( \beta = 104.43(3)^\circ \)
\( c = 14.793(5) \text{ Å} \) \( \gamma = 90^\circ \)
Volume 1108.3 Å³
Number of reflections for cell32 (20 < \( \theta < 22^\circ \))
Z 2
Density (calculated) 1.17 Mg/m³
Absorption coefficient 0.63 mm⁻¹
F(000) 425.22

B. Data collection

Crystal description colourless needle
Crystal size 0.44 x 0.06 x 0.08 mm
\( \theta \) range for data collection 0.00 to 0.00°
Index ranges \(-15 \leq h \leq 14, -7 \leq k \leq 7, \leq l \leq 18 \)
Reflections collected 3011
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Table 1 Atomic co-ordinates (x 10^4), equivalent isotropic displacement parameters (Å^2 x 10^3) and site occupancies for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

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Table 2 Bond lengths [Å] for (2R,2'S)-(4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically.

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Table 3 Bond Angles (°) for \((2R,2' S)\)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically.

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Symmetry transformations used to generate equivalent atoms.
Table 4 Anisotropic displacement parameters (\(\text{A}^2 \times 10^3\)) for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised chemically.

The anisotropic displacement factor exponent takes the form: 
\[-2 \pi^2 \left[ h^2 a^* U_{11} + \ldots + 2 \, h \, k \, a^* \, b^* \, U_{12} \right]\]

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X-ray crystal structure for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically
Crystal data and structure refinement for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically

A. Crystal data

Empirical formula \( \text{C}_{22}\text{H}_{33}\text{NO}_{5} \)

Formula weight 391.49

Wavelength 1.54178 Å

Temperature 220(2) K

Crystal system Monoclinic

Space group \( \text{P 2(1)} \)

Unit cell dimensions
\[
\begin{align*}
\text{a} &= 12.453(3) \text{ Å} & \alpha &= 90^\circ \\
\text{b} &= 6.2652(13) \text{ Å} & \beta &= 104.06(2)^\circ \\
\text{c} &= 14.866(3) \text{ Å} & \gamma &= 90^\circ \\
\end{align*}
\]

Volume 1125.1(4) Å\(^3\)

Number of reflections for cell43 (20 < \( \theta < 22^\circ \))

\( Z = 2 \)

Density (calculated) 1.156 Mg/m\(^3\)

Absorption coefficient 0.656 mm\(^-1\)

\( F(000) = 424 \)

B. Data collection

Crystal description Colourless rod

Crystal size 0.86 x 0.08 x 0.08 mm

\( \theta \) range for data collection 3.06 to 60.03°

Index ranges \(-13 \leq h \leq 13, 0 \leq k \leq 6, 0 \leq l \leq 16\)

Reflections collected 2216

Independent reflections 1813 [R(int) = 0.0521]

Scan type Omega-theta

Absorption correction Psi-scans (\( T_{\text{min}} = 0.555, T_{\text{max}} = 0.782 \))
C. Solution and refinement

Solution          Direct (SHELXS-97 (Sheldrick, 1990))
Refinement type    Full-matrix least-squares on $F^2$
Program used for refinement SHELXL-97
Hydrogen atom placement difmap and geometric
Hydrogen atom treatment mixed
Data / restraints / parameters 1813/1/368
Goodness-of-fit on $F^2$ 1.014
Conventional R [F>4σ(F)] R1 = 0.0433 [1393 data]
Weighted R ($F^2$ and all data) wR2 = 0.1144
Absolute structure parameter 0.2(5)
Extinction coefficient 0.0048(9)
Final maximum $δ/σ$ 0.187
Weighting scheme calc $w=1/[s^2(Fo^2)^2+(0.0672P)^2+0.0250P]$ where $P=(Fo^2+2Fc^2)/3$
Largest diff. peak and hole 0.155 and -0.157 e. Å$^{-3}$
Table 1 Atomic co-ordinates ($x \times 10^4$), equivalent isotropic displacement parameters ($Å^2 \times 10^3$) and site occupancies for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically. $U_{eq}$ is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

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Table 2 Bond lengths [Å] for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically.

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Appendices
Table 3 Bond Angles[Å] for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically.

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Symmetry transformations used to generate equivalent atoms
Table 4 Anisotropic displacement parameters ($\text{A}^2 \times 10^3$) for \textit{(2R,2'S)-[4-\text{tert-butyl}-2-\text{iso-butyl-succinyl}]-phenylalanine methyl ester 127a} synthesised enzymatically.

The anisotropic displacement factor exponent takes the form: $-2 \pi^2 \left[ h^2 a^2 U_{11} + ... + 2 h k a^* b^* U_{12} \right]$

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<td>35(3)</td>
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<td>51(3)</td>
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<td>42(3)</td>
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<tr>
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<td>55(3)</td>
<td>5(3)</td>
<td>16(2)</td>
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Table 5 Hydrogen co-ordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for (2R,2'S)-[4-(tert-butyl)-2-iso-butyl-succinyl]-phenylalanine methyl ester 127a synthesised enzymatically.

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U(eq)</th>
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<td>H(161)</td>
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<td>852</td>
<td>892</td>
<td>148</td>
</tr>
<tr>
<td>H(162)</td>
<td>9859</td>
<td>2227</td>
<td>829</td>
<td>148</td>
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<tr>
<td>H(163)</td>
<td>8733</td>
<td>2484</td>
<td>65</td>
<td>148</td>
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<td>H(171)</td>
<td>9884</td>
<td>6092</td>
<td>1120</td>
<td>106</td>
</tr>
<tr>
<td>H(172)</td>
<td>8914</td>
<td>7163</td>
<td>1479</td>
<td>106</td>
</tr>
<tr>
<td>H(173)</td>
<td>8719</td>
<td>6495</td>
<td>424</td>
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<td>H(181)</td>
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<td>5107</td>
<td>1543</td>
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<td>H(182)</td>
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<td>1406</td>
<td>167</td>
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<td>H(183)</td>
<td>7094</td>
<td>4126</td>
<td>535</td>
<td>167</td>
</tr>
<tr>
<td>H(164)</td>
<td>8430</td>
<td>1878</td>
<td>173</td>
<td>116</td>
</tr>
<tr>
<td>H(165)</td>
<td>8239</td>
<td>794</td>
<td>1084</td>
<td>116</td>
</tr>
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<td>H(166)</td>
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<td>1200</td>
<td>978</td>
<td>116</td>
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<td>H(174)</td>
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<td>5909</td>
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<td>74</td>
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<td>10023</td>
<td>5031</td>
<td>796</td>
<td>74</td>
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<tr>
<td>H(176)</td>
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<td>6886</td>
<td>1201</td>
<td>74</td>
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<td>H(184)</td>
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<td>5737</td>
<td>1369</td>
<td>73</td>
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<td>H(185)</td>
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<td>3329</td>
<td>1660</td>
<td>73</td>
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<td>H(186)</td>
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<td>599</td>
<td>73</td>
</tr>
<tr>
<td>H(42)</td>
<td>9800(40)</td>
<td>2140(80)</td>
<td>3850(30)</td>
<td>49(13)</td>
</tr>
<tr>
<td>H(41)</td>
<td>10420(30)</td>
<td>4900(80)</td>
<td>3870(30)</td>
<td>30(11)</td>
</tr>
<tr>
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<td>12150(30)</td>
<td>3280(70)</td>
<td>4000(30)</td>
<td>35(11)</td>
</tr>
<tr>
<td>H(511)</td>
<td>11360(40)</td>
<td>790(80)</td>
<td>5260(30)</td>
<td>42(13)</td>
</tr>
<tr>
<td>H(512)</td>
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<td>1430(90)</td>
<td>5430(40)</td>
<td>63(16)</td>
</tr>
<tr>
<td>H(521)</td>
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<td>4230(70)</td>
<td>5530(30)</td>
<td>39(12)</td>
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<tr>
<td>H(531)</td>
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<td>6340(140)</td>
<td>4920(50)</td>
<td>110(30)</td>
</tr>
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<td>H(532)</td>
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<td>6950(110)</td>
<td>6040(40)</td>
<td>80(18)</td>
</tr>
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<td>H(533)</td>
<td>13250(50)</td>
<td>5160(100)</td>
<td>5690(40)</td>
<td>75(18)</td>
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</table>
\begin{table}
\centering
\caption{Table 5 cont.}
\begin{tabular}{cccc}
\hline
Atom & x & y & z & U(eq) \\
\hline
H(541) & 11550(50) & 1980(120) & 6880(40) & 100(20) \\
H(542) & 12820(50) & 2670(90) & 6990(40) & 72(17) \\
H(543) & 11950(40) & 4370(100) & 7120(40) & 62(18) \\
H(71) & 13080(60) & 260(120) & 3580(50) & 110(30) \\
H(81) & 12320(40) & -3520(80) & 3240(30) & 48(14) \\
H(831) & 7840(140) & -7840(140) & 3320(50) & 110(30) \\
H(832) & 15380(50) & -5840(120) & 4270(50) & 90(20) \\
H(833) & 15660(50) & -5550(120) & 3400(50) & 100(20) \\
H(91) & 11770(30) & -1630(70) & 1810(30) & 30(10) \\
H(92) & 12580(40) & -4010(90) & 1780(30) & 45(13) \\
H(111) & 12290(40) & 1770(80) & 1420(30) & 45(13) \\
H(121) & 13470(40) & 3720(110) & 840(40) & 60(18) \\
H(131) & 15100(40) & 2880(80) & 720(30) & 52(14) \\
H(141) & 15750(40) & -960(90) & 1170(30) & 64(15) \\
H(151) & 14530(30) & -3010(80) & 1770(30) & 29(11) \\
\hline
\end{tabular}
\end{table}
6.2.0. Appendix III

Publications
Enhancement of \textit{Candida antarctica} lipase B enantioselectivity and activity in organic solvents

Marie-Claire Parker,,*a† Stuart A. Brown,b Lindsey Robertsonb and Nicholas J. Turnerb

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\textsuperscript{b} Edinburgh Centre for Protein Technology, Department of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK EH9 3JJ

The enantioselectivity and catalytic activity of Novozym 435\textsuperscript{b} [\textit{Candida antarctica} lipase B (CALB)] in organic solvents was found to dramatically increase upon the addition of a non-reactive organic base, such as Et\textsubscript{3}N, to the reaction system.

It has been shown that the unusual microenvironment of enzymes in organic solvents can affect a number of parameters, including the degree of protein hydration,\textsuperscript{1,2} secondary structure,\textsuperscript{3} the susceptibility of the protein to inactivation and variations in the ionisation state\textsuperscript{4} of side-chain residues. Frequently, these differences have been shown to result in interesting changes in the enzymes, including reversal of substrate specificity and changes in stereoselectivity, although the underlying reasons remain poorly understood.

It is commonly accepted that the best predictor of enzyme catalytic activity in low water organic media is thermodynamic water activity (\(a_w\)). Over the past few years although much has been reported on enzyme enantioselectivity in organic media there are as yet no predictive rules available. Crude lipase preparations have proved to be simple and effective biocatalysts for kinetic resolutions,\textsuperscript{6} for example, using chiral carboxylic acids and alcohols. However, the low purity of these preparations (presence of other lipases and competing hydrolases) can, in specific reactions, lead to low and unpredictable enantioselective behaviour. This effect can be compounded when using organic solvents, due to the effect of different solvent properties on catalytic activity.

The starting point for the work described herein was the lipase (Lipozyme\textsuperscript{a} Mucor miehei) catalysed dynamic resolution of 4-substituted oxazol-5(4H)-ones, a reaction we have previously employed for the synthesis of enantiomerically pure (S)-L-tert-leucine.\textsuperscript{6} It was previously found that the modest enantioselectivity in toluene (ca. 68\% ee) could be enhanced (ca. 97\% ee) by the addition of a catalytic amount of Et\textsubscript{3}N to the reaction; the role of Et\textsubscript{3}N is not to facilitate racemisation of the substrate.

We decided to investigate this effect in more detail by using a commercially available immobilised lipase,\textsuperscript{§} Novozym 435 (\textit{Candida antarctica} lipase B\textsuperscript{b} (CALB)), since a larger substrate range could be tested with this enzyme. The catalytic activity and enantioselectivity of the alcoholysis of (±)-2-phenyl-4-benzoxazol-5(4H)-one 1 using butan-1-ol as the nucleophile (Scheme 1) was monitored\textsuperscript{7} under a range of reaction conditions, including controlled water activity. Hydration was controlled by equilibrating\textsuperscript{8} enzyme and solvent with the appropriate saturated salt solution\textsuperscript{7} of known thermodynamic water activity \(a_w\). Therefore a low \(a_w\) system will be one in which the solvent is poorly hydrated and the enzyme, similarly, has a low level of hydration, and at high \(a_w\) (e.g. 0.97) the solvent is near water saturation and the enzyme is fully hydrated (as would be found in an aqueous system). Table I shows the effect of hydration on the initial catalytic rate and enantioselectivity, in three different solvents, n-hexane, toluene and MeCN, either with or without Et\textsubscript{3}N.\textsuperscript{**}

It can immediately be seen that the lipase-catalysed reaction is very sensitive to water activity. The addition of a non-reactive organic base,\textsuperscript{† †} Et\textsubscript{3}N, to the reaction enhances significantly both the enantioselectivity and catalytic activity of the enzyme. Even low levels of hydration, present in the more nonpolar solvents such as n-hexane and toluene, are detrimental to the overall catalytic performance of CALB. We find that generally for optimum yield and enantioselectivity, both the enzyme and solvent should be rigorously dried prior to addition of Et\textsubscript{3}N. We were interested to see if addition of Et\textsubscript{3}N to a reaction already in progress and of poor enantioselectivity, could reverse this effect. As can be seen from Fig. 1, the addition of Et\textsubscript{3}N after 140 min immediately results in enhanced catalytic rate and enantioselectivity.

In order to examine the generality of the effect of Et\textsubscript{3}N we investigated a second reaction, namely the CALB-catalysed

\begin{equation}
\begin{array}{c}
\text{Ph} & \text{O} & \text{O} \\
\text{Ph} & \text{H} & \text{Ph} \\
\text{HN} & \text{CO}_2 \text{Bu} & + \text{HN} & \text{CO}_2 \text{H}
\end{array}
\end{equation}

Scheme 1

Table I. Effect of water activity on initial catalytic rate\textsuperscript{a} and enantiospecificity as a function of hydration, with and without Et\textsubscript{3}N

<table>
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<tr>
<th>Solvent\textsuperscript{c}</th>
<th>(a_w)</th>
<th>No Et\textsubscript{3}N</th>
<th>Et\textsubscript{3}N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial rate/nmol min\textsuperscript{-1} mg\textsuperscript{-1}</td>
<td>Ee (%)</td>
<td>Initial rate/nmol min\textsuperscript{-1} mg\textsuperscript{-1}</td>
</tr>
<tr>
<td>n-hexane (anhydrous)</td>
<td>0</td>
<td>26 (± 1.5)</td>
<td>85 (± 3)</td>
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<tr>
<td>n-hexane</td>
<td>0.69</td>
<td>4 (± 0.5)</td>
<td>55 (± 2)</td>
</tr>
<tr>
<td>n-hexane</td>
<td>0.97</td>
<td>1.5 (± 0.15)</td>
<td>30 (± 5)</td>
</tr>
<tr>
<td>toluene</td>
<td>0</td>
<td>15 (± 0.8)</td>
<td>85 (± 4)</td>
</tr>
<tr>
<td>toluene</td>
<td>0.22</td>
<td>3</td>
<td>61 (± 6)</td>
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<tr>
<td>MeCN\textsuperscript{d}</td>
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<td>&gt;90</td>
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<tr>
<td>MeCN\textsuperscript{d}</td>
<td>0.1 (0.5% v/v H\textsubscript{2}O)</td>
<td>NR\textsuperscript{e}</td>
<td>5 (± 0.3)</td>
</tr>
<tr>
<td>MeCN\textsuperscript{d}</td>
<td>0.4 (2% v/v H\textsubscript{2}O)</td>
<td>NR\textsuperscript{e}</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Initial rate for (S)-butyl ester enantiomer 2. \textsuperscript{b} Results reported are the average of three separate measurements. \textsuperscript{c} Note I. \textsuperscript{d} Ref. 8. \textsuperscript{e} No reaction.

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reaction between 1-phenylacetoxycarbonyl-2-methylcyclohexene and butan-2-yl yielding 2-methylcyclohexanone and butyl phenylacetate.\(^{5,15}\) Using n-hexane (\(a_w = 0\)) and MeCN (0.5% H\(_2\)O, \(a_w = 0.1\)) as the solvents, we observed that the addition of Et\(_3\)N to the solvent resulted in a dramatic increase in the catalytic activity. An approximate 200-fold increase in activity was observed in MeCN (\(a_w = 0.1\)) and a 700-fold one for that in n-hexane (\(a_w = 0.97\)). The higher activity found in n-hexane is presumably due to a more intimate contact between the enzyme and Et\(_3\)N in a more nonpolar environment. Similarly, the activation effect for (\(\pm\))-2-phenyl-4-benzyloxazol-5(4H)-one ring-opening in MeCN is similar to that described above and is expected to be a result of less Et\(_3\)N adsorption to the enzyme in MeCN.

The ability of organic bases to increase the enantioselectivity of lipase-catalysed reactions in water-saturated organic solvents has previously been reported.\(^{10-13}\) In some cases, this effect has been attributed to the formation of an ion-pair between the base and any by-product acid. Using electrospray ionisation mass spectrometry (ESI-MS),\(^{1+}\) we have detected the formation of carboxylic acid 3 during the course of the oxazoline reaction at intermediate to high water activities (e.g. \(a_w = 0.69-0.97\)).

We have also found that addition of acid 3 to an already hydrated system results in loss of activity, which can be fully recovered upon addition of an organic base, presumably via formation of an ion pair. Ion pair formation is observed in both low and high dielectric non-hydrogen bonding solvents such as n-hexane and MeCN. In a high dielectric, non-hydrogen bonding solvent such as MeCN, where the acid was found to be more soluble, we find experimentally that dissolution of acid 3 in n-hexane and MeCN occurs upon addition of Et\(_3\)N, thus removing acid from the immediate microenvironment of the enzyme. However, the enhancement of catalytic performance and enantioselectivity for rigorously dried samples, and those of low water activity (\(a_w < 0.7\)) where we find no evidence for hydrolysis over the course of the initial rate measurement, cannot be explained in terms of hydrolysis products affecting enantioselectivity, since for an unrelated substrate, an activating effect on the catalytic activity has been demonstrated.

The addition of co-solvents, such as DMF and DMSO, was found to solubilise the acid and thus it was anticipated that they would perform a similar role to Et\(_3\)N in removing any acid from the immediate vicinity of the enzyme. Both DMF and DMSO were chosen as additives to the bulk organic solvent (toluene at \(a_w = 0.22\)). Although both DMF and DMSO increased the enantioselectivity of the reaction by 3-55% ee, there was no significant effect on the catalytic rate as found with Et\(_3\)N. Since the solvation of the carboxylic acid by these co-solvents occurs by a different mechanism to that of Et\(_3\)N, i.e. the additives are unable to form ion-pairs, they have limited use in reducing the overall effect.

The role of Et\(_3\)N therefore appears to be dual in nature, i.e. increasing both the enantioselectivity and catalytic activity of lipase-catalysed reactions. The addition of Et\(_3\)N therefore provides an additional strategy for improving the enantioselectivity of lipase-catalysed reactions. We are currently investigating this effect with other lipolytic enzymes.

We are grateful to Boehringer Mannheim, Germany, for the generous gift of lipase samples. The BBSC is acknowledged for a David Phillips Fellowship (M. C. P.) and a studentship (S. A. B.).

**Notes and References**

† E-mail: m.parker@chem.gla.ac.uk
‡ The thermodynamic water activity (\(a_w\)) describes the mass action effect of water on hydrolytic equilibria and also describes the partitioning of various water phases that can compete for water binding (ref. 1).

§ Polycrystalline gel electrophoresis of CALB desorbed from the solid support exhibited a single band corresponding to the reported molecular weight of CALB (33 KDa) (ref. 6).

¶ (\(\pm\))-2-Phenyl-4-benzyloxazol-5(4H)-one \(1.00\) mmol was placed in a 4 ml screw top vial together with the solvent, (either anhydrous or hydrated), butan-1-ol (0.24 mmol, 1.5 equiv.) CALB (40 mg) and Et\(_3\)N (14 mol%). The reaction vial was shaken at 230 rpm on a rotary shaker at 37 °C and the progress and ee (%) of the reaction were monitored by chiral HPLC (Chiralcel-OD, 250 × 4.6 mm, Malinckrodt Baker, n-hexane–PrOH (90:10 v/v), UV detection \(\lambda = 254\) nm).

**Candida antarctica** lipase B (CALB) was received as an immobilised preparation (Novozym 435, Boehringer Mannheim, Germany) and was dehydrated over P\(_2\)O\(_5\) (at room temp.) for 2–3 days. Rehydration of dried lipase to the desired water activity (\(a_w\)) was carried out using saturated salt solutions (equilibration period 48–72 h). (\(\pm\))-2-Phenyl-4-benzyloxazol-5(4H)-one \(1\) was stored over P\(_2\)O\(_5\) at 0 °C; anhydrous solvents were stored over freshly reactivated 3 Å or 4 Å molecular sieves. The water content of dried solvents was measured using Karl Fischer water titration (ref. 15) and found to be < 0.001 wt%. Solvents were hydrated separately from the enzyme using the same water equilibration procedure as described above, approximately 24 h before use.

**Control reactions** showed that no detectable ester (as judged by HPLC) was formed in the absence of enzyme, either with or without Et\(_3\)N, over a 48 h analysis period.

†† Other organic bases give very similar results to Et\(_3\)N, e.g. DABCO and lutidine. Insoluble inorganic bases, e.g. KHCO\(_3\) and K\(_2\)CO\(_3\), had no effect and did not result in the high catalytic rate and enantioselectivity observed with the soluble organic bases.

¶¶ Electrospray ionisation mass spectrometry (ESI-MS) and atmospheric chemical ionisation (APCI) were performed on a Micromass Platform II spectrometer (cone voltage 20 V).


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