A Linker Approach to Heterocyclic Amino Acids

By

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Abstract

Polypeptide Nucleic Acids, PNAs, are analogues of DNA and have the potential to bind to DNA by base-pairing and hence act as therapeutic agents. Amino acids carrying heterocycles in their side-chains are valid targets as natural products and as components of these potential therapeutic agents (PNAs) for use in living organisms. The aim of this investigation was to synthesise a range of heterocyclic amino acids, that could be used in the formation of PNAs. The proteinogenic amino acids, serine and cysteine and the unnatural amino acids, homocysteine, 2,3-diaminopropionic acid and 2,4-diaminobutyric acid, have been used in the formation of said heterocyclic amino acids via a C-X bond (where X=C, S, O or N) in a linker chain.

It was decided to approach the synthesis of heterocyclic amino acids by way of a linker approach, joining the ready-formed heterocycle with an amino acid. Once the amino acids had been suitably protected several different methods were attempted in order to form heterocyclic amino acids. To form a carbon-carbon (X=C) bond in the linker chain, radical and organocuprate conjugate addition reactions and hydroboration and metathesis coupling were attempted. The formation of a linker containing a carbon-heteroatom bond (X=S, O or N) was investigated using a substitution approach.

Where X=C, radical and organocuprate conjugate addition onto a methylene oxazolidinone amino acid template with N-(ω-haloalkyl) heterocycle derivatives (prepared by Mitsunobu coupling or phase transfer catalysis) produced none of the desired products. There was evidence of reduced product indicating that radicals had been formed, and evidence also of lithium/halogen exchange, leading to a number of undesired side reactions. The hydroboration and metathesis coupling reactions, again using a methylene oxazolidinone amino acid template, this time with N-vinyl heterocycle derivatives, also proved fruitless, possibly be due to steric factors.

Far greater success was had with formation of C-X bonds by substitution where X=S, O or N. The substitution reactions involved heteroatoms on the amino acids side chains reacting with N-(haloalkyl) heterocycle derivatives. Yields of the reactions involving cysteine were over 60% in many of the cases and new heterocyclic amino acids were prepared by this method.

Acknowledgements

I would like to thank firstly all members of the Jones group past and present for their friendship and helpfulness, all my other friends and family for their patience, my partner for his support throughout my research, all the technical staff for their assistance and guidance in NMR, MS, CHN etc, all the members of the organic research labs and all the other members of staff that have helped me during my research and finally Prof. Ray Jones for his help and guidance in the completion of this thesis.

Contents

Abbreviations

AIBN	2,2-Azobis(2-methylpropionitrile)
9-BBN	9-Borabicyclo(3.3.1)nonane
TTA	t-Butyltrichloroacetimidate
DBU	Diazabicyclo(5.4.0)undec-7-ene
DCB	1,3-Dichlorobutane
DCM	Dichloromethane
DhbtOH	3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
DNA	Deoxyribonucleic Acid
DIAD	Diisopropyl Azodicarboxylate
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
EtOH	Ethanol
EtOAc	Ethyl Acetate
Pfp	Pentafluorophenol
PTC	Phase Transfer Catalysis
PNA	Polypeptide Nucleic Acid
Oxone®	Potassium Peroxymonosulfate
RNA	Ribonucleic Acid
RT	Room Temperature
RBF	Round-Bottomed Flask
THF	Tetrahydrofuran
TFA	Trifluoroacetic Acid
TMSCl	Trimethylsilyl Chloride / Chlorotrimethylsilane
TPP	Triphenylphosphine

1. Introduction

1.1 Aim of Research

Amino acids carrying heterocycles in their side-chains are valid synthetic targets as natural products and as components of potential therapeutic agents for use in living organisms. Heterocyclic amino acids, as in Figure 1.1, can also be used in the formation of the prospective therapeutic agents Polypeptide Nucleic Acids, PNAs, hence how these amino acids are made is of great importance. The aim of the investigation reported in this thesis was to synthesise a range of heterocyclic amino acids, which could be used in the formation of PNAs.

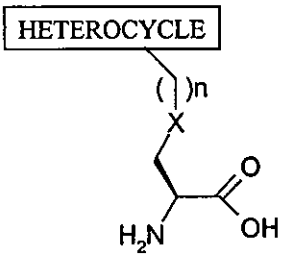


Figure 1.1 Heterocyclic Amino Acid (X=heteroatom or Carbon)

1.2 Amino Acids

As the name suggests an amino acid possesses both a carboxyl and an amino group attached to the same carbon atom. α-Amino acids have the general formula, $RCH(NH_2)COOH$, where R=H or an organic group possibly containing a heteroatom.[1] It is

the R group that determines the properties of the amino acid[2] and hence what makes them potential therapeutic agents. For this investigation only α-amino acids have been used and the configuration of the α-amino acids (L or D) can play an important role in the formation of a potential therapeutic agent.[1] In proteins, the amino acids have the L-configuration, corresponding to (S) in all but cysteine.

If the R group contains a heterocycle, any heterocycle could be used to form a synthetic amino acid, but in nature there are only two heterocycles found in proteinogenic amino acids (imidazole in histidine and indole in tryptophan). Amongst non-proteinogenic amino acids particular (but not exclusive) interest is in the five main heterocycles found in nucleic acids, and their incorporation into amino acids.

1.3 Nucleic Acids

A nucleic acid is an organic compound found in all living cells that consists of a chain of nucleotides, which are themselves made of a purine or pyrimidine base linked to a phosphorylated ribose sugar.[3] These bases are shown in Figure 1.2.

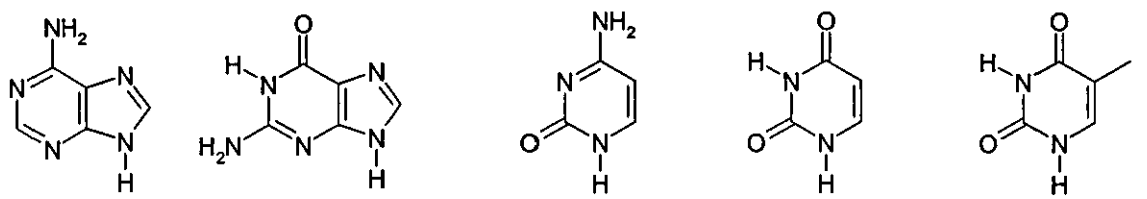


Figure 1.2 The Five Naturally Occurring Nucleobases

Nucleic Acids as Therapeutic Agents

3'-Deoxy-2',3'-didehydrothymidine is a well known and promising candidate for AIDS chemotherapy and further investigations into 2',3'-didehehydro-2',3'-dideoxynucleosides has led to further research involving other nucleobases.[4] The 3'-deoxy-2',3'-didehydrothymidine and precursor to other 2',3'-didehydro-2',3'-dideoxynucleosides are shown in Figure 1.3. There are several other nucleosides and nucleoside analogues which have potential for use as therapeutic agents.

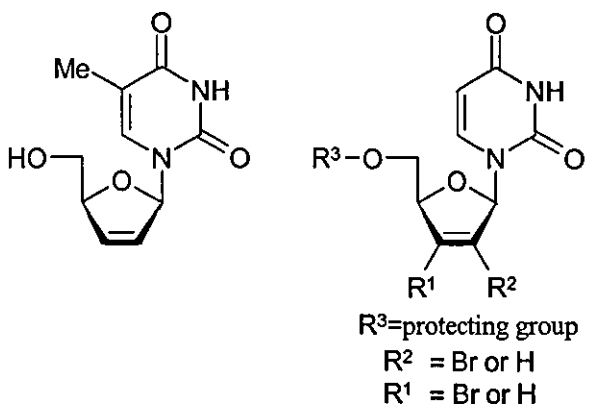


Figure 1.3 3'-deoxy-2',3'-didehydrothymidine and
2'- or 3'-bromo-3'-deoxy-2',3'-didehydrouridine

1.4 Heterocyclic Amino Acids as Natural Products

One reason heterocyclic amino acids are of interest is because of the number of them in natural products. The earliest of these isolated by Gmelin: N-thyminyl-L-alanine from a type of rose and called willardiine after the rose.[5] These naturally occurring heterocyclic amino acids have been successfully synthesised with yields up to 78%.[6]

Another couple of more recent heterocyclic examples are given below:

Baldwin et al. have investigated a number of non-proteinogenic α-amino acids based around the α-amino acid L-lathyrine (Figure 1.4).[7]

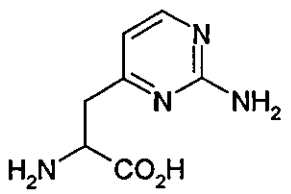


Figure 1.4 L-Lathyrine and other α-Amino Acids Based on this System

Young et al. have also investigated the use of pyroglutamic acid derivatives (Figure 1.5) as possibilities in the treatment of illnesses such as Alzheimers, epilepsy and ischaemia.[8]

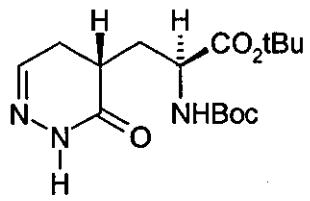


Figure 1.5 Pyrazine/Glutamic Acid Derivative

Further examples of heterocyclic amino acids are given later in this chapter.

Sialyl Oligosaccharides.

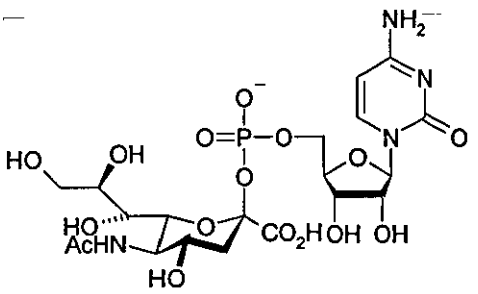


Figure 1.6 CMP-sialic acid

Although not a heterocyclic amino acid this shows heterocycles attached to a system that is similar to those PNAs are designed to mimic. Cytidine 5'-monophosphate-sialic acid (CMP-sialic acid) (Figure 1.6) links to the ends of glycoconjugates to form sialyl oligonucleotides which play an important role in cell differentiation, immune responses to bacterial and viral infections and other cell recognition events.[9] The inhibitors of sialyltransferases, that transfer sialic acid from CMP-sialic acid to galactose or other residues of oligosaccharides, would be excellent candidates as anti-inflammatory agents, anti-metasastic agents and agents for therapy of auto-immune diseases (Figure 1.7).

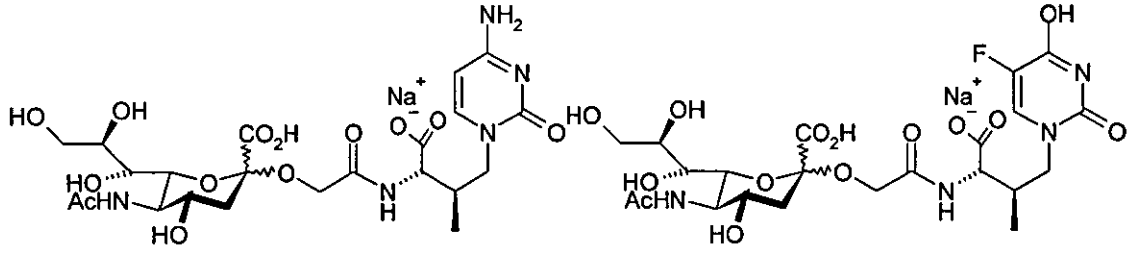


Figure 1.7 Mimetics of sialic acid

It has also been noted that, in the area of therapeutics, protein backbones have been modified like that of ketoamides[10] –NH-CO-CO- and in PNA research as described in greater detail later.

Pyrrole-Imidazole Polyamides - Distamycin

An example of where heterocycles have been used within the backbone similar to that of PNA is Distamycin, a natural product that contains three N-methylpyrrole amino acids and binds in the minor groove of DNA. This natural compound inhibits DNA-dependent processes but is of limited use because of its toxicity.[1] Distamycin analogues have therefore been of great interest as therapeutic agents. The base distamycin molecule is shown in Figure 1.8 and some of the variations shown in Figure 1.9.

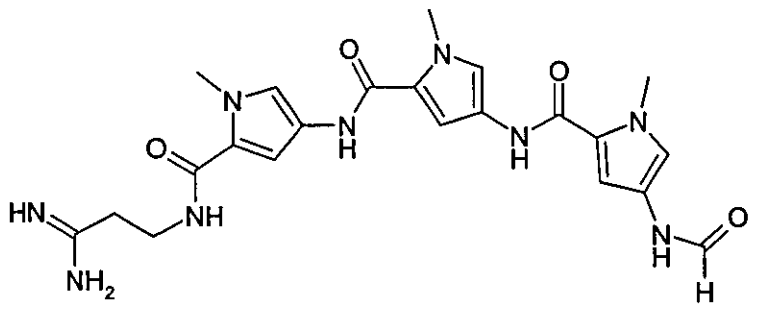


Figure 1.8 Distamycin

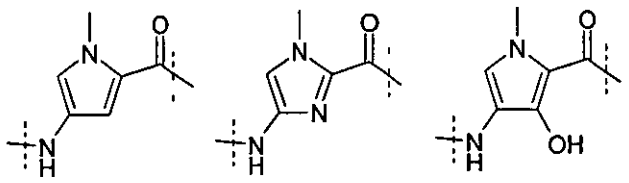


Figure 1.9 Distamycin and its Derivatives

1.5 Peptides and Peptide Bonds

The bond between an α-amino nitrogen and α-carboxyl carbon atom is known as a peptide bond and is formed with the loss of water (Figure 1.10). The linking of several amino acids by such peptide bonds produces a (poly)peptide; long polypeptide chains are known as proteins, and may function as enzymes.

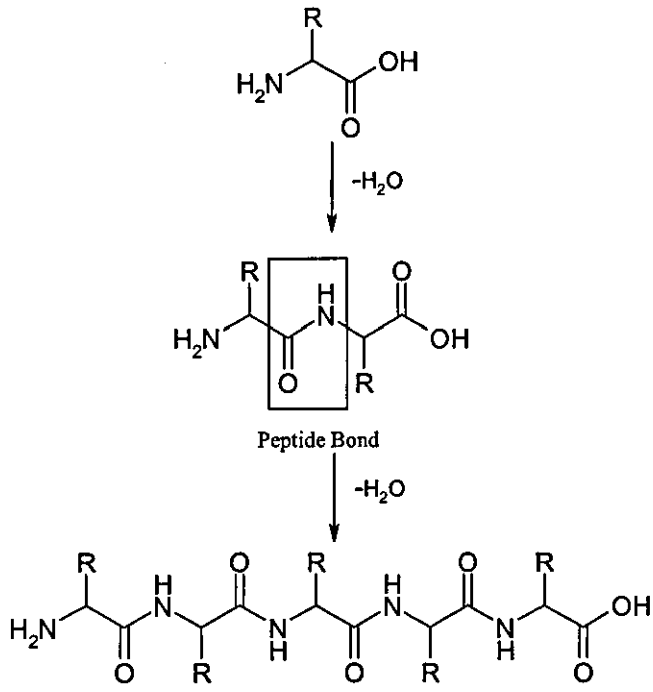


Figure 1.10 Peptide or Polypeptide

A large number of smaller peptides that occur naturally are thought to be the by-products of enzymatic reactions on proteins by tissue autolysis, bacterial action and digestive processes.[11] Other naturally occurring peptides have a clearer physiological role as coenzymes, hormones or antibiotics.

1.5.1 Polypeptide Nucleic Acid

It becomes obvious why heterocyclic amino acids are of such interest when PNA and DNA are compared as in Figure 1.11.

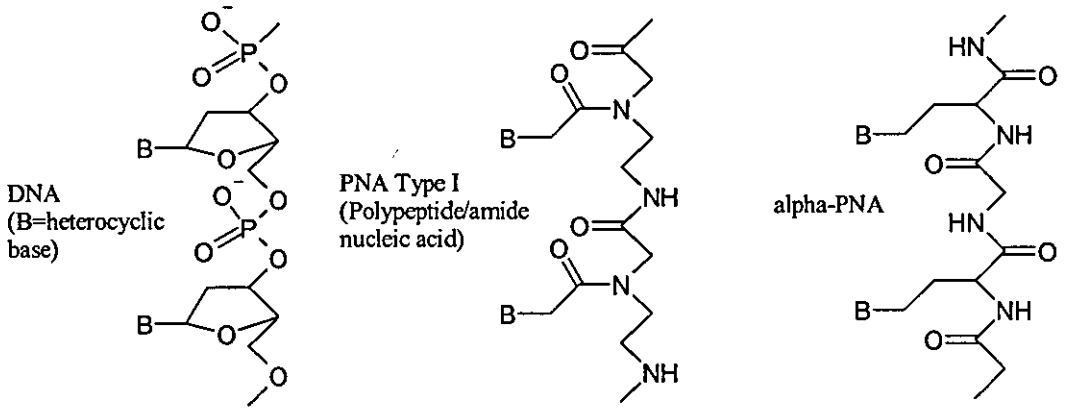


Figure 1.11 Comparison of DNA and PNA

DNA consists of a chain of ribose sugars with negatively charged phosphates and either purine or pyrimidine bases as side-chains. Amino acid derived chains with nucleic acid bases in their side-chains are monomers for the man-made analogous chain PNA with one major difference between this chain and the DNA chain, PNA contains an electrically neutral backbone where the phosphodiester backbone has been replaced by a polyamide or peptide backbone.

The first PNA macromolecules that were investigated over 40 years ago comprised of glycine and 1,2-diaminoethane with N-acyl side chains.[12,13,14] Since then, PNA investigation has been split into two main categories; Type I and Type II.[15]

PNA type I consists of N-(aminoalkyl)amino acid units with the nucleobases attached to the nitrogen in the backbone, the first of these, the N-(aminoethyl)glycine backbone, is shown in Figure 1.11; PNA type II consists of amino acid residues carrying the nucleobase in their side chain, again shown in Figure 1.11 as α-PNA, these amino acid residues can be interspersed with simple amino acids (e.g. glycine (illustrated) or valine) to give the correct length between each nucleobase or pseudonucleobase for bonding to DNA or RNA.[15]

PNAs containing heterocycles that are not naturally occurring have also been investigated previously, for example, adenine has been replace by 2,6-diaminopurine and this has been shown to enhance the PNAs tendency to hybridise, possibly due to its stronger pairing with thymine through three hydrogen bonds.[15]

PNAs are analogues of DNA and have the potential to bind to DNA by base-pairing (Figure 1.12).

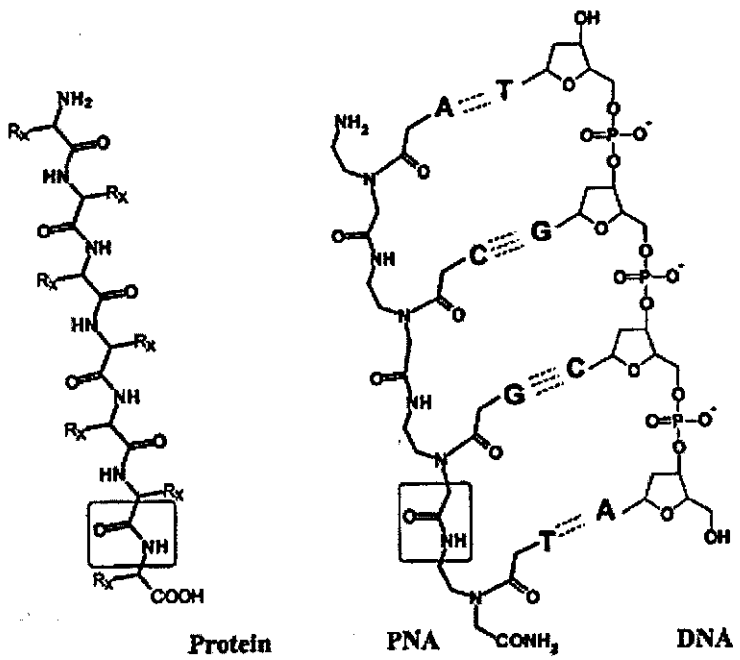


Figure 1.12 PNA-DNA base pairing (ref: Pseudo-Peptides in Drug Discovery, Ed. P.E. Nielsen, Wiley-VCH, (2004) 154)

They form a novel class of therapeutic agents as nucleic acids store and copy information within a living organism therefore information can be transferred to DNA using the synthetic nucleic acid chain, PNA.[16,17] In essence heterocyclic amino acids can induce biological activity when incorporated into peptides or peptide like structures.

1.5.2 DNA

DNA is usually found in the body in the form of a double helix (Figure 1.13).

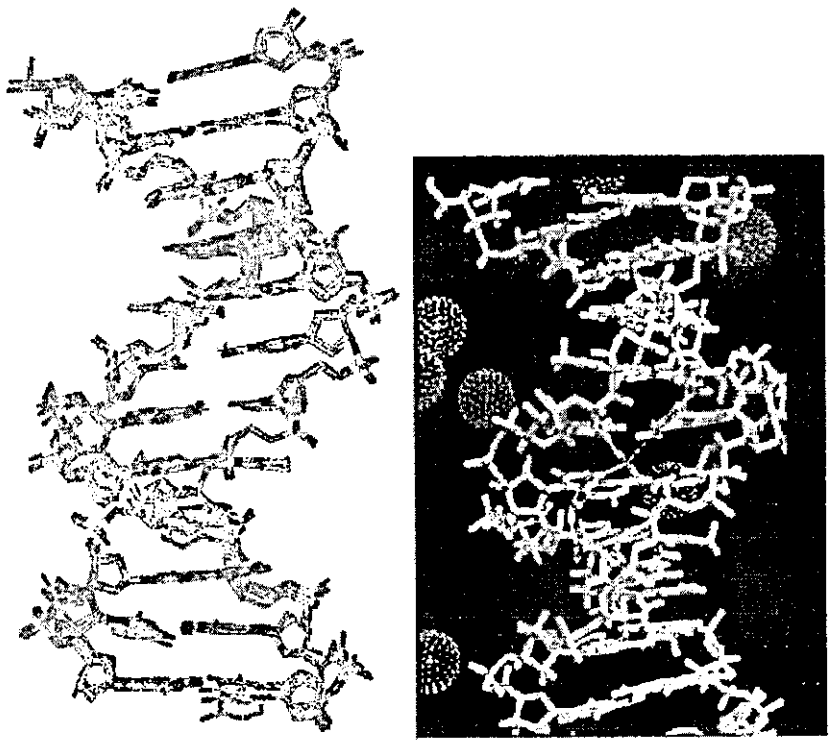
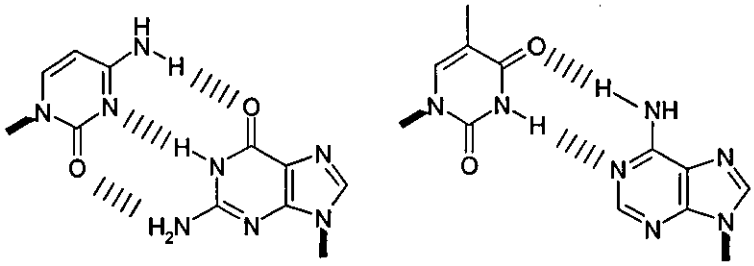


Figure 1.13 The Double helix formation of DNA. (ref: K.J.McConnell, D.L.Beveridge, DNA structure: What's in Charge?, J. Mol. Biol, (2000) 304, 803).

Two factors play a part in this "dimer" formation. One is regioselectivity, which manifests itself in DNA as shape complementarity; the two strands of DNA can come together like puzzle pieces: when the sequencing is not right then it will be very unlikely that the two

strands in question will fit together. The other is that of hydrogen bonding, which in the case of DNA, is specific between pairs of bases and these are known as Watson-Crick base pairs (Figure 1.14).



Cytosine-Guanine Thymine-Adenine

Figure 1.14 Watson-Crick base pairing

The insertion of molecules into the DNA system to disrupt the DNA sequence and to insert another molecule to either gather information from the DNA strand, or containing different information in a therapeutic manner, can be investigated with the use of PNA, which can contain both the spatial and polar properties necessary to form a PNA/DNA duplex.

PNA-DNA recognition again takes place by Watson-Crick base pairing[18] and it seems the DNA-PNA is more thermally stable than that of DNA-DNA and the rate of hybridisation is at least as fast as that of DNA-DNA duplex formation, possibly faster due to the lack of electrostatic repulsion between PNA and DNA. It is also possible that introduction of positive charges in PNA may improve the stability. In one study,[19] the -O-(PO_2-)-O- linkers of DNA are replaced by -NH-C(=NH_2^+)-NH- or -NH-C(=S^+CH_3)-NH- linkers in the PNA backbone. These linkers have shown increased hybridisation association rates, suggesting potential utility in diagnostics, biomolecular probes and antisense/antigene therapeutics.

To assist in the binding, specificity and uptake into cells, the insertion of stereogenic centres has been suggested.[20] The investigation into this has shown that the introduction of chiral centres into the PNA backbone does not alter the tendency of PNA and duplexes to form P-helixes but can limit the ability of PNA to form other conformations and increases the recognition selectivity.[20] The structure of a standard PNA-DNA duplex is that of a P-helix, this has the same specificity and selectivity as a DNA-DNA duplex but not exactly the same shape.

Incorporation of chirality or placing conformational constraints into PNA may also be important in discrimination of PNA-DNA bonding.[19] The base has often been appended to nitrogen in the chain (Figure 1.11), giving it no chirality, so that a way of introducing chirality is by linking the base to a carbon atom in the PNA backbone. It has been shown that the chiral PNA, oxy-peptide nucleic acid (OPNA) (Figure 1.15), does bind to complementary DNA and PNA strands[21] and it was noted once again that the hybridisation with DNA was greater than that with PNA and was demonstrated by strand replacement of PNA-OPNA to DNA-OPNA.

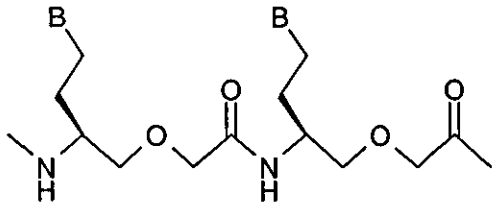


Figure 1.15 Chirality in PNA (In this case OPNA)

With OPNA chirality has been introduced, and this is the same for α-PNAs as shown in Figure 1.11. It is necessary for this chirality to be present and hence the amino acids that are to be used in this investigation will also impart chirality into the molecule. The

α-aminooxy acids are considered as β-peptide mimics, and can be built up into OPNA's and can be made from α-amino acids.

The molecular mechanism for binding-induced gene expression of PNA will have implications for the gene therapy in humans. Abnormal gene expression has been linked to many age related diseases and others such as cancer, several drugs have been developed to induce gene expression, but none so far have induced specific genes.[22]

1.6 Applications of PNA

In living organisms and the environment there are a number of natural products containing amino acids: an example of their use by a living organism is that of mediation by amino acids of synaptic excitation. The use of PNAs in living organisms has also been in gene therapeutics[23] as an agent for antisense and antigene applications in cell culture[24,25,26] due to their high chemical and biological stability.

Peptides are able to adopt well defined and controlled secondary structures which can in theory be used as scaffolds to place and orientate pharmacophores and create new molecules with interesting biological activities, hence the interest in building helical compounds with PNA.

Although 'naked' PNAs have been shown to down-regulate receptor activities upon injection of the PNAs into the brains of rats and hence into nerve cells,[24] the main problem in these cases is the inefficient uptake of PNA into cells, which is combated in a number of ways as explained later. From studies with mice[24] it has been shown that PNA has been

able to reach the target DNA within the cell and it is possible that targeting the nucleus of a cell with PNA is not as compromised as suggested by in vitro experiments, and so PNA investigation is worthwhile.

Human Telomerase

The inhibition of human telomerase activity is another example involving PNA, in which PNA is targeted to the RNA component of the enzyme.[27] Telomeres are guanine-rich regions of DNA located at the ends of eukaryotic chromosomes and are essential for preserving the stability of the genome and cell viability by preventing aberrant recombination and degradation of DNA. These telomeres, which have a telomeric sequence in human DNA of $(TTAGGG)_n$, are maintained by telomerase, an RNA dependent DNA polymerase. In healthy tissue these regions show little or no activity and the interest in them is because telomerase activity has been detected in tumour biopsies. Although the RNA telomerase region has been shown as a target for inhibition by antisense oligonucleotides, PNA was found to be a more effective antisense inhibitor of telomerase and the inhibition of human telomerase activity has been demonstrated using specific PNA recognition. The studies have shown that it is possible to enhance the inhibitory properties of relatively short PNAs by the use of a peptide fragment and the use of a cationic peptide has probably contributed to inhibition by increasing the stability of the complexes formed between PNA and the RNA region.

HIV Protein Inhibition

HIV is unfortunately a well known and widespread virus which has seen a large amount of investigation over the years.[1] One method for the treatment for people suffering from HIV could involve the use of PNAs. The disruption of the HIV-1 virus replication has been investigated by the use of a macromolecule, which incorporates a PNA-type backbone as shown in Figure 1.16. The molecule works by blocking the interaction of the HIV-1 protein with RNA. The tests have proven that this will work at nanomolecular concentrations and the problem of cell delivery has been broached by the use of a partly naturally occurring compound.

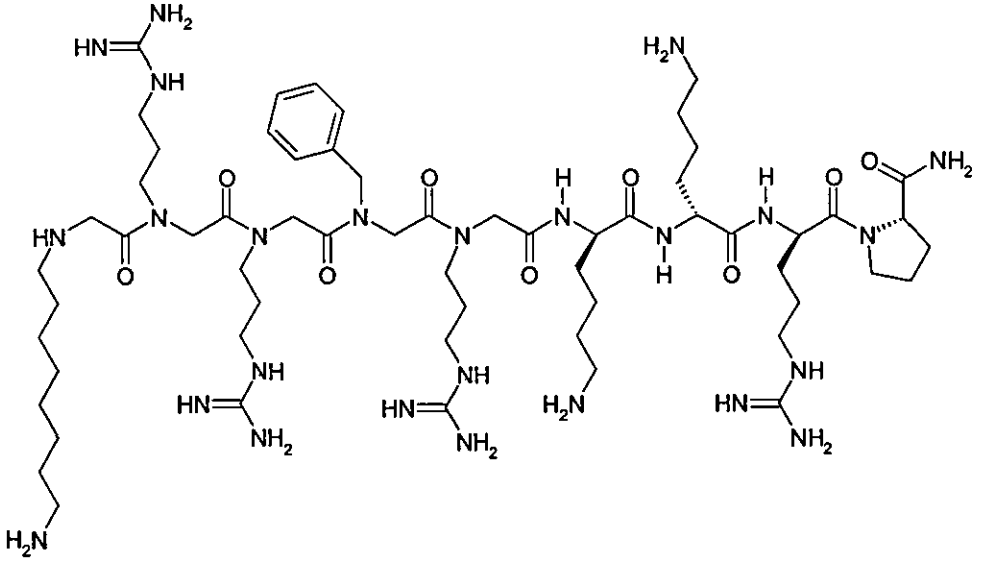


Figure 1.16 Macromolecule Inhibitor to HIV-1 virus

Gene Inhibition and Activation

Gene expression can be activated or inhibited by the presence of PNA. PNA binds to a wide range of DNA sites with high affinity and will competitively displace DNA from its double helix. In this way it is potentially useful in the treatment of cancer as shown by cultured and in vivo studies of cancerous kidney cells.[28] Polyamides can also be used with RNA and target viral genes normally transcribed by RNA polymerase. This has been shown in human blood lymphocytes where treatment with two polyamides in combination inhibited viral replication by 99%.

The human T-cell leukaemia virus has also been studied and the promoter of the virus inhibited. The polyamide is targeted to a sequence that is also recognised by bacterial gyrase and hence this inhibited the gyrase catalysed cleavage of the DNA.

PNA investigation

PNA can also be used in an investigative way. An investigation of the substrate specificity of helicases[29] involved PNAs being 'sent in' to substitute for one strand of DNA to see if specific enzyme/DNA interactions were necessary for the unravelling of the DNA helix.

The chemical, biological and genetic properties of DNA are obviously important for the development of new PNAs. It has been noticed that the structure of PNA-DNA intermediates is via Watson-Crick base pairing as shown by the parameters for PNA/DNA-PNA triplexes.[30] One method for the investigation of gene sequencing is by the invasion of

a DNA strand by PNA as a tool in genetic diagnostics.[31] Genome sequencing has revealed the identities of many genes that encode proteins whose functions are unknown.[25] Oligonucleotide mimics and PNAs are used as synthetic tools to inhibit gene expression at these sites.[25,32] The advantage of PNAs is that their uncharged neutral backbone does not inhibit their hybridisation as there is no interstrand electrostatic repulsion, which means there can be high affinity between PNA and either DNA or RNA.

1.6.1 Mechanism and Methods for PNA Invasion

When a PNA molecule replaces one of the DNA strands in double stranded DNA it does so by strand invasion with the displaced DNA strand forming a D-loop structure at the PNA binding site.[22] This D-loop is comparable in size to the length of PNA that has invaded (Figure 1.17).

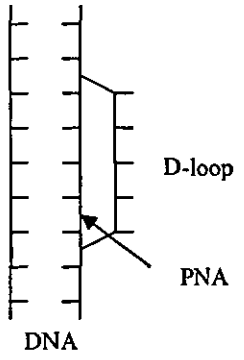


Figure 1.17 D-loop formation

Alternatively two PNAs insert into DNA and both sides of the double stranded DNA can become bound (Figure 1.18).

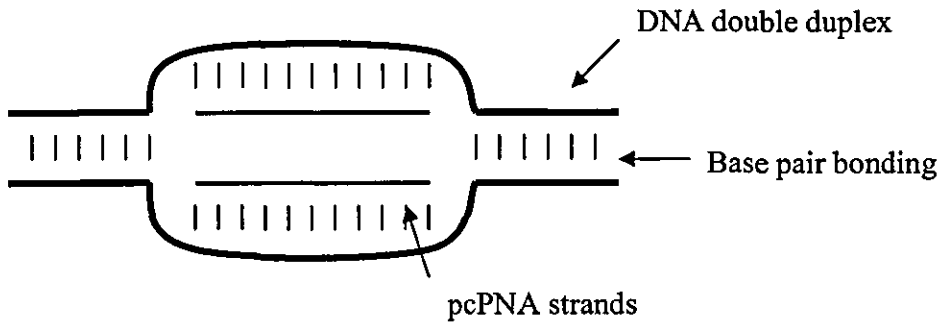


Figure 1.18 Invasion of PNA into DNA double duplex

For the invasion of the PNA molecule, experiments have shown that the molecule needs to be at least a 12mer in length and for sequence-specific binding they need to be longer, at least a 14mer.[22]

Cellular Delivery

The cellular delivery of PNAs into cells is an important factor, if they cannot be put into cells for therapeutic use then they cannot be of value as antisense or antigene agents. It is the pseudopeptide backbone of PNA that makes it stable in biological fluids, as it is not degraded by nucleases or peptidases (or other cellular enzymes) and gives PNA-DNA duplexes their thermal stability.[33] The uptake of a drug containing PNA may be limited by biological barriers, since they are polar molecules which diffuse poorly though the lipid bilayer of cells.[1]

It is also the backbone that makes it difficult to get PNA into the nucleus of a cell. This is due to the high salt concentration and pH dependency of PNA as demonstrated in several investigations where formation of the PNA-DNA duplex has been slow.[33]

Despite this PNA has been heavily investigated as it improves hybridisation characteristics of DNA and RNA. Initial studies into PNA were in cell-free environments and showed promising inhibition of transcriptional and translational processes. The investigation into the potential of PNA as a gene therapeutic has however been hampered by its poor uptake into cells, the problem being that PNA is a large hydrophilic molecule that does not cross lipid membranes easily. A variety of cellular delivery systems have been developed to aid in the investigation and use of PNAs within a cellular system.

Unmodified PNAs can be delivered into a cell by a number of methods,[33] the first and simplest chemical method to perform is that of microinjection. The PNA in injected directly into the cell nucleus and although this is an excellent tool for assessing the validity of PNA as an antisense agent, it is a laborious technique that can only be applied to small-scale experimental set-ups.

Electroporation is a more feasible method for the transfer of PNA, which has shown good results in the inhibition of telomerase.

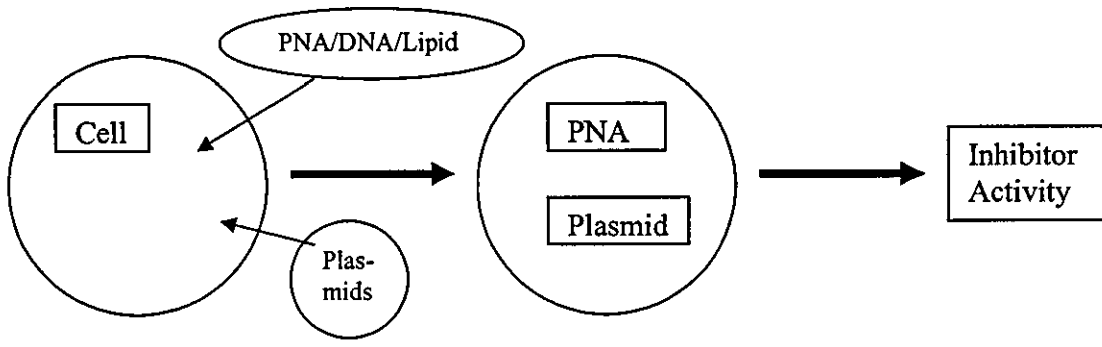


Figure 1.19 Introduction of PNA into cells.

Co-transfection with DNA allows the PNA to transfect the cell as it is hybridised to partially complementary DNA in the presence of cationic lipids (Figure 1.19) and this method has produced some promising results.[26] It is also possible to permeabilise the cell by the use of streptolycin-O to make the cell permeable to PNA. Another, possibly toxic, method is to use very high concentrations of PNA which encourages a limited uptake, this has shown promising results but the advantages of this method are probably outweighed by the disadvantages.

PNA can also be modified chemically by some kind of conjugation technique.[33] The conjugation of PNA to a lipophilic moiety, adamantyl-acetic acid or triphenylphosphonium ion, was done to promote PNA transfection by the use of cationic liposomes. This method has produced varying results and is unreliable since it is not possible to predict which type of cells the conjugate will be able to enter. Another method of conjugation has been used to some effect and it is that of conjugation to peptides. These so-called 'Trojan' peptides are a class of largely amphiphilic cationic/hydrophobic peptides that have been reported to have the ability to transport molecules across the cells membrane and show great potential

in the development of PNA-based antibiotics. Conjugation of PNAs to cell-specific receptor ligands has less potential and where PNA did target the desired cells there was little or no effect of PNA as in some cases the PNA was exclusively found in the vesicular compartments and not in the nucleus. Cationic β-peptides derived from HIV-1 proteins have been reported to permeate through the cell membrane and to mediate the transport of peptides, proteins and oligo-DNAs into target cells.[1] Arginine-rich amino acid residues have also undergone cellular uptake, and these arginine-rich compounds can be used to carry therapeutic agents with them with some flexibility in terms of side chain composition. Also PNA/DNA chimeras have been shown to form more stable duplexes with DNA, possibly due to lack of electrostatic repulsion, and have helped to combat difficulty with strand invasion.[30,31,34]

An investigation involving the testing of 18 antisense PNAs with a number of target genes and at a number of positions has shown that only one PNA in only one site on the DNA chain is necessary to cause an effect; an example of this is that of the significant inhibition of luciferase activity by one PNA at the extreme 5'-end of DNA, which blocked 80% of activity when delivered to cells as a PNA-DNA-Lipid complex. This luciferase investigation has also shown that if the DNA-PNA duplex is too stable for the PNA to be released when inside the cell or if the DNA-PNA duplex is too weak for the PNA to make it inside the cell, then inhibition or activity cannot occur.

Some other examples of problems and targets, possibly treatable by PNA, and how it has been delivered into the cell are:

Human Prostate Cancer / Telomerase – 15mer PNA – Cationic Liposomes;

Lymphoma / RNA – 15mer PNA – Electroporation;

E. Coli / Ribosomal RNA – 10-15mer PNA – Direct Delivery.

PNA/DNA stability

The helical structures of DNA and PNA are very similar, as shown in Figure 1.20, below:

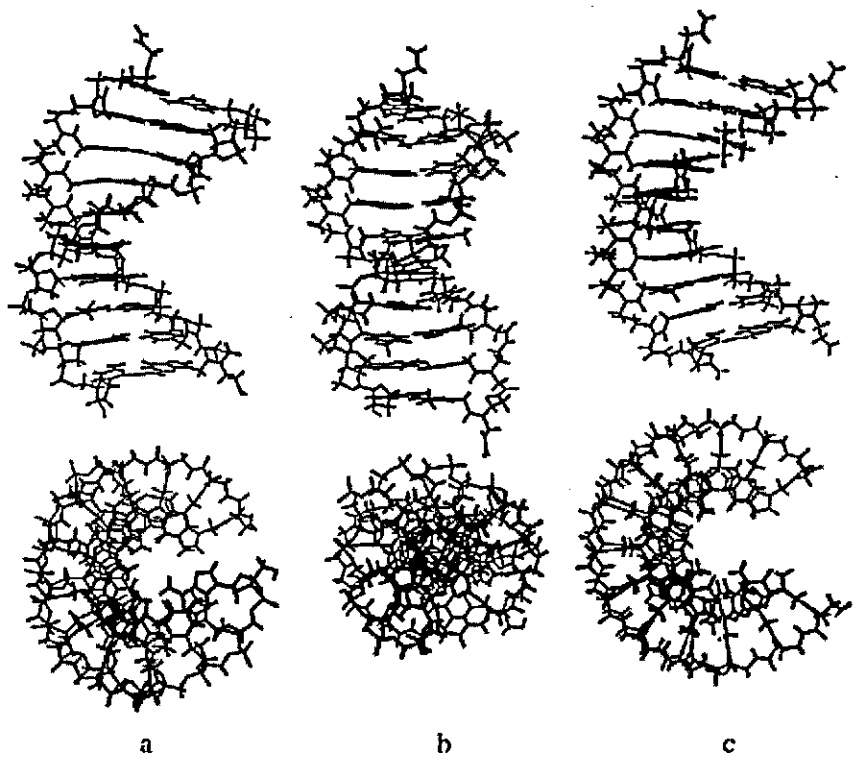
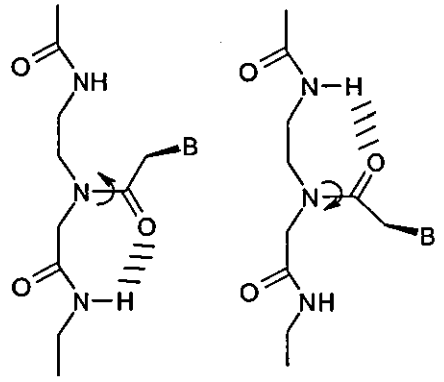


Figure 1.20 Comparison of DNA and PNA Duplexes Pseudo Peptides (ref: S. Sen, L. Nilsson, J. Am. Chem. Soc., (1998) 120, 619-631)

The stability of PNA-DNA duplexes may rely on the strength of the interresidue hydrogen bonding[35] within the PNA units as these stabilise the helical PNA strand when in this conformation (Figure 1.21), but this has been disputed by other investigators.[30] This is a perfect example of how PNA still requires a large amount of investigation.



Interresidue and Intraresidue

Figure 1.21 H-bonding in PNA

It has been noted that DNA-PNA and RNA-PNA duplexes are thermally more stable than their DNA/RNA-DNA/RNA counterparts. Due to the flexibility of PNA it is more able to conformationally adapt to the more rigid DNA or RNA.[24] It is this lack of conformational and configurational restraints that has led to the postulation that the binding occurs in an orientation dependent manner and PNA can bind to DNA from both the 3' and 5' ends of the DNA.[32]

One variation in the PNA backbone that has been tried is that of placing a methyl group on the nitrogen in order to test the structure-activity relationships. With the methyl group on the second nitrogen there is no possibility of intraresidue hydrogen bonding and this changes the hydrophilicity and hydration properties. It was demonstrated that PNA duplexes can accommodate up to ~30% of N-methyl substitutions in the backbone without a major loss in stability.[36]

Conformational heterogeneity between chains can arise due to alternative conformations possessing similar energies. This is where constraints on the backbone become necessary to form homogeneity between conformations.

PNA Structure

PNAs have been looked at in a number of different variations to try to solve the problems of solubility and ambiguity, mainly involving a different range of molecules in the backbone chain of the PNA. Investigations by a number of groups[32,37,38,39] have shown that PNA/DNA binding can be enhanced or controlled by:

1) Inserting some kind of fixed chirality into the molecule. The chirality of the chain is dependent on the configuration of the stereogenic centre on the amino acid, in naturally occurring amino acids this is the L-configuration.

2) Having some kind of charge in either the side chain or the main chain. The type of charge can help the solubility of the PNA molecule and is in line with the relatively low solubility of some uncharged PNAs.[40]

3) The pH of the environment the of the PNA strand. The binding rate is fastest in a slightly acidic environment with specificity being at its greatest around neutral and in some cases slightly basic pH.

4) The electrostatic and ionic strengths of the PNA. The molecular dynamics of the PNA/DNA system have been studied and have shown stability in the DNA/PNA duplex[41] which does not always contain a β-helix structure. This is most likely to be due to the electrostatically different backbone in PNA. It was also noted in this publication that

PNA/PNA duplexes did maintain a stable and well-defined double helix formation, showing the base pairings themselves are sufficient to form a stable double helix structure.

5) The temperature of the system.

6) The hydrogen - bonding of the system.

7) The rigidity of the backbone itself (Figure 1.22).[42]

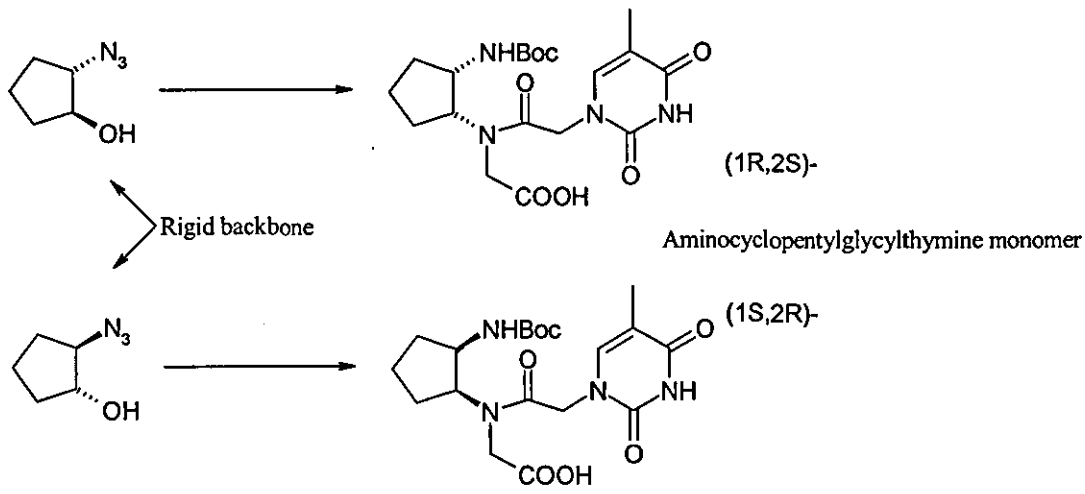


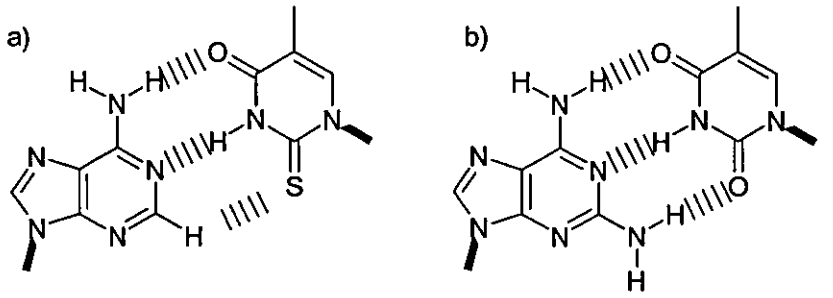
Figure 1.22 Formation of monomer for PNA containing a rigid backbone

8) The concentration of PNA. It has also been noted that for the formation of PNA/DNA the best rate is obtained by using two PNA molecules for every one DNA.[39] It would clearly be better if this could be decreased for financial and product formation reasons.

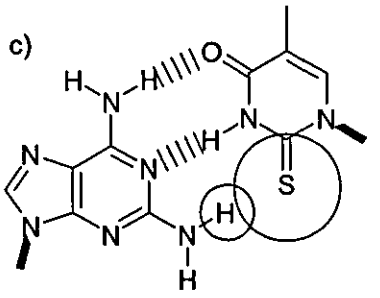
Structural Variations

As far as variants in the main chain is concerned it has been found that α-PNAs have shown better solubility than some other earlier PNAs, so further investigation into this is justified. Previous investigations into PNA/DNA interactions[43,44,45] have also involved, not

just the five naturally occurring nucleobases, but variations on a theme, such as PNA containing 2,6-diaminopurines and 2-thiouracils (Figure 1.23).



Interaction of a) Adenine and Thiouracil and b) Diaminopurine and Uracil



Interaction showing c) the steric clash of the Diaminopurine and Thiouracil

Figure 1.23 Pseudocomplementary PNAs[43,44,45]

These specific PNAs have been given the name pseudocomplementary PNAs and have been shown to work in a similar way to PNA and in some cases the pseudo-Watson-Crick pairings have greater strength than the natural pairings. These investigations have been successful for duplex-DNA invasion under low ionic concentrations, in cells however the ionic concentration will be greater and hence could potentially cause a problem for use in 'real life', as investigations have shown that PNA strand invasion is inhibited by NaCl.[32]

1.6.2 Formation of PNA Monomers

PNA type I monomers have been made in a number of different ways, depending on the type of PNA that is being made.

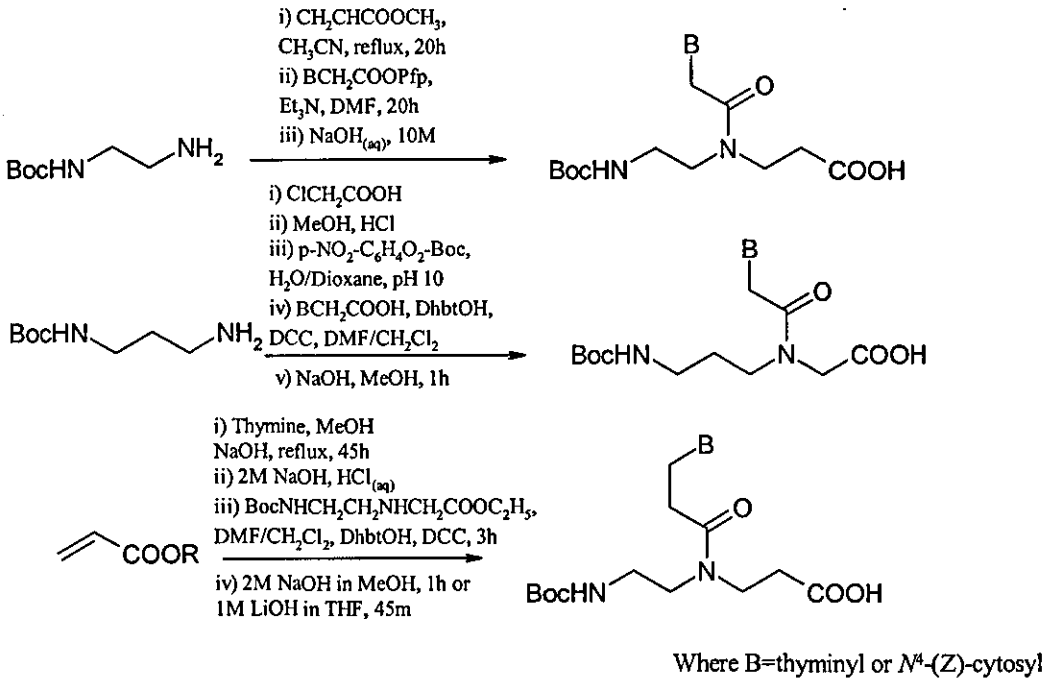


Figure 1.24 Methods of making PNA type I monomers[46]

In Figure 1.24 three different ideas for PNA are shown, the first is that of an extra carbon in what will become the backbone of PNA, the second contains two additional carbons and the third contains an additional carbon within the side chain. These have been investigated in the formation of a DNA duplex, but it was found that the original PNA formed the best duplex, with this area still being open to further investigation.[46]

A lot of the methods of synthesis are derived from pyrimidine and purine alkylation chemistry.

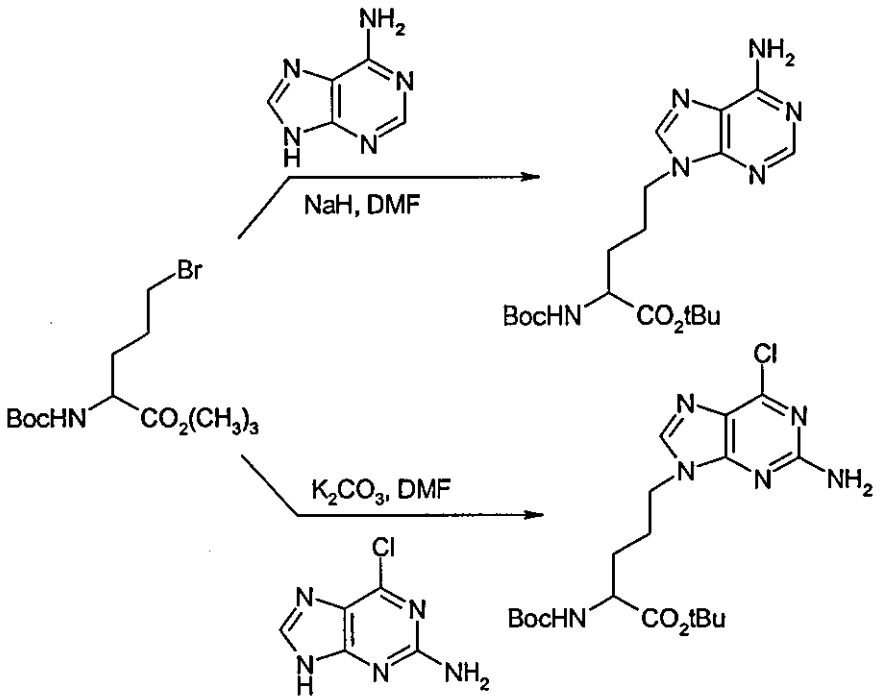


Figure 1.25 Method of making PNA type II monomer

One method for monomers used for PNA type II involved the use of halogens on the amino acid side chains for linkage to the heterocycles as shown in Figure 1.25. This will be discussed further in Chapter 2.

In the investigation described in this thesis linkage of the heterocycle to the amino acid will be looked at via a heteroatom as well as an all carbon chain. One method previously reported where the linkage chains contain a heteroatom is the use of iodine-promoted nucleosidation between an O-methylthiomethyl ether of serine and a heterocycle,[47] as shown in Figure 1.26.

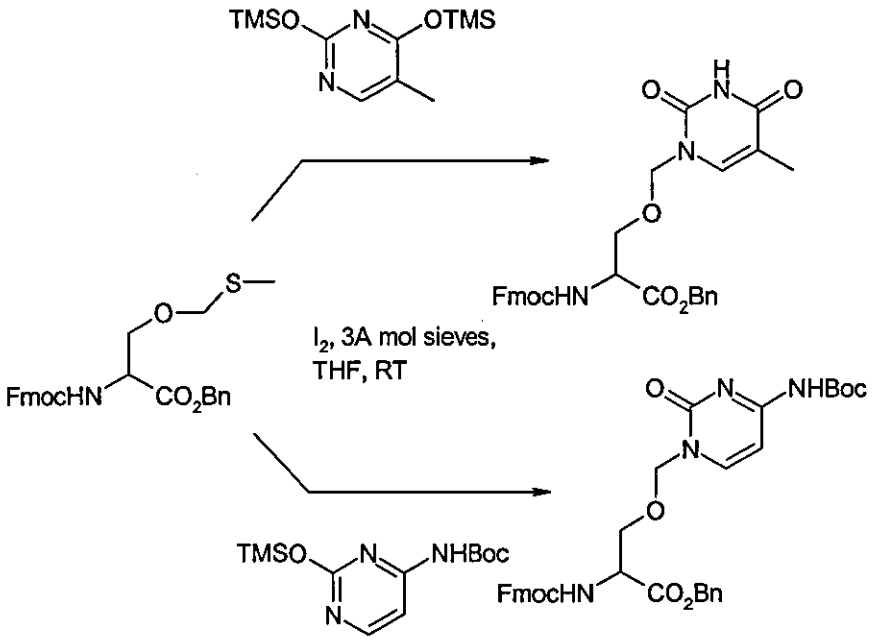


Figure 1.26 Iodine Promoted Reaction Scheme[47]

1.7 Heterocyclic Amino Acid Synthesis

There are various potential methods of forming heterocyclic amino acids and the methods used in this investigation have been detailed in the Results and Discussion section of this report. Methods for the formation of a C-C bond between the amino acid and the heterocycle can include: a glycine enolate approach; a radical approach; or a Mitsunobu approach.[48,49,50] Other methods involving the formation of the heterocylic ring whilst on the amino acid are also viable (e.g. Diels-alder reactions, etc) but these have not been studied in this investigation. It is also possible to use a heteroatom within the chain joining the heterocycle and the amino acid.

The same basic structure for a heterocyclic amino acid has been the target for each of the bonding methods used, and this is shown below in Figure 1.27:

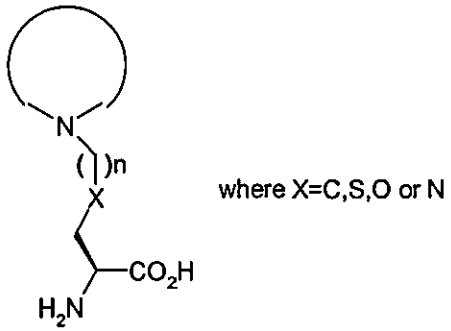


Figure 1.27 Heterocyclic Amino Acid

1.8 Protection of the Amino Acid

Before any reaction involving the bringing together of the amino acid and the heterocycle can take place there must be protection of some of the vulnerable groups.

In this investigation the proteinogenic amino acids serine, cysteine and homocysteine, and the unnatural amino acids 2,3-diaminopropionic acid and 2,4-diaminobutyric acid have been used, and hence have required protection from any undesired side reactions. A selection of possible protection methods is given below.

α-Amino Protection:[51,52]

Alkoxycarbonyl protection - amino groups are easily converted to alkoxycarbonylamino derivatives:

Benzyloxycarbonyl (Z, $C_6H_5CH_2OCO$-) – removed under acidic conditions;

t-Butoxycarbonyl (Boc, $C(CH_3)_3)OCO$-) – easy to add using base, easily removed by TFA;

2-(4-Biphenyl)isopropoxycarbonyl (Bpoc, $(C_6H_5)_2CHCH(CH_3)OCO$-) - more acidic, can be removed by catalytic hydrolysis;

9-Fluorenylmethoxycarbonyl (Fmoc, $C_{13}H_8CH_2OCO$-) - cleaved by piperidine/DMF;

Triphenylmethyl (Trt, $(C_6H_5)_3CO$-) - direct tritylation of amino acids using the chloride;

2-Nitrophenylsulphenyl (Nps, $C_6H_5(NO_2)S$-).

α-Carboxy Protection:[51,52]

Esterification

Methyl and ethyl esters - not affected by HBr, AcOH, TFA, catalytic hydrogenolysis, thiols, amines or organic solvents, can be removed by saponification or hydrazinolysis;

Benzyl esters - formed from 4-toluenesulfonic acid and benzyl alcohol, easier to cleave due to steric factors and conjugation;

t-Butyl esters - stable in free base form, inert to electrophilic and nucleophilic attack;

Phenyl esters - useful where acid sensitive groups are present, removed by catalytic hydrogenolysis.

For the investigations involving a carbon-carbon linkage, the protection of the two moieties was facilitated in one case by the formation of an oxazolidinone system as shown in Figure 1.28.

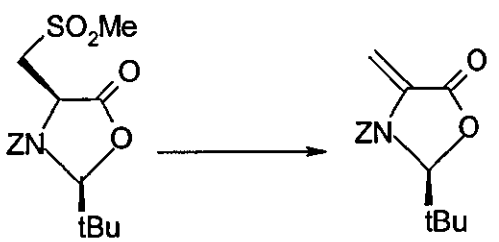


Figure 1.28 Oxazolidinone Protection of Amino Acid

This involved an internal esterification to protect the carbonyl group and benzyl chloroformate to protect the amino moiety by forming an alkoxycarbonyl.

In other cases involving a carbon-heteroatom linage, protection was by Boc protection of the amino group and simple ethyl ester protection of the acid as shown in Figure 1.29.

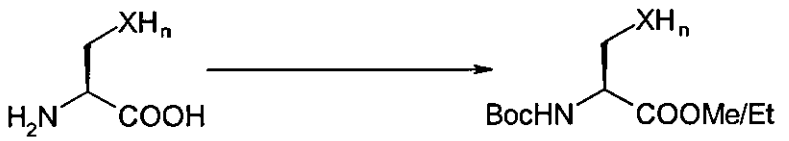


Figure 1.29 Boc/Ester Protection of Amino Acid

The actual procedures used and the reasoning behind them are explained in detail in the Results and Discussion and the Experimental sections of this thesis.

The amino acid is not the only compound that needs to be protected. In some cases the heterocycles also need protection as some had more than one reactive centre (in this investigation this was a nitrogen). This other reactive centre was either protected by Boc or a benzoyl group. The reaction to attach a haloalkyl group to the heterocycle (to become the linkage in the side chain) was done under Mitsunobu conditions. Further details of this are discussed and detailed in the Results and Discussion and the Experimental sections.

2 Results and Discussion

2.1 Heterocyclic Amino Acid Formation

This project has been to investigate heterocyclic amino acid formation by forming a C-X bond (where X= C, S, O, N) in the chain linking the amino acid backbone to the intact heterocycle. The linkage strategies are introduced below.

There are many methods that could be used to form heterocyclic amino acids by this strategy, for example: radical or organocuprate conjugate addition, hydroboration or metathesis coupling, substitution and Mitsunobu reactions or glycine enolate approaches. These are all alternatives to building up the heterocyclic ring from a functionalised amino acid side chain.

2.1.1 The Formation of Heterocyclic Amino Acids

There are a number of ways in which heterocyclic amino acids have been made in the past and some methods have involved heterocyclic ring formation on the amino acid side chain (e.g. Diels-Alder reactions, etc). These methods have not been covered in this thesis as it was decided that the research would focus on methods of forming the linkage between a preformed heterocycle and the amino acid units. Previous research into this area includes a glycine enolate approach, the Mitsunobu approach[53] and a radical approach.

2.1.1.1 Glycine Enolate Approach

This approach uses specifically glycine as the amino acid component of the heterocyclic amino acid and the heterocycle adds with the complete side chain.

The use of a glycine enolate has been explored previously by Seebach and Fritzi,[54,55] shown below in Figure 2.1a, and by Myers *et al.*[56,57] and in our group by Berthelot and Jones[58] with a pseudoephedrine-based method as shown in Figure 2.1b.

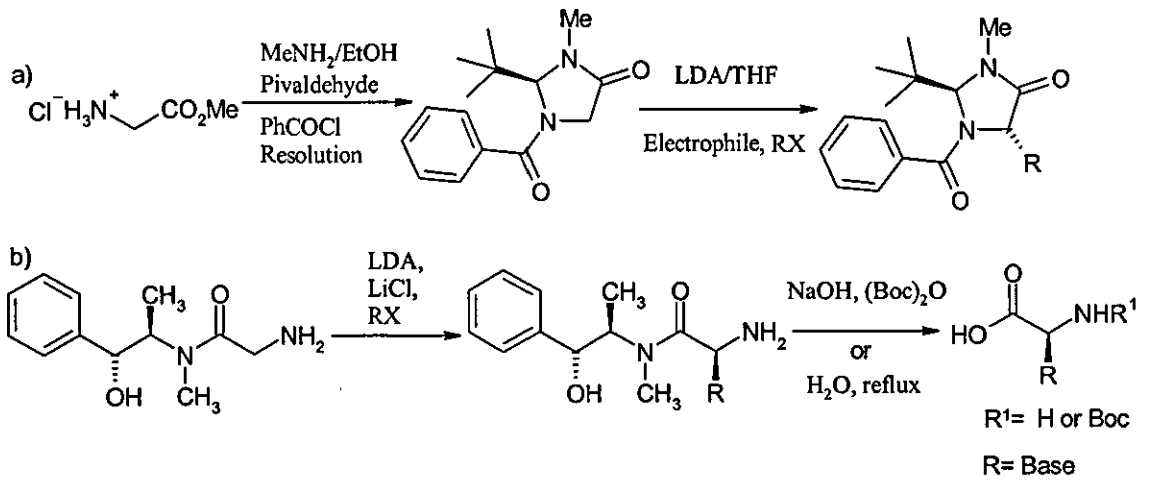


Figure 2.1a and b Alkylation of enolate

The drawback with this method, so far investigated, is the poor e.e. values and due to competition between substitution and elimination in haloalkylnucleobases under strongly basic conditions, amino acids with nucleobases in the side chain could not be formed.

2.1.1.2 Mitsunobu Approach

This approach is to the opposite extreme and links an amino acid with a complete side chain to the heterocycle. Serine (or another amino acid containing an alcohol group in the side chain) is reacted with a pyrimidine/purine base under Mitsunobu conditions to give the corresponding heterocyclic amino acid. This has been reported by Kuwahara[50,59] and Altmann *et al.*[48,60] for serine-based derivatives used in two different PNA structures as in Figures 2.2 and 2.3.

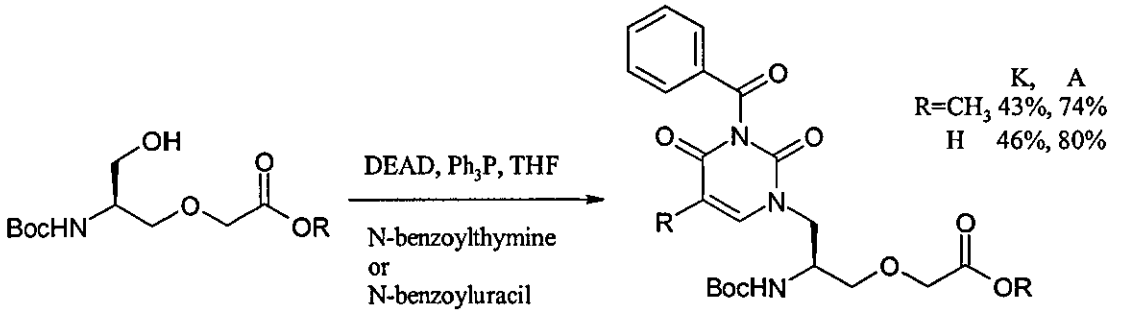


Figure 2.2 Use of Mitsunobu reaction in the formation of heterocyclic amino acids

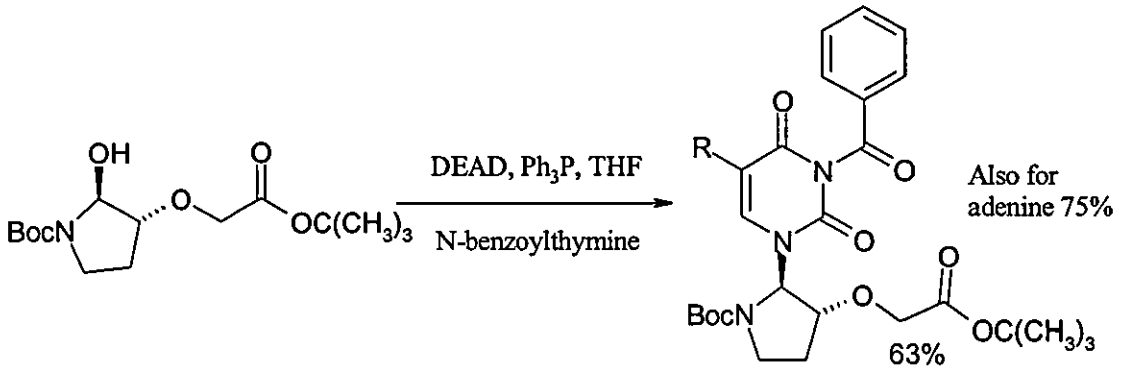


Figure 2.3 Other PNA preparations

One limitation with the Mitsunobu approach is the availability of suitable hydroxyl-functionalised side chains, with serine as the usual amino acid source for these reactions.

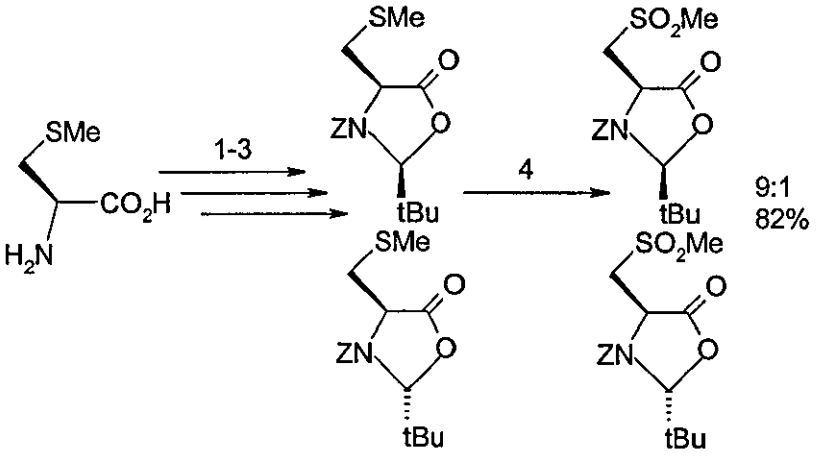
2.2 Heterocyclic Amino Acids for this Investigation

After looking at previous research, it was decided to focus on bond forming methodology where part of the side chain is situated on the amino acid moiety and part on the heterocycle.

For ease of explanation the methods investigated have been split into two separate groups: C-C bond formation and C-S/O/N bond formation.

2.2.1 Carbon-Carbon Bond Formation

The first step for this research was to synthesise a chiral amino acid template suitable for a range of carbon-carbon bond forming reactions. An oxazolidinone as a substrate for conjugate addition and various coupling procedures was selected, its synthesis being shown in Figures 2.4 and 2.5.[58]



1. NaOH, 5 min
2. ^{t}BuCHO, Hexane, Reflux, 24 h
3. Benzyl chloroformate, DCM, 0°C / RT, 36 h
   then $NaHCO_3$(aq), 4 h
4. Acetonitrile, Oxone in Water

Figure 2.4 (2S,4R)-3-Benzyloxycarbonyl-2-tert-butyl-4-methyleneoxazolidin-5-one and (2R, 4R) isomer

The amino acid derivative (R)-S-methylcysteine was stirred with sodium hydroxide solution to give the corresponding carboxylate salt. After evaporating to dryness, the salt was suspended in hexane with 2,2-dimethylpropanal and refluxed with Dean-Stark water removal to form a Schiff base at the amino moiety. The Schiff base then underwent cyclisation promoted by addition of benzyl chloroformate to acylate the imine nitrogen atom, hence protecting both the amino and acid moieties of the amino acid. This protection resulted in the formation of two diastereoisomers that could be distinguished by NMR spectroscopy but were difficult to separate using column chromatography. Both isomers were cleaned of excess benzyl chloroformate using an extraction procedure and then oxidised to the sulfones. Oxidation was achieved by adding an aqueous solution of Oxone® to a solution of the diastereoisomers in acetonitrile. Problems initially occurred at this stage, possibly due to the presence of residual benzyl alcohol formed from the benzyl chloroformate. This was resolved by removing the alcohol under vacuum at a raised

temperature for a longer time than previously thought necessary. The sulfone diastereoisomers were easier to separate at this stage using column chromatography on silica gel to give the major *syn*-isomer as a pale yellow liquid. This was not easy to crystallise using solvents, but it was serendipitously discovered that crystals were formed by leaving the product in the refrigerator for a few nights after removal of the solvents. The ratio of the two diastereoisomers could be determined by measuring the integrals of the SO_2Me signals in the ^1H NMR spectrum, which occurred at δ 2.87ppm for the minor *anti* isomer and δ 3.11ppm for the major *syn* isomer and by comparison of the masses of the diastereoisomers after column chromatography (the isomers being shown in Figure 2.4). The two isomers were distinguished by running n.O.e's of the pure isomers at the H-2 and H-4 frequencies. The ratio of the two isomers was found to be around 9:1 (*syn:anti*).

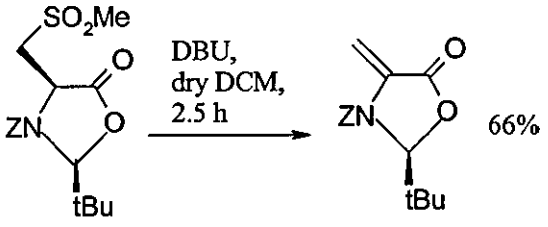


Figure 2.5 Oxazolidinone template

The elimination of the sulfone moiety to generate the desired double bond, needed for the proposed carbon-carbon bond forming reactions, was instigated using the strong hindered base, DBU, which was added dropwise to a solution of the *syn*-sulfone in dry DCM. This gave a relatively clean product, shown in Figure 2.5, which was further purified by filtering through a short column of silica gel. It was clear that the elimination reaction had taken place due to the disappearance of the proton NMR signal at δ 3.11ppm (SO_2Me protons).

Again the product proved difficult to crystallise, but the clear liquid formed clear crystals of (2S)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-methyleneoxazolidin-5-one (the oxazolidinone template) when it was left in the refrigerator for a few nights. The overall yield, based on the starting S-methylcysteine derivative, for this sequence of reactions, was good at 54%.

Use of Oxazolidinone Template

As explained briefly in the introduction, the linkage between the amino acid and the heterocycle can contain only carbons or a heteroatom and the oxazolidinone was used for the all-carbon approaches.

There are many different methods that may be used for C-C bond formation, and several of these have been looked at in this investigation. Each method is introduced before the discussion of the results for each method tried.

2.3 Radical Reactions

The first step in a radical chain reaction is always that of initiation, i.e. the formation of the radical for use in the reaction. There several ways in which a radical reaction can be initiated for use in synthesis.[51,61]

The first (and most common) is by thermolysis. Several compounds containing weak bonds can be broken down using heat to form a free radical initiator. Three main ones are di-t-butyl peroxide at 140°C, azobisisobutyronitrite (AIBN) at 90°C and t-butyl hyponitrite at 50°C. The initiator radical performs an atom abstraction on the reaction substrate to generate a chain carrier radical.

The second way to initiate a radical reaction is by photolysis. UV or visible light carries enough energy to break some bonds, with the type of bond in question determining the amount of energy that the light must have to break it.

Another way is by radiolysis. High energy electromagnetic radiation with α and β particles can promote a chemical change by ionisation. This method is often too energetic for selective synthesis.

Once a radical reaction has been initiated, the carrier radicals in the reaction can also react in different ways.[51,61] The proposal on this occasion is conjugate addition to the oxazolidinone template followed by H abstraction to continue the chain reaction, to give the addition product as in Figure 2.6.

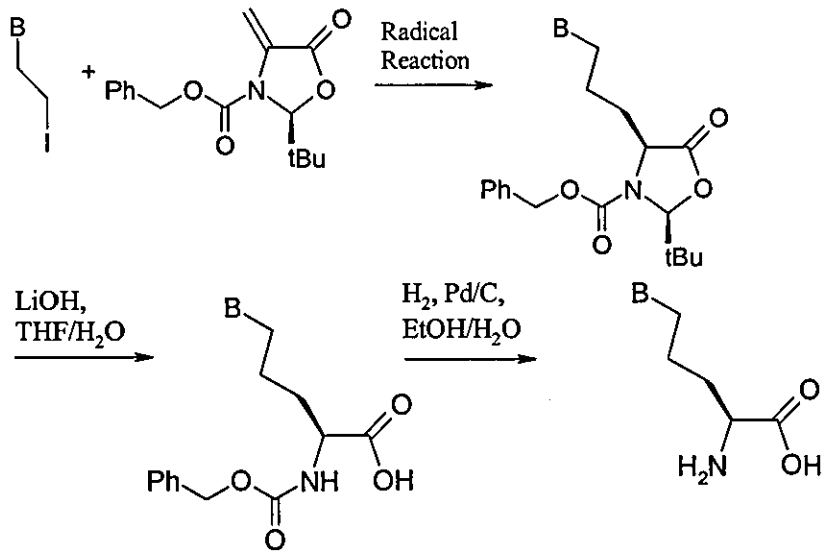


Figure 2.6 The planned reaction pathway for the formation of heterocyclic amino acids via radical conjugate addition

For this investigation the radical procedure was carried out in a similar way for each reaction. This involved the use of AIBN to form a radical by thermolysis which in turn

goes on to form a tributyl tin radical from Bu_3SnH, either added directly or formed in situ from Bu_3SnCl and $NaCNBH_3$.[62]

The proposed full pathway to amino acids is shown in Figure 2.6, with the addition product then being deprotected to give the heterocyclic amino acid.

For the radical reactions it was necessary to have a good leaving group, a halogen (e.g. iodine), at the position the radical formation was required, therefore iodoalkyl derivatives of the heterocycles were the most desirable. The simplest method available was that of Mitsunobu coupling to form the bromoalkyl derivative using a bromoalcohol, followed by halide exchange. The iodide formation could not be performed directly as the desired iodoalcohols were not readily available.

2.3.1 N-iodoalkyl derivatives

N3-Benzoyluracil

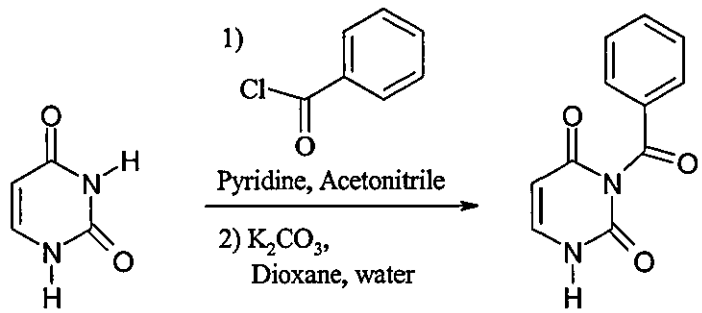


Figure 2.7 Benzoyl Protection of Uracil

The first heterocycle to be derivatised for addition to the oxazolidinone template by a radical reaction was uracil. Uracil contains two nitrogen atoms in the molecule which are

open to alkylation by the Mitsunobu method. The nitrogen atom at the N1 position is slightly more reactive than that at the N3 position, but since this difference is small it was necessary to protect at the N3 position (Figure 2.7) to only allow the Mitsunobu reaction to occur at the N1 position and prevent any undesired side reactions.

Uracil was suspended in a solution of acetonitrile/pyridine (5:1 v/v) with benzoyl chloride being added dropwise at 0°C due to the exothermic reaction. After 2 h the suspension had disappeared to give an orange solution. This was checked by ^{1}H NMR spectroscopy, which showed a change from 2 NH peaks in the δ10.5ppm region to a single peak, and by mass spectrometry and confirmed to be the N1-benzoyluracil, so the solution was left to stir overnight to form N1,N3-dibenzoyluracil. This was again confirmed by ^{1}H NMR spectroscopy which now showed no NH peaks in the δ 10.5ppm region. The dibenzoylated compound proved difficult to separate from the pyridine solvent even after extensive use of the rotary evaporator under reduced pressure. DCM and water were added to the 'slush' which produced a white suspension that was filtered off to give the dibenzoylated product as the solid component.

The N1 benzoyl group was preferentially removed by hydrolysis using potassium carbonate in water/dioxane (1:1 v/v) to give the desired N3-benzoyluracil product in 72% yield (Figure 2.7).

N3-Benzoyl-N1-(2-bromoethyl)uracil

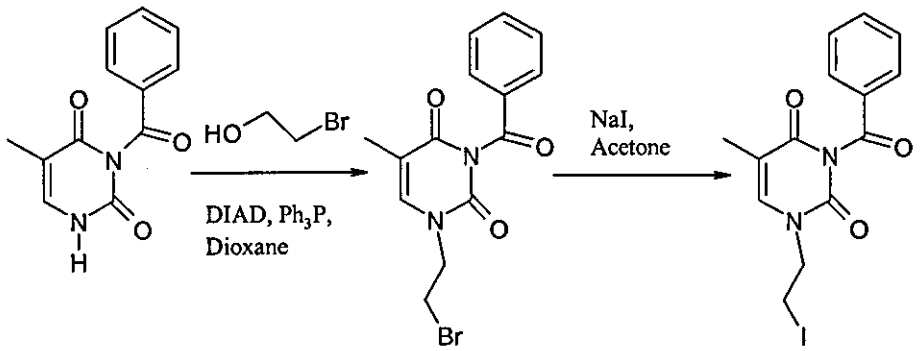


Figure 2.8 Bromoethyluracil

For the first reaction to form a haloalkylheterocycle, it was decided to derivatise the protected uracil with a haloethyl chain. A bromoethyl group was added to N3-benzoyluracil at the N1 position under Mitsunobu conditions, as shown in Figure 2.8.

N3-Benzoyluracil, 2-bromoethanol and triphenylphosphine (TPP) were stirred in dry dioxane with DIAD added dropwise over 1 h at ~5°C, just above the freezing temperature of dioxane. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure and the desired N1-alkylated product was purified using column chromatography. The first attempt gave a low yield of 27% and this was attributed to the solvent not being sufficiently dry. Indeed a second attempt with more stringently dried dioxane gave a yield of 95% for the desired product, N3-benzoyl-N1-(2-bromoethyl)uracil, and further reactions after this gave reproducible yields in the high 80's at least.

N3-benzoylated-N1-(2-iodoethyl) uracil

To form the iodoethyl derivative rather than the bromoethyl derivative a simple halogen exchange is needed and the so-called Finkelstein reaction was utilised, which takes advantage of NaI being soluble in acetone whereas NaBr is less soluble. The halogen exchange from the bromo to the iodoalkyl was orchestrated by reluxing the bromoalkyl derivative in dry acetone with dry NaI. This gave the desired N3-benzoyl-N1-(2-iodoethyl)uracil in a yield of 71% (Figure 2.8). For this reaction it proved very important to ensure that the solvent was completely dry or else the yields were severely reduced, as in the first attempt which gave a yield of 36% where the acetone was only distilled once. For all the Finkelstein reactions after this the acetone solvent was either distilled twice, or anhydrous acetone bought fresh from the suppliers was used and either of these gave the higher yields of around 71%.

N1-(2-Bromoethyl)benzimidazole

The N-alkylation reactions were also performed using benzimidazole as this heterocycle has a similar structure to purines but does not need protection.

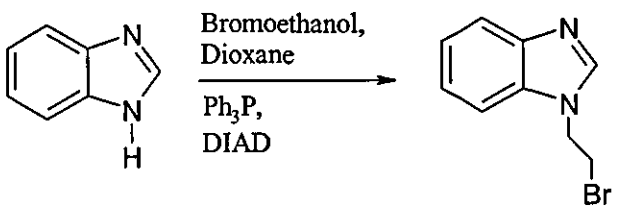


Figure 2.9 N1-(2-Bromoethyl)benzimidazole

Although the uracil derivative is useful for looking at the reactions where a natural heterocycle is present, it was decided that it would be easier for the initial test reactions for coupling to an amino acid, to use a heterocycle that does not need protection, but has a similar structure, such as benzimidazole. Any methods that prove fruitful for the benzimidazole derivative could also be used for the uracil and the other naturally occurring heterocycles.

Benzimidazole was bromoalkylated under Mitsunobu conditions, shown in Figure 2.9. The benzimidazole, 2-bromoethanol and TPP were stirred in dry dioxane with DIAD being added dropwise over 1 h. After being left to stir at room temperature overnight the solvent was removed under reduced pressure. The crude product was purified by column chromatography to give N1-(2-bromoethyl)benzimidazole with a yield of 98%.

N1-(2-Iodoethyl)benzimidazole

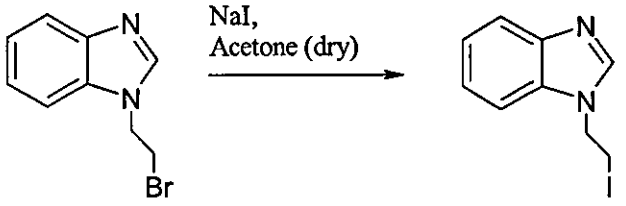


Figure 2.10 N1-(2-Iodoethyl)benzimidazole

The Finkelstein halogen exchange reaction was performed with doubly distilled dry acetone and the reaction was performed in the same manner as previously. The N1-(2-bromoethyl)benzimidazole and NaI were refluxed in dry acetone, which gave N1-(2-iodoethyl)benzimidazole in 75% yield, as shown in Figure 2.10. The product was clearly identified using mass spectrometry, with the increased mass for the iodo compound and the

two mass peaks for the two isotopes at ~272 and the second at ~273, and by ^{1}H NMR spectroscopy with the shift of the signal for the CH_2 protons adjacent to the halogen further upfield by about 0.5ppm. The yield was however lower than expected even though the acetone was distilled twice and the NaI dried in the oven before use, but the yield was not so significantly decreased as to be synthetically unusable.

2.3.2 Radical Conjugate Addition of Oxazolidinone and N-Haloalkyl heterocycles

Radical addition of Uracil Derivative and Oxazolidinone Template

An outline of the reaction scheme for the conjugate addition of the haloalkyluracil to the oxazolidinone template is shown in Figure 2.11.

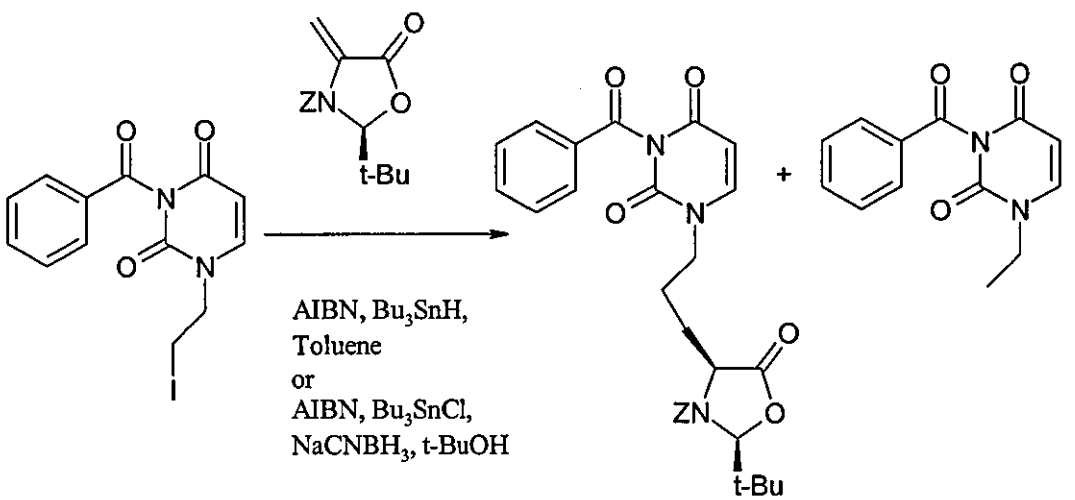


Figure 2.11 Radical Reaction: Uracil

Using Tributyltin Hydride

The oxazolidinone template, N3-benzoyl-N1-(2-iodoethyl)uracil and AIBN in toluene were heated at 80°C under nitrogen with tributyltin hydride added dropwise (Figure 2.11). The mixture was then refluxed overnight, however, no addition product was isolated. The reduced product, N3-benzoyl-N1-ethyluracil, was present, as seen by ^{1}H NMR spectroscopy, but could not be isolated. The presence of the reduced product indicated that the desired radical had been formed, but had gone on to perform hydrogen abstraction rather than conjugate addition.

Using Tributyltin Chloride

The oxazolidinone template and N3-benzoyl-N1-(2-iodoethyl)uracil, AIBN, sodium cyanoborohydride and tributyltin chloride were refluxed in tert-butanol under a positive atmosphere of nitrogen (Figure 2.11). The mixture was refluxed overnight then separated by column chromatography. It was necessary to separate without any confirmation of any addition product being present because the large number of peaks in the ^{1}H NMR spectrum made it impossible to analyse, a major source of these peaks being the tributyltin-containing components of the reaction. The presence of the organotin compounds also caused problems with the column chromatography of the mixture due to the tendency of the tin compounds to streak, meaning a second column was always necessary. The fractions collected may have contained a trace of the desired product but even if it was present it was inseparable even on several attempts at the reaction and separations.

Radical addition of Benzimidazole Derivative and the Oxazolidinone Template

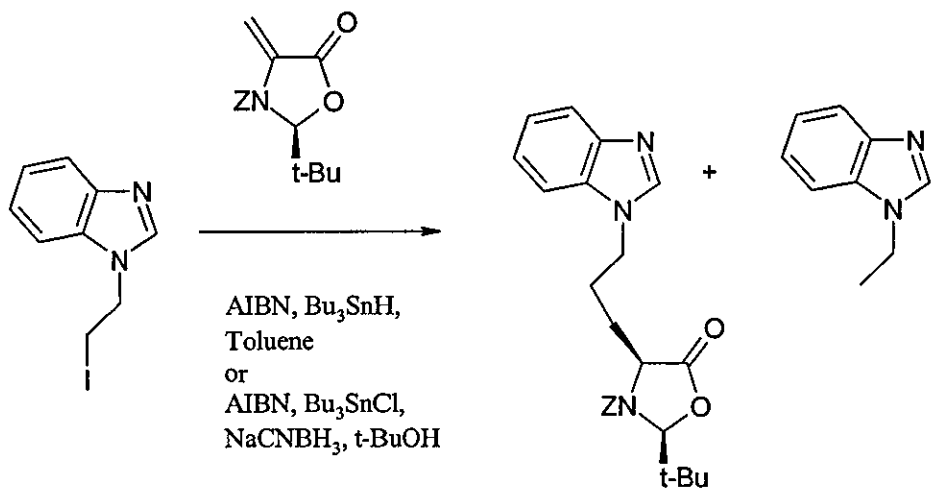


Figure 2.12 Radical reaction: Benzimidazole

Using Tributyltin Chloride

The tributyltin chloride method was tried first, as the chloride is a lot easier to use than the hydride. The N1-(2-iodoethyl)benzimidazole, oxazolidinone template, AIBN, sodium cyanoborohydride and tributyltin chloride were refluxed under nitrogen in tert-butanol for up to 48 h (Figure 2.12). Two different protocols were tried, either that of adding AIBN only at the start or adding it in portions every hour for up to 6 h.

Neither method produced any of the desired product but both the reactions produced greater than 40% of the reduced product N1-ethylbenzimidazole, both being assessed after 24 h and again after 48 h.

Using Tributyltin Hydride

N1-(2-Bromoethyl)benzimidazole, the oxazolidinone and AIBN were heated under a positive atmosphere of nitrogen in toluene after the dropwise addition of tributyltin hydride (Figure 2.12). After 24 h and 48 h the ^{1}H NMR spectra were again difficult to interpret so no conclusions were possible until the mixture had been separated by column chromatography twice.

There was a large amount of reduced product (Figure 2.12), 45% after 24 h and 46% after 48 h, and there was the possibility of a trace of the desired product but if so it proved impossible to separate, especially from the organotin residues, and hence this was not a viable reaction. The results are shown in Table 2.1.

Uracil / Benzimidazole	Tin Hydride or Chloride	AIBN	Time of Assessment (h)	Yield Reduced
Uracil	Hydride	At Start Only	24	Trace
			48	Trace
	Chloride		24	57%
			48	52%
Benzimidazole	Chloride	At Start Only	24	44%
			48	48%
		Every hour for 6 h	24	42%
			48	43%
	Hydride	At Start Only	24	45%
			48	46%

Table 2.1 Oxazolidinone with Benzimidazole

As mentioned previously the presence of the reduced product as the major product in the majority of the reactions shows that a radical is almost definitely being formed, but this is not leading to the desired conjugate addition product.

Radical reaction using a greater excess of the oxazolidinone template

The radical reactions described above used 5 equivalents of the oxazolidinone template and these had resulted in none of the desired product, so it was suggested that if a greater excess of the oxazolidinone was present then the ethylheterocycle radical would stand a greater chance of reaction with the oxazolidinone than of picking up a hydrogen atom.

N1-(2-Iodoethyl)benzimidazole and AIBN were stirred with up to 20 equivalents of the oxazolidinone template for up to 48 h (Figure 2.12). After the necessary two column chromatographic separations there was none of the desired conjugate addition product present for any of the experiments up to 20 equivalents, there was however still the presence of the reduced product in approximately the same quantities as for the previous reactions. The oxazolidinone starting material was also recovered, but the organotin compounds could not be fully separated from this so it could not be used again for any further reactions and the actual recovery could not be calculated precisely, but has been estimated to be quantitative.

Reaction using only the Sodium Cyanoborohydride

It was thought that the sodium cyanoborohydride may be causing the problem in the tributyltin chloride reactions as these reactions produced the most amount of reduced product in the uracil reactions.

To test this possibility the reaction was performed containing only the N1-(2-iodoethyl) benzimidazole and sodium cyanoborohydride, which were refluxed in tert-butanol under nitrogen for up to 48 h. After column chromatography it was seen that there had however been no reduction and only starting materials were obtained after the 48 h at reflux.

Radical reaction using the N1-(2-Bromoethyl)benzimidazole

It was also possible that the iodoethyl heterocycles could have been the cause of the problem, in that they may have been too reactive. The radical reaction was attempted with N1-(2-bromoethyl)benzimidazole, as in Figure 2.13, to see if this would react more slowly and hence be less likely to form the reduced product.

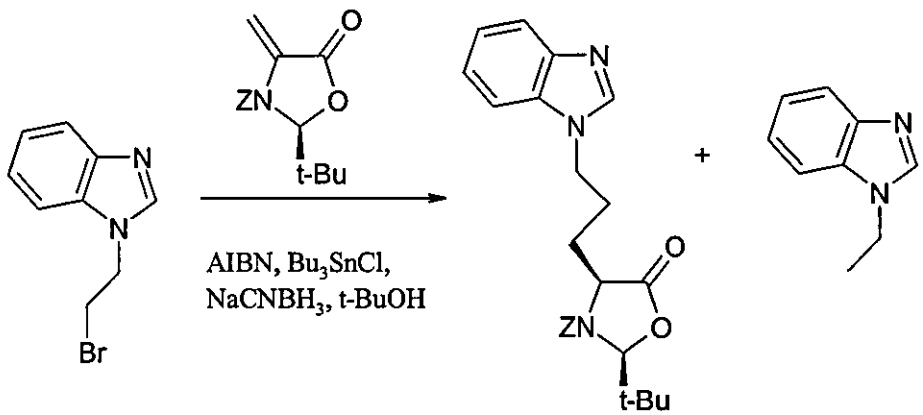


Figure 2.13 Radical reaction with N1-(2-Bromoethyl)benzimidazole

The same conditions were used as for the iodoethyl heterocycle reactions using the tributyltin chloride, as shown in Figure 2.13. This also proved fruitless as the bromoethylbenzimidazole was not reactive enough to even form the radical and only starting materials were recovered after 24 h of reaction time.

2.3.3 Summary of Radical Reactions

The radical reactions have produced none of the desired conjugate addition products. Although there have been signs that the radicals have been formed, they have picked up hydrogen atoms earlier in the reaction sequence than desired and only the reduced products have been seen.

2.4 Other Metal-Mediated Reactions

For both the hydroboration and cross-metathesis methods, to be discussed later, it was necessary to form an N-vinyl heterocycle.[63,64] This was easily achieved via the N1-(2-chloroethyl)benzimidazole.

Two attempts were made to form the chloroethyl precursor, the first was using a phase-transfer catalyst, the second using the Mitsunobu method. All of the following reactions were performed under nitrogen.

N1-(2-Chloroethyl)benzimidazole

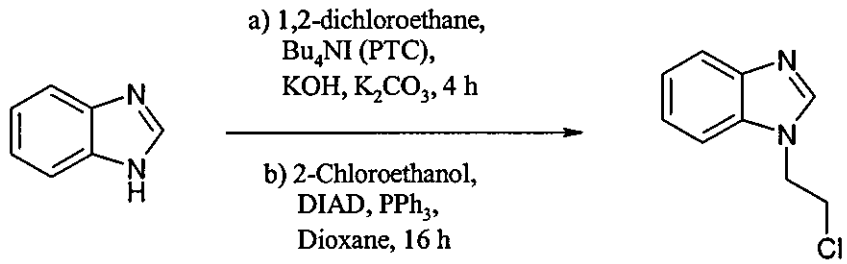


Figure 2.14 Formation of N1-(2-chloroethyl)benzimidazole

Phase-transfer catalyst method

Benzimidazole was added to a mixture of 1,2-dichloroethane, which acted as both the solvent and the reagent, tetrabutylammonium iodide as the phase transfer catalyst (PTC), KOH and K_2CO_3 (Figure 2.14). The reaction was stirred at room temperature for 4 h, so it does have the advantage of being a relatively fast reaction. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the desired product came off easily and cleanly but only gave a 54% yield.

Mitsunobu method

Since the Mitsunobu method had worked so well for the bromoalkyl derivatives of benzimidazole, this method was tried for the chloroalkyl derivatives. To a stirred solution of benzimidazole, 2-chloroethanol and triphenylphosphine in dioxane at 0°C was added DIAD slowly over 3 h (Figure 2.14). This was then allowed to warm to room temperature

and stirred for a further 16 h. After removing the solvent under reduced pressure the product was put through flash chromatography, from which it was separated quickly and relatively cleanly to give the desired product with a yield of 80%.

For both the above reactions it was necessary to ensure that the reactions had gone to completion (judged by ^{1}H NMR spectroscopy). Where the reaction had not gone to completion the products were difficult to separate from the starting materials.

Vinyl Benzimidazole

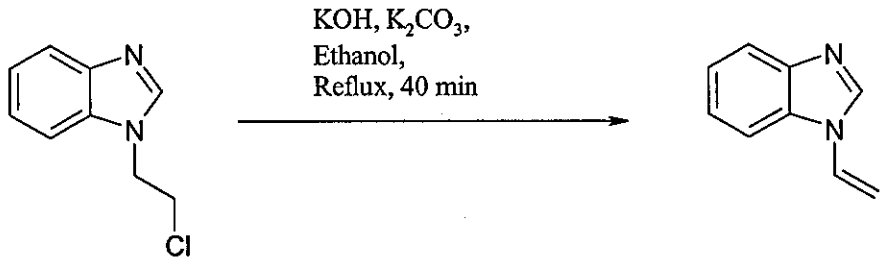


Figure 2.15 Formation of vinyl benzimidazole

N1-(2-Chloroethyl)benzimidazole was then used in the formation of N-vinyl benzimidazole, shown in Figure 2.15. The N1-(2-chloroethyl)benzimidazole, KOH and K_2CO_3 were stirred in ethanol at reflux for 40 min. After work-up the product was purified using flash chromatography, from which the vinyl benzimidazole eluted cleanly, with a very good yield of 97%.

2.4 Hydroboration

As an alternative to radical C-C formation, use of a boron linker was proposed, partly because boron is reportedly found in a large number of saturated and unsaturated heterocycles.[65] If a boron-containing reagent could be incorporated into the chain linking the heterocycle to the amino acid, this in itself would be a novel compound or, to form a C-C bond, the boron can be easily removed using a number of reactions. The proposed linkage is shown in Figure 2.16.

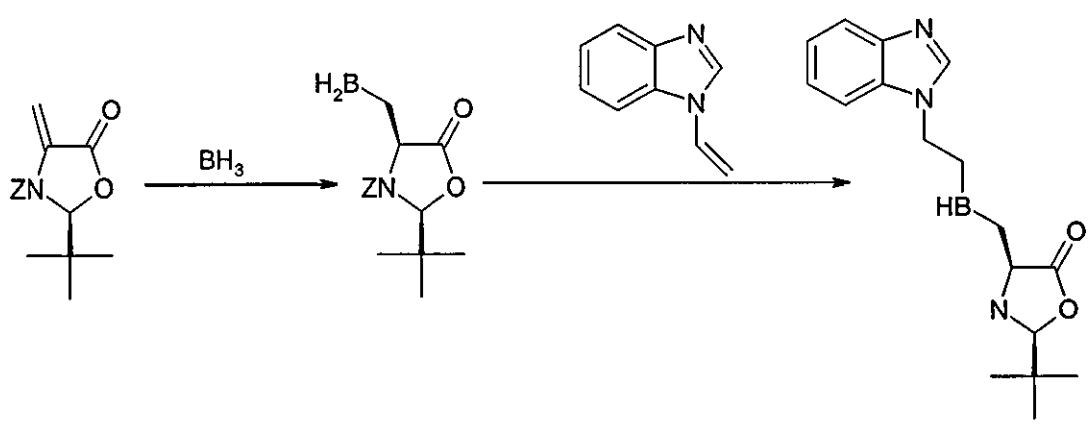


Figure 2.16 Planned Cross-Hydroboration of Oxazolidinone Template and Vinyl Benzimidazole to form a Linkage Containing Boron

Boron in Organic Reactions

Borane is a reducing agent and can add to C-C double bonds. In the past this has led to effective routes to functionalizing alkenes.[66] It is in this capacity that it has been proposed that a borane compound is used to form the boron-containing heterocyclic amino acid.

Complexes of the borane are useful as they provide the borane in its monomer form and allow measurement for 1:1 quantitative additions as well as making the molecule soluble in other solvents. These complexes are usually with diethyl ether, THF or methyl sulfide.[67]

There is facile addition of a B-H bond to the C=C bond. This is due to the cis-addition of BH via a four membered transition state,[67] and it is this process that is known as hydroboration. This is shown in Figure 2.17 below:

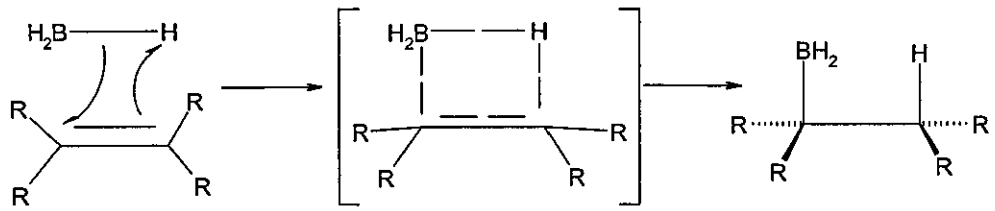


Figure 2.17 Hydroboration mechanism - cyclic

Hydroboration is best used for slightly hindered olefins where the boron is most likely to add regioselectively on one end of the double bond, the less hindered terminus;[68] anti-Markovnikov addition can usually be easily accomplished by the use of boron.[67] Obviously steric factors are a major influence but not the only one: delocalisation can cause the borane to go to the more hindered end and electronic factors can play a role in the cyclic transition state. Most double bonds will react with boranes, from mono- to tetra-substituted, although the more hindered the compound the fewer molecules can bond to boron. Steric factors also play an important part where the number and size of substituents on the boron are concerned.[69] For less substituted boron atoms the reactions can be done at lower temperatures,[67] this reduces the number of molecules bonding to a single boron atom.

There are a number of different ways to convert the organoborane into a different functional group.[67] For the present case, once the boron has been added, the removal of boron is envisaged to form a C-C bond between two different moieties (the protected amino acid and the heterocycle). To do this the methods that could be used are:

Coupling reaction - organoboranes + alkali + silver nitrate at 0°C, even in the best case, this reaction will only give a maximum yield of 50% due to side couplings.[67,70,71,72]

Use of a haloform anion (Figure 2.18) - Facile reaction with haloforms in presence of strong sterically demanding bases, this reaction does however extend the chain by one carbon.[67]

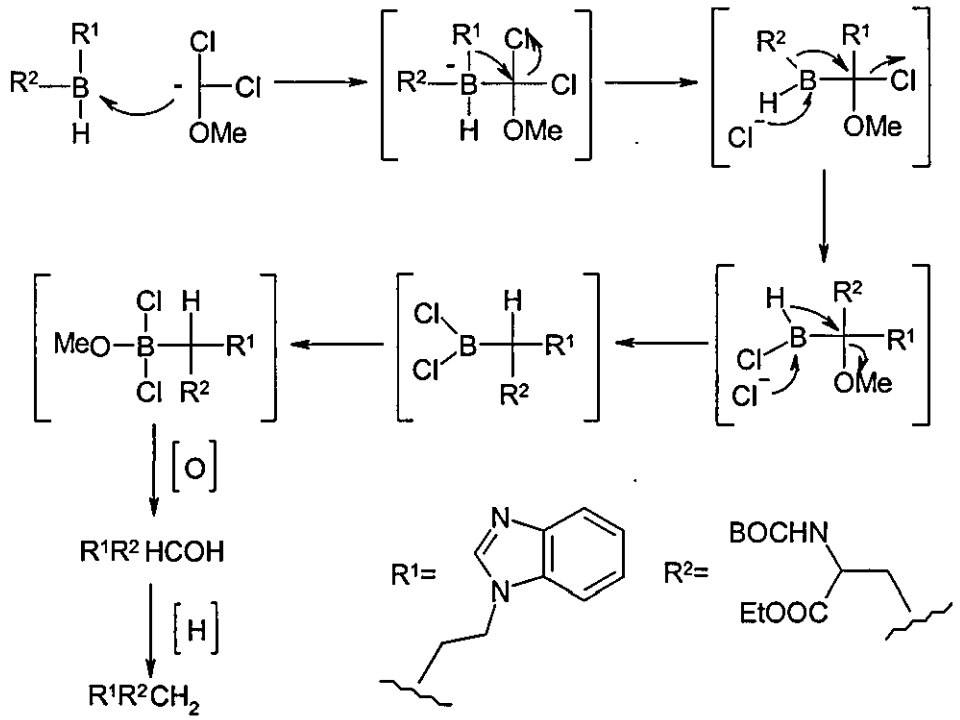


Figure 2.18 Proposed Removal of Boron to form Heterocyclic Amino Acid

Functional groups might cause a problem in hydroboration depending on the group in question but it has been noted by Brown[67] that ester groups are not in competition with the reduction of the double bond. It has also been shown by Sucrow *et al.* that even conjugated esters (of which there is one present in the oxazolidinone template) do also undergo hydroboration and do not cause a complication.[73] The proposed reaction scheme is shown in Figure 2.19.

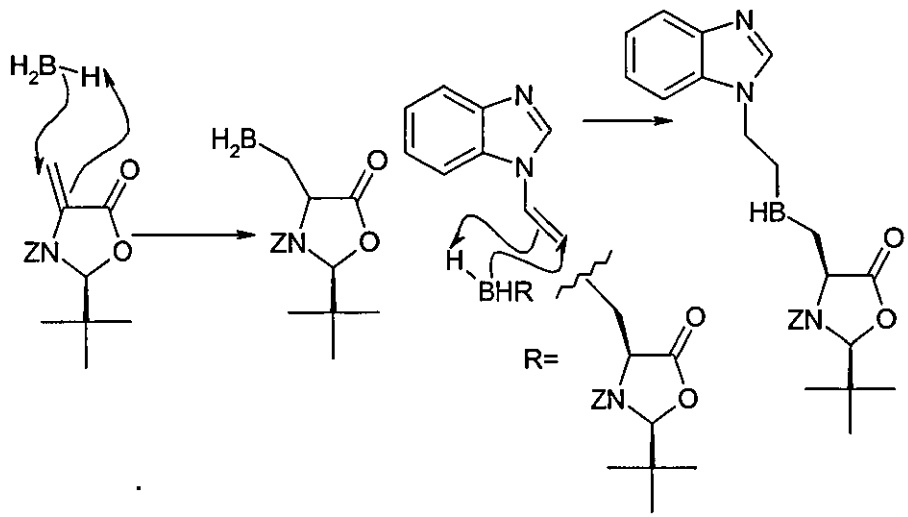


Figure 2.19 Proposed Reaction Scheme for Hydroboration of the Oxazolidinone Template and a Vinyl Heterocycle, in this case Vinylbenzimidazole

Advantages and Disadvantages

One major potential advantage of hydroboration is that the reactants for hydroboration are less toxic than the tin components often used in radical reactions. On the other hand N-vinyl benzimidazole looks like a classic reagent to undergo hydroboration, the oxazolidinone is however, more hindered and so could pose more problems. A syn-oxazolidinone is expected as hydroboration should occur at the least hindered face (Figure

2.18/2.19). The initial hydroboration reaction was planned on the oxazolidinone template to provide a relatively hindered borane that might only add to one N-vinyl benzimidazole.

Oxazolidinone Template Reactions with Borane Complexes

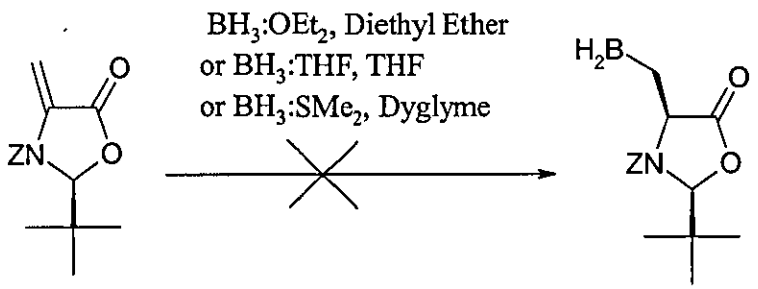


Figure 2.20 Reaction of borane complexes with oxazolidinone template

The oxazolidinone template was stirred vigorously in solvent (diethyl ether, THF or diglyme) in a flame-dried round-bottomed flask under nitrogen and cooled to 0°C. Borane-ether complex, borane-tetrahydrofuran complex or borane-methyl sulfide (respectively with solvent) was added to the mixture over 1 h (Figure 2.20). The mixture was warmed to 30°C for 48 h and monitored using TLC. No reaction was indicated using TLC so the temperature was increased to reflux but again no reaction was shown by TLC up to 48 h. After this time, excess borane was destroyed using water. There was however no reaction of the oxazolidinone with the borane in any of the complexes used, as shown by TLC and ^{1}H NMR spectroscopy.

Oxazolidinone Template Reactions with 9-BBN

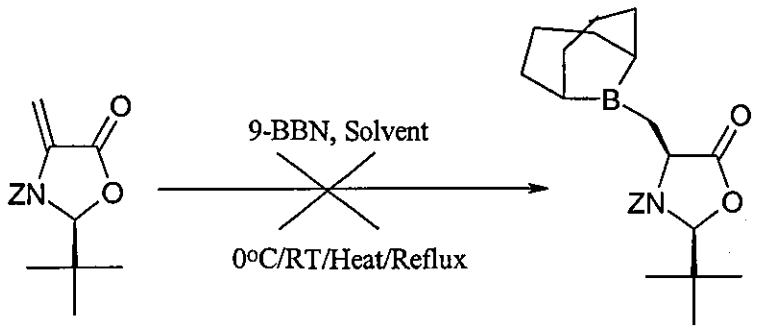


Figure 2.21 Reaction of 9-BBN with Oxazolinone Template

In parallel with the borane complex reactions, 9-BBN was also investigated as a hydroboration reagent. To a flame-dried round-bottomed flask was added the oxazolidinone template and 9-BBN in solvent (DCM, diethyl ether or THF) under nitrogen (Figure 2.21). This was stirred at 0°C for 36 h and was monitored using TLC. When no reaction had occurred after this time, the temperature was raised to room temperature and the mixture stirred for a further 36 h, again monitored by TLC. Unlike the borane complexes, 9-BBN is supplied as a dimer, so keeping the reaction at 0°C might not be beneficial as the compound is likely to stay as a dimer, but allowing the 9-BBN to warm up would allow it to form the monomer and hence it would be able to react. There was however still no reaction and the temperature was raised again to 30°C, then to reflux. There was no reaction of the oxazolidinone with 9-BBN at reflux after 36 h in DCM, diethyl ether or THF as shown by TLC and ^{1}H NMR spectroscopy.

Coupling Reaction

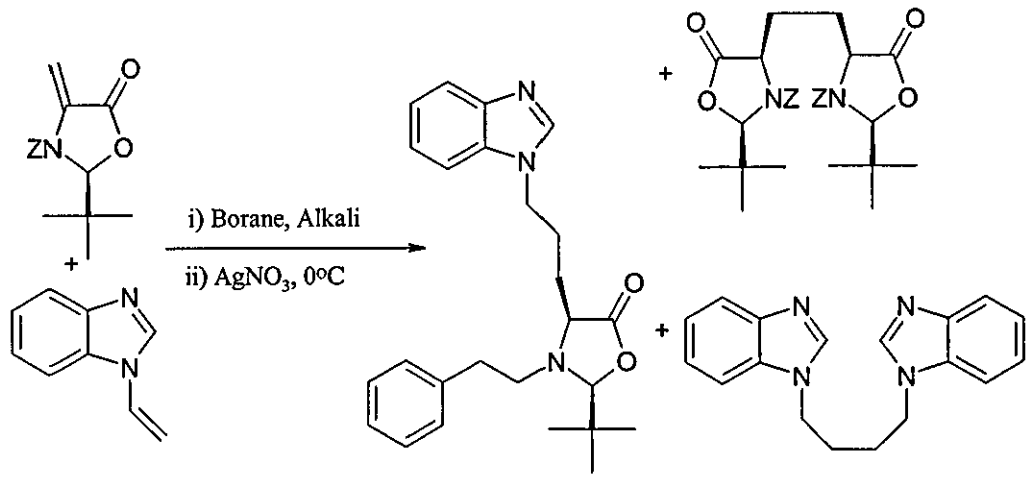


Figure 2.22 Coupling reaction involving borane and oxazolidinone template

This reaction, as shown in Figure 2.22, was performed in parallel to the other hydroboration attempts as described above on the oxazolidinone. To a flame-dried flask was added the oxazolidinone template and N-vinyl benzimidazole in THF under nitrogen. This was cooled to 0°C again to prevent undesired side reactions, then the BH_3:THF complex was added and the reaction mixture stirred for a further 12 h. KOH in methanol was then added followed by $AgNO_3$ solution at room temperature. After 2 h KOH was added and the mixture refluxed for 4 h. The reaction mixture was then poured onto water to destroy unreacted borane, acidified to pH 2 with HCl, filtered, impurities extracted from the precipitate and the precipitate was then recrystallised.

The advantage of this reaction would be that it is a one-pot reaction so time is saved, the disadvantage is the unwanted side reactions. However none of the desired cross-coupling products were seen by NMR spectroscopic examination of the crude material.

2.5.1 Summary of Hydroboration

If this method had worked the regiochemical effects would have added the borane in the desired anti-Markovnikov fashion, however, it has been seen that the oxazolidinone template has not reacted with the borane. This could be due to steric factors: the size of the oxazolidinone molecule could be preventing the attack by the borane, whether it be as a complex or as 9-BBN. At this stage it was decided not to try further hydroboration of the N-vinyl benzimidazole as the oxazolidinone hydroboration had failed to work.

2.6 Cross Metathesis

Cross metathesis is of interest as an alternative approach as, like the radical reaction, it is a straight bonding of carbon atoms. The metathesis reaction is where two alkenes are brought together at their double bonds and these bonds become interchanged.[74] The term cross metathesis refers to the reaction of two different alkene groups, although this is only one utilization of this method. Other uses include: ring closing metathesis and ring opening metathesis.[75] Metathesis was originally looked at from a mechanistic standpoint and polymer synthesis, only recently have investigations into its use in the synthesis of complex organic molecules become more common.[75,76] The plan was to investigate cross metathesis between the methylene oxazolidinone and an alkenyl heterocycle. This type of reaction has been investigated before with amino acids, but with different variations.[77,78]

The Metathesis Reaction

The catalysts used for this type of reaction contain well defined single component metal carbene complexes of ruthenium or molybdenum and are usually sourced commercially as Grubbs (Figure 2.23) or Schrock catalysts respectively.[75,76] These catalysts are however sensitive to air and moisture and have to be kept under nitrogen or argon in flame-dried vessels.[75]

The example of the general process below is for two different alkenes (it could very well be a single olefin) and one problem is strikingly obvious, the number of different possible molecules created.

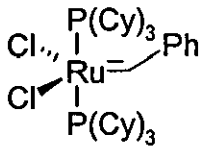


Figure 2.23 Grubbs Catalyst

In the example in Figure 2.24 only one of the facial reactions is shown but the total number of olefins that can come out of the reaction mixture is 14 if you include the starting olefins that may not have reacted.[74]

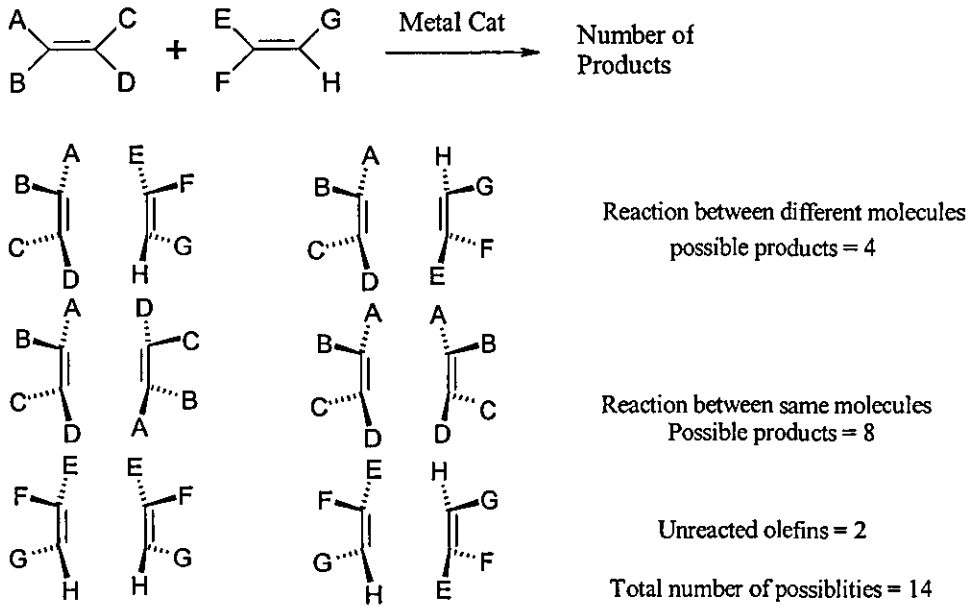


Figure 2.24 The Number of Possible Products from a Cross Metathesis Reaction

Steric hindrance can play an important role in reducing the number of unwanted side reactions and this is relevant in this investigation due to the size of the oxazolidinone molecule which is quite large. Exceptionally large groups can stop a reactant metathesising with itself, allowing a greater possibility for the desired cross-metathesis between two separate compounds.[79] Another way of controlling the reactions is by using an excess of one component. If the more reactive alkene is added in excess, there is more reactant able to combine with the less reactive component, which obviously means an increase in the yield of the desired compound but also an increase in undesired side reaction. This method is however only feasible if the more reactive component is relatively inexpensive.[75,79] Electronic factors can also play a part with the more electron deficient alkenes having a slower reaction.

Cross Metathesis Mechanism

The generally accepted mechanism for metathesis, as shown in Figure 2.25, is one involving a chain of successive 2+2 cycloadditions and cycloreversions between alkenes and metal carbenoids.[74,76]

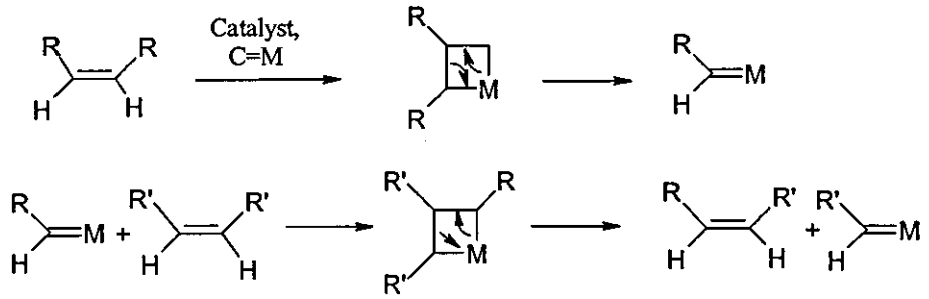


Figure 2.25 Mechanism for Cross Metathesis Reaction

It has also been noted that different groups will react at different rates; as a general guideline: $=CH_2 > =CHCH_2R > =CHCHR_2 > =CR_2$

Reactants containing allyl groups undergo cross-metathesis reaction in reasonable yields[80] for a range for different groups attached to the allyl group, including nitrogen containing groups.

2.6.1 Cross Metathesis Reactions

Two test reactions involving allyl bromide instead of the heterocycle, and the N-vinyl benzimidazole itself, were performed to check whether the oxazolidinone template would

undergo cross metathesis with another molecule. The Grubbs II Catalyst (Figure 2.23) was used for all the cross metathesis investigations.

Cross Metathesis with Allyl Bromide

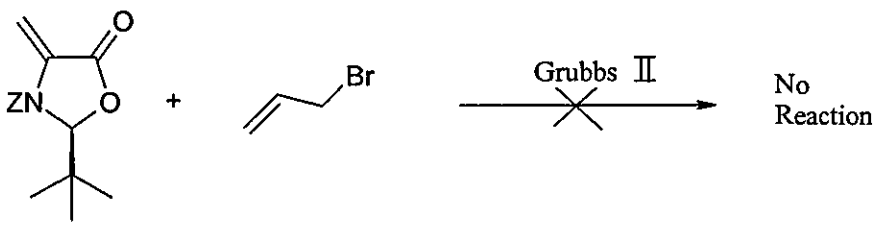


Figure 2.26 Cross metathesis reaction with oxazolidinone template and allyl bromide

Oxazolidinone and Grubbs II catalyst (10%mol) were place in a flame-dried round-bottomed flask in DCM under nitrogen. Allyl bromide was then added and the reaction mixture stirred at reflux for up to 48 h (Figure 2.26). The solvent was removed under reduced pressure. There was no point in performing any purification procedure as the ^{1}H NMR spectrum showed only oxazolidinone starting material and no cross metathesis product.

Cross Metathesis with N-Vinyl Benzimidazole

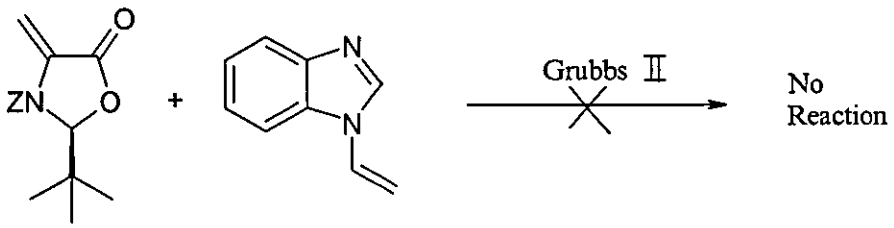
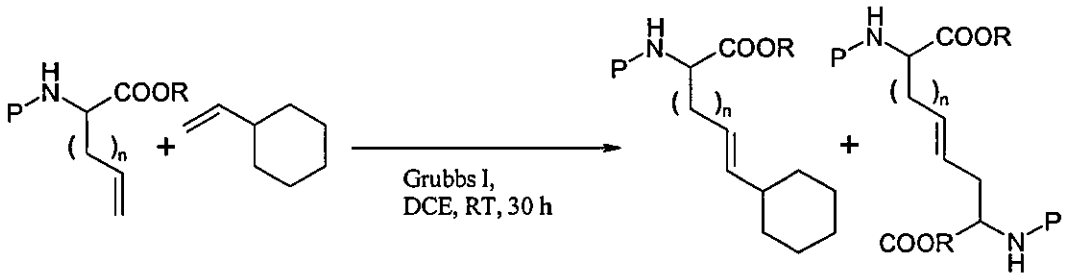


Figure 2.27 Cross metathesis reaction of oxazolidinone template and N-vinyl benzimidazole

Oxazolidinone and Grubbs II catalyst were place in a flame-dried round-bottomed flask in DCM under nitrogen (Figure 2.27). N-Vinyl benzimidazole was then added and the reaction mixture stirred at reflux for up to 48 h. The solvent was removed under reduced pressure. Once again there was no point in performing any purification procedure as the ^{1}H NMR spectrum showed only oxazolidinone and benzimidazole starting materials and no cross metathesis product.

The N-vinyl benzimidazole was not tried again after the discovery of a paper on cross metathesis of nitrogen containing systems by Vernall and Abell.[81] This further background on the use of cross metathesis in the formation of novel amino acids made interesting reading. With amino acids and peptides, a substituent bearing a terminal double bond can be attached to the α-carbon, amino acid side chain or the amino or the carboxyl groups. Glycine has been used as a scaffold for cross metathesis and reactions have shown that vinylglycine does not readily undergo cross-metathesis. This is likely to be due to the steric inaccessibility of the double bond to the catalyst. Further experiments have shown

that a longer chain has generally meant improved cross metathesis.[77] This is shown in Figure 2.28.



n	P	R	Yield % Cross-Metathesis	Yield % Self-Metathesis
0	Boc	Me	7	0
1	Boc	ᵗBU	49	16
1	Boc	Me	45	17
2	Boc	Me	66	28
2	Fmoc	Me	58	25
2	Fmoc	H	58	18

Figure 2.28 Example of the use of Cross Metathesis in Amino Acid Substituent Formation and how Steric and Chemical Factors may play a part.

This problem has also been demonstrated with amines[78] and amides.[82] In light of this information it is unsurprising that the oxazolidinone and N-vinyl benzimidazole proved unreactive substrates.

2.6.2 Summary of Cross Metathesis

There was no reaction of the oxazolidinone with the Grubbs II catalyst. As with hydroboration, this is likely to be due to steric factors. The Grubbs catalyst is a large molecule and so steric hindrance is even more likely to be the cause.

2.7 Organocuprates

An alternative approach is to use a carbon nucleophile in a conjugate addition to the oxazolidinone template. As the carbon centre at which an attack will take place is soft, a soft carbon nucleophile reagent is needed, which is why organocuprates were considered. The planned reaction is shown in Figure 2.29. It was proposed to prepare the cuprate via lithum-halogen exchange from the haloalkyl heterocycles that were already to hand, followed by transmetallation.

To make the oxazolidinone template was time consuming, so test conjugate addition reactions were performed using methyl acrylate instead of using the oxazolidinone template as an acceptor and a haloalkylbenzimidazole as a cuprate precursor. If the desired addition product was formed in the test reactions the next step would be to try the reaction using the oxazolidinone template.

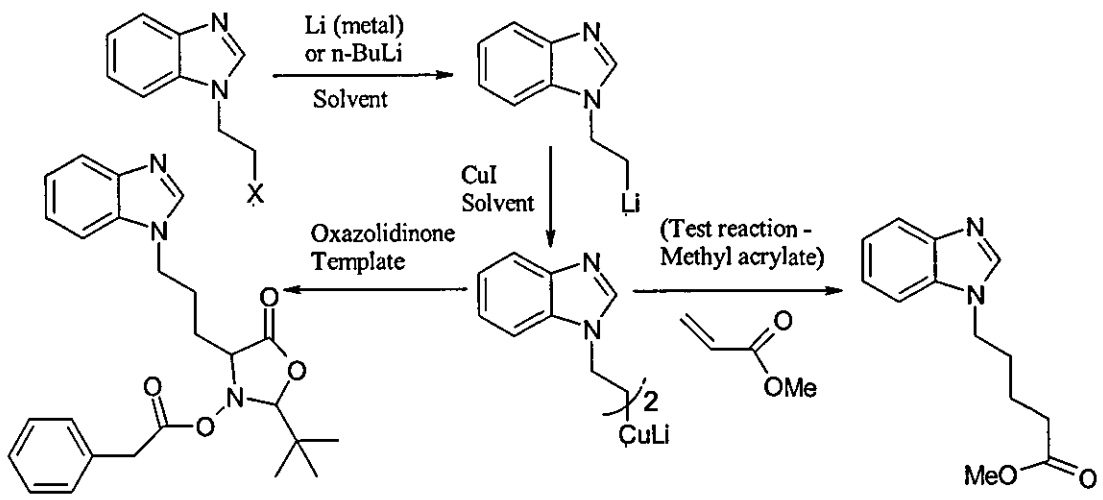


Figure 2.29 Planned Reactions involving Organocuprates, Haloalkylbenzimidazole and either the Oxazolidinone Template or Methyl Acrylate

2.7.1 Methods for the Formation of Organocuprates

Metal-Halogen Exchange

Alkyl halides can react with some metals to form organometallic compounds.[65,74,83] This method is famously used to form Grignard reagents, but these are not the only compounds that can be made this way. The halide can be iodo, bromo or chloro with decreasing reactivity in that order and can be primary, secondary, tertiary or aryl. Other functional groups may cause problems in the reaction. Alcohols, amines and carboxylic acids may be present but must be in the form of a salt, whereas ketones, aldehydes, cyanides, nitro and ester groups will interfere with the metal-halogen exchange reaction. Although the products of a lithium-halogen exchange reaction can be stored in a freezer at -20°C overnight, the best results are obtained from immediate use of the reagent. Problems in a reaction may arise if the reaction is very slow, then there is competition from the Wurtz reaction,[74,84] although is quite rare with lithium.

The Wurtz reaction is the generation of metal alkyls from electropositive metals and alkyl halides resulting in the coupling of the alkyl halides.

$2RX + 2M\ (Na\ or\ Li) \rightarrow RR + 2MX$

The mechanism is either a 2 step polar reaction (as below), or a radical process.

$RX + 2M \rightarrow R^{-}M^{+} + MX$

$R^{-}M^{+} + RX \rightarrow RR + MX$

The mechanism for the metal-halogen exchange reaction for the organocuprates and Grignard reagents is not fully understood, but it is believed to involve radicals.[74]

Transmetallation

Transmetallation involves replacement of one metal in an organic compound with another metal and can be used when the first metal is below the replacement metal in the electrochemical series. This method ensures that the organometallic compound is free from possible halide. Sometimes however the reaction needed is one to replace the more electropositive metal, this can be done using metal halides and is one of the most common methods for making organocuprate reagents. Two moles of an organolithium are reacted with one mole of copper(I) iodide at low temperatures in either ether or tetrahydrofuran[74,85,86] as shown in the following equation, to generate a lithum dialkylcuprate.

$$CuI + 2\ H_3C{-}Li \longrightarrow H_3C{-}Cu^{-}{-}CH_3\ Li^{+} + LiI$$

Organocuprate Reactivity

Organocuprates add to α,β-unsaturated aldehydes, ketones and to a lesser extent esters to give conjugate addition products. This is in contrast to Grignard reagents, which tend to afford 1,2-addition. The organic component of the cuprate can be alkyl, vinyl or aryl. One characteristic of this reaction is that only one of the organic groups is transferred, however, using a mixed reagent involving one slowly transferred group will overcome this if the reagent is expensive, for example: R(R'C≡C)CuLi (where R'= tBu or nPr), R(O-tBu)CuLi or R(PhS)CuLi.[74] The heterocycles to be used in this investigation are not expensive, so it was decided that two equivalents of the heterocycles would be used to form the standard

dialkyl cuprates. There is usually little or no competition from 1,2-addition to the C=O. Substitution at the carbonyl carbon aids in increasing yields.[74] The mechanisms for these reactions have not been determined fully (Figure 2.30).

There could be a cyclic mechanism or a SET process:[65]

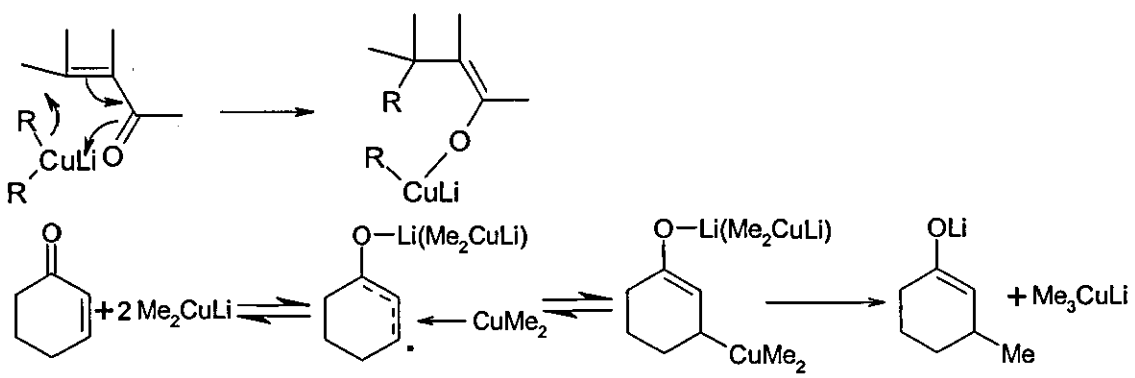


Figure 2.30 Possible Reaction Mechanisms for Organocuprate Reactions

Organocuprate couplings would simply not take place without the presence of a Lewis acid counter ion, either MgX^+ or Li^+. When lithium reacts with an alkyl halide, the compound formed does not exist as a free organolithium, but as a complex.[87]

There can be many variations in organocuprate protocols as to the type of solvent, purity of copper(I) precursor, use of catalytic processes and one-pot reactions[88,89]

One modification to the reaction scheme is to add either trimethylchlorosilane or trifluoroborane etherate.[74] These help the reaction in two different ways: the TMSCl will help to trap enolate anion intermediates as they form and hence help to force the conjugate addition reaction to completion and the $BF_3.Et_2O$ helps to establish an equilibrium by forming $RLi.BF_3$ and the more reactive $R_3Cu_2Li_2$ from $(R_2CuLi_2)_2$.

There are certain practical points that should be remembered in organocuprate chemistry:[65,87]

To obtain a lithium halogen exchange reaction it is essential to have the metal surface exposed as the surface of lithium quickly tarnishes in air;[87]

All organocuprate reactions should be done under nitrogen or argon;

All materials (chemicals and glassware) for the reaction must be completely dry as these reactions are very moisture sensitive;

The temperature of the reaction is also important, organocuprate reactions are usually performed between -78°C and 0°C, probably to hinder elimination and reduce other side reactions. The reactions are also usually performed in THF or ether.

2.7.2 Lithium Halogen Exchange Reactions

Use of Lithium Metal for Lithium/Halogen exchange

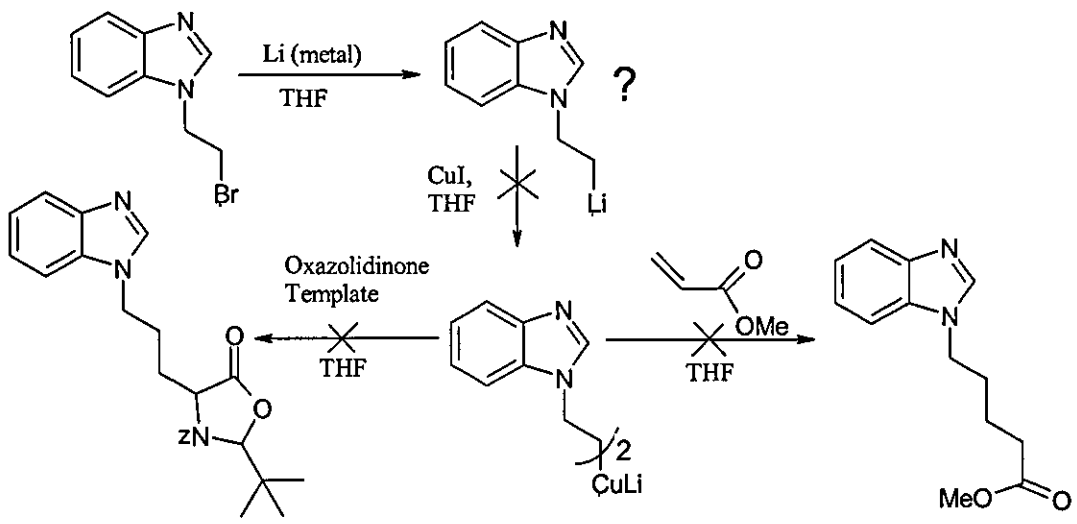


Figure 2.31 Organocuprate Reaction Involving Lithium Metal

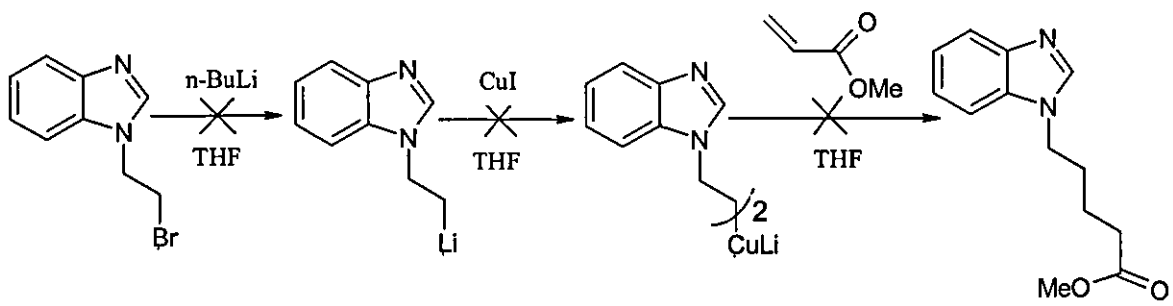
The reaction of lithium with N1-(2-bromoethyl)benzimidazole was initially attempted in diethyl ether but there was no reaction at the initial stage where the lithium metal is used so this was abandoned and THF was used instead and the scheme is shown in Figure 2.31. Finely cut lithium was placed in a flame-dried round-bottomed flask and suspended in THF under nitrogen. N1-(2-Bromoethyl)benzimidazole dissolved in THF was added slowly to the round-bottomed flask and constantly stirred at room temperature. There was then a colour change in the reaction mixture, once this colour change had begun the reaction was either a) cooled to approximately -10°C, b) kept at room temperature, or c) cooled down to -78°C. Any N1-(2-bromoethyl)benzimidazole not added before the colour change was now added. After 2 h below 0°C, the solution was cooled further to -78°C then siphoned through glass wool into another flame-dried round-bottomed flask at -78°C and kept under nitrogen. A pre-cooled suspension of copper(I) iodide in THF was then added slowly to the stirred mixture and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, and then cooled again to -78°C. Over this time the mixture turn from an orange/red colour to a deep red, almost black colour and the copper iodide disappeared. Methyl acrylate was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to room temperature and was stirred for a further 20 h at room temperature. The final colour for the reaction mixture was a dark green colour. The reaction mixture was filtered and the solvent removed under reduced pressure. An attempt at separation of the mixture was then made by flash column chromatography but no addition product was found in any of the fractions, and with further attempts at the reaction no separation was pursued as the crude ^{1}H NMR spectra showed no sign of the desired conjugate addition product.

Different temperatures were tried for the lithium-halogen exchange because the initial attempts of cooling to about -10°C gave dealkylated benzimidazole back from the reaction, presumably by β-elimination of the alkyl lithium. Attempt b) at RT and c) at -78°C gave the N-ethyl and N-vinyl benzimidazoles by elimination of HBr from N1-(2-bromoalkyl)benzimidazole by initially formed lithioalkylbenzimidazole.

This reaction was performed a number of times with slight variations. As well as changes in the temperature already described, chloroethyl and iodoethyl benzimidazole were used, but these either gave no reaction or no desired product. The reaction was also tried using TMSCl to aid the reaction but this also gave none of the desired products.

The reactions were also repeated using the oxazolidinone template, but this also gave none of the desired product, and it was decided to move on to try with n-butyl lithium.

n-Butyl-lithium



2.32 Organocuprate Reaction Involving n-Butyl-lithium

N-Butyl lithium was used as a possibly more reactive alternative to lithium metal in these circumstances, to promote rapid organolithium formation, the scheme being shown in Figure 2.32. To a flame-dried round-bottomed flask a solution of N1-(2-

bromoethyl)benzimidazole (or N1-(2-chloroethyl)benzimidazole) in dry ether or THF was added under nitrogen at -78°C. To this solution fresh n-butyl lithium (up to 3 mol equiv) was added and the reaction mixture stirred for 2 h at -78°C (or until there was a colour change). A pre-cooled suspension of copper(I) iodide in ether or THF was then added slowly to the solution and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, before cooling again to -78°C. Methyl acrylate was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to RT and stirred for a further 20 h at RT. The reaction was then worked up using saturated ammonium chloride solution and extracted using ether. Separation was by flash column chromatography.

Although there was a colour change for all the reactions tried, none of the variations gave any of the desired product and methyl acrylate was the only starting material recovered. Even after an attempt at separating the compounds the ^{1}H NMR spectra were inconclusive as the mixture proved to be inseparable.

2.7.3 Summary of Organocuprate Reactions

Lithium form	benzimidazole derivative	Solvent	Temperature (centigrade)	TMSCl	Occurrence
Li metal	Br	Ether	RT to -20	No	No Reaction
	Br	THF	RT to -20	No	Reduction and Elimination products
	Br	THF	RT	Yes	Gives Benzimidazole starting material
	Br	THF	-10	Yes	Gives Benzimidazole starting material
	Br	THF	-78	Yes	Reduction and Elimination products
	Cl	Ether	RT to -20	No	No Reaction
	Cl	THF	RT to -20	No	No Products
	I	THF	RT to 0	No	No Reaction
n-BuLi	Br	Ether	-78	No	Colour change, methyl acrylate back
	Br	THF	-78	No	Colour change, methyl acrylate back
	Cl	Ether	-78	No	Colour change, methyl acrylate back
	Cl	THF	-78	No	Colour change, methyl acrylate back

Table 2.2 Organocuprate reaction summary

Table 2.2 shows the reactions performed and their outcome. Despite the change in colour indicating some kind of reaction, none of the desired product was formed. The reduction and elimination products from the benzimidazole are likely to be due to the lithium metal reacting too slowly with the bromo compounds and so allowing enough time for the reduction/elimination reaction of the alkyl lithium and residual alkyl bromide. There is a possibility that there were a number of undesired side reactions with using n-butyl lithium,

such as deprotonation at the carbon between the nitrogen atoms on the benzimidazole. These side reactions have not however led to any identifiable products and the ^{1}H NMR spectra are very inconclusive, containing a large number of peaks that makes them difficult to interpret.

Given the lack of success with radicals, hydroboration, cross-metathesis and organocuprate approaches to forming C-C bonds as a link between amino acids and a heterocyclic side-chain and not wishing to repeat the work of other people with different methods, it was decided to shift to C-X bonds as the linkage.

2.8 Carbon-Heteroatom Bond Formation

The linkage between the heterocycle and the amino acid does not have to consist of just carbons, there can be other atoms present such as heteroatoms in the chain itself. This has been reported using different methods to form other PNAs[90,91] or DNA type compounds.[92]

The following reaction scheme shows the planned investigation into carbon-heteroatom linkage. Amino acids with nucleophilic heteroatoms in their side-chains were required since the haloalkylheterocycles were already in hand as electrophiles. Initially a reaction without base present was considered, but it was decided that the lone pairs on the heteroatoms would not be sufficient for the nucleophilic substitution reaction to take place. It was planned that the base would remove a proton from the heteroatom to give a more reactive nucleophile, then for nucleophilic attack to take place. The general reaction scheme is shown in Figure 2.33.

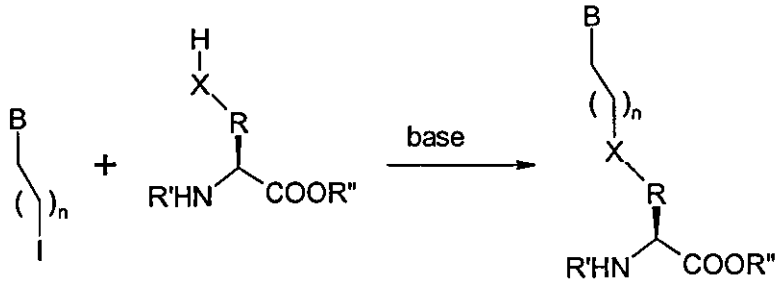


Figure 2.33 Formation of carbon-heteroatom linkage

The following amino acids were selected as potential candidates for this type of reaction, the naturally occurring L-cysteine, L-serine and the non-proteogenic 2,3-diaminopropionic acid, 2,4-diaminobutyric acid and homocysteine as in Figure 2.34.

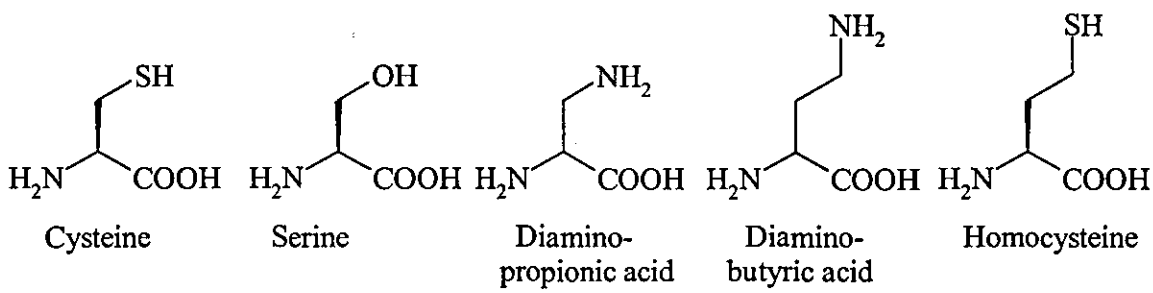


Figure 2.34 Suitable amino acids

The first reactions to be investigated used L-cysteine because the thiol group should be the most nucleophilic and least basic of SH, OH and NH_2.

2.8.1 Cysteine

Ester protection

The L-cysteine carboxyl group was protected as the ethyl ester as this ester was available commercially.

Protection of Amino Group

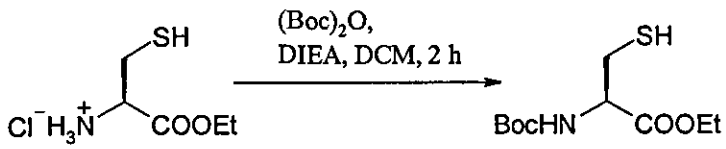


Figure 2.35 Boc protection of L-cysteine

To protect the amino group the t-butyloxycarbonyl function was selected, since it would be easily removed for couplings with other amino acids. To a dry round-bottomed flask was added cysteine ethyl ester, $(Boc)_2O$ and DIEA in dry DCM. The reaction was then stirred for 2 h at room temperature and aqueous work up and purification gave Boc-cysteine ethyl ester in 94% yield (Figure 2.35).

2.8.1.1 Reactions of Cysteine with Iodoalkyl Heterocycles

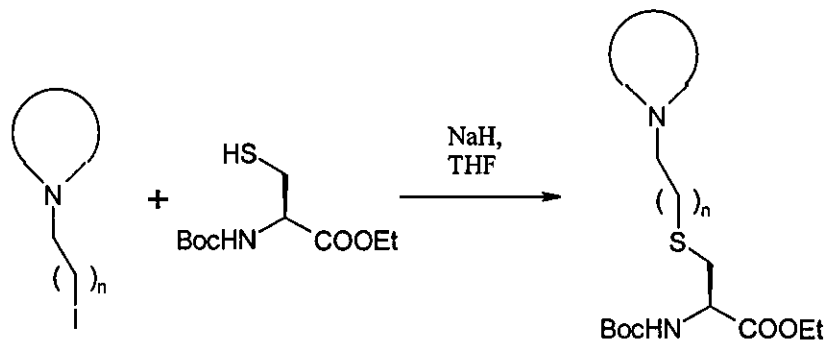


Figure 2.36 Substitution Reactions

The above scheme, Figure 2.36, shows the general reaction plan for the substitution reactions. The protected cysteine in dry THF is added to a flame-dried round-bottomed

flask which was cooled via an ice bath to 0°C. NaH is then added followed by the iodoethyl compound after a set time and the reaction allowed to warm to room temperature before refluxing overnight. The product is obtained by aqueous work up and column chromatography.

Reaction of Cysteine with N1-(2-Iodoethyl)benzimidazole

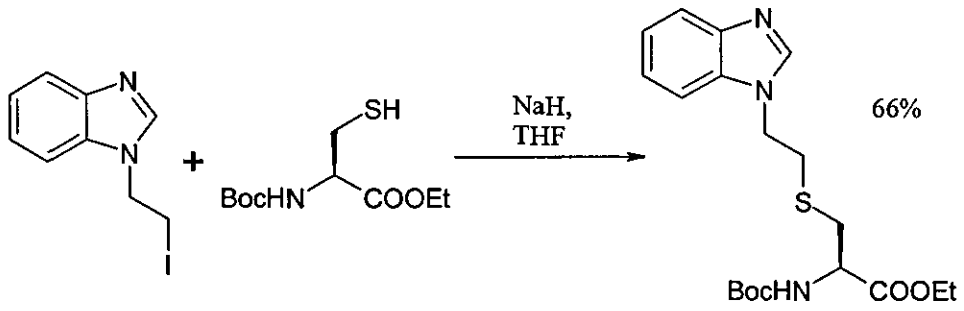


Figure 2.37 Boc-cysteine ethyl ester with N1-(2-iodoethyl)benzimidazole

Protected cysteine in dry THF was added to a flame-dried round-bottomed flask which was cooled to 0°C and under nitrogen. NaH was then added. In the first reaction the N1-(2-iodoethyl)benzimidazole was added immediately to the reaction mixture after the NaH, before the mixture was allowed to warm and then heated at reflux overnight. This first reaction gave some of the desired alkylation product, but more of the elimination product, N-vinyl benzimidazole and the cysteine starting material. The planned reaction scheme is shown in Figure 2.37. It was thought from these results that the N-vinyl benzimidazole had been formed from reaction of the iodoethyl compound directly with the NaH (Figure 2.38), implying that the reaction between NaH and N1-(2-iodoethyl)benzimidazole is very rapid and hence the NaH did not have time to deprotonate the protected cysteine.

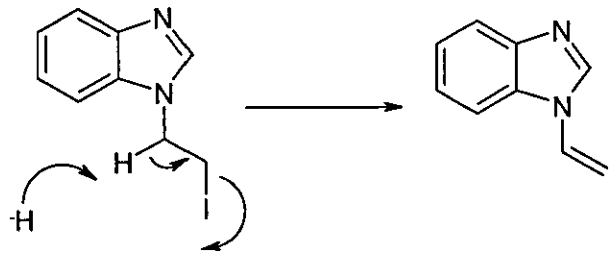


Figure 2.38 Elimination to N-Vinylbenzimidazole

A number of reactions were therefore performed to test whether the time at which the N1-(2-iodoethyl)benzimidazole was added after the NaH addition had an effect on the reaction, these results are shown in Table 2.3 below.

Time (min)	Yield of S-benzimidazole Boc-cysteine ethyl ester
0	46%
5	56%
10	62%
15	64%
20	66%
30	65%
45	67%
60	56%

Table 2.3 Cysteine with N1-(2-Iodoethyl)benzimidazole Reactions

It can be seen that for the first 20 minutes, the longer after the addition of NaH that N1-(2-iodoethyl)benzimidazole is added the better the yield of the desired alkylation product, showing that a delay in adding the iodo compound is beneficial in the formation of the desired alkylation product, although after 15 min the increase in yield becomes negligible. The product constitutes a novel S-linked heterocyclic amino acid as shown in Figure 2.37.

After the successful investigations with N1-(2-iodoethyl)benzimidazole, a range of reactions were performed using the same general procedure with other heterocycles.

Cysteine with Uracil Derivative

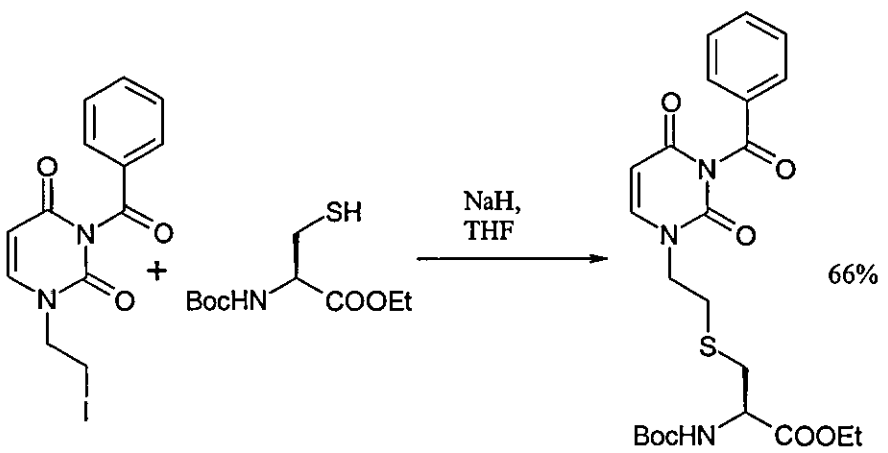


Figure 2.39 Boc-cysteine ethyl ester with N3-benzoylated-N1-(2-iodoethyl)uracil

This reaction was performed as detailed above and is shown in Figure 2.39, with the N3-benzoylated-N1-(2-iodoethyl)uracil being added to the reaction mixture 20 min after the NaH. The initial reaction with the Boc cysteine ethyl ester gave a lower than expected yield and so the time between NaH addition and addition of the uracil derivative was varied. The optimum time for the addition of the uracil derivative (to the nearest 5 min) was found to be 5 min, the yields for the various times are shown in Table 2.4.

Time (h)	Yield of S-uracil Boc-cysteine ethyl ester
0	67%
5	70%
10	66%
15	64%
20	62%
30	57%
45	55%
60	55%

Table 2.4 Uracil derivative with Cysteine Reactions

Thymine Reactions

It was first necessary to protect the thymine and to form the N3-benzoyl-N1-(2-iodoethyl)thymine, by the same protection/alkylation sequence developed for uracil.

N3-Benzoyl-N1-(2-iodoethyl)thymine

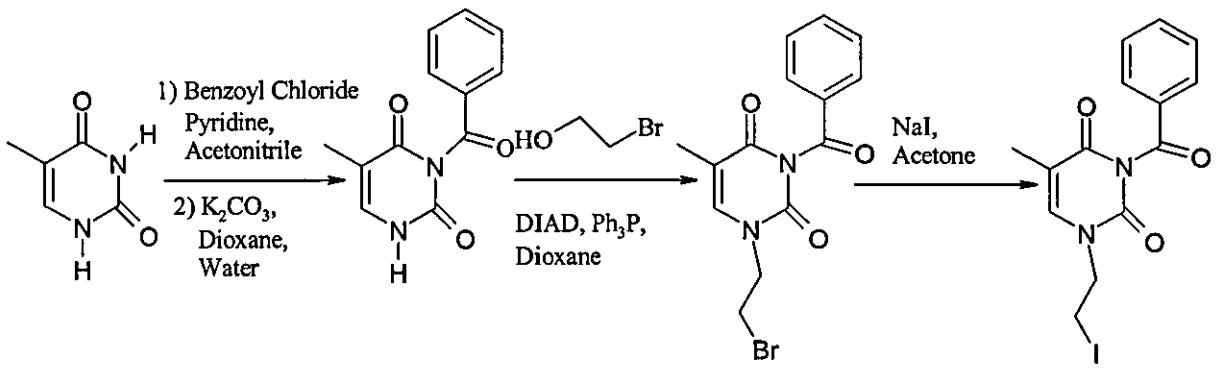


Figure 2.40 Preparation of Thymine Derivative for Reaction with Amino Acid

The same scheme was employed as for uracil previously and is shown in Figure 2.40. Firstly, thymine was protected at the N3 position using a suspension of thymine in a

solution of acetonitrile/pyridine with benzoyl chloride being added dropwise at 0°C. After 2 h the suspension had disappeared to give an orange solution. The solution was left to stir overnight to form N1,N3-benzoylthymine. This was confirmed by ^{1}H NMR spectroscopy which now showed no NH peaks in the δ 10.5ppm region. The dibenzoylated compound again proved difficult to separate from the pyridine solvent so DCM/water was added to the 'slush' which produced a white suspension. The solvent was filtered off to give the dibenzoylated product. The N1 benzoyl group was preferentially removed using potassium carbonate in water/dioxane (1:1 v/v) to give the desired N3-benzoylthymine product in 71% yield.

The bromoethyl group was then added. N3-benzoylthymine, 2-bromoethanol and triphenylphosphine (TPP) were stirred in dry dioxane with DIAD added dropwise over 1 h at 5°C. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure and the desired N1-alkylated product was purified using column chromatography. This gave a yield of 56% for the desired product, N3-benzoyl-N1-(2-bromoethyl)thymine, and further reactions after this also gave high yields.

To form the iodoethyl derivative from the bromoethyl derivative a simple halogen exchange, Finkelstein reaction, was again needed. N3-Benzoyl-N1-(2-bromoethyl)thymine was heated at relux in dry acetone with dry NaI to give the N3-benzoyl-N1-(2-iodoethyl)thymine as the desired product in a yield of 54% (Figure 2.40).

Cysteine with Thymine Derivative

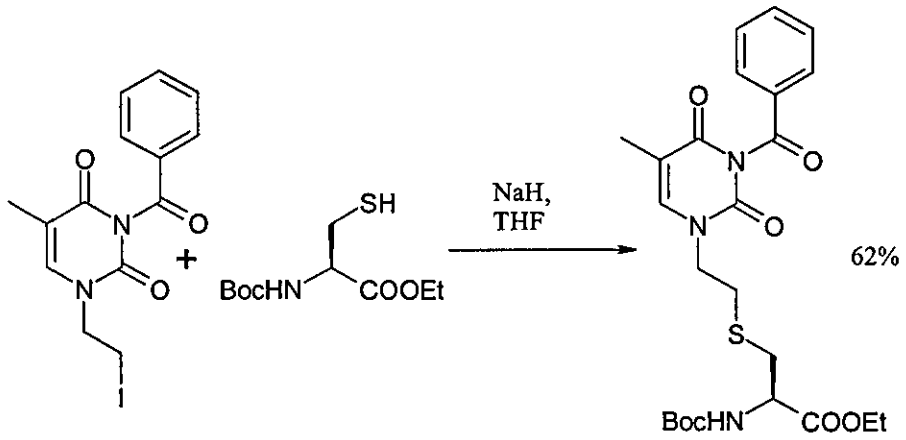


Figure 2.41 Boc-cysteine ethyl ester with N3-benzoylated-N1-(2-iodoethyl)thymine

This reaction was performed as previously detailed for the uracil derivative and is shown in Figure 2.41, with the N3-benzoylated-N1-(2-iodoethyl)thymine being added to the reaction mixture 20 min after the NaH. The initial reaction with the Boc-cysteine ethyl ester gave a lower than expected yield again and so the time the thymine derivative was added after the NaH was again varied. The optimum time for the addition of the thymine derivative was again found to be 5 minutes after the NaH, as shown in Table 2.5.

Time (h)	Yield of S-thymine Boc-cysteine ethyl ester
0	60%
5	80%
10	62%
15	57%
20	56%
30	54%
45	54%
60	55%

Table 2.5 Thymine derivative with Cysteine Reactions

Indole Reactions

Another heterocycle which was tried in this S-alkylation sequence, but not found in nature was indole. Like benzimidazole, indole does not need to be protected before it is used, and the bomoalkylation was tried directly on indole (Figure 2.42).

The indole was firstly reacted under the Mitsunobu conditions, as described previously. Indole, 2-bromoethanol and TPP were stirred in dry dioxane with DIAD added dropwise over 1 h at 5°C. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure. The crude ^{1}H NMR spectrum showed no sign of the bromoalkylated product and revealed only the starting materials. The unreacted TPP was removed, first by crystallization and then column chromatography and the starting material was retrieved quantitatively. It was concluded that the nitrogen on the indole was not acidic enough to react under Mitsunobu conditions.

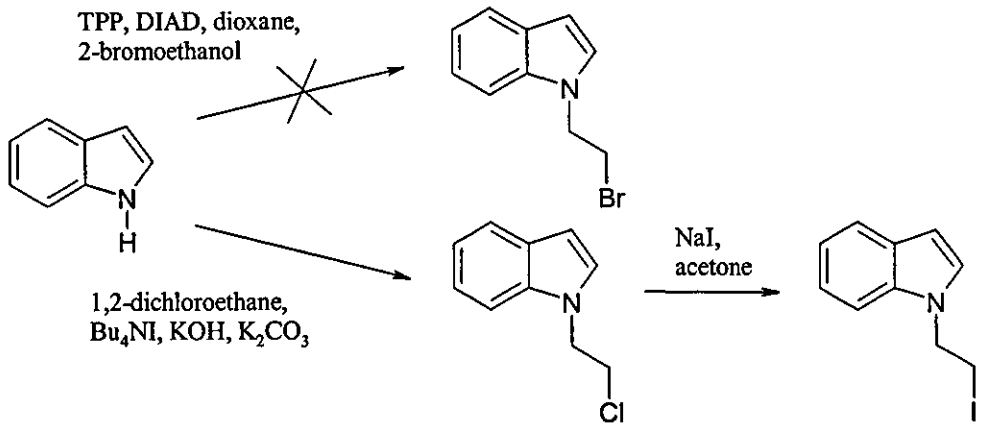


Figure 2.42 Formation of N-(2-Iodoethyl)indole

The phase transfer catalyst (PTC) method was then tried using 1,2-dichloroethane. Indole was added to a mixture of 1,2-dichloroethane, tetrabutylammonium iodide, solid KOH and K_2CO_3. The reaction was stirred at room temperature for 24 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the desired product separated easily and cleanly to give an 81% yield of N-(2-chloroethyl)indole.

It was then possible to do the halogen exchange (Finkelstein) reaction on the chloride, instead of the bromide. The reaction conditions used were the same, but the reaction times were longer. Chloroethylindole was heated at reflux in the dark in dry acetone with dry NaI. This gave the desired N-(2-iodoethyl)indole in a yield of 64% (Figure 2.42).

Cysteine with Iodoethylindole

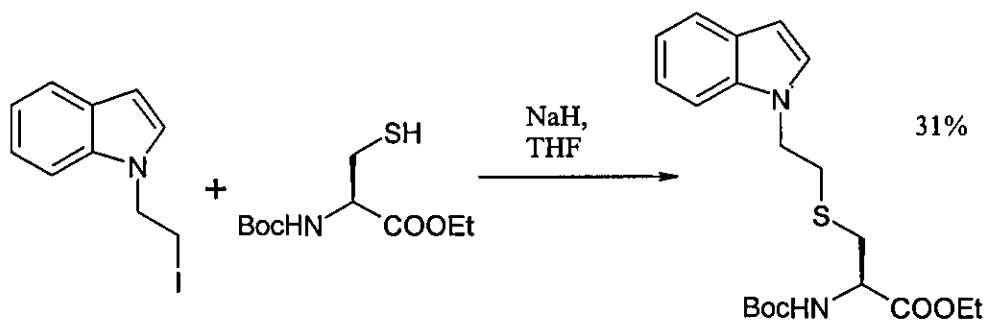


Figure 2.43 Boc-cysteine ethyl ester with N-(2-iodoethyl)indole

This reaction was performed as previously detailed using the protected cysteine, with the iodoethylindole being added to the reaction mixture 10 min after the NaH. After purification by column chromatography the desired S-alkylation product was obtained in a

yield of 31%. Subsequent reactions were performed, but with no improvement in yield, although it is unclear why the yield is only moderate (Figure 2.42).

Purine Reactions

Purine was also chosen as a heterocycle to be investigated because, although it does not occur naturally, it does possess the same ring structure as the nucleobases adenine (which has been investigated later) and guanine. Purine did not need to have any of its sites protected and so could be directly bromoalkylated (Figure 2.44).

Purine was bromoalkylated under Mitsunobu conditions. Purine, 2-bromoethanol and TPP were stirred in dry dioxane with DIAD added dropwise over 1 h at 5°C. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure and the mixture worked up to give the desired product N9-(2-bromoethyl)purine in a yield of 73%.

The purine was also chloroalkylated under the PTC conditions for comparison. Purine was added to a mixture of 1,2-dichloroethane, tetrabutylammonium iodide, solid KOH and K_2CO_3. The reaction was stirred at room temperature for 24 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the desired product, N9-(2-chloroethyl)purine, again separated easily and cleanly to give a 59% yield.

The Finkelstein halogen exchange reaction was then performed on both haloalkyl purines using dry acetone and NaI to give the desired product. The yield for the bromide to the iodide halogen exchange was 79% and the yield from the chloride to the iodide halogen exchange was 64% (Figure 2.44).

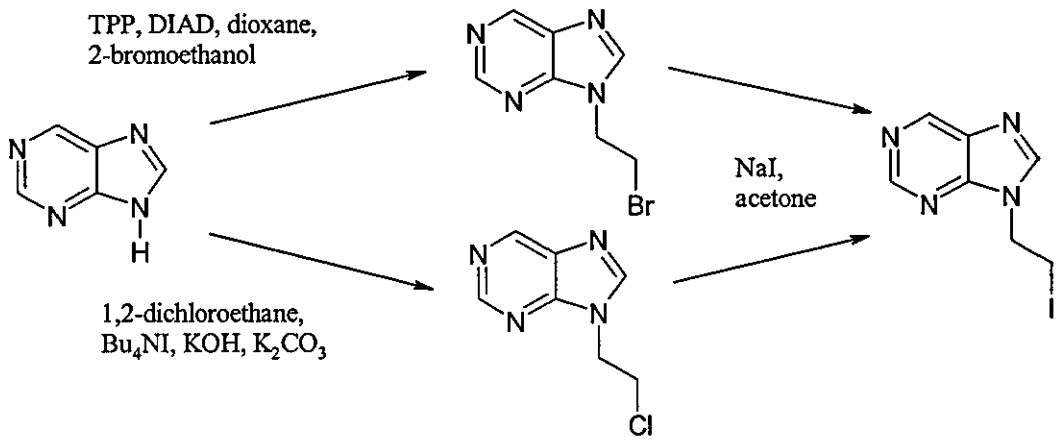


Figure 2.44 Formation of N9-(2-Iodoethyl)purine

It was decided that subsequent reactions to form the iodoalkyl compounds would be via the Mitsunobu method as this gave the higher yields both to form the haloalkyl compound and for the halogen exchange.

Cysteine with N9-(2-Iodoethyl)purine

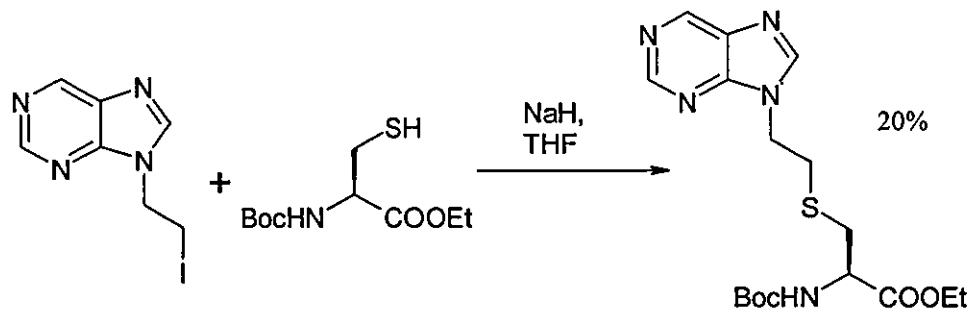


Figure 2.45 Boc-cysteine ethyl ester with N9-(2-iodoethyl)purine

This reaction was performed as previously detailed, with the N9-(2-iodoethyl)purine being added to the reaction mixture 10 min after the NaH was added to the protected cysteine.

After purification by column chromatography the desired S-alkylation product was obtained in a yield of 20% (Figure 2.45). Subsequent reactions were performed, but with no improvement on this yield, although it is again unclear why such a low yield was obtained.

Adenine Reactions

Although the reactions with purine had not proved to be high yielding, some of the desired heterocyclic amino acid derivative had been obtained so adenine was prepared for reaction with the protected cysteine. It was first necessary to protect the C-6 amino substituent of adenine, which can be done using the Boc protecting group.

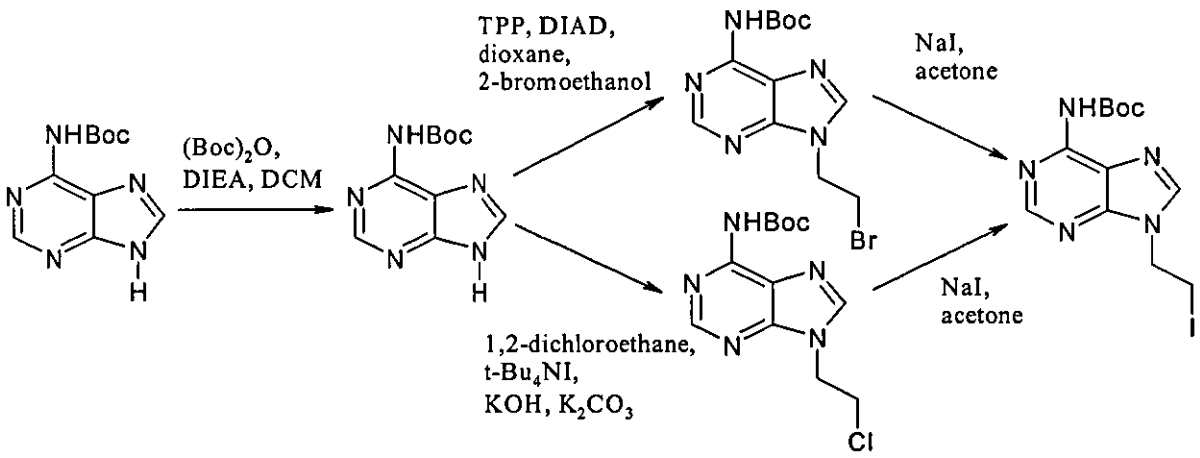


Figure 2.46 Formation of Boc Protected N9-(2-Iodoethyl)adenine

To a dry round-bottomed flask was added adenine, $(Boc)_2O$ and DIEA in dry DCM. The reaction was then stirred for 2 h at room temperature and the product was purified after aqueous work up to give Boc-adenine in quantitative yield, shown in Figure 2.46.

The next step involved the bromoalkylation of the protected adenine. This reaction had been performed before[57] and it has been seen previously that the reaction does not have a very good yield. The same method was used as previously under Mitsunobu conditions. Boc-Adenine, 2-bromoethanol and TPP were stirred in dry dioxane with DIAD added dropwise over 1 h at 5°C. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure followed by some purification by column chromatography. The initial crude yield was less than 30%, so the crude products were reacted a second time under the same conditions and the total yield was calculated to be 37%. It was decided not to try for a third time as it was thought the yield would not increase by a sufficient amount to warrant such action.

The chloroalkylation was also tried using the same method as previously, using a phase transfer catalyst. This however only gave an impure product which was inseparable from the mixture in about 59%.

The Finkelstein halogen exchange reaction was then performed using dry acetone and NaI to give the desired product via halogen exchange of the bromide to the iodide with a yield of 51% (Figure 2.46).

Cysteine with N9-(2-Iodoethyl)-Boc-adenine

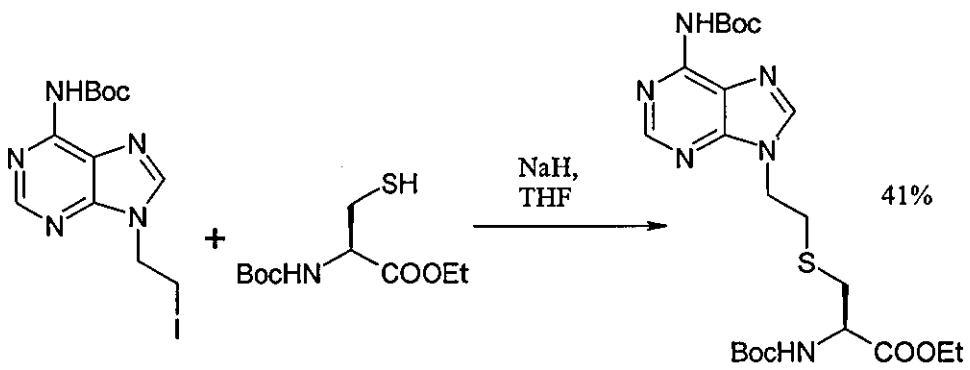


Figure 2.47 Boc-cysteine ethyl ester with N9-(2-iodoethyl)-Boc-adenine

This reaction was performed as previously detailed using the protected cysteine, with the N9-(2-iodoethyl)-Boc-adenine being added to the reaction mixture 10 min after the NaH. After purification by column chromatography the desired S-alkylation product, Figure 2.47, was obtained in a yield of 41%. Although still not a large yield this was higher than expected based on the purine results.

Benzotriazole Reactions

Benzotriazole was the next heterocycle to be investigated. This threw up a problem in that the reaction could occur at either nitrogen site, at N1 or N2 (Figure 2.48).

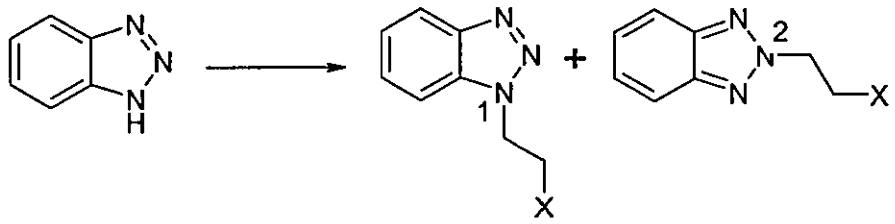


Figure 2.48 Haloalkylation at N1 and N2 sites

Statistically one may naively predict that the reaction should occur in 2:1 ratio N1 to N2. In practise this has occurred for a number of reactions,[93] and N1-alkylation was the initial target, although the N2 product could also be used in the formation of a heterocylic amino acid. The ratio of the N1:N2 products may in practice differ depending on the method of forming the haloalkyl compound.

Bromo/Chloro-ethylbenzotriazole

Being the first method used in the formation of haloalkyl heterocycles for this investigation, the Mitsunobu method was the first method tried for benzotriazole (Figure 2.49). Benzotriazole, 2-bromoethanol and TPP were stirred in dry dioxane with DIAD added dropwise over 3 h at 5°C. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure and the mixture worked up to give the N1-(2-bromoethyl)benzotriazole in a yield of only 27% and the N2-(2-bromoethyl)benzotriazole in a yield of 56%. The ratio of N1:N2 alkylation was the opposite to what was expected at 1:2 and the isomers were identified using NMR spectroscopy. The ^{1}H NMR spectra showed symmetry in the aromatic region for the N2 compound, which was also backed up by NOE spectra.

The benzotriazole was also chloroalkylated by the PTC protocol to test the ratio of the chloroethyl products (Figure 2.49). Benzotriazole was added to a mixture of 1,2-dichloroethane, tetrabutylammonium iodide, solid KOH and K_2CO_3, and the reaction was stirred at room temperature for 24 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the products separated off easily and cleanly to give the N1-alkylated compound in 66% yield and the N2 compound was not isolated. The selectivity between the two products was not as for the Mitsunobu reaction, but was still not as expected statistically.

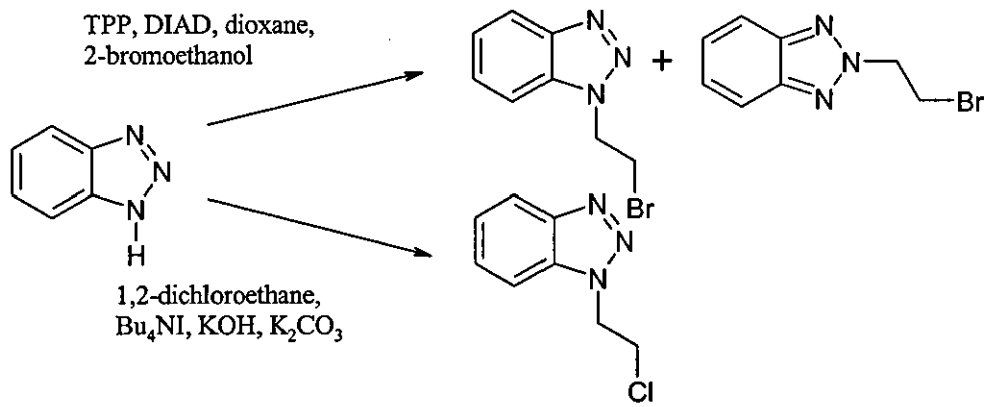


Figure 2.49 Bromoalkylation and Chloroalkylation of Benzotriazole

The Finkelstein halogen exchange reaction was then performed on all three haloalkyl compounds formed using dry acetone and NaI to give the desired products (Figure 2.50) via the halogen exchange of the bromide or chloride to the iodide in all cases:

N1 Bromo to N1 Iodo ethyl benzotriazole = 95%

N1 Chloro to N1 Iodo ethyl benzotriazole = 94%

N2 Bromo to N2 Iodo ethyl benzotriazole = 97%

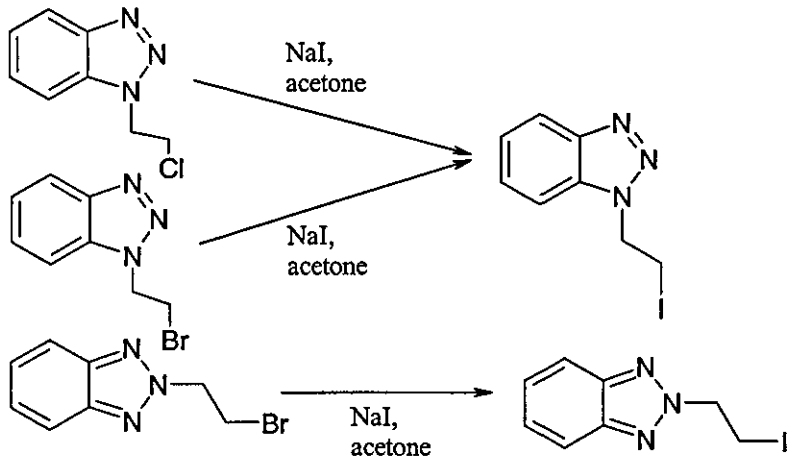


Figure 2.50 Formation of Iodoethylbenzotriazoles

Cysteine with Iodoethylbenzotriazoles

Both of the iodoethylbenzotriazoles in reaction with the protected cysteine gave good results.

These reactions were performed as previously detailed, with the iodoethylbenzotriazoles being added to the reaction mixture 10 min after the NaH. After purification by column chromatography, the desired S-alkylation products were obtained in a yield of 75% from the N1-series and in a yield of 74% for the N2-series (Figure 2.51).

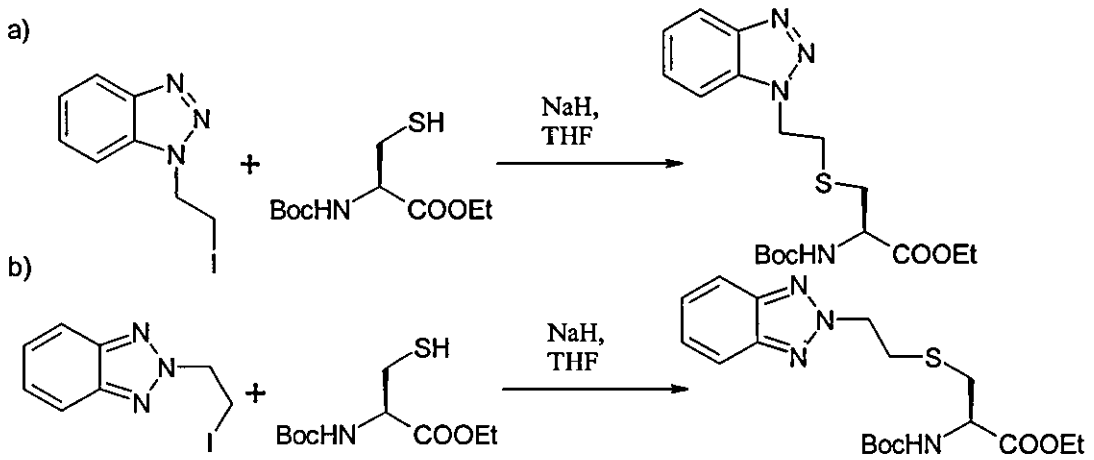


Figure 2.51 a) N1-Iodoethylbenzotriazole with Boc-cysteine ethyl ester
b) N2-Iodoethylbenzotriazole with Boc-cysteine ethyl ester

Having prepared a number of heterocylic amino acids based on haloethyl heterocycles, it was decided to extend the studies to use longer linkers available from ω-halopropyl or butyl heterocycles, but first it was necessary to prepare suitable substrates.

Iodopropyl and Iodobutyl Benzimidazole Reactions

N1-(3-Iodopropyl)benzimidazole can, like N1-(2-iodoethyl)benzimidazole, be formed via both the Mitsunobu and PTC methods. Both methods were tried in order to identify which gave the highest yield of the N1-(3-iodopropyl)benzimidazole.

N1-(3-Bromopropyl)benzimidazole

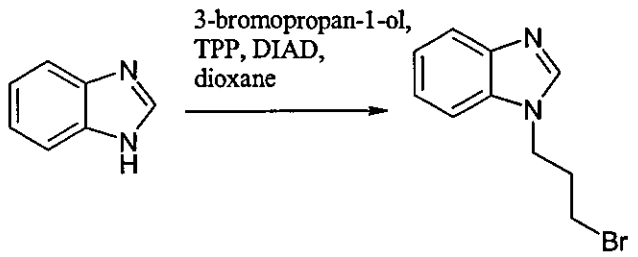


Figure 2.52 Formation of N1-(3-Bromopropyl)benzimidazole

Benzimidazole was N-bromoalkylated under Mitsunobu conditions (Figure 2.52). The benzimidazole, 3-bromopropanol and TPP were stirred in dry dioxane with DIAD being added dropwise over 1 h. After being left to stir at room temperature overnight the solvent was removed under reduced pressure. The crude product was purified by column chromatography to give the N1-(3-bromopropyl)benzimidazole with a yield of 78%.

N1-(3-Chloropropyl)benzimidazole

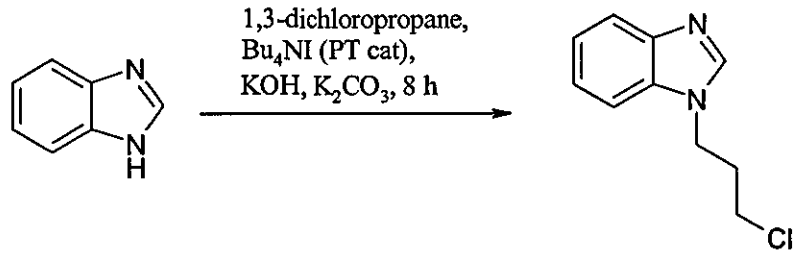


Figure 2.53 Formation of N1-(3-chloropropyl)benzimidazole

Benzimidazole was added to a mixture of 1,3-dichloropropane, tetrabutylammonium iodide, KOH and K_2CO_3. The reaction was however stirred for longer at room temperature

for 8 h (Figure 2.53). After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the desired product came off easily and cleanly but still only gave a 62% yield.

N1-(3-Iodopropyl)benzimidazole

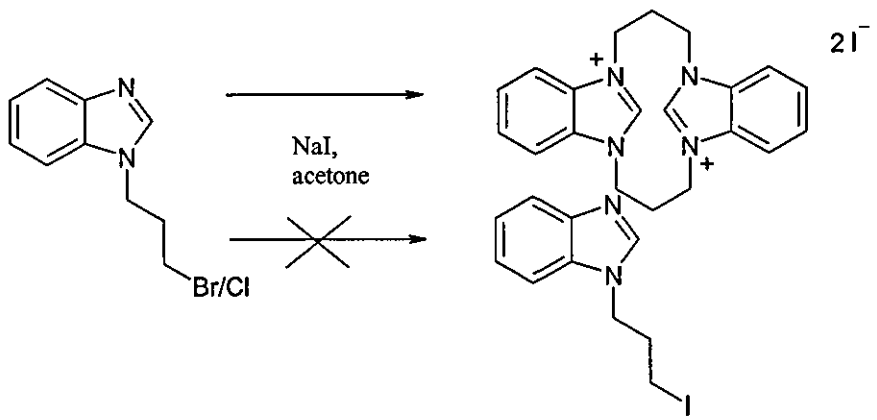


Figure 2.54 Attempted conversion of Bromo to N1-(3-Iodopropyl)benzimidazole

The Finkelstein halogen exchange reaction was then performed using dry acetone and NaI (Figure 2.54). However the desired product via the halogen exchange of either the chloride or bromide to the iodide did not form. After careful examination by NMR spectroscopy and mass spectometry it was concluded that a macrocyclic double quaternary salt had been formed, in a quantitative yield. The ^{1}H NMR spectrum clearly showed the presence of triplets and a quintet, meaning that the iodo group had not been reduced or eliminated as has been see for other reactions, and the mass spectrum gave ions of m/z 317.30 and 158.93 from LC/MS. The ion at 317.30 corresponds to loss of a proton from the doubly charged quarternary salt, while 158.93 corresponds to the mass/charge ratio of the doubly charged cyclic salt.

N1-(4-Chlorobutyl)benzimidazole

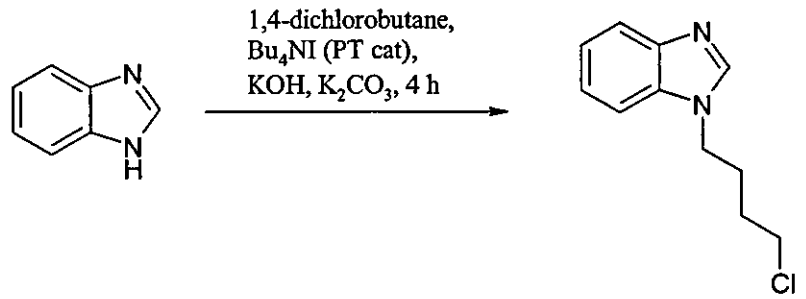


Figure 2.55 Formation of N1-(4-chlorobutyl)benzimidazole

Benzimidazole was added to a mixture of 1,4-dichlorobutane, tetrabutylammonium iodide, KOH and K_2CO_3 as shown in Figure 2.55. The reaction was stirred at room temperature for 8 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where the desired product came off easily and cleanly but only gave a 58% yield.

N1-(4-Iodobutyl)benzimidazole

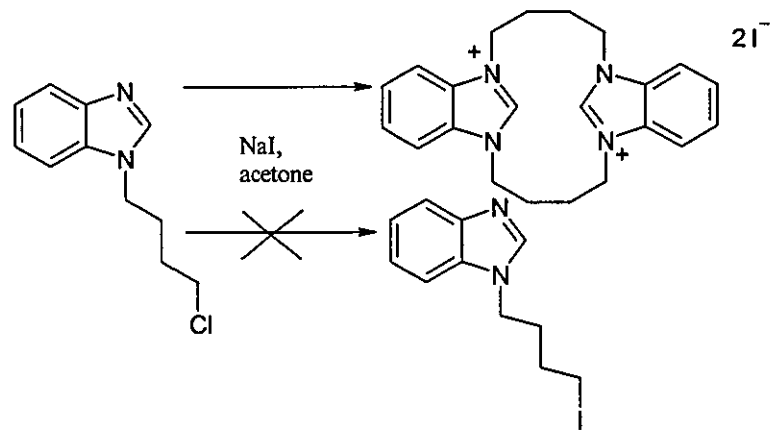


Figure 2.56 Bromo to Iodobutylbenzimidazole

The Finkelstein reaction was once again performed using NaI and acetone at reflux for 4 h (Figure 2.56). After careful examination by NMR spectroscopy and mass spectometry it was concluded that a quaternary salt had again been formed, in a quantitative yield. The NMR clearly showed the quintets and triplets as would be present in the quaternary salt, and the mass spectrum gave the ions at masses of 173.04 and 345.64, equivalent to the mass/charge ratio of the doubly charged salt and the double salt after the loss of a proton. The lack of success in preparing N-(3-iodopropyl) or N-(4-iodobutyl)benzimidazole led to further investigation with the other heterocycles.

Further Indole Reactions

Next the formation of N-(3-iodopropyl) and N-(4-iodobutyl)indole was attempted. This was tried only via the PTC method as the Mitsunobu method had not been successful for the ethyl derivatives and the length of the alkyl group should make little or no difference under Mitsunobu conditions.

N-(3-Iodopropyl)indole

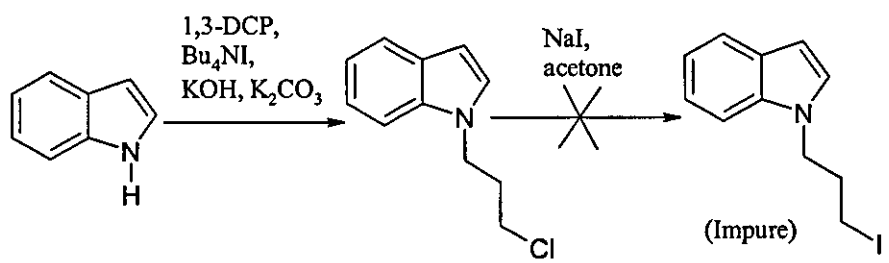


Figure 2.57 Formation of Chloro (and Iodo) propylindole

Indole was added to a mixture of 1,3-dichloropropane, tetrabutylammonium iodide, KOH and K_2CO_3. The reaction was then stirred at 50°C for 24 h and it was noted that the reaction had formed a dark brown colour. After the inorganics had been filtered off and the organic portion worked up, the mixture was put through flash chromatography, where a major product was isolated, in a yield of 61%, which proved to be the N-(3-chloropropyl)indole as shown in Figure 2.57.

The Finkelstein halogen exchange reaction was then performed using dry acetone and NaI to give the desired product in an impure state via halogen exchange of the chloride to the iodide with an approximate yield of 66% as an dark brown oil (Figure 2.56). The product was inseparable from the mixture and no substitution reactions were attempted.

N-(4-Chlorobutyl)Indole

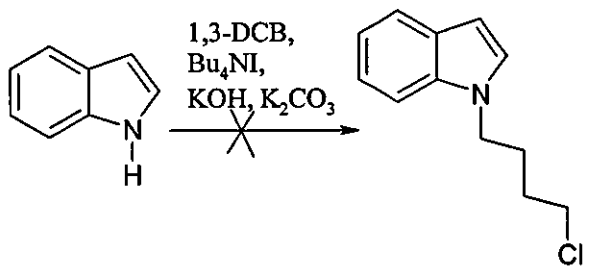


Figure 2.58 Formation of Chlorobutylindole

Indole was added to a mixture of 1,4-dichlorobutane, tetrabutylammonium iodide, KOH and K_2CO_3 (Figure 2.58). The reaction was stirred at room temperature for 48 h. After the inorganics had been filtered off and the organic portion worked up, the ^1H NMR spectrum was unclear and so flash chromatography was performed. After careful examination by NMR spectroscopy and mass spectometry of the different fractions, it was concluded that it

was not be possible for any chlorobutylindole to be separated from the mixture and so it would not be possible to form the corresponding iodo compound.

Further Reactions Involving Benzotriazole

As the investigations into the formation of iodoethylbenzotriazoles had given varying results depending on which reaction had been used, for the formation of the isometric iodopropylbenzotriazole compounds, both the Mitsunobu and the PTC methods were used.

Bromopropylbenzotriazole

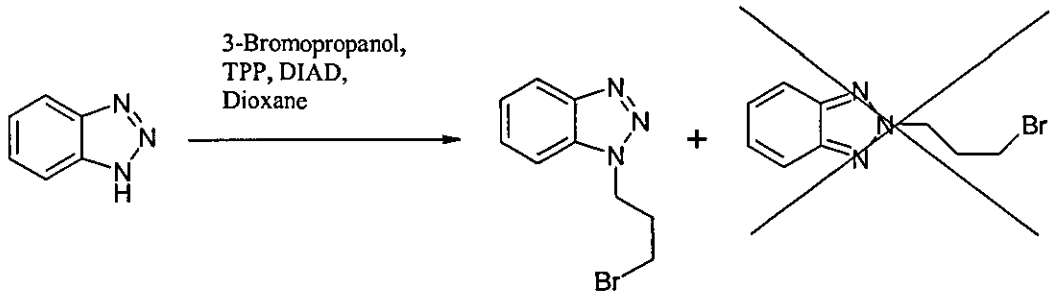


Figure 2.59 Benzotriazole with 3-Bromopropanol

Benzotriazole was bromoalkylated under Mitsunobu conditions. Benzotriazole, 3-bromopropanol and TPP were stirred in dry dioxane with DIAD being added dropwise over 1 h. After being left to stir at room temperature overnight the solvent was removed under reduced pressure. The crude product was purified by column chromatography to unexpectedly give only the N1-(3-bromopropyl)benzotriazole isomer with a yield of 78% (Figure 2.59).

Chloropropylbenzotriazole

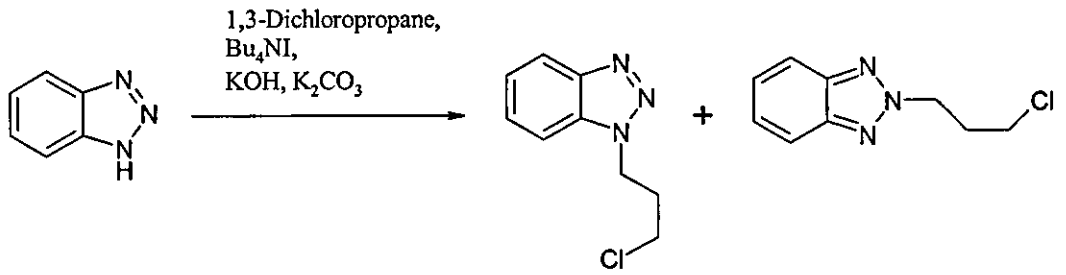


Figure 2.60 Benzotriazole with 1,3-dichloropropane

Benzotriazole was added to a mixture of 1,3-dichloropropane, tetrabutylammonium iodide, KOH and K_2CO_3 and the reaction was stirred at room temperature for 24 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was subjected to column chromatography, where both products were separated easily and cleanly to give the N1 alkylated compound in 52% yield and the N2 compound in a smaller yield of 24% (Figure 2.60). For the chloropropyl reaction the ratio of N1:N2 alkylation is 2:1, the statistical ratio that was not seen previously for the chloroethyl and bromoethyl reactions. Again this was confirmed by the use of NMR techniques.

Iodopropylbenzotriazole.

As previously the Finkelstein halogen exchange reaction was then performed using dry acetone and NaI, at reflux for 48 h, to give the desired product via the halogen exchange of the chloride to the N-(3-iodopropyl)benzotriazoles (Figure 2.61).

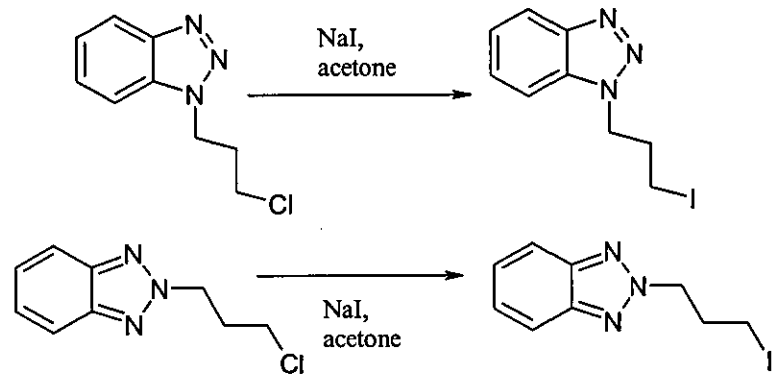


Figure 2.61 Chloro to Iodopropylbenzotriazoles

The yields vary for each case and in each case cream coloured crystals were formed:

N1-chloro to N1-(3-iodopropyl)benzotriazole = 77%

N2-chloro to N2-(3-iodopropyl)benzotriazole = 65%.

Chlorobutylbenzotriazole

As for the chloropropyl derivative above, only the chlorobutyl alkylation using the PTC method was performed (Figure 2.62).

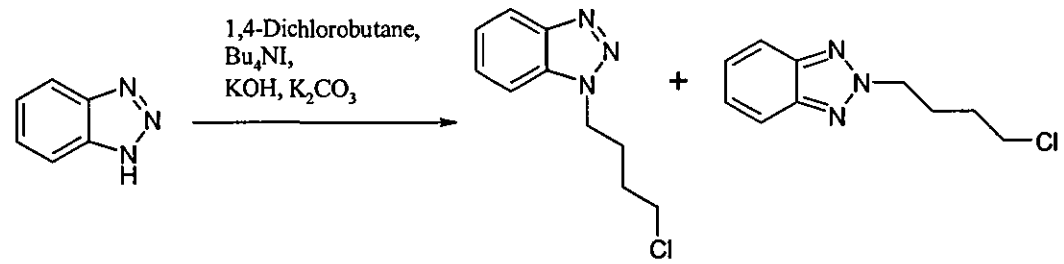


Figure 2.62 Formation of Chlorobutylbenzotriazoles

Benzotriazole was added to a mixture of 1,4-dichlorobutane, tetrabutylammonium iodide, KOH and K_2CO_3. The reaction was stirred at room temperature for 24 h. After the inorganics had been filtered off and the organic portion worked up, the mixture was subjected to column chromatography, where both products were separated easily and cleanly to give the N1-alkylated compound in 24% yield and the N2 compound in an almost equivalent yield of 21%. For the chlorobutyl reaction the ratio of N1:N2 was therefore 1:1.

Iodobutylbenzotriazole

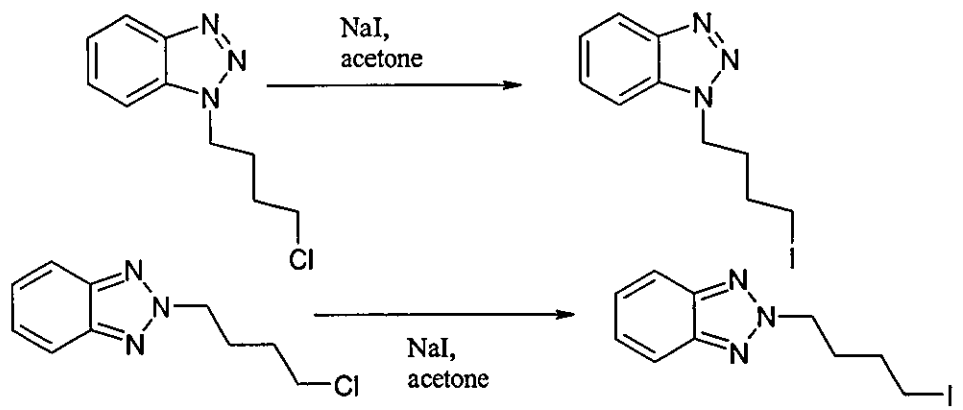


Figure 2.63 Chloro to Iodobutylbenzotriazoles

The Finkelstein halogen exchange reaction was again performed using dry acetone and NaI, at reflux for 48 h, to give the desired N-(4-iodobutyl) products via halogen exchange of the chloride to the iodide (Figure 2.63). The N1-(4-iodobutyl)benzotriazole was formed in a yield of 70%, and the N2-(4-iodobutyl)benzotriazole also in a yield of 70%. This reaction was again simple to perform with good yields of the products being formed.

Cysteine with N-(3-Iodopropyl)benzotriazole

The cysteine alkylations with N-(3-iodopropyl)benzotriazole reactions were both performed as for the previous nucleophilic substitution reactions. N-(3-Iodopropyl)benzotriazole was added to the reaction mixture 10 min after the NaH was added to the protected cysteine. Purification by column chromatography then gave the desired product.

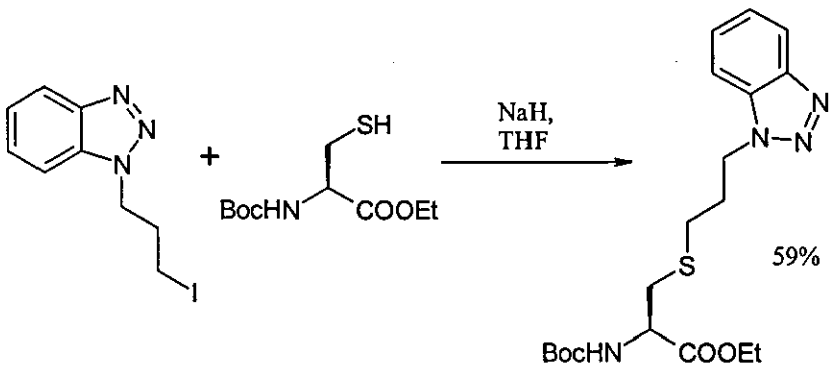


Figure 2.64 Reaction of Boc-cysteine ethyl ester with N1-(3-iodopropyl)benzotriazole

N-Boc-S-[3-(Benzotriazol-1-yl)propyl] cysteine ethyl ester was obtained in a yield of 59% (Figure 2.64). This was a reasonable yield although a second column was necessary to completely purify the desired product.

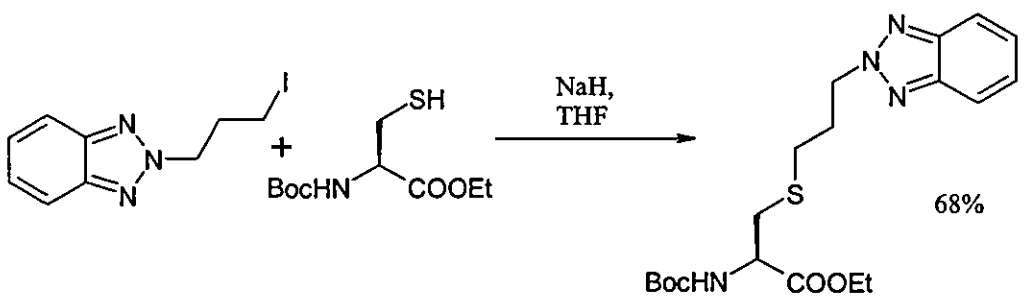


Figure 2.65 Reaction of Boc-cysteine ethyl ester with N2-(3-iodopropyl)benzotriazole

N-Boc-S-[3-(Benzotriazol-2-yl)propyl] cysteine ethyl ester was obtained in a yield of 68% (Figure 2.65). This was a reasonable yield although again a second column was necessary to completely purify the desired product.

Cysteine with N-(4-Iodobutyl)benzotriazole

The cysteine with N-(4-iodobutyl)benzotriazole reactions were both performed as for the previous nucleophilic substitution reactions. N-(4-Iodobutyl)benzotriazole was added to the reaction mixture 10 min after the NaH was added to the protected cysteine. Purification by column chromatography then gave the desired product.

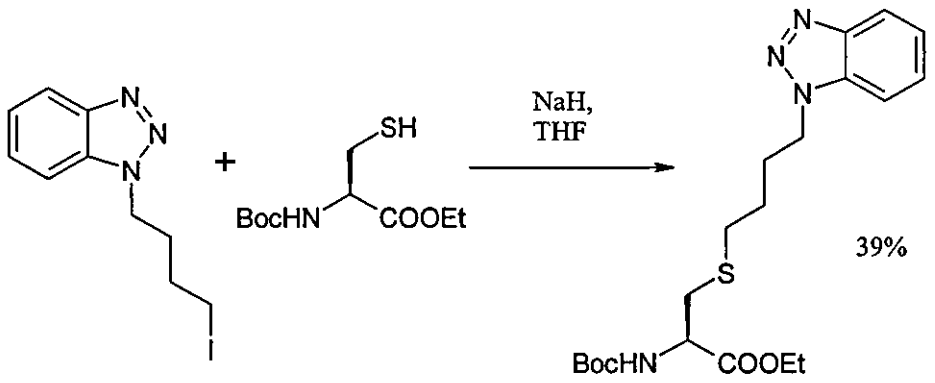


Figure 2.66 Reaction of Boc-cysteine ethyl ester with N1-(4-iodobutyl)benzotriazole

N-Boc-S-[3-(Benzotriazol-1-yl)butyl] cysteine ethyl ester was obtained in a yield of 39% (Figure 2.66). Subsequent reactions were performed, but the yield did not improve above this, although it is unclear why such a low yield was obtained.

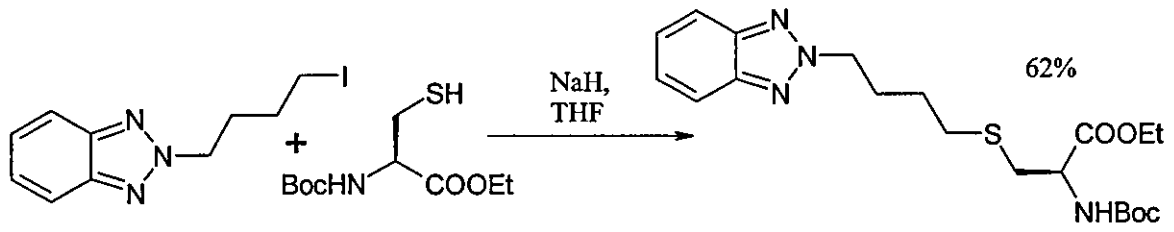


Figure 2.67 Reaction of Boc-cysteine ethyl ester with N2-(4-iodobutyl)benzotriazole

N-Boc-S-[3-(Benzotriazol-2-yl)butyl] cysteine ethyl ester was obtained in a yield of 61% (Figure 2.67). This was a much better yield than for the N1 compound, although it is unclear why.

Further Adenine Reactions

Due to time constraints it as decided that, being one of the five nucleobases, adenine, rather than purine would be investigated with the longer linker.

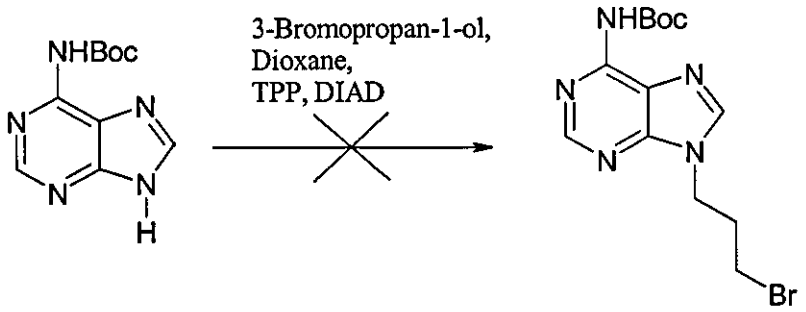


Figure 2.68 Formation of N9-(3-Bromopropyl)-Boc-adenine

It was shown from the previous reactions involving adenine and the Mitsunobu reaction that some problems may occur, and it was thought that as for the haloethyl reactions, there

would be a similar problem. Under Mitsunobu conditions, Boc-adenine, 3-bromopropanol and TPP were stirred in dry dioxane with DIAD added dropwise over 1 h at 5°C, Figure 2.68. The mixture was allowed to warm to room temperature and was stirred overnight to give a yellow solution. The solvent was then removed under reduced pressure followed by some purification by column chromatography. No N9-(3-bromopropyl)-(Boc)adenine was found, so the crude products were reacted a second time, and then a third time, under the same conditions, however none of the desired product was found and there was no further investigation with adenine.

2.8.1.2 Summary of Cysteine Reactions

The reactions of cysteine with various heterocycles has been successful with a good variety of heterocyclic systems and linker chain lengths. These variations have been successfully employed to generate novel S-linked protected heterocyclic amino acids as shown in Figures 2.69 and 2.70.

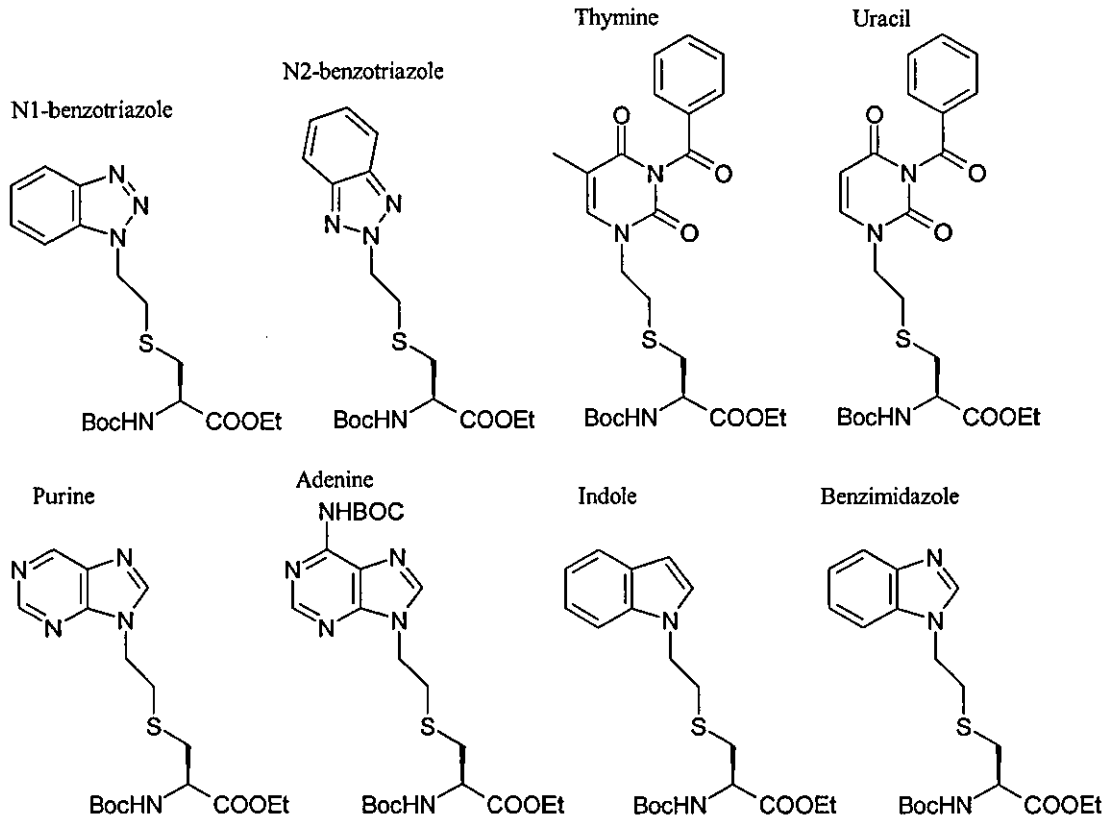
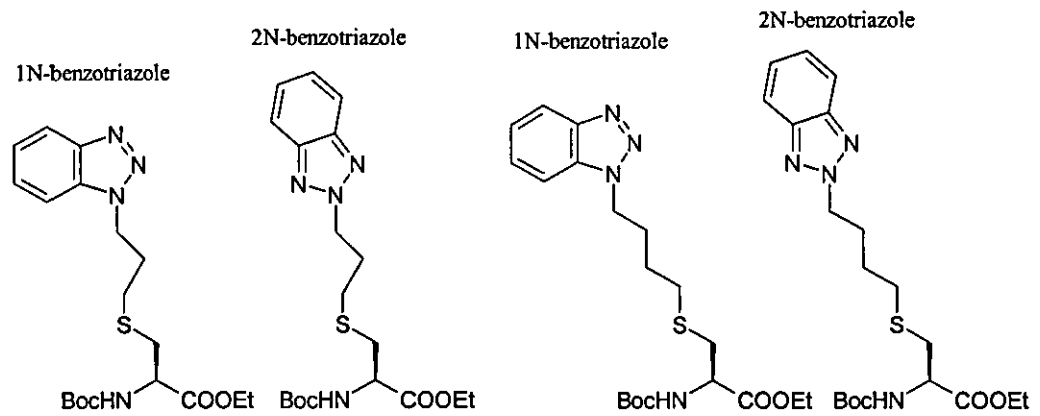


Figure 2.69 Ethyl-linked Cysteine Products



Figur 2.70 Propyl- and Butyl-linked Products

2.8.2 Serine

Serine was an obvious choice for use in the substitution reactions as another heteroatom-containing amino acid, providing an O-linker. The same route as with cysteine was used in the protection of serine by esterification on the acid moiety and by the use of a Boc group on the amino moiety.

2.8.2.1 Ester Protected Serine

Protection of Acid group by Esterification

The methyl ester of L-serine was available comercially, so no ester protection reaction at the acid moiety was necessary.

Protection of Amino Group

The Boc protection of serine was performed as for the Boc protection of the amino group on cysteine.[94]

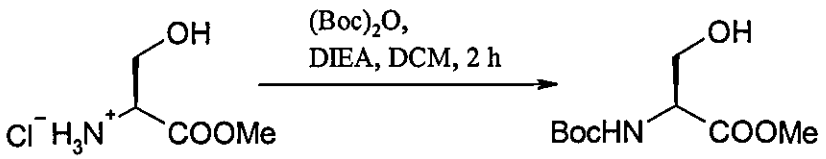


Figure 2.71 Boc protection of serine

To a dry round-bottomed flask was added serine methyl ester, $(Boc)_2O$ and DIEA in dry DCM. The reaction was then stirred for 2 h at room temperature and the product was purified after aqueous work up to give Boc serine methyl ester in 94% yield (Figure 2.71).

Reaction of Boc Serine Methyl Ester with N1-(2-Iodoethyl)benzimidazole

The first substitution reaction that was attempted with Boc serine methyl ester was again with N1-(2-iodoethyl)benzimidazole. To a flame-dried round-bottomed flask was added the protected serine in THF which was then cooled before the addition of NaH. The N-(2-iodoethyl)benzimidazole was then added after 5 to 10 min. After refluxing overnight the reaction was worked up as before (Figure 2.72). No substitution products from reaction of the protected serine derivative and N1-(2-iodoethyl)benzimidazole were seen.

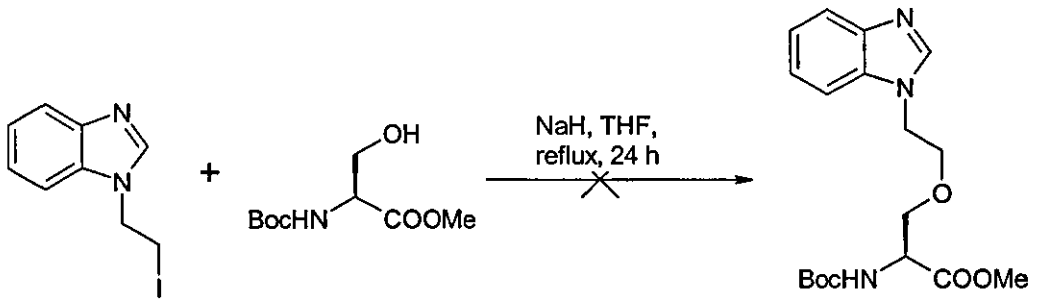


Figure 2.72 Planned Reaction for Addition of Serine to N1-(2-iodoethyl)benzimidazole

Initially it was thought that the benzimidazole derivative was being added to the reaction too soon after the addition of the NaH so this was investigated further by varying the time at which the N1-(2-iodoethyl)benzimidazole was added after the NaH.

The N1-(2-iodoethyl)benzimidazole was added directly after the NaH (at 0 min) or at 15, 30, 45 and 60 min after the addition of the NaH. In all cases none of the desired product was seen and the results are shown in Table 2.5 below:

Time (min)	Results
0	Vinyl benzimidazole + other inseparable material
15	Vinyl benzimidazole + other inseparable material
30	Iodoethyl benzimidazole + Vinyl benzimidazole + other inseparable material
45	Iodoethyl benzimidazole + Vinyl benzimidazole + other inseparable material
60	Iodoethyl benzimidazole + other inseparable material

Table 2.5 Times after addition of NaH for the N1-(2-iodoethyl)benzimidazole addition

It was suggested that the nucleophilic oxygen on the side-chain was attacking the ester group on another protected serine, shown in Figure 2.73, and hence not forming the desired addition product.

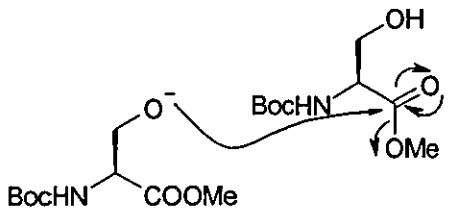


Figure 2.73 Nucleophilic nature of Oxygen

This could happen to form a number of different molecular mass ogliomers based on the same 'monomer' serine unit, which could explain the messy NMR spectra. The alkoxide

side-chain is less nucleophilic in S_N2 reactions than the thiolate of deprotonated cysteine, and is 'harder'.

t-Butyl Ester Protected Serine

It was thought that a bulkier ester group may prevent the deprotonated oxygen attacking the ester moiety, so the use of a t-butyl group was investigated (Figure 2.74).[95]

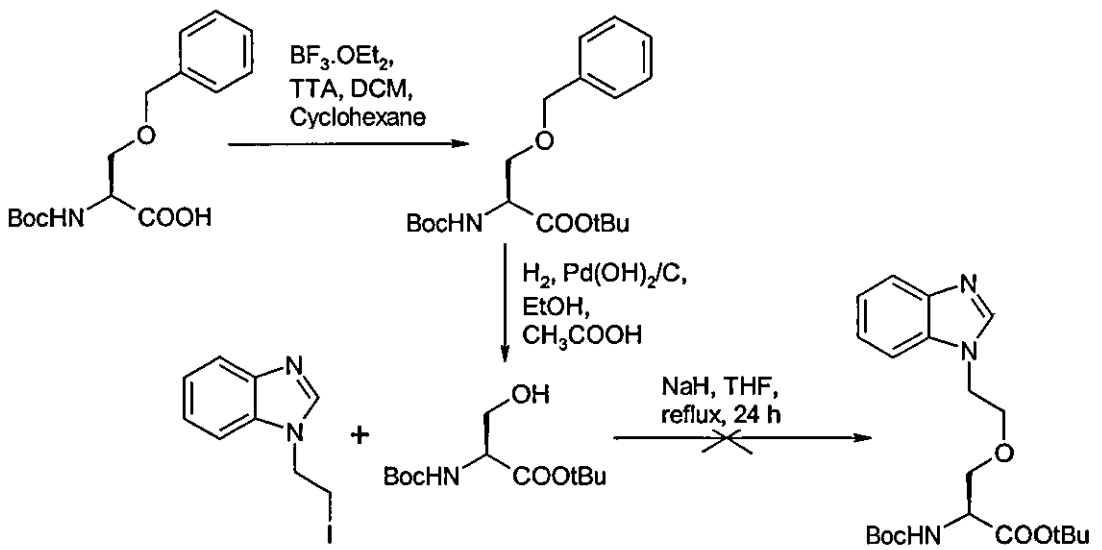


Figure 2.74 Preparation of t-butyl protected serine and reaction with N1-(2-iodoethyl)benzimidazole

Commercially available O-benzyl-Boc-L-serine in DCM was stirred in a round-bottomed flask under nitrogen. To this was added t-butyltrichloroacetimidate (TTA) in dry cyclohexane followed by BF_3:OEt_2 and the mixture was stirred for 14 h at room temperature. $NaHCO_3$ was then added to neutralise the acid. The solvent was removed under reduced pressure and the desired t-butyl ester purified by column chromatography in

a yield of 99%. The benzyl group was then removed in hydrogen saturated $EtOH/CH_3COOH$ under a hydrogen atmosphere in the presence of $Pd(OH)_2/C$ (10%) catalyst to give a quantitative yield of Boc-L-serine t-butyl ester (Figure 2.74).

The same reactions were then performed on the Boc serine t-butyl ester as for the Boc serine methyl ester. As shown in Figure 2.75, a flame-dried round-bottomed flask containing the serine derivative in THF was cooled before the addition of NaH and then the N1-(2-iodoethyl)benzimidazole after varying time periods as summarised in Table 2.6. The use of the t-butyl ester did not seem to aid in the formation of the desired substitution product and it did not seem to matter at what time after the NaH the N1-(2-iodoethyl)benzimidazole was added, the ^1H NMR spectra of the reactions were still complex and the products could not be separated. A summary is shown in Table 2.6.

Time (min)	Results
0	Vinyl benzimidazole + other inseparable material
15	Vinyl benzimidazole + other inseparable material
30	Vinyl benzimidazole + other inseparable material
45	Iodoethyl benzimidazole + Vinyl benzimidazole + other inseparable material
60	Iodoethyl benzimidazole + Vinyl benzimidazole + other inseparable material

Table 2.6 Times after addition of NaH for the N1-(2-iodoethyl)benzimidazole addition

Although complex, the ^1H NMR spectra did however show a lack of any peak at δ1.5 and 2.0, meaning that both the t-butyl ester and Boc groups have disappeared. An explanation for the former has already been shown for the methyl ester. Despite the bulkiness of the

t-butyl group the nucleophilic nature of the deprotonated oxygen means it is attacking at the ester to give an ester exchange.

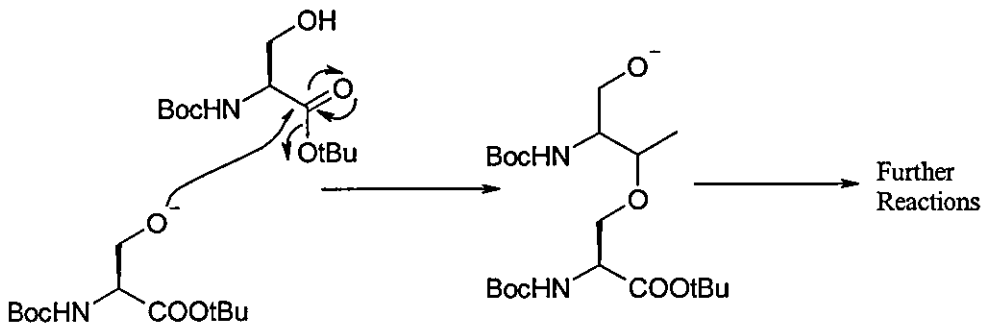


Figure 2.75 The Nucleophilic Nature of Oxygen

2.8.2.2 Amide Protected Serine

It was at this time that a paper was found which appeared to support this explanation for the problems that had been experienced with serine. Katoh *et al.*[96] (Figure 2.76) have tried to use the method of alkylating a nucleophilic oxygen using a haloester in just such a way as in this investigation, but also encountered the same problems as surmised above in Figure 2.76. It was also shown in this paper that an iodo group, if present, is eliminated under these conditions and does not take part in any substitution reaction. Katoh solved the problem to some degree by the use of different groups, which was not feasible for this case.

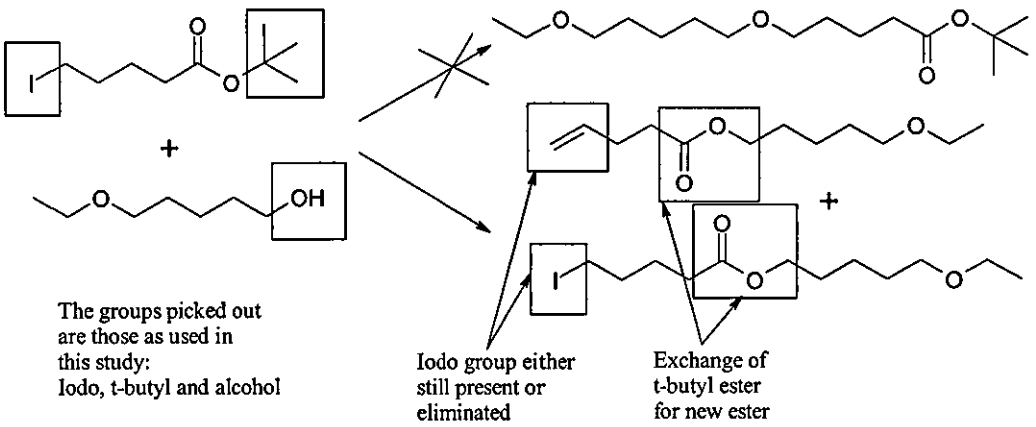


Figure 2.76 The ester exchange reaction as shown by Katoh *et al.*[96]

Amide Protection of Serine

It was then decided that an amide should be tried as a replacement to the ester protection, to reduce the carbonyl electrophilicity. The procedure for amidation of serine is not overly complicated. Methanol was saturated with ammonia at 78°C and Boc serine methyl ester was then added. This mixture was then shaken at room temperature overnight and the product obtained in 98% yield simply by removal of the ammonia and solvent under reduced pressure (Figure 2.77). The reaction could be easily followed by the use of IR spectroscopy as the carbonyl peak moves from 1740 to 1685 cm^{-1}.

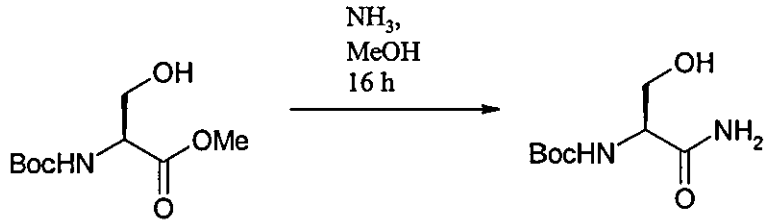


Figure 2.77 Amide protection of serine

The Boc serine amide was then reacted with N1-(2-iodoethyl)benzimidazole by the same method as for the previous experiments, using THF and NaH, but again none of the desired substitution product was seen (Figure 2.78), nor any N-vinyl benzimidazole. The ^{1}H NMR spectra were unclear as were the TLCs and it was decided not to pursue this approach any further.

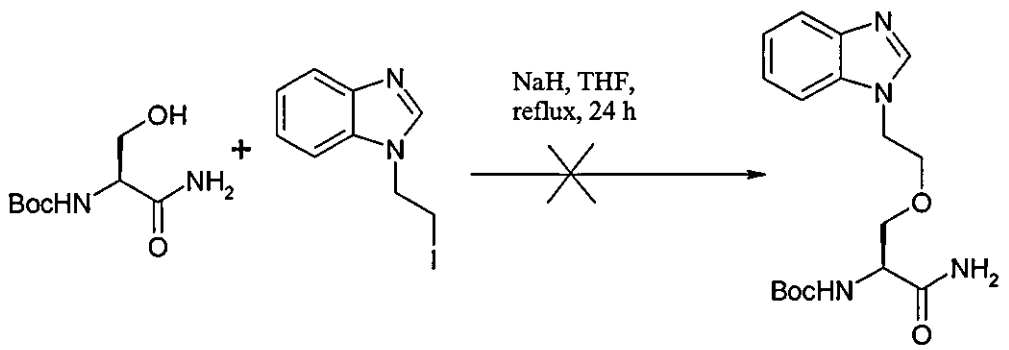


Figure 2.78 Reaction of iodoethyl benzimidazole with Boc serine amide

It was possible that the NH of the secondary amide group could have been causing a problem and so an attempt was made at protecting the serine carboxylic acid moiety using a tertiary amide.

Boc serine piperidine amide

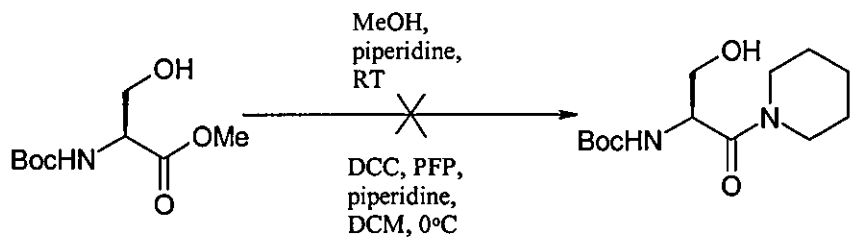


Figure 2.79 Piperidine protection of serine

The first method of piperidine amide formation from Boc-serine methyl ester, by reaction of piperidine in MeOH at room temperature, was tried without success (Figure 2.79). Before any further investigation could take place a method was found for the substitution using a serine derivative which did not involve the protection of the acid moiety.

2.8.2.3 Boc Only Protected Serine

Sugano and Miyoshi[97] have reported the side-chain benzylation of Boc-serine using DMF and NaH, i.e. having the carboxylic acid group present as the carboxylic salt. It was decided to try this method for serine with N1-(2-iodoethyl)benzimidazole.

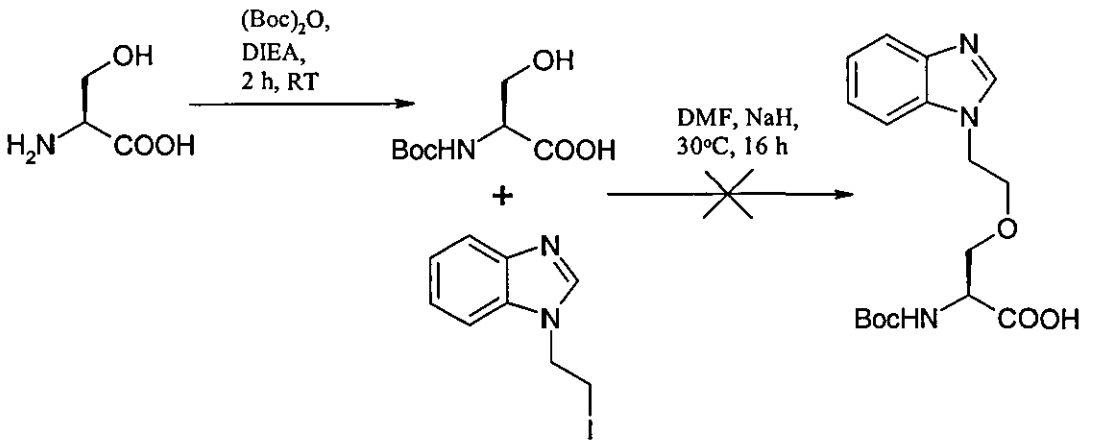


Figure 2.80 Boc-serine with N1-(2-Iodoethyl)benzimidazole

L-Serine was first protected using the Boc procedure as outlined previously, using $(Boc)_2O$ and DIEA in dry DCM at room temperature for 2 h. This gave a quantitative yield of Boc protected serine at 100% (Figure 2.80).

The next stage involving the substitution reaction was performed similar to the reactions involving THF/NaH but using DMF due to solubility problems of the Boc-amino acid in THF. To a flame-dried round-bottomed flask was added Boc-serine in DMF under nitrogen. This was cooled to 0°C and NaH (2 equiv) was added. The reaction was then allowed to warm slowly to room temperature and all the NaH was given time to react before the addition of N1-(2-iodoethyl)benzimidazole. The reaction was then stirred overnight at 30°C. The solvent was removed under reduced pressure and the residue dissolved in H_2O/ether. The ether was separated off and, upon acidification, the aqueous layer was extracted with EtOAc. The solvents were removed from both the ether and EtOAc solutions under reduced pressure.

With a reaction time of 30 min between Boc-serine and NaH the only product obtained was N-vinyl benzimidazole in both the ether and EtOAc solutions and the recovery of the Boc protected serine from the water layer, despite the acidification. It was decided that the NaH needed more time to react with serine so the time that the NaH was given to react was raised to 90 min.

With the 90 min reaction the ^{1}H NMR spectrum of the EtOAc extract showed no sign of the desired product, but some N1-(2-iodoethyl)benzimidazole starting material was seen. The ether layer for both reactions showed only the elimination product, vinyl benzimidazole. The water layer was also examined for product by ^{1}H NMR spectroscopy but again there was no product seen.

From these results it is possible to say that the 30 min time was too short and when the N1-(2-iodoethyl)benzimidazole was added the residual NaH simply caused elimination. The 90 min delay time showed that not all of the N1-(2-iodoethyl)benzimidazole was eliminated (presumably as less NaH remained) and was probably a reasonable time for addition of the reactant. It was decided to perform the reaction in the same manner as the earlier alkylations, using THF at reflux despite the solubility problems.

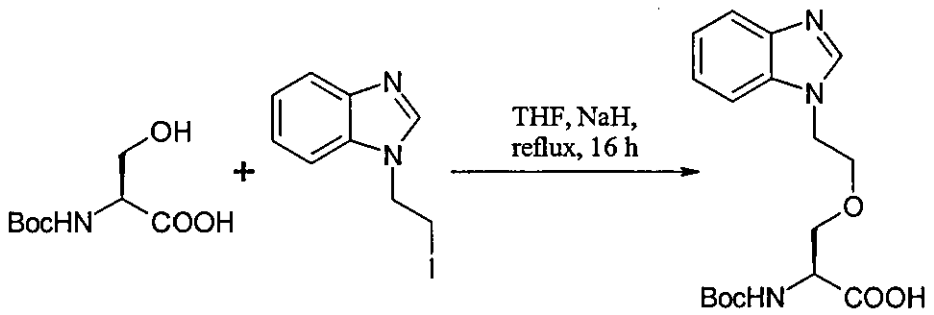


Figure 2.81 Reaction of Boc serine with iodoethyl benzimidazole

To a flame-dried round-bottomed flask was added Boc-serine in THF under nitrogen. This was cooled to 0°C and NaH (2 equiv) added. The reaction was allowed to warm to room temperature and after 90 min N1-(2-iodoethyl)benzimidazole was added and the reaction was then refluxed for 16 h (Figure 2.81). The solvent was removed under reduced pressure and the residue dissolved in H_2O/ether, the ether layer separated off and the water acidified and extracted with EtOAc. The solvents in both cases were removed under reduced pressure. The ether layer contained only N-vinyl benzimidazole, but the EtOAc layer was more promising and the ^1H NMR spectrum showed signs of a different product that could be the desired product, but it needed to be purified. After column chromatography of the residue from the EtOAc layer the ^1H NMR spectrum showed a promising spectrum of the

desired O-alkylation product although not yet completely clear. Support was given by the mass spectra where LCMS which uses ESMS and FAB showed peaks at masses 349 (±1H) and 247 (±1H), respectively. The former is the mass of the desired addition product and the latter corresponds to the mass if the Boc group has been removed in the mass spectrometer. The yield for this reaction was however low at only 35%.

2.8.2.4 Summary of Serine Reactions

After attempts to perform the O-alkylation of serine with N1-(2-iodoethyl)benzimidazole by first protecting the acid moiety, it was eventually found that a promising result was obtained from the reaction with no protection on the acid moiety. Although it was never possible to obtain the completely pure product, these were promising results for any future work with serine that may be performed in this area of research.

2.9 2,3-Diaminopropionic Acid

Another analogous compound to the cysteine that contains a heteroatom is 2,3-diaminopropionic acid, which would provide N-linked heterocyclic amino acids.
Like the other amino acids used, the amino and acid moieties can be protected using Boc and ester respectively. The 2,3-diaminopropionic acid reactions were run in parallel to those of serine described above.

Esterification

The racemic 2,3-diaminopropionic acid was converted into the methyl ester by heating at reflux in methanol in the presence of acid. The first attempt used acetyl chloride as the source of HCl.[98] To a flame-dried multi-necked round-bottomed flask under nitrogen was added the amino acid in dry methanol. This was then cooled in an ice bath to 0°C before the addition of acetyl chloride due the exothermic nature of the reaction. Acetyl chloride was then added slowly to the reaction, which was then allowed to warm to room temperature. The reaction was then heated at reflux for 48 h before purification of methyl ester hydrochloride salt by crystallisation. Although this reaction gave a very pure product, it unfortunately gave a very poor yield of only 20%. Many methods for forming the ester in the literature have used thionyl chloride (Figure 2.82), so this was the next reaction attempted.

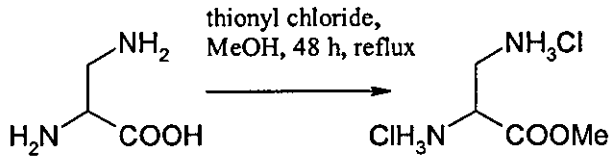


Figure 2.82 Methyl Ester Protection of 2,3-Diaminopropionic Acid

The same procedure was used for the addition of thionyl chloride as for acetyl chloride. The amino acid and dry methanol were added to a flame-dried multi-neck round-bottomed flask, which was then cooled in an ice bath to 0°C. The thionyl chloride was added slowly and the reaction mixture was then allowed to warm to room temperature before being heated at reflux for 4 h. Upon examination of the products after this time, the reaction had

gone a good way to completion, but starting material was left so another quantity of thionyl chloride was added at 0°C and then the reaction was refluxed for a further 24 h. This time the reaction had gone to completion and no starting material was left. The methyl ester bis-hydrochloride was purified by crystallisation in a 92% yield.[99,100,101]

Boc-protection / Lactam formation

The amino protection of diaminopropionic acid was more troublesome than for the oxygen or sulphur-containing amino acids as it is necessary to protect only one amino group of two which have comparable nucleophilic reactivity. Figure 2.83 below, highlights the problem of the two amino groups.

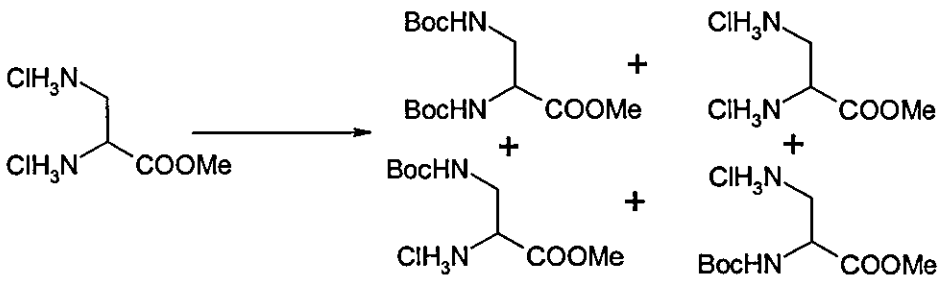


Figure 2.83 Possible Products of Boc Protection

Boc-protection was initially attempted on the simple ester with one equivalent of $(Boc)_2O$ to confirm that both amino groups would become protected. To a dry round-bottomed flask was added the methyl 2,3-diaminopropanoate hydrochloride salt, DIEA and $(Boc)_2O$ in dry DCM under nitrogen and the mixture was stirred for 2 h. The crude product was isolated and examined using ^1H NMR spectroscopy and mass spectrometry. The ^1H NMR spectrum showed what looked like two equal singlets at ~δ1.5 of 18 protons, and LCMS contained a

peak at 318.3, which confirm that some of the sample had become diprotected. A singly protected compound would have a single peak at ~δ1.5 and a mass of 253.7. It was therefore necessary to find a method where only one of the amino groups is protected and the formation of a β-lactam (Figure 2.84) was therefore considered.[98,102,103]

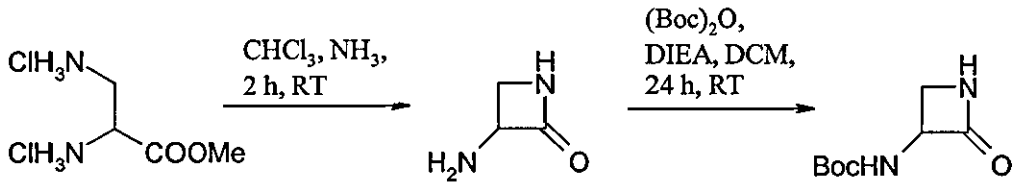


Figure 2.84 Formation of Lactam ring and Boc protection

There have been a number of other routes reported,[102,103,104] but due to time constraints the simplest method was tried as performed by Jones and Crockett to form the corresponding 5-membered ring system.[98] Chloroform was saturated with ammonia gas and the diamino ester bis-hydrochloride salt was added to the saturated solution, which was then shaken for 3 h at room temperature. The chloroform and ammonia gas were removed to leave the product, hopefully the β-lactam. It was difficult to characterise the product as the white solid produced was only soluble in water and although ^{1}H NMR spectra seemed to show the correct peaks, no NH peaks were visible in D_2O due to exchange. An IR spectrum of the compound was run as a KBr disc and this showed a movement of the carbonyl peak. It was decided to attempt to add a Boc group to any 'free' amino group in the hope that the compound would become more soluble. This was attempted using the same method as before in dry DCM with DIEA and $(Boc)_2O$. The product was slightly more soluble than before and the ^{1}H NMR analysis could be run in methanol. An LCMS of the product was also recorded. The ^{1}H NMR spectrum was not clean but the major peaks were those of the

desired product and the mass spectrum showed a clear peak at 186.4 (±1H). The product showed only one spot by TLC but although a short column was run it was difficult to clean up the product and the LCMS still showed masses at the desired 186.4 (±1H) and some diprotected product at 318.6 (±1H). It was decided to move on from the 2,3-diaminopropionic acid and look at the 2,4-diaminobutyric acid.

2.10 2,4-Diaminobutyric Acid

2,4-Diaminobutyric acid can be looked at in the same way as 2,3-diaminopropionic acid. It was decided to use the method to make the 5-membered lactam ring to selectively protect one amino group as this procedure has been previously successfully performed, probably due to less strain on the ring structure. The same route was used as for the four-membered lactam ring attempt, but with the use of commercial racemic 2,4-diaminobutyric acid.

Esterification

Firstly the methyl ester was formed using thionyl chloride and dry methanol at reflux for 48 h via the same method as described previously for 2,3-diaminopropionic acid, which gave the desired bis-hydrochloride product in a quantitative yield as white crystals (Figure 2.85).

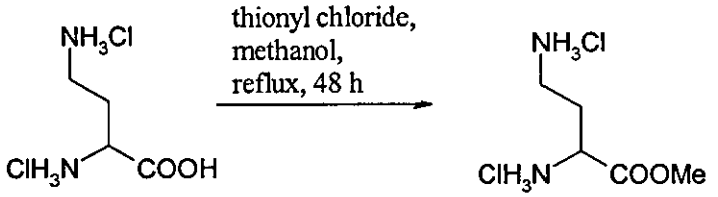


Figure 2.85 Ester protection of 2,4-diaminobutyric acid

Lactam Formation

2,4-Diaminobutyric acid ester can then be cyclised by shaking in ammonia-saturated chloroform for 3 h at room temperature. The product was gained in a yield of 86% after the removal of the solvents under reduced pressure. This product was difficult to analysis since, as had been found with the four-membered ring, it only dissolved in water. The IR analysis was performed using a KBr disc and this again showed a movement of the carbonyl peak from around 1710 to 1690cm^{-1}, indicating that the ester peak had changed to a lactam (Figure 2.86). ^{1}H NMR spectroscopy was also performed, signals at δ3.25 and δ3.38 for the methylene groups were present, but as the analysis was performed in D_2O no amino peaks were present.

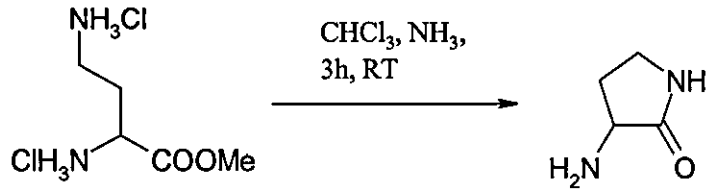


Figure 2.86 Lactam formation of 2,4-Diaminobutyric acid

Boc protection of Lactam

The 'free' amino group substituent of the presumed lactam was then protected using Boc in the hope that this would aid solubility. The Boc protection was performed in the same manner as before, by the use of $(Boc)_2O$ and DIEA in dry DCM, for up to 24 h at room temperature (Figure 2.87).

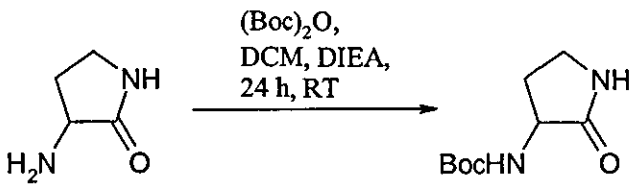


Figure 2.87 Boc protection of 5-membered Lactam

The ^1H NMR spectrum showed the Boc group to be present in the correct ratio with the lactam peaks by comparison of NMR integrals, with the Boc resonance occurring at δ1.63, but the Boc group did not seem to help solubility, and the NMR spectra had to be performed in D_2O so it was still not possible to see the NH peaks.

Reaction of 5-membered Lactam with N1-(2-Iodoethyl)benzimidazole

It was decided to try the N-alkylation of the lactam, despite the solubility problems making it difficult to analyse, with N1-(2-iodoethyl)benzimidazole (Figure 2.88) to see if the lactam could be reacted in the presence of the carbamate.

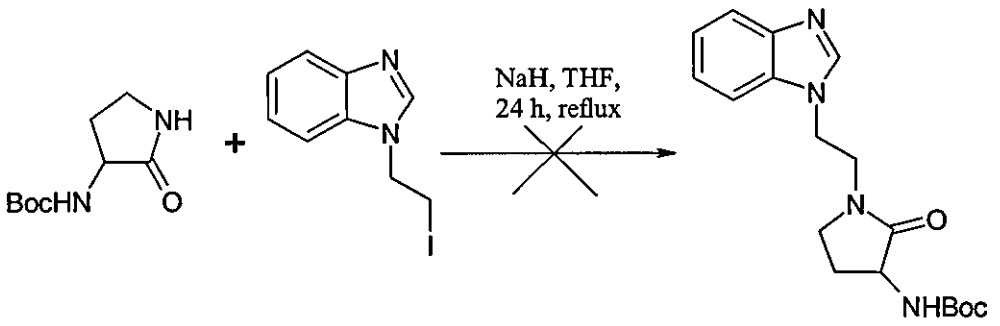


Figure 2.88 Reaction of 5-membered Lactam ring with N1-(2-Iodoethyl)benzimidazole

The lactam was suspended in dry THF in a flame-dried round-bottomed flask and cooled in an ice bath, NaH was then added and allowed to react for about 30 min, before the slow addition of the N1-(2-iodoethyl)benzimidazole. The mixture was then heated at reflux for 24 h. After work-up none of the substitution product was seen. N-Vinyl benzimidazole was the major product seen, which was disappointing as it was hoped that adding the N1-(2-iodoethyl)benzimidazole slowly would allow time for the desired substitution reaction to take place. In this case it is possible that the deprotonation of the nitrogen in the lactam ring is not occurring and the N1-(2-iodoethyl)benzimidazole simply reacts with the NaH.

Opening of the Lactam

Due to the failure of the lactam to react with N1-(2-iodoethyl)benzimidazole it was decided to open the ring and attempt the alkylation reaction again but using the singly Boc-protected 2,4-diaminobutyric acid.

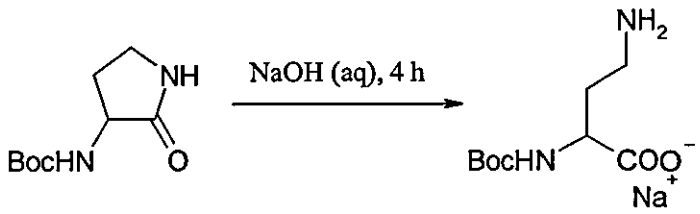


Figure 2.89 Opening of the Lactam ring

The 5-membered lactam ring was opened using a basic system, shown in Figure 2.89, as the use of an acid would remove the Boc protecting group. The lactam ring was stirred in aqueous NaOH for 4 h. The product, presumably as the salt, was obtained in a quantitative yield by the removal of water under reduced pressure. This was supported by ^{1}H NMR spectroscopy, where the CH_2 peaks had moved from δ3.25 and δ3.38 to δ2.91 and δ3.09, and IR spectometry where the lactam peak at 1690cm^{-1} was no longer present, and there was a peak at 1616cm^{-1}.

Reaction of Boc-2,4-Diaminobutyric Acid Sodium Salt with N1-(2-Iodoethyl)benzimidazole

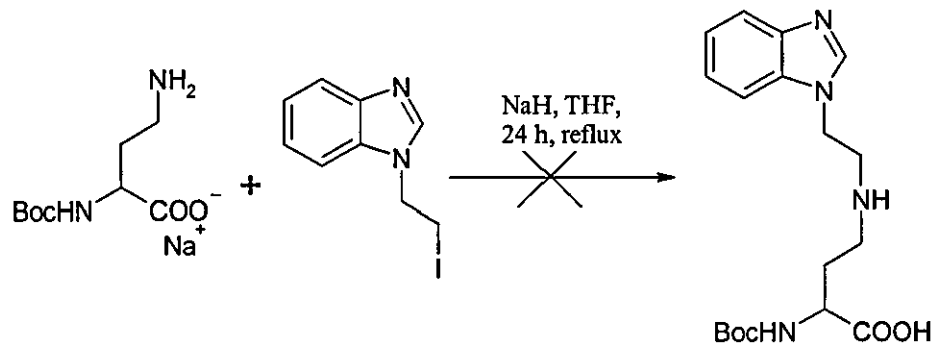


Figure 2.90 Reaction of Boc protected diaminobutyric acid and N1-(2-iodoethyl)benzimidazole

It was next attempted to use the crude salt in a substitution reaction with N1-(2-iodoethyl)benzimidazole. In a flame-dried round-bottomed flask under nitrogen was added the salt in THF which was then cooled to 0°C before the addition of the NaH (1equiv). The N1-(2-iodoethyl)benzimidazole was then added after 15 min and reaction warmed to room temperature and refluxed for 24 h (Figure 2.90). After work up the product was analysed using ^{1}H NMR spectroscopy, but this again showed no sign of the desired product, only vinyl benzimidazole from the elimination reaction with NaH. The failure may be due to deprotonation at the carbonyl moiety, or that the lactam formation/opening had not occurred as had been hoped.

2.11 Summary for Diamino Reactions

The major disadvantage of the diamino acid compounds is that of protection, the acid moiety can be easily protected in the same fashion as for the serine and cysteine compounds, but due to the presence of two amino groups the Boc protection of the α-amino group is more difficult. Even if the desired groups had been protected (which was difficult to confirm due to the insolubility of the compounds in organic solvents), the final substitution reaction was unsuccessful.

2.12 Homocysteine

Due to the success with cysteine itself, it was decided to investigate the use of homocysteine in these reactions, the added advantage being that if the reactions did work

this would open up the possibility of various chain lengths on the amino acid side of the heteroatom.

Homocysteine protection

Due to the lack of time available, racemic homocysteine was obtained commercially, ready protected as Boc-homocysteine methyl ester.

Boc-Homocysteine methyl ester with N1-(2-Iodoethyl)benzimidazole

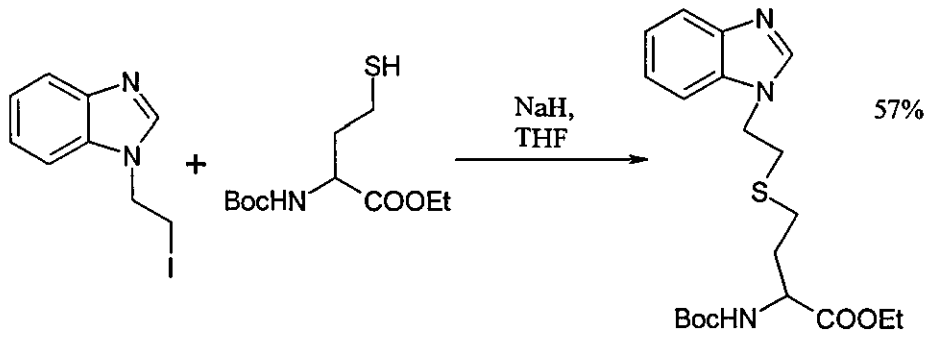


Figure 2.91 Boc-Homocysteine methyl ester with N1-(2-Iodoethyl)benzimidazole

As for the other alkylation reactions, to a dry round-bottomed flask was added the NaH, dry THF and protected homocysteine at 0°C and under nitrogen. These were stirred together for 15 min and allowed to warm before the addition of N1-(2-iodoethyl)benzimidazole. The reaction was then heated at reflux for 24 h. After this time the THF was removed under reduced pressure and the reaction worked up to leave the crude product that was purified by column chromatography to give the desired S-linked alkylation product in 57% yield (Figure 2.91).

The ^{1}H NMR spectrum showed all the necessary peaks, however the mass spectrum showed no sign of the required molecular mass at 393. However peaks at 261 from ES mass spectrometry and 362 from EIMS correspond to the reacted but deprotected amino acid with the loss of the Boc group and methoxy group and the methoxy group only, respectively.

2..13 Deprotection

Finally the protected cysteine ethyl-linked benzimidazole amino acid was deprotected as an example to test the protocols to give the deprotected heterocyclic amino acids.

Ester Removal

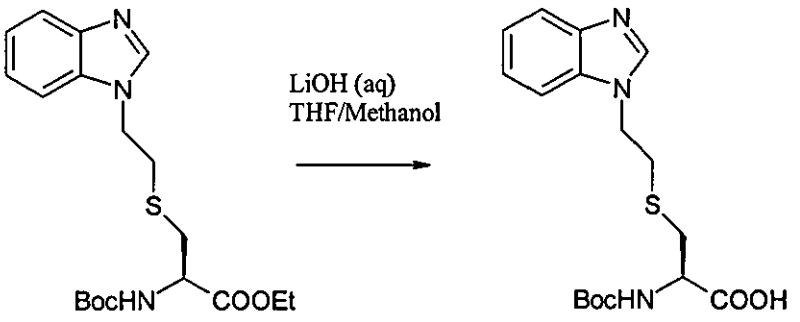


Figure 2.92 Ester deprotection

The deprotection of the carboxylic acid group was performed using LiOH(aq) in THF/methanol stirring for 8 h.[105] The organic solvents were removed under reduced

pressure, the water layer acidified, and the product extracted by EtOAc to afford the deprotected acid, obtained as a white solid in 97% yield (Figure 2.92).

Boc Removal

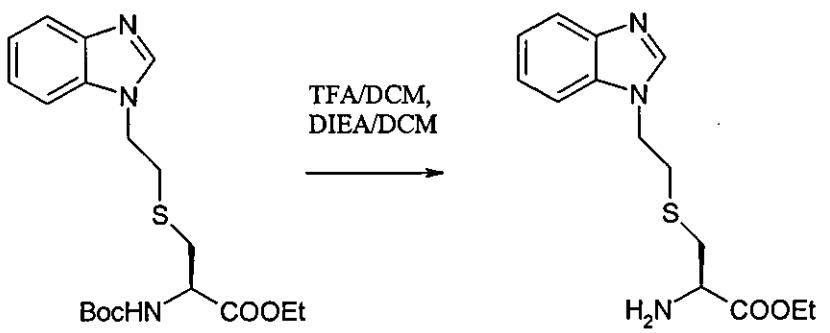


Figure 2.93 Boc deprotection

The Boc group was removed under acidic conditions using 50% TFA/DCM for 2 h followed by 5% DIEA to neutralise the acid.[106] The solvents were removed under reduced pressure to give the Boc-deprotected product in 86% yield (Figure 2.93). The removal of the TFA and DIEA under reduced pressure was difficult and was aided by the addition of ethanol to the mixture as a high boiling solvent to take the higher boiling compounds out of the mixture as it evaporated. For the complete removal of the TFA and DIEA, a short column was run, followed by removal of solvents under reduced pressure for about 8 h.

These protocols could be applied to all of the heterocyclic amino acids formed in this investigation to enable selective incorporation into peptide sequences.

2.14 Conclusion

After initial problems with the formation of the oxazolidinone template, as a method of amino acid protection, it was successfully synthesised in a reasonable overall yield. The other, simpler methods of amino acid protection also gave excellent yields of the protected amino acids. Almost all the heterocyclic derivatives that were attempted were successfully synthesised, although some had quite low yields, the majority were formed cleanly with very good, even excellent yields, which were then used in the attempted linker formation of heterocyclic amino acids.

The radical and organocuprate conjugate addition reactions involving the oxazolidinone template with N1-(2-haloethyl)benzimidazole (as well as the uracil derivative) produced none of the desired products. There was evidence of reduced product indicating that radicals had been formed in the radical reactions, and evidence also of lithium/halogen exchange, leading to a number of undesired side reactions, in the organocuprate reactions. The hydroboration and metathesis coupling reactions, involving the oxazolidinone template with N-vinyl heterocycle derivatives, also proved fruitless, steric factors may have been a problem for these methods.

The carbon-heterocycle bond-forming substitution reaction involving serine indicated the desired product had been formed, with yields of the carbon-heterocycle bond-forming substitution reactions involving cysteine being over 60% in many of the cases and several new heterocyclic amino acids were prepared by this method.

3 Future Work

The work in this thesis is based on two protected amino acid systems only, the oxazolidinone template for the carbon-carbon bonds and Boc protected amino acids for the heteroatom-carbon bonds in the heterocycle carrying side chain. The heterocycle derivatives used throughout this work have all been obtainable through the use of haloalkyl substituents as in Figure 3.1.

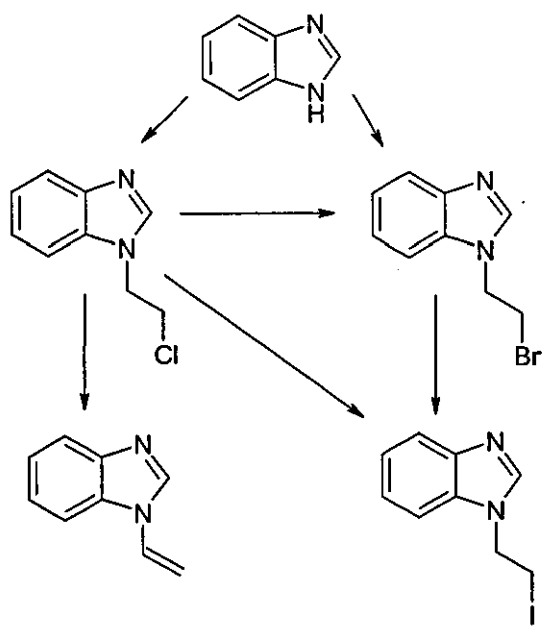


Figure 3.1 Haloalkyl Heterocycle Derivatives

Future work in this area could be by the use of different heterocycle derivatives with different amino acid derivatives.

3.1 Expansion of Current Work

As the work with cysteine has produced a number of promising results it would be possible to carry on to produce further heterocyclic amino acids containing sulphur in the linkage chain. It would also be possible to take the compounds created so far and create a new range of compounds by the addition of oxygen to make the corresponding sulfoxides or sulfones. An example of this is given in Figure 3.2.

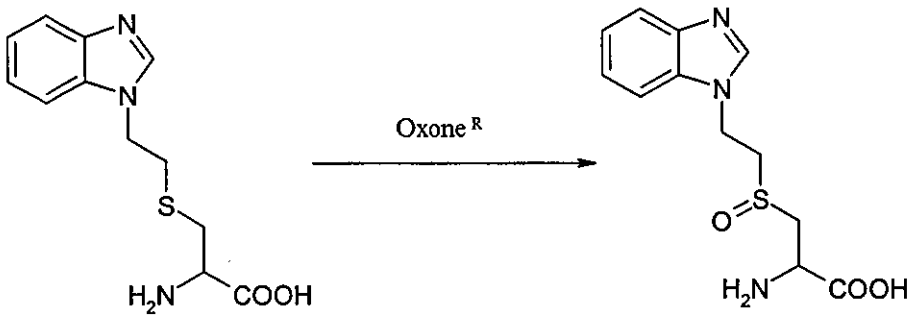


Figure 3.2 Oxidation of Sulphur

As well as work with cysteine, the work with serine could be continued. Some promising results were observed and with further investigation some respectable yields could be obtained as previously indicated in section 2.8.2.

Substitution Reactions

Another method based on the successfully utilised alkylation method in the formation of C-S bonds is the reversal of functionalities as exemplified in Figure 3.3.

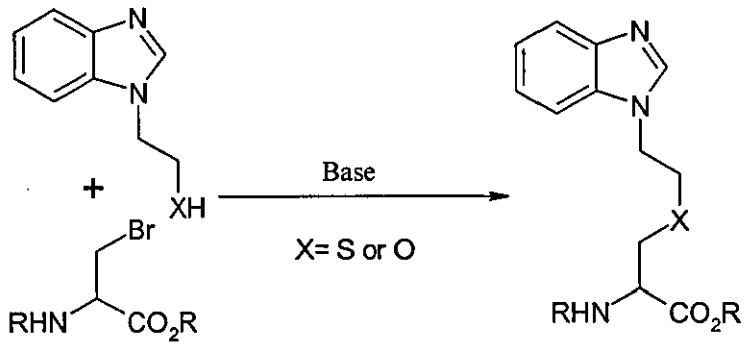


Figure 3.3 Reversed Substitution Reactions

Lactam Formation

The formation of a C-N linkage could still be performed by initial lactam formation for the selective αamino acid protection. A further method for the formation of the lactam, as previously attempted from 2,3-diaminopropanoic acid, has been described by Miller *et al.* [103,104,107,108] and utilises serine as the starting material as shown in Figure 3.4.

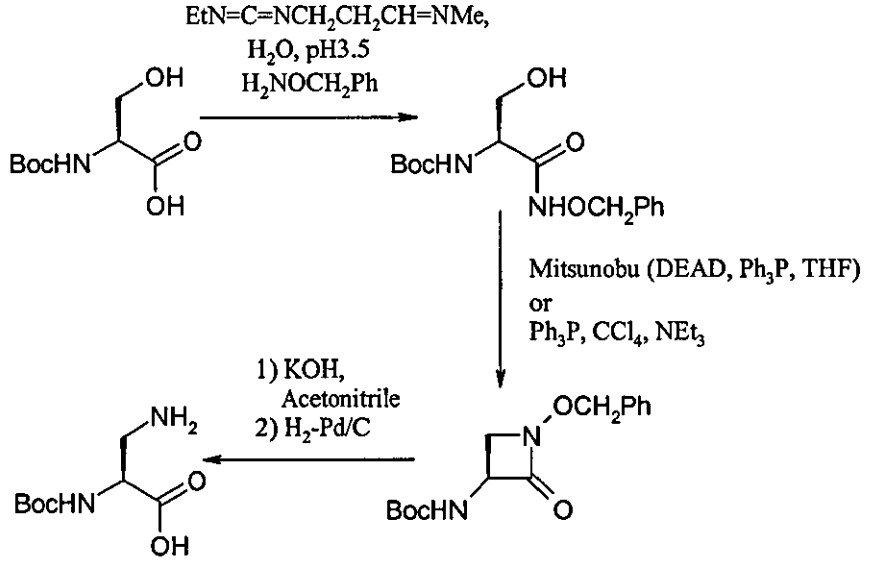


Figure 3.4 Formation of β-Lactam

One advantage of this method is that the use of ammonia gas is not necessary. Another major advantage over any others is that the optically pure starting material is readily available. This would provide a mono-protected diamine suitable for alkylation reactions.

3.2 Other Methods Utilising the same Starting Material

Addition of Sulphur to Double Bond

A well studied method for the addition of sulphur (thiol) to double bonds is by the use of radicals,[97,98,109] however little work has been done involving amino acids. Radicals have been looked at previously in this thesis for the formation of C-C bonds, and the general idea behind radical couplings has been introduced previously.

It should be possible to form a C-S bond using the *N*-Boc-cysteine alkyl ester with a vinyl or allyl heterocycle as shown in Figure 3.5.

Figure 3.5 Cysteine and Vinylbenzimidazole

This method is the almost guaranteed anti-Markovnikov addition to the less hindered or terminal end of the double bond as illustrated above, since this would proceed via the most

stable carbon radical. On a further positive note the planned reactions involve terminal double bonds, and it has been seen that terminal double bonds are the most reactive towards radical reactions.

Methods Involving a Different Template

During the course of this research programme, information was also found on αdisubstituted amino acids, and although the aim of the investigation was not the formation of α-disubstituted derivatives, at least one of the methods used could have the potential to be used for monoα-substituted amino acids.

Previous disubstituted work has been carried out by Obrecht *et al.*[110,111,112] into the formation of (*R*)-4-(bromoethyl)-4-methyl-2-phenyloxazol-5(4*H*)-one and (*S*)-4-(bromoethyl)-4-methyl-2-phenyloxazol-5(4*H*)-one (Figure 3.6). In this work, a racemic iodomethyl oxazolidinone was resolved by separation of diastereoisomers.

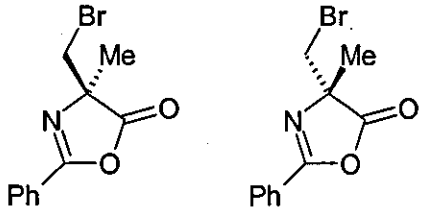


Figure 3.6 (*R*)-4-(Bromoethyl)-4-methyl-2-phenyloxazol-5(4*H*)-one and (*S*)-4-(Bromoethyl)-4-methyl-2-phenyloxazol-5(4*H*)-one

Figure 3.7 shows an outline for future work involving the preparation of an optically active iodomethyl glycine template, following a similar scheme as Orbrecht.[110,111,112]

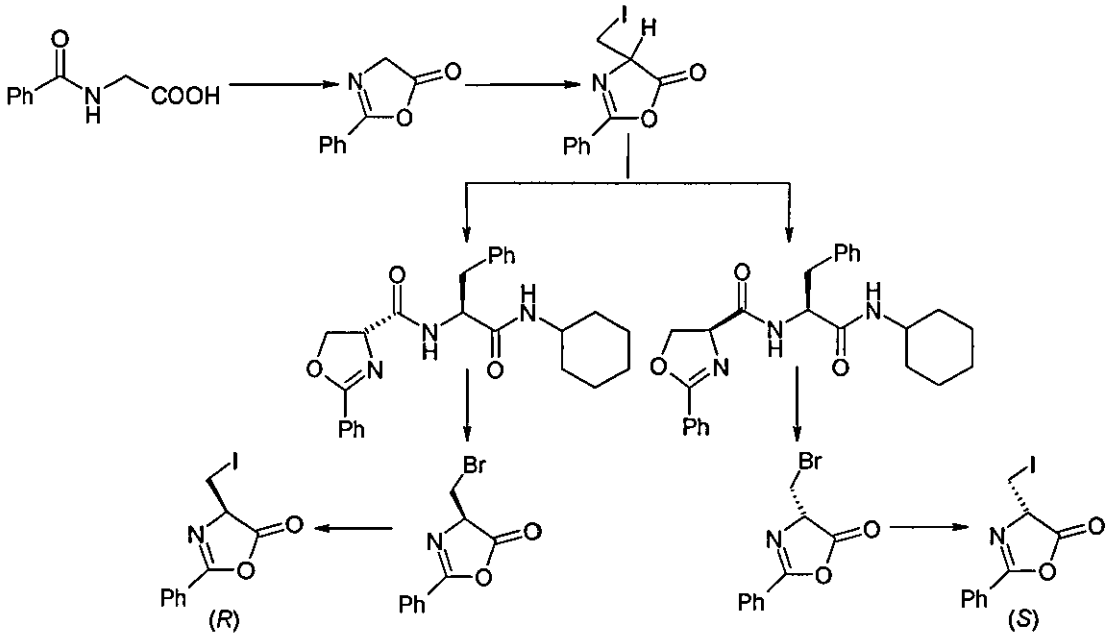


Figure 3.7 Plan for Forming (*R*)-4-(iodomethyl)-2-phenyloxazol-5(4*H*)-one and (*S*)-4-(iodomethyl)-2-phenyloxazol-5(4*H*)-one

The next stage could be substitution or radical reactions as shown in Figure 3.8.

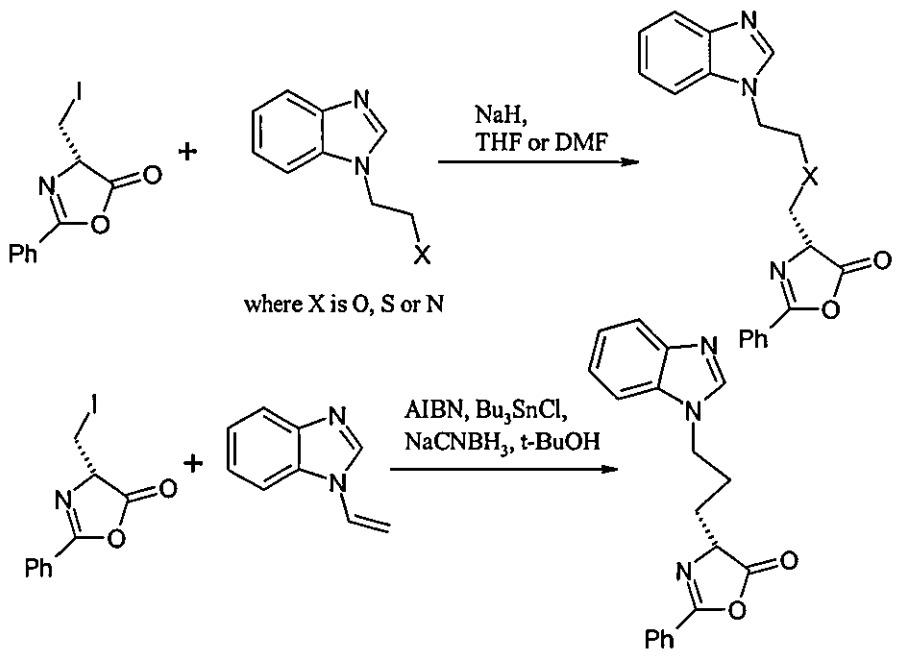


Figure 3.8 Substitution and Radical Reactions

Suzuki Coupling

This again involves the use of a boron derivative, in the form of a boronic acid, and palladium as a catalyst but it would not be necessary to attach the boron directly to the oxazolidinone template, which had proved to be problematic. A suggested Suzuki coupling is shown in Figure 3.9. It may then be possible to reduce the double bonds in the product by hydrogenation.

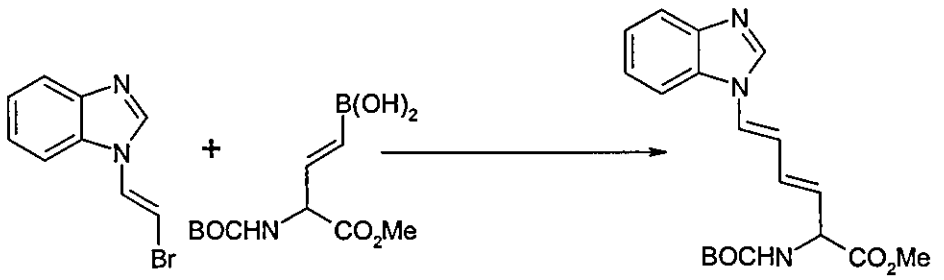


Figure 3.9 Suzuki Coupling

Experimental

General Experimental Details

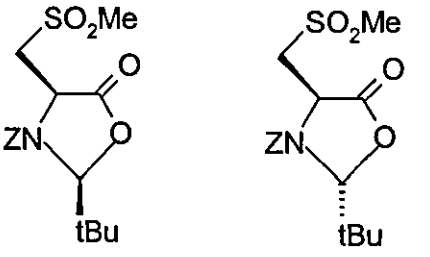
Starting materials were supplied by either Aldrich, Lancaster or Avacado and were used without purification unless otherwise stated.
Solvents were dried where necessary in the following manners:
THF – pre-dried in presence of potassium carbonate then distilled from sodium metal and benzophenone under a positive atmosphere of nitrogen;
EtOAc – distilled over calcium chloride;
DCM – distilled over calcium hydide (anhydrous);
1,4-Dioxane – Shaken over potassium hydroxide;

Column chromatography was performed using silica gel 60 (40-63 μ, 230-400 mesh, 60 A);
Thin layer chromatography (TLC) performed on Merck 0.2 mm silica 60 F_{254} coated UV active aluminium sheets;
Melting Points were measured on a Stuart Scientific Bibby SMP3 Melting Point Apparatus;
The Optical Rotations were performed on a polar 2001 using a 1 cm sample vessel using DCM as the solvent;
The Elemental Analysis was performed on a Perkin-Elmer CHN-2400;
The Molecular Mass was measured on a JEOL SX102 at Loughborough University and by the ESPRC national spectrometry unit at Swansea University, by either EI (Electron Impact) or FAB (Fast Atom Bombardment);
Liquid Chromatography – Mass Spectrometry (LC-MS) was performed using a Waters 600 controllers instrument, a Waters Symmetry C8 3.5 μm 4.6x50 nm column and a Waters 996 photodiode array detector attached to a Micromass Platform mass spectrometer which uses ES (Electrospray) ionization;
Infrared Spectra was obtained using either a Perkin-Elmer Paragon 1000 FTIR spectrometer for neat samples on NaCl discs (DCM used as a 'transport' solvent where

necessary) or a Shimadzu FTIR-8300 for KBr discs and recorded in the range 5000-500 cm^{-1};

In most cases the ^{1}H and ^{13}C NMR analysis was performed at 400 MHz on a Bruker DPX-400 and the remaining ^{1}H NMR at 250 MHz on a Bruker AC-250. The solvents used were $CDCl_3$, DMSO-d6, MeOH-d4 or acetone-d6 and the actual solvent used is specified in each experiment. Chemical shifts are quoted in ppm (parts per million) and the coupling constants (*J* values) in Hz (hertz). The following abbreviations have been used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad).

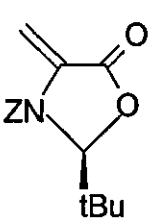
(2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one and (2R,2R)-isomer[58]



S-Methyl-(R)-cysteine (4.00 g, 29.60 mmol) was treated with aqueous sodium hydroxide (1.18 g, 29.60 mmol in 20 cm^3 of water). After 5 min the solution was evaporated to dryness under reduced pressure to leave a creamy white solid. 2,2-Dimethylpropanal (3.21 cm^3, 29.60 mmol) in hexane (44 cm^3) was added and the suspension was stirred and heated under reflux with Dean-Stark water removal for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the residual creamy white solid suspended in dry dichloromethane (40 cm^3). Benzyl chloroformate (6.58 cm^3, 50.00 mmol) was added dropwise to the stirred suspension at 0°C over 3 h. The mixture was stirred for a further 36 h at room temperature before adding aqueous sodium bicarbonate (10%w/v, 15 cm^3) and stirring for a further 4 h. The layers were then separated and the

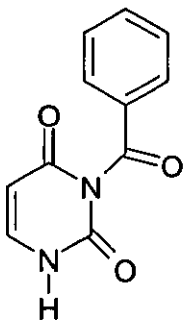
dichloromethane solution dried ($MgSO_4$), filtered and evaporated. The residue was stirred in acetonitrile (30 cm^3), then oxone® (14.60 g, 23.00 mmol) in water (75 cm^3) was added and the solution stirred for 24 h. Water (30 cm^3) was then added and the mixture extracted with dichloromethane (3 × 30 cm^3), the combined extracts were dried ($MgSO_4$), filtered and evaporated. Purification of the products was by column chromatography (ethyl acetate–hexane, 9:1 v/v to pure ethyl acetate on silica gel). This gave the title compounds: *anti*-diastereoisomer as a clear oil and the desired *syn*–diastereoisomer as a yellow oil, white crystals from ethyl acetate (5.14 g, 52%), m.p. 150-151°C (lit.,[58] m.p. 151-152°C). Found: MH^+ 370.1308; $C_{17}H_{23}NO_6S$ requires: MH^+ 370.1324. ν_{max} (NaCl/cm^{-1}) 2970 (CH_2), 1791 (NC=O), 1722 (conjugated C=O), 1482 (Ar), 1398 (CMe_3), 1308 (C-O), 1129 (SO_2Me), 1040 (C-O), 968 (CH), 737 and 698 (ArH). δ_H (400 MHz, $CDCl_3$) 0.95 (9 H, s, $C(CH_3)_3$), 3.11 (3 H, s, SO_2Me), 3.39 (1 H, dd, *J* 3.6, 15.3 Hz, $CHHSO_2Me$), 3.51 (1 H, dd, *J* 7.9, 15.3 Hz, $CHHSO_2Me$), 4.99 (1 H, dd, *J* 3.6, 7.9 Hz, $CHCH_2$) 5.22 (2 H, 2 x d, *J* 12.0, 13.4 Hz, CH_2Ph), 5.62 (1 H, s, $CHCMe_3$), 7.38 (5 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 24.6 (CMe_3), 37.8 (CMe_3), 43.0 (SO_2Me), 53.5 ($CHCH_2$), 58.4 (CH_2SO_2Me) 68.8 (CH_2Ph), 96.0 ($CHCMe_3$), 128.6, 128.7, 128.8 (ArCH), 130.3 (ArC), 156.4, 168.5 (C=O).

(2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(ene)oxazolidin-5-one[58]



(2S,4R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one (2.00 g, 5.93 mmol) in dry DCM (50 cm^3) was treated dropwise with DBU (1.77 cm^3, 11.85 mmol) and stirred at 0°C over 1 h. Water was then added and the organic layer was separated, washed with water, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude product was filtered though a short column of silica gel using DCM to yield the product as a colourless gel which solidified when left overnight under high vacuum as white crystals (1.14 g, 66%), m.p. 66-67°C (lit.,[58] m.p. 69-71°C), $[\alpha]^{25}_D$ 68.0 (c 1 in DCM) (lit.,[57] 65.4, c 0.4 in EtOH). Found: C,65.9, H,6.3, N,4.6%; $C_{16}H_{19}NO_4$ requires: C,66.4, H,6.6, N,4.8%. Found: M^+ 289.1310; $C_{16}H_{19}NO_4$ requires: M^+ 289.1314. m/z: 232, 188, 176, 92. ν_{max} (NaCl/cm^{-1}) 2969 (CH_2), 1794 (conjugated C=O), 1727 (NC=O), 1656 (C=C), 1397, 1326 (CMe_3), 1269, 1011 (C-O) and 759m, 697m (ArH). δ_H (400 MHz, $CDCl_3$) 0.93 (9 H, s, CMe_3), 5.26 (2 H, s, CH*H*Ph and $CHCMe_3$), 5.68 (2 H, s, $C=CH_2$), 5.72 (1 H, s, C*H*HPh), 7.38 (5 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 24.3 (CMe_3), 38.7 (CMe_3), 68.8 (CH_2Ph), 94.0 ($CHCMe_3$), 104.3 ($C=CH_2$), 128.6, 128.7, 128.8 (ArCH), 130.2 (ArC), 134.7 ($C=CH_2$), 152.4 (O(*C*=O)-N), 164.5 (C=C-*C*=O).

N3-Benzoyluracil[113]



Benzoyl chloride (11.30 cm^3, 88.40 mmol) was added dropwise to a stirred suspension of uracil (5.00 g, 44.20 mmol) in a mixture of dry acetonitrile/pyridine (100 cm^3 5:1 v/v). After leaving to stir for 24 h at RT the solvents were removed under reduced pressure. The residue was then treated with K_2CO_3 in dioxane/water (0.25 M, 100 cm^3, 1:1 v/v) for 2 h. The solution was filtered, washed with acetonitrile (50 cm^3), and dried under vacuum to give the title compound as a white powder (7.03 g, 72%), m.p. 170-171°C (lit.,[113] m.p. 148-149°C). Found: M^+ 216.0531; $C_{11}H_8N_2O_3$ requires: M^+ 216.0535. m/z: 188, 146, 105. ν_{max} (NaCl/cm^{-1}) 3321 (N-H), 2922 (C=C-H), 1747 (C-(C=O)-N), 1705 (Ph-(C=O)-N), 1652 (N-(C=O)-N), 1417, 1068 and 801 (ArH). δ_H (400 MHz, DMSO-d6) 5.73 (1 H, d, *J* 8.0 Hz, CHCO), 7.60 (2 H, t, *J* 8.0 Hz, ArH), 7.65 (1 H, d, *J* 8.0 Hz, ArH), 7.78 (1 H, t, *J* 8.0 Hz, CHN), 7.95 (2 H, d, *J* 8.0 Hz, ArH), 11.59 (1 H, br, NH). δ_C (100 MHz, DMSO-d6) 100.4 (C-6), 129.8, 130.5 (ArCH), 131.7 (ArC), 135.7 (ArCH), 143.7 (C-5), 150.4, 163.3, 170.4 (C=O).

N-(2-Bromoethyl) heterocycle (Method A)

Heterocycle (1 mol equiv), triphenylphosphine (1.2 mol equiv) and 2-bromoethanol (1.2 mol equiv) were suspended in dry dioxane (50 cm^3 per mmol of heterocycle) at 5°C, to which was added DIAD (1.2 mol equiv) dropwise over 3 h. The mixture was stirred under nitrogen at room temperature to give a clear solution. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the title compound as a yellow oil, which was in some cases recrystallised to give the product.

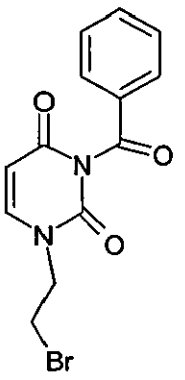
N-(2-Iodoethyl) heterocycle (Method B)

N-(2-Haloethyl) heterocycle (1 mol equiv) and dry sodium iodide (5 mol equiv) were heated in dry acetone (50 cm^3 per mmol of heterocycle) at reflux overnight under nitrogen in the dark. After cooling, the acetone was removed under reduced pressure and the residue taken up in ethyl acetate/water (1:1 v/v), the organic layer was separated and the aqueous layer was extracted with ethyl acetate twice more. The organic layers were combined and washed twice with sodium thiosulfate solution (2% w/v), then dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to yield the title compound.

N-(2-Chloroethyl)heterocycle (Method C)

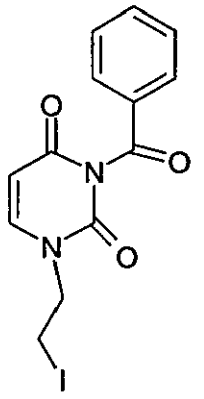
Heterocycle (1 mol equiv.) was added to a mixture of 1,2-dichloroethane, which acted as both the solvent and the reagent, tetrabutylammonium iodide (5%) as the phase transfer catalyst (PTC), KOH (6 mol equiv.) and K_2CO_3 (2.5 mol equiv.). The reaction was stirred at room temperature for 4 h. The inorganics filtered off and the organic portion washed (H_2O, 5 cm^3 per mmol), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The mixture was then put through flash column chromatography to isolated the title compound.

3-Benzoyl-1-(2-bromoethyl)uracil[58]



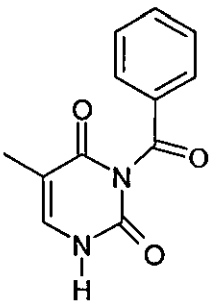
Prepared according to method A, using 3-benzoyluracil (5.00 g, 2.30 mmol), triphenylphosphine (7.24 g, 2.76 mmol) and 2-bromoethanol (1.96 cm^3, 2.76 mmol) in dry dioxane (100 cm^3) at 5°C, with DIAD (5.43 cm^3, 2.76 mmol) to give the title compound as a white solid (7.07 g, 95%), m.p. 177-179°C (lit.,[58] m.p. 183-184°C). Found: M^+ 321.9959; $C_{13}H_{11}{}^{79}BrN_2O_3$ requires: M^+ 321.9953. m/z: 296, 294, 277, 254, 252, 215, 188, 105. ν_{max} (NaCl/cm^{-1}) 2920 (C=C-H), 2850 (CH_2), 1747 (C-(C=O)-N), 1704 (Ph-(C=O)-N), 1662 (N-(C=O)-N), 1437 (CH_2), 1346, 1241, 760, 697 (ArH) and 685 (C-Br). δ_H (400 MHz, $CDCl_3$) 3.69 (2 H, t, *J* 5.6 Hz, CH_2Br), 4.16 (2 H, t, *J* 5.6 Hz, CH_2N), 5.83, (1 H, d, *J* 8.0 Hz, CHC=O), 7.33 (2 H, d, *J* 8.0 Hz, ArH), 7.51 (2 H, t, *J* 8.0 Hz, ArH), 7.67 (1 H, d, *J* 8.0 Hz, ArH), 7.93 (1 H, d, *J* 8.0 Hz, CHN). δ_C (100 MHz, $CDCl_3$) 29.4 (CH_2Br), 51.3 (CH_2N), 101.9 (C-6), 129.3, 130.5 (ArCH), 132.1 (ArC), 135.2 (ArCH), 144.8 (C-5), 149.7, 159.9, 168.1 (C=O).

3-Benzoyl-1-(2-iodoethyl)uracil[58]



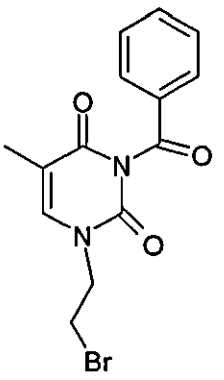
Prepared according to method B, using 3-benzoyl-1-(2-bromoethyl)uracil (5.00 g, 1.56 mmol) and dry sodium iodide (11.71 g, 7.81 mmol) in dry acetone (100 cm^3) to yield the title compound as a pale yellow solid (2.11 g, 36%), m.p. 185-187°C (lit.,[58] m.p. 190-191°C). Found: C,42.3, H,3.4, N,7.9%; $C_{13}H_{11}IN_2O_3$ requires: C,42.2, H,3.0, N,7.6%. Found: M^+ 369.9807; $C_{13}H_{11}IN_2O_3$ requires: M^+ 369.9814. m/z: 342, 277, 215, 188, 155, 105. ν_{max} (NaCl/cm^{-1}) 2920 (C=C-H), 2850 (CH_2) 1747 (C-(C=O)-N), 1705 (Ph-(C=O)-N), 1664 (N-(C=O)-N), 1436 (CH_2), 1254, 722 and 695 (ArH). δ_H (400 MHz, $CDCl_3$) 3.48 (2 H, t, *J* 6.4 Hz, CH_2I), 4.11 (2 H, t, *J* 6.4 Hz, CH_2N), 5.84 (1 H, d, *J* 8.0 Hz, CHC=O), 7.28 (2 H, d, *J* 8.0 Hz, ArH), 7.51 (2 H, t, *J* 8.0 Hz, ArH), 7.66 (1 H, d, *J* 8.0 Hz, CHN), 7.93 (1 H, d, *J* 8.0 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 22.9 (CH_2I), 51.8 (CH_2N), 102.0 (C-6), 129.2, 130.5 (ArCH), 133.3 (ArC), 135.9 (ArCH), 144.2 (C-5), 149.1, 162.5 and 171.1 (C=O).

N3-Benzoylthymine[113]



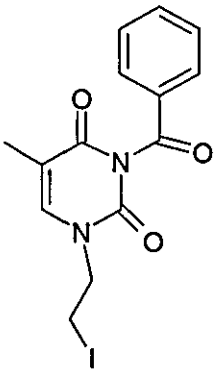
Benzoyl chloride (10.11 cm^3, 79.40 mmol) was added dropwise to a stirred suspension of thymine (5.00 g, 39.70 mmol) in a mixture of dry acetonitrile/pyridine (100 cm^3, 5:1 v/v). After leaving to stir for 24 h at RT the solvents were removed under reduced pressure. The residue was then treated with K_2CO_3 in dioxane/water (0.25 M, 100 cm^3, 1:1 v/v) for 2 h. The solution was filtered, washed with acetonitrile (50 cm^3), and dried under vaccum to give the title compound as a white powder (6.46 g, 71%), m.p. 148-149°C (lit.,[113] m.p. 150-152°C). Found: M^+ 230.0687; $C_{12}H_{10}N_2O_3$ requires: M^+ 230.0691. m/z: 203, 146, 105. ν_{max} (NaCl/cm^{-1}) 3398 (N-H), 2933 (C=C), 1745 (C-(C=O)-N), 1700 (Ph-(C=O)-N), 1596 (N-(C=O)-N), 1511, 1441, 1381 (CH_3), 1233, 907 and 730 (ArH). δ_H (400 MHz, DMSO-d6) 1.82 (3 H, s, CH_3), 7.54 (1 H, s, C=CHN), 7.60 (2 H, t, *J* 7.6 Hz, ArH), 7.78 (1 H, t, *J* 7.6 Hz, ArH), 7.94 (2 H, d, *J* 7.6 Hz, ArH). δ_C (100 MHz, DMSO-d6) 11.7 (CH_3), 107.9 (CCH_3), 129.4, 130.2 (ArCH), 131.3 (ArC), 135.3, (ArCH), 138.7 (C-6), 149.9, 163.5, 170.1 (C=O).

3-Benzoyl-1-(2-bromoethyl)thymine[58]



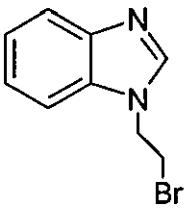
Prepared according to method A, using N3-benzoylthymine (5.00 g, 21.70 mmol), triphenylphosphine (6.84 g, 2.60 mmol) and 2-bromoethanol (1.85 cm^3, 2.60 mmol) in dry dioxane (100 cm^3) at 5°C, with DIAD (5.13 cm^3, 26.01 mmol) to give the title compound as a white solid (7.32 g, 56%), m.p. 166-167°C (lit.,[58] m.p. 183-184°C). Found: M^+ 336.0112; $C_{14}H_{13}{}^{79}BrN_2O_3$ requires: M^+ 336.0109. m/z: 310, 308, 277, 254, 252, 230, 188, 105. ν_{max} (NaCl/cm^{-1}) 3390, 2898 (CH_2), 2342, 1746 (C-(C=O)-N), 1699 (Ph-(C=O)-N), 1662 (N-(C=O)-N), 1462, 1414 (CH_2), 1429, 1376 (CH_3), 720 (ArH) and 672 (C-Br). δ_H (400 MHZ, $CDCl_3$) 1.98 (3 H, s, CH_3), 3.44 (2 H, t, *J* 6.6 Hz, CH_2Br), 4.07 (2 H, t, *J* 6.6 Hz, CH_2N), 7.13 (1 H, d, *J* 1.2 Hz, CHN), 7.50 (2 H, t, *J* 7.4 Hz, ArH), 7.65 (1 H, t, *J* 7.4 Hz, ArH), 7.93 (2 H, d, *J* 7.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 11.6 (CH_3), 36.7 (CH_2Br), 50.4 (CH_2N), 109.7 (*C*CH_3), 128.3, 129.6 (ArCH), 130.6 (ArC), 134.2 (ArCH), 139.3 (C-6), 148.7, 162.1, 167.8 (C=O).

3-Benzoyl-1-(2-iodoethyl)thymine[58]



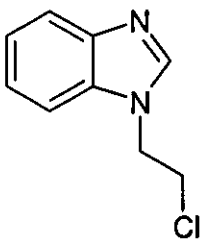
Prepared according to method B, using 3-benzoyl-1-(2-bromoethyl)thymine (5.00 g, 14.83 mmol) and dry sodium iodide (11.11 g, 74.15 mmol) in dry acetone (100 cm^3) to yield the title compound as a pale yellow solid (3.11 g, 54%), m.p. 145-147°C (lit.,[58] m.p. 138-139°C). Found: M^+ 383.9968; $C_{14}H_{13}IN_2O_3$ requires: M^+ 383.9972. m/z: 356, 277, 215, 230, 155, 105. ν_{max} (NaCl/cm^{-1}) 2954 (C=C), 1743 (C-(C=O)-N), 1702 (Ph-(C=O)-N), 1640 (N-(C=O)-N), 1451 (CH_3), 1411 (CH_2), 1363, 730 (ArH) and 598 (C-I). δ_H (400 MHz, $CDCl_3$) 2.04 (3 H, s, CH_3), 3.57 (2 H, t, *J* 6.8 Hz, CH_2I), 4.33 (2 H, t, *J* 6.8 Hz, CH_2N), 7.47 (2 H, t, *J* 7.4 Hz, ArH), 7.62 (1 H, t, *J* 7.4 Hz, ArH), 7.66 (1 H, d, *J* 2.6 Hz, CHN), 7.75 (2 H, d, *J* 7.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 13.2 (CH_3), 29.4 (CH_2I), 42.0 (CH_2N), 112.3 (CCH_3), 128.6, 129.3, (ArCH), 132.8 (ArC), 134.0 (ArCH), 140.7 (C-6), 149.9, 162.9, 169.7 (C=O).

N1-(2-Bromoethyl)benzimidazole[58]



Prepared according to method A, using benzimidazole (2.00 g, 16.93 mmol), triphenylphosphine (5.33 g, 20.31 mmol) and 2-bromoethanol (1.44 cm^3, 20.31 mmol) in dry dioxane (100 cm^3) at 5°C with DIAD (4.00 cm^3, 20.31 mmol) to yield the title product as colourless crystals (3.75 g, 98%), m.p. 77-79°C. Found: M^+ 223.9950, $C_9H_9{}^{79}BrN_2$ requires: M^+ 223.9949. m/z: 145, 131, 118, 104, 90. ν_{max}(NaCl/cm^{-1}) 3345, 2980 (CH_2), 2360, 1719 (conjugated C=N), 1497, 1438 (CH_2), 1246, 1119, 745, 722 (ArH) and 696 (C-Br). δ_H (400 MHz, $CDCl_3$) 3.71 (2 H, t, *J* 6.0 Hz, CH_2Br), 4.61 (2 H, t, *J* 6.0 Hz, CH_2N), 7.23-7.62 (4 H, m, ArH), 7.90 (1 H, s, CH). δ_C (100 MHz, $CDCl_3$) 39.2 (CH_2Br), 45.1 (CH_2N), 109.2 (CH), 119.3, 123.1, 129.0, 130.4 (ArCH), 133.7, 144.9 (ArC).

N1-(2-Chloroethyl)benzimidazole[114,115]

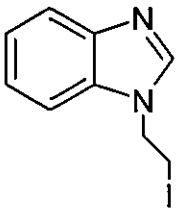


Prepared according to method C, using benzimidazole (1.00 g, 8.47 mmol) in 1,2-dichloroethane (70 cm^3) with tetrabutylammonium iodide (0.05 g, 5%), KOH (2.85 g, 50.79 mmol) and K_2CO_3 (2.93 g, 21.16 mmol). After stirring for 4 h, work up gave title

product (0.85 g, 54%), m.p. 76-78°C (lit.,[114,115] m.p. 84-86°C). Found: C,60.3, H,5.2, N,16.0%; $C_9H_9ClN_2$ requires: C,59.8, H,5.0, N,15.5%. Found: M^+ 180.0458; $C_9H_9{}^{35}Cl\,N_2$ requires: M^+ 180.0454. m/z: 144, 131, 117, 104, 90. ν_{max} (NaCl/cm^{-1}) 3404, 3056 (ArH), 2960 (CH_2), 2359, 1615 (C=N), 1494, 1437 (CH_2), 1257, 1118, 744, 721 (ArH) and 663 (C-I). δ_H (400 MHz, $CDCl_3$) 3.76 (2 H, t, *J* 6.0 Hz, CH_2Cl), 4.42 (2 H, t, *J* 6.0 Hz, CH_2N), 7.22-7.45 (3 H, m, ArH,), 7.59 (1 H, m, ArH), 7.89 (1 H, s, CH). δ_C (100 MHz, $CDCl_3$) 42.5 (CH_2Cl), 47.0 (NCH_2), 109.6 (CH), 121.0, 122.9, 129.0, 132.4 (ArCH), 133.3, 143.7 (ArC).

Prepared by a modification to method A, using benzimidazole (1.00 g, 8.47 mmol), 2-chloroethanol (0.82 g, 10.16 mmol) and triphenylphosphine (2.66 g, 10.16 mmol) in dry dioxane (20 cm^3) at 5°C, with DIAD (2.00 cm^3, 2.05 g, 10.16 mmol). After stirring for 16 h, work up gave title product (1.23 g, 80%) 72-74°C (lit.,[114] m.p. 84-86°C). Found: C,60.4, H,5.0, N,16.1%; $C_9H_9{}^{35}ClN_2$ requires: C,59.8, H,5.0, N,15.5%. Data as for N1-(2-chloroethyl)benzotriazole using method C.

N1-(2-Iodoethyl)benzimidazole

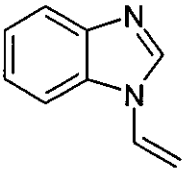


Prepared according to method B, using N1-(2-bromoethyl)benzimidazole (2.00 g, 8.88 mmol) and dry sodium iodide (6.66 g, 44.42 mmol) in dry acetone (100 cm^3). Work

up gave the title compound as a pale yellow solid (1.82 g, 75%). Data as for N1-(2-iodoethyl)benzotriazole using chloro derivative.

Prepared according to method B, using N1-(2-chloroethyl)benzimidazole (2.00g, 10.83 mmol) and dry sodium iodide (8.12 g, 54.25 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (2.12 g, 72%). Found: M^+ 271.9808; $C_9H_9IN_2$ requires: M^+ 271.9808. m/z: 144, 131, 118, 104, 90. ν_{max} (NaCl/cm^{-1}) 3315, 2980 (CH_2), 1653, 1506 (C=N), 1374 (CH_2), 1240, 1108, 698 (ArH), 511 (C-I). δ_H (400 MHz, $CDCl_3$) 3.51 (2 H, t, *J* 7.2 Hz, CH_2I), 4.58 (2 H, t, *J* 7.2 Hz, CH_2N), 7.34-7.53 (3 H, m, ArH,), 7.83 (1 H, m, ArH), 7.99 (1 H, s, CH). δ_C (100 MHz, $CDCl_3$) 44.2 (CH_2I), 53.1 (NCH_2), 109.9 (CH), 121.3, 121.9, 124.9, 130.2 (ArCH), 140.8, 146.4 (ArC).

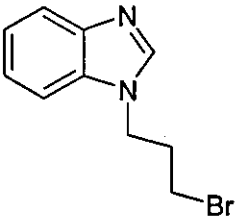
N1-Vinylbenzimidazole



N1-(2-Chloroethyl)benzimidazole (0.50 g, 2.71 mmol), KOH (3.66 g, 64.99 mmol) and hydroquinone (0.02 g, 0.14 mmol) were stirred in ethanol (50 cm^3) at reflux for 40 min. After this time the alcohol was removed under reduced pressure and the organic material extracted from the residue using DCM. The extract was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. Purification using flash chromatography gave the title product as a clear colourless liquid (0.39 g, 97%). Found: M^+ 144.0688, $C_9H_8N_2$ requires: M^+ 144.0686. m/z: 118, 91. ν_{max} (NaCl/cm^{-1}) 3389, 3089 (C=C-H), 1731, 1697,

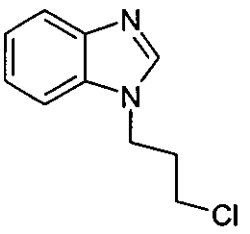
1610 (C=N, C=C), 1479, 1285, 886 and 742 (ArH). δ_H (250 MHz, $CDCl_3$) 5.08 (1 H, dd, *J* 9.0, 9.0 Hz, CH*H*), 5.53 (1 H, dd, *J* 15.7, 16.0 Hz, C*H*H), 7.11 (1 H, dd, *J* 9.0, 15.7 Hz, NCH), 7.33 (2 H, m, ArH), 7.53 (1 H, m, ArH), 7.80 (1 H, m, ArH), 8.12 (1 H, s, NCHN). δ_C (100 MHz, $CDCl_3$) 109.0 (NCHN), 117.2 (CH_2), 120.8 (NCH), 121.1, 122.4, 123.1, 124.8 (ArCH), 143.1, 145.2 (ArC).

N1-(3-Bromopropyl)benzimidazole



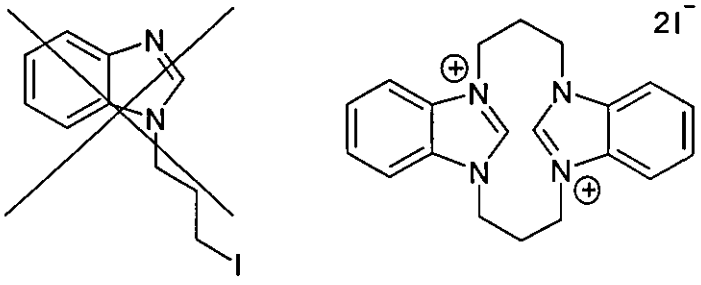
Prepared by modification to method A, using benzimidazole (1.00 g, 8.47 mmol), triphenylphosphine (2.66 g, 10.15 mmol) and 3-bromopropanol (0.72 cm^3, 10.15 mmol) in dry dioxane (100 cm^3) at 5°C with DIAD (4.00 cm^3, 20.31 mmol) to yield the title product as an oil (1.58 g, 78%). Found: MH^+ 238.9920; $C_{10}H_{11}{}^{79}BrN_2$ requires: MH^+ 239.0106. m/z: 160, 118, 104, 90. δ_H (400 MHz, $CDCl_3$) 2.10 (2 H, m, CH_2), 3.54 (2 H, t, *J* 6.3 Hz, CH_2Br), 4.20 (2 H, t, *J* 6.6 Hz, CH_2N), 7.23 (2 H, m, ArH,), 7.74 (2 H, m, ArH,), 7.83 (1 H, s, CH). δ_C (100 MHz, $CDCl_3$) 27.0 (CH_2), 29.6 (CH_2Br), 34.0 (NCH_2), 109.6 (CH), 120.5, 122.1, 123.0, (ArCH), 133.7 (ArC), 142.9 (ArCH), 143.9 (ArC).

N1-(3-Chloropropyl)benzimidazole



Prepared by modification to method C, using benzimidazole (1.00 g, 8.47 mmol) in 1,3-dichloropropane (70 cm^3) with tetrabutylammonium iodide (0.05 g, 5%), KOH (2.85 g, 50.79 mmol) and K_2CO_3 (2.93 g, 21.16 mmol). After stirring for 8 h, work up gave title product as an oil (1.04 g, 62%). Found: M^+ 194.0609; $C_{10}H_{11}{}^{35}ClN_2$ requires: M^+ 194.0608. m/z: 159, 118, 104, 90. δ_H (400 MHz, $CDCl_3$) 2.23 (2 H, m, CH_2Cl), 3.38 (2 H, t, *J* 6.5 Hz, CH_2N), 4.32 (2 H, t, *J* 6.5 Hz, CH_2), 7.23 (2 H, m, ArH,), 7.74 (2 H, m, ArH,), 7.83 (1 H, s, CH). δ_C (100 MHz, $CDCl_3$) 22.7 (CH_2), 32.2 (CH_2Cl), 46.4 (NCH_2), 102.8 (CH), 121.1, 128.9, 129.2, 133.6 (ArCH), 138.2, 147.3 (ArC).

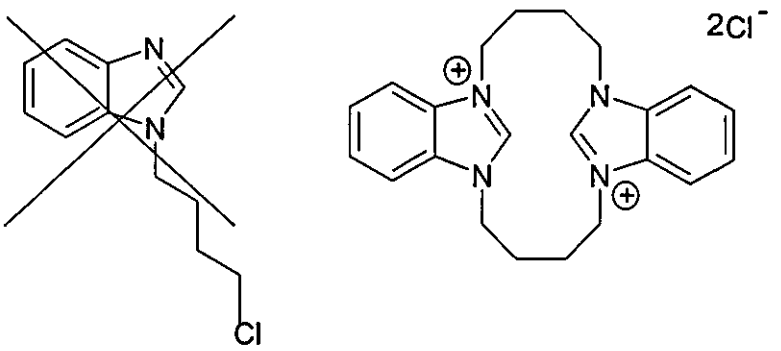
N1-(3-Iodopropyl)benzimidazole - Quarternary Salt



Prepared by modification to method B, using N1-(2-bromopropyl)benzimidazole and (1.00 g, 4.20 mmol) and dry sodium iodide (3.15 g, 21.01 mmol) in dry acetone (100 cm^3). Also by using N1-(2-chloropropyl)benzimidazole and (1.00 g, 5.16 mmol) and dry sodium

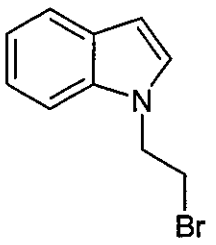
iodide (3.86 g, 25.78 mmol) in dry acetone (100 cm^3). Work up in both cases did not give the title compound, but gave what is believed to be the dimeric quaternary salt. Found: M^+ 158.93, 317.30; $C_{10}H_{11}IN_2$ requires: M^+ 286.00, but a double quaternary cation $C_{20}H_{22}N_4$ requires: 159.09 (M^{2+}), 317.18 ($[MH]^+$). δ_H (250 MHz, $CDCl_3$) 1.90 (2 H, br, CH_2), 4.90 (4H, t, *J* 6.9 Hz, CH_2), 7.22 - 7.78 (4H, m, ArH), 7.95 (1H, s, NCH).

N1-(4-Chlorobutyl)benzimidazole – Quaternary Salt



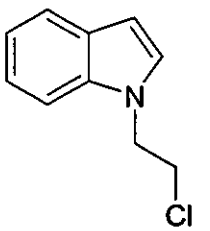
Prepared by modification to method C, using benzimidazole (1.00 g, 8.47 mmol) in 1,4-dichlorobutane (70 cm^3) with tetrabutylammonium iodide (0.05 g, 5%), KOH (2.85 g, 50.79 mmol) and K_2CO_3 (2.30 g, 21.16 mmol). After stirring for 8 h, work up did not give the title compound, but gave what is believed to be the dimeric quaternary salt. Found: M^+ 173.04, 345.64; $C_{11}H_{13}{}^{35}ClN_2$ requires: M^+ 208.08, but dimeric quarternary cations, $C_{22}H_{26}N_4$ requires: 173.09 (M^{2+}), 345.19 ($[M-H]^+$). δ_H (400 MHz, DMSO-d6) 2.08 (4 H, br, CH_2), 4.57 (4 H, t, *J* 5.6 Hz, CH_2), 7.67 (2 H, m, ArH), 8.34 (2 H, m, ArH), 9.77 (1 H, s, NCH). δ_C (100 MHz, DMSO-d6) 24.9 (CH_2), 26.0 (CH_2), 29.7 (CH_2), 46.2 (NCH_2), 113.8 (CH), 126.4, 126.8 (ArCH), 130.1 (ArC), 141.5, 142.1 (ArCH), 144.6 (ArC).

N-(2-Bromoethyl)indole



Prepared according to method A, using indole (1.00 g, 34.14 mmol), triphenylphosphine (2.69 g, 40.97 mmol) and 2-bromoethanol (0.73 cm^3, 40.97 mmol) in dry dioxane (100 cm^3) and DIAD (2.017 cm^3, 40.97 mmol). After workup this yielded none of the desired product, and quantitative amounts of the indole starting material was retrieved.

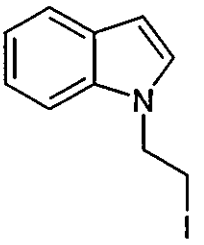
N-(2-Chloroethyl)indole



Prepared according to method C, using indole (2.00 g, 17.07 mmol), 1,2-dichloroethane (150 cm^3), tetrabutylammonium bromide (0.10 g, 5%), KOH (5.75 g, 0.10 mol) and K_2CO_3 (5.90 g, 0.04 mol). After stirring for 24 h, work up this gave title product as a brown oil (2.46 g, 81%). Found: C, 66.8, H, 5.9, N, 7.6%; $C_{10}H_{10}ClN$ requires: C, 66.8, H, 5.6, N, 7.8%. Found: MH^+ 180.0510; $C_{10}H_{10}{}^{35}ClN$ requires: MH^+ 180.0579. m/z: 144, 116, 90. ν_{max} (NaCl/cm^{-1}) 3407, 3286, 3050 (C=C), 2959 (CH_2), 1614 (C=C), 1454 (CH_2), 1334, 1093 and 742 (C-Cl). δ_H (400 MHz, $CDCl_3$) 3.84 (2 H, t, *J* 6.6 Hz, CH_2), 4.48 (2 H, t, *J* 6.6 Hz, CH_2), 6.61 (1 H, m, CH), 7.18 - 7.28 (3 H, m, ArH, CH), 7.43 (1 H, d, *J* 7.9 Hz, ArH),

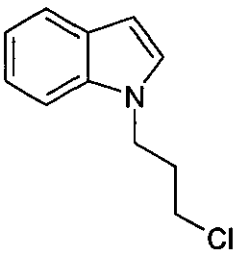
(1 H, d, *J* 7.9 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 42.5 (CH_2Cl), 48.0 (NCH_2), 102.0 (CH), 111.0 (NCH), 120.0, 121.9, 124.2 (ArCH), 127.9 (ArC), 128.8 (ArCH), 135.8 (ArC).

N-(2-Iodoethyl)indole



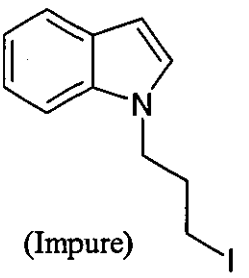
Prepared according to method B, using N-(2-chloroethyl)indole (1.40 g, 7.79 mmol) and dry sodium iodide (5.84 g, 38.97 mmol) in dry acetone (100 cm^3). After work up this gave the title compound as a dark brown oil (1.36 g, 64%). Found: C,44.3, H,3.9, N,5.0%; $C_{10}H_{10}IN$ requires: C,44.3, H,3.9, N,5.2%. Found (LCMS): MH^+ 272.03; $C_{10}H_{10}IN$ requires: MH^+ 272.00. m/z: 130, 117, 90. ν_{max} (NaCl/cm^{-1}) 3031 (C=C-H), 2924 (CH_2), 1563 (C=C), 1439 (CH_2), 1329, 1234, 1126, 743, 729 (ArH) and 504 (C-I). δ_H (400 MHz, $CDCl_3$) 3.44 (2 H, t, *J* 7.7 Hz, CH_2), 4.55 (2 H, t, *J* 7.7 Hz, CH_2), 6.60 (1 H, m, CH), 7.41-7.92 (3 H, m, ArH, CH), (1 H, d, *J* 7.5 Hz, ArH), (1 H, d, *J* 7.5 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 44.3 (CH_2I), 59.2 (NCH_2), 101.9 (CH), 106.7 (NCH), 109.7 (ArC), 119.2, 127.3, 127.8, 134.1 (ArCH), 145.5 (ArC).

N-(3-Chloropropyl)indole



Prepared by modification to method C, using indole (2.00 g, 17.07 mmol), 1,3-dichloropropane (150 cm^3), tetrabutylammonium bromide (0.10 g, 5%), KOH (5.75 g, 0.10 mol) and K_2CO_3 (5.90 g, 0.04 mol). Purification by flash column chromatography gave title product as a brown oil (2.01 g, 61%). Found: MH^+ 194.0823, $C_{11}H_{12}{}^{35}ClN$ requires: MH^+ 194.0738. m/z: 158, 118, 91. ν_{max} (NaCl/cm^{-1}) 3421, 3054 (C=C-H), 2970, 2862 (CH_2), 2218, 1718, 1589 (C=C), 1437 (CH_2), 1191, 1119, 1070, 746, 720 (ArH) and 695 (C-Cl). δ_H (400 MHz, $CDCl_3$) 2.35 (2 H, q, *J* 6.4 Hz, CH_2), 3.55 (2 H, t, *J* 6.4 Hz, CH_2Cl), 4.42 (2 H, t, *J* 6.4 Hz, NCH_2), 6.75 (1 H, d, *J* 3.14 Hz, CH), 7.28 (1 H, d, *J* 3.14 Hz, CH), 7.38 (1 H, t (dd), *J* 7.1, 8.1Hz, ArH), 7.46 (1 H, t (dd), *J* 7.1, 8.1 Hz, ArH), 7.56 (1 H, d, *J* 8.1 Hz, ArH) 7.80 (1 H, d, *J* 8.1 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 30.4 ($CH_2CH_2CH_2$), 42.2 (CH_2Cl), 43.1 (NCH_2), 101.7 (CH), 109.5 (NCH), 117.4, 119.7, 121.6, 122.1 (ArCH), 133.7, 136.1 (ArC).

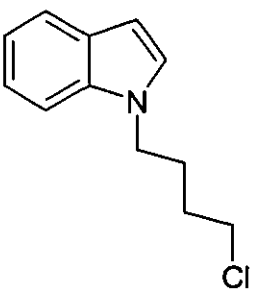
N-(3-Iodopropyl)indole



(Impure)

Prepared by modification to method B, using N-(3-chloropropyl)indole (2.00 g, 10.32 mmol) and dry sodium iodide (7.74 g, 51.62 mmol) in dry acetone (100 cm^3). The work up gave a dark brown oil of an impure mixture containing the desired product. Limited values due to impurities. Found: MH^+ 286.01; $C_{11}H_{12}IN$ requires: MH^+ 286.01. No IR run due to impurity of mixture. δ_H (400 MHz, $CDCl_3$) 2.10 (2 H, m, CH_2), 3.29 (2 H, t, *J* 6.6 Hz, CH_2Cl), 4.42 (2 H, t, *J* 6.2 Hz, NCH_2), 6.76 (1 H, d, *J* 3.1 Hz, CH), 7.21 (1 H, d, *J* 3.1 Hz, CH), 7.35 (1 H, m, ArH), 7.50 (1 H, m, ArH), 7.58 (1 H, d, *J* 7.8 Hz, ArH) 7.69 (1 H, t, *J* 7.8 Hz, ArH).

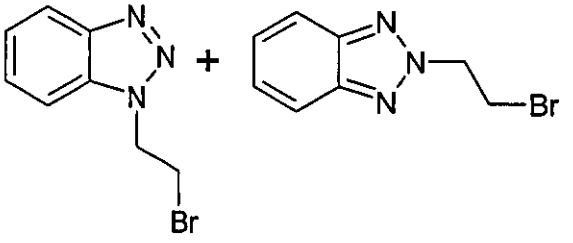
N-(4-Chlorobutyl)indole



Prepared by modification to method C, using indole (2.00 g, 17.07 mmol), 1,4-dichlorobutane (150 cm^3), tetrabutylammonium bromide (0.10 g, 5%), KOH (5.75 g, 0.10 mol) and K_2CO_3 (5.90 g, 0.04 mol). The work up gave a dark brown oil of an impure

mixture containing the desired product. Limited values due to impurities. Found (LCMS): M^+ 208.08; $C_{12}H_{14}{}^{35}ClN$ requires: M^+ 208.08. δ_H (400 MHz, $CDCl_3$) 1.83 (2 H, m, CH_2), 2.06 (2 H, m, CH_2), 3.57 (2 H, t, *J* 6.5 Hz, CH_2Cl), 4.21 (2 H, t, *J* 6.9 Hz, CH_2N), 6.61 (1 H, d, *J* 3.1 Hz, CH), 7.16 (1 H, d, *J* 3.1 Hz, CH), 7.22 (1 H, t (dd), *J* 7.5 Hz, ArH), 7.32 (1 H, t (dd), *J* 7.5 Hz, ArH), 7.43 (1 H, d, *J* 7.5 Hz, ArH), 7.75 (1 H, d, *J* 7.5 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 27.7 ($CH_2CH_2CH_2CH_2Cl$), 29.9 ($CH_2CH_2CH_2CH_2Cl$), 44.6 (CH_2Cl), 45.7 (NCH_2), 101.3(CH), 109.4 (NCH), 119.5, 121.1, 121.6, 127.9 (ArCH), 128.7, 136.0 (ArC).

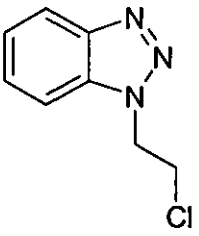
N1-(2-Bromoethyl)benzotriazole and N2-(2-Bromoethyl)benzotriazole



Prepared according to method A, using benzotriazole (2.00 g, 16.79 mmol), triphenylphosphine (5.28 g, 20.15 mmol) and 2-bromoethanol (1.43 cm^3, 20.15 mmol) in dry dioxane (100 cm^3) with DIAD (3.97 cm^3, 4.07 g, 20.15 mmol). After workup and chromatography this yielded the title products. N1-(2-bromoethyl)benzotriazole: yellow crystals (1.03 g, 27%), m.p. 107°C (lit.,[116] m.p. 119-120°C). Found: C,42.2, H,3.8, N,18.2%; $C_8H_8BrN_3$ requires: C,42.5, H,3.6, N,18.6%. Found: M^+ 224.9902 $C_8H_8{}^{79}BrN_3$ requires: M^+ 224.9902. m/z: 146, 132, 119. ν_{max} (NaCl/cm^{-1}) 3446, 2929 (CH_2), 1650 (C=N), 1444 (CH_2), 1329, 1249, 740 (ArH) and 624 (C-Br). δ_H (400 MHz, $CDCl_3$) 3.88 (2 H, t, *J* 6.6 Hz, CH_2Br), 5.03 (2 H, t, *J* 6.6 Hz, CH_2N), 7.41 (1 H, t (dd), *J* 7.4, 8.4 Hz, ArH),

7.54 (1 H, t (dd), *J* 6.8, 7.4 Hz, ArH), 7.60 (1 H, d, *J* 8.4 Hz, ArH), 8.00 (1 H, d, *J* 6.8 Hz, ArH) δ_C (100 MHz, $CDCl_3$) 28.9 (CH_2Br), 49.3 (CH_2N), 109.2 (ArCH), 120.2 (ArCH), 124.2 (ArCH), 127.8 (ArCH), 133.3 (ArC), 145.8 (ArC). N2-(2-bromoethyl)benzotriazole: yellow crystals (2.13 g, 56%), m.p. 62-64 (lit.,[117] m.p. 59-60°C). Found: C,43.0, H,3.2, N,18.6%; $C_8H_8BrN_3$ requires: C,42.5%, H,3.6%, N,18.6%. Found: M^+ 224.9902 $C_8H_8{}^{79}BrN_3$ requires: M^+ 224.9902. m/z: 146, 132, 119. ν_{max}(NaCl/cm^{-1}) 3414, 2960 (CH_2), 1648 (C=N), 1583, 1454 (CH_2), 1284, 886, 743 (ArH) and 630 (C-Br). δ_H (400 MHz, $CDCl_3$) 3.98 (2 H, t, *J* 6.7 Hz, CH_2Br), 5.12 (2 H, t, *J* 6.7 Hz, CH_2N), 7.42 (2 H, m, ArH), 7.88 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 28.1 (CH_2Br), 57.3 (CH_2N), 118.2 (ArCH), 126.7 (ArCH), 144.5 (ArC).

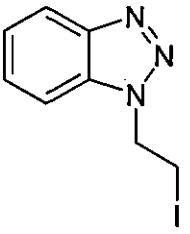
N1-(2-Chloroethyl)benzotriazole



Prepared according to method C, using benzotriazole (2.00 g, 16.79 mmol), 1,2-dichlorothane (70 cm^3), tetrabutylammonium bromide (0.10 g, 5%), KOH (5.65 g, 0.10 mol) and K_2CO_3 (5.80 g, 0.04 mol). Work up gave the title product, N1-(2-chloroethyl)benzotriazole, as white crystals (2.01 g, 66%), m.p. 118°C (lit.,[118] m.p. 108-109°C). Found: MH^+ 182.0478; $C_8H_8{}^{35}ClN_3$ requires: MH^+ 182.0480. m/z: 146, 131, 119. ν_{max} (NaCl/cm^{-1}) 3424, 2938 (CH_2), 1638 (C=N), 1457 (CH_2), 1316, 1274, 1228, 1164,

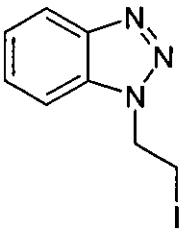
767, 747 (ArH) and (C-Cl). δ_H (400 MHz, $CDCl_3$) 4.06 (2 H, t, *J* 6.2 Hz, CH_2Cl), 4.96 (2 H, t, *J* 6.2 Hz, CH_2N), 7.41 (1 H, t, *J* 8.4 Hz, ArH), 7.55 (1 H, t, *J* 6.8 Hz, ArH), 7.61 (1 H, d, *J* 6.8 Hz, ArH), 8.10 (1 H, d, *J* 8.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 42.3 (CH_2Cl), 49.5 (CH_2N), 109.3, 120.2, 124.1, 127.8 (ArCH), 133.5, 145.9 (ArC). None of the isomeric N2-(2-chloroethyl)benzotriazole was isolated.

N1-(2-Iodoethyl)benzotriazole from Bromide



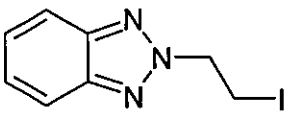
Prepared according to method B, using N1-(2-bromoethyl)benzotriazole (1.00 g, 4.42 mmol) and dry sodium iodide (3.32 g, 22.12 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (1.15 g, 95%), m.p. 106°C. Found: MH^+ 273.9836; $C_8H_8IN_3$ requires: MH^+ 273.9836. m/z: 146, 132, 118. ν_{max} (NaCl/cm^{-1}) 3352, 3018 (C=C-H), 2350, 1722 (C=N), 1613, 1435 (CH_2), 1311, 1072, 944 (C=C), 747 (ArH) and 665 (C-I). δ_H (400 MHz, $CDCl_3$) 3.67 (2 H, t, *J* 7.4 Hz, CH_2I), 5.02 (2 H, t, *J* 7.4 Hz, CH_2N), 7.36 (1 H, d, *J* 6.6 Hz, ArH), 7.48 (2 H, m, ArH), 8.04 (1 H, d, *J* 8.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 30.2 (CH_2I), 50.8 (CH_2N), 109.5, 120.8, 124.9, 128.3 (ArCH), 133.8, 146.4 (ArC).

N1-(2-Iodoethyl)benzotriazole from Chloride



Prepared according to method B, using N1-(2-chloroethyl)benzotriazole (2.00 g, 11.01 mmol) and dry sodium iodide (8.22 g, 55.05 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (2.82 g, 94%), m.p. 102°C. Found: MH^+ 273.9836; $C_8H_8IN_3$ requires: MH^+ 273.9836. m/z: 146, 132, 118. ν_{max} (NaCl/cm^{-1}) 3446, 3018 (C=C-H), 2359, 1785 (C=N), 1613, 1455 (CH_2), 1310, 1240, 1160, 944 (C=C), 747 (ArH) and 665 (C-I). δ_H (400 MHz, $CDCl_3$) 3.65 (2 H, t, *J* 7.3 Hz, CH_2I), 5.00 (2 H, t, *J* 7.3 Hz, CH_2N), 7.39 (1 H, t (dd), *J* 6.1, 6.5 Hz, ArH), 7.52 (2 H, m, ArH), 8.08 (1 H, d, *J* 8.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 30.3 (CH_2I), 50.7 (CH_2N), 109.6, 120.9, 124.7, 128.3 (ArCH), 133.4, 146.5 (ArC).

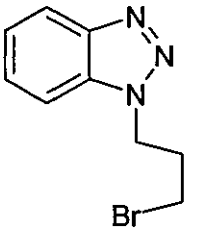
N2-(2-Iodoethyl)benzotriazole from Bromide



Prepared according to method B, using N2-(2-bromoethyl)benzotriazole (2.10 g, 9.29 mmol) and dry sodium iodide (6.96 g, 46.45 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (2.46 g, 97%), m.p. 66-69°C. Found: M^+ 272.9757; $C_8H_8IN_3$ requires: M^+ 272.9755. m/z: 146, 131, 120. ν_{max} (NaCl/cm^{-1}) 3424,

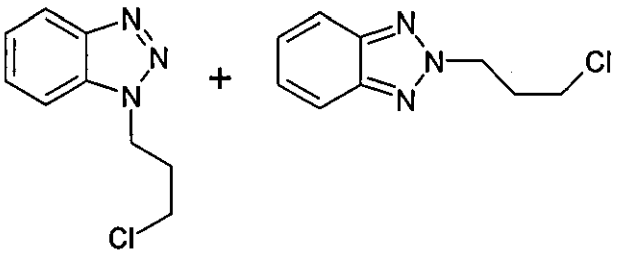
3030 (C=C-H), 1636 (C=N), 1439 (CH_2), 1234, 1130, 849 (C=C), 747 (ArH) and 598 (C-I). δ_H (400 MHz, $CDCl_3$) 3.75 (2 H, t, *J* 7.3 Hz, CH_2I), 5.09 (2 H, t, *J* 7.3 Hz, CH_2I), 7.42 (2 H, m, ArH), 7.88 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 31.3 (CH_2I), 59.3 (CH_2N), 119.2 (ArCH), 127.8 (ArCH), 145.5 (ArC).

N1-(3-Bromopropyl)benzotriazole



Prepared by modification to method A, using benzotriazole (2.00 g, 33.58 mmol), triphenylphosphine (10.57 g, 40.30 mmol) and 3-bromopropanol (3.64 cm^3, 44.30 mmol) in dry dioxane (100 cm^3) with DIAD (7.93 cm^3, 40.30 mmol). After workup this yielded the title product, N1-(3-bromopropyl)benzotriazole as a yellow oil (3.15 g, 78%). Found: MH^+ 240.0153; $C_9H_{10}{}^{79}BrN_3$ requires: MH^+ 240.0136. m/z: 160, 132, 119. ν_{max} (NaCl/cm^{-1}) 3345, 2900 (CH_2), 1660 (C=N), 1542, 1447 (CH_2), 1287, 1140, 821 (ArH) and 707 (C-Br). δ_H (400 MHz, $CDCl_3$) 2.33 (2 H, tt, *J* 6.8, 7.3 Hz, CH_2) 3.28 (2 H, t, *J* 6.8 Hz, CH_2Cl), 4.75 (2 H, t, *J* 7.3 Hz, CH_2N), 7.37 (1 H, m, ArH), 7.46 to 7.58 (2 H, m, ArH), 8.01 (1 H, d, *J* 7.9 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 27.8 (CH_2), 32.4 (CH_2Cl), 51.9 (CH_2N), 109.7, 121.2, 123.7, 126.5 (ArCH), 136.3, 144.4 (ArC). None of the isomeric N2-(3-bromopropyl)benzotriazole was isolated.

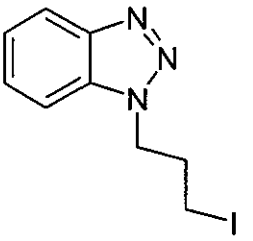
N1-(3-Chloropropyl)benzotriazole and N2-(3-Chloropropyl)benzotriazole



Prepared by modification to method C, using benzotriazole (2.00 g, 16.79 mmol), 1,3-dichloropropane (100 cm^3), tetrabutylammonium bromide (0.10 g, 5%), KOH (5.65 g, 0.10 mol) and K_2CO_3 (5.80g, 0.04 mol). Work up and chromatography gave title products. N1-(3-chloropropyl)benzotriazole: cream oil (1.59 g, 52%). Found: C,55.9, H,4.7, N,21.8%; $C_9H_{10}ClN_3$ requires: C,55.2, H,5.1, N,21.5%. Found: MH^+ 196.0642; $C_9H_{10}{}^{35}ClN_3$ requires: MH^+ 196.0642. m/z: 160, 133, 120. ν_{max} (NaCl/cm^{-1}) 3408, 2879 (CH_2), 1687 (C=N), 1523, 1438 (CH_2), 1202, 765 (ArH) and 720 (C-Cl). δ_H (400 MHz, $CDCl_3$) 2.45 (2 H, dt, *J* 6.0, 6.5 Hz, CH_2) 3.49 (2 H, t, *J* 6.0 Hz, CH_2Cl), 4.79 (2 H, t, *J* 6.5 Hz, CH_2N), 7.34 (1 H, t, *J* 8.3 Hz, ArH), 7.48 (1 H, t, *J* 8.3 Hz, ArH), 7.58 (1 H, d, *J* 8.3 Hz, ArH), 8.03 (1 H, d, *J* 8.3 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 32.4 (CH_2), 41.5 (CH_2Cl), 44.7 (CH_2N), 109.2, 120.0, 124.0, 127.5 (ArCH), 133.2, 145.9 (ArC). N2-(3-chloropropyl)benzotriazole: yellow oil (0.72 g, 24%). Found: C,54.9, H,5.2, N,20.9%; $C_9H_{10}ClN_3$ requires: C,55.2%, H,5.1%, N,21.5%. Found: M^+ 195.0560; $C_9H_{10}{}^{35}ClN_3$ requires: M^+ 195.0563. m/z: 146, 133, 119. ν_{max} (NaCl/cm^{-1}) 3298, 2901 (CH_2), 1732, 1647 (C=N), 1519, 1468 (CH_2), 1232, 1111, 1047, 770 (ArH) and 723 (C-Cl). δ_H (400 MHz, $CDCl_3$) 2.53 (2 H, dt, *J* 6.2, 6.5 Hz, CH_2), 3.56 (2 H, t, *J* 6.2 Hz, CH_2Cl), 4.89 (2 H, t,

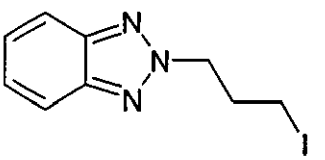
J 6.5 Hz, CH_2N), 7.35 (2 H, m, ArH), 7.84 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 32.6 (CH_2), 41.4 (CH_2Cl), 53.4 (CH_2N), 117.9, 126.4 (ArCH), 144.4 (ArC).

N1-(3-Iodopropyl)benzotriazole from Chloride



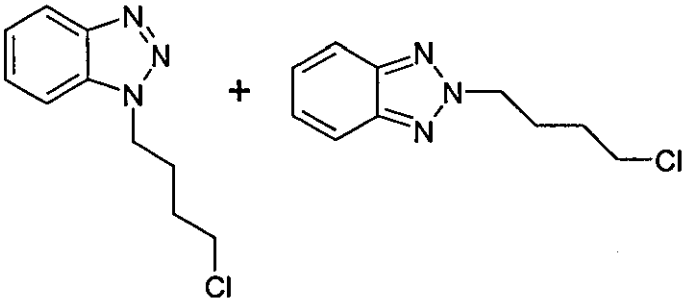
Prepared by modification to method B, using N1-(2-chloropropyl)benzotriazole (1.50 g, 8.26 mmol) and dry sodium iodide (6.19 g, 41.30 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (1.73 g, 77%), m.p. 98-99°C. Found: MH^+ 285.9887; $C_9H_{10}IN_3$ requires: MH^+ 285.9843. m/z: 160, 132, 119. ν_{max} ($NaCl/cm^{-1}$) 3423, 2958 (CH_2), 1610 (C=C), 1464 (CH_2), 1357, 1205, 739 (ArH) and 544 (C-I). δ_H (400 MHz, $CDCl_3$) 2.44 (2 H, dt, *J* 6.4, 6.5 Hz, CH_2), 3.12 (2 H, t, *J* 6.5 Hz, CH_2I), 4.73 (2 H, t, *J* 6.4 Hz, CH_2N), 7.01 (1 H, t, *J* 7.0 Hz, ArH), 7.39 (1 H, t, *J* 7.0 Hz, ArH), 7.44 (1 H, d, *J* 8.1 Hz, ArH), 7.68 (1 H, d, *J* 8.1 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 3.5 (CH_2I), 33.3 (CH_2), 45.9 (CH_2N), 109.3, 118.3, 121.5, 124.5 (ArCH), 127.0, 136.8 (ArC).

N2-(3-Iodopropyl)benzotriazole from Chloride



Prepared by modification to method B, using N2-(3-chloropropyl)benzimidazole (3.20 g, 17.62 mmol) and dry sodium iodide (13.20 g, 88.10 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow oil (2.94 g, 65%). Found: M^+ 285.9856, $C_9H_{10}IN_3$ requires: M^+ 285.9843. m/z: 160, 133, 119. ν_{max} (NaCl/cm^{-1}) 3463, 2962 (CH_2), 1615 (C=N), 1448 (CH_2), 1278, 1145, 969 (C=C), 751 (ArH) and 568 (C-I). δ_H (400 MHz, $CDCl_3$) 2.58 (2 H, dt, *J* 6.4, 6.7 Hz, CH_2), 3.15 (2 H, t, *J* 6.7 Hz, CH_2I), 4.81 (2 H, t, *J* 6.4 Hz, CH_2N), 7.37 (2 H, m, ArH), 7.82 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 4.2 (CH_2I), 30.6 (CH_2), 50.9 (CH_2N), 115.7 (ArCH), 128.2 (ArCH), 138.1 (ArC).

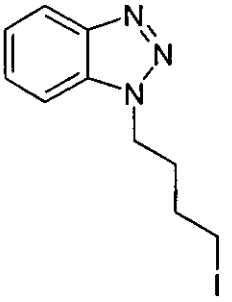
N1-(4-Chlorobutyl)benzotriazole and N2-(4-Chlorobutyl)benzotriazole



Prepared by modification to method C, using benzotriazole (6.00 g, 50.37 mmol) 1,3-dichlorobutane (150 cm^3), tetrabutylammonium bromide (0.30 g, 5%), KOH (16.96 g, 0.30 mol) and K_2CO_3 (17.40 g, 0.13 mol). Work up gave title product. N1-(4-chlorobutyl)benzotriazole: brown crystals (2.56 g, 24%), m.p. 104-106°C. Found: MH^+ 210.0794; $C_{10}H_{12}{}^{35}ClN_3$ requires: MH^+ 210.0793. m/z: 175, 137, 121. ν_{max}(NaCl/cm^{-1}) 3051 (C=C), 2958 (CH_2), 1566, 1445 (CH_2), 1327, 1264, 770 (ArH) and 736 (C-Cl). δ_H (400 MHz, $CDCl_3$) 1.85 (2 H, m, CH_2), 2.17 (2 H, m, CH_2) 3.56 (2 H, t, *J* 6.4 Hz, CH_2Cl),

4.68 (2 H, t, *J* 6.9 Hz, CH_2N), 7.38 (1 H, t, *J* 7.1 Hz, ArH), 7.51 (2 H, m, ArH), 8.05 (1 H, d, *J* 8.4 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 26.8, 29.4 (CH_2CH_2), 44.1 (CH_2I), 47.3 (CH_2N), 109.2, 120.1, 123.9, 127.4 (ArCH), 132.8, 146.0 (ArC). N2-(4-chlorobutyl) benzotriazole: brown crystals (2.23 g, 21%), m.p. 87-88°C. Found: MH^+ 210.0791; $C_{10}H_{12}{}^{35}ClN_3$ requires: MH^+ 210.0793. m/z: 175, 137, 121. ν_{max}(NaCl/cm^{-1}) 3305, 2980 (CH_2), 1699 (C=N), 1504 (CH_2), 1385, 1240, 1107, 761 (ArH) and 723 (C-Cl). δ_H (400 MHz, $CDCl_3$) 1.81 (2 H, m, CH_2), 2.28 (2 H, m, CH_2), 3.55 (2 H, t, *J* 6.4 Hz, CH_2I), 4.76 (2 H, t, *J* 6.8 Hz, CH_2N), 7.36 (2 H, m, ArH), 7.87 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 27.1, 29.3, (CH_2), 44.1 (CH_2I), 55.6 (CH_2N), 118.0 (ArCH), 126.3 (ArCH), 144.3 (ArC).

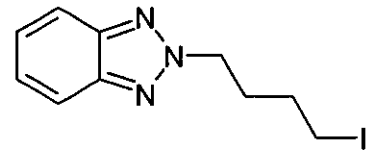
N1-(4-Iodobutyl)benzotriazole



Prepared by modification to method B, using N1-(4-chlorobutyl)benzotriazole (2.00 g, 9.54 mmol) and dry sodium iodide (7.15 g, 47.69 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow oil (2.01 g, 70%). Found: C,39.5, H,4.0, N,13.6%; $C_{10}H_{12}IN_3$ requires: C,39.9, H,4.0, N,14.0%. Found: M^+ 301.0073; $C_{10}H_{12}IN_3$ requires: M^+ 301.0076. m/z: 174, 146, 120. ν_{max}(NaCl/cm^{-1}) 3030 (C=C), 2934 (CH_2), 1601, 1462 (CH_2), 1137, 756 (ArH) and 526 (C-I). δ_H (400 MHz, $CDCl_3$) 1.83 (2 H, m,

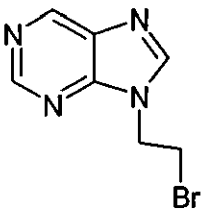
CH_2), 2.13 (2 H, m, CH_2), 3.18 (2 H, t, *J* 6.7 Hz, CH_2I), 4.68 (2 H, t, *J* 6.9 Hz, CH_2N), 7.33 (1 H, t, *J* 6.2 Hz, ArH), 7.48 (2 H, m, ArH), 8.06 (1 H, d, *J* 7.2 Hz, ArH). δ_C (100 MHz, $CDCl_3$) 6.9(CH_2I), 32.5, 35.4 (CH_2), 55.5 (CH_2N), 119.2, 123.8, 124.1, 128.9 (ArCH), 137.0, 142.7 (ArC).

N2-(4-Iodobutyl)benzotriazole



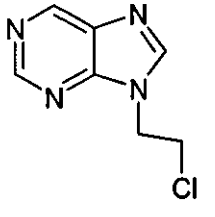
Prepared by modification to method B, using N2-(4-chlorobutyl)benzimidazole (2.00 g, 9.54 mmol) and dry sodium iodide (7.15 g, 47.69 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow solid (2.00 g, 70%). Found: C,39.3%, H,4.1, N14.3%; $C_{10}H_{12}IN_3$ requires: C39.9, H4.0, N14.0%. Found: M^+ 301.0069; $C_{10}H_{12}IN_3$ requires: M^+ 301.0070. m/z: 174, 147, 120. ν_{max}(NaCl/cm^{-1}) 3101 (C=C), 2944 (CH_2), 1599, 1445 (CH_2), 1221, 732 (ArH) and 511 (C-I). δ_H (400 MHz, $CDCl_3$) 1.84 (2 H, q, *J* 6.9, 7.3 Hz CH_2), 2.16 (2 H, dt, *J* 6.8, 7.3 Hz, CH_2), 3.22 (2 H, t, *J* 6.9 Hz, CH_2I), 4.77 (2 H, t, *J* 6.8 Hz, CH_2N), 7.36 (2 H, m, ArH), 7.48 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 5.8(CH_2I), 30.1, 30.8 (CH_2), 55.3 (CH_2N), 118.0, 126.4 (ArCH), 144.3 (ArC).

N9-(2-Bromoethyl)purine[119]



Prepared according to Method A, using purine (0.50 g, 4.20 mmol), triphenylphosphine (1.32 g, 5.04 mmol) and 2-bromoethanol (0.36 cm^3, 5.04 mmol) in dry dioxane (100 cm^3) and DIAD (0.99 cm^3, 5.04 mmol). After workup this yielded the title product as a brown solid (0.62 g, 73%), m.p. 188-190°C (lit.,[119] m.p. 195°C). Found: M^+ 227.9751; $C_7H_7{}^{79}BrN_4$ requires: M^+ 227.9834. m/z: 144, 117, 91. ν_{max}(NaCl/cm^{-1}) 3289 (C=C-H), 2979 (CH_2), 1700, 1496, 1456 (CH_2), 1267, 1219, 1108 (C=C) and 746 (C-Br). δ_H (400 MHz, $CDCl_3$) 3.68 (2 H, t, *J* 5.5 Hz, CH_2Br), 4.58 (2 H, t, *J* 5.5 Hz, CH_2N), 8.14 (1 H, s, ArH), 8.74 (1 H, s, ArH) 8.92 (1 H, s, ArH). δ_C (100 MHz, $CDCl_3$) 55.0 (CH_2Br), 60.3 (CH_2N), 138.8, 141.6 (ArCH), 143.7 (ArC), 155.6 (ArCH), 160.8 (ArC).

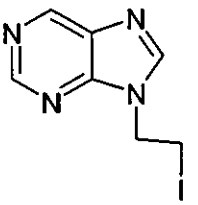
N9-(2-Chloroethyl)purine



Prepared according to Method C, using Purine (0.50 g, 4.20 mmol) in 1,2-dichlorothane (50 cm^3) with tetrabutylammonium iodide (0.03 g, 5%), KOH (1.41 g, 25.19 mmol) and K_2CO_3 (1.45 g, 10.50 mmol). Purification gave the title product as a yellow oil (0.39 g, 59%). Found: MH^+ 183.0548; $C_7H_7{}^{35}ClN_4$ requires: MH^+ 183.0437. m/z: 144, 117, 91.

ν_{max} (NaCl/cm^{-1}) 3411, 3082 (C=C-H), 2962 (CH_2), 1594 , 1502, 1408 (CH_2), 1305, 1200, 1096 (C=C) and 643 (C-Cl). δ_H (400 MHz, $CDCl_3$) 3.91 (2 H, t, *J* 5.6 Hz, CH_2Cl), 4.59 (2 H, t, *J* 5.6 Hz, CH_2N), 8.16 (1 H, s, ArH,), 8.91 (1 H, s, CH), 9.09 (1 H, s, ArH). δ_C (100 MHz, $CDCl_3$) 42.2 (CH_2Cl), 45.6 (NCH_2), 134.1 (ArC), 145.8, 148.7, (ArCH), 151.1 (ArC), 153.2 (ArCH).

N9-(2-Iodoethyl)purine

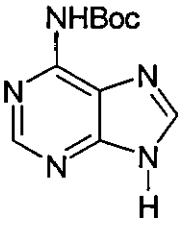


Prepared according to method B, using N9-(2-bromoethyl)purine (0.25 g, 1.23 mmol) and dry sodium iodide (0.92 g, 6.16 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow oil in a yield (0.24 g, 79%). Found MH^+ 274.9748; $C_7H_7IN_4$ requires: MH^+ 274.9795. m/z: 145, 117, 90. ν_{max} (NaCl/cm^{-1}) 3425, 2983 (CH_2), 1642, 1496, 1436 (CH_2), 1312, 1118 (C=C), 720 (ArH) and 520 (C-I). δ_H (400 MHz, $CDCl_3$) 3.61 (2 H, t, *J* 6.5 Hz, CH_2Cl), 4.66 (2 H, t, *J* 6.5 Hz, CH_2N), 8.16 (1 H, s, ArH,), 8.95 (1 H, s, CH), 9.14 (1 H, s, ArH),. δ_C (100 MHz, $CDCl_3$) 23.2 (CH_2I), 45.9 (NCH_2), 135.0 (ArC), 144.9, 148.8, (ArCH), 152.0 (ArC), 152.6 (ArCH).

Prepared according to method B, using N9-(2-chloroethyl)purine (0.25 g, 1.58 mmol) and dry sodium iodide (1.18 g, 7.88 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow oil (0.25 g, 64%). Found MH^+ 274.9750; $C_7H_7N_4I$ requires:

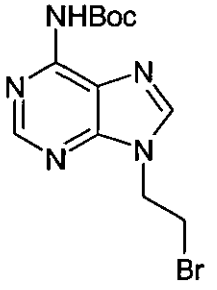
MH^+ 274.9795. m/z: 144, 117, 91. ν_{max} (NaCl/cm^{-1}) 3385, 2976 (CH_2) 1644, 1489, 1455 (CH_2), 1340, 1311, 1121 (C=C), 693 (ArH) and 514 (C-I). δ_H (400 MHz, $CDCl_3$) 3.61 (2 H, t, *J* 6.5 Hz, CH_2Cl), 4.67 (2 H, t, *J* 6.5 Hz, CH_2N), 8.15 (1 H, s ArH,), 8.95 (1 H, s, CH), 9.13 (1 H, s, ArH). δ_C (100 MHz, $CDCl_3$) 23.9 (CH_2I), 44.7 (NCH_2), 135.3 (ArC), 143.2, 148.5, (ArCH), 150.9 (ArC), 153.9 (ArCH).

6-Boc-aminopurine[120]



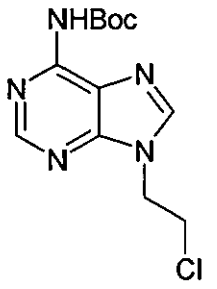
To a dry round-bottomed flask was added adenine (2.00 g, 14.80 mmol), $(Boc)_2O$ (3.23 g, 14.80 mmol) and DIEA (3.84 cm^3, 22.20 mmol) in dry DCM (100 cm^3). The reaction was then stirred for 2 h at room temperature. The solution was washed with H_2O (20 cm^3 x 3), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give 6-Boc-aminopurine in quantitative yield (3.46 g), m.p. 280-282°C (lit.,[120] m.p. 280-300°C). Found: M^+ 235.1069; $C_{10}H_{13}N_5O_2$ requires: M^+ 235.1069. m/z: 134, 119, 90. ν_{max}(NaCl/cm^{-1}) 3174, 2922, 1697 (C=N), 1437 (CH_3), 1386, 1118, 721 and 540. δ_H (400 MHz, $CDCl_3$) 1.71 (9 H, s, $C(CH_3)_3$), 1.85 (1 H, br, CNH), 5.78 (1 H, br, NHBOC), 8.51 (1 H, s, ArH), 8.52 (1 H, s, N=CH). δ_C (100 MHz, $CDCl_3$) 27.9 (CMe_3), 37.6 (CMe_3), 144.6 (N=CH), 147.9, 151.8 (ArC), 155.4 (ArCH), 161.5 (ArC-N), 204.6(C=O).

N9-(2-Bromoethyl)-6-Boc-aminopurine



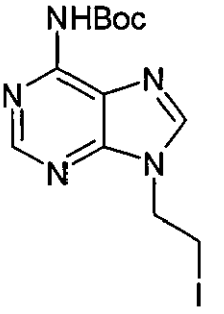
Prepared according to method A, using 6-Boc-aminopurine (3.45 g, 14.67 mmol), triphenylphosphine (4.616 g, 17.60 mmol) and 2-bromoethanol (1.24 cm^3, 17.60 mmol) in dry dioxane (100 cm^3) with DIAD (3.47 cm^3, 17.60 mmol). After workup this yielded the title product as colourless crystals (1.88 g, 37%), m.p. 197°C (lit.,[64] m.p. 195-197°C). Found: M^+ 341.04; $C_{12}H_{17}{}^{79}BrN_5O_2$ requires: M^+ 341.05. m/z: 241, 133, 118, 90. δ_H (400 MHz, $CDCl_3$) 1.63 (9 H, s, $C(CH_3)_3$), 3.82 (2 H, t, *J* 7.8 Hz, CH_2Br), 4.41 (2 H, t, *J* 7.8 Hz, CH_2N), 6.30 (1 H, br, NHBOC), 8.19 (1 H, s, ArH), 8.47 (1 H, s, ArH). δ_C (100 MHz, $CDCl_3$) 31.9 (CMe_3), 38.2 (CMe_3), 38.6 (CH_2Br), 51.9 (CH_2N), 137.5 (N=CH), 147.2, 149.3 (ArC), 157.8 (ArCH), 161.2 (ArC-N) 216.4 (C=O).

N9-(2-Chloroethyl)-6-Boc-aminopurine



Prepared according to method C, using 6-benzyloxycarbonylaminopurine (0.50 g, 2.13 mmol) in 1,2-dichloroethane (50 cm^3), tetrabutylammonium iodide (0.03 g, 5%), KOH (0.72 g, 12.75 mmol) and K_2CO_3 (0.73 g, 5.31 mmol). The material could not be further purified and work up gave title product as an impure yellow oil (0.39 g, 59%). δ_H (400 MHz, $CDCl_3$) 1.58 (9 H, s, $C(CH_3)_3$), 3.93 (2 H, t, *J* 5.5 Hz, CH_2Cl), 4.54 (2 H, t, *J* 5.5 Hz, CH_2N), 5.61 (1 H, br, NHBoc), 7.89 (1 H, s, ArH), 8.36 (1 H, s, ArH).

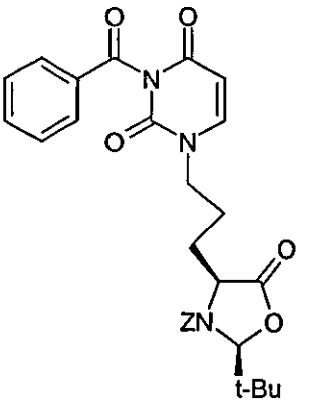
N9-(2-Iodoethyl)-N4-Boc-adenine



Prepared according to method B, using N9-(2-bromoethyl)6-Boc-aminopurine (1.60 g, 4.68 mmol) and dry sodium iodide (3.50 g, 23.38 mmol) in dry acetone (100 cm^3). Work up gave the title compound as a pale yellow oil (0.92 g, 51%). Found (LCMS): M^+ 389.05; $C_{12}H_{17}IN_5O_2$ requires: M^+ 389.05. m/z: 288, 134, 118, 90. ν_{max}(NaCl/cm^{-1}) 3419, 1642 (C=N), 1516 (C=C), 1436 (CH_2), 1373 (CH_3), 1277, 1108, 741. δ_H (400 MHz, $CDCl_3$) 1.39 (9 H, s, $C(CH_3)_3$), 3.57 (2 H, t, *J* 6.6 Hz CH_2I), 4.51 (2 H, t, *J* 6.6 Hz, CH_2N), 5.99 (1 H, br, NH), 7.80 (1 H, s, ArH,), 8.34 (1 H, s, CH), 8.87 (1 H, s, ArH). δ_C (100 MHz, $CDCl_3$) 28.2 (*CMe*$_3$), 29.9 (CH_2I), 38.2 (*C*Me_3), 51.7 (CH_2N), 139.1 (N=CH), 148.7, 150.7 (ArC), 155.8 (ArCH), 163.9 (ArCN) 213.4 (C=O).

Carbon – Carbon bond forming reactions

Conjugate radical addition of Uracil Derivative to Oxazolidinone:



Method A

3-Benzoyl-1-(2-iodoethyl)uracil (1.00 g, 2.69 mmol), (2S,2R)-3-benzyloxycarbonyl-2-*tert*-butyl-4-(ene)oxazolidin-5-one (1.56 g, 5.39 mmol), tributyltin chloride (0.18 cm^3, 0.22 g, 0.67 mmol), sodium cyanoborohydride (0.33 g, 5.39 mmol) and AIBN (0.04 g, 0.27 mmol) were stirred under reflux under nitrogen for 6 h: 1) with AIBN only added at the start; 2) with further additions of AIBN (0.04 g, 0.27 mmol) every hour, then refluxed for 24 h or 44 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (EtOAc:Hexane 1:4 to 4:1 v/v).

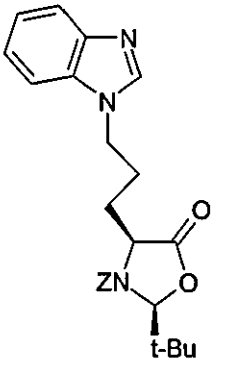
Only the oxazolidinone starting material and the reduced product, 3-benzoyl-1-ethyluracil, was found for the reactions at 24 h [1) 56% and 2) 54%]. δ_H (250 MHz, $CDCl_3$) 1.32 (3 H, t, *J* 7.3 Hz, CH_3), 3.84 (2 H, q, *J* 7.3 Hz, CH_2), 7.34-7.54 (5 H, m, ArH), 7.97 (1 H, br,

N=CH) 8.04 (1 H, br, CHC=O). The reactions at 48 h both produced the reduction product again at 1)57%, 2)52%.

Method B

3-Benzoyl-1-(2-iodoethyl)uracil (1.00 g, 2.69 mmol), (2S,2R)-3-benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (1.56 g, 5.39 mmol) and AIBN (0.04 g, 0.27 mmol) were stirred at reflux under nitrogen with tributyltin hydride (0.09 cm^3, 0.09 g, 0.32 mmol) added dropwise over 1 h and heating continued for 6 h or 24 h. The solvent was removed under reduced pressure. Reduction and addition products were not isolated, although possibly a trace was observed in the mixture, by NMR spectroscopy.

Conjugate radical addition of N1-(2-Iodoethyl)benzimidazole to Oxazolidinone:



Method A

N1-(2-Iodoethyl)benzimidazole (0.10 g, 0.37 mmol), (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.21 g, 0.74 mmol), tributyltin chloride (0.03 cm^3, 0.67 mmol), sodium cyanoborohydride (0.05 g, 0.09 mmol) and AIBN (0.006 g,

0.04 mol) were stirred at reflux under nitrogen for 6 h or 24 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (EtOAc:Hexane 1:4 to 4:1 v/v) to yield only the oxazolidinone starting material and the reduced N1-ethylbenzimidazole product (42% after 6 h and 45% after 12 h). Found: M^+ 146.0842; $C_9H_{10}N_2$ requires M^+ 146.0844. ν_{max} (NaCl/cm^{-1}) 3062, 2980, 2360, 1714, 1498, 1460, 1228, 745. δ_H (250 MHz, $CDCl_3$) 1.34 (3 H, t, *J* 7.3 Hz, CH_3), 4.18 (2 H, q, *J* 7.3 Hz, CH_2), 7.46-7.58 (4 H, m, ArH), 7.98 (1 H, s, N=CH).

Method B

N1-(2-Iodoethyl)benzimidazole (0.10 g, 0.37 mmol), (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.13 g, 0.44 mmol) and AIBN (0.006 g, 0.04 mmol) were stirred under reflux under nitrogen with tributyltin hydride (0.12 cm^3, 0.44 mmol) added dropwise over 1 h and heating continued for 6 h or 24 h. The solvent was removed under reduced pressure and the products purified under column chromatography (EtOAc:Hexane 9:1-1:9 v/v). The oxazolidinone starting material and the reduced N1-ethylbenzimidazole product was obtained as a white gum (45% and 46%). δ_H (250MHz, $CDCl_3$) as above.

Conjugate radical addition to Oxazolidinone using N1-(2-Bromoethyl)benzimidazole

N1-(2-Bromoethyl)benzimidazole (0.10 g, 0.44 mmol), (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.51 g, 1.78 mmol), tributyltin chloride (0.06 cm^3, 1.63 mmol), sodium cyanoborohydride (0.11 g, 0.22 mmol) and AIBN (0.01 g, 0.09 mol) were stirred at reflux under nitrogen for 48 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (EtOAc:Hexane 1:4 to 4:1 v/v) to yield only the oxazolidinone and N1-(2-bromoethyl)benzimidazole starting materials.

Reaction between N1-(2-Iodoethyl)benzimidazole and Cyanoborohydride

N1-(2-Iodoethyl)benzimidazole (0.10 g, 0.37 mmol), sodium cyanoborohydride (0.05 g, 0.09 mmol) and AIBN (0.006 g, 0.04 mmol) were stirred at reflux under nitrogen for 48 h. The solvent was removed by reduced pressure and the residue purified by flash chromatography (EtOAc:Hexane 1:4 to 4:1 v/v) to yield only the unchanged N1-(2-iodoethyl)benzimidazole starting material.

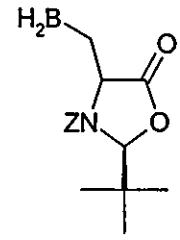
Conjugate radical addition with 20 equivalent of Oxazolidinone

N1-(2-Iodoethyl)benzimidazole (0.10 g, 0.37 mmol), (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (4.26 g, 7.35 mmol), tributyltin chloride (0.03 cm^3, 0.67 mmol), sodium cyanoborohydride (0.05 g, 0.09 mmol) and AIBN (0.006 g, 0.04 mol) were stirred at reflux under nitrogen for 48 h. The solvent was removed under

reduced pressure and the residue purified by flash chromatography (EtOAc:Hexane 1:4 to 4:1 v/v) to yield only the oxazolidinone starting material and the reduced N1-ethylbenzimidazole product in 43% yield.

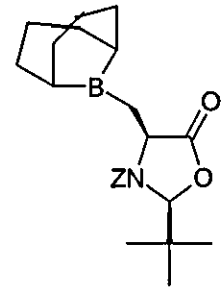
Hydroboration with Borane complexes

Hydroboration with diethylether, THF or dimethyl sulphide complex



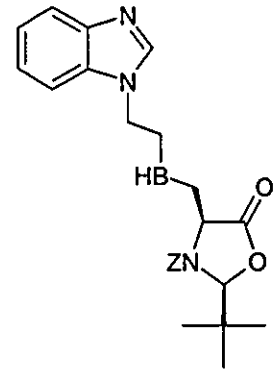
(2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one (0.40 g, 1.38 mmol) was stirred vigorously in diethyl ether (100 cm^3) in a flame-dried round-bottomed flask under nitrogen and cooled to 0°C. Borane diethylether complex (0.28 cm^3, 5.53 mmol), borane tetrahydrofuran complex (0.28 cm^3, 5.53 mmol) or borane methylsulfide (0.52 cm^3, 5.53 mmol) was added to the mixture over 1 h. The mixture was warmed to 30°C for 48 h then to reflux for 48 h. Water (1 cm^3) was then added and the organic layer separated off, dried ($MgSO_4$) and the solvent removed under reduced pressure. No hydroboration products were seen from any of the reactions.

Hydroboration with 9-BBN



To a flame-dried round-bottomed flask was added (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.05 g, 0.17 mmol) and 9-BBN (0.04 g, 0.17 mmol) in solvent [DCM, diethyl ether or THF (50 cm^3)] under nitrogen. This mixture was stirred at 0°C for 36 h and was monitored using TLC. When no reaction had occurred after this time, the temperature was raised to RT and the mixture stirred for a further 36 h, again monitored by TLC. As no reaction was observed, the temperature was raised again to 30°C, then reflux. Water was added and organic layer separated off, dried ($MgSO_4$) and the solvent removed under reduced pressure. There was no reaction of the oxazolidinone with 9-BBN at reflux after 36 h in DCM, diethyl ether or THF.

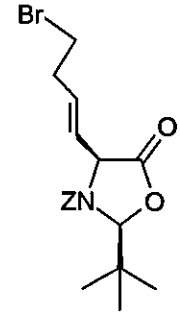
Coupling Reaction



To a flame dried flask was added N-vinylbenzimidazole (0.30 g, 2.07 mmol) and (2S,2R)-3-benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one (0.6 g, 2.07 mmol) in THF (20 cm^3) under nitrogen. This was cooled to 0°C and BH_3:THF complex (1M, 1.0369cm^3) added and the reaction mixture stirred for a further 12 h. KOH in methanol was then added (2 M, 10 cm^3) followed by $AgNO_{3(aq)}$ (5 M, 2 cm^3) at RT. After 2 h, KOH (3.00 g) was added and the mixture refluxed for 4 h. The reaction mixture was then poured onto water (50 cm^3), acidified to pH 2 with HCl, filtered and the precipitate extracted with boiling acetone. The acetone was filtered and evaporated off, but no product of the reaction of the heterocycle with the oxazolidinone template was isolated.

Cross Metathesis

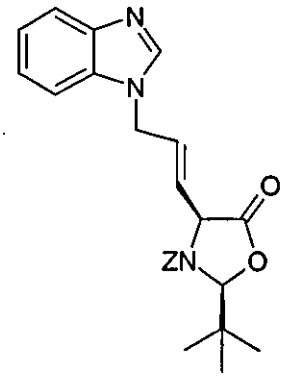
Cross Metathesis of Oxazolidinone Template with Allyl Bromide



(2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one (0.05 g, 0.17 mmol) and Grubbs catalyst (II) (0.001 g, 10 mol%) were place in a flame-dried round-bottomed flask in DCM (20 cm^3) under nitrogen. Allyl bromide (0.21 g, 1.73 mmol) was then added and the reaction mixture stirred at reflux for up to 48 h. The

solvent was removed under reduced pressure. None of the desired products were seen, only oxazolidinone starting material was recovered.

Cross Metathesis of Oxazolidinone Template with N1-Vinylbenzimidazole



(2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.05 g, 0.17 mmol) and Grubbs catalyst (II) (0.001 g, 10 mol%) were place in a flame-dried round-bottomed flask in DCM (20 cm^3) under nitrogen. N1-Vinylbenzimidazole (0.05 g, 0.35 mmol) was then added and the reaction mixture stirred at reflux for up to 48 h. The solvent was removed under reduced pressure. No purification was performed as the ^1H NMR spectrum showed only oxazolidinone and N1-vinylbenzimidazole starting materials and no cross metathesis product.

Organocuprates

Lithium/Halogen Exchange (Methyl Acrylate Acceptor)

Finely cut lithium (0.003 g, 0.44 mmol) was placed in a flame-dried round-bottomed flask and suspended in THF (40 cm^3) under nitrogen. N1-(2-Bromoethyl)benzimidazole (0.10 g, 0.44 mmol), in THF (10 cm^3), was added slowly to the round-bottomed flask and the solution stirred at room temperature. Once a colour change had occurred the reaction was either a) cooled to approximately -10°C, b) kept at RT, or c) cooled to -78°C. Any N1-(2-bromoethyl)benzimidazole not added before the colour change was then added. After 2 h the solution was cooled further (as necessary) to -78°C then siphoned through glass wool into another flame-dried round-bottomed flask at -78°C and kept under nitrogen. A pre-cooled suspension of copper(I) iodide (0.04 g, 0.22 mmol) in THF (10 cm^3) was then added slowly to the stirred mixture and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, before cooling again to -78°C. Methyl acrylate (0.02 cm^3, 0.33 mmol) was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to RT and stirred for a further 20 h at RT. The reaction mixture was filtered and the solvent removed under reduced pressure. The mixture was then subject to flash column chromatography but no addition product was found in any of the fractions, and with further attempts at the reaction no separation was pursued as the crude ^1H NMR spectra showed no sign of the desired conjugate addition product.

Initial attempts of cooling to a) -10°C gave the β-elimination product, dealkylated benzimidazole. Attempts at b) RT and c) -78°C gave the alternative elimination products of ethyl and vinyl benzimidazoles.

The data for the ethyl product is as detailed for N1-ethylbenzimidazole, an undesired product from the attempted radical reaction of the oxazolidinone template and N1-(2-iodoethyl)benzimidazole. The data for the vinyl product is as detailed previously for N1-vinylbenzimidazole.

This reaction was repeated using N1-(2-chloroethyl)benzimidazole (0.08 g, 0.44 mmol) and N1-(2-iodoethyl)benzimidazole (0.12 g, 0.44 mmol) as well as the addition of TMSCl (0.44 mmol), but these gave none of the desired product giving complex TLC results and inconclusive ^{1}H NMR spectra.

Lithium/Halogen Exchange (Oxazolidinone Acceptor)

Finely cut lithium (0.003 g, 0.44 mmol) was placed in a flame-dried round-bottomed flask and suspended in THF (40 cm^3) under nitrogen. N1-(2-Bromoethyl)benzimidazole (0.10 g, 0.44 mmol), in THF (10 cm^3), was added slowly to the round-bottomed flask and the solution stirred at room temperature. Once a colour change had occurred the reaction was either a) cooled to approximately -10°C, b) kept at RT, or c) cooled to -78°C. Any N1-(2-bromoethyl)benzimidazole not added before the colour change was then added. After 2 h the solution was cooled further (as necessary) to -78°C then siphoned through glass wool into another flame-dried round-bottomed flask at -78°C and kept under nitrogen. A pre-cooled suspension of copper(I) iodide (0.04 g, 0.22 mmol) in THF (10 cm^3) was then added

slowly to the stirred mixture and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, before cooling again to -78°C. (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl)oxazolidin-5-one (0.06 g, 0.22 mmol) was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to RT and stirred for a further 20 h at RT. The reaction mixture was filtered and the solvent removed under reduced pressure. The mixture was then subject to flash column chromatography but no addition product was found in any of the fractions, and with further attempts at the reaction no separation was pursued as the crude ^{1}H NMR spectra again showed no sign of the desired conjugate addition product.

The reactions at a) -10°C gave the β-elimination product, dealkylated benzimidazole and at b) RT and c) -78°C gave the alternative elimination products of ethyl and vinyl benzimidazoles, both obtained as white gums.

Again, the data for the ethyl product is as detailed for N1-ethylbenzimidazole, an undesired product from the attempted radical reaction of the oxazolidinone template and N1-(2-iodoethyl)benzimidazole. The data for the vinyl product is as detailed previously for N1-vinylbenzimidazole.

The addition of TMSCl (0.44 mmol), also gave none of the desired product, giving complex TLC results and inconclusive ^{1}H NMR spectra.

_n_-Butyllithium (Methyl Acrylate Acceptor)

To a flame-dried round-bottomed flask a solution of a) N1-(2-bromoethyl)benzimidazole (0.10 g, 0.44 mmol) or b) N1-(2-chloroethyl)benzimidazole (0.08 g, 0.44 mmol) in 1) dry

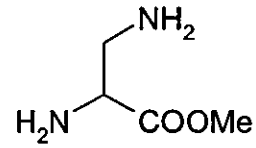
ether or 2) THF (40 cm^3) was added under nitrogen at -78°C. To this solution fresh *n*-butyllithium (0.09 g, 1.47 mmol) was added and the reaction mixture stirred for 2 h at -78°C (or until there was a colour change). A pre-cooled suspension of copper(I) iodide (0.04 g, 0.22 mmol) in ether or THF (10 cm^3) was then added slowly to the solution and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, before cooling again to -78°C. Methyl acrylate (0.02 g, 0.22 mmol) was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to RT and stirred for a further 20 h at RT. The reaction was then worked up using saturated ammonium chloride solution (20 cm^3) and extracted using ether (20 cm^3). This was then dried ($MgSO_4$) and the solvent evaporated off under reduced pressure. Separation was attempted by flash column chromatography. The starting material methyl acrylate was the only compound recovered from any of the reaction combinations. Even upon an attempt at separating the compounds the ^1H NMR spectra were inconclusive as the mixture proved to be inseparable.

_n_-Butyllithium (Oxazolidinone Acceptor)

To a flame-dried round-bottomed flask a solution of a) N1-(2-bromoethyl)benzimidazole (0.10 g, 0.44 mmol) or b) N1-(2-chloroethyl)benzimidazole (0.08 g, 0.44 mmol) in 1) dry ether or 2) THF (40 cm^3) was added under nitrogen at -78°C. To this solution fresh *n*-butyllithium (0.09 g, 1.47 mmol) was added and the reaction mixture stirred for 2 h at -78°C (or until there was a colour change). A pre-cooled suspension of copper(I) iodide (0.04 g, 0.22 mmol) in ether or THF (10 cm^3) was then added slowly to the solution and the temperature slowly raised to -10°C over 1 h and then to 0°C over 10 min, before cooling

again to -78°C. (2S,2R)-3-Benzyloxycarbonyl-2-*tert*-butyl-4-(methylsulfonylmethyl) oxazolidin-5-one (0.06 g, 0.22 mmol) was then added and the solution stirred for 2 h at -78°C before being allowed to warm slowly to RT and stirred for a further 20 h at RT. The reaction was then worked up using saturated ammonium chloride (20 cm^3) solution and extracted using ether (20 cm^3). This was then dried ($MgSO_4$) and the solvent evaporated off under reduced pressure. Separation was attempted by flash column chromatography. The starting material of the oxazolidinone template was again the only compound recovered from any of the reaction combinations. Even upon an attempt at separating the compounds the ^1H NMR spectra were again inconclusive as the mixture proved to be inseparable.

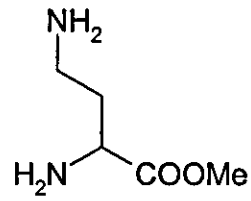
DL-Methyl 2,3-diaminopropanoate[98]



DL-2,3-Diaminopropionic acid (1.20 g, 8.54 mmol) and dry methanol (100 cm^3) were added to a flame-dried multi-neck round-bottomed flask, which was then cooled in an ice bath to 0°C. Thionyl chloride (0.62 cm^3, 85.38 mmol) was added slowly and the reaction mixture was then allowed to warm to RT before heating at reflux for 24 h. The methyl ester bis-hydrochloride was purified by crystallisation (1.20 g, 92%), m.p. 91°C. Found: MH^+ 119.0813; $C_4H_{10}N_2O_2$ requires: MH^+ 119.0815. m/z: 102, 81. ν_{max} (NaCl/cm^{-1}) 3304, 2928 (CH_2), 1698 (C=O), 1456, 1198, 1055, 598. δ_H (400 MHz, DMSO-d^6) 3.32

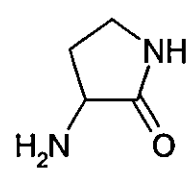
(2 H, dd, *J* 5.8, 6.1 Hz, CH_2), 3.78 (3 H, s, CH_3), 4.44 (1 H, t, *J* 6.1 Hz CH), 8.90 (4 H, br, 2 x NH_2). δ_C (100 MHz, DMSO- d^6) 37.1 (CH_2), 50.0 (CH), 53.4 (OMe), 167.0 (C=O).

DL-Methyl 2,4-diaminobutyrate[121]



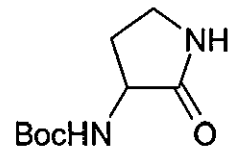
DL-2,4-Diaminobutyric acid (0.50 g, 4.23 mmol) and dry methanol (100 cm^3) were added to a flame-dried multi-neck round-bottomed flask, which was then cooled in an ice bath to 0°C. Thionyl chloride (0.31 cm^3, 4.23 mmol) was added slowly and the reaction mixture was then allowed to warm to RT before heating at reflux for 48 h. The methyl ester bis-hydrochloride salt was purified by crystallisation (0.54 g, 96%), m.p. 182°C (lit.,[121] m.p. 239°C). Found: MH^+ 133.0974; $C_5H_{12}N_2O_2$ requires: MH^+ 133.0971. m/z: 91, 86. ν_{max} (NaCl/cm^{-1}) 3201 (NH_2), 2931 (CH_2), 1744 (C=O), 1636 (NH_2), 1493 1151 (CH_2), 941 and 598 (CH). δ_H (400 MHz, CD_3OD) 2.27 (2 H, br, CH_2), 3.27 (2 H, br, NCH_2), 3.90 (3 H, s, CH_3), 4.29 (2 H, br, CH). δ_C (100 MHz, CD_3OD) 29.2 (CH_2), 34.7 (CH_2), 51.7 (CH), 54.5 (OMe), 170.0 (C=O).

Attempted Preparation of 3-Aminopyrrolidin-2-one



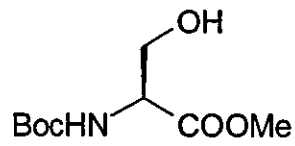
Chloroform (50 cm^3) was saturated with ammonia gas and methyl 2,4-diaminobutyrate (0.50 g, 3.78 mmol) was added to the saturated solution, which was then shaken for 3 h at RT. The chloroform and ammonia gas were removed under reduced pressure to give the presumed lactam product (0.33 g, 86%). ν_{max} (NaCl/cm^{-1}) 3425 (NH_2), 1652, 1635 (C=O), 1404 (CH_2), 1140, 974 and 689 (Ar). δ_H (400 MHz, D_2O) 2.05 (1 H, br, CHC*HH*), 2.98 (1 H, br, $NHCH_2$), 4.31 (1 H, br, CH,). δ_C (100 MHz, D_2O) 28.6 (CH_2), 36.7 (NCH_2), 52.7 (CH), 173.0 (C=O).

Attempted Preparation of 3-Boc-Aminopyrrolidin-2-one



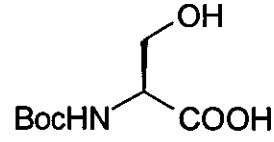
To a dry round-bottomed flask was added lactam (0.40 g, 4.00 mmol), DIEA (0.83 cm^3, 4.79 mmol) and $(Boc)_2O$ (0.87 g, 4.79 mmol) in dry DCM (20 cm^3) under nitrogen and the solution was stirred for 2 h. The solvents were removed under reduced pressure to give the presumed Boc protected lactam (0.73 g, 91%). ν_{max} (NaCl/cm^{-1}) 2982 (CH_2), 1810 (C=O), 1756 (C=O), 1372 (CMe_3), 1213, 1120 (CO), 1071, 845 and 775 (Ar). δ_H (400MHz, MeOD) 1.56 (9 H, s, $C(CH_3)_3$), 2.23 (2 H, m, CHCH_2), 3.30 (2 H, m, CH_2), 3.80 (1 H, t, *J* 6.2 Hz, CH). δ_C (100 MHz, MeOD) 22.2 (CH_2), 28.3 (NCH_2), 37.6 (CMe_3), 53.2 (CH), 114.5 (CMe_3) 173.9, 215.4 (C=O).

N-Boc-Serine Methyl Ester.[120]



To a dry round-bottomed flask was added serine methyl ester (1.00 g, 6.43 mmol), $(Boc)_2O$ (1.40 g, 6.43 mmol) and DIEA (1.34 cm^3, 7.71 mmol) in dry DCM (100 cm^3). The reaction was then stirred for 2 h at room temperature. The solution was washed with H_2O (20 cm^3 x 3), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give N-Boc-serine methyl ester (1.24 g, 94%), m.p. °C (lit.,[120] m.p. °C). Found: MH^+ 220.1187; $C_9H_{17}NO_5$ requires: MH^+ 220.1185. m/z: 164, 120. ν_{max} (NaCl/cm^{-1}) 3393 (OH), 2977 (CH_2), 1754, 1691 (C=O), 1531 (N-O), 1366 (OH), 1162 (C-O), 1063 (C-H), 849 and 779 (CH_2). δ_H (400 MHz, $CDCl_3$) 1.43 (9 H, s, $C(CH_3)_3$), 3.76 (1 H, s, OCH_3), 3.91 (2 H, m, CH_2), 4.23 (1 H, br, CH), 5.59 (1 H, d, *J* 7.6 Hz, NH). δ_C (100 MHz, $CDCl_3$) 27.4 (OMe), 28.3 (CMe_3), 52.6 (CH), 55.7 (CMe_3), 63.3 (CH_2), 155.8, 171.4 (C=O).

N-Boc-Serine.[121]

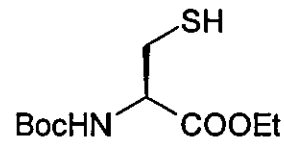


To a dry round-bottomed flask was added serine (1.00 g, 9.52 mmol), $(Boc)_2O$ (2.08 g, 9.52 mmol) and DIEA (1.99 cm^3, 11.42 mmol) in dry DCM (100 cm^3). The reaction was then stirred for 2 h at room temperature. The solution was washed with H_2O (20 cm^3 x 3), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give N-Boc-serine in quantitative yield (1.95 g), m.p. 98°C (lit.,[121] m.p. 91-95°C). Found: MH^+

230.1030; $C_8H_{15}NO_5$ requires: MH^+ 230.1028. m/z: 128, 114. ν_{max} (NaCl/cm^{-1}) 3405 (OH), 2979 (CH_2), 1807, 1731 (C=O), 1591 (N-O), 1371 (OH), 1117 (C-O), 951 (C-H), 844, 775 and 666 (CH_2). δ_H (400 MHz, $CDCl_3$) 1.39 (9 H, s, $C(CH_3)_3$), 3.02 (1 H, br, OH), 3.82 (2 H, m.br, CH_2), 4.23 (1 H, d, *J* 1.3 Hz, CH), 5.47 (1 H, d, *J* 6.9 Hz, NH). δ_C (100 MHz, $CDCl_3$) 14.1 (CMe_3), 21.0 (CH), 56.3 (CMe_3), 63.7 (CH_2), 155.9, 169.9 (C=O).

Formation of Sulphur containing Heterocyclic Amino Acids

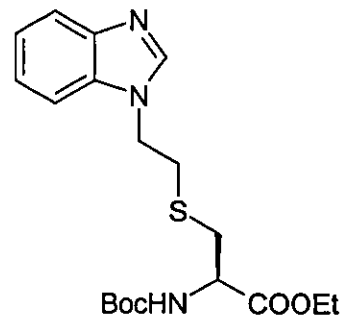
N-Boc-cysteine-O-ethyl ester



To a dry round-bottomed flask was added N-BOC-cysteine-O-ethyl ester (10.00 g, 53.86 mmol), $(Boc)_2O$ (11.76 g, 53.86 mmol) and DIEA (11.18 cm^3, 64.63 mmol) in dry DCM (200 cm^3). The reaction was then stirred for 2 h at room temperature. The solvent was then washed with 1 M HCl (100 cm^3 x 2), 1 M $NaHCO_3$ (100 cm^3 x 2), and H_2O (100 cm^3 x 2), dried ($MgSO_4$), and the solvent removed under reduced pressure. Work up gave the Boc-cysteine methyl ester as off white oil (12.62 g, 94%). Found: MH^+ 250.1108; $C_{10}H_{19}NO_4$ requires: MH^+ 250.1108. m/z: 211, 193, 135. ν_{max} (NaCl/cm^{-1}) 3367, 2979, 1712, 1507, 1368, 1165, 1029, 861, 737. δ_H (400 MHz, $CDCl_3$) 1.22 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.38 (9 H, s, $C(CH_3)_3$), 2.90 (2 H, br, CH_2S), 4.17 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.50 (1 H, br,

CH), 5.43 (1 H, br, NH). δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 27.2 (CH_2S), 28.2 (CMe_3), 56.2 (CH), 68.9 (OCH_2) 80.2 (CMe_3)170.4 (C=O).

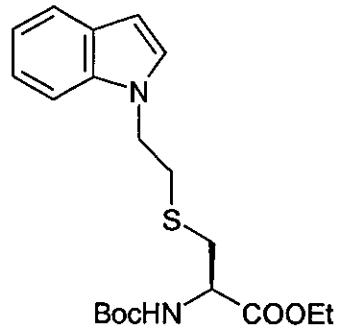
N-Boc-S-[2-(benzimidazol-1-yl)ethyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.10 g, 0.20 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel followed by N-Boc-(S)-cysteine ethyl ester (0.50 g, 2.01 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 20 min before the addition of N1-(2-iodoethyl)benzimidazole (2.18 g, 8.02 mmol), after which the reaction mixture was heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure (ethyl acetate/hexane). Purification was by column chromatography. The title product was formed as a yellow oil (0.44 g, 66%). Found C,58.2, H,6.8, N,10.4%; $C_{19}H_{27}N_3O_4S$ requires C,58.0, H,6.9, N,10.7%. Found: MH^+ 394.1802; $C_{19}H_{27}N_3O_4S$ requires: MH^+ 394.1801. m/z: 338, 279, 220, 192, 160, 146, 118. ν_{max} (NaCl/cm^{-1}) 3352, 3223 (CON-H), 2976 (CH_2), 1708 (C-C=O), 1494 (N-C=O) 1458 (C=N), 1366 (-$C(CH_3)_3$), 1250, 1165,

1024 (CH_2-S-CH_2), 744 and 721 (ArH). δ_H (400 MHz, $CDCl_3$) 1.18 (3 H, t, *J* 7.2 Hz, CH_2CH_3), 1.36 (9 H, s, CMe_3), 2.88 (2 H, dd/dd, *J* 6.3, 6.6 Hz, SCH_2), 2.91 (2 H, t, *J* 7.0 Hz, SCH_2), 4.11 (2 H, q, *J* 7.2 Hz, CH_2CH_3), 4.29 (2 H, t, *J* 7.0 Hz, NCH_2), 4.41 (1 H, br, CH), 5.23 (1 H, br, NH), 7.20-7.75 (4 H, m, ArH), 7.88 (1 H, s, N=CH). δ_C (100 MHz, $CDCl_3$) 14.2 (CH_2CH_3), 28.3 (CMe_3), 32.3, 34.8 (CH_2SCH_2), 42.9 (CMe_3), 44.9 (CH_2N), 61.9 (CH_2CH_3), 109.4 (NCH), 120.5, 122.4, 123.1, 128.6, 132.1 (ArCH), 133.3 (ArC), 143.7 (ArC), 155.2, 170.7 (*C*=O).

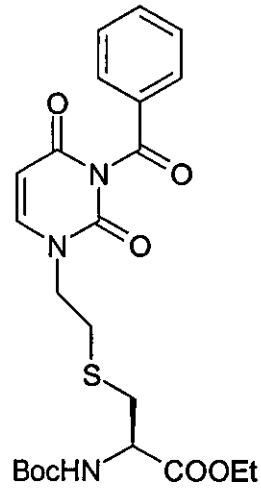
N-BOC-S-[2-(Indol-1-yl)ethyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.10 g, 0.20 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel and followed by N-Boc-(S)-cysteine ethyl ester (0.50 g, 2.01 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 10min before the addition of N-(2-iodoethyl)indole (2.17 g, 8.02 mmol), after which the reaction mixture was heated at reflux for 48 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O

(20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title product was formed as a yellow oil (0.24 g, 31%). Found: M^+ 347.1431; $C_{20}H_{28}N_2O_4S$ requires M^+ 392.1771; $C_{18}H_{23}N_2O_3S$ requires M^+ 347.1431 (loss of ethyl ester). m/z: 291, 249, 232, 203, 176, 146. δ_H (400 MHz, $CDCl_3$) 1.23 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.36 (9 H, s, CMe_3), 1.73 (2 H, br, SCH_2), 2.92 (2 H, br, SCH_2), 4.12 (2 H, br, NCH_2), 4.14 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.49 (1 H, br, CH), 5.29 (1 H, br, NH), 7.09-7.26 (2 H, m, ArH), 7.37-7.51 (2 H, m, ArH) 7.58-7.63 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 14.1 (CH_2CH_3), 28.3 (CMe_3), 29.7 (CH_2SCH_2), 30.3 (CMe_3), 31.9 (CH_2SCH_2), 45.1 (CH_2N), 61.8 (CH_2CH_3), 107.8 (CH), 126.4 (ArC), 128.4, 128.6, 129.2, 131.9, 132.2 and 132.9 (ArCH), 143.1 (ArC), 157.6, 179.9 (*C*=O).

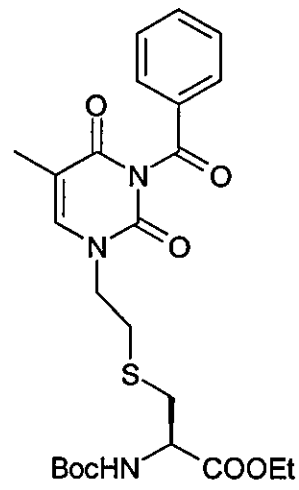
N-Boc-S-[2-(3-benzoyluracil-1-yl)ethyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.10 g, 0.20 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane

decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel followed by N-Boc-(S)-cysteine ethyl ester (0.50 g, 2.01 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 15 min before the addition of N1-(2-iodoethyl)-3-benzoyluracil (2.98 g, 8.02 mmol), after which the reaction was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title product was formed as a yellow oil (0.62 g, 64%). Found (LCMS): MH^+ 272.29; $C_{23}H_{29}N_3O_7S$ requires: MH^+ 493.19; $C_{11}H_{16}N_2O_4S$ MH^+ requires 272.08 (loss of NHBOC and benzoyl protection). m/z: 243, 188. Found: C,56.8, H,5.8, N,9.3%; $C_{23}H_{29}N_3O_7S$ requires C,56.2, H,5.9, N,8.5%. ν_{max} (NaCl/cm^{-1}) 3416 (CONH), 2930 2857 (CH_2), 2357, 1713 (C-C=O), 1650 (N-C=O), 1555(N-C=O), 1453 (CH_2), 1249, 1162, 1047 and 667 (ArH). δ_H (400 MHz, $CDCl_3$) 1.19 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.95 (9 H, s, CMe_3), 3.13 (1 H, t, *J* 6.6 Hz, SCH_2), 3.35 (2 H, t, *J* 6.4 Hz, SCH_2), 4.04 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.86 (3 H, m, NCH_2 and CH), 5.72 (1 H, d, *J* 8.0 Hz, COCH=CH), 7.19-7.62 (6 H, m, ArH, NCH=CH). δ_C (100 MHz, $CDCl_3$) 7.1 (CH_2CH_3), 20.7 (CMe_3), 50.0, 52.4 (CH_2SCH_2), 59.2 (CH_2CH_3), 62.1 (CH_2N), 64.9 (CMe_3), 68.3 (CH), 100.5 (NCH=CH), 127.2 (ArCH), 128.0 (CH=CHCO), 130.5 (ArCH), 143.7 (ArC), 148.3, 155.4, 161.2, 167.4, 169.9 (C=O).

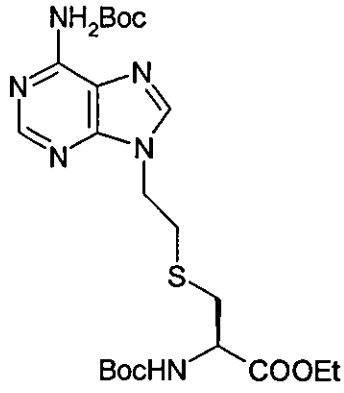
N-Boc-S-[2-(3-Benzoylthymin-1-yl)ethyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.10 g, 0.20 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel followed by N-Boc-(S)-cysteine ethyl ester (0.50 g, 2.01 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 20 min before the addition of N1-(2-iodoethyl)-3-benzoylthymine (3.09 g, 8.02 mmol), after which the reaction was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title product was formed as a yellow oil (0.61 g, 62%). Found: M^+ 388.16; $C_{24}H_{31}N_3O_7S$ requires: M^+ 506.20; $C_{19}H_{20}N_2O_5S$ requires 388.11. m/z: 185, 127. Found: C,56.8, H,6.4, N,9.0%; $C_{24}H_{31}N_3O_7S$ requires C,57.0, H,6.2, N,8.3%. νmax (NaCl/cm^{-1}) 3284 (CONH), 2978, 2930 (CH_2), 1745 (C-C=O), 1698, 1657 (N-C=O), 1454 (CH_2), 1367, 1251, 1175, 668 (ArH). δ_H (400 MHz, $CDCl_3$) 1.19 (3 H, t, *J* 6.6 Hz, CH_2CH_3), 1.75 (9 H, s, CMe_3),

1.81 (3 H, s, CH_3), 3.26 (1 H, t, *J* 7.8 Hz, SCH_2), 3.39 (2 H, t, *J* 7.1 Hz, SCH_2), 4.11 (2 H, q, *J* 6.6 Hz, CH_2CH_3), 4.16 (2 H, t, *J* 7.1 Hz, NCH_2), 4.68 (1 H, t, *J* 8.5 Hz, CH), 5.71 (1 H, s, C=CH), 7.24-7.95 (5 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 11.2 (CH_2CH_3), 11.7 ($COCH_3$) 27.3 (CMe_3), 40.9, 48.3 (CH_2SCH_2), 54.0 (CMe_3), 56.8 (CH_2N), 65.9 (CH_2CH_3), 99.0 (CH), 106.5 (CH_2CH_3), 107.6 (CCH_3=CH), 135.3 (CCH_3=*CH*), 135.7, 140.3 (ArCH), 150.1 (ArC), 160.0, 162.6, 163.1, 163.2, 163.4 (*C*=O).

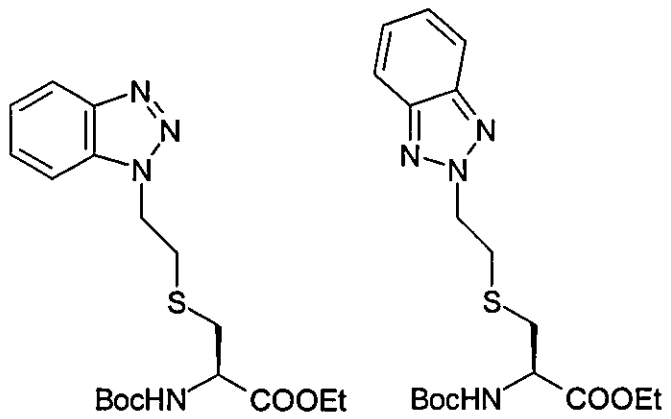
N-Boc-S-[2-(6-t-Butyloxycarbonylaminopurin-9-yl)ethyl]-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.10 g, 0.20 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. 25 cm^3 of dry THF was added to the reaction vessel followed by N-Boc-cysteine ethyl ester (0.50 g, 2.01 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 10 min before the addition of N-Boc-S-[2-(6-t-Butyloxycarbonylaminopurin-9-yl)ethyl]-cysteine ethyl ester (3.12 g, 8.020mmol) after which the reaction mixture was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated, washed with H_2O (20 cm^3), dried ($MgSO_4$), and

the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title product was formed as a yellow oil (0.42 g, 41%). ν_{max} (NaCl/cm^{-1}) 3419, 2979 (CH_2), 1738 (C=O), 1701 (C=N), 1657 (C=C), 1455, 1367 (CH_2), 1252, 1175, 1026, 807, 764, 734 and 668 (ArH). δ_H (250 MHz, $CDCl_3$) 1.14 (3 H, t, *J* 7.2 Hz, CH_2CH_3), 1.44 and 1.47 (18 H, 2 x s, 2 x CMe_3), 1.69 to 1.81 (4 H, m, CH_2SCH_2), 4.14 (2 H, q, *J* 7.2 Hz, CH_2CH_3), 4.24 (3 H, dd, *J* 2.5, 2.9 Hz, NCH_2), 6.00 (1 H, m, CH), 6.48 (2 H, br, 2 x NH), 7.50 and 7.58 (2 H, s, ArH).

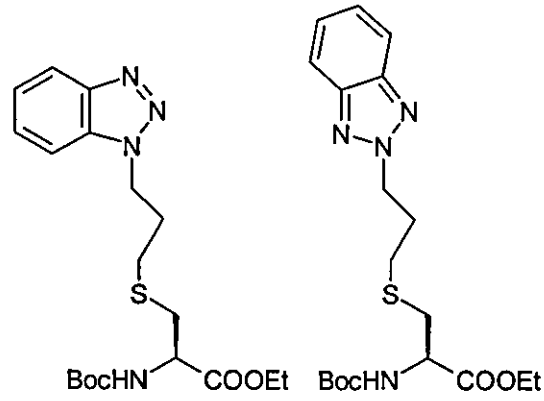
N-Boc-S-[2-(Benzotriazol-1-yl)ethyl]-(S)-cysteine ethyl ester and N-Boc-S-[2-(Benzotriazol-2-yl)ethyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.04 g, 0.08 mmol, 60% in mineral oil), which was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel followed by N-Boc-S-cysteine ethyl ester (0.20 g, 0.80 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 10 min before the addition of a) N1-(2-iodoethyl)benzotriazole (0.88 g, 3.21 mmol) and b) N2-(2-iodoethyl)benzotriazole

(0.88 g, 3.21 mmol), the reaction mixture was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title products were formed as an: a) yellow oil (0.25 g, 75%). Found: MH^+ 395.1750; $C_{18}H_{26}N_4O_4S$ requires: MH^+ 395.1752. m/z: 360, 338, 294, 250, 191, 176. ν_{max} (NaCl/cm^{-1}) 3444, 2915 (CH_2), 2065, 1634 (C=N), 1495 (CH_2), 1393, 1247, 1162, 746 and 668 (ArH). δ_H (400 MHz, $CDCl_3$) 1.24 (3 H, t, *J* 7.0 Hz, CH_2CH_3), 1.60 (9 H, s, CMe_3), 3.09 (2 H, br t, *J* 6.9 Hz, SCH_2), 3.22 (2 H, t, *J* 6.7 Hz, SCH_2), 4.08 (2 H, q, *J* 7.0 Hz, CH_2CH_3), 4.74 (2 H, t, *J* 6.9 Hz, NCH_2), 4.84 (1 H, t, *J* 6.7 Hz, CH), 5.23 (1 H, br, NH), 7.26 to 7.48 (3 H, m, ArH), 7.98 (1 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 12.3 (CH_2CH_3), 29.9 (CMe_3), 33.2 and 35.2 (CH_2SCH_2), 54.0 (NCH_2), 58.5 (CH_2CH_3), 64.0 (CMe_3), 111.9 (NCH), 128.6, 130.6, 131.1, 139.1 (ArCH), 142.1, 146.9 (ArC), 170.2, 184.6 (C=O); b) yellow oil (0.25 g, 74%). Found: MH^+ 395.1751; $C_{18}H_{26}N_4O_4S$ requires: MH^+ 395.1752. m/z: 360, 338, 294, 250, 191, 176. ν_{max} (NaCl/cm^{-1}) 3368, 2976 (CH_2), 1717, 1688 (C=N), 1506, 1455 (CH_2), 1165, 1128, 1025, 854 and 749 (ArH). δ_H (400 MHz, $CDCl_3$) 1.29 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.47 (9 H, s, CMe_3), 3.00 (2 H, br t, *J* 6.9 Hz, SCH_2), 3.29 (2 H, t, *J* 7.1 Hz, SCH_2), 4.23 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.55 (1 H, br, CH), 4.93 (2 H, t, *J* 6.9 Hz, NCH_2), 5.44 (1 H, br, NH), 7.40 and 7.88 (4 H, 2 x m, ArH). δ_C (100 MHz, $CDCl_3$) 14.1 (CH_2CH_3), 28.3 (CMe_3), 32.1 and 34.7 (CH_2SCH_2), 44.9 (NCH_2), 55.8 (CH_2CH_3), 61.8 (CMe_3), 118.0 (NCH), 126.5, 133.3 (ArCH), 144.4 (ArC), 177.9, 180.2 (C=O);

N-Boc-S-[2-(Benzotriazol-1-yl)propyl]-(S)-cysteine ethyl ester and N-Boc-S-[2-(Benzotriazol-2-yl)propyl]-(S)-cysteine ethyl ester

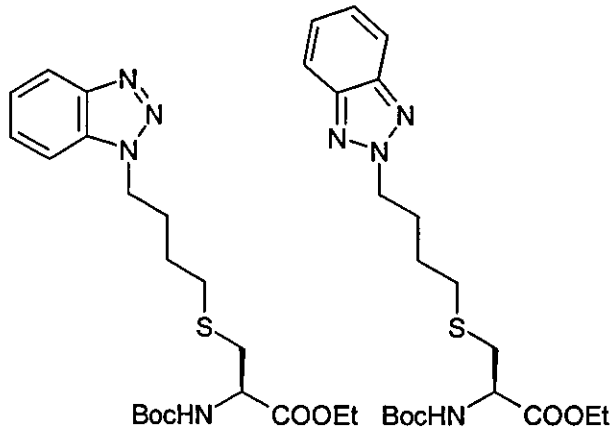


To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.04 g, 0.08 mmol, 60% in mineral oil), this was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel and followed by N-Boc-S-cysteine ethyl ester (0.20 g, 0.88 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 10 min before the addition of a) N1-(3-iodopropyl)benzotriazole (0.92 g, 3.21 mmol) and b) N2-(3-iodopropyl)benzotriazole (0.921g, 3.208mmol), the reaction mixture was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane. The title products were formed as a: a) yellow oil (0.20 g, 59%). Found: M^+ 408.1832; $C_{19}H_{28}N_4O_4S$ requires: M^+ 408.1833. m/z: 322, 250, 214, 198, 158, 144, 130, 118. ν_{max} (NaCl/cm^{-1}) 3447, 2990 (CH_2), 1636, 1556 (C=N), 1432 (CH_2), 1102, 1014, 823 and 692 (ArH). δ_H (400 MHz, $CDCl_3$) 1.22 (3 H, m,

CH_2CH_3), 1.43 (9 H, s, CMe_3), 1.76 (2 H, br, CH_2), 2.31 (2 H, t, *J* 7.0 Hz, SCH_2), 2.74 (2 H, t, *J* 7.0 Hz, SCH_2), 4.22 (2 H, m, CH_2CH_3), 4.66 (2 H, t, *J* 7.0 Hz, NCH_2), 5.23 (1 H, br, CH), 5.97 (1 H, br, NH), 7.31 to 7.47 (3 H, m, ArH), 8.00 (1 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 12.5 (CH_3), 16.9 (CH_2), 26.1 (CH), 27.8 (CMe_3), 32.1 and 37.1 (CH_2SCH_2), 42.9 (NCH_2), 59.9 (CH_2CH_3), 65.3 (CMe_3), 129.5, 130.2 (ArCH), 131.3 (ArC), 135.4, 143.3 (ArCH), 150.0 (ArC), 162.9, 170.0 (C=O); b) yellow oil (0.235g, 68%). Found: MH^+ 409.1913; $C_{19}H_{28}N_4O_4S$ requires: MH^+ 409.1911. m/z: 322, 250, 214, 184, 158, 144, 130, 118. ν_{max} (NaCl/cm^{-1}) 3447, 2987 (CH_2), 1653, 1559 (C=N), 1457 (CH_2), 1372, 1164, 1112, 1031, 807 and 668 (ArH). δ_H (400 MHz, $CDCl_3$) 1.24 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.38 (9 H, s, CMe_3), 1.70 (2 H, br, CH_2), 2.50 (2 H, t, *J* 6.5 Hz, SCH_2), 3.09 (2 H, t, *J* 6.6 Hz, SCH_2), 4.12 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.70 (2 H, t, *J* 6.5 Hz, NCH_2), 4.78 (1 H, br, CH), 5.00 (1 H, br, NH), 7.32 and 8.00 (4 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 12.3 (CH_3), 25.6 (CMe_3), 26.5 (CH), 27.9 (CH_2), 28.5 (NCH_2), 31.4 (SCH_2), 35.4 (CMe_3), 46.2 (SCH_2), 54.1 (CH_2CH_3), 118.3 (ArC), 122.3 and 125.8 (ArCH), 164.8 and 177.1 (C=O).

N-Boc-S-[2-(Benzotriazol-1-yl)butyl]-(S)-cysteine ethyl ester and

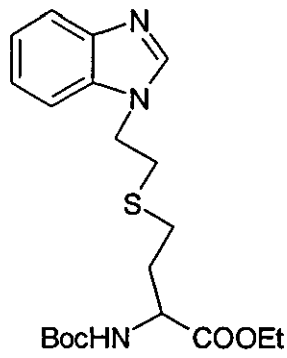
N-Boc-S-[2-(Benzotriazol-2-yl)butyl]-(S)-cysteine ethyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.04 g, 0.08 mmol, 60% in mineral oil), this was washed for 5min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (25 cm^3) was added to the reaction vessel and followed by N-Boc-S-cysteine ethyl ester (0.20 g, 0.88 mmol) in dry THF (25 cm^3). The reaction mixture was allowed to warm over a period of 10 min before the addition of a) N1-(2-iodobutyl)benzotriazole (0.97 g, 3.21 mmol) and b) N2-(2-iodobutyl)benzotriazole (0.97 g, 3.21 mmol), the reaction mixture was then heated at reflux for 16 h. The THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100 cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title products were formed as a: a) yellow oil (0.13 g, 39%). Found: M^+ 422.2013; $C_{20}H_{30}N_4O_4S$ requires: M^+ 422.1989. m/z: 340, 214, 198, 173, 158, 130. ν_{max} (NaCl/cm^{-1}) 3425, 2928 (CH_2), 1708 (C=N), 1495 (CH_2), 1367, 1280, 1165, 1025, 729 and 667 (ArH). δ_H (400 MHz, $CDCl_3$) 1.24 (3 H, t, *J* 7.2 Hz, CH_2CH_3), 1.43 (9

H, s, CMe_3), 1.68 (2 H, m, CH_2), 2.17 (2 H, m, CH_2), 2.58 (2 H, t, *J* 7.3 Hz, SCH_2), 2.92 (2 H, t, *J* 4.9 Hz, SCH_2), 4.18 (2 H, q, *J* 7.2 Hz CH_2CH_3), 4.67 (2 H, m, NCH_2 and CH), 5.34 (1 H, br, NH), 7.37 to 7.53 (2 H, m, ArH), 7.85 and 8.04 (2 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 11.8 (CH_3), 19.6, 20.0 (CH_2CH_2), 25.2 (CMe_3), 28.2 (CH), 31.2 and 35.4 (CH_2SCH_2), 46.8 (NCH_2), 66.3 (CH_2CH_3), 67.2 (CMe_3), 133.4, 133.9 (ArCH), 134.1, 140.7 (ArCH), 146.7, 150.0 (ArC), 168.8, 176.5 (C=O); b) yellow oil (0.20 g, 61%). Found: MH^+ 423.2063, $C_{20}H_{30}N_4O_4S$ requires: MH^+ 423.2067. m/z: 340, 214, 184, 173, 158, 130. ν_{max} (NaCl/cm^{-1}) 3418, 2928 (CH_2), 1710 (C=N), 1510 (CH_2), 1321, 1157, 1066, 747 and 624 (ArH). δ_H (400 MHz, $CDCl_3$) 1.23 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 1.43 (9 H, s, CMe_3), 1.66 (2 H, m, CH_2), 2.21 (2 H, m, CH_2), 2.49 (2 H, t, *J* 7.2 Hz, SCH_2), 2.58 (2 H, br, SCH_2), 4.15 (2 H, q, *J* 7.1 Hz, CH_2CH_3), 4.47 (1 H, br, CH), 4.73 (2 H, t, *J* 6.2 Hz, NCH_2), 5.28 (1 H, br, NH), 7.35 and 7.84 (4 H, m, ArH). δ_C (100 MHz, $CDCl_3$) 9.9 (CH_3), 24.9 (CMe_3), 27.0 and 29.1 (CH_2CH_2), 30.3 (CH), 31.0, 40.2 (CH_2SCH_2), 45.4 (NCH_2), 63.2 (CH_2CH_3), 70.6 (CMe_3), 122.7, 126.5 (ArCH), 133.1 (ArC), 171.4, 173.6 (C=O).

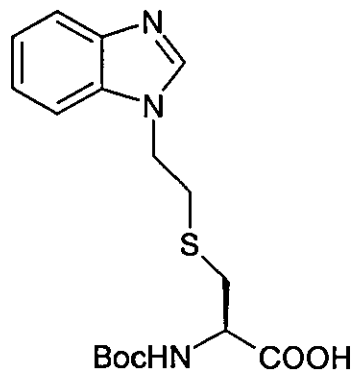
N-Boc-S-[2-(Benzimidazol-1-yl)ethyl]-(S)-homocysteine methyl ester



To a flame-dried RBF under a positive atmosphere of nitrogen was added NaH (0.01 g, 0.10 mmol, 60% in mineral oil), this was washed for 5 min with hexane, the hexane decanted, and then cooled to 0°C. Dry THF (15 cm^3) was added to the reaction vessel and followed by N-Boc-S-homocysteine methyl ester (0.10 g, 0.40mmol) in dry THF (15 cm^3). The reaction was allowed to warm over a period of 20 min before the addition of N1-(2-iodoethyl)benzimidazole (2.18 g, 8.02 mmol), the reaction mixture was then heated at reflux for 16 h. THF was then removed under reduced pressure and the residue taken up in DCM/H_2O (100cm^3, 1:1 v/v). The DCM layer was separated off, washed with H_2O (20 cm^3), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification was by column chromatography (ethyl acetate/hexane). The title product was formed as a cream oil (0.090 g, 57%). Found: MH^+ 363.16; $C_{19}H_{27}N_3O_4S$ requires: MH^+ 394.16; $C_{18}H_{24}N_3O_3S$ requires: MH^+ 363.14 – OMe deprotected. ν_{max} (NaCl/cm^{-1}) 3357, 2977 (CH_2), 1710 (C=N), 1496 (CH_2), 1367, 1250, 1165, 1024 and 744 (ArH). δ_H (400 MHz, $CDCl_3$) 1.38 (9 H, s, CMe_3), 1.89 (2 H, br, $CHCH_2$), 2.20 (2 H, br, SCH_2), 2.66 (2 H, t, *J* 7.8 Hz, SCH_2), 3.69 (3 H, s, CH_3), 3.85 (1 H, br, CH), 4.32 (2 H, br, NCH_2), 5.06 (1 H, br, NH), 7.32 (2 H, m, ArH), 7.73 (2 H, m, ArH), 8.37 (1 H, s, N=CH).

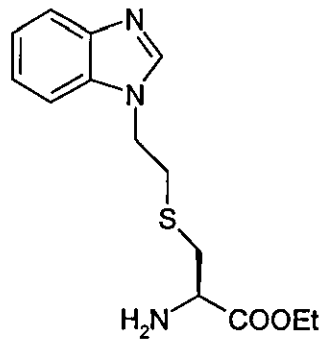
Deprotection Of Heterocylic Amino Acids

N-Boc-S-[2-(benzimidazol-1-yl)ethyl]-(S)-cysteine (Ester deprotection)



N-Boc-S-[2-(Benzimidazol-1-yl)ethyl]-(S)-cysteine ethyl ester (0.5 g, 1.27 mmol) was dissolved in THF/methanol (50 cm^3, 3:1 v/v) and the solution cooled in an ice bath. $LiOH_{(aq)}$ (1 M sol, 200 mol%) was then added and the mixture stirred for 8 h after it had warmed to room temperature. The organics were removed under reduced pressure, the water layer acidified by dilute HCl to pH 4 and the product extracted by EtOAc (20 cm^3 x 3). After the extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure, the deprotected product was obtained as a white solid (0.45 g, 97%). Found: MH^+ 365.96; $C_{17}H_{23}N_3O_4S$ requires: MH^+ 366.15. ν_{max} (NaCl/cm^{-1}) 3412, 2925 (CH_2), 1711 (C=O (acid)), 1514 (NC=O), 1368, 1256, 1160 (C-O), 909 (CH), 734 and 647 (ArH). δ_H (250 MHz, $CDCl_3$) 1.43 (9 H, s, CMe_3), 3.04-3.08 (4 H, m, CH_2SCH_2,), 4.52 (1 H, br, CH), 4.67 (2 H, t, *J* 7.2 Hz, NCH_2), 5.64 (1 H, br, NH), 7.50–7.52 (4 H, m, ArH), 7.96 (1 H, s, N=CH).

S-[2-(benzimidazol-1-yl)ethyl]-(S)-cysteine ethyl ester (Amino deprotection)



N-Boc-S-[2-(benzimidazol-1-yl)ethyl]-(S)-cysteine ethyl ester (0.5 g, 1.27 mmol) was added to TFA/DCM (50 cm^3, 1:1 v/v) and the mixture stirred for 2 h. DIEA/DCM (50 cm^3, 5:95 v/v) was then added to neutralise the acid. The solvents were removed under reduced pressure to give the Boc deprotected product run through a short silica column to give the free amine (0.37g, 86%). Found: MH^+ 294.47; $C_{14}H_{19}N_3O_2S$ requires: MH^+ 294.13. ν_{max} (NaCl/cm^{-1}) 3415, 2981 (O-CH_2-), 2356 (NH_2^+), 1766 (C=O(OC_2H_5)), 1475 (CH_2), 1371 (CH_3), 1118 (C-S-C), 844 (CH), 732 and 664 (ArH). δ_H (250 MHz, $CDCl_3$) 1.22 (3 H, t, *J* 7.1 Hz, CH_2CH_3), 3.07-3.09 (4 H, m, CH_2SCH_2,), 4.18 (2 H, q, *J* 7.1 Hz, CH_2CH_3) 4.35 (1 H, t, *J* 5.6 Hz, NCH), 4.66 (1 H, t, *J* 6.5 Hz, NCH_2), 6.11 (2 H, br, NH_2), 7.49–7.63 (4 H, m, ArH), 7.95 (1 H, s, N=CH).

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