A Versatile Chemo-Enzymatic Route to Enantiomerically Pure β-Branched-α-Amino Acids

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Declaration

This thesis is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Edinburgh and is my own composition. Unless otherwise stated, the work described was carried out by myself and is original. The contents of this thesis have not been previously submitted, in whole or in part, for any degree at this or any other university.
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Lastly I would like to thank my parents and Sally for putting up with me through the highs and lows, what more can I say?
Abstract

Enantiomerically pure β-branched-α-amino acids are important synthetic targets and a challenging group of compounds to prepare. A series of diastereoisomers of β-methyl-β-phenylalanine analogues have been prepared in enantiomerically pure form using a combination of chemo-and bio-catalysis.

Starting from L-threonine methyl ester and proceeding through the vinyl halide with Suzuki chemistry, a range of β,β-disubstituted didehydroamino acids were obtained as their (Z)-isomers. Asymmetric hydrogenation of these alkenes, using either the [Rh(R,R)-Et-DuPhos(COD)]BF₄ or [Rh(S,S)-Et-DuPhos(COD)]BF₄ catalyst, followed by hydrolysis, yielded two of the four possible sets of diastereoisomers of the β-branched amino acid. Subsequent stereoinversion, using a stereoselective amino acid oxidase in combination with a non-selective reducing agent, furnished the remaining two sets of diastereomers.
Abbreviations

AAO  Amino acid oxidase
Ac   Acetyl
BINAP 2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
Boc  Tertiary-butyloxy carbonyl
BPE  1,2-Bis(phospholano)ethane
br.  Broad
CAMP Cyclohexyl(ortho-methoxyphenyl)methyl phosphine
CAr  Aromatic carbon
Cbz  Benzyloxy carbonyl
ChiraPhos  2,3-Bis(diphenylphosphino)butane
COD  Cyclooctadiene
d   Doublet
DABCO 1,4-diazo bicyclo[2.2.2]octane
DAST (Diethylamino)sulfur trifluoride
DBU  1,8-Diazobicyclo[5.4.0]undec-7-ene
DCC  1,3-Dicyclohexylcarbodiimide
DCM  Dichloromethane
dd  Doublet of doublets
DDAA Didehydroamino acid
d.e. Diastereomeric excess
DEAD Diethyl azodicarboxylate
DIEA Diisopropylethylamine
DIPAMP 1,2-Bis(ortho-anisylphenylphosphino)ethane
DMAP 4-Dimethylaminopyridine
DOPA 3-(3,4-dihydroxyphenyl)alanine
DuPhos 1,2-Bis(phospholano)benzene
E Phos  Electrophile
EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
e.e. Enantiomeric excess
EI  Electron impact
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>eq.</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ES-</td>
<td>Negative ion electrospray</td>
</tr>
<tr>
<td>ES+</td>
<td>Positive ion electrospray</td>
</tr>
<tr>
<td>FAB</td>
<td>Fast atom bombardment</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>Hex</td>
<td>Hexane</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropylalcohol</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MePhe</td>
<td>Methylphenylalanine</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl</td>
</tr>
<tr>
<td>MTBE</td>
<td>Tertiary-Butyl methyl ether</td>
</tr>
<tr>
<td>NAPHOS</td>
<td>2,2'-Bis(diphenylphosphinomethyl)-1,1'-binaphthyl</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Nu</td>
<td>Nucleophile</td>
</tr>
<tr>
<td>PAMP</td>
<td>Phenyl(ortho-methoxyphenyl)methyl phosphine</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Palladium on activated carbon</td>
</tr>
<tr>
<td>pkD-AAO</td>
<td>Porcine kidney D-amino acid oxidase</td>
</tr>
<tr>
<td>PNB</td>
<td>para-Nitrobenzoyl</td>
</tr>
<tr>
<td>p-TSA</td>
<td>para-Toluenesulfonic acid</td>
</tr>
<tr>
<td>quin</td>
<td>Quintet</td>
</tr>
<tr>
<td>Rgd-AAO</td>
<td>Rhodotorula gracilis D-amino acid oxidase</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SFC</td>
<td>Supercritical fluid chromatography</td>
</tr>
<tr>
<td>svl-AAO</td>
<td>Snake venom L-amino acid oxidase</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetra-normal-butylammonium fluoride</td>
</tr>
<tr>
<td>TBDMS-OTf</td>
<td>Tertiary-Butyldimethylsilyl trifluoromethanesulfonate</td>
</tr>
</tbody>
</table>
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
TLC  Thin layer chromatography
TRAP 2,2'-Bis[1-(dialkylphosphino)ethyl]1,1'-biferrocene
Ts  Tosyl
TvD-AAO  *Trigonopsis variabilis* D-amino acid oxidase
Contents

1 Introduction .............................................................................................................. 1
  1.1 β-Branched amino acids ..................................................................................... 1
    1.1.1 Overview .................................................................................................. 1
    1.1.2 Unnatural amino acids in pharmaceuticals ................................................. 1
    1.1.3 Unnatural amino acids in peptides ........................................................... 2
    1.1.4 Synthesis of β-branched amino acids ......................................................... 4
  1.2 Didehydroamino acids ....................................................................................... 11
    1.2.1 Overview .................................................................................................. 11
    1.2.2 Routes to didehydroamino acids ............................................................... 12
      1.2.2.1 β-elimination reactions ....................................................................... 12
      1.2.2.2 Hydrolysis of oxazolone ..................................................................... 15
      1.2.2.3 Wittig related routes .......................................................................... 16
      1.2.2.4 Other routes to didehydroamino acids ............................................... 17
  1.3 Asymmetric hydrogenation ............................................................................... 19
    1.3.1 Overview .................................................................................................. 19
    1.3.2 The history of asymmetric hydrogenation ............................................... 20
    1.3.3 Mechanism of asymmetric hydrogenation ................................................. 26
  1.4 Stereoinversions ............................................................................................... 29
    1.4.1 Overview .................................................................................................. 29
    1.4.2 Enzymes in chemistry .............................................................................. 29
    1.4.3 Kinetic resolution .................................................................................... 30
    1.4.4 Dynamic kinetic resolution ..................................................................... 30
    1.4.5 Deracemisation ....................................................................................... 31
    1.4.6 Amino acid oxidases .............................................................................. 33
2 Results and Discussion ......................................................................................... 38
  2.1 Hydrogenation of didehydroamino acids prepared via oxazolones .................. 38
    2.1.1 Overview .................................................................................................. 38
    2.1.2 Imine synthesis ....................................................................................... 39
    2.1.3 Oxazolone synthesis .............................................................................. 40
    2.1.4 Didehydroamino acid synthesis .............................................................. 42
2.1.5 N-Acetyl to N-Boc conversion ................................................. 43
2.1.6 (E/Z)-Alkene asymmetric hydrogenations .............................. 44
2.1.7 Conclusion .............................................................................. 49
2.2 Threonine approach ........................................................................ 50
  2.2.1 Overview .................................................................................. 50
  2.2.2 Dehydration of threonine ........................................................... 50
  2.2.3 Bromination ............................................................................. 52
  2.2.4 Suzuki chemistry with vinyl bromides ...................................... 53
  2.2.5 Iodination ................................................................................ 55
  2.2.6 Suzuki chemistry of vinyl iodides ............................................. 55
  2.2.7 (Z)-Alkene asymmetric hydrogenations .................................... 56
  2.2.8 Hydrolysis ............................................................................... 57
  2.2.9 (E)-Alkene asymmetric hydrogenation ..................................... 58
  2.2.10 Conclusion ............................................................................. 62
2.3 Stereoinversions ............................................................................ 63
  2.3.1 Overview ................................................................................. 63
  2.3.2 Oxidation assay ...................................................................... 63
  2.3.3 Optimisation ............................................................................ 65
  2.3.4 L-AAO stereoinversion ............................................................... 67
  2.3.5 D-AAO stereoinversion ............................................................... 68
  2.3.6 Stereoinversion of (2R,3R)-amino acids .................................... 70
  2.3.7 Scale-up .................................................................................. 72
  2.3.8 Conclusions ........................................................................... 73
2.4 Application of project - Synthesis of an altemicidin mimic .......... 75
  2.4.1 Overview ................................................................................ 75
  2.4.2 Retrosynthetic strategy .............................................................. 76
  2.4.3 Scaffold branch ...................................................................... 78
  2.4.4 Sulfonamide branch ................................................................. 80
  2.4.5 The coupling of the two branches ........................................... 81
  2.4.6 Screening ............................................................................... 82
  2.4.7 Conclusions ........................................................................... 83
3 Experimental

3.1 General procedures

3.2 Experimental procedures – Synthesis of β-branched-α-amino acids

3.2.1 General procedure for synthesis of imines

3.2.2 General procedure for synthesis of oxazolones

3.2.3 General procedure for the synthesis of didehydroamino acids

3.2.4 Threonine dehydration approach

3.2.5 General procedure for Suzuki coupling

3.2.6 General procedure for high pressure hydrogenations

3.2.7 Asymmetric hydrogenation of (E)-alkenes

3.2.8 Asymmetric hydrogenation of (Z)-alkenes

3.2.9 General procedure for hydrolysis of amino acid methyl esters

3.2.10 General procedure for stereoinversions (HPLC scale)

3.2.11 Stereoinversion on a 1g scale

3.3 Experimental procedures - Altemicidin mimic project

3.3.1 General procedure for N-Boc to N-Z conversion

3.3.2 General procedure for the synthesis scaffold branch

3.3.3 General procedure deprotection of the scaffold branch

3.3.4 General procedure for coupling of the two branches

3.3.5 General procedure for mimic (Z)-deprotection

4 References


1 Introduction

1.1 β-Branched amino acids

1.1.1 Overview

β-Branched-α-amino acids are useful synthetic targets as they are the constituents of some pharmaceuticals and peptides.¹ By the replacement of amino acids in a biologically active peptide with unnatural β-branched amino acids, the added bulk of the β-substituent can affect the conformation of peptides allowing a powerful approach to studying proteins.²

The introduction of β-substituents gives the amino acid two stereogenic centres at the C(2) and C(3) position and results in four isomers, the threo (2R,3S) and (2S,3R) pair and the erythro (2R,3R) and (2S,3S) pair (Figure 1.1).

Whereas the natural β-branched amino acids such as threonine and isoleucine are produced through biosynthetic pathways, the unnatural amino acids in their enantiomerically pure form have traditionally been synthesised through the use of the chiral pool or chiral auxiliaries. With recent developments such as asymmetric hydrogenation of didehydroamino acids and the use of enzymes, a greater variety of unnatural amino acids can be readily produced.

1.1.2 Unnatural amino acids in pharmaceuticals

As more and more drugs are designed to target enzymes, hormones or receptors, chirality is of great importance in drug development. It has recently been stated that the worldwide sales of formulated drugs in single enantiomer dosage form is growing at an annual rate of 13%.³ Roughly a quarter of all drugs sold are single
enantiomer drugs of which there are numerous examples containing unnatural amino acids. L-DOPA 1 is used to reduce some of the symptoms of Parkinson's disease such as trembling, rigidity and slow movements. D-Penicillamine 2 is used for the treatment of arthritis. Many marketed drugs contain unnatural amino acid components. D-Phenylglycine 3 and its p-hydroxy analogue 4 are used in the broad spectrum antibiotics ampicillin and amoxicillin. D-2-Naphthylalanine 5, found in the drug Naferelin, is used in the treatment of high blood pressure (Figure 1.2).

As many pharmaceutical drugs contain amino acids, the production of enantiomerically pure amino acids is an increasing area of research. The introduction of unnatural amino acids or D-amino amino acids allows for a greater range of more potent pharmaceuticals to be developed by structure based design.

1.1.3 Unnatural amino acids in peptides

Selective incorporation of unnatural amino acids into proteins has become a powerful tool for protein design. A number of techniques are available for the introduction of amino acids into the protein. The earliest of these methods is site-directed mutagenesis which allows the replacement of any amino acid in the primary sequence of a protein by one of the twenty natural amino acids. This method does not allow for the incorporation of unnatural amino acids, of which a vast number have
been produced to be introduced. To achieve this, auxotrophs have been used. These are bacterial strains that are unable to produce one or more amino acids for themselves. By adding the unnatural amino acid during the growth of the auxotrophs, the amino acids are incorporated into the expressed protein. There is no control over where the amino acid is situated. More recently, unnatural amino acids have been incorporated into peptides using nonsense (stop codon) suppression methodology (Figure 1.3). In such a method a stop codon (TAG) is incorporated at the position coding for the residue of interest using site directed mutagenesis. A suppressor tRNA that recognises the stop-codon is prepared and acylated with the unnatural amino acid and then the two species added to a translation system from which the modified protein is obtained.

To allow for a variation in not only the amino acid residues present but their individual stereochemistry, direct chemical synthesis is a viable choice. Modern peptide synthesis through chemical ligation and expressed protein ligation allow for proteins of up to 200 residues to be prepared.

By introducing unnatural amino acids into petides, a variety of structural aspects can be investigated. The introduction of β-branched amino acids, especially the topographically constrained β-methylphenylalanine analogues, into proteins can effect the conformational constraints of the peptide and perhaps alter its biological activity. By the correct change of amino acid, features such as polarity and hydrophobicity of the peptide can be altered. As biological molecules do not usually contain fluorine, the use of fluorinated amino acids is of particular interest. The
incorporation of fluorine containing amino acids into proteins can lead to very stable protein folds as well directing highly specific protein-protein interactions. This has been demonstrated when the stability of the collagen triple helix is significantly increased by the replacement of hydroxyproline with fluoroproline.\(^\text{12}\)

1.1.4 Synthesis of β-branched amino acids

β-Branched amino acids are produced in biosynthetic pathways (Scheme 1.1).\(^\text{13}\) Threonine 7 is generated in a five step sequence starting from aspartate 6. Threonine is present in the biosynthesis of isoleucine. Threonine is deaminated to α-ketobutyrate 8 by threonine deaminase and this reacts with pyruvate 9 in five enzymatic steps to generate isoleucine 11. Valine 10 is produced by a similar sequence except that the pyruvate reacts initially with another pyruvate molecule.

**Scheme 1.1** Biosynthetic pathway to natural β-branched amino acids

When it comes to discussing the synthesis of the unnatural β,β-disubstituted amino acids, the focus is when one of the branches is a methyl group and the other branch is the variable such as in β-methylphenylalanine (β-MePhe) and analogues with
varying aromatic substituents. Optically pure aziridines 13 produced from 12 have been shown to be important building blocks in the synthesis of \(\alpha\)-amino acids and their derivatives (Scheme 1.2).\(^{14}\) It has been shown that the use aziridines can be used in the production of \(\beta\)-methylphenylalanine.\(^{15}\) \((2S,3R)-14\) was produced by this method although the diastereomer \((2S,3S)-14\) was obtained as a minor by-product.

An alternative approach from Riera's group in the same year led to the production of two diastereomers of \(\beta\)-methylphenylalanine although the route involved many steps (Scheme 1.3).\(^{16}\) This stereodivergent approach starts from an epoxide 15 that is ring opened with Me\(_2\)CuCNLi\(_2\) to the \(\alpha\)-hydroxy acid which was converted to 16. The synthesis becomes divergent at this point with the synthesis of the mesylates \((2R,3R)-17\) and \((2S,3R)-17\) which are readily converted to the N-Boc protected amino acids \((2R,3R)-18\) and \((2S,3R)-18\) respectively.
There is not much literature precedence for the synthesis of all four isomers of β-methylphenylalanine analogues. Hruby’s group have shown that any isomer of β-MePhe can be produced by a combination of starting with a chiral carboxylic acid to introduce one stereogenic centre and further reaction in the presence of an oxazolidinone to produce the other (Scheme 1.4). In this case the chiral starting material was from 3-phenylbutyric acid, which itself was obtained by resolution from the racemic mixture and converted to an anhydride, and the oxazolidinone obtained from D-phenylalanine. There was a problem with the displacement reaction with the organic azide producing what was thought to be bis-azidomethane as a by-product, which on one occasion caused a major explosion. In a follow up paper, the route is slightly modified for large scale synthesis utilising a different oxazolidinone. However, a (resolving) classical crystallisation was still required. Although was obtained on multi-gram quantities and each step gave

**Scheme 1.3 Epoxide approach**
high yields, only 50% yield would ever be possible for any given isomer due to the resolution step.

Scheme 1.4 β-Methylphenylalanine from auxiliary route

Using an auxiliary approach Hruby’s group has synthesised all four isomers of a series of β-branched acids including indole-protected β-methyltryptophan $^{22,21}$ β-methylyrosine $^{23,22}$ β-methyl-3-(2'-naphthyl)alanine $^{24,23}$ and β-isopropylphenylalanine $^{25}$ (Figure 1.4).$^{24}$
There are several methods in the literature that generate each isomer of a β-branched amino acid from a mixture of all four isomers. This can be done either enzymatically or chemically. All four isomers of β-MePhe \( \text{21} \) can be obtained through the stereospecific hydrolysis of a mixture of all four isomers of the N-carbamoyl-β-methylphenylalanine \( \text{26} \) (Scheme 1.5).\(^\text{25}\) Using the L- or D-carbamoylase enzyme, the \textit{threo} isomers were hydrolysed. \((2R,3R)-\text{21}\) was also obtained through the D-carbamoylase although the reaction was significantly slower than with \((2R,3S)-\text{21}\). The other \textit{threo} isomer \((2S,3S)-\text{21}\) was obtained by chemical hydrolysis.

\[ \text{22} \quad \text{23} \]
\[ \text{24} \quad \text{25} \]

\( \text{HN} \quad \text{H}_2\text{N} \quad \text{H}_2\text{N} \quad \text{H}_2\text{N} \)
\( \text{Me} \quad \text{Me} \quad \text{Me} \quad \text{Me} \)
\( \text{22} \quad \text{23} \quad \text{24} \quad \text{25} \)
\( \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \quad \text{CO}_2\text{H} \)

\( \text{HO} \quad \text{Me} \quad \text{Me} \quad \text{Me} \)

\( \text{Figure 1.4} \ a) \ \beta\text{-methyltryptophan, } b) \ \beta\text{-methyltyrosine, } c) \ \beta\text{-methyl-3-(2\text{'-naphthyl}) alanine, } d) \ \beta\text{-isopropylphenylalanine} \)
A chemical separation of all four isomers of β-methyl-3-(2-naphthyl)alanine 24 has recently been achieved by Rivier’s group. This bulky amino acid is used to enhance the potency and selectivity of their octa-peptide scaffold by introducing conformational restraint. The mixture of the four isomers was readily obtained and the erythro and threo pairs of isomers were isolated through crystallisation. The separate enantiomers were obtained through crystallisation of the Boc protected enantiomeric pair. With these separation techniques there is only a theoretical yield of 25% that can be obtained for any one isomer.

The routes discussed are either specific for a given isomer/isomer pair of the β-methylphenylalanine analogues or, if all four isomers are required, relatively long and low yielding. An alternative synthetic strategy for all four isomers of a given analogue would be a general route to a common intermediate and then introduce molecular diversity and stereochemistry.
This has been demonstrated recently by the isolation of all four isomers of $\beta$-methylphenylalanine 21 through the use of semipreparative HPLC.\textsuperscript{27} Using the (E) and (Z) isomers of oxazolone 27 as a starting point in stereodivergent synthesis (Scheme 1.6), the threo and erythro pairs of 29 were obtained. HPLC was used to separate out the individual isomers.

\textbf{Scheme 1.6} HPLC aided production of four isomers of $\beta$-MePhe

The key intermediate in this route was the didehydroamino acid 28. Didehydroamino acids can also be reduced with an asymmetric catalyst providing an efficient route to enantiomerically pure amino acids.
1.2 Didehydroamino acids

1.2.1 Overview

Arguably one of the most useful strategies to producing enantiomerically pure β-branched α-amino acids is by the asymmetric hydrogenation of didehydroamino acids (Figure 1.5). Bis-phosphine complexes have been developed which catalyse such reactions to give high yields and high ees. Didehydroamino acids themselves are a synthetically challenging series of compounds to produce and this means that this route to amino acid synthesis is often overlooked in favour of the use of chiral auxiliaries.

Apart from being a useful precursor to amino acids, several of these didehydroamino acids have been found in the series of naturally occurring bacterial antimicrobial peptides known as Lantibiotics. Examples include the 34 amino acid residue peptide food preservative nisin produced by *Lactococcus lactis* and the 32 amino acid residue peptide antimicrobial compound subtilin from *Bacillus subtilis*. Both these small peptides contain a varying amount of the dehydroalanine and dehydrobutyryne (β-methyldehydroalanine) residues.

Didehydroamino acids are useful synthetically as the structure allows for both nucleophilic and electrophilic attack (Figure 1.6). S- and N-nucleophiles readily attack the double bond whereas O-nucleophiles do not react. Electrophilic attack at the β-centre can also be achieved such as in the reaction of such enamides with N-bromosuccinimide. The double bond of the didehydroamino acids also allow for cyclisation reactions such as Diels-Alder or 1,3-dipolar cycloadditions and various reduction reactions to give amino acids.
1.2.2 Routes to didehydroamino acids

1.2.2.1 β-elimination reactions

Didehydroamino acids bearing two different β-substituents can exist in two isomeric forms, (E) and (Z). Synthetic routes usually produce the (Z)-isomer as it is the more thermodynamically stable. The (Z)-isomer is more favourable for synthetic chemists as these are good substrates for asymmetric hydrogenation giving high enantiomeric excesses.28 Under identical conditions, the (E)-isomer reacts slower and gives lower ees. The (E)-isomer can also be produced separately but many of the methods produce a mixture of the two isomers that need to be separated.

One of the most widely used route to didehydroamino acids comes from β-elimination reactions. Early examples in the literature involve the dehydration of serine derivatives to give didehydroalanine 31. By protecting both the acid and amino group, the hydroxyl group can be converted into a more favourable leaving group and dehydrated. The first example of this was reported by Rothstein37 where the protected serine 30 was chlorinated and treated with ammonia (Scheme 1.7).

The dehydration process can be driven by heat as an alternative to chemical treatment. Heating a β-alkylsulphinyl derivative of serine 32, the didehydroalanine 33 can be produced in high yields (Scheme 1.8).38
Expanding this methodology to threonine derivatives allows for the introduction of a β-methyl group. Until recently this dehydration was achieved through the conversion of the hydroxyl group into a p-toluenesulfonate followed by base induced elimination. Other methods involve the direct dehydration of β-hydroxy amino acids using expensive reagents such as DAST (Scheme 1.9).

Nugent reported that threonine can be dehydrated by acetic anhydride in the presence of a base to give the alkene product in high yields and (Z)-selectivity in the case of threonine (Scheme 1.10). Dehydration of N-Boc serine and N-Boc threonine proceeds similarly with Boc anhydride to give the N,N-diBoc protected didehydroamino acid.

As the dehydration reactions all result in unsubstituted or methyl-substituted didehydroamino acids, there is clearly very little variation in the products. However, these alkenes can be very useful for palladium chemistry such as in the Heck reaction with aryl or vinyl iodides. This highly efficient reaction results in didehydroamino acids with great scope for variation and retention of stereochemistry. With large aromatic diversity when using dehydroalanine, the stereochemistry is
predominantly (Z). When using 35 the reaction proceeds with retention of stereochemistry. (Scheme 1.11).

\[
\text{Scheme 1.11 Heck reaction}
\]

Alkenes such as 35 and 36 are also very useful as they can be readily brominated which allows for further substitution through Suzuki chemistry (Scheme 1.12).\textsuperscript{34,45} In the case of dehydroalanine 36, bromination results in the (Z)-isomer of the vinyl bromide 37 whereas the bromination of dehydromethylalanine 35 results in a 1:1 mixture of (E/Z) isomers of 38. These isomers can be separated by column chromatography.\textsuperscript{34}

\[
\text{Scheme 1.12 Bromination}
\]

Vinyl bromides are very useful Suzuki substrates and react well with aryl or vinyl boronic acids. With didehydroamino acids, the use of Suzuki coupling allows for a great range of \(\beta,\beta\)-disubstituted didehydroamino acids to be produced as single isomers with retention of alkene geometry (Scheme 1.13).
Another important route to didehydroamino acids is the condensation of an aldehyde or ketone with glycine via oxazolones 41 which themselves are usually produced by a variation on the Erlenmeyer condensation reaction. This synthesis involves the reaction of an N-acyl glycine 39 with an aldehyde or ketone 40 in the presence of acetic anhydride as the cyclising agent (Scheme 1.14).

This condensation is typically performed using aldehydes and generally results in the single (Z)-isomer of 41. Using lead acetate as a modifier it is possible to perform the condensation with acetophenones. Using a substituted acetophenone, a mixture of isomers is obtained if the substituent is an electron withdrawing group. Electron donating groups tend to give the (Z)-isomer. This route has its problems as the reaction requires refluxing in acetic anhydride and this means that low boiling aldehydes and ketones cannot be used. An alternative to using such harsh conditions has been demonstrated by Kumar who used the activated carbonyl in the form of an imine 42 (Scheme 1.15). This also required a change of cyclisation method as imines are unstable in acidic conditions.
Oxazolones are ring opened by the nucleophilic attack of hydroxide, alcohols, amines or amino acids to give the N-protected didehydroamino acids, esters, amides or peptides respectively with retention of alkene configuration (Scheme 1.16).\textsuperscript{49}

1.2.2.3 Wittig related routes

As shown in the oxazolone route, coupling of glycine derivatives can be a potentially useful reaction. This has also been demonstrated well by Kinoshita's group who have developed several routes to didehydroamino acids from \(\alpha\)-tosylglycine derivatives. The first route uses \(\alpha\)-tosylglycine derivatives \(\text{43}\) in a reaction with DBU and nitro compounds (Scheme 1.17).\textsuperscript{50} This led to both mono and disubstituted acids \(\text{44}\) with good (Z)-selectivity.

In a follow-up paper, \(\alpha\)-tosylglycine derivatives were again used, this time in a Wittig-type reaction. A wide variety of monosubstituted derivatives \(\text{45}\) have been
produced by this method with excellent yields and again good (Z)-selectivity (Scheme 1.18).

Scheme 1.18 Wittig-type reaction

In a related reaction, the Wittig-Horner reaction has been used in the production of mono- and disubstituted didehydroamino acids 48. In this case N-benzyloxycarbonyl-2-(diethoxyphosphinyl)-glycine ethyl ester 47 can be readily prepared on a kilogram scale from N-benzyloxycarbonyl-2-ethoxyglycine ethyl ester 46 in 95% yield.29 The Wittig-Horner reaction of 47 with ketones works well and gives high yields (Scheme 1.19) of didehydroamino acids 48. The (Z)-selectivity is further increased by the presence of DBU.52

Scheme 1.19 Wittig-Horner type reaction

1.2.2.4 Other routes to didehydroamino acids

In addition to these three genres of reaction there are also numerous other reactions that result in didehydroamino acids such as using oxo acids and amides,53 azido compounds,54 and aziridines.55 The condensation of aldehydes or ketones with isocyanooacetates 49 is another useful route to N-formylidehydroamino acids 51 via oxazolones 50 (Scheme 1.20).56 This mild methodology allows for the use of sensitive aromatic and aliphatic carbonyl compounds and the N-formyl group can be converted into an N-acetylidehydroamino acid through the acylation of the potassium salt of the N-formylaminoacylate to give the N-formyl-N-acylidehydroamino acid ester and subsequent cleavage of the formyl group.
Scheme 1.20 Isocyanooacetate condensation
### Introduction

#### 1.3 Asymmetric hydrogenation

#### 1.3.1 Overview

Biologically active compounds such as pharmaceutical drugs and advanced materials such as liquid crystals are common place in our daily lives. The biological responses from many pharmaceuticals come from molecular and chiral recognition. This can pose a problem such as in the much publicised case of thalidomide during the 1960’s where both enantiomers perform different biological functions. In the past when chirality was needed, chemists used either biochemical processes or resolution of racemic mixtures. Another important drug, the rare amino acid L-DOPA 1 for the treatment of Parkinson’s disease relied on a key resolution step in the Hoffman-LaRoche process (Scheme 1.21).

![Scheme 1.21 Hoffman-LaRoche L-DOPA process](image)

In industry, resolution steps become very expensive with numerous recycling loops or crystallisations. Thus, large scale productions of monosodium L-glutamate, L-lysine and L-menthol have traditionally been made by biochemical routes even though the racemic forms are made relatively easily through synthetic chemistry. In recent times, with the invention of chiral phosphine ligands, asymmetric hydrogenation of unsaturated bonds has become a very commercially viable route to
the synthesis of a number of enantiomerically pure compounds. The advantages of asymmetric hydrogenation are the cheap, environmentally friendly reducing agent, hydrogen, and the chiral multiplication in which a single molecule of the chiral catalyst can generate thousands of stereogenic centres.

1.3.2 The history of asymmetric hydrogenation

Hydrogenation is a core technology in chemistry. The process was initiated at the end of the 19th century by P. Sabatier who used fine particles of nickel as heterogeneous catalysts. 62

An early report for an asymmetric catalyst for hydrogenation came from Akabori in a 1956 *Nature* paper. 63 Here it was reported that a heterogeneous asymmetric catalyst was prepared by the adsorption of palladium chloride on silk fibroin fibre and reducing the complex. Using this catalyst, the protected glutamic acid 52 was produced with a very slight enantiomeric excess (Scheme 1.22).

![Scheme 1.22 First example of asymmetric induction in a hydrogenation reaction](image)

This was only useful in terms of scientific interest. Another key development a decade later came from the development of the tris(triphenylphosphine) halorhodium(I) complex and its subsequent use as a homogeneous catalyst in catalytic hydrogenations. 64 Around the same time methods were found for the production of chiral phosphanes. In 1968 the first major breakthrough was achieved by Knowles 65 when the triphenylphosphine of Wilkinson’s catalyst was replaced with a chiral counterpart to give 53 (Figure 1.7).

![Figure 1.7 Methylpropylphenylphosphine](image)
Introduction

It was found that hydrogenation of α-phenylacrylic acid at 60°C with the methylpropylphenylphosphine catalyst yielded optically active hydrotrropic acid with an enantiomeric excess of 15%. Shortly after this publication Horner also published the use of methylpropylphenylphosphine on a substituted styrene. Generally low enantiomeric excesses were obtained in the hydrogenation of α-ethylstyrene and α-methoxystyrene but the concept was there to be developed. The issue was now to match ligand to substrate. Knowles was motivated by the discovery that L-DOPA was effective in the treatment of Parkinson's disease. The synthesis of this compound in the Hoffman LaRoche process proceeded via a prochiral enamide which was hydrogenated to give the DL-DOPA. It was found that the enamide hydrogenated much faster than they had expected for such a highly substituted alkene and so the hydrogenation of the simpler molecule, α-acetamidocinnamic acid, became the basis for the structure-action relationship. From this, a series of phosphines with various alkyl groups were tried with not too much effect. The first success was with the introduction of o-anisoyl group instead of one of the phenyl groups. This molecule PAMP 54 (Figure 1.8) gave e.e. of up to 58% after modification of the conditions. Further modification led to the CAMP ligand 55 (Figure 1.8) which gave up to 88% e.e. This ligand was then used in the Monsanto L-DOPA process (Scheme 1.23).
Introduction

Scheme 1.23 Monsanto L-DOPA process

Around the same time Kagan reported the first use of a diphosphine-rhodium catalytic system DIOP (Figure 1.9) that gave up to 72% e.e. for the hydrogenation of α-acetamidocinnamic acid. This catalyst was then applied to a series of enamides with over 60% e.e.

Until this time it had been thought that the chirality on the phosphorus was important in achieving high enantiomeric excesses. Kagan had shown that chirality on the carbon backbone of the C₂ symmetric diphosphine could also achieve high enantioselectivities. This discovery led to a wave of new bis-phosphine ligands. Knowles then came up with his own chelating bis-phosphine ligand, DIPAMP (Figure 1.10) in 1975 that led to over 90% e.e. in the hydrogenation of α-acetamidoacrylic acids. It was soon employed in the L-DOPA process as it gave 95% e.e. in the key hydrogenation step. Another bis-phosphine ligand to show up at
this time was the ChiraPhos ligand 58 (Figure 1.10) developed by Bosnich in 1977 which gave almost complete optical purity in the hydrogenation of a series of enamides.\textsuperscript{72}

![Figure 1.10 a) DIPAMP and b) ChiraPhos](image)

At this stage no-one had investigated the effect of the enamide geometry on reaction substrate and product stereochemistry. It took further studies by Knowles with the new DIPAMP ligand in the hydrogenation of $\alpha$-acylaminoacrylic acids\textsuperscript{59,73} to find that (Z)-substrates hydrogenate 15-100 times faster and give greater enantiomeric excesses than their (E)-counterparts (Scheme 1.24).

![Scheme 1.24 (Z) vs. (E) hydrogenation](image)

A third class of chiral compound came about with the development of NAPHOS 60 (Figure 1.11) that contained an axial element of chirality.\textsuperscript{74} Although the enantiomeric excesses were not great with this ligand (54% for the hydrogenation of $\alpha$-acylamidoacrylic acid) a very similar ligand developed three years later revolutionised asymmetric hydrogenations. It was Noyori’s BINAP ligand 61 developed in 1980 (Figure 1.11)\textsuperscript{75,76} that really showed the full potential of

23
asymmetric hydrogenation with extremely high yields and e.e. on a wide variety of substrates. The ruthenium complexes are superior to the rhodium complexes, which give opposite enantioselectivity and lower ee's. The complexes are far superior to previous catalysts because they are fully aromatic which provides polarisability and enhances Lewis acidity of the complex. The complex is also available in the (R)-and (S)-form allowing for the formation of (R)-and (S)-hydrogenation products respectively.

![Figure 1.11 a) (S)-NAPHOS, b) (S)-BINAP and c) (R)-BINAP](image)

This ligand led the field of asymmetric hydrogenation and only one other major development has since occurred. In 1993 Burk’s group published what is arguably the most effective asymmetric hydrogenation catalyst DuPhos. (Figure 1.12) Along with the BPE ligand, DuPhos is an electron rich C₂-symmetric bis(phospholane) and works with near to 100% e.e. with substrate to catalyst ratios of 10000 up to 50000.

![Figure 1.12 a) DuPhos and b) BPE](image)

These catalysts allowed the hydrogenation of both (E)-and (Z)-trisubstituted enamides providing amino acids with very high enantiomeric excesses and the same stereochemical configuration as the catalyst. The ability to vary the groups of the phospholane allows for fine tuning of the catalyst to the steric demands of the substrate. The first studies into the hydrogenation of β,β-disubstituted amino acids
were performed with these catalysts. The (Z)-enamides were all hydrogenated using both catalysts with varying e.e. depending on the alkyl group attached to the ligand. It was generally found that smaller groups increased the selectivity. The (E)-alkene substrates however were not reduced at all with the Rh-DuPhos catalyst and lower enantioselectivities were obtained with the Rh-BPE catalyst compared to the (Z)-alkenes.

Since the mid 1990's there have been a large number of asymmetric hydrogenation catalysts developed all boasting higher and higher ee's. These include both monophosphine and bis-phosphine ligands. Of some interest is the TRAP ligand 64 (Figure 1.13) which shows the ability to hydrogenate (E)-alkenes with up to 80% e.e.

However this still falls short of being of great practical importance. An interesting observation was noted by Cativiela when the hydrogenation of (Z)-acetamidoacrylic acid methyl ester 65 using an iridium PAMP catalyst yielded a mixture of (Z)-and (E)-derived diastereomers 66 (Scheme 1.25). There was no further comment and there has been no mention in any other papers of this observation.

Until the ideal air stable catalyst is developed capable of delivering high enantioselectivity with high reaction rates for both (E)-and (Z)-β,β-disubstituted
alkenes with a very high turnover, alternative methods to obtain amino acids derived from asymmetric hydrogenation of \((E)\)-alkenes will be of great interest.

1.3.3 Mechanism of asymmetric hydrogenation

After nearly half a century, a wide range of catalysts have now been developed to allow for the hydrogenation of enamides with high selectivity. The question has always been asked of where the enantioselectivity originates. Much work has been put into the studies of the intermediates of the hydrogenation process through X-ray characterisation and NMR studies. The general mechanistic scheme for asymmetric hydrogenation was described well by Landis (Scheme 1.26).\(^{81,82}\)

**Scheme 1.26 General mechanism for asymmetric hydrogenation**

The mechanism describes the coordination of the substrate to the activated complex and its further reaction in two cycles, the "major manifold" and "minor manifold"
which are diastereomeric to each other. This scheme can also be applied to other bisphosphine ligands. In solution the alkene ligand, norbornadiene or cyclooctadiene, is hydrogenated and loses its affinity for rhodium and is displaced with solvent molecules. The catalytic cycle begins with the displacement of the solvent molecules with the enamide substrate 67. The chelation of the substrate to the rhodium is side on with the alkene double bond and the amide carbonyl oxygen electron lone pair. At this point there is an equilibrium between these two complexes 68 and 69, one of which is predominant (the “major” substrate complex 68). The major complex is more stable than the minor substrate 69. The less stable minor complex reacts faster with the oxidative addition of hydrogen in the first irreversible step and thus dictates the enantioselection. There is then a migratory insertion of one hydrogen into the alkene bond followed by reductive elimination to give the product. In this case the major product (S)-70 is obtained by the minor complex in the minor manifold cycle.

As the origin of enantioselection is not fully understood there have been a few proposals to predict the stereochemical outcome of rhodium catalysed hydrogenation reactions. The first quadrant diagram by Knowles based on DIPAMP 57 (Figure 1.14) shows the chiral environment of the rhodium atom divided into two symmetry related quadrants. This is based on looking at the complex along the phosphorus-rhodium-phosphorus plane as would an approaching substrate. It was suggested that a linear prochiral substrate would lie more easily along the face-exposed aryls rather than the edge-exposed aryls which are depicted by the shaded segments.

![Figure 1.14 a) Knowles' quadrants](image)

It can be seen that the linear prochiral acetamidoacrylate can lie unhindered on its re face. The carboxyl group is in an unhindered quadrant and so it can pick up the hydrogen to become L-alanine. The si face sits with its carboxyl group in the shaded quadrant and is not hydrogenated to give D-alanine. It can be seen how this can be
applied to higher amino acids where the β-group would occupy an unhindered quadrant in the favoured (Z)-form. This rule was found to be unsuitable for the electron rich catalysts such as Rh-DuPhos. With this in mind, the quadrant rule was modified to be made applicable to catalysts with both backbone chirality and P-stereogenic catalysts (Figure 1.15). In this rule, any catalyst possessing bulky substituents on the phosphorus in the upper left and lower right quadrants would produce (R) hydrogenation products whereas the opposite arrangement would give (S) hydrogenation products.

\[
\begin{array}{|c|c|}
\hline
L & S \\
S & L \\
\hline
\end{array}
\quad
\begin{array}{|c|c|}
\hline
S & L \\
L & S \\
\hline
\end{array}
\]

\[L \text{ and } S \text{ represent large and small steric hindrance}\]

\(\text{(R)-Product} \quad \text{(S)-Product}\)

\text{Figure 1.15 Modified quadrants}

Using this modified rule it is possible to predict the outcome of the asymmetric hydrogenation for any given catalyst and help in the design of future catalysts (Figure 1.16).

\[\begin{array}{c}
\text{BINAP, CHIRAPHOS etc} \\
\text{δ-conformer} \\
\text{→ (R)-product}
\end{array}\quad
\begin{array}{c}
\text{BINAP, CHIRAPHOS etc} \\
\text{λ-conformer} \\
\text{→ (S)-product}
\end{array}\]

\[\begin{array}{c}
\text{(R,R) DIPAMP} \\
\text{δ-conformer} \\
\text{→ (S)-product}
\end{array}\quad
\begin{array}{c}
\text{DuPhos} \\
\text{δ-conformer} \\
\text{→ (R)-product}
\end{array}\]

\text{Figure 1.16 Prediction of hydrogenation products}
1.4 Stereoinversions

1.4.1 Overview

Stereoinversion is a modification of deracemisation, an enzymatic route along with dynamic kinetic resolution that can theoretically give up to 100% yields of a single isomer from a racemate. These routes are an alternative to chemical routes such as asymmetric synthesis using chiral auxiliaries to achieve chirality or the chiral pool where nature has provided the stereochemistry that is used in the synthesis. It has been shown that the chemical route of asymmetric hydrogenation process for β,β-disubstituted amino acid is only efficient for the (Z)-alkene substrates. Stereoinversion is required to produce the amino acids that would be obtained through the hydrogenation of (E)-alkenes (Scheme 1.27).

![Scheme 1.27 Where asymmetric hydrogenation fails](image)

1.4.2 Enzymes in chemistry

Enzymes are a favourable alternative to chemical steps as they are naturally selective as they are themselves chiral, being made of L-amino acid residues and they require only mild reaction conditions. There are a vast number of enzymatic reactions now commonly used in laboratory preparations. Of interest to this project is the deracemisation process, from which stereoinversion is derived. To fully appreciate deracemisation it is important to look at some of the various resolution steps in chemistry.
1.4.3 Kinetic resolution

Kinetic resolution, where one enantiomer is more readily converted to product than its enantiomer, has been known since 1858 (Figure 1.17). Pasteur discovered that the D-enantiomer of ammonium tartrate was metabolised faster than the L-enantiomer when fermented in the presence of *Penicillium glaucum*.

![Figure 1.17 Kinetic resolution](image)

The hydrolase enzymes, such as lipases and proteases, are the most popular class of enzyme for resolution steps and are regularly used in industry. These resolution reactions can be very efficient but have a main drawback is that only a 50% yield can be obtained in any given reaction and the unfavoured enantiomer is considered waste or needs to be recycled in an additional racemisation step and could make processes costly.

1.4.4 Dynamic kinetic resolution

Dynamic kinetic resolution was the natural progression from kinetic resolution and the advantages it holds over kinetic resolution allow for widespread use in the lab and in industry. This process requires a chiral substrate which undergoes racemisation by chemocatalysis. Only one of these isomers reacts in the enzymatic step and therefore only one product forms with possible yield of 100% (Figure 1.18).

![Figure 1.18 Dynamic kinetic resolution](image)

This can be demonstrated in ring opening of 2-phenyloxazolin-5-ones 72 (Scheme 1.28). From the racemic substrate, it was possible to produce either enantiomer of 73 in high enantiomeric excesses by using a suitable lipase.
Although classical diastereomeric crystallisations and kinetic resolutions are the most prevalent in large scale syntheses at the present, the dynamic resolution approach is becoming a far more favourable option, although drawbacks do exist. In industry, enzymes are not available off-the-shelf for a desired reaction and may be incompatible with industrial demands. The use of directed evolution and high throughput screening allow for biocatalysts to be tailored for a specific reaction.\(^3\)

### 1.4.5 Deracemisation

Dynamic kinetic resolution is just one form of deracemisation but a potentially more useful variation comes from a cyclic oxidation and reduction stereoinversion process. The enrichment of one isomer under stereoinversion conditions was first demonstrated in by Hafner 1971\(^{85}\) by reaction of D-alanine, pig kidney D-amino acid oxidase and sodium borohydride in which L-alanine was formed via the imine intermediate. The first report of the reaction that was termed deracemisation came from Faber\(^{86}\) who described the possibility of converting racemic mixtures of secondary alcohols and amines to single enantiomers by the use of the oxidation and reduction cycles (Scheme 1.29).
Introduction

Scheme 1.29 Deracemisation through oxidation and reduction cycles

The practical feasibility of this type of reaction was demonstrated in the 1990’s by Soda with high yields and enantioselectivity obtained in the production of the cyclic amino acids proline\(^{87}\) and pipecolic acid\(^{88}\) using amino acid oxidases and sodium borohydride. Based on Faber’s report and Hafner and Soda’s observations, work was carried out within the Turner group to develop this process (Scheme 1.30).

Scheme 1.30 Stereoinversion

Faber\(^{89}\) and Carnell\(^{90}\) had made some progress in this area but the reactions involve many steps. The aim within the Turner group was to use a one pot reaction that contains both the oxidation and reduction steps \textit{in situ}. Work has been carried out within the group on both amines\(^{91}\) and amino acids. In the field of deracemisation of amino acids, it was found that using sodium cyanoborohydride was more efficient than sodium borohydride as the reductant.\(^{92}\) This is because sodium borohydride is highly reactive in water and raises the pH of the reaction mixture to \(\sim\)pH 10 which starts to irreversibly denature the enzyme. A whole series of acyclic D-amino acids were deracemised or stereoinverted with D-AAO and the cyanoborohydride reducing agent. These results were further expanded to show that the family of amine-borane complexes\(^{93}\) and catalytic transfer hydrogenation (CTH) with Pd/C and ammonium formate\(^{94}\) were effective at reducing the imines in such deracemisation reactions.\(^{95}\)
1.4.6 Amino acid oxidases

The D- and L-amino acid oxidase enzymes are part of the flavoprotein family. These enzymes catalyse the oxidative deamination of their corresponding amino acids \text{74} to yield the \(\alpha\)-keto acid \text{75}, ammonia and hydrogen peroxide (Scheme 1.31). This type of enzyme used flavin adenine dinucleotide (FAD) as a co-factor, which is re-oxidised by molecular oxygen and eliminates the need for additional cofactors.

\begin{equation}
\begin{align*}
\text{D-AAO} & : \quad \text{R} \quad \text{H}_2\text{N} \quad \text{CO}_2\text{H} \quad \rightarrow \quad \text{R} \quad \text{HN} \quad \text{CO}_2\text{H}^\prime \\
\text{E-FAD} & : \quad \text{O}_2 \quad \text{H}_2\text{O}_2
\end{align*}
\end{equation}

\begin{equation}
\begin{align*}
\text{L-AAO} & : \quad \text{R} \quad \text{H}_2\text{N} \quad \text{CO}_2\text{H} \quad \rightarrow \quad \text{R} \quad \text{HN} \quad \text{CO}_2\text{H}^\prime \\
\text{E-FAD} & : \quad \text{O}_2 \quad \text{H}_2\text{O}_2
\end{align*}
\end{equation}

Scheme 1.31 Action of amino acid oxidases

L-Amino acid oxidases are widely distributed in many different organisms and snake venom L-amino acid oxidases are very well studied. The function of these enzymes is not fully understood although they play a role in inducing apoptosis and affecting platelets, possibly through the production of locally high concentrations of hydrogen peroxide. It is thought that L-amino acid oxidases from bacterial, fungal and plant species are involved in the utilisation of nitrogen sources. L-Amino acid oxidases are usually non-covalently associated homodimeric (Figure 1.19a) FAD-binding glycoproteins with a molecular mass around 10-150KDa. The crystal structure of snake venom L-amino acid oxidase\textsuperscript{98} shows that each monomer comprises three domains, a FAD-binding domain, a substrate binding domain and a helical domain (Figure 1.19b). A funnel is formed between the substrate binding domain and the helical domain and provides substrate access to the active site.
Introduction

D-Amino acid oxidase is ubiquitous, found in numerous eukaryotic organisms including yeasts, fungi, insects, amphibians, reptiles, birds and mammals. Although there are a large number being reported very few have been isolated and characterised. It is thought that the presence of the enzyme in microorganisms such as yeasts is related to their ability to use D-amino acids for growth. In higher organisms it is unclear what the physiological role of D-amino acid oxidases is. The structure of the active holoenzyme is a monomer (Figure 1.20), although it exists in solution as a monomer-dimer and even higher molecular aggregate in equilibrium depending on concentration and form.

The active site is formed by hydrophobic residues with a cavity calculated to be around 160Å³ which corresponds to the volume occupied by an amino acid with a four carbon side chain. The limited size of the active-site is apparently related to the
presence of a loop or "lid" which covers the ligand and blocks access to the active site.

Mechanistic studies have been performed on D-amino acid oxidase from pig kidney.\textsuperscript{100,101} The enzyme catalyses the oxidation step and the imine intermediate is hydrolysed non-enzymatically. There are three different proposed mechanisms for substrate oxidation while the mechanism of oxygen reduction to hydrogen peroxide is still unknown. In the carbanion mechanism (Scheme 1.32) a carbanion 77 is formed by the abstraction of the $\alpha$-proton by a basic group in the active site and the lone pair attacks the flavin 76 N(5) position. The imino acid 78 forms and the flavin N(5) picks up the proton.

![Scheme 1.32 Carbanion mechanism](image)

The other two proposals are direct hydride transfer mechanisms. One involves an active site base abstracting the $\alpha$-proton (Scheme 1.33a). The imino acid would be formed as hydride transfer to the flavin N(5) occurs. The alternative direct hydride mechanism (Scheme 1.33b) involves the concerted transfer of the $\alpha$-proton to the flavin N5 as the imino acid is formed.
Introduction

The structure of the D-amino acid oxidase from *Rhodotorula gracilis* in its reduced form has been determined to high resolution (Figure 1.21) and shows great evidence for the hydride transfer mechanism.¹⁰²

Scheme 1.33 a) hydride transfer from the α-amino group, b) direct hydride transfer

A view along the flavin plane (Fig 1.21b) shows that the α-proton of D-alanine is pointing towards the flavin N(5) which is required for direct hydride transfer. The carbanion theory can also be rejected as there is no functional group present that
might act as an acid-base catalyst required to abstract the $\alpha$-proton. The active site has not been resolved for the L-AAO but has been modelled based on crystal structures of the enzyme in the presence of the phenylalanine (Figure 1.22). This model also shows that the $\alpha$-proton is in the direction of the N(5) atom on the flavin so it could be assumed that a similar mechanism is involved.

**Figure 1.22** Model of L-AAO active site with phenylalanine
2 Results and Discussion

2.1 Hydrogenation of didehydroamino acids prepared via oxazolones

2.1.1 Overview

In a 1988 Perkin Transactions 1 paper by Cabeza et al in which the hydrogenation of (Z)-β-MePhe didehydroamino acid 79 was reported using various iridium complexes, it was noted that with one of the catalysts this reaction only gave a 41% yield of (2R,3S)-80. The footnote suggested a 46% yield of the (E)-alkene derived amino acid, (2R,3R)-80, was also observed (Scheme 2.1).^80

Scheme 2.1 Iridium complex catalysed hydrogenation

There were no comments about this one-off obscure result and no groups have looked into this reaction further. A similar result was recently seen in our group when the hydrogenation of ~1:1.5 (E/Z)-79 gave predominantly one isomer of the four possible isomers of 80 depending on catalysts (Scheme 2.2).^103

Scheme 2.2 Hydrogenation of a (E/Z) alkene mix of isomers
This had been expanded to show that this is repeatable with a series of bis-phosphine catalysts. With this in mind, the initial aim of the project was to investigate the asymmetric hydrogenation of (E/Z) isomeric mixes of a series of didehydroamino acids (DDAAs) 83. The route to such DDAAs was via the methanolysis of oxazolones 82 (Scheme 2.3), themselves produced under mild Erlenmeyer synthesis from protected glycines 81. This allowed for a wide range of β-substituents and N-protecting groups to be readily incorporated into the DDAAs 83.

![Scheme 2.3 Overview of oxazolone route](image)

### 2.1.2 Imine synthesis

It has been previously reported that the activation of the carbonyl compound for addition to the oxazolone ring can be achieved by conversion into an imine. 48 This imine then reacts in an Erlenmeyer condensation reaction to form the substituted oxazolone.

A series of imines 85 were readily synthesised by the condensation of ketones 84, activated by p-TSA, with aniline refluxed with azeotropic removal of water under Dean-Stark conditions (Scheme 2.4). Aniline was used due to its high boiling point making it suitable for Dean-Stark conditions.
This heating was usually maintained for 24 hours and concentration of this mixture gave the crude imine 85 as an oily solid. Purification of 85 proved to be a problem due to their sensitivity to acidic conditions ruling out column chromatography. The instability of imines in water ruled out simple extraction. Several methods were tried to purify the imine enough to use in the oxazolone reaction. At the very least it was important to remove all traces of aniline as that could compete in the subsequent reaction. The most effective method found was to redissolve the crude imine in acetonitrile and continuously extract into hexane. Using this method yields between 41% and 95% were regularly achieved. Although the imine obtained was not very pure it was sufficient for the next step.

2.1.3 Oxazolone synthesis

The chosen route to the production of oxazolones was the mild two step Erlenmeyer condensation conditions. All of the protected glycine derivatives were commercially available except 4-methoxybenzamidoacetic acid 88, which was synthesised in 47% yield by the reaction of anisoyl chloride 86 with glycine 87 in the presence of sodium hydroxide (Scheme 2.5).

The acid group of 81 was activated using carboxylic acid reagents 89. These resulted in rapid ring closure to the oxazolone intermediate 90 (Scheme 2.6). Monitoring this reaction by TLC showed that the cyclisation was complete in about 1 hour.
Results and Discussion

The two step sequence was completed by the addition of the imine to the oxazolone and heating under reflux overnight (Scheme 2.7). Two series of oxazolones were produced. By cyclising a series of different N-protected glycines 81 and reacting with a single imine 85a, one series 91a-d were produced.

![Diagram of oxazolone synthesis](image)

Scheme 2.7 Oxazolone synthesis

The other series of oxazolones were produced by the cyclisation of only one glycine derivative, hippuric acid to give 92, and reacting with series of different imines 85b-e (Scheme 2.8). This led to oxazolones with varying 13-substituents 93a-d.

![Diagram of various 13-substituents](image)

Scheme 2.8 Various 13-substituents

Using DCC as the cyclising agent was inconvenient as the reaction was low yielding and the by-product of the reaction was the insoluble dicyclohexyl urea that was removed by filtration through celite. A little remained in solution at all times and was found in small quantities in the NMR of the oxazolones, even after column chromatography or recrystallisation. An alternative coupling agent was required and
EDCI proved to be a suitable replacement. The easily protonated nitrogen present made the urea by-product readily soluble in the weak acid wash of the work-up. The oxazolones 91a-d obtained from N-benzoyl glycines contained varying (E)/(Z) isomeric ratios except for the case where the β-substituent is a tert-butyl group. In this case, the single isomer is obtained possibly because of the steric bulk of the group.

The two isomers could be distinguished by NMR and allowed the ratios to be determined. Previous work into this area suggests that the major isomer in each case is the (Z)-isomer and this has been backed up by crystallographic data. The oxazolone derived from N-acetyl glycine was the (Z)-isomer of 91d. Although the oxazolone obtained could be purified by column chromatography the yields were still low. It is undetermined whether the low yield derives from the first or second part of the reaction. The yields were generally better when the unsubstituted 2-phenyl group was present such as on 91a and 93. Having electron withdrawing or electron donating substituents on the aromatic group, such as 91b and 91c respectively, did not affect the yield as both were equally bad. It was expected that the opposite electronic effects of these two groups would have an opposite effect on the amide oxygen giving different yields. Having a methyl group in the 2-position such as 91d also resulted in decreased yield.

2.1.4 Didehydroamino acid synthesis

The esters of didehydroamino acids 94a-h were produced in high yields by alcoholysis of the oxazolones 82 with sodium methoxide in methanol (Scheme 2.9). It was decided that the methyl esters were to be produced to avoid complications associated with transesterification in the reaction solvent of the hydrogenation experiments, methanol.

![Scheme 2.9 Methanolysis](image-url)
The methanolysis route was chosen as previous work suggests high yields and ease of purification by column chromatography. The reaction involves the addition of the oxazolone to a stirring solution of sodium methoxide in methanol at room temperature. The reaction generally took 3-6 hours as monitored by TLC and the yields varied from a poor 46% to a good 79%. These yields, coupled with the poor yields from the oxazolone step proved that this route was insufficient for a practical synthetic route. The \((E)/(Z)\) isomeric ratio of the didehydroamino acids determined by NMR remained similar to the ratio of the oxazolone isomers suggesting the alkene bond remains untouched in the reaction (Table 2.1). HPLC could have been used to determine these ratios and it would be expected that a closer match would have been found.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>R</th>
<th>R'</th>
<th>Oxazolone ((E)/(Z))</th>
<th>DDAA ((E)/(Z))</th>
</tr>
</thead>
<tbody>
<tr>
<td>94a</td>
<td>Ph</td>
<td>Ph</td>
<td>1.59:1</td>
<td>1.27:1</td>
</tr>
<tr>
<td>94b</td>
<td>Ph</td>
<td>(p-\text{NO}_2)-C(_6)H(_4)-</td>
<td>1.46:1</td>
<td>1.56:1</td>
</tr>
<tr>
<td>94c</td>
<td>Ph</td>
<td>(p-\text{MeO}-C(_6)H(_4)-</td>
<td>5.0:1</td>
<td>3.69:1</td>
</tr>
<tr>
<td>94d</td>
<td>Ph</td>
<td>iPr</td>
<td>1.54:1</td>
<td>1.68:1</td>
</tr>
<tr>
<td>94e</td>
<td>Ph</td>
<td>tBu</td>
<td>1:0</td>
<td>1:0</td>
</tr>
<tr>
<td>94f</td>
<td>(p-\text{NO}_2)-C(_6)H(_4)-</td>
<td>Ph</td>
<td>1.80:1</td>
<td>2.49:1</td>
</tr>
<tr>
<td>94g</td>
<td>(p-\text{MeO}-C(_6)H(_4)-</td>
<td>Ph</td>
<td>2.53:1</td>
<td>2.31:1</td>
</tr>
<tr>
<td>94h</td>
<td>Me</td>
<td>Ph</td>
<td>1:0</td>
<td>1:0</td>
</tr>
</tbody>
</table>

Table 2.1 Isomer ratio comparison

2.1.5 \textit{N}-Acetyl to \textit{N}-Boc conversion

To further broaden the range of \textit{N}-protecting groups to be examined the single isomer \textit{N}-acetyl DDAA was converted to the \textit{N}-Boc derivative. It was important to do the direct conversion as the oxazolone route was unfeasible due to the extra oxygen in the carbamate stopping ring formation. Recent studies by Burk showed a mild amide to carbamate conversion. He showed that it worked for a series of amino acids, so it was decided to see if it would work on didehydroamino acids.
Results and Discussion

Under Burk's conditions, 94a was refluxed with Boc anhydride in the presence of DMAP in THF for 4 hours. The cooled solution was treated with hydrazine in methanol and stirred overnight to yield the Boc protected DDAA 95 in moderate (79%) yield. NMR showed a single isomer, presumed (Z) as was the starting material (Scheme 2.10).

As this reaction has been shown to work for a series of amino acids by Burk, it seems likely that the same applies for most didehydroamino acids and is a practical alternative to deprotection followed by reprotection of the amino acid.

Attempts were made to convert the N-Ac group to an N-Cbz group. It was not possible to use the same method as the Cbz anhydride only gives the Cbz carbonate. Several methods were tried without success. The first was a direct conversion by treatment with TBDMS-OTf followed by reaction with benzyl bromide and TBAF. The second route was deprotection of the N-Ac group with TFA followed by reaction with benzyl chloroformate. It is unknown why this did not work and the idea was abandoned.

2.1.6 (E/Z)-Alkene asymmetric hydrogenations

Following the oxazolone approach, a whole series of DDAAs were produced with varying (E)/(Z) ratios and these were hydrogenated under identical conditions to see how the various functional groups would influence the reaction.

Before any asymmetric hydrogenations could be performed it was important to develop an assay to determine the isomer ratio of the hydrogenation products. Using Pd/C as the non-selective catalyst all the substrates were subjected to hydrogenation at 120psi in methanol with 10% catalyst. The protected amino acids were obtained by filtration and concentration. By NMR it was clear that there were two diastereomers present and the diastereomeric ratio could be determined. Under non-selective reducing conditions it would be expected that the d.e. would be the same as
the \((E)/(Z)\) ratio in the starting material. As the Pd/C hydrogenations of 96 give four isomers of 97 (Scheme 2.11), chiral HPLC using the Chiracel OD-H column was used to determine the retention time of each isomer. This allows for easy monitoring of the asymmetric hydrogenation reactions.

![Scheme 2.11 Hydrogenation products](image)

Scheme 2.11 Hydrogenation products

The first substrate to be examined was the 94a that was initially found to exploit the dynamic equilibrium process \((R=R'=\text{Ph})\). It was decided to use the \([\text{Rh}(R,R)-\text{Et-DuPhos(COD)})\text{BF}_4\) and \([\text{Rh}(S,S)-\text{Et-DuPhos(COD)})\text{BF}_4\) catalysts which were used previously within the group. Assigning the peaks to the relative isomer was simple as the \((R,R)\) catalysts give predominantly \((2R,3S)\)-amino acids and \((S,S)\) catalysts give predominantly \((2S,3R)\)-amino acids.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Peak 1 %</th>
<th>Peak 2 %</th>
<th>Peak 3 %</th>
<th>Peak 4 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd/C</td>
<td>18.3</td>
<td>30.8</td>
<td>18.0</td>
<td>32.9</td>
</tr>
<tr>
<td>([\text{Rh}(R,R)-\text{Et-DuPhos(COD)})\text{BF}_4)</td>
<td>15.3</td>
<td>0.9</td>
<td>17.7</td>
<td>66.1</td>
</tr>
<tr>
<td>([\text{Rh}(S,S)-\text{Et-DuPhos(COD)})\text{BF}_4)</td>
<td>22.0</td>
<td>61.1</td>
<td>16.9</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.2 Peak areas for the hydrogenation of 93a

The results clearly show enrichment of one \((Z)\)-alkene derived isomer and suppression of its enantiomer in both cases with the asymmetric catalyst (Table 2.2). From previous results, it was expected that the ratio of \((E)\)-alkene derived amino acids would decrease but that was not the case. From the NMR of the asymmetric
hydrogenation products it can clearly be seen that both diastereomeric pairs are present, further evidence that the reactions did not work as hoped.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Alkene d.e. %a</th>
<th>Total (Z)-productb</th>
<th>Total (E)-productb</th>
<th>Product d.e. %</th>
<th>Product e.e. %c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd/C</td>
<td>12 63.8%</td>
<td>36.2%</td>
<td>28</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>[Rh(R,R)-Et-DuPhos(COD)]BF₄</td>
<td>12 67.0%</td>
<td>33.0%</td>
<td>34</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>[Rh(S,S)-Et-DuPhos(COD)]BF₄</td>
<td>12 61.1%</td>
<td>38.9%</td>
<td>22</td>
<td>&gt;99</td>
<td></td>
</tr>
</tbody>
</table>

Determined by NMR, b Determined by HPLC, c(Z)-derived product

Table 2.3 Product stereochemistry from hydrogenation of 94a

From these results in Table 2.3 it can be seen that the asymmetric catalysts are very enantioselective but only towards the (Z)-isomer as the literature describes. There is only a mild enrichment of (Z)-alkene derived amino acids and this may be due to the inaccuracy of the alkene d.e. determined by NMR. The (E)-alkene derived amino acids were obtained in only slight enantiomeric excess mixture. The difference may be due to slight variations in absorbance between the threo and erthro isomers but no calibration was carried out.

In the early studies into this area it was suggested that the para group on the N-benzoyl group may influence the proposed equilibrium. With this in mind, the p-nitro and p-methoxybenzoyl protected didehydroamino acids 94f and 94g (Figure 2.1) were subjected to hydrogenation.

Under the high pressure asymmetric hydrogenation conditions the p-nitrobenzoyl didehydroamino acid 94f formed a mixture of products, other than those expected, shown by TLC and NMR, possibly resulting from the reduction of the nitro group.

Figure 2.1 p-substituted benzoyl protected DDAA
The desired amino acids could not be isolated from the by-products due to the small amounts used and the HPLC could not resolve any peaks. The results from the hydrogenation of the \( p \)-methoxybenzoyl didehydroamino acid 94g are summarised in Table 2.4 and show similar findings to those described earlier for the hydrogenation of 94a. In each case it is noted that the d.e. of the product is less than that of the starting material. This is most obvious with the Rh-(S,S)-Et-DuPhos hydrogenation where only a very small diastereomeric excess is obtained.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Alkene d.e. %</th>
<th>Total (Z)-product</th>
<th>Total (E)-product</th>
<th>Product de %</th>
<th>Product ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd/C</td>
<td>54</td>
<td>65.0%</td>
<td>35.0%</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>[Rh(S,S)-Et-DuPhos(COD)]BF₄</td>
<td>54</td>
<td>51.3%</td>
<td>48.7%</td>
<td>3</td>
<td>98</td>
</tr>
<tr>
<td>[Rh(R,R)-Et-DuPhos(COD)]BF₄</td>
<td>54</td>
<td>68.0%</td>
<td>32.1%</td>
<td>36</td>
<td>92</td>
</tr>
</tbody>
</table>

\(^{a}\) Determined by NMR, \(^{b}\) Determined by HPLC, \(^{c}\)(Z)-derived product

Table 2.4 Product stereochemistry for hydrogenation of 94g

Similar results came from the attempted asymmetric hydrogenation of didehydroamino acids bearing different \( \beta \)-substituents (Figure 2.2).

![Figure 2.2 Various \( \beta \)-substituents](image)

The \((E)/(Z)\) isomer mixes of these three DDAAs were all hydrogenated at high pressure. As was found in the case of the protecting groups, the presence of the \( p \)-nitrophenyl 94b led to a mixture of products that could not be separated. The results from the other experiments showed that there was no significant enrichment of \((Z)\)-alkene derived amino acids (Table 2.5). Again, the inaccuracy of the isomeric ratio is shown as the Pd/C catalysed hydrogenation shows a different ratio to the alkene, although this may also be attributed to the HPLC being uncalibrated for the difference in absorbance by the two diasteromers.
Table 2.5 Product and starting material stereochemistry for hydrogenations of 94c and 94d

It seemed clear at this point that the nature of the protecting group or β-substituent was not going to influence the course of the reaction. The previous observations of isomer enrichment may possibly have been caused by a contamination of either the substrate or catalyst. To test this, a series of high pressure hydrogenations were performed with various dopants added to the reaction mixture. These were generally based on reagents or by-products associated in the production of the DDAA substrates as they may not have been fully removed in the purification step by the previous researcher. Small amounts of aniline, sodium methoxide, acetic acid, acetophenone, imine, hippuric acid, DCC, EDCI, dicyclohexylurea and p-TSA were added in separate experiments but it was found that these had either no effect on the overall (Z)-selectivity of the reaction or poisoned the catalyst such that the reaction failed to proceed to completion.

The only substrate that did show signs of being involved in an equilibrium process was β-tert-butyl didehydroamino acid 94e (Figure 2.3).
The oxazolone route yielded 94e as a single geometric isomer, presumed (Z). Hydrogenation of this single isomer gave a mix of four isomers of the amino acid (Table 2.6).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Peak 1% (2S,3R)</th>
<th>Peak 2% (2S,3S)</th>
<th>Peak 3% (2R,3R)</th>
<th>Peak 4% (2R,3S)</th>
<th>d.e. %</th>
<th>e.e. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd/C</td>
<td>24.2</td>
<td>26.1</td>
<td>24.0</td>
<td>25.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[Rh(R,R)-Et-DuPhos(COD)] BF₄</td>
<td>25.6</td>
<td>1.5</td>
<td>4.3</td>
<td>68.6</td>
<td>88</td>
<td>46</td>
</tr>
<tr>
<td>[Rh(S,S)-Et-DuPhos(COD)] BF₄</td>
<td>58.6</td>
<td>3.5</td>
<td>3.0</td>
<td>34.9</td>
<td>87</td>
<td>25</td>
</tr>
</tbody>
</table>

*enantiomeric excess of (Z)-derived product*

**Table 2.6 Peak area and stereochemistry for hydrogenation of 94e**

It is clear from these results that there is an unusual process going on to result in four isomers from the Pd/C hydrogenation, which is supposedly always *cis* addition. Either there is an equilibrium process in solution which is strongly in favour of the (Z)-isomer and this is the form that the alkene takes when crystallising, or it is possible that the large steric bulk of the tert-butyl group effects the binding to the Pd/C catalyst surface changing the mechanism and allowing for both *cis* and *trans* addition of the hydrogen. The tert-butyl group could also affect the way the substrate binds to the Rh-DuPhos catalyst, resulting in both a low enantioselectivity and perhaps some *trans* addition.

2.1.7 Conclusion

From the experiments performed, the only clear conclusion is that the dynamic equilibrium approach is not easily repeatable. The route to the hydrogenation substrates gives only a low yield and the hydrogenations show no enrichment of the (Z)-alkene derived amino acids. It is possible that the previously observed reactions may not have gone fully to completion and so only the (Z)-isomer, which is known to react quicker, has reacted and hence given an apparent enrichment. If the reaction was allowed to complete the (E)-isomer would react and change the de’s. This could be tested by producing the pure (Z)- and (E)-βMePhe DDAA and subjecting them to asymmetric hydrogenation. However, there are no plans to take this work further at present.
2.2 Threonine approach

2.2.1 Overview

It has been shown that the work into dynamic equilibrium around the alkene bond was not reproducible. To get around this it was decided that asymmetric hydrogenation of a single alkene isomer would give the required amino acid in enantiomerically pure form. There are several routes to didehydroamino acids that result in the single isomer and it was decided to follow a threonine dehydration route (Scheme 2.12). This route requires the dehydration of threonine methyl ester hydrochloride 98 with acetic anhydride followed by halogenation. Suzuki chemistry on the vinyl halide 99 allows for a variety of substituents to be incorporated at the β-position.

![Scheme 2.12 Threonine overview](image)

Asymmetric hydrogenation of the single isomer alkenes using the Rh-DuPhos catalyst would give the desired enantiomerically pure protected amino acid. A simple deprotection would give the pure amino acid as the hydrochloride salt.

2.2.2 Dehydration of threonine

The dehydration of threonine methyl ester 98 is well documented and convenient as threonine methyl ester hydrochloride is an inexpensive starting material. The existing procedures include both step-wise and direct dehydration. The step-wise
dehydration involves the conversion of the alcohol into an isolated intermediate containing a better leaving group such as p-toluenesulfonate which is then eliminated using a base in a separate reaction.\textsuperscript{106} The direct approach uses expensive reagents such as DAST,\textsuperscript{40} diisopropylcarbodiimide\textsuperscript{107} or $N,N$-carbonyldiimidazole.\textsuperscript{108} Nugent et al. reported a method whereby L-threonine methyl ester 98 was dehydrated using refluxing acetic anhydride and a base.\textsuperscript{41} With this method 102 could be obtained through a simple distillation. This was the method that was adopted for this project. L-Threonine hydrochloride was refluxed in 16eq of acetic anhydride 99 and 6eq of sodium acetate for 3 hours. The mechanism (Scheme 2.13) involves the nucleophilic attack of both the amine group and the hydroxyl group on the acetic anhydride to give the intermediate 100. Nugent reported that an authentic sample of the intermediate 100 was cleanly dehydrated to 102. Although the scheme shows the second acetylation of the nitrogen on 101 occurring before the dehydration and perhaps acting as a driving force, it may be that the formation of the double bond allows the amide nitrogen to attack the acetic anhydride.

![Scheme 2.13 Threonine dehydration](image)

This procedure was worked up by vacuum distillation of 102 and resulted in regular yields of over 70% with 80% being the best obtained. NMR showed that only one
Results and Discussion

isomer was present and comparisons with the literature showed that this is the (Z)-isomer.

Having obtained good yields of 102, the next step was to remove a single acetyl group. This was achieved by reacting the 102 compound with triethylamine in refluxing methanol (Scheme 2.14).41

![Diagram of Acetyl group removal](image)

**Scheme 2.14 Acetyl group removal**

The triethylamine removes a single acetyl group and reacts no further with the stable amide and gives 103 in good yields after column chromatography. NMR confirms that the (Z)-configuration is retained.

2.2.3 Bromination

It was decided at this stage that halogenation followed by Suzuki chemistry was to be the chosen route over direct Heck chemistry on this substrate (Scheme 2.15). Heck reactions retain the geometry of the double bond and would have resulted in (E)-alkene 104 substrates which react slowly under asymmetric hydrogenation conditions.

![Diagram of Heck reaction](image)

**Scheme 2.15 Heck reaction**

For the Suzuki chemistry it was decided that the best substrate for these reactions would be the vinyl bromide 107 for which there is little precedence. The brief comments in the literature suggest that a two step procedure should give the vinyl bromide as a 1:1 mix of (E) and (Z) isomers.34,45 These isomers were assigned after
Results and Discussion

subsequent Suzuki chemistry and the ability of the (Z)-alkene to be readily hydrogenated under asymmetric catalysis

Scheme 2.16 Bromination

The reaction of 102 with NBS 105 in DCM at room temperature follows a nucleophilic attack route as opposed to a radical route to give the imine intermediate 106 (Scheme 2.16). This mixture was allowed to react overnight and TLC confirmed that the starting material had all been consumed. The imine intermediate was then treated with a tertiary amine base. DABCO was used initially but better yields were achieved through the use of triethylamine. Column chromatography of the crude mixture gave both isomers of 107 in turn in about 24% yield each which was disappointing. Having isolated both isomers of the vinyl bromide was potentially useful as Suzuki chemistry could give both isomers of each alkene. If asymmetric hydrogenation could be tailored to both these isomers instead of just the (Z)-alkenes, all four isomers of the desired amino acids could be produced.

2.2.4 Suzuki chemistry with vinyl bromides

The conditions for the Suzuki chemistry have been previously described by Burk using the same vinyl bromide substrate.34 The conditions of these Suzuki reactions vary from the traditional methods as they are much milder. It is important to use only a weak base as the more usual hydroxide bases may lead to hydrolysis of the ester group. The temperature of the reactions was also lower than traditional methods to reduce the risk of scrambling the double bond (Scheme 2.17).

The reactions proceeded well. For example, (E)-107 and its treatment with 1.5eq of boronic acid, 2eq of sodium carbonate and 10 mol% of palladium acetate in ethanol
at 55°C for 4 hours gave \((E)-108a-f\) in reasonable yield after column chromatography.

When the same conditions were applied to \((Z)-107\) the reactions gave varying to zero yields of \((Z)-108a-f\) (Scheme 2.18).

At this point, due to lack of material, and the low yields in making more of the substrate, it was decided not to continue with this bromoalkene approach. A literature search found that Boc anhydride dehydration of Boc-threonine 109 and its subsequent one-pot deprotection and bromination gave excellent yields of mainly \((Z)-bromoalkene 110\) (Scheme 2.19). This result was put down to the effect of TFA in the system favouring the \((Z)\)-isomer formation.

Several attempts were made at following the protocol but the dehydration reaction did not work as the paper suggested and attempted workup never resulted in the isolation of 110. The second step could not be attempted as the material was not pure enough to continue.
2.2.5 Iodination

Development of the methodology for the bromination under acidic conditions allowed the yield of the (Z)-vinyl bromide (Z)-107 to increase from 24 to 35%. However, as this was not a very effective Suzuki substrate it was decided to use the more reactive (Z)-vinyl iodide. Under the halogenating conditions described previously for the bromination reactions, and using NIS, only a 17% of isolated (Z)-vinyl iodide was obtained and the same amount of the (E)-isomer was also obtained. Adding TFA to the reaction solvent led to an increase to a 40% yield of the (Z)-isomer 112. A small amount (~8%) of the (E)-isomer was also isolated.

It is thought that the acidic conditions protonate an oxygen in the succinimide 111 promoting the nucleophilic attack and increasing the yield (Scheme 2.20). It is unknown how the addition of TFA leads to (Z)-selectivity.

![Scheme 2.20 Acid catalysed iodination](image)

2.2.6 Suzuki chemistry of vinyl iodides

Having obtained 112 in a reasonable yield, it was subjected to Suzuki reactions with the same set of boronic acid substrates as used previously (Scheme 2.21). The more reactive iodo group led to the reactions working with good yields in 4 hours after
workup and column chromatography. The alkenes 108 were verified as the single (Z)-isomers by NMR.

\[ \text{Scheme 2.21 Suzuki chemistry} \]

2.2.7 (Z)-Alkene asymmetric hydrogenations

The set of (Z)-substrates obtained through Suzuki chemistry were all hydrogenated with 1 mol% of both enantiomers of the [Rh-Et-DuPhos(COD)]BF\(_4\) catalyst in methanol. It was known that these are good substrates for DuPhos hydrogenations (Scheme 2.22).\(^\text{28}\)

\[ \text{Scheme 2.22 [Rh-Et-DuPhos(COD)]BF}_4 \text{ hydrogenation of (Z)-alkenes} \]

With the Rh-DuPhos catalyst, it was found that all of the (Z)-substrates were hydrogenated to completion giving >99% yields of 113 that were generally >95% e.e. in an overnight reaction at 100 psi. The pure amino acids were easily isolated by concentration of the crude reaction mixture and running the mixture down a short column of silica. As the reaction went to completion there were no main by-products to cause contamination as the Rh-DuPhos residues did not elute on the column.
2.2.8 Hydrolysis

The asymmetric hydrogenation reactions had resulted in six pairs of isolated enantiomers of the protected amino acids. These were all shown by NMR to be diastereomerically pure and HPLC and optical rotation confirmed that they were all close to enantiomerically pure. The protected amino acids 113 were all deprotected under the refluxing hydrochloric acid method described by McCord et al (Scheme 2.23). After refluxing the protected amino acid in 4M HCl for a few hours, decolourising charcoal was added before filtering and concentrating. Trituration with acetone gave pure 114 as the hydrochloride salt. There was a slight concern that such harsh conditions would lead to scrambling of the configuration of the α-hydrogen but NMR confirmed that the amino acids were diastereomerically pure.

The amino acids obtained were all also determined by HPLC to be enantiomerically and diastereomerically pure. It appears that the workup led to a certain amount of enantiomeric enrichment (Table 2.7) presumable as an effect of crystallisation.
Results and Discussion

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>Yield %</th>
<th>Ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-113a</td>
<td>Ph</td>
<td>88</td>
<td>99</td>
</tr>
<tr>
<td>(2S,3R)-113a</td>
<td>Ph</td>
<td>89</td>
<td>99</td>
</tr>
<tr>
<td>(2R,3S)-113b</td>
<td>p-F-C₆H₄⁻</td>
<td>78</td>
<td>99</td>
</tr>
<tr>
<td>(2S,3R)-113b</td>
<td>p-F-C₆H₄⁻</td>
<td>76</td>
<td>99</td>
</tr>
<tr>
<td>(2R,3S)-113c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>(2S,3R)-113c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>(2R,3S)-113d</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>78</td>
<td>99</td>
</tr>
<tr>
<td>(2S,3R)-113d</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>83</td>
<td>99</td>
</tr>
<tr>
<td>(2R,3S)-113e</td>
<td>p-MeO-C₆H₄⁻</td>
<td>66</td>
<td>99</td>
</tr>
<tr>
<td>(2S,3R)-113e</td>
<td>p-MeO-C₆H₄⁻</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>(2R,3S)-113f</td>
<td>2-Naphthyl⁻</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>(2S,3R)-113f</td>
<td>2-Naphthyl⁻</td>
<td>87</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.7 Yields and e.e. of amino acids obtained for hydrolysis of 113a-f

2.2.9 (E)-Alkene asymmetric hydrogenation

It is documented in the literature that (E)-β,β-disubstituted didehydroamino acids do not hydrogenate with high e.e. using Rh-DuPhos as the asymmetric catalyst.²⁸ During the course of Suzuki chemistry a series of such (E)-alkenes had been produced. It was decided to attempt hydrogenation under Rh-DuPhos conditions to verify the results previously published. With this in mind, the entire set of (E)-substrates were hydrogenated under 120psi of hydrogen in methanol for 90 hours with [Rh(R,R)-Et-DuPhos(COD)]BF₄ (Scheme 2.24).

![Scheme 2.24](image-url)
Table 2.8 [Rh(R,R)-Et-DuPhos(COD)]BF₄ hydrogenation of (E)-alkenes

The enantiomeric excesses were very low as expected (Table 2.8). A variety of rhodium catalysts bearing different ligands were therefore tested against a single (E)-alkene substrate (E)-108c to determine whether the nature of the ligand determines selectivity towards this alkene (Scheme 2.25). Under the standard hydrogenation conditions (E)-108c was reduced using a range of rhodium catalysts.

Table 2.9 summarises the results from the catalyst screening. The PhanePhos and Monophosphine ligands gave a near racemic mixture, determined by supercritical fluid chromatography (SFC). The Rh-Ferrotane catalysts showed some minor selectivity as did the Rh-DuPhos catalysts. The BPE ligand system showed a greater selectivity which agrees with the literature. In all cases it was found that the substituents on the ligand determined the selectivity of the catalysts. In the case of the Rh-DuPhos system it was found that having iPr substituents increased selectivity more than a methyl group, which itself was more selective than ethyl.
Results and Discussion

The set of Rh-BPE catalysts proved to be the most effective. In this case it was found that the iPr side group was the least effective but the recently published electron rich Rh-Ph-BPE proved to be modestly effective.\textsuperscript{110} An e.e. of 74% was very encouraging as that gives 87% of the desired isomer that in theory could be isolated by a simple recrystallisation.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Rh((R,R))-Me-BPE(COD)]BF(_4)</td>
<td>67</td>
</tr>
<tr>
<td>[Rh((R,R))-Et-BPE(COD)]BF(_4)</td>
<td>37</td>
</tr>
<tr>
<td>[Rh((S,S))-iPr-BPE(COD)]BF(_4)</td>
<td>13</td>
</tr>
<tr>
<td>[Rh((R,R))-Ph-BPE(COD)]BF(_4)</td>
<td>74</td>
</tr>
<tr>
<td>[Rh((R,R))-Me-DuPhos(COD)]BF(_4)</td>
<td>26</td>
</tr>
<tr>
<td>[Rh((R,R))-Et-DuPhos(COD)]BF(_4)</td>
<td>3</td>
</tr>
<tr>
<td>[Rh((S,S))-iPr-DuPhos(COD)]BF(_4)</td>
<td>33</td>
</tr>
<tr>
<td>[Rh((R,R))-Me-Ferrotane(COD)]BF(_4)</td>
<td>25</td>
</tr>
<tr>
<td>[Rh((R,R))-Et-Ferrotane(COD)]BF(_4)</td>
<td>9</td>
</tr>
<tr>
<td>[Rh((S,S))-tBu-Ferrotane(COD)]BF(_4)</td>
<td>12</td>
</tr>
<tr>
<td>[Rh((S))-Ph-(S)iPr-monophosphine (COD)]PF(_6)</td>
<td>6</td>
</tr>
<tr>
<td>[Rh((R))Phanephos(COD)]BF(_4)</td>
<td>3</td>
</tr>
</tbody>
</table>

\textbf{Table 2.9} Catalyst screen for the hydrogenation of (E)-108c

As the PhBPE was shown to be a promising ligand, this catalyst was used for the asymmetric hydrogenation for the set of (E)-substrates (Scheme 2.26).

\textbf{Scheme 2.26} [Rh\((R,R)\)-Ph-BPE(COD)]BF\(_4\) hydrogenation of (E)-alkenes
The Rh-PhBPE catalyst proved to be effective against most of the (E)-substrates (Table 2.10). It was noted that the (R)-catalyst gave the (2R)-product. This was unexpected and the phenyl group side has a higher priority than the methyl and ethyl chain in the assignment of the stereogenic centre of the catalyst. According to the quadrant model an examples in the literature, it has been shown that the (R) catalyst should give the (2S)-product.

The β-phenyl and β-naphthyl substrates did not react under these conditions. This was only attempted once due to the limited availability of the catalyst and it may have just been because of insufficient agitation in the case of the β-phenyl substrate. The β-naphthyl substrate did not work with either the Rh-DuPhos or Rh-BPE catalysts so presumably the (E)-isomer will not hydrogenate under any asymmetric conditions.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>Yield</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-108a</td>
<td>Ph</td>
<td>No reaction</td>
<td>N/A</td>
</tr>
<tr>
<td>(E)-108b</td>
<td>p-F-C₆H₄</td>
<td>100</td>
<td>79</td>
</tr>
<tr>
<td>(E)-108c</td>
<td>p-CF₃-C₆H₄</td>
<td>100</td>
<td>74</td>
</tr>
<tr>
<td>(E)-108d</td>
<td>p-MeO-C₆H₄</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>(E)-108e</td>
<td>3,5-DiMe-C₆H₃</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>(E)-108f</td>
<td>2-Naphthyl</td>
<td>No reaction</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2.10 [Rh(R,R)-Ph-BPE(COD)]BF₄ hydrogenation of (E)-alkenes

It has been shown that the Rh-DuPhos catalyst is not good for (E)-alkenes but the new Rh-Ph-BPE catalyst was showing encouraging signs. The Rh-Ph-BPE was tested for use in the asymmetric hydrogenation of the (Z)-alkenes (Scheme 2.27).
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>Yield %</th>
<th>ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Z)-108a</td>
<td>Ph</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>(Z)-108b</td>
<td>p-F-C₆H₄⁻</td>
<td>100</td>
<td>62</td>
</tr>
<tr>
<td>(Z)-108c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>No reaction</td>
<td>N/A</td>
</tr>
<tr>
<td>(Z)-108d</td>
<td>p-MeO-C₆H₄⁻</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>(Z)-108e</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>100</td>
<td>58</td>
</tr>
<tr>
<td>(Z)-108f</td>
<td>2-Naphthyl⁻</td>
<td>100</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 2.11 [Rh(R,R)-Ph-BPE(COD)]BF₄ hydrogenation of (Z)-alkenes

Although all the yields were excellent for the isolated product (Table 2.11), HPLC analysis showed that the enantiomeric excesses were very disappointing. Again, the Rh-Ph-BPE catalyst seems to show the opposite selectivity profile of the Rh-DuPhos catalyst and it could be possible to optimise the reaction conditions to favour higher enantioselectivities although lack of catalyst prevented this from being attempted.

2.2.10 Conclusion

The threonine approach proved an effective route to methyl (Z)-2-(N-acetylamino)-but-2-enoate, an effective precursor for palladium chemistry.

Attempted bromination of to methyl (Z)-2-(N-acetylamino)-but-2-enoate gave a low yield of a 1:1 (E) and (Z) mix of the vinyl bromide which were separable. It was found that only the (E)-isomers underwent Suzuki chemistry effectively so a modified iodination reaction was developed to give almost exclusively the (Z)-isomer of the vinyl iodide. The (Z)-vinyl iodides underwent Suzuki chemistry with high yields. The asymmetric hydrogenation process using Rh-DuPhos as the catalyst was shown only to work well for the (Z)-alkenes giving good yields and ees. The desired amino acids were thus obtained through simple acid hydrolysis of the hydrogenation products and resulted in enantiopure amino acids.

An alternative catalyst was found for use on the (E)-alkenes. The Rh-Ph-BPE catalyst gave results that were fairly promising although still lower than literature values. It was also found to give the opposite enantiomer of product to that expected and previously published.
2.3 Stereoinversions

2.3.1 Overview

As previously described, two of the four isomers of each amino acid could be produced using asymmetric hydrogenation. The proposed route to the corresponding diastereomers was through the use of stereoinversion using an amino acid oxidase (Scheme 2.28).\(^9^2\)

![Scheme 2.28 Stereoinversion process](image)

This process is based on the deracemisation process,\(^9^5\) in this case starting from a single enantiomer of the amino acid 115 instead of the racemate. In the first cycle one enantiomer of the amino acid is oxidised by the corresponding amino acid oxidase enzyme to the imine intermediate 116. This intermediate is non-selectively reduced \textit{in situ} to a racemic mixture (RS)-115. The subsequent cycles involve the oxidation of the isomer that is the preferred substrate for the enzyme followed by non-selective reduction. With each successive cycle the amount of the desired substrate increases such that after 7 cycles there is almost exclusively one stereoisomer.

2.3.2 Oxidation assay

Before any stereoinversions could be attempted, it was necessary to establish whether the β-branched-α-amino acids were suitable substrates for the amino acid oxidase enzymes. The amino acids produced were unnatural and therefore may not be accepted by the enzyme. The oxidation assay (Scheme 2.29) consisted of reacting
Results and Discussion

a solution of the amino acid in an assay mixture with the enzyme and horseradish peroxidase.

\[
\begin{align*}
\text{R} & \quad \text{H}_2\text{O} \\
\text{H}_2\text{NCO}_2\text{R} & \quad \text{H}_2\text{O}_2
\end{align*}
\]

\[\text{N}_2\_\text{FAD} \quad \text{FADH}\]

\[\text{O}_2 \quad \text{H}_2\text{O}_2\]

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

\[\text{0} \quad \text{N'} \quad \text{Peroxidase}\]

\[\text{NMe} \quad 2\text{H}_2\text{O} \quad \text{H}_2\text{N}\]

\[\text{119} + \text{118}\]

\[\text{O}_2 \quad \text{NMe} \quad \text{CO}_2\text{H}\]

\[\text{120} \quad \text{CO}_2\text{H}\]

\[\text{121} \quad \text{Quinoneimine dye} \quad \lambda_{\text{max}}=510\text{nm}\]

**Scheme 2.29 Mechanism of oxidation assay**

The oxidation assay mixture consisted of phosphate buffer, amino antipyrine and tribromohydrobenzoic acid. The amino acid 117 reacts with the amino acid oxidase in the presence of oxygen, releasing hydrogen peroxide as a by-product. The amino antipyrine 118 is converted to a reactive nitrene species 119 by the peroxidase enzyme driven by the hydrogen peroxide released in the oxidation process. The nitrene reacts with the tribromohyrobenzoic acid 120 to produce a quinimine dye 121 that absorbs UV light at 510nm and is pink in colour. The intensity of the colour can be monitored over time to give an estimate of the enzyme activity towards the substrate. In these studies it was just necessary to establish whether the reaction would occur so only the visible colour change was looked for and compared to a control. It was found that all the substrates reacted with the appropriate enzyme to produce the pink colour signalling a positive test. Just by looking it was clear to see that the different substrates were oxidised at different rates. Several attempts were made to monitor these reactions over time with a UV detector but the results were
never consistent or accurate. From these studies all that could be established was an approximate order of reactivity (Scheme 2.30).

![Scheme 2.30 Estimated order of reactivity with enzyme](image)

The relative rates determined by UV detection for the *T. variabilis* were even less accurate as the *T. variabilis* is immobilised on a resin and the mixture was not a homogeneous solution. The substrates bearing the phenyl group appeared to be the quickest reacting by eye but the time lapse between starting the reaction and monitoring meant that the reaction may have passed the quickest stage by the time monitoring began. Hence, the main conclusion to be drawn from the assay is that all the substrates could be oxidised by their appropriate enzyme.

### 2.3.3 Optimisation

It was decided that the first reactions to be tested for stereoinversion and to help optimise conditions would be the stereoinversion of (2S,3R)-114 obtained from the [Rh(S, S)-Ph-BPE(COD)]BF₄ catalysed asymmetric hydrogenation experiments. The initial guidelines followed involved treating a buffered solution of the substrate with the enzyme in the presence of catalase to remove any hydrogen peroxide produced and reducing agent. The reactions were to be carried out on a small scale with samples taken and filtered through protein concentrators for HPLC analysis when required. The commercially available snake venom L-AAO was used. The first
choice of reducing agent was ammonium formate with palladium on charcoal as this combination had shown very encouraging results in the group’s deracemisation projects. The reverse phase chiral column Chirex 3126 Penacillamine D was used to monitor these reactions. As it was two diastereomers that needed separating, it was very straightforward determining an assay on the HPLC and both substrates were well separated. This column had further backed up the oxidation assay by showing that \( (2S,3R)-1 \) is fully oxidised by the snake venom L-AAO in the presence of no reducing agent.

When the enzymatic reaction was attempted with the ammonium formate and palladium on charcoal present the signals on the HPLC were suppressed. The HPLC was also tried using an achiral C18 column as the products are diastereomers and in theory should separate. Again there was no signal so a different reducing agent was needed to allow the signal to be seen on the chiral HPLC. Sodium borohydride was tried but any reaction failed to occur. The HPLC showed a slight decrease in starting material peak but no product peak emerging. This is thought to be due to the by-product of the reaction, sodium hydroxide, being produced and raising the pH and denaturing the enzyme. Using sodium cyanoborohydride was the obvious choice and led to the first success. The reaction did not go to completion but there were the first signs of product formation. \( (2S,3R)-1 \) was partly converted into \( (2R,3R)-1 \) with a 58% d.e. and 43% yield. By increasing the amount of enzyme complete conversion was achieved although the yields were low. Several alternative reducing agents were tested and the conditions refined (Table 2.12).

<table>
<thead>
<tr>
<th>Reducing agent</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCNBH$_3$</td>
<td>41</td>
</tr>
<tr>
<td>DMAP:BH$_3$</td>
<td>33</td>
</tr>
<tr>
<td>NH$_3$:BH$_3$</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 2.12 Reducing agent comparison

All of the reactions shown in the table went to completion and the peaks were about 20 minutes apart on the HPLC. The use of amine-borane complexes as reductants for deracemisation has been investigated within the group. A significant increase in
yield was noticed with the switch to ammonia-borane complex as the reductant. The increase in yield is thought to be due to the solubility of the complex in aqueous conditions. At this point it was not known if the catalase and buffer were really necessary for good reactions. When the stereoinversion reaction was attempted without the buffered solution or catalase the results were slightly better, an 83% yield with complete conversion. This was an especially important factor as it reduces the amount of by-products that need removing in a larger scale reaction work-up. Attention was focussed on the complete stereoinversion of the set of substrates obtained in the asymmetric hydrogenation reactions. A problem was immediately discovered when it was found that not all the substrates showed up on the Penacillamine D column. The Chirobiotic T chiral column was more suited for the separation of amino acids and gave the enantiopurity of 114 described earlier.

2.3.4 L-AAO stereoinversion

The snake venom L-AAO used in the optimisation of conditions was used in the conversion of the set of L-substrates in an overnight reaction (Scheme 2.31). A 5mM solution of substrate was shaken with the enzyme and 16eq. of ammonia-borane complex. It was found that the β-naphthyl and the β-4-methoxyphenyl substrate required an extra 24 hours to react fully.

In general it was found that the results from the L-AAO stereoinversions were extremely high yielding with excellent enantiomeric excesses (Table 2.13). Although several of the substrates took longer to react, they usually ended up with good yields. This demonstrates a high substrate acceptance by the enzyme. There was one less reactive substrate, the β-dimethylphenyl substrate (2S,3R)-114e which only gave a
49% de and 71% yield after 60hrs. This may be due to the steric effect of the two methyl groups.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>de %</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2S,3R)-114a</td>
<td>Ph</td>
<td>&gt;99</td>
<td>83</td>
</tr>
<tr>
<td>(2S,3R)-114b</td>
<td>p-F-C₆H₄⁻</td>
<td>&gt;99</td>
<td>85</td>
</tr>
<tr>
<td>(2S,3R)-114c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>&gt;99</td>
<td>92</td>
</tr>
<tr>
<td>(2S,3R)-114d</td>
<td>p-MeO-C₆H₄⁻</td>
<td>&gt;99 after 40hr</td>
<td>80</td>
</tr>
<tr>
<td>(2S,3R)-114e</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>49% after 60 hr</td>
<td>71</td>
</tr>
<tr>
<td>(2S,3R)-114f</td>
<td>2-Naphthyl</td>
<td>&gt;99 after 40hr</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2.13 Snake venom L-AAO stereoinversions

2.3.5 D-AAO stereoinversion

The stereoinversion of the (2R,3S) D-amino acids obtained from the [Rh(R,R)-Et-DuPhos(COD)]BF₄ catalysed hydrogenations were then attempted using the more reactive of the D-AAO enzymes, from pig kidney, determined during the oxidation assay. The conditions were the same as for the L-AAO stereoinversions: a 5mM solution of substrate was treated with enzyme and 16eq of ammonium-borane complex and allowed to react overnight (Scheme 2.32).

The results were not as expected (Table 2.14) as only half of the substrates were accepted by the enzyme contradicting the oxidation assay which suggested they should all work. It appears that the nature of the aromatic group determines whether the reaction occurs or not.
Results and Discussion

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>de %</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-114a</td>
<td>Ph</td>
<td>63</td>
<td>73</td>
</tr>
<tr>
<td>(2R,3S)-114b</td>
<td>p-F-C₆H₄⁻</td>
<td>&gt;99</td>
<td>85</td>
</tr>
<tr>
<td>(2R,3S)-114c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>(2R,3S)-114d</td>
<td>p-MeO-C₆H₄⁻</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>(2R,3S)-114e</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>(2R,3S)-114f</td>
<td>2-Naphthyl</td>
<td>0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2.14 Pig kidney D-AAO stereoinversions

The problem was overcome as there are several D-AAO enzymes available and the group had some *T. variabilis* donated as a free sample from Sandoz. Under identical conditions the set of substrates were subjected to the stereoinversion procedure using the immobilised enzyme (Scheme 2.33). This had the advantage that it is easily removed from the reaction mixture by a simple filtration rather than using the protein concentrators that are required for the lyophilised enzymes.

The change of enzyme resulted in very impressive conversions (Table 2.15). All reactions went fully to completion and the products were obtained in good yields. The commercial aspects of this reaction are very important as there is potential for catalyst recycling and ease of larger scale reaction workup.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ar</th>
<th>de %</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-114a</td>
<td>Ph</td>
<td>&gt;99</td>
<td>69</td>
</tr>
<tr>
<td>(2R,3S)-114b</td>
<td>p-F-C₆H₄⁻</td>
<td>&gt;99</td>
<td>68</td>
</tr>
<tr>
<td>(2R,3S)-114c</td>
<td>p-CF₃-C₆H₄⁻</td>
<td>&gt;99</td>
<td>81</td>
</tr>
<tr>
<td>(2R,3S)-114d</td>
<td>p-MeO-C₆H₄⁻</td>
<td>&gt;99</td>
<td>80</td>
</tr>
<tr>
<td>(2R,3S)-114e</td>
<td>3,5-DiMe-C₆H₃⁻</td>
<td>&gt;99</td>
<td>72</td>
</tr>
<tr>
<td>(2R,3S)-114f</td>
<td>2-Naphthyl</td>
<td>&gt;99</td>
<td>81</td>
</tr>
</tbody>
</table>

Table 2.15 *Trigonopsis variabilis* D-AAO stereoinversions

2.3.6 Stereo inversion of (2R,3R)-amino acids

It has been shown that the stereoinversion process is a very versatile reaction with extremely encouraging results shown on the HPLC. To further show the effectiveness of this reaction, it was decided that (2R,3R)-3-methylphenylalanine (2R,3R)-114a, obtained commercially as the Boc-protected amino acid, was to be stereoinverted. This amino acid is the equivalent of the amino acid produced from the less favoured (E)-alkene hydrogenation. In this process the resulting amino acid product is the same as the hydrogenation of the (Z)-alkene and will hopefully demonstrate how the β-stereocentre effects the stereoinversion reaction.

The commercially available Boc protected (2R,3R)-β-methylphenylalanine (2R,3R)-114a was deprotected in high yield to give the hydrochloride salt. When this amino acid was subjected to the reaction conditions of stereoinversions described earlier using *TvD*-AAO, complete conversion occurred but the yield was found to be very low (Scheme 2.34). (2R,3S)-114a was previously obtained from the asymmetric hydrogenations in fairly good yields.
The proposed mechanisms for the amino acid oxidase enzymes involve the oxidation of the amino acid to the imine whilst bound to the enzyme. The imine is then released and hydrolysed in the aqueous media. In the case of stereoinversion there must be competing reactions of the imine with either the water or the dissolved reducing agent. Given that the imines produced by the two diastereomers are enantiomers of each other, and therefore chemically the same in a non-chiral environment, it would be expected that they would react at the same rate with ammonia borane. This suggests that the enzyme is oxidising one diastereomer much faster than the other diastereomer and also faster than the rate of imine reduction. This would result in more of the imine being hydrolysed rather than reduced and although an overall conversion would occur, the yield would be greatly reduced. It is therefore clear that the stereochemistry of the β-centre is very important in determining yield.

Stereoinversion of (2R,3R)-114a using pig kidney D-AAO resulted in a marked difference. Here an 83% yield with complete conversion was obtained, again demonstrating enzyme substrate specificity. When this result was coupled with the effective return stereoinversion of (2S,3R)-114a to (2R,3R)-114a using L-AAO described earlier a complete interconversion of β-methylphenylalanine was obtained (Scheme 2.35).
2.3.7 Scale-up

To show that the stereoinversion process was not limited to HPLC scale chemistry, it was decided to perform the reaction on a large scale, thus obtaining the product in solid form that can be fully characterised. The commercially available (2R,3R)-114a was used for convenience. The problem was that an effective large scale stereoinversion would require a large amount of enzyme. The pig kidney D-AAO has been shown to be far superior to the *Trigonopsis variabilis* but the expense make it far less feasible. As *Trigonopsis variabilis* D-AAO was available cheaply in large quantities, the conditions for the reaction had to be refined. It was found that increasing the amount of ammonia borane to 80 eq for the reaction of (2R,3R)-114a led to an increase of yield to 50% yield. Perhaps the more concentrated ammonia-borane solution reduced the amount of water excess and allows more of the imine to be reduced rather than hydrolysed. The 50% yield seemed reasonable enough to allow the scale-up to be attempted. It was necessary to increase the concentration of the amino acid solution or the one gram of solid would need to be reacted in 1 litre of water. This would pose major problems for workup. It was decided to do the reaction on a 25mM scale which only requires 200ml of water. The envisaged problem is that 80eq of ammonia-borane relates to 11.5g which is very costly and the by-products have to be removed at the end. With the theory that it is the concentration of ammonia-borane rather than the number of molar equivalents in mind, the reaction was performed with about 20eq of ammonia-borane (Scheme 2.36). This effectively provides a similar concentration in the reaction mixture as the 80eq did on the small 5mM scale.
The reaction was shaken at 37°C for 96 hours over a weekend before HPLC confirmed that complete reaction had occurred with an estimated yield of 54%. The workup of this reaction involved filtering off the enzyme, freeze drying and removing the unreacted ammonia-borane by filtration from hot ethanol. The ethanolic solution was concentrated the residue recrystallised using ethyl acetate and ethanol to give the (2S,3R)-114 in a 55% yield. The NMR showed that (2S,3R)-114 was free from contaminants and the optical rotation was close to literature comparisons. To fully show that the correct diastereomer had been made a small amount was converted to the hydrochloride salt and the NMR compared to the hydrochloride salt of (2R,3R)-114a and an authentic sample of (2S,3R)-114a hydrochloride salt obtained after the asymmetric hydrogenation reactions. Both the proton and carbon NMR confirmed that the desired isomer was obtained. The key shift in the proton NMR was the position of the β-protons. This proton changes from syn to anti configuration resulting in a shift in the quintuplet of 0.1 ppm. As the yield obtained from the work-up was similar to the yield obtained from the HPLC, it can be assumed that the HPLC results from the other stereoinversions give an accurate representation of the yield that would be expected on a larger scale reaction.

2.3.8 Conclusions

It has been demonstrated that both D and L-amino acid oxidase enzymes are effective in stereoinverting the α-proton on a large range of β-branched amino acids showing great potential for a further range of substrates. Initial studies showed how the choice of reducing agent can effect the reaction. A series of such amino acids with varying β-substituents have been stereoinverted with high yields and complete conversion. The range of substrates subjected to these reactions show that there is great scope for
a wider series of substrates. The configuration of the β-stereocentre has been shown to be important in the activity of the enzyme and the studies resulted in complete interconversion of two diastereomeric β-branched-α-amino acids. To show that the amino acids produced were the desired isomers, the reaction was performed on a synthetic rather than analytical scale and the product fully characterised. This demonstrated that the yield obtained through the small scale reactions matched what was obtained synthetically. This project could be taken forward with the synthetic scale production of all isomers of all six substrates. For the D-amino acid substrates, this could easily be achieved on the large scale as the enzyme is immobilised on resin allowing for ease of use and purification. The stereoinversion of the L-amino acid substrates would require the development of an active immobilised L-amino acid oxidase enzyme. This project could be expanded by changing the nature of the β-substituents and concluded with the development of a series of simple drug mimics such as analogues of L-DOPA, where the extra β-substituent may add to the bioactivity of the compound.
2.4 Application of project - Synthesis of an altemicidin mimic

2.4.1 Overview

This project has demonstrated a novel approach to the synthesis of unnatural amino acids. It has been described earlier how unnatural amino acids can be utilised in the studies of peptides and in the production of pharmaceuticals. To demonstrate a potential use of β-branched amino acids in pharmaceuticals a mimic of an analogue of the natural antibiotic altemicidin 122\textsuperscript{111} (Figure 2.4) was envisaged.

![Figure 2.4 Altemicidin and two analogues](image)

Altemicidin's two analogues SB-203207 123 and SB 203208 124\textsuperscript{112,113} are natural inhibitors of both bacterial and mammalian tRNA synthetases, whilst altemicidin is a novel acaricidal and anti-tumour agent and has been the subject of total synthesis.\textsuperscript{114} In the synthesis of such analogues, the methods currently available for the construction of the hexahydroazaindene core are not practical for providing a broad range of analogues for screening.

The analogue 124 has two β-branched amino acids. There is an isoleucine attached to a sulfonamide branch and a β-methylphenylalanine attached to the core of the molecule. The core is difficult to produce, however it is similar in structure to one of the eight isomers of a scaffold 125 developed at Chirotech (Figure 2.5).\textsuperscript{115}
With this in mind, a mimic 126 was designed based around the scaffold 125 (Figure 2.6). The scaffold cyclopentane mimics the cyclopentane of the hexahydroazaindene core with the same stereochemistry to which the side chains can be easily attached.

The mimic 126 contains the same sulfonamide branch with a single (S,S) isomer of isoleucine at the end matching the stereochemistry of 124. It was decided to make the mimic with all four isomers of β-methylphenylalanine and examine the effects of their different diastereomers when screened for cytotoxicity.

2.4.2 Retrosynthetic strategy

As this is a fairly large molecule, it was best to devise the route retrosynthetically using the simplest chemistry possible (Scheme 2.37) to achieve a convergent synthesis. The best starting point is to split 126 in two with a simple amide coupling of 127 to the 128 with all the amine groups protected using benzyloxycarbonyl groups.
The scaffold 125 and β-methylphenylalanine 129 can be split by a simple ester coupling and the sulfonamide can be separated into two starting materials with a sulfonamide coupling. The only problem foreseen at this stage was the synthesis of the small sulfonamide and whether it would nucleophilically attack an activated acid group.
2.4.3 Scaffold branch

The route to the scaffold branch was through the ester coupling of the \( N \)-protected amino acid 130 to the orthogonally \( N \)-protected scaffold 125 (Scheme 2.38).

\[ \begin{align*}
\text{130} & \xrightarrow{\text{HCl (1.25 M in MeOH)}} \text{125} \\
& \xrightarrow{\text{ZCl, 3 eq. NaOH, THF}} \text{60-84\%} \\
\text{132} & \xrightarrow{\text{HCl (1.25 M in MeOH)}} \text{131} \\
& \xrightarrow{\text{2-Chloro-1-methylpyridinium iodide, TEA, DMAP, DCM}} \text{42-65\%}
\end{align*} \]

Scheme 2.38 Scaffold branch route

The 4 isomers of the 130 were deprotected and reprotected with a benzyloxyacybonyl group. Coupling the protected amino acid to the hydroxy group of 125 was not as straightforward as expected. Early attempts at the esterification using EDCI as the acid activating agent in an overnight reaction led to no product. A different choice of activating agent was required. The solution to this problem came in the form of Mukaiyama’s reagent 2-chloro-1-methylpyridinium iodide 134.\textsuperscript{116} For application in the Staudinger reaction (Scheme 2.39), it is suggested that Mukaiyama’s salt in the presence of a carboxylic acid generates a ketene 135 by the abstraction of the \( \alpha \)-proton.\textsuperscript{117} If this were true in the case of this ester coupling a diastereomeric mix of 133 would be obtained which would be clearly visible in NMR. As only one isomer was observed an alternative mechanism is proposed. As it is amino acids being activated as opposed to carboxylic acids, the difference in acidity of the \( \alpha \)-proton may affect the course of reaction. In the experimental method, the acid is activated in
DCM and triethylamine. After stirring for an hour, DMAP was introduced and the reaction stirred over a weekend. In the proposed mechanism, it is thought that the triethylamine facilitates the attack of the Mukaiyama's salt forming an ester bond giving 136. Introducing the DMAP creates a more favourable leaving group allowing the nucleophilic attack of the alcohol 137 (Scheme 2.40).

Scheme 2.39 Mukaiyama's salt in the Staudinger reaction

Scheme 2.40 Proposed mechanism for Mukaiyama's salt coupling reaction
Workup gave 131 in 42-65% yield which was reasonable. The Boc group on the scaffold was then removed by hydrolysis with high yields to give the scaffold branch 132 as the hydrochloride salt.

2.4.4 Sulfonamide branch

The route designed to produce the sulfonamide branch was based around the coupling of the sulfonamide to isoleucine (Scheme 2.41).

The sulfonamide was prepared from the commercially available chlorosulfonylacetyl chloride 138 and was protected as the ester by reaction with ethanol. This only formed the ester at the carboxyl group, leaving the sulfonyl chloride untouched. The amine group was introduced by bubbling ammonia gas through a solution of the sulfonyl chloride 139. Only when ammonia gas was used did the reaction work although the yield was fairly poor. The coupling of 140 to L-isoleucine 141 was achieved by using DCC as the activating agent in the presence of DMAP and using pyridine as the solvent. Initially, there were doubts as to whether the amine group of 140 would couple to the acid group but, as the nitrogen is not tied up in a true amide bond, the reaction worked giving a 50% yield of 142. There was no
scrambling of the $\alpha$-centre on the isoleucine. Hydrolysis of the ester group using lithium hydroxide gave 143.

### 2.4.5 The coupling of the two branches

The final task in the synthesis of the mimic 124 was the coupling of the two branches 143 and 132 (Scheme 2.42).

These are both extremely bulky groups and it was unsure whether they would couple well. The initial attempt at the coupling used EDCI, but the crude mixture obtained...
from the reaction contained no trace of 144 by LC-MS. It was then decided to repeat
the conditions used for the sulfonamide coupling. Using DCC as the coupling agent,
a low yield of each isomer of 144 was obtained after chromatography.
Due to the bad separation on the column, too little was obtained pure to get a high
field NMR so characterisation was obtained by LC-MS. The final deprotection to
give 126 was then achieved using hydrogenolysis with palladium on charcoal. A
90psi atmosphere was used in an overnight reaction and crude 126 obtained by
filtration and concentration. Although impure, COSY NMR on a 400MHz machine
allowed 126 to be characterised, and along with LC-MS, it was confirmed 126 had
been produced.

2.4.6 Screening

Having produced all four isomers of 126, they were subjected to screening for
cytotoxicity. In vitro cytotoxicity assays detect the degree to which a treatment is
toxic to cells by measuring the viability of a population of cells in culture. Cell
viability is most often defined experimentally by the integrity of the outer cell
membrane. If the membrane is full of "holes", it allows the passage of substances
that would usually be excluded. Some dyes such as trypan blue or propidium iodide
are usually excluded from viable cells but they can enter the cell through holes in the
damaged membrane and stain the cytoplasm and thus can be used as an indicator of
nonviable cells. The leakage of components out of the cytoplasm through holes in the
damaged membrane can also show membrane integrity loss in both in vivo and in
vitro experiments.
The three assay methods used on the mimic 126 were the CytoTox-ONE
homogeneous membrane integrity assay, the Celltiter-Glo Luminescent Cell
Viability assay and the Apo-ONE homogeneous caspase-3/7 assay. 

The CytoTox-ONE assay measures the release of lactate dehydrogenase (LDH)
through the damaged membrane by conversion of Resazurin into the fluorescent
compound resorufin.
The Celltiter-Glo measures the amount of ATP present in culture which is directly
proportional to the number of viable cells. The assay generates a glow-type
luminescent signal.
The Apo-ONE assay detects caspase-3 and caspase-7 that play key effector roles in apoptosis though cleavage of specific substrates important for downstream apoptotic events. The buffer supplied lyses mammalian cells and supports optimal caspase activity. The supplied substrate is cleaved when caspase-3/7 is preset resulting in an intensely fluorescent compound when excited at 499nm.

Both the Celltiter-Glo and CytoTox-ONE were performed using the Vero cell line from African green monkey kidney cells and the Human hepatoma cell line, Hep G2. The Apo-ONE assay was performed on HepG2.

It was found in every case that the mimic had no effect on either cell line. This was a fairly disappointing result and suggests that the 6-membered heterocycle on the natural product is vital to the biological activity. It would be possible by the use of molecular modelling programs to determine how the core of the mimic 126 differs from the core of natural product 124 and further design the mimic to replicate the core better.

2.4.7 Conclusions

A synthetic route was designed to allow for the production of four isomers of a natural product mimic. The route was based around a cyclopentyl scaffold that closely resembled the core of the natural product which has a known anti-tumour activity but the screening of the mimics for cytotoxicity proved negative. A molecular model of both the mimic and the natural product would probably show how the second ring system folds and the position of any functional groups and how this differs from the mimic. From this it would be possible to add functionality to the methyl ester group to mimic these functional groups and hopefully achieve activity when screened. This would require a new synthetic strategy and with the low yield already achieved overall for the reaction will probably will not be taken forward.
3 Experimental

3.1 General procedures

IR absorption spectra were recorded on a Jasco-FT/IR-410 Spectrophotometer using standard techniques. $^1$H and $^{13}$C-NMR spectra were recorded on a Varian Gemini or Bruker AC250 spectrometer. Chemical shifts reported in ppm. The symbol * designates different isomer/diastereomeric pair. Mass spectra were recorded on a Micromass Platform-2 under electrospray conditions or Magnetic Sector MS250 machine for FAB-MS.

Optical rotation was measured on an AA1000 polarimeter from Optical Activity Ltd (measurements made at the sodium D-line). Concentrations given in g/100ml

Column chromatography was carried out using Fisher silica 60A, particle size 35-70 micron).

Melting points were carried out on a Gallenkamp melting point apparatus with no corrections.

All solvents and reagents are standard laboratory grade and were obtained from commercial sources. The porcine kidney and snake venom amino acid oxidases were obtained from Sigma and the *Trigonopsis variabilis* D-amino acid oxidase was obtained from Sandoz.
3.2 Experimental procedures – Synthesis of β-branched-α-amino acids

Synthesis of 4-methoxybenzamido acetic acid

To a solution of glycine (1.15g, 0.015mol) in sodium hydroxide (40ml, 2M) at 0°C was added p-anisoyl chloride (2.87g, 0.017mol) and sodium hydroxide (40ml, 2M) simultaneously over 10 minutes. The reaction mixture was allowed to warm to room temperature and stirred until complete dissolution occurred (90 minutes). The mixture was cooled to 0°C and acidified to pH 3 with concentrated HCl and the reaction mixture cooled in a freezer overnight. The pale yellow solid collected was purified by recrystallisation from ethanol/water to give pale yellow crystals (1.51g, 47%).

M.p. 152-154°C, lit. 158-160°C; νmax (nujol) 1572 (NH bend), 1686cm⁻¹ (C=O amide); δH (200MHz, CDCl₃) 3.78 (3H, s, OCH₃), 3.85 (2H, d J=6.0, CH₂), 6.95 (2H, d J=9.0, ArH), 7.83 (2H, t J=9.0, ArH); m/z (FAB) 210 (MH⁺).

3.2.1 General procedure for synthesis of imines

A mixture of aniline, ketone and p-TSA were heated under Dean-Stark conditions in toluene for 24 hours. The mixture was concentrated, taken up in acetonitrile and continuously extracted into hexane. The hexane solution was concentrated to the product that was of sufficient purity for the next stage. Further purification by column chromatography would have resulted in breakdown of the product.
Experimental

Synthesis of $N$-(phenyl)phenylmethylideneamine 83a

The general procedure was followed using aniline (6.38ml, 0.068mol), acetophenone (9.60ml, 0.082mol), $p$-TSA (0.002g, 0.012mmol) and toluene (80ml). The workup yielded a yellow/orange oil (12.60g, 95%).

$\nu_{\text{max}}$ (nujol) 1639 cm$^{-1}$ (C=N); $\delta_{H}$ (200MHz, CDCl$_3$) 2.25 (3H, s, CH$_3$), 6.81-8.03 (10H, m, ArH); $m/z$ (FAB) 196 (MH$^+$).

Synthesis of 1-(4-Nitro-phenyl)ethylidene-phenylamine 85b

The general procedure was followed using aniline (6.38g, 0.068mol), 4'-nitroacetophenone (13.53g, 0.082mol), $p$-TSA (0.002g, 0.012mmol) and toluene (80ml). Workup yielded a yellow solid (6.75g, 41%).

M.p. 75-77°C; $\nu_{\text{max}}$ (nujol) 1644 (C=N), 1514 (NO$_2$), 1342 cm$^{-1}$ (NO$_2$); $\delta_{H}$ (200MHz, CDCl$_3$) 2.28 (1H, s, CH$_3$), 2.67* (2H, s, CH$_3$), 6.65-8.34 (9H, m, ArH); $\delta_{C}$ (63MHz, CDCl$_3$) 17.4 (CH$_3$), 26.8* (CH$_3$), 118.9, 123.7, 128.9 (CAr), 141.2, 150.2, 163.5, 196.2 (C); $m/z$ (FAB) 241 (MH$^+$).
Experimental

Synthesis of [1-(4-Methoxyphenyl)-ethylidene]-phenylamine 85c

The general procedure was followed using aniline (6.38g, 0.068mol), 4'-methoxyacetophenone (12.24g, 0.082mol), p-TSA (0.002g, 0.012mmol) and toluene (80ml).
Workup yielded a pale yellow solid (11.30g, 74%).
M.p. 85-87°C (EtOH); $\nu_{\text{max}}$ (nujol) 1633cm$^{-1}$ (C=N); $\delta_H$ (200MHz, CDCl$_3$) 2.18 (3H, s, CH$_3$), 3.85 (3H, s, OCH$_3$), 6.75-7.92 (9H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 17.6 (CH$_3$), 55.8 (OCH$_3$), 123.5, 114.0, 120.0, 129.3, 129.4 (C$_{Ar}$), 132.6, 152.3, 162.0, 165.0 (C); m/z (FAB) 226 (MH$^+$).

Synthesis of (1,2-Dimethylpropylidene)phenylamine 85d

The general procedure was followed using aniline (6.38g, 0.068mol), 3-methyl-2-butanone (7.05g, 0.082mol), p-TSA (0.002g, 0.012mmol) and benzene (80ml).
Workup yielded a yellow oil (8.76g, 80%).
$\nu_{\text{max}}$ (film) 1661cm$^{-1}$ (C=N); $\delta_H$ (200MHz, CDCl$_3$) 1.28 (6H, d $J=6.8$Hz, (CH$_3$)$_2$), 1.81 (3H, s, CH$_3$), 2.67 (1H, sept $J=6.8$Hz, CH), 6.74-7.40 (5H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 17.4 (CH$_3$), 20.3 (CH), 39.6 (CH), 119.6, 123.3, 129.3 (C$_{Ar}$), 152.2, 176.6 (C); m/z (ES$^+$) 162 (MH$^+$).
Synthesis of Phenyl-(1,2,2-trimethylpropylidene)amine 85e

The general procedure was followed using aniline (6.38g, 0.068mol), pinaclone (10.23g, 0.082mol), p-TSA (0.002g, 0.012mmol) and toluene (80ml). Workup yielded an orange oil (6.78g, 57%).

\[ \nu_{\text{max}} \text{ (film)} \ 1651 \text{ (C=N), } \delta_{\text{H}} \text{ (200MHz, CDCl}_3) \ 1.31 \text{ (9H, s, (CH}_3)_3) , \ 1.82 \text{ (3H, s, CH}_3) , \ 6.70-7.40 \text{ (5H, m, ArH) ; } \delta_{\text{C}} \text{ (63MHz, CDCl}_3) \ 15.7 \text{ (CH}_3) , \ 28.2 \text{ ((CH}_3)_3) , \ 123.0 \text{, } 119.5 \text{, } 129.3 \text{ (C}_\text{Ar}) , \ 152.6 , \ 177.9 \text{ (C)} ; \ m/z \text{ (ES}^+) \ 176(\text{MH}^+) \text{.} \]

3.2.2 General procedure for synthesis of oxazolones

Coupling agent DCC or EDCI was added to a suspension of the N-protected glycine methyl ester in DCM and stirred at room temperature until the mixture turned clear (approx. 1 hour). Imine was added and the mixture heated at 53°C until TLC (hexane/ethyl acetate 4:1) showed no further change.

Synthesis of (E/Z)-4-(1-phenyl)ethylidene-2-phenyloxazol-5(4H)-one 91124

The general procedure was followed using DCC (7.84g, 0.038mol), hippuric acid (3.74g, 0.021mol), DCM (50ml) and imine 85a (3.70g, 0.019mol).
Experimental

On completion (4 hours) the reaction mixture was cooled, filtered over celite, washed four times with KHSO₄ (4x100ml, 0.3M), dried over MgSO₄ and concentrated to a dark orange oil. The crude oxazolone was further purified by wet flash column chromatography over silica (hexane/ethyl acetate 30:1 eluent) to yield yellow crystals (3.54g, 71%).

\[ \nu_{\text{max}} \text{(nujol)} \ 1757 \text{ (C=N), 1788cm}^{-1} \text{ (C=O)}; \delta_{\text{H}} \ (200MHz, \text{CDCl}_3) \ 2.68 \ (1.17H, \text{ s, CH}_3), \ 2.79^* \ (1.86H, \text{ s, CH}_3), \ 7.25-7.60 \ (7H, \text{ m, ArH}), \ 7.85-8.13 \ (3H, \text{ m, ArH}); m/z \ (\text{FAB}) \ 264 \ (\text{MH}^+). \]

Synthesis of \((\text{E/Z})-\text{4-(1-phenyl)ethylidene-2-(4-nitrophenyl)oxazol-5(4H)-one}\)

The general procedure was followed using DCC (7.84g, 0.038mol), 4-nitrohippuric acid (4.70g, 0.021mol), DCM (50ml) and 85a (3.70g, 0.019mol).

On completion (4 hours) the reaction mixture was cooled, filtered over celite, washed four times with KHSO₄ (4x100ml, 0.3M), dried over MgSO₄ and concentrated to a dark brown gummy solid. The crude oxazolone was further purified by wet flash column chromatography over silica (hexane/ethyl acetate 30:1) to yield yellow crystals (0.81g, 14%).

\[ \nu_{\text{max}} \text{(nujol)} \ 1766 \text{ (C=N), 1790cm}^{-1} \text{ (C=O)}; \delta_{\text{H}} \ (200MHz, \text{CDCl}_3) \ 2.70 \ (1.07H, \text{ s, CH}_3), \ 2.83^* \ (1.93H, \text{ s, CH}_3), \ 7.47-8.38 \ (9H, \text{ m, ArH}); \delta_{\text{C}} \ (63MHz, \text{CDCl}_3) \ 18.6 \ (\text{CH}_3), \ 23.1^* \ (\text{CH}_3), \ 123.8, \ 123.9, \ 128.0, \ 128.1, \ 128.5, \ 128.6, \ 129.8, \ 130.1, \ 130.6 \ (\text{CH}_A), \ 136.6, \ 138.4, \ 149.8, \ 152.7, \ 155.6, \ 158.3, \ 165.7 \ (\text{C}); m/z \ (\text{FAB}) \ 309 \ (\text{MH}^+); \text{ found (FAB) 309.08758 (MH}^+) \text{, C}_{17}\text{H}_{13}\text{N}_{2}\text{O}_4 \text{ requires 309.08753.}\]
Experimental

Synthesis of (E/Z)-4-(1-phenyl)ethylidene-2-(4-methoxyphenyl)oxazol-5(4H)-one

The general procedure was followed using EDCI (1.74g, 0.009mol), 4-methoxyhippuric acid (1.05g, 0.005mol), DCM (15ml) and 85a (0.88g, 0.0045mol). On completion (4.5 hours) the mixture was cooled to room temperature, added to DCM (120ml), washed with KHSO₄ (2x60ml/0.3M), dried over MgSO₄ and concentrated to a yellow oily solid. The crude oxazolone was further purified by wet flash column chromatography over silica to yield yellow crystals (180mg, 12%).

vₘₐₓ (nujol) 1759 (C=N), 1782cm⁻¹ (C=O); δ₂ (200MHz, CDCl₃) 2.56 (0.85H, s, CH₃), 2.71* (2.15H, s, CH₃), 3.81* (2.15H, s, OCH₃), 3.82 (0.85H, s, OCH₃), 6.88-8.00 (9H, m, ArH); δₐ (63MHz, CDCl₃) 18.0 (CH₃), 55.5 (OCH₃), 114.1, 127.9, 128.0, 129.6, 129.8 (C₆H₅), 117.9, 131.2, 138.9, 147.3, 160.1, 163.1, 166. (C); m/z (FAB) 293 (MH⁺); found (FAB) 294.11299 (MH⁺), C₁₈H₁₆NO₃ requires 294.11302.
Experimental

Synthesis of (Z)-4-(1-phenyl)ethylidene-2-methyloxadiazol-5(4H)-one 91d

The general procedure was followed using EDCI (3.26g, 0.017mol), N-acetylglycine (1.11g, 9.00mmol), DCM (30ml) and 85a (1.67g, 8.60mmol).

On completion (overnight) the mixture was cooled to room temperature, added to DCM (240ml), washed with KHSO₄ (2x160ml/0.3M), dried over MgSO₄ and concentrated to a gummy brown solid. The crude oxazolone was further purified by wet flash column chromatography over silica to yield a cream coloured solid (380mg, 22%).

M.p. 109-111°C; νₛₐₓ (nujol) 1766 (C=N), 1794 cm⁻¹ (C=O); δₗ (200MHz, CDCl₃) 2.21 (3H, s, C=CH₃), 2.62 (3H, s, CH₃), 7.18-7.64 (5H, m, ArH); δᵣ (63MHz, CDCl₃) 15.1 (C=CH₃), 18.2 (CH₃), 128.0, 129.2, 129.6 (Cᵣ), 130.28, 138.67, 148.72, 162.39, 166.73 (C); m/z (FAB) 202 (MH⁺); found (FAB) 202.08629 (MH⁺); C₁₂H₁₂NO₂ requires 202.08680.
Experimental

**Synthesis of (E/Z)-4-[1-(4-Nitrophenyl)ethylidene]-2-phenyl-4H-oxazol-5-one 93a**

The general procedure was followed using EDCI (2.50, 0.013mol), hippuric acid (1.97g, 0.011mol), DCM (25ml) and 85b (2.40g, 0.010mol).

On completion (4 hours), the solution was cooled to room temperature, added to DCM (260ml), washed with KHSO₄ (2x100ml, 0.3M), dried over MgSO₄ before concentrating to a gummy orange solid. Wet flash column chromatography (Hexane/ethyl acetate 10:1) yielded yellow crystals (0.52g, 17%).

\[ \nu_{\text{max}} (\text{KBr}) 1645(\text{C=N}), 1803\text{cm}^{-1}(\text{C=O}); \delta_{\text{H}} (200\text{MHz, CDCl}_3) 2.73 (1.22\text{H, s, CH}_3), 2.87^* (1.78\text{H, s, CH}_3), 7.52-8.42 (9\text{H, m, ArH}); m/z (\text{FAB}) 309 (\text{MH}^+). \]

**Synthesis of (E/Z)-4-[1-(4-Methoxyphenyl)ethylidene]-2-phenyl-4H-oxazol-5-one 93b**

The general procedure was followed using EDCI (2.50g, 0.013mol), hippuric acid (1.97g, 0.011mol), DCM (25ml) and 85c (2.25g, 0.010mol).
On completion (overnight), the solution was cooled to room temperature, added to DCM (260ml), washed with KHSO₄ (2x100ml, 0.3M), dried over MgSO₄ before concentrating to a yellow solid. Wet flash column chromatography (Hexane/ethyl acetate 10:1) yielded yellow crystals (2.01g, 69%).

ν_max (nujol) 1634 (C=N), 1755 cm⁻¹ (C=O); δ_H (200MHz, CDCl₃) 2.73 (0.50H, s, CH₃), 2.86* (2.50H, s, CH₃), 3.94 (0.50H, s, OCH₃), 3.97* (2.50H, s, OCH₃), 7.05-8.18 (9H, m, ArH); δ_C (63MHz, CDCl₃) 18.3 (CH₃), 55.9 (OCH₃), 113.9, 128.2, 129.2, 132.6, 132.9, (C₆H₅), 126.4, 131.6, 149.1, 160.1, 161.6, 167.6 (C); m/z (FAB) 294 (MH⁺).

**Synthesis of (E/Z)-4-(1,2-Dimethylpropylidene)-2-phenyl-4H-oxazol-5-one 93c**

The general procedure was followed using EDCI (3.86g, 0.020mol), hippuric acid (3.05g, 0.017mol), DCM (40ml) and H₂SO₄ (2.50g, 0.016mol).

On completion (overnight), the solution was cooled to room temperature, added to DCM (350ml), washed with KHSO₄ (2x150ml, 0.3M), dried over MgSO₄ before concentrating to an oily orange solid. Wet flash column chromatography (Hexane/ethyl acetate 8:1) yielded a pale yellow solid (2.08g, 59%).

ν_max (KBr) 1657(C=N), 1720cm⁻¹ (C=O); δ_H (200MHz, CDCl₃) 1.23 (2.36H, d J=6.8Hz, (CH₃)₂), 1.24* (3.64H, d J=6.8Hz, (CH₃)₂), 2.35 (1.18H, s, CH₃), 2.40* (1.82H, s, CH₃), 3.87* (0.61H, quin. J=6.8Hz, CH), 4.24 (0.39H, quin. J=6.8Hz, CH), 7.34-8.14 (4H, m, ArH); m/z (FAB) 230 (MH⁺); found (FAB) 230.11815 (MH⁺), C₁₄H₁₃NO₂ requires 230.11810.
Experimental Synthesis of (Z)-2-Phenyl-4-(1,2,2-trimethylpropylidene)-4H-oxazol-5-one 93d

The general procedure was followed using EDCI (2.50g, 0.013mol), hippuric acid (1.97g, 0.012mol), DCM (25ml) and 85e (1.75g, 0.010mol).

On completion (48 hours), the solution was cooled to room temperature, added to DCM (260ml), washed with KHSO4 (2x100ml, 0.3M), dried over MgSO4 before concentrating to a gummy orange solid. Wet flash column chromatography (Hexane/ethyl acetate 1:1) yielded a gummy orange solid (0.26g, 11%).

νmax (KBr) 1648cm⁻¹ (C=N); δH (200MHz, CDCl3) 1.50 (9H, s, (CH3)3, 2.53 (3H, s, CH3), 7.54-8.16 (5H, m, ArH); δC (63MHz, CDCl3) 15.9 (CH3), 29.4 ((CH3)3), 127.8, 128.6, 132.1 (C Ar), 126.1, 130.8, 156.9, 165.6, 166.9 (C); m/z (FAB) 244 (MH⁺); found (FAB) 244.1334 (MH⁺), C15H18NO2 requires 244.13375.

3.2.3 General procedure for the synthesis of didehydroamino acids

The oxazolone was added to a stirring solution of sodium methoxide in methanol. The mixture was stirred at room temperature until TLC showed no further change.

Synthesis of (E/Z)-Methyl-2-benzamido-3-phenylbut-2-enoate 94a
The general procedure was followed using 91a (2.0g, 7.60mmol), sodium methoxide (0.041g, 7.50mmol) and methanol (50ml).

On completion (4 hours) the resulting mixture was acidified to pH 5 with 2M HCl, concentrated to an oily solid that was dissolved in DCM (50ml), washed with water (30ml), dried over MgSO₄ and concentrated to a pale yellow solid. Wet flash column chromatography (Hexane/ethyl acetate 4:1) yielded white crystals (1.20g, 53%).

\[ \nu_{\text{max}} \text{ (nujol) } 1518 \text{ (N-H bend), } 1640 \text{ (C=O amide), } 1720 \text{ (C=O ester), } 3319 \text{ cm}^{-1} \text{ (N-H stretch); } \delta_{\text{H}} (200\text{MHz, CDCl}_3) 2.19 \text{ (1.32H, s, CH}_3\text{), } 2.32* \text{ (1.68H, s, CH}_3\text{), } 3.46 \text{ (1.32H, s, OCH}_3\text{), } 3.87* \text{ (1.68H, s, OCH}_3\text{), } 7.20-7.90 \text{ (10H, m, ArH); } m/z \text{ (FAB) 296 (MH}^+).\]

**Synthesis of (E/Z)-2-Benzoylamino-3-(4-nitrophenyl)but-2-enoic acid methyl ester 94b**

The general procedure was followed using oxazolone 93a (0.50g, 1.62mmol), sodium methoxide (8.8mg, 0.16mmol) and methanol (15ml).

On completion (6 hours) the mixture was acidified to pH 5 with 2M HCl and concentrated to an off-white solid that was dissolved in DCM (40ml), washed twice with water (40ml) dried over MgSO₄ and concentrated to an off-white solid. Wet flash column chromatography (DCM/ethyl acetate 20:1) yielded an off white powder (0.40g, 72%).

\[ \nu_{\text{max}} \text{ (nujol) } 1508 \text{ (NH bend), } 1646 \text{ (C=O amide), } 1719 \text{ (C=O ester), } 3215 \text{ cm}^{-1} \text{ (NH stretch); } \delta_{\text{H}} (200\text{MHz, CDCl}_3) 2.27 \text{ (1.83H, s, CH}_3\text{), } 2.48* \text{ (1.17H, s, CH}_3\text{), } 3.59 \text{ (1.83H, s, CO}_2\text{CH}_3\text{), } 3.95* \text{ (1.17H, s, CO}_2\text{CH}_3\text{), } 7.33-8.32 \text{ (9H, m, ArH); } \delta_{\text{C}} (63\text{MHz, CDCl}_3) 21.0 \text{ (CH}_3\text{), } 22.8* \text{ (CH}_3\text{), } 52.6 \text{ (CO}_2\text{CH}_3\text{), } 59.9* \text{ (CO}_2\text{CH}_3\text{), } 123.9, 129.0, 132.7, 132.8, (C}_{\text{Ar}}, 138.7, 147.6, 165.6, 166.3 \text{ (C); } m/z \text{ (FAB) 341 (MH}^+).\]
Experimental

Synthesis of (E/Z)-2-Benzoylamino-3-(4-methoxyphenyl)but-2-enoic acid methyl ester 94c

\[ \text{CO}_2\text{Me} \]

The general procedure was followed using oxazolone 93b (1.00g, 3.41mmol), sodium methoxide (17.7mg, 0.34mmol) and methanol (30ml).

On completion (4 hours) the mixture was acidified to pH 5 with 2M HCl and concentrated to an off-white solid that was dissolved in DCM (50ml), washed twice with water (50ml) dried over MgSO₄ and concentrated to an off-white solid. Wet flash column chromatography (DCM/ethyl acetate 20:1) yielded a white solid (0.68g, 61%).

\[ \nu_{\text{max}} \text{ (KBr)} 1508 \text{ (NH bend)}, 1647 \text{ (C}=\text{O amide)}, 1720 \text{ (C}=\text{O ester)}, 3235\text{cm}^{-1} \text{ (NH stretch)}; \delta_{\text{H}} \text{ (200MHz, CDCl}_3\text{)} 2.20 \text{ (0.64H, s, CH}_3\text{)}, 2.34* \text{ (2.36H, s, CH}_3\text{)}, 3.56 \text{ (0.64H, s, OCH}_3\text{)}, 3.84* \text{ (2.36H, s, OCH}_3\text{)}, 3.86 \text{ (0.64H, s, CO}_2\text{CH}_3\text{)}, 3.90* \text{ (2.36H, s, CO}_2\text{CH}_3\text{)}, 6.91-7.67\text{(9H, m, ArH); m}/z \text{ (FAB) 326 (MH}^+\text{).}

Synthesis of (E/Z)-2-Benzoylamino-3,4-dimethylpent-2-enoic acid methyl ester 94d

\[ \text{CO}_2\text{Me} \]

The general procedure was followed using oxazolone 93e (1.00g, 4.37mmol), sodium methoxide (24.0mg, 0.48mmol) and methanol (25ml).

On completion (3 hours) the mixture was acidified to pH 5 with 2M HCl and concentrated. The residue was dissolved in DCM (40ml), washed twice with water
Experimental

(40ml), once with brine (40ml) dried over MgSO₄ and concentrated to a pale yellow solid. Wet flash column chromatography (DCM/ethyl acetate 1:1) yielded a white solid (0.81g, 71%).

\[ \nu_{\text{max}} \text{(KBr)} 1521 \text{ (NH bend)}, 1644 \text{ (C=O amide)}, 1722 \text{ (C=O ester)}, 3306 \text{ cm}^{-1} \text{ (NH stretch)}; \delta_H \text{ (200MHz, CDCl₃)} 1.12 \text{ (6H, m, (CH₃)₂)}, 1.85 \text{ (1.12H, s, CH₃)}, 2.12^* \text{ (1.88H, s, CH₃)}, 3.07 \text{ (0.37H, quin. J=6.8Hz, CH)}, 3.66^* \text{ (0.63H, quin. J=6.8Hz, CH)}, 3.82(1.12H, s, CO₂CH₃), 3.84^*(1.88H, s, CO₂CH₃), 7.33-7.93 \text{ (5H, m, ArH)}; \delta_C \text{ (63MHz, CDCl₃)}: 13.9 \text{ (CH₃)}, 20.3 \text{ ((CH₃)₂)}, 21.1^* \text{ ((CH₃)₂)}, 30.5 \text{ (CH)}, 31.7^* \text{ (CH)}, 52.3 \text{ (CO₂CH₃)}, 127.7, 129.1, 132.3 \text{ (C₆H₅)}, 120.5, 134.3, 153.9, 166.3 \text{ (C)}; m/z \text{ (FAB)} 262 \text{ (MH⁺)}; found (FAB) 262.14381 \text{ (MH⁺)}, C₁₅H₂₀N₂O₃ \text{ requires 262.14431.}

Synthesis of (Z)-2-Benzoylamino-3,4,4-trimethylpent-2-enoic acid methyl ester 94e

\[
\begin{align*}
\text{The general procedure was followed using oxazolone 93d (0.25g, 1.03mmol),} \\
\text{sodium methoxide (5.6mg, 0.10mmol) and methanol (18ml).} \\
\text{On completion (3 hours) the mixture was acidified to pH 5 with 2M HCl and} \\
\text{concentrated to a yellow solid that was dissolved in DCM (25ml), washed twice with} \\
\text{water (25ml), once with brine (25ml) dried over MgSO₄ and concentrated to a yellow} \\
\text{solid. Wet flash column chromatography (DCM/ethyl acetate 10:1) yielded a white} \\
\text{solid (0.18g, 64%).}
\end{align*}
\]

\[ \nu_{\text{max}} \text{(KBr)} 1638 \text{ (C=O amide)}, 1719 \text{ (C=O ester)}, 3336 \text{ cm}^{-1} \text{ (NH stretch)}; \delta_H \text{ (200MHz, CDCl₃)} 1.31 \text{ (9H, s, tBu)}, 2.01 \text{ (3H, m, CH₃)}, 3.85 \text{ (3H, s, CO₂CH₃)}, 7.51-7.89 \text{(5H, m, ArH)}; \delta_C \text{ (63MHz, CDCl₃)}: 17.6 \text{ (CH₃)}, 29.4 \text{ (tBu)}, 52.0 \text{ (CO₂CH₃)}, 127.0, 128.6, 131.9 \text{ (C₆H₅)}, 36.6, 122.9, 133.3, 147.4, 165.5, 166.9 \text{ (C)}; \text{ (FAB) 276 (MH⁺)}; \text{ found (FAB) 276.13060 (MH⁺)}, C₁₆H₂₂N₂O₃ \text{ requires 276.15997.}
Experimental

Synthesis of (E/Z)-Methyl-2-(4-nitrobenzamido)-3-phenylbut-2-enoate 94f

The general procedure was followed using 91b (0.23g, 0.75mmol), sodium methoxide (4.1mg, 0.075mmol) and methanol (5ml).
On completion (3 hours) the resulting mixture was acidified to pH 5 with 2M HCl concentrated to an oily solid that was dissolved in DCM (5ml), washed with water (3ml), dried over MgSO4 and concentrated to a yellow solid. Wet flash column chromatography (DCM/ethyl acetate 30:1) yielded yellow crystals (117mg, 46%).

ν<sub>max</sub> (nujol) 1524 (NH bend), 1643 (C=O amide), (1727 (C=O ester), 3150cm<sup>-1</sup> (NH stretch); δ<sub>H</sub> ( 200MHz, CDCl<sub>3</sub>) 2.22 (0.86H, s, CH<sub>3</sub>), 2.38* (2.14H, s, CH<sub>3</sub>), 3.47 (0.86, s, OCH<sub>3</sub>), 3.88* (2.14H, s, OCH<sub>3</sub>), 7.20-8.34 (9H, m, ArH); δ<sub>C</sub> (63MHz, CDCl<sub>3</sub>) 20.7 (CH<sub>3</sub>), 22.1* (CH<sub>3</sub>), 51.9 (OCH<sub>3</sub>), 52.3* (OCH<sub>3</sub>), 122.9, 123.7, 126.9, 127.1, 127.9, 128.0, 128.2, 128.5, 128.9 (C<sub>Ar</sub>), 138.6, 138.7, 139.5, 140.5, 149.6, 163.6, 165.1 (C); m/z (FAB) 341 (MH<sup>+</sup>); found (FAB) 341.11378 (MH<sup>+</sup>);
C<sub>18</sub>H<sub>17</sub>N<sub>2</sub>O<sub>5</sub> requires 341.11374.

Synthesis of (E/Z)-Methyl-2-(4-methoxy)benzamido)-3-phenylbut-2-enoate 94g

The general procedure was followed using 91c (0.16g, 0.55mmol), sodium methoxide (2.9mg, 0.11mmol) and methanol (10ml).
On completion (3.5 hours) the resulting mixture was acidified to pH 5 with 2M HCl concentrated to a gummy solid that was dissolved in DCM (10ml), washed with water (5ml), dried over MgSO4 and concentrated to a pale brown solid. Wet flash column chromatography (DCM/ethyl acetate 20:1) yielded white crystals (96mg, 54%).

$\nu_{\text{max}}$ (nujol) 1522 (N-H bend), 1658 (C=O amide), 1731 (C=O ester), 3300cm$^{-1}$ (N-H stretch); $\delta_H$ (250MHz, CDCl$_3$) 2.17 (0.69H, s, CH$_3$), 2.29* (2.31H, s, CH$_3$), 3.44 (0.69H, s, OCH$_3$), 3.79* (2.31H, s, OCH$_3$), 3.84 (0.69H, s, CO$_2$CH$_3$), 3.85* (3.31H, s, CO$_2$CH$_3$), 6.79-7.87 (9H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 20.3 (CH$_3$), 52.1 (OCH$_3$), 55.2 (CO$_2$CH$_3$), 113.7, 127.4, 128.8, 128.9, 129.2 (C$_{Ar}$), 123.9, 125.4, 135.8, 139.6, 162.4, 164.8, 165.7 (C); m/z (FAB) 326 (MH$^+$); found (FAB) 326.13886 (MH$^+$); C$_{19}$H$_{20}$N$_2$O$_4$ requires 326.13923.

**Synthesis of (Z)-Methyl-2-methylamido-3-phenylbut-2-enoate 94h$^{127}$**

![Structural formula](image)

The general procedure was followed using 91d (0.25g, 1.13mmol), sodium methoxide (6.1mg, 0.11mmol) and methanol (15ml).

On completion (4 hours) the resulting mixture was acidified to pH 5 with 2M HCl concentrated to a gummy solid that was dissolved in DCM (15ml), washed with water (7ml), dried over MgSO4 and concentrated to a pale brown solid. Wet flash column chromatography (DCM/ethyl acetate 1:1) yielded white crystals (230mg, 79%).

M.p. 144-146°C, lit.$^{127}$ 141-142°C; $\nu_{\text{max}}$ (nujol) 1532 (N-H bend), 1657 (C=O amide), 1720 (C=O ester), 3177cm$^{-1}$ (N-H stretch); $\delta_H$ (200MHz, CDCl$_3$) 1.85 (3H, s, CH$_3$), 2.24 (3H, s, Ac), 3.80 (3H, s, CO$_2$CH$_3$), 6.56 (1H, s br., NH), 7.32-7.41 (5H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 20.5 (CH$_3$), 22.4 (Ac), 52.0 (CO$_2$CH$_3$), 127.2, 128.0, 128.7 (C$_{Ar}$), 123.5, 137.5, 139.8, 165.5, 168.6 (C); m/z (FAB) 234 (MH$^+$); found (FAB) (MH$^+$) 234.11316; C$_{13}$H$_{16}$NO$_3$ requires 234.11302.
Experimental

Synthesis of (Z)-Methyl-2-(tert-butyloxyamido)-3-phenylbut-2-enoate

Methyl(E/Z)-2-methylamido-3-phenylbut-2-enoate 94h (0.14g, 0.58mmol) and N, N-dimethyl-4-aminopyridine (14mg, 0.12mmol) were dissolved in THF (2ml). Di-tert-butyl dicarbonate (0.27ml, 1.15mmol) were added and the mixture heated under reflux for 4 hours.

The reaction mixture was cooled to room temperature and methanol (2ml) was added. Hydrazine mono-hydrate (0.12ml, 2.3mmol) was added and the mixture stirred overnight.

The reaction mixture was poured into DCM (5ml) and washed with HCl (5ml, 1M), CuSO₄ (5ml), NaHCO₃ (5ml) before drying over MgSO₄ and concentrating. The resulting white crystals (134mg, 79%).

M.p. 97-99°C; νmax (nujol) 1627 (N-H bend), 1703 (C=O carbamate), 1729 (C=O ester), 3246cm⁻¹ (N-H stretch); δH (200MHz, CDCl₃) 1.39 (9H, s, (CH₃)₃); 2.23 (3H, s, CH₃), 3.84 (3H, s, CO₂CH₃), 5.79 (1H, s br., NH), 7.20-7.43 (5H, m, ArH); δC (63MHz, CDCl₃) 20.5 (CH₃), 28.0 ((CH₃)₃), 51.9 (CO₂CH₃), 127.4, 127.8, 128.7 (C₆H₅), 80.5, 123.7, 140.0, 153.1, 165.9 (C); m/z (EI) 291 (M⁺); found (EI) (M⁺) 291.14706; C₁₆H₂₁NO₄ require 291.14706.
3.2.4 Threonine dehydration approach

Synthesis of (Z)-2-Diacetylaminobut-2-enoic acid methyl ester

A mixture of L-threonine methyl ester hydrochloride (22.0g, 0.129mol) and sodium acetate (65.0, 0.793ml) in acetic anhydride (195ml) was heated under reflux for 2 hours.

The mixture was concentrated and the residue partitioned between ether (350ml) and water (350ml). The extracted ether layer was further washed with water (100ml) and concentrated to an orange oil. Vacuum distillation gave a pale yellow oil (20.53g, 80%).

B.p. 91-93°C at 0.5mmHg, Lit. 41 88-92°C at 0.5Torr; $\nu_{\text{max}}$ (KBr) 1662 (C=O amide), 1720 cm$^{-1}$ (C=O ester); $\delta_H$ (200MHz, CDCl$_3$) 1.84 (3H, d $J$=7Hz, CH$_3$), 2.38 (6H, s, Ac$_2$), 3.84 (3H, s, CO$_2$CH$_3$), 7.27 (1H, q $J$=7Hz, CH); m/z (ES$^+$) 200 (MH$^+$), 222 (MNa$^+$).

Synthesis of (Z)-2-Acetylaminobut-2-enoic acid methyl ester

A mixture of enamide 102 (10.00, 0.050mol) and triethylamine (0.69g, 6.9mmol) in methanol (150ml) was heated under reflux overnight.

The reaction mixture was concentrated and purified by wet flash chromatography (DCM/ethyl acetate 1:1) to yield a white solid (6.67g, 85%).

$\nu_{\text{max}}$ (KBr) 1519 (NH bend), 1664 (C=O amide), 1726 (C=O ester), 3260cm$^{-1}$ (NH stretch); $\delta_H$ (200MHz, CDCl$_3$) 1.84 (3H, d $J$=7Hz, CH$_3$), 2.20 (3H, s, CH$_3$ amide), 3.83 (3H, s, CO$_2$CH$_3$), 6.87 (1H, q $J$=7Hz, CH), 6.94 (1H, s br., NH); (ES$^+$) 158 (MH$^+$), 180 (MNa$^+$).
Experimental Synthesis of \((E/Z)-2\text{-Acetylamino-3-bromobut-2-enolic acid methyl ester (E/Z)-107}^34\)

NBS (6.85g, 39.0mmol) was added to a solution of alkene 102 (5.50g, 35.0mmol) in DCM (30ml) and the mixture stirred at room temperature overnight. Triethylamine (4.91ml, 35.0mmol) was added and the mixture stirred at room temperature for 2 hours.

The mixture was diluted with DCM (30ml) and washed once with HCl (0.1M, 50ml), twice with water (2x50ml) and once with brine (50ml) before drying over MgSO_4_ and concentrating. Wet flash column chromatography (hexane/ethyl acetate 1:1) of the crude vinyl bromide gave first the \((Z)\)-isomer (2.49g, 24%) followed by the \((E)\)-isomer (2.42g, 24%) as yellow solids. The isomers were assigned after subsequent Suzuki and hydrogenation experiments.

\((Z)\)-isomer: M.p. 118-120°C; \(v_{\text{max}}\) (KBr) 1518 (NH bend), 1660 (C=O amide), 1720 (C=O ester), 3245cm\(^{-1}\) (NH stretch); \(\delta_H\) (200MHz, CDCl\(_3\)) 2.04 (3H, s, CH\(_3\)), 2.48 (3H, s, Ac), 3.76 (3H, s, CO\(_2\)CH\(_3\)), 6.95 (1H, s br., NH); \(\delta_C\) (63MHz, CDCl\(_3\)) 22.7 (CH\(_3\)), 24.4 (Ac), 52.6 (CO\(_2\)CH\(_3\)), 123.1, 127.0, 162.8, 167.9 (C); \(m/z\) (FAB) 236 + 238 (MH\(^+\)); found (FAB) 235.99206 (MH\(^+\)), C\(_7\)H\(_{11}\)BrNO\(_3\) requires 235.99223.

\((E)\)-isomer: M.p. 113-115°C; \(v_{\text{max}}\) (KBr) 1499 (NH bend), 1662 (C=O amide), 1728 (C=O ester), 1268cm\(^{-1}\) (NH stretch); \(\delta_H\) (200MHz, CDCl\(_3\)) 2.35 (3H, s, CH\(_3\)), 2.65 (3H, s, Ac), 4.08 (3H, s, CO\(_2\)CH\(_3\)), 7.58 (1H, s br., NH); \(\delta_C\) (63MHz, CDCl\(_3\)) 22.6 (CH\(_3\)), 25.8 (Ac), 52.4 (CO\(_2\)CH\(_3\)), 123.5, 125.6, 164.5, 168.4 (C); \(m/z\) (ES\(^+\)) 236 + 238 (MH\(^+\)), 258 + 260 (MNa\(^+\)).

Synthesis of \((Z)\)-2-Acetylamino-3-iodobut-2-enolic acid methyl ester \(112\)

\(\text{Synthesis of (Z)-2-Acetylamino-3-iodobut-2-enolic acid methyl ester 112}\)
NIS (12.9g, 0.057mol) was added to a stirring solution of alkene 102 (7.50g, 0.048mol) in 2% TFA in DCM (250ml with 5ml TFA). The mixture was stirred at room temperature overnight. The mixture was cooled in an ice bath before triethylamine (20ml) was added slowly. The mixture was allowed to stir for a further hour. The mixture was concentrated and taken up in DCM, washed with 1MKHSO₄, water and brine before concentrating and running down a column of silica (DCM/ethyl acetate 1:1) to obtain a yellow/brown solid (5.41g, 40%).

M.p. 114-116°C; \( \nu_{\text{max}} \) (KBr) 1518 (NH bend), 1659 (C=O amide), 1717 (C=O ester), 3249 cm\(^{-1}\) (NH stretch); \( \delta_H \) (250MHz, CDCl₃) 2.15 (3H, s, CH₃), 2.80(3H, s, Ac), 3.84 (3H, s, CO₂CH₃), 6.91(1H,s br., NH); \( \delta_C \) (63MHz, CDCl₃) 22.7 (CH₃), 28.8 (Ac), 52.5 (CO₂CH₃), 102.1, 131.4, 161.4, 168.1 (C); \( m/z \) (FAB) 284 (MH\(^+\)); found (FAB) 283.97830 (MH\(^+\)), C\(_{7}\)H\(_{11}\)NO₃ requires 283.97837.

### 3.2.5 General procedure for Suzuki coupling

A mixture of haloalkene, boronic acid (1.5eq), palladium acetate (0.1eq) and sodium carbonate (2eq) in ethanol was heated at 55°C for 4 hours. The mixture was concentrated and taken up in DCM/water (1:1). The DCM layer was further washed once with water, once with brine and dried over MgSO₄ before concentrating to the crude didehydroamino acid.

**Synthesis of \((E)-2\)-Acetylamino-3-phenylbut-2-enoic acid methyl ester** \((E)-108a\)\(^{28}\)

![Chemical structure](image)

The general procedure was followed using \((E)-107\) (500mg, 2.12mmol), phenylboronic acid (388mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml).
Experimental

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (288mg, 58%).

\[ \delta_H \ (200\text{MHz, } \text{CDCl}_3) \ 2.05 \ (3H, \text{ s, } \text{CH}_3), \ 2.07 \ (3H, \text{ s, } \text{CH}_3 \text{ amide}), \ 3.36 \ (3H, \text{ s, } \text{CO}_2\text{CH}_3), \ 7.07-7.65 \ (5H, \text{ m, } \text{ArH}); \ m/z \ (\text{ES}^+) \ 234 \ (\text{MH}^+), \ 256 \ (\text{MNa}^+). \]

Synthesis of (E)-2-Acetylamino-3-(4-fluorophenyl) but-2-enoic acid methyl ester (E)-108b

![Structure of (E)-2-Acetylamino-3-(4-fluorophenyl) but-2-enoic acid methyl ester](structure_image)

The general procedure was followed using (E)-107 (500mg, 2.12mmol), 4-fluorophenylboronic acid (445mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (378mg, 71%).

M.p. 164-165 °C; \( \nu_{\text{max}} \) (KBr) 1503 (NH bend), 1658 (C=O amide), 1730 (C=O ester), 3283 cm\(^{-1}\) (NH stretch); \( \delta_H \) (200MHz, \text{CDCl}_3) 1.98 (3H, s, \text{CH}_3), 2.04 (3H, s, Ac), 3.34 (3H, s, \text{CO}_2\text{CH}_3), 6.85-7.27 (4H, m, ArH); \( \delta_C \) (63MHz, \text{CDCl}_3) 21.6 (\text{CH}_3), 22.8 (Ac), 51.7 (\text{CO}_2\text{CH}_3), 114.9 (d J_{CF}=21.6Hz, C_{Ar}), 128.8 (d J_{CF}=8.1Hz, C_{Ar}), 124.0, 136.6, 139.0 (C), 162.1 (d J_{CF}=247.5Hz), 165.7, 168.7 (C); \( m/z \) (ES\(^+) \ 252 \ (\text{MH}^+), \ 274 \ (\text{MNa}^+). \)

Synthesis of (E)-2-Acetylamino-3-(4-trifluoromethylphenyl) but-2-enoic acid methyl ester (E)-108c

![Structure of (E)-2-Acetylamino-3-(4-trifluoromethylphenyl) but-2-enoic acid methyl ester](structure_image)
The general procedure was followed using \((E)-107\) (500mg, 2.12mmol), 4-trifluoromethylphenylboronic acid (604mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml). Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (558mg, 87%).

M.p. 152-154°C; \(\nu_{\text{max}}\) (KBr) 1523 (NH bend), 1661 (C=O amide), 1728 (C=O ester), 3276cm\(^{-1}\) (NH stretch); \(\delta_{\text{H}}\) (200MHz, CDCl\(_3\)) 2.34 (3H, s, CH\(_3\)), 2.36 (3H, s, Ac), 3.64 (3H, s, CO\(_2\)CH\(_3\)), 7.42 (1H, s br., NH), 7.46-8.15 (4H, m, ArH); \(\delta_{\text{C}}\) (200MHz, CDCl\(_3\)) 21.8 (CH\(_3\)), 22.9 (Ac), 51.1 (CO\(_2\)CH\(_3\)), 125.0, 127.4 (C\(_{\text{Ar}}\)), 124.3, 139.5, 144.5, 165.1, 168.9 (C); \(m/z\) (ES\(^{+}\)) 324 (MNa\(^{+}\)).

**Synthesis of \((E)-(2-Acetylamino-3-4-methoxyphenyl) but-2-enoic acid methyl ester \((E)-108d\)**

The general procedure was followed using \((E)-107\) (500mg, 2.12mmol), 4-methoxyphenylboronic acid (483mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml). Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (334mg, 60%).

M.p. 110-113°C; \(\nu_{\text{max}}\) (KBr) 1514 (NH bend), 1659 (C=O amide), 1703 (C=O ester), 3496cm\(^{-1}\) (NH stretch); \(\delta_{\text{H}}\) (200MHz, CDCl\(_3\)) 2.25 (3H, s, CH\(_3\)), 2.29 (3H, s, Ac), 3.61 (3H, s, OCH\(_3\)), 3.95 (3H, s, CO\(_2\)CH\(_3\)); \(\delta_{\text{C}}\) (63MHz, CDCl\(_3\)) 20.9 (CH\(_3\)), 22.3 (Ac), 51.2 (OCH\(_3\)), 54.6 (CO\(_2\)CH\(_3\)), 112.8, 127.9 (C\(_{\text{Ar}}\)), 122.7, 132.2, 139.1, 165.7, 168.3 (C); \(m/z\) (ES\(^{+}\)) 286 (MNa\(^{+}\)).
Synthesis of (E)-2-Acetylamino-3-(3,5-dimethylphenyl) but-2-enoic acid methyl ester (E)-108e

The general procedure was followed using (E)-107 (500mg, 2.12mmol), 3,5-dimethylphenylboronic acid (477mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml).
Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (378mg, 68%).

M.p. 183-187°C; $\nu_{\text{max}}$ (KBr) 1537 (NH bend), 1657 (C=O amide), 1734 (C=O ester), 3222 cm$^{-1}$ (NH stretch); $\delta_H$ (250MHz, CDCl$_3$) 2.02 (3H, s, CH$_3$), 2.07 (3H, s, Ac), 2.20 (6H, s, (CH$_3$)$_2$), 3.36 (3H, s, CO$_2$CH$_3$), 6.70-6.84 (3H, m, ArH), 7.36 (1H, s, NH); $\delta_C$ (63MHz, CDCl$_3$) 21.0 ((CH$_3$)$_2$), 21.4 (CH$_3$), 22.8 (Ac), 51.6 (CO$_2$CH$_3$), 124.7, 136.9 (C$_{Ar}$), 124.1, 129.3, 140.1, 140.4, 166.1, 168.7 (C); m/z (FAB) 262 (MH$^+$); found (FAB) 262.14440 (MH$^+$), $C_{15}H_{20}NO_3$ requires 262.14432.

Synthesis of (E)-2-Acetylamino-3-naphthalen-2-y1-but-2-enoic acid methyl ester (E)-108f

The general procedure was followed using (E)-107 (500mg, 2.12mmol), 2-naphthaleneboronic acid (547mg, 3.18mmol), palladium acetate (48mg, 0.212mmol), sodium carbonate (450mg, 4.24mmol) and ethanol (15ml).
Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (406mg, 68%).
M.p. 184-186°C; \( \nu_{\text{max}} \) (KBr) 1518 (NH bend), 1678 (C=O amide), 1720 (C=O ester), 1318 cm\(^{-1} \) (NH stretch); \( \delta_{\text{H}} \) (250MHz, CDCl\(_3\)) 2.37 (3H, s, CH\(_3\)), 2.40 (3H, s, Ac), 2.54 (3H, s, CO\(_2\)CH\(_3\)), 7.46-8.10 (7H, m, ArH); \( \delta_{\text{C}} \) (63MHz, CDCl\(_3\)) 21.6 (CH\(_3\)), 22.8 (Ac), 51.7 (CO\(_2\)CH\(_3\)), 125.4, 125.8, 126.0, 126.1, 127.6, 127.8 (C\(_{\text{Ar}}\)), 124.1, 132.5, 132.9, 138.1, 139.8, 166.0, 168.8 (C); \( m/z \) (FAB) 284 (MH\(^+\)); (FAB) Found 284.12903 (MH\(^+\)), C\(_{17}\)H\(_{18}\)NO\(_3\) requires 284.12867.

**Synthesis of (Z)-2-Acetylamino-3-phenylbut-2-enoic acid methyl ester (Z)-108a\(^{127}\)**

![Diagram](image)

The general procedure was followed using 112 (800mg, 2.82mmol), phenylboronic acid (518mg, 4.24mmol), palladium acetate (64mg, 0.282mmol), sodium carbonate (600mg, 5.66mmol) and ethanol (25ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (480mg, 73%).

For characterisation, see oxazolone approach.

**Synthesis of Synthesis of (Z)-2-Acetylamino-3-(4-fluorophenyl) but-2-enoic acid methyl ester (Z)-108b\(^{78}\)**

![Diagram](image)

The general procedure was followed using 112 (500mg, 1.77mmol), 4-fluorophenylboronic acid (371mg, 2.65mmol), palladium acetate (40mg, 0.177mmol), sodium carbonate (375mg, 3.53mmol) and ethanol (15ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (341mg, 77%).
M.p. 105-107°C; v_{max} (KBr) 1509 (NH bend), 1654 (C=O amide), 1723 (C=O ester), 3280 cm^{-1} (NH stretch); δ_{H} (250MHz, CDCl₃) 2.09 (3H, s, CH₃), 2.45 (3H, s, Ac), 4.02 (3H, s, CO₂CH₃), 6.70 (1H, s br., NH), 7.23-7.46 (4H, m, ArH); δ_{C} (63MHz, CDCl₃) 21.2 (CH₃), 23.0 (Ac), 52.6 (CO₂CH₃), 116.2 (d, J_{CF}=21.6Hz, C_{Ar}), 129.6 (d, J_{CF}=8.2Hz, C_{Ar}), 124.2, 136.3, 137.9 (C), 164.7 (d, J_{CF}=252Hz, CF), 165.9, 169.1 (C); m/z (FAB) 252 (MH⁺); found (FAB) 252.10362 (MH⁺), C₁₃H₁₅FNO₃ requires 252.10360.

Synthesis of (Z)-2-Acetylamino-3-(4-trifluoromethylphenyl)but-2-enoic acid methyl ester (Z)-108c

The general procedure was followed using 112 (400mg, 1.41mmol), 4-trifluoromethylphenylboronic acid (403mg, 2.12mmol), palladium acetate (32mg, 0.141mmol), sodium carbonate (300mg, 2.83mmol) and ethanol (15ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (356mg, 84%).

M.p. 118-121°C; v_{max} (KBr) 1508 (NH bend), 1682 (C=O amide), 1709 (C=O ester), 3306 cm^{-1} (NH stretch); δ_{H} (250MHz, CDCl₃) 1.96 (3H, s, CH₃), 2.36 (3H, s, Ac), 3.90 (3H, s, CO₂CH₃), 6.57 (1H, s br., NH), 7.33-7.73 (4H, m, ArH); δ_{C} (63MHz, CDCl₃) 20.4 (CH₃), 22.5 (Ac), 52.2 (CO₂CH₃), 125.7, 127.7 (C_{Ar}), 124.2, 137.4, 143.8, 165.1, 168.7 (C); m/z (FAB) 302 (MH⁺); found (FAB) 302.10071 (MH⁺), C₁₄H₁₅F₃NO₃ requires 302.10040.
Synthesis of (Z)-2-Acetylamino-3-(4-methoxyphenyl)but-2-enoic acid methyl ester (Z)-108d

The general procedure was followed using 112 (500mg, 1.77mmol), 4-methoxyphenylboronic acid (403mg, 2.65mmol), palladium acetate (40mg, 0.177mmol), sodium carbonate (375mg, 3.53mmol) and ethanol (15ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (338mg, 73%).

M.p. 106-108°C, Lit.127 124-126°C; v_max (KBr) 1519 (NH bend),1637 (C=O amide), 1720 (C=O ester), 3244 cm⁻¹ (NH stretch); δ_H (200MHz, CDCl₃) 1.99 (3H, s, CH₃), 2.32 (3H, s, Ac), 3.91 (3H, s, CO₂CH₃), 6.55 (1H, s br., NH), 6.97-7.34 (4H, m, ArH); δ_C (63MHz, CDCl₃) 20.5 (CH₃), 22.5 (Ac), 52.0 (CO₂CH₃), 55.1 (OCH₃), 114.0, 128.7 (C_Ar), 123.2, 131.7, 137.4, 159.3, 165.7, 168.6 (C); m/z (FAB) 264 (MH⁺); found (FAB) 264.12316 (MH⁺), C₁₄H₁₈NO₄ requires 264.12358.

Synthesis of (Z)-2-Acetylamino-3-(3,5-dimethylphenyl)but-2-enoic acid methyl ester (Z)-108e

The general procedure was followed using 112 (400mg, 1.41mmol), 3,5-dimethylphenylboronic acid (318mg, 2.12mmol), palladium acetate (32mg, 0.141mmol), sodium carbonate (300mg, 2.83mmol) and ethanol (15ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a white solid (301mg, 82%).
Experimental

M.p. 124-126°C; $\nu_{\text{max}}$ (KBr) 1527 (NH bend), 1648 (C=O amide), 1728 (C=O ester), 3238 cm$^{-1}$ (NH stretch); $\delta_{\text{H}}$ (250MHz, CDCl$_3$) 2.08 (3H, s, CH$_3$), 2.40 (3H, s, Ac), 2.50 (6H, s, (CH$_3$)$_2$), 6.71 (1H, s br., NH), 7.14-7.27 (3H, m, ArH); $\delta_{\text{C}}$ (63MHz, CDCl$_3$) 20.4 (CH$_3$), 21.1 ((CH$_3$)$_2$), 22.5 (Ac), 52.0 (CO$_2$CH$_3$), 124.8, 129.7 (C$_{\text{Ar}}$), 123.4, 136.9, 138.4, 139.6, 165.5, 168.4 (C); m/z (FAB) 262 (MH$^+$); (FAB) Found 262.14480 (MH$^+$), C$_{15}$H$_{20}$NO$_3$ requires 267.14432.

Synthesis of (Z)-2-Acetylamino-3-naphthalen-2-yl-but-2-enoic acid methyl ester (Z)-108f

The general procedure was followed using 112 (800mg, 2.82mmol), 2-naphthaleneboronic acid (729mg, 4.24mmol), palladium acetate (64mg, 0.242mmol), sodium carbonate (600mg, 5.66mmol) and ethanol (25ml).

Wet flash column chromatography (DCM/ethyl acetate 1:1) gave a pale yellow solid (575mg, 72%).

M.p. 132-135°C; $\nu_{\text{max}}$ (KBr) 1509 (NH bend), 1650 (C=O amide), 1720 (C=O ester), 3298 cm$^{-1}$ (NH stretch); $\delta_{\text{H}}$ (250MHz, CDCl$_3$) 1.78 (3H, s, CH$_3$), 2.28 (3H, s, Ac), 3.77 (3H, s, CO$_2$CH$_3$), 6.52 (1H, s br., NH), 7.28-7.80 (7H, m, ArH); $\delta_{\text{C}}$ (63MHz, CDCl$_3$) 20.5 (CH$_3$), 22.4 (Ac), 52.1 (CO$_2$CH$_3$), 125.2, 126.4, 126.5, 127.6, 127.9, 128.5 (C$_{\text{Ar}}$), 123.9, 132.7, 133.1, 137.2, 137.3, 165.5, 168.6 (C); m/z (FAB) 284 (MH$^+$); found (FAB) 284.12934 (MH$^+$), C$_{17}$H$_{18}$NO$_3$ requires 284.12867.

3.2.6 General procedure for high pressure hydrogenations

A mixture of substrate and catalysts in degassed methanol with a magnetic stirrer were placed into the multi-well Baskerville hydrogenation bomb that was placed on a magnetic stirrer hotplate. The apparatus was pressurised and vented three times to operating pressure with nitrogen followed by repeating the procedure with hydrogen. The vessel was then pressurised with hydrogen and the reactions allowed to stir at
room temperature for the required length of time. At the end of reaction, the vessel was vented and pressurised three times with nitrogen before opening.

Pd/C reactions were worked up by filtration and concentration to the product. Asymmetric reactions were worked up by concentration and running the residue through a short column of silica (ethyl acetate).

**Pd/C hydrogenation of (E/Z)-2-benzamido-3-phenylbut-2-enoate 94a**

![Chemical structure of 94a](image)

The general procedure was followed using alkene 94a (50mg, 0.17mmol), Pd/C (10%, 5.0mg) and methanol (5ml) at 120psi for 21 hours. Workup yielded a white solid (50mg, 100%).

δ<sub>H</sub> (200MHz, CDCl<sub>3</sub>) 1.50 (1.63H, d J=7.4Hz, CH<sub>3</sub>), 1.56* (1.37H, d J=7.0Hz, CH<sub>3</sub>), 3.37 (0.44H, quin. J=6.6Hz, CH), 3.59* (0.56H, quin. J=7.0Hz, CH), 3.67 (1.69H, d J=1.5Hz, CH<sub>3</sub>), 3.80* (1.31H, d J=1.5Hz, CH<sub>3</sub>), 5.04 (1H, m, CH), 6.69 (0.38H, d br. J=8.2Hz, NH), 7.25* (0.62H, d br. J=8.2Hz, NH), 7.27-7.84 (10H, m, ArH).

Chiracel OD; Gradient: Hex/IPA (98:2 to 80:20) over 30mins, 254nm, 30°C; R<sub>t</sub> (min) 12.77 (18.3%), 13.76 (30.8%), 16.07 (18.0%), 16.72 (32.9%).

**[Rh(S,S)-Et-DuPhos(COD)]BF<sub>4</sub> hydrogenation of (E/Z)-2-benzamido-3-phenylbut-2-enoate 94a**

![Chemical structure of 94a](image)
The general procedure was followed using alkene 94a (50mg, 0.17mmol), [Rh(S,S)-Et-DuPhos(COD)]BF₄ (1.1mg, 0.0017mmol) and methanol (5ml) at 120psi for 90 hours. Workup yielded a white solid (32mg, 64%).

δ_H (200MHz, CDCl₃) 1.50 (1.2H, d J=7Hz, CH₃), 1.51* (1.8H, d J=7Hz, CH₃), 3.38-3.60 (1H, m, CH), 3.68 (1.2H, s, CO₂CH₃), 3.81* (1.8H, s, CO₂CH₃), 5.10 (1H, m, CH), 6.46 (0.4H, d br. J= 7.8Hz, NH), 6.74* (0.6H, d br. J= 8.2Hz, NH), 7.26-7.87 (10H, m, ArH).

Chiracel OD; Gradient Hex/IPA (98:2 to 80:20) over 30 mins, 254nm, 30°C; R_t (min) 12.82 (22.0%), 13.76 (61.1%), 16.15 (16.9%).

[Rh(R,R)-Et-DuPhos(COD)]BF₄ hydrogenation of (E/Z)-2-benzamido-3-phenylbut-2-enolate 94a

The general procedure was followed using alkene 94a (50mg, 0.17mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (1.1mg, 0.0017mmol) and methanol (5ml) at 120psi for 90 hours. Workup yielded a white solid (50mg, 100%).

δ_H (200MHz, CDCl₃) 1.50 (1H, d J=7.0Hz, CH₃), 1.55* (2H, d J=7.4Hz, CH₃), 3.38-3.63 (1H, m, CH), 3.68 (2H, s, CO₂CH₃), 3.81* (1H, s, CO₂CH₃), 5.10 (1H, m, CH), 6.43 (0.33H, d br. J=9.0Hz, NH), 6.71* (0.67H, d br. J=8.2Hz, NH), 7.26-7.86 (10H, m, ArH).

Chiracel OD; Gradient Hex/IPA (98:2 to 80:20) over 30 mins, 254nm, 30°C; R_t (min) 12.81 (15.3%), 13.82 (0.9%), 15.94 (17.7%), 16.51 (66.1%).
Pd/C hydrogenation of (E/Z)-2-Benzoylamino-3-(4-methoxyphenyl)but-2-enoic acid methyl ester 94c

The general procedure was followed using alkene 94c (50mg, 0.15mmol), Pd/C (10%, 5.0mg) and methanol (5ml) at 120psi overnight. Workup yielded a white solid (50mg, 100%).

δH (200MHz, CDCl3): 1.51 (3H, d J=7.0Hz, CH3), 3.36 (1H, quin J=7.0Hz, CH), 3.68 (3H, s, OCH3), 3.85 (3H, s, CO2CH3), 5.04 (1H, dd J=6.6&7.8Hz, CH), 6.75 (1H, d br. J=8.2Hz, NH), 6.92-7.94 (9H, m, ArH).

Chiracel OD; Gradient Hex/EtOH (98:2 to 90:10) over 30mins, 254nm, 20°C; Rf (min) 14.01 (6.8%), 15.25(42.9%), 16.75(7.0%), 18.36(43.3%).

[Rh(S,S)-Et-DuPhos(COD)]BF4 hydrogenation of (E/Z)-2-Benzoylamino-3-(4-methoxyphenyl)but-2-enoic acid methyl ester 94c

The general procedure was followed using alkene 94c (50mg, 0.15mmol), [Rh(S,S)-Et-DuPhos(COD)]BF4 (1.0mg, 0.0015mmol) and methanol (5ml) at 120psi for 90 hours. Workup followed by wet flash column chromatography (hexane/ethyl acetate 2:1) yielded a white solid (31mg, 62%).
[Rh(R,R)-Et-DuPhos(COD)]BF₄ hydrogenation of (E/Z)-2-Benzoylamino-3-(4-methoxyphenyl) but-2-enoic acid methyl ester 94c

\[
\begin{align*}
\delta_H (200MHz, CDCl₃): &\ 1.53 (3H, d J=7.0Hz, CH₃), 3.37 (1H, quin J=7.0Hz, CH), \\
&\ 3.70 (3H, s, OCH₃), 3.87 (3H, s, CO₂CH₃), 5.06 (1H, dd J=6.6&7.8Hz, CH), 6.67 \\
&\ (1H, d br. J=8.2Hz, NH), 6.90-7.85 (9H, m, ArH).
\end{align*}
\]

Chiracel OD; Gradient Hex/EtOH (98:2 to 90:10) over 30mins, 254nm, 20°C; Rᵣ (min) 14.09 (6.7%), 15.29(86.0%), 16.41(5.2%), 18.19(2.1%).

The general procedure was followed using alkene 94c (50mg, 0.15mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (1.0mg, 0.0015mmol) and methanol (5ml) at 120psi for 113 hours
Workup yielded a white solid (50mg, 100%).

\[
\begin{align*}
\delta_H (200MHz, CDCl₃): &\ 1.53 (3H, d J=7.0Hz, CH₃), 3.37 (1H, quin J=7.0Hz, CH), \\
&\ 3.70 (3H, s, OCH₃), 3.87 (3H, s, CO₂CH₃), 5.06 (1H, dd J=6.6&7.8Hz, CH), 6.67 \\
&\ (1H, d br. J=8.2Hz, NH), 6.90-7.85 (9H, m, ArH).
\end{align*}
\]
Pd/C hydrogenation of (\(E/Z\))-2-Benzoylamino-3,4-dimethylpent-2-enoic acid methyl ester 94d

The general procedure was followed using alkene 94d (25mg, 0.10mmol), Pd/C (10%, 2.5mg) and methanol (2.5ml) at 120psi for 120 hours.
Workup yielded a white solid (24mg, 96%).

\[\delta_H (200MHz, CDCl_3) 1.02 (6H, m, (CH_3)_2), 1.14 (3H, m, CH_3), 1.65 (1H,m,CH), 1.90 (1H, m, CH(CH_3)_2), 3.82 (3H, d, CO_2CH_3), 4.90 (0.5H, dd J=6.5 and 8.5H, CH), 5.11* (0.5H, dd J=4.4 and 7.1Hz, CH), 6.55* (0.5H, d br. J=9.3Hz , NH), 6.74 (0.5H, d br. J=8 , NH), 7.40-7.86 (5H,m,ArH).\]

Chiracel OD-H: 0-5%EtOH in Hexane over 30mins, 254nm, 10°C, R_t (min) 20.71 (24.3%), 21.30 (25.6%), 22.32 (24.9%), 23.33 (25.2%).

\([\text{Rh}(S,S)-\text{Et-DuPhos(COD))BF}_4\) hydrogenation of (\(E/Z\))-2-Benzoylamino-3,4-dimethylpent-2-enoic acid methyl ester 94d

The general procedure was followed using alkene 94d (25mg, 0.12mmol), \([\text{Rh}(S,S)-\text{Et-DuPhos(COD))BF}_4\) (1.1mg, 0.0012mmol) and methanol (2.5ml) at 120psi for 130 hours.
Workup yielded a white solid (10mg, 40%).

\[\delta_H (200MHz, CDCl_3) 1.02 (6H, m, (CH_3)_2), 1.14 (3H, m, CH_3), 1.65 (1H, m, CH), 1.90 (1H, m, CH(CH_3)_2), 3.82 (3H, d, CO_2CH_3), 4.90 (0.36H, dd J=6.5 and 8.5H, CH).\]
CH), 5.11* (0.64H, dd $J=4.4$ and 7.1Hz, CH), 6.55* (0.64H, d br. $J=9.3$Hz, NH), 6.74 (0.36H, d br. $J=8.0$Hz, NH), 7.40-7.86 (5H, m, ArH).

Chiracel OD-H: 0-5%EtOH in Hexane over 30mins, 254nm, 10°C, $R_t$ (min) 19.46 (4.1%), 20.05 (22.8%), 21.41 (56.4%), 22.89 (16.6%).

\[
[Rh(R,R)-Et-DuPhos(COD)]BF_4 \text{ hydrogenation of } (E/Z)-2-Benzoylamino-3,4-dimethylpent-2-enoic acid methyl ester \ 94d
\]

The general procedure was followed using alkene 94d (25mg, 0.12mmol), [Rh(R,R)-Et-DuPhos(COD)]BF_4 (1.1mg, 0.0012mmol) and methanol (2.5ml) at 120psi for 130 hours.

Workup yielded a white solid (23mg, 92%).

$\delta^H$ (200MHz, CDCl_3) 1.02 (6H, m, (CH_3)_2), 1.14 (3H, m, CH_3), 1.65 (1H, m, CH), 1.90 (1H, m, CH(CH_3)_2), 3.82 (3H, d, CO_2CH_3), 4.90 (0.33H, dd $J=6.5$ and 8.5Hz, CH), 5.11* (0.67H, dd $J=4.4$ and 7.1Hz, CH), 6.55* (0.67H, d br. $J=9.3$Hz, NH), 7.40-7.86 (5H, m, ArH).

Chiracel OD-H: 0-5%EtOH in Hexane over 30mins, 254nm, 10°C, $R_t$ (min) 19.39 (56.7%), 20.03 (16.0%), 21.50 (4.8%), 22.84 (22.5%).
Experimental

Pd/C hydrogenation of (Z)-2-Benzoylamino-3,4,4-trimethylpent-2-enoic acid methyl ester 94e

The general procedure was followed using alkene 94e (50mg, 0.18mmol), Pd/C (10%, 5.0mg) and methanol (5ml) at 120psi for 20 hours. Reaction was incomplete so 5mg more catalyst added. After a further 22 hours the reaction was stopped. Workup yielded 46mg (92%) of a clear oil. Wet flash column chromatography (hexane/ethyl acetate 4:1) gave a white solid (36mg, 72%).

$\delta_H$ (200MHz, CDCl$_3$) 1.07 (12H, m, tBu + CH$_3$), 2.01 (1H, m, CH), 3.81 (3H, s, CO$_2$CH$_3$), 5.06 (0.5H, dd $J$=4.5&8.6Hz, CH), 5.28* (0.5H, dd $J$=2.0&9.8Hz, CH), 6.53 (0.5H, d br. $J$=10.2Hz, NH), 6.75* (0.5H, d br. $J$=7.4Hz, NH), 7.50-7.89 (5H, m, ArH).

Chiracel OD; Gradient Hex/IPA (98:2 to 70:30) over 30mins, 254nm, 5°C; R$_t$ (min) 11.89 (24.2%), 13.58 (26.1%), 19.22 (24.0%), 21.33 (25.7%).

[Rh(S,S)-Et-DuPhos(COD)]BF$_4$ hydrogenation of (Z)-2-Benzoylamino-3,4,4-trimethylpent-2-enoic acid methyl ester 94e

The general procedure was followed using alkene 94e (50mg, 0.18mmol), [Rh(S,S)-Et-DuPhos(COD)]BF$_4$ (1.2mg, 0.0018mmol) and methanol (5ml) at 120 psi. No reaction after 24 hours so 1mg more catalyst was added. After 110 hours reaction was still incomplete.
Experimental

The mixture was concentrated and wet flash column chromatography (hexane/ethyl acetate 4:1) yielded a gummy white solid (8mg, 16%).

$\delta_H$ (200MHz, CDCl$_3$) 0.93 (12H, m, tBu + CH$_3$), 1.95 (1H, m, CH), 3.69 (3H, s, CO$_2$CH$_3$), 5.16 (1H, dd $J$=2.0&10.0Hz, CH), 6.37 (1H, d br. $J$=9.7Hz, NH), 7.19-7.28 (5H, m, ArH).

Chiracel OD; Gradient Hex/IPA (98:2 to 70:30) over 30mins, 254nm, 5°C; $R_t$ (min) 11.84 (58.6%), 13.83 (3.5%), 19.62 (3.0%), 21.60 (34.9%).

[Rh($R,R$)-Et-DuPhos(COD)]BF$_4$ hydrogenation of (Z)-2-Benzoylamino-3,4,4-trimethyldipent-2-enoic acid methyl ester 94e

\[
\text{Me} \quad \text{Me} \\
\text{Me} \quad \text{CO}_2\text{Me}
\]

The general procedure was followed using alkene 94e (40mg, 0.15mmol), [Rh($R,R$)-Et-DuPhos(COD)]BF$_4$ (1.0mg, 0.0015mmol) and methanol (5ml) at 120psi for 113 hours

Workup yielded of a gummy white solid (11mg, 27%).

$\delta_H$ (200MHz, CDCl$_3$) 1.08 (12H, m, tBu + CH$_3$), 2.03 (1H, m, CH), 3.83 (3H, s, CO$_2$CH$_3$), 5.29 (1H, dd $J$=2.0&10.0Hz, CH), 6.52 (1H, d br. $J$=9.7Hz, NH), 7.53-7.90 (5H, m, ArH).

Chiracel OD; Gradient Hex/IPA (98:2 to 70:30) over 30mins, 254nm, 5°C; $R_t$ (min) 12.15 (25.6%), 13.88 (1.5%), 20.52 (4.3%), 23.17 (68.6%).
Pd/C hydrogenation of (E/Z)-2-(4-methoxy)benzamido)-3-phenylbut-2-enoate 94g

The general procedure was followed using alkene 94g (50mg, 0.15mmol), Pd/C (10%, 5.0mg) and methanol (5ml) at 120psi for 72 hours.
Workup yielded a white solid (48mg, 96%).

δ_H (200MHz, CDCl_3) 1.47 (1.5H, d J=7.4Hz, CH_3), 1.52* (1.5H, d J=7.0Hz, CH_3), 3.35 (0.45H, m, CH), 3.54* (0.55H, m, CH), 3.66 (1.5H, s, OCH_3), 3.80* (1.5H, s, OCH_3), 3.90 (1.5H, s, CO_2CH_3), 3.92* (1.5H, s, CO_2CH_3), 5.02 (1H, m, CH), 6.30 (0.5H, d br. J=8.0Hz, NH), 6.61* (0.5H, d br. J=8.0Hz, NH), 6.94-7.82 (9H, m, ArH).

Chiracel OD; Isocrat Hex/IPA (80:20), 20 mins, 254nm, 5°C; R_t (min) 7.55 (32.15%), 8.46 (17.80%), 14.70 (17.25%), 17.52 (32.80%).

[Rh(S,S)-Et-DuPhos(COD)]BF_4 hydrogenation of (E/Z)-2-(4-methoxy) benzamido)-3-phenylbut-2-enoate 94g

The general procedure was followed using alkene 94g (50mg, 0.15mmol), [Rh(S,S)-Et-DuPhos(COD)]BF_4 (1.0mg, 0.0015mmol) and methanol (5ml) at 120psi for 110 hours.
Workup followed by wet flash column chromatography (hexane/ethyl acetate 2:1) yielded a white solid (13mg, 26%).
Experimental

$\delta_H$ (200MHz, CDCl$_3$) 1.50 (1H, d $J=7.0$Hz, CH$_3$), 1.55* (2H, d $J=7.8$Hz, CH$_3$), 3.36-3.63 (1H, m, CH), 3.68 (2H, s, OCH$_3$), 3.82* (1H, s, OCH$_3$), 3.93 (2H, s, CO$_2$CH$_3$), 3.95* (2H, s, CO$_2$CH$_3$), 5.10 (1H, m, CH), 6.32 (0.32H, d br. $J=8.2$Hz, NH), 6.60* (0.67H, d br. $J=8.2$Hz, NH), 6.96-7.83 (9H, m, ArH).

Chiracel OD; Isocrat Hex/IPA (80:20), 20 mins, 254nm, 5°C; R$_r$ (min) 7.95 (2.7%), 8.86 (12.9%), 14.38 (19.1%), 16.95 (65.3%).

$[\text{Rh}(R,R)-\text{Et-DuPhos(COD)}]BF_4$ hydrogenation of (E/Z)-2-(4-methoxy)benzamido)-3-phenylbut-2-enoate 94g

The general procedure was followed using alkene 94g (50mg, 0.15mmol), (R,R)-EtDuPhos (1.0mg, 0.0015mmol) and methanol (5ml) at 120psi for 113 hours

Workup yielded a clear gum (49mg, 98%).

$\delta_H$ (200MHz, CDCl$_3$) 1.49 (1.5H, d $J=7.1$Hz, CH$_3$), 1.52* (1.5H, d $J=7.4$Hz, CH$_3$), 3.35-3.61 (1H, m, CH), 3.66 (1.5H, s, OCH$_3$), 3.80* (1.5H, s, OCH$_3$), 3.91 (1.5H, s, CO$_2$CH$_3$), 3.91* (1.5H, s, CO$_2$CH$_3$), 5.12 (1H, m, CH), 6.35 (0.5H, d br. $J=8.6$Hz, NH), 6.65* (0.5H, d br. $J=8.6$Hz, NH), 6.94-7.83 (9H, m, ArH)

Chiracel OD; Isocrat Hex/IPA (80:20), 20 mins, 254nm, 5°C; R$_r$ (min) 7.57 (50.8%), 8.42 (27.7%), 13.37 (21.1%), 15.61 (0.5%).
3.2.7 Asymmetric hydrogenation of (E)-alkenes

Synthesis of erythro-2-Acetylamino-3-phenylbutyric acid methyl ester *threo*-108a

The general procedure was followed using alkene (E)-108a (20mg, 0.080mmol), Pd/C (2.0mg) and methanol (2.0ml) at 120psi for 22 hours. Workup yielded a white gummy solid (20mg, 100%).

\[
\begin{align*}
\delta_H \text{ (250MHz, CDCl}_3) & \quad 1.49 \text{ (3H, d } J=7.2Hz, \text{ CH}_3), \quad 2.10 \text{ (3H, s, Ac), } 3.51 \text{ (1H, quin. } J=6.0Hz, \text{ CH), } 3.85 \text{ (3H, s, CO2CH}_3), \quad 4.99 \text{ (1H, m, CH), } 6.70 \text{ (1H, d br. } J=8.2Hz, \text{ NH), } 7.27-7.47 \text{ (4H, m, ArH).}
\end{align*}
\]

Chiracel OD, 1ml/min, 2%EtOH in hexane, 210nm, 15°C; R (min) 13.82 (49.9%), 16.80 (50.1%).

Synthesis of (2R,3R)-2-Acetylamino-3-phenylbutyric acid methyl ester (2R,3R)-108a

The general procedure was followed using alkene (E)-108a (100mg, 0.40mmol), [Rh(R,R)-Et-DuPhos(COD)]BF$_4$ (2.6mg, 0.004mmol) and methanol (5.0ml) at 120psi for 90 hours. Workup yielded a white gum (100mg, 100%).

\[
\begin{align*}
\delta_H \text{ (250MHz, CDCl}_3) & \quad 1.49 \text{ (3H, d } J=7.2Hz, \text{ CH}_3), \quad 2.10 \text{ (3H, s, Ac), } 3.51 \text{ (1H, quin. } J=6.0Hz, \text{ CH), } 3.85 \text{ (3H, s, CO2CH}_3), \quad 4.99 \text{ (1H, m, CH), } 6.70 \text{ (1H, d br. } J=8.2Hz, \text{ NH), } 7.27-7.47 \text{ (4H, m, ArH).}
\end{align*}
\]
Chiracel OD, 1ml/min, 2%EtOH in hexane, 210nm, 15°C; R<sub>t</sub> (min) 13.82 (54.9%), 16.80 (45.1%).

**Synthesis of erythro-2-Acetylamino-3-(4-fluorophenyl)but-2-enolic acid methyl ester erythro-113b**

\[
\begin{align*}
\text{Me} & \quad \text{H} \\
\text{AcH} & \quad \text{H} \\
& \quad \text{CO}_2\text{Me} \\
\end{align*}
\]

The general procedure was followed using alkene (E)-108b (20mg, 0.080mmol), Pd/C (2.0mg) and methanol (2.0ml) at 120psi for 22 hours.

Workup yielded a white gummy solid (20mg, 100%).

\[\delta_H (400MHz, CDCl_3) 1.31 (3H, d J=7Hz, CH_3), 1.97 (3H, s, Ac), 3.35 (1H,quin. J=7Hz, CH), 3.71 (3H, s, CO_2CH_3), 4.85 (1H, m, CH), 6.70 (1H,d br. J=9Hz, NH), 6.95-7.15 (4H, m, ArH).\]

SFC: Chiralpak AD; 15% MeOH in CO<sub>2</sub>, 254nm; R<sub>t</sub> (min) 1.82 (51.4%), 2.96 (48.6%).

**Synthesis of (2R,3R)-2-Acetylamino-3-(4-fluorophenyl)but-2-enolic acid methyl ester (2R,3R)-113b using [Rh(R,R)-Et-DuPhos(COD)]BF<sub>4</sub>**

\[
\begin{align*}
\text{Me} & \quad \text{H} \\
\text{AcH} & \quad \text{H} \\
& \quad \text{CO}_2\text{Me} \\
\end{align*}
\]

The general procedure was followed using alkene (E)-108b (100mg, 0.40mmol), [Rh(R,R)-Et-DuPhos(COD)]BF<sub>4</sub> (2.6mg, 0.004mmol) and methanol (5.0ml) at 120psi for 90 hours.

Workup yielded a yellow gum (100mg, 100%).
δ_H (400MHz, CDCl_3) 1.31 (3H, d J=7Hz, CH_3), 1.97 (3H, s, Ac), 3.36 (1H, quin. J=7Hz, CH), 3.71(3H, s, CO_2CH_3), 4.83 (1H, m, CH), 6.65 (1H, d br. J=9Hz, NH), 6.95-7.15 (4H, m, ArH).

SFC: Chiralpak AD; 15% MeOH in CO_2, 254nm; R_t (min) 1.88 (55.0%), 2.97 (45.0%).

Synthesis of (2R,3R)-2-Acetylamino-3-(4-fluorophenyl)but-2-enoic acid methyl ester (2R,3R)-13b using [Rh(R,R)-Ph-BPE(COD)]BF_4

The general procedure was followed using alkene (E)-108b (10.0mg, 0.04mmol), [Rh(R,R)-Ph-BPE(COD)]BF_4 (0.5mg) and methanol (2.0ml) at 100psi for 20 hours. Workup yielded a yellow gum (10mg, 100%).

SFC: Chiralpak AD; 15% MeOH in CO_2, 254nm; R_t (min) 1.88(89.5%), 2.97(10.5%).

Synthesis of erythro-2-Acetylamino-3-(4-trifluoromethylphenyl)but-2-enoic acid methyl ester erythro-113c

The general procedure was followed using alkene (E)-108c (20mg, 0.066mmol), Pd/C (2.0mg) and methanol (2.0ml) at 120psi for 22 hours. Workup yielded a white gummy solid (20mg, 100%).

δ_H (400MHz, CDCl_3) 1.34 (3H, d J=7Hz, CH_3), 1.97 (3H, s, Ac), 3.43 (1H, quin. J=7Hz, CH), 3.72 (3H, s, CO_2CH_3), 4.92 (1H, m, CH), 5.75 (1H, d br. J=9Hz, NH), 7.25-7.58 (4H, m, ArH)

SFC: Chiralpak AD; 15% MeOH in CO_2, 254nm; R_t (min) 2.40 (50.3%), 3.11 (49.7%).
Synthesis of (2R,3R)-2-Acetylamino-3-(4-trifluoromethylphenyl)but-2-enoic acid methyl ester (2R,3R)-113c using [Rh(R,R)-Et-DuPhos(COD)]BF₄

The general procedure was followed using alkene (E)-1108c (100mg, 0.33mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (2.2mg, 0.0033mmol) and methanol (5.0ml) at 120psi for 90 hours. Workup yielded a white solid (97mg, 95%).

δ_H (400MHz, CDCl₃) 1.34 (3H, d J=7Hz, CH₃), 1.98 (3H, s, Ac), 3.43 (1H, quin. J=7Hz, CH), 3.72 (3H, s, CO₂CH₃), 4.92 (1H, m, CH), 5.69 (1H, d br. J=9Hz, NH), 7.21-7.58 (4H, m, ArH).
SFC: Chiralpak AD; 15% MeOH in CO₂, 254nm; R_t (min) 2.39 (50.0%), 3.04 (50.0%).

Synthesis of (2R,3R)-2-Acetylamino-3-(4-trifluoromethylphenyl)but-2-enoic acid methyl ester (2R,3R)-113c using [Rh(R,R)-Ph-BPE(COD)]BF₄

The general procedure was followed using alkene (E)-108c (8.0mg, 0.03mmol), [Rh(R,R)-Ph-BPE(COD)]BF₄ (0.5mg) and methanol (2.0ml) at 100psi for 72 hours. Workup yielded a white gum (8mg, 100%).

SFC: Chiralpak AD; 15% MeOH in CO₂, 254nm; R_t (min) 2.39 (87.0%), 3.04 (13.0%).

Synthesis of erythro-2-Acetylamino-3-(4-methoxyphenyl)but-2-enoic acid methyl ester erythro-113d
The general procedure was followed using alkene \((E)-108d\) (20mg, 0.076mmol), Pd/C (2.0mg) and methanol (2.0ml) at 120psi for 22 hours. Workup yielded a white gummy solid (20mg, 100%).

\[ \delta_H (400MHz, \text{CDCl}_3) 1.31 (3H, d J=7Hz, \text{CH}_3), 1.97 (3H, s, \text{Ac}), 3.33 (1H, \text{quin. } J=7Hz, \text{CH}), 3.71 (3H, s, \text{CO}_2\text{CH}_3), 3.80 (3H, s, \text{OCH}_3), 4.79 (1H, m, \text{CH}), 6.78 (1H, d br. J=9Hz, \text{NH}), 6.81-7.06 (4H, m, \text{ArH}). \]

SFC: Chiralpak AD; 15% MeOH in CO2, 254nm; \(R_t\) (min) 2.60 (50.3%), 4.70 (49.7%).

Synthesis of \((2R,3R)-2\text{-Acetylamino-3-}(4\text{-methoxyphenyl})\text{but-2-enoic acid methyl ester (2R,3R)-113d using [Rh(R,R)-Et-DuPhos(COD)]BF}_4^{78}\]

The general procedure was followed using alkene \((E)-108d\) (100mg, 0.38mmol), \([\text{Rh(R,R)-Et-DuPhos(COD)}]\text{BF}_4\) (2.5mg, 0.0038mmol) and methanol (5.0ml) at 120psi for 90 hours. Workup yielded an off white solid (96mg, 94%).

\[ \delta_H (400MHz, \text{CDCl}_3) 1.31 (3H, d J=7Hz, \text{CH}_3), 1.97 (3H, s, \text{Ac}), 3.33 (1H, \text{quin. } J=7Hz, \text{CH}), 3.71(3H, s, \text{CO}_2\text{CH}_3), 3.79 (3H, s, \text{OCH}_3), 4.80 (1H, m, \text{CH}), 6.62 (1H, d br. J=9Hz, \text{NH}), 6.81-7.06 (4H, m, \text{ArH}) \]

SFC: Chiralpak AD; 15% MeOH in CO2, 254nm; \(R_t\) (min) 2.43 (62.5%), 4.60 (37.5%).

Synthesis of \((2R,3R)-2\text{-Acetylamino-3-}(4\text{-methoxyphenyl})\text{but-2-enoic acid methyl ester (2R,3R)-113d using [Rh(R,R)-Ph-BPE(COD)]BF}_4^{78}\]

The general procedure was followed using alkene \((E)-108d\) (10.0mg, 0.04mmol), \([\text{Rh(R,R)-Ph-BPE(COD)}]\text{BF}_4\) (0.5mg) and methanol (2.0ml) at 100psi for 20 hours. Workup yielded a white gum (10mg, 100%).
Experimental

SFC: Chiralpak AD; 15% MeOH in CO₂, 254nm; Rₜ (min) 2.43 (87.5%), 4.60 (12.5%).

Synthesis of erythro-2-Acetylamino-3-(3,5-dimethylphenyl)but-2-enoic acid methyl ester erythro-113e

The general procedure was followed using alkene G(E)-108e (20mg, 0.077mmol), Pd/C (2.0mg) and methanol (2.0ml) at 120psi for 22 hours. Workup yielded a white gummy solid (20mg, 100%).

δ_H (400MHz, CDCl₃) 1.30 (3H, d J=7Hz, CH₃), 1.95 (3H, s, Ac), 2.29 (6H, s, (CH₃)₂), 3.27 (1H, quin. J=7Hz, CH), 3.71 (3H, s, CO₂CH₃), 4.78 (1H, m, CH), 5.65 (1H, d br. J=9Hz, NH), 6.71-6.88 (3H, m, ArH)

SFC: Chiralpak AD; 15% MeOH in CO₂, 254nm; Rₜ (min) 1.81 (50.0%), 7.43 (50.0%).

Synthesis of (2R,3R)-2-Acetylamino-3-(3,5-dimethylphenyl)but-2-enoic acid methyl ester (2R,3R)-113e using [Rh(R,R)-Et-DuPhos(COD)]BF₄

The general procedure was followed using alkene (E)-108e (100mg, 0.38mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (2.5mg, 0.0038mmol) and methanol (5.0ml) at 120psi for 90 hours. Workup yielded an off white solid (96mg, 94%).

126
δ\textsubscript{H} (400MHz, CDCl\textsubscript{3}) 1.31 (3H, d \textit{J}=7Hz, CH\textsubscript{3}), 1.96 (3H, s, Ac), 2.31 (6H, s, (CH\textsubscript{3})\textsubscript{2}), 3.26 (1H, quin. \textit{J}=7Hz, CH), 3.71 (3H, s, CO\textsubscript{2}CH\textsubscript{3}), 4.78 (1H, m, CH), 5.61 (1H, d br. \textit{J}=9Hz, NH), 6.70-6.89 (3H, m, ArH)

SFC: Chiralpak AD; 15% MeOH in CO\textsubscript{2}, 254nm; \textit{R} \textsubscript{t} (min) 2.40 (63%), 9.71 (37%).

**Synthesis of (2R,3R)-2-Acetylamino-3-(3,5-dimethylphenyl)but-2-enoic acid methyl ester (2R,3R)-113e using [Rh(R,R)-Ph-BPE(COD)]BF\textsubscript{4}**

The general procedure was followed using alkene (E)-108e (10.0mg, 0.04mmol), [Rh(R,R)-Ph-BPE(COD)]BF\textsubscript{4} (0.5mg) and methanol (2.0ml) at 100psi for 20 hours. Workup yielded a white gum (10mg, 100%).

SFC: Chiralpak AD; 15% MeOH in CO\textsubscript{2}, 254nm; \textit{R} \textsubscript{t} (min) 2.40 (90.0%), 9.71 (10.0%).

### 3.2.8 Asymmetric hydrogenation of (Z)-alkenes

**Synthesis of threo-2-Acetylamino-3-phenylbutyric acid methyl ester threo-113a**

![Threo-2-Acetylamino-3-phenylbutyric acid methyl ester](image)

The general procedure was followed using alkene (Z)-108a (50mg, 0.22mmol), Pd/C (10%, 5.0mg) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a white solid (50mg, 100%).

δ\textsubscript{H} (200MHz, CDCl\textsubscript{3}) 1.44 (3H, d \textit{J}=7.4Hz, CH\textsubscript{3}), 2.07 (3H, s, CH\textsubscript{3}), 3.22 (1H, quin. \textit{J}=7.0Hz, CH), 3.63 (3H, s, OCH\textsubscript{3}), 4.81 (1H, dd \textit{J}=6.6&9.0Hz, CH), 6.09 (1H, d br. \textit{J}=8.6Hz, NH), 7.20-7.41 (5H, m, ArH).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; \textit{R} \textsubscript{t} (min) 16.76 (49.8%), 20.66 (50.2%).
Experimental

Synthesis of (2R,3S)-2-Acetylamino-3-phenylbutyric acid methyl ester (2R,3S)-113a

The general procedure was followed using alkene \((Z)-108a\) (300mg, 1.29mmol), \([Rh(R,R)-Et-DuPhos(COD)]BF_4\) (8.4mg, 0.013mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a white solid (300mg, 99%).

M.p. 82-86°C; \(\nu_{max}\) (Film) 1541 (NH bend), 1657 (C=O amide), 1743 (C=O ester), 3289 cm\(^{-1}\) (NH stretch); \((200MHz, CDCl_3)\) 1.47 (3H, d \(J=7.4Hz, CH_3\)), 2.06 (3H, s, COCH\(_3\)), 3.25 (1H, quin. \(J=7.0Hz, CH\)), 3.62 (3H, s, CO\(_2\)CH\(_3\)), 4.87 (1H, dd \(J=6.6&9.0Hz, CH\)), 6.20 (1H, d br. \(J=8.6Hz, NH\)), 7.21-7.41 (5H, m, ArH); \(m/z\) (ES\(^{+}\)) 236 (MH\(^{+}\)); \([\alpha]^{21}_D\) -47.4° (c=0.27, CHCl\(_3\)).

Chiral OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; \(R_t\) (min) 16.34 (99.3%), 20.22 (0.7%).

Synthesis of (2S,3R)-2-Acetylamino-3-phenylbutyric acid methyl ester (2S,3R)-113a

The general procedure was followed using alkene \((Z)-108a\) (300mg, 1.29mmol), \([Rh(S,S)-Et-DuPhos(COD)]BF_4\) (8.4mg, 0.013mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded white solid (300mg, 99%).
M.p. 81-84°C; \( \nu_{\text{max}} \) (Film) 1544 (NH bend), 1657 (C=O amide), 1744 (C=O ester), 3285 cm\(^{-1}\) (NH stretch); \( \delta_H \) (200MHz, CDCl\(_3\)) 1.47 (3H, d \( J=7.4 \)Hz, CH\(_3\)), 2.06 (3H, s, COCH\(_3\)), 3.25 (1H, quin. \( J=7.0 \)Hz, CH), 3.62 (3H, s, CO\(_2\)CH\(_3\)), 4.87 (1H, dd \( J=6.6\&9.0 \)Hz, CH), 6.20 (1H, d br. \( J=8.6 \)Hz, NH), 7.21-7.41 (5H, m, ArH); \( m/z \) (ES\(^+\)) 236 (MH\(^+\)); [\( \alpha \)\(^{21}\)\(_D\)] +49.3° (c=0.14, CHCl\(_3\)).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; \( R_l \) (min) 15.25 (0.1%), 21.20 (99.9%).

**Synthesis of threo-2-Acetylamino-3-(4-fluorophenyl)butyric acid methyl ester**

\[ \text{threo-113b} \]

The general procedure was followed using alkene (Z)-108b (20mg, 0.080mmol), Pd/C (10%, 2mg) and methanol (2ml) at 100psi for 72 hours. Workup yielded a pale yellow solid (20mg, 100%).

\( \delta_H \) (200MHz,CDCl\(_3\)) 1.43 (3H, d \( J=7.4 \)Hz, CH\(_3\)), 2.08 (3H, s, Ac), 3.26 (1H, quin. \( J=7.2 \)Hz, CH), 3.65 (3H,s,CO\(_2\)CH\(_3\)), 4.86 (1H,dd \( J=6.8\&9.0 \)Hz,H), 6.02 (1H,d br. \( J=8.4 \)Hz, NH), 7.01-7.40 (4H, m, ArH).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 6.8 °C; \( R_l \) (min) 17.88 (51.0%), 26.95 (49.0%).

129
Experimental

Synthesis of (2R,3S)-2-Acetylamino-3-(4-fluorophenyl)butyric acid methyl ester (2R,3S)-113b

The general procedure was followed using alkene (Z)-108b (300mg, 1.20mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (7.9mg, 0.012mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 92-95 °C; νmax (KBr) 1512 (NH bend), 1652 (C=O amide), 1738 (C=O ester), 3316cm⁻¹ (NH stretch); δH (250MHz,CDC13) 1.28 (3H, d J=7.2Hz, CH₃), 1.94 (3H, s, Ac), 3.14 (1H, quin. J=7.1Hz, CH), 3.50 (3H, s, CO₂CH₃), 4.71 (1H, dd J=6.7 & 8.9Hz, CH), 6.00(1H, d br. J=8.8Hz, NH) 6.87-7.20 (4H, m, ArH); δC (63MHz,CDC13) 17.5 (CH₃), 23.5 (Ac), 42.4 (CH), 52.5 (CO₂CH₃), 57.9 (CH), 115.6 (d JCF=21.2Hz, C₆H₅), 129.4 (d, JCF=7.9Hz, C₆H₅), 137.2 (C), 162.2 (d JCF=245.7Hz, CF), 170.0, 172.3 (C); m/z (FAB) 254 (MH⁺); found (FAB) 254.11962 (MH⁺), C₁₃H₁₇FN₂O₃ requires 254.11925.

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 6.8 °C; Rᵣ (min) 17.49 (99.0%), 27.05 (1.0%).

Synthesis of (2S,3R)-2-Acetylamino-3-(4-fluorophenyl)butyric acid methyl ester (2S,3R)-113b

The general procedure was followed using alkene (Z)-108b (300mg, 1.20mmol), [Rh(S,S)-Et-DuPhos(COD)]BF₄ (7.9mg, 0.012mmol) and methanol (5ml) at 100psi for 72 hours.
Workup yielded a pale yellow solid (300mg, 99%).

M.p. 92-94°C; $\nu_{\text{max}}$ (KBr) 1511 (NH bend), 1651 (C=O amide), 1738 (C=O ester), 3316 cm\(^{-1}\) (NH stretch); $\delta_{H}$ (250MHz, CDCl\(_3\)) 1.28 (3H, d $J=7.2$Hz, CH\(_3\)), 1.94 (3H, s, Ac), 3.14 (1H, quin. $J=7.1$Hz, CH), 3.50 (3H, s, CO\(_2\)CH\(_3\)), 4.71 (1H, dd $J=6.7$ & 8.9Hz, CH), 6.00 (1H, d br. $J=8.8$Hz, NH) 6.87-7.20 (4H, m, ArH); $\delta_{C}$ (63MHz, CDCl\(_3\)) 17.5 (CH\(_3\)), 23.5 (Ac), 42.4 (CH), 52.5 (CO\(_2\)CH\(_3\)), 57.9 (CH), 115.6 (d $J_{CF}=21.2$Hz, C\(_{Ar}\)), 129.4 (d, $J_{CF}=7.9$Hz, C\(_{Ar}\)), 137.2 (C), 162.2 (d $J_{CF}=245.7$Hz, CF), 170.0, 172.3 (C); $m/z$ (FAB) 254 (MH\(^{+}\)); found (FAB) 254.11925 (MH\(^{+}\)), C\(_{13}\)H\(_{17}\)FNO\(_3\) requires 254.11925.

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 6.8 °C; R\(_{t}\) (min) 26.08% (100%).

**Synthesis of threo-2-Acetylamino-3-(4-trifluoromethylphenyl)butyric acid methyl ester threo-113c**

![Structure](somerichbridge.com.png)

The general procedure was followed using alkene (Z)-108c (20mg, 0.066mmol), Pd/C (10%, 2mg) and methanol (2ml) at 100psi for 72 hours. Workup yielded a white solid (10mg, 50%).

$\delta_{H}$ (250MHz, CDCl\(_3\)) 1.32 (3H, d $J=7.2$Hz, CH\(_3\)), 1.94 (3H, s, Ac), 3.22 (1H, quin. $J=7.1$Hz, CH), 3.51 (3H, s, CO\(_2\)CH\(_3\)), 4.78 (1H, dd $J=6.6$ & 8.8Hz, CH), 6.01 (1H, d br. $J=8.4$Hz, NH), 7.20-7.50 (4H, m, ArH).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5 °C; R\(_{t}\) (min) 16.46 (49.8%), 20.03 (50.2%).
Synthesis of (2R,3S)-2-Acetylamino-3-(4-trifluoromethylphenyl)butyric acid methyl ester (2R,3S)-113c

The general procedure was followed using alkene (Z)-108c (300mg, 1.00mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (6.6mg, 0.010mmol) and methanol (5ml) at 100psi for 72 hours.
Workup yielded a white solid (300mg, 99%).

M.p. 75-77 °C; \( \nu_{\text{max}} \) (KBr) 1564 (NH bend), 1654 (C=O amide), 1741 (C=O ester), 3318 cm⁻¹ (NH stretch); \( \delta_H \) (250MHz, CDCl₃) 1.32 (3H, d J=7.2Hz, CH₃), 1.94 (3H, s, Ac), 3.22 (1H, quin. J=7.1Hz, CH), 3.51 (3H, s, CO₂CH₃), 4.78 (1H, dd J=6.6 & 8.8Hz, CH), 6.01 (1H, d br. J=8.4Hz, NH), 7.20-7.50 (4H, m, ArH); \( \delta_C \) (63MHz, CDCl₃) 16.7 (CH₃), 23.0 (Ac), 42.5 (CH), 52.1 (CO₂CH₃), 57.1 (CH), 125.2, 127.9 (C₆H₄), 145.2, 169.6, 171.5 (C); \( m/z \) (FAB) 304 (MH⁺); found (FAB) 304.11621 (MH⁺), C₁₄H₁₇F₃NO₃ requires 304.11605; \([\alpha]_{D}^{21}\) -49.5° (c=0.63, CHCl₃).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; \( R_1 \) (min) 16.05 (99.2%), 21.66 (0.8%).

Synthesis of (2S,3R)-2-Acetylamino-3-(4-trifluoromethylphenyl)butyric acid methyl ester (2S,3R)-113c

The general procedure was followed using alkene (Z)-108c (300mg, 1.00mmol), [Rh(S,S)-Et-DuPhos(COD)]BF₄ (6.6mg, 0.010mmol) and methanol (5ml) at 100psi for 72 hours.
Workup yielded a white solid (300mg, 99%).
Experimental

M.p. 73-75°C; $\nu_{\text{max}}$ (KBr) 1538 (NH bend), 1653 (C=O amide), 1741 (C=O ester), 3317 cm$^{-1}$ (NH stretch); $\delta_H$ (250MHz, CDCl$_3$) 1.32 (3H, d $J=7.2$Hz, CH$_3$), 1.94 (3H, s, Ac), 3.22 (1H, quin. $J=7.1$Hz, CH), 3.51 (3H, s, CO$_2$CH$_3$), 4.78 (1H, dd $J=6.6$ & 8.8Hz, CH), 6.01(1H, d br. $J=8.4$Hz, NH), 7.20-7.50 (4H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 16.7 (CH$_3$), 23.0 (Ac), 42.5 (CH), 52.1 (CO$_2$CH$_3$), 57.1 (CH), 125.2, 127.9 (C$_{Ar}$), 145.2, 169.6, 171.5 (C); m/z (FAB) 304 (MH$^+$); found (FAB) 304.11558 (MH$^+$), C$_{14}$H$_{17}$F$_3$NO$_3$ requires 304.11605; $[\alpha]^{21}_D$ +41.7° (c=0.79, CHCl$_3$).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; $R_t$ (min) 17.00 (0.9%), 20.37 (99.1%).

Synthesis of threo-2-Acetylamino-3-(4-methoxyphenyl)butyric acid methyl ester threo-113d

The general procedure was followed using alkene (Z)-108d (20mg, 0.038mmol), Pd/C (10%, 2mg) and methanol (2ml) at 100psi for 72 hours.

Workup yielded a pale yellow gummy solid (20mg, 100%).

$\delta_H$ (250MHz, CDCl$_3$) 1.29 (3H, d $J=7.2$Hz, CH$_3$), 1.93 (3H, s, Ac), 3.07 (1H, quin. $J=7.1$Hz, CH), 3.50 (3H, s, CO$_2$CH$_3$), 3.79 (3H, s, OCH$_3$), 4.69 (1H, dd $J=6.7$ & 8.8Hz, CH), 6.02 (1H, d br. $J=9.0$Hz, NH), 6.69-7.03 (4H, m, ArH).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5 °C; $R_t$ (min) 21.39 (51.0%), 26.98 (49.0%).
Experimental

Synthesis of (2R,3S)-2-Acetylamino-3-(4-methoxyphenyl)butyric acid methyl ester (2R,3S)-113d

The general procedure was followed using alkene (Z)-108d (300mg, 1.14mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (7.5mg, 0.011mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 101-105 °C; \( \nu_{\text{max}} \) (KBr) 1514 (NH bend), 1650 (C=O amide), 1738 (C=O ester) cm\(^{-1}\) (NH stretch); \( \delta_H \) (250MHz, CDCl₃) 1.29 (3H, d \( J=7.2 \text{Hz}, \text{CH}_3 \)), 1.93 (3H, s, Ac), 3.07 (1H, quin. \( J=7.1 \text{Hz}, \text{CH} \)), 3.50 (3H, s, CO₂CH₃), 3.79 (3H, s, OCH₃), 4.69 (1H, dd \( J=6.7 \& 8.8 \text{Hz}, \text{CH} \)), 6.02 (1H, d br. \( J=9.0 \text{Hz}, \text{NH} \)), 6.69-7.03 (4H, m, ArH); \( \delta_C \) (63MHz, CDCl₃) 16.6 (CH₃), 22.5 (Ac), 41.3 (CH), 51.3 (CO₂CH₃), 54.5 (OCH₃), 57.1 (CH), 113.1, 127.9 (C₆H₅), 132.4, 158.0, 169.0, 171.4 (C); \( m/z \) (FAB) 266 (MH\(^+\)); found (FAB) 266.13870 (MH\(^+\)), C\(_{14}\)H\(_{20}\)N\(_{0}\)O\(_{4}\) requires 266.13923; [\( \alpha \)]\(_{D}\)\(^{21}\) -48.0° (c=0.32, CHCl₃).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; \( R_t \) (min) 21.36 (98.0%), 27.71 (2.0%).

Synthesis of (2S,3R)-2-Acetylamino-3-(4-methoxyphenyl)butyric acid methyl ester (2S,3R)-113d

The general procedure was followed using alkene (Z)-108d (300mg, 1.14mmol), [Rh(S,S)-Et-DuPhos(COD)]BF₄ (7.5mg, 0.011mmol) and methanol (5ml) at 100psi for 72 hours.

134
Workup yielded a pale yellow solid (300mg, 99%).

M.p. 108-110 °C; v_{max} (KBr) 1514 (NH bend), 1650 (C=O amide), 1738 (C=O ester), 3252cm^{-1} (NH stretch); δ_{H} (250MHz, CDCl_3) 1.29 (3H, d J=7.2Hz, CH_3), 1.93 (3H, s, Ac), 3.07 (1H, quin. J=7.1Hz, CH), 3.50 (3H, s, CO_2CH_3), 3.79 (3H, s, OCH_3), 4.69 (1H, dd J=6.7 & 8.8Hz, CH), 6.02 (1H, d br. J=9.0Hz, NH), 6.69-7.03 (4H, m, ArH); δ_{C} (63MHz, CDCl_3) 16.6 (CH_3), 22.5 (Ac), 41.3 (CH), 51.3 (CO_2CH_3), 54.5 (OCH_3), 57.1 (CH), 113.1, 127.9 (C_Ar), 132.4, 158.0, 169.0, 171.4 (C); m/z (FAB) 266 (MH^+); found (FAB) 266.13926 (MH^+), C_{14}H_{19}N_{0.5}O_{4} requires 266.13923; [α]_{21}^{D} +45.0° (c=0.38, CHCl_3).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; R_{t} (min) 22.52 (3.3%), 26.34 (96.7%).

**Synthesis of threo-2-Acetylamino-3-(3,5-dimethyphenyl)butyric acid methyl ester threo-113e**

![Diagram of the molecule](image)

The general procedure was followed using alkene (Z)-108e (20mg, 0.071mmol), Pd/C (10%, 2mg) and methanol (2ml) at 100psi for 72 hours. Workup yielded a white gummy solid (10mg, 50%).

δ_{H} (250MHz, CDCl_3) 1.29 (3H, d J=7.2Hz, CH_3), 1.92 (3H, s, Ac), 2.17 (6H, s, (CH_3)_2), 3.03 (1H, quin. J=7.1Hz, CH), 3.49 (3H, s, CO_2CH_3), 4.69 (1H, dd J=6.8 & 8.9Hz, CH), 6.00 (1H, s br. J=8.8Hz, NH), 6.69-6.80 (3H, m, ArH).

Chiracel OD-H, 1ml/min, 2-10%IPA in hexane over 30mins, 210nm, 5 °C; R_{t} (min) 17.13 (47.2%), 19.25 (52.8%) (dropped to baseline).
Experimental

Synthesis of (2R,3S)-2-Acetylamino-3-(3,5-dimethylphenyl)butyric acid methyl ester (2R,3S)-113e

The general procedure was followed using alkene (Z)-108e (300mg, 1.14mmol), [Rh(R,R)-Et-DuPhos(COD)]BF₄ (7.6mg, 0.011mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 115-118 °C; v_max (KBr) 1540 (NH bend), 1650 (C=O amide), 1739 (C=O ester), 3320 cm⁻¹ (NH stretch); δ_H (250MHz, CDCl₃) 1.29 (3H, d J=7.2Hz, CH₃), 1.92 (3H, s, Ac), 2.17 (6H, s, (CH₃)₂), 3.03 (1H, quin. J=7.1Hz, CH), 3.49 (3H, s, CO₂CH₃), 4.69 (1H, dd J=6.8 & 8.9Hz, CH), 6.00 (1H, s br. J=8.8Hz, NH), 6.69-6.80 (3H, m, ArH); (63MHz, CDCl₃) 16.9 (CH₃), 21.2 ((CH₃)₂), 23.0 (Ac), 42.4 (CH), 51.7 (CO₂CH₃), 57.5 (CH), 125.3, 137.6 (Cᵗ), 128.7, 140.8, 169.5, 171.9 (C); (FAB) 264 (MH⁺); found (FAB) 264.15971 (MH⁺), C₁₅H₂₂NO₃ requires 264.15997; [α]²°D⁻⁷₅.₇⁰ (c=0.44, CHCl₃).

Chiracel OD-H, 1ml/min, 2-10%IPA in hexane over 30mins, 210nm, 5°C; Rᵣ (min) 17.25 (99.8%), 19.65 (0.2%).
Experimental

Synthesis of $(2S,3R)$-2-Acetylamino-3-(3,5-dimethylphenyl)butyric acid methyl ester $(2S,3R)$-113e

The general procedure was followed using alkene $(Z)$-108e (300mg, 1.14mmol), $[\text{Rh}(S,S)$-Et-DuPhos(COD)]BF$_4$ (7.6mg, 0.011mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 112-114 °C; $\nu_{\text{max}}$ (KBr) 1541 (NH bend), 1650 (C=O amide), 1740 (C=O ester), 3319 cm$^{-1}$ (NH stretch); $\delta_H$ (250MHz, CDCl$_3$) 1.29 (3H, d $J=7.2$Hz, CH$_3$), 1.92 (3H, s, Ac), 2.17 (6H, s, (CH$_3$)$_2$), 3.03 (1H, quin. $J=7.1$Hz, CH), 3.49 (3H, s, CO$_2$CH$_3$), 4.69 (1H,dd $J=6.8$ & 8.9Hz, CH), 6.00 (1H, s br. $J=8.8$Hz, NH), 6.69-6.80 (3H, m, ArH); $\delta_C$ (63MHz, CDCl$_3$) 16.9 (CH$_3$), 21.2 ((CH$_3$)$_2$), 23.0 (Ac), 42.4 (CH), 51.7 (CO$_2$CH$_3$), 57.5 (CH), 125.3, 137.6 (C$_{Ar}$), 128.7, 140.8, 169.5, 171.9 (C); (FAB) 264 (MH$^+$); found (FAB) 264.15971 (MH$^+$), C$_{15}$H$_{22}$NO$_3$ requires 264.15997; $[\alpha]^{21}_D$ +71.6° (c=0.71, CHCl$_3$).

Chiracel OD-H, 1ml/min, 2-10%IPA in hexane over 30mins, 210nm, 5°C; $R_t$ (min) 18.93 (100%).

Synthesis of threo-2-Acetylamino-3-naphthalen-2-ylbutyric acid methyl ester threo-113f

The general procedure was followed using alkene $(Z)$-108f (20mg, 0.071mmol), Pd/C (10%, 2mg) and methanol (2ml) at 100psi for 72 hours.

137
Workup yielded a pale yellow solid (20mg, 100%).

δ_H (250MHz, CDCl_3) 1.40 (3H, d J=7.2Hz, CH_3), 1.91 (3H, s, Ac), 3.28 (1H, quin. J=7.1Hz, CH), 3.44 (3H, s, CO_2CH_3), 4.83 (1H, dd J=6.9 & 8.9Hz, CH), 6.01 (1H, d br. J=8.6Hz, NH), 7.18-7.74 (7H, m, ArH).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5 °C; R_t (min) 21.78 (49.7%), 25.91 (50.3%).

Synthesis of (2R,3S)-2-Acetylamino-3-naphthalen-2-ylbutyric acid methyl ester (2R,3S)-113f

The general procedure was followed using alkene (Z)-108f (300mg, 0.99mmol), [Rh(R,R)-Et-DuPhos(COD)]BF_4 (6.5mg, 0.010mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 65-69°C; v_max (KBr) 1544 (NH bend), 1639 (C=O amide), 1739 (C=O ester), 3267cm^{-1} (NH stretch); δ_H (250MHz, CDCl_3) 1.40 (3H, d J=7.2Hz, CH_3), 1.91 (3H, s, Ac), 3.28 (1H, quin. J=7.1Hz, CH), 3.44 (3H, s, CO_2CH_3), 4.83 (1H, dd J=6.9 & 8.9Hz, CH), 6.01 (1H, d br. J=8.6Hz, NH), 7.18-7.74 (7H, m, ArH); δ_C (63MHz, CDCl_3) 16.5 (CH_3), 22.4 (Ac), 42.2 (CH), 51.3 (CO_2CH_3), 56.9 (CH), 125.0, 125.1, 125.4, 125.7, 127.0, 127.4 (C_Ar), 131.9, 132.4, 138.0, 169.1, 171.4 (C); m/z (FAB) 286 (MH^+); found (FAB) 286.14219 (MH^+), C_{17}H_{20}NO_3 requires 286.14432; [α]^{21}_D -58.4° (c=0.69, CHCl_3).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; R_t (min) 21.08 (99.7%), 26.62 (0.3%).
Synthesis of \((2S,3R)-2\text{-Acetylamino-3-naphthalen-2-yl}\text{butyric acid methyl ester} \ (2S,3R)-113f\)

The general procedure was followed using alkene \((Z)-108f\) (300mg, 0.99mmol), [Rh(S,S)-Et-DuPhos(COD)]BF₄ (6.5mg, 0.010mmol) and methanol (5ml) at 100psi for 72 hours.

Workup yielded a pale yellow solid (300mg, 99%).

M.p. 70-72 °C; \(\nu\)max (KBr) 1544 (NH bend), 1639 (C=O amide), 1738 (C=O ester), 3266cm⁻¹ (NH stretch); \(\delta_H\) (250MHz, CDCl₃) 1.40 (3H, d \(J=7.2\)Hz, CH₃), 1.91 (3H, s, Ac), 3.28 (1H, quin. \(J=7.1\)Hz, CH), 3.44 (3H, s, CO₂CH₃), 4.83 (1H, dd \(J=6.9\) & 8.9Hz, CH), 6.01 (1H, d br. \(J=8.6\)Hz, NH), 7.18-7.74 (7H, m, ArH); \(\delta_C\) (63MHz, CDCl₃) 16.5 (CH₃), 22.4 (Ac), 42.2 (CH), 51.3 (CO₂CH₃), 56.9 (CH), 125.0, 125.1, 125.4, 125.7, 127.0, 127.4 (C₆H₅), 131.9, 132.4, 138.0, 169.1, 171.4 (C); \(m/z\) (FAB) 286 (MH⁺); found (FAB) 286.14475 (MH⁺), \(C_{17}H_{20}NO₃\) requires 286.14432; [\(\alpha\)]D²¹ +50.5° (c=0.83, CHCl₃).

Chiracel OD-H, 1ml/min, 5%IPA in hexane, 210nm, 5°C; Rₜ (min) 22.09 (0.9%), 25.09 (99.1%).

3.2.9 General procedure for hydrolysis of amino acid methyl esters

Hydrochloric acid (4N) was added to a stirring solution of the N-acetyl didehydroamino acid methyl ester in acetone. The mixture was heated under reflux for 3 hours before the mixture was treated with activated charcoal and filtered. Concentration of the solution followed by trituration with acetone (unless otherwise stated) gave the desired amino acid as the hydrochloride salt.
Synthesis of (2R,3S)-2-Amino-3-phenylbutyric acid hydrochloride (2R,3S)-114a

The general procedure was followed using (2R,3S)-113a (250mg, 1.06mmol), acetone (2.5ml) and HCl (20ml) and yielded a white solid (201mg, 88%).

M.p.195-198°C; $\nu_{\text{max}}$ (nujol) 1603 (amino acid II), 1731 (amino acid I), 1951 and 2535 (NH$_3^+$ overtones and combinations), 2975 (OH), 3437 cm$^{-1}$ (NH$_3^+$); $\delta_H$ (250MHz, D$_2$O) 1.29 (3H, d $J=7.3$Hz, CH$_3$), 3.80 (1H, quin., $J=5.9$Hz, CH), 4.70 (1H, d $J=5.7$Hz, CH), 7.16-7.40 (5H, m, ArH); $\delta_C$ (63MHz, D$_2$O) 15.1 (CH$_3$), 40.1 (CH), 59.2 (CH), 128.2, 128.5, 129.6 (CAr), 139.5 171.6 (C); $m/z$ (FAB) 180 (MH$^+$); found (FAB) 180.10245 (MH$^+$), C$_{10}$H$_{14}$NO$_2$ requires 180.10245; $[\alpha]_{D}^{22}$ -5.0° (c=0.50, H$_2$O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; $R_t$ (min) 7.69 (0.2%), 9.55 (99.8%).

Synthesis of (2S,3R)-2-Amino-3-phenylbutyric acid hydrochloride (2S,3R)-114a$^{27}$

The general procedure was followed using (2S,3R)-113a (250mg, 1.06mmol), acetone (2.5ml) and HCl (20ml) and yielded a white solid (204mg, 69%).

M.p. 190-192°C, lit 214°C; $\nu_{\text{max}}$ (nujol) 1603 (amino acid II), 1731 (amino acid I), 1951 and 2535 (NH$_3^+$ overtones and combinations), 2977 (OH), 3437 cm$^{-1}$ (NH$_3^+$); $\delta_H$ (250MHz, D$_2$O) 1.23 (3H, d $J=7.3$Hz, CH$_3$), 3.32 (1H, quin., $J=5.9$Hz, CH), 4.01 (1H, d $J=5.7$Hz, CH), 7.12-7.22 (5H, m, ArH); $\delta_C$ (63MHz, D$_2$O) 15.1 (CH$_3$), 40.1
Experimental

(CH), 59.2 (CH), 128.2, 128.5, 129.6 (C<sub>Ar</sub>), 139.5 171.6 (C); m/z (FAB) 180 (MH<sup>+</sup>);
found (FAB) 180.10256 (MH<sup>+</sup>), C<sub>10</sub>H<sub>13</sub>NO<sub>2</sub> requires 180.10245; [α]<sub>D</sub> +5.0° (c=0.50, H<sub>2</sub>O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R<sub>t</sub> (min) 7.56 (99.9%), 9.99 (0.1%).

Synthesis of (2R,3S)-2-Amino-3-(4-fluorophenyl)butyric acid hydrochloride (2R,3S)-114b

![Chemical Structure](image)

The general procedure was followed using (2R,3S)-113b (253mg, 1.0mmol), acetone (2.5ml) and HCl (20.0ml) and yielded a white solid (181mg, 78%).

M.p. 190-192°C; \(\nu_{\text{max}}\) (KBr) 1605 (amino acid II), 1732 (amino acid I), 2531 (NH<sub>3</sub><sup>+</sup> overtones and combinations), 2992 (OH), 3448cm<sup>-1</sup> (NH<sub>3</sub><sup>+</sup>); \(\delta_H\) (200MHz, D<sub>2</sub>O) 1.38 (3H, d \(J=7.2\)Hz, CH<sub>3</sub>), 3.49 (1H, quin. \(J=6.2\)Hz, CH), 4.17 (1H, d \(J=5.8\)Hz, CH), 7.05-7.34 (4H, m, ArH); \(\delta_C\) (63MHz, D<sub>2</sub>O) 15.5 (CH<sub>3</sub>), 39.5 (CH), 59.2 (CH), 116.2 (d \(J_{CF}=21.5\)Hz, C<sub>Ar</sub>), 130.0 (d \(J_{CF}=8.4\)Hz, C<sub>Ar</sub>), 135.3 (C), 162.5 (d \(J_{CF}=244.0\)Hz, CF), 171.5 (C); m/z (FAB) 198 (MH<sup>+</sup>); found (FAB) 198.09337 (MH<sup>+</sup>), C<sub>10</sub>H<sub>13</sub>NO<sub>2</sub> requires 198.09303; [α]<sub>D</sub> -4.5° (c=1.00, H<sub>2</sub>O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R<sub>t</sub> (min) 7.85 (0.3%), 9.21 (99.7%).

Synthesis of (2S,3R)-2-Amino-3-(4-fluorophenyl)butyric acid hydrochloride (2S,3R)-114b

![Chemical Structure](image)
The general procedure was followed using (2S,3R)-113b (253mg, 1.0mmol), acetone (2.5ml) and HCl (20.0ml) and yielded a white solid (178mg, 76%).

M.p. 186-190°C; ν\text{max} (KBr) 1604 (amino acid II), 1732 (amino acid I), 1950 and 2530 (NH₃⁺ overtones and combinations), 2990 (OH), 3449cm⁻¹ (NH₃⁺); δ_H (200MHz, D₂O) 1.41(3H, d J=7.2Hz, CH₃), 3.51 (1H, quin. J=6.2Hz, CH), 4.18 (1H, d J=6.0Hz, CH), 7.08-7.36 (4H, m, ArH); δ_C (63MHz, D₂O) 15.4 (CH₃), 39.4 (CH), 59.1 (CH), 116.2 (d J_CF=21.5Hz, C_Ar), 130.0 (d J_CF=8.4Hz, C_Ar), 135.4 (C), 162.5 (d J_CF=244.0Hz, CF), 171.6 (C); m/z (FAB) 198 (MH⁺); found (FAB) 198.09318 (MH⁺), C_{10}H_{13}FN_O₂ requires 198.09303; [α]_{D}^{22} +4.5° (c=1.00, H₂O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; Rₜ (min) 7.69 (99.6%), 9.67 (0.4%).

Synthesis of (2R,3S)-2-Amino-3-(4-trifluoromethylphenyl)butyric acid hydrochloride (2R,3S)-114c

![Chemical structure](Image)

The general procedure was followed using (2R,3S)-113c (250mg, 0.83mmol), acetone (2.5ml) and HCl (20.0ml) and ether trituration yielded a white solid (168mg, 71%).

M.p. 183-186°C; ν\text{max} (KBr) 1623 (amino acid II), 1733 (amino acid I), 2998 (OH), 3443cm⁻¹ (NH₃⁺); δ_H (200MHz, D₂O) 1.36(3H, d J=6.8Hz, CH₃), 3.51(1H, quin. J=6.2Hz, CH), 4.07 (1H, d J=6.0Hz, CH), 7.41-7.67 (4H, m, ArH); δ_C (63MHz, D₂O) 15.3 (CH₃), 40.1 (CH), 58.9 (CH), 126.7, 128.8 (C_Ar), 143.9, 171.3 (C); m/z (FAB) 248 (MH⁺); found (FAB) 248.09024 (MH⁺), C_{11}H_{13}F₃NO₂ requires 248.08984; [α]_{D}^{22} -6.0° (c=1.00, H₂O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; Rₜ (min) 11.06 (100%).

142
Synthesis of (2S,3R)-2-Amino-3-(4-trifluoromethylphenyl)butyric acid hydrochloride (2S,3R)-114c

The general procedure was followed using (2S,3R)-113c (250mg, 0.83mmol), acetone (2.5ml) and HCl (20.0ml) and yielded a white solid (198mg, 84%).

M.p. 181-184°C; ν_{max} (KBr) 1622 (amino acid II), 1733 (amino acid I), 2998 (OH), 3443cm\(^{-1}\) (NH\(^+_3\)); δ\(H\) (200MHz, D\(_2\)O) 1.40 (3H, d, J=6.8Hz, CH\(_3\)), 3.54 (1H, quin. J=6.2Hz, CH), 4.14 (1H, d, J=6.0Hz, CH), 7.43-7.69 (4H, m, ArH); δ\(C\) (63MHz, D\(_2\)O) 15.3 (CH\(_3\)), 40.1 (CH), 58.9 (CH), 126.7, 128.8 (C\(_{Ar}\)), 143.9, 171.3 (C); m/z (FAB) 248 (MH\(^+\)); found (FAB) 248.08999 (MH\(^+\)), C\(_{11}\)H\(_{13}\)F\(_3\)NO\(_2\) requires 248.08984; [\(\alpha\)]\textsuperscript{22}_D +7.0° (c=1.00, H\(_2\)O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R\(_f\) (min) 7.51 (100%).

Synthesis of (2R,3S)-2-Amino-3-(4-methoxyphenyl)butyric acid hydrochloride (2R,3S)-114d

The general procedure was followed using (2R,3S)-113d (250mg, 0.95mmol), acetone (2.5ml) and HCl (20.0ml) and yielded a white solid (154mg, 66%).

M.p. 210-213°C; ν_{max} (KBr) 1611 (amino acid II), 1732 (amino acid I), 1942 and 2532 (NH\(^+_3\) overtones and combinations), 2988 (OH), 3442cm\(^{-1}\) (NH\(^+_3\)); δ\(H\) (200MHz, D\(_2\)O) 1.35 (3H, d, J=7.4Hz, CH\(_3\)), 3.45 (1H, quin. J=6.4Hz, CH), 4.04 (1H, d, J=5.4Hz, CH), 6.95-7.28 (4H, m, ArH); δ\(C\) (63Hz, D\(_2\)O) 15.4 (CH\(_3\)), 39.4
Experimental

(CH), 55.8 (OCH₃), 59.2 (CH), 114.9, 129.5 (C₆H), 131.9, 158.9, 171.6 (C); m/z (FAB) 210 (MH⁺); found (FAB) 210.11298 (MH⁺), C₁₁H₁₆NO₃ requires 210.11302; [α]²²D -6.0° (c=1.00, H₂O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R₁ (min) 9.83 (0.1%), 11.93 (99.9%).

Synthesis of (2S,3R)-2-Amino-3-(4-methoxyphenyl)butyric acid hydrochloride (2S,3R)-114d

The general procedure was followed using (2S,3R)-113d (250mg, 0.95mmol), acetone (2.5ml) and HCl (20.0ml) and yielded a white solid (155mg, 67%).

M.p. 211-213°C; ν_max (KBr) 1611(amino acid II), 1731 (amino acid I), 1947 and 2535 (NH₃⁺ overtones and combinations), 2990 (OH), 3442cm⁻¹ (NH₃⁺); δ_H (200MHz, D₂O) 1.35(3H, d J=7.4Hz, CH₃), 3.45 (1H, quin. J=6.4Hz, CH), 4.04 (1H, d J=5.4Hz, CH), 6.95-7.29(4H, m, ArH); δ_C (63Hz, D₂O) 15.4 (CH3), 39.4 (CH), 55.8 (OCH₃), 59.2 (CH), 114.9, 129.5 (C₆H), 131.9, 158.9, 171.6 (C); m/z (FAB) 210 (MH⁺); found (FAB) 210.11318 (MH⁺), C₁₁H₁₆NO₃ requires 210.11302; [α]²²D +5.0° (c=1.00, H₂O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R₁ (min) 9.88 (100%).
Experimental

Synthesis of (2R,3S)-2-Amino-3-(3,5-dimethylphenyl)butyric acid hydrochloride (2R,3S)-114e

The general procedure was followed using (2R,3S)-114e (250mg, 0.95mmol), acetone (2.5ml) and HCl (20.0ml) and trituration with ether yielded a white solid (205mg, 89%).

M.p. 183-186°C; \( \nu_{\text{max}} \) (KBr) 1607 (amino acid II), 1733 (amino acid I), 3435 cm\(^{-1} \) (NH\(_3\)); \( \delta_H \) (200MHz, D\(_2\)O) 1.32 (3H, d \( J=7.4 \text{Hz} \), CH\(_3\)), 2.21 (6H, s, (CH\(_3\))\(_2\)), 3.38 (1H, quin. \( J=6.0 \text{Hz} \), CH), 4.03 (1H, d \( J=5.4 \text{Hz} \), CH), 6.91-6.98 (3H, m, ArH); \( \delta_C \) (63MHz, D\(_2\)O) 15.2 (CH\(_3\)), 20.8 ((CH\(_3\))\(_2\)), 40.0 (CH), 59.1 (CH), 126.0, 129.8 (C\(_{Ar}\)), 139.7, 171.5 (C); \( m/z \) (FAB) 208 (MH\(^+\)); found (FAB) 208.13378 (MH\(^+\)), C\(_{12}\)H\(_{18}\)NO\(_2\) requires 208.13375; [\( \alpha \)]\(^{22}_D\) -6.0° (c=1.00, H\(_2\)O).

Chirobiotic T, 40%EthOH in water, 0.7ml/min, 210nm, room temp; R\(_t\) (min) 10.01 (0.5%), 12.81 (99.5%).

Synthesis of (2S,3R)-2-Amino-3-(3,5-dimethylphenyl)butyric acid hydrochloride (2S,3R)-114e

The general procedure was followed using (2S,3R)-114e (250mg, 0.95mmol), acetone (2.5ml) and HCl (20.0ml) and trituration with ether yielded a white solid (170mg, 73%).
M.p. 180-185°C; \( \nu_{\text{max}} \) (KBr) 1608 (amino acid II), 1733 (amino acid I), 2975 (OH), 3437 cm\(^{-1}\) (NH\(^+\)); \( \delta_H \) (200MHz, D\(_2\)O) 1.33 (3H, d \( J=7.4 \) Hz, CH\(_3\)), 2.24 (6H, s, (CH\(_3\))\(_2\)), 3.43 (1H, quin. \( J=6.0 \) Hz, CH), 4.04 (1H, d \( J=5.4 \) Hz, CH), 6.95-7.00 (3H, m, ArH); \( \delta_C \) (63MHz, D\(_2\)O) 15.2 (CH\(_3\)), 20.8 ((CH\(_3\))\(_2\)), 40.0 (CH), 59.1 (CH), 126.0, 129.8 (C\(_A\)), 139.7, 171.5 (C); \( m/z \) (FAB) 208 (MH\(^+\)); found (FAB) 208.13419 (MH\(^+\)), C\(_{12}\)H\(_{18}\)NO\(_2\) requires 208.13375; \([\alpha]^{22}_D +4.0^\circ\) (c=1.00, H\(_2\)O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; \( R_t \) (min) 9.15 (99.5%), 14.83 (0.5%).

**Synthesis of (2R,3S)-2-Amino-3-naphthalen-2-ylbutyric acid hydrochloride (2R,3S)-114f**

![Structure](image)

The general procedure was followed using \((2R,3S)-1113f\) (250mg, 0.88mmol), acetone (2.5ml) and HCl (20.0ml) and yielded an off-white solid (158mg, 68%).

M.p. 200-203°C; \( \nu_{\text{max}} \) (KBr) 1588 (amino acid II), 1770 amino acid I), 1978 (NH\(^+\) overtones and combinations), 2981 (OH), 3419 cm\(^{-1}\) (NH\(^+\)); \( \delta_H \) (200MHz, D\(_2\)O) 1.46 (3H, d \( J=7.2 \) Hz, CH\(_3\)), 3.64 (1H, quin. \( J=6.8 \) Hz, CH), 4.22 (1H, d \( J=5.4 \) Hz, CH), 7.41-7.91 (7H, m, ArH); \( m/z \) (FAB) 230 (MH\(^+\)); found (FAB) 230.11857 (MH\(^+\)), C\(_{14}\)H\(_{16}\)NO\(_2\) requires 230.11810; \([\alpha]^{22}_D -10.0^\circ\) (c=1.00, H\(_2\)O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; \( R_t \) (min) 17.12 (100%).
Experimental

Synthesis of (2S,3R)-2-Amino-3-naphthalen-2-ylbutyric acid hydrochloride (2S,3R)-114f

The general procedure was followed using (2S,3R)-113f (250mg, 0.88mmol), acetone (2.5ml) and HCl (20.0ml) and yielded an off-white solid (203mg, 87%).

M.p. 205-207°C; \[\nu_{\text{max}}\text{ (KBr)}\] 1588 (amino acid II), 1770 (amino acid I), 1977 (NH\(_3^+\) overtones and combinations), 2982 (OH), 3419 cm\(^{-1}\) (NH\(_3^+\)); \(\delta\)\(_H\) (200MHz, D\(_2\)O) 1.47 (3H, d \(J=7.2\text{Hz},\text{CH}\(_3\)\)), 3.66 (1H, quin. \(J=6.8\text{Hz},\text{CH}\)), 4.19 (1H, d \(J=5.4\text{Hz},\text{CH}\)), 7.43-7.93 (7H, m, ArH); \(m/z\) (FAB) 230 (MH\(^+\)); found (FAB) 230.11834 (MH\(^+\)), C\(_{14}\)H\(_{16}\)NO\(_2\) requires 230.11810; [\(\alpha\)]\(^{22}_D\) +10.0° (c=1.00, H\(_2\)O).

Chirobiotic T, 40%EtOH in water, 0.7ml/min, 210nm, room temp; R\(_t\) (min) 8.87 (100%).

3.2.10 General procedure for stereoinversions (HPLC scale)

A mixture of amino acid substrate, the appropriate amino acid oxidase and borane-ammonia complex in water were shaken at 30°C. The protein was removed either by filtration \((Tv)\) or using a protein concentrator \((pk, sv)\) before each HPLC run.

Synthesis of \((2R,3R)-2-Amino-3-phenylbutyric acid (2R,3R)-114a using snake venom L-AAO\(^{27}\)

The general procedure was followed using \((2S,3R)-114a\) (4.3mg, 0.02mmol), svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).
Experimental

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t =0hr 7.00 (100%); t=20hr 8.88 (100%); 83% Yield, >99% d.e.

Synthesis of (2R,3R)-2-Amino-3-(4-fluorophenyl)butyric acid (2R,3R)-114b using snake venom L-AAO

The general procedure was followed using (2S,3R)-114b (4.7mg, 0.02mmol), svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t =0hr 6.79 (100%); t=20hr 8.69 (100%); 85% Yield, >99% d.e.

Synthesis of (2R,3R)-2-Amino-3-(4-trifluoromethylphenyl)butyric acid (2R,3R)-114c using snake venom L-AAO

The general procedure was followed using (2S,3R)-114c (5.6mg, 0.02mmol), svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t =0hr 6.48 (100%); t=20hr 7.94 (100%); 92% Yield, >99% d.e.
Synthesis of (2R,3R)-2-Amino-3-(4-methoxyphenyl)butyric acid (2R,3R)-114d using snake venom L-AAO

The general procedure was followed using (2S,3R)-114d (4.9mg, 0.02mmol), svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t =0hr 7.56 (100%); t=20hr 7.71 (2.7%), 9.95 (97.3%); t=40hr 10.13 (100%); 80% Yield, >99% d.e.

Synthesis of (2R,3R)-2-Amino-3-(3,5-dimethylphenyl)butyric acid (2R,3R)-114e using snake venom L-AAO

The general procedure was followed using (2S,3R)-114e (4.9mg, 0.02mmol), svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t =0hr 7.28 (100%); t=20hr 7.28 (52.8%), 8.26 (47.2%); t=40hr 7.43 (32.6%), 8.41 (67.4%); t=64hr 7.36 (25.5%), 8.27 (74.5%); 49% d.e.
Synthesis of \( (2R,3R)-2\text{-amino-3-naphthalen-2-ylbutyric acid} \) \( (2R,3R)-114f \) using snake venom L-AAO

\[
\text{The general procedure was followed using } (2S,3R)-114f \ (5.3\text{mg, 0.02mmol}), \text{ svL-AAO (10mg), borane-ammonia complex (10mg, 0.32mmol) and water (4ml).}
\]

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: \( t =0\text{hr 9.08 (100%)}; \ t=20\text{hr 9.82 (1.2%)}, 11.96 (98.8%); \ t=40\text{hr 12.50 (100%)}; \ 80\% \text{Yield, >99\% d.e.} \)

Synthesis of \( (2S,3S)-2\text{-amino-3-phenylbutyric acid} \) \( (2S,3S)-114a \) using pig kidney D-AAO

\[
\text{The general procedure was followed using } (2R,3S)-114a \ (2.2\text{mg, 0.01mmol}), \text{ pkD-AAO (2.5mg), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).}
\]

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: \( t =0\text{hr 7.62 (8.2%)}, 9.52 (91.9%); \ t=24\text{hr 7.00 (79.7%)}, 9.88 (20.4%); \ 73\% \text{ Yiel}, 63\% \text{ d.e.} \)
Synthesis of (2S,3S)-2-Amino-3-(4-fluorophenyl)butyric acid (2S,3S)-114b using pig kidney D-AAO

The general procedure was followed using (2R,3S)-114b (2.3mg, 0.01mmol), pkD-AAO (2.5mg), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirex 3126 Penacillamine D; 1.2ml/min, 1mMCuSO4 in 30%MeOH, 254nm: t=0hr 41.46 (100%); t=20hr 71.33 (100%); 77% Yield, >99% d.e.

Synthesis of (2S,3S)-2-Amino-3-(4-trifluoromethylphenyl)butyric acid (2S,3S)-114c using pig kidney D-AAO

The general procedure was followed using (2R,3S)-114c (2.8mg, 0.01mmol), pkD-AAO (2.5mg), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t=0hr 7.60 (11.03%), 9.03 (88.97%); t=72hr 7.10 (98.9%), 9.71 (1.1%); 96% Yield, 89% d.e.
Synthesis of (2S,3S)-2-Amino-3-phenylbutyric acid (2S,3S)-114a using *Trigonopsis variabilis* D-AAO

The general procedure was followed using (2R,3S)-114a (2.2 mg, 0.01 mmol), TyD-AAO (5 drops of slurry), borane-ammonia complex (5 mg, 0.16 mmol) and water (2 ml).

Chirobiotic T; 0.7 ml/min, 40% EtOH, 210 nm: t=0 hr 6.60 (0.2%), 8.82 (99.8%); t=20 hr 6.40 (99.8%), 8.94 (0.2%); 69% Yield, >99% d.e.

Synthesis of (2S,3S)-2-Amino-3-(4-fluorophenyl)butyric acid (2S,3S)-114b using *Trigonopsis variabilis* D-AAO

The general procedure was followed using (2R,3S)-114b (2.3 mg, 0.01 mmol), TyD-AAO (5 drops of slurry), borane-ammonia complex (5 mg, 0.16 mmol) and water (2 ml).

Chirobiotic T; 0.7 ml/min, 40% EtOH, 210 nm: t=0 hr 8.25 (100%); t=20 hr 6.33 (100%); 68% Yield, >99% d.e.
Experimental

Synthesis of (2S,3S)-2-Amino-3-(4-trifluoromethylphenyl)butyric acid (2S,3S)-114c using *Trigonopsis variabilis* D-AAO

The general procedure was followed using (2R,3S)-114c (2.8mg, 0.01mmol), Tvd-AAO (5 drops of slurry), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t=0hr 6.30 (1.0%), 7.82 (99.0%); t=20hr 6.05 (100%); 81% Yield, >99% d.e.

Synthesis of (2S,3S)-2-Amino-3-(4-methoxyphenyl)butyric acid (2S,3S)-114d using *Trigonopsis variabilis* D-AAO

The general procedure was followed (2R,3S)-114d (2.5mg, 0.01mmol), Tvd-AAO (5 drops of slurry), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: t=0hr 6.99 (0.2%), 9.13 (99.8%); t=20hr 6.76 (100%); 81% Yield, >99% d.e.
Synthesis of (2S,3S)-2-Amino-3-(3,5-dimethylphenyl)butyric acid (2S,3S)-114e using *Trigonopsis variabilis* D-AAO

![Chemical Structure](image)

The general procedure was followed using \((2R,3S)\)-104e (2.4mg, 0.01mmol), *TvD-AAO* (5 drops of slurry), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: \(t=0\)hr 6.73 (2.8%), 10.44 (97.2%); \(t=20\)hr 6.53 (100%); 72% Yield, >99% d.e.

Synthesis of (2S,3S)-2-Amino-3-naphthalen-2-ylbutyric acid (2S,3S)-114f using *Trigonopsis variabilis* D-AAO

![Chemical Structure](image)

The general procedure was followed using \((2R,3S)\)-114f (2.7mg, 0.01mmol), *TvD-AAO* (5 drops of slurry), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirobiotic T; 0.7ml/min, 40%EtOH, 210nm: \(t=0\)hr 8.02 (1.9%), 13.02 (98.1%); \(t=20\)hr 7.83 (100%); 80% Yield, >99% d.e.
Synthesis of (2S,3R)-2-Amino-3-phenylbutyric acid (2S,3R)-114a using pig kidney D-AAO

The general procedure was followed using (2R,3R)-114a (2.2mg, 0.01mmol), pkD-AAO (2.5mg), borane-ammonia complex (5mg, 0.16mmol) and water (2ml).

Chirex 3126 Penacillamine D; 1.2ml/min, 1mMCuSO4 in 30%MeOH, 254nm: t=0hr 21.58 (5.6%), 59.73 (94.4%); t=20hr 22.49 (100%), 83% Yield, >99% d.e.

3.2.11 Stereoinversion on a 1g scale

A mixture of (2S,3R)-114a (1.00g, 4.64mmol), ammonia borane complex (3.10g, 100mmol), Trigonopsis variabilis (10ml of immobilised slurry, 222.7 U/g dry weight) in water (200ml) was shaken at 37°C for 96hr. HPLC showed complete conversion with 54% yield. The mixture was filtered and freeze dried. The residue was taken up in ethanol and filtered to remove unreacted ammonia borane. Concentration of the ethanolic solution gave the crude salt that was recrystallised from ethanol/ethyl acetate to give a white solid (455mg, 55%).

M.p. 188°C decomp.; δH (250MHz, D2O) 1.42 (3H, d J=7.3Hz, CH₃), 3.52 (1H, quin. J=6.8Hz, CH), 4.21 (1H, d J=5.4Hz, CH), 7.32-7.45 (5H, m, ArH); δC (63MHz, D₂O) 15.2 (CH₃), 40.1 (CH), 59.2 (CH), 128.3, 128.6, 129.6 (C₀), 139.6, 171.7 (C); [α]²⁰_D 4.2° (c=0.83, CHCl₃).

HPLC: Chirobiotic T; Rₜ (min): t=0hr 10.33(100%), t=96hr 8.46(100%).
3.3 Experimental procedures - Altemicidin mimic project

Synthesis of Chlorosulfonylecetic acid ethyl ester 139

\[
\begin{align*}
\text{EtO} & \quad \text{SO}_2 \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

To a stirring solution of chlorosulfonylacetyl chloride (2.18 g, 0.0123 mol) in MTBE (15 ml) at 0°C was added a solution of ethanol (0.71 ml in 15 ml MTBE) slowly. The mixture continued to stir for 1 hour in the thawing ice bath before concentrating to the crude product that was used directly for the next step (2.24 g, 98%).

\[\delta_H (400MHz, CDCl_3): 1.36 (3H, t J=9Hz, \text{CH}_3), 4.34 (2H, q J=8Hz, \text{CH}_2\text{CH}_3), 4.60 (2H, s, \text{CH}_2).\]

Synthesis of Sulfamoylacecetic acid ethyl ester 140

\[
\begin{align*}
\text{EtO} & \quad \text{SO}_2 \\
\text{NH}_2 & \quad \text{EtO}
\end{align*}
\]

Ammonia gas was bubbled through a solution of 139 (300 mg, 1.61 mmol) in MTBE (5 ml) at 0°C for 10 minutes. The mixture continued to stir for 1 hour in the thawing ice bath.

The mixture was filtered and the filtrate was concentrated to a pale yellow solid. (147 mg, 55%).

\[m/z (ES^-) 166 (M-1)^-\]

Synthesis of (2-Benzylxycarbonylamino-3-methylpentanoylsulfamoyl)acetic acid ethyl ester 142

\[
\begin{align*}
\text{EtO} & \quad \text{SO}_2 \\
\text{NH}_2 & \quad \text{EtO}
\end{align*}
\]

A mixture of 140 (200 mg, 1.20 mmol), (Z)-isoleucine (477 mg, 1.80 mmol), DCC (371 mg, 1.80 mmol) and DMAP (220 mg, 1.80 mmol) in pyridine (10 ml) was stirred at 42°C overnight.
The mixture was filtered through celite and concentrated. The residue was taken up in DCM (20ml), washed once with KHSO₄ (0.3M, 20ml), once with sat. sodium bicarbonate solution (20ml), once with sat. copper sulphate solution (20ml) and once with brine before drying over MgSO₄ and concentrating to a clear oil. Wet flash column chromatography (heptane/ethyl acetate 4:1) gave a clear oil (250mg, 50%).

δ_H (400MHz, MeOD): 0.85 (3H, t J=7Hz, CH₂CH₃), 0.91 (3H, d J=7Hz, CH₃), 1.10 (1H, m, CH₂CH₃), 1.21 (3H, t J=7Hz, CH₃CH₂O), 1.49 (1H, m, CH₂CH₃), 3.95-4.22 (5H, m, CH+CH₂SO₂+OCH₂CH₃), 5.00 (2H, m, CH₂Ph), 5.45 (1H, s br, NH), 7.20-7.40 (5H, m, ArH); (ES⁺) 437 (MNa⁺).

**Synthesis of (2-Benzylloxycarbonylamino-3-methyl-pentanoylsulfamoyl)acetic acid 143**

To a stirring solution of 142 (150mg, 0.363mmol) in methanol/water (4:1, 10ml) at 0°C was added lithium hydroxide monohydrate (23mg, 0.545mmol) and the mixture allowed to stir in the thawing ice bath for 2 hours.

The mixture was concentrated and the residue taken up in water (10ml), made basic with NaOH (1M) and washed with MTBE (10ml). The aqueous layer was acidified with HCl (1M) and washed twice with MTBE (2x15ml). The combined organics were washed with brine, dried over MgSO₄ and concentrated to a clear gum (125mg, 89%).

δ_H (400MHz, MeOD): 0.81 (3H, t J=7Hz, CH₂CH₃), 0.88 (3H, d J=7Hz, CH₃), 1.09 (1H, m, CH₂CH₃), 1.40 (1H, m, CH₂CH₃), 1.75 (1H, m, CH), 3.99 (1H, d J=8Hz, CH), 4.30 (2H, s, CH₂SO₂), 4.97 (2H, s, CH₂Ph), 7.10-7.31 (5H, m, ArH); (ES⁺) 409 (MNa⁺).
3.3.1 General procedure for N-Boc to N-Z conversion

2-tert-Butoxycarbonylamino-3-phenylbutyric acid (500mg, 1.79mmol) was stirred in HCl solution (1.25M in MeOH, 15ml) at 40°C for 2 hours.

The mixture was concentrated, taken up in water (20ml) and washed once with MTBE (15ml). The water layer was concentrated and taken up in NaOH (1M, 5ml) and THF (5ml) and cooled to 0°C. Benzyl chloroformate (0.28ml in 5ml THF) was added and the mixture stirred at room temperature overnight.

The mixture was concentrated, taken up in aq. NaOH and washed with MTBE. The aqueous layer was acidified with HCl (1M) and washed twice with MTBE. The organics were washed with brine, dried over MgSO₄ and concentrated.

Synthesis of (2R,3S)-2-Benzylloxycarbonylamino-3-phenylbutyric acid (2R,3S)-125

The general procedure gave a white solid (450mg, 80%).

δ_H (400MHz, CDCl₃) 1.40 (3H, d J=7Hz, CH₃), 3.32 (1H, m, CH), 4.72 (1H, m, CH), 5.07 (2H, m, CH₂), 5.24 (1H, d br. J=9Hz, NH), 7.21-7.40 (10H, m, ArH); m/z (ES⁻) 312 (M-1)⁻.

Synthesis of (2S,3R)-2-Benzylloxycarbonylamino-3-phenylbutyric acid (2S,3R)-125

The general procedure gave a white solid (335mg, 60%).

δ_H (400MHz, CDCl₃) 1.39 (3H, d J=7Hz, CH₃), 3.31 (1H, m, CH), 4.62 (1H, m, CH), 5.08 (2H, m, CH₂), 5.23 (1H, d br. J=9Hz, NH), 7.16-7.36 (10H, m, ArH); m/z (ES⁻) 312 (M-1)⁻.
Synthesis of (2R,3R)-2-Benzylloxycarbonylamino-3-phenylbutyric acid (2R,3R)-125

The general procedure gave a white solid (470mg, 84%).

\[ \delta_H (400\text{MHz, CDCl}_3) \ 1.40 \ (3\text{H, d } J=7\text{Hz, CH}_3), 3.43 \ (1\text{H, m, CH}), 4.59 \ (1\text{H, m, CH}), 4.99 \ (1\text{H, d br. } J=9\text{Hz, NH}), 5.08 \ (2\text{H, s, CH}_2), 7.11-7.33 \ (10\text{H, m, ArH}); \ m/z \ (\text{ES}^-) \ 312 \ (\text{M}-1)'.

Synthesis of (2S,3S)-2-Benzylloxycarbonylamino-3-phenylbutyric acid (2S,3S)-125

The general procedure gave a white solid (423mg, 76%).

\[ \delta_H (400\text{MHz, CDCl}_3): 1.41 \ (3\text{H, d } J=7\text{Hz, CH}_3), 3.43 \ (1\text{H, m, CH}), 4.59 \ (1\text{H, m, CH}), 4.97 \ (1\text{H, d br. } J=9\text{Hz, NH}), 5.08 \ (2\text{H, s, CH}_2), 7.12-7.35 \ (10\text{H, m, ArH}); \ m/z \ (\text{ES}^-) \ 312 \ (\text{M}-1)'.

3.3.2 General procedure for the synthesis scaffold branch

A solution of (Z)-protected amino acid (200mg, 0.639mmol) and scaffold (166mg, 0.639mmol) in DCM (20ml) was treated with 2-chloror-1-methylpyridinium iodide (212mg, 0.831mmol) followed by triethylamine (0.18ml). After 30 minutes DMAP (40mg, 0.320mmol) was added and the solution stirred at room temperature over a weekend.

The solution was treated with HCl (0.1M, 20ml). The aqueous layer was removed and further washed with DCM. The combined organics were washed with brine, dried over MgSO\textsubscript{4} and concentrated. Wet flash column chromatography of the residue (heptane/ethyl acetate 4:1) gave the product.

Synthesis of scaffold branch (2R,3S)-133

The general procedure gave a clear gum (148mg, 42%).
δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>): 1.22 and 1.35 (3H, d, CH₃), 1.42 (9H, d, (CH₃)<sub>3</sub>), 1.60 (1H, m, ring CH₂), 1.82 (1H, m, ring CH₂), 2.08 (1H, m, ring CH₂), 2.31 (1H, m, ring CH₂), 2.80 (1H, m, CHCO₂CH₃), 3.24 (1H, m, CH), 3.44 (1H, m, CH), 3.67 and 3.70 (3H, s, CH₃CO₂), 4.02 (1H, m, CH), 4.50 (1H, m, CHNH), 5.05 (2H, m, CH₂Ph), 5.30 (1H, m, CH), 7.45-7.08 (10H, m, ArH); <i>m/z</i> (ES<sup>+</sup>) 577 (MNa<sup>+</sup>).

**Synthesis of scaffold branch (2S,3R)-133**

The general procedure gave a clear gum (160mg, 45%).

<i>m/z</i> (ES<sup>+</sup>) 577 (MNa<sup>+</sup>).

**Synthesis of scaffold branch (2R,3R)-133**

The general procedure gave a white solid (230mg, 65%).

δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>): 1.36 (3H, d, CH₃), 1.46 (9H, s, (CH₃)<sub>3</sub>), 1.87 (1H, m, ring CH₂), 2.10 (1H, m, ring CH₂), 2.30 (2H, m, ring CH₂), 2.85 (1H, m, CH), 3.23 (1H, m, CHPh), 3.70 (3H, s, OCH₃), 4.04 (1H, m, CH), 4.50 (1H, m, CHNH), 5.07 (2H, m, CH₂Ph), 5.20 (1H, m, CH), 7.17-7.47 (10H, m, ArH); (ES<sup>+</sup>) 577 (MNa<sup>+</sup>).

**Synthesis of scaffold branch (2S,3S)-133**

The general procedure gave a white solid (193mg, 55%).

(ES<sup>+</sup>) 577 (MNa<sup>+</sup>).

### 3.3.3 General procedure deprotection of the scaffold branch

![Diagram](image.png)

Scaff-MePhe (100mg, 0.181mmol) was stirred in HCl solution (1.25M in MeOH, 3ml) at 40°C overnight.

The reaction mixture was concentrated to the product that was used crude for the next step.
Synthesis of scaffold branch hydrochloride (2R,3S)-132
The general procedure gave a pale yellow solid (78mg, 88%).

Synthesis of scaffold branch hydrochloride (2S,3R)-132
The general procedure gave a pale yellow solid (80mg, 90%).

Synthesis of scaffold branch hydrochloride (2R,3R)-132
The general procedure gave a pale yellow solid (72mg, 81%).

$\delta_1$ (400MHz, MeOD) 1.19 (3H, d, CH$_3$), 2.02 (2H, m, ring CH$_2$), 2.35 (2H, m, ring CH$_2$), 2.95 (1H, m, CH), 3.09 (1H, m, CH), 3.62 (3H, s, OCH$_3$), 4.35 (1H, m, CH), 4.88 (2H, s, CH$_2$), 5.16 (1H, s, CH), 7.06-7.35 (10H, m, ArH).

Synthesis of scaffold branch hydrochloride (2S,3S)-132
The general procedure gave a pale yellow solid (75mg, 84%).

3.3.4 General procedure for coupling of the two branches

A mixture of 132 (64mg, 0.130mmol), 143 (50mg, 0.130mmol), DCC (41mg, 0.200mmol) and DMAP (24mg, 0.200mmol) in pyridine (3ml) was stirred at 42°C overnight.

The mixture was concentrated and the residue taken up in DCM (10ml), washed with KHSO$_4$ (0.3M, 5ml), sat. sodium bicarbonate (5ml), sat. copper sulphate (5ml) and brine before drying over MgSO$_4$ and concentrating. Wet column flash.
Experimental chromatography of the residue (heptane/ethyl acetate 4:1 to 0:1) gave the product which was taken forward without further purification.

**Synthesis protected mimic (2R,3S)-144**
The general procedure gave a white solid (24mg, 22%).

\[ m/z \ (ES^+) \ 845 \ \text{(MNa}^+) \].

**Synthesis protected mimic (2S,3R)-144**
The general procedure gave a white solid (25mg, 23%).

\[ m/z \ (ES^+) \ 845 \ \text{(MNa}^+) \].

**Synthesis protected mimic (2R,3R)-144**
The general procedure gave a white solid (40mg, 37%).

\[ m/z \ (ES^+) \ 845 \ \text{(MNa}^+) \].

**Synthesis protected mimic (2S,3S)-144**
The general procedure gave a white solid (23mg, 21%).

\[ m/z \ (ES^+) \ 845 \ \text{(MNa}^+) \].

### 3.3.5 General procedure for mimic (Z)-deprotection

![Diagram of the mimic molecule]
A solution of (Z)-Mimic (20mg, 0.024mmol) and Pd/C (2mg) in methanol was stirred at room temperature under 90psi of hydrogen overnight. The mixtures were concentrated to the product. Further purification was impractical due to the small quantities involved.

Synthesis of Altemicidin mimic (2R,3S)-126

The general procedure gave a clear gum (13mg, 100%).

δ_H (400MHz, MeOD) 0.87 (3H, t J=7Hz, CH_3H_2), 0.96 (3H, d J=7Hz, CH_3), 1.17 (1H, m, CH_2CH_3), 1.32 (3H, d J=7Hz, CH_3), 1.49 (1H, m, CH_2CH_3), 1.88 (1H, m, CH), 2.07 (2H, m, ring CH_2), 2.30 (2H, m, ring CH_2), 2.75 (1H, m, CH), 2.97 (1H, m, CH), 3.52 (3H, s, OCH_3), 3.59 (2, m, CH_2SO_2), 3.92 (1H, m, CH), 4.03 (1H, m, CH), 4.21 (1H, m, CH), 7.11-7.32 (5H, m, ArH); m/z (ES^+) 555 (MH^+).

Synthesis of Altemicidin mimic (2S,3R)-126

The general procedure gave a clear gum (13mg, 100%).

δ_H (400MHz, MeOD) 0.87 (3H, t J=7Hz, CH_3H_2), 0.96 (3H, d J=7Hz, CH_3), 1.18 (1H, m, CH_2CH_3), 1.38 (3H, d J=7Hz, CH_3), 1.50 (1H, m, CH_2CH_3), 1.81 (1H, m, CH), 1.98 (2H, m, ring CH_2), 2.22 (2H, m, ring CH_2), 2.89 (1H, m, CH), 3.19 (1H, m, CH), 3.52 (3H, s, OCH_3), 3.65 (2, m, CH_2SO_2), 3.95 (1H, m, CH), 4.03 (1H, m, CH), 4.21 (1H, m, CH), 7.11-7.32 (5H, m, ArH); m/z (ES^+) 555 (MH^+).

Synthesis of Altemicidin mimic (2R,3R)-126

The general procedure gave a clear gum (12mg, 92%).

δ_H (400MHz, MeOD) 0.84 (3H, t J=7Hz, CH_3H_2), 0.90 (3H, d J=7Hz, CH_3), 1.05 (1H, m, CH_2CH_3), 1.22 (3H, d J=7Hz, CH_3), 1.48 (1H, m, CH_2CH_3), 1.80 (1H, m, CH), 1.95 (2H, m, ring CH_2), 2.19 (2H, m, ring CH_2), 2.87 (1H, m, CH), 3.06 (1H, m, CH), 3.52 (3H, s, OCH_3), 3.60 (2, m, CH_2SO_2), 3.68 (1H, m, CH), 3.93 (1H, m, CH), 4.19 (1H, m, CH), 7.11-7.32 (5H, m, ArH); m/z (ES^+) 555 (MH^+).

Synthesis of Altemicidin mimic (2S,3S)-126

The general procedure gave a clear gum (13mg, 100%).
$\delta_H$ (400MHz, MeOD) 0.85 (3H, t $J=7$Hz, $CH_3H_2$), 0.91 (3H, d $J=7$Hz, $CH_3$), 1.13 (1H, m, $CH_2CH_3$), 1.31 (3H, d $J=7$Hz, $CH_3$), 1.48 (1H, m, $CH_2CH_3$), 1.85 (1H, m, $CH$), 1.99 (2H, m, ring CH$_2$), 2.30 (2H, m, ring CH$_2$), 2.91 (1H, m, CH), 3.26 (1H, m, CH), 3.52 (3H, s, OCH$_3$), 3.63 (2H, m, CH$_2$SO$_2$), 3.92 (1H, m, CH), 4.00 (1H, m, CH), 4.21 (1H, m, CH), 7.16-7.32 (5H, m, ArH); $m/z$ (ES$^+$) 555 (MH$^+$).
4 References


Appendix 1
Both D- and L-β- and γ-substituted α-amino acids can be interconverted to their respective L- and D-diestereoisomers by treatment with an enantioselective amino acid oxidase and a chemical reducing agent.

Enantioselectively pure β-substituted α-amino acids are valuable building blocks for the synthesis of peptidomimetics and enzyme inhibitors. The incorporation of a β-substituent into an α-amino acid (e.g. β-methylphenylalanine) generally leads to restricted conformational flexibility in peptides offering the prospect of developing high affinity ligands for receptors. Methods for the preparation of β-substituted α-amino acids include resolution, asymmetric catalysis, and also asymmetric synthesis employing chiral auxiliaries. A particularly versatile approach involves the catalytic asymmetric hydrogenation of β,β-disubstituted dehydroamino acids using chiral phosphine ligands. Although such substrates tend to undergo hydrogenation slowly, Burk et al. have developed appropriate ligands (e.g. Me-DuPHOS) that result in high enantioselectivities. However even these ligands tend to be more selective and reactive towards (Z)-configured β-substituted enamides compared to the (E)-isomers resulting in preferential access to only two of the four possible diastereoisomers. In addition, a number of natural β-substituted α-amino acids are more readily available in one diastereomeric form than the other (cf. L-isoleucine vs. D-α-isoisoleucine). With this in mind we sought to develop methodology for interconverting diastereoisomers of amino acids bearing two stereogenic centres.

Recently we have developed a general and practical method for the stereoinversion of α-amino acids and amines. The overall process relies upon the combined action of an enantioselective oxidase and a non-selective chemical reducing agent (e.g. Pb/C–HCO₂NH₂, amine : borane; sodium borohydride/cyanoborohydride). For example, the use of an L-α-amino acid oxidase (l-AAO) enables conversion of a racemic mixture of α-amino acids to the optically pure α-enantiomer (Scheme 1).

Provided that the amino acid oxidase is highly enantioselective then only 7 oxidation/reduction cycles are required to achieve an e.e. of >99% with yields ranging between 80–99%.[11,12]

Extension of this approach to α-amino acids containing one or more additional stereogenic centres would provide a method for interconverting diastereoisomers rather than enantiomers. Herein we demonstrate the success of this approach using a range of different amino acid oxidases and chemical reducing agents.

Initially it was important to establish that β-substituted α-amino acids were indeed substrates for the various L- and D-amino acid oxidases. Thus (2R,3S)-2-amino-3-methylhexanoic acid 2 and (2R,3S)-β-methyl-phenylalanine 3 were prepared in racemic form, and as a mixture of diastereomers (ca. 40% d.e.), from the corresponding racemic aldehydes via the Strecker reaction. Each substrate was subjected to stereoinversion using a-α-amino acid oxidase from Trigonopsis variabilis and either NaBH₄ or NaCNBH₃ as the reducing agent (Scheme 2). The progress of the reactions was monitored by chiral HPLC allowing the concentration of all four stereoisomers to be assessed during the reaction. For both substrates, rapid consumption of the (2R)-diastereoisomers was observed, albeit at slightly different rates, with concomitant production of the (2S)-isomers (Table 1). For substrate 2, the reaction was found to be complete (>99% e.e. for each diastereomer) after 18 h using NaCNBH₃ and only 3.5 h with the more reactive NaBH₄. At the end of the reaction a 1 : 1 mixture of the (2S,3S)- and (2S,3R)-isomers was observed (NB the final ratio of diastereoisomers is independent of the initial diastereomeric composition generated via the Strecker reaction and always approaches 1 : 1). In contrast to 2, (2R,3S)-β-methyl-phenylalanine 3 was found to react more slowly although again the use of NaBH₄ enabled complete conversion after 6 h. The reduced rate with this substrate may be a consequence of partial inhibition of the reaction by some 3-methylphenylpyruvic acid produced via hydrolysis of the intermediate imine.[14]

Having established the basic reaction conditions we next tested the generality of the stereoinversion procedure by examining two different amino acid oxidases and an alternative enantiomer of the oxidase.

![Scheme 1](image1.png)

**Scheme 1** Deracemisation of an α-amino acid αL-1 using a combination of an L-α-amino acid oxidase (l-AAO) and reducing agent.

<table>
<thead>
<tr>
<th>Substrate (%)</th>
<th>Yield (%)</th>
<th>e.e. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>63</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>48*</td>
</tr>
<tr>
<td>6</td>
<td>86</td>
<td>50*</td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate (%)</th>
<th>Yield (%)</th>
<th>e.e. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>63</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
<td>&gt;99</td>
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<tr>
<td>4</td>
<td>69</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>48*</td>
</tr>
<tr>
<td>6</td>
<td>86</td>
<td>50*</td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

* denotes e.e. of one of the diastereomers indicating incomplete reaction.
reducing agent (NH$_3$ : BH$_3$) which has previously been shown to be effective. In this case we chose to use enantiomerically pure (2R,3R)-3 and (2S,3R)-3 as substrates and successfully interconverted the pair of diastereoisomers using ß-AAO from porcine kidney and ß-AAA from snake venom (Scheme 3).

In addition to NH$_3$ : BH$_3$, catalytic transfer hydrogenation (Pd/C-HCO$_2$NH$_4$) is also compatible with the oxidase reactions. Both reagents were examined for their ability to convert l-isoleucine 4 to d- allo-isoleucine 5 in the presence of ß-AAA from Proteus myxofaciens (Scheme 4). This enzyme has been over-expressed in Escherichia coli$^{13}$ and can be used either as a whole cell biocatalyst or partially disrupted cells. The use of NH$_3$ : BH$_3$ (40 equiv.) with disrupted cells yielded d- allo-isoleucine 5 in 87% yield and 99% d.e. whereas by comparison Pd/C-HCO$_2$NH$_4$ with intact cells gave 5 in 61% yield and 96% d.e. The use of snake venom L-AOO with NH$_3$ : BH$_3$ resulted in a modest improvement (89% yield; >99% d.e.).

The use of snake venom L-AAO with NH$_3$ : BH$_3$ resulted in rapid conversion to (2R,4S)-6 (66% yield; >99% e.e. and d.e.). The ß-AAO catalysed oxidation of (2R,4S)-6 proceeded rapidly suggesting that the presence of a ß-methyl group has relatively little effect on the reaction rate compared to simple ß-amino acids.

In conclusion we have developed a novel procedure for interconverting ß- and ß-substituted ß-amino acid diastereoisomers using amino acid oxidases in combination with a range of chemical reducing agents. The procedure appears highly versatile in that so far we have examined four different AAOs and four different reducing agents and in all cases the reactions proceeded in good to high yield and excellent stereoselectivities.

We are grateful to the BBSRC and GlaxoSmithKline (A.E.)/DowPharma (G.R.) for CASE awards and to Great Lakes for a postdoctoral fellowship (F.R.A.). We also acknowledge financial support from the Wellcome Trust.

Notes and references

12. The final e.e. of the product from a deracemisation reaction is determined by the ratio of rate constants k$_1$ / k$_2$ for oxidation of the ß and ß-enantiomers since the latter are in equilibrium via the imino acid. Hence the e.e. is identical to that achieved by a standard kinetic resolution process. The e.e. is also given by (E - 1)/(E + 1) as for dynamic kinetic resolutions: H. Stecher and K. Faber, Synthesis, 1997, 1998, 8574.
Appendix 2
Enantiomerically pure $\beta$-branched $\alpha$-amino acids constitute valuable building blocks for the synthesis of modified peptides possessing activity as enzyme inhibitors.\(^1\) The incorporation of bulky $\beta$-substituents into an amino acid lead to conformational restriction in peptides and allows for the development of high affinity ligands for receptors. Existing methods for the synthesis of the individual stereoisomers of $\beta$-methyl $\alpha$-amino acids generally rely upon either stereoselective approaches employing chiral auxiliaries\(^2\) or alternatively nonselective synthesis followed by separation of the isomers by fractional recrystallization of diastereomeric salts.\(^3\) Although high enantiomeric excesses can be achieved via the latter process, the limiting factor is the maximum yield of 50%. An attractive, recently developed alternative is the catalytic asymmetric hydrogenation of $\beta,\beta$-disubstituted dihydroamino acids using chiral bis-phosphine ligands.\(^4\) However, it has been found that although the (Z)-aryl-didehydroamino acids undergo fast and highly selective hydrogenation, the corresponding (E)-isomers react more slowly and with much lower selectivities, resulting in access to only two of the four possible stereoisomers.\(^5\) Herein, we report a combined chemoenzymatic method for the synthesis of all four stereoisomers of a series of enantiomerically pure $\beta$-methyl-$\beta$-arylalalanine analogues.

We have recently developed a method for the deracemization of $\alpha$-amino acids\(^6\) and amines\(^7\) via a cyclic oxidation-reduction sequence and have shown that it can be extended to the stereo-inversion of $\beta$- and $\gamma$-substituted $\alpha$-amino acids.\(^8\) The catalytic cycle involves the combined action of an enantioselective amino acid oxidase (AAO), which oxidizes the $\alpha$-amino acid to the corresponding imine, together with a nonselective reducing agent (e.g., ammonia-borane), which effects reduction back to the starting material. For example, the (2R,3R)- and (2S,3R)-isomers of $\beta$-methylphenylalanine 1a were successfully interconverted in 82% yield by this method in high yield and enanto/diastereoselectivity of the substrates, and thus, we turned to the o-AAO from $\gamma$-substituted $\alpha$-amino acids.\(^9\) Since the (2S,3R)- and (2R,3S)-pair of diastereomers are easily accessed via catalytic asymmetric hydrogenation of the (Z)-didehydroamino acids as described above, we envisaged using the AAO stereoinversion procedure to convert them, respectively, to the less accessible (2R,3R)- and (2S,3S)-diastereoisomers.

Methyl (Z)-2-(N-acetylamino)but-2-enoate 3 was prepared by dehydration of $\ell$-threonine methyl ester 2 according to the method of Nugent et al.\(^6\) Subsequent bromination using $N$-bromosuccinimide followed by triethylamine gave the vinyl bromide 4 ($X = Br$) in 48% yield as a 1:1 mixture of (E/Z)-isomers\(^10\) which could be separated (Scheme 2). However, the subsequent Suzuki couplings were generally found to proceed in higher yields with the (E)- rather than the (Z)-isomers, and hence, we decided to examine the more reactive vinyl iodide, which to our knowledge has not previously been reported.\(^11\)

Treatment of (Z)-3 with $N$-iodosuccinimide followed by Et$_3$N yielded 5 as a 1:1 mix of (E/Z)-isomers. However, addition of 2% TFA to the solvent CH$_2$Cl$_2$\(^12\) gave 5 in 48% yield predominantly as the (Z)-isomer (Z/E = 5:1). The (Z)-isomer was separated by chromatography and then subjected to a range of Suzuki couplings (Scheme 3), which proceeded in good yields (Table 1) to give a range of (Z)-didehydroamino acids (6a–f).

Asymmetric hydrogenation of the (Z)-didehydroamino acids 6a–f was performed at 100 psi hydrogen for 18 h in methanol using either the [Rh($R,R$)-Et-DuPhos(COD)]BF$_3$ or [Rh($S,S$)-Et-DuPhos(COD)]BF$_3$ catalyst to yield the (2R,3S)-7a–f from (2S,3R)-7a–f diastereoisomers respectively (Scheme 4). Purification through a short column of silica resulted in quantitative yields of the products 7a–f with ee’s >98%, with the exception of (2R,3S)-7f (ee = 96%) and (2S,3R)-7f (ee = 93%).\(^13\) Deprotection by refluxing in 4 M HCl gave the enantiomerically pure amino acids 1a–f as the hydrochloride salts in high yields and >99% ee after trituration with acetone (Table 2).

We initially screened the (2R,3S)-isomers of 1a–f against d-AAO from pig kidney, which we had previously shown to be effective for the stereoinversion of (2R,3S)-1a to (2S,3S)-1a.\(^8\) However, this particular enzyme was unable to transform all of the substrates, and thus, we turned to the d-AAO from Trigonopsis

---

\(^{1}\) University of Edinburgh.  
\(^{2}\) Dowpharma.
fl,-disubstituted didehydroamino acids 6a-f followed by stereo-

ecesses. The vinyl iodide (Z)-5 is used as a unique building block

inversion using n- and L-AAO's. Further studies aimed at broad-

to access all 23 products via asymmetric hydrogenation

rate = 0.7 mL/min. Cuantant EtOE-tH = 40:60; sec Supporting Infomsa

determined by reverse-phase chiral I-IPLC (Chirobiotie T column; flow

All enantiomeric and diasteromeric excesses for the reactions were

Table 1. Yields of 6a-f from Suzuki Couplings

<table>
<thead>
<tr>
<th>product</th>
<th>Ar</th>
<th>yield %</th>
</tr>
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<tbody>
<tr>
<td>6a</td>
<td>C6H5</td>
<td>81</td>
</tr>
<tr>
<td>6b</td>
<td>p-F-C6H4</td>
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<td>6d</td>
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</tr>
<tr>
<td>6e</td>
<td>2,5-di-Me-C6H3</td>
<td>82</td>
</tr>
<tr>
<td>6f</td>
<td>p-MeO-C6H4</td>
<td>73</td>
</tr>
</tbody>
</table>

Table 2. Yields and Enantiomeric Excesses of (2R,3S)-1a-f and (2S,3S)-1a-f

<table>
<thead>
<tr>
<th>substrate</th>
<th>Ar</th>
<th>product</th>
<th>yield %</th>
<th>ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-7a</td>
<td>C6H5</td>
<td>(2R,3S)-7a 1a</td>
<td>88 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2S,3R)-7a</td>
<td>C6H5</td>
<td>(2S,3R)-7a 1a</td>
<td>89 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2R,3S)-7b</td>
<td>4-F-C6H4</td>
<td>(2R,3S)-7b 1b</td>
<td>78 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2S,3R)-7b</td>
<td>4-F-C6H4</td>
<td>(2S,3R)-7b 1b</td>
<td>76 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2R,3S)-7c</td>
<td>4-Cl-C6H4</td>
<td>(2R,3S)-7c 1e</td>
<td>81 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2S,3R)-7c</td>
<td>4-Cl-C6H4</td>
<td>(2S,3R)-7c 1e</td>
<td>84 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2R,3S)-7d</td>
<td>2-naphthyl</td>
<td>(2R,3S)-7d 1d</td>
<td>68 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2S,3R)-7d</td>
<td>2-naphthyl</td>
<td>(2S,3R)-7d 1d</td>
<td>87 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2R,3S)-7e</td>
<td>2,5-di-Me-C6H3</td>
<td>(2R,3S)-7e 1e</td>
<td>89 &gt;99</td>
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<tr>
<td>(2S,3R)-7e</td>
<td>2,5-di-Me-C6H3</td>
<td>(2S,3R)-7e 1e</td>
<td>73 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2R,3S)-7f</td>
<td>4-MeO-C6H4</td>
<td>(2R,3S)-7f 1f</td>
<td>66 &gt;99</td>
<td></td>
</tr>
<tr>
<td>(2S,3R)-7f</td>
<td>4-MeO-C6H4</td>
<td>(2S,3R)-7f 1f</td>
<td>67 &gt;99</td>
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Supporting Information Available: Experimental procedures for the preparation of vinyl iodoide Z-5, asymmetric hydrogenation reactions, and the stereoinversion procedures (PDF). This material is available
free of charge via the Internet at http://pubs.acs.org.

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previously been reported. Hoerner, R. S.; Askim, D.; Volante, R. P.;
(12) Silva, N. O.; Abreu, A. S.; Ferreira, P. M. T.; Monteiro L. S.; Queiroz,
(13) All enantiomeric and diastereomeric excesses for the reactions were
determined by reverse-phase chiral HPLC (Chirobiotie T column; flow
rate = 0.7 mL/min, eluant EtOH/H2O = 40:60; see Supporting Information
for full details).
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anneing this approach to other classes of chiral compounds are
ongoing and will be reported in due course.

varia

Finally, (2R,3R)-1a was converted to (2S,3R)-1a on a preparative
scale, using d-AAO from T. variabilis and ammonia–borane
complex as the reducing agent, yielding the isolated product in 55%
yield. Workup of these reactions is very straightforward, involving
simply filtration to remove the enzyme catalyst, followed by
evaporation and recrystallization from ethanol/ethyl acetate.

In summary, we have developed a highly versatile and efficient
route to all but one of a set of 24 β-methylarylalanine analogues
1a-f in high yields and excellent enantiomeric/diasteromeric
excesses. The vinyl iodoide (Z)-5 is used as a unique building block
to access all 23 products via asymmetric hydrogenation of the (Z)
β-disubstituted dihydroxyamo acids 6a-f followed by stereo-

The preparation of (2S,3R)-1a-f using d- and L-AAO

Table 3. Stereoversions of 1a-f Using d- and L-AAO

<table>
<thead>
<tr>
<th>substrate</th>
<th>Ar</th>
<th>enzyme</th>
<th>product</th>
<th>yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-1a</td>
<td>C6H5</td>
<td>d-AAO</td>
<td>(2S,3S)-1a 1a</td>
<td>69</td>
</tr>
<tr>
<td>(2R,3S)-1b</td>
<td>C6H4+2-F</td>
<td>d-AAO</td>
<td>(2S,3S)-1b 1b</td>
<td>68</td>
</tr>
<tr>
<td>(2S,3S)-1c</td>
<td>C6H4+2-Cl</td>
<td>d-AAO</td>
<td>(2S,3S)-1c 1c</td>
<td>81</td>
</tr>
<tr>
<td>(2R,3S)-1d</td>
<td>2-naphthyl</td>
<td>d-AAO</td>
<td>(2S,3S)-1d 1d</td>
<td>80</td>
</tr>
<tr>
<td>(2R,3S)-1e</td>
<td>C6H4(2,5-DiMe)</td>
<td>d-AAO</td>
<td>(2S,3S)-1e 1e</td>
<td>72</td>
</tr>
<tr>
<td>(2R,3S)-1f</td>
<td>C6H4+2-OH</td>
<td>d-AAO</td>
<td>(2S,3S)-1f 1f</td>
<td>81</td>
</tr>
<tr>
<td>(2S,3S)-1a</td>
<td>C6H5</td>
<td>L-AAO</td>
<td>(2S,3S)-1a 1a</td>
<td>83</td>
</tr>
<tr>
<td>(2S,3S)-1b</td>
<td>C6H4+2-F</td>
<td>L-AAO</td>
<td>(2S,3S)-1b 1b</td>
<td>85</td>
</tr>
<tr>
<td>(2S,3S)-1c</td>
<td>C6H4+2-Cl</td>
<td>L-AAO</td>
<td>(2S,3S)-1c 1c</td>
<td>92</td>
</tr>
<tr>
<td>(2S,3S)-1d</td>
<td>2-naphthyl</td>
<td>L-AAO</td>
<td>(2S,3S)-1d 1d</td>
<td>80</td>
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<tr>
<td>(2S,3S)-1e</td>
<td>C6H4(2,5-DiMe)</td>
<td>L-AAO</td>
<td>(2S,3S)-1e 1e</td>
<td>71</td>
</tr>
<tr>
<td>(2S,3S)-1f</td>
<td>C6H4+2-OH</td>
<td>L-AAO</td>
<td>(2S,3S)-1f 1f</td>
<td>80</td>
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