Cycloaddition routes to pyrazole and pyrazoline amino acids

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Cycloaddition Routes to Pyrazole and Pyrazoline Amino Acids

A thesis submitted in fulfilment of the requirements for the award of the degree

Doctor of Philosophy

From

Loughborough University

by

Laura Elizabeth Seager, MChem

June 2009

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Abstract

In recent years, the design and synthesis of structures that can potentially mimic the properties of the peptide bond have been of great interest to biological chemists. We are investigating the synthesis of novel pyrazoline-based structures as potential peptide mimetics. The pyrazoline unit is assembled by 1,3-dipolar cycloaddition of nitrile imines, which are generated \textit{in-situ} from hydrazonyl chlorides. We have investigated two routes to afford the hydrazonyl chloride: 1) \textit{via} a hydrazone; and 2) \textit{via} a hydrazide both of which have resulted in the successful synthesis of the desired pyrazolines. Subsequent syntheses have been carried out using a variety of different dipoles and dipolarophiles.

We have taken approach 1 and used this to synthesize a pyrazole as one major enantiomer.

![Pyrazole structure]

This pyrazole has been subject to peptide couplings to form a complete peptide mimetic.
NMR studies have been carried out on the synthesized peptide mimetic to determine the degree of hydrogen bonding and β-turn characteristics.
Acknowledgements

First and foremost I must thank my supervisor Professor Ray Jones for his guidance and support. He has proven to be everything that I wanted in a PhD supervisor and I am eternally grateful for the help he has given me over the last 4 years. With that I must thank all of the members of the Jones Group who I have seen progress throughout their studies and go on to successful careers in chemistry.

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I would also like to thank the following Chemistry Department technical staff; John Kershaw, Alistair Daley, Andy Kowalski and Dr Mark Elsegood, for their help with collaborating data for this thesis. Special thanks must go to Dr Mark Edgar for letting me be “trustee” of the department NMR facility and for teaching me all that a student needs to know (and possibly more) about NMR.

Finally I must thank my parents Malcolm and Julie for supporting me in everything that I do and also for letting me convince them that a PhD was the right path to take.
Abbreviations

Å Ångström
Ar Aromatic
Bn Benzyl
Boc tert-Butyloxycarbonyl
br Broad
CAN Ceric ammonium nitrate
Cbz Benzoyloxycarbonyl
d Doublet
DCC N,N'-Dicyclohexylcarbodiimide
DCM Dichloromethane
DEAD Dethyl azodicarboxylylate
DEPT Distortionless Enhancement by Polarisation Transfer
DIBAL Diisobutylaluminium hydride
DIEPA Diisopropylethylamine
DMAP 4-(Dimethylamino)pyridine
DMF N,N-Dimethylformamide
DMS Dimethyl sulphide
DMSO Dimethyl sulfoxide
EDCI 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EI Electron ionisation
FAB Fast atom bombardment
Fmoc Fluorenylmethyloxycarbonyl
h Hour(s)
HOBt N-Hydroxybenzotriazole
HOMO Highest occupied molecular orbital
HPLC High Performance Liquid Chromatography
IR Infra-red
J Coupling constant (NMR spectroscopy)
K Kelvin
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>LDA</td>
<td>Lithium diisopropyl amide</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>NCS</td>
<td>N-Chlorosuccinimide</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
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<td>q</td>
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<td>RT</td>
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<td>Triplet</td>
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<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THAC</td>
<td>Tetrahexylammonium chloride</td>
</tr>
<tr>
<td>Ts</td>
<td>para-Toluenesulfonyl</td>
</tr>
<tr>
<td>VT</td>
<td>Variable Temperature</td>
</tr>
</tbody>
</table>
4.0 Experimental
4.1 General Procedures
4.2 Experimental Procedures

5.0 References

Appendix I X-Ray Crystal Data
Appendix II HPLC Spectra and Data
1.0 Introduction

1.1 1,3-Dipolar Cycloaddition

1,3-Dipolar cycloaddition is a classic reaction in organic synthesis. The scope of this type of reaction and its popularity has been confirmed in the last ten to twenty years by the number of books and reviews that have been published on the subject.\textsuperscript{1,2} 1,3-Dipolar cycloaddition can be described simply as the addition of a 1,3-dipole to an unsaturated system such as an alkene for the synthesis of five-membered heterocycles. If we look historically at the 1,3-dipolar cycloaddition reaction we have to go as far back as 1883 when Curtius discovered diazoacetic ester,\textsuperscript{3} a 1,3-dipole, but it was then his younger colleague, Buchner who studied the reactions of diazoacetic ester with \(\alpha,\beta\)-unsaturated esters\textsuperscript{4} and therefore went on to describe the first 1,3-dipolar cycloaddition reaction. In a matter of years Beckmann, and Werner and Buss, discovered nitrones\textsuperscript{5} and nitrile oxides\textsuperscript{6} respectively. Therefore the evolution of 1,3-dipolar cycloaddition reactions has been over 100 years in the making, during which time many more dipoles have been discovered and classified.

It was Huisgen who, in the 1960's, established the general application of 1,3-dipoles in organic synthesis.\textsuperscript{7} This, along with work by Woodward and Hoffman regarding the concept of conservation of orbital symmetry\textsuperscript{8} led to a new understanding of the mechanism of concerted cycloadditions and in addition gave chemists the ability to predict relative reactivity and regioselectivity.
In simple terms, a 1,3-dipole can be shown as an $a\,b\,c$ structure that reacts with, for example, alkenes to give a five-membered heterocycle (Fig 1).  

![Fig 1](image)

**Allyl anion type**

![Fig 2](image)

**Propargyl/allenyl anion type**
The term 1,3-dipole was given because it is impossible to write electron paired resonance structures for these species without incorporating charges. This does not mean that the compounds are particularly polar as the charges are not localized.

1,3-Dipoles can be categorized into two different types (Fig 2). The allyl anion type is characterized by four electrons in three parallel p\textsubscript{z} orbitals which are perpendicular to the plane of the dipole and that the 1,3-dipole is bent. This allyl anion type 1,3-dipole can be drawn as resonance structures, two in which the three atoms have an electron octet and two in which \( a \) or \( c \) has an electron sextet. The central atom \( b \) can either be nitrogen, oxygen or sulfur, but must have a lone pair.

The second type is known as the propargyl/allenyl anion type whereby an extra \( \pi \) orbital is located in the plane orthogonal to the allenyl anion type molecular orbital. Therefore the former orbital is not involved in the resonance structures and reactions of this dipole. In the case of the propargyl/allenyl anion the central atom \( b \) is limited only to nitrogen.

The vast majority of 1,3-dipoles consist mainly of elements from groups IV, V and VI in the periodic table. As the main elements used are carbon, nitrogen and oxygen, this leads to a possible 18 dipoles; 12 of the allyl anion type and 6 of the propargyl/allenyl anion type (Fig 3).
Allyl anion type

Nitrones

Azomethine Imines

Carbonyl Ylides

Carbonyl Imines

Azomethine Ylides

Carbonyl Oxides

Azimines

Nitrosimines

Azoxy Compounds

Nitrosoxides

Nitro Compounds

Ozone
Propargyl/allenyl anion type

<table>
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<th>Propargyl/Allenyl Anion Type</th>
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Nitrile Oxides

Diazalkanes

Nitrile Imines

Azides

Nitrile Ylides

Nitrous Oxide

Fig 3 Different Dipole Structures

The transition state of the concerted 1,3-dipolar cycloaddition reaction is controlled by the frontier molecular orbitals of the substrates. It was Sustmann who classified 1,3-dipolar cycloaddition reactions into three types based upon the relative frontier molecular orbital energies between the dipole and the dipolarophile (Fig 4).\(^\text{10}\)
Fig 4 Molecular orbital description of the three types of interaction

For type I, 1,3-dipolar cycloaddition reactions the dominant interaction is that of the HOMO<sub>dipole</sub> with the LUMO<sub>dipolarophile</sub>. For type II, the FMO energies of the dipole and dipolarophile are similar and therefore this implies that both HOMO-LUMO interactions are important. Finally, 1,3-dipolar cycloaddition reactions of type III are dominated by the interaction between the LUMO<sub>dipole</sub> and HOMO<sub>dipolarophile</sub>.

Certain dipoles have been categorized into these types. 1,3-Dipolar cycloaddition reactions of type I are common with dipoles such as azomethine ylides and azomethine imines. Reactions of nitrones generally come under the category of type II along with nitrile oxides, but some classify these as borderline type III since nitrile oxides have low lying HOMO energies. Finally, dipoles such as ozone and nitrous oxide come under the type III heading. These groupings are not exclusive as the introduction of...
electron-donating and electron-withdrawing substituents on the dipole or the alkene can alter the frontier molecular orbital energies.

Reactions are therefore favoured if one component is strongly nucleophilic and the other is strongly electrophilic. Therefore, for a given 1,3-dipole, the dominant interaction is more likely to be of type I for dipolarophiles with conjugative electron-withdrawing groups and for type III, cycloadditions are most likely to take place with enol ethers, enamines and other electron-rich dipolarophiles.

Arguably the most well known and commonly taught example of a 1,3-dipolar cycloaddition reaction is the ozonolysis of an alkene bond. The reagent for this type of reaction is ozone $\text{O}_3$. Ozone is a symmetrical bent molecule with a central positively charged oxygen and two terminal oxygen atoms carrying the negative charge; it is a typical 1,3-dipole. The mechanism for the reaction is shown below (Scheme 1)

![Scheme 1](image)

The product of the cycloaddition reaction is a very unstable compound due to the weak O-O single bond. It immediately decomposes by a reverse 1,3-dipolar cycloaddition to give a simple aldehyde, or ketone, and a new 1,3-dipole known as a carbonyl oxide. This, being a 1,3-dipole, adds to the carbonyl compound in a third cycloaddition step. It is possible that it could add back reversing the way that it was formed but it prefers to
add with the opposite regiochemistry with the nucleophilic oxyanion attacking the carbon atom of the carbonyl group as shown below (Scheme 2).

Scheme 2

The product of this 1,3-dipolar cycloaddition reaction is known as an ozonide. This is often decomposed using dimethyl sulfide, which attacks the ozonide to give DMSO and two molecules of aldehyde (Scheme 3).

Scheme 3

The 1,3-dipolar cycloaddition reaction has proved useful in the synthesis of the drug celecoxib. Celecoxib is an NSAID (non-steroidal anti-inflammatory drug) that is used in the treatment of osteoarthritis, rheumatoid arthritis and menstrual symptoms and is marketed under the brand name Celebrex.
A common approach to the synthesis of this structure involves the reaction of 4-sulfamidophenylhydrazine with a diketone (Scheme 4).

However this leads to a regioisomeric mixture of pyrazoles which then requires recrystallization to give the desired regioisomer. A publication by Oh has shown that celecoxib can be synthesised from a nitrile imine via a 1,3-dipolar cycloaddition strategy (Scheme 5).
In conclusion this type of reaction is extremely versatile for the synthesis of a number of heterocycles. For that reason, and for the purpose of this thesis, it was considered very favourable when considering strategies for the synthesis of novel heterocyclic peptide mimetics.
1.2 Peptides

Peptides are sequences of amino acids linked together via the formation of an amide bond. The smallest possible peptide could be formed by the condensation reaction of the acid terminus of one amino acid and the amine terminus of the other amino acid to give what is known as a dipeptide; many can link together to form a polypeptide (Scheme 6).

Scheme 6

In general, these chains of linked amino acid residues are considered peptides if they are up to 50 amino acids in length; anything above this can be classified as proteins. The peptide unit formed is a relatively rigid, planar structure due to the restricted rotation around the C-N bond. This means that two isomers should be possible – a cis and a trans. In nature, nearly all peptide units are found to be trans. This is not surprising when comparing the cis and trans structures as the cis structure can be seen to be more crowded.

The driving force of peptide formation is the elimination of water and also the fact the peptide bond is stable. This can be understood by looking at the hybridisation of the atoms in the peptide bond 2.
As the nitrogen atom is next to the sp² hybridised carbon of the carbonyl group, the lone pair of electrons on the nitrogen can be conjugated with the carbonyl group. This is known as resonance. It is this phenomenon, whereby the lone pair and π electrons in the peptide bond are delocalised as represented by extreme resonance forms that adds to the stability of the amide bond (Fig 5).

It is also the hybridisation of the nitrogen atom itself that adds to the stability of the peptide bond. The nitrogen of an amine is sp³ hybridised whereas the nitrogen atom of an amide or peptide bond is sp² hybridised. In the sp² hybridised nitrogen, the lone pair is delocalised and thus is unavailable, therefore the amide nitrogen has little nucleophilic chemistry (Fig 6).
1.3 Peptide Mimetics

For a number of years now, peptide mimetics have become of great interest to both organic and medicinal chemists due to the advantages they have over physiologically active peptides. Biological tests on a number of peptides and proteins have shown their potential for use in drug form but the use of natural peptides as drugs is limited due to a number of factors. They have low metabolic stability towards proteolysis and poor absorption after ingestion, which is mainly due to their high molecular mass. Also they undergo rapid excretion through the liver and kidneys. These factors have led organic and medicinal chemists to design and synthesise molecules that can mimic the biological activity of natural peptides but also overcome the problems discussed above. These types of molecules are known as peptide mimetics.

A peptide mimetic is described as a compound that, as the ligand of a receptor, can imitate or block the biological effect of a peptide at the receptor level.\textsuperscript{12} For example, morphine 3, an opioid alkaloid, is a classic example of a nonpeptide ligand that has been found to mimic the effects of β-endorphin, a peptide consisting of 31 amino acid residues.
The tetrapeptide 4 was identified as a potent reversible inhibitor of ICE (interleukin-1β converting enzyme). Interleukin 1β contributes to the pathogenesis of inflammatory diseases such as rheumatoid arthritis and osteoarthritis. The X-ray crystal structure of the compound bound in the active site of ICE was published and defined key spatial, lipophilic and hydrogen bonding interactions for potent ICE inhibition. These were highlighted and include a lipophilic moiety (the tyrosine side chain), a ‘bent’ dipeptide central core, a hydrogen bonding backbone and an aspartic acid-like cysteine trap.\textsuperscript{13}

\begin{center}
\includegraphics[width=0.5\textwidth]{tetrapeptide.png}
\end{center}

Rigid peptide mimetic compounds have been identified as potent reversible and irreversible inhibitors. One compound of significance was Pralnacasan\textsuperscript{®} 5, which was characterised as a selective reversible inhibitor for ICE and was progressed into late stage clinical trials.

\begin{center}
\includegraphics[width=0.5\textwidth]{peptide_mimetic.png}
\end{center}
Pralnacasan® incorporates a bicyclic core to mimic the central dipeptide region and to constrain the structure into a conformation that maintains the key hydrogen bonding points.

When designing molecules as potential peptide mimetics there are a few requirements that exist in terms of their desired pharmacological properties. They must have good metabolic stability, good bioavailability, high receptor affinity and minimal side effects. In order to meet this brief there are guidelines that can be followed to give the best possible chance of achieving the above.\(^\text{14}\)

If possible, the majority of the peptide backbone should be replaced as long as bond surrogates have shown some retained biological activity or peptide bonds are not at the forefront of the most bioactive conformation. It is important that peptide side chains are retained as far as possible as it may be these groups that are recognised by receptors. For example, if replacing an amino acid residue such as lysine, initially this should be replaced by a mimetic that has a primary amine present at the end of an alkyl chain. Finally conformational flexibility should either be maintained in mimetic structures so that they are able to adopt conformations matching that of the original peptide, or constrained to bioactive conformations.

Looking from a structural point of view there are many ways in which it is possible to mimic a peptide, from simply making changes to the amino acid side chains to creating structures that have no peptide structural character at all.

A very simple method of modifying the amino acid is to replace natural amino acid residues with unnatural amino acid residues, that is swapping L- for D-amino acids.
Other ways of modifying amino acids include exchange for $\alpha,\beta$-unsaturated, cyclic and $\beta$-amino acids. The structures of some analogues of phenylalanine and tryptophan are shown below (Fig 7). \(^{12}\)

![Peptide mimetic analogues of phenylalanine and tryptophan](image)

Fig 7 Peptide mimetic analogues of phenylalanine and tryptophan

Replacing individual units for other atoms can also modify the peptide backbone. Examples of appropriate changes are given in Table 1. \(^{15}\)
As said previously, it is important to maintain conformation when designing peptide mimetics. This can be achieved by the introduction of bridges between different parts of
the molecule. Bridging can take place within a single amino acid residue e.g. 6, or the bridge can be a link between two side chains, e.g. 7. This can be achieved, for example, by the incorporation of lactams or piperazinones and the compounds are known as dipeptide mimetics. It is also possible to have bridges that link two peptide backbone units, e.g. 8, or even link a side chain with a backbone, as in 9.\textsuperscript{15}

![Chemical structures](image)

When considering maintaining the conformational structure of a peptide, it should be noted that natural peptides have secondary structures such as turns and folds. Turns are segments of a polypeptide chain where the peptide chain reverses its overall direction. Perhaps the most significant turning point is the $\beta$-turn. It can be described as any tetrapeptide sequence in which the $\text{Ca}^{-1} - \text{Ca}^{+3}$ distance in a nonhelical region is less than 7 Å.\textsuperscript{16} This type of turn is formed from four amino acid residues and is stabilized
by a hydrogen bond interaction between the carbonyl of the first amino acid and the proton situated on the NH of the fourth amino acid (Fig 8).

![Fig 8](image-url)

Another type of peptide turn is called a γ-turn which is made up of only three amino acid residues, where the stabilizing H-bond is between the carbonyl of the first residue and the proton on the NH of the third amino acid (Fig 9).

![Fig 9](image-url)

From the examples shown below (Fig 10), it is clear that mimicking the β-turn can be achieved by structures that are recognizable derivatives of peptides whereas some structures are clearly non-peptidic.15
Modification to the peptide backbone can lead to an increase in the biological half life in comparison with that of the parent compound. Due to the many synthetic possibilities, peptide mimetics can be categorized due to the type of modification to the peptide backbone.15 Carbapeptides are those where the NH-C=O unit of the amide bond is replaced by a carbon group such as CH$_2$C=O. This group can also be extended to hydroxyethylene and carba modifications. Compounds containing dipeptide-analogous hydroxyethylene groups are among the most active inhibitors of renin and HIV-1 protease. Umezawa and co-workers have isolated the arphenamines, both naturally occurring ketomethylene analogues (Fig 11).17
Synthetic approaches for the synthesis of hydroxyethylene dipeptide analogues have been reported. One example of a synthesis is by Fray et al. They start with a Boc-protected amino aldehyde, which is treated with the lithium derivative of ethyl propiolate. The lactone is formed after hydrogenation and is then metalated and treated with methylallyl bromide. The \( \alpha \)-substituted lactone is formed by a final hydrogenation, and can then go on to ring open with suitable nucleophiles to form linear peptide analogues (Scheme 7).
Azapeptides are another class of backbone-modified peptides that have proven to be of importance in the pharmaceutical industry. Here the α-CH group of one or more of the amino acid residues is replaced by a nitrogen atom with retention of the original side chain (Fig 12).

Peptoids are oligomeric analogues that contain N-alkylated glycines joined together in a peptide-like manner. The α-CHR groups have been replaced by NR groups and the
NH groups replaced by CH₂ units. The position of the side chains and carbonyl groups of the original peptide chains remains unchanged. Peptoids are not chiral since the side chains corresponding to the normal amino acids are attached to the N atoms and only achiral CH₂ groups are present instead of NH groups (Fig 13).

\[
\begin{align*}
\text{Fig 13}
\end{align*}
\]

Conformational studies have shown that peptoids have much greater conformational flexibility than peptides. This is due to the absence of the CO-HN hydrogen bonds, as there are no NH bonds present in the peptoid chain.

Peptide mimetics have also been categorized based on the nature of their structure as opposed to the way in which the structure has been modified.¹²

Opioids are a class of peptide mimetics based around the structure of morphine, the main constituent of opium. Countless numbers of morphine derivatives have been synthesized in an aim to develop a more nonaddictive analgesic. With the help of these
compounds it was possible to discover various opioid receptors in animal organisms. It was later shown that the opium alkaloids were mimetics of the endogenous opioids. Naltrindole 10 was shown to mimic the action of the peptide sequence 11.\textsuperscript{12}

\begin{center}
\begin{tikzpicture}
\end{tikzpicture}
\end{center}


\textbf{11}

Tachykinins are peptides with a length of ten or eleven amino acids that act as neurotransmitters or neuromodulators in various parts of the central and peripheral nervous systems. These peptides possess a common C-terminal sequence Phe-X-Gly-Leu-Met-NH\textsubscript{2}. The best known and most investigated of these substances is substance P and was discovered in 1931 by von Euler and Gaddam.\textsuperscript{19} The receptors for substance P are designated NK 1-3. The first nonpeptide antagonist for the NK 1 receptor is 12 which emerged from a screening process. Other potent nonpeptide antagonists for the NK 1 receptor are 13 and the steroid derivative 14.\textsuperscript{20,21}
Somatostatin 15 is a cyclic tetradecapeptide which is formed in the hypothalamus and inhibits the release of growth in the pituitary gland.\textsuperscript{22} It has also been found in the secretory cells of the intestine and the pancreas. Compound 16 was described as the first nonpeptide somatostatin mimetic.\textsuperscript{23}

\[
\text{H - Ala - Gly - Cys - Lys - Asn - Phe - Phe - Trp}
\]

\[
\text{HO - Cys - Ser - Thr - Phe - Thr - Lys}
\]
Literature searches show that there have been many significant contributions to the field of peptide mimetics.

Novel approaches have shown designs involving organic templates. The pioneering work of Fiegel introduced the phenoxanthiin ring system as a template as well as a range of biphenyl systems as a method of stabilizing the β-structure in peptide loops.

Templates of this kind have also been developed by Muller and Obrecht. Other groups have reported studies of linear and cyclic peptides that can be made to assemble into β-hairpin structures to various extents, either in organic solvents or water.
Templates derived from proline and related derivatives have proven to be attractive due to their non-aromatic and polar character since extended aromatic systems tend to be highly immunogenic. Robinson and co-workers have carried out significant studies in this area. Examples of some proline based templates are shown (Fig. 14)

![Template 1](image1)

![Template 2](image2)

Fig 14

The synthetic schemes for these templates are shown below.
Template 1

(a) O₃ then Ph₂PCHCOOMe; 100%, (b) H₂, Pd; 93%, (c) DMAP, toluene, reflux; 79%, (d) LDA, THF then 'BuOOC-\(N=\)N-COO'Bu; 88%, (e) LDA, THF then 'BuCOOH, (f) TFA, CH₂Cl₂, (g) H₂, Pt, H₂O₂; 68%, (h) Fmoc - Cl, dioxane, aq. Na₂CO₃; 61%

Scheme 8
Template 2

\[ \text{COOH} \rightarrow \text{a-d} \]

Scheme 9

\[ X = \text{N}_3, \text{R} = \text{tBu} \]

\[ X = \text{NHfoc}, \text{R} = \text{H} \]

(a) SOCl\textsubscript{2}, MeOH; 100\%; (b) LDA, -78 °C, then BrCH\textsubscript{2}COO\textsuperscript{t}Bu; 80\%; (c) MeOH/ H\textsubscript{2}O, LiOH.H\textsubscript{2}O; 83\%; (d) H-Pro(\text{OH})-OMe.HCl, \text{Pr\textsubscript{2}}EtN, HBTU, CH\textsubscript{2}Cl\textsubscript{2} and separation of diastereoisomers; 42\%; (e) H\textsubscript{2}, Pd/C, EtOAc; 83\%; (f) TosCl, Pyr; 91\%; (g) NaN\textsubscript{3}, DMF, 78 °C; 91\%; (h) H\textsubscript{2}, Pd/C, EtOAc; 100\%; (i) FmocCl, \text{Pr\textsubscript{2}}EtN, CH\textsubscript{2}Cl\textsubscript{2}; 98\%; (j) H\textsubscript{2}O/TFA; 100\%
1.4 Nitrogen-Containing Heterocyclic Peptide Mimetics

Heterocycles can be very useful tools for the construction of peptide or β-turn mimics. The nature of these structures can force the molecule to curve like the β-turn structure to encourage hydrogen bonding and the rigidity of the heterocycle means that they are not broken down easily; a highlighted problem with peptides.

To look at examples of N-heterocyclic peptide mimetics it is sensible to start with some examples studied within the Jones group.

In 1995 a paper published by Jones with Crockett, Gilbert and Rees describes the synthesis and application of imidazolines as amide bond replacements. The similarities between the amide bond and imidazoline structure were highlighted including the presence of two heteroatoms, similar configuration of double bonds, similar hydrogen bonding possibilities and similar steric properties (Fig 15).

There had been previous reports in the literature by Jones on imidazolines of type I as an amide bond replacement. For the purpose of the more recent paper it was decided that imidazolines of type II would be synthesised. The R group of imidazoline type II is
no longer attached to the imidazoline ring, and hence is differently conformationally constrained.

The strategy for the synthesis of the imidazoline was to carry out a condensation reaction of the N-protected amino acid 18 with the diamino compound 19.

![Chemical structure](image)

The synthesis of the diamino compound 19 is shown (Scheme 10).

![Scheme 10](image)

The N-protected amino acid was converted to the S-methylthioimidate salt using a previously reported method. The N-benzyloxycarbonyl protected amino acid was
converted to the piperidine amide via the pentafluorophenyl ester, then treatment with Lawesson's reagent gave the thioamide. This was converted to the S-methylthioimidate salt by treatment with methyl iodide. The condensation reaction was carried out with the diamino compound to give the required imidazoline (Scheme 11).

![Scheme 11]

More recently in 2007, Sachetti reported the synthesis of two benzimidazoles as tetrapeptide mimics 20 and 21.³⁰
The synthesis of both was carried out using a different strategy to that of Jones and both benzimidazoles were synthesised in few steps (Scheme 12).

Scheme 12

A paper by Jones in 2000 described the synthesis of 3-(1-aminoalkyl)isoxazole-4-carboxylic acids as peptide bond replacements. Isoxazoles are five membered
heterocycles containing two adjacent heteroatoms; one being oxygen and the other nitrogen.\(^{22}\)

The strategy for this synthesis is of particular interest, in connection with this thesis, as the heterocyclic framework was constructed via a 1,3-dipolar cycloaddition from the nitrile oxide and the enaminoester (Scheme 13).

(a) \(\text{i-Bu}_2\text{AlH, toluene, \(-78\) }^\circ\text{C}\); (b) \(\text{NH}_2\text{OH.HCl, NaOAc, EtOH-H}_2\text{O, }60\) \(^\circ\text{C}\); (c) \(\text{i-ButOCl, CHCl}_3, 0\) \(^\circ\text{C}\); (d) \(\text{Et}_3\text{N, CHCl}_3, \text{relux}\)

Scheme 13
With the isoxazole synthesised, the ester and amine functionalities could be deprotected, in turn, and coupled to amino acids. This showed the ability of such an isoxazole to be incorporated into a peptide chain.

Over the past few years there have been many published examples of heterocyclic peptide mimetics including a variety of nitrogen containing heterocycles.

In 2005, Gmeiner and Bitterman reported the synthesis of spirocyclic β-lactams as potent β-turn inducing peptide mimetics.32 This account is of particular interest as the synthesis starts with (S)-proline, a proteinogenic amino acid and shows the utilisation of a single heteroatom ring system.

The synthesis of the spirocyclic model begins with the synthesis of an enantiopure α-vinylproline derivative (Scheme 14).

Scheme 14
By a simple amide bond formation and an intramolecular Mitsunobu reaction followed by aminolysis, the spirocyclic \( \beta \)-lactam is formed (Scheme 15).

(a) HATU, DIPEA, NMP, GlyOMe.HCl, rt, 30 min; (b) 1. \( \text{O}_3 \), CH\(_2\)Cl\(_2\), -78 °C, 2. NaBH(OAc)\(_3\), rt, 6-24 h; (c) DEAD, PPh\(_3\), THF, rt, 2.5-5 h; (d) CH\(_3\)NH\(_2\), EtOH, 0 °C, 40 min.

Scheme 15

In a later paper published in 2007, Gmeiner reported the synthesis of another proline derived \( \beta \)-turn mimetic 25.
This spirobarbiturate structure was designed in such a way that the NH atoms of the spirobarbiturate ring could be exploited to incorporate different substituents which may serve as molecular probes exploring binding pockets of complementary target proteins. There are examples in the literature of more heteroatom-rich ring systems, such as oxadiazoles, as peptidomimetic building blocks.

In 2007, Dolenc reported the synthesis of twelve new 1,2,4-oxadiazole-based compounds that incorporate a protected amine and a carboxyl or ester group, thus serving as potential peptide mimetic building blocks.\(^{34}\) Again the synthesis starts with a proteinogenic amino acid which has retained its stereochemistry throughout the sequence (Scheme 16).
There is no shortage of bicyclic ring examples of peptide mimetics in the literature. This may be due to the way bicyclic scaffolds can encourage any appropriate substituents to hydrogen bond thus mimicking structural features of β-turns.

In 2006, Lubell reported the synthesis of enantiopure C6-functionalized pyrrolizidine amino acids as potential dipeptide mimetics (Fig 16).35

Upon synthesis of the bicyclic structure, the hydroxyl group can be manipulated to be in the R or S configuration depending upon the stereochemistry of the acetyl protected oxygen in the starting material.

The synthesis was carried out in four steps from an α-acetoxy ketone (Scheme 17).
Scheme 17

Here the 6R stereoisomer was formed by starting with the 5R α-acetoxy ketone; starting with the 5S α-acetoxy ketone would therefore yield the 6S bicycle.

There are examples in the literature of bicyclic peptide mimetics constructed from larger heterocyclic ring systems.

Peptide mimetics based around 2-pyridone scaffolds have proven to be useful as they inhibit the hepatitis C virus (HCV) NS3 serine protease. They have been shown to act as inhibitors of human rhinovirus (HRV) 3C protease. In general, ring fused 2-pyridone scaffolds are also present in compounds with many biological applications such as anti-cancer agents, ACE inhibitors and inhibitors of Aβ - peptide aggregation.

Almqvist, Åberg, and Sellstedt contributed in this area by reporting the design and synthesis of peptide mimetics based upon the substituted bicyclic 2-pyridones 26.36
Hruby et al. reported the synthesis of a pyrimidine/piperazine fused bicycle 27 in 2008.\textsuperscript{37}

The synthesis was carried out starting with a mono protected diamine via an acid catalysed cyclisation to give the bicyclic product as one diastereoisomer (Scheme 18).
Scheme 18

(a) Boc-L-Phe-OH, BOP, HOBt, NMM, DMF; (b) TFA, CH₂Cl₂; (c) 2,2-diethoxyacetaldehyde, THF; (d) NaBH(OAc)₃, AcOH, THF; (e) Cbz-Cl, DIEA, CH₂Cl₂; (f) formic acid; (g) H₂, Pd/C, MeOH
1.5 Pyrazolines

2-Pyrazolines, or 4,5-dihydropyrazoles, are five membered heterocycles containing two adjacent nitrogen atoms with the general structure shown (Fig 17).

![Fig 17 Basic structure of pyrazoline](image)

There are three tautomeric pyrazoline structures, shown below, but the 2-pyrazolines are by far the most common (Fig 18).

![Fig 18 The Tautomeric Pyrazoline Structures](image)

The first reported synthesis of a pyrazoline was by Knorr and Blank in 1885 where they described the slow reduction of 1,3-diphenyl-5-methylpyrazole with sodium and ethanol. In 1887 Fisher and Knoevenagel reported the first synthesis of a pyrazoline from aryl hydrazines by the reaction of phenylhydrazine and acrolein. It was then Curtius and Wirsing who first synthesized pyrazoline itself from the reaction of acrolein with hydrazine.
The standard syntheses of pyrazolines presently either involve the conjugate addition and subsequent cyclisation of hydrazines with $\alpha,\beta$-unsaturated carbonyl compounds (Scheme 19), or proceed via a 1,3-dipolar cycloaddition reaction of nitrile imines with olefins that preferably possess electron-withdrawing groups (Scheme 20). 

Scheme 19

Scheme 20

Nitrile oxide and nitrone 1,3-dipolar cycloadditions have been exploited in the construction of enantiopure five membered heterocycles. Despite the utility of enantiopure pyrazolines in organic synthesis and some interesting applications of related products, the stereoselective synthesis of pyrazolines has only become useful in the last few years. A 1998 review by Gothelf and Jørgensen stated that "only very few
studies have been performed in the field of asymmetric 1,3-dipolar cycloadditions involving nitrile imines”.\(^1\) In 2005, Molteni published a review on stereoselective cycloadditions of nitrile imines as a source of enantiopure heterocycles.\(^4\)

The behaviour of N-4-methylphenyl-C-methoxycarbonyl nitrile imines towards a series of enantiopure acrylamides was investigated. It was found that the diastereoisomeric ratio ranged from 58:42 to 83:17 depending upon the chiral auxiliary connected to the dipolarophile. It was found that bulkier acrylamides gave better results as a possible outcome of lower conformational flexibility. It was also found that the base, whether chiral or achiral, and the presence of salts such as LiCl as potential complexing agents had little or no influence on the diastereoselectivity (Scheme 21).

\[ \text{Scheme 21} \]

Cycloaddition of the nitrile imine 28 with 29 gave a 75:25 mixture of diastereoisomers in a yield of 60%.\(^4\) In terms of regioselectivity, the formation of just the 5-substituted
4,5-dihydropyrazoles is in agreement with the HOMO-dipole (LUMO-dipolarophile) control which is typical for cycloaddition of nitrile imines with dipolarophiles that bear electron-withdrawing groups (Scheme 22).

Scheme 22

Pyrazolines, as with other nitrogen containing heterocycles are of great interest to medicinal and organic chemists due to their proven biological activity. In the literature there are reported examples of pyrazoline derived compounds that have shown anti-diabetic,\textsuperscript{43} antidepressant\textsuperscript{44} and anti-inflammatory activity.\textsuperscript{45} There are also examples reported that have shown ability to inhibit enzymes related to Alzheimer’s and Parkinson’s diseases.\textsuperscript{46}

In a 2007 paper by Conti, the synthesis of 1,3,5-trisubstituted pyrazolines was described as part of a project aimed at the discovery of new NMDA (N-methyl-D-aspartate) antagonists.\textsuperscript{47} 1,3,5-Trisubstituted pyrazolines and pyrazoles are of great interest as synthetic targets as they form the basis of numerous drugs including Viagra and Celebrex. The synthesis of the 1,3,5-trisubstituted pyrazoline, 5-substituted-3-dimethoxyphosphono-2-pyrazoline 30, was accomplished by a 1,3-dipolar cycloaddition from a nitrile imine. The presence of the phosphonate moiety has been shown to be responsible for an increase in potency (Scheme 23).
There are examples of pyrazoline-containing natural products reported in the literature. In 2005, Kelly and co-workers reported the total synthesis of nigellicine 31, a natural product that was first isolated in 1985 from the seeds of *Nigella sativa*.

**Scheme 23**
Pyrazole ring structures are also of great interest and there is evidence of their synthesis via a 1,3-dipolar cycloaddition route in the literature.

A paper by Oh describes the synthesis of celecoxib 34 by 1,3-dipolar cycloaddition.\textsuperscript{11} Celecoxib is the active molecule in the widely prescribed drug Celebrex, a COX-2 inhibitor. A common approach for the synthesis of celecoxib is to react 4-sulfamidophenylhydrazine 32 with diketone 33 (Scheme 24).

![Scheme 24](image)

In this example, and others, a regioisomeric mix of both pyrazoles is often produced therefore further recrystallization techniques are required to obtain the correct regioisomer. The example described by Oh utilises the 1,3-dipolar cycloaddition reaction to give a totally regioisomeric product.

Due to the extensive number of publications that show the presence of pyrazoles and pyrazolines in drug targets and natural products, along with great interest from biological chemists, it was felt that this type of heterocycle was worthy of further research, in particular in connection with heterocyclic peptide mimetics.\textsuperscript{49}
2.0  Aim of the Project

The aim of this project is to incorporate all of the topics covered in the introduction leading to the synthesis of pyrazolines as potential peptide mimetics with the general structure 35, as an extension of work carried out previously in the group on substituted isoxazoles and azomethine imines.\textsuperscript{31,50}

![Diagram of compound 35]

The imine bond potentially acts as a carbonyl mimic therefore the peptide bond is replaced and the peptide chain is allowed to carry on through ring structure therefore mimicking the dipeptide structure 36. So not only are we creating a peptide mimetic but also, due to the presence of the protected amine and acid functionalities, we aim to synthesise novel non-proteinogenic amino acids.

![Diagram of compound 36]
The initial aim is to synthesize these types of pyrazolines from N-protected proteinogenic amino acids via routes involving either a hydrazone 37 or a hydrazide 38.

As discussed previously, pyrazolines can be formed by 1,3-dipolar cycloaddition. In order to do this a dipole must be generated in the form of a nitrile imine 40. This is done by the treatment of a hydrazonyl chloride 39 with base (Scheme 25).

The dipole that is formed in situ can then be subject to a suitable dipolarophile and by the mechanism shown the desired heterocycle is formed (Scheme 26).
In more specific terms, the aim of this thesis is to investigate the scope of the 1,3-dipolar cycloaddition reaction leading to the synthesis of compound 35 and to investigate the potential in variation of the group $R^2$ situated on N-1 of the heterocyclic ring.

Scheme 26
3.0 Results and Discussion

3.1 Synthesis via a Hydrazone Route

We initially had two strategies for the synthesis of the pyrazolines, the first of these proceeding via a hydrazone route. The method employed for the synthesis of the pyrazoline is a 1,3-dipolar cycloaddition. This type of cycloaddition relies on the reaction of a suitable dipole, here in the form of a nitrile imine, with a dipolarophile. Nitrile imines are formed in situ by the reaction of hydrazonyl chlorides (Scheme 27) with base.

Scheme 27
As the retrosynthetic analysis shows, our first task was to synthesise an N-protected amino acid ester. We chose L-alanine 41 as our starting amino acid as the presence of the methyl group may have given us early indications of any steric effects. L-Alanine was esterified using acetyl chloride in dry methanol, a procedure already established within the group to give L-alanine methyl ester 42 in 96% yield (Scheme 28).\(^{51}\)

\[
\begin{align*}
\text{Me} & \quad \text{CO}_2\text{H} \\
\text{NH}_2 & \\
\text{41} & \\
\end{align*}
\]

\[
\begin{align*}
\text{Me} & \quad \text{Cl} \\
\text{MeOH, } & \Delta, 24\text{ h} \\
96\% & \\
\text{Me} & \quad \text{CO}_2\text{Me} \\
\text{NH}_2 & \\
\text{42} & \\
\end{align*}
\]

**Scheme 28**

The next step was amine protection. We decided to try two common N-protecting groups in order to establish whether there could be preference of one over the other further into the synthesis. tert-Butyloxycarbonyl (Boc) and benzyloxycarbonyl (Cbz) protecting groups were employed as these types of N-protection had also been carried out in the group previously.\(^{51}\) This gave the Boc 43 and Cbz 44 protected amino acid esters in yields of 96% and 53%, respectively (Schemes 29 & 30).

\[
\begin{align*}
\text{Me} & \quad \text{CO}_2\text{Me} \\
\text{NH}_2 & \\
\text{42} & \\
\text{Me} & \quad \text{CO}_2\text{Me} \\
\text{NHBoc} & \\
\text{43} & \\
\end{align*}
\]

**Scheme 29**
Both the Boc 43 and Cbz 44 L-alanine methyl esters were reduced to their respective aldehydes 45 and 46 in 93% yield using DIBAL-H in dry toluene at -78 °C (Schemes 31 & 32).

As these first few synthetic steps were performed quite smoothly we were soon ready to attempt the synthesis of the phenylhydrazones. This was done using a common method used as a test for the presence of carbonyl compounds. The crude aldehydes were
dissolved in a little ethanol and added to a solution of phenylhydrazine hydrochloride and sodium acetate in water. After heating at approximately 70 °C for 15 minutes the Boc-protected phenylhydrazone precipitated from solution; this happened more rapidly on cooling in an ice bath. The solid produced was filtered and dried to give [1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester 47 in a yield of 81% (Scheme 33).

Scheme 33

The Cbz-protected phenylhydrazone was not able to be isolated in the same way as it formed as an oil in the reaction solution. Attempts were made to extract and purify this oil but they were unsuccessful so, due to its ease of formation and high purity, we decided to carry the synthesis forward using the Boc-protected hydrazone 47.

Formation of isoxazoles had been reported and carried out using a 1,3-dipolar cycloaddition previously in the group. These reaction conditions were applied to the Boc-protected phenylhydrazone with the aim of forming the desired pyrazoline in one pot. These reactions are summarised in Table 2.

The hydrazone 47 was C-chlorinated using N-chlorosuccinimide (NCS) at 0 °C before heating to reflux for 17h. After this period the dipolarophile was added in the form of ethyl acrylate followed by Et3N over a period of 3h and the reaction mixture was left to
reflux for a further 6h (Entry 1). Previous members of the group had found that, in the isoxazole series from chlorinated oximes, better yields could be achieved by slower addition of the base. We tried adding Et₃N over longer periods of time (Entries 2-3) but still only a complex mixture (CM) was seen. We decided to try two other dipolarophiles with stronger electron-withdrawing groups than ethyl acrylate. The chlorination and cycloaddition was carried out as before except N-phenylmaleimide and diethyl fumarate were employed as dipolarophiles (Entries 4-5) but analysis showed once again that the reaction had not worked.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Dipolarophile</th>
<th>Base</th>
<th>Addition Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CHCl₃</td>
<td>Ethyl Acrylate</td>
<td>Et₃N</td>
<td>3h</td>
<td>CM</td>
</tr>
<tr>
<td>2</td>
<td>CHCl₃</td>
<td>Ethyl Acrylate</td>
<td>Et₃N</td>
<td>4h</td>
<td>CM</td>
</tr>
<tr>
<td>3</td>
<td>CHCl₃</td>
<td>Ethyl Acrylate</td>
<td>Et₃N</td>
<td>4h</td>
<td>CM</td>
</tr>
<tr>
<td>4</td>
<td>CHCl₃</td>
<td>N-Phenylmaleimide</td>
<td>Et₃N</td>
<td>3h</td>
<td>CM</td>
</tr>
<tr>
<td>5</td>
<td>CHCl₃</td>
<td>Diethyl Fumarate</td>
<td>Et₃N</td>
<td>3h</td>
<td>CM</td>
</tr>
</tbody>
</table>

Table 2 Summary of Attempted 1,3-Dipolar Cycloaddition Reactions

After this disappointment we went back to the literature to find work by Torssell and his co-workers where they had reported the synthesis of pyrazolines in one pot from hydrazones.⁵³

We applied this methodology to the Boc-protected phenylhydrazone whereby C-chlorination was carried out using NCS for 1h at 60 °C before addition of ethyl acrylate.
and KHCO₃ as the base. The reaction mixture was stirred at 70 °C for 20h and purification gave 5-(1-tert-butoxycarbonylamino-ethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester 48 in a yield of 41% as a 1:1 mixture of diastereoisomers, as confirmed by chiral phase HPLC (Scheme 34). Et₃N was tried as an alternative base to give 48 in a comparable yield of 41%, again as a 1:1 diastereomeric mixture.

Scheme 34

Both reactions were regioselective giving us the ethyl ester in the required position. This was confirmed by obtaining a crystal structure of the pyrazoline (Fig 19) which shows that only one diastereoisomer had crystallised out. This shows an S configuration at the chiral centre of C(13) and an R configuration at the newly formed chiral centre of C(1). Unfortunately on further attempts, we were unable to form a crystallised product that could be separated from the diastereomeric mixture.
After establishing this methodology to synthesise the pyrazolines we decided to look at modified dipoles which would give us pyrazolines with different groups at N-1 of the heterocyclic ring.

We thought that it would be useful to prepare pyrazolines whereby the N-1 atom of the pyrazoline heterocycle was protected by amine protecting groups with a view to cleaving these off at a later stage and replacing them with different alkyl chains or groups with interesting functionality. N-(2-tert-Butoxycarbonylaminopropylidene)hydrazinecarboxylic acid tert-butyl ester 49 and N-(2-tert-Butoxycarbonylaminopropylidene)hydrazinecarboxylic acid benzyl ester 50.
were prepared from N-protected amino acid aldehyde 45 using the required protected hydrazines in toluene over a period of 24h in yields of 27% and 48%, respectively (Schemes 35 & 36).

![Scheme 35](image)

We attempted to chlorinate and cycloadd to these hydrazones as before using NCS as the chlorinating reagent and ethyl acrylate as the dipolarophile in the presence of KHCO₃ as the base. Table 3 gives a summary of these reactions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hydrazone</th>
<th>R</th>
<th>% Yield</th>
<th>Cycloaddition Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>Boc</td>
<td>27</td>
<td>C-Chlorinated Product</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>Cbz</td>
<td>48</td>
<td>C-Chlorinated Product</td>
</tr>
</tbody>
</table>

Table 3 Summary of Attempted Cycloaddition Reactions with 16 and 17
For both of the hydrazones, only the hydrazonyl chloride derivatives of these compounds was recovered. This was confirmed by the loss of the appropriate signal in the $^1$H NMR spectra. For the Boc- and Cbz-protected hydrazones we were unsure as to why these reactions would not work. We had hoped that these electron-withdrawing groups would render the NH proton more acidic and enhance the dipole formation but this did not appear to be the case.

Also, Molteni published work in which an investigation had been carried out into the effects of functional groups situated para on the phenyl ring of phenylhydrazones (Scheme 37).$^{54}$

![Scheme 37](image)

The results (Table 4) show that for the more electron-withdrawing para-substituents of the phenyl ring, much longer reaction times are needed resulting in much lower yields.
These findings lead us to believe that, due to the electron-withdrawing nature of the carbonyl-containing Boc and Cbz groups, the 1,3-dipolar cycloaddition would not take place as the nitrile imine has reduced reactivity. However if we are seeing the C-chlorinated compounds as products then this could suggest that the dipoles are never actually formed.

Attempts were made to prepare pyrazolines with a less electron-deficient dipolarophile to provide a substituent on the pyrazoline that we could then oxidise to the carboxylic acid. Both 49 and 50 were chlorinated as before with NCS before addition of allyl alcohol, as a more electron-rich dipolarophile, but neither of these reactions gave cycloaddition products.

This led us to look at different protocols for preparing the pyrazolines that we had already managed to synthesise. Many of the methods that we came across required the isolation of the hydrazonyl chloride before cycloaddition. In order to do this we used a method previously reported by Patel in which the Corey-Kim reagent, an N-

<table>
<thead>
<tr>
<th>R</th>
<th>Time/Min</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>Me</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>MeO</td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td>Br</td>
<td>70</td>
<td>68</td>
</tr>
<tr>
<td>NO2</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4
chlorosuccinimide-dimethyl sulfide complex previously used for the oxidation of alcohols to carbonyl compounds, was employed.\textsuperscript{55,56}

The Corey-Kim reagent was prepared \textit{in situ} according to the reported procedure by addition of dimethyl sulfide (DMS) to N-chlorosuccinimide at 0 °C. Treatment of this complex with the Boc-protected phenylhydrazone 47 at -78 °C, with warming to room temperature over a period of 3h resulted in the formation of the desired hydrazonyl chloride in 55% yield. The mechanism suggested by Patel in his paper is shown below (Scheme 38).

\begin{center}
\textbf{Scheme 38}
\end{center}

The first step is assumed to be loss of a proton from the nitrogen atom. The Corey-Kim reagent then undergoes nucleophilic attack by the nitrogen atom of the hydrazone resulting in the loss of succinimide. The double bond of the hydrazone rearranges forcing removal of dimethyl sulfide and resulting in the generation of a carbocation.
This then undergoes attack by the counteranion, $\text{Cl}^-$, to give $51$, which can tautomerise to give the hydrazonyl chloride $52$.

Confirmation of the formation of the hydrazonyl chloride was given by $^1\text{H}$ NMR analysis where the disappearance of the appropriate proton signal is observed. By carrying out this methodology we were able to isolate the hydrazonyl chloride and attempt more cycloadditions.

A paper by Molteni, who has carried out work on a large number of reactions involving the synthesis of pyrazolines from nitrile imines, suggested the use of $\text{AcOAg}$ as the base and used much milder conditions to those we had used previously.\textsuperscript{57} The hydrazonyl chloride $52$ produced by the Corey-Kim route was reacted with ethyl acrylate in the presence of $\text{AcOAg}$ at room temperature (Scheme 39).

\[
\begin{align*}
\text{Me} & \quad \text{NHPh} \\
\text{NHBoc} & \quad \text{Cl} \\
\xrightarrow{\text{AcOAg}} & \\
toluene, 2-20\text{h}, \text{RT} & \\
15-25\% & \\
\text{Me} & \quad \text{NHPh}
\end{align*}
\]

Scheme 39

The reaction time was varied from 2 to 20 h with the pyrazoline $48$ only being formed in 15% yield at best. We tried adding another 1.5 eq of $\text{AcOAg}$ after a reaction time of 1 h before leaving the reaction for a further 19 h but in this case a yield of only 25% could be achieved after purification. Again regioselectivity was maintained but the product was still formed as a mixture of diastereoisomers.
In another of the papers by Molteni, 1,3-dipolar cycloadditions of nitrile imines were carried out in aqueous media in the presence of tetrahexylammonium chloride (THAC) as a catalyst.\textsuperscript{54} We carried out this reaction on our Boc-protected hydrazonyl chloride, prepared by the Corey-Kim procedure, in 0.1M aqueous sodium hydrogen carbonate with a catalytic amount of THAC and ethyl acrylate as the dipolarophile. The reaction mixture was shaken for 2h and an orange solid was formed that could be collected by suction filtration to give 48 in a yield of 57\% (Scheme 40).

\[
\begin{array}{c}
\text{Me} \quad \text{NHPh} \\
\text{NHBoc} \\
\text{Cl}
\end{array}
\xrightarrow{\text{0.1M aq. base, THAC, 2h, RT}}
\begin{array}{c}
\text{Me} \quad \text{NHPh} \\
\text{NHBoc} \\
\text{CO}_2\text{Et}
\end{array}
\]

\textbf{Scheme 40}

This method meant that all impurities were filtered off with the aqueous medium therefore no further purification of the product was needed. It is thought that due to the hydrophobic nature of the organic reagents they are forced into close association whereas the catalyst acts as a phase transfer catalyst that carries the basic agent from the aqueous medium to the organic aggregate.
3.2 Synthesis via Hydrazide Route

A retrosynthetic analysis for the second approach to the pyrazolines is shown in Scheme 48. As seen for the hydrazone route, the pyrazoline is to be formed by the 1,3-dipolar cycloaddition of a nitrile imine with a suitable dipolarophile, and the nitrile imine is to be formed, as before, by the treatment of a suitable hydrazonyl chloride with base. The difference is in the generation of the hydrazonyl chloride, this time from an acyl hydrazide (Scheme 41).

Scheme 41

The first task at hand was to prepare N-protected amino acids. The amino acid of choice was again L-alanine 41 with di-tert-butyl dicarbonate and benzyl chloroformate being used to add N-terminal protecting groups. These reactions were carried out as performed previously in the group to give N-tert-butyloxycarbonyl-L-alanine 53 and N-benzylloxycarbonyl-L-alanine 54 (Schemes 42 & 43).
After this we were ready to prepare the desired hydrazides. Originally this was done using phenylhydrazine hydrochloride in the presence of dicyclohexylcarbodiimide (DCC) as the peptide coupling agent along with 1-hydroxybenzotriazole (HOBT) as the carboxyl activating agent (Schemes 44 & 45).

Scheme 43

Scheme 44

Scheme 45
This was an extremely capricious reaction and was difficult to repeat to the desired purity, therefore an alternative method was sought. Zhang and his co-workers carried out an investigation into the preparation of carboxylic acid hydrazides.\textsuperscript{59} He found that the order of addition of the reagents had an effect on the outcome of the reaction. A stepwise reaction in which the acid is first converted to an activated intermediate followed by reaction with the chosen hydrazine gave the best results. This stepwise method minimizes the number of side reactions as it avoids the coexistence of the hydrazine with the peptide coupling agent. We applied this methodology to our work whereby HOBT was first of all added to a solution of the N-protected alanine in dimethylformamide. This time 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was used as the coupling agent as it is easier to handle than DCC and the byproducts are easier to remove from the product. This was added in one portion after the addition of HOBT. After 2 hours the reaction mixture was poured into a solution of phenylhydrazine in dimethylformamide whilst maintaining a temperature of 0-10 °C (Schemes 46 & 47).

![Scheme 46](image-url)
By forming the intermediates in this stepwise manner and carrying out a reverse mode of addition, the formation of side products is suppressed and the desired hydrazides, 55 and 56 were formed in yields of 50% and 56% respectively.

The synthesis of these N-protected phenylhydrazides meant that we were able to attempt the chlorination of these products followed by cycloaddition.

A number of reagents can be used for the chlorination of amide bonds or hydrazides, such as PCl₅, POCl₃, and CCl₄ in the presence of triphenylphosphine. Some of these methods have been found to be quite harsh and a drawback is that the excess of chlorinating agent and reagent-derived byproducts have to be removed. Nevertheless we decided to try some of these more classical methods as a means of obtaining the desired hydrazonyl chlorides. The results of these experiments are summarised in Table 5.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Chlorinating Reagent</th>
<th>Solvent</th>
<th>Temp</th>
<th>Hours</th>
<th>Base</th>
<th>Hours</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc</td>
<td>PCl₅</td>
<td>Toluene</td>
<td>Δ</td>
<td>3</td>
<td>-</td>
<td>CM</td>
</tr>
<tr>
<td>2</td>
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*temperature changed to 70° C

**Table 5 Summary of conditions used for chlorination of 56**

Initially PCl₅ was used as the chlorinating reagent (Entry 1) whereby the Boc-protected phenylhydrazide was heated to reflux in toluene. This led to a complex mixture (CM) in which nothing could be identified or isolated. We decided that as chlorination and cycloaddition *via* the hydrazone route was carried out in one pot, we would try this for the hydrazide route. Therefore we tried chlorinating again with PCl₅ (Entry 2) and CCl₄/PPh₃ (Entry 3) for the period of time indicated before adding the base and ethyl acrylate again as the dipolarophile. The reaction mixtures were left for a further 20h, as done for the hydrazone route, at the temperatures indicated but again these led to complex mixtures. We decided that as ethyl acetate was the solvent of choice for the hydrazone route we would attempt the chlorination and cycloaddition in this solvent using the bases indicated (Entries 4-5). On analysis no cycloaddition product was seen.
We then decided that to be certain that the chlorination of the hydrazides had worked, we would try to isolate the hydrazonyl chloride (Entries 6-8) before carrying out the cycloaddition reaction. Again PCl₃ was used as the chlorinating reagent but as before a complex mixture was seen in both cases. CCl₄ in the presence of PPh₃ was tried again but left to react at room temperature for a much longer period of time than before. It appeared that as we thought, the chlorination of the Boc-protected hydrazide had taken place to give 57, and this was also seen when performed with the Cbz-protected hydrazide to give 58 (Schemes 48 & 49).

![Scheme 48](image)

Having isolated the Boc- and Cbz- protected phenylhydrazonyl chlorides, it was decided to carry these through to the cycloaddition in crude form as problems had been encountered with their purification with the hydrazone route. A mechanism for C-chlorination is shown (Scheme 50).
The cycloaddition was carried out for the Boc and Cbz phenylhyrazonyl chlorides, as was done for the hydrazone route, whereby KHCO₃ was added to a solution of the hyrazonyl chloride in the presence of ethyl acrylate as the dipolarophile. After purification 5-(1-tert-butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester 48 was isolated in 31% yield as a 1:1 mixture of diastereoisomers. No cycloaddition product was obtained from the Cbz protected hyrazonyl chloride.
3.3 Conclusion

In conclusion, we have been able to synthesise our target molecule via the two routes that we proposed, a hydrazone 47 and a hydrazide 56 route.

![Chemical structures](image)

We have looked at different amine protecting groups to see if there was a preference as to which one gave us the best yields. It was found that the Boc-protected amino acids gave the best results. It is thought that the Cbz-protected hydrazones and hydrazides do not undergo cycloaddition reactions as the conditions for chlorination involve the presence of the Cl⁻ ion. It is known that Cbz is removed from amine groups with the use of Br⁻, therefore it is logical to think that the chloride ion may work in the same way.

We have been able to use a number of methodologies to synthesise the target molecules whereby the chlorination step and 1,3-dipolar cycloaddition reactions can be carried out in "one pot" or in a stepwise manner. We have also trialled different bases to see if they can have an influence on the yield of the cycloaddition reactions.

Taking into account factors such as ease and time of reaction, number of steps and yield, the hydrazone route has proven to be the best. For the purpose of this thesis, any further cycloadditions will be undertaken via the hydrazone route.
3.4 Diastereoselective Cycloaddition Reactions

As mentioned earlier, it had been realised that on cycloaddition of the hydrazonyl chloride with ethyl acrylate, the formation of the new chiral centre led to the product 48 being an inseparable mixture of diastereoisomers in a 1:1 ratio. This was confirmed earlier by chiral phase HPLC, although normal HPLC would have shown this. It can also be identified in the $^1$H NMR spectra of pyrazoline 48 as two overlapping doublets of doublets for the proton on the ethyl ester substituted carbon can be seen representing each diastereoisomer.

This was the case for all of the methods carried out to form the pyrazoline 48. It was decided that we needed to find modifications to try and influence the diastereoselectivity of the reaction.

The first method that was tried was the inclusion of a chiral auxiliary on the dipolarophile. Molteni reported the 1,3-dipolar cycloaddition reactions of hydrazonyl chlorides with proline acrylamides as the proline could easily be removed to leave the free carboxylic acid. Molteni reported yields of up to 70% with the proline acrylamide as a chiral auxiliary but the best diastereoselectivity ratio achieved was 67:33. In our case this would give a pyrazoline with an amino acid already coupled at
the acid terminus of the molecule. Before carrying out the synthesis of the proline acrylamide, a test reaction was carried out using N,N-dimethylacrylamide as the dipolarophile (Scheme 51).

Scheme 51

The reaction was carried out under the preferred cycloaddition conditions of in situ chlorination with N-chlorosuccinimide and cycloaddition with KHCO₃ as the base. This gave the pyrazoline 59 in a yield of 25% and a 1:1 mixture of diastereoisomers. As this reaction was a success, synthesis of the proline acrylamide was carried out.

L-Proline was esterified with methanol in the presence of thionyl chloride to give the methyl ester 60 in 100% yield. This was subsequently converted to the acrylamide 61 by a literature procedure to give the desired product in 43% yield (Scheme 52).
With the acrylamide in hand, chlorination and cycloaddition of the hydrazone 47 was carried out to give the pyrazoline 62 in 15% yield (Scheme 53). Although this cycloaddition was achieved, the inclusion of L-proline on the dipolarophile did not influence the diastereoselectivity of the reaction as the products were again a 1:1 mixture of diastereoisomers.

Scheme 53

It was thought that by using a different starting amino acid with a bigger side chain, the diastereoselectivity of the reaction could be influenced from the amine side of the molecule. Thus the amine group of L-valine methyl ester 63 was protected using di-tertiary-butyl dicarbonate in a yield of 78%. The Boc-protected amine 64 was then reduced to the aldehyde 65 using DIBAL at -78 °C in a yield of 76%. This was used immediately and converted to the phenylhydrazone 66 using phenylhydrazine hydrochloride and sodium acetate. The hydrazone was subject to the normal chlorination and KHCO₃ cycloaddition conditions to give the pyrazoline 67 in 20% yield but yet again as an inseparable 1:1 mixture of diastereoisomers (Scheme 54).
One way of avoiding this problem was to start with an amino acid that had no chiral centre at all, the only one of these being glycine (Scheme 55). Again the amine group of glycine methyl ester hydrochloride 68 was protected with di-tertiary-butyl dicarbonate to give the Boc-protected amino acid 69 in 81% yield. This was then reduced to give the aldehyde 70 which was used directly as before to form the phenylhydrazone 71. Chlorination and cycloaddition with ethyl acrylate was carried out to give the
pyrazoline 72 in a poor 8% yield. As there is only one chiral centre in the molecule this was now a mixture of enantiomers.

Scheme 55

As changing the starting amino acid proved unsuccessful in influencing diastereoselectivity, it was decided that attention would be turned back to the
dipolarophile. A variety of ester activated dipolarophiles were tried (Scheme 56) and the results are summarised in the table below (Table 6).

\[
\begin{array}{cccccc}
\text{Entry} & \text{R}^1 & \text{R}^2 & \text{R}^3 & \% \text{Yield} & \text{Ratio of Diastereoisomers} \\
73 & \text{CO}_2\text{Bu} & \text{H} & \text{H} & 27 & 1:1 \\
74 & \text{CO}_2\text{Bu} & \text{Me} & \text{H} & 25 & 1:1 \\
75 & \text{CO}_2\text{Me} & \text{H} & \text{CO}_2\text{Me} & 12 & 1:1 \\
\end{array}
\]

Table 6

Tertiary-butyl acrylate and tertiary-butyl methacrylate were tried as it was thought that the size of the butyl group may lead to preferential cycloaddition from one face but this was not the case as a 1:1 mixture of diastereoisomers was seen in both cases. Dimethyl fumarate was chosen for similar reasons to see if one approach for cycloaddition would be preferred over the other but again the product was a 1:1 mixture of diastereoisomers.
3.5 Conclusion

In conclusion, we have looked at a number of factors to influence the diastereoselectivity of the reaction including the use of different starting amino acids and the use of different dipolarophiles. We have also looked at using a chiral dipolarophile as this would give us a product that was already coupled to an amino acid. In all cases the pyrazolines were isolated, thus extending the set of pyrazolines prepared. However they were still isolated as 1:1 mixtures of diastereoisomers except for the glycine based compound.
3.6 Synthesis of Pyrazoles

It had been queried earlier that the presence of diastereoisomers may have been due to the amino acid aldehydes racemising over a period of time.\textsuperscript{62} It was felt that the integrity of this chiral centre needed to be proven in order to confirm the origin of the diastereoselectivity.

This was done by carrying out the cycloaddition step with the alkyne methyl propiolate as the dipolarophile. This meant that no new chiral centre would be formed in the molecule and the reaction should lead to only one enantiomer. The chlorination and cycloaddition were carried out as normal, using the NCS and KHCO\textsubscript{3} methods respectively, to give the pyrazole 76 in 25% yield (Scheme 57).

\begin{center}
\includegraphics[width=\textwidth]{scheme57.png}
\end{center}

Scheme 57

As this yield was slightly disappointing the cycloaddition was repeated under aqueous media conditions as this had proven to be better yielding in the past. The hydrazonyl chloride 52 was synthesised and isolated as previously before using the Corey – Kim reagent.\textsuperscript{55} Reaction with methyl propiolate in 0.1M NaHCO\textsubscript{3} in the presence of...
tetrahexylammonium chloride gave the pyrazole 76. Under these conditions the pyrazole 76 was formed in a better yield of 40% (Scheme 58).

Scheme 58

3.6.1 HPLC Analysis

The pyrazole 76 was analysed using HPLC with a chiral column. It was apparent that there was one large peak present with what appeared to be a small shoulder at the base. On further manipulation of the chromatography the separation was improved to show two peaks. It was thought that one of these peaks was a major enantiomer with a small presence of the other enantiomer (Fig 20 – Appendix II)).
In order to further confirm that this was case the whole synthesis was carried out using a racemic mixture of D and L-alanine methyl ester hydrochloride 77 (Scheme 59). Initially the amine was group was protected again using di-tertiary-butyl dicarbonate to give the desired product 78 in 90% yield. This was then reduced using DIBAL at -78°C to give the aldehyde 79 in 95%. The aldehyde was used immediately to synthesise the phenylhydrazone 80 in 88% yield which was then subject to the usual chlorination and cycloaddition conditions to give the enantiomeric mixture of pyrazoles 81 in 30% yield.
Scheme 59

This enantiomer mixture was then subject to chiral HPLC chromatography (Fig 21).
By overlaying the chromatograms it was shown that the initial trace was indeed from the L-series showing one major enantiomer with a small presence of the minor enantiomer (Fig 22).

Thus it appears that the amino acid derived chiral centre is very largely preserved during the synthetic sequence, with epimerisation limited to < 1%.

The pyrazole 76 was then subject to deprotections and peptide couplings.

3.7 Peptide Couplings

Firstly the methyl ester was hydrolysed using conditions reported by Molteni. The pyrazole was dissolved in aqueous tetrahydrofuran and 2M NaOH and left for 3h at room temperature. After work up, the free acid 82 was seen in almost quantitative yield. This was pure enough to be used directly for coupling to another amino acid. It was decided that glycine methyl ester hydrochloride would be used as the peptide coupling
could be carried out without the use of any racemisation suppression agent and there would be no introduction of another chiral centre to the molecule. The peptide coupling was carried out using EDCI as the coupling agent in anhydrous diethyl ether to give the product 83 in a yield of 46%, coupled at the acid terminus (Scheme 60).

![Diagram of chemical reactions](attachment:image.png)

**Scheme 60**

It was also possible to deprotect the amine terminus of the pyrazole 76. Trifluoroacetic acid was used to remove the Boc-group and gave the amine as a trifluoroacetate salt which was converted to the hydrochloride salt 84 by treatment with 2M hydrochloric acid in a yield of 65%. This hydrochloride salt was treated with triethylamine to yield the free amine. The peptide coupling was then carried out using Boc-glycine 85 with
EDCI again used as the coupling agent (Scheme 61) to afford 86, albeit in an unoptimised yield of 13%.

Scheme 61

The next logical step to follow this synthesis was to hydrolyse ester 86 and carry out a further coupling reaction to obtain the fully coupled peptide mimetic.

The pyrazole 86 was treated with 2M NaOH at room temperature (Scheme 62) as before.
The hydrolysis step afforded the product 87 in a yield of 70%.

The pyrazole acid 87 was next subject to the peptide coupling conditions with glycine methyl ester (Scheme 63).

This gave the target peptide mimetic 88 coupled at both the amine and acid terminii in a yield of 33%. 
3.7.1 NMR Studies

Whilst we have been able to synthesise the target molecule 88 we need to prove that this molecule could potentially act as a peptide mimetic.

In order to do this we need to look at characteristics of peptide chains and see if 88 shows any of these characteristics. One of the most notable characteristics of the peptide chain is the way that the chain is forced to bend and turn due to the interacting hydrogen bonds (Fig 23).

![Image of hydrogen bonding interactions in a β-turn structure]

**Fig 23 Hydrogen Bonding Interactions in a β-Turn Structure**

For our molecule to be considered as a peptide turn mimetic, it would have to bend and incorporate hydrogen bonding interactions as shown (Fig 24).
One method of confirming the structure and the presence of any hydrogen bonding interactions is X-ray crystallography but this relies on the product being crystalline and growing crystals for analysis, and only applies to the solid state. Another method for identification of potential hydrogen bonding uses $^1$H NMR spectroscopy. Samuel Gellman has made a great contribution in the area of using spectroscopic techniques, such as NMR, to determine the degree of hydrogen bonding in molecules. This technique is can be used to investigate the solution conformation of our peptide mimetic.

The rate at which an amide proton exchanges between its various hydrogen bonded and non-hydrogen bonded states is rapid on the NMR timescale, therefore the lone signal observed represents the weighted average of signals for that particular proton in its different environments. NMR studies documented in the literature have shown that an intramolecularly hydrogen bonded NH proton displays a chemical shift of around 7-8 ppm whereas a free NH proton is around 6 ppm. The NH monitored in our pyrazole occurs at approximately 7 ppm which is within the shift range for intramolecular hydrogen bonding.
VT $^1$H NMR experiments were run on the peptide mimetic 88. Recordings were taken at five degree intervals over the range 273-333 K in CDCl$_3$. The spectra were run at fairly low concentrations (5nM) to minimise any possible intermolecular amide-amide hydrogen bonding.

The change in amide proton chemical shift is shown plotted as a function of temperature (Fig 25).

Fig 25 Amide NH Proton Shifts as a Function of Temperature

In the literature it has been suggested if there is a change in temperature, this should have little effect on the chemical shifts of protons that are involved in an intramolecular
hydrogen bond. It is found that those hydrogens that are exposed to the solvent tend to exhibit a larger temperature coefficient (> 4 ppb/K) than those that are intramoleculary hydrogen bonded (< 3 ppb/K).

The proton studied lies within the relevant chemical shift range suggested for a intramoleularly hydrogen bonded proton but the chemical shift changes quite significantly with temperature i.e. the temperature coefficient (Δδ/ΔT) calculated from the data plotted is -5.9 ppb/K. This value is outside the range of values suggested for a proton that is intramoleularly hydrogen bonding.

3.8 Conclusion

In conclusion, the target molecule has been synthesised as a pyrazole 76 instead of a pyrazoline.

The pyrazole 76 has been subject to amine and ester deprotections. We have then been able to carry out peptide couplings at both terminii to give the individually coupled product as well as the fully coupled product 88.
We have carried out variable temperature $^1$H NMR studies to show if the molecule adopts a β-turn-like conformation through intramolecular hydrogen bonding. The studies have shown that this is unlikely but without further proof this idea cannot be discounted.
3.9 Reversed Functionality

It was proposed that the pyrazolines could be synthesised with "reversed functionality" where the acid and the amine functionalities would be on opposite carbons to the heterocycles prepared so far. The retrosynthetic analysis for this strategy is shown below (Scheme 64).

Scheme 64

The first synthetic steps leading to the synthesis of a suitable benzyl hydrazone have recently been reported in the literature (Scheme 65).53

Scheme 65
The synthesis was repeated to give the benzylhydrazone 91 in a yield of 77%. The same conditions were used in an attempt to synthesise the phenylhydrazone but they just gave starting material in the form of the chlorinated product 90. As an alternative ethyl glyoxylate 92 was treated with phenylhydrazine hydrochloride and sodium acetate in aqueous ethanol as done previously to give the phenylhydrazone 93 in a yield of 56% that required no further purification (Scheme 66).

Scheme 66

It was now possible to start carrying out cycloaddition reactions.

With the knowledge of previous cycloaddition reactions involving hydrazones with chiral centres and also reactions involving the formation of new chiral centres it was decided that, initially, cycloaddition reactions would be carried out with alkynes. In the first instance, the dipolarophile of choice was to be derived from glycine. It is possible to synthesise amino-acid based alkynes via the Corey – Fuchs synthesis but as it had been decided that glycine would be trialled initially, the required functionality could be achieved by using propargylamine as the dipolarophile. In order to make direct comparison with previous work, the amine group of propargylamine was protected with
di-tertiary-butyl dicarbonate and the phenylhydrazone was used as the source of the dipole. The chlorination and cycloaddition steps were carried out using the preferred conditions but although chlorination appeared to have taken place the cycloaddition reaction did not. After this, a series of cycloaddition reactions were investigated using different dipoles and dipolarophiles (Scheme 67).

![Scheme 67](image)

These are summarised in the table below (Table 7).
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**Table 7 Cycloaddition of 93 and 91 with various dipolarophiles**

To summarise, none of the reactions carried out using the phenylhydrazone were successful. As outlined above the cycloaddition was first attempted using Boc-protected propargylamine 105 as the dipolarophile (Entry 1). This was prepared under standard Boc-protection conditions in dichloromethane to give prop-2-ynylcarbamic acid tert-butyl ester 105 in a yield of 96% (Scheme 68).
As the Boc-protected propargylamine did not work we decided to try both propargyl and allyl alcohol (Entries 2 and 3) as the alcohol group could potentially be converted to give the amine functionality, but neither of these cycloadditions worked. Unprotected allylamine was tried to see if the presence of the Boc-protecting group was influencing the reactivity of the dipolarophile but again this reaction was unsuccessful (Entry 4).

We decided to try the chlorinations and cycloadditions again with propargyl alcohol and allylamine but this time the reactions were carried out in toluene and heated to reflux for the cycloaddition steps (Entries 5 and 6). NMR analysis showed that these conditions did not produce my pyrazole.

The phenylhydrazone was converted to the hydrazonyl chloride using the Corey – Kim conditions. This was isolated and the cycloaddition step was carried out with silver acetate as the base and in toluene at room temperature (Entry 7). These conditions had proven to be successful in publications by Molteni and in previous cycloadditions reported in this thesis but again these conditions did not work.

Propargylamine was protected using p-toluenesulfonyl chloride as it was thought that this would make the alkyne bond less electron-rich and aid in the cycloaddition reaction. N-Propargyl-p-toluenesulfonamide 105a was obtained in a yield of 77% (Scheme 69). The cycloaddition reaction was carried out using this dipolarophile but again this proved unsuccessful (Entry 8).
We next decided to turn our attention to the benzylhydrazone of ethyl glyoxylate as it had been shown in the literature that cycloaddition reactions had been carried out with this compound for the formation of pyrazolines.\textsuperscript{53} Initially it was decided that a couple of these literature procedures would be repeated to check that pyrazolines could be formed using this type of dipole. The benzylhydrazone of ethyl glyoxylate was subject to chlorination using NCS as normal and then the cycloaddition step was carried out using ethyl acrylate and styrene as dipolarophiles. The cycloadditions with ethyl acrylate and styrene gave pyrazolines 102 and 103 as reported in the literature in yields of 78 and 29\%, respectively (Entries 9 and 10).

In the literature, the cycloaddition reaction was also carried out using allyl alcohol.\textsuperscript{53} The paper states that the product was extremely difficult to purify and was therefore directly converted to the acid. It was thought that the alcohol could be converted to the
amine. Direct transformations from the crude alcohol product of allyl alcohol cycloaddition to the amine were attempted with no success. We tried to purify the presumed alcohol functionalised product to prove that this was produced in the synthesis but this proved tricky as reported.

As the reported reactions with ethyl acrylate and styrene had worked, the cycloaddition step was carried out with allylamine as the dipolarophile (Entry 11) to give what was assumed to be the pyrazoline 104 in 47% yield (Scheme 70).

Scheme 70

With this desired cycloaddition product assumed to be in hand it was thought that we could go ahead and attempt peptide couplings with another amino acid at the amine terminus. The coupling was initially carried out using Boc-glycine as before but proved to be unsuccessful. On further analysis of the $^1$H NMR spectra of the presumed cycloadduct it was found that the desired pyrazoline product had not been formed, due to the absence of the characteristic doublet of doublet signal seen in the spectra of previous structures.

A paper by Molteni in 2004 discussed potential by-products of a 1,3-dipolar cycloaddition with a hydrazonyl chloride acting as the dipole precursor and allyl
alcohol as the dipolarophile. The four possible products of this reaction (106 - 109), promoted by the silver ion, are shown below (Scheme 71).

![Scheme 71](image)

Scheme 71

We were initially convinced that the desired pyrazoline 104 had been obtained as mass spectrometric analysis was able to give us an accurate mass that matched that of the pyrazoline 104. However the possible by-product 108 reported by Molteni from allyl alcohol has the same molecular mass as the pyrazoline 109, therefore suggesting that synthesis of a by-product was likely in our allylamine reaction.

The rationale for the formation of the by-product 108 is that it is highly likely that the formation of the required nitrile imine is promoted by the high affinity of the silver ion for Cl⁻ and proceeds via the nitrilium cation 110. Normally this cation goes on to lose a proton and form the correct nitrile imine for dipolar cycloaddition to give the correct pyrazoline. However, it is possible that allyl alcohol capture gives rise to carbocation 111 which then evolves to give 108 (Scheme 72).
Scheme 72

This rationale can be applied to our synthesis to determine the possible by-product (Scheme 73).
Although this rationale is in agreement with literature reports, the argument reported by Molteni depends on the affinity of the Cl⁻ ion to the Ag⁺ ion to form the nitrilium cation which captures the allyl alcohol. This in turn goes on to form the by-product via a secondary carbocation intermediate.

In our example there are no ions present that would encourage the loss of the Cl⁻ ion and encourage the formation of the by-product so it remains uncertain as to the structure of the product of our allylamine reaction.
3.10 Conclusion

In conclusion, we have been able to synthesise a number of pyrazolines with "reversed" functionality. However, none of the products formed have proven to be suitable candidates for peptide mimetics. It appears that the reactive nature of the dipole can give rise to potential side reactions and by-products.
3.11 Debenzylation Reactions

As we had the ethyl acrylate cyclisation product in hand it was thought that if the benzyl group of N-1 could be removed then this may give an opportunity to put different functional groups onto the ring at this position, or even extend the peptide mimetic chain from this N-terminus (Scheme 74).

![Scheme 74](image)

There are many examples in the literature for the removal of benzyl groups from amines even though this can be a notoriously tricky reaction. The first conditions that we tried were those reported by Rewolinski involving treatment with base followed by exposure to oxygen. This method was of particular interest as the reaction conditions were applied to N-heterocyclic compounds such as 112, 113 and 114.
The proposed mechanism for this type of debenzylation is shown (Scheme 75).

Scheme 75

The pyrazoline 102 was dissolved in DMSO and potassium tertiary butoxide was added whilst stirring at room temperature. Oxygen was bubbled through the reaction solution for 10 minutes before quenching with saturated ammonium chloride (Scheme 76).
After work up and analysis it was found that these conditions proved far too harsh and the ring appeared to decompose as the $^1$H NMR spectra showed no sign of ring protons being present. It is suggested in an earlier publication by Kawakami and Suzuki that heterocycles which incorporate either nitro or ester groups in the molecule were not tolerant of the reaction conditions.\textsuperscript{70} In these cases the starting materials appear to decompose to a complex mixture of products as soon as they come into contact with the KO$^\text{t}$Bu/DMSO mixture, prior to the addition of oxygen, thus confirming our findings.

A publication by Davies and co-workers reported the use of cerium ammonium nitrate (CAN) for the removal of benzyl groups.\textsuperscript{71} An example of this application is shown (Scheme 77).
These conditions were tried with the styrene cyclisation product 103 as it was thought that there was potentially less functionality for the reagent to react with. The pyrazoline was stirred for 3 hours with CAN at room temperature in aqueous acetonitrile. A yellow oil was produced that solidified after a period of time (Scheme 78).

\[ \text{EtO}_2\text{C} \quad \text{Bn} \quad \text{N-N} \quad \text{EtO}_2\text{C} \quad \text{Ph} \]

\[ \text{MeCN-H}_2\text{O (5:1), RT, 3h} \]

\[ \rightarrow \]

\[ \text{EtO}_2\text{C} \quad \text{N-NH} \quad \text{EtO}_2\text{C} \quad \text{Ph} \]

Scheme 78

\(^1\text{H}\) NMR analysis showed that the pyrazoline ring was still intact and also both phenyl rings were still present. The signals for the protons at C-2 and C-3 had disappeared. It was thought that the actual product was the pyrazole due to the appearance of a single aromatic proton peak in the appropriate region of the \(^1\text{H}\) NMR spectrum. Also a mass spectrum of the product was obtained along with an accurate mass which gave the correct mass for the proposed pyrazole 103\text{a} (Scheme 79).

\[ \text{EtO}_2\text{C} \quad \text{Bn} \quad \text{N-N} \quad \text{EtO}_2\text{C} \quad \text{Ph} \]

\[ \text{MeCN-H}_2\text{O (5:1), RT, 3h} \]

\[ \rightarrow \]

\[ \text{EtO}_2\text{C} \quad \text{Bn} \quad \text{N-N} \quad \text{EtO}_2\text{C} \quad \text{Ph} \]

Scheme 79
Although this was thought to be a potentially useful reaction, without the removal of the benzyl group this molecule could not be utilised as a peptide mimetic. With that in mind we concentrated our efforts on finding ways to remove the benzyl group and leave the free amine.

The use of palladium catalyst and hydrogen as de-benzylating agents has been widely reported in the literature. This gave us reason to utilise this method in order to de-benzylate 103.

The pyrazoline 103 was dissolved in methanol and treated with Pd(OH)$_2$ at room temperature under a hydrogen atmosphere (Scheme 80).

Scheme 80

After 19h the reaction mixture was filtered through a pad of Celite. The resulting filtrate was concentrated under reduced pressure but the product obtained was confirmed by $^1$H NMR spectroscopy to be the starting pyrazoline.
Another reported procedure involving the use of palladium reagents as a means of de-benzylation is catalytic transfer hydrogenation.\textsuperscript{72}

The pyrazoline 103 was stirred in absolute ethanol and acetic acid. The reaction mixture was treated with 10\% Pd-C followed by 1,4-cyclohexadiene under a nitrogen atmosphere (Scheme 81).

\begin{center}
\includegraphics[width=\textwidth]{Scheme81.png}
\end{center}

Scheme 81

After 17h the reaction mixture was again filtered through a pad of celite and the resulting filtrate was concentrated under reduced pressure to give an orange oil that was confirmed by \textsuperscript{1}H NMR spectroscopy to be the starting material.

3.12 Conclusion

In conclusion, a number of known de-benzylation methodologies have been applied to the synthesised pyrazolines. None have these have led to the desired de-benzylated product. This may be due to the number and type of functionalities that are incorporated into the heterocyclic structures. It is neccessary to find a method that will work in a chemoselective manner and just complete the removal of the N-benzyl group.
3.13 Attempted Synthesis with \( \beta \)-alanine

As we had successfully carried out the synthesis of novel pyrazoles and pyrazolines with a number of proteinogenic amino acids, we felt that it was necessary to look at non-proteinogenic amino acids.

It was decided that we would attempt to carry out the complete synthesis of a pyrazole with \( \beta \)-alanine as the starting amino acid to see if an extra carbon in the side chain would have an influence on the 1,3-dipolar cycloaddition reaction. Also it was thought that this extra carbon in the side chain may provide extra flexibility and encourage hydrogen bonding.

We already had \( \beta \)-alanine ethyl ester hydrochloride 115 to hand in the group so the first reaction to carry out was Boc-protection of the amine terminus. This was carried out as already reported in dichloromethane for 17h (Scheme 82).

\[
\begin{align*}
\text{BocO, Et}_3\text{N} & \quad \text{DCM, 17h} \\
& \quad 0 \degree \text{C} - \text{RT} \\
\text{66%}
\end{align*}
\]

After workup, the Boc-protected \( \beta \)-alanine ethyl ester 116 was given in a yield of 66 \%

The next step was the formation of the aldehyde using DIBAL. This was done using the same procedure as carried out for the other amino acids in anhydrous toluene (Scheme 83).
Here the Boc-protected aldehyde 117 was given in a 100% yield and was used for the next step without further purification.

The final step to carry out before attempting the cycloaddition reaction was the formation of the hydrazone analogue. Again this was carried out using the same method as described before in aqueous ethanol (Scheme 84).

The resulting hydrazone 118 formed as a precipitate in solution. After filtration and drying the product was afforded in a yield of 47%.

As the first three steps were performed in a straightforward manner, we were able to begin the 1,3-dipolar cycloaddition reaction of 118 with methyl propiolate in an attempt to synthesise 119.
The synthesis of 119 was attempted under the usual chlorination and cycloaddition conditions using NCS as the chlorinating agent and KHCO$_3$ as the base. Upon completion of the reaction and purification of the product it was found that the pyrazole had not been synthesised and only the starting material remained. The reaction was repeated a couple of times to see if there had been some error in work up or purification but on analysis of all of the isolated products, none of them were proven to be the desired pyrazole.

Therefore it was decided that more work was needed in this area to find suitable methodology to complete the 1,3-dipolar cycloaddition and then to carry out further manipulations.

3.14 Conclusion

In conclusion, we have been able to successfully synthesise the required precursors for 1,3-dipolar cycloaddition from β-alanine ethyl ester hydrochloride. On attempting the cycloaddition reaction using tried and tested methodology, we were not able to obtain the desired pyrazole. Further work is needed on the cycloaddition reaction to find suitable conditions to obtain the required product.
3.15 Final Conclusion

We have succeeded in completing the synthesis of our target pyrazoline 48 by a number of methods using different bases and different strategies.

![Chemical Structure 48](image1)

It has been shown that the reaction is regioselective but is not diastereoselective as 48 was always obtained as a 1:1 mixture of diastereoisomers.

In order to overcome this problem we have looked at different amino acid-derived dipoles and varied dipolarophiles that may have an influence on the diastereoselectivity but none of these proved to be successful. As questions were asked about the integrity of the chiral centre provided by the starting amino acid we turned our attention to proving that this was not the case and prepared pyrazole 76 as essentially the one enantiomer.

![Chemical Structure 76](image2)
We have been able to subject 76 to further manipulations in order to achieve the fully coupled peptide mimetic 88.

![Chemical structure of 88]

We have carried out variable temperature NMR studies to ascertain whether this structure adopts the same folding structure as peptides due to intramolecular hydrogen bonding although this has so far proven to be inconclusive.

Attempts have been made to synthesise pyrazoles and pyrazolines with "reversed functionality" that could be subject to deprotection and coupling reactions in the same vein as 76. However we have not been able to complete the synthesis of a suitable target. We have taken some of the reversed functionality candidates and attempted to remove the benzyl group by a number of deprotection methods but none have these have given the deprotected product.

Finally we have looked at carrying out a complete synthesis of the pyrazole 119 using a non-proteinogenic β-amino acid as our starting point.
Further work is needed in this area to find a suitable methodology to obtain the desired product.
4.0 Experimental

4.1 General information

Solvents and Reagents

All solvents and reagents were purified by standard techniques as reported in Perrin. D.D.; Armarego, W. L. F., Purification of Laboratory Chemicals, 3rd edition. Pergamon Press, Oxford, 1998 or used as supplied from commercial sources as appropriate.

Reagent chemicals were purchased from Aldrich Chemical Company Ltd., Lancaster Chemical Synthesis Ltd. and Acros (Fisher) Chemicals Ltd. Commercially available reagents were used as supplied, without further purification unless otherwise stated. Air- and moisture-sensitive reactions were carried out using glassware that had been dried overnight in an oven at 240 °C.

Solvents where necessary, were dried and stored over 4Å molecular sieves prior to use. Molecular sieves were activated at 240°C over a period of 3 days. Light petroleum (P.E. 40-60) refers to the fraction of the light petroleum ether which boils between 40-60 °C. DCM refers to dichloromethane.
Chromatographic Procedures

Analytical thin layer chromatography (TLC) was conducted using aluminium or glass backed plates coated with 0.25 mm silica containing fluorescer. Plates were visualised by quenching of UV light (254 nm) as well as through staining with 1% w/v potassium permanganate in aqueous alkaline solution followed by heat where appropriate. Flash chromatography was conducted using Merck Kieselgel (70-230 Mesh ASTM) as the stationary phase unless otherwise stated. Samples were applied as saturated solutions in the appropriate solvent. Pressure was applied to the column by use of hand bellows.

High Performance Liquid Chromatography (HPLC) was carried out on a Chrom Elite Automated HPLC system with a chiral reverse phase column. Samples were run at 0.5ml/min in a 99:1 v/v solution of hexane : isopropyl alcohol.

FT-IR

Infra-red spectroscopy (IR) was conducted in the range of 4000-600 cm⁻¹, using a Perkin-Elmer Fourier Transform Paragon 1000 spectrophotometer (with internal calibration). Samples were dissolved in an appropriate solvent and applied as a thin film to the NaCl plates.
$^1$H NMR

Proton magnetic resonance spectra ($^1$H NMR) were recorded at 400 MHz on a Bruker DPX-400 spectrometer as solutions in CDCl$_3$ unless otherwise specified. Chemical shifts ($\delta_H$) are quoted as parts per million (ppm) and are referenced to tetramethylsilane (TMS) as the internal standard. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q) multiplet (m) and broad (br). Assignment of individual proton signals was assisted by analysis of $^1$H COSY spectra and nOe data. Coupling constants ($J$ values) are reported in hertz (Hz). Diastereoisomer ratios were calculated from the integration of suitable peaks in the $^1$H NMR spectra.

$^{13}$C NMR

Carbon magnetic resonance spectra ($^{13}$C NMR) were recorded at 100 MHz using a DPX-400 spectrometer as solutions in CDCl$_3$ unless otherwise specified. Chemical shifts ($\delta_C$) are quoted as parts per million (ppm) and are referenced to tetramethylsilane (TMS) as the internal standard. Assignment of individual carbon signals was assisted by DEPT and HMQC data.
Mass Spectra

Mass spectra (high/low resolution) were recorded using a JEOL SX 102 instrument, with modes of ionisation being indicated as electron impact (EI) and fast atom bombardment (FAB) with only the molecular ion, molecular ion fragments and major peaks being reported. Analysis was performed by Mr J. Kershaw, Department of Chemistry, Loughborough University.

Other Data

Melting points where appropriate were determined using an electrical 9100 Thermal Melting point instrument and are uncorrected. Yields (unless otherwise stated) are quoted for isolated pure products. Combustion analysis data were recorded by Mr A. Daley, Department of Chemistry, Loughborough University.
4.2 Experimental Procedures

L- Alanine methyl ester hydrochloride [42]51

To a suspension of L-alanine (17.60 g, 0.198 mol) in dry methanol (125 ml) at 0 °C, acetyl chloride (66.24 g, 60 ml, 0.84 mmol, 4.3 eq) was added dropwise over 1h with stirring. The reaction mixture was heated to reflux for 20h, after which the solvents were removed under reduced pressure to yield a viscous colourless oil. This formed a white solid after trituration (14.67 g, 93%) that was used without further purification; mp. 109-111 °C (lit.35 109-111 °C); [α]D = 6.4 (c 0.10, MeOH); δH (400 MHz; CDCl₃), 1.41 (3H, d, J 7.2, CH₂CH), 3.75 (3H, s, OCH₃), 4.08 (1H, br s, CH₂CH), 8.53 (3H, br s, NH₂⁺); δC (100 MHz; CDCl₃) 15.6 (CH₃), 48.5 (CH), 52.7 (OCH₃), 171.3 (C=O); νmax (CHCl₃)/cm⁻¹ 3233 (NH), 2932 (aliphatic CH), 2865 (aliphatic CH), 1680 (C=O)
N-tert-Butyloxycarbonyl-L-alanine methyl ester [43]^{st}

To L-alanine methyl ester hydrochloride (10.00 g, 71.67 mmol) in dichloromethane (360 ml) at 0 °C, di-tert-butyl dicarbonate (16.42 g, 75.25 mmol, 1.0 eq) was added. The mixture was stirred for 15 min before triethylamine (14.49 g, 19.96 ml, 143.34 mmol, 2 eq) was added and the reaction mixture stirred for 17 h. The mixture was then washed with citric acid solution (2M, 2 x 150 ml) and saturated brine (2 x 150 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a light yellow oil (14.0 g, 96%). [α]D = -48.0 (c 0.10, CHCl3); δH (400 MHz; CDCl3) 1.38 (3H, d, J 7, CH3CH), 1.45 (9H, s, (CH3)J), 3.75 (3H, s, OCH3), 4.33 (1H, m, CH3CH), 4.97 (1H, br s, NH); δc (100 MHz; CDCl3) 18.5 (CH3), 28.3 (C(CH3)3), 49.1 (CH), 52.3 (OCH3), 155.1 (C=O), 173.9 (C=O); νmax (CHCl3)/cm⁻¹ 3265 (NH), 2957 (aliphatic CH), 2866 (aliphatic CH), 1750 (C=O), 1690 (C=O)
N-Benzylloxycarbonyl-L-alanine methyl ester [44]^51

To a biphasic mixture of L-alanine methyl ester hydrochloride (5.04 g, 36.13 mmol) and potassium hydrogen carbonate (14.41 g, 143.91 mmol, 4.0 eq) in water (150 ml) and ethyl acetate (175 ml) at 0 °C was added dropwise over 30 min with vigorous stirring benzyl chloroformate (7.41 g, 6.2 ml, 43.44 mmol, 1.2 eq). The reaction mixture was left to stir at room temperature for 16 h. The aqueous phase was acidified to pH 1 with concentrated hydrochloric acid, separated and further extracted with ethyl acetate (150 ml). The organic phases were combined, washed with saturated brine (2 x 200 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil which was purified by chromatography on silica gel, eluting with light petroleum : ethyl acetate (3:1 v/v) to yield the title compound as a colourless oil (4.61 g, 53%). [α]D = -34.3 (c 1.1, MeOH); δH (400 MHz; CDCl3) 1.32 (3H, d, J 7, CH3CH), 3.65 (3H, s, OCH3), 4.31 (1H, m, CH2CH), 5.02 (2H, s, OCH2Ph), 5.34 (1H, br s, NH), 7.31 (5H, s, Ph-H); δC (100 MHz; CDCl3) 18.5 (CH3), 49.6 (CH), 52.4, (OCH3), 66.9 (CH2), 128.1 (Ph-CH), 128.3 (Ph-CH), 128.4 (Ph-CH), 136.3 (Ph-C), 155.7 (C=O), 173.52 (C=O); v_max (CHCl3)/cm^-1 3338 (NH), 2952 (aliphatic CH), 1749 (C=O), 1699 (C=O)
L-2-(N-tert-butyloxycarbonylamino)propanal [45]^{73}

To L-N-tert-butyloxycarbonylalanine methyl ester (2.64 g, 13.06 mmol) in dry toluene (60 ml) at -78 °C under nitrogen over 1h, DIBAL-H (1.0M in toluene, 44.28 ml, 44.28 mmol, 2.5 eq) was added dropwise with stirring, with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5h. Methanol (14 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (50 g) in water (200 ml), and stirred vigorously for 1.5h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (2.1 g, 93%) that was used without further purification. $\delta_H$ (400 MHz; CDCl$_3$) 1.34 (3H, d, J 7, CH$_3$CH), 1.45 (9H, s, C(CH$_3$)$_3$), 4.63 (1H, m, CH$_3$CH), 5.10 (1H, br s, NH), 9.57 (1H, s, CHO); $\delta_C$ (100 MHz; CDCl$_3$) 14.7 (CH$_3$), 28.3 (C(CH$_3$)$_3$), 55.5 (CH), 155.4 (C=O), 199.9 (CHO); $\nu_{max}$ (CHCl$_3$)/cm$^{-1}$ 3347 (NH), 2978, 2934 (aliphatic CH), 1682 (C=O).
L-2-(N-benzylxycarbonylamino)propanal [46] 73

To N-Benzylxycarbonyl-L-alanine methyl ester (2.15 g, 9.05 mmol) in dry toluene (30 ml) at -78 °C under nitrogen over 1h, DIBAL-H (1.0M in toluene, 22.14 ml, 22.14 mmol, 2.4 eq) was added dropwise with stirring, with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5h. Methanol (14 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (50g) in water (200 ml), and stirred vigorously for 1.5h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (1.7 g, 93%) that was used without further purification. δH (400 MHz; CDCl3) 1.36 (3H, d, J 7.6, CH3CH), 4.30 (1H, br s, CH3CH), 5.12 (2H, s, OCH2Ph), 5.37 (1H, br s, NH), 7.17 – 7.35 (5H, m, Ph-H), 9.54 (1H, s, CHO); δC (100 MHz; CDCl3) 13.6 (CH3), 63.6 (CH), 69.6 (CH2), 127.3 (Ph-CH), 127.4 (Ph-CH), 128.7 (Ph-CH), 140.9 (Ph-C), 157.5 (C=O), 200.6 (CHO); vmax (CHCl3)/cm⁻¹ 3335 (NH), 2974 (aliphatic CH), 1697 (C=O).
L-[1-Methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester [47]

To phenylhydrazine hydrochloride (3.28 g, 22.68 mmol, 1.5 eq) and sodium acetate (4.98 g, 60.71 mmol, 4 eq) in water, was added the crude L-2-(N-tert-butyloxycarbonylamino)propanal (2.60 g, 15.01 mmol) in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The resulting precipitate was filtered and dried to give an orange solid (3.19 g, 81%) that was used without further purification; mp. 118-120 °C; δH (400 MHz; CDCl3) 1.29 (3H, d, J 7, CH3CH), 1.40 (9H, s, C(CH3)3), 4.34 (1H, br s, CH3CH2), 5.00 (1H, br s, NH), 6.77 (1H, t, J 6.8, Ph-H), 6.92 (2H, d, J 6, Ph-H), 7.00, (1H, br s, CH=N), 7.18 (2H, t, J 7.2 Ph-H), 8.22 (1H, br s, NH); δC (100 MHz; CDCl3) 28.4 (C(CH3)3), 112.6 (PhC), 120.0 (PhC), 129.3 (PhC), 144.9 (C=N), 155.3 (C=O); vmax (CHCl3)/cm⁻¹ 3383 (NH), 1691 (C=O), 1606 (C=N), 1165 (C-O), 753 (PhCH); m/z (FAB) 263.1629 (M⁺ C₁₄H₂₁N₃O₂ requires 263.1634), 263 (33), 208 (53), 147 (100), 93 (41), 57 (60)
L-N'- (2-tert-Butyloxycarbonylamino) propylidene) hydrazinecarboxylic acid benzyl ester [50]

A solution of L-2-(N-tert-butyloxycarbonylamino)propanal (2.4 g, 13.90 mmol) and benzyl carbazate (2.38 g, 14.06 mmol, 1.0 eq) was left to stand in toluene (20 ml) for 20h. The product separated out as a white solid which was collected by suction filtration to yield the title compound (2.14 g, 48%) as a white solid; mp 101-103 °C; $[\alpha]_D = 13.2 \, (c \ 1.14, \text{CHCl}_3)$; $\delta_H$ (400 MHz; CDCl$_3$) 1.25 (3H, d, J 6.8, CH$_3$CH), 1.37 (9H, s, C(CH$_3$)$_3$), 4.03 (1H, br s, CH$_2$CH), 5.14 (2H, s, CH$_2$Ph), 7.26-7.30 (5H, m, Ph-H), 7.97 (1H, br s, NH); $\delta_C$ (100 MHz; CDCl$_3$) 18.91 (CH$_3$), 28.4 (C(CH$_3$)$_3$), 47.44 (CH), 67.4 (CH$_2$-Ph), 128.2 (PhC), 128.4 (PhC), 128.6 (PhC), 135.8 (C=N), 136.0 (C=O), 155.2 (C=O); $\nu_{max}$ (KBr)/cm$^{-1}$ 3329 (NH), 3264 (NH), 1712 (C=O), 1521 (C=N), 1165 (C=O), 737 (PhCH); m/z (EI) 322.1770 (M$^+$ C$_{16}$H$_{25}$N$_3$O$_4$ requires 322.1767), 307 (27), 154 (100), 137 (49), 136 (57), 91 (31).
L-N'-2-tert-Butoxycarbonylaminopropylidene)hydrazinecarboxylic acid tert-butyl ester [49]

A solution of L-2-(N-tert-butyloxycarbonylamino)propanal (2.10 g, 12.16 mmol) and tert-butyl carbazate (1.61 g, 12.15 mmol, 1.0 eq) was left to stand in toluene (20 ml) for 20h. No precipitate was seen so the reaction mixture was concentrated under reduced pressure to give a yellow oil, which was treated with methanol and water to give a white solid (0.94 g, 27%) that was collected by suction filtration; mp 118-120 °C; (Found C, 54.5; H, 8.3; N, 14.2. C₁₃H₂₅N₃O₄ requires C, 54.3; H, 8.8; N, 14.6%); [α]D = -11.6 ( c 1.03, CHCl₃); δH (400 MHz; CDCl₃) 1.26 (3H, d, J 6.8, CH₃CH), 1.37 (9H, s, C(CH₃)₃), 1.43 (9H, s, C(CH₃)₃), 4.30 (1H, br s, CH₂CH), 5.19 (1H, br s, NH), 7.19 (1H, s, HC=N), 7.72 (1H, br s, NH); δC (100 MHz; CDCl₃) 19.0 (CH₃), 28.3 (C(CH₃)₃), 28.4 (C(CH₃)₃), 47.37 (CH), 136.0 (C=N), 155.3 (C=O), 156.3 (C=O); vₘₚ (KBr)/cm⁻¹ 3348 (NH), 1711 (C=O), 1535 (C=N), 1164 (C-O), 779 (PhCH); m/z (EI) 288.1928 (M⁺, C₁₃H₂₅N₃O₄ requires 288.1923), 176 (38), 154 (100), 136 (27), 57 (22).
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [48]

To a solution of L-[1-Methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.24 g, 4.72 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.71 g, 5.35 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Ethyl acrylate (0.918 g, 1.0 ml, 9.16 mmol, 1.9 eq), potassium hydrogen carbonate (2.41 g, 23.97 mmol, 5.1 eq) and a few drops of water were added and the reaction mixture was left to stir at 70° C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a dark orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (7:1 v/v) to yield the title compound (0.72 g, 41%) in an inseparable 1:1 mixture of diastereoisomers, as an orange solid; mp 93-95 °C; (Found C, 62.6; H, 7.2; N 11.8. C₁₉H₂₇N₃O₄ requires C, 63.1; H, 7.5; N, 11.6%); δH (400 MHz; CDCl₃) 1.16 (3H, t, J 7.2, CO₂CH₂CH₃), 1.35 (3H, d, J 7.2, CH₃CH), 1.38 (9H, s, C(CH₃)₃), 2.99 (1H, dd, J 7.2 & 17.6, CH₂), 3.24 (1H, dd, J 12.4 & 17.6, CH₂), 4.14 (2H, q, J 7.2, CH₂CH₃), 4.43 (1H, m, CH₃CH), 4.54 (1H, dd, J 7.2 & 12.4 CHCO₂CH₂CH₃), 4.57 (1H, dd, J 7.2 & 12.4, CHCO₂CH₂CH₃), 5.00 (1H, br s, NH), 6.78 (1H, t, J, Ph-H), 6.93 (2H, d, J 8.4, Ph-H), 7.18 (2H, t, J 7.6, Ph-H); δC (100 MHz; CDCl₃) 14.2 (CH₃) 21.09 (CH₃), 28.2
((CH₃)₃C), 40.15 (CH₂), 46.08 (CH), 61.7 (CH₂), 62.43 (CH), 113.0 (Ph-CH), 113.0 (Ph-CH), 119.7 (Ph-CH), 119.8 (Ph-CH), 129.0 (Ph-CH), 129.1 (Ph-C), 145.3 (C=N), 171.2 (C=O), 171.5 (C=O); νmax (CHCl₃)/cm⁻¹ 3354 (NH), 1599 (C=N), 1708 (C=O), 1168 (C-O), 750 (PhCH); m/z (EI) M⁺362.2073 (C₁₉H₂₇N₃O₄ requires 362.2080), 171 (12), 154 (24), 147 (19), 123 (21), 111 (28), 109 (35), 95 (54), 81 (54), 69 (85), 57 (100), 55 (99).
Preparation of hydrazonyl chloride [52] by Corey-Kim Chlorination

To N-chlorosuccinimide (2.70 g, 20.27 mmol, 3.0 eq) in dichloromethane (30 ml) at 0 °C was added dimethyl sulfide (3.3 g, 3.0 ml, 42.24 mmol, 6.3 eq) over 5min. After stirring for 15min the reaction mixture was further cooled to −78 °C. L-[1-Methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.76 g, 6.67 mmol) in dichloromethane (20 ml) was added and the reaction mixture was stirred at this temperature for 1h, then slowly allowed to warm to room temperature over a period of 3h. Cold water (70 ml) was added, and the organic layer was washed with cold water (70 ml), saturated brine solution (70 ml), saturated aqueous sodium sulphate (70 ml) and water (100 ml). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to give a dark orange oil (1.3g, 55%) that was used without further purification. δH (400 MHz; CDCl3) 1.39 (12H, s, CH3CH & (CH3)3C), 4.59 (1H, m, CH3CH), 4.85 (1H, br s, NH), 6.79 (1H, t, J 7.4, Ph-H), 6.92 (2H, d, J 8.8, Ph-H), 7.16 (2H, t, J 7.6, Ph-H); δC (100 MHz; CDCl3) 19.6 (CH3), 28.4 (C(CH3)3), 52.3 (CH), 113.2 (Ph-CH), 121.0 (Ph-CH), 129.3 (Ph-CH), 143.5 (Ph-C), 143.4 (C=N), 154.8 (C=O); νmax (CHCl3)/cm⁻¹ 3372 (NH), 2359 (=NH), 1700 (C=O), 1602 (C=N),
1165 (C-O);  m/z (FAB) M 297.1238 (C_{14}H_{20}N_{3}O_{2}Cl requires 297.1244), 297 (42), 241 (71), 181 (100), 154 (66), 136 (48), 57 (83)
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [48]

A solution of the hydrazonyl chloride 52 (1.30 g, 3.65 mmol) and ethyl acrylate (0.46 g, 0.5 ml, 4.58 mmol, 1.3 eq) in dry toluene (50 ml) under nitrogen was treated with silver acetate (1.22 g, 7.31 mmol, 2.0 eq) under stirring at room temperature for 21h. The undissolved material was filtered off and the solvent reduced under reduced pressure to give a brown oil. This was purified using column chromatography (3:2 ethyl acetate : light petroleum to yield an orange oil (0.20 g, 15%) as a mixture of inseperable diastereoisomers (1:1).

NMR Data consistent with those of 48 listed earlier.
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [48]

The hydrazonyl chloride 52 (1.30 g, 3.65 mmol), ethyl acrylate (1.0 ml) and tetrahexylammoniumchloride (0.14 g, 0.36 mmol, 0.1 eq) in sodium hydrogen carbonate solution (0.1M, 20 ml) was stirred for 20h. The reaction mixture was filtered by suction filtration, the solid material washed with water and dried to give an orange solid (0.69 g, 57%) that required no more purification. The product was an inseparable mixture of diastereoisomers (1:1).

NMR Data consistent with those of 48 listed earlier.
N-tert-Butyloxycarbonyl-L-alanine [53] 

![Chemical Structure](image)

To a solution of L-alanine (6.45 g, 72.40 mmol) in 1,4-dioxane, water and 1M sodium hydroxide (280 ml) (2:1:1 v/v/v) at 0 ºC was added di-tert-butyl dicarbonate (17.86 g, 81.83 mmol, 1.1 eq). The solution was stirred for 90 min at 0 ºC then allowed to warm to room temperature and stirred for a further 30 min. The reaction mixture was concentrated under reduced pressure to approx. 70 ml then cooled to 0 ºC. Ethyl acetate (70 ml) was added and the biphasic mixture acidified to pH 3 with a solution of potassium hydrogen sulfate (2M). The aqueous layer was separated, then extracted with ethyl acetate (2x50 ml). The organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was recrystallised from light petroleum and ethyl acetate to yield a white solid (9.47 g, 69%); mp 83-85 ºC (lit. 79-83 ºC); [α]D = -48.0 (c 0.10, CHCl₃); δH (400 MHz; CDCl₃) 1.39 (12H, m, CH₃CH₂C(CH₃)₃), 4.11 (1H, br s, CHCH₃), 4.97 (1H, br s, NH); δC (100 MHz; CDCl₃) 18.3 (CH₃), 28.3 (C(CH₃)₃), 49.1 (CH), 155.5 (C=O), 177.7 (C=O); νmax (CHCl₃)/cm⁻¹; 3321 (NH), 2979 (aliphatic CH), 1724 (C=O), 1700 (C=O), 1164 (C-O)
N-Benzoxycarbonyl-L-alanine [54] 58

To a solution of L-alanine (9.97 g, 111.97 mmol) and sodium hydroxide (9.1 g, 227.5 mmol) in water (100 ml) was added benzyl chloroformate (19.40 g, 16.23 ml, 113.72 mmol, 1.0 eq) dropwise at 0 °C with the simultaneous addition of sodium hydroxide in water over a period of 1h. The mixture was stirred for a further 16h. After this period the solution was carefully acidified to pH 1 with concentrated hydrochloric acid. The suspension was then extracted with ethyl acetate (2 x 70ml), and the organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the title compound as a white solid (24.1 g, 96%); mp 83-85 °C (lit. 74-82-84 °C); [α]D = -14.2 (c 2.0, CHCl₃); δH (400 MHz; CDCl₃) 1.25 (3H, d, J 7, CHCH₃), 4.00 (1H, br s, CHCH₃), 5.02 (2H, s, PhCH₂), 7.36 (5H, s, Ph-H), 7.56 (1H, d, J 7, NH); δC (100 MHz; CDCl₃) 18.4 (CH₃), 49.5 (CH), 67.2 (CH₂), 128.2 (Ph-CH), 128.3 (Ph-CH), 128.6 (Ph-C), 135.7 (Ph-C), 155.9 (C=O), 177.8 (C=O); νmax (CHCl₃)/cm⁻¹; 3333 (NH), 1701 (C=O), 1534 (C-O)
N-Benzylloxycarbonyl-L-alanine N²-phenylhydrazide [55]

To a solution of L-N-benzyloxycarbonylalanine (1.02 g, 4.57 mmol) in DMF (20 ml) was added 1-hydroxybenzotriazole hydrate (0.78 g, 5.77 mmol, 1.3 eq) followed by EDCI (1.00 g, 5.22 mmol, 1.1 eq). The reaction mixture was stirred for 2h at room temperature before being slowly added to a solution of phenylhydrazine (1.21 g, 1.1 ml, 11.18 mmol, 2.4 eq) in DMF (20 ml) at 0 °C. After 0.5h, water (40 ml) was added and the organic phase was extracted with ethyl acetate (2 x 40 ml). The organic phases were combined, washed with saturated sodium hydrogen carbonate solution (40 ml), dried over magnesium sulfate, filtered and concentrated under reduced pressure to give an orange solid. This was recrystallised from dichloromethane/hexane to yield the title compound as a white solid (0.71 g, 50%); mp 152-154 °C (lit. 154.5-155.5 °C); [α]_D = +4.2 (c 0.17, MeOH); δ_H (400 MHz; CDCl₃) 1.45 (3H, d, J 6.8, CH₃CH), 4.36 (1H, m, CHCH₃), 5.15 (2H, s, CH₂Ph-H), 5.38 (1H, br s, NH), 6.09 (1H, br s, NH), 6.81 (2H, d, J 8, Ph-H), 6.92 (1H, t, J 7.6, Ph-H), 7.23 (2H, t, J 7.6, Ph-H), 7.37 (5H, br s, CH₂Ph-H), 8.21 (1H, br s, NH); δ_C (100 MHz; CDCl₃) 18.0 (CH₃), 49.2 (CH), 67.3 (CH₂), 112.9 (Ph-C), 113.5 (Ph-C), 121.3 (Ph-C), 128.2 (Ph-C), 128.4 (Ph-C), 128.6 (Ph-C), 129.2 (Ph-C), 129.6 (Ph-C), 147.6 (C=O), 172.4 (C=O)
To a solution of L-N-tert-butyloxycarbonylalanine (3.25 g, 17.15 mmol) in DMF (40 ml) was added 1-hydroxybenzotriazole (3.08 g, 22.80 mmol, 1.3 eq) followed by EDCI (3.60 g, 18.78 mmol, 1.1 eq). The reaction mixture was stirred for 2h at room temperature before being slowly added to a solution of phenylhydrazine (3.12 g, 2.9 ml, 29.47 mmol, 1.7 eq) in DMF (20 ml) at 0 °C. After 0.5h, water (40 ml) was added and the organic phase was extracted with ethyl acetate (40 ml). The organic phases were combined, washed with saturated sodium hydrogen carbonate (40 ml), dried over magnesium sulfate, filtered and concentrated under reduced pressure to give an orange solid. This was recrystallised from dichloromethane/hexane to yield the title compound as a white solid (2.7 g, 56%); mp 148-150 °C (lit.75 151-152 °C); [α]D = -46.8 (c 1.17, CHCl3); δH (400 MHz; CDCl3) 1.41 (3H, d, J 6.8, CH3(CH), 1.48 (9H, s, C(CH3)3), 4.25 (1H, br s, CH3(CH), 6.06 (1H, br s, NH), 6.82 (2H, d, J 7.6, Ph-H), 6.89 (1H, t, J 7.6, Ph- H), 7.22 (2H, t, J 7.2, Ph-H), 8.33 (1H, br s, NH); δC (100 MHz; CDCl3) 17.7 (CH3), 28.3 (C(CH3)3), 48.6 (CH), 113.6 (Ph-C), 121.2 (Ph-C), 129.2 (Ph-C), 147.7 (Ph-C), 155.8 (C=O), 172.7 (C=O)
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [48]

To a solution of N-tert-butyloxycarbonyl-L-alanine N^2-phenylhydrazide (0.56 g, 2.02 mmol) in dry acetonitrile (20 ml) under nitrogen was added triphenylphosphine (2.53 g, 9.65 mmol, 4.8 eq) followed by carbon tetrachloride (1.594 g, 1.0 ml, 10.36 mmol, 5.1 eq). The reaction mixture was left to stir at room temperature for 17h. Saturated brine (20 ml) was added and the organic phase was separated, dried over magnesium sulfate, filtered and concentrated under reduced pressure to give an orange oil. This was dissolved in ethyl acetate (15 ml) along with ethyl acrylate (2.30 g, 2.5 ml, 22.92 mmol, 11.3 eq). Triethylamine (5.08 g, 7.0 ml, 50.22 mmol, 24.9 eq) was added and the reaction mixture was stirred at 70 °C for 20h. After this period the reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil which was purified by column chromatography on silica gel, eluting with light petroleum : ethyl acetate (7:1 v/v) to yield the title compound as an orange solid (0.22 g, 31%). The products was given as an inseperable mixture of diastereoisomers (1:1).

NMR Data consistent with those of 48 listed earlier.
[1-(5-Dimethylcarbamoyl-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)ethyl]carbamic acid tert-butyl ester [59]

To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (2.64 g, 10.04 mmol) in ethyl acetate (25 ml) at 60 °C was added N-chlorosuccinimide (1.61 g, 12.06 mmol, 1.2 eq). The reaction mixture was left to stir for 1h. N-Dimethylacrylamide (1.92 g, 2.0 ml, 19.41 mmol, 1.9 eq), potassium hydrogen carbonate (5.06 g, 50.54 mmol, 5.0 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (1:1 v/v) to yield the title compound (2.1 g, 58%) as an orange oily solid which was an inseparable mixture of diastereoisomers (1:1); δH (400 MHz; CDCl3) 1.38 (12H, br s, CH3CH & C(CH3)3), 2.94 & 3.00 (6H, s, N(CH3)2), 4.42 (1H, br s, CHCH3), 4.71 (1H, m, CH), 5.17 (1H, br s, NH), 6.75 (1H, q, J 5.2, Ph-H), 6.86 (1H, t, J 8, Ph-H), 7.09 (1H, d, J 8.8, Ph-H), 7.15 (2H, t, J 8.4, Ph-H); δC (100 MHz; CDCl3) 19.1 (CH3), 28.4 (C(CH3)3), 35.6 (NCH3), 37.4 (NCH3), 38.8 (CH2), 46.1 (CH), 62.1 (CH), 112.7
(Ph-C), 113.8 (Ph-C), 119.5 (Ph-C), 128.9 (Ph-C), 129.3 (Ph-C), 145.3 (C=N), 166.6
(C=O), 170.5 (C=O); $\nu_{\text{max}}$ (CHCl$_3$/cm$^{-1}$) 3305 (NH), 2976 (aliphatic CH), 1708 (ester
C=O), 1655 (amide C=O), 1599 & 1503 (arene C=C), 1171 (ester C-O); $m/z$ (EI) M
360.2168 (C$_{19}$H$_{28}$N$_4$O$_3$ requires 360.2161), 302 (24), 257 (21), 243 (22), 232 (41), 205
(26), 172 (29), 171 (100), 57 (31)
L-Proline methyl ester hydrochloride [60]

To a solution of L-proline (3.31 g, 28.73 mmol) in methanol (50 ml) was added thionyl chloride (5.21 g, 3.2 ml, 43.86 mmol, 1.5 eq) dropwise at 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 4 h. Excess methanol was removed under reduced pressure. The residue was treated with ether (20 ml) and concentrated under reduced pressure to yield the title compound as a pale yellow oil (4.7 g, 100%). \([\alpha]_D = -34.0 (c 1.11, \text{MeOH})\), \((\text{lit}^{74} = -33.0 (c 1.00 \text{H}_2\text{O})\)); \(\delta_H (400 \text{ MHz; DMSO}) 1.87-2.01 (4H, m, \text{CH}_2\text{CH}_2\text{CH}_2), 2.22 - 2.27 (2H, m, \text{CH}_2\text{CH}_2\text{CH}_2), 3.75 (3H, s, \text{CO}_2\text{CH}_3), 9.19 (1H, s, \text{CHCO}_2\text{CH}_3), 10.59 (1H, s, \text{NH}); \delta_C (100 \text{ MHz; DMSO}) 23.08 (\text{CH}_2\text{CH}_2), 29.94 (\text{CH}), 45.04 (\text{CH}_3), 58.54 (\text{CH}_2), 170.24 (\text{C=O})\); \(\nu_{\text{max}} (\text{CHCl}_3)/\text{cm}^{-1} 3337 (\text{NH}), 2952 (\text{aliphatic CH}), 1749 (\text{ester C=O}), 1174 (\text{C-O})\).
N-Acryloyl-L-proline methyl ester [61] 61

![Diagram of N-Acryloyl-L-proline methyl ester]

To a solution of L-proline methyl ester hydrochloride (4.41 g, 6.64 mmol), triethylamine (5.39 g, 7.42 ml, 53.28 mmol, 2 eq) and DMAP (0.65 g, 5.32 mmol, 0.8 eq) in dichloromethane (100 ml) at 0 °C, was added acryloyl chloride (2.89 g, 2.60 ml, 31.93 mmol, 1.2 eq). The mixture was stirred at room temperature for 19.5 h and then washed with 1M HCl (3 x 50 ml) followed by 1M NaHCO3 (3 x 50 ml). The organic layers were combined, dried over MgSO4 and concentrated under reduced pressure to give a pale yellow oil. This was purified by column chromatography on silica gel eluting with ethyl acetate : acetone (2:1 v/v) to give a colourless oil (2.08 g, 43%). [α]D = -30.4 (c 1.23 CHCl3); δH (400 MHz; CDCl3) 2.04 – 2.25 (4H, m, CH2CH2), 3.61 – 3.80 (2H, m, NCH2) 4.55 (1H, dd, J 3.6 & 8.4, CHCO2CH3); Rotational isomer A 3.74 (3H. s, CO2CH3), 5.67 (1H, dd, J 2.4 & 10, CH), 6.26 (1H, dd, J 10 & 16.4, CH), 6.37 (1H, dd, J 2 & 16.4, CH); Rotational isomer B 3.75 (3H, s, CO2CH3), 5.72 (1H, dd, J 2.4 & 10, CH), 6.38 (1H, dd, J 2.4 & 16.8, CH), 6.49 (1H, dd, J 10.4 & 16.8, CH); δC (100 MHz; CDCl3) 24.7 (CH2), 29.1 (CH2), 46.4 (CH2), 52.1 (CH3), 58.8 (CH), 128.3 (CH2), 128.0 (CH), 164.4 (C=O), 172.5 (C=O); νmax (CHCl3)/cm⁻¹ 3467 (NH), 2953, 2880 (aliphatic CH), 1741 (ester C=O), 1643 (amide C=O), 1197 (C-O).
1-[5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carbonyl]pyrrolidine-2-carboxylic acid methyl ester [62]

To a solution of the hydrazone (0.77 g, 2.92 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.42 g, 3.14 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. N-Acryloyl-L-proline methyl ester (1.08 g, 5.88 mmol, 2.0 eq), potassium hydrogen carbonate (1.48 g, 14.78 mmol, 5.1 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (2:1 v/v) to yield the title compound (0.20 g, 15%) as a yellow oil which was an inseperable mixture of diastereoisomers (1:1). $\delta_H$ (400 MHz; CHCl₃) 1.35 – 1.40 (13H, m, C(CH₃)₃ & CH₂CH₂), 1.37 (3H, d, $J$ 6.8, CHCH₃), 2.94 (1H, dd, $J$ 9.2 & 17.6, CH), 3.00 (1H, dd, $J$ 9.6 & 18, CH), 3.32 (1H, dd, $J$ 12.8 & 17.6, CH), 3.50 – 3.58 (3H, m, CHCO₂CH₃ & CH₂), 3.63 (3H, s, CO₂CH₃), 4.46 – 4.50 (1H, m, CHCH₃), 4.60 (1H, dd, $J$ 9.6 & 16, CH), 4.61 (1H, dd, $J$ 9.2 & 15.2, CH), 4.94 (1H, br s, NH), 6.78 – 6.81 (1H, m, Ph-H), 6.88 – 6.96 (2H, m, Ph-H), 7.13 – 7.18 (2H, m,
Ph-H; $\delta_C$ (100 MHz; CHCl$_3$) 24.7 (CH$_2$), 28.2 (CH$_3$), 28.4 (C(CH$_3$)$_3$), 29.3 (CH$_2$), 46.3 (CH), 46.8 (CH$_2$), 48.6 (CH$_2$), 52.4 (CH$_3$), 58.9 (CH), 59.7 (CH), 122.9 (Ph-C), 124.4 (Ph-C), 127.7 (Ph-C), 129.2 (Ph-C), 137.1 (C=N), 160.9 (C=O), 171.9 (C=O); $\nu_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$ 3433 (NH), 2977, 2879 (aliphatic CH), 1743, 1702 (ester C=O), 1656 (amide C=O), 1173 (C-O) 1051 (C-O); $m/z$ (FAB) M 444.2367 ($C_{23}H_{32}N_4O_5$ requires 444.2373), 328 (65), 232 (52), 172 (21), 171 (100), 57 (16)
N-tert-Butyloxy carbonylglycine methyl ester [69]\textsuperscript{76}

\[ \text{CO}_2\text{Me} \quad \text{CO}_2\text{Me} \]
\[ + \quad - \]
\[ \text{NH}_3\text{Cl} \quad \text{NHBOc} \]

To glycine methyl ester hydrochloride (5.00 g, 39.86 mmol) in dichloromethane (180 ml) at 0 °C, di-tert-butyl dicarbonate (9.09 g, 41.65 mmol, 1.0 eq) was added. The mixture was stirred for 15 min before triethylamine (7.95 g, 11.0 ml, 78.57 mmol, 2.0 eq) was added and the reaction mixture stirred for 17 h. The mixture was then washed with citric acid solution (2M, 2 x 75 ml) and saturated brine (2 x 75 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a clear oil (7.3 g, 81%). \( \delta \text{H}(400 \text{ MHz}; \text{CDCl}_3) \): 1.38 (9H, s, C(CH\text{3})\text{3}), 3.68 (3H, s, CO\text{2}CH\text{3}), 3.85 (2H, d, J 5.2, CH\text{2}), 5.03 (1H, br s, NH); \( \delta \text{C}(100 \text{ MHz}; \text{CDCl}_3) \): 28.2 (C(CH\text{3})\text{3}), 42.3 (CH\text{2}), 52.2 (CH\text{3}), 155.9 (C=O), 171.0 (C=O); \( \nu \text{max} (\text{CHCl}_3) \): 3371 (NH), 2978, 2935 (aliphatic CH), 1701, 1755 (ester C=O), 1369 (ester C-O)
L-N-tert-Butyloxy carbonylvaline methyl ester [63]^{76}

To L-valine methyl ester hydrochloride (5.00 g, 29.84 mmol) in dichloromethane (180 ml) at 0 °C, di-tert-butyl dicarbonate (6.89 g, 31.57 mmol, 1.1 eq) was added. The mixture was stirred for 15 min before triethylamine (5.78 g, 8.0 ml, 57.22 mmol, 1.9 eq) was added and the reaction mixture stirred for 17 h. The mixture was then washed with citric acid solution (2M, 2 x 75 ml) and saturated brine (2 x 75 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a clear oil (6.2 g, 78%). $[\alpha]_D^0 = 184.71 \ (c \ 1.57, \ CHCl_3); \ \delta_H \ (400 \ MHz; \ CHCl_3) \ 0.85 \ (7H, \ dd, \ J \ 6.8 \ & 26.4, \ CH(CH_3)_2), \ 1.38 \ (9H, \ s, \ C(CH_3)_3), \ 3.67 \ (3H, \ s, \ CO_2CH_3), \ 4.14 - 4.17 \ (1H, \ m, \ CHCH(CH_3)_2), \ 4.97 \ (1H, \ br \ s, \ NH); \ \delta_C \ (100 \ MHz; \ CDCl_3) \ 28.3 \ (C(CH_3)_3), \ 31.1 \ (CH(CH_3)_2), \ 52.0 \ (CH_3) \ 79.7 \ (CH), \ 146.8 \ (CH), \ 155.7 \ (C=O), \ 172.9 \ (C=O); \ \nu_{max} \ (CHCl_3)/cm^{-1} \ 3367 \ (NH), \ 2970, \ 2935, \ 2877 \ (aliphatic \ CH), \ 1742, \ 1743 \ (ester \ C=O), \ 1369 \ (ester \ C-O).
N-tert-butyloxycarbonyl ethanal [70]°

To N-tert-butyloxycarbonylglycine methyl ester (3.99 g, 17.71 mmol) in dry toluene (60 ml) at -78 °C under nitrogen was added dropwise with stirring, over 1h, DIBAL-H (1.0M in toluene, 44.28 ml, 44.28 mmol, 2.5 eq) with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5h. Methanol (14 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (50 g) in water (200 ml), and stirred vigorously for 1.5h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (3.4 g, 98%) that was used without further purification. $\delta_H$ (400 MHz; CDCl$_3$) 1.38 (9H, s, C(CH$_3$)$_3$), 3.98 (2H, d, J 5.1, CH$_2$), 9.56 (1H, s, CHO); $\delta_C$ (100 MHz; CDCl$_3$) 28.4 (C(CH$_3$)$_3$), 51.3 (CH$_2$), 171.3 (C=O), 197.5 (C=O); $\nu_{max}$ (CHCl$_3$/cm$^{-1}$) 3356 (NH), 2978, 2931 (aliphatic CH), 1613 (aldehyde C=O), 1519 (amide C=O), 1369 (ester C-O)
L-3-(N-Benzyloxycarbonylamino)methyl butanal [65]^{78}

To L-N-tert-butyloxycarbonylvaline methyl ester (2.37 g, 8.86 mmol) in dry toluene (30 ml) at -78 °C under nitrogen was added dropwise with stirring, over 1h, DIBAL-H (1.0M in toluene, 22.00 ml, 22.00 mmol, 2.5 eq) with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5h. Methanol (7 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (25 g) in water (100 ml), and stirred vigorously for 1.5h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (1.6 g, 76%) that was used without further purification. \( \delta_H^{400\text{MHz; CDCl}_3} \): 0.86 (3H, d, \( J 6.8, \text{CH}(CH_3)_2 \)), 0.95 (3H, d, J 6.8, CH(CH\(_3\))\(_2\)), 1.37 (9H, s, C(CH\(_3\))\(_3\)), 2.22 (1H, m, CHCH(CH\(_3\))\(_2\)), 4.16 (1H, m, CHCH(CH\(_3\))\(_2\)), 5.09 (1H, br s, NH), 9.55 (1H, s, CHO); \( \delta_C^{100\text{MHz; CDCl}_3} \): 19.02 (CH\(_3\)), 19.09 (CH\(_3\)), 21.41 (CH), 28.36 (C(CH\(_3\))\(_3\)), 58.00 (CH), 155.86 (C=O), 200.41 (C=O); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1}\): 3340 (NH), 2970, 2932, 2877 (aliphatic CH), 1705 (aldehyde C=O), 1512 (amide C=O), 1366 (ester C=O)
To phenylhydrazine hydrochloride (3.40 g, 22.81 mmol, 1.1 eq) and sodium acetate (6.98 g, 85.09 mmol, 4 eq) in water, was added N-tert-butyloxycarbonyl ethanal (4.16 g, 21.26 mmol) in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The reaction mixture was then cooled and extracted with chloroform (2 x 50 ml). The organic layers were combined and washed with saturated brine (2 x 50 ml), dried over MgSO₄ and concentrated under reduced pressure to give an orange oil. This was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (3:1) to give the desired product as an orange oil (3.19 g, 81%); δH (400 MHz; CDCl₃) 1.40 (9H, s, C(CH₃)₃), 3.90 (2H, br s, CH₂), 5.02 (1H, br s, NH), 6.74 – 6.77 (2H, m, Ph-H), 6.90 – 6.92 (1H, m, Ph-H), 6.99 (1H, br s, CH), 7.15 – 7.19 (2H, m, Ph-H), 7.35 (1H, br s, NH); δC (100 MHz; CHCl₃) 28.2 (C(CH₃)₃), 45.7 (CH₂), 112.7 (Ph-C), 120.0 (Ph-C), 121.3 (Ph-C), 127.8 (Ph-C), 129.2 (Ph-C), 147.6 (C=N); νmax (CHCl₃)/cm⁻¹ 3418 (NH), 2976 (aliphatic CH), 1693 (amide C=O), 1601 (NH), 1166 (C-O); m/z (FAB) M 249.1473 (C₁₃H₁₉N₃O₂ requires 249.1478), 194 (21), 193 (27), 133 (35), 93 (25), 57 (100).
L-[2-Methyl-1-(phenylhydrazonomethyl)propyl]carbamic acid tert-butyl ester [66]

To phenylhydrazine hydrochloride (1.16 g, 8.02 mmol, 1.2 eq) and sodium acetate (2.24 g, 27.36 mmol, 3.4 eq) in water, was added L-3-(N-benzyloxy carbonylamino)methyl butanal (1.60 g, 6.73 mmol) that was dissolved in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The resulting precipitate was filtered and dried to give an orange solid (1.92 g, 96%) that was used without further purification; mp 152.5 – 153.5 °C; [α]D = -60.3 (c 1.04, CHCl3); δH (400 MHz; CDCl3) 0.88 (6H, dd, J 2.0 & 7.2, CH(CH3)2), 2.06 (1H, m, CHCH(CH3)2), 4.12 (1H, m, CHCH(CH3)2), 5.13 (1H, br s, NH), 6.77 (1H, t, J 7.2, Ph-H), 6.92 (2H, d, J 7.2, Ph-H), 6.99 (1H, br s, CH), 7.17 (2H, t, J 7.6, Ph-H), 7.38 (1H, br s, NH); δC (100 MHz; CDCl3) 17.6 & 18.8 (CH(CH3)2), 28.4 (C(CH3)3), 50.8 (CH(CH3)2), 112.6 (Ph-C), 119.8 (Ph-C), 119.9 (Ph-C), 129.2 (Ph-C), 129.2 (Ph-C), 138.1 (CH), 145.0 (C=N), 155.8 (C=O); νmax (CHCl3)/cm⁻¹ 3290 (NH), 2965 (aliphatic CH), 1697 (ester C=O), 1602 & 1495 (arene C=C), 1168 (ester C-O); m/z (FAB) M 291.1949 (C16H25N2O2 requires 291.1947), 291 (42), 236 (57), 235 (100), 192 (41), 175 (98), 93 (30), 92 (24), 57 (51)
5-(tert-Butoxycarbonylaminomethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [72]

To a solution of the L-[1-Methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.23 g, 4.93 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.71 g, 5.32 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Ethyl acrylate (0.92 g, 1.0 ml, 9.19 mmol, 1.9 eq), potassium hydrogen carbonate (2.42 g, 24.17 mmol, 4.9 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (7:1 v/v) to yield the title compound (0.13 g, 8%) as an orange oil which was an inseperable mixture of diastereoisomers (1:1); δ_H (400 MHz; CDCl₃) 1.16 (3H, t, J 6.8, CO₂CH₂CH₃), 1.39 (9H, s, C(CH₃)₃), 2.99 (1H, dd, J 7.2 & 17.6, CH), 3.25 (1H, dd, J 12.4 & 17.6, CH), 4.02 (2H, d, J 5.6, CH₂), 4.14 (2H, q, J 7.2, CO₂CH₂CH₃), 4.54 (1H, dd, J 7.2 & 12.4, CH), 4.57 (1H, dd, J 7.2 & 12.4, CH), 4.97 (1H, br s, NH), 6.79 (1H, t, J 7.6, Ph-H), 6.92 (2H, d, J 8.0, Ph-H), 7.16-7.20 (2H, m, Ph-H); δ_C (100 MHz; CDCl₃) 14.1 (CHCH₃), 28.2 (C(CH₃)₃), 39.9 (CH₂), 39.3 (CH), 40.0 (CH), 61.8 (CH₂), 62.1
(CH), 113.0 (Ph-C), 119.8 (Ph-C), 126.0 (Ph-C), 128.6 (Ph-C), 129.1 (Ph-C), 145.2
(C=N), 155.6 (C=O), 171.5 (C=O); \nu_{\text{max}} (\text{CHCl}_3)/\text{cm}^{-1} 3355 (\text{NH}), 2976 (\text{aliphatic CH}),
1700 (\text{ester C=O}), 1501 (\text{arene C=C}), 1166 (\text{ester C-O}); \ m/z (\text{FAB}) M 347.1849
(\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_4 \text{requires 347.1845}), 347 (43), 292 (45), 291 (41), 290 (26), 231 (100), 157
(93), 57 (61)
5-(1-tert-Butoxycarbonylamino-2-methylpropyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid ethyl ester [67]

To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.41 g, 4.81 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.73 g, 5.47 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Ethyl acrylate (0.92 g, 1.0 ml, 9.19 mmol, 1.9 eq), potassium hydrogen carbonate (2.44 g, 24.37 mmol, 5.1 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (5:1 v/v) to yield the title compound (0.37 g, 20%) as an orange oil which was an inseperable mixture of diastereoisomers (1:1); νmax δH (400 MHz; CDCl3) 0.94 (6H, br d, J 6.4, CH(CH₃)₂), 1.02 (3H, t, J 6.8, CO₂CH₂CH₃), 1.46 (9H, s, C(CH₃)₃), 2.05 (1H, m, CH(CH₃)₂), 3.03 (1H, dd, J 6.8 & 17.6, CH), 3.28 (1H, dd, 12.4 & 18, CH), 4.20 (2H, q, J 7.2, CO₂CH₂CH₃), 4.50 (1H, br s, CHCH(CH₃)₂), 4.61 (1H, dd, J 6.8 & 12.4, CH), 4.62 (1H, dd, J 6.4 & 12.4, CH), 6.85 (1H, t, J 7.2, Ph-H), 7.00 (2H, d, J 7.6, Ph-H), 7.25 (2H, t, J 7.2, Ph-H); δC (100 MHz; CDCl3) 14.1 (CH₃), 19.5 ((CH₃)₂), 28.2 (C(CH₃)₃), 28.4 (CHCH₃), 61.7 (CH₂), 61.8 (CH), 62.0 (CH), 113.1 (Ph-C), 119.7 (Ph-
C), 129.1 (Ph-C), 145.4 (C=N), 171.5 (C=O); (CHCl₃)/cm⁻¹ 3345 (NH), 2974 (aliphatic CH), 1701 (ester C=O), 1600 & 1501 (arene C=C), 1168 (ester C-O); m/z (FAB) M 389.2312 (C₂₁H₃₁N₄O₄ requires 389.2315), 389 (21), 316 (32), 273 (75), 260 (70), 246 (26), 199 (100), 57 (46)
To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.29 g, 4.92 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.76 g, 5.69 mmol, 1.2 eq). The reaction mixture was left to stir for 1h. tert – Butyl acrylate (1.14 g, 1.3 ml, 8.89 mmol, 1.8 eq), potassium hydrogen carbonate (2.43 g, 24.27 mmol, 4.9 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (6:1 v/v) to yield the title compound (0.52 g, 27%) as an orange solid which was an inseparable mixture of diastereoisomers (1:1); mp 85.5 – 88.3 °C; δH (400 MHz; CDCl3) 1.32 & 1.33 (9H, s, C(CH3)J), 1.39 (12H, br s, CHCH3 & C(CH3)J), 3.20 (1H, dd, J 12.4 & 16.8, CH), 4.46 (2H, m, CHCH3 & CH), 5.13 (1H, br s, NH), 6.77 (1H, t, J 7.6, Ph-H), 6.86 (1H, d, J 9.2, Ph-H), 6.94 (1H, d, J 8, Ph-H), 7.17 (2H, t, J 7.2, Ph-H); δC (100 MHz; CDCl3) 27.5 (CH3), 27.9 (C(CH3)J), 28.4 (C(CH3)J), 62.8 (CH), 63.0 (CH), 113.1 (Ph-C), 114.2 (Ph-C), 128.9 (Ph-C), 129.0 (Ph-C), 145.4 (C=N), 155.2
(C=O), 170.5 (C=O); $\nu_{\text{max}}$ (CHCl₃)/cm⁻¹ 3416 (NH), 2976 (aliphatic CH), 1701 (ester C=O), 1600 (NH), 1499 (arene C=C), 1152 (ester C-O); $m/z$ (FAB) M 389.2312 ($C_{21}H_{31}N_3O_4$ requires 389.2315), 288 (24), 266 (32), 251 (35), 232 (88), 217 (85), 205 (42), 171 (100), 57 (40)
5-(1-tert-Butoxycarbonylaminoethyl)-3-methyl-2-phenyl-3,4-dihydro-2H-pyrazole-3-carboxylic acid tert-butyl ester [74]

![Chemical structure](image)

To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.30 g, 4.92 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.74 g, 5.54 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. tert-Butyl methacrylate (1.40 g, 1.6 ml, 9.85 mmol, 2.0 eq), potassium hydrogen carbonate (2.42 g, 24.17 mmol, 4.9 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (5:1 v/v) to yield the title compound (0.50 g, 25%) as an orange solid which was an inseparable mixture of diastereoisomers (1:1); mp 109.3 - 112.7 °C; δH (400 MHz; CDCl3) 1st diastereoisomer 1.36 (9H, d, J 3.6 C(CH3)3), 1.47 (9H, d, J 1.2 C(CH3)3), 1.53 (3H, d, J 5.6, CHCH3), 4.47 (1H, br s, CHCH3); 2nd diastereoisomer 1.47 (9H, d, J 3.6, C(CH3)3), 1.53 (3H, d, J 7.2, CHCH3) 4.49 (1H, br s, CHCH3); both diastereoisomers 2.86 (1H, dd, J 3.2 & 17.2, CH), 3.31 (1H, dd, J 4 & 17.2, CH), 6.85 (1H, t, J 7.2, Ph-H), 6.96 (1H, d, J 6.8, Ph-H), 7.03 (1H, d, J 8, Ph-H), 7.16 (1H, d, J 9.2, Ph-H), 7.21 (1H, t J 7.2, Ph-H); δC (100 MHz; CDCl3) 19.3 (CH3), 21.2 (CH3), 27.7
(C(CH₃)₃), 28.4 (C(CH₃)₃), 39.5 (CH₂), 46.1 (CH), 50.1 (CH), 114.8 (Ph-C), 115.7 (Ph-C), 120.0 (Ph-C), 124.6 (Ph-C), 144.3 (C=N), 155.3 (C=O), 172.1 (C=O); \( \nu_{\text{max}} \)

(CHCl₃)/cm⁻¹ 3375 (NH), 2977 (aliphatic CH), 1718 (ester C=O), 1598 & 1496 (arene C=C), 1166 (ester C-O); \( m/z \) (FAB) M 403.2466 (C₂₂H₃₃N₃O₄ requires 403.2471), 403 (26), 302 (35), 280 (44), 265 (36), 246 (91), 231 (70), 219 (53), 185 (100), 57 (29)
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-3,4-dihydro-2H-pyrazole-3,4-dicarboxylic acid dimethyl ester [75]

To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.30 g, 4.94 mmol) in ethyl acetate (15 ml) at 60 °C was added N-chlorosuccinimide (0.74 g, 5.54 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Dimethyl fumarate (1.40 g, 9.71 mmol, 2.0 eq), potassium hydrogen carbonate (2.44 g, 24.37 mmol, 4.9 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (5:1 v/v) to yield the title compound (0.25 g, 12%) as an orange oil which was an inseperable mixture of diastereoisomers (1:1); $\delta_H$ (400 MHz; CDCl$_3$) 1.39 (12H, br s, CHCH$_3$ & C(CH$_3$)$_3$), 3.70 (3H, s, CO$_2$CH$_3$), 3.73 (3H, s, CO$_2$CH$_3$), 4.14 (1H, dd, J 2.8 & 10.4, CH), 4.61 (1H, m, CHCH$_3$), 4.98 (1H, dd, J 7.2 & 10.4, CH$_2$), 6.83 (1H, t, J 7.2, Ph-H), 6.90 (1H, d, J 9.2, Ph-H), 6.97 (1H, d, J 8.0, Ph-H), 7.14 (1H, d, J 8.8, Ph-H), 7.20 (1H, t, J 8.4, Ph-H); $\delta_C$ (100 MHz; CDCl$_3$) 20.1 (CH$_3$), 28.3 (C(CH$_3$)$_3$), 46.1 (CH), 49.7 (CH$_2$), 53.1 (CH$_3$), 53.2 (CH$_3$), 56.5 (CH), 65.6 (CH), 113.4 (Ph-C), 114.6 (Ph-C),
120.6 (Ph-C), 129.1 (Ph-C), 143.1 (C=N), 168.4 (C=O), 170.1 (C=O), 170.4 (C=O); $v_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$: 3403 (NH), 2977 (aliphatic CH), 1741 (ester C=O), 1716 (ester C=O), 1598 (NH), 1497 (arene C=C), 1167 (ester C-O); $m/z$ (FAB) M$^+$ 406.1972 (C$_{20}$H$_{28}$N$_3$O$_6$ requires 406.1978), 324 (31), 323 (46), 290 (63), 289 (100), 263 (40), 229 (69), 197 (33), 57 (33)
To a solution of L-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (0.70 g, 2.65 mmol) in ethyl acetate (10 ml) at 60 °C was added N-chlorosuccinimide (0.40 g, 3.00 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Methyl propiolate (0.5 g, 0.5 ml, 5.95 mmol, 2.2 eq), potassium hydrogen carbonate (1.37 g, 13.68 mmol, 5.2 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (7:1 v/v) to yield the title compound (0.21 g, 25%) as an orange oil; [α]_D = -43.5 (c 1.15, MeOH) ; δH (400 MHz; CDCl₃) 1.38 (9H, s, C(CH₃)₃), 1.46 (3H, J 6.8, CHCH₃), 3.71 (3H, s, CO₂CH₂CH₃), 4.86 (1H, br s, CHCH₃), 5.09 (1H, br s, NH), 6.84 (1H, s, CH), 7.33-7.41 (5H, m, Ph-H) ; δC (100 MHz; CDCl₃) 21.8 (CH₃), 28.2 (C(CH₃)₃), 45.0 (CH), 52.1 (CO₂CH₃), 110.0 (CH), 126.0 (Ph-C), 128.6 (Ph-C), 128.7 (Ph-C), 133.6 (C), 140.1 (C=N), 155.2 (C=O), 159.3 (C=O); νmax (CHCl₃)/cm⁻¹ 3349 (NH), 2975 (aliphatic CH), 1717 (ester C=O), 1597 & 1499 (arene C=C), 1237 & 1167 (C-O); m/z
(EI) M 345.1644 (C_{13}H_{23}N_{3}O_{4} requires 345.1688), 289 (100), 244 (62), 230 (64), 57 (44)
A solution of the hydrazonyl chloride 52 (1.41 g, 3.96 mmol), methyl propiolate (1.0 g, 1.0 ml, 11.89 mmol, 3.0 eq) and tetrahexylammonium chloride (0.15 g, 0.36 mmol, 0.1 eq) in sodium hydrogen carbonate (0.1M, 20 ml) was stirred for 20h. The reaction mixture was then taken up in dichloromethane (75 ml) and washed with water (100 ml), dried over sodium sulfate and concentrated under reduced pressure to give an orange oil. This was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (2:1 v/v) to give an orange oil (0.54 g, 40 %); $\delta_H$ (400 MHz; CDCl$_3$) 1.38 (9H, s, C(CH$_3$)$_3$), 1.46 (3H, d, J 6.4, CHCH$_3$), 3.71 (3H, s, CO$_2$CH$_3$), 4.85 (1H, br s, CHCH$_3$), 5.23 (1H, br s, NH), 6.84 (1H, s, CH), 7.33 – 7.40 (5H, m, Ph-H)

NMR Data are consistent with those of 76 listed above.
To DL-alanine methyl ester hydrochloride (10.00 g, 71.67 mmol) in dichloromethane (360 ml) at 0 °C, di-tert-butyl dicarbonate (15.60 g, 71.48 mmol, 1.0 eq) was added. The mixture was stirred for 15min before triethylamine (14.52 g, 20.0 ml, 143.49 mmol, 2.0 eq) was added and the reaction mixture stirred for 17h. The mixture was then washed with citric acid solution (2M, 2 x 150 ml) and saturated brine (2 x 150 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a clear oil (14.6 g, 90 %). δH (400 MHz; CDCl3) 1.32 (3H, d, J 7.2, CHCH₃), 1.38 (9H, s, C(CH₃)₃), 3.68 (3H, s, CO₂CH₃), 4.25 (1H, m, CHCH₃), 5.18 (1H, br s, NH); δC (100 MHz; CDCl₃) 18.5 (CH₃), 28.5 (C(CH₃)₃), 52.3 (CH₃), 79.7 (CH), 155.5 (C=O), 174.0 (C=O); vmax (CHCl₃)/cm⁻¹ 3363 (NH), 2940, 2882, (aliphatic CH), 1728 (amide C=O), 1689 (ester C=O), 1365 (ester C-O)
(DL)-2-(N-tert-Butyloxycarbonylamino)propanal [79]

To DL-N-tert-butyloxycarbonylalanine methyl ester (2.61 g, 12.84 mmol) in dry toluene (30 ml) at -78 °C under nitrogen was added dropwise with stirring, over 1h, DIBAL-H (1.0M in toluene, 44.00 ml, 44.00 mmol, 3.4 eq), with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5h. Methanol (14 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (25 g) in water (200 ml), and stirred vigorously for 1.5h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (2.1 g, 95 %) that was used without further purification. δ_H (400 MHz; CDCl_3) 1.31 (3H, d, J 7.6, CHCH₃), 1.45 (9H, s, C(CH₃)₃), 4.20 (1H, br s, CHCH₃), 5.33 (1H, br s, NH), 9.54 (1H, s, CHO); δ_C (100 MHz; CDCl_3) 21.4 (CH₃), 28.4 (C(CH₃)₃), 55.5 (CH), 125.3 (Ph-CH), 128.2 (Ph-CH), 129.0 (Ph-CH), 137.8 (Ph-C), 155.5 (C=O), 200.0 (CHO); ν_max (CHCl_3)/cm⁻¹ 3426 (NH), 2934 (aliphatic CH), 1736 (C=O)
DL-[1-Methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester [80]

\[ \text{CHO} \quad \text{NHBOc} \quad \rightarrow \quad \text{NHBOc} \quad \text{NHBoc} \]

To phenylhydrazine hydrochloride (3.13 g, 21.64 mmol, 1.6 eq) and sodium acetate (4.56 g, 55.59 mmol, 4.0 eq) in water, was added DL-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (2.40 g, 13.9 mmol) that was dissolved in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The resulting precipitate was filtered and dried to give an oily orange solid (3.18 g, 88%) that was used without further purification; \( \delta_H \) (400 MHz; CDCl₃) 1.36 (3H, d, J 7.2 CHCH₃), 1.51 (9H, s, C(CH₃)₃), 4.50 (1H, br s, CHCH₃), 5.24 (1H, br s, NH), 6.82-6.89 (2H, m, Ph-H), 7.00-7.02 (1H, m, Ph-H), 7.12 (1H, s, CH), 7.25-7.29 (2H, m, Ph-H), 7.53 (1H, br s, NH); \( \delta_C \) (100 MHz; CDCl₃) 19.2 (CHCH₃), 28.4 (C(CH₃)₃), 41.4 (CH), 112.6 (Ph-C), 112.6 (Ph-C), 119.6 (Ph-C), 119.9 (Ph-C), 129.2 (Ph-C), 139.8 (CH), 145.3 (C=N), 151.1 (C=O); \( \nu_{\text{max}} \) (CHCl₃)/cm⁻¹ 3410, 3302 (NH), 2978 (aliphatic CH), 1694 (ester C=O), 1601 (C=N); \( m/z \) (EI) M 263.1637 (C₁₄H₂₁N₃O₂ requires 263.1634) 207 (28), 108 (41), 93 (100), 92 (47), 77 (44), 57 (66)
5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-2H-pyrazole-3-carboxylic acid methyl ester [81]

To a solution of DL-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid tert-butyl ester (1.36 g, 4.77 mmol) in ethyl acetate (25 ml) at 60 °C was added N-chlorosuccinimide (0.81 g, 6.07 mmol, 1.3 eq). The reaction mixture was left to stir for 1h. Methyl propiolate (1.0 g, 1.0 ml, 11.89 mmol, 2.5 eq), potassium hydrogen carbonate (2.62 g, 26.17 mmol, 5.5 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give an orange oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (7:1 v/v) to yield the title compound (0.50 g, 30 %) as an orange waxy solid. δ_H (400 MHz; CDCl_3) 1.38 (9H, s, C(CH_3)_3), 1.46 (3H, d, J 6.8, CHCH_3), 3.71 (3H, s, CO_2CH_3), 4.42 (1H, br s, CHCH_3), 5.10 (1H, br s, NH), 6.84 (1H, s, CH), 7.32 – 7.39 (5H, m, Ph-H); δ_C (100 MHz; CDCl_3) 21.1 (CHCH_3), 28.2 (C(CH_3)_3), 45.0 (CH), 52.1 (CO_2CH_3), 110.0 (CH), 126.0 (Ph-C), 128.7 (Ph-C), 133.8 (C), 140.1 (C=N), 155.2 (C=O), 159.5 (C=O); υ_{max} (CHCl_3)/cm^{-1} 3349 (NH), 2977 (aliphatic CH), 1713 (ester C=O), 1598 & 1500
(arene C=C), 1166 (ester C-O); \textit{m/z} (EI) M 345.1644 (C\textsubscript{18}H\textsubscript{23}N\textsubscript{3}O\textsubscript{4} requires 345.1689), 289 (100), 244 (62), 230 (64), 57 (44)
(S)-5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-2H-pyrazole-3-carboxylic acid methyl ester (0.75 g, 2.18 mmol) was stirred at room temperature in a mixture of tetrahydrofuran (20 ml) and 2M NaOH (20 ml) for 3 h. After this period aqueous HCl was added to pH 3, the mixture was extracted with ethyl acetate (2 x 50 ml) and the combined organic layers were washed with water (50 ml). The organic layers were dried over sodium sulfate and concentrated under reduced pressure to give the title compound as an orange oil (0.70 g, 97 %) that was used without further purification. δ_H (400 MHz; CDCl3) 1.38 (9H, s, C(CH3)3), 1.45 (3H, d, J 6.4, CHCH3), 4.85 (1H, m, CHCH3), 6.84 (1H, s, CH), 7.33-7.40 (5H, m, Ph-H); δ_C (100 MHz; CDCl3) 21.1 (CH3), 28.4 (C(CH3)3) 44.3 (CH), 114.2 (CH), 126.0 (Ph-C), 128.6 (Ph-C), 128.6 (Ph-H), 164.8 (C=O), 171.3 (C=O); ν_max (CHCl3)/cm^-1 3345 (NH), 1666 (C=O), 1652 (C=O), 1527 (C=N); m/z (El) M 331.1526 (C17H21N3O4 requires 331.1532), 289 (88), 275 (38), 244 (67), 230 (100), 216 (31), 77 (25), 57 (67)
(S)-([5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-2H-pyrazole-3-carbonyl]amino)acetic acid methyl ester [83]

\[
\begin{align*}
&\text{NHBOc} \\
&\text{Ph} \\
&\text{CO}_2H \\
&\text{NHBOc} \\
&\text{Ph} \\
&\text{CO}_2\text{Me}
\end{align*}
\]

5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-2H-pyrazole-3-carboxylic acid (0.60 g, 1.81 mmol), glycine methyl ester hydrochloride (0.29 g, 2.27 mmol, 1.3 eq) and EDCI (0.48 g, 2.50 mmol, 1.4 eq) were dissolved in anhydrous diethyl ether (90 ml), cooled to 0 °C and stirred for 1 h. The reaction mixture was then warmed to room temperature and stirred for a further 1 h. After this period the reaction mixture was again cooled to 0 °C and Et₃N (0.3 ml was added over a period of 0.5 h. When the addition was complete the reaction mixture was warmed to room temperature and stirred for a further 17 h. Water (25 ml) was added to dissolve any precipitated material, the organic layer separated and the aqueous extracted with ethyl acetate (2 x 10 ml). The combined organic phases were dried over magnesium sulfate, filtered and evaporated to give a dark orange residue. The crude product was purified by column chromatography on silica gel using hexane and ethyl acetate (1:1 v/v) as eluent to give an orange oil (0.33 g, 46 %); $\delta_H$ (400 MHz; CDCl₃) 1.38 (9H, s, C(CH₃)₃), 1.46 (3H, d, J 6.8, CHCH₃), 3.71 (3H, s, CO₂CH₃), 4.12 (2H, s, CH₂CO₂Me), 4.85 (1H, m, CHCH₃), 5.09 (1H, br s, NH),
6.72 (1H, s, NH), 6.84 (1H, s, CH), 7.33 – 7.41 (5H, m, Ph – H); δC (100 MHz; CDCl3)
21.3 (CH3), 28.3 (C(CH3)3), 43.8 (CH), 44.4 (CH2), 52.1 (OCH3), 119.5 (CH), 126.0
(Ph-CH), 127.6 (Ph-CH), 128.6 (Ph-CH), 128.8 (Ph-CH), 129.6 (Ph-CH), 133.8 (C),
140.0 (C=N), 153.8 (C=O), 159.4 (C=O), 168.7 (C=O); νmax (CHCl3)/cm⁻¹ 3371, 3330
(NH), 1756 (amide C=O), 1674 (C=O), 1521 (C=N); m/z (El) M 402.1913 (C20H26N4O5
requires 402.1903)
tert-Butoxycarbonylaminoacetic acid [85]^{65}

To a solution of glycine (5.11 g, 68.05 mmol) in 1,4-dioxane, water and 1M sodium hydroxide (280 ml) (2:1:1 v/v/v) at 0 °C was added di-tert-butyl dicarbonate (14.58 g, 66.80 mmol, 1.0 eq). The solution was stirred for 90 min at 0 °C then allowed to warm to room temperature and stirred for a further 30 min. The reaction mixture was concentrated under reduced pressure to approx. 70cm^3 then cooled to 0 °C. Ethyl acetate was added and the biphasic mixture acidified to pH 3 with a solution of potassium hydrogen sulfate (2M). The aqueous layer was separated, then extracted with ethyl acetate (2x50cm^3). The organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a white solid that was recrystallised from hexane : ethyl acetate (1:1 v/v) to give a white solid (7.3 g, 61%); mp 90.2 – 92.5 °C (lit^{65} 86-89 °C); δH (400 MHz; CDCl3) 1.48 (9H, s, C(CH₃)₃), 3.94 (1H, s, OH), 3.99 (2H, br s, CH₂), 5.06 (1H, br s, NH); δC (100 MHz; CDCl3) 28.2 (C(CH₂)), 42.2 (CH₂), 156.1 (C=O), 174.0 (C=O); νmax (CHCl₃)/cm⁻¹ 3364 (OH), 2982, 2936 (aliphatic CH), 1713 (amide C=O), 1694 (C=O), 1527 (NH)
(S)-1-(5-Methoxycarbonyl-1-phenyl-1H-pyrazol-3-yl)ethylammonium chloride [84]

\[
\text{NHBoc}
\]

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

\[
\text{NH}_3\text{Cl}
\]

(S)-5-(1-tert-Butoxycarbonylaminoethyl)-2-phenyl-2H-pyrazole-3-carboxylic acid methyl ester (0.94 g, 2.72 mmol) was dissolved in dichloromethane (70 ml), trifluoroacetic acid (14.0 ml) was added and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure then 2M HCl (20 ml) was added and the reaction mixture was stirred for 1 h. The solvent was removed under reduced pressure. Water (20 ml) and ethyl acetate (20 ml) were added, the two phases separated and the aqueous layer collected. The water was removed and the residue taken up in toluene (10 ml) which was then removed under reduced pressure to give a brown solid (0.50 g, 65 %); \( \delta_H \) (400 MHz; d6-DMSO) 1.58 (3H, d, \( J \) 6.8, CHCH₃), 3.85 (3H, s, CO₂CH₃), 4.54 (1H, m, CHCH₃), 7.28 (1H, s, CH), 7.46-7.53 (5H, m, Ph-H), 8.59 (3H, br s, NH₂); \( \delta_C \) (100 MHz; DMSO) 18.9 (CH₃), 52.3 (CO₂CH₃), 110.5 (CH), 125.7 (Ph-C), 128.7 (Ph-C), 133.8 (C=O), 140.0 (C=N); \( \nu_{\text{max}} \) (CHCl₃)/cm⁻¹ 3410 (NH), 2924 (aliphatic CH), 1736 (ester C=O), 1389 (ester C-O); \( m/z \) (El) M (–HCl) 245.1170 (C₁₃H₁₅N₃O₂ requires 245.1164), 231 (15), 230 (100), 77 (9).
(S)-5-[1-(2-tert-Butoxycarbonylaminoacetylamino)ethyl]-2-phenyl-2H-pyrazole-3-carboxylic acid methyl ester [86]

(S)-5-[(2-Methoxycarbonyl-1-phenyl-1H-pyrazol-3-yl)ethyl]ammonium chloride (0.49 g, 1.23 mmol) was suspended in DCM (60 ml). Et₃N (0.36 g, 0.5 ml, 3.59 mmol, 2.9 eq) was added and the reaction mixture was stirred for 10 min. Water (50 ml) was added and the organic layer was separated, dried over magnesium sulfate and concentrated under reduced pressure to give the free amine (0.32 g), which was used immediately in the coupling reaction.

tert-Butoxycarbonylaminoacetic acid (0.29 g, 1.66 mmol, 1.3 eq) and EDCI (0.28 g, 1.46 mmol, 1.2 eq) were dissolved in anhydrous DCM (30 ml) and stirred at 0 °C for 10 min. The free amine (0.32 g, 1.23 mmol) in anhydrous DCM (10 ml) was added and the solution was allowed to reach room temperature. The reaction mixture was then stirred for a further 19 h. After this period, water (50 ml) was added and the organic layer separated. This was dried over magnesium sulfate and concentrated under reduced pressure to give a dark orange oil. This was purified by column chromatography in
silica gel using hexane : ethyl acetate (1:1 v/v) as the eluent to give an orange oil. (0.06 g, 13 %); [α]D = -52.9 (c 0.74, CHCl3); δH (400 MHz; CDCl3); 1.35 (9H, s, C(CH3)3), 1.48 (3H, d, J 6.8, CHCH3), 3.71 (3H, s, CO2CH3), 3.75 (2H, br d, CH2), 5.16 (1H, br s, CHCH3), 6.68 (1H, br s, NH), 6.84 (1H, s, CH), 7.32 - 7.41 (5H, m, Ph-H); δC (100 MHz; CDCl3) 21.3 (CH3), 28.3 (C(CH3)3), 43.8 (CH), 44.5 (CH2), 52.1 (CO2CH3), 110.1 (CH), 126.0 (Ph-C), 128.6 (Ph-C), 128.8 (Ph-C), 133.7 (C), 140.0 (C), 140.0 (C=N), 153.8 (C=O), 159.4 (C=O), 168.7 (C=O); vmax (CHCl3)/cm⁻¹ 3367, 3321 (NH), 1717 (amide C=O), 1666 (C=O), 1527 (C=N); m/z (El) M 402.1908 (C20H26N4O5 requires 402.1903), 402 (41), 346 (55), 244 (76), 230 (84), 229 (100), 57 (43)
(S)-5-[1-(2-tert-Butoxycarbonylaminoacetylamino)ethyl]-2-phenyl-2H-pyrazole-3-carboxylic acid [87]

(S)-5-[1-(2-tert-Butoxycarbonylaminoacetylamino)ethyl]-2-phenyl-2H-pyrazole-3-carboxylic acid methyl ester (0.18 g, 0.44 mmol) was stirred at room temperature in a mixture of tetrahydrofuran (20 ml) and 2 M NaOH (20 ml) for 3 h. After this period aqueous HCl was added to pH 3, the mixture was extracted with ethyl acetate (2 x 50 ml) and the combined organic layers were washed with water (50 ml). The organic layers were dried over sodium sulfate and concentrated under reduced pressure to give the title compound as an orange oil (0.12g, 70 %) that was used without further purification. δH (400 MHz; CDCl3) 1.40 (12H, s, C(CH3)3 & CHCH3), 3.87 (2H, br s, CH2), 5.05 (1H, m, CHCH3), 5.36 (1H, br s, NH), 6.84 (1H, s, CH), 7.33-7.40 (5H, m, Ph-H); δC (100 MHz; CDCl3) 21.1 (CH3), 28.2 (C(CH3)3), 43.6 (CH), 44.2 (CH2), 110.1 (CH), 125.9 (Ph-C), 128.8 (Ph-C), 129.6 (Ph-C), 133.7 (Ph-C), 139.9 (C=CH), 153.8 (C=N), 156.2 (C=O), 160.7 (C=O), 174.9 (C=O); νmax (CHCl3)/cm⁻¹ 3324 (NH), 2977, 3931 (aliphatic CH) 1716 (C=O), 1683, (C=O), 1652 (C=O), 1557 (C=N); m/z (FAB)
M+ 389.1825 (C_{19}H_{24}N_{4}O_{5} requires 389.1817), 347 (39), 176 (79), 154 (100), 137 (63), 136 (86), 107 (29), 89 (21), 57 (37)
(S)-{(5-[1-(2-tert-Butoxycarbonylaminoacetylamino)ethyl]-2-phenyl-2H-pyrazole-3-carbonyl]amino)acetic acid methyl ester [88]

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

(S)-5-[1-(2-tert-Butoxycarbonylaminoacetylamino)ethyl]-2-phenyl-2H-pyrazole-3-carboxylic acid (0.20 g, 0.53 mmol), glycine methyl ester hydrochloride (0.08 g, mmol, 1.3 eq) and EDCI (0.16 g, mmol, eq) were dissolved in anhydrous diethyl ether (30 ml), cooled to 0 °C and stirred for 1 h. The reaction mixture was then warmed to room temperature and stirred for a further 1 h. After this period the reaction mixture was again cooled to 0 °C and Et₃N (0.1 ml) was added over a period of 0.5 h. When the addition was complete the reaction mixture was warmed to room temperature and stirred for a further 17 h. Water (25 ml) was added to dissolve any precipitated material, the organic layer separated and the aqueous extracted with ethyl acetate (2 x 10 ml). The combined organic phases were dried over magnesium sulfate, filtered and evaporated to give a dark orange residue. The crude product was purified by column chromatography on silica gel using hexane and ethyl acetate (1:1 v/v) as eluent to give an orange oil (0.03 g, 33 %); \( \delta_H \) (400 MHz; CDCl₃) 1.35 (9H, s, C(CH₃)₃), 1.41 (3H, d, J 6.8, CHCH₃), 3.68 (2H, s, CH₂), 3.70 (2H, s, CH₂) 3.79 (H, s, CO₂Me), 5.12 (1H, br s, NH), 5.11 (1H, br s, NH), 5.49 (1H, m, CHCH₃), 6.82 (1H, s, CH), 7.13 (1H, br s,
NH) 7.27 - 7.43 (5H, m, Ph - H); δ C (100 MHz; CDCl₃) 21.3 (CH₃), 28.3 (C(CH₃)₃), 43.8 (CH), 44.4 (CH₂), 51.8 (CH₂), 52.1 (OCH₃), 119.5 (CH), 126.0 (Ph-CH), 127.6 (Ph-CH), 128.6 (Ph-CH), 128.8 (Ph-CH), 129.6 (Ph-CH), 133.8 (C), 140.0 (C), 153.8 (C=O), 156.15 (C=O), 159.4 (C=O), 168.7 (C=O); ν max (CHCl₃)/cm⁻¹ 3308 (NH), 1756 (amide C=O), 1739 (amide C=O), 1692 (C=O), 1662 (C=O), 1597 (C=N); m/z (FAB) M+ 459.2130 (C₂₂H₂₉N₅O₆ requires 459.2118)
Ethyl 2-chloro-2-ethoxyacetate [90]^{53}

Phosphorus pentachloride (4.50 g, 21.61 mmol, 1.1 eq) was added at 0 °C to ethyl diethoxymëacetate (3.46 g, 3.50 ml, 19.62 mmol) over a period of 10 min. After this period the reaction mixture was heated at 80° C for 19 h. Dichloromethane (50 ml) was added and the reaction mixture was washed with water (3 x 50 ml), dried over magnesium sulfate and concentrated under reduced pressure to give a yellow liquid. This was purified by vacuum distillation to give a clear colourless liquid (2.34 g, 72%); δ_H (400 MHz; CDCl₃) 1.31 – 1.36 (6H, m, CO₂CH₂CH₃ & COCH₂CH₃), 3.68 (1H, dq, J 6.8 & 7.2, CH), 4.04 (1H, dq, J 7.2 & 7.2, CH), 4.30 (2H, q, J 6.8, CH₂CH₃), 5.82 (1H, s, CH); δ_C (100 MHz; CDCl₃) 14.3 (CH₂), 62.6 (CH₃), 66.4 (CH₃), 88.6 (CH₂), 167.4 (C=O); νₘₐₓ (CHCl₃)/cm⁻¹ 2986, 2901 (aliphatic CH), 1759 (C=O), 1373 (ester C-O), 779 (C-Cl).
(Benzylhydrazono)acetic acid ethyl ester [91]^3

Benzylhydrazine dihydrochloride (4.26 g, 21.84 mmol,) was suspended in water (10 ml) and the pH adjusted to approximately 4. Ethyl 2-chloro-2-ethoxyacetate (4.19 g, 25.14 mmol, 1.2 eq) in dioxane (25 ml) was added in portions at 0 °C. The reaction mixture was stirred at room temperature for 1 h then neutralized to pH 8 with 1 M aqueous sodium hydroxide and concentrated under reduced pressure to half the volume. Water (30 ml) was added and the resulting emulsion extracted with dichloromethane (2 x 30 ml). The organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil. This was purified by column chromatography on silica gel eluting with diethyl ether : dichloromethane (4:1 v/v) to give the title compound as a yellow oil (4.0 g, 77%), that was used without further purification. δH (400 MHz; CDCl3) 1.32 (3H, t, J 6.8, CO2CH2CH3), 4.27 (2H, q, J 6.8, CO2CH2CH3), 4.40 (2H, d, J 4.4, CH2-Ph), 6.78 (1H, br s, NH), 6.87 (1H, br s, CH), 7.28 - 7.38 (5H, m, Ph-H); δC (100 MHz; CDCl3) 14.2 (CH3), 51.5 (CH2), 67.0 (CH2), 97.5 (CH), 127.4 (Ph-C), 127.7 (Ph-C), 128.5 (Ph-C), 128.6 (Ph-C), 135.3 (C=N), 167.6 (C=O); νmax (CHCl3)/cm⁻¹ 3333 (NH), 2901 (aliphatic CH), 1705 (ester C=O), 1551 (NH), 1496 (C=N).
To phenylhydrazine hydrochloride (4.25 g, 29.39 mmol, 1.4 eq) and sodium acetate (6.24 g, 62.33 mmol, 2.9 eq) in water, was added ethyl glyoxylate (2.2 g, 2.0 ml, 21.55 mmol) that was dissolved in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The resulting precipitate was filtered and dried to give a yellow solid (2.11 g, 56%) that was used without further purification; mp 130.9 – 132.3 °C; δH (400 MHz; CDCl3) 1.38 (3H, t, J 7.2, CO2CH2CH3), 4.34 (2H, q, J 7.2, CO2CH2CH3), 7.01 (1H, t, J 7.2, Ph-H), 7.10 (1H, s, CH), 7.19 (2H, d, J 7.6, Ph-H), 7.32 (2H, t, J 7.6, Ph-H), 8.39 (1H, br s, NH), δC (100 MHz; CDCl3) 14.2 (CH3), 61.0 (CH2), 113.9 (Ph-C), 122.4 (Ph-C), 122.7 (CH), 125.6 (Ph-C), 129.4 (Ph-C), 164.5 (C=O); νmax (CHCl3)/cm⁻¹ 3241 (NH), 3063 (aromatic CH), 2978, 2909 (aliphatic CH), 1705 (ester C=O), 1551 (C=N)
1-Benzyl-4,5-dihydro-1H-pyrazole-3,5-dicarboxylic acid diethyl ester [102]

\[
\begin{align*}
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\text{N-H} & \quad \text{N-H} \\
\text{N} & \quad \text{N} \\
\text{Bn} & \quad \text{Bn} \\
\text{Cl} & \quad \text{Cl} \\
\end{align*}
\]

To a solution of (benzylhydrazono)acetic acid ethyl ester (1.06 g, 5.13 mmol) in ethyl acetate (20 ml) at 60 °C was added N-chlorosuccinimide (0.74 g, 5.54 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Ethyl acrylate (0.92 g, 1.0 ml, 9.23 mmol, 1.8 eq), potassium hydrogen carbonate (2.59 g, 25.87 mmol, 5.0 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (3:2 v/v) to yield the title compound (1.22 g, 78 %) as a yellow oil. \(\delta_H (400 \text{ MHz}; \text{CDCl}_3) 1.17 \times 3 (H, t, J 7.2, \text{CO}_2\text{CH}_2\text{CH}_3), 1.27 \times 3 (H, t, J 7.2, \text{CO}_2\text{CH}_2\text{CH}_3), 3.12 \times 2 (H, d, J 12.4, \text{CH}_2\text{-Ph}), 3.98 \times 1 (H, t, J 4.0, \text{CH}), 4.04 \times 2 (H, q, J 7.2, \text{CO}_2\text{CH}_2\text{CH}_3), 4.23 \times 2 (H, q, J 7.2, \text{CO}_2\text{CH}_2\text{CH}_3), 4.51 \times 1 (H, d, J 14.8, \text{CH}), 4.73 \times 1 (H, d, J 14.8, \text{CH}), 7.20 - 7.28 \times 5 (H, m, \text{Ph-H}); \delta_C (100 \text{ MHz}; \text{CDCl}_3) 14.1 (\text{CH}_3), 14.4 (\text{CH}_3), 36.1 (\text{CH}_2), 49.5 (\text{CH}), 59.8 (\text{CH}_2), 61.5 (\text{CH}_2), 61.6 (\text{CH}_2), 64.8 (\text{CH}_3), 128.6 (\text{Ph - CH}), 128.7 (\text{Ph - CH}), 129.2 (\text{Ph - CH}), 135.5 (\text{Ph - C}), 138.2 (\text{C= N}), 170.0 & 170.8 (\text{C= O}); \nu_{\text{max}} (\text{CHCl}_3)/\text{cm}^{-1} 2982 (\text{aliphatic CH}), 1740, 1701 (\text{ester C= O}), 1559 (\text{C= N})
1-Benzyl-5-phenyl-4,5-dihydro-1H-pyrazole-3-carboxylic acid ethyl ester [103]

To a solution of (benzylhydrazono)acetic acid ethyl ester (1.49 g, 7.23 mmol) in ethyl acetate (20 ml) at 60 °C was added N-chlorosuccinimide (1.03 g, 7.71 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Styrene (1.99 g, 2.2 ml, 19.14 mmol, 2.6 eq), potassium hydrogen carbonate (3.67 g, 36.66 mmol, 5.1 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (9:1 v/v) to yield the title compound (0.61 g, 29%) as a yellow oil; δ_H (400 MHz; CDCl_3) 1.27 (3H, t, J 6.8, CO_2CH_2CH_3), 2.82 (1H, dd, J 13.6 & 17.2, CH), 3.25 (1H, dd, 12.4 & 17.2, CH), 4.24 (2H, q, J 6.8, CO_2CH_2CH_3), 4.38 (1H, t, J 12.4, CH), 5.20 (2H, s, CH_2 –Ph), 7.09 (2H, d, J 6, Ph-H), 7.17 – 7.31 (10H, m, Ph-H); δ_C (100 MHz; CDCl_3) 13.1 (CH_3), 40.3 (CH_2), 52.4 (CH_2), 59.9 (CH_2), 67.0 (CH), 126.5 (Ph-CH), 126.6 (Ph-CH), 127.2 (Ph-CH), 127.4 (Ph-CH), 127.8 (Ph-CH), 127.9 (Ph-CH), 128.1 (Ph-CH), 130.2 (Ph-CH), 132.0 (Ph-CH), 134.5 (Ph-C), 136.7 (Ph-C), 138.5 (C=N), 158.6 (C=O); v_max (CHCl_3)/cm^{-1} 3063, 3028 (aromatic CH), 2982, 2932 (aliphatic CH), 1740 (ester C=O), 1551 (C=N)
Attempted Synthesis of 5-Aminomethyl-1-benzyl-4,5-dihydro-1H-pyrazole-3-carboxylic acid ethyl ester [104]

To a solution of (benzylhydrazono)acetic acid ethyl ester (0.50 g, 2.43 mmol) in ethyl acetate (20 ml) at 60 °C was added N-chlorosuccinimide (0.35 g, 2.62 mmol, 1.1 eq). The reaction mixture was left to stir for 1h. Allylamine (0.23 g, 0.3 ml, 4.00 mmol, 1.6 eq), potassium hydrogen carbonate (1.19 g, 11.89 mmol, 4.9 eq) and a few drops of water were added and the reaction mixture was left to stir at 70 °C for 20h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography on silica gel eluting with light petroleum : ethyl acetate (4:1 v/v) to yield a product (0.30 g, 47%) as a yellow oil; δH (400 MHz; CDCl3) 1.35 (3H, t, J 6.4, CO2CH2CH3), 4.12 (2H, br s, CH2NH2), 4.33 (2H, q, J 7.2, CO2CH2CH3), 5.13 (2H, dd, J 20.8 & 34, CH2), 5.88 (1H, m, CH), 7.23 – 7.33 (3H, m, Ph – H), 7.66 – 7.68 (2H, m, Ph – H), 8.41 (2H, s, NH2); δC (100 MHz; CDCl3) 14.1 (CH3), 46.8 (CH2), 62.7 (CH2), 116.5 (CH2), 128.4 (Ph-C), 128.7 (Ph-C), 129.8 (Ph-C), 133.4 (C=N), 134.7 (Ph-C), 158.1 (C=O); νmax (CHCl3)/cm⁻¹ 3391 (NH), 1736 (C=O), 1586 (C=N)
Prop-2-ynylcarbamic acid tert-butyl ester [105]\textsuperscript{82}

![Chemical structure](image)

To propargylamine hydrochloride (0.65 g, 8.35 mmol) in dichloromethane (60 ml) at 0 °C, di-tert-butyl dicarbonate (1.95 g, 8.93 mmol, 1.1 eq) was added. The mixture was stirred for 15 min before triethylamine (1.67 g, 2.3 ml, 16.50 mmol, 2.0 eq) was added and the reaction mixture stirred for 17 h. The mixture was then washed with citric acid solution (2M, 2 x 30 ml) and saturated brine (2 x 30 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a clear oil (1.25 g, 96 %). $\delta_{\text{H}}$ (400 MHz; CDCl$_3$) 1.38 (9H, s, C(CH$_3$)$_3$), 2.15 (1H, t, J 2.8, CHCCH$_2$), 3.85 (2H, br s, CHCCH$_2$), 4.84 (1H, br s, NH); $\delta_{\text{C}}$ (100 MHz; CDCl$_3$) 28.5 (C(CH$_3$)$_3$), 30.3 (CH$_2$), 85.2 (CH), 146.7 (CCH), 156.3 (C=O); $\nu_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$ 3306 (NH), 2982 (aliphatic CH), 1701 (ester C=O)
N–Propargyl–p–toluenesulfonamide [105a] 81

![Chemical structure](image)

To a solution of dichloromethane (40 ml) and p–toluenesulfonyl chloride (1.92 g, 10.10 mmol, 1.0 eq) was added propargylamine hydrochloride (0.93 g, 10.19 mmol) followed by triethylamine (2.18 g, 3.0 ml, 21.52 mmol, 2.1 eq). The reaction mixture was stirred at room temperature for 12 h. After this period the reaction mixture was diluted with dichloromethane and washed with 1 M hydrochloric acid (50 ml). The organic phase was dried over magnesium sulfate and concentrated under reduced pressure to give a white solid (1.64 g, 77 %) that was used without further purification. mp 73.4 – 75.2 °C; δ_H (400 MHz; CDCl₃) 2.09 (1H, t, J 2.8, CH₂), 2.40 (3H, s, CH₃), 3.79 (2H, dd, J 2.4 &, 5.46, CH₂), (1H, t, J 6.0, NH), 7.29 (2H, d, J 8.0, Ph–H), 7.77 (2H, d, J 8.0, Ph–H); δ_C (100 MHz; CDCl₃) 21.4 (CH₃), 32.8 (CH₂), 78.1 (CH), 127.4 (Ph–C), 129.7 (Ph–C), 136.5 (C), 143.84 (CCH); ν_max (CHCl₃)/cm⁻¹ 3282 (NH), 3067 (aromatic CH), 1157 (S=O);
N-tert-Butyloxycarbonyl β-alanine ethyl ester [116]

To β-alanine ethyl ester hydrochloride (4.63 g, 30.14 mmol) in dichloromethane (180 ml) at 0 °C, di-tert-butyl dicarbonate (6.84 g,) was added. The mixture was stirred for 15 min before triethylamine (11.0 ml,) was added and the reaction mixture stirred for 17 h. The mixture was then washed with citric acid solution (2 M, 2 x 75 ml) and saturated brine (2 x 75 ml), then dried over magnesium sulfate and concentrated under reduced pressure to yield a clear colourless yellow oil (6.6 g, 66%). δ<sub>H</sub> (400 MHz; CDCl<sub>3</sub>) 1.27 (3H, t, J<sub>7.2</sub>, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.42 (9H, s, (CH<sub>3</sub>)<sub>3</sub>), 2.50 (2H, t, J 6, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.73 (2H, q, J 6, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.97 (2H, q, J 7.2, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 5.07 (1H, br s, NH); δ<sub>C</sub> (100 MHz; CDCl<sub>3</sub>) 14.2 (CH<sub>3</sub>), 28.5 (C(CH<sub>3</sub>)<sub>3</sub>), 34.6 (CH<sub>2</sub>), 36.1 (CH<sub>2</sub>), 60.6 (CH<sub>2</sub>), 155.8 (C=O), 172.5 (C=O); ν<sub>max</sub> (CHCl<sub>3</sub>) cm<sup>-1</sup> 3357 (NH), 2976, 2932 (aliphatic CH), 1693 (C=O)
(3-Oxopropyl)carbamic acid tert-butyl ester [117]

To N-tert-butyloxycarbonyl-β-alanine ethyl ester (3.88 g, 17.86 mmol) in dry toluene (60 ml) at -78 °C under nitrogen over 1 h, DIBAL-H (1.0 M in toluene, 44.0 ml, 44.0 mmol, 2.5 eq) was added dropwise with stirring, with the aid of a syringe pump. After the addition the reaction mixture was stirred for a further 0.5 h. Methanol (14 ml) was added, and the reaction mixture was poured into a solution of Rochelle salt (50 g) in water (200 ml), and stirred vigorously for 1.5 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 100 ml). The organic layers were then combined and washed with saturated brine (3 x 100 ml), dried over magnesium sulfate and concentrated under reduced pressure to yield a colourless oil (3.6 g, 100%) that was used without further purification. \[\delta_H (400 \text{ MHz}; \text{CDCl}_3) 1.35 (9H, s, C(CH_3)_3), 2.59 (2H, t, J 6, CH_2CH_2CHO), 3.32 (2H, q, J 6, CH_2CH_2CHO), 5.08 (1H, br s, NH), 9.69 (1H, s, CHO); \delta_C (100 \text{ MHz}; \text{CDCl}_3) 28.5 (\text{C(CH}_3)_3), 32.7 (\text{CH}_2), 44.2 (\text{CH}_2), 137.8 (\text{C=O}), 156.3 (\text{C=O}), 201.5 (\text{CHO}); \nu_{\text{max}} (\text{CHCl}_3)/\text{cm}^{-1} 3357 (\text{NH}), 2976, 2932 (\text{aliphatic CH}), 1693 (\text{C=O}); m/z (\text{EI}) M 173.1084 (C_8H_{15}NO_3 \text{ requires } 173.1052), 155 (37), 136 (39), 118 (48), 102 (40), 57 (100)\]
[3-(Phenylhydrazono)propyl]carbamic acid tert-butyl ester [118]

To phenylhydrazine hydrochloride (6.04 g, mmol, eq) and sodium acetate (6.17 g, mmol, eq) in water, was added the crude (3-oxopropyl)carbamic acid tert-butyl ester (3.60 g, 20.79 mmol) in ethanol (5 ml). The solution was warmed to 70 °C for 10 min and cooled in an ice bath. The resulting precipitate was filtered and dried to give an orange solid (2.69 g, 47%) that was used without further purification. δH (400 MHz; CDCl3) 1.36 (9H, s, C(CH3)3), 2.41 (2H, q, J 4.8, CH2CH2), 3.34 (2H, q, J 6, CH2CH), 6.77 (1H, t, J 7.2, Ph-H), 6.88 (2H, d, J 7.6, Ph-H), 7.00 (1H, t, J 4.8, CH=N), 7.17 (2H, t, J 6.8 Ph-H), 7.20 (1H, s, NH); δC (100 MHz; CDCl3) 28.4 (C(CH3)3), 32.7 (CH2), 37.7 (CH2), 79.3 (CH), 112.5 (Ph-C), 112.8 (Ph-C), 119.8 (Ph-C), 129.2 (Ph-C), 137.1 (CHN), 145.1 (C), 156.0 (C=O); νmax (CHCl3)/cm⁻¹ 3354 (NH), 2977, 2929 (aliphatic CH), 1690 (C=O) 1610 (C=N); m/z (El) M 263.1755 (C14H21N2O2 requires 263.1734), 208 (37), 199 (41), 155 (37), 154 (42), 136 (29), 93 (24), 57 (95).
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Appendix I  X-Ray Crystal Data of 48
Table 1. Crystal data and structure refinement for rcfj20.

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<th>Identification code</th>
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<td>Radiation, wavelength</td>
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<td>( b = 11.4316(4) ) Å</td>
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<tr>
<td></td>
<td>( c = 16.8254(6) ) Å</td>
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<tr>
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<td>Calculated density</td>
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<td>F(000)</td>
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Crystal colour and size: colourless, 0.36 x 0.09 x 0.04 mm$^3$

Reflections for cell refinement: 2323 (θ range 2.91 to 27.48°)

Data collection method: Bruker-Nonius 95mm CCD camera on ω-goniostat

θ range for data collection: 3.02 to 27.52°

Index ranges: h -6 to 6, k -14 to 14, l -21 to 21

Completeness to θ = 27.52°: 99.2%

Intensity decay: 0%

Reflections collected: 11290

Independent reflections: 2363 ($R_{int}=0.0376$)

Reflections with F^2>2σ: 2223

Absorption correction: semi-empirical from equivalents

Min. and max. transmission: 0.970 and 0.997

Structure solution: direct methods

Refinement solution: Full-matrix least-squares on F^2

Weighting parameters: a, b = 0.0316, 0.2720

Data / restraints / parameters: 2363 / 1 / 244

Final R indices [F^2>2σ]:

R1 = 0.0351, wR2 = 0.0811

R indices (all data):

R1 = 0.0388, wR2 = 0.0833

Goodness-of-fit on F^2:

1.080

Absolute structure parameter: 10(10) Friedels merged, chirality known from SM.

Extinction coefficient: 0.027(6)

Largest and mean shift/su:

0.000 and 0.000

Largest diff. peak and hole:

0.192 and -0.167 e A$^{-3}$
Table 2. Atomic coordinates and equivalent isotropic displacement parameters (Å²)
for rcfj20. $U_{eq}$ is defined as one third of the trace of the orthogonalized $U^i$ tensor.

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Table 3. Bond lengths [Å] and angles [°] for rcfj20.

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<td>C(8)–C(9)–C(4)</td>
<td>120.01(19)</td>
<td></td>
</tr>
<tr>
<td>O(1)–C(10)–C(1)</td>
<td>124.50(19)</td>
<td></td>
</tr>
<tr>
<td>C(10)–O(2)–C(11)</td>
<td>116.06(14)</td>
<td></td>
</tr>
<tr>
<td>N(3)–C(13)–C(3)</td>
<td>109.50(17)</td>
<td></td>
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<td>C(3)–C(13)–C(14)</td>
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<tr>
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<td></td>
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<td>N(3)–C(15)–O(4)</td>
<td>109.63(17)</td>
<td></td>
</tr>
<tr>
<td>O(4)–C(16)–C(17)</td>
<td>110.8(2)</td>
<td></td>
</tr>
<tr>
<td>C(17)–C(16)–C(19)</td>
<td>113.82(19)</td>
<td></td>
</tr>
<tr>
<td>C(17)–C(16)–C(18)</td>
<td>110.0(3)</td>
<td></td>
</tr>
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N(1)–C(3)–N(2) = 107.90(17)°
C(4)–N(2)–C(1) = 124.78(17)°
N(2)–C(1)–C(10) = 110.44(16)°
C(10)–C(1)–C(2) = 110.75(17)°
N(1)–C(3)–C(2) = 114.60(18)°
C(2)–C(3)–C(13) = 122.10(17)°
N(2)–C(4)–C(5) = 120.80(18)°
C(6)–C(5)–C(4) = 119.90(19)°
C(6)–C(7)–C(8) = 119.14(19)°
C(8)–C(9)–C(4) = 120.01(19)°
O(1)–C(10)–C(1) = 124.50(19)°
C(10)–O(2)–C(11) = 116.06(14)°
N(3)–C(13)–C(3) = 109.50(17)°
C(3)–C(13)–C(14) = 113.15(17)°
O(3)–C(15)–N(3) = 124.75(19)°
N(3)–C(15)–O(4) = 109.63(17)°
O(4)–C(16)–C(17) = 110.8(2)°
C(17)–C(16)–C(19) = 113.82(19)°
C(17)–C(16)–C(18) = 110.0(3)°
Table 4. Hydrogen coordinates and isotropic displacement parameters (Å$^2$) for rcfj20.

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U</th>
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<tr>
<td>H(1)</td>
<td>0.0891</td>
<td>0.8740</td>
<td>0.6292</td>
<td>0.024</td>
</tr>
<tr>
<td>H(2A)</td>
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<td>0.9561</td>
<td>0.7319</td>
<td>0.027</td>
</tr>
<tr>
<td>H(2B)</td>
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<td>0.9344</td>
<td>0.7934</td>
<td>0.027</td>
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<tr>
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<td>1.1408</td>
<td>0.5900</td>
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<td>0.028</td>
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<tr>
<td>H(7)</td>
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<td>0.9301</td>
<td>0.4282</td>
<td>0.028</td>
</tr>
<tr>
<td>H(8)</td>
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<td>0.7818</td>
<td>0.4567</td>
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<tr>
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<td>0.8092</td>
<td>0.5544</td>
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<tr>
<td>H(11A)</td>
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<td>0.5214</td>
<td>0.7017</td>
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<tr>
<td>H(11B)</td>
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<td>0.5977</td>
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<tr>
<td>H(12A)</td>
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<td>0.6276</td>
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<td>H(12B)</td>
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<td>0.5606</td>
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<td>0.043</td>
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<tr>
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<td>1.1931</td>
<td>0.7578</td>
<td>0.024</td>
</tr>
<tr>
<td>H(14A)</td>
<td>0.3858</td>
<td>1.3043</td>
<td>0.7692</td>
<td>0.044</td>
</tr>
<tr>
<td>H(14B)</td>
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<td>1.3680</td>
<td>0.7813</td>
<td>0.044</td>
</tr>
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<td>H(14C)</td>
<td>0.1654</td>
<td>1.3191</td>
<td>0.6951</td>
<td>0.044</td>
</tr>
<tr>
<td>H(3)</td>
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<td>1.158(2)</td>
<td>0.8787(14)</td>
<td>0.025</td>
</tr>
<tr>
<td>H(17A)</td>
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<td>0.9505</td>
<td>1.0182</td>
<td>0.058</td>
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<td>1.0307</td>
<td>0.9824</td>
<td>0.058</td>
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<td>H(17C)</td>
<td>-0.4071</td>
<td>1.0004</td>
<td>1.0758</td>
<td>0.058</td>
</tr>
<tr>
<td>H(18A)</td>
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<td>1.1773</td>
<td>1.1281</td>
<td>0.083</td>
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<tr>
<td>H(18B)</td>
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<td>0.083</td>
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</tr>
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<td>H(19C)</td>
<td>-0.3953</td>
<td>1.2332</td>
<td>1.0991</td>
<td>0.061</td>
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Table 5. Torsion angles [°] for rcfj20.

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<th>Bond</th>
<th>Value</th>
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<td>C(3)-N(1)-N(2)-C(4)</td>
<td>173.58(18)</td>
</tr>
<tr>
<td>C(4)-N(2)-C(1)-C(10)</td>
<td>64.8(3)</td>
</tr>
<tr>
<td>C(4)-N(2)-C(1)-C(2)</td>
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<tr>
<td>N(2)-C(1)-C(2)-C(3)</td>
<td>10.30(19)</td>
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<tr>
<td>N(2)-N(1)-C(3)-C(2)</td>
<td>1.5(2)</td>
</tr>
<tr>
<td>C(1)-C(2)-C(3)-N(1)</td>
<td>-8.0(2)</td>
</tr>
<tr>
<td>N(1)-N(2)-C(4)-C(9)</td>
<td>-166.15(18)</td>
</tr>
<tr>
<td>N(1)-N(2)-C(4)-C(5)</td>
<td>16.4(3)</td>
</tr>
<tr>
<td>N(2)-C(4)-C(5)-C(6)</td>
<td>174.58(19)</td>
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<tr>
<td>C(4)-C(5)-C(6)-C(7)</td>
<td>1.0(3)</td>
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<td>C(6)-C(7)-C(8)-C(9)</td>
<td>-1.8(3)</td>
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<tr>
<td>N(2)-C(4)-C(9)-C(8)</td>
<td>-175.11(18)</td>
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<tr>
<td>N(2)-C(1)-C(10)-O(1)</td>
<td>48.7(3)</td>
</tr>
<tr>
<td>N(2)-C(1)-C(10)-O(2)</td>
<td>-132.87(18)</td>
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<tr>
<td>O(1)-C(10)-O(2)-C(11)</td>
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<td>54.0(2)</td>
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<td>N(1)-N(2)-C(4)-C(5)</td>
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<tr>
<td>C(9)-C(4)-C(5)-C(6)</td>
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</tr>
<tr>
<td>C(5)-C(6)-C(7)-C(8)</td>
<td>1.3(3)</td>
</tr>
<tr>
<td>C(7)-C(8)-C(9)-C(4)</td>
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</tr>
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<td>N(1)-C(3)-C(13)-N(3)</td>
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<tr>
<td>N(1)-C(3)-C(13)-C(14)</td>
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<td>C(3)-C(13)-N(3)-C(15)</td>
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<td>C(13)-N(3)-C(15)-O(3)</td>
<td>1.8(3)</td>
</tr>
<tr>
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<td>0.3(4)</td>
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<tr>
<td>C(15)-O(4)-C(16)-C(17)</td>
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</tr>
<tr>
<td>C(15)-O(4)-C(16)-C(18)</td>
<td>-179.4(2)</td>
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Table 6. Hydrogen bonds for rcfj20 [Å and °].

<table>
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<tr>
<th>Bond</th>
<th>d(D-H)</th>
<th>d(H...A)</th>
<th>d(D...A)</th>
<th>&lt;(DHA)</th>
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</thead>
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<tr>
<td>N(3)-H(3)...O(3)</td>
<td>0.85(3)</td>
<td>2.14(3)</td>
<td>2.937(2)</td>
<td>156(2)</td>
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</table>

Symmetry operations for equivalent atoms
' x+1,y,z
Appendix II  HPLC Data and Spectra of 76 and 81

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\farah\Data\Yohan\les 193 231007a.dat
Method: C:\EZChrom Elite\Enterprise\Projects\ben\Method\99.1 0.5ml 50min Laura.met
Acquired: 23/10/2007 10:43:34
Printed: 23/10/2007 11:41:54

<table>
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<th>UV Results</th>
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<th>Area</th>
<th>Area %</th>
<th>Height</th>
<th>Height %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2188830</td>
<td>93.00</td>
<td>182113</td>
<td>93.44</td>
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<tr>
<td>UV Results</td>
<td>39.573</td>
<td>1646835</td>
<td>7.00</td>
<td>12793</td>
<td>6.56</td>
</tr>
</tbody>
</table>

Totals    | 23535665       | 100.00 | 194906 | 100.00 |

Retention Time

Area

Area %

Height

Height %
Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\farah\Data\Yohan\les racemic 221007.dat
Method: C:\EZChrom Elite\Enterprise\Projects\ben\Method\99.1 0.5ml 50min Laura.met
Acquired: 22/10/2007 15:36:23
Printed: 23/10/2007 09:43:12

UV Results

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<th>Height %</th>
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</thead>
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<td>80534</td>
<td>52.61</td>
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<td>39.520</td>
<td>11332943</td>
<td>51.65</td>
<td>72542</td>
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<tr>
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