Heterocycles in peptide chemistry

Thesis

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Heterocycles in Peptide Chemistry

By

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Declaration

I declare that the work presented in this thesis is the result of my own investigations, and where the work of others is cited, it is fully acknowledged. The work described in this thesis has not been or is not being submitted elsewhere for any other degree.

..........................................................  
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..........................................................  
Professor R. C. F. Jones (supervisor)

..........................................................  
Dr J. N. Iley (co-supervisor)
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Abstract

The synthesis of 5-membered heterocyclic rings that bear both amine and carboxylic acid functional groups has been investigated using a 1,3-dipolar cycloaddition reaction strategy. These molecules, on incorporation into a chain of amino acids, have the potential to restrict the conformational freedom of the peptide.

Cycloaddition of a nitrile oxide, derived from a Boc-protected naturally-occurring α-amino acid, with a pyrrolidine enamine led to a Boc-protected 3-aminoalkylisoxazole amino acid ester. The nitrogen and carbon termini of this isoxazole were coupled to other α-amino acids. Analysis of the dipeptide from coupling to (S)-alanine indicated that the integrity of the chiral centre of the isoxazole had been retained during the synthesis. Molecular modelling of a tripeptide unit incorporating the isoxazole showed that the presence of the ring had, as intended, restricted the conformational freedom of the molecule.

Analogous cycloadditions using azomethine imines as the dipole yielded the corresponding tetrahydropyrazoles (pyrazolidines). These dipoles were generated by reaction of an aldehyde with a 1,2-disubstituted hydrazine, followed by elimination of the elements of water from the resulting aminol. Reaction with a dipolarophile bearing an electron-withdrawing substituent gave predominantly the 4-substituted pyrazolidine. A study of the scope of the reaction found that, although the required carboxylic acid group could easily be incorporated by use of methyl acrylate as the dipolarophile, it proved impossible to attach an amine group to the ring using this methodology. However, by using one of the nitrogen atoms in the ring as the N-terminus, two pyrazolidines with protected amine and carboxylic acid groups were prepared, and these can be thought of as conformationally restricted β-amino acids.
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# Abbreviations

## General Terms

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<th>Description</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>Alloc</td>
<td>Allyloxycarbonyl</td>
</tr>
<tr>
<td>AMPA</td>
<td>2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>b.p.</td>
<td>boiling point</td>
</tr>
<tr>
<td>Bu</td>
<td>n-Butyl</td>
</tr>
<tr>
<td>'Bu</td>
<td>tert-Butyl</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionisation</td>
</tr>
<tr>
<td>COSY</td>
<td>$^1$H-$^1$H correlation spectroscopy</td>
</tr>
<tr>
<td>CTP</td>
<td>D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH$_2$</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DEAD</td>
<td>Diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DIBAL</td>
<td>Diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DCU</td>
<td>Dicyclohexylurea</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>D-S</td>
<td>Dean and Stark</td>
</tr>
</tbody>
</table>
EDCI  1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EI    Electron Impact
ES    Electrospray
Et    Ethyl
FAB   Fast Atom Bombardment
FMO   Frontier Molecular Orbital
HOMO  Highest Occupied Molecular Orbital
HPLC  High Performance Liquid Chromatography
i.d.  internal diameter
Im    Imidazole
LG    Leaving Group
LH-RH Luteinising Hormone-Releasing Hormone
LUMO  Lowest Unoccupied Molecular Orbital
Me    Methyl
MO    Molecular Orbital
m.p.  melting point
NCS   N-Chlorosuccinimide
NMDA  N-Methyl-D-aspartic acid
NMR   Nuclear Magnetic Resonance
nOe   Nuclear Overhauser Effect
PG    Protecting Group
Ph    Phenyl
ppm   parts per million
iPr   iso-Propyl
RGDS  Arg-Gly-Asp-Ser
TBDMS tert-Butyldimethylsilyl
TCTP  D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH₂
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
Tic  Tetrahydroisoquinoline-3-carboxylic acid
TLC  Thin Layer Chromatography
TMS  Tetramethylsilane
p-tolyl  para-methylphenyl
pTs  para-toluenesulfonyl
Z  benzyloxycarbonyl

Amino Acid Nomenclature

Amino acids are given three letter codes as abbreviations. In peptides the abbreviation of each residue is separated by a hyphen, which can be formally considered as the amide bond. For example, an alanine molecule bonded to the N-terminus of glycine is abbreviated to Ala-Gly. Protecting groups that may be attached are also indicated, e.g. alanine with a Z protecting group on nitrogen is represented as Z-Ala. Side chain protecting groups are written in brackets, e.g. Tyr(Me) for a tyrosine residue with the OH group of the phenyl ring protected as the methyl ether.

Below are listed the three letter codes for the 20 amino acids commonly found in peptides and proteins. Also included are the abbreviations of other amino acids (both naturally-occurring and synthetic) that will be encountered in this work. Unless stated, all amino acids have S stereochemistry (except cysteine which is R) as this is the naturally-occurring enantiomer.
<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>-CH₃</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>-(CH₃)₃N-H-NH₂</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>-CH₂CO₂H</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Asp</td>
<td>-CH₂SH</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>-CH₂CH₂CO₂H</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glu</td>
<td>&lt;Glu</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>-CH₂C-H-NH₂</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>-H</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>-CH₂H</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>-CH(CH₃)CH₂CH₃</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>-CH₂CH(CH₃)₂</td>
</tr>
<tr>
<td>Name</td>
<td>Abbreviation</td>
<td>R</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>----</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>(-\text{(CH}_2\text{)}_4\text{NH}_2)</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>(-\text{CH}_2\text{CH}_2\text{SCH}_3)</td>
</tr>
<tr>
<td>Norleucine(^1)</td>
<td>Nle</td>
<td>(-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>Pen</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>(-\text{CH}_2\text{phenyl})</td>
</tr>
<tr>
<td>Proline(^2)</td>
<td>Pro</td>
<td>(-\text{Proline}^2)</td>
</tr>
<tr>
<td>Sarcosine(^2)</td>
<td>Sar</td>
<td>(-\text{CH}_3\text{NH}\text{CO}_2\text{H})</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>(-\text{CH}_2\text{OH})</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>(-\text{CH}(\text{OH})\text{CH}_3)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>(-\text{Tryptophan}^2)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>(-\text{Tyrosine}^2)</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>(-\text{CH}(\text{CH}_3)\text{2})</td>
</tr>
</tbody>
</table>

1. Not a naturally-occurring amino acid
2. Full structure shown for clarity
Chapter 1:

Introduction
1.1 Background

Peptides are chains of up to 50 amino acids linked head-to-tail by amide bonds (Scheme 1.1) (longer sequences are usually referred to as proteins). They are of great importance in nature, as they play a key role in many biological processes such as digestion, respiration and the immune system. Examples include insulin, synthesised in the pancreas and responsible for lowering blood sugar levels, angiotensin and Substance P, both of which control blood pressure, and thyrotropin, present in the thyroid gland.

![Scheme 1.1](image)

Being so commonplace in living systems, peptides would appear to be an ideal choice for use as potential pharmaceutical agents and pesticides. However, naturally-occurring peptides suffer from a number of limitations that prevent their use in such applications. These include poor absorption into the bloodstream, rapid degradation in the gastrointestinal tract due to proteolysis of the amide bond and a lack of specificity of activity caused by conformational flexibility.

To overcome these problems, chemists began to explore the possibility of modifying natural peptides in an attempt to eliminate the problems associated with their stability whilst retaining the biological activity. Such analogues can function by either mimicking the naturally-occurring molecule – an agonist – or by blocking a receptor site, rendering it inactive, an antagonist. Molecules that contain a peptide backbone which has been modified are known as pseudopeptides\(^1\) or peptidomimetics.\(^2\) These are defined as
substances that possess a similar secondary structure to a natural peptide and can therefore act in the same manner. The secondary structure of a peptide refers to the arrangement of the backbone in regular patterns, thus controlling the orientation of the side chains. This is critical for recognition of the molecule at a receptor site, and therefore peptidomimetics can not only act as stable, biologically active compounds, but can also provide some insight into the conformation a molecule must adopt if it is to exhibit such activity.

The first laboratory synthesis of a peptide hormone was carried out by Du Vigneaud\textsuperscript{3} in 1953 who successfully prepared oxytocin, a cyclic peptide comprising eight amino acids that is responsible for uterine muscle contraction during labour. Further studies\textsuperscript{4} revealed that removal of the N-terminal amino group – to give desamino-oxytocin – resulted in the biological activity of the molecule being doubled, an observation attributed to an increased stability towards enzymatic degradation.

Desamino-oxytocin was the first example of a peptidomimetic, and further research soon followed. Early work concentrated on the addition or deletion of amino acids or the exchange of one residue for another. This field of chemistry has since become a more sophisticated science with specifically designed non-peptidic units being incorporated into chains of amino acids. These units usually replace two amino acids as the amide bond which links two residues in the parent molecule is replaced by some other functional group. Examples of amide bond replacements include C=C double bonds\textsuperscript{1.1,5} hydroxyethylene groups\textsuperscript{1.2,6} ketomethylene groups\textsuperscript{1.37} and reduced amides\textsuperscript{1.48} (Fig. 1.1).
A number of reviews of peptidomimetics has been published\textsuperscript{2, 9-19} and the field of peptidomimetic research is now so vast that an exhaustive review is unrealistic. Therefore only peptidomimetics containing a heterocyclic ring system will be considered here, and even these will be limited to selected examples.

A simple, straight-chain peptide backbone consists of three types of single bond (Fig. 1.2), each with a dihedral angle associated with it; the $\alpha$-carbon-carbonyl carbon bond ($\psi$), the amide bond ($\omega$) and the $\alpha$-carbon-nitrogen bond ($\phi$). Rotation is possible about all of these (although the amide bond has a degree of double bond character, restricting it somewhat) giving rise to a number of possible conformations.
Introducing a ring system can limit or prevent rotation about one or more of these bonds, reducing the range of conformations that the molecule can adopt. The ring arises by either tethering two points on the peptide backbone, usually involving extension of the side chain, or by replacing an entire section of the backbone with a cyclic molecule. The presence of a ring in a peptidomimetic usually serves one of two functions. It can stabilise the conformation of the molecule by increasing its rigidity, or it can act as a mimic for a structural feature such as a turn or helix. In nature, structural features such as turns are usually induced by the insertion of a proline residue (Fig. 1.3), which possesses a bridge between the nitrogen and α-carbon atoms.

![Fig. 1.3](image)

1.2 Features of Secondary Structure

It is appropriate at this point to consider the features of secondary structure to which we have already alluded. The four main structural features are α-helices, β-pleated sheets, β-turns and γ-turns. Due to the complex and extended structure of α-helices and β-pleated sheets, only a few examples of molecules which can mimic them or initiate their formation are known. The comparatively straightforward structure of reverse turns, and the fact that they often occur at points in peptides which are significant with regard to the molecule’s biological activity, means that they are the focus of the majority of secondary structure mimetics. The difference between the two kinds of turn is described below.
The presence of a $\beta$-turn causes the peptide backbone to go back upon itself. Any sequence of four amino acid units in a non-helical region of the peptide where the $\alpha$-C atoms of the first and fourth residues are $\leq 7\text{Å}$ apart is defined as a $\beta$-turn (Fig. 1.4). This rather broad definition means that many different types of $\beta$-turn are known (called Type I, Type II etc.), though they will not be detailed here. Another feature worth noting is the hydrogen bonding that takes place between the carbonyl oxygen of the first residue and the N-H of the fourth.

\[ \text{Fig. 1.4} \]

A $\gamma$-turn performs a similar task to a $\beta$-turn. However, whereas a $\beta$-turn comprises 4 amino acid residues, a $\gamma$-turn is made up of only 3 (Fig. 1.5). These are less common than $\beta$-turns although they are still an important structural feature.

\[ \text{Fig. 1.5} \]
1.3 Analogues of Single Amino Acids

One of the simplest amino acids with a heterocycle as part of the backbone is a tetrahydroisoquinoline carboxylic acid (Tic) (Fig. 1.6). It results from insertion of a methylene bridge between the amine and the 2-position of the side chain phenyl ring of a phenylalanine molecule.

![Fig. 1.6](image)

An example of the effect that this restraint can have on the biological activity of a compound can be demonstrated by the work of Kazmierski and Hruby.\textsuperscript{27} Previously, they had designed a potent opioid receptor antagonist called CTP (Fig. 1.7). By replacing the N-terminal D-Phe residue with its more restricted Tic analogue, TCTP was synthesised, a molecule with a much higher level of selectivity than CTP for one type of receptor over another (>9000 fold for the $\mu$ receptor against the $\delta$ receptor).

![Fig. 1.7](image)

Other examples of conformationally restricted analogues of single amino acids include the tryptophan mimetics \textsuperscript{1.5} (Fig. 1.8) and an azaTic phenylalanine derivative \textsuperscript{1.6}.\textsuperscript{29}
1.4 Lactams

Attachment of an amino acid side-chain to the nitrogen atom of an adjacent residue, to form a pseudodipeptide, results in a lactam. Many cyclic peptidomimetics contain a lactam of some description as it allows retention of the amide bond. As mentioned earlier, the hydrogen bonding abilities of the amide bond are important in terms of the secondary structure of the molecule. Keeping the amide bond prevents disruption of these hydrogen bonds.

Freidinger and colleagues have carried out extensive research into simple lactams including the synthesis of an analogue of Luteinising Hormone-Releasing Hormone (Fig. 1.9). This involved the replacement of the Gly-Leu dipeptide unit with the γ-lactam 1.7 (Scheme 1.2) synthesised by cyclisation of 1.8, the methyl sulfonium salt of Boc-Met-Leu-OMe. Biological tests showed LH-RH mimic 1.9 to be 2.4 times as potent as the parent
hormone. The enhanced activity of 1.9 suggests that the bioactive conformation contains a β-turn.

\[
\begin{align*}
\text{BocHN} & \quad \text{CO}_2\text{CH}_3 \\
\text{H} & \quad \text{N} \\
\text{CO}_2\text{H} & \quad \text{BocHN} \\
\text{1.7} & \quad \text{1.8} \\
\text{i, NaH} & \quad \text{1.9} \\
\end{align*}
\]

Scheme 1.2

1.5 Rings Containing More Than One Heteroatom

\[
\begin{align*}
\text{1.10} \\
\text{Fig. 1.10}
\end{align*}
\]
Horwell and colleagues, having designed 1.10 (Fig. 1.10), an antagonist for the NK₁ neuropeptide receptor, then prepared a conformationally restricted analogue 1.11 (Scheme 1.3) containing a piperazinone ring,³¹ which is effectively an open chain peptide containing an ethylene bridge between the nitrogen atoms of the two amide bonds. The synthesis of 1.11 involved a Mitsunobu cyclisation of 1.12 to form the heterocyclic intermediate 1.13 (Scheme 1.3).

\[
\begin{align*}
\text{HO-} & \text{N-} \text{Ph} \\
\text{H₂N-} & \text{C-} \text{NH} \\
i, \text{ DEAD, PPh₃} & \\
\end{align*}
\]

On testing the ability of 1.11 to bind to the NK₁ receptor, it was found that the cyclic analogue was $10^4$ times less potent than the acyclic compound 1.10. A comparison of the
crystal structures of 1.10 and 1.11 revealed that although the backbone of the piperazinone mimetic has a similar conformation to the acyclic compound, the orientation of the side chains is significantly altered. It is this that is presumed to be responsible for the decrease in biological activity.

![iRGDS](image)

*Fig. 1.11*

The tetrapeptide sequence Arg-Gly-Asp-Ser (RGDS) (Fig. 1.11) of fibrinogen is crucial in its binding to a glycoprotein called GP IIb/IIIa. This results in platelet aggregation, which can lead to thrombosis, therefore any molecule which can mimic RGDS and block the receptor site has potential for use in the treatment of this condition. Stilz, Beck, Jablonka and Just designed and synthesised a heterocyclic peptidomimetic 1.14 (Scheme 1.4) which features a carbonyl bridge between the α-nitrogen atoms of the arginine and glycine residues, giving rise to a dioxotetrahydroimidazole (a hydantoin), as well as a phenyl ring as a rigid analogue of the arginine side-chain. The cyclisation occurred via the acid catalysed intramolecular attack at a carbonyl group by a urea nitrogen atom to give the hydantoin 1.15, which was then converted to the mimic 1.14.
In the peptidomimetics highlighted so far, the peptide backbone has been left intact. Some mimics though, replace the peptide backbone with a different structural unit (see Fig. 1.1). In these cases, the side chains are frequently conserved, as they are the portions of the molecule that are important for binding to a receptor site. Peptidomimetics of this type often have enhanced stability towards proteolytic degradation as the readily hydrolysed amide bond is no longer present. However, such compounds may no longer be able to form the crucial intramolecular hydrogen bonds that are present in the parent peptide. For this reason, many peptidomimetics display only subtle changes in order that they retain many of the bonding properties of the original peptide.
Falorni, Dettori and Giacomelli have carried out enantiospecific syntheses of amino acids 1.16 containing an oxazole ring (Scheme 1.5). Although this does not possess an amide bond, the ring does contain both a nitrogen and an oxygen atom which have the same positioning as an amide relative to the rest of the peptide backbone (Fig. 1.12). Although the lack of an N-H group will prevent hydrogen bonding to another carbonyl group, it is still possible for the oxygen atom to act as a hydrogen bond acceptor.

![amide oxazole](image)

Fig. 1.12

The ring was prepared by a rhodium-catalysed reaction of amide 1.17 (derived from an N-protected α-amino acid) and diazocompound 1.18 (Scheme 1.5). The resulting β-carbonyl amide 1.19 then underwent dehydrative cyclisation to the oxazole.

![Scheme 1.5](image)

i, Rh₂(OAc)₄; ii, PPh₃, I₂, Et₃N.
By selecting appropriate functionality at $R^1$, the oxazole ring-containing fragment 1.16 can either be attached to the side chain ($R^1=\text{CO}_2\text{Bn}, R^2=\text{Boc}$) or form part of the backbone ($R^1=\text{Ph}, R^2=\text{Z}$) of a pseudopeptide.

Thiazoles can be employed similarly, sulfur having similar chemical properties to oxygen. These ring systems have been found to be present in a number of cytotoxic cyclic peptides isolated from marine animals, e.g. Ascidiacyclamide,$^{34}$ Patellamide A,$^{35}$ B$^{36}$ and C,$^{36}$ and Ulithiacyclamide.$^{37,38}$ Shioiri and colleagues devised a synthesis for optically pure amino acids containing a thiazole ring from an $\alpha$-amino aldehyde 1.20 and the methyl ester of cysteine 1.21, a naturally-occurring amino acid (Scheme 1.6).$^{39}$ After attack of the aldehyde carbonyl group by the cysteine nitrogen, the same carbon atom was attacked by the sulfur atom of the cysteine side chain. Loss of the elements of water gave the thiazolidine 1.22 as a racemic mixture that upon oxidation with manganese dioxide yielded the product 1.23. The stereochemistry at the chiral centre of the $\alpha$-aminoaldehyde was retained during the cyclisation.

\[
\begin{align*}
R & \quad \text{H} \\
\text{O} & \quad \text{NH}_2 \\
\text{N} & \quad \text{H} \\
\text{1.20} & \\
+ & \\
\text{R} & \quad \text{H} \\
\text{SH} & \quad \text{CO}_2\text{CH}_3 \\
\text{1.22} & \\
\text{NHZ} & \quad \text{CO}_2\text{CH}_3 \\
\text{1.23} & \\
\text{1.21} & \\
\text{i, MnO}_2 &
\end{align*}
\]

Scheme 1.6
Analogue 1.24 (Scheme 1.7), which was designed as an HIV protease inhibitor, contains an imidazole ring in place of an amide bond. Retention of the NH group in the backbone is desirable for hydrogen-bonding to the carbonyl of a glycine residue in the receptor site, whilst the lone pair of electrons on the other ring nitrogen atom offers further hydrogen bonding via a water molecule to another site on the receptor. The molecule also contains an acyclic amide bond replacement, a hydroxyethylene group (Fig. 1.1). The imidazole ring was synthesised by cleavage of the isoxazole in 1.25 by hydrogenation to form 1.26 (Scheme 1.7). Base-catalysed intramolecular attack on the amide carbonyl by the free amine and elimination of the elements of water formed 1.27, which was then converted to the inhibitor 1.24.

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

\[
\begin{align*}
\text{BocHN} & \quad \text{TBDMSO} \\
\text{Ph} & \quad \text{O} \\
1.26 & \quad \text{NH}_2 \\
\text{Ph} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{BocHN} & \quad \text{TBDMSO} \\
\text{Ph} & \quad \text{O} \\
1.27 & \quad \text{NH}_2 \\
\text{Ph} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{Ph} & \quad \text{O} \\
\text{Z-Val-NH} & \quad \text{H} \\
1.24 & \quad \text{Pr} \\
\text{Ph} & \quad \text{OH} \\
\text{Ph} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{BocHN} & \quad \text{TBDMSO} \\
\text{Ph} & \quad \text{O} \\
1.24 & \quad \text{H} \\
\text{Ph} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
i, \text{H}_2, \text{Pd-C}; & \quad \text{ii, NaOH};
\end{align*}
\]

Scheme 1.7

Peptidomimetic 1.24 and a number of analogues were tested for their inhibition of HIV-1 protease. Also tested were the analogous triazole and thiazole containing mimics 1.28 and 1.29 (cf. 1.24), both derived from the protected hydroxyethylene pseudodipeptide 1.30.
The triazole 1.28 was prepared by condensation of the amide 1.30 with $N,N$-dimethylformamide dimethyl acetal to form 1.31. Nucleophilic attack by hydrazine in the presence of acid then generated the triazole ring. The thiazole was synthesised by reacting the amide 1.30 with Lawesson's reagent to give thioamide 1.32, which was condensed with $N,N$-dimethylformamide dimethyl acetal as before. $S$-Alkylation of the product with 1-bromo-2-butanone was followed by spontaneous cyclisation to form the thiazole. All three ring systems were found to be effective replacements for an amide bond, however 1.24 was found to be significantly more potent than 1.28 and 1.29 as an inhibitor of HIV-1 protease. This was attributed to the imidazole ring being a better hydrogen bond acceptor than the thiazole or triazole.

\[ \text{Scheme 1.8} \]

1. (CH$_3$)$_2$NCH(OCH$_3$)$_2$;  
2. H$_2$NNH$_2$.H$_2$O;  
3. aq. NaOH;  
4. Lawesson's reagent;  
5. BrCH$_2$COEt
The structural similarity between oxazoles, thiazoles and imidazoles was utilised by Singh, Gordon, Morgan and colleagues, who prepared peptidomimetics containing each of these three ring systems from the same \(\beta\)-oxodipeptide unit $1.33$ (Scheme 1.9).\(^{44,45}\) To form the thiazole $1.34$, Lawesson's reagent was used to thionate the carbonyl group. Cyclisation occurred spontaneously with none of the intermediate thioamide being isolated. The best yields of the imidazole $1.35$ were obtained with ammonia when the water generated during ring formation was removed azeotropically. The oxazole $1.36$ meanwhile, was formed by the action of triphenylphosphine and a base, the elements of water being eliminated in the ring-forming process.

Scheme 1.9

\[ \text{i, Lawesson's reagent; ii, NH}_4\text{OH, AcOH; iii, Ph}_3\text{P, DBU.} \]
All three peptidomimetics were used as replacements for the Trp and Phe residues of the neurokinin antagonist H-Pro-Trp-Phe-Trp-Leu-Phe-NH\textsubscript{2} 1.37, the amide bond between the two residues effectively being substituted by a 5-membered azole ring.\textsuperscript{46} In each case an increase in biological activity was observed; however, the thiazole-containing peptide derived from 1.34 was found to be the most potent, with a 10-fold increase over 1.37.

![cis-trans](image)

**Fig. 1.13**

Johnson and Yu synthesised a series of peptidomimetics containing a tetrazole ring to act as a replacement for a cis amide bond (Fig. 1.13).\textsuperscript{47} Although not as common as the trans configuration, the cis amide plays an important part in peptide folding. The ring-forming step in the synthesis of peptidomimetic 1.38 involved conversion of a simple dipeptide ester 1.39 to the imidoyl chloride 1.40 using phosphorus pentachloride, followed by treatment with hydrogen azide to give the tetrazole 1.38 (Scheme 1.10).

![Scheme 1.10](image)
Unfortunately, epimerisation at the chiral centre of the N-terminal amino acid occurred during the cyclisation. However, further research found that this could be suppressed by the addition of quinoline during the formation of 1.40. Epimerisation, this time at the chiral centre of the C-terminal amino acid, also took place if the tetrazole dipeptide was subjected to treatment with base. Therefore, although Johnson and Yu had found that the best yields were obtained when a phthaloyl group was used to protect the N-terminal amine, this proved to be impractical as its cleavage required basic conditions. Zabrocki and co-workers, in their preparation of bradykinin analogues containing the tetrazole amide bond replacement, employed a Z group - cleaved by hydrogenation - to protect the N-terminus and a benzyl ester - removed under acidic conditions - to protect the C-terminus, thus retaining the stereochemical purity of the molecule.

The conformationally restricted amino acid 1.41, which contains a pyrrole ring, was synthesised by Abell, Hoult and Jamieson, also as a surrogate for a cis amide bond.

Formation of the ring was brought about by reaction of the hydrochloride salt of an amino acid methyl ester 1.42 with 1,4-dimethoxytetrahydrofuran 1.43 (Scheme 1.11). Formylation of the resulting pyrrole 1.44 with trimethyl orthoformate followed by reductive amination with ammonium acetate and sodium cyanoborohydride then introduced the amine function to give the mimetic 1.41. N- and C-Terminal protecting groups can then be attached as required making 1.41 a more versatile cis amide bond mimic than the tetrazole 1.38 described earlier.
Cycloadditions are frequently used to construct carbocyclic and heterocyclic rings as they usually allow a high degree of stereocontrol to be exercised over the final product. The field of peptidomimetic research includes a number of cases of heterocycles that have been constructed using a cycloaddition reaction; some examples synthesised by Diels-Alder reactions and 1,3-dipolar cycloadditions will now be considered.

1.7.1 Peptidomimetics Constructed Using a Diels-Alder Reaction

The Diels-Alder reaction involves reaction of a diene (containing two double bonds) and a dienophile (which possesses just one) (Scheme 1.12) resulting in the formation of a 6-membered ring.
Captopril (Fig. 1.14) is a peptidomimetic used to control high blood pressure.\textsuperscript{51} As part of the effort to discover even more potent mimetics to treat this condition, Hassall and coworkers used a Diels-Alder reaction between triazole \textbf{1.45} or \textbf{1.46} and the diene \textbf{1.47} as a key step in the synthesis of the tetrahydrotriazolopyridazinediones \textbf{1.48} and \textbf{1.49} (Scheme 1.13), which are conformationally restricted analogues of captopril.\textsuperscript{52}
The di-substituted azanorbornane 1.50 (Scheme 1.14) is a β-turn mimic designed to model the Trp-Phe sequence of a cyclic peptide that exhibits a high affinity for a neuropeptide receptor (NK-2). As well as imitating the side chain orientation of Trp and Phe, the dipole moment of 1.50 also closely matches that of the hexapeptide it is designed to replace. Whilst 1.50 does not display the same affinity for the NK-2 receptor, it does display the same affinity for the NK-1 receptor as the hexapeptide, suggesting that the Trp-Phe section of the molecule is responsible for binding to this site. The synthesis of the azanorbornane 1.50 involved an aza-Diels-Alder reaction of cyclopentadiene with the dienophile 1.51 (Scheme 1.14), which is derived from the condensation of benzylamine with phenylglyoxal 1.52.
1.7.2 Peptidomimetics Constructed Using a 1,3-Dipolar Cycloaddition

1,3-Dipolar cycloadditions are used in the synthesis of 5-membered rings containing one or more hetero-atoms (see Section 1.10). The perhydroisoindol-4-one 1.53 (Scheme 1.15) is a non-peptidic antagonist of Substance P. A key step in its synthesis was a 1,3-dipolar cycloaddition involving the azomethine ylid 1.54 and cyclohex-2-enone 1.55 to produce the perhydroisoindol-4-one 1.56 in 84% yield. As the dipole is C₂-symmetrical, only one regioisomer can arise from the reaction.

\[
\begin{array}{c}
\text{Ph} \quad \text{Ph} \\
\text{1.55} \\
\text{H₂C⁻} \\
\text{H₂C⁺} \\
\text{Ph} \\
\end{array} \quad \text{1.54} \quad \xrightarrow{\text{+}} \quad \begin{array}{c}
\text{Ph} \quad \text{Ph} \\
\text{1.56} \\
\text{H} \\
\text{Ph} \\
\end{array}
\]

Scheme 1.15

Harwood and co-workers have carried out research into the enantioselective synthesis of proline derivatives from α-amino acids, again by the 1,3-dipolar cycloaddition of an azomethine ylid (Scheme 1.16). (S)-Valine 1.57 was converted to the morpholinone template 1.58 in which the original chirality has been used to generate a new chiral centre. On formation of the azomethine ylid 1.59, by reaction with formaldehyde, the chiral centre
at C-3 derived from the valine was destroyed; however, the presence of the second chiral centre allowed stereocontrol at C-3 during cycloaddition. Hydrogenation of the resulting cycloadduct 1.60 gave the proline derivative 1.61, which possesses the same stereochemistry at C-2 as the starting amino acid 1.57.

Further research has investigated the use of other aldehydes to introduce functionality at the 5-position of the proline ring,\(^{57}\) as well as intramolecular cycloadditions with a tethered dipolarophile to exert greater stereocontrol over the reaction.\(^{58}\) Cycloadditions have also been carried out using a morpholinone template bearing a phenyl group at C-3 instead of iso-propyl, which had the effect of increasing both the yield and the diastereocontrol.\(^{59}\)
The orthogonally protected spirocyclic pseudodipeptide 1.64 was designed as a scaffold for combinatorial libraries. One of the intermediates in its preparation is the tricyclic pyrazoline 1.63, formed by the intramolecular dipolar cycloaddition of an azomethine imine 1.62 (Scheme 1.17). By having the dipolarophile tethered to the dipole, a high degree of regiocontrol was observed. This is because the twisting of the intermediate 1.62 that would be required to form the other regioisomer would raise the energy of the transition state to a prohibitive level.
Isoxazolidines have been used as proline analogues by Vasella and Voeffray. They devised an asymmetric synthesis of the (3S)-enantiomer of the 5-oxaprol ine 1.67 by means of the 1,3-dipolar cycloaddition of ethylene to the N-glycosyl nitrone 1.66 derived from the oxime of D-mannose 1.65 and tert-butyl glyoxylate (Scheme 1.18). By using the oxime of D-ribose 1.68, the (3R)-5-oxaprol ine 1.69 can also be prepared.

**Scheme 1.18**
NMBA 1.71, AMPA 1.72 and kainic acid 1.73 all mimic glutamic acid 1.70 (Fig. 1.15). The hydroxyisoxazolinylprolines (±)1.74 and (±)1.75 were prepared as conformationally restricted analogues of these compounds (1.74 being regarded as a mimic of NMBA and 1.75 of AMPA and kainic acid, the spacing between the backbone carboxylic acid group and the side-chain OH functionality being similar). Their syntheses involved the 1,3-dipolar cycloaddition of bromonitrile oxide 1.76 (derived from the oxime 1.77) to the unsaturated proline derivative 1.78 (Scheme 1.19). The resultant fused bicyclic products 1.79, 1.80 and 1.81, which are simply regio- and stereoisomers from the cycloaddition (the diastereoisomer of 1.79 was not detected), were then treated with sodium hydroxide to hydrolyse the ester and to convert the bromine substituent to a hydroxyl group. Trifluoroacetic acid (TFA) removed the Boc group to realise the peptidomimetics 1.74 and 1.75. The action of sodium hydroxide also altered the stereochemistry of 1.81, positioning the ester group on to the opposite face of the pyrrolidine ring to the isoxazole, meaning that both 1.80 and 1.81 gave rise to the final product 1.75.
Scheme 1.19

An isoxazoline ring, constructed by the 1,3-dipolar cycloaddition of nitrile oxide 1.82 (Scheme 1.20), has also been used as a conformationally constrained version of Val-Pro.63
Cycloaddition of 1.82 with methyl acrylate produced syn and anti diastereoisomers of 1.83, although these were separable by recrystallisation after coupling to the methyl ester of methionine. The methionine-coupled product was then incorporated into a series of tetra- and hexapeptides to produce analogues of known immunomodulating peptides. Similarly, a stereoselective method for synthesising the isoxazoline 1.84 using an N-acryloyl derivative of Oppolzer’s camphor sultam has been reported.64

1.8 Previous Research - Imidazolines

Previous research in the group carried out at Nottingham 65-69 has focussed on the construction of imidazolines 1.85 (Fig. 1.16) for insertion into peptide backbones. An imidazoline can be regarded as a cyclic version of an amidine 1.86, which bears a close structural relationship to an amide 1.87.

![Fig. 1.16](Image)

This was thought to be a suitable replacement for an amide bond as it features a similar arrangement of hetero-atoms and double bonds as well as similar hydrogen bonding capabilities. In addition to the conformational constraints introduced by the ring, the replacement of the amide bond with a more basic functional group should increase the resistance of the molecules to proteolytic hydrolysis.

The initial strategy for preparing peptidomimetics with the imidazoline ring was to use a convergent approach, the ring being assembled as the final stage of the synthesis.65
Several peptidomimetics were formed in this way, including 1.88, an analogue of enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) with the imidazoline ring replacing the amide bond between the two glycine residues (Scheme 1.21).

```
\[
\begin{align*}
\text{Z-Tyr(Bn)NH}_{2}\text{Cl}^- & \quad + \quad \text{Phe-Leu-OMe} \\
\text{H} & \quad \text{H}_2\text{N} \\
\text{Et} & \quad \text{O}
\end{align*}
\]

i, MeOH, 65°C.

Scheme 1.21
```

Further work involved ring construction as the initial stage followed by coupling to other amino acids in a divergent approach. Amino acid side chains were introduced by use of appropriately substituted diamines. Cyclisation to form the imidazoline 1.89 was carried out by reaction of the S-methylthioimidate 1.90 with the diamine 1.91 (Scheme 1.22). Using standard peptide coupling reactions, the imidazoline 1.89 (a conformationally restricted replacement for Trp-Nle) was incorporated into a mimic of the CCK-4 analogue H-Trp-Nle-Asp-Phe-NH₂.
Racemisation occurred at the chiral centre of the thioimidate during ring formation. However, separation of the Boc-protected imidazolines 1.92 allowed preparation and isolation of both diastereoisomers of the CCK analogue 1.93 as trifluoroacetate salts.

1.9 Aims and Targets

This project aims to extend this area of research by synthesising other 5-membered heterocyclic rings bearing amine and carboxylic acid functional groups. These molecules can then be incorporated into peptides in order to restrict their conformational freedom. The ring systems that will be studied are isoxazoles 1.94 (Fig. 1.17) and tetrahydropyrazoles 1.95. Isoxazoles are fully unsaturated, hetero-aromatic derivatives of the isoxazolines studied by Kim et al. that were discussed earlier.
Apart from the absence of any double bonds, tetrahydropyrazoles are structurally similar to isoxazoles, having two adjacent nitrogen atoms instead of a nitrogen atom and an oxygen atom. The presence of amine and carboxylic acid ring substituents gives target molecules with a general structure of 1.95 or 1.96 (Fig.1.18).

Target 1.96 has a substitution pattern in which the amine and acid functions are arranged in a similar manner to the natural dipeptide 1.98. In the second general target 1.97, the amine and carboxylic acid groups have one less atom between them than in the natural dipeptide 1.98. For this reason, molecules of this type might be expected to have a more pronounced effect on peptide backbone conformation than those of type 1.96. Comparison of the intended targets with the generic dipeptide 1.98 also shows that the amide bond has been replaced. This should increase the stability towards proteolytic degradation.
1.10 Synthetic Strategy

The strategy for forming the 5-membered heterocyclic rings will employ a 1,3-dipolar cycloaddition reaction. This comprises a three-atom component, the dipole, and a dipolarophile which contributes two atoms to the ring (Scheme 1.23). Such reactions have found widespread use in organic synthesis as they provide an easy and controlled route to 5-membered heterocycles.

![Scheme 1.23](image)

The dipole, as its name suggests, bears both a positive and a negative charge, although it is charge neutral overall. In reaction schemes the dipole is usually drawn so that the constituent atoms each have a full octet in their valence shell. This resonance structure does not highlight the 1,3-reactivity of the species as the charges are on adjacent atoms. This reactivity is best represented by the sextet resonance structure shown in Fig. 1.19.

![Fig. 1.19](image)

For this resonance to occur, the central atom (b) in the dipole must carry a lone pair of electrons to stabilise the dipole. Carbon cannot fulfil this requirement and so the dipole must contain a hetero-atom such as nitrogen at this position. Many dipoles contain more than one atom which is not carbon.
A necessity of the dipolarophile is that it contains a multiple bond, as a pair of π-electrons is required to form a bond to the dipole. If the dipolarophile contains a triple bond (two pairs of π-electrons), then the cyclic product will contain a double bond between atom d and e (Scheme 1.23).

The mechanism of cycloaddition is generally believed to be a concerted process, as proposed by Huisgen.\textsuperscript{71} Other mechanisms, such as the formation of diradical intermediates have been postulated,\textsuperscript{72} but these do not readily account for the stereospecificity observed in such reactions.

The regiochemistry of 1,3-dipolar cycloadditions is best explained by frontier molecular orbital (FMO) theory. It is the relative energy levels of these molecular orbitals (MOs) and the size of their coefficients that control the reactivity and regioselectivity of the dipolar cycloaddition. Three classifications of 1,3-dipolar cycloaddition have been identified, as shown in Fig. 1.20.\textsuperscript{73}

\begin{center}
\begin{tabular}{ccc}
\textbf{Dipole} & \textbf{Dipolarophile} & \textbf{Dipole} \\
\textbf{Dipolarophile} & \textbf{Dipole} & \textbf{Dipolarophile} \\

\begin{tikzpicture}
\draw [->] (0,0) -- (0.5,0);
\draw [->] (1,0) -- (1.5,0);
\draw [->] (0,0) -- (0,-0.5);
\draw [->] (0,0) -- (0,0.5);
\draw [->] (1,0) -- (1,-0.5);
\draw [->] (1,0) -- (1,0.5);
\node at (0,0) {L};
\node at (1,0) {L};
\node at (0,-0.5) {H};
\node at (1,-0.5) {H};
\end{tikzpicture} & \begin{tikzpicture}
\draw [->] (0,0) -- (0.5,0);
\draw [->] (1,0) -- (1.5,0);
\draw [->] (0,0) -- (0,-0.5);
\draw [->] (0,0) -- (0,0.5);
\draw [->] (1,0) -- (1,-0.5);
\draw [->] (1,0) -- (1,0.5);
\node at (0,0) {L};
\node at (1,0) {L};
\node at (0,-0.5) {H};
\node at (1,-0.5) {H};
\end{tikzpicture} & \begin{tikzpicture}
\draw [->] (0,0) -- (0.5,0);
\draw [->] (1,0) -- (1.5,0);
\draw [->] (0,0) -- (0,-0.5);
\draw [->] (0,0) -- (0,0.5);
\draw [->] (1,0) -- (1,-0.5);
\draw [->] (1,0) -- (1,0.5);
\node at (0,0) {L};
\node at (1,0) {L};
\node at (0,-0.5) {H};
\node at (1,-0.5) {H};
\end{tikzpicture} \\
\textbf{HOMO controlled} & \textbf{HOMO/LUMO controlled} & \textbf{LUMO controlled} \\
\textbf{Type I} & \textbf{Type II} & \textbf{Type III} \\
\end{tabular}
\end{center}

\textbf{H} = \text{HOMO} \\
\textbf{L} = \text{LUMO}

Fig. 1.20
If the highest occupied molecular orbital (HOMO) of the dipole and the lowest unoccupied molecular orbital (LUMO) of the dipolarophile are the two MOs closest in energy, then this will be the dominant interaction and the cycloaddition is said to be HOMO controlled (Type I). Alternatively, if the two closest energy levels are the LUMO of the dipole and the HOMO of the dipolarophile, then the reaction is said to be LUMO controlled (Type III). A third class of cycloaddition occurs when the difference between the energy levels is the same or comparable and the reaction is under the control of both HOMO and LUMO (Type II).

The regioselectivity is often determined by the size of the orbital coefficients at the termini of the two closest FMOs (i.e. the ones which are said to control the reaction). The dipole and dipolarophile react in such a way that the larger (terminal) coefficients of the two components align. This maximises the orbital overlap, which gives rise to the lower energy transition state (Fig. 1.21a). Should the dipole and dipolarophile react the other way around, less overlap occurs and a higher energy transition state forms (Fig. 1.21b). If the terminal coefficients of the FMOs of a component are similar in magnitude, then one transition state is not significantly favoured over the other and little or no regioselectivity is observed.

![Fig. 1.21](image)

The magnitude of the coefficients is dependent on the constituent atoms at the reaction centre and the nature of their substituents (e.g. electron-withdrawing). Houk and co-
workers have applied perturbation theory to calculate the co-efficients of the HOMOs and LUMOs of a variety of dipoles and dipolarophiles allowing predictions to be made on the regioselectivity of a particular cycloaddition.

The goals of the project have now been outlined and the general structure of the target molecules identified. A strategy for the synthesis of these target molecules is also in place. Identification of the exact targets and discussion of the efforts made to synthesise them will be presented in the following chapters.
Chapter 2: Results and Discussion - Isoxazoles
2.1 Introduction

The isoxazole ring system 2.1 was initially chosen for incorporation into a non-proteinogenic amino acid. This heterocycle can be formed by the 1,3-dipolar cycloaddition of a nitrile oxide 2.2 and a dipolarophile 2.3 containing a triple bond, or its equivalent (Scheme 2.1).

\[
\begin{align*}
N = O & \quad \iff \quad R^1\equiv R^2\equiv R^3 \\
\text{2.1} & \quad \text{2.2} & \quad \text{2.3}
\end{align*}
\]

Scheme 2.1

Nitrile oxides are widely used in organic synthesis, as they are the most common way to prepare isoxazoles and isoxazolines. These compounds are of great synthetic importance as they can be converted, using a range of reductive sequences, into a variety of functional groups, for example β-hydroxy ketones 2.4, γ-amino-alcohols 2.5 and α,β-unsaturated ketones 2.6 (Scheme 2.2). By gaining access through a ring system, the functionality is masked but can be uncovered subsequently as required.

\[
\begin{align*}
\text{2.4} & \quad \text{2.5} & \quad \text{2.6}
\end{align*}
\]

Scheme 2.2
Previous experience in our group with the synthesis of heterocyclic triones, such as the 3-acyltetramic acid and 3-acyl-4-hydroxypyridone groups of natural products, led us to choose isoxazoles as the first target ring system. The β,β'-tricarbonyl moiety 2.7 (Fig. 2.1) found in the heterocyclic triones (drawn here in the enol form) is extremely polar and reactive, but can be masked by means of an isoxazole ring, as in 2.8. This permits other reactions to be carried out on the molecule without affecting the trione system.

![Fig. 2.1]

Nitrile oxides are usually generated in one of two ways. The first involves the loss of water from a primary nitro-compound (Scheme 2.3).

![Scheme 2.3]

A number of dehydrating agents has been used, the classical example being the Mukaiyama and Hoshino method, which employs phenyl isocyanate (Scheme 2.4). However this has the disadvantage of creating diphenylurea 2.9 as a by-product which can often prove difficult to remove. Other reagents that accomplish this dehydration include para-toluenesulfonic acid, ethyl chloroformate, and di-tert-butyl dicarbonate (Boc₂O) with 4-dimethylaminopyridine (DMAP).
A second method for generating nitrile oxides employs the oxidation of an aldoxime using, for example, manganese dioxide.\textsuperscript{87} Alternatively, the oxime can be halogenated at the oxime carbon atom by reagents such as chlorine gas, sodium hypochlorite,\textsuperscript{88} Chloramine T\textsuperscript{89} or an N-halosuccinimide.\textsuperscript{90} Treatment with base results in dehydrohalogenation, forming the dipole (Scheme 2.5).
Since nitrile oxides bear two charged atoms they are extremely reactive, and as such are not stable enough to be isolated. Even when stored in the absence of other reagents they will readily undergo an irreversible dimerisation, and for this reason they are usually generated in situ, for example by the addition of a base to a solution of a chlorinated aldoxime in the presence of a dipolarophile.

2.2 Disconnection

The Boc-protected 3-aminoalkylisoxazole amino acid ester 2.10 was selected as the target molecule and a possible disconnection is shown in Scheme 2.6. It can be viewed as a conformationally constrained replacement for a dipeptide unit containing an alanine residue (Fig. 2.2). In addition to the absence of an amide bond (improving stability towards hydrolytic cleavage of the molecule), the isoxazole has one less atom between the N- and C-termini, a feature that will further restrict the flexibility of the backbone.
The selection of an acid-labile group at the N-terminus of 2.10, and a base-sensitive one at the C-terminus was made to allow the two ends of the amino acid to be deprotected orthogonally. The third major class of protecting group, those that are removed by hydrogenation, are of little value here as the conditions required for their removal will result in the isoxazole ring being destroyed by reductive N-O bond cleavage.

The presence of a substituent (here a methyl group) at the 5-position of the target isoxazole 2.10 will be a key factor in directing the ester to the desired 4-position on the ring. Without this substituent, the required dipolarophile would be a mono-substituted alkynoate ester. Such monosubstituted alkynes are known, almost without exception, to result exclusively or predominantly in the alkyne substituent occupying the 5-position of the resultant isoxazole ring upon reaction with nitrile oxides.

The amine functionality is located on the 2-position of an alkyl chain because, as will be seen, this is ultimately derived from a naturally-occurring \( \alpha \)-amino acid. In this case the amino acid chosen was \( S \)-alanine, the simplest natural example that contains a chiral centre.
The first of the two components required for the cycloaddition is thus the dipole 2.11 (shown in Scheme 2.6 as both a 1,3-charged species and as the more stable octet resonance structure), which would be generated by the loss of HCl from the hydroximoyl chloride 2.13. This process will be effected by treatment with base as mentioned previously. An alternative way of viewing hydroximoyl chlorides is as C-chlorooximes, which are readily prepared from standard oximes, in this case 2.14. The oxime could be prepared from an \( \alpha \)-aminoaldehyde 2.15. These are widely used as intermediates in a range of reactions and a great number of methods for their preparation is known. The aldehyde can be accessed by reduction of the ester 2.16, which is a di-protected amino acid (here, \( S \)-alanine 2.17).

\[
\begin{align*}
\text{2.11} & \quad \iff \quad \text{2.13} & \iff \quad \text{2.14} \\
\text{2.15} & \iff \quad \text{2.16} & \iff \quad \text{2.17} \\
\text{2.18} & \iff \quad \text{2.19} & \iff \quad \text{2.20}
\end{align*}
\]

Scheme 2.7

Scheme 2.8
The other fragment of the target molecule 2.10, represented by the double-bonded synthon 2.12, will be derived from the pyrrolidine enamine 2.18 (Scheme 2.8). Reaction of 2.18 with the dipole 2.11 will result in a 2-isoxazoline which will eliminate a pyrrolidine molecule across the bond between the 4 and 5 positions to give the desired product. This sequence will be discussed in more detail later.

This enamine is a more useful synthetic equivalent than the simple alkyne due to the enhanced regioselectivity it offers. Reaction of the nitrile oxide 2.11 with ethyl but-2-ynoate 2.21 (Scheme 2.9) should result in predominantly the required product 2.10, however some of the undesired regioisomer 2.22 would also be expected to be formed.

Attaching an electron-donating group (here, the enamine nitrogen atom) to the opposite end of the double bond to the electron-withdrawing group (the carboxylate ester), results in formation of the 4-carboxyisoxazole as the major product. On cycloaddition, it has been found that the electron-donating substituent will prefer to be located at the 5-position of the product heterocycle, which enhances the lesser preference of an electron-
withdrawing group for location at the 4-position of the ring. This can be attributed to the electron-donating group altering the coefficients of the FMOs of the dipolarophile.

As previously mentioned, the cycloaddition of a nitrile oxide with 2.21 will give rise to two regioisomers (Scheme 2.9); the 4-carboxyisoxazole 2.10 formed as a result of the HOMO controlled reaction (see Chapter 1, Section 1.10), and the 5-carboxyisoxazole 2.22 from the LUMO controlled cycloaddition (Fig. 2.3). (The relative magnitudes and signs of the FMOs of nitrile oxides and dipolarophiles bearing an electron-withdrawing group have previously been determined by Houk and colleagues). 74

![Fig. 2.3](image)

Attaching an electron-donating group to the multiple bond of the dipolarophile will alter the coefficients of both the HOMO and LUMO in the manner shown in Fig. 2.4. 98

Although the difference in magnitude of the two coefficients of the LUMO has increased, they will still react with the HOMO of the dipole to give the same product as before, the 4-carboxyisoxazole. However in the HOMO of the dipolarophile, the larger of the two coefficients is now at the same end of the multiple bond as the ester group (previously it was at the opposite end), and so the LUMO controlled cycloaddition will now also give the
4-carboxyisoxazole. Therefore, both the HOMO and LUMO controlled reactions yield the same product.

Following cycloaddition, elimination across the 4,5-bond of an initially formed isoxazoline results in the isoxazole (Scheme 2.10). If the eliminated molecule is stable enough then this process can occur spontaneously, driven by the stability of the aromatic ring that is formed.

Scheme 2.10
The strategy of using a doubly-bonded "push-pull" dipolarophile, which then undergoes elimination, was first reported by Stork and McMurry, who used pyrrolidine enamines of \( \beta \)-keto esters to synthesise 4-carboxyisoxazoles. Since then, it has been adopted a number of times to obtain isoxazoles with electron-withdrawing substituents at the 4-position, such as nitrile and nitro groups (Schemes 2.11 & 2.12).

![Scheme 2.11](image)

![Scheme 2.12](image)

Species other than enamines have been used to direct electron-withdrawing groups, e.g. triphenylphosphonium salts (Scheme 2.13); however, studies have shown that the best yields and regioselectivities are obtained by using enamines, such as those of pyrrolidine or morpholine.

![Scheme 2.13](image)
The planned synthesis of the enamine 2.18 was via the dehydrative addition of an amine to a β-keto ester. The required β-keto ester in this case is ethyl acetoacetate 2.19, which is commercially available, and although this will react with any secondary amine, pyrrolidine 2.20 was selected due to its well-publicised success in such applications.

2.3 Synthesis of the Hydroximoyl Chloride

\[
\begin{align*}
\text{O} & \quad \overset{\text{i}}{\rightarrow} & \quad \text{O} \\
\text{NH}_2 & \quad \rightarrow & \quad \text{OMe} \\
2.17 & \quad \rightarrow & \quad 2.23 \\
\text{NH}_3\text{Cl}^- & \quad \rightarrow & \quad \text{NHBoc} \\
2.23 & \quad \rightarrow & \quad 2.16 \\
\text{NH} & \quad \rightarrow & \quad \text{Cl} \\
& \quad \rightarrow & \\
2.13 & \quad \rightarrow & \quad 2.14 \\
\text{NH} & \quad \rightarrow & \quad \text{H} \\
& \quad \rightarrow & \\
& \quad \rightarrow & \\
& \quad \rightarrow & \\
& \quad \rightarrow & \\
2.14 & \quad \rightarrow & \quad 2.15 \\
\text{O} & \quad \rightarrow & \quad \\
\end{align*}
\]

i, AcCl, MeOH, 0°C, 72h; ii, Et3N, Boc2O; iii, DIBAL, -78°C; iv, NH2OH.HCl, NaOAc; v, tBuOCl.

Scheme 2.14

\( S\)-Alanine 2.17 was converted into its methyl ester 2.23 (Scheme 2.14) in order to create a good leaving group for the subsequent reduction to the corresponding aldehyde. Esterification was accomplished using anhydrous methanol as both the solvent and the source of the methoxy nucleophile. Acetyl chloride was also present, which, upon reaction with methanol, generated hydrochloric acid as catalyst for the reaction. The acetyl chloride was added dropwise to methanol that had been cooled to 0°C, before addition of the amino acid in one portion and stirring of the solution at 0°C. Despite the presence of 4 equivalents of acetyl chloride, the reaction proceeded sluggishly, taking 72-96h to reach
completion. Even so, this method was found to be more successful than dissolving the amino acid in methanol, cooling to 0°C for the addition of acetyl chloride, then heating at reflux. In both cases the acetyl chloride had to be added very slowly, or fumes of HCl gas would begin to escape from the reaction flask. The desired ester was obtained in a virtually quantitative yield as the hydrochloride salt, with no purification required other than removal of the solvent.

N-Protection of the amino ester was achieved by treatment with triethylamine and Boc₂O (Scheme 2.14). On dissolving the ester hydrochloride 2.23 in dichloromethane (DCM) (in which it was only sparingly soluble) and cooling to 0°C, two equivalents of base were added dropwise, one to neutralise the hydrochloride and give the free amine, the second to remove a proton from the nitrogen atom at a later stage of the reaction. Boc₂O (as a solution in DCM) was then added dropwise, and the solution stirred for 16h at room temperature, by which time the reaction had reached completion. Only a very slight excess of Boc₂O was used, as removing unreacted material at the end of the reaction proved difficult. Repeated washing with a 1M solution of citric acid was required in order to eliminate all traces of Boc₂O. Despite the volatile nature of the reaction solvent, on its removal under reduced pressure the product 2.16 was usually obtained as an oil, which would only crystallise after standing for several days, presumably due to traces of trapped tert-butanol.

The N-protected ester 2.16 was reduced to the aldehyde 2.15 by means of diisobutylaluminium hydride (DIBAL) using a procedure developed by Rich and co-workers. DIBAL (1M solution in toluene) was added to a solution of the ester in dry toluene at -78°C. Care was needed to prevent the desired aldehyde from being further reduced to the corresponding alcohol; consequently the reaction was carefully monitored
by thin layer chromatography (TLC). As soon as all of the starting material had been consumed (approximately 45 min after hydride addition had been completed), the excess hydride was quenched with methanol. A saturated solution of sodium potassium tartrate (Rochelle salt) was used in the reaction work up, the tartrate anion forming a complex with the aluminium ion to ensure that it remained in the aqueous layer. On adding the reaction mixture to the Rochelle salt solution, a thick emulsion formed which gradually dissipated on stirring vigorously for 2 h. This was presumably due to the aluminium ion complexing to the tartrate whilst still dissolved in the organic phase, and subsequently partitioning to the aqueous phase.

A $^1$H NMR spectrum of the crude aldehyde showed that it was virtually pure, although there were small traces of impurities, the major of which was assumed to be the alcohol. However, it was decided that purification would not be practical, since $\alpha$-amino aldehydes are unstable towards racemisation. This loss of chirality occurs via keto-enol tautomerism (Scheme 2.15), a process studied by Ito and co-workers who found that racemisation is accelerated when the aldehyde is subjected to column chromatography. This was attributed to the acidic nature of silica gel and an acid-catalysed mechanism for the tautomerism was postulated.

![Scheme 2.15](image)

In order to minimise this problem the crude aldehyde 2.15 was immediately converted to the oxime 2.14 (Scheme 2.14). The aldehyde, dissolved in ethanol, was added to an excess of hydroxylamine hydrochloride and sodium acetate in water. After heating at 60-70°C for
10 min the reaction mixture was left to cool overnight, during which time the oxime crystallised from the solution. Purification of the oxime by recrystallisation from aqueous ethanol, a common solvent for such procedures, proved awkward due to the extreme solubility of the oxime in ethanol. In addition, the purified product retained significant amounts of moisture and, since anhydrous conditions were required for the subsequent cycloaddition, prolonged drying over phosphorus pentoxide in a vacuum dessicator was necessary. An attempt was also made to recrystallise the oxime from chloroform and hexane. This yielded a white crystalline solid as before but this time no drying was required. Combustion analysis showed a higher degree of purity than the earlier samples, however the melting point was found to be considerably lower than that of material crystallised from ethanol and water (125°C compared to 136°C).

Thus the oxime 2.14 was obtained as a white solid which is thought to be more stable towards racemisation than its aldehyde precursor. This was attributed to the fact that it can be purified by recrystallisation, thus avoiding the need for contact with any acidic or basic substances, such as silica or alumina, which may cause racemisation (see Scheme 2.15). This oxime exists as a 2:1 mixture of E and Z isomers (calculated from the ratio of the integrals of the $^1$H NMR spectrum), though it was not possible to determine which of these was the major product. NMR studies showed that, even on heating to 70°C, there was no alteration in the E:Z ratio. Presumably, the lone pair of electrons on the nitrogen atom presents a significant energy barrier to rotation about the double bond (Scheme 2.16). However, the presence of E:Z isomers proved not to be a problem, as both isomers are able to undergo transformation to the nitrile oxide dipole.
To chlorinate 2.14 at the carbon of the C=N double bond, a source of Cl+ was required. Larsen and Torssell\textsuperscript{104} have reported N-chlorosuccinimde (NCS) for chlorination of oximes as a convenient alternative to chlorine gas, obtaining good yields by reaction at room temperature in the presence of a trace of pyridine. Since the addition of base may lead to dehydrochlorination of the chlorinated product and generate the dipole before it is needed, the reaction was attempted in the absence of pyridine. On heating at reflux with NCS in dry chloroform for 2h, it was found that whilst one of the two starting materials visible by TLC (presumed to be the two geometric isomers of the oxime) was readily converted, the other was significantly less reactive, some remaining even after heating for 16h. Other workers have found one isomer of an oxime significantly more reactive than the other towards N-halogenated succinimidnes. In trying to brominate an oxime with N-bromosuccinimde, Kim and colleagues\textsuperscript{54} found one isomer to be completely unreactive.

As an alternative, \textit{tert}-butyl hypochlorite\textsuperscript{97}, synthesised from household bleach (effectively a solution of sodium hypochlorite), acetic acid and \textit{tert}-butanol\textsuperscript{105} was selected. On dissolving the oxime in chloroform and cooling to <5°C, the \textit{tert}-butyl hypochlorite was added, turning the solution a deep blue. After 45min the colour had turned to green and TLC analysis showed that both \textit{E} and \textit{Z} oximes had reacted and no starting material remained. The resulting hydroximoyl chloride 2.13 was not isolated, but used directly in the cycloaddition reaction.
2.4 Synthesis of the Enaminoester Dipolarophile

The required dipolarophile, ethyl 3-pyrrolidinobut-2-enoate 2.18 (arbitrarily drawn as the E-isomer), was synthesised by dissolving ethyl acetoacetate 2.19 and pyrrolidine 2.20 in toluene and heating at reflux in a flask fitted with a Dean and Stark trap (Scheme 2.17). When no further water separated, the solvent was removed under reduced pressure to give the enamine 2.18 in quantitative yield. A slight excess of pyrrolidine was used to ensure that all of the \( \alpha \)-keto ester was consumed, thus avoiding the problem of having to remove it at the end of the reaction (b.p. 181°C). Care was needed when storing the product as, when left in air, it slowly hydrolyses back to the starting materials. However, the product could be kept for many months without noticeable decomposition, if stored in a freezer in a flask flushed with nitrogen.

2.5 Cycloaddition to form Isoxazole 2.10

Due to the anticipated high reactivity of the nitrile oxide 2.11, it was generated \textit{in situ}. Having formed the hydroximoyl chloride 2.13 in chloroform solution, 2-3 equivalents of the dipolarophile 2.18 were added to the reaction mixture and the solution heated to reflux. Triethylamine was added slowly, causing the loss of HCl from 2.13 and forming the dipole 2.11 (Scheme 2.18). Since the desired isoxazole 2.10 was the product isolated, the dipole
2.11 must then have attacked the enamine, presumably to form an intermediate 2-isoxazoline 2.24 that underwent the anticipated spontaneous elimination of a pyrrolidine molecule. However, no direct evidence for the postulated intermediate 2-isoxazoline 2.24 was obtained.

\[
\begin{align*}
\text{i, Et}_3\text{N, CHCl}_3 & \\
\text{Scheme 2.18}
\end{align*}
\]

Since nitrile oxides are known to dimerise readily, steps were taken to minimise the possibility of this unwanted reaction occurring. By having an excess of the enamine present, the chances of the dipole reacting with it (rather than another molecule of dipole) were enhanced. The slow addition of base meant that only a small amount of the dipole was present in the flask at any one time, again improving the statistical probability of reaction occurring with the enamine. By adding triethylamine over a period of approximately 4-5h, a yield of 65% (from the oxime) has been attained. A slower rate of addition was found not to improve the efficiency of the reaction, whilst adding the base in one portion caused a drop in the yield to around 30-35%.
Only one cycloaddition product was isolated from the reaction, demonstrating the high regioselectivity of the reaction with the enaminoester dipolarophile. This is believed to be the 4-carboxyisoxazole 2.10, based on reported precedent, and on previous research in the group involving the synthesis of pyrroloisoxazolone 2.25 (Fig. 2.5), the structure of which has been confirmed by X-ray crystallography.

A successful synthesis of a ring-containing amino acid derivative had now been completed. However, in order to be of potential use in peptides, it was necessary to see whether it was possible to selectively couple the isoxazole to other amino acids to construct pseudopeptides.

2.6 Coupling to other Amino Acids

In order to couple two amino acids, protecting groups are needed so that unwanted side reactions do not occur. For example, in Scheme 2.19, coupling the two unprotected amino acids could result in four different dipeptides, as the amino group of 2.26 is just as likely to react with the carboxylic acid function of 2.27 as vice versa. Alternatively, either 2.26 or 2.27 could react with another molecule of the same type to form homodimers.
By protecting the amine function of one amino acid and the carboxylic acid group of the other, a clean reaction is ensured with only one possible product (Scheme 2.20).

In addition to suitable protecting groups on the amino acids to be coupled, a so-called “coupling reagent” is required to transform the hydroxyl function into a better leaving group. The classic coupling reagent is dicyclohexylcarbodiimide (DCC) 2.28 which converts the acid into a type of activated ester 2.29 (Scheme 2.21). A disadvantage of DCC is that dicyclohexylurea (DCU) 2.30 is formed as a by-product, which can prove difficult to remove at the end of the reaction.
To circumvent this problem, a variant of DCC was chosen, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC or EDCI) (Fig. 2.6). This has a similar mode of action to DCC, however the by-product formed is water-soluble and so can simply be washed out on work-up.
Before attachment of an amino acid to the nitrogen terminus of the isoxazole 2.10 was possible, the Boc protecting group had to be removed. This was accomplished using a solution of trifluoroacetic acid (TFA) in DCM (1:10 v/v) (Scheme 2.22). After stirring for 2h at room temperature, no starting material was visible on analysis by TLC. Having removed the solvent, the oily product was dissolved and stirred in a solution of 2M hydrochloric acid for 30min. This converted the trifluoroacetate salt into the more easily handled hydrochloride salt 2.31, an off-white solid.

The first coupling reaction attempted was to an N-protected glycine derivative. N-Boc-glycine 2.32 was prepared by reaction of glycine with Boc₂O and a base. The reaction was carried out by dissolving glycine in a mixture of 1,4-dioxane, water and 1M aqueous sodium hydroxide, cooling the solution to 0°C then adding Boc₂O. After stirring for 90min at 0°C and 30min at room temperature, the reaction was worked up with citric acid. Extraction with ethyl acetate gave the crude product, which was purified by
recrystallisation from a mixture of hexane and ethyl acetate, resulting in a 47% yield of the protected amino acid 2.32. This reaction was performed once; thus yields have not been optimised.

The N-protected glycine 2.32 and EDCI were dissolved in anhydrous diethyl ether along with the hydrochloride salt of the deprotected isoxazole 2.31, which remained as a suspension (Scheme 2.22). On cooling to 0°C, triethylamine was added to the reaction mixture to generate the free amine and the mixture was stirred for 16h at room temperature. By the end of the reaction, a white suspension was still present, the isoxazole salt being replaced by triethylamine hydrochloride and the urea by-product from EDCI, both of which are insoluble in ether. Following addition of water to remove the amine hydrochloride and urea by-products, and purification by column chromatography, the dipeptide 2.34 was obtained as a colourless gum in a somewhat disappointing 39% yield; however several repetitions of the reaction gave similar results. A slightly different procedure was adopted involving reaction of the deprotected isoxazole 2.31 and triethylamine in DCM for 10min. Addition of water and separation of the organic phase yielded the free amine. This was added (as a solution in anhydrous DCM) to a solution of N-Boc-glycine and EDCI in anhydrous DCM, which had previously been stirring for 10min. This mixture was stirred for 16h and worked up as in the original procedure. The result was an improvement in the yield to a moderate 57%, still lower than might be expected for this type of reaction.

In order to check whether the stereochemical integrity of the isoxazole had been retained during its synthesis, a coupling to alanine was attempted. Since the isoxazole and its glycine-coupled derivative contain only one chiral centre, any racemisation during the synthetic sequence will produce two enantiomers, which have identical chemical and
physical properties (apart from the sign of their optical rotation) and are indistinguishable by NMR spectroscopy. By introducing a second chiral centre, the possibility arises of different diastereoisomers with different chemical and physical properties. If enantiomerically pure alanine is used, then the presence of diastereoisomers would necessarily be due to a loss of chirality in the isoxazole amino acid unit.

Coupling to form 2.35 was carried out in a similar manner to the synthesis of 2.34, by the slow addition of triethylamine to a mixture of the N-protected amino acid (in this case (S)-N-Boc-alanine 2.33), EDCI and the ethyl ester hydrochloride of the isoxazole 2.31 in anhydrous DCM (Scheme 2.22). Like the protected glycine derivative, 2.33 was prepared by the addition of Boc₂O to an ice-cooled solution of (S)-alanine in 1,4-dioxane, water and 1M aqueous sodium hydroxide. After stirring at 0°C for 90min and room temperature for 30min, the mixture was treated with a solution of potassium hydrogen sulfate. This resulted in a yield of 2.33 of 37%, lower than that obtained in synthesis of the glycine derivative, which employed a citric acid work up. However, as with the synthesis of N-Boc-glycine, the reaction was performed once and is unoptimised.

Reaction of (S)-N-Boc-alanine 2.33, EDCI and the deprotected isoxazole 2.31, followed by treatment with water and purification by column chromatography, realised the product 2.35 as a white solid in 74% yield. Only one product arising from a coupling reaction was obtained from the column chromatography, and analysis by ¹H and ¹³C NMR spectroscopy showed it to be made up of only one compound (Figs. 2.7 and 2.8).
As diastereoisomers can have very similar NMR spectra, the dipeptide was also analysed by reverse-phase high performance liquid chromatography (HPLC) (Fig. 2.9). In addition it was analysed on a Hypercarb column designed for the separation of diastereoisomers. In both cases, there was no sign of more than one product being present, indicating that synthesis of the isoxazole amino acid derivative 2.31 had been completed with no loss of optical activity at any stage.

![Fig. 2.9](image)

2.8 Coupling at the C-Terminus

To attach amino acids to the carboxyl terminus of the isoxazole 2.10, it was first necessary to hydrolyse the ethyl ester to give the carboxylic acid 2.36 (Scheme 2.23). Having experienced problems with incomplete conversion and low yields when using lithium hydroxide, the conditions used to effect hydrolysis were to dissolve both the ester 2.10 and potassium hydroxide in ethanol and heat the solution to reflux for 16h. This was followed by addition of citric acid and extraction with DCM, to give the carboxylic acid 2.36 in 97% yield.
Coupling to a C-protected alanine derivative (to probe the stereochemical purity as previously described) was carried out by the slow addition of triethylamine to an ice-cooled solution of carboxylic acid 2.36, EDCI and (S)-alanine methyl ester hydrochloride 2.38 in anhydrous DCM. Following addition of water and extraction with ethyl acetate, purification by column chromatography yielded what appeared to be a single product on analysis by TLC. However the $^1$H NMR spectrum (Fig. 2.10), whilst corresponding to the desired product 2.40, appeared to show that the product was a mixture of diastereoisomers. In addition to the signals at $\delta$H 4.7, 5.2 and 5.4 ppm, which are due to the CH protons attached to the 2 chiral centres and the NH next to the Boc group, respectively, there are similar shaped signals at 4.9, 5.1 and 5.8 ppm suggesting a compound of similar structure. These signals are unlikely to be caused by one of the unreacted starting materials being present, as the signals for the 5-methyl group of the isoxazole ring and the methyl ester have the same integral value, showing them to be present in a 1:1 ratio. Measurement of the integrals in the NMR spectrum shows that the two diastereoisomers are present in a ratio of approximately 5:1. Other impurities are also present, which were not removable by column chromatography.
Fig. 2.10

The presence of a mixture of diastereoisomers was attributed to the conditions employed for the ester hydrolysis being too severe and racemisation of the isoxazole amino acid occurring. Lithium hydroxide in a water/THF/ethanol solution (1:1:1 v/v/v) was used as a milder alternative, the reaction mixture being stirred at room temperature rather than heated at reflux. The reaction proceeded extremely slowly, and after stirring for 16h approximately one sixth of the starting material remained (as measured by the ratio of integrals of the two 5-CH₃ signals in the ¹H NMR spectrum). As a prolonged reaction time did not achieve complete conversion, the coupling reaction with (S)-alanine methyl ester hydrochloride 2.38 (Scheme 2.23) was carried out using the crude carboxylic acid 2.36. This was accomplished by generating the free amine of alanine methyl ester from the hydrochloride salt using triethylamine, then adding this (as a solution in anhydrous DCM) to an ice-cooled solution prepared by stirring carboxylic acid 2.36 and EDCI in anhydrous DCM for 10min. Only one product, with ¹H and ¹³C NMR spectra corresponding to the
desired dipeptide $2.40$, was isolated from column chromatography, in a yield of 24%.

Analysis of the $^1$H and $^{13}$C spectra (Figs. 2.11 & 2.12) showed that only one diastereoisomer of $2.40$ had been formed, as there are no additional signals in the $^1$H NMR spectrum (cf. Fig. 2.10) and the $^{13}$C NMR spectrum does not indicate the presence of another product. The purity of $2.40$ was confirmed when only one peak was observed on analysis by reverse phase HPLC and on passage through a column of Hypercarb.

![Fig. 2.11]
Coupling to glycine ethyl ester hydrochloride 2.37, to give the dipeptide 2.39 (Scheme 2.23), was also attempted. This was initially carried out by the slow addition of triethylamine to an ice-cooled mixture of the carboxylic acid 2.36, EDCI and glycine ethyl ester hydrochloride 2.37 in anhydrous ether. The method of generating glycine ethyl ester as the free base by treatment of the hydrochloride salt 2.37 with triethylamine, then adding it to a solution prepared by stirring 2.36 and EDCI for 10 min in anhydrous DCM was also tried. Both methods resulted in yields of the final product 2.39 of approximately 40%.

The moderate yields for these reactions suggest that both the amine and acid functions of the isoxazole suffer from a degree of steric hindrance making attack at these centres slightly more difficult than in ordinary amino acids. Since one reason for synthesising these molecules is to inhibit the conformational freedom of the acid and amine groups, this is perhaps not unexpected. In addition, the carboxy group of the isoxazole-4-carboxylates
is deactivated by conjugation to the oxygen atom of the ring; esters for example, may alternatively be regarded as vinylogous carbonates (Fig. 2.13).

![Fig. 2.13](image)

2.9 Further Couplings

In order to demonstrate that there are no limitations on couplings to the isoxazole, the tripeptide 2.41 was prepared by two different routes (Scheme 2.24), namely N-coupling followed by C-coupling (Route A) and C-coupling followed by N-coupling (Route B).

To couple the dipeptide 2.34 to glycine ethyl ester hydrochloride 2.37 (Route A), the ester substituent on the isoxazole was first hydrolysed in a manner similar to that described previously, using lithium hydroxide in a water/THF/ethanol solution (1:1:1 v/v/v). Although one seventh of the starting material 2.34 remained, the crude carboxylic acid was reacted with EDCI in an ice-cooled solution of anhydrous DCM. To this solution was added glycine ethyl ester (as a solution in anhydrous DCM) formed by reaction of glycine ethyl ester hydrochloride 2.37 with triethylamine. The yield for the coupling reaction to produce 2.41 was rather low (28%), and again this was attributed to both the hindered environment of the carboxylic acid group and its deactivation.
For Route B, removal of the Boc group from the amino group of 2.39 was required. Deprotection with TFA did not occur as smoothly as before, with significant loss of starting material through degradation. Therefore, a solution of acetyl chloride in ethanol was used to generate the necessary acidic conditions. Analysis by TLC showed that all of the starting material had reacted after 6h, and the hydrochloride salt of the amine was obtained in quantitative yield. The hydrochloride salt was converted to the free amine using triethylamine, and then added (as a solution in anhydrous DCM) to a solution of $N$-
Boc-glycine 2.32 and EDCI which had been cooled to 0°C. The product 2.41 was obtained in 10% yield, the low value being partly attributed to the small scale of the reaction and therefore mechanical loss of material during work up and purification. Analysis by TLC and $^1$H and $^{13}$C NMR spectroscopy showed the product to be identical to that obtained from reaction of 2.34 and glycine ethyl ester 2.37, described earlier (Route A).

No X-ray crystal structure analysis of the tripeptide 2.41 was possible since attempts to recrystallise the product proved unsuccessful. In order to gain some idea of the possible conformations of the product, molecular modelling studies were undertaken using the modelling program Spartan 4.1, run on a Silicon Graphics Indy workstation. Fig. 2.14 shows the minimum energy geometry obtained from a calculation using the AM1 semi-empirical method when the three amide bonds are locked in the trans conformation. (The alkoxy groups at either end have been replaced with methyl groups in order to simplify the calculation). Whilst not necessarily the lowest energy conformation that is possible, it was the lowest that could be found in these studies. It has a calculated standard enthalpy of formation of $-517\text{kJmol}^{-1}$, between 3 and 37kJmol$^{-1}$ more stable than conformations located from calculations performed on the molecule when the starting geometry was altered (e.g. cis amide bonds).
The model indicates that the presence of the isoxazole ring has restricted conformational flexibility in the peptide, the two ends of the molecule being forced much closer together than might reasonably be expected in a chain of four acyclic amino acids. The distance between the α-carbon atoms of the two glycine residues, which can be considered as the first and fourth residues of the sequence (as the aminoalkylisoxazole unit is a dipeptide mimic), is 5.4 Å. Since a β-turn has been defined as a tetrapeptide sequence in which the αC(1)-αC(4) distance is ≤7 Å, 25 the tripeptide 2.41 does possess some of the characteristics of a β-turn. However, any hydrogen bonding between the first and fourth residues would
seem to be impossible in this conformation as the hydrogen atoms of the amido groups point away from the oxygen atoms of the carbonyl groups. For intramolecular hydrogen bonding to occur they would need to point inwards. Another feature of the molecule that is apparent from the model is the planar nature of the isoxazole ring, arising from its aromaticity.

2.10 An Alternative Target

\[
\begin{align*}
\text{NH} & \quad \text{Boc} \\
\text{H} & \quad \text{CO}_2\text{Et}
\end{align*}
\]

\[2.42\]

\[
\begin{align*}
\text{NH} & \quad \text{Boc} \\
\text{C} & \quad \text{O}
\end{align*}
\]

\[2.43\]

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{C} & \quad \text{O}
\end{align*}
\]

\[2.44\]

Scheme 2.25

Attempts were also made to synthesise the 5-aminoalkylisoxazole 2.42, which carries the same substituents as 2.10 but with the groups at the 3- and 5-positions reversed. This effectively exchanges the nitrogen and oxygen atoms in the ring, which, it was speculated, may alter the charge density distribution around the ring. A retrosynthetic analysis using the cycloaddition of a nitrile oxide is shown in Scheme 2.25.

The dipole for the proposed cycloaddition 2.44 was to be generated by the dehydration of nitroethane. A test reaction to form 2.45 (Scheme 2.26), which was carried out by slow addition of nitroethane (to minimise nitrile oxide dimerisation) to a solution of methyl acrylate, Boc₂O and DMAP in hexane and acetonitrile (4:1 v/v), resulted in an 86% yield of the desired product 2.45. This showed that the combination of DMAP and Boc₂O as used by Basel and Hassner was an effective dehydrating agent.
Previous work\textsuperscript{106} has found that enamines such as 2.43 cannot be synthesised by reaction of pyrrolidine with the corresponding $\beta$-keto ester if the carbon atom adjacent to the ketone carbonyl group is a branch point, so a modified approach was required. The envisaged route to the dipolarophile was as depicted in Scheme 2.27. The pyrrolidine enamine 2.43 would be prepared from the alkyne 2.46, a step taken to enhance the regioselectivity as mentioned earlier. The alkyne would be derived ultimately from the $\alpha$-aminoaldehyde 2.15 used previously, and would be prepared \textit{via} the dibrominated alkene 2.47 using the Corey-Fuchs reaction.\textsuperscript{107}
2.11 Attempts to Synthesise the Dipolarophile

By adding the aldehyde 2.15 to an ice-cooled mixture of carbon tetrabromide, zinc and triphenylphosphine and stirring at room temperature for 24h\(^{108}\), the alkene 2.47 was produced in a 50% yield (Scheme 2.28). However, subsequent dehydrobromination and bromine-lithium exchange using butyl lithium, followed by acylation with ethyl chloroformate to form the alkyne 2.46, proved a somewhat capricious reaction despite reports in the literature of it being successfully carried out in excellent yields.\(^{109}\) On some occasions, yields of 58% were obtained, although 30-35% was more usual and quite often none of the alkyne at all was recovered. Attempts to add pyrrolidine across the triple bond by heating the amine and alkyne 2.46 in ethanol\(^{110}\) also proved difficult with complete conversion to the enamine 2.43 proving elusive. Since no purification was possible, due to the susceptibility of the enamine to hydrolysis, the cycloaddition was attempted on the mixture of enamine and alkyne. This was carried out in a similar manner to the test
reaction, by addition of nitroethane to a solution of enamine 2.43, Boc₂O and DMAP in hexane and acetonitrile (4:1 v/v). Approximately 17% of the isoxazole 2.42 was isolated from the reaction mixture, however it contained significant impurities which could not be removed by column chromatography.

Due to the problems encountered in forming the enamine 2.43, a cycloaddition between nitroethane and the alkyne 2.46 was attempted (Scheme 2.28), again by slow addition of nitroethane to a mixture of the alkyne, Boc₂O and DMAP in a hexane/acetonitrile (4:1 v/v) solution. However, no cycloadduct was isolated.

As a more accessible alternative to the enamine 2.43, the tert-butyldimethylsilyl (TBDMS) enol ether 2.52 (arbitrarily drawn as the E-isomer) was prepared as shown in Scheme 2.29.
The β-keto ester 2.51 was synthesised according to a procedure reported by Pollet and Gelin and used by a previous worker in the group. Ethyl hydrogen malonate 2.49 and iso-propylmagnesium bromide (formed by treatment of 2-bromopropane with magnesium in anhydrous THF) were stirred at room temperature for 90 min, then at 40°C for 30 min in anhydrous THF to give magnesium enolate 2.50. On cooling to 0°C, a solution of the activated ester 2.48 in anhydrous THF, prepared by reaction between N-Boc-alanine and 1,1'-carbonyldiimidazole at room temperature in anhydrous THF, was added and the mixture stirred for 16 h at room temperature. The resultant β-keto ester 2.51 was converted to the silyl enol ether 2.52 with sodium hydride and TBDMS chloride. However, the attempted cycloaddition, performed by the addition of nitroethane to a solution of silyl enol ether 2.52, Boc₂O and DMAP in a hexane/acetonitrile (4:1 v/v) failed, with 48% of the β-keto ester 2.51 being recovered.

At this point, efforts to synthesise the 5-aminoalkylisoxazole 2.42 were abandoned. It was decided that the final product would be unlikely to have a significantly different overall shape or electron distribution to the 3-aminoalkyl isoxazole 2.10 already made, and to spend more time attempting to overcome the synthetic difficulties would not be worthwhile.

2.12 Conclusions

A protected amino acid containing a heterocyclic ring 2.10 has been successfully synthesised, no loss of optical activity in the substituent at the 3-position of the ring being observed. It has been shown that it is possible to selectively couple other amino acids at either the C- or N-terminus of the molecule, although the yields of these couplings are often low. This is presumably due to steric hindrance and, in coupling to the C-terminus,
deactivation of the acid group through conjugation with the ring. On attachment of an amino acid to either end of the heterocyclic molecule, a peptide chain was formed with the isoxazole ring incorporated into the peptide backbone. Molecular modelling suggested that the presence of the isoxazole ring significantly restricted the conformational flexibility of the backbone.
Chapter 3:

Results and Discussion -
Tetrahydropyrazoles
3.1 Introduction

Based upon the work with isoxazoles, efforts were next concentrated towards the synthesis of an alternative heterocyclic ring system bearing amine and acid functions. Replacing the oxygen atom of the isoxazole ring with a nitrogen atom produces pyrazoles 3.1 (Fig. 3.1), the saturated derivatives of which are pyrazolidines (tetrahydropyrazoles) 3.2. It is with these saturated versions, which have the potential to bear three chiral centres, at the 3-, 4- and 5- positions, that this work is concerned.

Like isoxazoles, pyrazolidines can be formed by a 1,3-dipolar cycloaddition. The required dipole for such a reaction is an azomethine imine 3.3, which adds to a multiply bonded molecule 3.4 (Scheme 3.1). Highly functionalised molecules can be constructed in this way, although many of the reported examples of azomethine imine cycloadditions have been intramolecular in order to gain some control over the stereo- and regiochemistry of the product.114-116
The approach that we adopted was the addition of a hydrazine 3.5 to an aldehyde 3.6, as developed by Oppolzer (Scheme 3.1).\textsuperscript{117} Reaction of the aldehyde 3.6 with the hydrazine 3.5 in toluene heated to reflux in a flask fitted with a Dean-Stark trap, results in formation of an intermediate aminol 3.7, that loses the elements of water in a 1,3-elimination to give the dipole 3.3 (Scheme 3.2).

Although virtually any aldehyde can be used in the reaction, certain restrictions are placed on the nature of the nitrogen substituents of the hydrazine. For example, R\textsuperscript{2} is usually an alkyl or aryl group; it cannot be a hydrogen atom otherwise elimination across the C-N bond to form a hydrazone will occur. R\textsuperscript{3}, however, should be an electron-withdrawing group in order to stabilise the negative charge on the adjacent nitrogen atom.
3.2 Initial Work

Before attempting to synthesise amino acids containing a tetrahydropyrazole ring, a test reaction was attempted to ensure the viability of the cycloaddition. Our initial target was 3.8 (Scheme 3.3), to be formed by cycloaddition of the azomethine imine 3.9 with methyl acrylate. This dipolarophile was chosen because it contains the ester group required for the cycloadditions that will be tried later to form amino acid analogues.

The dipole was to be formed from reaction between 2-methylpropanal and \(N^1\)-acetyl-\(N^2\)-methylhydrazine 3.10. This hydrazine has the simplest alkyl group possible (methyl) at one nitrogen atom, and acetyl, a simple electron-withdrawing group, at the other. The aldehyde 2-methylpropanal was chosen as it possesses a similar branched geometry at the \(\alpha\)-carbon to the amino aldehyde 2.15 used in the isoxazole work. If this cycloaddition were found to be successful, then 2-methylpropanal could be replaced by aminoaldehyde 2.15, and a pyrazolidine with both acid and amine functions could be synthesised.

Though both the dipolarophile and aldehyde are commercially available, the accessibility of unsymmetrical 1,2-disubstituted hydrazines is more problematic. Simple targets, such as 3.10 can be prepared by reaction of methylhydrazine with an ester,\(^{118}\) giving
predominantly the 1,2-disubstituted product. Unfortunately the reaction suffers from a number of problems and limitations. The first of these is that in addition to the 1,2-disubstituted hydrazine 3.11, a significant amount of the 1,1-disubstituted product 3.12 is also formed (Scheme 3.4).

If the source of the electron-withdrawing group is more reactive than an ester, then the 1,1-disubstituted hydrazine is the exclusive product. This restricts the choice of groups that can be attached to the unsubstituted nitrogen centre using this reaction. This tendency for reaction to take place more readily at the substituted nitrogen atom is perhaps not surprising as the nitrogen centre to which the methyl group is attached is the more nucleophilic.

Another problem is that the reaction is extremely slow, taking several days to go to completion. Despite these drawbacks, acetylation of methylhydrazine with ethyl acetate was chosen as the method to prepare 3.10 since it was the most convenient preparation available. The low yields returned were not considered to be especially significant due to the low cost of the starting materials.
The reaction was performed in ethanol, heated to reflux for 72h under an atmosphere of nitrogen (Scheme 3.5). No effort was made to purify the ethyl acetate, as the most likely contaminants, namely ethanol, water and acetic acid, have all been found to catalyse the reaction.\textsuperscript{119} After heating, the solvent was removed under reduced pressure and the resultant viscous liquid purified by distillation to give a colourless oil which solidified when stored in a freezer. Analysis by NMR spectroscopy and comparison of the chemical shifts obtained with published values\textsuperscript{119} showed the product to be predominantly the desired 1,2-disubstituted hydrazine with traces of the 1,1-product also present. On allowing the product to warm to room temperature, part of it melted and some remained as a solid. Removal of the liquid component of the mixture by filtration gave a white solid which proved to be pure $N^1$-acetyl-$N^2$-methylhydrazine 3.10, formed in 49% yield. Care had to be taken at all stages of the purification to keep the product under an inert atmosphere, as contact with air for even a few minutes resulted in decomposition, noticeable by the product gradually turning yellow.

\begin{align*}
\text{H}_3\text{C}-\text{N}=\text{N}-\text{CH}_3 \quad &\rightarrow \quad \text{H}_3\text{C}-\text{N}^+\text{N}^-\text{CH}_3 \\
\text{3.10} &\quad + \\
\text{H}_3\text{C} &\rightarrow \quad \text{H}_3\text{C} \\
\text{CH}_3 &\rightarrow \quad \text{CO}_2\text{CH}_3
\end{align*}

Scheme 3.6

\text{CO}_2\text{CH}_3

i, toluene, reflux, 2h; ii, $\text{CO}_2\text{CH}_3$
With the hydrazine to hand, an attempt was made at the cycloaddition (Scheme 3.6). The aldehyde, 2-methylpropanal and N\textsuperscript{1}-acetyl-N\textsuperscript{2}-methylhydrazine 3.10 were dissolved in toluene and heated together to reflux in a flask fitted with a Dean and Stark trap to remove the water released by dipole formation. After two hours, methyl acrylate was added and the solution heated at reflux for a further 16h. Initially, the dipolarophile was not added earlier in order to reduce the possibility of it reacting with the hydrazine in a competing Michael addition (Scheme 3.7). However, this proved to be an unnecessary precaution as a subsequent experiment where all 3 reagents were added at once produced virtually the same yields and ratios of products.

![Scheme 3.7](image)

On removal of the solvent, and purification by column chromatography, two products were obtained, whose \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were both consistent with the desired cycloadducts. The first of these was a single compound, obtained in 25% yield, which, when studied using 2-dimensional NMR spectroscopy, was assigned as the 4-methoxycarbonylpyrazolidine 3.13. This conclusion was based on the couplings between the protons attached to the ring. A \textsuperscript{1}H-\textsuperscript{13}C correlation spectrum was used to ascertain that the protons with chemical shifts of 3.6 and 4.1ppm were attached to the same carbon atom and hence were those of the methylene group (Fig. 3.2).
The protons at approximately 3.0 and 3.7 ppm (the latter signal is in the same region as that of the ester methyl group and so determination of its exact chemical shift is difficult) were both bonded to carbon atoms that a DEPT spectrum showed to be tertiary. These protons are therefore those attached to the heterocyclic ring carbon atoms that carry the isopropyl and ester substituents. The $^1$H-$^1$H correlation (COSY) spectrum (Fig. 3.3) shows that these two protons couple to each other (although it was not possible to determine the coupling constant), something that would only be possible if they were on adjacent carbon atoms. This means that the isopropyl and ester substituents are also on adjacent carbon atoms, and the methyl ester can therefore be assigned to the 4-position of the ring, meaning that the structure of the cycloaddition product is 3.13. No coupling of the CH of the isopropyl group to the adjacent ring CH was observed in the COSY NMR spectrum. This is presumably due to the dihedral angle between the two protons being close to 90°, which, according to the Karplus relationship should result in no visible coupling.
The other cycloadduct was a mixture of two compounds in a 5:4 ratio - calculated from the integrals of the $^1$H NMR spectrum - that together accounted for a further 25% yield. These were separated by normal-phase preparative HPLC, although significant amounts of the product were lost during purification. The major component, when analysed by NMR spectroscopy was also attributed as a 4-methoxycarbonyl product 3.13. A $^1$H-$^{13}$C correlation spectrum showed that the protons at 3.68 and 4.37 ppm were those of the methylene group (Fig. 3.4).
Again the signals at 2.94 and 3.05ppm, the other two protons attached to the ring and found to be methine CHs, showed coupling to each other on analysis of the COSY NMR spectrum (Fig. 3.5). (The magnitude of the coupling constant could not be assigned, although the proton attached to the same carbon of the ring as the isopropyl group appears as a doublet of doublets with $J$ values of 3.9 and 8.8Hz).

The 4-substituted product is therefore present as 2 different stereoisomers, one having the alkyl group and methyl ester in a syn arrangement, the other as the anti. It was not possible to unambiguously determine the relative stereochemistries, however based on results found in later reactions with benzaldehyde (Section 3.6), the product which did not require purification beyond simple column chromatography will be tentatively assigned as the syn isomer 3.16 (Fig. 3.6) and the other, which was further purified by HPLC, as the anti 3.17.

![Fig. 3.6](image)

The minor isomer isolated from the preparative HPLC column was obtained in insufficient quantities to properly analyse by NMR spectroscopy, however $^1$H and $^{13}$C NMR spectra were recorded along with a mass spectrum. This showed that the product had the same relative molecular mass as 3.16 and 3.17 and that the product was a mixture of diastereoisomers and/or rotational isomers which would arise from restricted rotation about the amide bond of the $N$-acetyl group. It was therefore tentatively assumed to be the 5-methoxycarbonylpyrazolidine 3.14.
The observed preference for formation of the 4-carboxy pyrazolidines over the 5-substituted product is a contrast to that reported by Oppolzer who, on reaction of ethyl acrylate with the 1,2-disubstituted hydrazine 3.18 and formaldehyde (Scheme 3.8), isolated 65% of the 5-ethoxycarbonyl pyrazolidine 3.19 and 7.6% of the 4-substituted product 3.20.\textsuperscript{117}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\chemfig{H3C-N-N-H + \quad \text{i} \quad -H_2O \quad \rightarrow \quad \text{ii} \quad H3C-N-N-H}};
\end{tikzpicture}
\end{center}

i, toluene, reflux; ii, CO\textsubscript{2}Et, 2-4h.

\textbf{Scheme 3.8}

However, other reports of cycloadditions involving azomethine imines, where one nitrogen atom bears an alkyl group and the other an electron-withdrawing group, have found that they react with acrylate esters to give predominantly the 4-substituted product. Huisgen and co-workers found that reaction of the azomethine imine 3.21 with ethyl acrylate gave the 4-carboxy product 3.22 in 85% yield (Scheme 3.9).\textsuperscript{120}
More recently, Husson and colleagues used the cyclic hydrazine 3.23, which on undergoing a cycloaddition with benzaldehyde and methyl acrylate resulted in a 74% yield of the 4-methoxycarbonylpyrazolidine 3.24 (Scheme 3.10).\textsuperscript{121}

Also isolated from the reaction mixture was a product whose NMR and mass spectra correspond to the Michael addition product 3.15 mentioned earlier. This material accounted for a further 46% of the hydrazine used in the reaction. Interestingly, this value dropped to 9% when all three reagents were added at once, each of the two fractions containing cycloadducts still being obtained in yields of around 20%.
To summarise, the test reaction resulted in formation of both syn and anti diastereoisomers of the 4-methoxycarbonylpyrazolidine 3.13. One, believed to be the syn product 3.16, was obtained in 25% yield whilst the other, presumed to be the anti isomer 3.17, required purification by HPLC and was isolated in an 11% yield. The 5-methoxycarbonylpyrazolidine 3.14 was also isolated from the reaction mixture (1%), possibly as a mixture of syn and anti diastereoisomers. Most of the hydrazine that did not react to form a pyrazolidine was found to have undergone a Michael addition reaction with methyl acrylate to form 3.15 (Scheme 3.7).

3.3 Mechanism of Dipole Formation

\[
\begin{align*}
\text{Ph} & \quad \text{N} \quad \text{N} \quad \text{O} \\
\text{CH}_3 & \quad \text{N} \quad \text{H} \quad \text{H} \\
3.25 & + \\
\text{O} & \quad \text{H} \\
\text{CH}_3 & \quad \text{O} \\
3.26 & \\
\end{align*}
\]

\[
\begin{align*}
i & , \text{toluene, 110°C, molecular sieve} \\
3.27 & \\
3.28 & \\
\end{align*}
\]

Scheme 3.11

In order to gain some understanding of the mechanism of the cycloaddition, or more specifically the dipole formation, the progress of the reaction was monitored by \(^1\)H NMR spectroscopy. 2-Methylpropanal and \(N^1\)-acetyl-\(N^2\)-benzylhydrazine 3.25 (for synthesis see
section 3.10) were both dissolved in fully deuterated toluene and mixed in an NMR tube along with 4Å molecular sieves (Scheme 3.11). A spectrum recorded approximately 5 minutes after the two components were added to each other showed that in addition to the two starting materials, another product had been formed that corresponded to the aminol 3.26. This was indicated by the presence of a second acetyl group CH₃ resonance and the formation of two new doublets at chemical shifts of less than 1ppm in addition to the doublet from the 2-methylpropanal (Fig. 3.7).

These are caused by the methyl groups of the isopropyl substituent which, unlike in the starting aldehyde, are non-equivalent in the aminol 3.26 due to the presence of a chiral centre in the molecule. Finally, the signal from the methylene group of the hydrazine fragment, which is a singlet with a chemical shift of 3.81ppm in the starting material 3.25, appears as two doublets at 3.57 and 4.01ppm, since in the aminol the two protons are
diastereotopic. This is another feature arising from the presence of a chiral centre in the molecule.

It is worth noting here that no signals corresponding to the enamine 3.28 were observed. This would be apparent by the methyl groups originating from the aldehyde appearing as two singlets and the appearance of a signal due to the proton attached to the C=N double bond.

Following this initial analysis, the sealed NMR tube and its contents were then heated at 110°C in an oil bath. At various intervals the tube was removed from the bath and a 1H NMR spectrum again recorded. These spectra showed that even after heating for 72h the ratio of the starting materials to aminol did not change.

A second experiment was carried out, this time with two equivalents of methyl acrylate added to the tube. Analysis by 1H NMR spectroscopy immediately after addition of the reactants showed only a trace of the aminol in the mixture, the presence of the dipolarophile seeming to inhibit its formation (Fig. 3.8(a)).

Again the sample was heated at 110°C and analysed at regular intervals. After 21h (Fig. 3.8(b)) peaks at around 3.2ppm, that are characteristic of the cyclic product 3.29 (Scheme 3.12), became evident. The disappearance of the singlet at 3.85ppm from the methylene group in the hydrazine indicated the progress of the reaction and after 72h of heating the reaction was adjudged to have gone to completion (Fig. 3.8(c)) as the integral underneath this singlet remained unchanged on further heating.
Fig. 3.8
These two experiments suggest that the aldehyde and the hydrazine 3.25 exist in equilibrium with the aminol 3.26 (Scheme 3.12) and that this is itself in equilibrium with the dipole 3.27, with very little dipole present under normal circumstances. By removing the water that is formed and having a dipolarophile present that removes the dipole 3.27 from the system, it is possible to force the equilibria towards the products.

This hypothesis is consistent with the work of Huisgen\textsuperscript{122} who also assumed the presence of an equilibrium between starting materials, an aminol and a dipole which could be intercepted by a dipolarophile (Scheme 3.13).
3.4 Reactions with Functionalised Aldehydes

Having successfully carried out the test reaction, the next step was to try and introduce an amine function into the reactants in order to create an amino acid. The planned strategy for achieving this was to use an aldehyde bearing a nitrogen atom (Scheme 3.14), a suitable
example being the $\alpha$-aminoaldehyde 2.15 used in the isoxazole work. This would afford a 3-(1-aminoalkyl)-4-carboxyprazolidine 3.30.

However, on heating the aldehyde 2.15, the hydrazine 3.10 and methyl acrylate to reflux in toluene with the removal of water, none of the desired product was obtained. Instead, two products were visible by TLC, one of which proved to be an inseparable mixture of products that we were unable to further identify. The other was a mixture of two compounds, probably either stereoisomers or rotational isomers caused by restricted rotation about the amide bond of the $N$-acetyl group. Purification by HPLC isolated one of the products from this mixture, and its $^1$H NMR spectrum indicated that the product contained elements of both the aldehyde and hydrazine, but no methyl ester signal from the dipolarophile was apparent. In addition to signals for the $N$-methyl, $N$-acetyl and tert-butyl groups were a doublet and a multiplet with similar chemical shifts to the CH$_3$CH fragment of the aldehyde 2.15, suggesting that this part of the molecule had remained intact. The only other signals were an NH (7.0ppm) and a broad signal (5.4ppm) caused by a CH. The oxazoline 3.31 is consistent with this spectrum and the EI mass spectrum contained a peak corresponding to the protonated molecular ion together with peaks corresponding to the loss of acetyl, $^1$Bu and $^1$BuO fragments. The product was isolated in 27% yield, assuming that it is the oxazoline 3.31.

![Scheme 3.15](image-url)
The probable mechanism of formation of this product is shown in Scheme 3.15. The hydrazine 3.10 and aldehyde 2.15 react together to form an intermediate hydrazinium salt that, instead of deprotonating to form the dipole and reacting with the dipolarophile, loses the alternative carbamate NH proton and undergoes a cyclisation. Intramolecular reactions are generally faster than intermolecular ones, and the cycloaddition is known to proceed relatively slowly (Section 3.3), so the oxazoline 3.31 is formed apparently in preference to the desired pyrazolidine 3.30. To overcome this problem, an alternative protecting group was sought. The use of a benzyl group instead of Boc should prevent the cyclisation occurring since the carbonyl group that is necessary for the unwanted side-reaction is no longer present. Attaching two benzyl groups to the nitrogen atom means that the required aldehyde is \(N,N\)-dibenzyllalaninal 3.32, which has been synthesised previously\(^\text{123}\), so its preparation was preceded (Scheme 3.16).

\[
\begin{align*}
\text{3.33} & \xrightarrow{i} \text{3.34} & \xrightarrow{\text{ii}} \text{3.35} \\
\text{3.32} & \\
\end{align*}
\]

\(i, \text{BnBr, } K_2\text{CO}_3; ii, \text{LiAlH}_4; iii, (\text{CH}_3)_2\text{SO}, (\text{COCl})_2, \text{Et}_3\text{N}\)

Scheme 3.16
By converting the carboxylic acid group of S-alanine 3.33 to a benzyl ester, the desired protecting groups could be attached in one step. Tribenzylation was carried out using benzyl bromide and potassium carbonate, the reaction being performed in ethanol. As with the conversion of alanine to its methyl ester (Chapter 2), the reaction proceeded extremely slowly despite the use of an alkyl halide instead of an alcohol. After stirring for 48h at 20°C, water was added to the reaction mixture and the solution extracted with ethyl acetate, the addition of sodium chloride being necessary to separate the aqueous and organic layers. The organic layers were washed with a 1M solution of sodium hydroxide and the crude product purified by column chromatography to give the N,N-diprotected benzyl ester 3.34 in 38% yield. Unreacted amino acid, which would have been lost in the aqueous layer during work up, partly explains this low yield. The reaction was not attempted again, so no efforts were made to secure an improvement on it. However, since protecting groups such as benzyl are usually attached and removed with high yields (it is for their ease of manipulation that they are chosen), a more efficient conversion should certainly be possible.

The ester was then reduced to the alcohol 3.35 in anhydrous THF at -60°C using 15 molar equivalents of lithium aluminium hydride. After 1h, the reaction was quenched with a saturated solution of ammonium chloride in water and THF. Column chromatography realised the product in an unoptimised 65% yield. Oxidation of the alcohol 3.35 to the aldehyde was performed using DMSO, oxalyl chloride and triethylamine (the Swern reaction) and resulted in an 83% yield of the aldehyde 3.32. Since this was found to decompose at room temperature within 4 days, it was used immediately in a cycloaddition reaction (Scheme 3.17).
Again the attempted cycloaddition was carried out by dissolving all three reactants in toluene and heating at reflux for 16h with a Dean and Stark trap for the removal of water. Once more, upon solvent removal and purification, none of the desired product 3.37 was isolated. The only isolable product, formed in 6.5% yield, had a $^1$H NMR spectrum consistent with the 2,3-dihydro-1,3,4-oxadiazole 3.38.

This is formed by a cyclisation similar to that shown in Scheme 3.15 that resulted in formation of the oxazoline 3.31. However, this time it is the N-acetyl group which loses a proton and attacks the C=N double bond. The fact that this type of cyclisation is not observed in cycloadditions with 2-methylpropanal (or benzaldehyde - see section 3.6) suggests that its occurrence is due to the presence of an electronegative group in a $\beta$-position to the carbon atom of the C=N double bond. This could destabilise the dipole sufficiently to cause reaction with the oxygen atom of the acetyl group, resulting in
formation of 3.38 via the mechanism shown in Scheme 3.17, in preference to a cycloaddition with methyl acrylate to give the pyrazolidine 3.37.

The failure of the nitrogen-bearing aldehydes 2.15 and 3.32 led to cycloadditions being attempted with aldehydes bearing a functional group that could be converted subsequently to an amine (Scheme 3.18). Reactions of both 2-oxopropanal, 3.39, and ethyl glyoxalate, 3.40, were tried with hydrazine 3.10 and methyl acrylate in toluene at reflux but did not yield any cycloaddition products. The only product we were able to identify in each case was the Michael addition product 3.15 (Scheme 3.7), isolated in yields of 18 and 10% respectively.

\[
\begin{aligned}
&\text{H}_3\text{C}-\text{N}-\text{N}\text{H} \text{CH}_3 \\
&\quad + \\
&\quad \text{R}\text{CHO} \\
\end{aligned}
\]

\[\text{R} = \text{CH}_3, 3.39 \]
\[\text{R} = \text{OCH}_2\text{CH}_3, 3.40\]

Scheme 3.18

2-Benzylxypropanal 3.41 has an ether function α- to the aldehyde carbonyl group which has the potential to be converted to an amine. This aldehyde was formed in 36% yield (unoptimised) by the O-benzylation of ethyl lactate 3.42 with sodium hydride and benzyl bromide. Subsequent reduction of the ethyl ester 3.43 using DIBAL at low temperature (-50°C) furnished the aldehyde 3.41 in 25% yield (unoptimised) (Scheme 3.19).
However, reaction with the hydrazine 3.10 and methyl acrylate in toluene heated to reflux failed to produce any of the desired cycloadduct 3.44. The only product that could be isolated from the reaction (in a yield of 11%) had a $^1$H NMR spectrum consistent with the 2,3-dihydro-1,3,4-oxadiazole shown in Fig. 3.9, which has a structure analogous to 3.38.
3.5 A Different Approach to Dipole Formation

An alternative way to generate 1,3-dipoles is via the thermally or chemically induced 1,2-shift of a proton. This process is known as prototropy and has been used in a number of syntheses of heterocyclic molecules.\textsuperscript{126} For example, the approach has been used by Grigg in the formation of azomethine imines from the hydrazones of aromatic aldehydes and ketones, tautomerisation taking place during heating of the hydrazone in xylene at 150°C (Scheme 3.20).\textsuperscript{127}

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme320.png}
\end{center}

**Scheme 3.20**

Attempts were made to carry out a cycloaddition with the hydrazone 3.45, derived from 2-methylpropanal and acetyl hydrazide (Scheme 3.21). This should form the dipole 3.46 analogous to 3.9 (Scheme 3.3), the only difference being the absence of the N-methyl group. Hydrazone 3.45 was synthesised by heating the two reactants in toluene at reflux in a flask fitted with a Dean and Stark trap to remove the water that is generated during the
reaction. After 2h, the solvent was removed to give the desired product in a quantitative yield.

\[
\begin{align*}
\text{H}_3\text{C}-\text{N} = \text{N}-\text{H} + \text{CH}_3\text{C} = \text{O} &\rightarrow \text{N} = \text{N}-\text{CCH}_3 \\
3.45 &\rightarrow \\
\text{H}_3\text{C} - \text{N} = \text{C}(\text{CO}_2\text{CH}_3)_2 &\rightarrow \\
3.46
\end{align*}
\]

i, toluene, reflux, Dean and Stark trap, 16h; ii, xylene, reflux, 48h

Scheme 3.21

The hydrazone was then heated with methyl acrylate in degassed xylene at 150°C for 48h. However, on removal under reduced pressure of the solvent and any unreacted dipolarophile that remained, the only material that was recovered was the starting hydrazone 3.45.

Work has also been carried out by Kanemasa, Tsuge and colleagues on the use of hydrazones derived from aryl aldehydes as synthetic equivalents to azomethine imines. They found that on heating hydrazone 3.47 in toluene at reflux with methyl acrylate no
reaction took place. However, the use of \( N-(p\text{-tolyl})\)maleimide as the dipolarophile resulted in a 39% yield of the cycloadduct 3.48 (Scheme 3.22).

As this shows maleimide to be more reactive towards azomethine imines than acrylates, attempts were made to react a hydrazone with \( N\text{-methylmaleimide} \).

It should be mentioned that Kanemasa and Tsuge also carried out a cycloaddition with benzaldehyde, ethyl 3-benzyl carbazate 3.49 and \( N-(p\text{-tolyl})\)maleimide in order to compare the different methods of dipole generation (Scheme 3.23). They found in this case that a quantitative yield of the dipole was generated and a 73:27 mixture of endo:exo isomers of 3.50 was obtained.
In place of 2-methylpropanal acetylhydrazone 3.45, the experiment was attempted using the 4-butoxycarbonylhydrazone 3.51 (Scheme 3.24) since this bears closer resemblance to the hydrazone used by Kanemasa and Tsuge. This was prepared by the reaction of 4-butyloxycarbamate and 2-methylpropanal in toluene at reflux with the removal of water by means of a Dean and Stark trap. However, as before, heating the hydrazone 3.51 in xylene at reflux for 48h in the presence of N-methyl maleimide as dipolarophile did not yield any cycloadduct 3.52, the only identifiable products being the starting materials.
Clearly the generation of dipoles from hydrazones derived from aliphatic aldehydes is not possible by the action of heat alone. It has been found, again by Grigg and co-workers, that Lewis acids will assist the generation of the related azomethine ylide dipoles from arylidene imines of α-amino esters, due to the formation of a metallo-dipole (Scheme 3.25).
Further development of this methodology\textsuperscript{130} led to the addition of a base as well as the Lewis acid which, as might be expected from the set of equilibria outlined in Scheme 3.25, promotes the conversion of 3.54 to 3.55. Silver acetate was found to give the highest yields of cycloadducts (lithium bromide predominantly giving rise to the product of Michael addition to the dipolarophile), whilst the base of choice was triethylamine. On extending this approach to imines derived from aliphatic aldehydes,\textsuperscript{131} it was discovered that in addition to catalysing the cycloaddition process, the combination of silver acetate and triethylamine also suppresses the imine-enamine tautomerism (Scheme 3.26), which takes place in imines of aliphatic aldehydes bearing an α-hydrogen atom.

\[ \text{Scheme 3.26} \]

It was hoped that this principle could be extended to the hydrazone 3.45, which would then also form a metallo-dipole 3.56, as shown in Scheme 3.27 (cf. 3.53).
Thus 2-methylpropanal acetylhydrazone and methyl acrylate (2 equivalents) were heated, along with silver acetate (1.5 equivalents) and triethylamine (1 equivalent) in dry toluene. The reaction was carried out at room temperature and at 80°C, but in neither case was any product of a cycloaddition observed, starting material 3.45 being recovered. Reactions using 2-methylpropanal tert-butoxycarbonylhydrazone 3.51 gave a similar result.

3.6 Cycloadditions with Benzaldehyde

Having thus far only completed a successful cycloaddition with 2-methylpropanal, the tolerance of the reaction to other non-functionalised aldehydes was tested. Benzaldehyde was chosen as a suitable example, as it has been used successfully in azomethine imine cycloadditions by others. On reaction of benzaldehyde with a molar equivalent of $N^1$-acetyl-$N^2$-methylhydrazine 3.10 and 1.5 equivalents of methyl acrylate in toluene, heated at reflux for 72h in a flask fitted with a Dean and Stark trap, several products were obtained (Scheme 3.28).
Of these, **3.57** is believed to arise from dimerisation of the dipole to give the hexahydrotetrazine in 27% yield. The $^1$H NMR spectrum contains peaks due to the $N$-acetyl group, the $N$-methyl group, and the phenyl group. The only other peak, with the same chemical shift as two of the aromatic hydrogen atoms, was attributable to a CH group. An accurate mass measurement showed the molecular ion to have a mass consistent with that required for **3.57**. The formation of such products is not unknown; Oppolzer has reported that reaction of formaldehyde and $N^1$-methyl-$N^2$-phenylacetylhydrazine without a large excess of dipolarophile resulted in a significant quantity (74%) of the dimer **3.61** (Scheme 3.29).

**Scheme 3.28**

3.10 + \( \text{O} \) \( \text{Ph} \) \( \text{H} \) \( \text{H} \) \( \text{C} \) \( \text{O} \)

i, toluene, reflux, Dean and Stark trap

![Scheme 3.28](image-url)
Dorn also reported isolating "thermal dimers" as by-products of cycloaddition in his study of reactions between highly polar species,\textsuperscript{132} while Huisgen used the dimeric form of an azomethine imine as a means to store it, the molecule reverting to the dipole on gentle heating.\textsuperscript{122}

A second product, 3.15, (33\%) can be attributed to a Michael addition between the hydrazine and methyl acrylate, as observed in the earlier reaction with 2-methylpropanal (Scheme 3.7).

Finally, two products consistent with a cycloaddition reaction were isolated. One of these (22\%) was itself a mixture of two compounds, present in approximately a 9:2 ratio as measured by the integrals of the $^1$H NMR spectrum. Although apparently pure when analysed by TLC, an analytical-scale HPLC column confirmed the presence of two products. Some of the major isomer was isolated by preparative HPLC and assigned as a 4-methoxycarbonylpyrazolidine isomer on the basis of the $^1$H NMR and mass spectra. Unfortunately none of the minor product could be separated from the mixture. The regiochemistry was determined by 2-dimensional NMR studies. $^1$H-$^{13}$C NMR
spectroscopy showed that the ring protons with chemical shifts of 3.86 and 4.43ppm were part of the methylene group (Fig. 3.10).

Consequently, the protons at 3.45 and 4.47ppm must be attached to the carbon atoms in the ring that bear the substituents, a conclusion supported by a DEPT spectrum showing these carbon atoms to be tertiary. A COSY NMR spectrum (Fig. 3.11) showed that these two protons at the substituted positions on the ring couple to each other (\(J = 5.9\text{Hz}\)). This means that they must be attached to adjacent ring carbon atoms and so the product carries the methyl ester substituent in the 4-position, giving rise to the possible structures 3.58 or 3.59.
In order to discover the relative stereochemistry of the product, a study of the nuclear Overhauser effects (n.O.e.s) in the NMR spectrum was carried out (Fig. 3.12).

On irradiation of $H_B$, no enhancement of the signal due to $H_A$ was apparent, only of that due to one of the protons of the methylene group, $H_C$, which had an n.O.e. of about 6%. This was reinforced by a 5% enhancement of the signal due to $H_B$ when the sample was irradiated at the frequency of $H_C$. Irradiation of proton $H_A$ did not give rise to any n.O.e. although irradiation of $H_D$ did result in an enhancement of $H_A$ of around 9%. There was
also an enhancement of HD when HC was irradiated although this was not mirrored by
irradiation of HD. Due to the close proximity in chemical shift of the signals due to HA and
HC, it is difficult to put exact values on the magnitudes of enhancement they undergo.
However, it is the fact they are observable, rather than their magnitude, which is important
here.

The lack of any observable n.O.e. between HA and HB suggests that they are on opposite
faces of the ring, therefore the phenyl and ester groups must also be on opposing faces.
This anti configuration suggests that the identity of the product is 3.58. The assignment of
the relative stereochemistry was confirmed by the structure obtained from an X-ray
crystallographic study (Fig. 3.13).
This shows that in the solid state the dihedral angle between H_A and H_B, the two protons at the substituted positions on the ring, is 164.09°. The coupling constant between these two protons is 5.9Hz, significantly lower than might be expected from the Karplus relationship which predicts a \( J \) value of approximately 8.5Hz for a dihedral angle of 165°. This difference is due to the nitrogen atoms of the pyrazolidine ring. The presence of an electronegative substituent on the carbon atom bearing H_A means that the magnitude of the coupling constant between H_A and H_B is less than that predicted by the Karplus relationship. \(^{133}\) The methyl and acetyl groups attached to the two nitrogen atoms are also in an \textit{anti} arrangement, the lone pair on each of the nitrogens in the ring causing them to act as tetrahedral centres in a similar manner to carbon atoms. Thus each of the 4 ring substituents is \textit{anti} to the group adjacent to it.

Also isolated from the mixture of products was a pure compound with both \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra very similar to that of \textit{3.58}. Again analysis by 2-dimensional NMR spectroscopy showed that the two protons at 3.76 and 4.45ppm are attached to the same carbon atom (Fig. 3.14).

Thus, the ring protons attached to the points of substitution must be those with chemical shifts of 3.94 and 4.35ppm. Once more this was confirmed by a DEPT spectrum which showed that the carbon atoms to which these hydrogen atoms are bonded are tertiary. A \(^1\text{H}-^1\text{H}\) correlation spectrum (Fig. 3.15) showed coupling between the two protons (\( J \) 7.7Hz) implying that this product must also carry the ester group in the 4-position.
As the *anti* product had already been isolated and characterised it could be reasonably assumed that this product was the *syn* isomer 3.59. The coupling constant between H_A and H_B, the two protons at the substituted positions on the ring, is larger than that of the *anti* product 3.58. According to the Karplus relationship, this means that the dihedral angle between these protons is either greater than 165° (the angle in 3.58) or it is close to 0°. As the *syn* isomer is unlikely to have a larger dihedral angle between H_A and H_B than the *anti* isomer, it can be assumed that the latter is true. Experiments to determine any enhancements due to the n.O.e. proved inconclusive and attempts to obtain a structural analysis by X-ray crystallography were also unsuccessful. However as the product had been shown to be a 4-methoxycarbonylpyrazolidine, and the chemical shifts in the \textsuperscript{1}H NMR spectrum are different to those observed for the *anti* product 3.58, it will be assumed that the product is indeed the *syn* isomer 3.59.

Since both of the 4-substituted pyrazolidine diastereoisomers have been isolated and identified, the minor isomer that eluted with 3.59 on purification by flash chromatography was assumed to be the 5-substituted product 3.60. This is based on analysis of the \textsuperscript{1}H NMR spectrum of the mixture prior to purification by HPLC. An extra peak is visible in the region of the N-acetyl group (approximately 2.5ppm) and there is a multiplet at 4.8ppm, a similar chemical shift to the proton attached to the ester substituted carbon atom in 3.14, the 5-methoxycarbonyl pyrazolidine formed in the test reaction (Section 3.2). In addition the ratio of integrals suggests that the minor product contains an aromatic ring, an N-methyl group and a methyl ester.

To summarise, the cycloaddition with benzaldehyde yielded the *anti* 4-methoxycarbonyl pyrazolidine 3.58 in a yield of approximately 21% (6% isolated yield following purification by HPLC) and a 17% yield of the *syn* 4-methoxycarbonyl pyrazolidine 3.59.
The 5-methoxycarbonyl pyrazolidine 3.60 was formed in trace amounts but could not be isolated. Also formed were the Michael addition product 3.15 in 33% yield and 3.57, formed in 27% yield by dimerisation of the azomethine imine dipole. The formation of a mixture of stereoisomers of the 4-methoxycarbonyltetrahydropyrazole, together with apparently only one 5-substituted product, is in agreement with the cycloaddition carried out using 2-methylpropanal (Scheme 3.6). Further supporting evidence for this analysis is provided by work on the elucidation of the structure of hydroxycotinine.  

\[
\begin{align*}
\text{Nitrone 3.62 (Scheme 3.30) is analogous to the azomethine imine 3.66 formed by reaction} \\
\text{between benzaldehyde and the hydrazine 3.10 (Scheme 3.31)}
\end{align*}
\]
On reaction of the nitrone 3.62 with methyl acrylate (Scheme 3.30), three isoxazolidines were formed, the 5-substituted product 3.63 and the anti and syn isomers of the 4-substituted regioisomer, 3.64 and 3.65, respectively. Whilst determining the relative stereochemistry of 3.64 and 3.65 it was noted in the $^1$H NMR spectra that the peak for the CH$_3$ of the methyl ester appeared at 3.2ppm for the syn isomer and 3.7ppm for the anti. This compares favourably with values for the pyrazolidines 3.59 and 3.58 of 3.35ppm for the syn and 3.76ppm for the anti isomer.

The formation of two diastereoisomers 3.58 and 3.59 of the 4-methoxycarbonyl pyrazolidines can be rationalised by considering two possible transition states for the cycloaddition, exo and endo. If the azomethine imine dipole 3.66, formed by reaction of benzaldehyde with $N^1$-acetyl-$N^2$-methylhydrazine 3.10, is assumed to adopt an $E$ configuration, then an exo transition state, where the ester group of the dipolarophile points away from the dipole, will result in formation of the anti isomer 3.58 (Scheme 3.32). If the ester group is positioned beneath the dipole, the transition state is endo, and the product is the syn isomer 3.59 (Scheme 3.33). Should the dipole adopt a Z configuration then this will be reversed, 3.58 arising from an endo transition state and 3.59 from the exo.
From Schemes 3.32 and 3.33 the exo transition state appears to more favourable as there is less steric interaction between the methyl ester and the phenyl ring. However, the endo transition state is stabilised by a secondary π-orbital interaction between the central atom of the dipole and the double bond substituent of the dipolarophile (Fig. 3.16). The syn and anti isomers 3.58 and 3.59 are formed in approximately equal quantities, suggesting that the increased energy due to the steric hindrance of the endo transition state is offset by the stabilisation provided by the secondary orbital interactions, and that the two transition states are of roughly equal energy.
Having found that the cycloaddition would work with a second aldehyde, different conditions and ratios of reactants were tried to see if the yields of the tetrahydropyrazoles could be improved.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions</th>
<th>Molar Ratio of Reactants</th>
<th>Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PhCHO 3.10 Methyl Acrylate</td>
<td>3.57 3.58 3.59</td>
</tr>
<tr>
<td>1</td>
<td>Toluene, reflux, D-S trap</td>
<td>1 1 1.5</td>
<td>27% 22% 17%</td>
</tr>
<tr>
<td>2</td>
<td>Toluene, reflux, no trap</td>
<td>1 1 2</td>
<td>20% 5% 3%</td>
</tr>
<tr>
<td>3</td>
<td>Toluene, reflux, 1eq water</td>
<td>1 1 2</td>
<td>20% 4% 2%</td>
</tr>
<tr>
<td>4</td>
<td>Toluene, reflux, 3eq Et$_3$N</td>
<td>1 1 1.5</td>
<td>18% a a</td>
</tr>
<tr>
<td>5</td>
<td>Benzene, reflux, D-S trap</td>
<td>1 1 2</td>
<td>8% 18% 14%</td>
</tr>
<tr>
<td>6</td>
<td>Toluene, 20°C, mol. sieve</td>
<td>1 1 2</td>
<td>5% &lt;1% 0%</td>
</tr>
<tr>
<td>7</td>
<td>Toluene, reflux, D-S trap</td>
<td>2 1 2</td>
<td>16% 26% 24%</td>
</tr>
<tr>
<td>8</td>
<td>Toluene, reflux, D-S trap</td>
<td>1 2 2</td>
<td>b 25% 23%</td>
</tr>
</tbody>
</table>

*a Cycloadducts isolated as mixture. Not separated as aim of reaction was to minimise dimer formation. b Product formed but not isolated.

Despite the removal of water from the system seeming essential to drive forward the equilibrium between starting materials and dipole, cycloadditions of azomethine imines have been reported where an equivalent of water has been added.\textsuperscript{121} The reaction between benzaldehyde, $N^1$-acetyl-$N^2$-methylhydrazine 3.10 and methyl acrylate was attempted in the absence of a Dean and Stark trap (Entry 2) so that the water formed as a by-product.
was not removed from the reaction vessel, and also with a molar equivalent of water added to the mixture (again without a trap) (Entry 3). In both cases the yield of the cycloadducts fell significantly. The quantity of dipole dimer 3.57 was also reduced, although not to the same extent. As a consequence of the lower yields, over 40% of benzaldehyde was also retrieved. This compares with only a trace of unreacted aldehyde being recovered in the original reaction (Entry 1) (Scheme 3.28).

In order to reduce the quantity of the unwanted hexahydropyrazidine 3.57, the cycloaddition was carried out with the addition of 3 equivalents of triethylamine (Entry 4). This device has found success in suppressing dimerisation during cycloadditions involving azomethine imines; however, in this case it did not appear effective. Although the yield of pure material was slightly lower than previously, a mixture isolated following column chromatography contained more of the dimer, along with all three isomers of the pyrazolidine, 3.58, 3.59 and 3.60.

In order to see if less severe conditions could increase the yield of the desired products (i.e. 4-methoxycarbonyl isomers 3.58 and 3.59), the reaction was carried out in benzene (Entry 5). This boils at 79°C (cf. 105°C for toluene) and, like toluene, forms a low-boiling azeotrope with water enabling a Dean and Stark trap to remain an effective way to remove the by-product water. The lower temperature resulted in a small drop in the cycloadduct yields, although the amount of dimer that formed was markedly reduced. A further lowering of the reaction temperature, to 20°C, this time using a 4Å molecular sieve to remove water, gave only a trace of 3.58 and 3.59 and none of the 5-substituted isomer 3.60 (Entry 6).
The Michael addition that is in competition with the cycloaddition is a significant problem. To circumvent its formation, an extra molar equivalent of benzaldehyde was added. By having the aldehyde present in excess, the reaction between hydrazine and aldehyde, rather than hydrazine and dipolarophile, should be enhanced. However, only a slight increase in yield was recorded (Entry 7), being of the same order as for the initial synthesis (Scheme 3.28). Further, a doubling of the quantity of the hydrazine, so that complete conversion of the aldehyde to dipole can take place, even allowing for Michael addition, produced a similar, marginal improvement (Entry 8).

Overall, no significant gain in yield was achieved, though by having the hydrazine or aldehyde present in excess, a small improvement was noticed. Neither did the variation in conditions greatly affect the ratio of anti 4-methoxycarbonyl pyrazolidine 3.58 to its syn diastereoisomer 3.59.

3.8 Reaction with Other Dipolarophiles

As a further test of the reaction’s versatility, a different dipolarophile was used in place of methyl acrylate. Acrylonitrile was selected, as, like methyl acrylate, it has a monosubstituted double bond with an electron-withdrawing group attached. On reaction of 2 molar equivalents with benzaldehyde and N1-acetyl-N2-methylhydrazine 3.10 (1:1) in toluene heated to reflux for 72h, two cycloaddition products were obtained, 3.67 and 3.68, in 10 and 15% yields respectively (Scheme 3.34).
The identity of each compound was assigned by analysis of the 2-dimensional NMR spectra and by comparison with the 4-alkoxycarbonyl pyrazolidines 3.58 and 3.59 (Scheme 3.28). For the compound that was obtained as pure material after flash chromatography, the $^1$H-$^{13}$C correlation NMR spectrum showed that the protons with chemical shifts of 3.87 and 4.41 ppm were those of the methylene group (Fig. 3.17).

The protons with chemical shifts of 3.78 and 4.32 ppm are therefore those attached to the substituted carbon atoms of the ring. As the COSY NMR spectrum shows that these protons couple ($J$ 6.6 Hz) to each other (Fig. 3.18), they must be attached to adjacent ring carbon atoms and, therefore, so must the phenyl and nitrile substituents.
The nitrile substituent is therefore at the 4-position of the ring, *i.e.* 3.67 or 3.68. Its relative stereochemistry will be discussed shortly.

As in the case of the cycloaddition to methyl acrylate, the other cycloadduct was contaminated with traces of another product, which in this case proved inseparable by preparative HPLC. The $^1$H-$^{13}$C NMR correlation spectrum shows the protons of the methylene group to be those with chemical shifts of 3.84 and 4.49ppm (Fig. 3.19).

![Fig. 3.19](image)

The protons at 3.44 and 4.46ppm are therefore those attached to the same ring carbon atoms as the phenyl and nitrile substituents. A COSY NMR spectrum (Fig. 3.20) shows that these two protons couple to each other ($J$ 6.0Hz), implying that they are attached to adjacent carbon atoms and that this product also bears the nitrile substituent at the 4-position of the ring.
In order to determine the relative stereochemistry of these products their $^1$H NMR spectra were compared with those of the 4-methoxycarbonyl pyrazolidines 3.58 and 3.59. The main difference between the syn and anti 4-substituted pyrazolidines appears to be the chemical shift of the proton attached to the 4-position of the ring which is approximately 3.8-3.9 in the syn isomer but only 3.4-3.5ppm in the anti isomer. The $^1$H NMR spectrum of the 4-cyano pyrazolidine obtained in pure form showed that the chemical shifts of the hydrogen atoms attached to the ring were almost identical to those of the syn 4-methoxycarbonyl pyrazolidine 3.59 (Fig. 3.21). It was therefore assumed that the 4-cyano pyrazolidine also possessed syn relative stereochemistry, allowing it to be assigned the structure 3.68. The $^1$H NMR spectrum of the impure 4-cyano pyrazolidine was very similar to that of the anti 4-methoxycarbonyl pyrazolidine 3.58 (Fig. 3.22). It was therefore assigned as the anti pyrazolidine 3.67.
Fig. 3.21

X = Peak due to ethyl acetate - Ignore

3.59

3.68
Fig. 3.22

- Peak due to ethyl acetate - Ignore

X = Peak due to ethyl acetate - Ignore
In addition, the coupling constant between $H_A$ and $H_B$ was found to be larger in the 4-cyano pyrazolidine that was obtained in pure form than in the impure product (6.6Hz compared to 6.0Hz). In the case of the 4-methoxycarbonyl pyrazolidines the syn isomer 3.59 had a larger coupling constant between the $H_A$ and $H_B$ than the anti isomer 3.58. This also suggests that the product isolated in pure form was the syn 4-cyano pyrazolidine 3.68, and the impure compound was the anti 4-cyano pyrazolidine 3.67.

The impurity in 3.67 was assumed to be the 5-substituted pyrazolidine 3.69. This is based on the $^1$H NMR spectrum; this shows extra peaks in the region of the $N$-acetyl group (approximately 2.5ppm) and there is also a multiplet at 4.8ppm, similar to peaks observed in the $^1$H NMR spectra of the 5-substituted pyrazolidines 3.14 and 3.60 studied earlier. In addition, combustion analysis of the impure product showed good agreement with the calculated values, showing the impurity to have the same molecular formula as the desired product.

![Scheme 3.35](image_url)
To further investigate the scope of the reaction, cycloadditions with 1,2-disubstituted dipolarophiles were attempted. Dimethyl maleate was initially chosen; on reaction with the azomethine imine 3.66 (formed from benzaldehyde and $N^1$-acetyl-$N^2$-methylhydrazine 3.10) only one pure product 3.70 was isolated, in 11% yield (Scheme 3.35). This proved to have an all-syn relationship between the phenyl group and the two ester substituents when n.O.e. experiments were performed (Fig. 3.23).

![Fig. 3.23](image)

Irradiation of the signal due to $H_A$ gave a 9% enhancement of the resonance for $H_B$ and a 2% enhancement of the signal due to $H_C$. Irradiation of the sample at the frequency of $H_B$ resulted in n.O.e. enhancements for $H_A$ and $H_C$ of 9% and 10% respectively. Finally, applying radiation at the frequency of $H_C$ saw a signal enhancement of 7% to $H_B$ and just over 1% for $H_A$. Though the interaction between $H_A$ and $H_C$ is weak, the strong n.O.e. witnessed to both on irradiation of $H_B$ shows that all 3 protons are on the same side of the ring.

An additional 22% of a mixture of cycloaddition products was also obtained, although further purification was not possible. Only two diastereoisomers (and their enantiomers, which will appear identical in NMR spectroscopy) can arise from concerted reaction of the azomethine imine and dimethyl maleate, namely the all-syn isomer 3.70 and the isomer
with both ester substituents \textit{anti} to the phenyl substituent. However, since three different cycloadducts were obtained, under the reaction conditions of prolonged heating at high temperatures, conversion of some of the dimethyl maleate into dimethyl fumarate (its thermodynamically more stable geometric isomer) may have occurred before cycloaddition. This would lead to the possibility of additional diastereoisomers with the ester groups \textit{anti} to each other.

To further address this issue, the reaction was repeated with dimethyl fumarate (Scheme 3.36). This should also give only two diastereomeric products when observed by NMR spectroscopy, since the fumarate should not isomerise in the way that dimethyl maleate appears to do.

\begin{center}
\includegraphics[width=0.7\textwidth]{scheme3.36}
\end{center}

\textbf{Scheme 3.36}

On heating benzaldehyde, \textit{N}^1-\textit{acetyl-~-N}^2-\textit{methylhydrazine 3.10} and dimethyl fumarate in toluene at reflux for 72h in a flask fitted with a Dean and Stark trap, 38\% of the product 3.71, with the ester group in the 4-position \textit{anti} to the phenyl substituent, was isolated. The
relative stereochemistry was again determined by a study of the nuclear Overhauser effects (Fig. 3.24).

Fig. 3.24

Irradiation of the signal due to H_B showed no enhancement of the signals of either proton H_A or H_C, only of the resonances for the protons on the aromatic ring. H_C was enhanced by nearly 3% on irradiation of H_A, and this was matched by irradiating H_C to give an enhancement of H_A of just over 3%.

The *anti, anti* isomer 3.71 was also present as the major component of a further fraction that proved to be an inseparable mixture, the other component having an NMR spectrum consistent with the *syn, anti* diastereoisomer, 3.72. Assuming this to be the case, an extra 21% of 3.71 can be identified, along with 6% of 3.72. Since *anti, anti* isomer 3.71 is the least sterically hindered of the two possible products it might be expected to be the major product.

Having uncovered a cycloaddition that formed one cycloadduct with a reasonable degree of efficiency, the same dipole was then reacted with *N*-phenylmaleimide. This is known to be an extremely reactive dipolarophile, and on formation of the bicyclic product 3.73 it should be possible to break open the imide ring and form an ester group (Scheme 3.37).
This would then have potential in terms of forming the carboxylic acid group of a heterocyclic amino acid.

Unfortunately, the reaction between $N$-phenylmaleimide and the azomethine imine 3.66, derived from benzaldehyde and $N^1$-acetyl-$N^2$-methylhydrazine 3.10, (Scheme 3.38) resulted in only a 4% yield of the cycloadduct 3.74, possibly due to steric effects.

The relative stereochemistry of the product was once more deduced by means of n.O.e. enhancements (Fig. 3.25). A through-space interaction between $H_B$ and $H_C$ is observed. Irradiation of the signal for $H_B$ results in a 3% enhancement of that of $H_C$ whilst irradiation
at the frequency of $H_C$ enhances the signal due to $H_B$ by 4%. $H_A$ is not affected in either case, nor does irradiation at its frequency cause any enhancements in $H_B$ or $H_C$. This suggests an *anti* relationship between the phenyl ring and the imide.

![Diagram](image)

**Fig. 3.25**

An interesting feature of this molecule is that, in the $^1H$ NMR spectrum, the proton adjacent to the phenyl ring, $H_A$, appears as a sharp singlet, exhibiting no spin-spin splitting due to a coupling with $H_B$ that might have been expected. This suggests that the angle between the two protons is very close to 90°, which, according to the Karplus relationship, should result in no visible coupling. $H_B$ appears as a doublet; however, on closer inspection is found to be a doublet of doublets, although the NMR instrument is not quite sensitive enough to accurately record the magnitude of the coupling to $H_A$. The absence of any observable splitting of the signal due to $H_A$ is also presumably due to the limitations imposed by the resolution of the spectrometer.

As the most efficient cycloaddition achieved so far was to dimethyl fumarate, the difference in reactivity of the two ester substituents in the cycloadduct 3.71 was examined. If, on treatment of 3.71 with an amine, one ester reacted preferentially over the other, this could be of use as the carbon terminus in an amino acid (Scheme 3.39).
A simple amine, methylamine, was chosen and one equivalent of a methanolic solution was reacted with the diester 3.71. Since the reaction of amines with non-activated esters can be extremely slow, a catalyst was used. Cyanide has been reported as an extremely efficient and mild catalyst for the aminolysis of esters, so 0.1 molar equivalents of sodium cyanide were also added to the reaction mixture. After heating in an oil bath at 50-55°C for 16h, two new products were obtained. The $^1$H NMR spectrum of the first product (Fig. 3.26(b)), which was formed in 38% yield, revealed that one methyl ester peak had been lost when compared to the starting material (Fig. 3.26(a)), and a new doublet was observed near 2.8ppm caused by the CH$_3$ group of an N-methyl amide. Of the protons attached to the ring, only the doublet of doublets due to the proton in the 4-position had shifted significantly, from 4.03 to 4.78ppm, suggesting that reaction has occurred at the 4-position, to give the amide ester 3.75. The other product, formed in 33% yield, contained no methyl ester signals in the $^1$H NMR spectrum and two doublets near 2.8ppm (Fig. 3.26(c)).
Fig. 3.26

X = Peak due to ethyl acetate - ignore
The chemical shifts of the protons attached to the pyrazolidine ring were all different from the starting material (Fig. 3.26(a)). That of the proton attached to the same carbon as the phenyl ring had increased, from 4.58 to 4.75ppm, as had that of the proton at the 4-position, from 4.03 to 4.38ppm. The chemical shift of the proton attached to the carbon at the 5-position had dropped however, from 5.16 to 4.86pm. This product was assigned as the diamide 3.76. (The amine was in fact present in very slight excess, accounting for the total amount of methylamine found in the products being over 100%).

The fact that these two products were formed in almost equal yields indicates that there is little difference in reactivity between the esters and therefore the diester 3.71, despite being formed in relatively high yields, has limited use as the basis for a peptide mimetic.

3.9 An Alternative Nitrogen Terminus

![Diagram](image)

\[ \text{PG} \quad \text{ Protecting Group } \]

Scheme 3.40
Due to the lack of success in our attempts to incorporate a nitrogen-containing functional group that would become the N-terminus of the proposed heterocyclic amino acid, a slightly different strategy was adopted. It was decided to use one of the nitrogen atoms in the ring as the amine terminus of the amino acid (Scheme 3.40).

Tetrahydropyrazoles have been used before in peptide chemistry, with full use made of the ring nitrogens. Dutta and Morley\textsuperscript{136} incorporated the di-protected pyrazolidine 3.77 into a chain of amino acids to represent an aza-proline unit where the $\alpha$-CH of proline has been replaced by a nitrogen atom (Scheme 3.41).

![Chemical structure of 3.77 and reaction scheme]

\[ Z\overset{\text{N-N}}{\begin{array}{c} \text{Boc} \end{array}} \overset{i}{\rightarrow} Z\overset{\text{N-N}}{\begin{array}{c} \text{Boc} \\
\end{array}} \]

\[ \text{i, Br(CH}_2\text{)}_3\text{Br, NaH} \]

\textbf{Scheme 3.41}

More recently, Carreira has synthesised aza-prolines by a 1,3-dipolar cycloaddition between trimethylsilyldiazomethane 3.78 and a dipolarophile 3.79 bearing a camphor-based chiral auxiliary (Scheme 3.42)\textsuperscript{137}. With the diprotected pyrazolidine 3.80 in hand, peptide couplings were carried out.
In order to use one of the nitrogen atoms in the pyrazolidine ring as an N-terminus, it is necessary to replace either the methyl or acetyl group with a standard amino acid protecting group that can be easily removed. For this approach, different 1,2-disubstituted hydrazines are required.

### 3.10 Benzyl Group to Protect Nitrogen

Substituting the methyl group for benzyl defines \( N^1\)-acetyl-\( N^2\)-benzylhydrazine 3.25 as a target. Rather than using a low-yielding reaction to try and selectively acetylate benzylhydrazine, which is itself very expensive, it was decided to synthesise 3.25 via hydrazone formation and subsequent reduction of the double bond (Scheme 3.43).
To prepare the hydrazone 3.81, benzaldehyde and acetylhydrazine were dissolved in toluene and heated to reflux in a flask fitted with a Dean and Stark trap. After 16h, the reaction was stopped and, following removal of the solvent and unreacted benzaldehyde on a rotary evaporator, the pure product was obtained in 64% yield after column chromatography.

Attempts to reduce the C=N double bond of 3.81 with 1.5 molar equivalents of sodium cyanoborohydride (NaBH$_3$CN) and an excess of glacial acetic acid were unsuccessful, with only trace amounts of the required product being recovered. The reaction was attempted in acetonitrile at room temperature and by using acetic acid as the solvent. Increasing the amount of NaBH$_3$CN to 6 equivalents and heating the acetonitrile to reflux produced a similar result. A successful conversion was achieved by using para-toluenesulfonic acid (pTsOH) in place of acetic acid. The reaction was performed in tetrahydrofuran (THF)
with 4 equivalents of both reducing agent and acid. The pH of the reaction was controlled with bromocresol green. This indicator is blue at high pH levels but changes to a yellow-brown colour when a pH of around 3.5 is reached. This is the optimum level of acidity for reductions with NaBH₃CN and was maintained by the addition of a solution of pTsOH using a syringe. On completion of the reaction, the sodium salt of the acid, which had precipitated from the reaction mixture, was removed by filtration and the solution extracted with ethyl acetate. This yielded the cyanoborate salt 3.82, which was purified by column chromatography and subsequently converted to the free hydrazine 3.25 using a 1M solution of sodium hydroxide and heating to 60°C for 1h.

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

Scheme 3.44

When the cycloaddition reaction between 2-methylpropanal, the benzylhydrazine 3.25 and methyl acrylate was attempted (Scheme 3.44), heating at reflux in toluene for 16h did not give the desired product. This was attributed to N₁-acetyl-N₂-benzylhydrazine 3.25 being less reactive than N₁-acetyl-N₂-methylhydrazine 3.10. Indeed, the NMR experiments described in Section 3.3 revealed that a reaction time of 72h was required. Since the
temperature at which the reaction was performed was higher than the boiling points of both 2-methylpropanal and methyl acrylate, the reaction was carried out in a sealed tube. In place of a Dean and Stark trap, 4Å molecular sieves were used to remove the water. These reaction conditions yielded the desired product 3.29 as one stereoisomer, but the yield was extremely low (4%). Based on previously described cycloadditions (Schemes 3.6 and 3.28) this was assumed to be a 4-substituted isomer. The $^1$H NMR spectrum of 3.29 shows that a signal at approximately 3.1 ppm is due to two of the protons attached to the ring (Fig. 3.27).

![Fig. 3.27](image)

A $^1$H-$^{13}$C correlation spectrum shows that one of these protons is attached to the carbon bearing the isopropyl group (Hₐ) and the other is bound to the carbon atom carrying the ester substituent (Hₖ) (Fig. 3.28).
As these protons are indistinguishable by NMR spectroscopy it is not possible to ascertain the regiochemistry of the product by a COSY experiment. Coupling is observed between the signal at 3.1ppm and both protons of the methylene group (H_C & H_D), however it is not possible to deduce whether the coupling from H_C and H_D is to H_B alone, which would indicate the product was the 4-isomer, or to both H_A and H_B, suggesting the 5-isomer.

Even assuming cycloadduct 3.29 is the 4-substituted product, no assumptions can be made about the relative stereochemistry as studies of the n.O.e. will suffer from similar problems to 2-dimensional NMR spectroscopy. An enhancement of the peak at 3.1ppm could be due to interaction with either both or just one of H_A and H_B, making it impossible to determine whether they are on the same face of the ring or not. Also, unlike the pyrazolidines 3.58 and 3.59 with a phenyl group at the 3-position, which had different chemical shifts of the methyl ester depending on whether the product was syn or anti, the esters of both isopropyl derivatives 3.16 and 3.17 had chemical shifts of around 3.8ppm (as does 3.29).

3.11 Nitrogen Protection with a Boc Group

N^1-tert-Butoxycarbonyl-N^2-methylhydrazine 3.83, like N^1-acetyl-N^2-methylhydrazine 3.10, possesses the ability to stabilise the negative charge of the azomethine imine dipole, but it has the advantage over 3.10 in that it should be possible to cleave the Boc group on treatment with acid.
It was not possible to prepare the N-Boc-hydrazine 3.83 in a manner analogous to the acetylated hydrazine 3.10 (Scheme 3.45), i.e. nucleophilic attack on a butoxycarbonyl derivative by methylhydrazine and elimination of a leaving group, since the required reagent would be Boc₂O. Acid anhydrides are more reactive than esters and so the 1,1-disubstituted product 3.84 would result as discussed in Section 3.2.¹¹⁸

![Chemical structures](image.png)

Scheme 3.45

A solution to this problem was found in the approach employed by Gani and colleagues (Scheme 3.46).¹⁴¹ The substituted nitrogen of methylhydrazine is blocked by a benzyloxycarbonyl, or Z, group, a nitrogen protecting group requiring different conditions to Boc for deprotection. On reaction with Boc₂O, the Boc group can only react with the unsubstituted nitrogen atom. The Z group is then removed to give the desired asymmetrically disubstituted hydrazine, \(N¹\)-tert-butoxycarbonyl-\(N²\)-methylhydrazine 3.83.
Although a three-step synthesis, each of the steps in the sequence is a standard protection or deprotection reaction that should proceed in high yield.

\[
\begin{align*}
  \text{H}_2\text{C}-\text{N-NH}_2 & \quad \overset{i}{\rightarrow} \quad \text{H}_2\text{C}-\text{O-}
\end{align*}
\]

3.85

\[
\begin{align*}
  \text{H}_3\text{C}-\text{N-O-} & \quad \overset{\text{ii}}{\rightarrow} \quad \text{H}_3\text{C}-\text{N-NH}_2
\end{align*}
\]

3.86

\[
\begin{align*}
  \text{H}_3\text{C}-\text{N-O-} & \quad \overset{\text{iii}}{\rightarrow} \quad \text{H}_3\text{C}-\text{N-O-}
\end{align*}
\]

3.83

\[
\begin{align*}
  \text{i, } & \quad \text{H}_3\text{C}-\text{O-Cl} \quad ; \quad \text{ii, } \left(\text{H}_3\text{C}-\text{O-}\right)_2 \quad ; \quad \text{iii, } \text{H}_2, \text{Pd-C}
\end{align*}
\]

Scheme 3.46

\(N^1\)-Benzylxycarbonyl-\(N^1\)-methylhydrazine 3.85 was prepared by treating methylhydrazine in chloroform at 0°C with triethylamine followed by benzyl chloroformate. After stirring the mixture at 20°C for 16h the product was purified by distillation to give a colourless liquid in 57% yield. \(^1\)H NMR spectroscopy showed that the chemical shifts for the methyl group at 3.10ppm and for the N-H protons at 4.15ppm were in good agreement with published values for 3.85.\(^{142}\) By way of comparison, in the 1,2-disubstituted product the \(N\)-methyl group has a chemical shift of 2.63ppm and the NH protons appear between 5.0 and 6.0ppm.\(^{142}\)
Addition of a solution of Boc₂O in dichloromethane to the 1,1-disubstituted hydrazine 3.85 (dissolved in propan-2-ol) resulted in reaction at the unsubstituted nitrogen atom, which, following recrystallisation, gave the trisubstituted hydrazine 3.86 as a white solid in 53% yield. In the ¹H NMR spectrum of 3.86, the signals for the protons in the Boc methyl groups, the N-methyl group and the benzyl methylene group appear as broad singlets when the spectrum is recorded using deuterated chloroform as a solvent. On changing the solvent to fully deuterated dimethyl sulfoxide these signals each appear as two peaks indicating the presence of rotational isomers, caused by restricted rotation about the amide bond of the N-Boc group. (This phenomenon is not apparent in the ¹H NMR spectrum of 3.85 and so is presumably not due to the amide bond of the N-Z group). The Z protecting group was then removed by stirring a methanolic solution of 3.86 in the presence of a charcoal-supported palladium catalyst under an atmosphere of hydrogen to give the required disubstituted hydrazine 3.83. Although TLC analysis indicated that all of the starting material had reacted to form a single product, only 35% of 3.83 was isolated. This low yield is unoptimised as the reaction was only performed once, and is probably due to inadequate washing of the catalyst, resulting in some of the product being left in the slurry containing the catalyst and its support.

\[
\begin{align*}
3.83 & \quad + \\
+ & \quad \rightarrow \\
\text{O} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{3.87} & \quad \text{CO}_2\text{CH}_3
\end{align*}
\]

i, toluene, 72h, Dean and Stark trap

Scheme 3.47
Reaction of this hydrazine with an equimolar amount of benzaldehyde and 2 equivalents of methyl acrylate, heated to reflux in toluene for 72h, yielded the cycloaddition product 3.87 in an 18% yield (Scheme 3.47).

As with the N-benzylated pyrazolidine 3.29, exact determination of the regio- and stereochemistry of the product was not possible due to an overlap of signals in the $^1$H NMR spectrum (Fig. 3.29). (The 4-methoxycarbonyl isomer 3.87 is drawn merely from analogy with earlier results).

A $^1$H-$^{13}$C correlation spectrum shows that the two signals at 3.75ppm are due to $H_B$ and either $H_C$ or $H_D$ (Fig. 3.30).
As these two protons cannot be distinguished, COSY NMR and n.O.e. studies do not provide any useful information about the regiochemical structure of the product.

The N-Boc pyrazolidine 3.87 is presumed to be the 4-substituted product based on the results obtained from reactions with the N-methylhydrazine 3.10 (Scheme 3.28). In the syn and anti products from that reaction, 3.59 and 3.58, different chemical shifts for the methyl ester (3.2 and 3.7ppm respectively) were observed in the $^1$H NMR spectrum. A value of 3.4ppm in 3.87 is not sufficiently close to either of these to allow any speculation on the relative stereochemistry of the product.

3.12 Conclusions

The 1,3-dipolar cycloadditions of azomethine imine dipoles that have been described demonstrate that the reaction is subject to a number of restrictions. Although it has been shown that a variety of mono- and 1,2-disubstituted dipolarophiles can be used, the cycloaddition appears to be more sensitive to structural variations in the hydrazine and aldehyde. Exchanging the $N$-acetyl group for a $N$-tert-butoxycarbonyl group (effectively swapping a methyl group for O-tert-butyl) has little effect on the reaction, however replacing the $N$-methyl group with $N$-benzyl, which has considerably more steric bulk, resulted in a significant drop in yield, even with a longer reaction time. Whilst the reaction proceeded with 2-methylpropanal or benzaldehyde, no cycloadditions took place with an
aldehyde bearing a heteroatom functional group. Thus, it appears that the only pyrazolidines that can be synthesised using this approach are those bearing an alkyl or aryl substituent at the 3-position.

The pyrazolidines 3.29 and 3.87 both have nitrogen substituents that, in principle, are easy to remove independently of the methyl ester group protecting the carboxylic acid function. There is, therefore, potential for these compounds to be coupled to other amino acids, and to create a peptide chain with a pyrazolidine ring incorporated. Both of these molecules can be thought of as conformationally restricted β-amino acids, as there are two carbon atoms between the N-terminus nitrogen and the C-terminus carbon atom (Fig. 3.31).

\[
\begin{align*}
\text{β-Amino Acid} & \quad R^3=\text{Bn}, R^4=\text{COCH}_3, R^5=(\text{CH}_3)_2\text{CH}; 3.29 \\
& \quad R^3=\text{CH}_3, R^4=\text{CO}_2\text{C(\text{CH}_3)_3}, R^5=\text{Ph}; 3.87
\end{align*}
\]

Fig. 3.31
Chapter 4:

Experimental
Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter using a cell with a path length of 10mm. Combustion analyses were performed by MEDAC Ltd. (Englefield Green, Surrey). Accurate mass measurements were carried out by the EPSRC National Mass Spectrometry Service Centre (University of Wales, Swansea). Infrared spectra were recorded using a Perkin-Elmer 1710 FT-IR spectrometer. $^1$H NMR spectra were obtained at 400MHz on a JEOL JNM-EX400 or at 300MHz on a JEOL JNM-LA300. $^{13}$C NMR spectra were recorded at 100MHz on a JEOL JNM-EX400 or at 75MHz on a JEOL JNM-LA300. Chemical shifts are reported in parts per million (ppm) from tetramethylsilane (TMS) to two decimal places. Multiplicities are given as s-singlet, d-doublet, t-triplet, q-quartet, dd-doublet of doublets, ddd- doublet of doublet of doublets, m-multiplet and br.-broad signal. Coupling constants ($J$) are quoted in Hz to one decimal place. Low resolution mass spectra were recorded on a VG Micromass VG20-250 spectrometer or by the EPSRC National Mass Spectrometry Service Centre (University of Wales, Swansea) by electron impact (EI), chemical ionisation (CI), fast atom bombardment (FAB) or electrospray (ES). Crystallographic measurements were carried out by the EPSRC X-Ray Crystallography Service (University of Southampton).

All reagents were purified by distillation or recrystallisation where appropriate. Methanol and ethanol were dried using magnesium and iodine, THF and ether were dried over sodium wire or potassium lumps, and DCM was dried using sodium hydride, all according to the procedures described in "Purification of Laboratory Chemicals" by Perrin and Armarego. Anhydrous toluene was obtained commercially.

Column chromatography was carried out using Fluka Silica Gel 60 (220-440 mesh). TLC analysis was performed using Machery-Nagel Polygram SIL G/UV$_{254}$ plates with
fluorescent indicator and visualised by ultraviolet light or aqueous potassium permanganate.

Analytical HPLC analysis was carried out on a Waters W600 fitted with a Phenomenex Luna C\textsubscript{18} (2) column (25 cm x 4.6 mm i.d.) at 30°C and an ultraviolet detector set to 220 nm. A solvent system of water:acetonitrile was employed, with an initial ratio of 85:15 v/v rising to 45:55 v/v after 21 min. Preparative HPLC was performed using a Waters Delta Prep 3000 fitted with a Jones Apex 2 silica column (5 micron, 25 cm x 21 mm i.d.).
(S)-Alanine methyl ester hydrochloride (2.23)

\[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{COOCH}_3
\]

Acetyl chloride (111.50g, 1.42mol) was added dropwise to anhydrous methanol (200cm\(^3\)) at 0°C. Alanine (30.12g, 338.07mmol) was added and the solution was stirred for 72h at this temperature. The solvent was evaporated under reduced pressure to give the ester (45.68g, 97%) as a white solid, m.p. 105-107°C (lit., 104-112°C); \(\delta_H\) [400MHz; (CD\(_3\))\(_2\)SO] 1.44 (3H, d, 7.3Hz, CH\(_3\)), 3.74 (3H, s, CO\(_2\)CH\(_3\)), 4.05 (1H, q, J 7.3Hz, CHCH\(_3\)) and 8.77 (3H, s, NH\(_3\)); \(\delta_C\) [100MHz; (CD\(_3\))\(_2\)SO] 15.53 (CH\(_2\)CH), 47.79 (CH), 52.65 (CO\(_2\)CH\(_3\)) and 170.24 (C=O); m/z (EI) 104 (M\(^+\)-Cl, 5%), 88, and 44 (100).

(S)-N-tert-Butoxycarbonylalanine methyl ester (2.16)

\[
\text{CH}_3\text{CO}_2\text{CH}_3 \rightarrow \text{CH}_3\text{CO}_2\text{CH}\text{CH}\text{NH}\text{CH}_3
\]

(S)-Alanine methyl ester hydrochloride (10.01g, 71.72mmol) was dissolved in DCM (300cm\(^3\)) in a flask fitted with a calcium chloride drying tube. The solution was cooled to 0°C and triethylamine (14.52g, 143.49mmol) was added dropwise. Di-tert-butyl dicarbonate (17.27g, 79.13mmol) dissolved in DCM (15cm\(^3\)) was added in a similar manner, then the solution allowed to warm to room temperature and stirred for 16h. The solution was washed with citric acid solution (1M; 3x150cm\(^3\)), dried (MgSO\(_4\)) and the solvent removed under reduced pressure to yield the title compound as a white solid (10.57g, 73%), m.p. 31-33°C (lit., \(^{145}\) 33-34°C); \(\delta_H\) (400MHz; CDCl\(_3\)) 1.38 (3H, d, J 6.8
Hz, CH₃CH), 1.45 [9H, s, (CH₃)₃C], 3.74 (3H, s, CO₂CH₃), 4.32 (1H, m, CHCH₃) and 5.08 (1H, m, NH); δ c (100MHz; CDCl₃) 18.67 (CH₃CH), 28.33 [(CH₃)₃C], 49.18 (CH), 52.30 (CO₂CH₃), 79.85 [C(CH₃)₃], 155.13 (CONH) and 173.88 (CO₂CH₃); m/z (El) 203 (M⁺, 0.1%), 144, 130, 102, 88, 59, 57 (100), 44 (100) and 29.

(S)-N-tert-Butoxycarbonylalaninal (2.15)

(S)-N-tert-Butoxycarbonylalanine methyl ester (6.75g, 33.21mmol) was dissolved in anhydrous toluene (100cm³) under an atmosphere of nitrogen and cooled to -78°C. Diisobutylaluminium hydride (1M solution in toluene, 83cm³, 83.00mmol) was added over 90min. After addition was completed the solution was stirred for a further 1h then the reaction quenched with methanol (6cm³). The mixture was then poured into a saturated solution of potassium sodium tartrate (200g) in water (600cm³) and stirred vigorously at room temperature for 90min. The aqueous phase was separated and extracted with diethyl ether (3x75cm³). The organic extracts were combined, dried (MgSO₄) and the solvent was removed under reduced pressure to give the crude aldehyde as a white solid (4.58g, 80%), which was immediately converted to the oxime; δ h (400MHz; CDCl₃) 1.34 (3H, d, J 7.3Hz, CH₃CH), 1.46 [9H, s, (CH₃)₃C], 4.23 (1H, m, CHCH₃), 5.14 (1H, br.s, NH) and 9.57 (1H, s, CHO); δ c (100MHz; CDCl₃) 14.87 (CH₃CH), 28.29 [(CH₃)₃C], 55.54 (CH), 80.14 [C(CH₃)₃], 155.36 (CONH) and 199.80 (CHO).
A solution of (S)-N-tert-butoxycarbonylalaninal (4.58g, 26.44mmol) in ethanol (10cm³) was added to a solution of hydroxylamine hydrochloride (10.59g, 152.40mmol) and sodium acetate (20.72g, 252.59mmol) in water (50cm³). Ethanol was added to dissolve the resulting precipitate, then the solution was warmed to approximately 60°C for 10min. The solution was allowed to cool to room temperature then placed in a refrigerator for 16h. The resulting white precipitate was collected by filtration and recrystallised from water and ethanol to give a mixture of syn and anti geometric isomers of the oxime as a white solid (3.93g, 63% from the ester), m.p. 136-138°C (lit., 145-146°C); [α]D 25 33.0° (c 0.44, CH₃OH); Found: MNOH₂⁺, 206.1505. C₈H₁₆N₂O₃ requires MNOH₂⁺, 206.1505; ν_max (KBr)/cm⁻¹ 3346, 3221, 2982, 1684, 1531, 1446, 1328, 1164, 1052 and 896; Major isomer: δH [300MHz; (CD₃)₂SO] 1.11 (3H, d, J 6.9Hz, CH₃CH), 1.38 [9H, s, (CH₃)₂C], 4.61 (1H, m, CHCH₃), 6.55 (1H, d, J 5.9Hz, CH=N), 7.09 (1H, d, J 7.5Hz, NH) and 10.89 (1H, s, OH); δC [75MHz; (CD₃)₂SO] 17.37 (CH₃CH), 28.19 [(CH₃)₂C], 41.90 (CHCH₃), 77.87 [C(CH₃)₃], 152.65 (CH=N) and 154.86 (C=O); Minor isomer: δH [300MHz; (CD₃)₂SO] 1.35 (3H, d, J 7.0Hz, CH₃CH), 1.38 [9H, s, (CH₃)₂C], 4.13 (1H, m, CHCH₃), 7.03 (1H, d, J 8.4Hz, NH), 7.19 (1H, d, J 5.3Hz, CH=N) and 10.63 (1H, s, OH); δC [75MHz; (CD₃)₂SO] 18.63 (CH₃CH), 28.19 [(CH₃)₂C], 45.49 (CHCH₃), 77.87 [C(CH₃)₃], 150.59 (CH=N) and 154.86 (C=O); m/z (EI) 189 (MH⁺, 4%), 173, 132, 115, 88, 72, 59, 57 (100), 44 and 41.
**Ethyl 3-pyrrolidinobut-2-enoate (2.18)**

![Chemical Structure](image)

Ethyl acetoacetate (10.21g, 78.45mmol) and pyrrolidine (5.62g, 79.02mmol) were dissolved in toluene (150cm³) and heated at reflux under Dean and Stark conditions for 4h. The solvent was removed under reduced pressure to give the title compound (14.37g, 100%) as a brown solid with a melting point below ambient temperature that was used without further purification; $\nu_{\text{max}}$ (neat)/cm⁻¹ 3583, 2975, 2870, 1679, 1577, 1433, 1365, 1184 and 1059; $\delta_{\text{H}}$ (300MHz; CDCl₃) 1.24 (3H, t, $J$ 7.1Hz, CH₃CH₂), 1.93 (4H, m, NCH₂CH₂), 2.46 (3H, s, CH₃CN=CH), 3.30 (4H, br., NCH₂), 4.08 (2H, q, $J$ 7.1Hz, CH₂CH₃) and 4.45 (1H, s, CH=CN); $\delta_{\text{C}}$ (75MHz; CDCl₃) 14.76 (CH₃), 16.67 (CH₃), 25.18 (NCH₂CH₂), 47.89 (NCH₂), 58.09 (CH₂CH₃), 83.269 (CH=CN), 159.58 (CN=CH) and 169.24 (C=O).

**tert-Butyl hypochlorite**

![Chemical Structure](image)

In subdued light, a mixture of 2-methylpropan-2-ol (74cm³, 780mmol) and glacial acetic acid (49cm³, 860mmol) was added to an ice-cooled household bleach solution (1000cm³). After stirring for 3min, the organic layer was separated and washed with sodium carbonate solution (0.1M; 100cm³) and water (100cm³). Drying (CaCl₂) realised the title compound as a yellow oil (45.3g, 54%).
(S)-Ethyl 3-(1-tert-butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylate (2.10)

(S)-2-tert-Butoxycarbonylaminopropanaldoxime (1.00g, 5.31mmol) was dissolved in chloroform (200cm$^3$) and cooled to 0°C. tert-Butyl hypochlorite (0.63g, 5.82mmol) was added and the solution stirred for 45min. Ethyl 3-pyrrolidinobut-2-enoate (1.95g, 10.64mmol) was added and the solution heated to reflux. Triethylamine (0.60g, 5.88mmol) was then added over a period of 4-5h and the solution heated at reflux for another 16h. The solution was washed with citric acid solution (1M; 2x50cm$^3$) and saturated brine (50cm$^3$), then the organic layer dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane and ethyl acetate (2:1 v/v) as eluant to give the isoxazole as a yellow oil (1.04g, 66%); [$\alpha$]$_D^{26}$ -29.8° (c 1.13, CHCl$_3$); Found: C, 56.22; H, 7.42; N, 9.21%; MH$,^+$, 299.1617. C$_{14}$H$_{22}$N$_2$O$_5$ requires C, 56.36; H, 7.43; N, 9.39%; MH$,^+$, 299.1607; $\nu_{\max }$(CHCl$_3$)/cm$^{-1}$ 3443, 2983, 2935, 1713, 1607, 1505, 1456, 1369, 1306, 1240, 1166, 1112, 1050, 1032 and 861; $\delta$$_H$(400MHz; CDCl$_3$) 1.39 (3H, t, J 7.1Hz, CH$_3$CH$_2$), 1.44 [9H, s, (CH$_3$)$_3$C], 1.48 (3H, d, J 6.8Hz, CH$_3$CH), 2.66 (3H, s, 5-CH$_3$), 4.35 (2H, m, CH$_2$CH$_2$), 5.30 (1H, m, CHCH$_3$) and 5.68 (1H, d, J 8.3Hz, NH); $\delta$$_C$(100MHz; CDCl$_3$) 13.52 (5-CH$_3$), 14.19 (CH$_3$CH$_2$), 21.18 (CH$_3$CH), 28.38 [(CH$_3$)$_3$C], 43.84 (CHCH$_3$), 61.05 (CH$_2$CH$_3$), 79.50 [C(CH$_3$)$_3$], 107.405 (C), 154.99 (CONH), 162.12 (C), 164.67 (C) and 176.04 (C); $m/z$ (EI) 299 (MH$,^+$, 5%), 283, 225, 197, 183, 154, 137, 57 (100) and 43.
(S)-Ethyl 3-(1-aminoethyl)-5-methylisoxazole-4-carboxylate hydrochloride (2.31)

(S)-Ethyl 3-(1-tert-butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylate (1.16g, 3.89mmol) was dissolved in DCM (200cm³). Trifluoroacetic acid (20cm³) was added and the solution stirred for 2h. The solvent was removed under reduced pressure, then hydrochloric acid (2M; 30cm³) was added and the solution stirred for 1h. The solvent was removed under reduced pressure. Water (30cm³) and ethyl acetate (30cm³) were added to the residue, the two phases separated and the aqueous layer was collected. The water was removed on a rotary evaporator, then the product was dissolved in toluene (10cm³) which was removed under reduced pressure to yield the amine salt (0.86g, 94%) as a pale brown solid, m.p. 126°C (dec.); [α]_D^{25} -29.9° (c 1.03, CH₃OH); Found: C, 44.60; H, 6.28; N, 11.59%; M⁺-HCl 198.0999. C₉H₁₅N₂O₃Cl·0.5H₂O requires C, 44.36; H, 6.62; N, 11.50%; C₉H₁₅N₂O₃Cl requires M-HCl, 198.1004; ν_max (KBr)/cm⁻¹ 2878, 2645, 2518, 2025, 1713, 1600, 1522, 1475, 1381, 1349, 1281, 1134, 1042, 837 and 791; δ_H [400MHz; (CD₃)₂SO] 1.32 (3H, t, J 7.1Hz, CH₃CH₂), 1.57 (3H, d, J 6.8Hz, CH₃CH), 2.70 (3H, s, 5-CH₃), 4.30 (2H, m, CH₂CH₃), 4.76 (1H, m, CHCH₃) and 8.91 (3H, s, NH₃⁺); δ_C [100MHz; (CD₃)₂SO] 12.88 (5-CH₃), 13.79 (CH₃CH₂), 17.94 (CH₃CH), 43.07 (CHCH₃), 60.94 (CH₂CH₃), 107.06 (C), 160.79 (C), 161.25 (C) and 176.19 (C); m/z (FAB) 199 (M⁺-Cl, 100%), 182, 105, 61 and 45 (100).
Glycine (5.10g, 67.94mmol) was dissolved in a mixture of 1,4-dioxane, water and 1M sodium hydroxide solution (2:1:1 v/v/v) (280cm³) and cooled to 0°C. Di-tert-butyl dicarbonate (14.84g, 68.00mmol) was added and the solution stirred for 90min at 0°C followed by 30min at room temperature. The solvent was evaporated under reduced pressure to approx. 70cm³, then cooled to 0°C and covered with a layer of ethyl acetate (25cm³). Citric acid (13.45g, 70.01mmol) was added, then the aqueous layer was separated and extracted with ethyl acetate (2x50cm³). The combined organic layers were dried (MgSO₄) and the solvent was removed under reduced pressure. The crude product was recrystallised from hexane and ethyl acetate (1:1 v/v) to give the title compound as a white solid (5.56g, 47%), m.p. 87-89°C (lit., 88-89°C); Major rotational isomer: δₜ (400MHz; CDCl₃) 1.46 [9H, s, (CH₃)₃C], 3.97 (2H, br.s, CH₂), 5.14 (1H, br.s, NH) and 11.11 (1H, br.s, OH); δc (100MHz; CDCl₃) 28.29 [(CH₃)₃C], 42.23 (CH₂), 80.45 [C(CH₃)₃], 155.98 (CONH) and 174.93 (CO₂H); Minor rotational isomer: δₜ (400MHz; CDCl₃) 1.46 [9H, s, (CH₃)₃C], 3.91 (2H, br.s, CH₂), 6.86 (1H, s, NH) and 11.11 (1H, br.s, OH); δc (100MHz; CDCl₃) 28.29 [(CH₃)₃C], 43.40 (CH₂), 81.78 [C(CH₃)₃], 157.30 (CONH) and 173.99 (CO₂H); m/z (EI) 176 (MH⁺, 14%), 160, 120, 59 (100), 57 (100), 41 and 29.
To a solution of (S)-alanine (6.36g, 71.39mmol) in 1,4-dioxane, water and 1M sodium hydroxide (2:1:1 v/v/v) (280cm³) at 0°C was added di-tert-butyl dicarbonate (17.53g, 80.32mmol). The solution was stirred for 90min at 0°C then allowed to warm to room temperature and stirred for a further 30min. Using a rotary evaporator, the mixture was reduced to approx. 70cm³ then cooled to 0°C. Ethyl acetate (25cm³) was added and the biphasic mixture acidified to pH 3 with a solution of potassium hydrogen sulphate (2M). The aqueous layer was separated, then extracted with ethyl acetate (2x50cm³). The organic layers were combined, dried (MgSO₄) and the solvent was removed under reduced pressure. The crude product was recrystallised from hexane and ethyl acetate (1:1 v/v) to yield a white solid (4.96g, 37%), m.p. 79-81°C (lit., 81-82°C); Major rotational isomer: δH (400MHz; CDCl₃) 1.45 [12H, m, CH₃CH & (CH₃)₃C], 4.35 (1H, m, CHCH₃), 5.15 (1H, m, NH) and 11.44 (1H, br.s, OH); δC (100MHz; CDCl₃) 18.45 (CH₃CH), 28.31 [(CH₃)₃C], 49.12 (CH), 80.25 [C(CH₃)₃], 155.45 (CONH) and 178.03 (CO₂H); Minor rotational isomer: δH (400MHz; CDCl₃) 1.45 [12H, m, CH₃CH & (CH₃)₃C], 4.17 (1H, m, CHCH₃), 6.88 (1H, br.s, NH) and 11.44 (1H, br.s, OH); δC (100MHz; CDCl₃) 18.45 (CH₃CH), 28.31 [(CH₃)₃C], 50.22 (CH), 81.62 [C(CH₃)₃], 156.87 (CONH) and 177.36 (CO₂H); m/z (EL) 190 (MH+, 29%), 172, 144, 88, 59 and 57 (100).
(S)-Ethyl 3-[1-N-(tert-butoxycarbonylaminoacetyl)aminoethyl]-5-methylisoxazole-4-carboxylate (2.34)

(S)-Ethyl 3-(L-aminoethyl)-5-methylisoxazole-4-carboxylate hydrochloride (0.98g, 4.18mmol) was suspended in DCM (250cm³). Triethylamine (0.87g, 8.61mmol) was added and the mixture stirred for 10min. Water (50cm³) was then added, the organic layer separated and dried (MgSO₄) and the solvent removed under reduced pressure to yield the free amine (0.68g, 82%) which was used immediately in the coupling reaction.

N-tert-Butoxycarbonylglycine (0.79g, 4.51mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.96g, 5.01mmol) were dissolved in anhydrous DCM (150cm³) and stirred at 0°C for 10min. (S)-Ethyl 3-(1-aminoethyl)-5-methylisoxazole-4-carboxylate (0.68g, 3.43mmol) in anhydrous DCM (50cm³) was added, the solution allowed to reach room temperature and stirred for 16h. Water (50cm³) was added, the organic layer separated and dried (MgSO₄), and the solvent removed under reduced pressure. Purification by column chromatography using hexane and ethyl acetate (1:1 v/v) as eluant yielded the title compound as a colourless gum (0.85g, 70%; 57% from hydrochloride salt); [α]D²⁷ -31.3° (c 1.05, CHCl₃); Found: C, 53.64; H, 7.25; N, 11.67%; MH⁺, 356.1806. C₁₆H₂₅N₃O₆·0.1H₂O requires C, 53.80; H, 7.11; N, 11.76%; C₁₆H₂₅N₃O₆ requires MH⁺, 356.1821; v_max (CHCl₃)/cm⁻¹ 3428, 2984, 2935, 1713, 1607, 1500, 1456, 1369, 1308, 1167 and 1112; δ_H (400MHz; CDCl₃) 1.40 (3H, t, J 7.1Hz, CH₃CH₂), 1.44 [9H, s, (CH₃)₃C], 1.49 (3H, d, J 6.8Hz, CH₃CH), 2.66 (3H, s, 5-CH₃), 3.83 (2H, m, 161
NHCH₂), 4.36 (2H, m, CH₂CH₃), 5.52 (1H, br., NHCH₂), 5.61 (1H, m, CHCH₃) and 7.55 (1H, d, J 8.3Hz, NHCH); δₓ (100MHz; CDCl₃) 13.53 (5-CH₃), 14.19 (CH₃CH₂), 20.67 (CH₃CH), 28.29 [(CH₃)₃C], 42.19 (NHCH₂), 44.22 (CHCH₃), 61.21 (CH₂CH₃), 79.86 [C(CH₃)₃], 107.48 (C), 156.02 (NHCO₂), 162.20 (C), 164.01 (C), 168.78 (C) and 176.08 (C); m/z (FAB) 356 (MH⁺, 40%), 300, 256, 199, 57 (100), 44, 41 and 29.

(S,S)-Ethyl 3-[1-N-(2-tert-butoxycarbonylaminopropanoyl)aminoethyl]-5-methylisoxazole-4-carboxylate (2.35)

(S)-Ethyl 3-(1-aminoethyl)-5-methylisoxazole-4-carboxylate hydrochloride (0.50g, 2.13mmol), (S)-N-tert-butoxycarbonylalanine (0.88g, 4.65mmol), and EDCI (0.96g, 5.01mmol) were dissolved in anhydrous diethyl ether (80cm³) and cooled to 0°C. Triethylamine (0.24g, 2.37mmol) was added over period of 4h, then the solution allowed to warm to 20°C and stirred for 16h. Water (50cm³) was added, then the organic layer was separated, dried (MgSO₄) and the solvent removed under reduced pressure. Purification by column chromatography using a solvent system of hexane and ethyl acetate (1:1 v/v) yielded the title compound as a white solid (0.58g, 74%), m.p. 70-73°C; [α]₀²⁵ -49.0° (c 1.11, CHCl₃); Found: C, 54.59; H, 7.40; N, 10.94%; MH⁺, 370.1976. C₁₇H₂₇N₃O₆·0.25H₂O requires C, 54.60; H, 7.41; N, 11.24%; C₁₇H₂₇N₃O₆ requires MH⁺, 370.1978; vₘₐₓ (KBr)/cm⁻¹ 3338, 2983, 2935, 1726, 1687, 1659, 1605, 1526, 1458, 1367, 1335, 1254, 1168 and 1107; δₓ (300MHz; CDCl₃) 1.34 (3H, d, J 7.0Hz, CH₃CHCO), 1.39 (3H, t, J 7.1Hz, CH₃CH₂), 1.43 [9H, s, (CH₃)₃C], 1.49 (3H, d, J 7.0Hz, CH₃CHC=N), 2.66 (3H, s,
5-CH₃), 4.20 (1H, br., CHCONH), 4.35 (2H, m, CH₂CH₃), 5.23 (1H, br.d, J 6.6Hz, NHCO₂), 5.60 (1H, m, CHC=N) and 7.36 (1H, br., NHCHC=N); δC (75MHz; CDCl₃) 13.54 (5-CH₃), 14.20 (CH₃CH₂), 18.67 (CH₃CHCO), 20.77 (CH₃CHC=N), 28.30 [(CH₃)₃C], 42.30 (CHC=N), 50.16 (CHCO), 61.20 (CH₂CH₃), 79.81 [C(CH₃)₃], 107.45 (C), 155.34 (NHCO₂), 162.21 (C), 164.01 (C), 171.81 (C) and 176.10 (C); m/z (FAB) 370 (MH⁺, 60%), 314 (100), 270, 292, 199, 182 and 153.

(S)-3-(1-tert-Butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylic acid (2.36)

![Chemical structure](image)

To a solution of (S)-ethyl 3-(1-tert-butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylate (0.49g, 1.64mmol) in water, THF and ethanol (1:1:1 v/v/v) (7cm³) was added lithium hydroxide monohydrate (0.07g, 1.69mmol). The solution was stirred at 20°C for 16h, then citric acid (0.33g, 1.72mmol) added. The resulting precipitate was filtered off and the solvent removed under reduced pressure. The residue was partitioned between water (25cm³) and DCM (25cm³). The organic layer was dried (MgSO₄) and the solvent removed to yield the carboxylic acid as a white solid (0.29g, 65%), m.p. 146-147°C; [α]D₂² -22.1° (c 1.08, CH₃OH); Found: C, 53.46; H, 6.88; N, 9.97%; MH⁺, 271.1299. C₁₂H₁₈N₂O₅ requires C, 53.33; H, 6.71; N, 10.36%; MH, 271.1294; v_max (KBr)/cm⁻¹ 3348, 2983, 2588, 1692, 1597, 1530, 1479, 1368, 1335, 1248, 1164, 1137 and 1057; δH [400MHz; (CD₃)₂SO] 1.33 (3H, d, J 7.3Hz, CH₃CH), 1.36 [9H, s, (CH₃)₃C], 2.61 (3H, s, 5-CH₃), 5.07 (1H, m, CHCH₃) and 7.26 (1H, d, J 8.3Hz, NH); δC [100MHz; (CD₃)₂SO] 12.86 (5-CH₃), 19.99 (CH₃CH), 28.09 [(CH₃)₃C], 43.31 (CHCH₃), 77.89 [C(CH₃)₃].
107.44 (C), 154.64 (CONH), 162.98 (C), 165.53 (C) and 175.24 (C); m/z (FAB) 271 (MH+, 8%), 215, 171, 75, 61, 57, 49, 43 and 29.

(S)-3-(1-tert-Butoxycarbonylaminoethyl)-4-(ethoxycarbonylmethylaminocarbonyl)-5-methylisoxazole (2.39)

(S)-3-(1-tert-Butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylic acid (0.43g, 1.45mmol), glycine ethyl ester hydrochloride (0.25g, 1.79mmol) and EDCI (0.39g, 2.03mmol) were dissolved in anhydrous diethyl ether (80cm³), cooled to 0°C and stirred for 45min. The mixture was allowed to warm to room temperature and stirred for 1h, then cooled to 0°C and triethylamine (0.18g, 1.79mmol) was added over 3.5h. When addition was complete, the suspension was warmed to room temperature and stirred for a further 16h. Water (25cm³) was then added to dissolve any precipitated material, the ether layer separated and the aqueous layer extracted with ethyl acetate (2x10cm³). The combined organic extracts were dried (MgSO₄) and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel using hexane and ethyl acetate (1:1 v/v) as eluant to give the title compound as a white solid (0.20g, 39%), m.p. 132-133°C; [α]D²² -24.0° (c 0.51, CH₃OH); Found: C, 54.07; H, 7.15; N, 11.66%; MH⁺, 356.1871. C₁₆H₂₅N₃O₆ requires C, 54.07; H, 7.09; N, 11.82%; MH, 356.1821; νmax (KBr)/cm⁻¹ 3318, 2984, 2934, 1755, 1733, 1685, 1654, 1610, 1531, 1450, 1370, 1338, 1243, 1175, 1061 and 864; δH (400MHz; CDCl₃) 1.30 (3H, t, 17.1Hz, CH₃CH₂), 1.41 [9H, s, (CH₃)₃C], 1.54 (3H, d, J 7.3Hz, CH₃CH), 2.62 (3H, s, 5-CH₃), 4.06 (1H, d, J 5.4Hz,
(S,S)-3-(1-tert-Butoxycarbonylaminoethyl)-4-[1-methoxycarbonyl)ethylaminocarbonyl]-5-methylisoxazole (2.40)

An experimental procedure analogous to that used for the synthesis of (S)-ethyl 3-[1-N-(tert-butoxycarbonylaminoacetyl)aminoethyl]-5-methylisoxazole-4-carboxylate was adopted with the following reagents: (S)-alanine methyl ester hydrochloride (0.10g, 0.74mmol) and triethylamine (0.16g, 1.58mmol). On generating the free base it was dissolved in anhydrous DCM (25cm³) and added to (S)-3-(1-tert-butoxycarbonylaminoethyl)-5-methylisoxazole-4-carboxylic acid (0.09g, 0.35mmol) and EDCI (0.08g, 0.42mmol) dissolved in anhydrous DCM (15cm³). On purification, the title compound was isolated as a sticky white solid (0.03g, 24%); [α]D25 14.2° (c 1.37, CHCl₃); Found: MH⁺, 356.1836. C₁₆H₂₅N₃O₆ requires MH⁺, 356.1821; νmax (CHCl₃)/cm⁻¹ 3443, 3263, 2980, 1743, 1695, 1657, 1504, 1456, 1370, 1240, 1169 and 1054; δH (300MHz; CDCl₃) 1.41 [9H, s, (CH₃)₃C], 1.52 (3H, d, J 7.3Hz, CH₃CHCO₂), 1.56 (3H, d, J 7.0Hz, CH₃CH₂), 2.07 (3H, m, CH₂CO₂), 4.11 (1H, d, J 5.4Hz, CHHCO₂) (major rotational isomer), 4.24 (3H, m, CHHCO₂ & CH₂CH₃), 5.13 (1H, m, CHCH₃), 5.44 (1H, d, J 8.3Hz, NHCH) and 8.08 (1H, br., NHCH₂); δC (100MHz; CDCl₃) 12.69 (5-CH₃), 14.16 (CH₃CH₂), 20.78 (CH₃CH), 28.29 [(CH₃)₃C], 41.44 (NHCH₂), 42.81 (CHCH₃), 61.47 (CH₂CH₃), 80.49 [C(CH₃)₃], 111.28 (C), 155.87 (CONH), 162.14 (C), 162.89 (C), 169.71 (C) and 172.42 (C); m/z (FAB) 356 (MH⁺, 16%), 300, 256, 155, 85, 75, 65, 61, 57, 43, 35 and 29.
\( \text{CH}_3\text{CH}=\text{N}) \), 2.60 (3H, s, 5-\text{CH}_3), 3.77 (3H, s, \text{CO}_2\text{CH}_3), 4.69 (1H, m, \text{CHCO}_2), 5.16 (1H, m, \text{CH}=\text{N}), 5.38 (1H, d, \text{J} 8.3\text{Hz}, \text{NHCO}_2) \text{ and } 8.43 (1H, \text{br.}, \text{NHCHCO}_2); \delta_C (75\text{MHz}; \text{CDCl}_3) 12.58 (5-\text{CH}_3), 17.31 (\text{CH}_3\text{CHCO}_2), 20.90 (\text{CH}_3\text{CH}=\text{N}), 28.30 [(\text{CH}_3)_3\text{C}], 42.63 (\text{CH}=\text{N}), 48.41 (\text{CHCO}_2), 52.45 (\text{CO}_2\text{CH}_3), 80.55 [(\text{CH}_3)_3\text{C}], 111.48 (\text{C}), 155.95 (\text{NHCO}_2), 161.68 (\text{C}), 162.96 (\text{C}), 172.30 (\text{C}) \text{ and } 173.48 (\text{C}); m/z (\text{FAB}) 356 (\text{MH}^+, 30\%), 300, 256, 239, 179, 154, 136, 105, 91, 57 (100), 44, 41 \text{ and } 29.

\((S)-3-[1-N-(\text{\text{tert-Butoxycarbonylaminoacetyl})aminoethyl]-4-(\text{ethoxycarbonyl)methylaminocarbonyl})-5-methylisoxazole (2.41)\)

Route A:

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\text{(S)-Ethyl } 3-[1-N-(\text{tert-butoxycarbonylaminoacetyl)aminoethyl}-5-methylisoxazole-4-carboxylate was converted to the carboxylic acid using the procedure described for (S)-3-(1-\text{\text{tert-butoxycarbonylaminoethyl})-5-methylisoxazole-4-carboxylic acid. This was accomplished using the following quantities of reagents: (S)-ethyl 3-[1-N-(\text{tert-butoxycarbonylaminoacetyl)aminoethyl}-5-methylisoxazole-4-carboxylate } (0.15g, 0.42mmol), lithium hydroxide monohydrate (0.02g, 0.48mmol), water, \text{THF and ethanol (1:1:1 v/v/v) } (7cm^3) \text{ to give (S)-3-[1-N-(\text{tert-butoxycarbonylaminoacetyl)aminoethyl}-5-methylisoxazole-4-carboxylic acid (0.06g, 43%) as a colourless gum that was used directly; } \delta_H (300\text{MHz}; \text{CDCl}_3) 1.37 [12H, m, \text{CH}_3\text{CH} & (\text{CH}_3)_3], 2.61 (3H, s, 5-\text{CH}_3), 3.42-3.62 (2H, m, \text{CH}_2\text{NH}), 5.38 (2H, m, \text{CHCH}_3 & \text{NHCH}_2) \text{ and } 6.94 (\text{NHCH}).\]

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The acid was then converted to the desired product using the conditions employed for the synthesis of (S)-ethyl 3-[1-N-(tert-butoxycarbonylaminoacetyl)aminoethyl]-5-methylisoxazole-4-carboxylate using the following quantities of reagents: (S)-3-[1-N-(tert-butoxycarbonylaminoacetyl)aminoethyl]-5-methylisoxazole-4-carboxylic acid (0.29g, 0.89mmol) and EDCI (0.44g, 2.30mmol) in anhydrous DCM (40cm³). The free base, formed by reaction between glycine ethyl ester hydrochloride (4.98g, 35.68mmol) and triethylamine (7.26g, 71.75mmol), was added as a solution in anhydrous DCM (40cm³). The title compound was obtained as a white solid (0.10g, 27%), m.p. 96-99°C; [α]D^28 7.2° (c 1.31, CHCl₃); Found MH^+, 413.2068. C₁₈H₂₈N₄O₇ requires MH, 413.2036; ν_max (KBr)/cm⁻¹ 3323, 3116, 2972, 2935, 1723, 1667, 1632, 1578, 1539, 1455, 1408, 1282 and 1168; δH (300MHz; CDCl₃) 1.31 (3H, t, J 7.1Hz, CH₃CH₂), 1.45 [9H, s, (CH₃)₃C], 1.58 (3H, d, J 7.1Hz, CH₃CH), 2.61 (3H, s, 5-CH₃), 3.68-3.89 (2H, m, CH₂CO₂), 4.08-4.28 (4H, m, CH₂CONH & CH₂CH₃), 5.07 (1H, br., NHC₃O₂), 5.41 (1H, m, CHCH₃), 7.18 (1H, d, J 7.9Hz, NHCH) and 8.09 (1H, m, NHCH₂CO₂); δC (75MHz; CDCl₃) 12.53 (5-CH₃), 14.15 (CH₃CH₂), 20.35 (CH₃CH), 28.26 [(CH₃)₃C], 41.45 (CH₂CONH), 41.76 (CHCH₃), 44.07 (CH₂CO₂), 61.60 (CH₂CH₃), 80.37 [C(CH₃)₃], 111.46 (C), 155.93 (NHCO₂), 162.35 (C), 162.41 (C), 170.00 (C), 170.15 (C) and 172.17 (C); m/z (FAB) 413 (MH^+, 100%), 385, 357, 313, 282, 256, 210, 153 and 107.

Route B:
(S)-3-(1-tert-Butoxycarbonylaminoethyl)-4-(ethoxycarbonylmethylaminocarbonyl)-5-methylisoxazole (0.11g, 0.31mmol) was dissolved in anhydrous ethanol (25cm³) and cooled to 0°C. Acetyl chloride (0.12g, 1.58mmol) was added dropwise and the solution stirred for 6h. After this time, TLC analysis [eluted with hexane and ethyl acetate (1:1 v/v)] showed all of the starting material to have reacted, whereupon the solvent was removed under reduced pressure to give the free amine salt (0.09g, 100%) which was used without further purification. The reaction conditions used for the synthesis of (S)-ethyl 3-[1-N-(tert-butoxycarbonylaminoacetyl)aminoethyl]-5-methylisoxazole-4-carboxylate were again adopted using the following quantities: (S)-3-(1-aminoethyl)-4-(ethoxycarbonylmethylaminocarbonyl)-5-methylisoxazole hydrochloride (0.09g, 0.31mmol), N-tert-butoxycarbonylglycine (0.12g, 0.68mmol), EDCI (0.14g, 0.73mmol) and triethylamine (0.04g, 0.36mmol). The acid and coupling reagent were dissolved in anhydrous DCM (15cm³) and the free amine was added as a solution in the same solvent (15cm³). Purification led to the isolation of the title compound as a colourless gum (0.01g, 10%); data as for sample from Route A.

1,1-Dibromo-3-(tert-butoxycarbonylamino)but-1-ene (2.47)

\[
\begin{align*}
\text{NHBoc} & \quad \text{Br} \\
\text{H} & \quad \text{Br}
\end{align*}
\]

Carbon tetrabromide (4.31g, 13.00mmol), zinc (0.86g, 13.15mmol) and triphenylphosphine (3.45g, 13.15mmol) were all dissolved/suspended in anhydrous DCM (50cm³) and the mixture stirred vigorously at 0°C. A solution of (S)-N-tert-butoxycarbonylaminalaninal (0.68g, 3.93mmol) in dry DCM (10cm³) was added and the mixture stirred for 24h at 20°C. Pentane (50cm³) was added to the resultant dark brown
solution and the precipitate that formed was filtered off. This was then dissolved in DCM (75cm$^3$), pentane (75cm$^3$) was again added and the precipitate removed by filtration. This precipitate was again treated with pentane and DCM, filtered, and the organic extracts were combined and the solvent removed under reduced pressure. Purification of the crude material by column chromatography eluting with hexane and ethyl acetate (2:1 v/v) gave the alkene (0.65g, 50%) as a white solid, m.p. 85-86°C; $\delta_H$ (400MHz; CDCl$_3$) 1.24 (3H, d, $J$ 6.8Hz, CH$_3$CH), 1.45 [9H, s, (CH$_3$)$_3$C], 4.34 (1H, m, CHCH$_3$), 4.55 (1H, br., NH) and 6.34 (1H, d, $J$ 8.4Hz, CH=C); $\delta_C$ (100MHz; CDCl$_3$) 19.81 (CH$_3$CH), 28.37 [(CH$_3$)$_3$C], 49.38 (CHCH$_3$), 79.77 [C(CH$_3$)$_3$], 90.14 (CBr$_2$), 140.43 (CH=CBr$_2$) and 154.77 (C=O).

**Ethyl 4-(tert-butoxycarbonylamino)pent-2-ynoate (2.46)**

1,1-Dibromo-3-(tert-butoxycarbonylamino)but-1-ene (0.10g, 0.30mmol) was dissolved in anhydrous THF (10cm$^3$) and cooled to -78°C. $n$-BuLi (0.84cm$^3$ of a 1.1M solution in hexanes, 0.92mmol) was added and after stirring the solution for 45min, it was allowed to warm to 20°C and stirred for another 45min. On cooling to -60°C, ethyl chloroformate (0.05g, 0.42mmol) was also added and the solution again allowed to warm to 20°C where it was stirred for 16h. The reaction mixture was treated with water (5cm$^3$) then extracted with ether (3x10cm$^3$). The organic extracts were dried (MgSO$_4$) and the solvent was removed under reduced pressure to give the alkyne (0.05g, 74%) as a yellow oil; $\delta_H$ (400MHz; CDCl$_3$) 1.34 (3H, t, $J$ 7.2Hz, CH$_3$CH$_2$), 1.53 [9H, s, (CH$_3$)$_3$C], 1.57 (3H, d, $J$ 6.8Hz, CH$_3$CH), 4.28 (2H, q, $J$ 7.2Hz, CH$_2$CH$_3$) and 5.27 (1H, dq, $J$ 2.4 & 6.8Hz, CHCH$_3$), NH not observed; $\delta_C$ (100MHz; CDCl$_3$) 14.09 (CH$_3$CH$_2$), 20.51 (CH$_3$CH), 27.93
[(CH₃)₃C], 43.68 (CHCH₃), 63.03 (CH₂CH₃), 70.58 (C≡C), 82.69 (C), 83.35 (C), 151.32 (C=O) and 153.44 (C=O).

**Ethyl 4-(tert-butoxycarbonylamino)-3-oxo-pentanoate (2.51)**

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

\[
\begin{align*}
\text{NH} & \quad \text{Boc} \quad \text{CO}_2\text{Et}
\end{align*}
\]

(S)-N-tert-Butoxycarbonylalanine (1.75g, 9.25mmol) and 1,1'-carbonyldiimidazole (1.50g, 9.25mmol) were dissolved in anhydrous THF (35cm³) and stirred at 20°C for 24h.

To magnesium turnings (0.44g, 18.10mmol) in anhydrous THF (35cm³) heated to reflux was slowly added 2-bromopropane (1.99g, 16.18mmol). The mixture was heated at reflux for a further 2h.

A solution of ethyl hydrogen malonate (1.34g, 10.14mmol) in dry THF (40cm³) was cooled to 0°C and the solution of iso-propylmagnesium bromide added over 40 minutes. After stirring the reaction mixture for 90min at 20°C, it was warmed to 40°C and stirred for another 30min. On cooling to 0°C, the imidazolide solution was added and the solution allowed to warm to 20°C, whereupon it was stirred for 16h. Ethyl acetate (75cm³) was added and then the solution was washed with a citric acid solution (1M; 125cm³). It was then extracted with ethyl acetate (3x50cm³), the organic layers were combined, dried (MgSO₄) and the solvent was removed. The crude product was purified by column chromatography eluting with hexane and ethyl acetate (7:3 v/v) to give the title compound (1.36g, 57%); \(\delta_H\) (400MHz; CDCl₃) 1.21 (3H, t, J 7.2Hz, CH₃CH₂), 1.29 (3H, d, J 7.2Hz, CH₃CH), 1.38 (9H, s, [CH₃]₃C), 3.55 (2H, m, CH₂CO₂), 4.12 (2H, q, J 7.2Hz, CH₂CH₃).
4.30 (1H, m, CHCH₃) and 5.28 (1H, d, J 8.0Hz, NH); δC (100MHz; CDCl₃) 14.35 (CH₃CH₂), 17.03 (CH₃CH), 28.70 [(CH₃)₃C], 45.97 (CH₂CO₂), 55.57 (CHCH₃), 61.84 (CH₂CH₃), 80.18 [C(CH₃)₃], 155.09 (NHCO₂), 167.22 (CO₂CH₂) and 202.69 (C=O); m/z (EI) 260 (MH⁺, 18%), 231, 204, 186, 144, 115, 88, 57 (100), 44 (100), 41 and 29.

\[ \text{N}^1\text{-Acetyl-N}^2\text{-methylhydrazine (3.10)} \]

Methylhydrazine (3.52g, 76.41mmol) and ethyl acetate (6.77g, 76.78mmol) were dissolved in ethanol (6cm³) and heated at reflux under an atmosphere of nitrogen for 96h. The solvent was removed under reduced pressure (10mmHg) and the crude reaction mixture then distilled under vacuum, b.p. 68°C/0.3mmHg (lit., 119°C/12mmHg). After allowing the product to solidify in a freezer for 1-2h, it was filtered under a nitrogen atmosphere to give the desired product (3.29g, 49%) as a white solid, m.p. 40-43°C (lit., 119 38-42°C); Found: C, 36.99; H, 9.15; N, 29.23%; MH⁺, 89.0714. C₃H₇N₂O·0.5H₂O requires C, 37.10; H, 9.34; N, 28.85%; C₃H₇N₂O requires MH, 89.0715; νmax (KBr)/cm⁻¹ 3445, 3274, 1657, 1443 and 1375; δH (300MHz; CDCl₃) 1.96 (3H, s, CH₃CO) and 2.62 (3H, s, CH₃N); δC (75MHz; CDCl₃) 21.21 (CH₃CO), 39.36 (CH₃N) and 169.54 (C=O); m/z (EI) 89 (MH⁺, 7%), 88, 60, 46, 45 and 43.
1-Acetyl-4-methoxycarbonyl-2-methyl-3-(2-propyl)tetrahydropyrazole (3.16 & 3.17)

\[
\begin{align*}
N^1-\text{Acetyl}-N^2-\text{methylhydrazine} &\quad (0.50\text{g, } 5.67\text{mmol}) \\
\text{and 2-methylpropanal} &\quad (0.24\text{g, } 3.30\text{mmol})
\end{align*}
\]

were dissolved in toluene (50cm\(^3\)) and placed in a round-bottomed flask fitted with a Dean and Stark trap. The solution was heated at reflux under a nitrogen atmosphere for 2h. Heating was then stopped and methyl propenoate (1.00g, 11.66mmol) added whereupon the solution was heated at reflux for a further 16h under nitrogen. The solvent and excess dipolarophile were removed under reduced pressure and the crude material purified by column chromatography using hexane and ethyl acetate (1:1 v/v) as the solvent. This yielded one stereoisomer (Isomer A) of the title compound as a yellow oil (0.19g, 25%) Purification by normal-phase preparative HPLC using a solvent system of CH\(_3\)CN in water (30% v/v) resulted in isolation of the other diastereoisomer (Isomer B) (0.09g, 11%) along with the 5-methoxycarbonyl isomer (0.03g, 1%), both as yellow oils. 

\(N^1-(2-\text{Methoxycarbonylethyl})-N^1-\text{methyl}-N^2-\text{acetylhydrazine}\), the product of Michael addition between the hydrazine and methyl propenoate, was also isolated (0.45g, 46%) as a mixture of rotational isomers. Isomer A: Found: M\(^+\), 228.1474. C\(_{11}\)H\(_{20}\)N\(_2\)O\(_3\) requires M, 228.1474; \(\nu_{\text{max}}\) (CHCl\(_3\))/cm\(^{-1}\) 3670, 3441, 3013, 2925, 1740, 1647, 1439, 1373, 1245, 1185 and 1124; \(\delta_H\) (300MHz; CDCl\(_3\)) 0.83 (3H, d, \(J\ 6.6\text{Hz}, \text{CH}_3\text{CH}\)), 0.94 (3H, d, \(J\ 6.6\text{Hz}, \text{CH}_3\text{CH}\)), 1.70 [1H, m, \(\text{CH(CH}_3\text{)}_2\)], 2.20 (3H, s, \(\text{CH}_3\text{CON}\)), 2.59 (3H, s, \(\text{CH}_3\text{N}\)), 3.02 [1H, m, \(\text{CHCH(CH}_3\text{)}_2\)], 3.67 (1H, m, 5-\(\text{CHH}\)), 3.72 (1H, s, \(\text{CO}_2\text{CH}_3\)), 3.76 (1H, m, \(\text{CHCO}_2\)) and 4.20 (1H, m, 5-\(\text{CHH}\)); \(\delta_C\) (100MHz; CDCl\(_3\)) 18.02 (\(\text{CH}_3\text{CH}\)), 20.75 (\(\text{CH}_3\text{CON}\)), 21.09 (\(\text{CH}_3\text{CH}\)), 29.23 [\(\text{CH(CH}_3\text{)}_2\)], 44.80 (5-\(\text{CH}_2\)), 44.98 (\(\text{CHCO}_2\)), 46.57 (\(\text{CH}_3\text{N}\)), 52.00
(CO$_2$CH$_3$), 73.69 [CHCH(CH$_3$)$_2$], 170.51 (C=O) and 171.06 (C=O); m/z (EI) 228 (M$^+$, 13%), 197, 185 (100), 143, 125, 83 and 43. Isomer B: Found: M$^+$, 228.1474. C$_{11}$H$_{20}$N$_2$O$_3$ requires M, 228.1474; $\nu_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$ 2963, 1734, 1637, 1498, 1369, 1257 and 1185; $\delta_{\text{H}}$ (400MHz; CDCl$_3$) 0.93 (3H, d, $J$ 6.4Hz, CH$_3$CH), 0.99 (3H, d, $J$ 6.8Hz, CH$_3$CH), 1.50 [1H, m, CH(CH$_3$)$_2$], 2.16 (3H, s, CH$_3$CON), 2.60 (3H, s, CH$_3$N), 2.94 [1H, dd, $J$ 3.9 & 8.8Hz, CHCH(CH$_3$)$_2$], 3.05 (1H, m, CH$_2$CO), 3.68 (1H, m, 5-CHH), 3.75 (1H, s, CO$_2$CH$_3$) and 4.37 (1H, m, 5-CHH); $\delta_{\text{C}}$ (100MHz; CDCl$_3$) 19.11 (CF$_3$CH), 19.81 (CH$_3$CH), 20.57 (CH$_3$CON), 32.61 [CH(CH$_3$)$_2$], 44.22 (5-CH$_2$), 45.79 (CH$_3$N), 47.46 (CH$_2$CO$_2$), 52.49 (CO$_2$CH$_3$), 77.07 [CHCH(CH$_3$)$_2$], 170.88 (C=O) and 173.99 (C=O); m/z (EI) 228 (M$^+$, 24%), 197, 185 (100), 169, 143, 125, 83 and 43. 5-Substituted isomer: Found: C, 56.73; H, 8.74; N, 12.00%; M$^+$, 228.1474. C$_{11}$H$_{20}$N$_2$O$_3$0.25H$_2$O requires C, 56.75; H, 8.87; N, 12.03%; C$_{11}$H$_{20}$N$_2$O$_3$ requires M, 228.1474; $\nu_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$ 2960, 1749, 1646, 1438, 1420 and 1370; Major rotational isomer: $\delta_{\text{H}}$ (400MHz; CDCl$_3$) 0.92 (3H, d, $J$ 6.4Hz, CH$_3$CH), 1.05 (3H, d, $J$ 6.3Hz, CH$_3$CH), 1.73 [1H, m, CH(CH$_3$)$_2$], 2.06 (1H, m, 4-CHH), 2.18 (3H, s, CH$_3$CON), 2.50 (3H, s, CH$_3$N), 2.53 (1H, m, 4-CHH), 2.66 [1H, m, CHCH(CH$_3$)$_2$], 3.74 (1H, s, CO$_2$CH$_3$) and 4.61 (1H, m, CH$_2$CO); $\delta_{\text{C}}$ (100MHz; CDCl$_3$) 20.28 (CH$_3$CH), 20.87 (CH$_3$CON), 20.96 (CH$_3$CH), 28.73 [CH(CH$_3$)$_2$], 32.61 (4-CH$_2$), 46.25 (CH$_3$N), 52.40 (CO$_2$CH$_3$), 57.63 (CH$_2$CO$_2$), 72.33 or 73.52 [CHCH(CH$_3$)$_2$], 170.55 (C=O) and 172.36 (C=O); Minor rotational isomer: $\delta_{\text{H}}$ (400MHz; CDCl$_3$) 0.88 (3H, d, $J$ 6.8Hz, CH$_3$CH), 0.96 (3H, d, $J$ 6.8Hz, CH$_3$CH), 1.33 [1H, m, CH(CH$_3$)$_2$], 2.06 (1H, m, 4-CHH), 2.20 (3H, s, CH$_3$CON), 2.50 (3H, s, CH$_3$N), 2.53 (1H, m, 4-CHH), 2.66 [1H, m, CHCH(CH$_3$)$_2$], 3.76 (1H, s, CO$_2$CH$_3$) and 4.61 (1H, m, CH$_2$CO); $\delta_{\text{C}}$ (100MHz; CDCl$_3$) 19.44 (CH$_3$CH), 20.87 (CH$_3$CON), 20.96 (CH$_3$CH), 30.36 [CH(CH$_3$)$_2$], 31.27 (4-CH$_2$), 46.25 (CH$_3$N), 52.40 (CO$_2$CH$_3$), 58.16 (CH$_2$CO$_2$), 72.33 or 73.52 [CHCH(CH$_3$)$_2$], 171.18 (C=O) and 172.86 (C=O); m/z (EI) 228 (M$^+$, 24%), 197, 185 (100), 169, 143, 125, 83 and 43 (100). Michael addition product: Major rotational isomer: $\delta_{\text{H}}$ (300MHz; CDCl$_3$) 2.06
(3H, s, CH₃CO), 2.48-2.61 (2H, m, CH₂), 2.59 (3H, s, CH₃N), 2.86-3.07 (2H, m, CH₂), 3.69 (3H, s, CO₂CH₃) and 6.50 (1H, br.s, NH); δC (75MHz; CDCl₃) 19.63 (CH₃CO), 32.34 (CH₂), 47.37 (CH₃N), 51.84 (CH₂), 55.53 (CO₂CH₃), 172.36 (C=O) and 174.84 (C=O); Minor rotational isomer: δH (300MHz; CDCl₃) 1.90 (3H, s, CH₃CO), 2.48-2.61 (2H, m, CH₂), 2.66 (3H, s, CH₃N), 2.86-3.07 (2H, m, CH₂), 3.69 (3H, s, CO₂CH₃) and 6.54 (1H, br.s, NH); δC (75MHz; CDCl₃) 21.48 (CH₃CO), 32.40 (CH₂), 46.17 (CH₃N), 51.81 (CH₂), 54.01 (CO₂CH₃), 168.62 (C=O) and 173.20 (C=O); m/z (EI) 174 (M⁺, 1%), 143, 131, 116, 101, 59 (100) and 43.

N¹-Acetyl-N²-(2-tert-butoxy-4-methyl-2-oxazolin-5-yl)-N¹-methylhydrazine (3.31)

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-(2-propyl)tetrahydropyrazole was adopted using the following reagents: (S)-N-tertbutoxycarbonylalaninal (0.50g, 2.89mmol), N¹-acetyl-N¹-methylhydrazine (0.38g, 4.31mmol) and methyl propenoate (0.75g, 8.66mmol) in toluene (50cm³). Purification by column chromatography eluting with hexane and ethyl acetate (1:1 v/v), followed by further purification by normal phase HPLC using a solvent system of 30% (v/v) CH₃CN in water gave the oxazoline (0.19g, 27%); δH (400MHz; CDCl₃) 1.36 (3H, d, J 7.3Hz, CH₃CH), 1.46 [9H, s, (CH₃)₃C], 2.32 (3H, s, CH₃CO), 3.19 (3H, s, CH₃N), 4.45 (1H, br., CHCH₃), 5.39 (1H, br., CHNCH₃) and 7.03 (1H, br.s, NH); δC (100MHz; CDCl₃) 18.86 (CH₃CH), 21.43 (CH₃CO), 27.32 (CH₃N), 28.57 [(CH₃)₃C], 47.93 (CHCH₃), 79.55
\[
[C(\text{CH}_3)_3], \ 141.97 \ (\text{CHNCH}_3), \ 155.32 \ (\text{C} = \text{N}) \ \text{and} \ 172.60 \ (\text{C} = \text{O}); \ m/\ell \ (\text{EI}) \ 244 (\text{MH}^+, 75\%), \ 204, 201, 144, 128, 99 (100), 59, 57, 43 \ \text{and} \ 41.
\]

\(\text{(S)}-N,N\text{-Dibenzylalanine benzyl ester (3.34)}\)

\[
\text{S-Alanine (8.16g, 91.59mmol) and benzyl bromide (48.31g, 282.45mmol) were dissolved in ethanol (1500cm}^3\). \ A \ solution \ of \ potassium \ carbonate \ (50.66g, 366.54mmol) \ in \ water (350cm}^3\) \ was \ added, \ and \ the \ solution \ stirred \ at \ 20^\circ\text{C for 48h. Water (300cm}^3\) \ was \ added \ and \ the \ aqueous \ layer \ extracted \ with \ ethyl \ acetate (3x250cm}^3\). \ The \ combined \ organic extracts \ were \ washed \ with \ sodium \ hydroxide \ solution (2M; 300cm}^3\) \ and \ then \ water (3x300cm}^3\). \ Following \ drying (\text{MgSO}_4) \ and \ removal \ of \ the \ solvent \ under \ reduced pressure, \ the \ crude \ product \ was \ purified \ by \ column \ chromatography \ using \ a \ solvent system \ of \ hexane \ and \ ethyl \ acetate (8:1 v/v) \ to \ give \ the \ product \ as \ a \ colourless \ oil (12.50g, 38\%); \ \delta_H (300MHz; \text{CDCl}_3) \ 1.34 \ (3\text{H}, \ d, J 7.1Hz, \text{CH}_3\text{CH}), \ 3.55 \ (1\text{H}, \ q, J 7.1Hz, \text{CHCH}_3), \ 3.62 \ (2\text{H}, \ d, J 14.0Hz, \text{NCH}_2\text{H}), \ 3.82 \ (2\text{H}, \ d, J 14.0Hz, \text{NCH}_2\text{H}), \ 5.14 \ (1\text{H}, \ d, J 12.4Hz, \text{CO}_2\text{CH}_2\text{H}), \ 5.22 \ (1\text{H}, \ d, J 12.4Hz, \text{CO}_2\text{CH}_2\text{H}) \ \text{and} \ 7.17-7.40 \ (15\text{H}, \ m, \text{Ar-CH}); \ \delta_C (75MHz; \text{CDCl}_3) \ 14.92 \ (\text{CH}_3\text{CH}), \ 54.38 \ (\text{C}_6\text{H}_5\text{CH}_3), \ 56.17 \ (\text{CHCH}_3), \ 65.98 \ (\text{C}_6\text{H}_5\text{CH}_3), \ 126.89 \ (\text{Ar-CH}), \ 128.19 \ (\text{Ar-CH}), \ 128.22 \ (\text{Ar-CH}), \ 128.28 \ (\text{Ar-CH}), \ 128.55 \ (\text{Ar-CH}), \ 128.60 \ (\text{Ar-CH}), \ 136.11 \ (\text{Ar-C}), \ 139.79 \ (\text{Ar-C}) \ \text{and} \ 173.50 \ (\text{C} = \text{O}); \ m/\ell \ (\text{EI}) \ 359 (\text{M}^+, 5\%), \ 268, 224, 181, 132, 91 (100) \ \text{and} \ 65.\)
(S)-2-(Dibenzylamino)propanol (3.35)

(S)-N,N-Dibenzylalanine benzyl ester (0.50g, 1.39mmol) was dissolved in anhydrous THF (40cm³) and cooled to -60°C. LiAlH₄ (0.79g, 20.82mmol) was carefully added and the mixture stirred for 1h. The reaction was quenched with a saturated solution of ammonium chloride (1.58g, 29.54mmol) in water and THF (20cm³), and the resulting mixture extracted with DCM (3x50cm³). The organic extracts were combined, dried (MgSO₄) and the solvent removed under reduced pressure. Purification by column chromatography eluting with hexane and ethyl acetate (4:1 v/v) gave the title compound as a colourless oil (0.23g, 65%); δ_H (400MHz; CDCl₃) 0.97 (3H, d, _J_ 6.9Hz, CH₂CH), 2.99 (1H, m, CHHOH), 3.14 (1H, m, CHHOH), 3.34 (2H, d, _J_ 13.7Hz, NCHH), 3.44 (1H, m, CHCH₃), 3.81 (2H, d, _J_ 13.7Hz, NCHH) and 7.15-7.33 (10H, m, Ar-CH); δ_C (100MHz; CDCl₃) 8.67 (CH₃CH), 52.58 (CH₂), 54.15 (CHCH₃), 62.71 (CH₂), 126.90 (Ar-CH), 128.09 (Ar-CH), 129.22 (Ar-CH) and 139.26 (Ar-C); m/z (EI) 255 (M⁺, 13%), 240, 224 (100), 181, 132, 91 (100), 65 and 28.

(S)-N,N-Dibenzylalaninal (3.32)

Oxalyl chloride (0.17g, 1.38mmol) was dissolved in dry DCM (20cm³). The solution was cooled to -60°C and DMSO (0.22g, 2.82mmol) was added and the solution then stirred for
2 min. After this time, (S)-2-(dibenzylamino)propanol (0.17 g, 0.67 mmol) was added to the mixture, which was then stirred for 15 min. The solution was treated with triethylamine (0.41 g, 4.02 mmol), stirred for 5 min and allowed to warm to 20°C. Water (5 cm³) was added and the aqueous layer extracted with DCM (3 x 15 cm³). The organic extracts were washed with hydrochloric acid (0.3 M; 20 cm³), water (20 cm³), saturated brine (20 cm³) and water (20 cm³) again. Drying the organic layer (MgSO₄) and removing the solvent under reduced pressure yielded the aldehyde (0.14 g, 83%) as a yellow oil; δ H (400 MHz; CDCl₃) 1.17 (3H, d, J 6.9 Hz, CH₃CH), 3.32 (1H, q, J 6.9 Hz, CHCH₃), 3.56 (2H, d, J 14.2 Hz, NCHH), 3.72 (2H, d, J 14.2 Hz, NCHH), 7.21-7.43 (10H, m, Ar-CH) and 9.72 (CHO); δ C (100 MHz; CDCl₃) 6.79 (CH₃CH), 54.94 (CHCH₃), 62.87 (CH₂), 127.49 (Ar-CH), 128.62 (Ar-CH), 129.28 (Ar-CH), 139.01 (Ar-C) and 202.42 (C=O).

(S)-3-(1-Dibenzylaminoethyl)-2,5-dimethyl-4-oxo-1-pyrazoline (3.38)

(S)-N,N-Dibenzylalaninal (0.12 g, 0.47 mmol), N¹-acetyl-N²-methylhydrazine (0.05 g, 0.56 mmol) and methyl propenoate (0.33 g, 3.89 mmol) were dissolved in toluene (50 cm³) and heated at reflux in the presence of 4 Å molecular sieves for 16 h. Following removal of the solvent under reduced pressure and purification by column chromatography eluting with hexane and ethyl acetate (1:1 v/v), the only pure product isolated was the title compound as a yellow oil (0.01 g, 7%), δ H (400 MHz; CDCl₃) 1.29 (3H, d, J 7.4 Hz, CH₃CH), 2.30 (3H, s, CH₃C=N), 3.07 (3H, s, CH₃N), 3.63 (2H, d, J 10.3 Hz, C₆H₅CHH), 3.67 (1H, m, CHCH₃), 3.71 (2H, d, J 10.3 Hz, C₆H₅CHH), 6.94 (1H, d, J 5.0 Hz, NCHO) and 7.22-7.40 (10H, m, Ar-CH).
2-Benzylloxypropanal (3.41)

Methyl 2-benzylloxypropionate (1.98g, 10.19mmol) was dissolved in anhydrous toluene (40cm$^3$) and cooled to -50°C. DIBAL (10.16cm$^3$ of a 1.2M solution in toluene, 12.40mmol) was added dropwise then the solution stirred for 2.5h. A saturated solution of sodium metabisulfite (60cm$^3$) was added and the mixture was allowed to warm to 20°C. The layers were separated and the organic phase further extracted with saturated sodium metabisulfite solution (3x80cm$^3$). The combined aqueous layers were washed with ether (3x60cm$^3$) then cooled to 0°C and the pH raised to 11 with sodium hydroxide solution (8M). The solution was then extracted with ether (3x50cm$^3$), washed with saturated brine (75cm$^3$), dried (MgSO$_4$) and the solvent removed under reduced pressure. Purification by column chromatography using 9:1 (v/v) hexane:ethyl acetate as eluant gave the aldehyde as a colourless oil (0.41g, 25%); Found: MNH$_4^+$, 182.1183. C$_{10}$H$_{12}$O$_2$ requires MNH$_4^+$, 182.1181; $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3465, 3032, 2982, 2869, 2361, 1736, 1497, 1455, 1375 and 1094; $\delta_h$ (300MHz; CDCl$_3$) 1.31 (3H, d, $J$ 7.0Hz, CH$_3$), 3.87 (tH, dq, $J$ 1.7 & 7.0Hz, CHCH$_3$), 4.57 (1H, d, $J$ 11.7Hz, CHH), 4.64 (1H, d, $J$ 11.7Hz, CHH), 7.28-7.36 (SH, m, Ar-CH) and 9.65 (1H, d, $J$ 1.7Hz, CHO); $\delta_c$ (75MHz; CDCl$_3$) 15.26 (CH$_3$), 71.96 (CH$_2$), 79.41 (CHCH$_3$), 127.91 (Ar-CH), 128.04 (Ar-CH), 128.54 (Ar-CH), 137.37 (Ar-C) and 203.32 (CHO); m/z (Cl) 182 (MNH$_4^+$, 100%), 125, 108, 106, 91 and 61.
3-(1-Benzylxoyethyl)-2,5-dimethyl-4-oxo-1-pyrazoline (Fig. 3.9)

2-Benzylxopyranal (0.37g, 2.25mmol), \(N^1\)-Acetyl-\(N^2\)-methylhydrazine (0.20g, 2.27mmol) and methyl propenoate (0.40g, 4.66mmol) in toluene (25cm\(^3\)) were placed in a round-bottomed flask fitted with a Dean and Stark trap and heated at reflux for 24h. The solvent was removed under reduced pressure and the residue purified by column chromatography eluting with hexane and ethyl acetate (1:1 v/v). The only pure product isolated was the title compound as a yellow oil (0.06g, 11%), \(\delta_H\) (300MHz; CDCl\(_3\)) 1.40 (3H, d, \(J\) 6.4Hz, \(\text{CH}_3\)), 2.32 (3H, s, \(\text{CH}_3\text{C=NN}\)), 3.13 (3H, s, \(\text{CH}_3\text{N}\)), 4.18 (1H, quintet, \(J\) 6.4Hz, \(\text{CHCH}_3\)), 4.55 (2H, s, \(\text{C}_6\text{H}_5\text{CH}_2\)), 6.84 (1H, d, \(J\) 6.4Hz, NCHO) and 7.19-7.37 (5H, m, Ar-\(\text{CH}\)).

2-Methylpropanal Acetylhydrazone (3.45)

2-Methylpropanal (1.99g, 27.53mmol), acetylhydrazine (1.99g, 26.86mmol) and toluene (60cm\(^3\)) were placed in a flask fitted with a Dean and Stark trap. After heating at reflux for 2h, the solvent was removed under reduced pressure to yield the title compound (3.43g, 100%) as a colourless viscous oil; \(\delta_H\) (400MHz; CDCl\(_3\)) 1.10 [6H, d, \(J\) 6.8Hz, \((\text{CH}_3)_2\text{CH}\)], 2.24 (3H, s, \(\text{CH}_3\text{CO}\)), 2.50 [1H, m, \(\text{CH}(\text{CH}_3)_2\)], 7.17 (1H, d, \(J\) 5.5Hz, CH-N) and 10.34
2-Methylpropanal tert-Butoxycarbonylhydrazone (3.51)

The procedure for the synthesis of 2-methylpropanal acetylhydrazone was used with the following reagents: 2-methylpropanal (2.94g, 40.74mmol) and tert-butyl carbazate (4.98g, 37.68mmol). The product was isolated (3.44g, 49%) following column chromatography eluting with hexane and ethyl acetate (4:1 v/v); \( \delta \) (400MHz; CDCl₃) 1.09 [6H, d, \( J \) 6.8Hz, \((CH₃)₂CH]\), 1.50 [9H, s, \((CH₃)₃C]\), 2.58 [1H, m, \( CH(CH₃)₂ \)], 7.04 (1H, d, \( J \) 5.4Hz, \( CH=CH \) and 7.69 (1H, br.s, \( NH \)); \( \delta \)C (100MHz; CDCl₃) 19.90 [(CH₃)₂CH], 28.29 [(CH₃)₃C], 31.35 [CH(CH₃)₂], 80.92 [C(CH₃)₃], 152.27 and 152.60.

1-Acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole (3.58 & 3.59)

Benzaldehyde (1.20g, 11.31mmol), \( N^1 \)-acetyl-\( N^2 \)-methylhydrazine (1.05g, 11.92mmol) and methyl propenoate (1.53g, 17.77mmol) in toluene (70cm³) were placed in a flask fitted with a Dean and Stark trap. After heating at reflux for 72h, the solvent was removed using
a rotary evaporator and the crude product purified by column chromatography eluting with hexane:ethyl acetate (2:1 v/v) to give the syn diastereoisomer (0.50g, 17%) as a white solid, m.p. 47-50°C. The other diastereoisomer was also isolated (0.63g, 21%) but contained traces of an impurity. This was removed by further purification using normal-phase preparative HPLC with a solvent system of 0.375% methanol in DCM (v/v) to give the anti diastereoisomer (0.17g, 6%) as a white solid, m.p. 79-81°C. Also isolated from the reaction mixture was 1,4-diacetyl-2,5-dimethyl-3,6-diphenylhexahydrotetrazine (0.53g, 27%), formed by dimerisation of the dipole, as a white solid, m.p. 80-82°C. Syn isomer:

Found: C, 63.99; H, 6.96; N, 10.68%; M⁺, 262.1318. C₁₄H₁₈N₂O₃ requires C, 64.11; H, 6.92; N, 10.67%; M, 262.1317; νmax (KBr)/cm⁻¹ 2955, 2380, 1728, 1646, 1437, 1382, 1316, 1213, 1134, 1094, 732 and 697; δH (300MHz; CDCl₃) 2.16 (3H, s, CH₃CO), 2.74 (3H, s, CH₃N), 3.35 (3H, s, CO₂CH₃), 3.76 (1H, dd, J 9.7 & 11.6Hz, 5-CHH), 3.94 (1H, m, CHCO₂), 4.35 (1H, d, J 7.7Hz, C₆H₅CH), 4.45 (1H, dd, J 7.5 & 11.6Hz, 5-CHH), 7.05-7.14 (2H, m, Ar-CH) and 7.23-7.33 (3H, m, Ar-CH); δC (75MHz; CDCl₃) 21.08 (CH₃CO), 44.45 (5-CH₂), 46.17 (CH₃N), 47.84 (CHCO₂), 51.77 (CO₂CH₃), 72.36 (C₆H₅CH), 126.70 (Ar-CH), 127.95 (Ar-CH), 128.48 (Ar-CH), 137.90 (Ar-C), 170.06 (C=O) and 171.45 (C=O); m/z (EI) 262 (M⁺, 7%), 219 (100), 187, 159, 115, 91, 77, 57 and 43. Anti isomer:

Found: C, 64.12; H, 6.94; N, 10.61%; MH⁺, 263.1396. C₁₄H₁₈N₂O₃ requires C, 64.11; H, 6.92; N, 10.67%; MH, 263.1396; νmax (KBr)/cm⁻¹ 3456, 2956, 2889, 1738, 1656, 1464, 1412, 1382, 1208, 1053, 726 and 701; δH (300MHz; CDCl₃) 2.17 (3H, s, CH₃CO), 2.63 (3H, s, CH₃N), 3.45 (1H, m, CHCO₂), 3.76 (3H, s, CO₂CH₃), 3.86 (1H, m, 5-CHH), 4.43 (1H, m, 5-CHH), 4.47 (1H, d, J 5.9Hz, C₆H₅CH) and 7.27-7.37 (5H, m, Ar-CH); δC (75MHz; CDCl₃) 20.83 (CH₃CO), 44.51 (CH₃N), 45.02 (5-CH₂), 50.87 (CHCO₂), 52.62 (CO₂CH₃), 73.51 (C₆H₅CH), 126.68 (Ar-CH), 127.90 (Ar-CH), 128.77 (Ar-CH), 139.86 (Ar-C), 171.05 (C=O) and 172.72 (C=O); m/z (EI) 263 (MH⁺, 10%), 219, 187, 159, 131, 117, 115, 91, 77, 57 and 43 (100). Tetrazine: Found: C, 68.18; H, 6.87; N, 15.86%; MH⁺,
353.1986. $C_{20}H_{24}N_4O_2$ requires C, 68.16; H, 6.86; N, 15.89; MH, 353.1977; $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3020, 2975, 1682, 1602, 1573, 1462, 1411, 1398, 1142, 995, 762 and 698; $\delta_{\text{H}}$ (300MHz; CDCl$_3$) 2.46 (6H, s, CH$_3$CO), 3.34 (6H, s, CH$_3$N), 7.34-7.44 (6H, m, Ar-CH) and 7.59-7.72 (6H, m, Ar-CH and NCHN); (75MHz; CDCl$_3$) 21.59 (CH$_3$CO), 27.47 (CH$_3$N), 126.951 (Ar-CH), 128.74 (Ar-CH), 129.62 (Ar-CH), 134.81 (Ar-C), 138.72 (NCHN) and 172.88 (C=O); $m/z$ (EI) 176 (M$^+$, 5%), 133, 90, 89, 77, 63, 51 and 43 (100).

1-Acetyl-4-cyano-2-methyl-3-phenyltetrahydropyrazole (3.67 & 3.68)

![Chemical Structure]

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole was used with the following reagents: benzaldehyde (0.94g, 8.85mmol), $N^1$-acetyl-$N^2$-methylhydrazine (0.76g, 8.63mmol), propenonitrile (0.93g, 17.47mmol) and toluene (40cm$^3$). Purification yielded one pure diastereoisomer of the title compound (0.29g, 15%), assumed to have syn relative stereochemistry, as a pale yellow solid, m.p. 150-153°C. The other diastereoisomer (0.20g, 10%), presumed to be anti, contained traces of an impurity (which appeared to be the 5-cyano isomer) and was obtained as a yellow solid. Syn isomer: Found: C, 68.09; H, 6.64; N, 18.06%; M$^+$, 229.1216. $C_{13}H_{15}N_3O$ requires C, 68.10; H, 6.59; N, 18.32%; M, 229.1215; $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 2998, 2964, 2895, 2246, 1656, 1470, 1453, 1444, 1414, 1373, 758 and 712; $\delta_{\text{H}}$ (300MHz; CDCl$_3$) 2.21 (3H, s, CH$_3$CO), 2.72 (3H, s, CH$_3$N), 3.78 (1H, ddd, $J$ 5.6, 6.6 & 8.2Hz, CHCN), 3.87 (1H, dd, $J$ 8.2 & 11.4Hz, 5-CHH), 4.32 (1H, d, $J$ 6.6Hz, C$_2$H$_5$CH), 4.41 (1H, dd, $J$ 5.6 & 11.4Hz, 5-CHH), 7.25-7.27 (2H, m, Ar-CH) and 7.31-7.43 (3H, m,
Ar-CH); δc (75MHz; CDCl₃) 20.90 (CH₃CO), 34.90 (CHCN), 45.83 (CH₃N), 46.50 (5-CH₂), 72.29 (C₆H₅CH), 117.07 (CN), 127.00 (Ar-CH), 128.88 (Ar-CH), 128.94 (Ar-CH), 135.85 (Ar-C) and 172.09 (C=O); m/z (EI) 229 (M⁺, 5%), 186 (100), 159, 142, 133, 115, 91, 77, 57 and 43. *Anti* isomer: Found: C, 67.90; H, 6.57; N, 18.26%; M⁺, 229.1226. C₁₃H₁₅N₃O requires C, 68.10; H, 6.59; N, 18.32%; M, 229.1215; vmax (KBr)/cm⁻¹ 3035, 2994, 2963, 2922, 2883, 2802, 2247, 1662, 1496, 1398, 1344, 1319, 1216, 1110, 768, 738 and 702; δₜ (300MHz; CDCl₃) 2.14 (3H, s, CH₃CO), 2.75 (3H, s, CH₃N), 3.44 (1H, m, CHCN), 3.84 (1H, dd, J 7.1 & 11.9Hz, 5-CH₃), 4.46 (1H, d, J 6.0Hz, C₆H₅CH), 4.49 (1H, m, 5-CH₂H), 7.26-7.32 (2H, m, Ar-CH) and 7.34-7.42 (3H, m, Ar-CH); δc (75MHz; CDCl₃) 20.82 (CH₃CO), 34.96 (CHCN), 44.48 (CH₃N), 46.04 (5-CH₂), 74.21 (C₆H₅CH), 119.38 (CN), 126.52 (Ar-CH), 128.74 (Ar-CH), 129.11 (Ar-CH), 137.26 (Ar-C) and 171.55 (C=O); m/z (EI) 229 (M⁺, 1%), 186, 143, 133, 115, 91, 77, 57 and 43 (100).

1-Acetyl-4,5-dimethoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole (*all-syn*) (3.70)

![Chemical structure](image)

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole was used with the following reagents: benzaldehyde (0.56g, 5.31mmol), N¹-acetyl-N²-methylhydrazine (0.47g, 5.33mmol), dimethyl maleate (1.66g, 11.52mmol) and toluene (40cm³). Analysis of the products following purification revealed the presence of the tetrazine from dipole dimerisation (not isolated) and an inseparable mixture of stereoisomeric tetrahydropyrazoles (0.43g, 25%). The only pure product to be isolated was the *all-syn* tetrahydropyrazole (0.18g, 11%), a white solid m.p. 155-158°C;
Found: C, 59.78; H, 6.32; N, 8.58%; M⁺, 320.1372. C₁₆H₂₀N₂O₅ requires C, 59.99; H, 6.29; N, 8.74%; M, 320.1372; ν_max (KBr)/cm⁻¹ 3007, 2953, 1765, 1747, 1641, 1494, 1455, 1433, 1377, 1356, 1342, 1198, 1168, 1110, 741 and 700; δ_H (300MHz; CDCl₃) 2.33 (3H, s, CH₃CO), 2.78 (3H, s, CH₃N), 3.53 (3H, s, CO₂CH₃), 3.66 (3H, s, CO₂CH₃), 4.23 (1H, dd, J 7.9 & 10.1Hz, 4-CHCO₂), 4.50 (1H, d, J 7.9Hz, C₆H₅CH), 4.85 (1H, d, J 10.1Hz, 5-CHCO₂) and 7.24-7.38 (5H, m, Ar-CH); δ_C (75MHz; CDCl₃) 21.18 (CH₃CO), 45.23 (CH₃N), 51.18 (4-CHCO₂), 51.98 (CO₂CH₃), 52.24 (CO₂CH₃), 59.00 (5-CHCO₂), 71.65 (C₆H₅CH), 127.97 (Ar-CH), 128.01 (Ar-CH), 128.09 (Ar-CH), 136.08 (Ar-C), 167.93 (C=O), 168.74 (C=O) and 169.57 (C=O); m/z (EI) 321 (MH⁺, 2%), 278, 219, 159, 133, 115, 91, 77, 59 and 43 (100).

1-Acetyl-4,5-dimethoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole (anti-anti) (3.71)

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole was used with the following reagents: benzaldehyde (0.61g, 5.71mmol), N¹-acetyl-N²-methylhydrazine (0.51g, 5.79mmol), dimethyl fumarate (1.68g, 11.66mmol) and toluene (40cm³). Along with an 18:5 mixture of the title compound and the syn-anti diastereoisomer (0.49g, 27%), the title compound was isolated (0.69g, 38%) as a white solid, m.p. 152-154°C; Found: C, 59.89; H, 6.27; N, 8.71%; M⁺, 320.1368. C₁₆H₂₀N₂O₅ requires C, 59.99; H, 6.29; N, 8.74%; M, 320.1372; ν_max (KBr)/cm⁻¹ 2991, 2963, 2361, 1765, 1732, 1654, 1435, 1409, 1215, 764 and 708; δ_H (300MHz; CDCl₃) 2.27
(6H, s, CH₃CO & CH₃N), 3.71 (3H, s, CO₂CH₃), 3.81 (3H, s, CO₂CH₃), 4.03 (1H, dd, J 8.3 & 10.8Hz, 4-CHCO₂), 4.58 (1H, d, J 10.8Hz, C₆H₅CH), 5.16 (1H, d, J 8.3Hz, 5-CHCO₂) and 7.33-7.43 (5H, m, Ar-CH); δc (75MHz; CDCl₃) 20.95 (CH₃CO), 39.75 (CH₃N), 49.79 (4-CHCO₂), 52.84 (CO₂CH₃), 52.97 (CO₂CH₃), 62.33 (5-CHCO₂), 71.90 (C₆H₅CH), 128.82 (Ar-CH), 128.83 (Ar-CH), 128.85 (Ar-CH), 132.85 (Ar-C), 170.93 (C=O), 171.00 (C=O) and 171.18 (C=O); m/z (EI) 320 (M⁺, 12%), 278, 245, 217, 185, 159, 115, 91, 77, 59 and 43 (100).

2-Acetyl-3-methyl-6,8-dioxo-4,7-diphenyl-2,3,7-triazabicyclo[3.3.0]octane (anti-syn) (3.74)

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole was used with the following reagents: benzaldehyde (0.63g, 5.90mmol), N₁-acetyl-N₂-methylhydrazine (0.55g, 6.24mmol), N-phenylmaleimide (2.05g, 11.84mmol) and toluene (40cm³). Purification by column chromatography, eluting with hexane:ethyl acetate (3:2 v/v) yielded the product (0.08g, 4%) as a yellow solid, m.p. 239-241°C; Found: C, 68.43; H, 5.44; N, 11.58%; M⁺, 349.1422. C₂₀H₁₉N₃O₃ requires C, 68.75; H, 5.44; N, 12.02%; M, 349.1426; νmax (KBr)/cm⁻¹ 1719, 1653, 1499, 1386, 1199, 1158, 746 and 696; δH (300MHz; CDCl₃) 2.03 (3H, s, CH₃CO), 2.87 (3H, s, CH₃N), 4.02 (1H, d, J 8.6Hz, C₆H₅CHCHCO), 4.73 (1H, s, C₆H₅CH), 5.80 (1H, d, J 8.6Hz, NCHCO) and 7.18-7.53 (10H, m, Ar-CH); δc (75MHz; CDCl₃) 20.88 (CH₃CO), 47.55 (CH₃N), 53.11 (C₆H₅CHCHCO) 58.42 (NCHCO), 73.07 (C₆H₅CH), 125.82 (Ar-CH), 125.99 (Ar-
CH), 128.28 (Ar-CH), 129.07 (Ar-CH), 129.12 (Ar-CH), 129.39 (Ar-CH), 131.51 (Ar-C), 139.67 (Ar-C), 172.99 (C=O) and 175.80 (C=O); m/z (EI) 350 (MH\(^+\), 82%), 349 (100), 307, 159, 133, 115, 91, 77 and 43.

1-Acetyl-5-methoxycarbonyl-2-methyl-4-methylaminocarbonyl-3-phenyltetrahydropyrazole (3.75) & 1-Acetyl-2-methyl-4,5-bis(methylaminocarbonyl)-3-phenyltetrahydropyrazole (3.76)

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

1-Acetyl-4,5-dimethoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole (0.14g, 0.43mmol) was placed in a screw-cap vial along with sodium cyanide (2.7mg, 0.06mmol) and a solution of methylamine in methanol (2M; 0.22cm\(^3\), 0.44mmol). The mixture was heated in an oil bath at 50-55°C for 16h. The solvent was then removed under reduced pressure and the crude material purified by column chromatography using ethyl acetate as eluant to give the mono-amide product (0.05g, 38%) as a colourless oil and the di-amide product (0.04g, 33%) as a white solid, m.p. 208-210°C; Mono-amide: Found: MH\(^+\), 320.1614. C\(_{16}\)H\(_{21}\)N\(_3\)O\(_4\) requires MH, 320.1610; \(\nu_{\text{max}}\) (CHCl\(_3\))/cm\(^{-1}\) 3680, 3341, 2956, 1734, 1681, 1636, 1556, 1438, 1416 and 1247; \(\delta\)\(_{\text{H}}\) (300MHz; CDCl\(_3\)) 2.14 (3H, s, CH\(_3\)CO), 2.28 (3H, s, CH\(_3\)NN), 2.85 (3H, d, J 5.0Hz, CH\(_3\)NHCO), 3.66 (3H, s, CO\(_2\)CH\(_3\)), 4.54 (1H, d, J 11.5Hz,
C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}), 4.78 (1H, dd, J 7.5 & 11.5Hz, CHCONH), 5.08 (1H, d, J 7.5Hz, CHCO\textsubscript{2}), 7.33-7.44 (4H, m, Ar-CH & NH) and 7.46-7.50 (2H, m, Ar-CH); \[^{13}C\] (75MHz; CDCl\textsubscript{3}) 20.99 (CH\textsubscript{3}CO), 26.56 (CH\textsubscript{3}NHCO), 39.37 (CH\textsubscript{3}NN), 46.15 (CHCONH), 52.63 (CO\textsubscript{2}CH\textsubscript{3}), 63.29 (CHCO\textsubscript{2}), 71.46 (C\textsubscript{6}H\textsubscript{5}CH), 128.74 (Ar-CH), 128.79 (Ar-CH), 129.27 (Ar-CH), 132.95 (Ar-C), 169.80 (C=O), 172.04 (C=O) and 173.03 (C=O); \[^{1}H\] (300MHz; CDCl\textsubscript{3}) 2.13 (3H, s, CH\textsubscript{3}CO), 2.30 (3H, s, CH\textsubscript{3}NHCO), 2.76 (3H, d, J 4.7Hz, CH\textsubscript{3}NHCO), 2.84 (3H, d, J 5.0Hz, CH\textsubscript{3}NHCO), 4.38 (1H, dd, J 8.3 & 11.6Hz, 4-CH), 4.75 (1H, d, J 11.6Hz, C\textsubscript{6}H\textsubscript{5}CH), 4.86 (1H, d, J 8.3Hz, 5-CH), 7.04 (1H, br.d, J 4.7Hz, NH), 7.30-7.41 (5H, m, Ar-CH) and 7.65 (1H, br.d, J 5.0Hz, NH); \[^{13}C\] (75MHz; CDCl\textsubscript{3}) 21.16 (CH\textsubscript{3}CO), 26.43 (CH\textsubscript{3}NHCO), 26.58 (CH\textsubscript{3}NHCO), 39.69 (CH\textsubscript{3}NN), 46.57 (4-CHCONH), 63.96 (5-CHCONH), 69.58 (C\textsubscript{6}H\textsubscript{5}CH), 128.56 (Ar-CH), 128.74 (Ar-CH), 128.98 (Ar-CH), 133.65 (Ar-C), 170.16 (C=O), 171.56 (C=O) and 174.05 (C=O); m/z (Cl) 319 (MH\textsuperscript{+}, 100%), 275, 191, 145, 131, 120, 106, 91 and 77.

Benzaldehyde Acetylhydrazone (3.81)

\[
\begin{align*}
\text{HOOC-} & \quad \text{CH}_3 \\
\text{N} & \quad \text{C} \\
\text{N} & \quad \text{H} \\
\end{align*}
\]

Benzaldehyde (26.22g, 0.25mol) and acetylhydrazine (18.30g, 0.25mol) in toluene (300cm\textsuperscript{3}) were placed in a flask fitted with a Dean and Stark trap. After heating at reflux for 16h, the solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel using ethyl acetate as eluant to give the product as a
white solid, (25.72g, 64%) m.p. 136.5-138.0°C (lit.148 137-140°C); $\nu_{\text{max}}$ (KBr)/cm\(^{-1}\) 3187, 3048, 2979, 2868, 1675, 1608, 1400, 1343, 1139, 1023 and 952; $\delta_H$ (400MHz; CDCl\(_3\)) 2.42 (3H, s, CH\(_3\)), 7.40 (3H, m, Ar-CH), 7.68 (2H, m, Ar-CH), 7.89 (1H, s, CH=N) and 10.56 (1H, br.s, NH); $\delta_C$ (100MHz; CDCl\(_3\)) 20.39 (CH\(_3\)), 127.14 (Ar-CH), 128.71 (Ar-CH), 130.06 (Ar-CH), 133.92 (Ar-C), 144.05 (CH=N) and 177.52 (C=O).

$N^2$-Acetyl-$N^1$-benzylhydrazine-cyanoborate (3.82)

\[
\begin{align*}
\begin{array}{c}
\text{N} & \text{N} \\
\text{O} & \text{CH}_3 \\
\end{array} & \xrightarrow{\text{ }} & \\
\begin{array}{c}
\text{N} & \text{N} \\
\text{O} & \text{CH}_3 \\
\end{array}
\end{align*}
\]

To benzaldehyde acetylhydrazone (4.62g, 28.48mmol) and sodium cyanoborohydride (7.18g, 114.26mmol) in anhydrous THF (250cm\(^3\)) was added a few drops of bromocresol green indicator. para-Toluenesulfonic acid monohydrate (21.98g, 114.35mmol) in anhydrous THF (50cm\(^3\)) was slowly added to maintain a pH of around 3.5, indicated by a light brown colour. After addition was complete, the suspension was stirred for 1h, then the sodium para-toluenesulfonate that had precipitated was filtered off. The solution was diluted with ethyl acetate (200cm\(^3\)) and washed with saturated brine (150cm\(^3\)), saturated sodium bicarbonate solution (150cm\(^3\)), and a second portion of saturated brine (150cm\(^3\)). After drying (MgSO\(_4\)), the solvent was removed under reduced pressure to give the crude product which was purified by column chromatography on silica gel using toluene and THF (1:1 v/v) as eluant to give the pure hydrazine salt (4.02g, 70%) as a white solid, m.p. 130-131°C; Found: [M-H], 202.1155. $C_{10}H_{14}BN_3O$ requires M-H, 202.1152; $\nu_{\text{max}}$ (KBr)/cm\(^{-1}\) 3205, 3111, 3067, 2965, 2816, 2450, 2421, 1663, 1560, 1416, 1373, 1290, 1222 and 1134; $\delta_H$ [300MHz; (CD\(_3\))\(_2\)SO] 1.71 (3H, s, CH\(_3\)CO), 3.79 (1H, dd, J 4.6 & 13.0Hz, C\(_6\)H\(_5\)CH\(_2\)), 4.20 (1H, d, J 13.0Hz, C\(_6\)H\(_5\)CH\(_3\)), 7.30 (3H, m, Ar-CH), 7.42 (2H, m, Ar-CH), 136.5-138.0°C (lit.)
Ar-CH), 9.72 (1H, br., NH) and 10.40 (1H, br., NH); δC [75MHz; (CD$_3$)$_2$SO] 19.61 (CH$_3$CO), 59.17 (C$_6$H$_5$CH$_2$), 127.79 (Ar-CH), 128.55 (Ar-CH), 131.05 (Ar-CH), 131.51 (C), 138.70 (C) and 166.36 (C=O); m/z (ES) 202 ([M-H$^-$], 100), 175, 161, 132 and 83.

$N^1$-Acetyl-$N^2$-benzylhydrazine (3.25)

\[
\begin{align*}
\text{N-Acetyl-N'-benzylhydrazinium cyanoborate (0.15g, 0.74mmol) was dissolved in water (2cm}^3\text{), sodium hydroxide solution (1M; 0.82cm}^3\text{, 0.82mmol) added, and the solution heated at 60°C for 1h. Ethyl acetate (5cm}^3\text{) was added, the organic layer separated and dried (MgSO}_4\text{), then the solvent removed under reduced pressure to yield the free hydrazine as a white solid (0.11g, 91%), a mixture of rotational isomers, m.p. 77-78°C (lit.\textsuperscript{149} 78°C); Found: MH$^+$, 165.1031. C$_9$H$_{12}$N$_2$O requires MH, 165.1028; v$_{\text{max}}$ (KBr)/cm$^{-1}$ 3311, 3228, 3027, 1641, 1537, 1496 and 1372; Major isomer: δ$_H$ (400MHz; CDCl$_3$) 1.89 (3H, s, CH$_3$CO), 3.94 (2H, s, C$_6$H$_5$CH$_2$), 4.75 (1H, br., NH), 7.32 (5H, m, Ar-CH) and 7.57 (1H, br., NH); δC (100MHz; CDCl$_3$) 21.10 (CH$_3$CO), 55.72 (C$_6$H$_5$CH$_2$), 127.56 (Ar-CH), 128.49 (Ar-CH), 128.91 (Ar-CH), 137.58 (Ar-C) and 169.77 (C=O); Minor isomer: δ$_H$ (400MHz; CDCl$_3$) 2.01 (3H, s, CH$_3$CO), 3.86 (2H, s, C$_6$H$_5$CH$_2$), 4.75 (1H, br., NH), 7.32 (5H, m, Ar-CH) and 7.57 (1H, br., NH); δC (100MHz; CDCl$_3$) 19.46 (CH$_3$CO), 56.93 (C$_6$H$_5$CH$_2$), 127.96 (Ar-CH), 128.60 (Ar-CH), 128.71 (Ar-CH), 136.50 (Ar-C) and 176.00 (C=O); m/z (FAB) 165 (MH$^+$, 61%), 106, 91 (100) and 43. \end{align*}
\]
1-Acetyl-2-benzyl-4-methoxycarbonyl-3-(2-propyl)-tetrahydropyrazole (3.29)

\[ \begin{array}{c}
\text{Ph} - \text{N} - \text{N} - \text{O} \\
\text{CH}_3
\end{array} \rightarrow \begin{array}{c}
\text{Ph} - \text{N} - \text{N} - \text{O} \\
\text{CH}_3
\end{array} \]

\[ \text{CO}_2\text{CH}_3 \]

\[ N^1\text{-Acetyl}-N^2\text{-benzylhydrazine (0.32g, 1.95mmol), 2-methylpropanal (0.15g, 2.09mmol) and methyl propenoate (0.52g, 6.00mmol) were placed in a glass tube with a screw-cap. Toluene (10cm}^3\text{) and 4Å molecular sieves were also placed in the tube, which was then sealed. The solution was heated for 72h, after which the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with hexane and ethyl acetate (2:1 v/v) to give the product (0.03g, 4\%) as a yellow oil; Found: MH}^+, 305.1863. \text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_3 \text{ requires MH}, 305.1865; \nu_{\text{max}} \text{(CHCl}_3\text{)} cm^{-1} 3021, 2963, 1733, 1641, 1437, 1369, 1216 and 700; \delta_H \text{(300MHz, CDCl}_3\text{)} 0.83 (3H, d, J 6.9Hz, \text{CH}_3\text{CH}), 0.85 (3H, d, J 7.1Hz, CH}_3\text{CH}), 1.39 [1H, m, CH(CH}_3\text{)2}], 1.92 (3H, s, CH}_3\text{CON), 3.06-3.12 [2H, m, CHCH(CH}_3\text{)2 & CHCO}_2\text{)], 3.75 (1H, m, 5-CHH), 3.79 (3H, s, CO}_2\text{CH}_3), 3.83 (1H, d, J 11.9Hz, C}_6\text{H}_5\text{CHH), 3.90 (1H, d, J 11.9Hz, C}_6\text{H}_5\text{CHH), 4.52 (1H, dd, J 9.8 &12Hz, 5-CHH) and 7.26-7.38 (5H, m, Ar-CH); \delta_C \text{(75MHz, CDCl}_3\text{)} 18.99 (CH}_3\text{CH), 20.09 (CH}_3\text{CH), 20.72 (CH}_3\text{CON), 32.51 [CH(CH}_3\text{)2], 44.65 (5-CH}_2\text{), 47.56 (CHCO}_2\text{), 52.54 (CO}_2\text{CH}_3), 61.84 (C}_6\text{H}_5\text{CH}_2), 74.86 [CHCH(CH}_3\text{)2], 127.93 (Ar-CH), 128.45 (Ar-CH), 130.29 (Ar-CH), 136.29 (Ar-C), 171.59 (C=O) and 174.37 (C=O); m/z (El) 305 (MH}^+, 2\%), 261, 171, 111, 91 (100), 69, 65 and 43.\]
Triethylamine (4.21g, 41.61mmol) was added to methylhydrazine (1.84g, 39.85mmol) in chloroform (30cm³). The solution was cooled to 0°C and benzyl chloroformate (5.81g, 34.04mmol) was added over a period of 30min. The solution was stirred at 20°C for 16h, then washed with water (10cm³). The organic layer was separated, dried (MgSO₄) and the solvent evaporated. The residue was distilled under reduced pressure to give the required hydrazine (3.49g, 57%) as a colourless liquid, b.p. 120°C/0.6mmHg (lit., 98-102°C/0.3mmHg); Found: C, 60.00; H, 6.84; N, 15.09%; MH⁺, 181.0975. C₉H₁₂N₂O₂ requires C, 59.99; H, 6.71; N, 15.54%; MH, 181.0977; v max (neat)/cm⁻¹ 3334, 2953, 1703, 1627, 1455, 1394, 1352, 1213, 1165 and 1002; δH (300MHz; CDCl₃) 3.10 (3H, s, CH₃N), 4.15 (2H, br.s, NH₂), 5.13 (2H, s, C₆H₅CH₂) and 7.31-7.36 (5H, m, Ar-CH); δC (75MHz; CDCl₃) 38.38 (CH₃N), 67.59 (C₆H₅CH₂), 128.00 (Ar-CH), 128.14 (Ar-CH), 128.51 (Ar-CH), 136.47 (Ar-C) and 157.50 (C=O); m/z (CI) 198 (MNH₄⁺, 54%), 183, 181 (MH⁺, 100), 137, 135, 122, 120, 108, 106, 91, 64 and 61.

N₁-Benzoxycarbonyl-N₂-tert-butoxycarbonyl-N₁-methylhydrazine (3.86)

N₁-Benzoxycarbonyl-N₁-methylhydrazine (5.73g, 31.80mmol) was dissolved in propan-2-ol (40cm³) and cooled to 0°C. A solution of Boc₂O (7.71g, 35.33mmol) in DCM
(15cm³) was added dropwise, then the reaction mixture allowed to warm to 20°C and stirred for 16h. The solvent was removed under reduced pressure and the crude product purified by column chromatography eluting with hexane and ethyl acetate (3:1 v/v). Recrystallisation from hexane and ether (10:1 v/v) gave the tri-substituted hydrazine (4.75g, 53%) as a white solid, m.p. 58-59°C (lit.,¹⁵⁰ 61-62°C); Found: C, 60.08; H, 7.22; N, 10.03%; MH⁺, 281.1502. \( \text{C}_{14}\text{H}_{20}\text{N}_{20}\text{O}_{4} \) requires C, 59.99; H, 7.19; N, 9.99%; MH, 281.1501; \( \nu_{\text{max}} \) (KBr)/cm⁻¹ 3271, 3150, 2966, 1697, 1462, 1422, 1368 and 1154; \( \delta_{\text{H}} \) (300MHz; CDCl₃) 1.43 [9H, br.s, (CH₃)₃C], 3.19 (3H, s, CH₃N), 5.15 (2H, s, C₆H₅CH₂), 6.52 (1H, br.s, NH) and 7.32-7.34 (5H, m, Ar-CH); \( \delta_{\text{C}} \) (75MHz; CDC₁₃) 28.13 [(CH₃)₃C], 37.99 & 38.42 (CH₃N), 67.95 (C₆H₅CH₂), 81.61 [C(CH₃)₃], 127.99 (Ar-CH), 128.18 (Ar-CH), 128.47 (Ar-CH), 136.03 (Ar-C), 154.69 & 155.25 (C=O) and 156.38 & 156.76 (C=O); \( m/z \) (CI) 298 (MNH⁺, 21%), 281 (MH⁺, 2%), 242, 198, 183, 181, 147, 108 (100), 106 and 91.

\( \text{N}^1\text{-tert-Butoxycarbonyl-N}^2\text{-methylhydrazine (3.83)} \)

\( \text{N}^1\text{-Benzyloxycarbonyl-N}^2\text{-tert-butoxycarbonyl-N}^1\text{-methylhydrazine (2.00g, 7.14mmol)} \) was dissolved in methanol (40cm³). A catalyst of 10% palladium on charcoal (0.12g, 11.28mmol) was added and the solution was stirred at 20°C under an atmosphere of hydrogen for 16h. The catalyst was filtered off and washed with methanol (3x20cm³). The solvent was removed under reduced pressure and the product purified by column chromatography using a solvent of hexane and ethyl acetate (4:1 v/v) to give the title compound (0.36g, 35%) as a white solid m.p. 42-45°C (lit.,¹⁴¹ 46-49°C); \( \nu_{\text{max}} \) (KBr)/cm⁻¹
3321, 3260, 2983, 1699, 1559, 1489, 1368, 1274, 1253, 1175 and 1143; \( \delta_H \) (300MHz; CDCl\textsubscript{3}) 1.47 [9H, s, \((\text{CH}_3)_3\text{C}\)], 2.62 (3H, s, CH\textsubscript{3}N), 3.89 (1H, br.s, NHCH\textsubscript{3}) and 6.14 (1H, br.s, NHC=O); \( \delta_C \) (75MHz; CDCl\textsubscript{3}) 28.38 [\text{CH}_3\text{C}], 39.36 (CH\textsubscript{3}N), 80.46 [C(CH\textsubscript{3})\textsubscript{3}] and 156.66 (C=O).

1-\text{tert}-Butoxycarbonyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole

(3.87)

\[
\begin{array}{c}
\text{H}_3\text{C} \quad \text{N} \quad \text{N} \quad \text{O} \quad \text{O} \\
\text{H}_3\text{C} \quad \text{N} \quad \text{N} \quad \text{O} \quad \text{O} \\
\text{Ph} \quad \text{CO}_2\text{CH}_3
\end{array}
\]

The procedure for the synthesis of 1-acetyl-4-methoxycarbonyl-2-methyl-3-phenyltetrahydropyrazole was used with the following reagents: benzaldehyde (0.26g, 2.46mmol), \( \text{N}^1-\text{tert}-\text{butoxycarbonyl-N}^2-\text{methylhydrazine} \) (0.35g, 2.39mmol), methyl propenoate (0.42g, 4.89mmol) and toluene (25cm\textsuperscript{3}). Purification by column chromatography eluting with hexane and ethyl acetate (2:1 v/v) led to isolation of the \( \text{N}-\text{Boc tetrahydropyrazole} \) (0.14g, 18%) as a colourless oil; Found: \( M^+ \), 320.1735. \( \text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_4 \) requires \( M, 320.1736; \nu_{\text{max}} \text{ (CHCl}_3)/\text{cm}^{-1} 2982, 1739, 1710, 1390, 1369, 1312, 1169 and 1121; \( \delta_H \) (300MHz, CDCl\textsubscript{3}) 1.54 [3H, s, \((\text{CH}_3)_3\text{C}\)], 2.72 (3H, s, CH\textsubscript{3}N), 3.42 (3H, s, \text{CO}_2\text{CH}_3), 3.72 (2H, m, CHCO\textsubscript{2} & 5-CHH), 4.00 (1H, dd, J 10.8 & 14.3Hz, 5-CHH), 4.32 (1H, d, J 6.4Hz, C\text{C}_6\text{H}_5\text{CH}) and 7.17-7.30 (5H, m, Ar-CH); \( \delta_C \) (75MHz, CDCl\textsubscript{3}) 28.52 [\text{CH}_3\text{C}], 45.54 (CH\textsubscript{3}N), 46.36 (5-CH\textsubscript{2}), 48.33 (CHCO\textsubscript{2}), 51.65 (\text{CO}_2\text{CH}_3), 71.61 (C\text{C}_6\text{H}_5\text{CH}), 80.39 [\text{C(CH}_3)_3], 126.70 (Ar-CH), 127.69 (Ar-CH), 128.27 (Ar-CH), 137.85 (Ar-C), 154.79 (CON) and 170.19 (\text{CO}_2\text{CH}_3); \text{m/z} \text{ (EI)} 320 (M\textsuperscript{+}, 3%), 265, 219, 187, 159, 133, 115, 91, 77, 57 (100) and 41.
References


