Synthesis of Nucleic Acid-Binding Ligands

By

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Abstract

The most favoured amino acid in protein-nucleic acid interfaces is arginine due to the existence of the guanidinium group in its structure that can form good hydrogen-bonding geometries. In addition, the length of the side chain gives it a great deal of torsional freedom and therefore permits the guanidinium group to orient itself to interact with a wide range of targets through bidentate H-bonding. A torsional constraint for the guanidine group could be achieved by its conversion into a bicyclic framework, resulting in unidirectional hydrogen bonding with its targets and reducing the loss of the conformational entropy as a result of its binding. The aim of this research is to synthesise nucleic acid targeting oligopeptides which will include non-natural analogues of arginine as part of their sequence with the purpose of activating or inhibiting biological functions. The synthesis of different sized rings of bicyclic guanidine derivatives could be a means to control the hydrophobic interaction with DNA, with hydrophobicity increasing as the size of the ring of the bicyclic guanidine increases. In addition, the high basicity of guanidine leads to production of the corresponding guanidinium cation upon protonation. Therefore, the formation of guanidinium cation leads to hydrogen bonding and electrostatic interactions with anions like phosphate groups. So, the purpose of preparing differently sized rings of bicyclic guanidine in the current work is to compare their interactions with DNA. There are four themes in our research concerned with the design and synthesis of bicyclic guanidine derivatives and non-natural amino acids. The first theme concerns the synthesis of a protected bicyclic guanidine methylene amine that was achieved by utilising Boc-L-methioninol as a precursor and converting it to the corresponding azide. However, incorporation of this bicyclic guanidine into an amino acid moiety proved difficult and thus the modified amino acid was not formed. The second theme of the project considers a novel strategy to generate a bicyclic guanidine methylene alcohol with a variably sized ring in a high yield starting from an azido alkane amine and Boc-L-methionine as precursors. This modification to the route will open a promising way to produce the functionalised bicyclic guanidine. Bicyclic guanidine methylene chloride was synthesised that gave us a way to insert bicyclic guanidine into a tyrosine amino acid moiety so that a protected L-tyrosine possessing bicyclic guanidine with a differently sized ring could be synthesised. The third theme involved the new strategy towards the synthesis of bicyclic guanidine methylene amine followed by conjugation with Fmoc-Glu-OAll to produce a modified amino acid. The fourth, and final,

theme of our research includes the preparation of dimers of bicyclic guanidine. Furthermore, a crystal structure for the protected bicyclic guanidine intermediate in this theme has also been obtained.

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Abbreviation*s*

Ac	Acetyl
Ar	Aryl
BCG	Bicyclic guanidine
BH_3-DMS	Borane dimethylsulfide
Boc	*Tert*-butoxycarbonyl
Cbz	Benzyl carbamate
°C	Degrees Celsius
cm	Centimetre
DBU	1, 8-Diazabicyclo [5.4.0] undec-7-ene
DCC	*N*, *N*-dicyclohexylcarbodiimide
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIPEA	Diisopropylethyl amine
DMAP	4-Dimethylaminopyridine

DMC	2-Chloro-1, 3-dimethylimidazolinium chloride
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et_2O	Diethylether
Fmoc	Fluorenylmethyloxycarbonyl
g	Gram
H-tbd	1, 6, 8-triazabicyclo [5.3.0] dec-7-ene
H-tbn	1, 4, 6-triazabicyclo [4.3.0] non-6-ene
H-tbu	1, 6, 8-triazabicyclo [5.4.0] undec-7-ene
HCl	Hydrochloric acid
HOBt	1-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
hppH	1, 5, 7-triazabicyclo [4.4.0] dec-5-ene

$(\mathrm{Im})_2\mathrm{CS}$	*N, N*'-Thiocarbonyldiimidazole
IR	Infrared
m/z	Mass to charge ratio (in mass spectrometry)
Me	Methyl
MeOTf	Methyl trifluoromethanesulfonate
Mtt	4-Methyltrityl
n-BuLi	*n*- Butyllithium
$\mathrm{NEt_3}$	Triethylamine
NMR	Nuclear magnetic resonance
Pbf	2, 2, 4, 6, 7-Pentamethyl-2, 3-dihydrobenzofuran
$\mathrm{PbfNH_2}$	2, 2, 4, 6, 7-Pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide
$\mathrm{PPh_3}$	Triphenylphosphine
ppm	Parts per million
Py	Pyridine
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

r.t.	Room temperature
Red-Al	Sodium bis (2-methoxyethoxy) aluminumhydride
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
TBAF	Tetra-*n*-butylammonium fluoride
TBS/TBDMS	*Tert*-butyldimethylsilyl
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Tr	Triphenylmethyl chloride
tRNA	Transfer ribonucleic acid
Ts	4-toluene sulfonyl
vRNA	Virion ribonucleic acid
WC	Watson-Crick

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Chapter One
Introduction

1.1 Motivation

DNA can interact with ligands, e.g. from peptide libraries containing a non-natural amino acid, for the purpose of activating or inhibiting biological functions. Certain amino acids with basic residues, such as arginine, display selective binding for specific nucleobases. The significant functional group in the arginine amino acid is a guanidine group, which can play an important role in the recognition between peptides and their nucleic acid targets. The synthesis of functionalised and non-functionalised bicyclic guanidine has attracted increasing interest during the past few decades due to its properties and various uses in different fields of chemistry**.** Bioconjugation is an approach to introduce small molecules into an amino acid moiety that can achieve relatively quick syntheses of a wide range of compounds that contain the same sub-structure but with different steric and electronic properties. The insertion of modified amino acids into peptides through parallel chemistry is an important procedure in medicinal chemistry and biochemistry. In the following sections, the background of many subjects is discussed, related to the main goal of our research. The discussion starts with the general concepts of nucleic acids, then moves to their interactions with proteins and the involvement of particular amino acids, focussing especially on the interactions of the arginine residues in these complexes. The next section includes the properties of guanidine groups, bicyclic guanidine and then describes the strategies used to synthesise guanidine and bicyclic guanidine. Bioconjugation as a general concept is described, followed by the different linkages that have been used to conjugate small molecules to biomolecules. Finally, I describe the aim of my research.

1.2 Nucleic acids

Nucleic acids[1] (DNA and RNA) are molecules that are responsible for many crucial functions in organisms. Although nucleic acids were described in 1869 by Friedrich Miescher,[2] it was only in the 1940s that their significance as the holders of hereditary information became obvious.[3] They are found in a free state or linked to proteins to create nucleoproteins. Nucleic acids contain carbon, hydrogen, oxygen, phosphorus and nitrogen. Generally, there are two types of nucleic acid: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The main functions of DNA are to store, and transfer, genetic information. DNA also acts as a template to produce new DNA and RNAs; while protein synthesis occurs *via* RNAs. Numerous reactions are involved in the synthesis of such macromolecules, and this process is a challenge to the cell. The cell uses a modular method for the assembly of macromolecules by combining monomers called nucleotides. A phosphodiester linkage is used to join these monomers to synthesise a polynucleotide. There are three units forming a nucleotide: a nitrogenous base, a five-carbon sugar, and a phosphate group[4] as outlined in Figure 1.1.

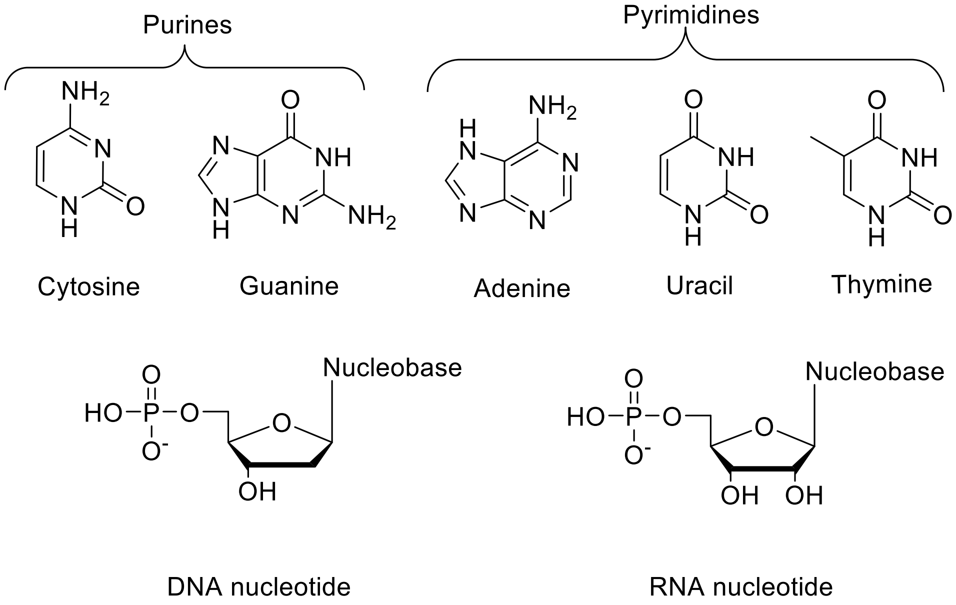


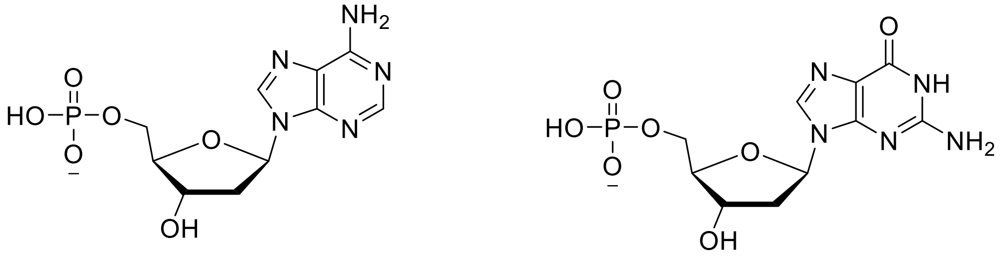
Figure 1.1: The structures of nucleobases, RNA nucleotides and DNA nucleotides.

1. Nitrogenous base: The nitrogenous bases[1] in DNA are adenine, guanine, cytosine and thymine while RNA consists of the same nitrogenous bases except it has uracil instead of thymine.

2. The five-carbon sugar is ribose in RNA and deoxyribose in DNA. Importantly, the position of the hydroxyl group at the 2′-carbon of ribose contributes effectively to the secondary structure of RNA. RNA can be distinguished from DNA chemically by utilising alkaline solutions, due to the possibility for the deprotonation of the hydroxyl group of the sugar moiety causing hydrolysis of RNA strands.[5]

3. The third part of a nucleotide is the phosphate group that results from the esterification of phosphoric acid *via* connection to the hydroxyl group of a sugar at C5 to generate a phosphate ester.

The nitrogenous bases are connected to the sugar through a glycosidic bond to form a nucleotide; C1 at the sugar connects to N1 in a pyrimidine or to N9 of a purine. Common structures for nucleotides in DNA are shown in Figure 1.2.[1]


Adenosine 5' monophosphate Guanosine 5' monophosphate

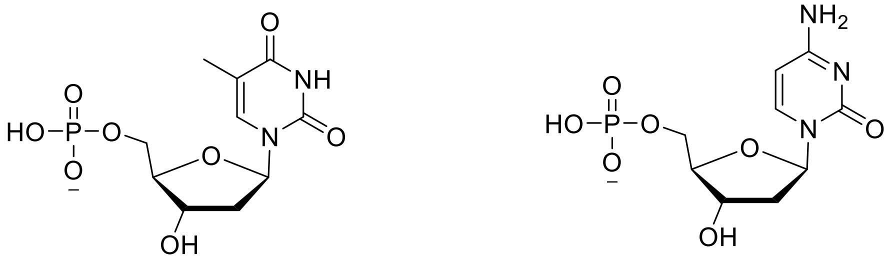

Thymidine 5' monophosphate Cytidine 5' monophosphate

Figure 1.2: Common structures for deoxynucleotides.

It was revealed that one chemical defect in DNA involved the easy loss of an amino group from cytosine, thus changing the genetic information. Normally, cytosine interacts with guanine through hydrogen bonding in DNA double strands, but if the amino group is lost and it subsequently forms uracil, the resulting nucleobases would have the propensity to pair with adenine which could lead to undesired mutation.[6] Lindahl concluded that the cell must have the ability to protect itself from this chemical defect. In 1974, he reported his detection of the bacterial enzyme that is responsible for the fixing of this damage to cytosine of DNA. This bacterial enzyme[7] is named uracil-DNA glycosylase.

In DNA and RNA, the phosphodiester linkage is formed by the connection of the 5′-phosphate group of one nucleotide with the 3′-hydroxyl of the sugar of the following nucleotide. Thus, the terminus of the resulting oligonucleotide derived from the 3′-hydroxyl of the sugar is named the 3′-end while that derived from the 5′-hydroxyl group is called the 5′-end. Consequently, their sequences are conventionally depicted from the 5′-end (left hand) to the 3′-end (right hand). Principally, RNA occurs as a single-strand molecule that folds itself in such a way as to make internal hydrogen bonding interactions that lead to characteristic secondary and tertiary structures, these then allow for a wide range of functional activities.[8, 9] Structurally, RNA exists in all major life forms as three forms: ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA). Ribosomal RNA represents approximately 80% of the total RNA in the cell and is complexed with protein to produce the ribosomes. The ribosome can be classified as two parts, the larger proportion is RNA that accounts for about 65% and the smaller proportion is protein that accounts for about 35% of the total ribosome.[10]

The second form of RNA is tRNA that represents about 15% of the whole RNA in a cell and it is found in many sequences, each one specifies a particular amino acid. The smallest part of RNA (less than 5%) belongs to mRNA which, in eukaryotes, is produced within the nucleus and transfers to the cytoplasm prior to protein synthesis. Functionally, the next step after producing mRNA is its translation into a protein. In this case, a "language" barrier exists. A 4-letter code (A, U, C, and G) is utilised to write the mRNA while a 20-letter code is used to make proteins. Codons are represented by three nucleotides at a time. Each codon possesses the information limited to one amino acid. Mathematically, more than 20 codons exist (64 possible codons) and there are only 20 amino acids that are directly genetically encoded with a codon. The overlap among of the codons is the reason that there are 64 codons, comprising only 61 codons for amino acids, with 3 stop codons.

DNA exists in the nucleus of the cell of eukaryotes and can also be found in organelles such as the mitochondria, but is free roaming around the cytoplasm of prokaryotes. In 1953, Watson and Crick introduced magnificent work on DNA structure by proposing an anti-parallel double helix structure of DNA. They reported that B-DNA is composed of two strands that are coiled around each other to generate a right-handed helix.[11] The specificity of the double helix would be achieved *via* hydrogen bonding interaction between the nitrogenous bases between the two strands. There are two grooves on the surface of the strands with different width and depth and they are known as major (wide and deep) and minor grooves (narrow and shallow)[12] as shown in Figure 1.3.

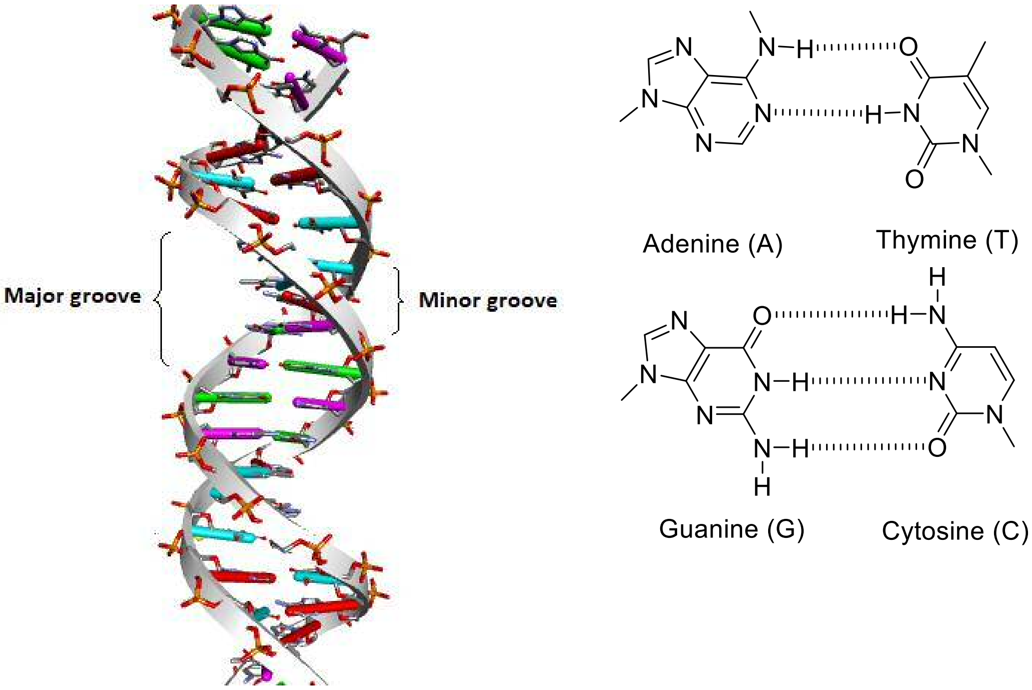


Figure 1.3: Major and minor groove of the B- helix and schematic of the hydrogen bonding between nitrogenous base pairs, PDB 5F9I.[13]

This discovery, the complementarity of the double helix, caused a fundamental shift in the biological sciences through understanding how genetic information can be precisely replicated. After Watson and Crick's deduction, it was discovered that DNA possesses many different structural forms. In addition, there are other forms of DNA double strands that could exist such as A and Z-DNA. The second type of DNA is A-form that also contains a right-hand double-helix like the B-form. The major groove in A-DNA tends to be narrow and deep while it is wide and shallow in the minor groove. A third type, Z-DNA has a remarkably different secondary structure from the other types of DNA. Structurally, the helix is left handed. The sugar–phosphate backbone takes on an asymmetrical zigzag. As mentioned before, RNA has a hydroxyl group at the carbon 2 position of the ribose sugar backbone and this prevents the formation of a B-form helix and triggers adoption of the A-form. The double helix of RNA is quite similar to the A-DNA form. The major groove of A-RNA is deeper and narrower than the minor groove thus leading to the minor groove being more accessible to contact with protein.

1.2.1 DNA-Protein interactions

Nucleic acids and proteins are two of the most significant biomolecules in living systems.[14] Small molecules and proteins can interact with DNA[15] through three distinguishable binding modes: external interaction with the sugar-phosphate backbone, internal interactions in the major or minor groove, and intercalation. Electrostatic interactions primarily occur on the sugar-phosphate backbone. The protein-nucleic acid complex is formed by interaction between the side-chains of amino acids of the protein with the nitrogenous bases and sugar-phosphate backbone. Hydrogen bonding is considered one of the most important forces in protein-DNA interaction, forming both sequence specific and non-sequence specific bonds between protein and DNA. The salient characteristics of DNA that can be recognised by protein are the major groove and minor groove, giving sequence specific interactions. Many forces contribute to binding in the protein-DNA structure. These forces include van der Waals, electrostatic forces, hydrogen bonding and water mediated hydrogen bonding. Many families of DNA-binding proteins have been identified[16] and each family's members possesses the same DNA-binding motif. These families involve helix-turn-helix (HTH) proteins, the homeodomains (HDs), zinc finger (coordinating) proteins, the steroid receptors, leucine zipper proteins and the helix-loop-helix proteins. The helix-turn-helix motif is considered the most known and most intensively studied motif that exists in DNA-binding proteins, both in prokaryotes and eukaryotes. RNA polymerase and DNA polymerase are enzymatic examples of natural proteins that bind to nucleic acid targets.[17] Another example of natural proteins that bind with DNA is zinc fingers (Figure 1.4). The chain of zinc fingers folds along the DNA or RNA segments, binding in the grooves and extending the amino acids' side chains to read the bases. Each zinc finger possesses an arginine amino acid extending from the alpha helix, which forms a hydrogen bond with Nitrogen 7 and also Oxygen 6 of the guanine that lies at the 3′ end of the binding site. The crystal structure of a zinc finger protein–DNA interaction, Zif268, was published in 1991.[18] Recent studies of structures in the Protein Data Bank (PDB) showed that the majority of all protein interactions with DNA minor grooves at AT-tracks, are formed by arginine or lysine.[19] The reagent *p*-hydroxyphenyl glyoxal (HPG) is widely used for determining if arginine side chains are included in mediating the binding between a protein and other ligands, by the modification of the guanidyl group when HPG reacts with exposed arginine residues.[20]

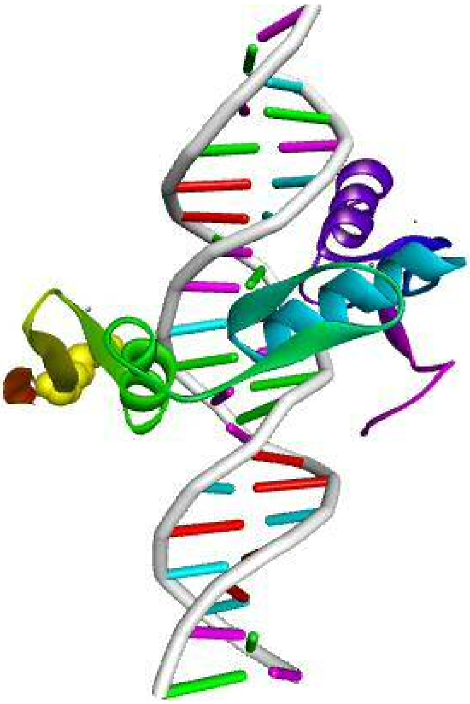


Figure 1.4: Biological assembly of DNA with zinc fingers, PDB ID 4F6M.[21]

Many scientists have utilised these features in an attempt to design chemical compounds which can interact with several sites of the nucleic acid target to inhibit the expression of specific genes. Distamycin and netropsin are representative examples of natural small molecule heterocyclic polyamides that display sequence-selective binding to DNA. Distamycin has three *N*-methyl pyrrole heterocyclic amides, while netropsin has two *N*-methyl pyrrole heterocyclic amides. They are selective for binding to AT rich sites in the minor groove of DNA because the 2-amino groups of guanine blocks distamycin from binding to GC base pairs in the minor groove by steric hindrance.[22] Figure 1.5 shows the interaction between distamycin and DNA. In addition, the recognition[23] of AT-rich regions by minor groove binders is achieved by a combination of hydrophobic interactions, hydrogen bonding and electrostatic interactions. Thus, these cationic polyamides[24] not only contribute to electrostatic interactions and hydrogen bonds with the polyanionic DNA, but also take part in van der Waals binding in the minor groove.

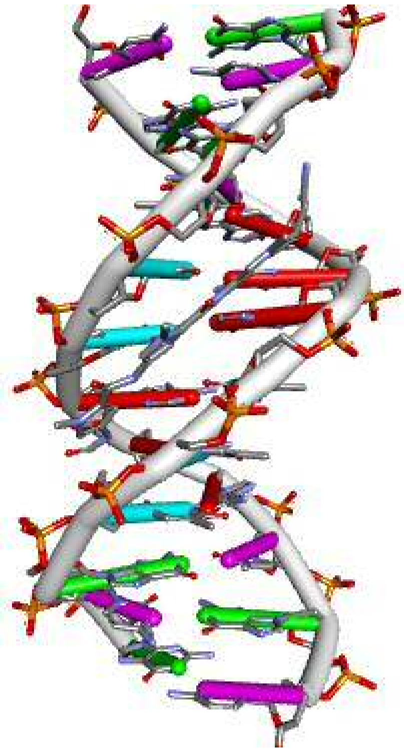


Figure 1.5: Distamycin-DNA binding, PDB ID 2DND.[25]

Seeman *et al.*[23] postulated that discrimination between different bases in a Watson-Crick double helix could be achieved by certain amino acids; thus, a molecular recognition code could be found. Seeman *et al*.[26] and then Hélène *et al*.[27] have suggested that the guanidine group in the arginine amino acid shows only hydrogen bond donors and could preferably form double H-bonds with the exposed edges of the guanine (Figure 1.6).

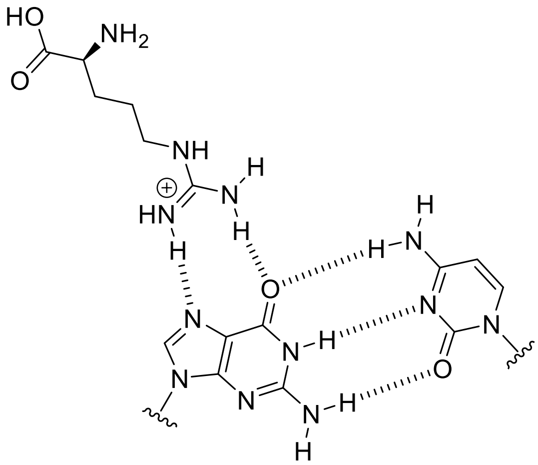


Figure 1.6: Seeman suggested double H-bonding between the guanidine group and the Hoogsteen face of guanine.

Pabo is another researcher who has supported this supposition; he predicted that direct contact was achieved between the alpha helix of the protein and the major groove of the DNA.[28] It was claimed by Matthews[29] that the code for recognition is not simple due to the complexity of different DNA-protein complexes. Based on the survey of the crystal structure of many DNA-transcription factor complexes, Suzuki demonstrated[30] those residues of some amino acids recognise a couple of chemical groups on the same DNA base and the interactions will be stronger in bidentate H-bonds than single H-bonding. Furthermore, it has been reported that Arg is more likely to be binding with guanine rather than any other of the nitrogenous bases, frequently through bidentate H-bonding. Gutfreund *et al.* agreed with Suzuki's hypothesis and also reported that there is a specific binding between arginine and guanine.[31] Luscombe *et al.* reported that the side chain of the arginine amino acid can be arranged in several geometries in order to donate dual H-bonds.[32] To enhance the impact of the investigation performed by Seeman *et al.*, Luscombe *et al*. have also reported that two possible interaction conformations could be observed: the end-on approach represents the most common conformation, which is contact with one or two of the distal nitrogen atoms $N^{\eta 1}$ and $N^{\eta 2}$ (Figure 1.7 A); and the side-on approach interacts with the side chain where the N^{ε} and $N^{\eta 2}$ are used for H-bond donation (Figure 1.7 B). Furthermore, it was observed that a single N atom in the guanidine group could donate two H-bonds and behave like a lysine residue to form bidentate interactions (Figure 1.7 C and D).

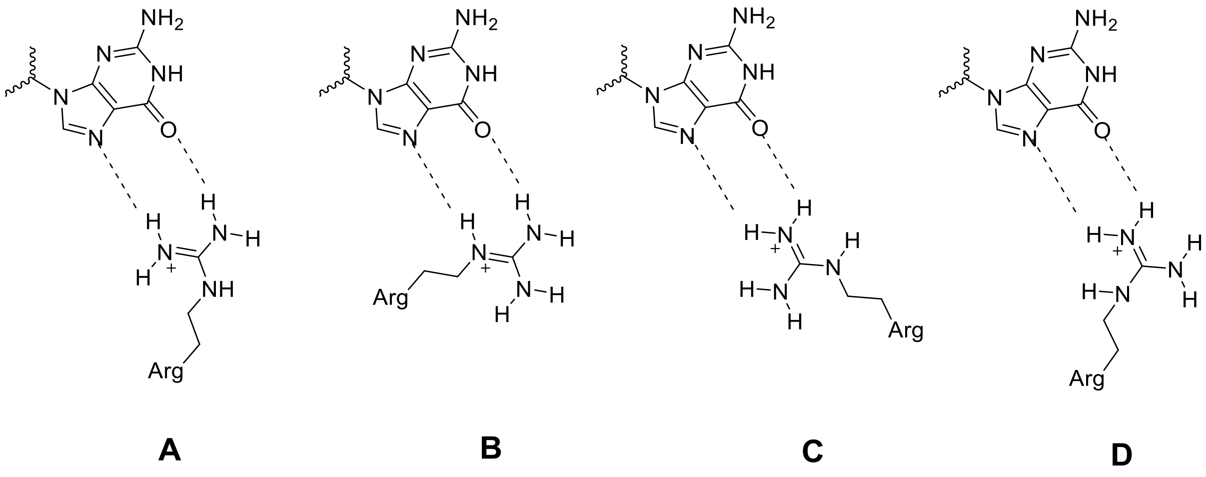


Figure 1.7: Bidentate interactions between arginine residue and the Hoogsteen edge of guanine noticed by Luscombe *et al.*[32]

Based on computational analysis of 26 non-homologous protein-DNA complexes, which were taken from the Protein Data Bank and Nucleic Acid Database (NDB), Jones *et al*. have emphasised that the interactions between protein and DNA take place predominantly in the major grooves of the complexes.[33] It is clear that the minor groove is too compressed to host common binding motifs. Other studies have suggested that the arginine amino acid has another feature which contributes to contact with DNA through electrostatic interactions i.e. between the positively charged side chain and the negatively charged phosphate backbone of the DNA.[31, 33, 34] These interactions contribute effectively to the stabilisation of the protein-DNA complex.[32, 35] In DNA-protein interactions, conformational change could be induced in the DNA's secondary structure and the level of deformation can be expected to range from a slight kink,[36] to the large bends noticed in the structure of the nucleosome.[37] It has been noticed that the arginine side chain interacts regularly within this cramped minor groove.[38] This minor groove binding has been mainly located in distorted protein-DNA complexes.[39]

1.2.2 Protein-RNA interactions

RNA-protein interactions play a significant role in many biological processes, such as translation and splicing. Structurally, protein-RNA complexes are much more diverse than protein-DNA complexes. The specific mechanisms of protein-DNA interactions are more well-understood than those of protein-RNA interactions.[16, 19, 40] Helical RNA is composed of Watson-Crick base pairings of the four nucleobases. Instead of the classic B-form helices usually found with DNA, RNA forms A-form helices due to the steric hindrance from the 2′ OH of ribose. Furthermore, the major groove in the classic A-form helices is deeper and less accessible than the major groove in B-form helices and thus inhibits base binding between the arginine and guanine Hoogsteen edge. Non-Watson-Crick pairs in an RNA duplex, such as the GU wobble, GG and AA, also have a higher potential of becoming unpaired. As a result of these non-WC base pairs loops, bulges and hairpins can form giving a flexible tertiary structure.[41] An understanding of the molecular recognition of protein-RNA binding has proved difficult, due to little statistical information being available owing to a lack of x-ray crystal structure data. Jones *et al*. have achieved a structural survey of 32 protein-RNA structures from the PDB.[42] They emphasised that arginine has a high tendency to be found in the binding site of protein-RNA complexes and that the interactions take place through hydrogen bonding with the phosphates. However, it has been reported that arginine residues can interact strongly with phosphate oxygen atoms through electrostatic interaction, without the suitable geometry to form hydrogen bonds being found.[43] Some researchers have suggested that arginine residues bind to the phosphate groups at the beginning and do not have hydrogen bonding specificity towards the bases.[42, 44, 45] On the other hand, another proposal shows that bidentate interactions could take place between arginine residues and the Hoogsteen edge of guanine at O6 and N7 positions which corresponds with the interactions in protein-DNA complexes, although this is much less common for RNA.[43, 46, 47, 48] As a result of NMR experiments including the TAR RNA complex, it has been observed that the base-specific binding of arginine residues with the Hoogsteen edge of guanine leads to the formation of a nucleobase triple.[41] Burke proposed that this binding is also necessary for aminoacyl-tRNA synthetases to catalyse the combination of an amino acid to its cognate tRNA in *Escherichia coli*.[49] However, it has been noted that unpaired cytosine also interacts with the arginine residue, through dual H-bonds, at the Watson-Crick base edge (Figure 1.8 A),[43, 46, 48, 50, 51] Nη^1 and Nη^2 of an arginine residue can donate H-bonds to N3 and O2 in cytosine. Other interactions of arginine residues with nitrogenous bases in RNA have been reported. Kim has explained that a bidentate interaction takes place between arginine and uracil.[51] This happened between O2 of uracil and Nε and Nη of the arginine residue (Figure 1.8 B).

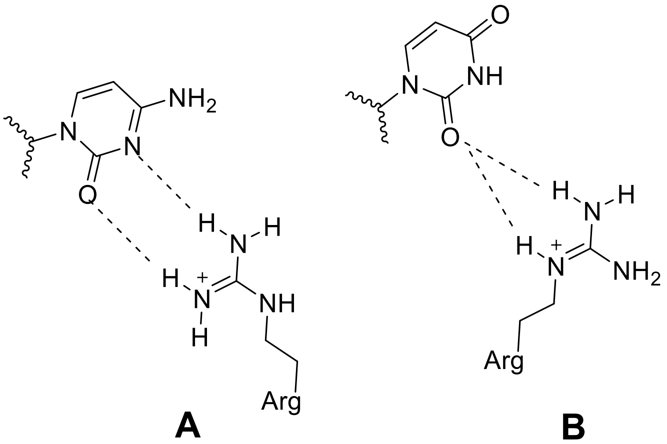


Figure 1.8: Bidentate interactions between cytosine and an arginine residue (A) and bidentate interactions between uracil and an arginine residue in protein-RNA complexes (B).

Several studies have been carried out to explain the nature of the binding in protein-RNA interactions, and conflicting results have justified the difficulty in recognising a specific code for the recognition of a protein for its RNA target. Other functional classes of RNA can interact with protein, as Jones has reported.[42] There are different classes of RNA that can interact with proteins namely messenger RNA (mRNA), transfer RNA (tRNA) and viral RNA (vRNA). These classes of RNA have a higher number of contacts compared with ribosomal RNA (rRNA) due to more frequent helical structures in rRNA.[45] However, it has been concluded that arginine has a much higher propensity than other amino acids to interact with different RNA functional classes.[44] Overall, this part of the introduction has discussed many issues concerned with the molecular recognition between a protein and its RNA target. It can be summarised that the arginine amino acid is most likely to be present at the binding interface and although there have been different particular interactions reported, most research concludes that base-specific interactions occur between arginine residues and the exposed edges of various base pairs.

1.2.3 Guanidines

Guanidine has attracted significant attention due to its useful applications and unique properties that cannot be easily replicated by other functional groups. It can, for example, contribute to the stabilisation of protein conformations through hydrogen bonding, due to the hydrophilic nature of the guanidine group.[52] There are three resonance structures of guanidinium and all are equivalent (Figure 1.9), thus, resulting in a large delocalisation energy and stabilisation of the positive charge. This leads to an increase in the basicity of guanidine. The pK_a of guanidine is 13.6 so it is considered the strongest common organic nitrogenous base.[53] In physiological conditions, guanidine is protonated and the cationic result (guanidinium) tends to be highly stable in an aqueous solution; therefore, it can form electrostatic interactions with opposite charges, such as that previously mentioned between arginine residue and the phosphate group in RNA and DNA.

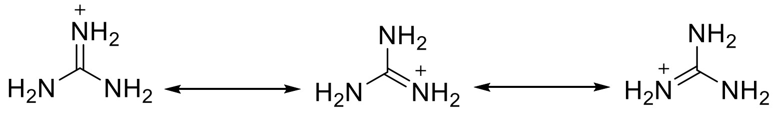


Figure 1.9: The resonance structures of the guanidinium cation.

Experimentally, it was recorded[53] that the protonation of guanidine occurs at the imino nitrogen because the other amino groups are less basic than the imine group atom by 30 kcal/mol. On the other hand, electron-donating substituents such as an alkyl group on the nitrogen atom in guanidines causes slight increases in its basicity while attaching electron-withdrawing groups such as OCH_3, $COCH_3$, CN and NO_2 results in the reverse effect. However, increasing the number of amino groups in the molecule causes a strong effect on the gas phase basicity. In general, molecules containing bi-guanidine groups are stronger bases than others having one guanidine. Table 1 shows the pK_a values for guanidine derivatives.

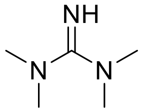
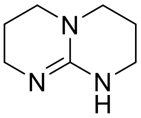
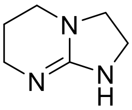
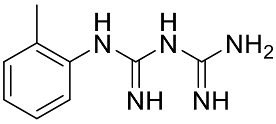
Base	pK_a (MeCN)	pK_a (EtOH)
	n.d.	17.1
	25.96	n.d.
	24.55	n.d.
	19.43	n.d.

Table 1: Experimental data of the pK_a values for guanidine derivatives. n.d. – not determined.[50]

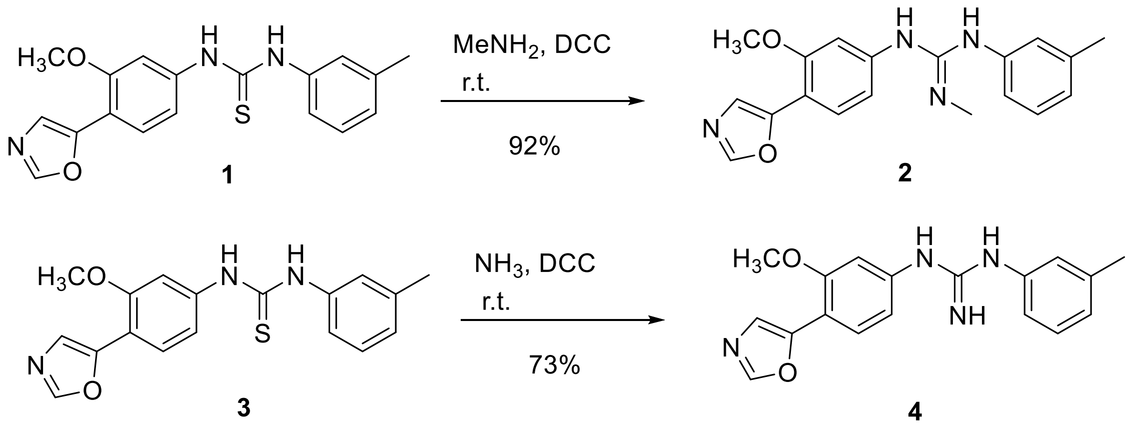
The following sections are about synthetic strategies towards substituted guanidines.

1.2.3.1 The synthesis of guanidine from thiourea

Several reagents have been used to convert a thiourea moiety into guanidinium functionalities. These reagents include *N*,*N*-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), mercury (II) chloride ($HgCl_2$), mercury (II) oxide (HgO) and 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent).

1.2.3.1.1 DCC coupling

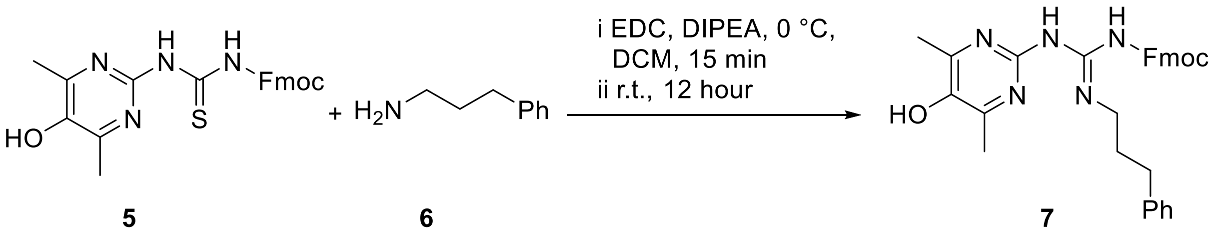
A novel guanidinium functionality has been synthesised[54] through DCC coupling by Iwanowicz and co-workers and these guanidines have since been used as inhibitors of inosine monophosphate dehydrogenase. The synthetic route to **2** and **4** are depicted in Scheme 1.1.



Scheme 1.1: Utilising DCC coupling to produce a novel guanidinium functionality.

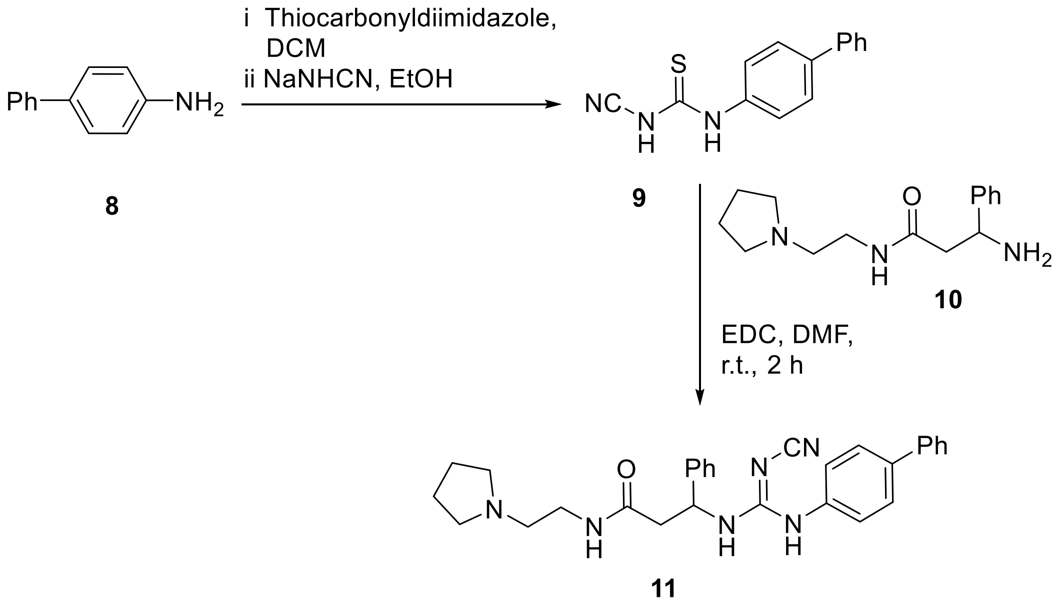
1.2.3.1.2 EDC coupling

Another approach to synthesise a guanidine derivative has been achieved by Jefferson and co-workers through the EDC coupling reagent as outlined in Scheme 1.2.[55]



Scheme 1.2: The synthetic route to bicyclic guanidine by using the EDC reagent.

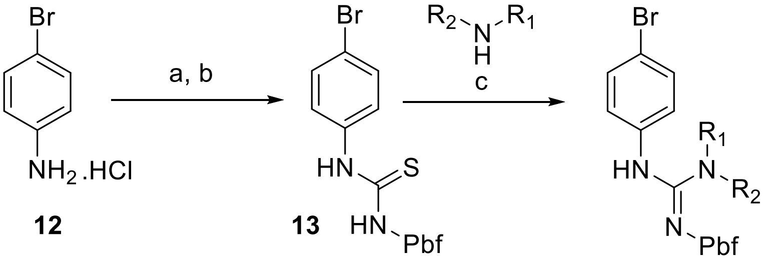
Furthermore, cyanoguanidine **11** was synthesised by Wolin and co-workers through EDC coupling as in Scheme 1.3.[56]



Scheme 1.3: The preparation of cyanoguanidines by Wolin and co-workers.[53]

1.2.3.1.3 Mukaiyama's Reagent

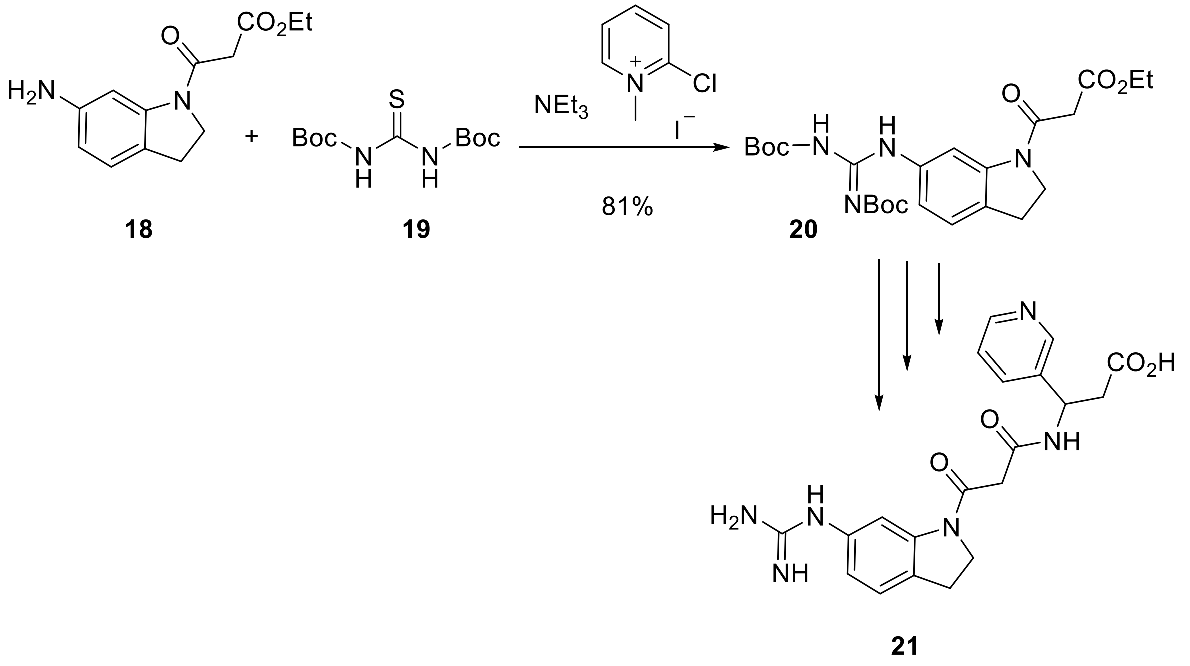
This approach has utilised Mukaiyama's Reagent (2-chloro-1-methylpyridinium iodide) to generate *N*,*N′*-substituted guanidine derivatives *via* an aromatic sulfonyl-activated thiourea intermediate as in Scheme 1.4.[57] The first step in this synthetic route consisted of the conversion of a primary amine to the corresponding pentafluorophenyl thiocarbamate. This was converted to an aryl sulfonyl-activated thiourea by using PbfNHK. The final step involved the production of the guanidine derivative by the treatment of **13** with a secondary amine in the presence of Mukaiyama's reagent in excellent yield.



14, R_1 = H, R_2 = cyclohexyl, Yield: 88%
15, R_1 = H, R_2 = *t*-butyl, Yield: 84%
16, R_1 = R_2 = ethyl, Yield: 85%
17, R_1 = R_2 = isopropyl, Yield: 88%

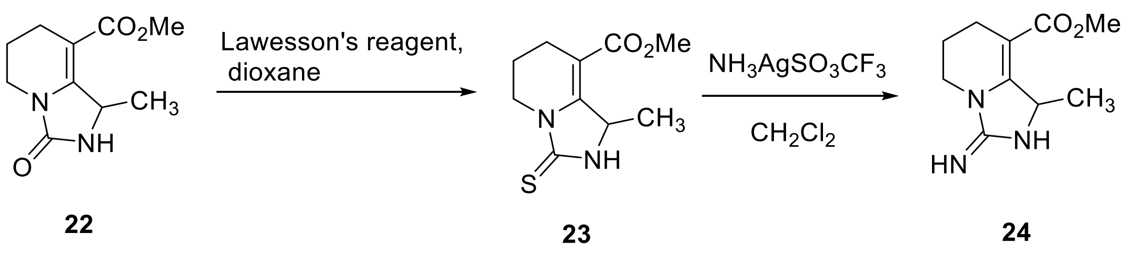
Scheme 1.4: Utilising Mukaiyama reagent to generate *N*,*N′*-substituted guanidine derivatives. a) Pentafluorophenyl chloroformate, DIPEA, CH_2Cl_2; b) Pbf-NH_2, potassium *t*-butoxide, DMSO; c) Mukaiyama's reagent, DIPEA, THF/DMF.

Nagashima *et al.*[58] have developed an efficient synthesis to produce malonamide derivatives as αvβ3 antagonists that contain a guanidine moiety in their structure as outlined in Scheme 1.5. The protocol involved the guanylation of compound **18** with *N*,*N*´-bis (*tert*-butoxycarbonyl) thiourea, by utilising Mukaiyama´s reagent and trimethylamine, to produce the *tert*-butoxycarbonyl (Boc) protected guanidine derivative **20**. The Boc protecting group was then removed by using 4 M HCl in dioxane to obtain the desired guanidine compound **21**.



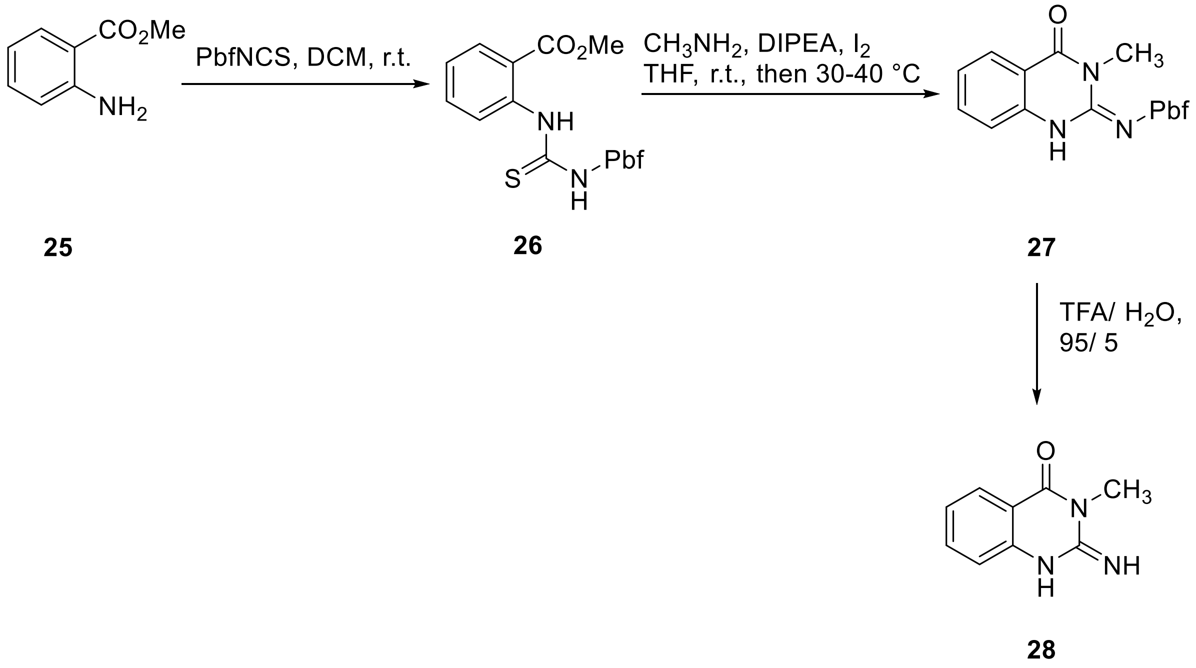
Scheme 1.5: The production of malonamide derivatives having the guanidine group in their structure.[58]

The initial work towards the synthesis of functional guanidine has since been advanced by Rao while attempting to produce anti-viral drug leads.[59] This synthetic route included preparation of a urea and then conversion to the corresponding thiourea **23** by using Lawesson's reagent in dioxane. Production of a cyclic guanidine **24** was achieved *via* subjecting the thiourea to $NH_3AgSO_3CF_3$ (Scheme 1.6).



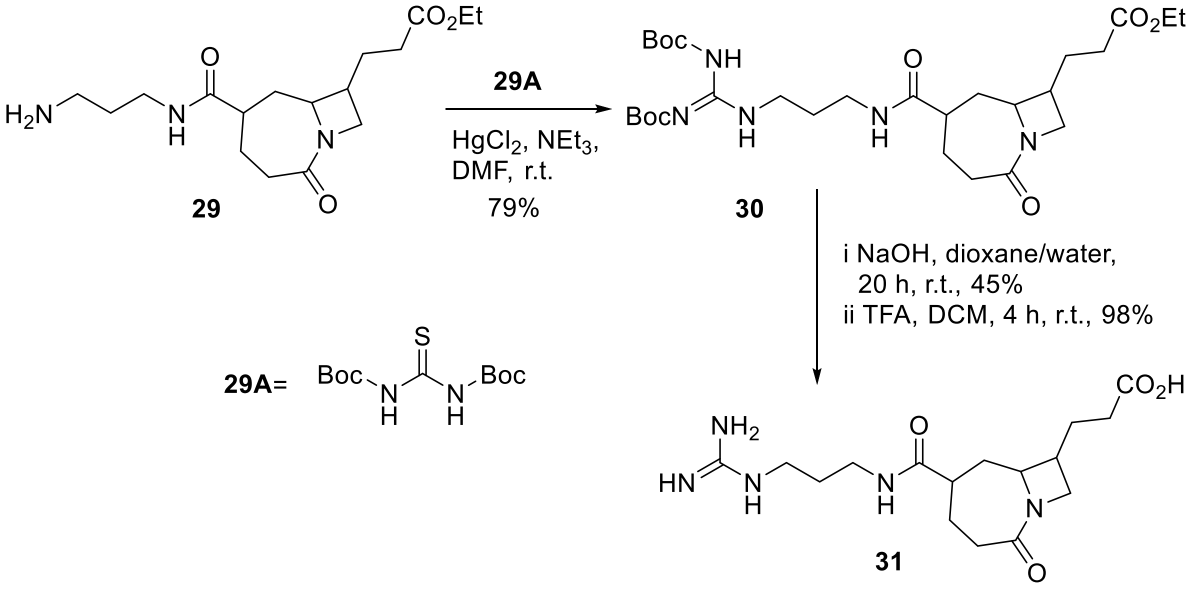
Scheme 1.6: Rao's methodology to synthesise cyclic guanidine.[59]

Another approach to synthesise a cyclic guanidine, this time possessing a quinazolinone heterocyclic skeleton has been reported by Jizhen *et al.*[60] This cyclic guanidine **28** was generated by the reaction between Pbf-activated thiourea **26** and a primary amine *via* iodine-mediated guanidinylation as outlined in Scheme 1.7.



Scheme 1.7: The synthetic route to cyclic guanidine **28**.

Girard *et al.* have published the preparation of a new bicyclic lactam having a guanidine group in the side chain (Scheme 1.8).[61] The production of compound **30** was achieved through the coupling of **29** with *N*,*N*´-bis-*t*-butoxycarbonylthiourea **29A** in the presence of $HgCl_2$. The final step involved the hydrolysis of the ester, by adding sodium hydroxide, followed by the removal of the protecting groups, by adding TFA, to generate 5-(guanidinocarboxamido propyl)-1-azabicyclo [5.2.0] nonan-2-one-8-propionic acid **31**.



Scheme 1.8: Synthesis of a bicyclic lactam having a guanidine group in the side chain.

1.2.4 The chemistry of bicyclic guanidine

It could be expected that the conversion of the guanidine group into a rigid bicyclic guanidine framework gives the guanidine a specific hydrogen bonding direction, in a single face, giving new properties to its interaction with DNA or RNA strands.[62] The formation of a rigid bicyclic guanidine framework gives new features to the guanidine; their impact is limited to three areas related to the steric and electronic features. The efficiency with which charge is distributed over the molecule is one of these significant characteristics. The charge could be negative for guanidinate anions or positive for guanidinium cations (Figure 1.10). The delocalisation of charge can be explained as a simple orbital overlap between the nitrogen lone-pairs and the p-orbital of the sp^2 carbon within the CN_3 unit. Constraining the substituents of the non-amidine nitrogen N (3) atom, into the ring system (tertiary nitrogen), leads to a preferable alignment for the lone-pair of this atom to be involved in the delocalisation scheme. However, the localised N (2)-C=N (1) is an essential fragment of neutral guanidines, and is where the majority of chemical processes shown by these molecules take place. The tertiary nitrogen N (3) therefore participates in specifying the electronic and physical properties of guanidines.

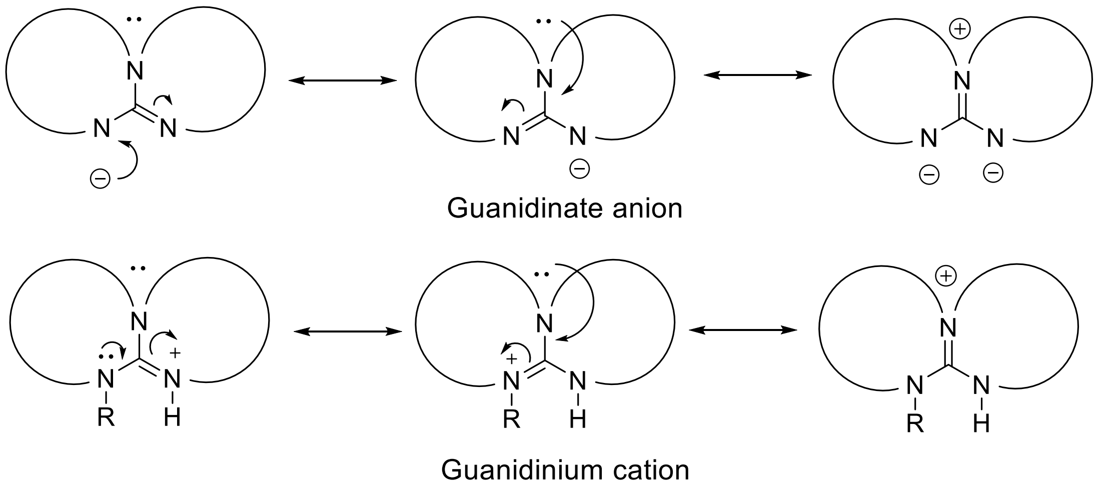


Figure 1.10: Resonance forms of the bicyclic guanidinate anion and bicyclic guanidinium cation.

The size of the rings of bicyclic guanidine imposes an additional geometric constraint on the molecule. This constraint determines the orientation of the frontier orbitals of the amidine nitrogen atoms. A wide projection band on purely geometric arguments is introduced by reducing the ring size from 6 to 5 membered rings. The differences among the molecular structures of guanidine, bicyclic guanidine (6-membered ring) and bicyclic guanidine (5-membered ring) are shown in Figure 1.11.

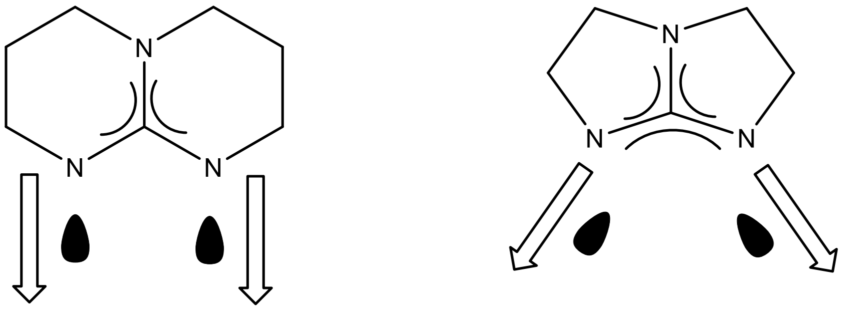


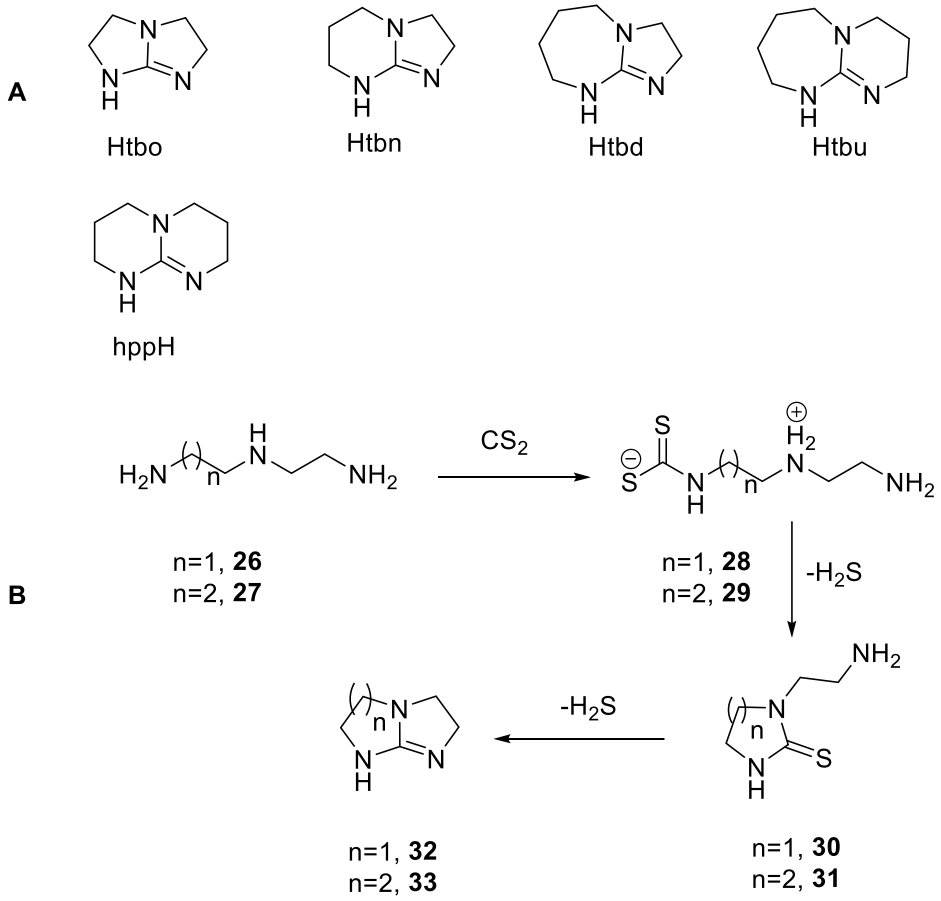
Figure 1.11: Comparison between the projection of frontier orbitals for the {6, 6} - and {5, 5} - bicyclic derivatives.

1.2.5 The synthesis of bicyclic guanidine

The synthesis of bicyclic guanidines has attracted remarkable attention over the past 60 years because of its uses in many fields of chemistry. Two strategies have been used to prepare the bicyclic guanidinium moiety. The earlier work implemented the cyclization of a mono-cyclic guanidine,[63-67] while the second strategy centred on attaching the central carbon atom of the guanidine to a triamine.[68-70]

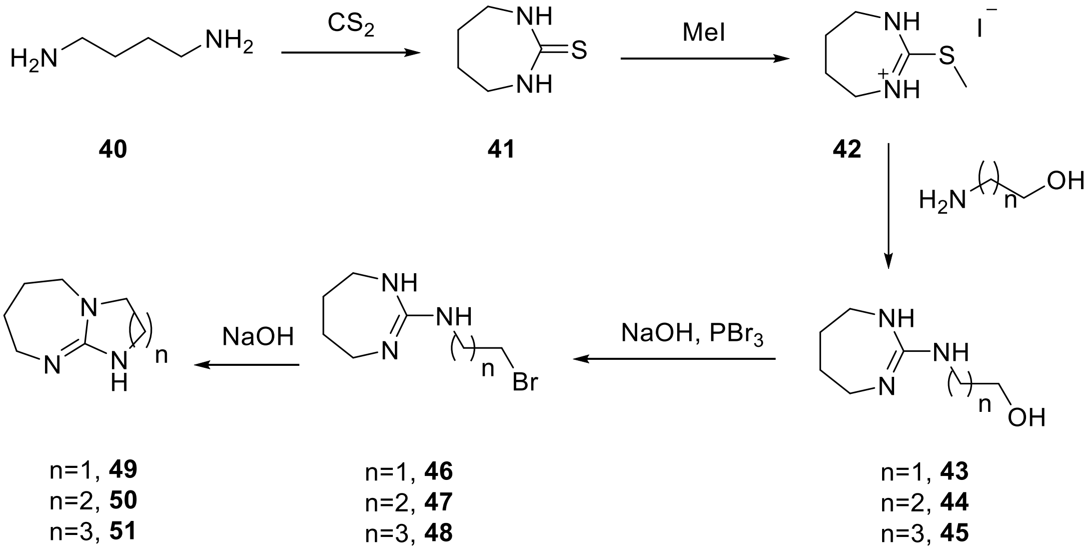
1.2.5.1 Differently sized rings for bicyclic guanidines

There are many different sized rings of unfunctionalised bicyclic guanidine and their abbreviations are as shown below in Scheme 1.9A.[62] Earlier work to synthesise non-functionalised bicyclic guanidines has used multi-step procedures,[65] or expensive starting materials.[70] In the patent literature, a simple method was published in 1990 to synthesise {6, 6} - and {5, 5} - guanidines, hppH and Htbo, that involved a one- pot method (Scheme 1.9 B).[71] As it is the simplest method to produce bicyclic guanidine, this procedure has been used commercially, driven by the potential use of the products in organocatalysis.[72] Producing a non-symmetric {6, 5} - bicyclic model has also been achieved by using the same procedure as mentioned above.[68]



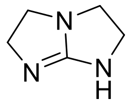
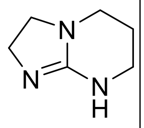
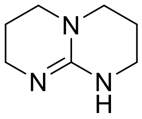
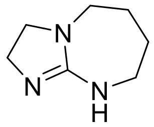
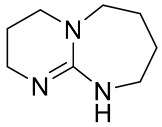
Scheme 1.9: **A**. Different sized rings of bicyclic guanidine, **B**. Preparation of H-tbo and H-tbn.

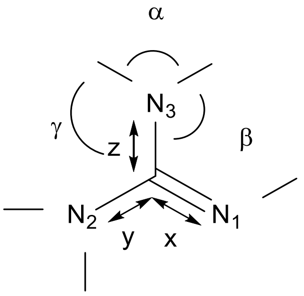
Another approach to construct differently sized rings of bicyclic guanidine has been reported as outlined in Scheme 1.10.[66] The synthesis of these compounds has been started by the reaction between carbon disulfide, and 1, 4-diaminobutane **40**, in ethanol to synthesise an thiourea **41** followed by methylation at the sulfur atom to generate the intermediate **42**. The reaction between compound **42** and ethanolic amine gave **43** that was converted to the corresponding halide **46** by treatment with PBr_3. Next, the generation of Htbu **49** was achieved by the treatment of **46** with NaOH, finally producing **50** and **51** by using propanol amine and butanol amine respectively.



Scheme 1.10: Production of differently sized rings of bicyclic guanidine.

X-ray diffraction data showed a comparison of structural features of the differently size rings of bicyclic guanidine including {5, 5}, {6, 5}, {6, 6}, {7, 5} and {7, 6} and are listed in Figure 1.12.[68] In the “N (2)-C=N (1)” amidine component, it could be clearly observed that the distribution of carbon-nitrogen bond distances is equal for each molecule and were clearly defined for the C–N (1) and C–N (2) bonds. However, for N (3), there are a wide range of values for the degree of pyramidalisation (DP%) that are finally affecting the value of ΔC–N. It is unlikely to form a planar configuration (higher DP% values) in the presence of nitrogen N (3) (tertiary amine) especially with the bicyclic guanidine having five-membered rings, such as H-tbo, H-tbn and H-tbd (n=5). This is in agreement with the retention of electron density at this position, thus, decreasing any delocalisation of these neutral compounds over the CN_3 core with greater values of ΔCN. However, these compounds have only recently been synthesised and they need further investigation,[66] especially the implication of the changes in charge delocalisation. This will lead to a different effect in these compounds on their neutral, cationic and anionic forms, which is expected to lead to changes in chemical behaviour.

	H-tbo 	H-tbn 	hppH 	H-tbd 	H-tbu 
C-N1	1.346(2)	1.290(3)	1.328(2)	1.233(2)	1.293(2)
C-N2	1.297(2)	1.347(3)	1.367(2)	1.354(2)	1.356(2)
C-N3	1.391(2)	1.383(3)	1.331(2)	1.355(2)	1.394(2)
DP%	26.58	18.19	0.11	20.74	4.20
ΔCN	0.048	0.057	0.039	0.062	0.090
Δ'CN	0.070	0.065	0.038	0.069	0.034



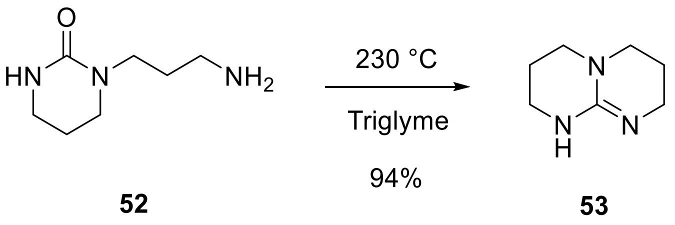
$\Delta CN\ /\text{Å} = y - x$

$\Delta' CN\ /\text{Å} = z - [\{x + y\} / 2]$

$DP\% = [360 - (\alpha + \beta + \gamma)] / 0.9$

Figure 1.12: Definition of parameters used in the comparison of structural data.[65]

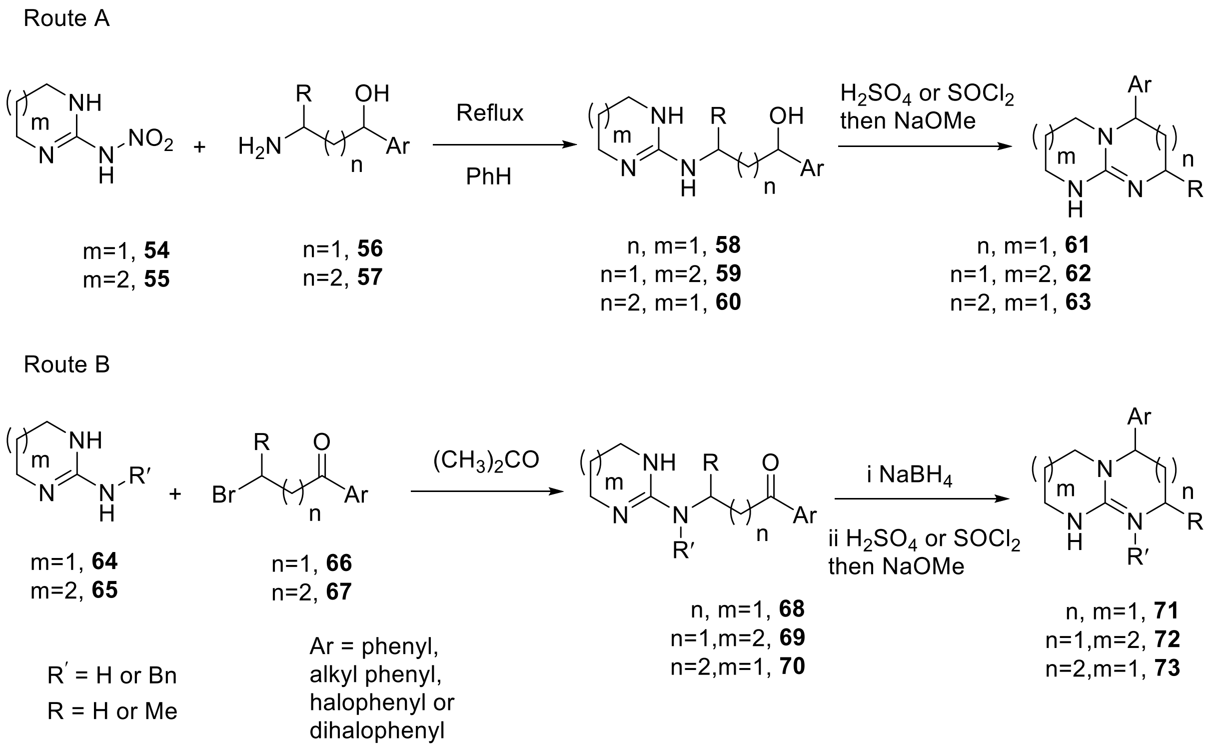
Another procedure to synthesise bicyclic guanidine is by using high-boiling point polyether solvents.[73] These harsh conditions involving high reaction temperatures (200-250 °C), and long reaction times (2-3 days), are applied to avoid the use of the hazardous reagent carbon disulfide (CS_2). The highest yields of 94% have been obtained when acyclic urea **52** was used as a starting material (Scheme 1.11).



Scheme 1.11: The synthesis of bicyclic guanidine from monocyclic urea.

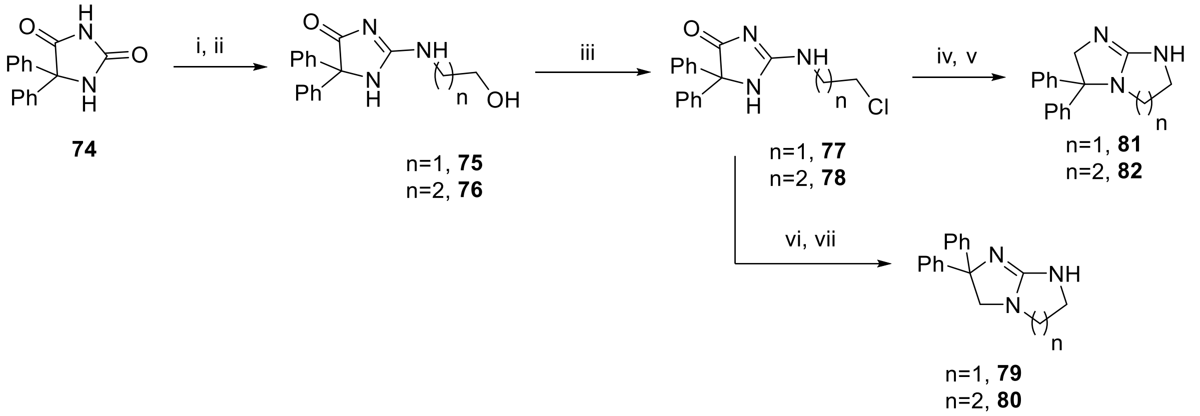
1.2.6 Substituted bicyclic guanidines

The synthetic route to produce a variety of aryl substituted bicyclic guanidines, has been described by Van Gelder *et al.*[74] The formation of these compounds was achieved through two steps. The first step is to produce the aryl substituted monocyclic alcohols (Scheme 1.12 A). The second is the formation of the bicyclic guanidine by condensation or de-halogenation. In the same way, the alkylation of the *N*-benzyl monocyclic guanidine leads to the production of benzyl substituted bicyclic guanidines (Scheme 1.12 B).



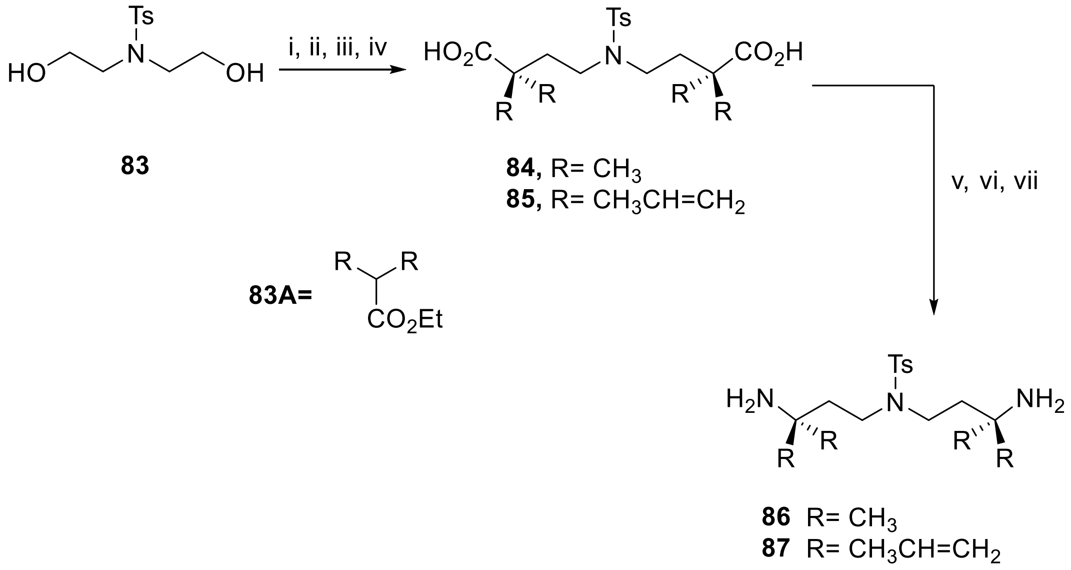
Scheme 1.12: The route to produce aryl substituted bicyclic guanidines by Van Gelder's synthesis.[74]

Another method to synthesise 2, 2- and 3, 3-diphenyl substituted {5, 5} bicyclic guanidines (**79** and **81** respectively) was described by Kosasayama *et al.* (Scheme 1.13).[67] Formation of monocyclic guanidino alcohol was achieved in the first step, and then conversion of the alcohol group to the chloride **77** and **78**, followed by intramolecular nucleophilic substitution then Red-Al reduction which produces the 2, 2-diphenyl derivatives **79** and **80**. Increasing the temperature of this reaction to 200 °C leads to the production of 3, 3-diphenyl derivatives **81** and **82** which were thus synthesised by using this method.



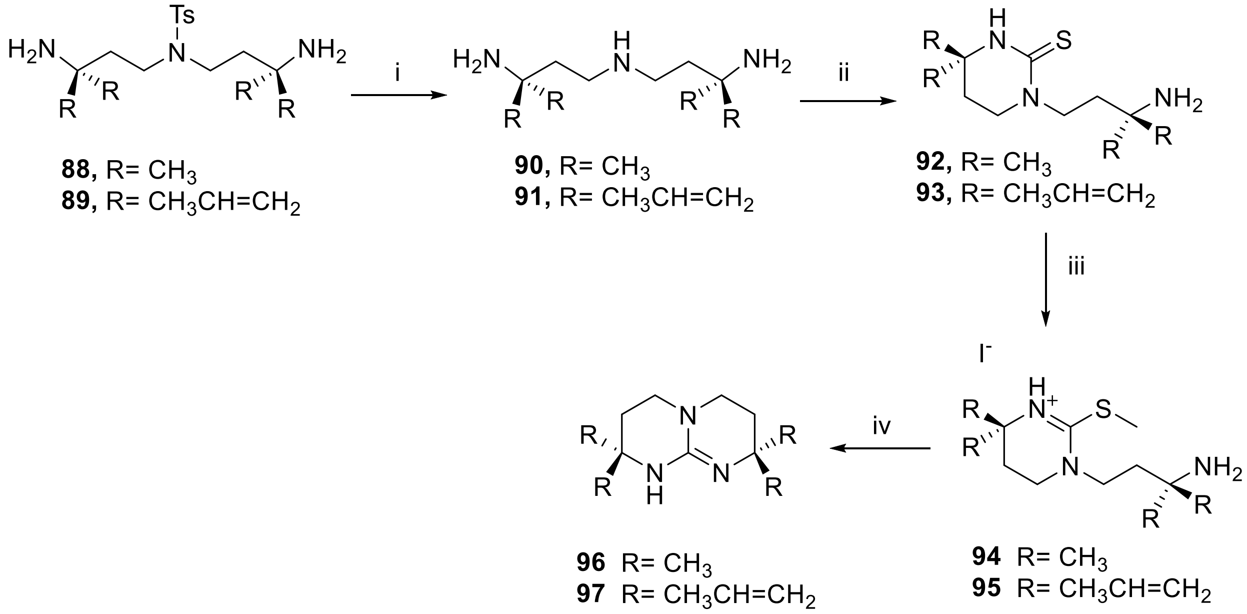
Scheme 1.13: Preparation of 2, 2 and 3, 3-diphenyl bicyclic guanidines. i. $(C_2H_5)_3OBF_4$, CH_2Cl_2, Δ (88%); ii. $H_2NCH_2(CH_2)_nOH$, EtOH, Δ (n=1 85%, n=2 88%); iii. $SOCl_2$, Δ (n=1 97%, n=2 94%); iv. NaH, DMF (n=1 87%, n=2 88%); v. $NaAlH_2(OCH_2CH_2OCH_3)_2$, THF, Δ (n=1 57%, n=2 62%); vi. NaH, DMF, Δ (n=1 88%, n=2 90%); vii. $NaAlH_2(OCH_2CH_2OCH_3)_2$, THF (n=1 29%, n=2 81%).

Schmidtchen described the first synthetic route to produce alkyl and allyl substituted bicyclic guanidine in 1980.[70] The first step in this synthetic route comprises the conversion of **83** to its corresponding di-iodide and then the resulting product was alkylated with **83A** (Scheme 1.14). The tetra-substituted di-ester product was converted to the corresponding di-acid **84** by an acid hydrolysis process. The next step included the preparation of the carboxylic azide followed by a Curtius rearrangement and hydrolysis, which yielded the first part in Schmidtchen's route to tetra-substituted diamines **86** and **87** respectively.



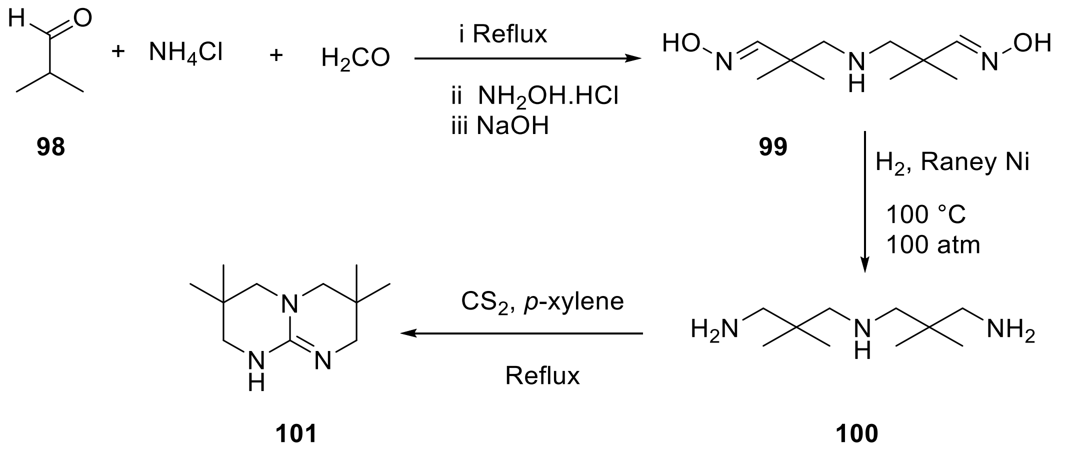
Scheme 1.14: Schmidtchen's route to produce the tetra-substituted diamine.[70] i. MsCl, TEA, CH_2Cl_2 (90%); ii. NaI, $(CH_3)_2CO$ (70%); iii. LDA, **83A**, THF, 0 °C; iv. KOH, H_2O, Δ (**84** = 75%, **85** = 73% 2 steps); v. Oxalyl chloride, DMF, PhH; vi. NaN_3, H_2O, $(CH_3)_2CO$, Δ, CCl_4; vii. 6 M HCl, H_2O, THF (**86** = 71%, **87** = 72% 3 steps).

The final step in Schmidtchen's protocol included the de-protection of the tosyl group to furnish a triamine. A thiouronium compound was then produced by the reaction between **90** and thiophosgene, followed by methylation to generate **94**. The final step to produce *α, α, α', α'*- [6, 6]-bicyclic guanidines **96** was achieved by treatment of **94** with sodium methoxide in methanol (Scheme 1.15).



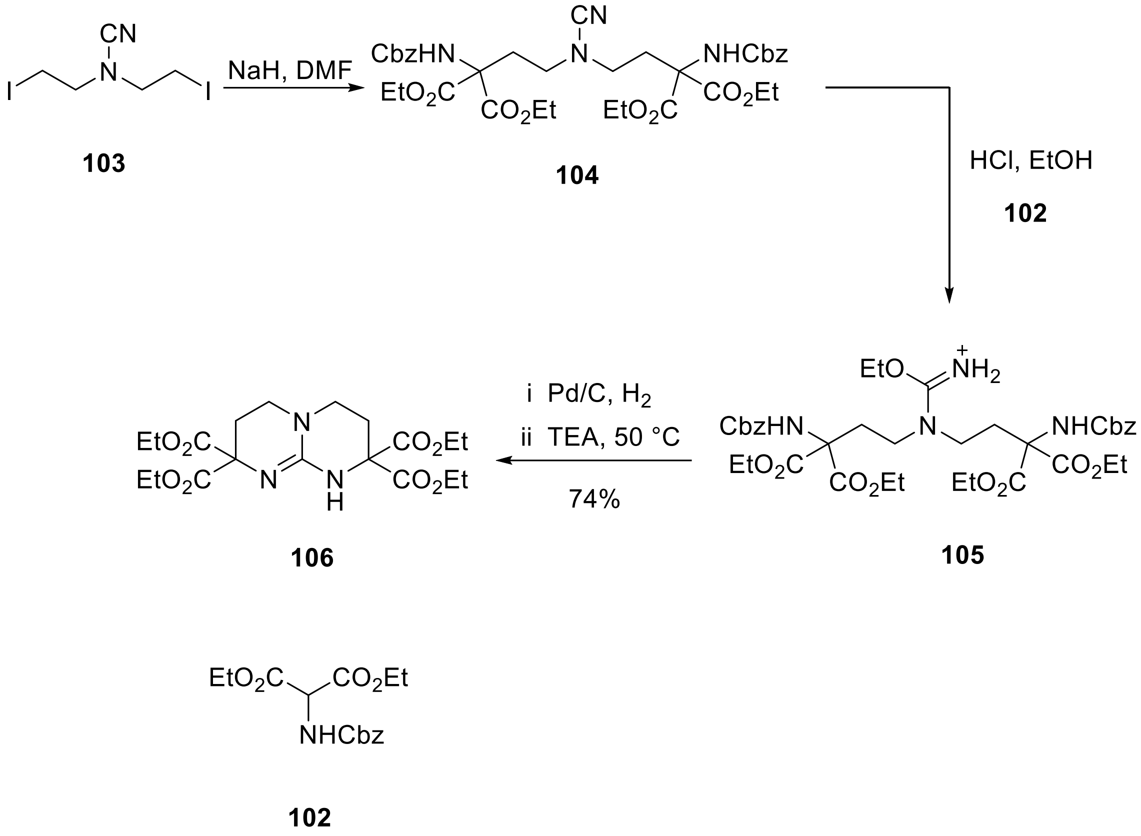
Scheme 1.15: The final steps in Schmidtchen's route to produce tetramethyl and tetrapropenyl α, α, α′, α′- [6, 6]-bicyclic guanidines. i. Li, NH_3 (l), THF (**90** = 75%, **91** = 81%); ii. $CSCl_2$, TEA, CH_2Cl_2 (**92** = 80%, **93** = 81%); iii. MeI, CH_3NO_2; iv. $NaOCH_3$, CH_3OH or KOt-Bu, t-BuOH (**96** = 79%, **97** = 70% 2 steps).

A synthetic route to methyl and ethyl β, β, β′, β′- [6, 6]-bicyclic guanidine derivatives has been published by Cotton and co-workers.[75] The synthetic route involved using a Mannich-style reaction to get an intermediate compound (tetra-methyl and ethyl triamine) followed by Raney® nickel hydrogenolysis, the final step being a cyclisation to obtain substituted bicyclic guanidine **101** (Scheme 1.16).



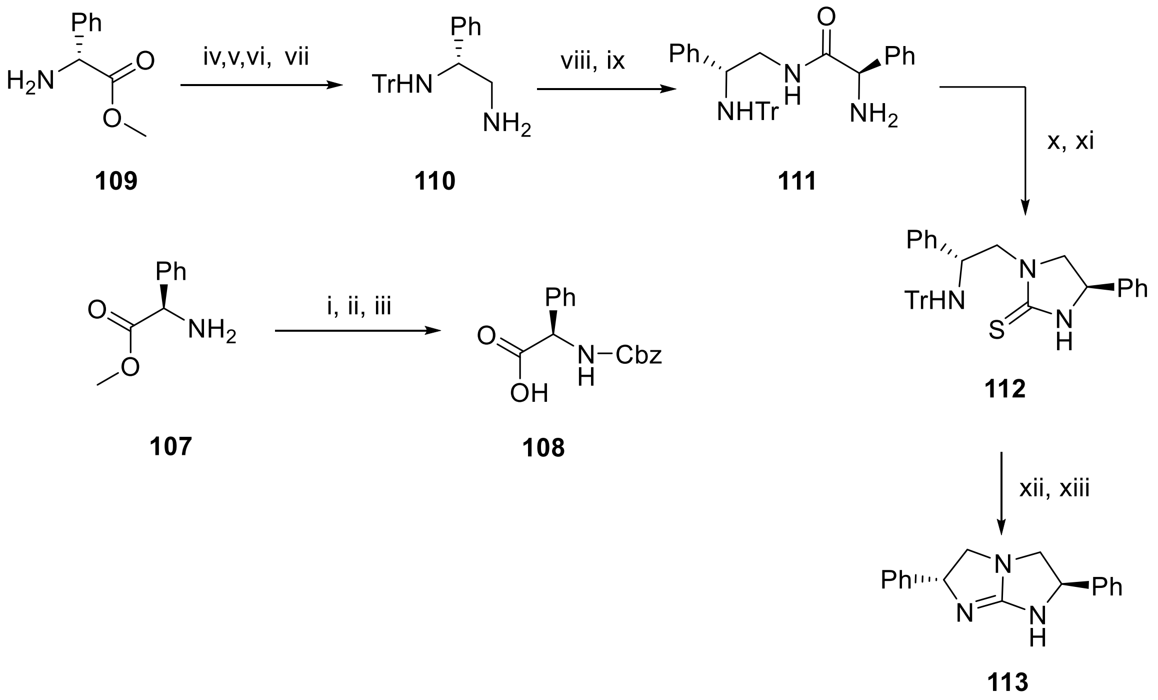
Scheme 1.16: The synthetic route to methyl and ethyl β, β, β′, β′- [6, 6]-bicyclic guanidines as reported by Cotton.[75]

Building on prior developments in the synthesis of substituted bicyclic guanidine, the bicyclic guanidino tetra ester has been synthesised by Jadhav *et al,*[76] using a Pinner reaction to produce the iminium intermediate **105** (Scheme 1.17). The next step included hydrogenolysis, after which the cyclisation was achieved by heating in TEA to generate bicyclic guanidine **106**.



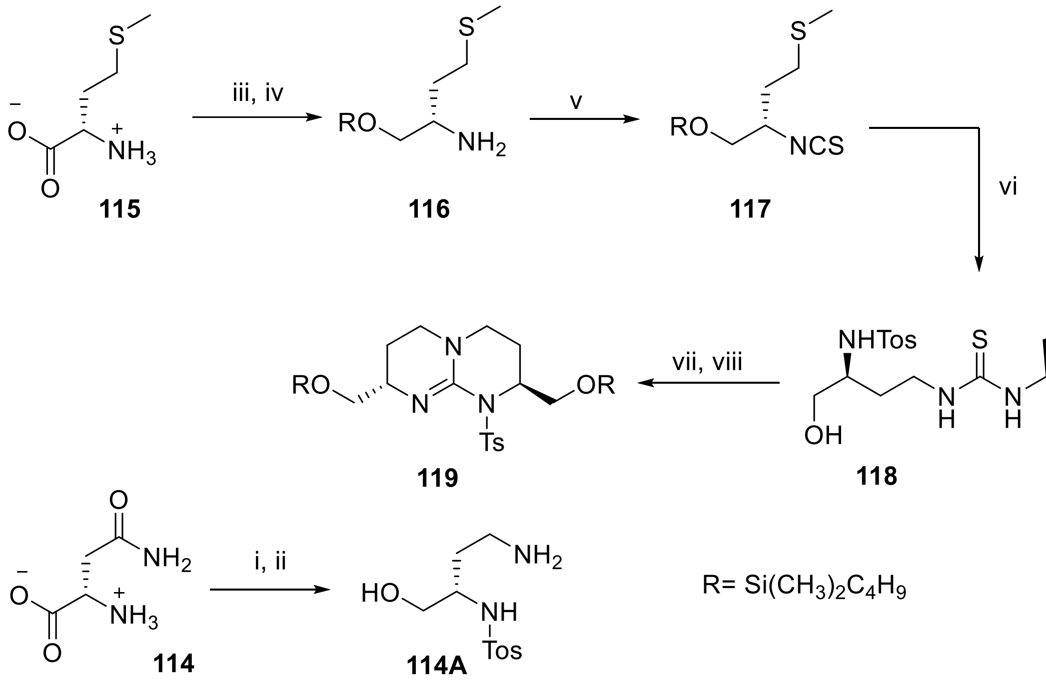
Scheme 1.17: The synthetic route to bicyclic guanidine tetraethyl ester.[76]

A C2 symmetric chiral bicyclic guanidine has been synthesised as early as 1989 by Corey and Ohtani (Scheme 1.18).[77] Many steps have been used in their synthetic route; the first step included using α-phenylglycine methyl ester as a starting material to synthesise a monoprotected diamine **110**, and an *N*-protected amino acid **108**. These are then coupled to produce substituted a triamine **111** followed by hydrogenolysis and reduction. The formation of thiourea **112** was achieved by reaction with thiophosgene and then cyclisation was induced by the methylation of the sulfur atom to yield the C2 symmetric chiral bicyclic guanidine **113**.



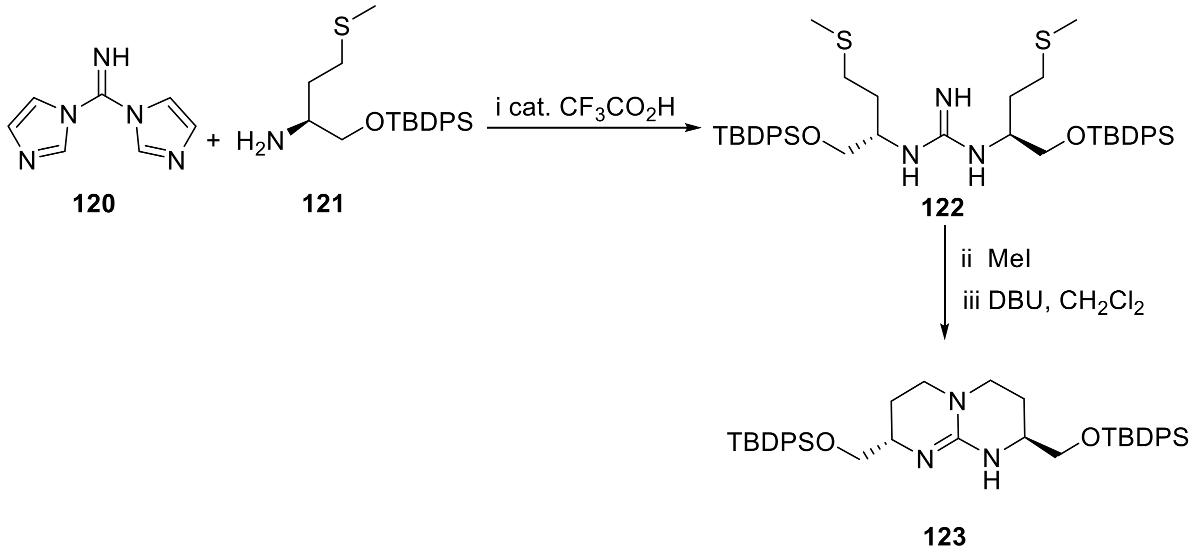
Scheme 1.18: Synthesis of chiral C2 symmetric chiral bicyclic guanidine by Corey et al.[77] i. H_2, PtO_2-H_2O, AcOH, 23 °C; ii. Cbz-Cl, Py, CH_2Cl_2, 0 °C, 1 h; iii. NaOH, H_2O/MeOH, 23 °C; iv. NH_3, MeOH, 0 °C, 3 h, 23 °C, 16 h, 84%; v. H_2, PtO_2-H_2O, AcOH, 23 °C, 40 h (87%); vi. TrCl, TEA, CH_2Cl_2, 23 °C, 97%; vii. $LiAlH_4$, Et_2O, reflux, 95%; viii. DCC, HOBt, **108**, THF, 77%; ix. H_2, 10% Pd/C, 1:1 THF/MeOH, 23 °C, 100%; x. Red-Al, PhH, reflux, 76%; xi. $CSCl_2$, Na_2CO_3, 1:1 CH_2Cl_2/H_2O, 95%; xii. MeI, MeOH, 60 °C, 100%; xii. DMF, 100 °C, 60%; xii. MeI, MeOH, 60 °C; xiii. DMF, 100 °C, (55% over 2 steps).

Schmidtchen and co-workers have synthesised asymmetric chiral bicyclic guanidines as anchor modules for oxoanionic functions i.e. the interaction of bicyclic guanidine with oxoanionic functions such as carboxylate.[78, 79] L-methionine and L-asparagine were starting materials in their synthetic route. L-methioninol was produced, silyl protected and then reacted with thiophosgene to obtain the corresponding isothiocyanate **117**. Next this was coupled to a tosyl protected diamino alcohol **114A** (prepared from L-asparagine) to obtain thiourea. Methylation of **118** at the sulfur atoms gave chiral bicyclic guanidine **119** (Scheme 1.19).



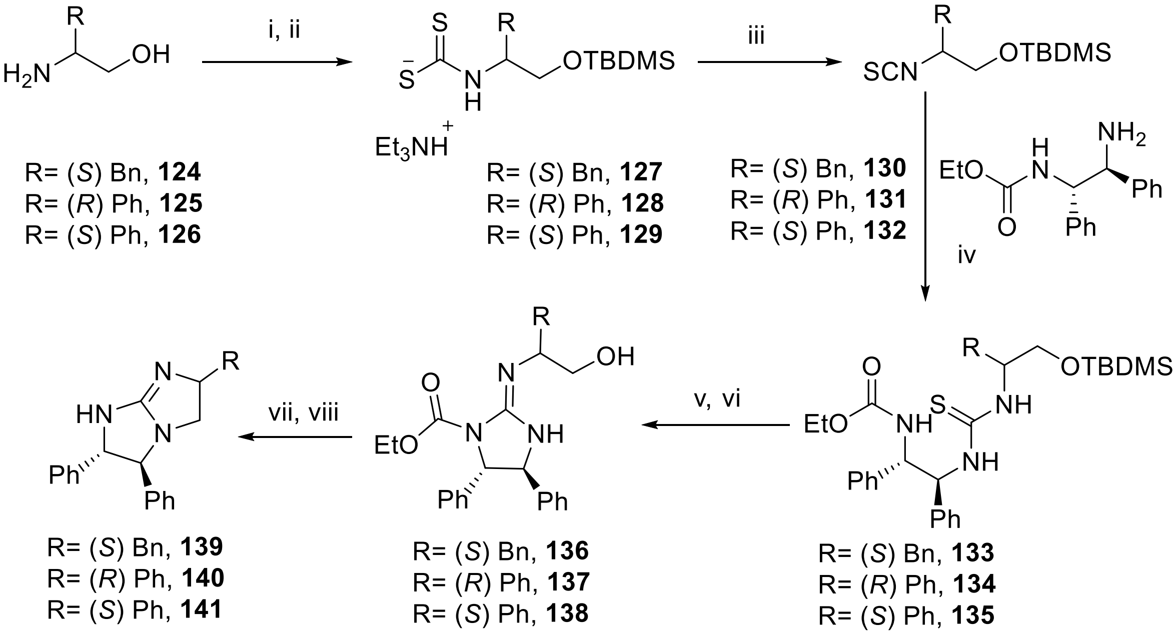
Scheme 1.19: Synthetic route to synthesise asymmetric chiral bicyclic guanidine. i.Ts-Cl, $(CH_3)_2CO$ (80%); ii. $LiBH_4$, TMS-Cl, THF (86%); iii. BH_3.DMS, THF (85%); iv. TBDPS-Cl, imidazole, CH_3CN (yield not given); v. $CSCl_2$, Na_2CO_3, CH_2Cl_2/H_2O (1:1) (87%); vi. **114A**, CH_3CN (85%); vii. TBDPS-Cl, imidazole, CH_2Cl_2 (91%); viii. a) TEA, MeOTf, b) TEA (87%).

Jadhav *et al.*[80] have reported another strategy to synthesise substituted bicyclic guanidino compounds as outlined in Scheme 1.20. This strategy included guanidinylation of L-α-methionine, and then induced cyclisation *via* methylation at the sulfur atom and the addition of an excess of base to obtain **123**.



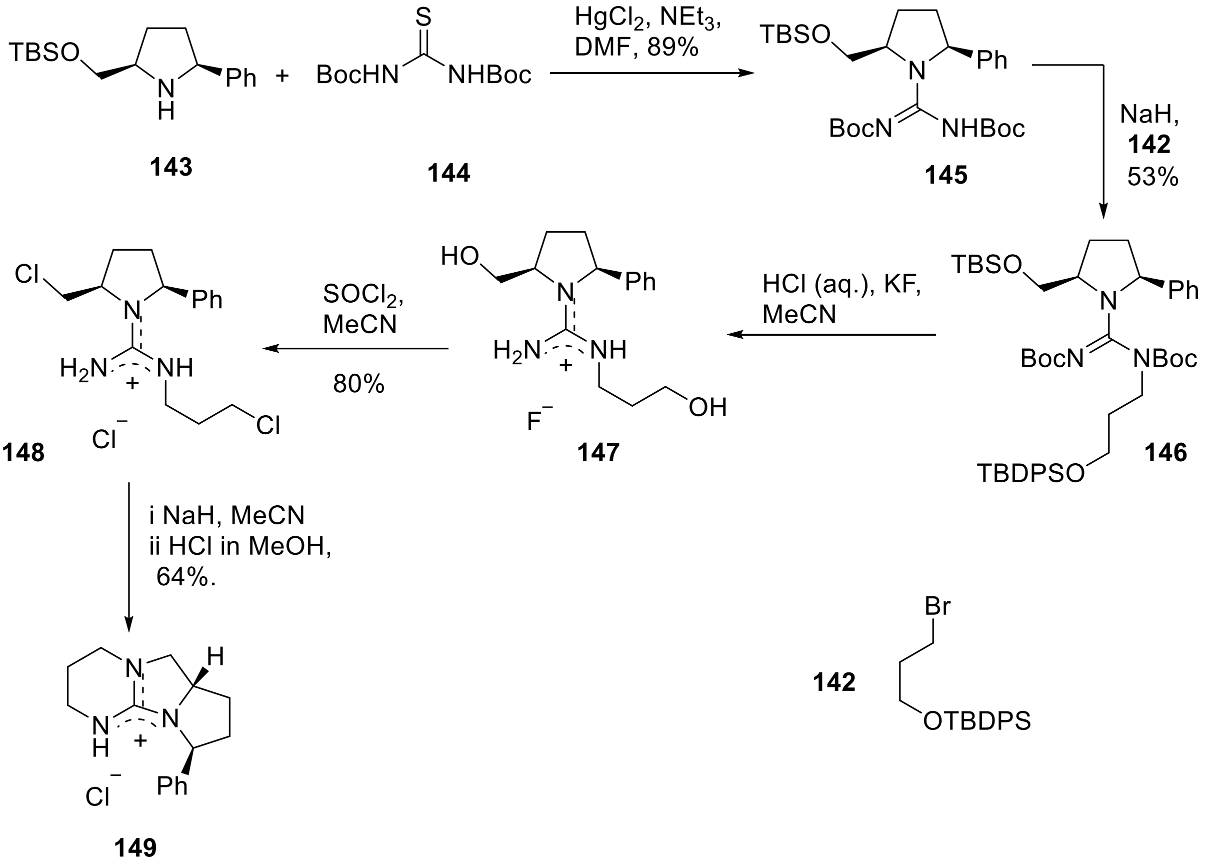
Scheme 1.20: Guanidinylation of L- methionine to synthesise di-substituted bicyclic guanidine.

The reagent 2-chloro-1,3-dimethylimidazolinium chloride (DMC) has been used by Isobe *et al.*[81] to synthesise diphenylbenzyl and triphenyl substituted bicyclic guanidines (Scheme 1.21). A number of silylated amino alcohols were converted in two steps to isothiocyanates **130**-**132** which were reacted with a protected diamine to synthesise thioureas **133**-**135**. Monocyclic guanidine **136**-**138** were produced from the thiourea by adding DMC, followed by removal of the silyl protecting group. A second treatment with DMC then afforded the bicyclic guanidines **139**-**141**.



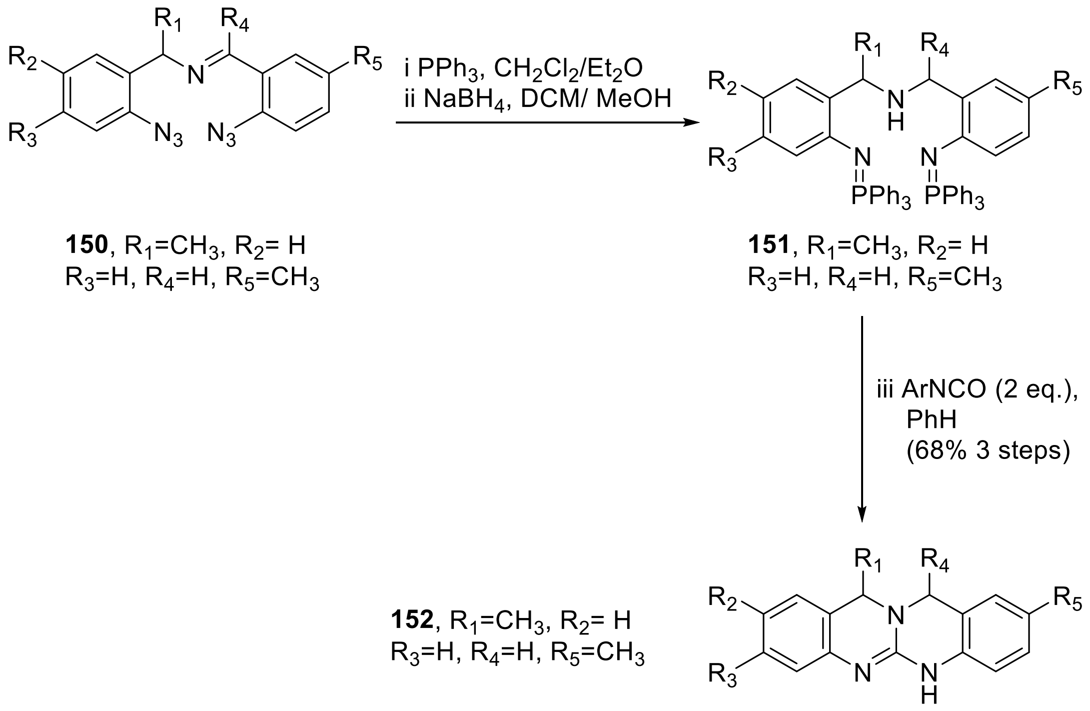
Scheme 1.21: Isobe's approach to produce triaryl substituted bicyclic guanidine.[81] i. TBDMS-Cl, TEA, DMAP, CH_2Cl_2 (100%); ii. CS_2, TEA, CH_2Cl_2; iii. DMC, TEA, CH_2Cl_2 (96% 2 steps); iv. CH_2Cl_2, reflux (98%); v. DMC, TEA, MeCN, reflux (88%); vi. TBAF, THF (96%); vii. DMC, TEA, MeCN, reflux (57%); viii. NaOMe, MeOH (89%).

Turočkin *et al.*[82] have reported the synthetic route to produce tricyclic guanidinium salts (Scheme 1.22), inspired by the strategy that Kim reported to generate a guanidine salt, using the reaction of bis-Boc-thiourea with pyrrolidine (**143**) in the presence of mercury chloride.[83] The resulting product **145** was reacted with compound **142**, in the presence of NaH in anhydrous DMF, to produce **146**. Removal of the protecting groups followed by chlorination gave compound **148**. The final product **149** was synthesised through intramolecular substitution by the treatment of **148** with strong base NaH.



Scheme 1.22: The synthesis of a chiral benzoguanidinium cation.

A synthetic route to bicyclic guanidine fused to aromatic rings and displaying variable substituents has been published by Molina as outlined in Scheme 1.23.[84, 85, 86] The core principle of this synthetic route involved the treatment of bis(iminophosphoranes) with aryl isocyanates or isothiocyanates to generate a wide variety of tetracyclic compounds which possess bicyclic guanidino functionality.



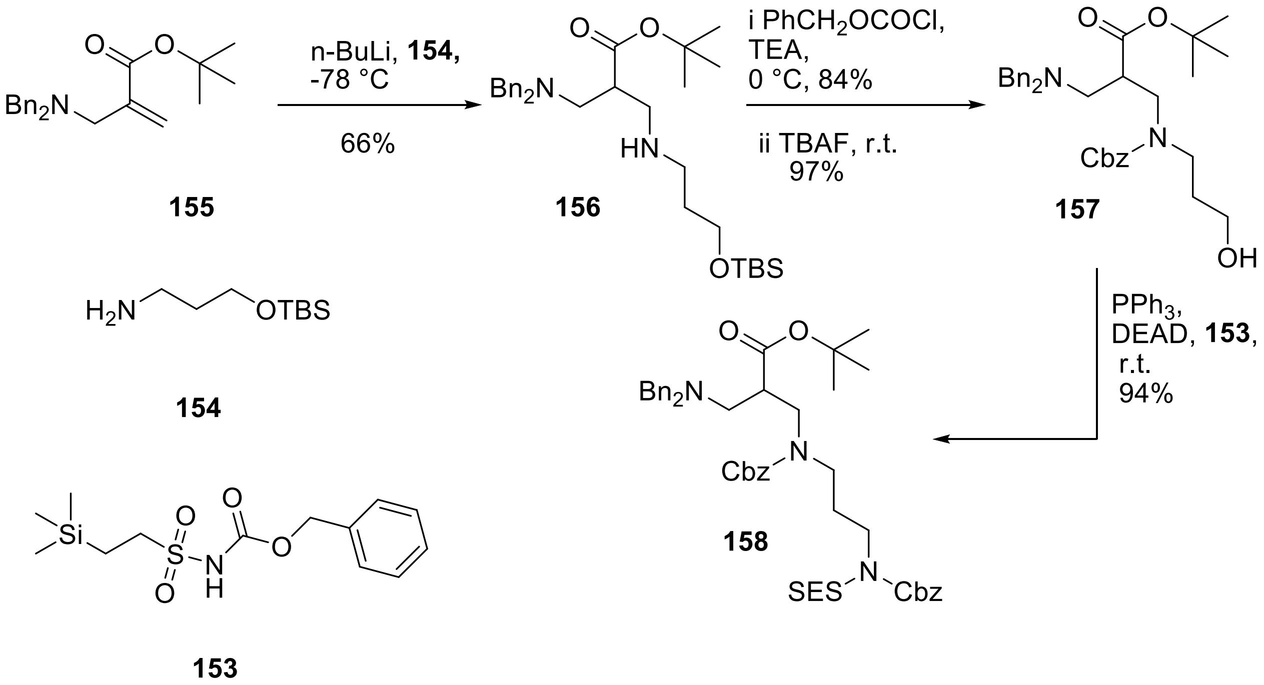
150, $R_1=CH_3$, $R_2=H$
$R_3=H$, $R_4=H$, $R_5=CH_3$

151, $R_1=CH_3$, $R_2=H$
$R_3=H$, $R_4=H$, $R_5=CH_3$

152, $R_1=CH_3$, $R_2=H$
$R_3=H$, $R_4=H$, $R_5=CH_3$

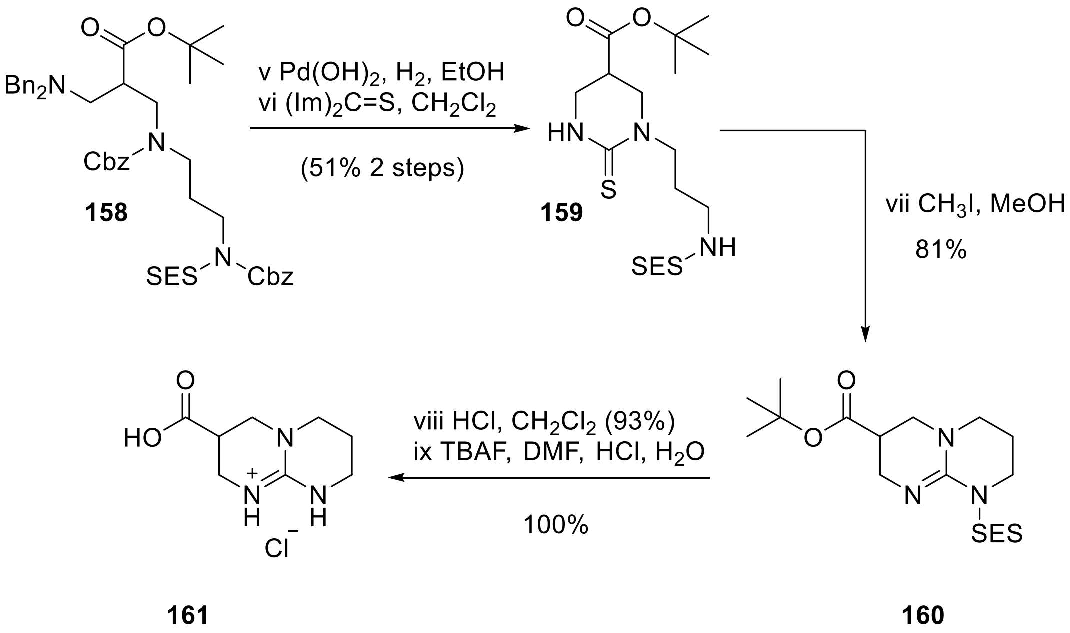
Scheme 1.23: Molina's synthesis of bicyclic guanidine derivatives.

Grillot and Hart have reported a procedure to synthesise a bicyclic guanidine functionalised at the C3 position as in Scheme 1.24.[87] The central consideration in their synthetic route is the production of substituted triamine **158**.



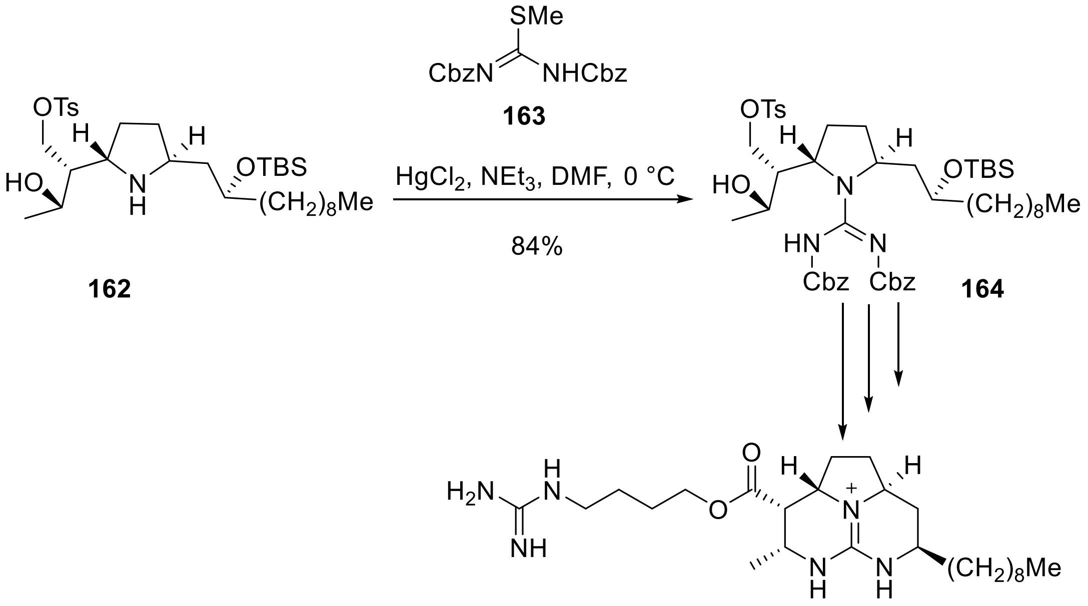
Scheme 1.24: The synthesis of substituted triamine **158**.[87]

Removal of the Cbz and benzyl protecting groups was achieved and then the thiourea compound **159** was produced. The final step involved methylation at the sulfur atom and then intramolecular nucleophilic substitution to form the bicyclic guanidine **160** followed by removal of the protecting groups to generate **161** as shown in Scheme 1.25.



Scheme 1.25: The synthesis of a bicyclic guanidine functionalised at the C3 position.

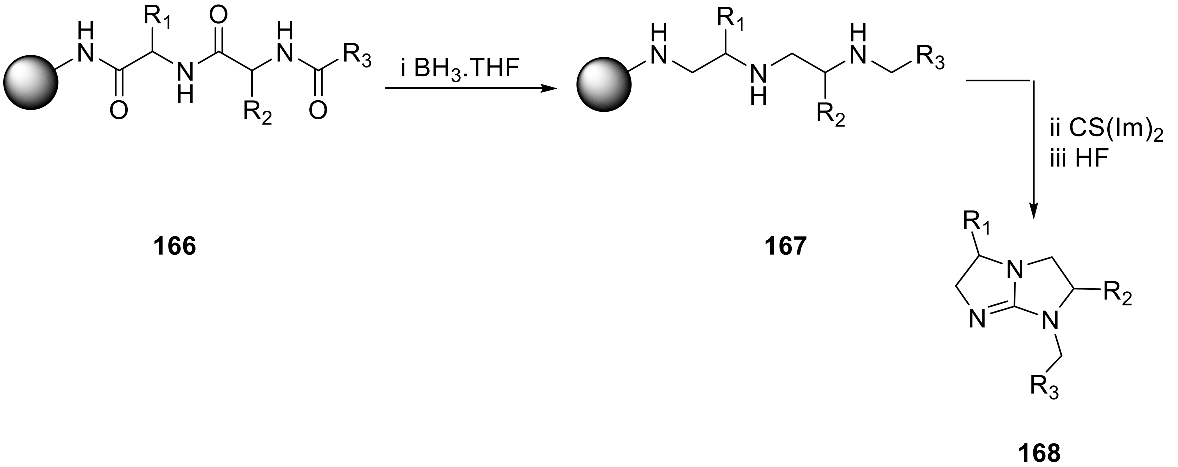
Batzelladines A – I are natural products that possess a polycyclic guanidine alkaloid in their structure. These natural products have been isolated by a SmithKline-Beecham group from Bahamian (batzelladines A – E) and Jamaican sponges (batzelladines F – I). [88, 89] The synthetic route to produce batzelladine D has been reported by Ishiwata and co-workers[90] as outlined in Scheme 1.26. Compound **164** has been produced by the treatment of the 2, 5- disubstituted pyrrolidine with bis-Z-2-methyl-2-isothiourea in the presence of mercury(II) chloride and triethylamine. The production of **165** has been successfully performed in 86% yield over 7 steps.



165

Scheme 1.26: The synthetic route to batzelladine D by Ishiwata *et al.*[90]

An interesting strategy involving the use of solid phase chemistry was applied to produce a series of functionalised bicyclic guanidines by Nefzi *et al.* as shown in Scheme 1.27.[91] By utilising borane as a reducing agent, tripeptides containing different functional groups were converted to their corresponding triamines. Cyclisation was then achieved by utilising thiocarbonyl diimidazole reagent and then removal from the resin led to the production of a library of trialkyl-substituted bicyclic guanidines. Later on, this strategy was used to synthesise libraries of bicyclic guanidines tri-substituted with a variety of amino acid side chains.[92]



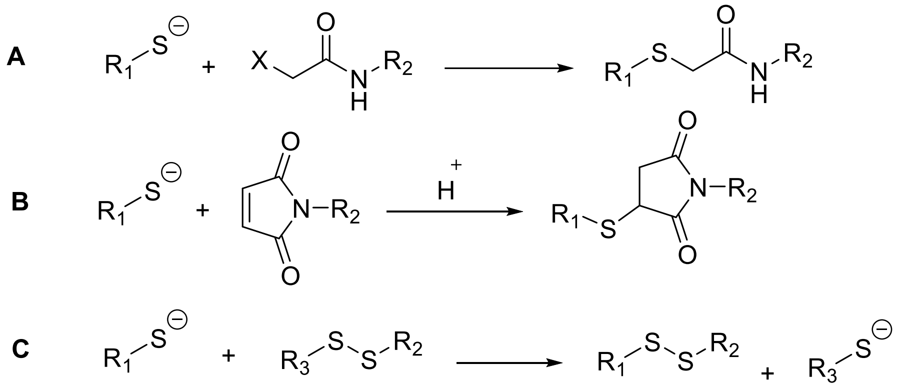
Scheme 1.27: The synthesis of functionalised bicyclic guanidine by utilising solid phase chemistry.

1.2.7 Synthetic modification of peptides and proteins

This synthetic modification can be achieved by first modification of an amino acid side chain, followed by incorporation of the amino acid into the peptide/peptidomimetic or protein by chemical or biochemical (translation) synthesis. Alternatively, the bioconjugation strategy can be followed in which peptide/protein is synthesised first, and then an amino acid selectively modified. This is easier to apply to proteins, as these are challenging to make by chemical synthesis. Bioconjugation plays an important role in bio-organic synthesis,[93] and this synthetic approach is utilised to covalently join a biomolecule to another component to produce a stable conjugate that combines the properties of both components. This technique gives researchers potential access to a wide range of natural or synthetically valuable products that prove difficult to synthesise by stepwise chemical synthesis.[94] The ability to produce complicated molecules by conjugation reactions that could be used in many fields such as therapeutics,[95] or diagnostics,[96] is of particular importance. A bioconjugation reaction should have a number of desirable properties,[97] namely: chemoselectivity and bio-harmony (non-toxic), does not perturb the system of interest in an undesirable way and bio-orthogonality. If the conjugation reaction is to take place in a cell, then the reagent must enter the cell and reach the target for modification while not reacting with other molecules in the cell. A goal of our project is to make bicyclic guanidine (BCG) mimics of arginine. To achieve this, the BCG needs to be attached to a peptide. One way of doing this is to use asymmetric synthesis, chiral auxiliaries and/or resolution to produce an enantiopure BCG amino acid from scratch. A potentially simpler method is to make use of the reactivity of a natural amino acid side chain through which to attach the BCG. An advantage is that the existing amino acid provides the chirality. A variety of bioconjugation reactions for functionalising amino acids have been reported in the literature, the chemistry is therefore well established. In the following sections, the conjugation chemistry for covalently attaching molecules to amino acids will be briefly reviewed.

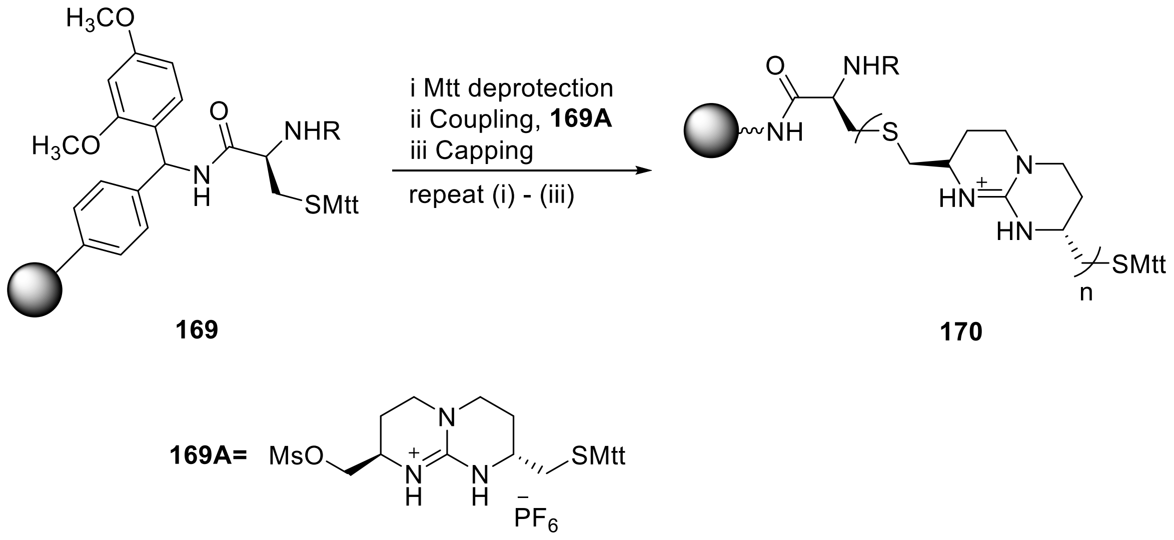
1.2.7.1 Thioether linkage

A good example of a potent nucleophile is the thiolate group in aqueous solution.[98] Thus, derivatives of proteins can be produced *via* the thiolate group of a cysteine side chain, and this approach is now considered a common protocol of bioconjugation.[99] Interestingly, due to their limited presence in natural proteins, site-specific conjugation can be generated at a unique cysteine moiety.[100] Scheme 1.28 shows bioconjugation through formation of either thioethers or disulfides by reaction of a halo acetamide, maleimide, or disulfide with cysteine.



Scheme 1.28: Bioconjugation through thioethers or disulfides. The treatment of thiolates with (A) a haloacetamide, (B) a maleimide, and (C) a disulfide, X= halogen.

Iodoacetamides were used to detect the presence of free cysteines in proteins because the thiolate on the cysteine moiety reacts more rapidly than other amino acid functional groups (Scheme 1.28 A).[101] Recent studies reported that chloroacetamides displayed greater specificity than iodoacetamides toward cysteine moieties.[93] In addition, maleimides could be used as electrophiles for thiol-mediated bioconjugation.[102, 103] Succinimidyl thioethers have been formed *via* Michael addition by the reaction between the thiolate and maleimide (Scheme 1.28 B).[98] Reactions A and B produced linkages that are stable *in vivo*. However, conjugation through a disulfide gives a linkage that is easily cleaved *in vivo* through reduction by glutathione. Thus, disulfides are best utilised specifically for *in vitro* applications, for example the crosslinking,[104, 105] and immobilisation,[106] of peptides and proteins. Martos *et al.*[107] have reported another approach for the generation of thioether-linked chiral bicyclic guanidinium oligomers that are utilised for cell internalisation purposes. A Merrifield-like peptide synthesis technique was used to investigate this goal as given in (Scheme 1.29) below.

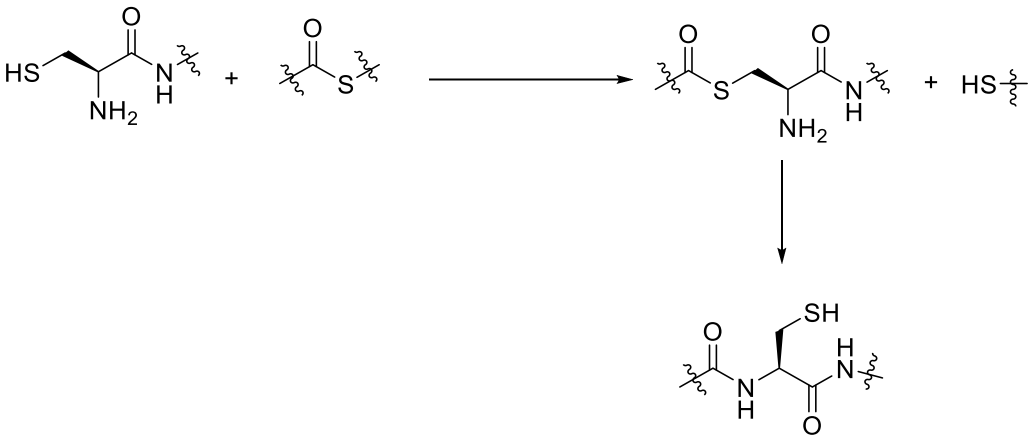


Scheme 1.29: The synthesis of thioether-linked chiral bicyclic guanidinium oligomers.

1.2.7.2 Amide bond linkage

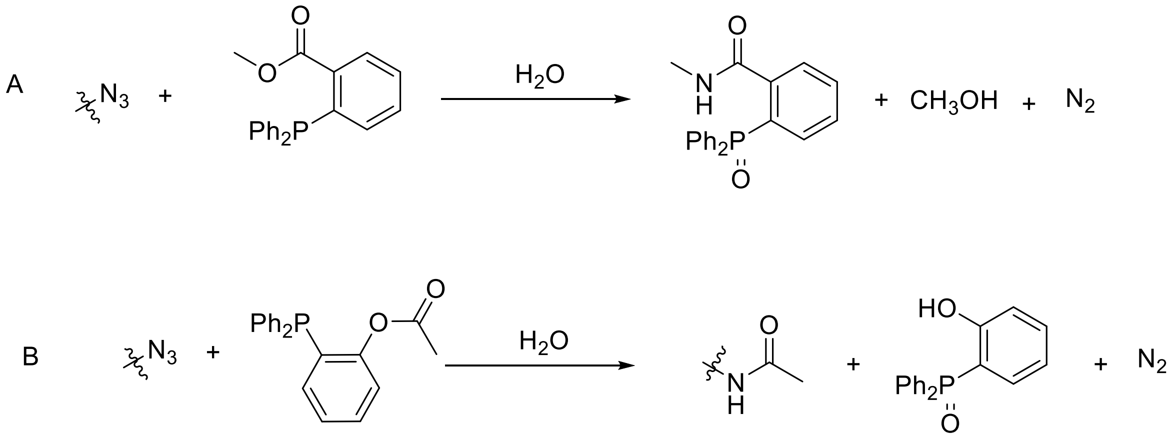
The most attractive feature of amide bond linkages is a long life of ca. 600 years at neutral pH.[108] Amide bonds are formed by the reaction of molecules possessing an amino group and compounds having an activated ester. The formation of an amide bond through amine containing amino acids on peptides can produce impure products because of the presence of several primary amines in peptides. An amide bond could be formed at the lysine moiety, or, at the *N*-terminus of the peptide and these possibilities can result in a complicated product mixture that leads to difficult purification, and hence an expensive bioconjugation strategy. The bioconjugation strategy named native chemical ligation offers another means to form an amide bond between peptides and proteins.[108] This approach has often been used to connect fluorescent molecules to proteins.[109]

A reaction can occur between the thiol of a *N*-terminal cysteine residue-possessing peptide and a thioester peptide to form an amide bond and regenerate the free thiol functional group. The reaction takes place through a trans-thioesterification followed by a *S*- to *N*- acyl migration, as in Scheme 1.30. The free thiol next to the new amide bond could also contribute a functional group for a second subsequent bioconjugation reaction, or produce undesired products *via* side reactions and dimerisation of the bioconjugate by disulfide bond formation. To resolve this problem, desulfurisation can be used to remove an unwanted cysteine,[110] provided that the peptide or protein does not naturally include cysteine as a requisite for its structure or function.



Scheme 1.30: The native chemical ligation method to produce amide bonds.

Another approach to produce bioconjugates *via* amide bond formation is by Staudinger ligation.[111] This approach is similar to the original Staudinger procedure, that involves the reduction of an azide group to an amine by using the reagent triphenylphosphine. The formation of an amide bond through the Staudinger ligation method involves conjugating a ligand derivatised with an azide group to another ligand possessing a phosphine group and an acyl group that it utilises for the production of the amide bond. The Staudinger ligation could be performed in a non-traceless manner (Scheme 1.31 A), leaving a phosphine oxide in the product, or in a traceless way (Scheme 1.31 B). The Staudinger ligation method has been used to introduce glycans onto cell surfaces. Generally, this technique is a very important strategy to generate homogenous bioconjugates that can be purified and characterised easily. The drawback of this method is the challenge of introducing non-natural azide-containing amino acids or carbohydrates into the cellular structure.[112, 113]



Scheme 1.31: (A) Staudinger ligation with a phosphine oxide in the conjugate (B) Staudinger ligation without a phosphine oxide in the conjugate.[112]

1.2.7.3 C-N Double bond conjugation

The formation of a carbon-nitrogen double bond is another approach that could be used in bioconjugation chemistry. This double bond is formed by the reversible reaction between an amine with an aldehyde or ketone functional group to produce an imine bond. Subsequently, the formation of a more stable linkage can be achieved by reducing the imine bond. This approach could be used specifically in order to connect carbohydrates to proteins.[114] Another strategy to generate a carbon-nitrogen double bond was achieved by the reaction between hydrazine or alkoxyamine compounds, with an aldehyde or ketone functional group, to generate hydrazone (C=N-N) and oxime (C=N-O) linkages. The stability of these linkages is higher than imine linkages but they can also be susceptible to hydrolysis. However, this strategy could also be applied in bioconjugation chemistry, especially in applications that do not need long term stability. An elegant example of this approach is the linkage between peptides and glass slides to generate microarrays to reveal antibodies in blood samples and cell adhesion assays.[115, 116] Figure 1.13 depicts the *E* geometry of the C-N double bond in an imine, hydrazine and oxime.

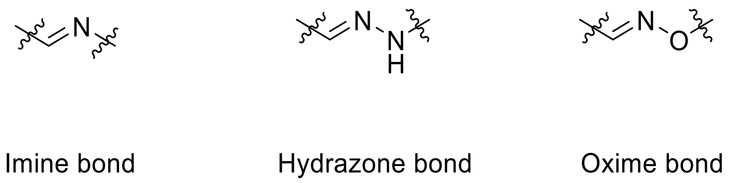
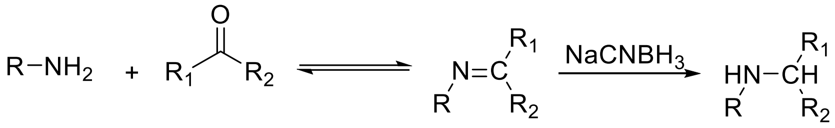


Figure 1.13: C-N Double bond bioconjugation.

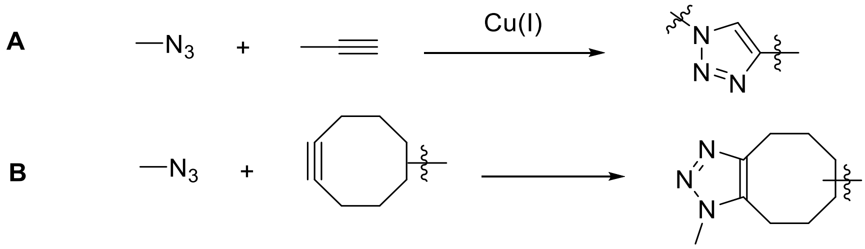
Typically, the Schiff base can be reduced to a secondary amine by utilising sodium cyanoborohydride; this reaction is called reductive amination as outlined in Scheme 1.32.



Scheme 1.32: The formation of a secondary amine by reductive amination.

1.2.7.4 Linkages by cycloaddition

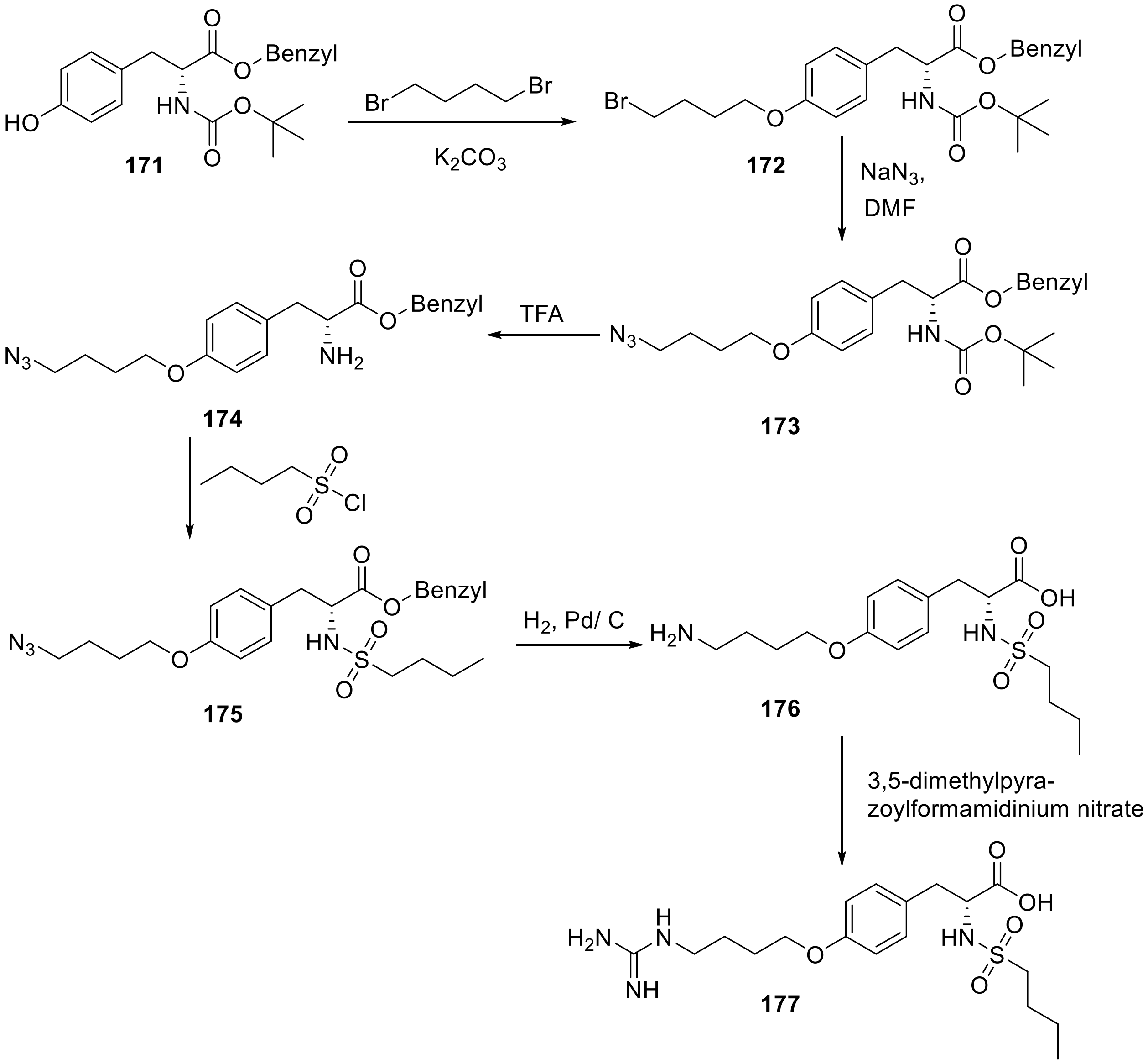
The usage of Cu(I) as a catalyst contributes to the acceleration of the Huisgen azide-alkyne 1,3-dipolar cycloaddition to produce a stable 1,4-disubstituted (1,2,3)-triazole linkage and this is one example of a "click reaction" (Scheme 1.33 A). Sharpless intended the term to be used for reliable, selective, mild reactions. This azide-alkyne reaction has many positive features that leads to its utilisation in generating bioconjugates; these features include purification by simple product filtration,[117] and high stability at elevated temperature and over a range of pH. Another advantage for the click reaction is that both reactants in the click reaction (the azide and alkyne functional groups) do not react with other functional groups found in biomolecules leading to the formation of homogenous conjugates. The rigidity of the formed triazole linkage leads to minimised interactions between the combined ligands, thus resulting in a low propensity to aggregate and minimising the potential for a reaction to occur between two ligands. However, the copper catalysed click reaction is not useful in *vivo* due to the presence of Cu (I) that leads to cytotoxicity.[98] The strain promoted reaction (Scheme 1.33 B) is a copper free alternative that is suitable for in *vivo* use.[118] The applications of the click reaction have included a wide range of bioconjugations, such as labelling of peptides, proteins and DNA.[118]



Scheme 1.33: (A) Cu (I)-catalysed Huisgen 1, 3-dipolar azide-alkyne cycloaddition and (B) strain-induced Huisgen 1, 3-dipolar azide-alkyne cycloaddition.

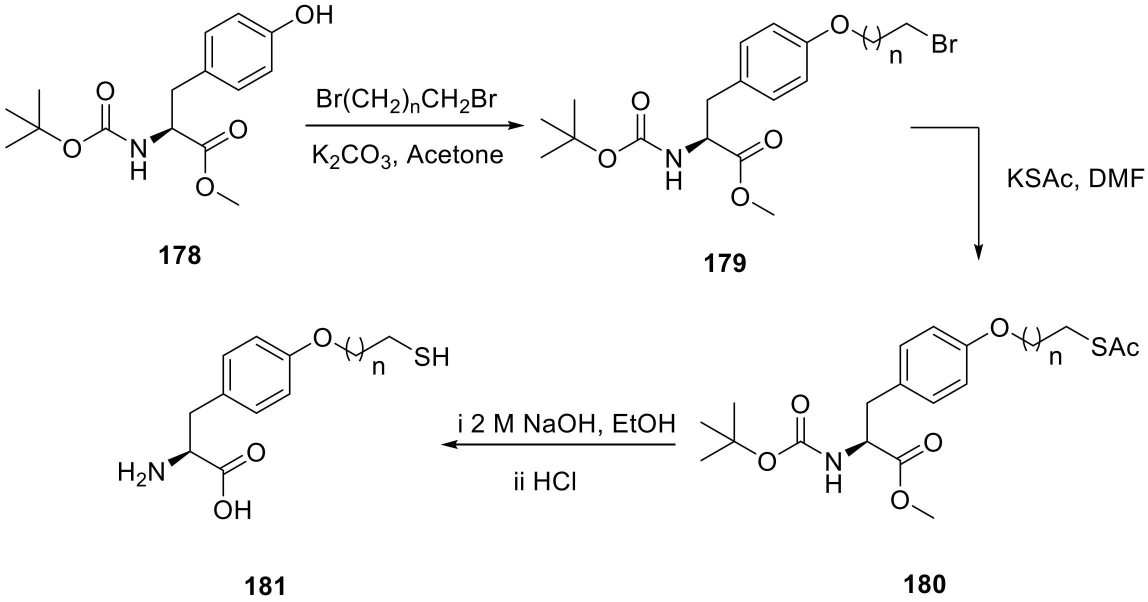
1.2.7.5 Ether linkage

The ether linkage has been used to produce organic peptidomimetic antagonists for inhibiting angiogenesis in inflamed and tumour tissues and metastases. The synthetic route for these compounds started with a protected tyrosine **171**, and then used a conjugation to attach an alkyl halide to the side chain, and ultimately gave guanidine **177**.[119] Scheme 1.34 shows one of the synthetic routes to small molecules that contribute to construction of a polypeptide.



Scheme 1.34: The synthesis of a non-natural amino acid through ether linkage.[119]

Recently, a non-canonical amino acid, possessing long side-chain thiols was produced through forming an ether linkage.[120] After that, a library of random β-lactamase mutants having these noncanonical amino acid was also constructed. Liu *et al.* reported that these noncanonical amino acids contribute to improved stability of the protein through the introduction of disulfide bridges. These bioconjugates have been synthesised by the reaction between a protected tyrosine amino acid with dibromo alkanes in the presence of potassium carbonate as outlined in Scheme 1.35. The resulting ether **179** was reacted with KSAc to produce compound **180**. Compound **181** was formed in two steps, the first step included hydrolysis of the thioacetate by adding 2 M NaOH followed by the second step of adding HCl to cleave off the Boc protecting group.



Scheme 1.35: The synthesis of a non-canonical amino acid through an ether linkage.[120]

1.2.8 The aim of this research

As described in the previous sections, arginine forms crucial interactions for the molecular recognition of nucleic acids by proteins. The formation of bicyclic guanidine provides a constraint on the orientation of the guanidine group which could then result in a smaller loss in conformational entropy on binding to its target. Furthermore, a rigid bicyclic framework on the guanidine group would produce only one mode hydrogen bond when it interacts with its targets. Thus, bicyclic guanidines may be superior building blocks to arginine for specific recognition of nucleic acids by small molecules. From the literature survey, it is observed that the synthesis of bicyclic guanidine has attracted growing attention due to their usage in many fields of chemistry. The present work considers the synthesis of differently sized rings of bicyclic guanidine, having alcohol or amine functional groups in the side chain and then conjugation of these functional groups with an amino acid moiety to produce modified amino acids possessing a bicyclic guanidine in the side chain. The next chapter deals with the design and synthesis of differently sized rings of bicyclic guanidine derivatives, the synthesis of modified amino acids and the formation of dimer-bicyclic guanidine.

Chapter Two

New Strategies to Synthesise Bicyclic Guanidine Derivatives and Functionalisation of Amino Acids

Results and Discussion

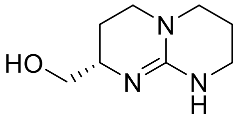
2.1 Introduction

Due to the specific properties of guanidinium on the side chain of arginine, it is considered an important component commonly used by proteins to recognise its nucleic acid target. These specific properties that involve exact patterns of hydrogen bonding and the high degree of basicity leads the guanidinium group to play an important role in the recognition between a protein/peptide and its nucleic acid target. The arginine amino acid side chain contains a three-carbon aliphatic straight chain linked to the guanidinium group, which gives much torsional freedom, a group that then interacts with its target molecules through dual H-bonding donation.

Converting the guanidine group into a rigid framework, bicyclic guanidine, would minimise the loss in conformational entropy on contacting its target and the hydrophobic hydrocarbon residues will certainly result in a well-defined structure. Thus, orienting the hydrogen bonding direction in only one mode with accurate positioning of the guest relative to the host structure, might produce the highest of degree of specificity when discriminating between a wide range of targets. However, the formation of a rigid bicyclic framework causes a decrease of rotational freedom about C-N bonds and total absence of geometric isomerisation of the C=N double bond. On the other hand, producing differently sized rings of bicyclic guanidine derivatives could contribute to the hydrophobic interaction with DNA as the hydrophobicity increases gradually with the increase of the size of the ring of bicyclic guanidine.

As it has been discussed in the introductory chapter, it was observed that the “N2-C=N1” amidine component for the different sized rings of bicyclic guanidine has the same distribution of carbon-nitrogen bond distances in all structures of non-functionalised bicyclic guanidine. On the other hand, it was noticed that the tertiary nitrogen N3 is responsible for attaining a different value for the degree of pyramidalization (DP%) which affects another parameter named Δ'CN. This parameter measures the contribution of N3 to the delocalisation of the “N2-C=N1” amidine. Looking back to the figure 1.12 in the introductory chapter, we can notice that the (DP%) of hppH (0.11) is less than that of H-tbu (4.20) so Δ'CN of hppH (0.038) is not too much bigger than that of H-tbu (0.034). Consequently, the geometry of the guanidine group of hppH is likely to be more planar than H-tbu and we can assume that our targets possess the same geometries. In addition, it is expected that the six membered ring of our targets do not have a chair conformation because the guanidine has a desirable planar shape.

However, it is important to realize that the high basicity of guanidine leads to production of the corresponding guanidinium cation upon protonation. Therefore, the formation of guanidinium cation leads to hydrogen bonding and electrostatic interactions with anions like phosphate groups. So, the purpose of preparing differently sized rings of bicyclic guanidine (Figure 2.1) in the current work is to compare their interactions with DNA.



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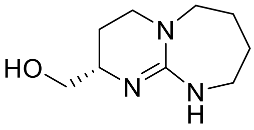


220

Figure 2.1: The structures of functionalised bicyclic guanidine [6,6] and [6,7].

In this chapter, there are four themes in our research concerned with design and synthesis of bicyclic guanidine derivatives and non-natural amino acids. The first theme discusses the synthesis of bicyclic guanidine methylene amine. As mentioned previously in the introductory chapter, functionalised bicyclic guanidine can be generated *via* the cyclisation of thiourea, as reported by Schmidtchen *et al.*[78] and Munster *et al.*[121] Our novel strategy for the synthesis of bicyclic guanidine included modification of this synthetic route. The goal is to synthesise a non-natural amino acid containing a bicyclic guanidine residue, so production of a bicyclic guanidine is needed with functional groups in the side chain to permit its introduction into the moiety of an amino acid. In our group, the protected bicyclic guanidine methylene iodide was synthesised by Steven Hill but encountered many problems incorporating the amino acid moiety.[122] It had a tosyl nitrogen protection (Figure 2.2), as an inexpensive model for Pbf. The tosyl group is not suitable for Fmoc solid phase synthesis though, as it cannot be removed using trifluoroacetic acid. One of the problems was that the alkyl iodide was unreactive towards nucleophiles which meant that attachment of the amino acid was not successful. Therefore, our concern was to find a new strategy to address this goal. The synthesis of bicyclic guanidines containing an amine group in the side chain was considered as one of the choices to investigate this goal. Then a reaction with Fmoc-glutamic acid or Fmoc-aspartic acid is necessary to prepare a non-natural amino acid through an amide linkage.

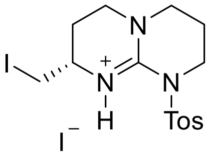


Figure 2.2: Protected bicyclic guanidine methylene iodide which has been prepared by Steven Hill.[122]

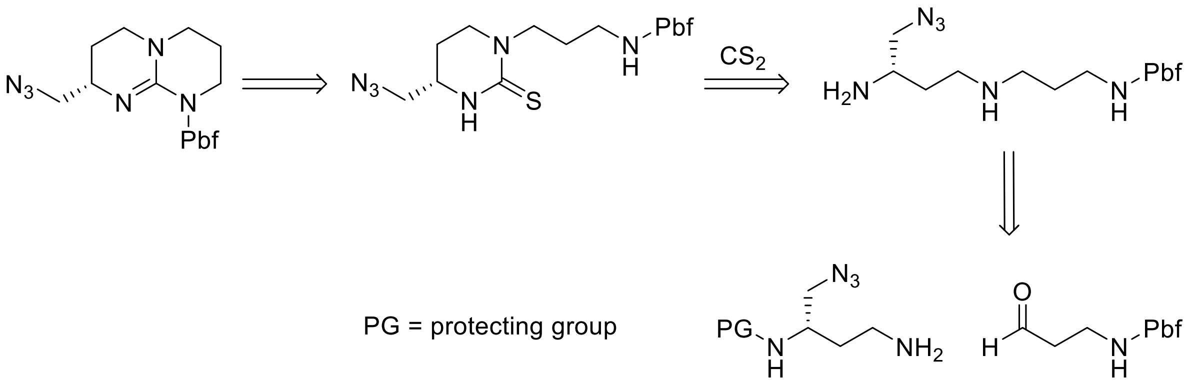
Producing bicyclic guanidines with differently sized rings is considered as the second goal of this project; 1, 3-Diaminopropane-Pbf **183** and 1, 4-diaminobutane-Pbf **187** have been synthesised; these then form the unsubstituted ring of their respective bicyclic guanidine. The second theme of our project is the novel strategy to generate a differently sized rings of bicyclic guanidine methylene alcohol (Scheme 2.14), in a high yield, starting from an azido alkane amine and Boc-L-methionine as precursors for the synthesis of these bicyclic guanidine derivatives. Our focus was concentrated on the thiourea intermediate **196** which was used to produce bicyclic guanidine. In the original procedure,[78] the conversion of the thiourea to the functionalised bicyclic guanidine was achieved in one pot and in low yield due to the formation of a monocyclic by-product. In our strategy, monocyclic isothiourea was first produced and then the bicyclic guanidine formation was subsequently triggered by the hydrogenation of an azide group in the monocyclic isothiourea. This modification of the route will open a promising way to produce the functionalised bicyclic guanidine. The third theme involved a new strategy toward the synthesis of bicyclic guanidine methylene amine followed by conjugation with Fmoc-Glu-OAll to generate a modified amino acid.

The fourth theme in our research includes the preparation of a dimer-bicyclic guanidine. Furthermore, the crystal structure of **233** was successfully determined from crystals produced by vapour diffusion during the routes to synthesise bicyclic guanidine derivatives.

2.2 Result and Discussion

2.2.1 Retrosynthetic analysis

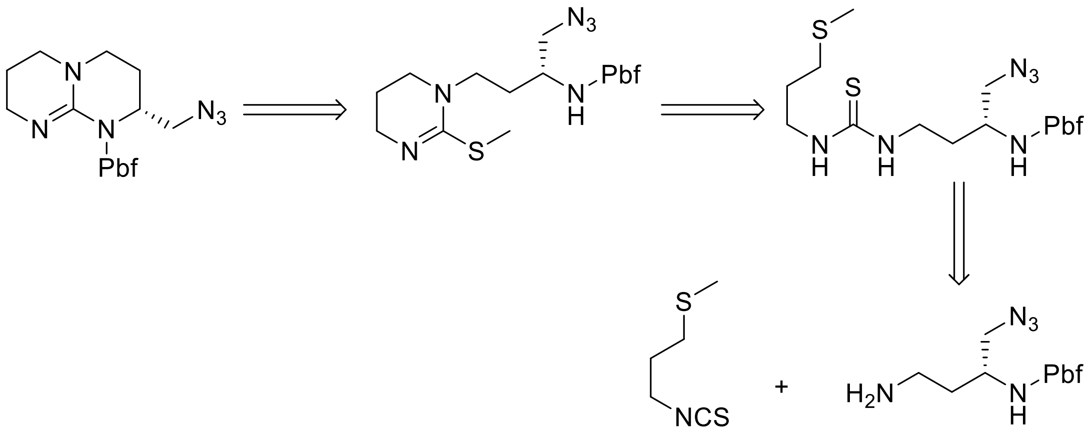
In this section, we approach the disconnection of functionalised bicyclic guanidine with Pbf protecting group for retrosynthetic analysis. We then propose three retrosynthetic routes towards functionalised bicyclic guanidine from simple starting materials. Retrosynthetic route A (Scheme 2.1) involved the disconnection of bicyclic guanidine to a substituted triamine intermediate that has been reported by Cotton and co-workers.[75] The key challenge in this approach is presented by the final step involving the cyclisation of thiourea especially considering that Cotton and co-workers have used a substituted triamine without a protecting group, which leads to decreased reactivity of the amine group and thus inhibition of the production of bicyclic guanidine. It was reported that the cyclisation of triamine by utilising CS_2 produced only monocyclised thiourea and the yield of the reaction was optimised by using a high boiling point solvent (triethylene glycol dimethyl ether) for three days.[123] However, the production of bicyclic guanidine from monocyclised isothiourea needs harsh conditions, thus, synthesising bicyclic guanidine-*N*-Pbf seemed implausible to achieve especially with the presence of a deactivating Pbf protecting group.



Scheme 2.1: Retrosynthetic route A for bicyclic guanidine derivative.

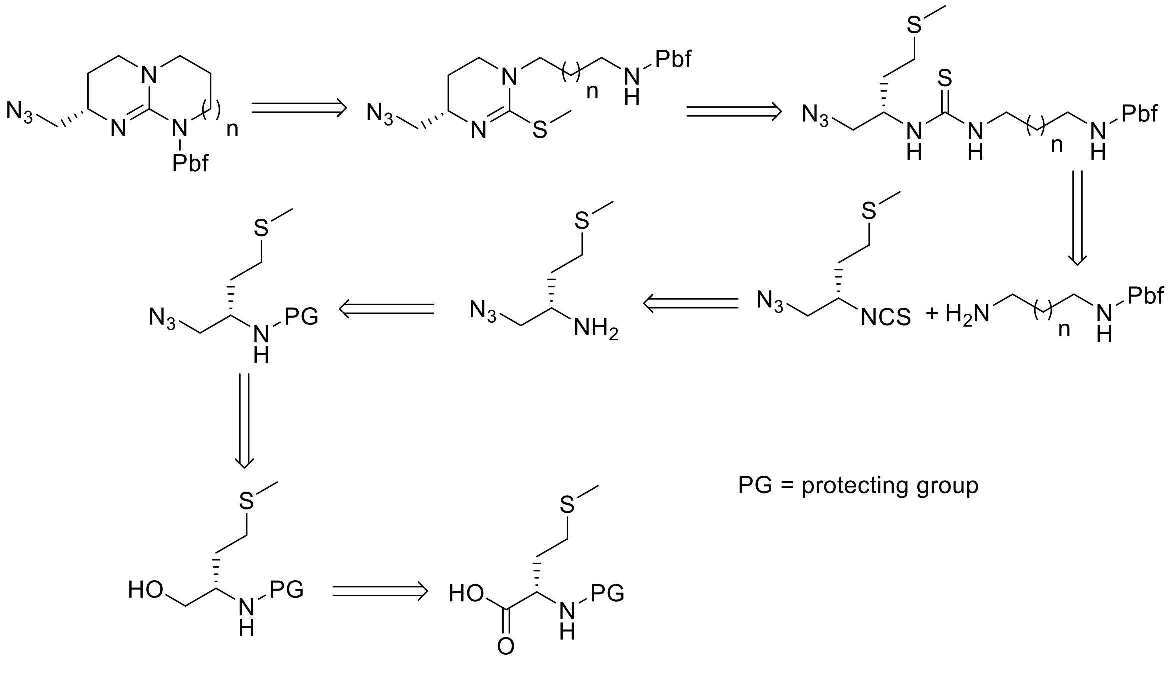
Retrosynthetic route B (Scheme 2.2), involved the disconnection of a bicyclic guanidine derivative that formed the monocyclised isothiourea as in Scheme 2.2. The key challenge of this approach is the cyclisation of the monocyclic intermediate. A second potential obstacle in this reaction is steric hindrance from the methylene azide group which might be expected to inhibit the production of bicyclic guanidine. Consequently, the existence of the azide and

Pbf group around the nucleophile lead us to avoid using this route to produce our target molecule.



Scheme 2.2: Retrosynthetic route B of bicyclic guanidine derivative.

Retrosynthetic route C in Scheme 2.3 included the disconnection of bicyclic guanidine methylene to a monocyclic isothiourea which could be obtained from a thiourea. The thiourea can be obtained from the coupling of an isothiocyanate and diamino alkane-Pbf where the isothiocyanate is derived from the corresponding azido methionine derivative. Azido methionine is accessible from protected-methioninol, which in turn can be obtained from the commercially available Boc-L-methionine which is a cheap source of the stereocentre.



PG = protecting group

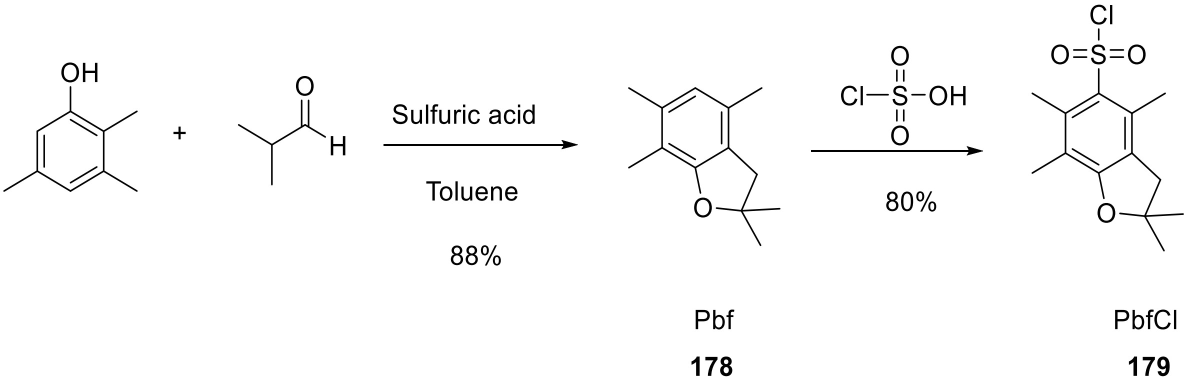
Scheme 2.3: Retrosynthetic route C of bicyclic guanidine derivative.

2.2.2 Novel strategy to synthesise functionalised bicyclic guanidine

It was noticed that the synthetic pathway proposed by Schmidtchen *et al.* was undesirably long to synthesise a bicyclic guanidine derivative, commencing from a starting material of Boc-L-methionine to reach the target.[78] Thus, we devised a novel strategy for the synthesis of the protected bicyclic guanidine methylene amine that had a shorter pathway than the previous synthetic route. Our new synthetic protocol depends on the conversion of the alcohol group of Boc-L-methioninol to a good leaving group by reaction with mesyl chloride in the presence of triethylamine. Then the resulting product was heated with sodium azide in dry dimethylformamide to generate the corresponding azide. Deprotection of the Boc group was achieved followed by the formation of isothiocyanate by the reaction with thiophosgene. Furthermore, diaminoalkane-Pbf is considered to be an important part of this synthetic route to generate of bicyclic guanidine rings of different sizes; so, if the alkane contains 3 carbon atoms, the unsubstituted ring of the bicyclic guanidine will be 6-membered while if it contains 4, it will be 7-membered. It is important to mention that the Pbf protecting group is the standard one that is used for Arg. It has the advantage that it is removed relatively rapidly with TFA. By introducing it at the start of the synthesis, it was hoped that this would reduce the need for protecting group manipulations later on. There was a precedent for the cyclisation reactions working, even with the sulfonamide protection (e.g. Steven Hill's work with tosyl).[122] The coupling of diaminoalkane-Pbf with the azido isothiocyanate resulted in the formation of an azido thiourea. The next step included the formation of bicyclic guanidine methylene azide *via* two steps. The first step was methylation at the sulfur atom and then the next step involved induced cyclisation. Finally, the hydrogenation of the azide group was used to obtain a bicyclic guanidine methylene amine **201**.

2.2.1.2 The synthesis of Pbf-Cl reagent

In synthetic organic chemistry, compounds need appropriate protection during reactions to achieve the clean formation of new compounds and prevent the formation of undesired by-products. Arginine is one of the compounds that needs protecting because of the propensity of the guanidine group to undergo side reactions due to its nucleophilicity. The main features of Pbf are its stability towards bases and catalytic hydrogenation and complete protection of guanidino side chain. In our synthetic protocol, the reagent PbfCl was used frequently and although it is commercially available, unfortunately, it is expensive and of variable quality. Therefore, it has been prepared as in Scheme 2.4 by using the cheap starting materials of isobutyraldehyde and 2, 3, 5-trimethyl phenol in the presence of sulfuric acid, as discussed in the literature.[124] Purification of this product has been achieved by Kugelrohr distillation to attain the white solid, Pbf, **178**.



Scheme 2.4: The synthesis of reagent PbfCl.

The chlorosulfonation of Pbf was performed by adding 2 equivalents of chlorosulfonic acid to the compound **178** at 0 °C.[125] It is important to mention that the duration of addition plays a significant role in obtaining a good yield as shown in the Table 2.1. After that, the mixture was poured into an iced solution of sodium bicarbonate to quench and then extracted with dichloromethane; thus, producing a silver solid PbfCl which has been used without further purification. This reagent was verified by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and IR spectroscopy, all of which showed consistency with the literature spectra.

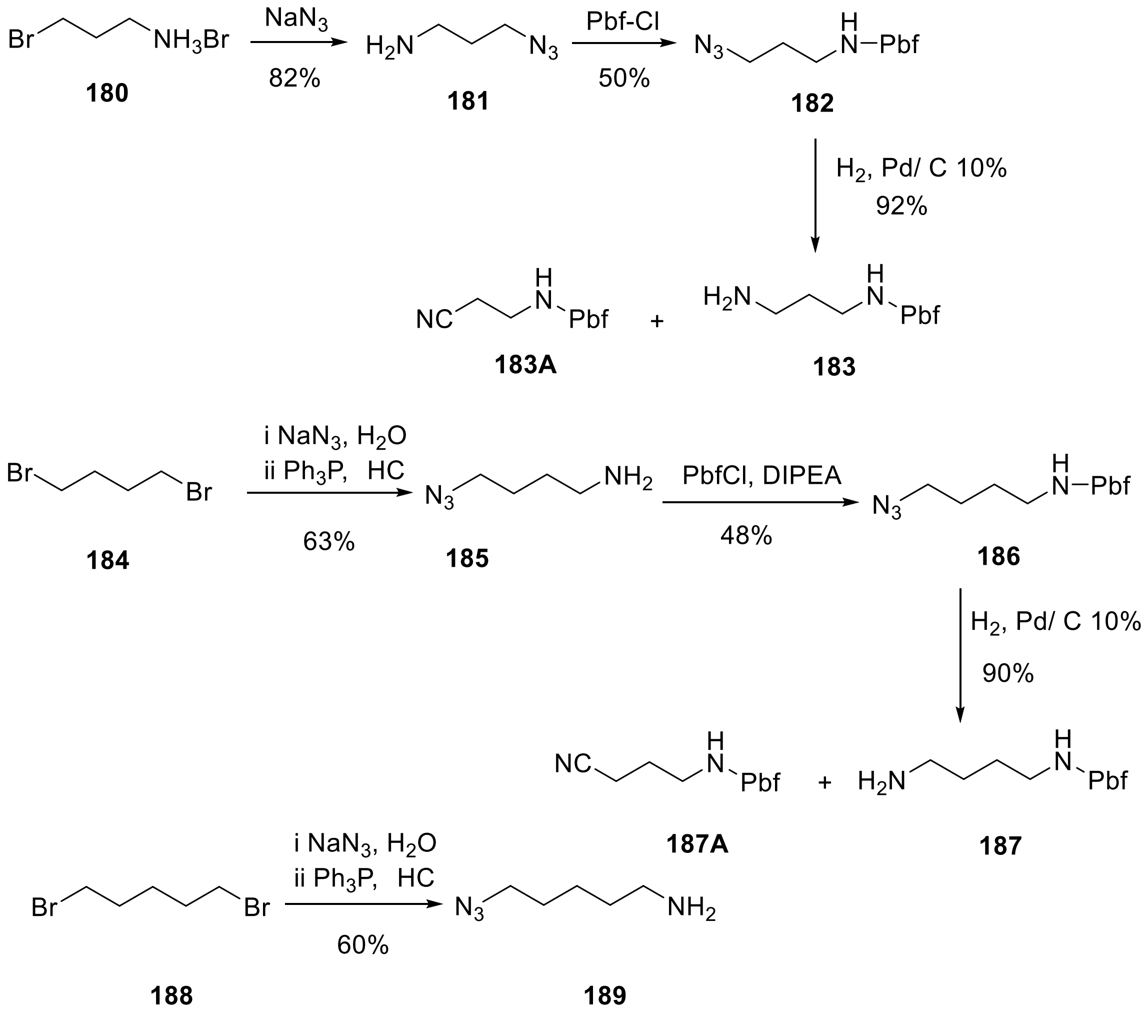
Entries	Duration of addition (min)	Yield (%)
1	30	50
2	60	65
3	120	70
4	150	75
5	180	80

Table 2.1: The conditions of PbfCl formation.

2.2.1.3 The synthesis of diamino alkane-Pbf

The production of diaminoalkane-Pbf with different alkyl chain lengths has been accomplished by using two different starting materials as shown in Scheme 2.5. The commercially available 3-bromo-1-amino-propane hydrobromide was reacted with sodium azide to produce 3-azidopropyl-1-amine *via* nucleophilic substitution. The resulting compound was protected with the Pbf protecting group. Compound **187** was synthesised by using 1,4-dibromobutane as a starting material, which was reacted with sodium azide to generate 1,4-diazidobutane. Selective hydrogenation was achieved by using a biphasic mixture of triphenyl phosphine in ether/ethyl acetate and aqueous HCl.[126] The initially formed azidoamine is protonated by the acid and partitions into the aqueous layer to prevent the over-reduction to diamine. After the organic layer is removed, the aqueous layer was washed with diethyl ether to remove the residual triphenylphosphine oxide and non-ionic organic reagents. A base was added to the aqueous layer followed by extraction with dichloromethane to give 1-azido-4-amino-butane **185** followed by adding PbfCl to generate compound **186**.

The hydrogenation of the azido compound **186** was achieved by using Pd on activated carbon in ethanol in a hydrogen atmosphere to obtain diamino alkane-Pbf **187**. Before embarking on alternative conditions, the yield was about 75%; then another attempt was used to improve the resulting yield by using methanol instead of ethanol and this gave a good yield of 92%.



Scheme 2.5: The synthesis of diaminoalkane-Pbf.

Another approach that was used to reduce azido alkane-Pbf was by using ammonium chloride and activated zinc in ethanol and water. After heating for 2 h, an ammonia solution was added. The advantages of this procedure are the short time (2 hours) taken to obtain the product and attaining a high yield 98%. A longer reaction time (12 hours) was required using the alternative approach (cat. hydrogenation) to complete consumption of starting material.

Interestingly, a crystal structure was obtained of Pbf-protected-3-aminopropanenitrile (**183A**) as a by-product contained within the crude protected-1,3-diamino-propane, **183**. Similarly, a crystal structure of protected-4-aminobutanenitrile (**187A**) was obtained from the crude sample of protected 1,4-diamino-butane, **187**. Palladium catalysed decomposition of azides to nitriles has been reported in the literature[127] and it appears that this reaction has competed with the desired hydrogenation. The samples were crystallised from EtOH/H_2O solution by vapour diffusion, and the polar solvent mixture has favoured crystallisation of the minor nitrile impurity in preference to the more polar amine. Compound **183A** and compound **187A** crystallised in the orthorhombic system space group $P2_12_12_1$. Full crystallographic data are included in Appendix 6.1 and 6.2.

It is preferable to discuss the propyl compound (Figure 2.3 A), as the R factor is lower and there is less disorder at the terminus of the alkyl chain. The structure is consistent with a nitrile and not the amine due to the linearity of atoms (CCN angle 179.2°) and short C-N distance (1.16 Å). The ^{1}H NMR spectrum recorded for **183A** using $CDCl_3$ as a solvent showed the appearance of a triplet peak at 3.10 ppm that corresponds to the terminal CH_2CN protons of **183A**. A comparison of the integration of this triplet with the integration of a singlet peak belonging to a methyl group showed the presence of 7% of the nitrile compound **183A** in the sample of crude amine sample.

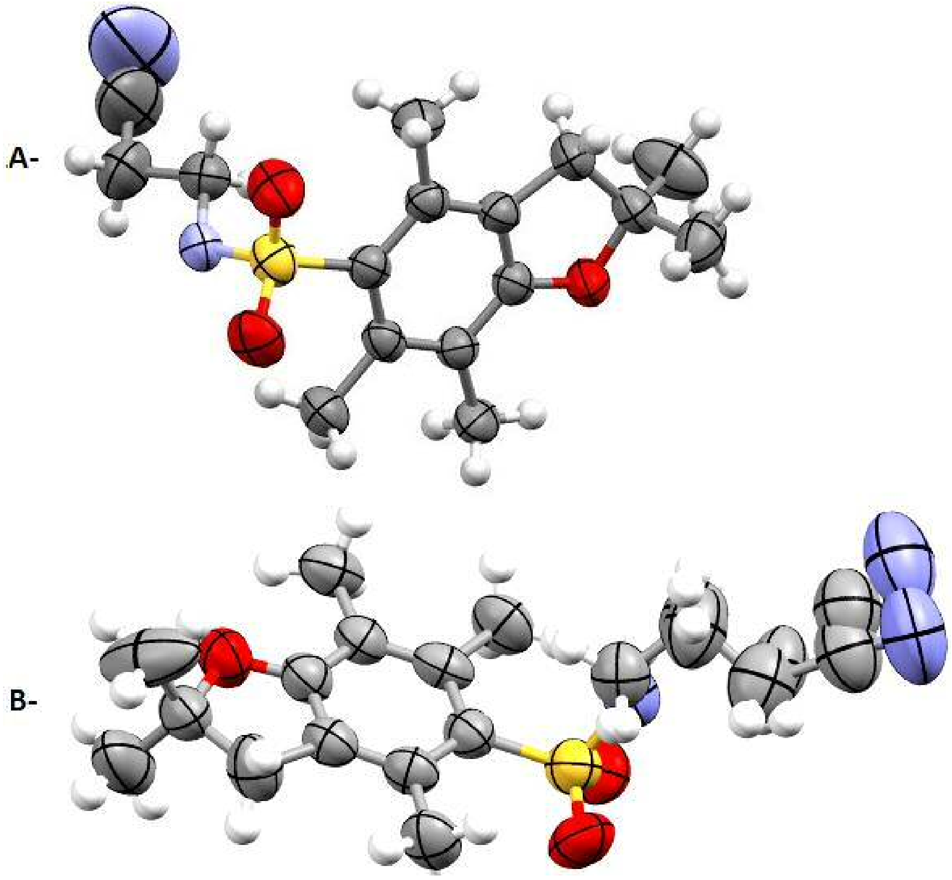
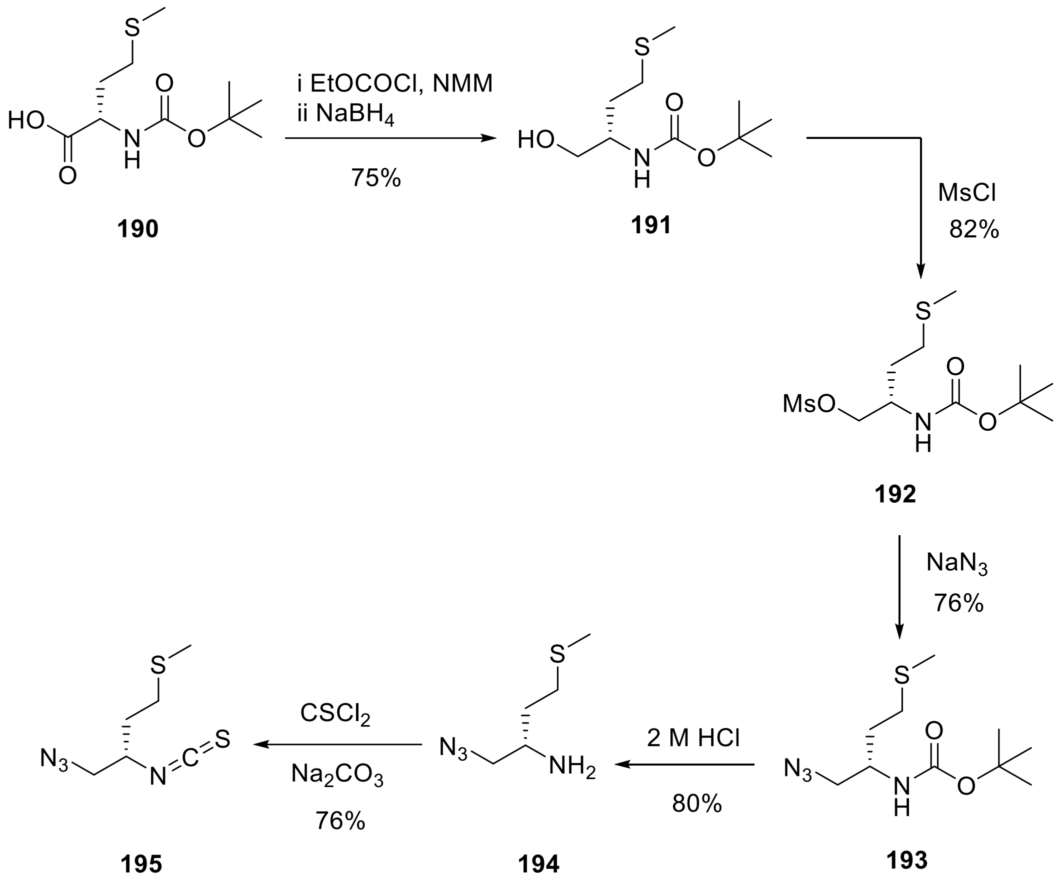


Figure 2.3: Thermal ellipsoid plot of A -**183A** and B -**187A** showing ellipsoids at the 50% probability level. **187A** is modelled as two conformations with occupancies of 0.61 and 0.39.

2.2.1.4 The synthesis of azido isothiocyanate

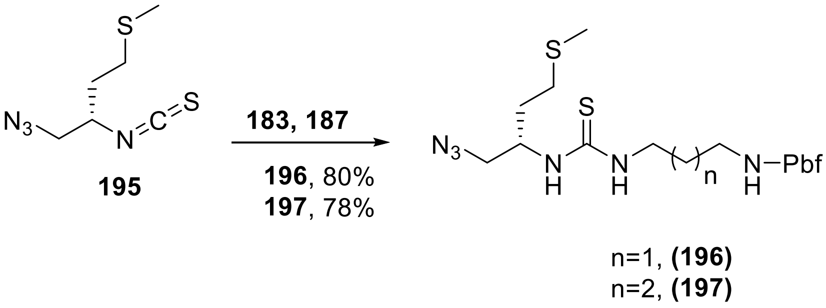
The synthetic route to prepare azido isothiocyanate **195** as shown (Scheme 2.6) started with the precursor Boc-L-methionine which was reduced to Boc-L-methioninol **191** by using two steps. The first step involved activation of the Boc-L-methionine by stirring it with ethyl chloroformate and *N*-methyl morpholine to form a mixed anhydride. The second step being reduction of this anhydride to the Boc-L-methionine by utilising the reducing agent sodium borohydride. The Boc-L-methioninol was collected in a yield of 75% and needed no further purification. Converting the alcohol group of the Boc-L-methioninol to a good leaving group was achieved by the reaction with mesyl chloride in triethylamine. To run this reaction successfully, anhydrous conditions were required under a nitrogen atmosphere. Azido methionine was synthesised *via* nucleophilic substitution by the heating of mesylated methioninol with sodium azide. The ^{1}H and ^{13}C NMR spectra of **193** recorded were in very good agreement with the literature spectra.[128] Deprotection of the Boc protecting group for **193** was then carried out *via* acidic conditions, by adding 2 M HCl. The ^{1}H and ^{13}C NMR spectra of **194** showed that peaks at 1.38 and 28.5 ppm for ^{1}H and ^{13}C NMR respectively, belonging to the Boc protecting group, were absent from the spectrum. In addition, the IR spectrum displayed a characteristic stretch for the azide group at 2096 cm^{-1}. Finally, the production of azido-isothiocyanate **195** proceeded by the reaction between compound **194** and thiophosgene in the presence of sodium carbonate in a relatively high yield. The ^{13}C NMR spectrum of this product displayed a resonance at 135 ppm, indicating the existence of the N=C=S functionality in this structure.



Scheme 2.6: The synthetic route to azido isothiocyanate **195**.

2.2.1.5 Thiourea azide formation

The formation of thiourea azide was achieved by the coupling of equimolar amounts of isothiocyanate azide and di-aminoalkyl-Pbf as in Scheme 2.7 below. The azido thiourea was generated in a good yield as a yellow foam.

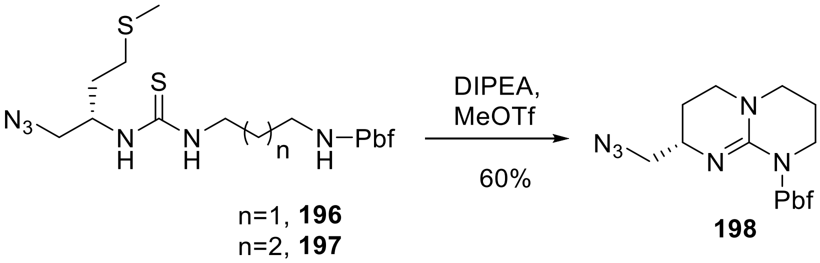


Scheme 2.7: The formation of azido thiourea **196** and **197**.

With regards to the conformation of the substituted thiourea, earlier studies reported that *N*, *N'*-disubstituted thioureas could be found in solution in different rotameric forms.[129] ^{1}H NMR spectra recorded for compound **196** showed splitting of some peaks which indicated the presence of a rotational isomer around CS. It is assumed that these rotamers generate the complexity in the NMR spectra. ^{1}H NMR spectra were recorded in the range from 30 °C to 50 °C in $CDCl_3$ and when the temperature increases, the signal became sharpened and more defined. The ^{13}C NMR spectra showed the peaks of CS at 182.1 and 181.4 ppm for azido thiourea **196** and **197** respectively; and also, the IR spectra recorded a characteristic azido stretch at 2096 cm^{-1} for **196** and 2093 cm^{-1} for **197**.

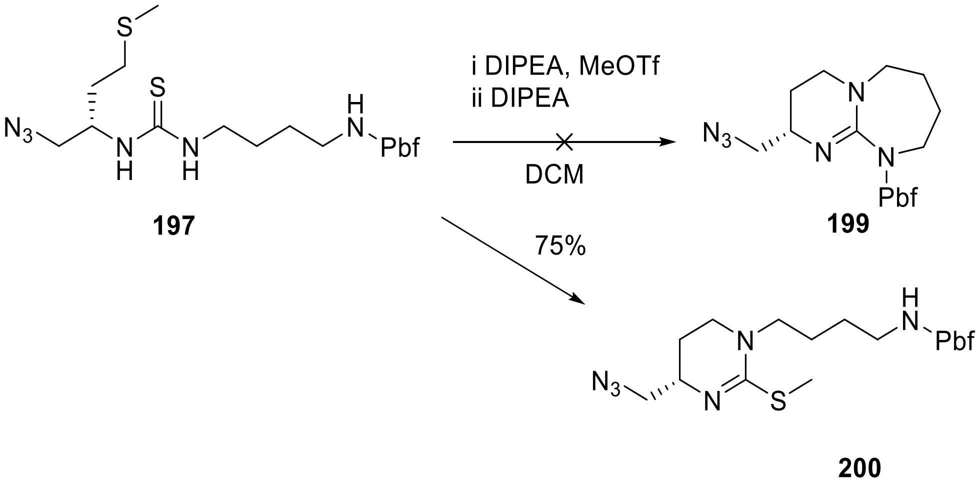
2.2.1.6 Bicyclic guanidine methylene azide

This thiourea compound has been utilised as a precursor for the generation of a bicyclic guanidine derivative through a one pot, two steps reaction as in Scheme 2.8. The first step included the methylation at the sulfur atom by adding methyl triflate at 0 °C and then cyclisation was induced by adding an excess of diisopropylethylamine to produce compound **198**. Unfortunately, the yield after purification was only a modest 60%.



Scheme 2.8: The synthesis of bicyclic guanidine methylene azide **198**.

The resulting product was characterized by ^{1}H NMR spectroscopy and the methyl sulfur peak at 2.06 ppm in compound **196** was noted as disappearing in compound **198**. The ^{13}C NMR spectrum showed a disappearing (C=S) peak at 181.4 ppm and the mass spectrometry result was consistent with the compound **198**. In an effort to produce bicyclic guanidines with different sized rings, the same procedure was used as in the previous product. Commencing from the compound **197**, cyclisation to produce compound **199** was attempted according to Scheme 2.9 below:



Scheme 2.9: Attempt at preparation of a bicyclic guanidine containing a 7-membered ring.

The cyclization of compound **197** using MeOTf and DIPEA yielded the monocyclized isothiourea **200** instead of the doubly cyclised product **199** (Scheme 2.9). Unfortunately, all attempts to achieve the production of this compound have not succeeded and stopped at the monocyclic stage. A high boiling point solvent (triethylene glycol dimethyl ether) was used for three days for this reaction to induce cyclisation of the second ring, but the process failed. As mentioned, the distance between the protected amine group and the thiocarbonyl group became bigger relative to compound **196** and resulted in generating only a monocyclic product.

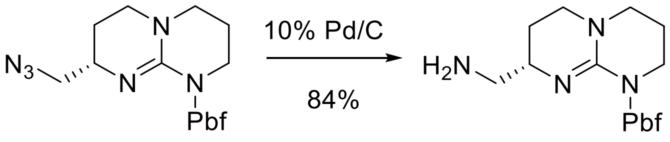
The low reactivity of the sulfonamide nitrogen represents another challenge in the ring closure reaction. The mass spectrum and ^{1}H NMR spectrum for the resulting product showed that only a monocyclic product had been produced. However, the double cyclised product, bicyclic guanidine containing 7- membered rings was not attained under any conditions. Table 2.2 shows the various conditions used in an attempt to synthesise **199**.

Entries	Reagent	Time, hours	Temperature, °C	Solvent	% of monocyclic
1	DIPEA, MeOTf	24	45	DCM	68
2	DIPEA, MeOTf	24	63	$CHCl_3$	70
3	DIPEA, MeOTf	48	45	DCM	72
4	DIPEA, MeOTf	48	216	Triglyme	73
5	DIPEA, MeOTf	72	216	Triglyme	75

Table 2.2: The conditions used to synthesise compound **199**.

2.2.1.7 The formation of bicyclic guanidine methylene amine

Hydrogenation of the compound **198** into the amine **201** was accomplished by using the catalyst Pd/C 10% in methanol under a hydrogen atmosphere to afford the amine in an 84% yield as in Scheme 2.10 below.

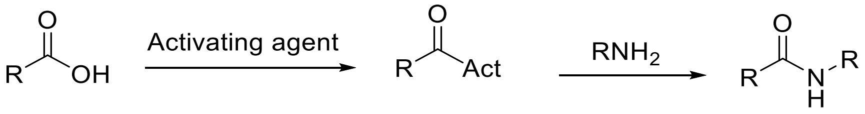


198 **201**

Scheme 2.10: The synthesis of bicyclic guanidine methylene amine.

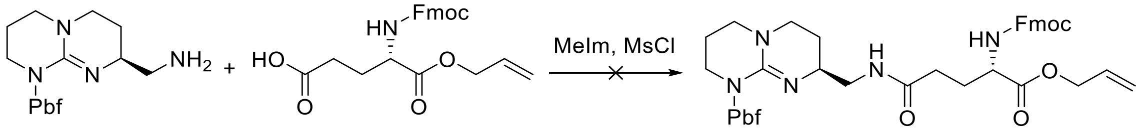
2.2.1.8 Attempts to synthesise a non-natural amino acid

Starting from a carboxylic acid, a common method to form an amide bond is by first reacting the acid with an activating agent. The resulting activated acid derivative is then reactive towards a nucleophilic attack (Scheme 2.11). This part is then more prone to nucleophilic attack by an amino group from another molecule to construct the desired amide.



Scheme 2.11: Activation of a carboxylic acid to form an amide.

Several activating agents were employed in attempts to synthesise a non-natural amino acid with a protected bicyclic guanidine side chain. Our design used a residue of Fmoc-Glu-OAll or Fmoc-Asp-OAll as an amino acid moiety to connect with the amine group in the side chain of our small molecule bicyclic guanidine. The first attempt included a modification of a procedure to synthesise aryl amides reported in the literature.[130] Methanesulfonyl chloride and *N*-methylimidazole in dichloromethane was used to activate the carboxylic acid in the Fmoc-Glu-OAll and then compound **201** was added to the resulting solution as in Scheme 2.12 below. The mixture was stirred for two hours, but unfortunately, the TLC indicated that there was no reaction over a 24-hour time period. It was suggested that the *R*f of the product might be very close to the *Rf* of the starting material so ^{1}H NMR and mass spectra were recorded and again showed that no product had been formed.

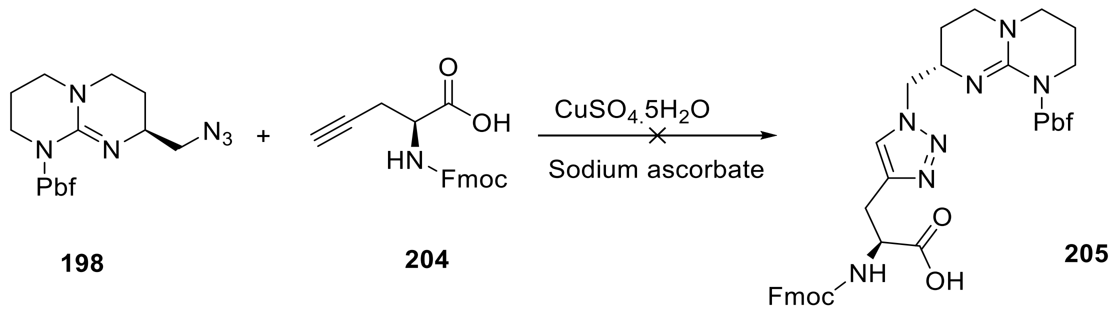


201 **202** **203**

Scheme 2.12: First attempt to synthesise a non-natural amino acid.

The second attempt was to use *N, N'*-dicyclohexylcarbodiimide as a reagent to activate Fmoc-Glu-OAll as an HOBt ester. Unfortunately, the desired non-natural amino acid was not obtained. The ^{1}H NMR spectrum showed the starting material's peaks and mass spectra emphasised that no product had been formed. A common procedure that is utilised to generate an amide bond in solid phase peptide synthesis by using HOBt and PyBOP in DMF was employed in our work, but again the result was not successful. In my opinion, there may be a strong intra-molecular hydrogen bonding between the sulfone in the Pbf protecting group and the amine group which may prevent the formation of an amide bond.

After this negative result, another approach was suggested as an alternate route to the non-natural amino acid, namely the "click reaction" of copper(I) catalysed azide-alkyne 1,3-dipolar cycloaddition. Compound **198** was mixed with sodium ascorbate as a reducing agent in the presence of $CuSO_4{\cdot}5H_2O$ then compound **204** was added,[131] as shown in Scheme 2.13 below.

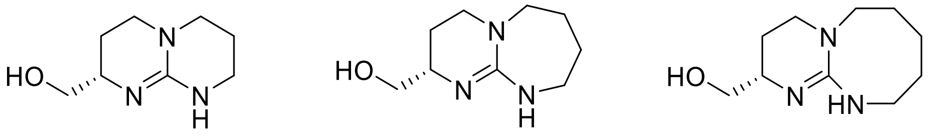


Scheme 2.13: Attempt to synthesise a non-natural amino acid by click reaction.

Unfortunately, the desired non-natural amino acid was not generated. The ^1H NMR spectrum of the attained material recorded that no product had been produced. The mass spectra showed that the isolated material is in fact only the starting material. Although the literature has reported that molecules having an arginine amino acid and alkyne could be conjugated by a click reaction to other molecules containing azide groups, this was not the case here surprisingly. In my opinion, the copper has probably chelated with the guanidine group diminishing its catalytic activity.

2.2.2 A novel synthetic protocol for functionalising the bicyclic guanidine

In this part, we will introduce a novel strategy to generate a functionalised bicyclic guanidine. This novel strategy is inspired by the synthetic route that was explained in section 2.2.1 and in the Schmidtchen *et al.* strategy to produce bicyclic guanidine derivatives.[78] This strategy was developed in an attempt to overcome the lack of reactivity of the protected amine group that was the main factor in producing a monocyclic isothiourea **200** as opposed to the desired product of a bicyclic guanidine derivative. There are two key challenges which have been discussed in the formation of bicyclic guanidine methylene amine; firstly, the monocyclic by-product that was formed during the cyclisation of compounds **197**. The second key challenge is the conversion of the alcohol group to the azide group in the first step, which was the modification of the Schmidtchen route in the previous chapter. The modification avoids the protection and deprotection steps and then conjugates the resulting bicyclic guanidine to the amino acid residue. In other words, the azide functional group behaves as a masked amine and thus avoids the use of a protecting group that would otherwise be necessary. The other potential advantage is the formation of bicyclic guanidine in higher yield due to avoidance of the reaction stopping at the monocyclic stage. Therefore, our novel strategy uses the precursors 1-amino-3-azido-propane **181**, 1-amino-4-azido-butane **185** and 1-amino-5-azido-pentane **189**. Three target compounds were initially suggested namely differently sized rings of bicyclic guanidine methylene alcohol including a six, seven and eight membered rings system as outlined in Figure 2.4.



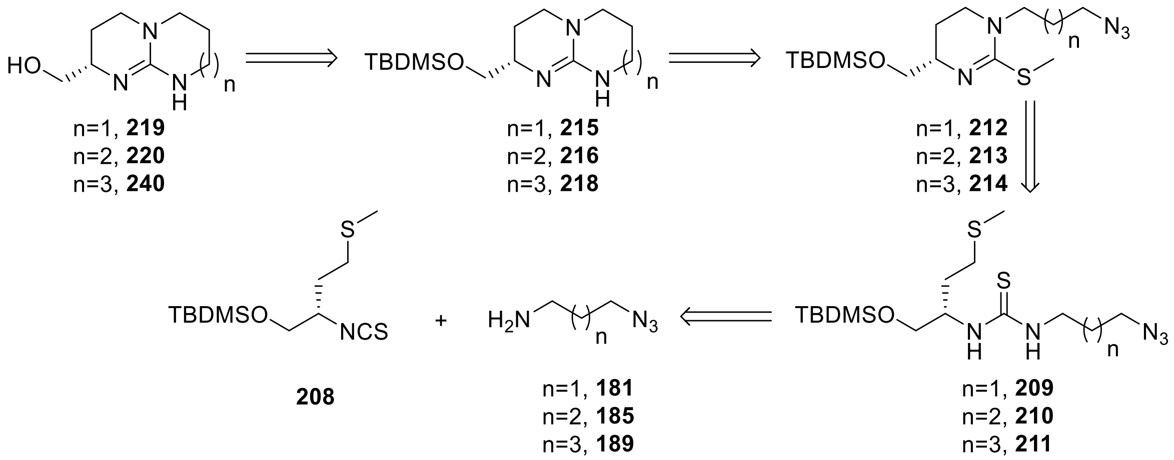
219 **220** **240**

Figure 2.4: Initial targets.

The next targets are the non-natural amino acids possessing these groups in their side chains.

2.2.2.1 Retrosynthetic analysis of bicyclic guanidine derivatives

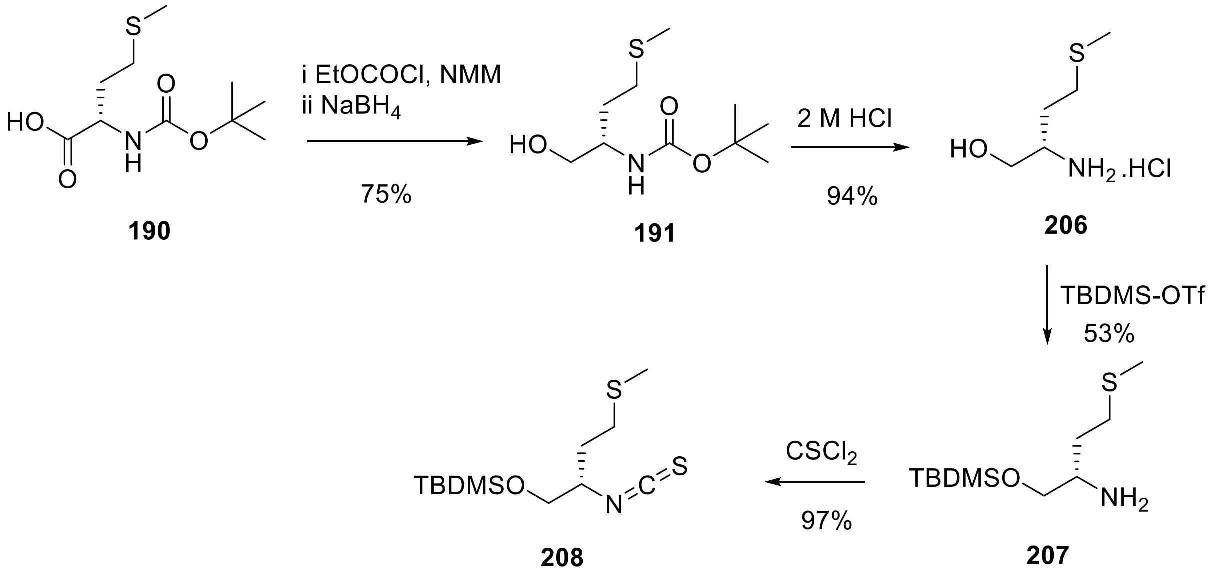
This section discusses a novel route to compound **219**. A retrosynthetic analysis of this compound is given in Scheme 2.14 below. Retrosynthetically, compound **219** can be generated from an isothiourea that could be also derived from a thiourea. The thiourea could be obtained from the coupling between isothiocyanate and compounds **181** or **185** or **189.**



Scheme 2.14: A retrosynthetic route for differently sized rings of bicyclic guanidine methylene alcohol.

2.2.2.2 The formation of silylated isothiocyanate

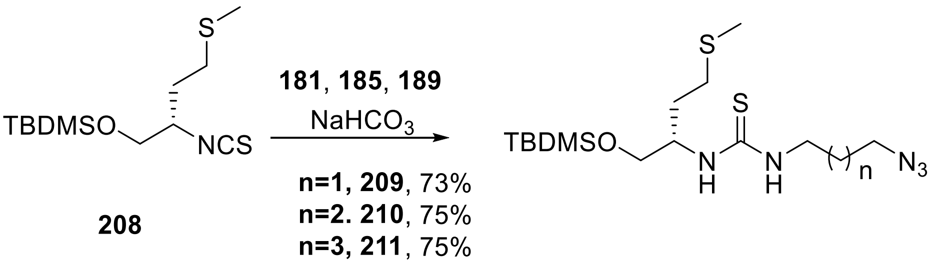
The production of silylated isothiocyanate **208** started from Boc-L-methionine as a chiral precursor. The first step included the preparation of compound **191** as described in Scheme 2.15. The next step was the de-protection of the Boc group *via* mild acidic conditions to attain the salt of the methioninol as a waxy solid in a 94% yield. The silyl protecting group was installed on the hydroxyl function of the methioninol by adding the reagent TBDMS-OTf in the presence of imidazole. It is clear that α-L-methioninol contains two functional groups and the silylation of the amino group would be much faster than at the desired alcohol, due to a quick silylation at the more nucleophilic amino residue and then sluggish migration to the alcohol group. It is expected that the pK_a of protonated methioninol is about 9-10, therefore, using a weak base such as imidazole (imidazolium pK_a = 5.5), would support this reaction by preventing any nucleophilic attack from the amino group. Compound **206** reacted with the TBDMS-OTf in the presence of imidazole in DCM at 0 °C. This reaction was accomplished easily and without issue. The purification of the crude product followed the procedure that was described by Jadhav *et al.*[80] which included utilising a complex extraction protocol to remove any by-products and thus resulting in a pure product. This work up involved washing the crude product with distilled water three times to remove the excess of imidazole and then dissolving it in a solution containing H_2O/ CH_3CN/ AcOH 60/40/2.5 mL This was followed by the important step of removing the remaining silanol by-product by washing with hexane. Taking into account that the silyl protecting group could be removed by a strong base, the solution was basified by adding the weak base sodium bicarbonate, and then it was extracted with DCM. Compound **207** was used in the following reaction without further purification. Due to the high yield that was obtained by utilising the original procedure to prepare the azido isothiocyanate **195**,[129] the same procedure was used again to generate silylated isothiocyanate **208** as in Scheme 2.16. Compound **207** was dissolved in DCM and the inorganic weak base $NaHCO_3$ was added following by the addition of thiophosgene.



Scheme 2.15: The synthetic route to compound **208**.

2.2.2.3 The formation of silylated thiourea

The synthesis involved the coupling of one equivalent of compound **208** with one equivalent of compound **181** in dry acetonitrile and in the presence of sodium bicarbonate. Once complete consumption of the starting material was noticed, the reaction was purified to obtain the compound **209** in a 73% yield as outlined in Scheme 2.16. The same chemistry was utilised to obtain compounds **210** and **211**.

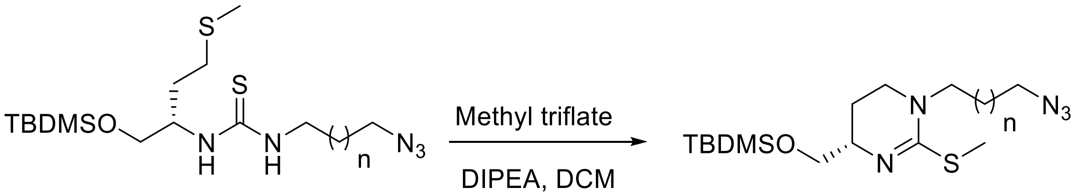


Scheme 2.16: The formation of silylated thiourea derivatives.

^{1}H NMR spectrum recorded for compounds **209, 210, 211** showed splitting of some peaks due to the presence of a rotational isomer around CS which was proved experimentally in compound **196**. The ^{1}H NMR spectra of silylated thioureas in $CDCl_3$ at room temperature are consistent with a static structure with two singlet methyl groups belonging to the silyl protecting group in different chemical environments, given the six hydrogen integrals at 0.01 ppm and nine hydrogen integrals at 0.88 ppm. The IR spectra of silylated thiourea was characterised by absorption at 2088 cm^{-1} belonging to the azide group; finally, ^{13}C NMR spectra have recorded a chemical shift at 180 ppm for C=S.

2.2.2.4 The production of monocyclic isothiourea

It was suggested that the bicyclic guanidine methylene azide **198** was installed *via* isolating the monocyclic intermediate, and subsequently the second cyclisation induced to generate the bicyclic guanidine. Therefore, our novel strategy included the synthesis of the monocyclic compound by utilising the same chemistry that was reported to prepare compound **198** through the methylation at the sulfur atom by using methyl triflate and then cyclisation was induced by adding an excess of DIPEA. Finally, these products were achieved in acceptable yields as in Scheme 2.17 below.



n=1, 209
n=2. 210
n=3, 211

n=1, **212,** 68%
n=2, **213**, 65%
n=3, **214**, 66%

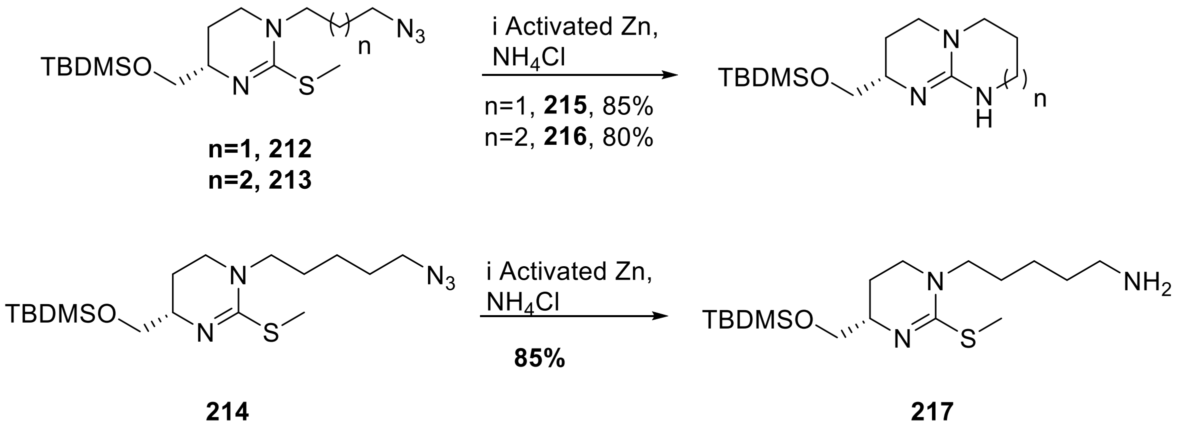
Scheme 2.17: The formation of monocyclic isothiourea.

In this route, three monocyclic isothiourea compounds have been synthesised depending on the amino alkyl azide used.

2.2.2.5 The formation of silylated bicyclic guanidine

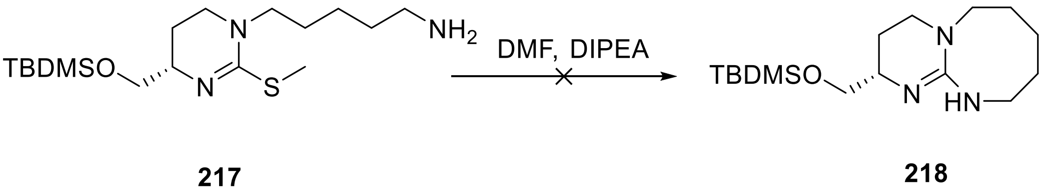
Our aim in this project is to synthesise a non-natural amino acid possessing a bicyclic guanidine in the side chain; thus, the central consideration included the formation of functionalised bicyclic guanidine in an easy and trouble-free procedure. Schmidtchen *et al.*

have synthesised functionalised bicyclic guanidine with two protecting groups: silyl and tosyl groups, which then need to be removed. Experimentally, there are many necessary steps to achieve functionalised bicyclic guanidine; the polarity of this compound is high and thus it is thought that there might be some problems in purification. In addition, deprotection of two protecting groups after many steps to reach the target would decrease the quantity of the final compound that is available to make a non-natural amino acid. Synthetically, utilising Pd on activated carbon under a hydrogen atmosphere is not the right choice to reduce the azide group in the monocyclic isothiourea due to the poisoning of palladium by the sulfur atom. The hydrogenation of the azide group of monocyclic thiourea was therefore accomplished by using activated zinc and ammonium chloride in an aqueous medium as in Scheme 2.18 below.[132] As monitored by TLC, heating the mixture under reflux for two hours was enough to complete the consumption of the starting material and then an ammonia solution was added to the resulting mixture. Modification in the workup was made, specifically by heating the mixture with ammonia solution for one hour instead of the 10 min described in the original procedure[132] to complete the cyclisation reaction and produce bicyclic guanidine. The original procedure included washing the crude product with water to remove the excess of ammonium chloride, however our bicyclic guanidine is slightly soluble in water, so the aqueous solvents were evaporated and then the product re-dissolved in methanol. Subsequently, the solid was filtered and the solvent was evaporated again to get the desired compound. Interestingly, it was thought that the hydrogenation of the azide group would produce the corresponding amine, but the silylated bicyclic guanidine was produced instead due to the reactivity of amino group formed by the hydrogenation of azide and the ammonia solution used in the work up. The major product of the reaction showed the expected mass for the guanidine product in the mass spectrum. The FTIR spectrum recorded showed the absence of an azide peak. The ^{13}C NMR spectrum however displayed the correct C=N resonance at 153.3 ppm.



Scheme 2.18: The formation of differently sized rings of bicyclic guanidine.

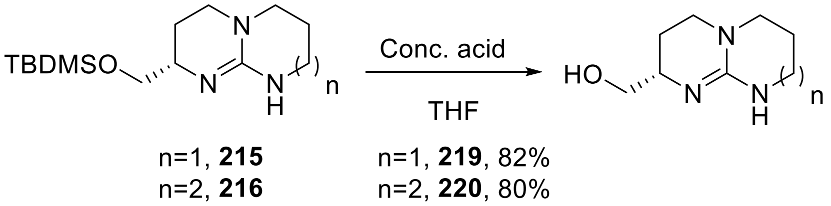
On the other hand, bicyclic guanidine having an eight membered ring **218** was not produced. ^{1}H, ^{13}C NMR and mass spectrum showed that the product is instead the compound **217**. This was confirmed by the FTIR spectrum showing the existence of primary amine. Many attempts have been made to produce bicyclic guanidine possessing an eight membered ring, including an attempt where the corresponding amine was dissolved in DMF and heated under reflux for one day in the presence of a weak base DIPEA as in Scheme 2.19, but unfortunately again no product was formed. Another attempt was done by using a strong base, sodium hydride, to induce cyclisation. However, all attempts did not succeed in obtaining the desired compound.



Scheme 2.19: Attempts to synthesise compound **218**.

2.2.2.6 Deprotection of silyl group

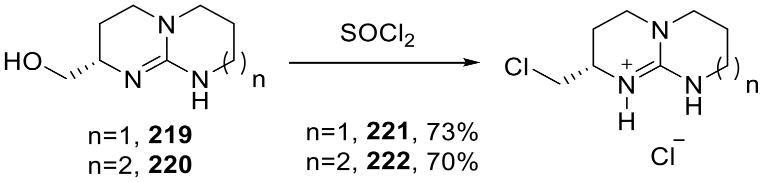
Removal of the silyl protecting group was accomplished by adding 12 M HCl (aq) in tetrahydrofuran as in Scheme 2.20 below. After the completion of the reaction, the solvent was evaporated (co-evaporated with ethanol). However, compounds **219** and **220** are soluble in water so the residue was washed with hexane and then DCM to obtain the pure product.



Scheme 2.20: The formation of bicyclic guanidine methylene alcohol.

2.2.2.7 The synthesis of bicyclic guanidine methylene chloride

Conversion of **219** and **220** to **221** and **222** respectively was carried out *via* a substitution reaction by adding thionyl chloride as in Scheme 2.21. At the end of this reaction, the resulting mixture was placed under a vacuum and the excess thionyl chloride was evaporated under reduced pressure.

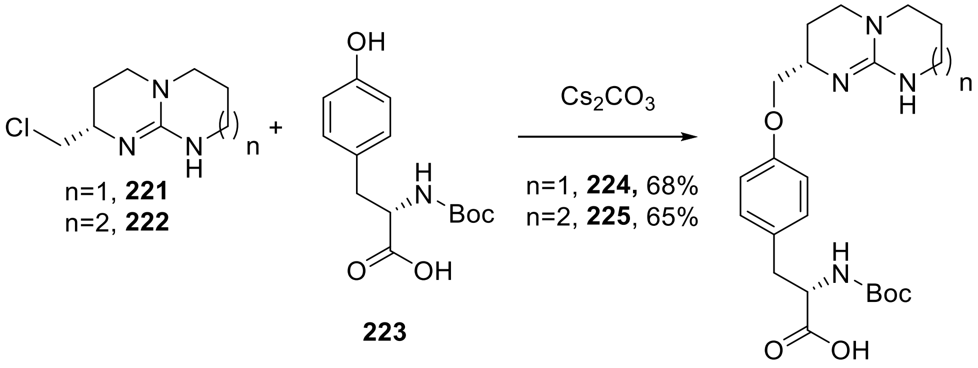


Scheme 2.21: Preparation of bicyclic guanidine methylene chloride.

The production of **221** and **222** is considered as the key point in our synthetic protocol towards synthesising a non-natural amino acid containing differently sized rings of bicyclic guanidine in the side chain. Thus, these products have been purified through flash column chromatography. High-resolution mass spectra for these compounds were identical with the calculated mass. ^{1}H and ^{13}C NMR spectra ^{1}H and ^{13}C NMR spectra and also FTIR were consistent with the structure of these compounds.

2.2.2.8 The formation of a non-natural amino acid

After completing the synthesis of compounds **221** and **222**, the plan was to find a suitable amino acid residue that could connect to our molecule. Therefore, it was proposed that Boc-L-tyrosine could react with **221** in the presence of caesium carbonate to produce a non-natural amino acid through nucleophilic substitution *via* Williamson ether synthesis. Although Boc-L-tyrosine is commercially available, it was prepared *via* a one pot process by the reaction between L-tyrosine and Boc-anhydride in the presence of triethylamine.[133-134] A non-natural amino acid was then prepared in a yield of 68% with compound **224** and 65% with compound **225** as in Scheme 2.22.[135]

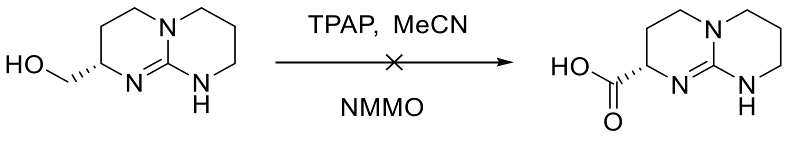


Scheme 2.22: The synthesis of a non-natural amino acid.

At the beginning of this reaction, deprotonation of the phenol group in Boc-L-tyrosine was achieved by adding caesium carbonate. Then, the phenoxide group was reacted with bicyclic compound **221** through an S_N2 substitution reaction.[135] The resultant high-resolution mass spectrum was identical with the calculated mass and also the peak of the carboxylic acid could be easily recognised at 12 ppm in the ^1H NMR spectra. ^{13}C NMR spectra recorded the peak of the carboxylic acid at 180 ppm.

2.2.2.9 Attempts to synthesise bicyclic guanidine carboxylic acid

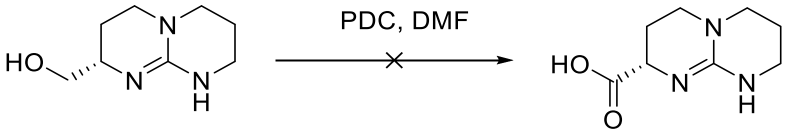
When we started this project, our attention concentrated on the design and synthesis of a non-natural amino acid with a bicyclic guanidine side chain. Producing compound **219** encouraged us to oxidise it to the corresponding carboxylic acid; then, the resulting product could react with the side chain of the amino acid lysine. Two different oxidation reagents were investigated to achieve this conversion. The first attempt used tetrapropylammonium perruthenate (TPAP) in acetonitrile in the presence of *N*-methylmorpholineoxide as in Scheme 2.23 below.[136] The mixture was stirred for one day. This reaction was monitored by TLC. The ^1H and ^{13}C NMR spectroscopy and also FTIR spectroscopy recorded that the result was just starting material and this experiment was proven to be unsuccessful.



219 **226**

Scheme 2.23: Attempt to synthesise bicyclic guanidine carboxylic acid.

Another attempt used the procedure of Corey and Schmidt that involved utilising pyridinium dichromate in DMF as in Scheme 2.24.[137] Unfortunately, this procedure was not successful as the result was again only starting material.

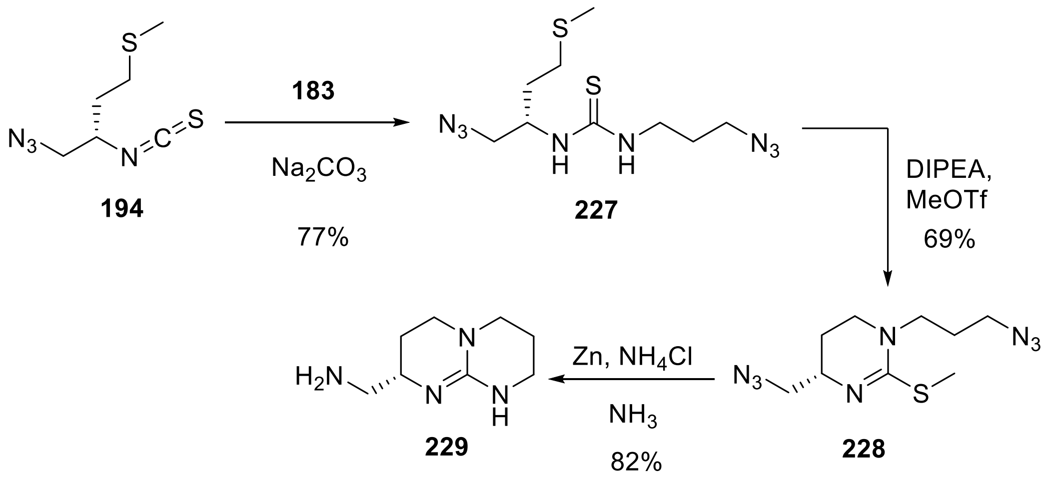


219 **226**

Scheme 2.24: Second attempt 2 to produce bicyclic guanidine carboxylic acid.

2.2.3 The synthesis of bicyclic guanidine methylene amine

The previous work to produce compound **219** pushed us towards another attractive synthetic route for functionalised bicyclic guanidine. To avoid the steps that are needed for protection and de-protection during the synthetic rote to **219**, our new protocol circumvented these steps by introducing an azide group instead of an alcohol group in compound **206**. This protocol has used the same chemistry that was used to synthesise compound **198** and **215**. This time the compound **194** was used as a precursor for the synthesis of compound **229**. Scheme 2.25 shows the synthetic route to produce **229**.

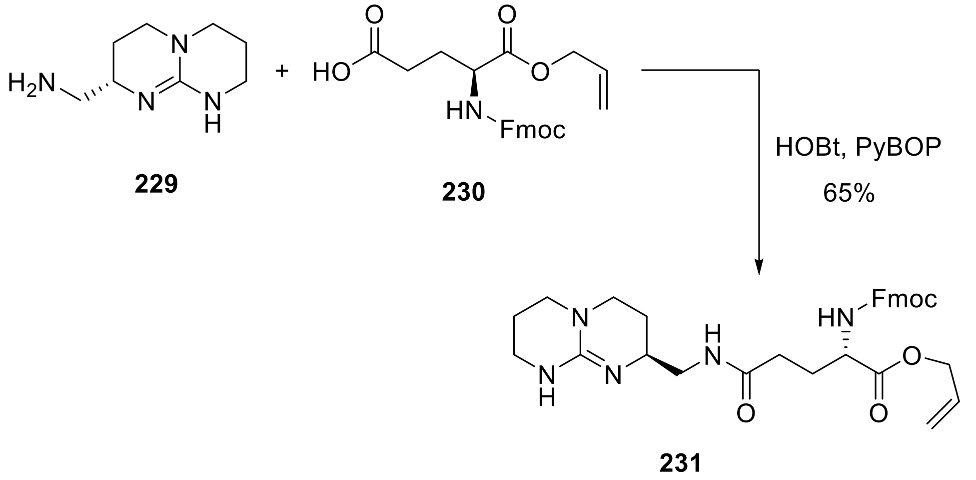


Scheme 2.25: The synthesis of bicyclic guanidine methylene amine.

Thus, the oily product for compound **229** was collected and characterised by ^{1}H and ^{13}C NMR spectroscopy which indicated that this oily compound is the target compound. The high-resolution mass spectrum was identical with the calculated *m*/*z* for compound **229** as well.

2.2.4 The functionalisation of amino acids

Due to the existence of the primary amine in compound **229**, the best choice was to conjugate it with an amino acid through a peptide bond. Thus, the most ideal option was determined to be Fmoc-Glu-OAll, which was used to conjugate with our molecule. The synthesis therefore included the reaction between **229** and Fmoc-Glu-OAll in the presence of HOBt and PyBOP to generate compound **231** as outlined in Scheme 2.26.

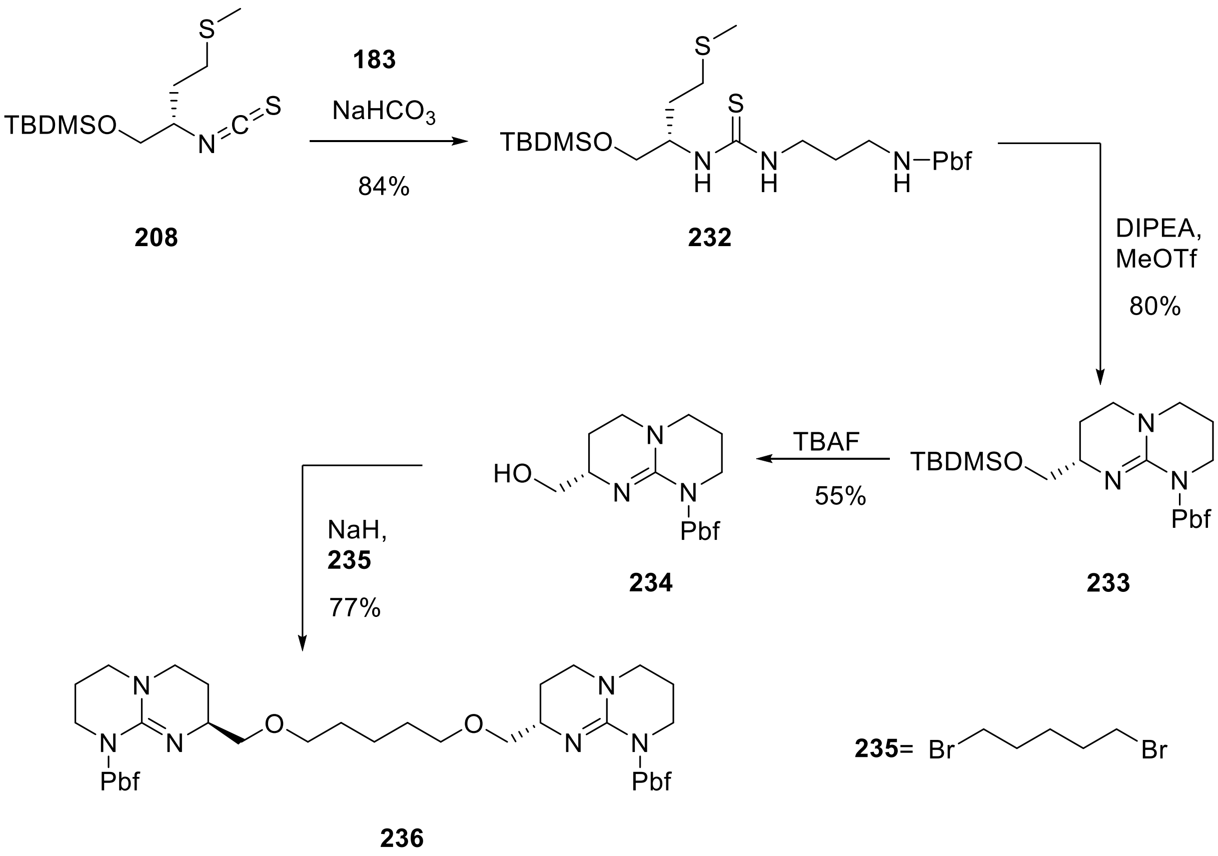


Scheme 2.26: The synthetic route to a non-natural amino acid.

This structure for **231** was verified in the ^1H, ^{13}C NMR and mass spectra recorded for this compound.

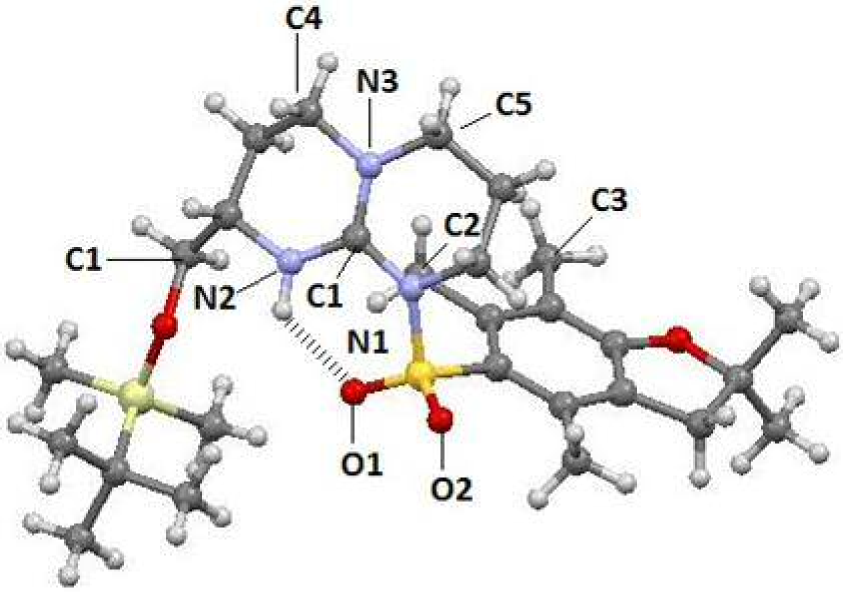
2.2.5 The synthesis of a dimer of bicyclic guanidine

The synthesis of molecules possessing two guanidine groups has attracted wide attention, especially in medicinal chemistry, due to the use of these molecules as antimicrobial drugs.[138] For example, some common antibiotic molecules contain two guanidine groups, such as streptomycin and dihydrostreptomycin. Therefore, our synthetic route is the same that was used by Schmidtchen *et al.* to produce **234** (Scheme 2.27). The synthetic route involved the reaction between silylated isothiocyanate **208** and compound **183** to produce thiourea **232**. Bicyclic guanidine derivative **233** was synthesised by the methylation of the sulfur atom in thiourea **232** and then cyclisation was achieved by heating under reflux with diisopropylethylamine. De-protection of silyl protecting group was then performed by adding *tetra*-*n*-butylammonium fluoride. Two equivalents of compound **234** was finally reacted with one equivalent of 1, 5-dibromopentane in the presence of a strong base, sodium hydride, to produce a dimer of bicyclic guanidine **236**.



Scheme 2.27: Synthesis of bicyclic guanidine dimer by reacting 2 equivalents of bicyclic guanidine methylene alcohol with one equivalent of 1, 5-dibromopentane.

Single crystals of compounds **233** were isolated by vapour diffusion from EtOH/H_2O solution. The X-ray structure of **233** indicated that the product had been successfully formed as its triflate salt. Focussing on the exocyclic methylene group, it can be seen that the atoms around the group are occupying an equatorial type position and the sulfonamide group and exocyclic CH_2 group are in close proximity to the Pbf protecting group. Interestingly the conformation of the Pbf is pointing the same way as the CH_2 which might lead to a steric hindrance towards the reaction of the substituted exocyclic methylene group with a wide range of functional groups. Selected atom distances to the carbon of the exocyclic methylene group of compound **233** are given in Table 2.3.



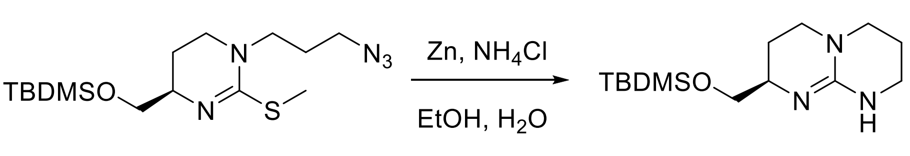
Atom A	Atom B	Distance Å
O1	C1	3.979
O2	C1	6.051
C2	C1	4.344
C3	C1	7.075

Table 2.3: The distance between selected atoms in the crystal structure of **233**, triflate omitted from the figure.

The C-N bond lengths of the guanidinium group of the compound **233** are C1-N3 = 1.327 Å, C1-N1 = 1.376 Å and C1-N2 = 1.321 Å. These lengths are consistent with delocalization of the lone pair of the bridge head nitrogen (N3) into the guanidinium group. The bond angles around N3 in **233** are C1-N3-C4 = 119.13°, C1-N3-C5 = 123.774° and C4-N3-C5 = 115.88°. The sum of the bond angles at both C1 (359.99°) and N3 (358.78°) shows that the geometry of these atoms is almost planar, also consistent with delocalization of electron density from the tertiary nitrogen N3 into the core of the ring. The calculated N2H – O1 distance is 2.073 Å so that there appears to be a hydrogen bond between these groups.

2.2.4 Stereochemical investigation

In our research Boc-L-methionine was utilised as a chiral precursor for the synthesis of bicyclic guanidine derivatives. No reaction occurred on the chiral centre during the reaction steps to produce the target. To verify that no racemisation of the configuration happened during the many conditions that had been used to produce the bicyclic guanidine derivatives, an experiment was performed to synthesise a genuine sample of the other enantiomer for comparison by chiral chromatography. This experiment involved the use of the commercially available Boc-D-methionine, followed by the same chemistry that had been used to synthesise compound **215**, thus, producing **238** as in Scheme 2.28. A chiral HPLC column with a stationary phase of cellulose tris (3, 5-dimethylphenylcarbamate) coated on 5 µm silica-gel, and mobile phase of isopropanol and hexane, was used to analyse the bicyclic guanidine samples derived from L- and D-methionine. At the end of the column, a UV-visible absorbance detector was used to detect elution of the compounds. The resulting chromatogram shows the retention time and peak area associated with each enantiomer. Identification of the substances was achieved through the retention time and the peak area indicates the concentration of the substances in the sample. In our experiment, this technique was used to verify the chiral integrity of the stereocentre of the bicyclic guanidine derivatives that had originated from Boc-L-methionine. At the beginning, 10 µL of D-silylated bicyclic guanidine solution (1 mg in 100 mL) was injected and the retention time was 5.73 min; then L-silylated bicyclic guanidine solution (1 mg in 100 mL) was injected and the retention time was 6.64 min. D and L isomers of bicyclic guanidine were mixed manually and each one showed a different retention time. The D isomer was not visible in the L- sample and the L isomer also was not visible in the D-sample, thus proving that only one isomer had been isolated.

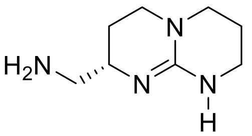


237 **238**

Scheme 2.28: Production of D-bicyclic guanidine derivatives.

2.2.5 Computational binding studies

The possible interactions of bicyclic guanidine derivatives and arginine (Figure 2.5) with nucleobases were modelled using the DFT method (ωB97x-D (dispersion corrected DFT) with 6-31+G** basis set) in the Spartan 10 software by Dr Jamie Platts. An investigation was performed into the effect of the cyclic structure of guanidine and whether this new cyclic substituent is going to make any difference or not. Calculations were performed in the gas-phase. Arginine and bicyclic guanidine were bonded to guanine and the binding energy for the arginine-guanine interaction was −170 kJ mol^{-1} while for the bicyclic guanidine-guanine interaction the energy was −154 kJ mol^{-1}. The binding energy for compound **302** with a guanine-cytosine base pair was −167.7 kJ mol^{-1}. The binding energy for compound **229**-guanine interaction was −153.8 kJ mol^{-1}, compound **219**-guanine was −157.8 kJ mol^{-1} and compound **304**-guanine was −176.7 kJ mol^{-1}. Thus, these results showed that most of the bicyclic guanidine derivatives' guanine interactions are not planar, theoretically (at least using this DFT method) the non-planar interaction appears to be more stable, despite this interaction initially being expected to be less favourable than a planar interaction. On the other hand, compound **304** might interact well with guanine. It could be concluded that the binding energies of the interaction of **219** and **229** with guanine-cytosine are approximately the same, −157.8 kJ mol^{-1} (BCG-OH), −153.8 kJ mol^{-1} (BCG-NH$_2$), but the binding energy of BCG-acetamide (−176.7 kJ mol^{-1}) indicates a more preferable interaction with guanine-cytosine. On the other hand, the binding energy of these models was calculated in the gas phase and the entropy factor was omitted in our calculations, so the result is only an approximation. Theoretically, the resulting angles between the planes of the bicyclic guanidine and nitrogenous bases during the interaction has suggested that the geometry of these molecules during the interaction may not be planar. Figure 2.6 shows the angle between **302** and guanine-cytosine as modelled by Jamie Platts.



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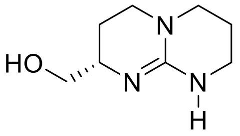

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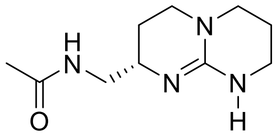

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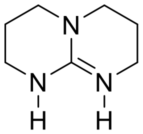
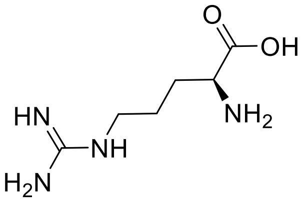


302



303

Figure 2.5: Bicyclic guanine derivatives and arginine that were modelled with guanine-cytosine.

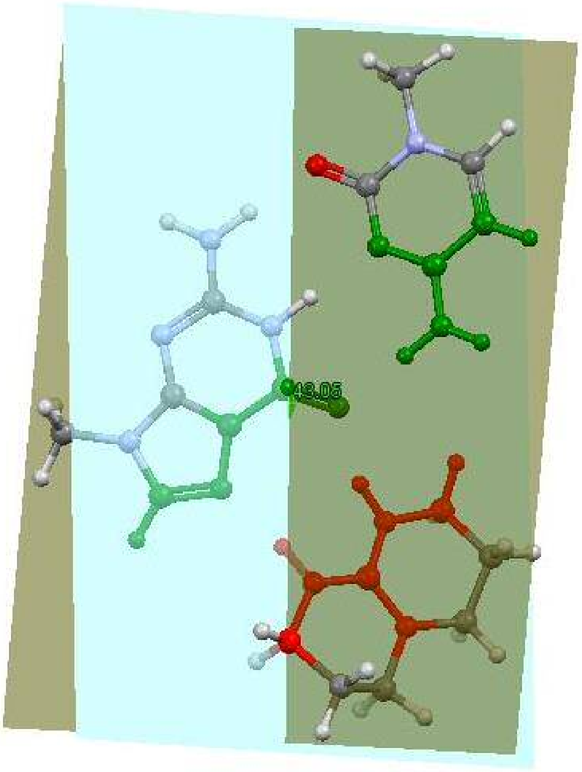


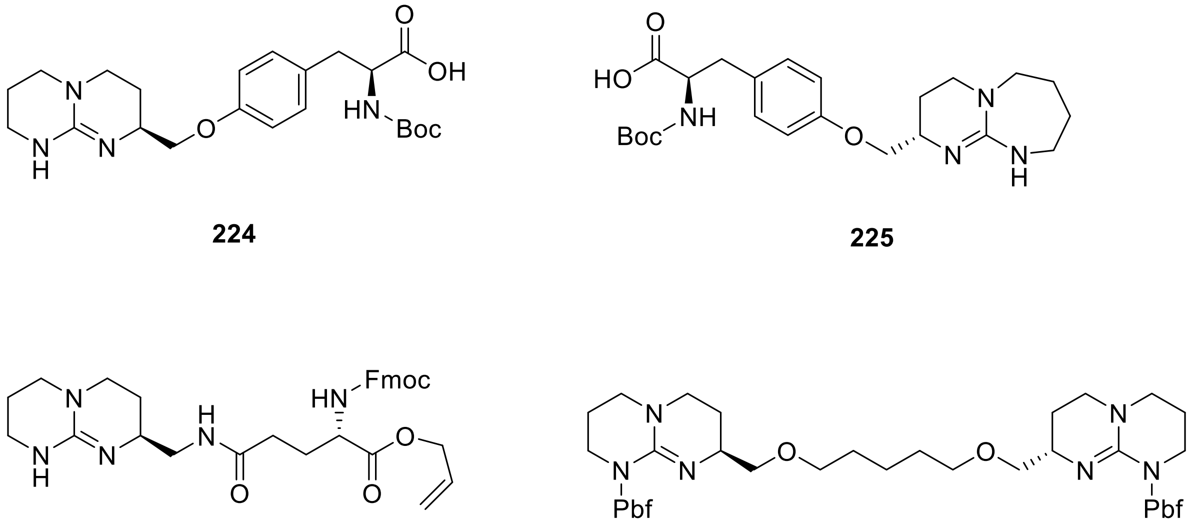
Figure 2.6: Angle between the planes of bicyclic guanidine and guanine-cytosine, green colour for selected atoms in guanine-cytosine and red colour for selected atoms (guanidine group) and two carbon atoms in bicyclic guanidine.

Chapter Three

Conclusions and Future Works

3.1 Conclusions

As described in the introductory chapter, bicyclic guanidines have many applications in a variety of fields in chemistry so our main aim was to find a smooth and easy synthetic route to bicyclic guanidine derivatives. A novel synthetic route to functionalised bicyclic guanidines has since been devised and thus a number of modified amino acids possessing bicyclic guanidine derivatives in their structures and dimer bicyclic guanidine **236** have been produced, as outlined in Figure 3.1.



224 **225** **231** **236**

Figure 3.1: The structure of new amino acids having bicyclic guanidine in their structures that were synthesised by our synthetic protocols.

The utility of a new approach for the synthesis of bicyclic guanidine methylene alcohol, as revealed by the synthesis of monocyclic isothiourea, has been demonstrated. A key feature is illustrated by the conversion of the azide group in the monocyclic isothiourea into bicyclic guanidine methylene alcohol *via* a hydrogenation process, which in turn triggered the production of bicyclic guanidine derivatives through intramolecular substitution. The synthesis of the thiourea intermediates can be highlighted as having the potential to be improved through minimising the use of protecting groups. A protection/deprotection event introduces at least two steps into the synthetic route in the Schmidtchen protocol to produce bicyclic guanidine derivatives. This incurs additional costs for chemical reagents and waste disposal, and generally causes a decreased overall yield. In our project, azides were used to serve as a protected amino group, thus, removing a 'protecting-group' method that was used in Schmidtchen's route.[78] Furthermore, the modification of the step for the cyclisation of thiourea was achieved by synthesising a monocyclic isothiourea containing azide group in the side chain. The hydrogenation of this azide triggered this molecule to form a bicyclic guanidine in a high yield.

Due to the sensitivity of the Pbf protecting group to acidic conditions, as described in the literature, the precursor diaminoalkane Pbf was first prepared by hydrogenation of the corresponding azide using Pd on activated carbon under hydrogen atmosphere. Alternative conditions were utilised to investigate the same reaction, but instead using activated zinc and ammonium chloride. This method successfully reduced the azide group to an amine while retaining the Pbf protecting group in the structure, and we can conclude that these reaction conditions did not cleave off the Pbf protecting group. Furthermore, the resulting product was formed in a high yield and short time (ca. 2 hours) when compared with catalytic hydrogenation using Pd on activated carbon.

We have demonstrated that a new series of modified amino acids having a bicyclic a guanidine in the side chain can be prepared through conjugation. These could allow for the preparation of highly diverse libraries based on the bicyclic guanidine scaffold. The most promising approach seems to be the modified amino acids having bicyclic guanidine conjugated to a tyrosine moiety through an ether linkage.

The synthesis of the Pbf-Cl reagent that is used to protect the amine group, from cheaper butyraldehyde and 2,3,5-trimethyl phenol precursors has also been described. These published synthetic methods allowed this reagent to be produced in a good yield with limited difficulty.

The production of a bicyclic guanidine with a seven-membered ring **199** in the presence of the Pbf protecting group has also proved difficult, while the corresponding compound without this bulky Pbf protecting group was successfully produced.

The synthesis of bicyclic guanidines containing an eight-membered ring **218** has proved unsuccessful despite a number of different reaction conditions being used to try to produce it.

The attempts to conjugate protected bicyclic guanidine methylene amine **201** with Fmoc-glu-OAll were not successful while the corresponding compound without a Pbf protecting group was successfully conjugated with Fmoc-Glu-OAll (Compound **231**). We can conclude that the presence of the bulky protecting group inhibited this reaction, preventing formation of the amide bond.

Attempting to produce a modified amino acid containing a bicyclic guanidine through cyclic addition (click reaction), *via* the reaction between **198** and **204** was not successful. Also attempting to oxidise the alcohol group in the side chain of bicyclic guanidine **219** to produce **226** was also not successful despite different reagents from literature procedures being tested.

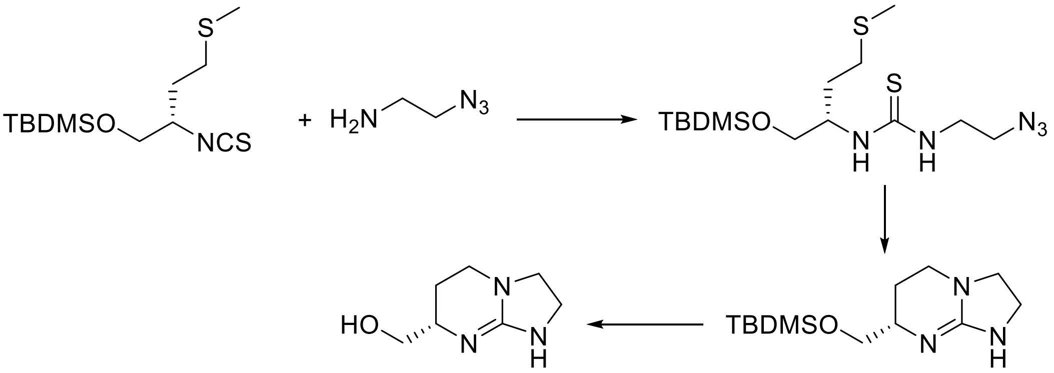
Computational chemistry was used *via* the DFT method to predict the nature of the bicyclic guanidine-nitrogenous bases' binding, and the results inferred that the resulting angles between these molecules may not be planar.

3.2 Future work

Incorporation into peptides of our modified amino acids that possess different sized bicyclic guanidine rings is the first priority for future work. Two peptide sequences will be synthesised, the first peptide sequence will include six amino acids (Fmoc-Ser(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH). The second peptide sequence will include the same amino acids sequence except for insertion of a modified amino acid having our target bicyclic guanidine instead of Fmoc-Arg(Pbf)-OH. The comparison between DNA binding affinity of peptides having arginine with comparable sequences having our arginine analogue could be investigated in the future.

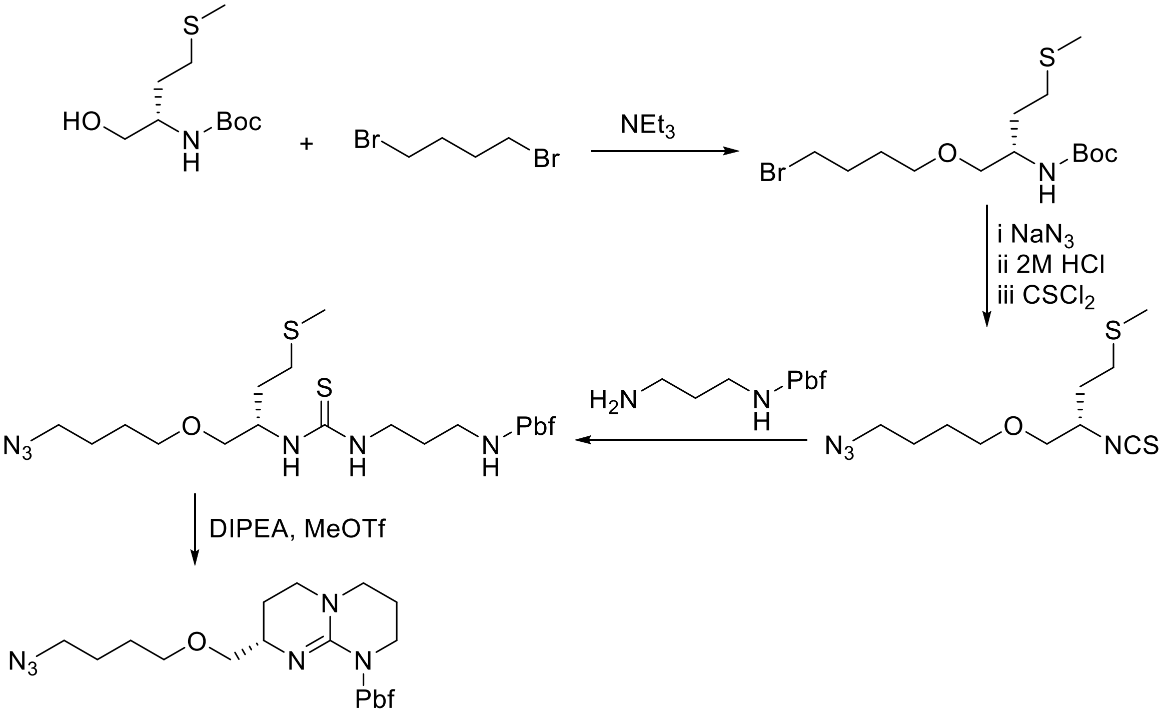
On the other hand, the bicyclic guanidine dimer research needs further investigation on two fronts, the first being the synthesis of the dimer of bicyclic guanidine with different lengths of side chain and achieving binding studies for these molecules relating to the hydrogen bonding and hydrophobic forces against nitrogenous bases. The other being the potential use of our synthesised bicyclic guanidine dimers as antimicrobials, which could also be studied in the future.

Furthermore, the same chemistry that was used to produce compound **219** could be used to generate bicyclic guanidines containing five membered rings as outlined in Scheme 3.1.



Scheme 3.1: Proposal to synthesise bicyclic guanidine containing five membered rings.

If this work is continued, I would recommend trying to synthesise a bicyclic guanidine having longer side chains (Scheme 3.2), which might overcome the barrier to producing a non-natural amino acid containing protected bicyclic guanidines. Interactions with the Pbf protecting group may be inhibiting the amine group in the side chain from forming an amide bond. Comparisons of the binding affinities of this flexible bicyclic guanidine and the synthesised rigid bicyclic guanidine could be made against nitrogenous bases.



Scheme 3.2: Proposal to synthesise bicyclic guanidine having a long side chain.

Modified amino acids **224** and **225** containing bicyclic guanidine in the side chain were successfully synthesised. Future work has therefore to focus primarily on incorporating these non-natural amino acids into peptides and then on binding studies for these peptides against nitrogenous bases.

Chapter Four
Experimental Part

4.1 Instrumentation and reagents for organic synthesis

4.1.1 Chemicals and solvents

Most of the solvents used throughout, including anhydrous dimethylformamide were purchased from commercial suppliers and used without further purification. Anhydrous tetrahydrofuran, dichloromethane and acetonitrile were collected from a solvent purification system. Chemicals were purchased from Acros, Aldrich, Fluka, Fluorochem, Molekula, Alfa Aesar and Novabiochem and were used without further purification.

4.1.2 Melting Points

Melting points were measured using a Gallenkamp melting point apparatus with samples in open capillary tubes.

4.1.3 Thin-Layer Chromatography

All reactions were monitored by thin-layer chromatography (TLC) which was performed on precoated aluminium sheets of Merck silica gel 60 F254 (0.20 m) and visualised by UV radiation (254 nm) or/and by staining with ceric ammonium molybdate solution (235 mL distilled H_2O, 12 g ammonium molybdate, 0.5 g ceric ammonium molybdate, 15 mL sulfuric acid), potassium permanganate solution (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% NaOH, 200 mL distilled H_2O) or iodine.

4.1.4 Column Chromatography

Manual column chromatography was performed using silica gel 60 (Merck, 230-400 mesh) under pressure (Flash Chromatography) or as gravitational column chromatography. The solvents used for the purification are indicated in the text and were purchased from Fisher Scientific as laboratory grade.

4.1.5 NMR Spectroscopy

All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) or a Bruker Ascend 500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C

NMR) or Bruker Fourier 300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR). Deuterated solvents $CDCl_3$, D_2O, CD_3OD and DMSO-d6 were used. Chemical shifts are given in parts per million using the residual solvent peak as a reference.· Coupling constants (*J*) values are quoted in Hertz (Hz).

4.1.6 Optical Rotation

Optical rotation was measured with a SCHMIDT and HAENSCH UniPO1 L polarimeter at 20 °C in a cuvette of 50 mm length with the sodium D-line (589.30 nm). HPLC grade chloroform was used to prepare the solution and the concentration is indicated in the data section.

4.1.7 Mass Spectrometry

Mass spectrometric measurements were performed by the EPSRC Mass Spectrometry Service Centre, Swansea University or by R. Jenkins/R. Hick/S. Waller at Cardiff University. Ions were generated by the atmospheric pressure chemical ionisation (APCI), electrospray (ES) or electron ionisation (EI). Mass fragments usually are given in atomic mass units per elementary charge (*m*/*z*) with relative abundance of the ions in percentage (%). The high-resolution mass spectrometry (HRMS) for most of the compounds was carried out at EPSRC Mass Spectrometry Service centre, Swansea University. The molecular ion peak values quoted for either molecular ion $[M]^+$, molecular ion plus hydrogen $[M+H]^+$, molecular ion plus ammonium ion $[M+NH_4]^+$ or molecular ion plus sodium $[M+Na]^+$.

4.1.8 IR Spectroscopy

IR spectra were recorded on a Shimadzu IR Affinity-1S instrument. Wavenumbers are quoted in cm^{-1}. All compounds were measured neat directly on the ATR crystal of the IR machine.

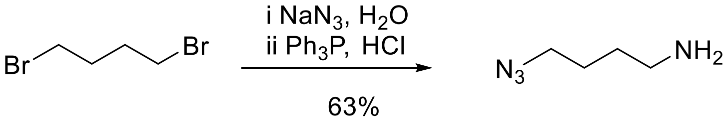
4.1.9 X-Ray Crystallography

Single-crystal XRD data were collected on an Agilent SuperNova Dual Atlas diffractometer with a mirror monochromator (using either Cu (λ= 1.5418 Å) or Mo (λ=0.7107 Å) radiation), equipped with an Oxford cryosystems cooling apparatus. Crystal structures were solved and refined using SHELX. Non-hydrogen atoms were refined with anisotropic displacement parameters. A riding model was used with U_{iso} set at 1.2 or 1.5 times the value of U_{eq} for the atom to which they are bonded.

4.1.10 Computational chemistry

The possible interactions of bicyclic guanidine derivatives and arginine with nucleobases were modelled using the DFT method (ωB97x-D (dispersion corrected DFT) with 6-31+G** basis set) in the Spartan 10 software by Dr Jamie Platts.

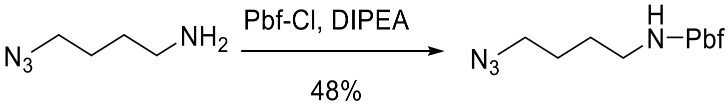
General procedure for the synthesis of azido alkyl amine from dibromo alkane



184 **185**

Sodium azide (10.4 mmol) was added to a solution of 1, n-dibromoalkane (3.4 mmol) in water (30 mL). The mixture was heated at 80 °C for 20 h. After that, the mixture was made basic by adding 2 M NaOH and then the aqueous phase was extracted with ether (3 × 10 mL). Triphenylphosphine (5 mmol) was added to the solution of diazidoalkane over one hour. A mixture of 1/1 ether/ethyl acetate (15 mL) and 5% HCl (15 mL) was added to the resulting product. The mixture was stirred for 24 h at r.t. The organic phase was removed and the aqueous phase washed with diethyl ether (3 × 10 mL). The resulting aqueous phase was made basic with 2 M sodium bicarbonate to pH 10 and then extracted again with dichloromethane (3 × 10 mL). The combined organic layers were dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting products did not need further purification.

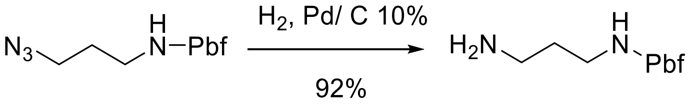
General procedure to protect organic amine with Pbf protecting group



185 **186**

The solution of Pbf-Cl **179** (300 mg, 1 mmol) and diisopropylethylamine (0.9 mL, 1.3 mmol) in dry DCM (25 mL) were added to the organic amine (200 mg, 1.7 mmol). The mixture was heated to reflux for 4 h under nitrogen. The mixture was washed with sat. aq. NH_4Cl, washed twice further with brine, dried over $MgSO_4$ and concentrated *in vacuo.* The crude product was purified by using flash column chromatography through silica gel using the eluent of hexane and ethyl acetate to get the protected organic amine**.**

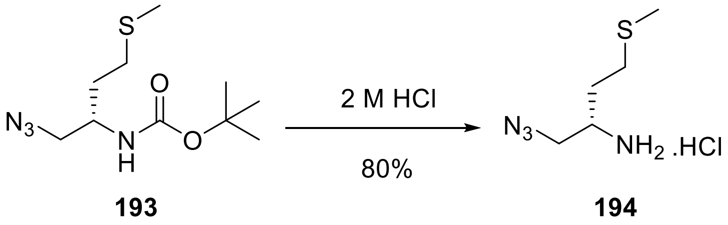
General procedure for the hydrogenation of azides to amines



182 **183**

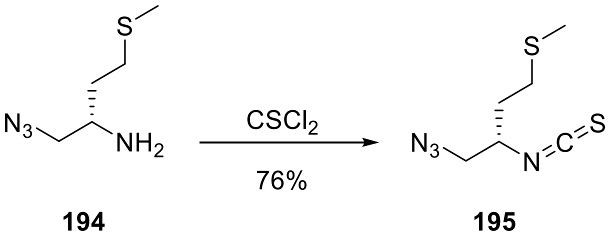
The appropriate azide compound (1.91 mmol) and 10% Pd/C (150 mg) were suspended in methanol. The resulting mixture was stirred at room temperature for 12 h under a hydrogen atmosphere. The result was filtered and washed with methanol through a Celite® filter. The solvent was evaporated under reduced pressure and then dried under high vacuum to get pure amine, no further purification was needed.

General procedure for deprotection of Boc protecting group



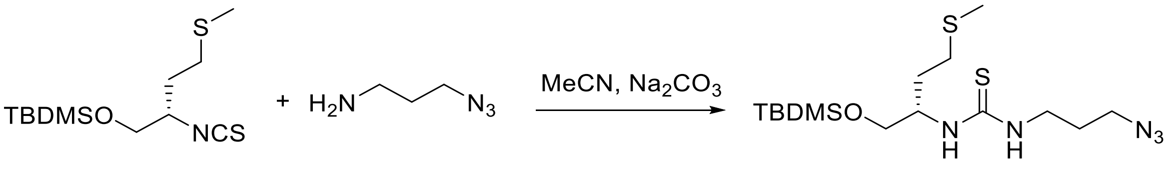
2 M HCl (30 mL) was added to the solution of the relevant compound having a Boc protecting group (11.53 mmol) in tetrahydrofuran (10 mL). The mixture was stirred vigorously for 4 h at room temperature. The solvent was evaporated (co-evaporation with ethanol) under reduced pressure to obtain the desired compound. No further purification was needed.

General procedure for the synthesis of isothiocyanate compounds from amines



The appropriate amine compound (7.49 mmol) and sodium bicarbonate (9.52 mmol) were suspended in dichloromethane (25 mL). Thiophosgene (8.95 mmol) was added to the resulting suspension. The mixture was stirred overnight at room temperature. Distilled water (20 mL) was added to this solution. The organic layer was collected and dried over $MgSO_4$. The organic solution was concentrated under reduced pressure to attain the desired compound.

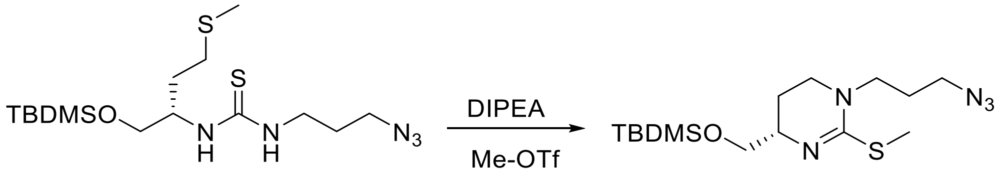
General procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amine



208 **181** **209**

The appropriate isothiocyanate (5.44 mmol) and amine (5.44 mmol) were dissolved in a dry acetonitrile (30 mL) and then sodium bicarbonate (11.32 mmol) was added. The mixture was heated under reflux with vigorous stirring overnight. The reaction was allowed to reach room temperature, filtered and washed with water (3 × 10 mL) and then with brine (3 × 10 mL). The solvent was evaporated under reduced pressure to obtain crude product. This residue was purified by using flash column chromatography.

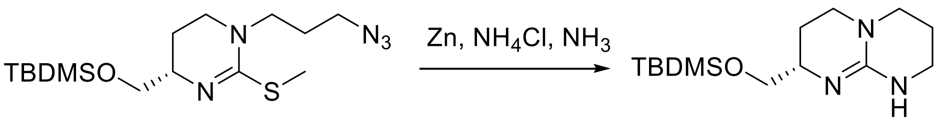
General procedure for the cyclisation of thiourea



212 **215**

The appropriate thiourea (2 g, 5.1 mmol) was dissolved in dry dichloromethane (30 mL). Diisopropylethylamine (0.32 mL, 0.23 g, 1.8 mmol) was added. Methyl triflate (1.3 mL, 1.88 g, 11.5 mmol) was added dropwise to the resulting solution at 0 °C. The mixture was stirred for 4 h at room temperature. Diisopropylethylamine 8.4 mL (6.2 g, 48.2 mmol) was then added and the mixture was heated under reflux for 24 h. It was cooled and then washed with NaOH (2 M) (× 2). The mixture was washed with water (× 3). The organic layer was dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain crude product. This residue was purified by using flash column chromatography.

General procedure for the hydrogenation of azides to amines by using activated zinc and ammonium chloride

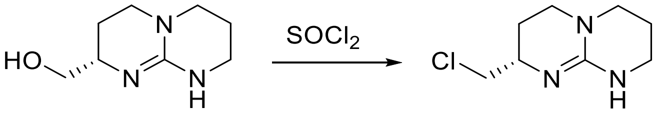


212 **215**

The appropriate azide compound (9.63 mmol) and ammonium chloride (22.49 mmol) were dissolved in ethanol (26 mL) and H_2O (9 mL) followed by the addition of activated zinc (12.84 mmol). The mixture was heated under reflux for 2 h and allowed to cool to room temperature; then ammonia solution (4 mL) was added with efficient stirring for hour. The solid was filtered; the solvent was evaporated under reduced pressure and the residue was dissolved in methanol (30 mL). The solid (ammonium chloride) was filtered again, the solvent was evaporated under reduced pressure again to obtain the amine compound. Flash column chromatography was used to acquire a pure product.

Note: activated zinc was synthesised by adding 2 M HCl (10 mL) to the zinc powder (5 g, 77 mmol) and then the mixture was stirred for 30 minutes followed by the filtration; then, the zinc was washed with acetone, ethanol, water and diethyl ether. The activated zinc was dried under high vacuum for 14 h.

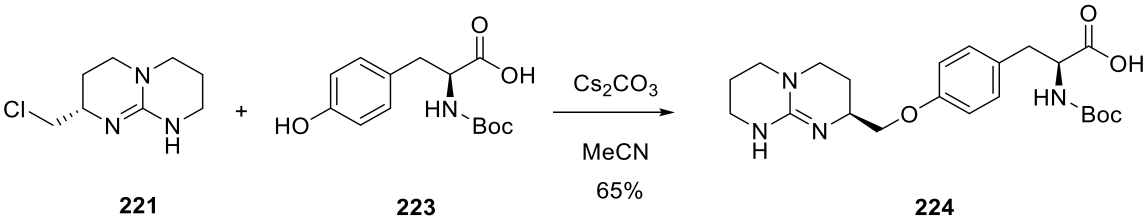
General procedure for the synthesis of alkyl halides from alcohols



219 **221**

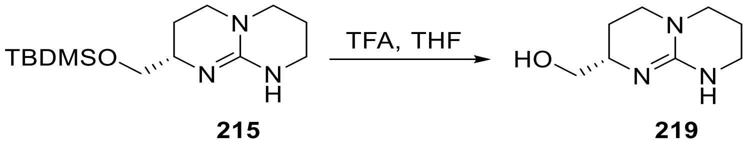
The appropriate alcohol compound (529 mg, 3.1 mmol) was dissolved in thionyl chloride (6 mL) under nitrogen atmosphere. The mixture was heated under reflux for 10 h. The resulting solution was allowed to cool to room temperature. Thionyl chloride was evaporated and then the residue was dissolved in dichloromethane; washed with water three times followed by washing with brine three times. The combined organics were dried over $MgSO_4$ and concentrated *in vacuo* to obtain the crude product. Flash column chromatography was used to acquire a pure product.

General procedure for the synthesis of modified amino acid having bicyclic guanidine in their moiety



Boc-L-tyrosine (312.2 mg, 1.1 mmol) and caesium carbonate (1.8 g. 5.54 mmol) were suspended in anhydrous acetonitrile (30 mL) under a nitrogen atmosphere. The mixture was stirred for 30 minutes. Then, a solution of alkyl halide (1.1 mmol) in acetonitrile (10 mL) was added to the resulting mixture. The mixture was heated under reflux overnight. The solid was removed by filtration, followed by evaporation of the solvent under reduced pressure from the filtrate. Purification of the residue was achieved by using flash column chromatography.

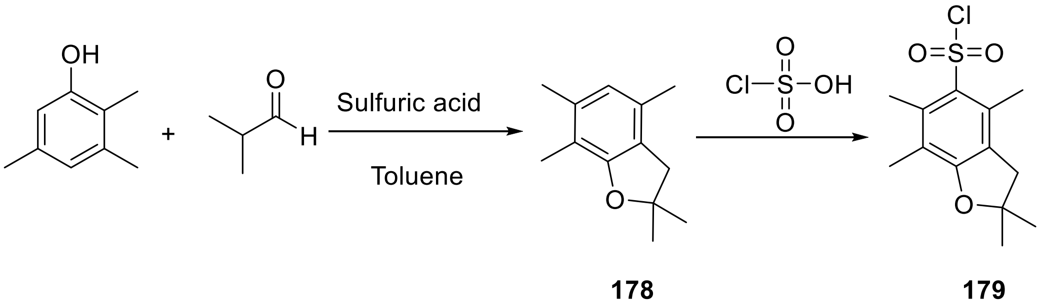
General procedure for the de-protection of silyl group by utilising trifluoroacetic acid



Trifluoroacetic acid (5 mL) was added to the solution of the silylated alcohol (750 mg, 2.64 mmol) in tetrahydrofuran (25 mL). The mixture was stirred vigorously for 5 h. The solvent was evaporated under reduced pressure. This product is soluble in water; thus, the residue was dissolved in water and then washed with dichloromethane three times. The aqueous solvent was re-evaporated under reduced pressure again (co-evaporation with ethanol) to obtain the alcohol.

4.2 Procedures

2, 2, 4, 6, 7-Pentamethyl-2, 3-dihydrobenzofuran-5-sulfonyl chloride (179)[139]



Sulfuric acid (0.9 g) was added to the solution of 2,3,5-trimethylphenol (30 g, 220.2 mmol) and isobutyraldehyde (24 mL, 264.4 mmol) in toluene (30 mL) and heated under reflux for 7 h, using a Dean-Stark apparatus to continually remove the water formed during the reaction. The reaction was monitored by TLC. The solvent was evaporated under reduced pressure to obtain the crude product. The pure product **178** was collected by using a short path vacuum distillation apparatus; (Kugelrohr) and the yield was 88%. Pbf **178** (16.74 g, 87 mmol) was dissolved in dry dichloromethane (200 mL). Chlorosulfonic acid (21.3 g, 183 mmol) was added to the solution dropwise at 0 °C for 1.5 h. The mixture was stirred at room temperature for 2 h and was then poured into an ice-cold solution of 5% sodium bicarbonate (150 mL). The mixture was extracted by dichloromethane. The combined organics were dried over $MgSO_4$ and then concentrated to obtain the product **179** as a silver solid (20 g, 80%). Further purification was not needed.

TLC (Silica gel, hexane/DCM, 9:1) R_f = 0.41 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[139]

m.p.= 45-46 °C (lit 44–46 °C)

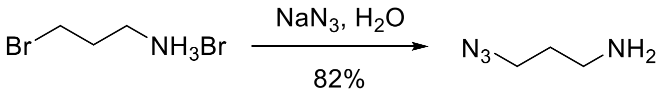
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (s, 2 H, CH_2), 2.19 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 2.07 (s, 3 H, CH_3), 1.46 (s, 6H, 2 CH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ 157.2 (ArC), 136.4 (ArC), 131.2 (ArC), 122.6 (ArC), 122.1 (ArC), 115.4 (ArC), 86.0 (C $(CH_3)_2$), 42.5 (CH_2), 28.7 (CH_3), 19.4 (CH_3), 18.3 (CH_3), 11.6 (CH_3).

FT-IR ($CHCl_3$ film, cm^{-1}) 2973 (s, aliphatic CH), 1457 (m), 1410 (s), 1285 (s), 1152 (s), 1088 (s).

ESI+ (*m/z*): 289 $[M+H]^+$

3-Azidopropan-1-amine (181)[140]



180 **181**

A mixture of compound **180** (2.2 g, 10 mmol) and sodium azide (1.8 g, 27.6 mmol) in water (10 mL) was heated at 80 °C for 24 h. The solution was cooled by using an ice bath; then dichloromethane (40 mL) and potassium hydroxide were added until the solution reached a pH of approximately 12. The aqueous layer was extracted with dichloromethane (2 × 10 mL); the combined organics were washed with brine (2 × 10 mL). Subsequently, the organic azide was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to obtain **181** (820 mg, 82%) as a colourless oil.

TLC (silica gel, 3:1 hexane/ EtOAc), R_f= 0.25 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[140]

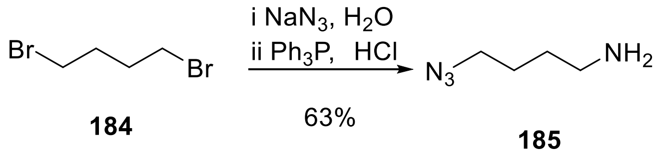
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.33 (t, 2H, $^3J_{HH}$ = 6.7 Hz), 2.84 (t, 2H, $^3J_{HH}$ = 6.8 Hz), 1.72-1.59 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 51.1 (CH_2), 38.1 (CH_2), 29.2 (CH_2).

FT-IR ($CHCl_3$ film, cm^{-1}) 3310 (br, s, NH), 2955 (s, aliphatic CH), 2090 (s, CN_3).

HRMS-AP+ (m/z): calcd. for $C_3H_9N_4$ $[M+H]^+$: 101.0822, found 101.0823

4-Azidobutan-1-amine (185)[141]



The general procedure for the synthesis of azido alkyl amine from dibromo alkane was followed to yield **185** as a colourless oil (244 mg, 63%).

TLC (silica gel, 3:1 hexane/ EtOAc), *R_f*= 0.22 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[141]

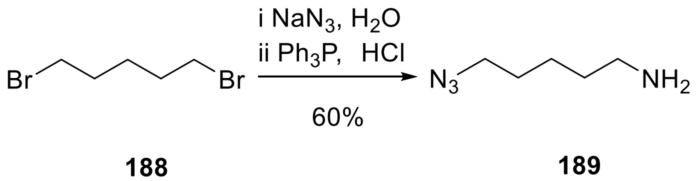
^{1}H NMR (400 MHz, CDCl$_3$) δ 5.26 (s, 2H, NH_2), 3.33-2.95 (t, 2H, $^3J_{HH}$=13.5 Hz, N_3CH_2), 2.75-2.61 (m, 2H, $^3J_{HH}$ =13.7 Hz, NH_2CH_2), 1.6-151 (m, 2H, $^3J_{HH}$ =13.5 Hz, $N_3CH_2CH_2$), 1.49-1.4 (m, 2H, $NH_2CH_2CH_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 53.2 (CH_2), 42.1 (CH_2), 31.2 (CH_2), 27.0 (CH_2).

FT-IR (CHCl$_3$ film, cm^{-1}) 3372 (br, s, NH), 2979 (s, aliphatic CH), 2093 (s, CN_3).

CI+ (*m/z*): 115.1 $[M+H]^+$

5-Azidopentan-1-amine (189)[142]



The general procedure for the synthesis of azido alkyl amine from dibromo alkane was followed to yield **189** as a colourless oil (261 mg, 60%).

TLC (silica gel, 3:1 hexane/ EtOAc), *R*$_f$= 0.32 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[142]

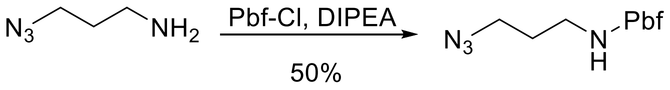
^{1}H NMR (300 MHz, CDCl$_3$) δ 3.34 (t, 2H, $^3J_{HH}$=6.4 Hz, N_3CH_2), 2.76-2.45 (t, 2H, $^3J_{HH}$=6.4 Hz, NH_2CH_2), 1.69-1.58 (m, 2H, $NH_2CH_2CH_2$), 1.57-1.46 (m, 2H, $N_3CH_2CH_2$), 1.5-1.33 (m, 2H, $NH_2CH_2CH_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 52.2 (CH_2), 42.3 (CH_2), 33.2 (CH_2), 28.0 (CH_2), 23.2 (CH_2).

FT-IR (CHCl$_3$ film, cm^{-1}) 3366 (br, s, NH), 2973 (s, aliphatic CH), 2098 (s, CN_3).

ESI+ (*m/z*): 129.1 $[M+H]^+$

***N*-(3-azidopropyl)-2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (182)**



181 **182**

The general procedure to protect organic amine with Pbf protecting group was followed to synthesise **182** as a white powder (1.4 g, 50%).

TLC (silica gel, 3:1 hexane/ EtOAc), *R*$_f$= 0.4 (UV)

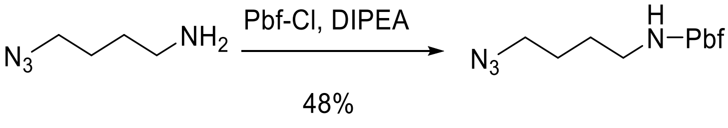
^{1}H NMR (400 MHz, CDCl$_3$) δ 4.70 (t, 1H, $^3J_{HH}$ = 6.4 Hz, NH), 3.40-3.22 (t, 2H, $^3J_{HH}$ = 6.4 Hz, N_3CH_2), 3.12-3.01 (m, 4H, $NHCH_2$, furan-CH_2), 2.57 (s, 3H, $ArCH_3$), 2.52 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$), 1.83-1.71 (m, 2H, $N_3CH_2CH_2$), 1.50 (s, 6H, $C(CH_3)_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.8 (ArC), 141.7 (ArC), 134.1 (ArC), 127.7 (ArC), 125.3 (ArC), 118.1 (ArC), 86.9 ($C (CH_3)_2$), 49.0 (CH_2), 43.2 (CH_2), 40.1 (CH_2), 28.8 (CH_2), 28.6 ($C (CH_3)_2$), 19.4 (CH_3), 17.7 (CH_3), 12.6 (CH_3).

HRMS-AP+ (m/z): calcd. for $C_{16}H_{25}N_4O_3S$ $[M+H]^+$: 353.1647, found 353.1650

FT-IR ($CHCl_3$ film, cm^{-1}) 3305 (br, s, NH), 2973 (s, aliphatic CH), 2095 (s, CN_3), 1575 (s), 1463 (s), 1413 (s), 1320 (s), 1259 (s), 1142 (s), 1093 (s), 998 (s).

N-(4-azidobutyl)-2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (186)



185 **186**

The general procedure to protect organic amine with Pbf protecting group was followed to synthesise **182** as a pale-yellow solid (299 mg, 48%).

TLC (silica gel, 3:1 hexane/ EtOAc), R_f = 0.3 (UV).

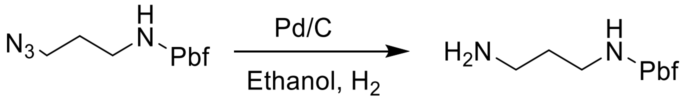
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (t, 1H, $^3J_{HH}$ = 6.4 Hz, NH), 3.37-3.25 (t, 2H, $^3J_{HH}$ = 6.4 Hz, N_3CH_2), 2.89-2.81 (m, 4H, $NHCH_2$, furan-CH_2), 2.49 (s, 3H, $ArCH_3$), 2.41 (s, 3H, $ArCH_3$), 2.15 (s, 3H, $ArCH_3$), 1.58 (quin, 2H, $^3J_{HH}$ = 3.4 Hz, $N_3CH_2CH_2$),1.5-1.42 (m, 2H, $NHCH_2CH_2$), 1.40 (s, 6H, $C(CH_3)_2$).

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8 (ArC), 141.5 (ArC), 134.4 (ArC), 127.2 (ArC), 125.31 (ArC), 118.2 (ArC), 87.8 ($C (CH_3)_2$), 49.0 (CH_2), 43.2 (CH_2), 40.1 (CH_2), 28.2 (CH_2), 27.0 (CH_2), 29.1 ($C (CH_3)_2$), 19.4 (CH_3), 18.1 (CH_3), 12.1 (CH_3).

ES- MS (*m/z*): 401 $[M+Cl]^-$

FT-IR ($CHCl_3$ film, cm^{-1}) 3309 (br, s, NH), 2979 (s, aliphatic CH), 2089 (s, CN_3), 1578 (s), 1464 (s), 1417 (s), 1323 (s), 1257 (s), 1144 (s), 1095 (s), 996 (s).

***N*-(3-aminopropyl)-2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (183)**



182 **183**

The general procedure for the hydrogenation of azides to amines was followed to yield **183** as a white foam (573 mg, 92%).

TLC (silica gel, 10% AcOH-EtOH), R_f = 0.31 (UV).

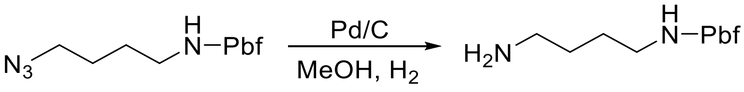
^{1}H NMR (400 MHz, CDCl$_3$) δ 5.12 (br, NH_2), 3.12 (m, 4H, $NHCH_2$, furan-CH_2), 2.85 (t, 2H, $^3J_{HH}$ = 6.6 Hz, NH_2CH_2), 2.55 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 2.09 (s, 3H, $ArCH_3$), 1.69-1.53 (quin, 2H, $NH_2CH_2CH_2$), 1.46 (s, 6H, C $(CH_3)_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.6 (ArC), 128.3 (ArC), 127.2 (ArC), 127.0 (ArC), 118.2 (ArC), 110.0 (ArC), 77.8 (C $(CH_3)_2$), 44.4 (CH_2), 42.6 (CH_2), 41.5 (CH_2), 33.1 (CH_2), 27.9 (C $(CH_3)_2$), 19.4 (CH_3), 16.9 (CH_3), 12.42 (CH_3).

ES+ MS (*m/z*): 327 $[M+H]^+$

FT-IR (CHCl$_3$ film, cm^{-1}) 3365 (s, NH), 2930 (s, aliphatic CH), 1569 (s, NH), 1460 (s), 1310 (s), 1144 (s), 1094 (s).

***N*-(4-aminobutyl)-2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (187)**



186 **187**

The general procedure for the hydrogenation of azides to amines was followed to yield **187** as a white foam (584.7 mg, 90%).

TLC (silica gel, 2:1 hexane/ EtOAc), R_f = 0.31 (UV, ninhydrin).

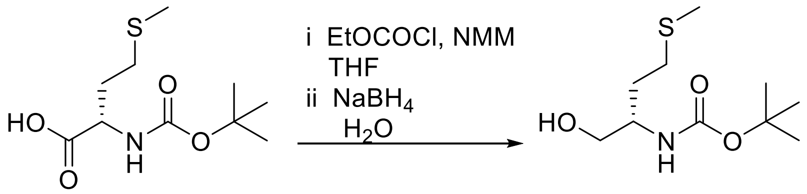
^{1}H NMR (400 MHz, CDCl$_3$) δ 5.06 (br, NH$_2$), 3.12 (m, 4H, NHCH$_2$, furan-CH$_2$), 3.0 (t, 2H, $^3J_{HH}$ = 6.6 Hz, NH$_2$CH$_2$), 3.0-2.89 (m, 2H, NHCH$_2$CH$_2$), 2.35 (s, 3H, ArCH$_3$), 2.48 (s, 3H, ArCH$_3$), 2.09 (s, 3H, ArCH$_3$), 1.53-1.52 (m, 2H, NH$_2$CH$_2$CH$_2$), 1.48 (s, 6H, C(CH$_3$)$_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.5 (ArC), 128.2 (ArC), 127.0 (ArC), 126.9 (ArC), 118.4 (ArC), 110.7 (ArC), 77.9 (C (CH$_3$)$_2$), 44.5 (CH$_2$), 42.8 (CH$_2$), 40.6 (CH$_2$), 41.6 (CH$_2$), 33.0 (CH$_2$), 27 (C (CH$_3$)$_2$), 19.2 (CH$_3$), 16.7 (CH$_3$) 12.4 (CH$_3$).

ESI+ MS (m/z): 341 [M+H]$^+$

FT-IR (CHCl$_3$ film, cm^{-1}) 3368 (s, NH), 2935 (s, aliphatic CH), 1567 (s, NH), 1464 (s), 1315 (s), 1146 (s), 1097 (s).

<u>(*S*)-*Tert*-Butyl 1-hydroxy-4-(methylthio) butan-2-ylcarbamate (191)</u>[143]



190 **191**

NMM (3.72 mL, 3.43 g, 34 mmol) was added to the solution of Boc-*N*-methionine (8.00 g, 34 mmol) in anhydrous tetrahydrofuran (45 mL). Ethyl chloroformate 3.6 mL (4.16 g, 38.4 mmol) was added dropwise to the resulting solution at 0 °C. The mixture was stirred continuously at 0°C for 1 h until it became a clear solution. It was then filtered, the solid washed with tetrahydrofuran and the solution was added dropwise to an ice-cold solution of sodium borohydride (3.63 g, 96 mmol) in water (40 mL) over a 20-min period. The reaction was stirred at 0 °C for 1 hour and then stirring continued overnight. Subsequently, HCl (1 M) was added to the solution until pH 4 was reached. The solution was made basic by adding sodium bicarbonate and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organics were washed with sat. sodium chloride solution, dried over MgSO$_4$ and concentrated *in vacuo* giving alcohol **191** as a colourless oil (6 g, 75%).

TLC (silica gel, 10:4 Ethyl acetate/ TEA), R_f= 0.28 (UV)

The spectroscopic data are in agreement with those recorded in the literature[143]

^{1}H NMR (250 MHz, CDCl$_3$) δ 4.85 (br d, 1H, $^3J_{HH}$ = 7. 6 Hz, NH), 3.73-3. 68 (m, 1H, NHCH), 3.64 (dd, 1H, $^2J_{HH}$ = 11.2 Hz, $^3J_{HH}$ = 3.9 Hz, HOCHH), 3.62 (dd, 1H, = 11.0 Hz, $^2J_{HH}$ = 5.2Hz, HOCHH), 2.58-2.43 (m, 2H, SCH$_2$), 2.06 (s, 3H, SCH$_3$), 1.91-1.79 (m, 2H, SCH$_2$CH$_2$), 1.47 (s, 9H, *t*-Bu).

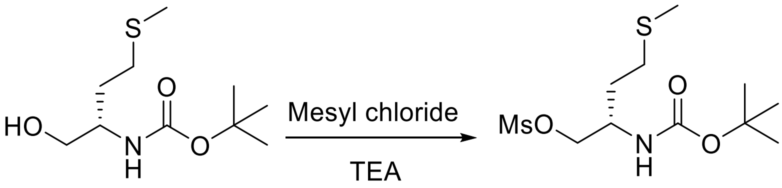
^{13}C NMR (125 MHz, CDCl$_3$) δ 157.1 (C=O), 80.1 (C (CH$_3$)$_3$), 66.1 (CH$_2$OH), 52.3 (CH), 32.0 (CH$_2$), 31.7 (CH$_2$), 28.5 (*t*-Bu), 15.7 (SCH$_3$).

ESI- MS (*m/z*): 270 [M+Cl]$^-$.

FT-IR (CHCl$_3$ film, cm^{-1}) 3360 (br s, NH), 2978 (m, aliphatic CH), 1685 (s, C=O), 1522 (s, NH), 1365 (m) 1245 (m), 1175 (s).

= −13.5 (c= 0.25, CHCl$_3$)

<u>(*S*)-2-(*tert*-butoxycarbonylamino)-4-(methylthio) butyl methanesulfonate (192)</u>[128]



191 **192**

Boc-L-methioninol **191** (5 g, 21.2 mmol) was dissolved in dry dichloromethane (30 mL) and triethylamine 5.8 mL (4.2 g, 42.4 mmol) was added to this solution. Mesyl chloride (1.96 mL (2.91 g, 25.44 mmol) was added dropwise to the resulting product at 0°C in a nitrogen atmosphere. The mixture was stirred at 0 °C for 30 minutes and then allowed to reach room temperature with vigorous stirring for 2 h. The mixture was washed with water three times. The combined organic layers were dried over MgSO$_4$. The solvent was evaporated under reduced pressure to obtain the crude product. Flash column chromatography was used to purify this product through silica gel and using the eluent of hexane and ethyl acetate to furnish **192** (5.5 g, 82%) as a yellow oil.

TLC (silica gel, 2/ 1 Hexane/ EtOAc), R_f= 0.2 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[128]

^{1}H NMR (300 MHz, MeOD) δ 4.85 (br d, 1H, $^3J_{HH}$ = 7.5 Hz, NH), 3.73-3.68 (m, 1H, NHCH), 3.85-3.66 (dd, 1H, $^2J_{HH}$ = 11.0 Hz, $^3J_{HH}$ = 3.6 Hz, OCHH), 3.62-3.56 (dd, 1H, = 11.0 Hz, $^2J_{HH}$ = 5.0 Hz, OCHH), 2.62–2.51 (m, 2H, SCH_2), 3.10 (s, 3H, CH_3SO_2), 2.05 (s, 3H, SCH_3), 1.91–1.75 (m, 2H, SCH_2CH_2), 1.42 (s, 9H, *t*-Bu).

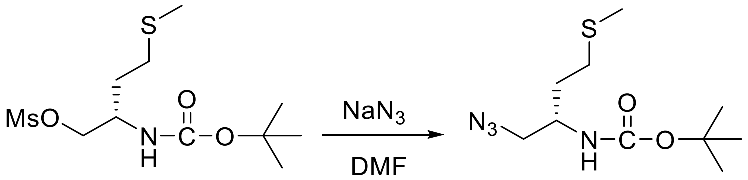
^{13}C NMR (75 MHz, CDCl$_3$) δ 155.2 (C=O), 80.1 (C $(CH_3)_3$), 70.9 (CH_2OS), 48.9 (CH), 37.3 (CH_2), 30.6 (CH_2), 29.2 (CH_2), 28.3 (*t*-Bu), 15.5 (SCH_3).

HRMS-AP+ (m/z): calcd. for $C_{11}H_{24}NO_5S_2$ $[M+H]^+$: 314.1096, found 314.1096

FT-IR (CHCl$_3$ film, cm^{-1}) 3355 (br s, NH), 2981 (m, aliphatic CH), 1677 (s, C=O), 1517 (s, NH), 1361 (m), 1251 (m), 1178 (s).

= −16 (c= 0.22, $CHCl_3$)

(*S*)-*Tert*-butyl 1-azido-4-(methylthio) butan-2-ylcarbamate (193)[128]



192 **193**

Sodium azide (2.06 g. 31.8 mmol) was added to the solution of **192** (5.00 g, 15.9 mmol) in dry dimethylformamide (30 mL). The mixture was heated under reflux for 4 h and was allowed to cool to room temperature. The solvent was evaporated under reduced pressure and then H_2O (30 mL) was added. The aqueous solution was extracted with dichloromethane three times. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. Purification of this compound was achieved by using flash column chromatography through silica gel and using the eluent of hexane and ethyl acetate to get **193** (3.2 g, 76%) as a pale-yellow oil.

TLC (silica gel, 2.2/ 0.8 Hexane/ EtOAc), *R$_f$*= 0.7 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[128]

^{1}H NMR (400 MHz, CDCl$_3$) δ 3.85-3.8 (m, 1H, NHCH), 3.38-3.32 (m, 2H, SCH$_2$), 2.65-2.45 (m, 3H, N$_3$CHH, SCH$_2$CH$_2$), 2.03 (s, 3H, SCH$_3$), 1.77-167 (m, 1H, N$_3$CHH), 1.38 (s, 9H, *t*-Bu).

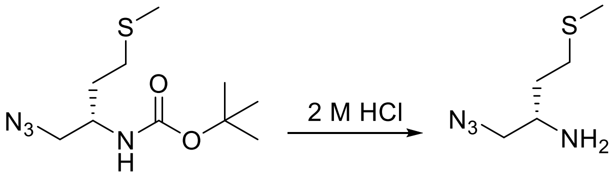
^{13}C NMR (125 MHz, CDCl$_3$) δ 155.2 (C=O), 79.4 (C (CH$_3$)$_3$), 57.6 (N$_3$CH$_2$), 52.3 (NHCH), 36.0 (CH$_2$), 31.7 (CH$_2$), 28.5 (*t*-Bu), 15.7 (SCH$_3$).

HRMS-EI+ (m/z): calcd. for $C_{10}H_{20}N_4O_2S$ $[M]^{\cdot+}$: 260.1307, found 260.1302

FT-IR (CHCl$_3$ film, cm^{-1}) 3476 (br s, NH), 2979 (m, aliphatic CH), 2122 (br s, N$_3$), 1753 (s, C=O), 1515 (s), 1166 (m), 843 (s), 762 (s)

= −38 (c= 0.21, CHCl$_3$)

(*S*)-1-azido-4-(methylthio) butan-2-amine (194)



193 **194**

The general procedure for deprotection of Boc protecting group was followed and then the mixture was made basic by adding sodium bicarbonate. The aqueous layer was extracted with dichloromethane three times. The combined organic layers were dried over magnesium sulfate and then concentrated *in vacuo* to yield **194** as a pale-yellow oil (1.47 g, 80%).

TLC (silica gel, 10/ 1 EtOAc/ MeOH), *R$_f$*= 0.33 (UV)

^{1}H NMR (300 MHz, CD$_3$OD) δ 3.86-3.82 (m, 1H, NHCH), 3.4-3.35 (m, 2H, SCH$_2$), 2.67-2.46 (m, 3H, N$_3$CHH, SCH$_2$CH$_2$), 2.05 (s, 3H, SCH$_3$), 1.79-1.68 (m, 1H, N$_3$CHH).

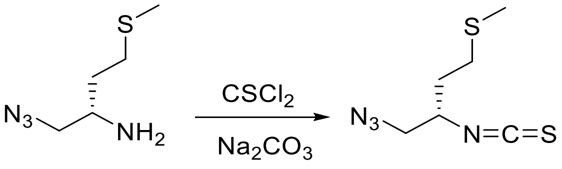
^{13}C NMR (75 MHz, MeOD) δ 59.3 (CH), 58.2 (CH_2N_3), 34.2 (CH_2), 30.1 (CH_2), 18.1 (CH_3).

HRMS-ESI+ (m/z): calcd. for $C_5H_{13}N_4S$ $[M-H]^+$: 161.0861, found 161.0857

FT-IR ($CHCl_3$ film, cm^{-1}) 3370 (br s, NH_2), 2919 (m, aliphatic CH), 2096 (br s, N_3), 1602 (s), 1499 (s)

= −25 (c= 0.18, $CHCl_3$)

(*S*)- (4-azido-3-isothiocyanato butyl) (methyl) sulfane (195)



194 **195**

The general procedure for the synthesis of isothiocyanate compounds from amines was followed to yield **195** as a golden oil (1.15 g, 76%).

TLC (silica gel, 10:0.1% $CHCl_3$/ Methanol), R_f= 0.4 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87-3.82 (m, 1H, SCNCH), 3.39-3.33 (m, 2H, SCH_2), 2.64-2.46 (m, 3H, N_3CHH, SCH_2CH_2), 2.12 (s, 3H, SCH_3), 1.94-1.88 (m, 1H, N_3CHH), 1.39 (s, 9H, *t*-Bu).

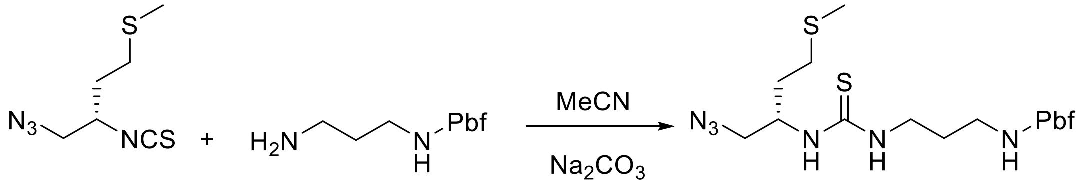
^{13}C NMR (125 MHz, $CDCl_3$) δ 136.1 (NCS), 54.2 (CH_2N_3), 53.3 (CH), 31.6 (CH_2), 30.7 (CH_2), 15.7 (CH_3).

HRMS-ES+ (*m/z*): calcd. for $C_6H_{11}N_4S_2$ $[M+H]^+$: 203.0425, found 203.0429

FT-IR ($CHCl_3$ film, cm^{-1}) 2925 (m, aliphatic CH), 2094 (br s, N_3), 2088 (s, NCS), 1605 (s), 1493 (s)

= −29 (c= 0.215, $CHCl_3$)

(*S*)-*N*-(3-(3-(1-azido-4-(methylthio) butan-2-yl) thioureido) propyl)-2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (196)



195 **183** **196**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **196** as a yellow foam (2.3 g, 80%).

TLC (Silica gel, 2:1 Hexane: EtOAc) R_f= 0.12 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84-3.71 (m, 3H, NHCH, $SCNHCH_2$), 3.70-3.45 (m, 1H, N_3CHH), 3.38-3.32 (m, 2H, SCH_2), 2.99-2.94 (m, 2H, SO_2NHCH_2), 2.96 (s, 2H, furan-CH_2), 2.65-2.58 (m, 2H, SCH_2CH_2), 2.52 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 2.10 (s, 3H, $ArCH_3$), 2.06 (s, 3H, SCH_3), 1.80-1.61 (m, 3H, N_3CHH, $NHCH_2CH_2$), 1.44 (s, 6H, $(CH_3)_2$).

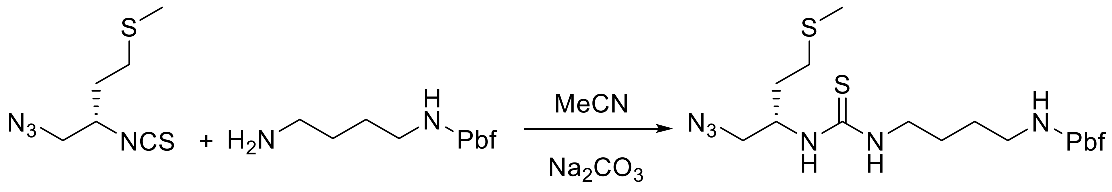
^{13}C NMR (300 MHz, $CDCl_3$) δ 182.1 (C=S), 160.5 (ArC), 139.2 (ArC), 135.2 (ArC), 127.0 (ArC), 125.1 (ArC), 118 (ArC), 87,1 ($C(CH_3)_2$), 54.2 (CH_2), 49.1 (CH_2), 45 (CH), 42.2 (CH_2), 39.5 (CH_2), 33.0 (CH_2),30.2 (CH_2), 28 (CH_2), 27.6 ($C(CH_3)_3$, 19.7 (CH_3), 18.0 (CH_3), 16.6 (CH_3), 13.5 (CH_3).

HRMS-ESI+ (m/z): calcd. for $C_{22}H_{36}N_6O_3S_3$ $[M-H]^+$: 529.2089, found 529.2100

FT-IR ($CHCl_3$ film, cm^{-1}) 3360 (br s, NH), 2950 (s, aliphatic CH), 2096 (s, N_3), 1550 (m), 1453 (m), 1302 (s), 1130 (s), 1089 (s).

= −31 (c= 1.1, $CHCl_3$)

(*S*)-*N*-(4-(3-(1-azido-4-(methylthio) butan-2-yl) thioureido) butyl)-2, 2, 4, 6,7-pentamethyl-2, 3-dihydrobenzofuran-5-sulfonamide (197)



195 **187** **197**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **197** as a yellow foam (261 mg, 78%).

TLC (Silica gel, 2:1 Hexane: EtOAc) R_f= 0.12 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85-3.74 (m, 3H, NHCH, $SCNHCH_2$), 3.73-3.47 (m, 1H, N_3CHH), 3.39-3.33 (m, 2H, SCH_2), 3.12-2.96 (m, 2H, SO_2NHCH_2), 2.97 (s, 2H, furan-CH_2), 2.67-2.61 (m, 2H, SCH_2CH_2), 2.53 (s, 3H, $ArCH_3$), 2.51 (s, 3H, $ArCH_3$), 2.11 (s, 3H, $ArCH_3$), 2.08 (s, 3H, SCH_3), 1.81-1.63 (m, 3H, N_3CHH, $SO_2NHCH_2CH_2$), 1.61-1.58 (m, 2H, $NCSNHCH_2CH_2$), 1.43 (s, 6H, $(CH_3)_2$).

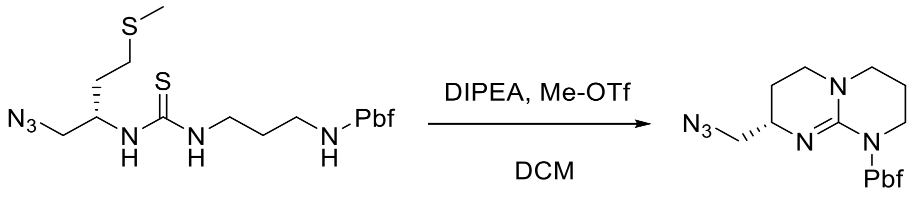
^{13}C NMR (300 MHz, $CDCl_3$) δ 181.4 (C=S), 161.3 (ArC), 138.8 (ArC), 136.1 (ArC), 127.2 (ArC), 125.3 (ArC), 118.3 (ArC), 87.2 ($C(CH_3)_2$), 54.3 (CH_2), 49.2 (CH_2), 45.1 (CH), 42.3 (CH_2), 39.6 (CH_2), 33.1 (CH_2), 30.3 (CH_2), 28.2 (CH_2), 26.4 (CH_2), 27.4 ($C(CH_3)_3$, 19.8 (CH_3), 18.1 (CH_3), 16.7 (CH_3), 13.7 (CH_3).

HRMS-ESI+ (m/z): calcd. for $C_{23}H_{39}N_6O_3S_3$ $[M+H]^+$: 543.2246, found 543.2251

FT-IR ($CHCl_3$ film, cm^{-1}) 3365 (br s, NH), 2952 (s, aliphatic CH), 2093 (s, N_3), 1552 (m), 1454 (m), 1303 (s), 1132 (s), 1090 (s).

= −33 (c= 1.2, $CHCl_3$)

(*S*)-8-(azidomethyl)-1-(2, 2, 4, 6, 7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)-2, 3, 4, 6, 7, 8-hexahydro-1H-pyrimido [1, 2-a] pyrimidine (198)



196 **198**

The general procedure for the cyclisation of thiourea was followed to synthesise **198** as a yellow foam (251.6 mg, 60%).

TLC (silica gel, 10: 0.1% $CHCl_3$: MeOH) 10: 0.1% R_f= 0.21 UV

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93-3.81 (m, 2H, SO_2NCH_2), 3.65-3.61 (m, 1H, N_3CHH), 3.33-3.22 (m, 1H, NCH), 3.15-3.1 (m, 4H, $NCHCH_2CH_2$, $SO_2NCH_2CH_2CH_2$), 3.00 (s, 2H, furan-CH_2),2.98-2.95 (m, 1H, N_3CHH), 2.50 (s, 3H, $ArCH_3$), 2.47 (s, 3H, $ArCH_3$), 2.13 (s, 3H, $ArCH_3$), 2.11-2.02 (m, 2H, $SO_2NCH_2CH_2$), 1.39-1.26 (m, 2H, $NCHCH_2$), 1.51 (s, 6H, 2 × $ArCH_3$).

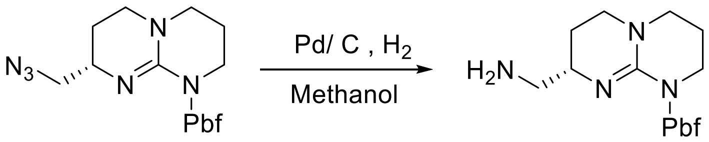
^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4 (ArC), 140.1 (ArC), 135.7 (ArC), 133.7 (ArC), 125.5 (ArC), 118.0 (ArC), 86.4 (C $(CH_3)_2$), 50.3 (CH), 48.3 (CH_2), 47.7 (CH_2), 43.2 (CH_2), 42.1 (CH_2), 40.3 (CH_2), 28.6 (CH_3), 28.3 (CH_3), 26.7 (CH_2), 23.4 (CH_2), 19.3 (CH_3), 17.2 (CH_3), 13.5 (CH_3).

HRMS-ES+ (*m/z*): calcd. for $C_{21}H_{30}N_6O_3S$ $[M+H]^+$: 447.2173, found 447.2168

FT-IR ($CHCl_3$ film, cm^{-1}) 2954 (s, aliphatic CH), 2091(s, N_3), 1634 (s,), 1426 (m), 1317 (s), 1139 (s), 1093 (s).

= −42 (c= 0.12, $CHCl_3$)

(*S*)-(1-(2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-ylsulfonyl)-2, 3, 4, 6, 7, 8-hexahydro-1H-pyrimido [1, 2-a] pyrimidin-8-yl) methanamine (201)



198 **201**

The general procedure for the hydrogenation of azides to amine was followed to yield **201** as a colourless oil (139 mg, 84%).

TLC (silica gel, 10: 0.5% $CHCl_3$: MeOH) *R*R_f= 0.32 UV

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93-3.81 (m, 2H, SO_2NCH_2), 3.78-3.71 (m, 1H, NH_2CHH), 3.33-3.22 (m, 1H, NCH), 3.21-3.1 (m, 5H, $NCHCH_2CH_2$, $SO_2NCH_2CH_2CH_2$, NH_2CHH), 3.03 (s, 2H, furan-CH_2), 2.51 (s, 3H, $ArCH_3$), 2.46 (s, 3H, $ArCH_3$), 2.14 (s, 3H, $ArCH_3$), 2.12-2.03 (m, 2H, $SO_2NCH_2CH_2$), 1.39-1.28 (m, 2H, $NCHCH_2$), 1.52 (s, 6H, 2 × $ArCH_3$).

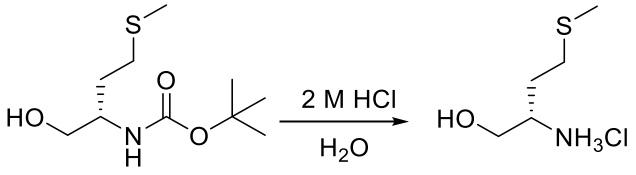
^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1 (ArC), 140.1 (ArC), 135.7 (ArC), 133.7 (ArC), 125.5 (ArC), 118.0 (ArC), 88.1 (C $(CH_3)_2$), 55.1 (CH), 49.2 (CH_2), 47.8 (CH_2), 42.3 (CH_2), 42.2 (CH_2), 41.3 (CH_2), 29.5 (CH_3), 28.4 (CH_3), 26.8 (CH_2), 24.2 (CH_2), 19.2 (CH_3), 17.5 (CH_3), 13.8 (CH_3).

HRMS-ES+ (*m/z*): calcd. for $C_{21}H_{33}N_4O_3S$ $[M+H]^+$: 421.2273, found 421.2276

FT-IR ($CHCl_3$ film, cm^{-1}) 3445 (br m, NH_2), 2953 (s, aliphatic CH), 1633 (s), 1426 (m), 1317 (s), 1140 (s), 1093 (s).

= −40 (c= 0.18, MeOH)

(*S*)-2-Amino-4-(methylthio) butan-1-ol hydrochloride (206)[122]



191 **206**

The general procedure for deprotection of Boc protecting group was followed to yield **206** as a pale yellow waxy solid (2.73 g, 94% yield).

TLC (silica gel, 10% AcOH/ EtOH), R_f = 0.46 (UV)

The spectroscopic data are in agreement with those recorded in the literature[122]

^{1}H NMR (400 MHz, D_2O) δ 3.87-3.82 (m, 1H, HOCHH), 3.70-3.65 (m, 1H, HOCHH), 3.49-3.43 (m, 1H, NH_2CH), 2.64 (m, 2H, SCH_2), 2.15 (s, 3H, SCH_3), 2.02-1.87 (m, 2H, SCH_2CH_2).

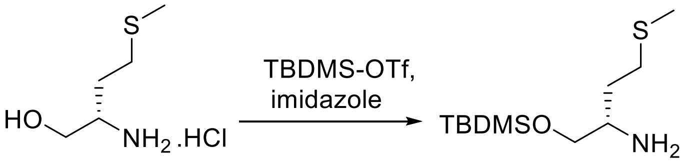
^{13}C NMR (100 MHz, D_2O) δ 60.7 (CH_2OH), 53.3 (CH), 28.8 (CH_2), 28.6 (CH_2), 14.6 (CH_3).

ESI+ MS (*m/z*): 172 $[M+H]^+$

FT-IR ($CHCl_3$ film, cm^{-1}) 3378 (br s, NH_2), 2917 (br s, aliphatic CH), 1604 (s, NH), 1497 (s), 1051 (s, C-O).

= −22 (c= 0.2, $CHCl_3$)

(*S*)-1-((*tert*-butyldimethylsilyl) oxy)-4-(methylthio) butan-2-amine (207)[122]



206 **207**

Methioninol hydrochloride **206** (2.28 g, 13.38 mmol) and imidazole (1.82 g, 26.76 mmol) were suspended in anhydrous acetonitrile. *Tert*-butyldimethylsilyl triflate 3.66 mL (4.22 g, 15.98 mmol) was then added dropwise to the resulting suspension at 0 °C under a nitrogen atmosphere. The mixture was stirred vigorously at this temperature for 2 h and then it was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was stirred with 2 M NaOH (50 mL) for 15 minutes and then extracted with dichloromethane three times. The organic phase was washed with water three times to remove the excess of imidazole. The solvent was evaporated under reduced pressure. The residue was dissolved in the solution of $H_2O/CH_3CN/CH_3COOH$ (60/40/2, 3 × 35 mL). The combined aqueous phases were washed with hexane three times and then acetonitrile was evaporated *in vacuo*. The solution was made basic by adding sodium bicarbonate. The aqueous solution was extracted with dichloromethane (3 × 10 mL). The combined organics were dried over $MgSO_4$ and evaporated *in vacuo* to obtain **207** (1.8 g, 53%) as a colourless oil. No further purification was needed.

TLC (silica gel, 10:1 $CHCl_3$/ MeOH), $R_f = 0.45$ (ninhydrin)

The spectroscopic data are in agreement with those recorded in the literature[122]

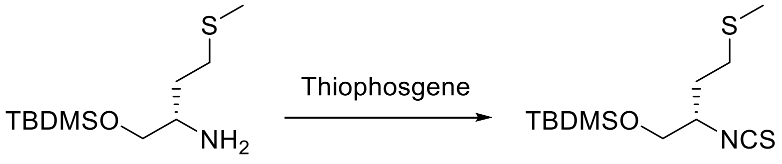
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57-3.51 (m, 1H, OCH*H*), 3.37 (m, 1H, OCH*H*), 2.88-2.84 (m, 1H, NH_2CH), 2.62-2.52 (m, 2H, SCH_2), 2.11 (s, 3H, SCH_3), 1.72-1.64 (m, 1H, SCH_2CHH), 1.52-1.44 (m, 1H, SCH_2CHH), 0.88 (s, 9H, *t*-Bu), 0.02 (s, 6H, $Si(CH_3)_2$).

^{13}C NMR (100 MHz, $CDCl_3$) δ 68.7 (CH_2OH), 52.3 (CH), 33.5 (CH_2), 31.7 (CH_2), 26.4 (*t*-Bu), 18.8 (C $(CH_3)_3$), 16.2 (CH_3), −5.1 ($Si(CH_3)_2$).

ESI+ MS (m/z): 250 $[M+H]^+$

FT-IR ($CHCl_3$ film, cm^{-1}) 3377 (s, NH_2), 2926 (s, aliphatic CH), 1474 (m, NH), 1258 (s, C-O), 1104 (s).

<u>(*S*)-*Tert*-butyl (2-isothiocyanato-4-(methylthio) butoxy) dimethylsilane (208)</u>[122]



207 **208**

The general procedure for the synthesis of isothiocyanate compounds from amines was followed to yield **208** as a brown oil (1.7 g, 97%).

TLC (silica gel, 2:1 hexane/ EtOAc), R_f = 0.45 (UV)

The spectroscopic data are in agreement with those recorded in the literature[122]

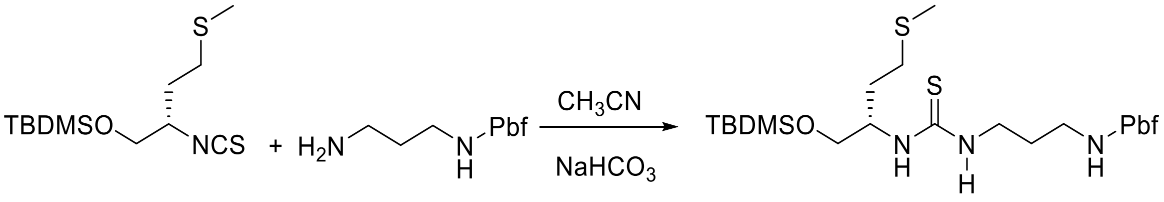
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89-3.87 (m, 1H, SCNCH), 3.74-3.69 (m, 2H, OCH_2), 2.55-2.63 (m, 1H, SCHH), 2.61-2.54 (m, 1H, SCHH), 2.11 (s, 3H, SCH_3), 1.88-1.86 (m, 2H, SCH_2CH_2), 0.81 (s, 9H, *t*-Bu), 0.01 (s, 6H, $Si(CH_3)_2$).

^{13}C NMR (100 MHz, $CDCl_3$) δ 135.0 (NCS), 65.4 (CH_2OH), 59.0 (CH), 31.6 (CH_2), 30.7 (CH_2), 25.9 (*t*-Bu), 18.4 (C $(CH_3)_3$), 15.7 (CH_3), -5.3 (Si $(CH_3)_2$).

FT-IR ($CHCl_3$ film, cm^{-1}) 2960 (s, aliphatic CH), 2088 (br s, NCS), 1135 (S, C-O), 830 (S)

CI+ (m/z): 291 $[M+H]^+$

(*S*)-2, 2, 4, 6, 7-pentamethyl-*N*-(2, 2, 3, 3-tetramethyl-6-(2-(methylthio) ethyl)-8-thioxo-4-oxa-7, 9-diaza-3-siladodecan-12-yl)-2, 3-dihydrobenzofuran-5-sulfonamide (232)



208 **183** **232**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and an amine compound was followed to yield **232** as a pale-yellow foam (0.833 g, 89.4%).

TLC (silica gel, 2:1 hexane/ EtOAc), R_f = 0.3 (UV)

^{1}H NMR (400 MHz, CDCl$_3$) δ 3.70-3.63 (m, 4H, OCH_2, $SCNHCH_2$), 2.97-2.92 (m, 2H, SO_2NHCH_2), 2.95 (s, 2H, furan-CH_2), 2.61-2.51 (m, 2H, SCH_2), 2.54 (s, 3H, $ArCH_3$), 2.47 (s, 3H, $ArCH_3$), 2.13 (s, 3H, $ArCH_3$), 2.09 (s, 3H, SCH_3), 1.83-1.55 (m, 5H, SCNHCH, SCH_2CH_2, $NHCH_2CH_2$), 1.46 (s, 6H, $(CH_3)_2$), 0.87 (s, 9H, *t*-Bu), 0.06 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$).

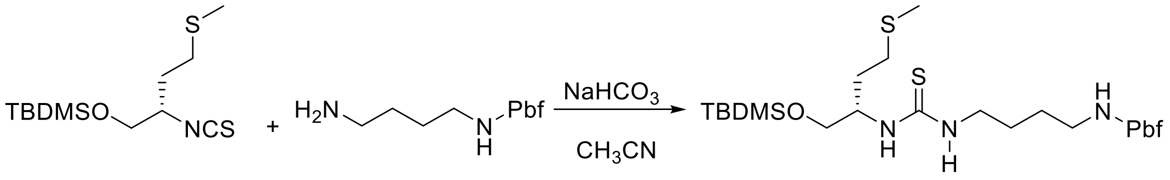
^{13}C NMR (100 MHz, CDCl$_3$) δ 182.6 (C=S), 162.1 (ArC), 155.3 (ArC), 139.3 (ArC), 135.7 (ArC), 125.4 (ArC), 118.2 (ArC), 86.7 ($C(CH_3)_2$), 43.3 (CH_2), 39.3 (CH_2), 34.6 (CH_2), 31.0 (CH_2), 28.8 ($C(CH_3)_2$), 25.0 (*t*- Bu), 19.7 (CH_3), 18.6 ($C(CH_3)_3$), 18.0 (CH_3), 15.6 (CH_3), 12.7 (CH_3), −5.1 ($Si(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{28}H_{52}N_3O_4S_3Si$ $[M+H]^+$: 618.2889 found 618.2892

FT-IR (CHCl$_3$ film, cm^{-1}) 3371 (br s, NH), 2954 (s, aliphatic CH), 1552 (m), 1459 (m), 1305 (s), 1135 (s), 1093 (s).

= −24 (c= 1.1, $CHCl_3$)

(*S*)-2, 2, 4, 6, 7-pentamethyl-N-(2, 2, 3, 3-tetramethyl-6-(2-(methylthio) ethyl)-8-thioxo-4-oxa-7, 9-diaza-3-silatridecan-13-yl)-2, 3-dihydrobenzofuran-5-sulfonamide (232A)



208 **187** **232A**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **232A** as a pale-yellow foam (0.89 g, 82%).

TLC (silica gel, 2:1 hexane/ EtOAc), R_f = 0.35 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66-3.52 (m, 4H, OCH_2, $SCNHCH_2$), 3.41-3.32 (m, 2H, SO_2NHCH_2), 2.92 (s, 2H, furan-CH_2), 2.9-2.81 (m, 2H, $SO_2NHCH_2CH_2$), 2.61-2.51 (m, 2H, SCH_2), 2.47 (s, 3H, $ArCH_3$), 2.41 (s, 3H, $ArCH_3$), 2.13 (s, 3H, $ArCH_3$), 2.04 (s, 3H, SCH_3), 1.83-1.55 (m, 5H, SCNHCH, SCH_2CH_2, $NHCH_2CH_2$), 1.41 (s, 6H, $(CH_3)_2$), 0.87 (s, 9H, *t*-Bu), 0.01 (s, 6H, $Si(CH_3)_2$.

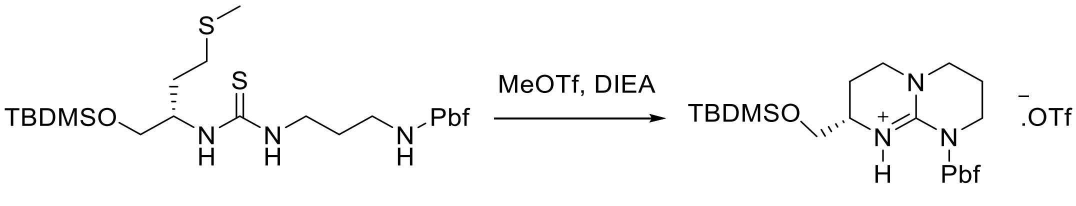
^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5 (C=S), 161.2 (ArC), 152.4 (ArC), 139.5 (ArC), 136.2 (ArC), 125.7 (ArC), 118.5 (ArC), 86.2 (C(CH3)2), 43.3 (CH_2), 38.9 (CH_2), 34.8 (CH_2), 33.2 (CH_2), 31.1 (CH_2), 28.9 ($C(CH_3)_2$), 25.7 (*t*- Bu), 18.9 (CH_3), 18.7 ($C(CH_3)_3$), 18.6 (CH_3), 15.7 (CH_3), 12.8 (CH_3), −5.3 ($Si(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{29}H_{52}N_3O_4S_3Si$ $[M-H]^-$: 630.2889 found 630.2862

FT-IR ($CHCl_3$ film, cm^{-1}) 3373 (br s, NH), 2951 (s, aliphatic CH), 1553 (m), 1457 (m), 1304 (s), 1134 (s), 1092 (s).

= −22 (c= 1.38, $CHCl_3$)

(*S*)-8-(((*tert*-butyldimethylsilyl) oxy) methyl)-1-((2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-yl) sulfonyl)-1, 3, 4, 6, 7, 8-hexahydro-2H-pyrimido [1, 2-a] pyrimidine (233)

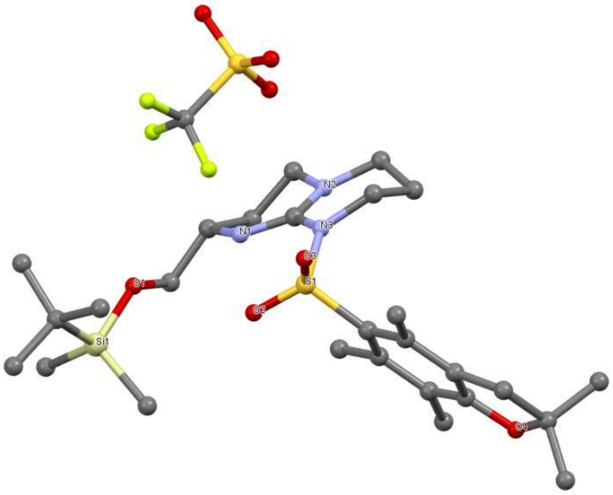


232 **233**

The general procedure for the cyclisation of thiourea was followed to obtain **233** as a yellow powder (64 mg, 80%).

TLC (silica gel, EtOAc: 1% TEA), R_f = 0.4 (UV, CAM).

X-Ray Crystallographic structure for **233**



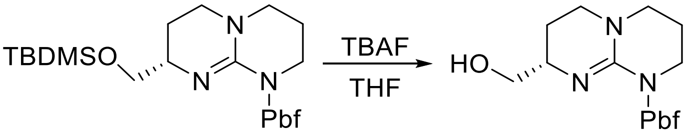
^{1}H NMR (400 MHz, CDCl$_3$) δ 3.92-3.79 (m, 2H, SO_2NCH_2), 3.62-3.60 (m, 1H, OCHH), 3.33-3.28 (m, 1H, NCH), 3.12-2.99 (m, 4H, $NCHCH_2CH_2$, $SO_2NCH_2CH_2CH_2$), 2.83-2.80 (m, 1H, OCHH), 3.00 (s, 2H, furan-CH_2), 2.52 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 2.13 (s, 3H, $ArCH_3$), 2.06-1.93 (m, 3H, $SO_2NCH_2CH_2$, $NCHCH_2$), 1,46 (s, 6H, $(CH_3)_2$), 1.37-1.29 (m, 1H, NCHCHH), 0.85 (s, 9H, *t*-Bu), 0.01 (s, 6H, $Si(CH_3)_2$).

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.1 (ArC), 143.2 (ArC), 137.7 (ArC), 132.7 (ArC), 124.3 (ArC), 117.1 (ArC), 86.4 (C(CH$_3$)$_2$), 60.3 (CH$_2$), 50.3 (CH), 48.3 (CH$_2$), 47.7 (CH$_2$), 43.2 (CH$_2$), 42.1 (CH$_2$), 40.3 (CH$_2$), 28.5 (CH$_3$), 28.5 (CH$_3$), 27.7 (CH$_2$), 25.9 (*t*-Bu), 23.3 (CH$_2$), 19.1 (CH$_3$), 18.1 (C(CH$_3$)$_3$), 17.2 (CH$_3$), 12.5 (CH$_3$), -5.3 (Si(CH$_3$)$_2$)

HRMS-ES+ (*m/z*): calcd. for $C_{28}H_{52}N_3O_4S_3Si$ $[M+H]^+$: 536.2978 found 536.2999

FT-IR (CHCl$_3$ film, cm^{-1}) 3445 (br m, NH), 2955 (s, aliphatic CH), 1636 (s,), 1426 (m), 1317 (s), 1136 (s), 1095 (s).

(*S*)-(9-((2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-yl) sulfonyl)-3, 4, 6, 7 , 8, 9-hexahydro-2H-pyrimido[1, 2-a] pyrimidin-2-yl) methanol (234)



233 **234**

Compound **233** (150 mg, 0.35 mmol) was dissolved in tetrahydrofuran (5 mL). TBAF (1 M in THF, 433 µl, 0.40 mmol) was added dropwise to the resulting mixture. The suspension was stirred for 20 h at room temperature. The solvent was evaporated under reduced pressure and the residue was washed with ether (3×10 mL). The result was concentrated *in vacuo* and purified by using flash column chromatography through silica gel and using the eluent of chloroform and methanol to get a white solid **234** after removal of solvent (81 mg, 55%).

TLC (silica gel, 50:1 CHCl$_3$/ MeOH), R_f = 0.36 (CAM)

^{1}H NMR (400 MHz, CD$_3$OD) δ 3.91-3.86 (m, 1H, SO$_2$NCHH), 3.76-3.72 (m, 1H, SO$_2$NCHH), 3.53-3.50 (m, 1H, HOCHH), 3.29- 3.24 (m, 1H, NCH), 3.18 (s, 2H, furan-CH$_2$), 3.15-3.10 (m, 2H, HOCHH, NCHCH$_2$CH$_2$), 3.06-3.04 (m, 2H, SO$_2$NCH$_2$CH$_2$CH$_2$), 2.96-2.92 (m, 1H, NCHCH$_2$CH$_2$), 2.32 (s, 3H, ArCH$_3$), 2.08-1.91 (m, 2H, SO$_2$NCH$_2$CH$_2$), 1.64-1.51 (m, 2H, NCHCH$_2$). 2.59 (s, 3H, ArCH$_3$), 2.58 (s, 3H, ArCH$_3$), 2.28-2.23 (m, 1H, OCH$_2$CHH), 2.22 (s, 3H, ArCH$_3$), 2.04-1.89 (m, 5H, NCHCH$_2$, SO$_2$NCH$_2$CH$_2$, OCH$_2$CHH), 1.57 (s, 6H, ArCH$_3$).

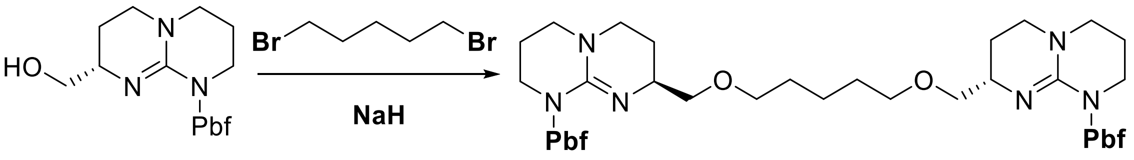
^{13}C NMR (100 MHz, CDOD) δ 159.3 (ArC), 144 (ArC), 138.5 (ArC), 134.8 (ArC), 124.6 (ArC), 117.2 (ArC), 86.5 ($C(CH_3)_2$), 60.2 (CH_2), 50.1 (CH), 48.4 (CH_2), 43.4 (CH_2), 42.3 (CH_2), 40.5 (CH_2), 28.6 (CH_3), 28.3 (CH_3), 27.8 (CH_2), 26 (*t*-Bu), 23.5 (CH_2), 19.1 (CH_3), 18.3 ($C(CH_3)_3$), 17.2 (CH_3), 12.6 (CH_3), -5.4 ($Si(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{21}H_{32}N_3O_4S$ $[M+H]^+$: 422.2114 found 422.2110

FT-IR ($CHCl_3$ film, cm^{-1}) 3478 (br m, OH), 2856 (m, aliphatic CH), 1593 (s), 1494 (m), 1313 (s), 1165(s), 1093 (m), 1037 (m).

= −45 (c= 0.14, MeOH)

1,5-bis(((S)-9-((2, 2, 4, 6, 7-pentamethyl-2, 3-dihydrobenzofuran-5-yl) sulfonyl)-3, 4, 6, 7, 8, 9-hexahydro-2H-pyrimido [1, 2-a] pyrimidin-2-yl) methoxy) pentane (236)



234 **236**

Sodium hydride (10 mg, 0.41 mmol) was added to the solution of compound **234** (90 mg, 0.21 mmol) in dichloromethane (20 mL). The mixture was stirred vigorously at room temperature for 30 min. 1,5-Dibromopentane (24 mg, 0.1 mmol) was added to the resulting product. The mixture was stirred at room temperature for 24 h and then H_2O (20 mL) was added. The aqueous layer was extracted with dichloromethane (3 × 10 mL). The organic layer was washed with water and then with brine. The combined organic layers were dried over $MgSO_4$ and then concentrated to get crude compound. The yield of compound **236** after purification through silica gel and using the eluent of chloroform and methanol was (73.5 mg, 77%) as a colourless oil.

TLC (silica gel, 50:1 $CHCl_3$/ MeOH), R_f = 0.22 (UV)

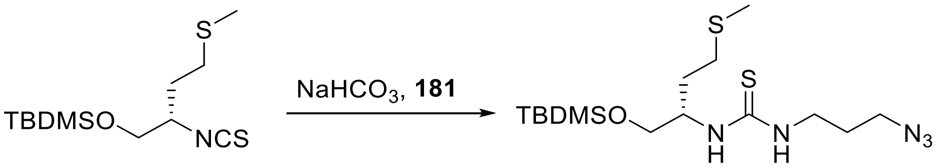
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56-3.46 (t, 4H, OCH_2CH_2, *J*= 7.6 Hz), 3.4-3.3 (m, 2H. NCH), 3.25-3.13 (m, 4H, $CHCH_2O$), 3.11-3.03 (m, 4H, $SNCH_2$), 2.8-2.91 (m, 4H, NCH_2), 3.06 (s, 4H, CH_2-Furan), 2.56 (s, 6H, $2CH_3$), 2.5 (s, 6H, $2CH_3$),1.9-1.81 (m, 4H, $NCHCH_2$) 2.13 (s, 6H, $2CH_3$), 1.74-153 (m, 10H, NCH_2CH_2, $OCH_2CH_2CH_2$) , 1.49 (s, 12H, $4CH_3$, Furan), 1.4-1.25 (m, 4H, OCH_2CH_2).

13C NMR (100 MHz, $CDCl_3$) δ 159.3 (ArC), 138.9 (ArC), 136.1 (ArC), 133.8 (ArC), 125.3 (C=N), 124.8 (ArC), 116.7 (ArC), 86.5 (C $(CH_3)_2$), 78.5 (CH_2), 76.1 (CH_2), 74.2 (CH_2), 54.3 (CH), 48.1 (CH_2), 45.3 (CH_2), 42.5 (CH_2), 41.2 (CH_2), 32.1 (CH_2), 29.3 (CH_3), 25.1 (CH_2), 22.4 (CH_2), 23.3 (CH_2), 19.2 (CH_3), 18.2 (CH_3), 17.1 (CH_3).

MS-ESI: ***m/z*** = 911.4 $[M+H]^+$

FT-IR ($CHCl_3$ film, cm^{-1}) 2866 (m, aliphatic CH), 1593 (s), 1494 (m), 1313 (s), 1137 (s, C-O), 1093 (m), 1037 (m).

(*S*)-1-(3-azidopropyl)-3-(1-((tert-butyldimethylsilyl) oxy)-4-(methylthio) butan-2-yl) thiourea (209)



208 **209**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **209** as a pale-yellow oil (0.83 g, 73%).

TLC (silica gel, 2:1 hexane/ EtOAc), *R$_f$*= 0.31 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67-3.56 (m, 3H, OCH_2, NHCH), 3.4-3.29 (m, 2H, $NHCH_2$), 2.53-2.45 (t, 2H, SCH_2, *J*=7.6 Hz) 2.03 (s, 3H, SCH_3), 1.81-1.72 (m, 2H, SCH_2CH_2), 1.6-151 (m, 4H, N_3CH_2, $N_3CH_2CH_2$), 0.82 (s, 9H, C $(CH_3)_3$), 0.01 (s, 6H, Si $(CH_3)_2$).

^{13}C NMR (300 MHz, $CDCl_3$) δ 181.1 (CS), 67.2 (CH_2), 55.1 (CH), 50.5 (CH_2), 49.1 (CH_2), 33.2 (CH_2), 29.2 (C $(CH_3)_3$), 27.1 (CH_3), 25.3 (C $(CH_3)_3$, 19.2 (CH_2), 13.5 (SCH_3), -5.3 ($Si(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{15}H_{34}N_5OS_2Si$ $[M+H]^+$: 392.1969, found 392.1969

FT-IR ($CHCl_3$ film, cm^{-1}) 3363 (br s, NH), 2952 (s, aliphatic CH), 2089 (s, N_3), 1551 (m), 1456 (m), 1303 (s), 1137 (s), 1090 (s).

= −25 (c= 0.14, $CHCl_3$)

(*S*)-1-(4-azidobutyl)-3-(1-((*tert*-butyldimethylsilyl) oxy)-4-(methylthio) butan-2-yl) thiourea (210)

208 **210**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **210** as a colourless oil (2.1 g, 75%).

TLC (silica gel, hexane: diethyl ether), R_f= 0.08 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.55 (m, 2H, OCH_2),3.41-3.39 (m, 2H $SCNHCH_2$), 3.29-3.21 (m, 3H, NHCH, SCH_2), 2.5-2.38 (m, 2H, $NHCH_2CH_2$), 1.99 (s, 3H, SCH_3), 1.89-1.52 (m, 4H, SCH_2CH_2, N_3CH_2), 1.51-1.49 (m, 2H, $N_3CH_2CH_2$), 0.89 (s, 9H, *t*-Bu), 0.01 (s, 6H, $Si(CH_3)_2$).

^{13}C NMR (100 MHz, MeOD) δ 182 (CS), 62.4 (CH_2), 58.3 (CH), 51.3 (CH_2), 31.1 (CH_2), 29,3 (C $(CH_3)_3$), 28.4 (CH_2), 26.1 (CH_2), 23.3 (CH_2), 22.9 (CH_2), 22.5 (*t*-Bu), 15.9 (SCH_3), -5.2 (Si $(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{16}H_{36}N_5OS_2Si$ $[M+H]^+$: 406.2125, found 406.2130

FT-IR ($CHCl_3$ film, cm^{-1}) 3368 (br s, NH), 2954 (s, aliphatic CH), 2087 (s, N_3), 1554 (m), 1453 (m), 1301 (s), 1135 (s), 1092 (s).

= −22 (c= 0.55, $CHCl_3$)

(*S*)-1-(5-azidopentyl)-3-(1-((*tert*-butyldimethylsilyl) oxy)-4-(methylthio) butan-2-yl) thiourea (211)

208 **211**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amino compound was followed to yield **211** as a colourless oil (518 mg, 75%).

TLC (silica gel, hexane: diethyl ether), R_f= 0.12 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68-3.58 (m, 2H, OCH_2), 3.27-3.16 (m, 3H, NCH, $NHCH_2$), 2.55-2.44 (m, 2H, SCH_2), 2.03 (s, 3H, SCH_3), 1.83-1.72 (m, 2H, SCH_2CH_2), 162-1.48 (m, 4H, N_3CH_2, $NHCH_2CH_2$), 1.4-131 (m, 2H, $N_3CH_2CH_2CH_2$), 1.29-1287 (m, 2H, $N_3CH_2CH_2$), 0.82 (s, 9H, *t*-Bu), 0.01 (s, 6H, $Si(CH_3)_2$).

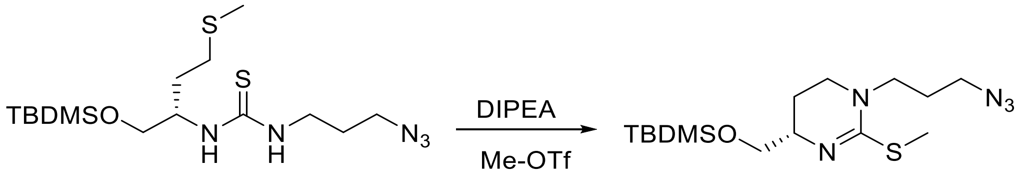
^{13}C NMR (100 MHz, MeOD) δ 181.9 (CS), 63.6 (CH_2), 58.2 (CH), 51.4 (CH_2), 50.2 (CH_2), 49.1 (CH_2), 48.3 (CH_2), 32.1 (CH), 27.5 (CH_2), 25.8 (*t*-Bu), 24.1 (CH_2), 18.2 (CH_2), 15.5 (SCH_3), −5.7 (Si $(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{17}H_{38}N_5OS_2Si$ $[M+H]^+$: 420.2287, found 420.2281.

FT-IR ($CHCl_3$ film, cm^{-1}) 3366 (br s, NH), 2952 (s, aliphatic CH), 2088 (s, N_3), 1556 (m), 1454 (m), 1302 (s), 1137 (s), 1095 (s).

= −30 (c= 0.54, $CHCl_3$)

(S)-1-(3-azidopropyl)-4-(((tert-butyldimethylsilyl) oxy) methyl)-2-(methylthio)-1, 4, 5, 6-tetrahydropyrimidine (212)



209 **212**

The general procedure for the cyclisation of thiourea was followed to obtain **212** (1.24 g, 68%) as a colourless oil.

TLC (silica gel, 2:1 hexane/ EtOAc), R_f= 0.3 (UV)

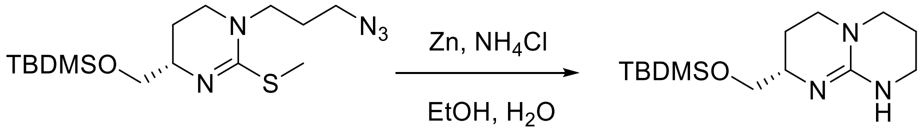
^{1}H NMR (300 MHz, MeOD) δ 3.8-3.71 (m, 2H, OCH_2), 3.4-3.31 (m, 4H, NCH_2, N_3CH_2), 3.25-3.1 (m, 3H, NCH_2CH_2, NCH), 2.21 (s, 3H, SCH_3), 1.61-1.48 (m, 4H, $N_3CH_2CH_2$, NCH_2), 0.82 (s, 9H, *t*-Bu), 0.01 (s, 6H, Si $(CH_3)_2$).

^{13}C NMR (300 MHz, $CDCl_3$) δ 154.6 (C=N), 67.7 (CH_2), 56.2 (CH), 52.9 (CH_2), 50.9 (CH_2), 49.1 (CH_2), 45.2 (CH_2), 27.2 (C $(CH_3)_2$), 26.1 (CH_2), 25.0 (C $(CH_3)_3$), 13.2 (SCH_3), −5.3 (Si $(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. For $C_{15}H_{32}N_5OSSi$ $[M+H]^+$: 358.2097, found 358.2106

= −33 (c= 0.26, $CHCl_3$)

(*S*)-8-(((*tert*-butyldimethylsilyl) oxy) methyl)-1, 3, 4, 6, 7, 8-hexahydro-2H-pyrimido [1, 2-a] pyrimidine (215)



212 **215**

The general procedure for the hydrogenation of azides to amines by using activated zinc and ammonium chloride was followed to yield **215** as white foam (2.3 g, 85%).

TLC (silica gel, 10:1 CH_3Cl/ MeOH), *R$_f$*= 0.66 (UV)

^{1}H NMR (400 MHz, MeOD) δ 3.61-3.52 (m, 2H, OCH_2), 3.45-3.35 (m, 2H, $NHCH_2$), 3.21-3.15 (m, 4H, NCH_2, NCH_2), 3.14-3.091 (m, 4H, $NHCH_2CH_2$), 1.82-1.68 (m, 2H, NCH_2CH_2, 0.84 (s, 9H, *t*-Bu), 0.01 (s, 6H, Si $(CH_3)_2$).

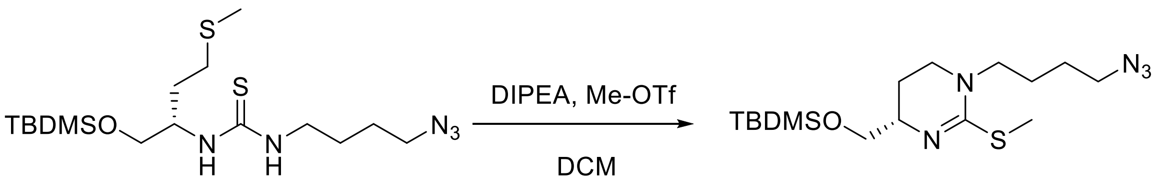
^{13}C NMR (100 MHz, MeOD) δ 153.5 (C=N), 68.3 (CH_2), 58.7 (CH), 45.7 (CH_2), 43.5 (CH_2), 42.3 (CH_2), 30.5 (C $(CH_3)_3$), 25.9 (*t*-Bu), 23.7 (CH_2), 21.3 (CH_2), −5.5 (Si $(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{14}H_{29}N_3OSi$ $[M+H]^+$: 284.2158, found 284.2166

FT-IR ($CHCl_3$ film, cm^{-1}) 3450 (br m, NH), 2953 (s, aliphatic CH), 1631 (s,), 1429 (m), 1317 (s), 1136 (s, C-O), 1093 (s).

= −45 (c= 0.133, $CHCl_3$)

(*S*)-1-(4-Azidobutyl)-4-(((*tert*-butyldimethylsilyl) oxy) methyl)-2-(methylthio)-1, 4, 5, 6-tetrahydropyrimidine (213)



210 **213**

The general procedure for the cyclisation of thiourea was followed to yield **213** as a colourless oil (1.1 g, 65%).

TLC (silica gel, diethyl ether), R_f= 0.52 (UV)

^{1}H NMR (300 MHz, MeOD) δ 3.9-3.73 (m, 2H, OCH_2), 3.6-3.34 (m, 4H, NCH_2, N_3CH_2), 3.31-2.9 (m, 3H, NCH_2CH_2, NCH), 2.21 (s, 3H, SCH_3), 1.64-1.49 (m, 4H, $N_3CH_2CH_2$, NCH_2), 1.39-1.28 (m, 2H, $NCHCH_2$), 0.83 (s, 9H, *t*-Bu), 0.01 (s, 6H, Si $(CH_3)_2$).

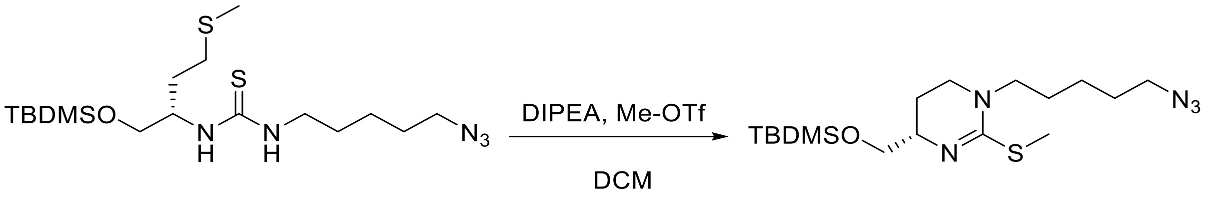
^{13}C NMR (300 MHz, $CDCl_3$) δ 151.0 (C=N), 68.2 (CH_2), 57.1 (CH), 53.3 (CH_2), 49.1 (CH_2), 48.8 (CH_2), 33.1 (C $(CH_3)_2$), 29.3 (CH_2), 28.2 (CH_2), 26.1 (C $(CH_3)_3$, 24.3 (CH_2), 12.2 (CH_3), -1 (Si $(CH_3)_2$.

HRMS-ES+ (*m/z*): calcd. for $C_{16}H_{34}N_5OSSi$ $[M+H]^+$: 372.2253, found 372.2258

FT-IR ($CHCl_3$ film, cm^{-1}) 3366 (br s, NH), 2955 (s, aliphatic CH), 2089 (s, N_3), 1552 (m), 1453 (m), 1303 (s), 1130 (s, C-O), 1090 (s).

= −35 (c= 0.14, $CHCl_3$)

(*S*)-1-(5-azidopentyl)-4-(((*tert*-butyldimethylsilyl) oxy) methyl)-2-(methylthio)-1, 4, 5, 6-tetrahydropyrimidine (214)



211 **214**

The general procedure for the cyclisation of thiourea was followed to yield **214** as a colourless oil (1.8 g, 66%).

TLC (silica gel, diethyl ether), R_f= 0.62 (UV)

^{1}H NMR (300 MHz, MeOD) δ 4.1-3.83 (m, 2H, OCH_2), 3.75-3.38 (m, 4H, NCH_2, N_3CH_2), 3.34-2.95 (m, 3H, NCH_2CH_2, NCH), 2.22 (s, 3H, SCH_3), 1.71-1.52 (m, 4H, $N_3CH_2CH_2$, NCH_2), 1.42-1.29 (m, 4H, $NCHCH_2$, $N_3CH_2CH_2CH_2$), 0.83 (s, 9H, *t*-Bu), 0.01 (s, 6H, Si $(CH_3)_2$).

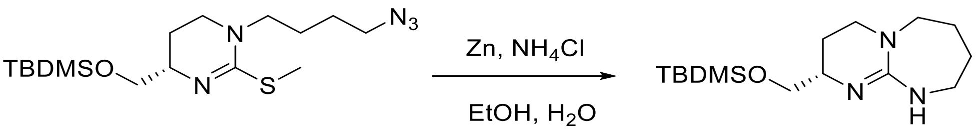
^{13}C NMR (300 MHz, $CDCl_3$) δ 158.1 (C=N), 62.3 (OCH_2), 52 (CH_2), 51.2 (CH_2), 50.5 (CH_2), 49.8 (CH_2), 40.2 (C $(CH_3)_3$), 29.1 (CH_2), 28.0 (CH_2), 27.6 (CH_2), 24.1 (C $(CH_3)_3$, 23.2 (CH_2), 18.2 (SCH_3), 0.4 (Si $(CH_3)_2$.

HRMS-ES+ (*m/z*): calcd. for $C_{17}H_{36}N_5OSSi$ $[M+H]^+$: 386.2415, found 386.2410

FT-IR ($CHCl_3$ film, cm^{-1}) 3363 (br s, NH), 2952 (s, aliphatic CH), 2091(s, N_3), 1553 (m), 1455 (m), 1302 (s), 1131 (s, C-O), 1091 (s).

= −38 (c= 0.2, $CHCl_3$)

(*S*)-2-(((*tert*-butyldimethylsilyl) oxy) methyl)-2, 3, 4, 6, 7, 8, 9, 10-octahydropyrimido [1, 2-a] [1, 3] diazepine (216)



213 **216**

The general procedure for the hydrogenation of azides to amines by using activated zinc and ammonium chloride was followed to yield **216** as a white foam (686.5 mg, 70%).

TLC (silica gel, EtOAc: 1% TEA) R_f= 0.11 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71-3.55 (dd, 2H, OCH_2), 3.51-3.32 (m, 4H, (NCH_2, $NHCH_2$), 3.1-2.89 (m, 2H, $NCHCH_2CH_2$), 1.82-2.21 (m, 4H, $NHCH_2CH_2CH_2$, $NHCH_2CH_2$), 1.52-1.5 (m, 3H, $NCHCH_2$, NCH), 0.83 (s, 9H, *t*-Bu), -0.01 (s, 6H, Si $(CH_3)_2$).

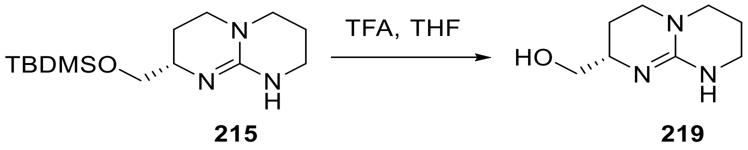
^{13}C NMR (300 MHz, $CDCl_3$) δ 158.2 (C=N), 62.5 (OCH_2), 54 (CH_2), 52.4 (CH_2), 51.3 (CH_2), 50.2 (CH_2), 41.5 (C $(CH_3)_3$), 25 (CH_2), 23.0 (CH_2), 22.6 (CH_2), 23.2 (C $(CH_3)_3$), -0.39 (Si $(CH_3)_2$).

HRMS-ES+ (*m/z*): calcd. for C_{15} H_{32} N_3 O Si $[M+H]^+$: 298.2315, found 298.2325

FT-IR ($CHCl_3$ film, cm^{-1}) 3452 (br m, NH), 2950 (s, aliphatic CH), 1634 (s,), 1430 (m), 1318 (s), 1137 (s, C-O), 1092 (s).

= −42 (c= 0.16, $CHCl_3$)

(*S*)- (3, 4, 6, 7, 8, 9-hexahydro-2H-pyrimido [1, 2-a] pyrimidin-2-yl) methanol (219)



The general procedure for the de-protection of silyl group by utilising trifluoroacetic acid was followed to yield **219** as a pale-yellow oil (366 mg, 82%).

TLC (silica gel, 5:2 MeOH/ DCM), R_f = 0.25 (CAM)

^{1}H NMR (400 MHz, MeOD) δ 3.21-3.11 (m, 3H, OCH_2, NCH), 3.09-3.0 (m, 2H, $NHCH_2$), 2.91-2.75 (m, 4H, NCH_2, NCH_2), 1.69-1.45 (m, 4H, $NHCH_2CH_2$, NCH_2CH_2).

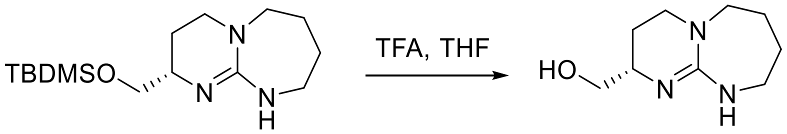
^{13}C NMR (100 MHz, MeOD) δ 150.4 (C=N), 63.5 (CH_2), 50.25 (CH), 46.2 (CH_2), 45.1 (CH_2), 37.8 (CH_2), 22.5 (CH_2), 20.6 (CH_2).

HRMS-ES+ (*m/z*): calcd. for $C_8H_{15}N_3O$ $[M+H]^+$: 170.1288, found 170.1283

FT-IR ($CHCl_3$ film, cm^{-1}) 3475 (br m, OH), 3380 (br, NH) 2859 (m, aliphatic CH), 1633 (s, NH), 1604 (C=N), 1590 (m), 1491 (m), 1315 (s).

= −75 (c= 0.12, MeOH)

(*S*)- (2, 3, 4, 6, 7, 8, 9, 10-octahydropyrimido [1, 2-a] [1, 3] diazepin-2-yl) methanol (220)



216 **220**

The general procedure for the de-protection of silyl group by utilising trifluoroacetic acid was followed to yield **220** as a pale-yellow oil (293 mg, 80%).

TLC (silica gel, 5:2 MeOH/ DCM), R_f = 0.21 (CAM)

^{1}H NMR (400 MHz, MeOD) δ 3.78-3.64 (m, 3H, OCH$_2$,NCH), 3.63-3.53 (m, 4H, NHCH$_2$, NCH$_2$), 3.16-3.08 (m, 2H, NCH$_2$), 2.11-2.01 (m, 2H, NHCH$_2$CH$_2$), 1.89-1.66 (m, 2H, NCH$_2$CH$_2$), 1.3 (m, 2H, NCH$_2$CH$_2$).

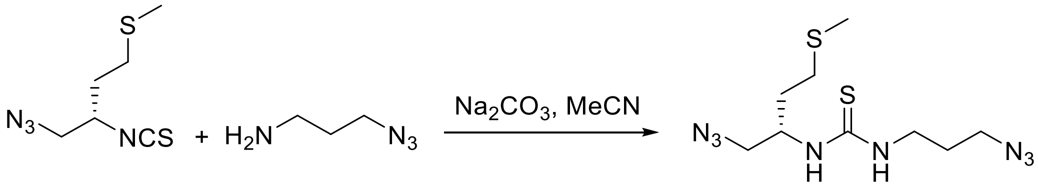
^{13}C NMR (100 MHz, MeOD) δ 151.5 (C=N), 63.0 (CH$_2$), 58.5 (CH), 48.5 (CH$_2$), 45.1 (CH$_2$), 38.3 (CH$_2$), 27.1 (CH$_2$), 25.3 (CH$_2$), 24.1 (CH$_2$).

HRMS-ES+ (*m/z*): calcd. for $C_9H_{18}N_3O$ $[M+H]^+$: 184.1444, found 184.1443

FT-IR (CHCl$_3$ film, cm^{-1}) 3469 (br m, OH), 3383 (br, NH) 2855 (m, aliphatic CH), 1636 (s, NH), 1608 (C=N), 1590 (m), 1491 (m), 1317 (s).

= −72 (c= 0.14, MeOH)

(*S*)-1-(1-azido-4-(methylthio) butan-2-yl)-3-(3-azidopropyl) thiourea (227)



194 **183** **227**

The general procedure for the synthesis of thiourea compounds by the reaction between isothiocyanate and amines was followed to yield **227** as a yellow oil (1.81 mg, 77%).

TLC (silica gel, 2:1 Hexane/ EtOAc), R_f = 0.17 (KMnO4)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53-3.45 (m, 2H, ($NHCH_2$), 2.83-2.75 (m, 1H, NHCH), 2.65-2.55 (m, 2H, SCH_3CH_2), 2.06 (s, 3H, SCH_3), 1.97-1.88 (m, 2H, $NHCHCH_2$), 179-1.72 (m, 4H, (N_3CH, $N_3CH_2CH_2$), 1.7-1.61 (m, 2H, N_3CH_2CH)

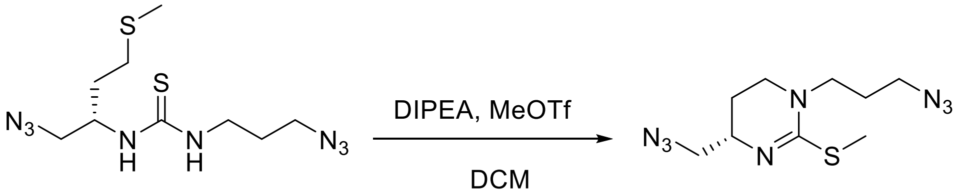
^{13}C NMR (125 MHz, $CDCl_3$) δ 182.4 (C=S), 59.2 (CH), 54.1 (CH_2), 49.7 (CH_2), 48.3 (CH_2), 32.5 (CH_2), 30.1 (CH_2), 28.3 (CH_2), 16.2 (CH_3).

HRMS-ES+ (*m/z*): calcd. for $C_9H_{18}N_8S_2$ $[M+H]^+$: 303.1174, found 303.1172

FT-IR ($CHCl_3$ film, cm^{-1}) 3368 (br s, NH), 2953 (s, aliphatic CH), 2088 (s, N_3), 1553 (m), 1450 (m), 1304 (s), 1094 (s).

= −29 (c= 0.20, $CHCl_3$)

(*S*)-4-(azidomethyl)-1-(3-azidopropyl)-2-(methylthio)-1, 4, 5, 6-tetrahydropyrimidine (228)



227 **228**

Azide **227** (1 g, 3.31 mmol) was dissolved in dichloromethane (30 mL). Diisopropylethylamine (0.21 mL) (155.82 mg, 1.2 mmol) was added to the resulting reaction. Methyl triflate (0.9 mL) (1.3 g, 7.94 mmol) was added dropwise at 0 °C. The mixture was stirred at r.t. for 4 h. Then diisopropylethylamine (5.5 mL) (4.08 g, 31.57 mmol) was added to the resulting mixture and heated under refluxed overnight. The mixture was cooled and then washed with 2M NaOH and H_2O. The organic layer was dried over $MgSO_4$ and then concentrated to obtain the crude product. Purification by using column chromatography purification through silica gel and using the eluent of hexane and ethyl acetate (1:1) afforded the product after removal of solvent as a yellow oil (612 mg, 69%).

TLC (silica gel, 1:1 Hexane/ EtOAc), R_f = 0.31 (UV)

^{1}H NMR (300 MHz, CDCl$_3$) δ 3.42-3.38 (m, 1H, NCH), 2.82-3.72 (m, 2H, NCH_2), 2.43 (s, 3H, SCH_3), 2.4-2.29 (m, 2H, $N_3CH_2CH_2CH_2$), 1.93-1.72 (m, 2H, $NCHCH_2$), 1.65-1.55 (m, 2H, N_3CH_2), 1.52-1.4 (m, 2H, $N_3CH_2CH_2$), 1.39-1.3 (m, 2H, N_3CH_2).

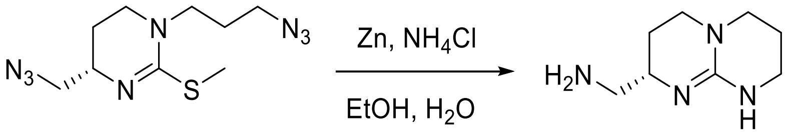
^{13}C NMR (125 MHz, CDCl$_3$) δ 167.0 (C=N), 63.1 (CH), 55.0 (CH_2), 49.2 (CH_2), 45.1 (CH_2), 35.0 (CH_2), 27.1 (CH_2), 18.2 (CH_2), 14.2 (SCH_3).

HRMS-ES+ (*m/z*): calcd. for $C_9H_{16}N_8S$ $[M+H]^+$: 269.1297, found 269.1285

FT-IR (CHCl$_3$ film, cm^{-1}) 2956 (s, aliphatic CH), 2090 (s, N_3), 1556 (m), 1452 (m), 1303 (s), 1092 (s).

= −33 (c= 0.28, $CHCl_3$)

(*S*)- (3, 4, 6, 7, 8, 9-hexahydro-2H-pyrimido [1, 2-a] pyrimidin-2-yl) methanamine (229)



228 **229**

The general procedure for the hydrogenation of azides to amines by using activated zinc and ammonium chloride was followed to yield **229** as a pale-yellow foam (322.6 mg, 82%).

TLC (silica gel, 5:2 MeOH/ DCM), R_f = 0.25 (CAM)

^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.45 (m, 2H, NHCH), 3.41-3.35 (m, 2H, NH_2CH_2), 3.31-3.24 (m, 2H, $NHCH_2$), 3.1-2.95 (m, 2H, NCH_2), 2.92-2.81 (m, 2H, NCH_2) 1.73-1.65 (m, 2H, NCH_2CH_2), 1.52-1.43 (m, 2H, NCH_2CH_2).

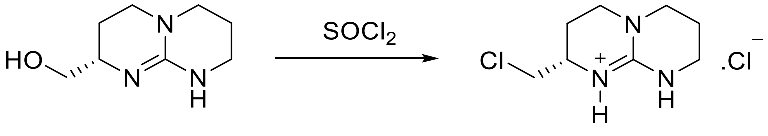
^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.1 (C=N), 63.0 (CH_2), 50.3 (CH), 45.1 (CH_2), 40.1 (CH_2), 38,4 (CH_2), 33.2 (CH_2), 22.0 (CH_2)

HRMS-ES+ (*m/z*): calcd. for $C_8H_{17}N_4$ $[M+H]^+$: 169.1453, found 169.1447

FT-IR ($CHCl_3$ film, cm^{-1}) 3477 (br m, NH_2), 2860 (m, aliphatic CH), 1631 (s, NH), 1603 (C=N) 1591(s), 1493 (m), 1317 (s), 1166 (s), 1098 (m), 1037 (m).

= −70 (c= 0.16, MeOH)

(*S*)-8-(chloromethyl)-1, 3, 4, 6, 7, 8-hexahydro-2H-pyrimido [1, 2-a] pyrimidine (221)



219 **221**

The general procedure for the synthesis of alkyl halides from alcohols was followed to yield **221** (423.3 mg, 73%) as a brown oily product.

TLC (silica gel, 10:0.2% DCM/ MeOH), R_f = 0.32 (UV)

^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.68-3.70 (m, H, NCH), 3.60-3.64 (m, 2H, CH_2Cl), 3.59-3.55 (m, 2H, $NHCH_2$), 3.46-3.51 (m, 2H, NCH_2), 3.39-3.44 (m, 2H, NCH_2), 1.69-1.65 (m, 2H, NCH_2CH_2)1.59-1.64 (m, 2H, NCH_2CH_2).

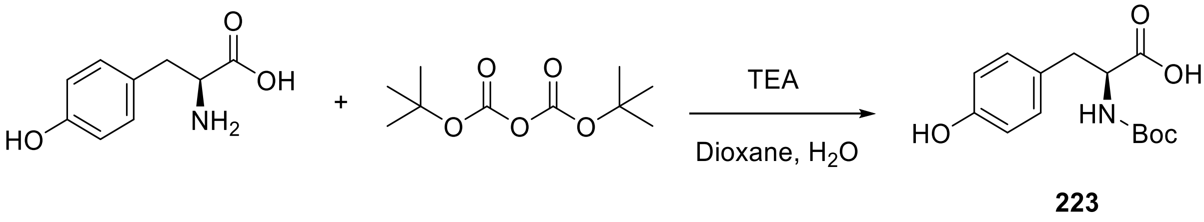
^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.1 (C=N), 59.1 (CH), 52.4 (CH_2), 47.3 (CH_2), 44.3 (CH_2), 41.1 (CH_2), 30.3 (CH_2), 26.0 (CH_2).

HRMS-ES+ (*m/z*): calcd. for $C_8H_{15}N_3$ $[M-H]^+$: 188.0955, found 188.0958

FT-IR ($CHCl_3$ film, cm^{-1}) 3472 (br s, NH), 2860 (m, aliphatic CH), 1633 (s, NH), 1605 (C=N) 1593(s), 1490 (m), 1315 (s).

= −52 (c= 0.15, MeOH)

(*S*)-2-(*tert*-butoxycarbonylamino)-3-(4-hydroxyphenyl) propanoic acid (223)[134]



L-tyrosine (7.4 g, 40.8 mmol) was suspended in 1/1 dioxane/ water (50 mL/ 50 mL). TEA (8.5 mL) was added to the resulting solution. Then Boc anhydride (11 g, 50.4 mmol) was added to the resulting reaction at 0 °C. After 30 min, the cold bath was removed and then the mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure and then dichloromethane (25 mL) and water (25 mL) were added. The aqueous layer was washed with dichloromethane three times. The aqueous layer was made acidic with 1 M HCl and then extracted with dichloromethane three times. Combined organic layers were dried over $MgSO_4$ and then the solvent was evaporated under reduced pressure to get a white foam of **223** (8.6 g, 75%), for which no further purification was needed.

TLC (silica gel, EtOAc), R_f = 0.35 (UV)

The spectroscopic data are in agreement with those recorded in the literature.[134]

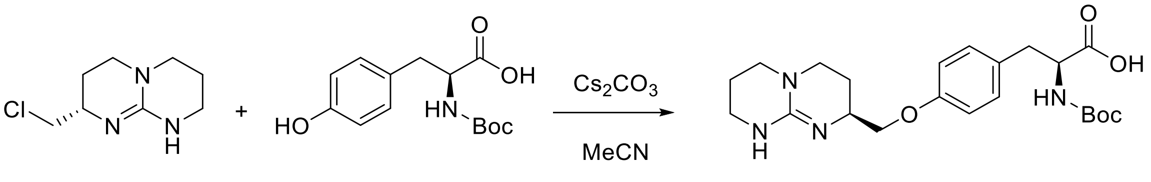
^{1}H-NMR (300 MHz, DMSO) δ 7.01 (d, 2H, 2ArH, *J*= 7.8 Hz), 6.66 (d. 2H, 2ArH, *J*=7.8 Hz), 4.02 (m, 1H, CH), 2.83 (m, 2H, $ArCH_2$), 1.33 (s, 9H, Boc)

^{13}C NMR (125 MHz, DMSO) δ 174.5 (COOH), 155.9 (C=O, Boc), 157.1 (COH), 130.4 (2ArC), 128.2 (ArC), 127.0 (ArC), 115.1 (2ArC), 78.1 (C (CH_3)), 56.1 (NHC), 36.2 (CH_2), 30.1 (C $(CH_3)_3$), 28.4 C $(CH_3)_3$.

FT-IR ($CHCl_3$ film, cm^{-1}) 3366 (br s, OH, phenolic), 3290 (br s, OH, COOH), 2930 (m, aliphatic CH), 1711 (s, C=O, COOH), 1480 (m), 1540 (m), 1367 (m), 1226 (s), 823 (s).

HRMS-ES+ (*m/z*): calcd. for $C_{14}H_{19}NNaO_5$ $[M+ Na]^+$: 304.1161, found 304.1169.

(*R*)-2-((*tert*-butoxycarbonyl) amino)-3-(4-(((S)-3, 4, 6, 7, 8, 9-hexahydro-2H-pyrimido [1, 2-a] pyrimidin-2-yl) methoxy) phenyl) propanoic acid (224)



221 **223** **224**

The general procedure for the synthesis of modified amino acid having bicyclic guanidine in their side chain was followed to yield **224** (323 mg, 68%) as a white foam.

TLC (silica gel, 10:1 DCM/ MeOH), R_f = 0.7 (UV)

^{1}H-NMR (300 MHz, CDCl$_3$) δ 6.8 (d, 2H, $^3J_{HH}$=7.8 Hz), 6.5 (d, 2H, $^3J_{HH}$=7.8 Hz), 4.69-4.58 (m, 1H, CHCOOH), 4.06-4.19 (m, 2H, NCHCH$_2$), 3.37-3.43 (m, 2H, NHCH), 2.8-3.1 (m, 2H, ArCH$_2$), 2.78-2.69 (m, 2H, NHCH$_2$), 2.68-2.63 (m, 2H, NCH$_2$), 2.62-2.56 (m,2H,NCH$_2$), 1.73-1.67 (m, 2H, NHCH$_2$CH$_2$), 1.59-1.49 (m, 2H, NCH$_2$CH$_2$), 1.39 (s, 9H, C(CH$_3$)$_3$).

^{13}C-NMR (100 MHz, CDCl$_3$) δ 174 (COOH), 157.8 (ArC), 155.3 (Boc, CO), 151.3 (C=N), 129.3 (2ArC), 126.8 (ArC), 114.5 (2ArC), 77.1 ($\underline{\text{C}}$(CH$_3$), 71.3 (CH$_2$), 68.1 (CH), 53.4 (CH), 49.5 (CH$_2$), 43.8 (CH$_2$), 37.8 (CH$_2$), 28.2 (CH$_2$), 27.6 (C(CH$_3$)$_3$), 24.6 (CH$_2$).

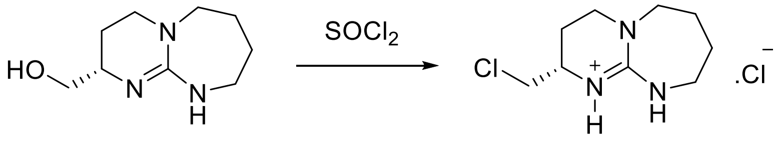
HRMS-ES+ (*m/z*): calcd. for C$_{22}$H$_{33}$N$_4$O$_5$ [M+H]$^+$: 433.2451, found 433.2451

FT-IR (CHCl$_3$ film, cm^{-1}) 3372(br s, NH), 3292 (br s, OH, COOH), 2933 (m, aliphatic CH), 1713 (s, C=O, COOH), 1482 (m), 1543 (m), 1365 (m), 1270 (s, C-O), 822 (s)

λ_{max} (EtOH)/nm: 285.5

= −67 (c= 0.1, MeOH)

(*S*)-2-(chloromethyl)-2, 3, 4, 6, 7, 8, 9, 10-octahydropyrimido [1, 2-a] [1, 3] diazepine (222)



220 **222**

The general procedure for the synthesis of alkyl halides from alcohols was followed to yield **222** (605.31 mg, 70%) as a brown oily product.

TLC (silica gel, 10:0.2% DCM/ MeOH), $R_f = 0.3$ (UV)

Chlorine %wt/wt: 29.77, found 29.38

^{1}H-NMR (300 MHz, CDCl$_3$) δ 3.71-3.65 (m, 1H, NCH), 3.63-3.51 (m, 2H, CH$_2$Cl), 2.8-2.91 (m, 2H, NHCH$_2$), 2.68-2.75 (m, 2H, NCH$_2$), 2.61-2.66 (m, 2H, NCH$_2$), 1.7-1.79 (m, 2H, NHCH$_2$CH$_2$), 1.66-1.69 (m, 2H, NCH$_2$CH$_2$), 1.56-1.5 (m, 2H, NCH$_2$CH$_2$).

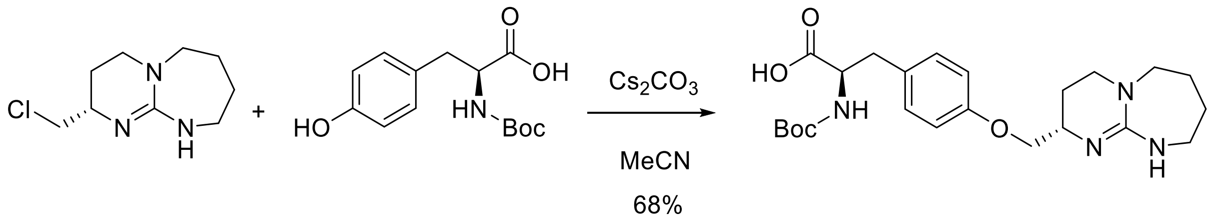
^{13}C-NMR (100 MHz, MeOD) δ 155.1 (C=N), 59.0 (CH), 50.3 (CH$_2$), 49.1 (CH$_2$), 48.3 (CH$_2$), 42.2 (CH$_2$), 33.2 (CH$_2$), 30.5 (CH$_2$), 28.6 (CH$_2$).

HRMS-ES+ (*m/z*): calcd. for $C_9H_{17}ClN_3$ $[M+H]^+$: 202.1106, found 202.1105

FT-IR (CHCl$_3$ film, cm^{-1}) 3476(br s, NH), 2869 (m, aliphatic CH), 1631 (s, NH), 1603 (C=N) 1590 (s), 1497 (m), 1313 (s).

= −55 (c= 0.22, MeOH)

(*R*)-2-((*tert*-butoxycarbonyl) amino)-3-(4-(((S)-2, 3, 4, 6, 7, 8, 9, 10-octahydropyrimido [1, 2-a] [1, 3] diazepin-2-yl) methoxy) phenyl) propanoic acid (225)



222 **223** **225**

The general procedure for the synthesis of modified amino acid having bicyclic guanidine in their moiety was followed to yield **225** (249 mg, 65%) as a white foam.

TLC (silica gel, 10:1 DCM/ MeOH), R_f =0.72 (UV)

^{1}H-NMR (300 MHz, CDCl$_3$) δ 6.9 (d, 2H, $^3J_{HH}$=7.8 Hz), 6.5 (d, 2H, $^3J_{HH}$=7.8 Hz), 4.7-4.65 (m, 1H, CHCOOH), 4.19-4.09 (m, 2H, NCHCH$_2$), 3.39-3.31 (m, 1H, NCH), 2.92-2.89 (m, 2H, ArCH$_2$), 2.8-3.1 (m, 2H, NHCH$_2$), 2.78-2.7 (m, 2H, NCH$_2$), 2.69-2.6 (m, 2H, NCH$_2$), 1.79-1.7 (m, 2H, NHCH$_2$CH$_2$), 1.66-1.59 (m, 2H, NCH$_2$CH$_2$), 1.45-1.57 (m, 2H, NCH$_2$CH$_2$), 1.41 (s, 9H, C(CH$_3$)$_3$.

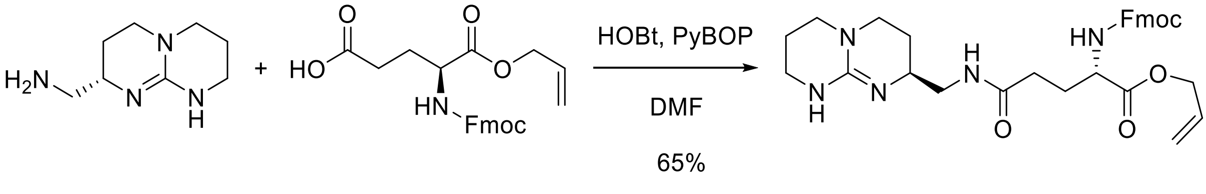
^{13}C-NMR (100 MHz, CDCl$_3$) δ 173 (COOH), 172.9 (ArC), 156.6 (Boc, CO), 155.3 (C=N), 130.2 (2ArC), 127.2 (ArC), 114.8 (2ArC), 79.3 (C(CH$_3$), 70.5 (CH$_2$), 69.1 (CH), 54.3 (CH), 50.1 (CH$_2$), 44.4 (CH$_2$), 38.2 (CH$_2$), 29.5 (CH$_2$), 28.1 (CH$_2$), 27.3 (C(CH$_3$)$_3$), 25.5 (CH$_2$).

HRMS-ES+ (*m/z*): calcd. for $C_{23}H_{35}N_4O_5$ $[M+H]^+$: 447.2607, found 447.2620

FT-IR (CHCl$_3$ film, cm^{-1}) 3374 (br s, NH), 3295 (br s, OH, COOH), 2931 (m, aliphatic CH), 1713(s, C=O, COOH), 1481 (m), 1540 (m), 1364 (m), 1273 (s, C-O), 821(s)

= −65 (c= 0.15, MeOH)

Allyl N2-(((9H-fluoren-9-yl) methoxy) carbonyl)-N5-(((S)-3, 4, 6, 7, 8, 9-hexahydro-2H-pyrimido [1, 2-a] pyrimidin-2-yl) methyl)-L-glutaminate (231)



229 **230** **231**

HOBt (221.5 mg, 1.64 mmol) and PyBOP (145.69 mg, 0.9 mmol) were added to a solution of Fmoc-Glu-OAll (670.7 mg, 1.64 mmol) in dry dimethylformamide (15 mL). The mixture was stirred at room temperature for 10 minutes. Compound **229** (278.5 mg, 1.64 mmol) was added to the resulting solution at 0 °C. The mixture was stirred at room temperature for 10 h. The solvent was evaporated under reduced pressure and then the residue was redissolved in dichloromethane. The solid was filtered. The solvent was evaporated *in vacuo* from the filtrate to give the crude product. The yield after purification through silica gel and using the eluent of DCM and MeOH (10:0.1) and removal of solvent was 633 mg (69%) as a pale-yellow oil.

TLC (silica gel, 10:0.1% DCM/ MeOH), R_f = 0.5 (UV)

^{1}H-NMR (300 MHz, MeOH) δ 7.79 (d, 2H, $^3J_{HH}$=7.5 Hz), 7.66 (d, 2H, $^3J_{HH}$=7.4 Hz), 7.43 (t, 2H, J=7.4 Hz), 7.33 (t, 2H, J=7.4 Hz), 5.97 (m, 1H, CH_2=CH), 5.26 (m, 2H, CH_2=CH) 4.73-4.71 (m, 2H, OCH_2), 4.71-4.69 (m, 2H, OCH_2 CH-All), 4.48-4.39 (m, 1H, furan), 4.38-4.28 (m, 1H, NHCH), 3.27-3.19 (m,1H,-NCH), 3.15-3.0 (m,2H,$NCHCH_2$), 2.71-2.69 (m, 2H, $NHCH_2$), 2.69-2.58 (m, 2H, NCH_2), 2.55-2.43 (m,2H,NCH_2), 2.13-2.09 (m, 2H, $NHCHCH_2$), 2.0-1.89 (m, 2H, $COCH_2$), 1.75-1.68 (m, 2H, NCH_2CH_2), 1.55-1.63 (m, 2H, NCH_2CH_2).

^{13}C-NMR (100 MHz, MeOH) δ 173.0 (CO-OH), 172.6 (CO-OAll), 161.3 (CO-Fmoc), 152.0 (C=N), 145.0 (2C, Fmoc-ArC), 143.0 (2C-Fmoc-ArC), 138.1 (C -Allyl), 128.3(2C-ArC), 127.2 (2C-ArC), 126.2 (2C-ArC), 122.4 (2C-ArC), 119.2 (C -Allyl), 70.5 (CH_2), 68.1 (CH_2), 59.0 (CH), 50.2 (CH_2), 49.4 (CH_2), 47.1 (CH-furan), 45.5 (CH), 44.3 (CH_2), 42.1 (CH_2), 35.3 (CH_2), 29.1 (CH_2), 26.5 (CH_2), 23.1 (CH_2).

HRMS-ES+ (*m/z*): calcd. for $C_{31}H_{38}N_5O_5$ $[M+H]^+$: 560.2873, found 560.2877

FT-IR ($CHCl_3$ film, cm^{-1}) 3340 (br s, NH), 3050 (s, OH, COOH), 2950 (m, aliphatic CH), 1750 (s, C=O, COOH), 1690 (s, C=C, amide), 1530 (m), 1450 (m), 1420 (m), 1340 (m), 1280 (s), 1250 (m).

= −72 (c= 0.22, MeOH)

Chapter Five
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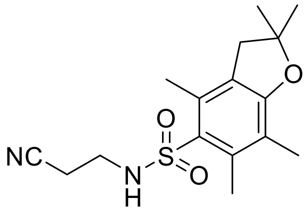
Chapter Six
Appendix

6.1 Crystallography data for **183A**

***N*-(2-cyanoethyl)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide 183A**



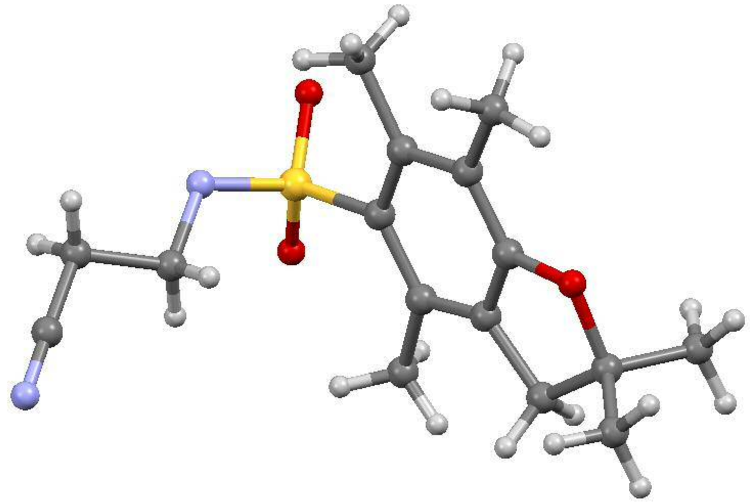


Table 1. Crystal data and structure refinement for **183A**.

Identification code	shelx	
Empirical formula	C16 H21 N2 O3 S	
Formula weight	321.41	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 28.292(3) Å	α= 90°.
	b = 10.8440(9) Å	β= 90°.
	c = 5.6300(4) Å	γ = 90°.
Volume	1727.2(3) $Å^3$	
Z	4	
Density (calculated)	1.236 Mg/m^3	
Absorption coefficient	0.201 mm^{-1}	
F(000)	684	
Crystal size	0.620 × 0.163 × 0.061 mm^3	
Theta range for data collection	3.439 to 30.058°.	
Index ranges	-34<=h<=36, -14<=k<=11, -7<=l<=5	
Reflections collected	8052	
Independent reflections	4118 [R(int) = 0.0414]	

Completeness to theta = 25.242°	99.7 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4118 / 0 / 204
Goodness-of-fit on F^2	1.023
Final R indices [I>2sigma(I)]	R1 = 0.0648, wR2 = 0.1421
R indices (all data)	R1 = 0.1241, wR2 = 0.1766
Absolute structure parameter	0.20(7)
Extinction coefficient	n/a
Largest diff. peak and hole	0.232 and -0.252 e.$Å^{-3}$

Table 2. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2× 10^3) for **183A**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	-334(2)	10198(5)	7394(9)	64(1)
C(2)	-50(2)	9327(5)	8922(10)	73(1)
C(3)	454(2)	9693(4)	8477(8)	54(1)
C(4)	453(2)	10543(4)	6644(8)	54(1)
C(5)	865(2)	9305(4)	9502(7)	54(1)
C(6)	1287(2)	9836(4)	8637(8)	54(1)
C(7)	1284(2)	10710(4)	6726(8)	54(1)
C(8)	856(2)	11060(4)	5705(7)	52(1)
C(9)	-548(2)	11213(6)	8854(12)	94(2)
C(10)	-697(3)	9580(8)	5842(12)	118(3)
C(11)	825(2)	8375(4)	11505(9)	72(1)
C(12)	1723(2)	11301(5)	5743(10)	76(2)
C(13)	821(2)	11930(5)	3640(9)	70(1)
C(14)	1890(2)	7455(5)	7042(10)	74(1)
C(15)	2239(2)	6741(6)	5577(15)	107(2)
C(16)	2012(4)	5653(8)	4620(20)	163(4)
N(1)	2115(1)	8577(4)	7903(8)	65(1)
N(2)	1826(5)	4786(9)	3870(30)	273(8)
O(1)	8(1)	10791(3)	5797(6)	70(1)
O(2)	2131(1)	10500(3)	10107(7)	86(1)
O(3)	1763(1)	8673(4)	11863(6)	89(1)
S(1)	1843(1)	9414(1)	9835(2)	68(1)

Table 3. Bond lengths [Å] and angles [°] for **183A**.

C(1)-O(1)	1.468(6)
C(1)-C(9)	1.501(7)
C(1)-C(10)	1.506(8)
C(1)-C(2)	1.509(7)
C(2)-C(3)	1.500(6)
C(2)-H(2A)	0.9700
C(2)-H(2B)	0.9700
C(3)-C(5)	1.364(6)
C(3)-C(4)	1.383(6)
C(4)-O(1)	1.373(5)
C(4)-C(8)	1.377(6)
C(5)-C(6)	1.412(6)
C(5)-C(11)	1.517(6)
C(6)-C(7)	1.434(6)
C(6)-S(1)	1.771(5)
C(7)-C(8)	1.392(6)
C(7)-C(12)	1.504(6)
C(8)-C(13)	1.501(6)
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(12)-H(12A)	0.9600
C(12)-H(12B)	0.9600
C(12)-H(12C)	0.9600
C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600
C(13)-H(13C)	0.9600
C(14)-N(1)	1.456(6)
C(14)-C(15)	1.503(8)
C(14)-H(14A)	0.9700

C(14)-H(14B)	0.9700
C(15)-C(16)	1.448(12)
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
C(16)-N(2)	1.157(12)
N(1)-S(1)	1.612(4)
O(2)-S(1)	1.441(4)
O(3)-S(1)	1.414(4)
O(1)-C(1)-C(9)	106.2(4)
O(1)-C(1)-C(10)	106.7(4)
C(9)-C(1)-C(10)	111.7(6)
O(1)-C(1)-C(2)	105.8(4)
C(9)-C(1)-C(2)	111.2(5)
C(10)-C(1)-C(2)	114.6(5)
C(3)-C(2)-C(1)	104.2(4)
C(3)-C(2)-H(2A)	110.9
C(1)-C(2)-H(2A)	110.9
C(3)-C(2)-H(2B)	110.9
C(1)-C(2)-H(2B)	110.9
H(2A)-C(2)-H(2B)	108.9
C(5)-C(3)-C(4)	121.6(4)
C(5)-C(3)-C(2)	131.0(4)
C(4)-C(3)-C(2)	107.4(4)
O(1)-C(4)-C(8)	123.2(4)
O(1)-C(4)-C(3)	113.1(4)
C(8)-C(4)-C(3)	123.7(4)
C(3)-C(5)-C(6)	116.6(4)
C(3)-C(5)-C(11)	117.2(4)
C(6)-C(5)-C(11)	126.1(4)
C(5)-C(6)-C(7)	121.6(4)
C(5)-C(6)-S(1)	120.9(3)
C(7)-C(6)-S(1)	117.5(3)
C(8)-C(7)-C(6)	119.6(4)
C(8)-C(7)-C(12)	116.7(4)
C(6)-C(7)-C(12)	123.6(4)
C(4)-C(8)-C(7)	116.8(4)
C(4)-C(8)-C(13)	119.9(4)

C(7)-C(8)-C(13)	123.2(4)
C(1)-C(9)-H(9A)	109.5
C(1)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(1)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(1)-C(10)-H(10A)	109.5
C(1)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(1)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(5)-C(11)-H(11A)	109.5
C(5)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(5)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(7)-C(12)-H(12A)	109.5
C(7)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	109.5
C(7)-C(12)-H(12C)	109.5
H(12A)-C(12)-H(12C)	109.5
H(12B)-C(12)-H(12C)	109.5
C(8)-C(13)-H(13A)	109.5
C(8)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5
C(8)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5
N(1)-C(14)-C(15)	109.0(5)
N(1)-C(14)-H(14A)	109.9
C(15)-C(14)-H(14A)	109.9
N(1)-C(14)-H(14B)	109.9
C(15)-C(14)-H(14B)	109.9
H(14A)-C(14)-H(14B)	108.3
C(16)-C(15)-C(14)	109.4(6)

C(16)-C(15)-H(15A)	109.8
C(14)-C(15)-H(15A)	109.8
C(16)-C(15)-H(15B)	109.8
C(14)-C(15)-H(15B)	109.8
H(15A)-C(15)-H(15B)	108.2
N(2)-C(16)-C(15)	179.2(12)
C(14)-N(1)-S(1)	119.1(4)
C(4)-O(1)-C(1)	107.7(4)
O(3)-S(1)-O(2)	118.0(2)
O(3)-S(1)-N(1)	107.5(2)
O(2)-S(1)-N(1)	105.2(2)
O(3)-S(1)-C(6)	108.2(2)
O(2)-S(1)-C(6)	109.3(2)
N(1)-S(1)-C(6)	108.2(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (Å^2× 10^3) for **183A**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

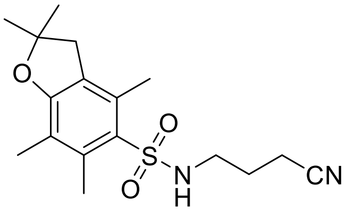
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	51(3)	74(3)	67(3)	0(3)	11(2)	-3(3)
C(2)	58(3)	69(3)	93(4)	12(3)	19(3)	4(3)
C(3)	56(3)	49(2)	57(2)	3(2)	11(2)	-1(2)
C(4)	55(3)	50(2)	57(2)	-6(2)	8(2)	1(2)
C(5)	60(3)	47(2)	57(2)	-2(2)	8(2)	-1(2)
C(6)	57(3)	51(2)	56(2)	-6(2)	7(2)	0(2)
C(7)	53(3)	53(2)	58(2)	-8(2)	11(2)	-5(2)
C(8)	57(3)	49(2)	52(2)	-1(2)	10(2)	2(2)
C(9)	100(5)	96(4)	86(4)	11(4)	25(3)	30(4)
C(10)	116(6)	146(6)	90(4)	0(5)	-12(4)	-52(5)
C(11)	75(4)	68(3)	74(3)	17(3)	4(3)	-3(3)
C(12)	65(3)	80(3)	83(3)	5(3)	14(3)	-7(3)
C(13)	73(4)	65(3)	72(3)	11(3)	25(3)	7(3)
C(14)	71(4)	67(3)	84(4)	3(3)	6(3)	-1(3)
C(15)	98(5)	81(4)	142(7)	-7(4)	12(4)	18(4)
C(16)	158(9)	95(5)	236(12)	-52(8)	64(8)	4(6)
N(1)	49(2)	66(2)	80(3)	2(2)	1(2)	2(2)
N(2)	252(13)	144(7)	423(19)	-147(10)	125(13)	-45(8)
O(1)	55(2)	76(2)	78(2)	16(2)	10(2)	6(2)
O(2)	72(2)	92(2)	94(2)	-16(3)	-13(2)	-21(2)
O(3)	81(3)	120(3)	66(2)	24(2)	-9(2)	0(2)
S(1)	60(1)	80(1)	64(1)	-1(1)	-6(1)	-4(1)

Table 5. Hydrogen coordinates (× 10^4) and isotropic displacement parameters (Å^2× 10^3) for **183A**.

	x	y	z	U(eq)
H(2A)	-131	9424	10586	88
H(2B)	-104	8477	8460	88
H(9A)	-309	11574	9844	141
H(9B)	-795	10881	9832	141
H(9C)	-676	11832	7820	141
H(10A)	-831	10176	4775	176
H(10B)	-942	9237	6817	176
H(10C)	-549	8934	4943	176
H(11A)	932	8744	12958	109
H(11B)	1017	7668	11148	109
H(11C)	502	8124	11676	109
H(12A)	1695	11376	4050	114
H(12B)	1993	10800	6121	114
H(12C)	1762	12105	6431	114
H(13A)	984	11585	2298	105
H(13B)	964	12704	4056	105
H(13C)	495	12058	3245	105
H(14A)	1616	7663	6084	89
H(14B)	1784	6959	8374	89
H(15A)	2356	7252	4291	128
H(15B)	2506	6501	6556	128

6.2 Crystallography data for **187A**

N*-(3-cyanopropyl)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-*sulfonamide **187A**



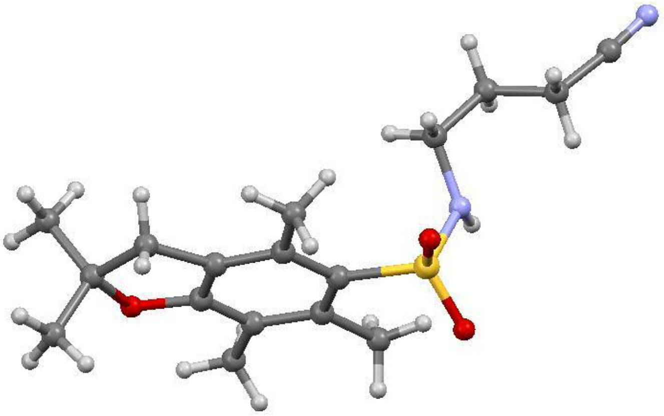


Table 1. Crystal data and structure refinement for **187A**.

Identification code	shelx	
Empirical formula	$C_{17}H_{24}N_2O_3S$	
Formula weight	336.44	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P\,2_1\,2_1\,2_1$	
Unit cell dimensions	a = 5.5730(15) Å	α= 90°.
	b = 11.201(5) Å	β= 90°.
	c = 29.510(7) Å	γ = 90°.
Volume	1842.1(10) Å^3	
Z	4	
Density (calculated)	1.213 Mg/m^3	
Absorption coefficient	0.191 mm^{-1}	
F(000)	720	
Crystal size	0.620 × 0.163 × 0.061 mm^3	
Theta range for data collection	3.638 to 29.890°.	
Index ranges	-5<=h<=7, -10<=k<=15, -38<=l<=41	
Reflections collected	8264	

Independent reflections	4391 [R(int) = 0.0689]
Completeness to theta = 25.242°	99.7%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4391 / 153 / 254
Goodness-of-fit on F^2	0.965
Final R indices [I>2sigma(I)]	R1 = 0.0794, wR2 = 0.1970
R indices (all data)	R1 = 0.1836, wR2 = 0.2741
Absolute structure parameter	-0.04(13)
Extinction coefficient	n/a
Largest diff. peak and hole	0.229 and -0.277 e.Å^{-3}

Table 2. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2× 10^3) for jer1605b. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	-88(13)	2854(8)	2784(3)	92(2)
C(2)	-1669(16)	2050(7)	2488(3)	104(2)
C(3)	-938(12)	2372(6)	2009(2)	76(2)
C(4)	900(12)	3211(6)	2034(3)	77(2)
C(5)	-1778(10)	1953(5)	1599(2)	73(2)
C(6)	-722(11)	2447(6)	1196(2)	75(2)
C(7)	1207(11)	3313(5)	1232(3)	78(2)
C(8)	1986(11)	3691(6)	1656(2)	71(2)
C(9)	1531(17)	2132(12)	3078(4)	187(6)
C(10)	-1522(19)	3761(11)	3024(4)	156(4)
C(11)	-3766(14)	1043(6)	1603(3)	97(2)
C(12)	2409(13)	3834(7)	818(3)	102(3)
C(13)	3976(13)	4586(6)	1711(3)	97(2)
N(1)	363(12)	1169(5)	428(2)	94(2)
O(1)	1493(9)	3511(4)	2467(2)	96(2)
O(2)	-1860(10)	3044(5)	367(2)	107(2)
O(3)	-3815(9)	1288(6)	702(2)	117(2)
S(1)	-1697(3)	2010(2)	653(1)	91(1)
C(14)	980(16)	33(7)	635(3)	100(2)
C(15)	2570(60)	-550(50)	289(13)	132(8)
C(16)	750(80)	-1000(50)	-43(12)	142(7)
C(17)	1640(80)	-1620(40)	-463(13)	146(7)
N(2)	2170(100)	-2330(30)	-746(11)	204(12)
C(14A)	980(16)	33(7)	635(3)	100(2)
C(15A)	2350(40)	-770(30)	308(7)	137(6)
C(16A)	1250(50)	-840(20)	-166(6)	122(5)
C(17A)	2870(60)	-1490(20)	-476(8)	148(6)
N(2A)	4260(60)	-1990(20)	-712(8)	203(10)

Table 3. Bond lengths [Å] and angles [°] for **187A**.

C(1)-C(10)	1.474(12)
C(1)-O(1)	1.482(9)
C(1)-C(9)	1.490(12)
C(1)-C(2)	1.533(11)
C(2)-C(3)	1.516(9)
C(2)-H(2A)	0.9700
C(2)-H(2B)	0.9700
C(3)-C(5)	1.378(9)
C(3)-C(4)	1.392(9)
C(4)-O(1)	1.360(8)
C(4)-C(8)	1.378(9)
C(5)-C(6)	1.439(8)
C(5)-C(11)	1.505(9)
C(6)-C(7)	1.452(9)
C(6)-S(1)	1.762(7)
C(7)-C(8)	1.392(9)
C(7)-C(12)	1.509(9)
C(8)-C(13)	1.504(9)
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(12)-H(12A)	0.9600
C(12)-H(12B)	0.9600
C(12)-H(12C)	0.9600
C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600
C(13)-H(13C)	0.9600
N(1)-C(14)	1.452(10)
N(1)-S(1)	1.626(6)
N(1)-H(12)	0.996(13)

O(2)-S(1)	1.437(5)
O(3)-S(1)	1.438(5)
C(14)-C(15)	1.498(13)
C(14)-H(14A)	0.9700
C(14)-H(14B)	0.9700
C(15)-C(16)	1.497(13)
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
C(16)-C(17)	1.502(13)
C(16)-H(16A)	0.9700
C(16)-H(16B)	0.9700
C(17)-N(2)	1.194(13)
C(15A)-C(16A)	1.529(12)
C(15A)-H(15C)	0.9700
C(15A)-H(15D)	0.9700
C(16A)-C(17A)	1.480(12)
C(16A)-H(16C)	0.9700
C(16A)-H(16D)	0.9700
C(17A)-N(2A)	1.181(12)

C(10)-C(1)-O(1)	106.5(7)
C(10)-C(1)-C(9)	115.0(10)
O(1)-C(1)-C(9)	106.1(6)
C(10)-C(1)-C(2)	111.6(7)
O(1)-C(1)-C(2)	105.9(6)
C(9)-C(1)-C(2)	111.1(8)
C(3)-C(2)-C(1)	103.7(6)
C(3)-C(2)-H(2A)	111.0
C(1)-C(2)-H(2A)	111.0
C(3)-C(2)-H(2B)	111.0
C(1)-C(2)-H(2B)	111.0
H(2A)-C(2)-H(2B)	109.0
C(5)-C(3)-C(4)	121.9(6)
C(5)-C(3)-C(2)	130.2(7)
C(4)-C(3)-C(2)	107.9(6)
O(1)-C(4)-C(8)	123.8(6)
O(1)-C(4)-C(3)	113.4(6)
C(8)-C(4)-C(3)	122.9(7)

C(3)-C(5)-C(6)	117.0(6)
C(3)-C(5)-C(11)	118.4(6)
C(6)-C(5)-C(11)	124.5(6)
C(5)-C(6)-C(7)	120.0(6)
C(5)-C(6)-S(1)	121.3(5)
C(7)-C(6)-S(1)	118.7(5)
C(8)-C(7)-C(6)	120.0(6)
C(8)-C(7)-C(12)	118.1(6)
C(6)-C(7)-C(12)	121.9(7)
C(4)-C(8)-C(7)	118.2(6)
C(4)-C(8)-C(13)	119.8(6)
C(7)-C(8)-C(13)	121.9(6)
C(1)-C(9)-H(9A)	109.5
C(1)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(1)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(1)-C(10)-H(10A)	109.5
C(1)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(1)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(5)-C(11)-H(11A)	109.5
C(5)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(5)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(7)-C(12)-H(12A)	109.5
C(7)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	109.5
C(7)-C(12)-H(12C)	109.5
H(12A)-C(12)-H(12C)	109.5
H(12B)-C(12)-H(12C)	109.5
C(8)-C(13)-H(13A)	109.5
C(8)-C(13)-H(13B)	109.5

H(13A)-C(13)-H(13B)	109.5
C(8)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5
C(14)-N(1)-S(1)	120.3(6)
C(14)-N(1)-H(12)	118(4)
S(1)-N(1)-H(12)	110(4)
C(4)-O(1)-C(1)	109.0(5)
O(2)-S(1)-O(3)	117.4(3)
O(2)-S(1)-N(1)	105.8(4)
O(3)-S(1)-N(1)	107.2(4)
O(2)-S(1)-C(6)	109.3(3)
O(3)-S(1)-C(6)	108.5(3)
N(1)-S(1)-C(6)	108.3(3)
N(1)-C(14)-C(15)	104(2)
N(1)-C(14)-H(14A)	111.0
C(15)-C(14)-H(14A)	111.0
N(1)-C(14)-H(14B)	111.0
C(15)-C(14)-H(14B)	111.0
H(14A)-C(14)-H(14B)	109.0
C(16)-C(15)-C(14)	101(2)
C(16)-C(15)-H(15A)	111.5
C(14)-C(15)-H(15A)	111.5
C(16)-C(15)-H(15B)	111.5
C(14)-C(15)-H(15B)	111.5
H(15A)-C(15)-H(15B)	109.3
C(15)-C(16)-C(17)	118(3)
C(15)-C(16)-H(16A)	107.7
C(17)-C(16)-H(16A)	107.7
C(15)-C(16)-H(16B)	107.7
C(17)-C(16)-H(16B)	107.7
H(16A)-C(16)-H(16B)	107.1
N(2)-C(17)-C(16)	165(4)
C(16A)-C(15A)-H(15C)	108.8
C(16A)-C(15A)-H(15D)	108.8
H(15C)-C(15A)-H(15D)	107.7
C(17A)-C(16A)-C(15A)	110.0(17)
C(17A)-C(16A)-H(16C)	109.7

C(15A)-C(16A)-H(16C)	109.7
C(17A)-C(16A)-H(16D)	109.7
C(15A)-C(16A)-H(16D)	109.7
H(16C)-C(16A)-H(16D)	108.2
N(2A)-C(17A)-C(16A)	177(3)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (Å^2×10^3) for **187A**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

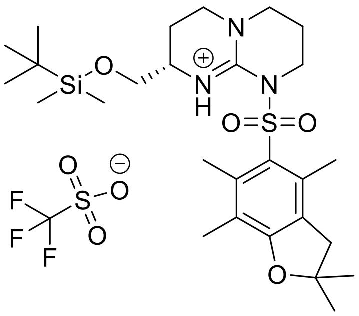
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	84(4)	117(6)	76(5)	-1(5)	11(4)	6(5)
C(2)	125(6)	105(5)	82(5)	7(5)	20(5)	-11(6)
C(3)	70(3)	79(4)	78(5)	9(3)	12(3)	4(3)
C(4)	73(4)	76(4)	82(5)	-8(4)	12(3)	0(3)
C(5)	68(3)	72(3)	80(5)	10(3)	10(3)	-5(4)
C(6)	71(3)	84(4)	71(5)	4(3)	5(3)	3(3)
C(7)	64(3)	67(4)	102(6)	7(3)	21(4)	7(3)
C(8)	69(3)	68(3)	77(4)	0(3)	9(3)	5(3)
C(9)	100(6)	269(15)	192(11)	140(11)	-34(7)	-30(9)
C(10)	120(7)	181(10)	165(10)	-89(8)	42(7)	-34(8)
C(11)	99(5)	94(5)	100(5)	0(4)	21(5)	-22(4)
C(12)	104(5)	110(5)	93(6)	20(5)	27(4)	-14(4)
C(13)	76(4)	88(5)	126(6)	-4(4)	19(4)	-3(4)
N(1)	110(5)	81(4)	91(5)	1(3)	13(4)	-1(4)
O(1)	102(3)	104(3)	80(4)	-6(3)	15(3)	-16(3)
O(2)	117(4)	115(3)	89(3)	28(3)	-10(3)	9(4)
O(3)	87(3)	151(5)	113(4)	8(4)	-15(3)	-35(4)
S(1)	85(1)	105(1)	83(1)	8(1)	-1(1)	-1(1)
C(14)	121(5)	87(5)	93(5)	4(4)	4(5)	-7(5)
C(15)	172(13)	97(13)	128(12)	2(10)	-27(11)	14(11)
C(16)	179(12)	109(11)	138(12)	-4(10)	-20(11)	8(10)
C(17)	179(14)	124(12)	135(12)	-10(11)	17(13)	20(13)
N(2)	280(30)	210(20)	126(17)	-25(17)	40(20)	10(20)
C(14A)	121(5)	87(5)	93(5)	4(4)	4(5)	-7(5)
C(15A)	182(11)	104(11)	124(10)	-11(9)	-24(9)	12(10)
C(16A)	171(10)	95(8)	100(10)	-9(8)	-18(9)	12(9)
C(17A)	181(13)	129(10)	134(11)	-11(9)	2(11)	21(11)
N(2A)	270(20)	181(16)	155(14)	-21(13)	31(16)	59(16)

Table 5. Hydrogen coordinates (× 10^4) and isotropic displacement parameters ($Å^2$× 10^3) for jer1605b.

	x	y	z	U(eq)
H(2A)	-1361	1213	2550	125
H(2B)	-3357	2214	2538	125
H(9A)	2533	1637	2892	281
H(9B)	586	1638	3275	281
H(9C)	2516	2655	3256	281
H(10A)	-479	4373	3139	233
H(10B)	-2360	3393	3272	233
H(10C)	-2660	4109	2819	233
H(11A)	-3958	732	1904	146
H(11B)	-3374	404	1399	146
H(11C)	-5235	1414	1507	146
H(12A)	1936	4653	784	154
H(12B)	1937	3391	555	154
H(12C)	4120	3790	854	154
H(13A)	4067	4833	2022	145
H(13B)	3660	5268	1523	145
H(13C)	5471	4229	1623	145
H(14A)	-443	-444	690	120
H(14B)	1827	149	918	120
H(15A)	3648	25	151	158
H(15B)	3491	-1195	420	158
H(16A)	-224	-327	-138	170
H(16B)	-297	-1549	115	170
H(14C)	-477	-369	730	120
H(14D)	1953	174	902	120
H(15C)	2430	-1570	434	164
H(15D)	3986	-478	281	164
H(16C)	-284	-1252	-150	147
H(16D)	962	-42	-280	147
H(12)	1700(90)	1670(50)	310(20)	100(20)

6.3 Crystallographic data for **233**

(*S*)-8-(((*tert*-butyldimethylsilyl) oxy) methyl) -1-((2, 2, 4, 6, 7-pentamethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl)-1, 3, 4, 6, 7, 8-hexahydro-2H-pyrimido [1,2-a] pyrimidine 233



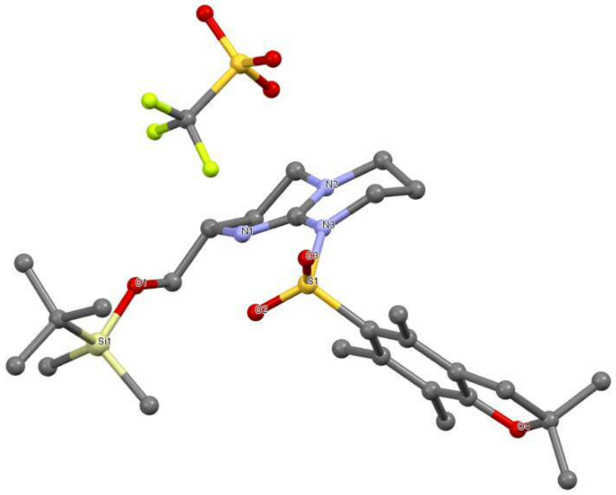


Table 1. Crystal data and structure refinement for **233**.

Identification code	jer1502	
Empirical formula	$C_{28}H_{46}F_3N_3O_7S_2Si$	
Formula weight	685.89	
Temperature	150(2) K	
Wavelength	1.54184 Å	
Crystal system	Monoclinic	
Space group	$P\,2_1$	
Unit cell dimensions	a = 8.5784(3) Å	α= 90°.
	b = 14.4797(5) Å	β= 96.052(4)°.
	c = 13.6961(5) Å	γ = 90°.
Volume	1691.75(10) $Å^3$	
Z	2	
Density (calculated)	1.346 Mg/m^3	
Absorption coefficient	2.317 mm^{-1}	
F(000)	728	
Crystal size	0.330 × 0.057 × 0.037 mm^3	
Theta range for data collection	4.457 to 73.830°.	
Index ranges	-10<=h<=10, -10<=k<=17, -16<=l<=16	
Reflections collected	6602	

Independent reflections	4492 [R(int) = 0.0226]
Completeness to theta = 67.684°	99.7%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4492 / 1 / 407
Goodness-of-fit on F^2	1.026
Final R indices [I>2sigma(I)]	R1 = 0.0369, wR2 = 0.0944
R indices (all data)	R1 = 0.0393, wR2 = 0.0970
Absolute structure parameter	0.004(14)
Extinction coefficient	n/a
Largest diff. peak and hole	0.639 and -0.455 e.Å^{-3}

Table 2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for **233**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	4903(4)	3475(3)	13994(3)	33(1)
C(2)	4708(7)	3823(4)	15024(3)	59(1)
C(3)	4752(8)	4299(4)	13300(4)	75(2)
C(4)	6546(5)	3073(4)	14004(5)	68(2)
C(5)	1353(5)	2945(4)	13702(4)	51(1)
C(6)	3768(6)	1480(3)	14332(3)	47(1)
C(7)	2759(4)	1732(3)	11814(3)	33(1)
C(8)	3644(4)	1581(2)	10935(2)	29(1)
C(9)	2892(4)	889(3)	10197(3)	33(1)
C(10)	4040(4)	680(3)	9468(2)	35(1)
C(11)	6034(3)	633(2)	10847(2)	24(1)
C(12)	6408(5)	-292(3)	9383(3)	45(1)
C(13)	7666(5)	-811(3)	10016(3)	50(1)
C(14)	8558(4)	-125(3)	10679(3)	41(1)
C(15)	8054(3)	-888(2)	12787(2)	25(1)
C(16)	6615(3)	-1379(2)	12654(2)	26(1)
C(17)	6587(3)	-2332(3)	12823(2)	27(1)
C(18)	8016(4)	-2752(2)	13105(3)	29(1)
C(19)	9422(3)	-2277(3)	13240(2)	30(1)
C(20)	9498(3)	-1334(3)	13095(2)	27(1)
C(21)	5054(3)	-923(3)	12335(3)	34(1)
C(22)	5107(4)	-2899(3)	12728(3)	34(1)
C(23)	11084(4)	-877(3)	13296(3)	35(1)
C(24)	10691(4)	-2964(3)	13566(4)	45(1)
C(25)	9847(4)	-3896(3)	13449(3)	39(1)
C(26)	10083(5)	-4485(3)	14356(3)	49(1)
C(27)	10219(5)	-4403(4)	12529(3)	53(1)
C(28)	8067(5)	3080(3)	10769(4)	53(1)
N(1)	5221(3)	1252(2)	11289(2)	27(1)
N(2)	5494(3)	299(2)	9974(2)	29(1)
N(3)	7459(3)	337(2)	11293(2)	30(1)
O(1)	3654(3)	2364(2)	12448(2)	36(1)
O(2)	6884(3)	785(2)	13001(2)	33(1)

O(3)	9591(3)	654(2)	12604(2)	38(1)
O(4)	8160(3)	-3672(2)	13293(2)	40(1)
O(5)	8250(4)	3440(3)	8962(3)	79(1)
O(6)	7404(5)	1905(3)	9413(3)	74(1)
O(7)	10089(4)	2370(3)	9773(3)	65(1)
Si(1)	3399(1)	2560(1)	13610(1)	31(1)
S(1)	8039(1)	303(1)	12509(1)	26(1)
S(2)	8497(1)	2651(1)	9591(1)	42(1)
F(1)	6552(4)	3308(3)	10729(4)	103(2)
F(2)	8339(4)	2461(3)	11485(2)	70(1)
F(3)	8888(5)	3838(3)	11022(3)	92(1)

Table 3. Bond lengths [Å] and angles [°] for **233**.

C(1)-C(3)	1.521(6)
C(1)-C(4)	1.523(6)
C(1)-C(2)	1.524(5)
C(1)-Si(1)	1.885(4)
C(5)-Si(1)	1.859(4)
C(6)-Si(1)	1.860(4)
C(7)-O(1)	1.429(4)
C(7)-C(8)	1.505(5)
C(8)-N(1)	1.468(4)
C(8)-C(9)	1.517(5)
C(9)-C(10)	1.505(5)
C(10)-N(2)	1.469(5)
C(11)-N(1)	1.322(4)
C(11)-N(2)	1.326(4)
C(11)-N(3)	1.376(4)
C(12)-N(2)	1.461(5)
C(12)-C(13)	1.512(7)
C(13)-C(14)	1.501(6)
C(14)-N(3)	1.487(4)
C(15)-C(16)	1.420(4)
C(15)-C(20)	1.421(4)
C(15)-S(1)	1.766(4)
C(16)-C(17)	1.399(5)
C(16)-C(21)	1.515(4)
C(17)-C(18)	1.386(5)
C(17)-C(22)	1.507(4)
C(18)-O(4)	1.361(4)
C(18)-C(19)	1.383(5)
C(19)-C(20)	1.383(5)
C(19)-C(24)	1.507(5)
C(20)-C(23)	1.512(4)
C(24)-C(25)	1.531(6)
C(25)-O(4)	1.477(4)
C(25)-C(26)	1.503(6)
C(25)-C(27)	1.522(6)
C(28)-F(3)	1.330(5)

C(28)-F(2)	1.331(6)
C(28)-F(1)	1.337(6)
C(28)-S(2)	1.803(5)
N(3)-S(1)	1.687(3)
O(1)-Si(1)	1.654(3)
O(2)-S(1)	1.436(2)
O(3)-S(1)	1.419(2)
O(5)-S(2)	1.434(4)
O(6)-S(2)	1.433(4)
O(7)-S(2)	1.421(4)

C(3)-C(1)-C(4)	108.9(5)
C(3)-C(1)-C(2)	108.0(4)
C(4)-C(1)-C(2)	108.0(4)
C(3)-C(1)-Si(1)	111.5(3)
C(4)-C(1)-Si(1)	109.8(3)
C(2)-C(1)-Si(1)	110.6(3)
O(1)-C(7)-C(8)	107.2(3)
N(1)-C(8)-C(7)	107.9(3)
N(1)-C(8)-C(9)	108.6(3)
C(7)-C(8)-C(9)	114.5(3)
C(10)-C(9)-C(8)	108.2(3)
N(2)-C(10)-C(9)	110.2(3)
N(1)-C(11)-N(2)	120.6(3)
N(1)-C(11)-N(3)	119.6(3)
N(2)-C(11)-N(3)	119.8(3)
N(2)-C(12)-C(13)	111.5(3)
C(14)-C(13)-C(12)	107.8(4)
N(3)-C(14)-C(13)	109.0(3)
C(16)-C(15)-C(20)	122.0(3)
C(16)-C(15)-S(1)	118.4(2)
C(20)-C(15)-S(1)	119.6(2)
C(17)-C(16)-C(15)	120.2(3)
C(17)-C(16)-C(21)	116.6(3)
C(15)-C(16)-C(21)	123.3(3)
C(18)-C(17)-C(16)	116.6(3)
C(18)-C(17)-C(22)	119.8(3)
C(16)-C(17)-C(22)	123.5(3)

O(4)-C(18)-C(19)	113.6(3)
O(4)-C(18)-C(17)	122.8(3)
C(19)-C(18)-C(17)	123.5(3)
C(20)-C(19)-C(18)	121.6(3)
C(20)-C(19)-C(24)	130.6(3)
C(18)-C(19)-C(24)	107.7(3)
C(19)-C(20)-C(15)	116.0(3)
C(19)-C(20)-C(23)	117.5(3)
C(15)-C(20)-C(23)	126.5(3)
C(19)-C(24)-C(25)	103.4(3)
O(4)-C(25)-C(26)	106.7(3)
O(4)-C(25)-C(27)	105.9(3)
C(26)-C(25)-C(27)	112.9(4)
O(4)-C(25)-C(24)	105.5(3)
C(26)-C(25)-C(24)	113.1(4)
C(27)-C(25)-C(24)	112.0(4)
F(3)-C(28)-F(2)	108.5(5)
F(3)-C(28)-F(1)	107.0(4)
F(2)-C(28)-F(1)	106.9(4)
F(3)-C(28)-S(2)	111.5(3)
F(2)-C(28)-S(2)	113.0(3)
F(1)-C(28)-S(2)	109.8(4)
C(11)-N(1)-C(8)	125.5(3)
C(11)-N(2)-C(12)	123.7(3)
C(11)-N(2)-C(10)	119.1(3)
C(12)-N(2)-C(10)	115.9(3)
C(11)-N(3)-C(14)	118.3(3)
C(11)-N(3)-S(1)	127.1(2)
C(14)-N(3)-S(1)	114.0(2)
C(7)-O(1)-Si(1)	125.3(2)
C(18)-O(4)-C(25)	108.1(3)
O(1)-Si(1)-C(5)	109.80(18)
O(1)-Si(1)-C(6)	109.57(18)
C(5)-Si(1)-C(6)	109.0(2)
O(1)-Si(1)-C(1)	103.44(15)
C(5)-Si(1)-C(1)	113.2(2)
C(6)-Si(1)-C(1)	111.72(19)
O(3)-S(1)-O(2)	118.05(16)

O(3)-S(1)-N(3)	104.85(15)
O(2)-S(1)-N(3)	107.64(13)
O(3)-S(1)-C(15)	110.15(15)
O(2)-S(1)-C(15)	111.31(16)
N(3)-S(1)-C(15)	103.60(16)
O(7)-S(2)-O(6)	114.5(3)
O(7)-S(2)-O(5)	114.3(2)
O(6)-S(2)-O(5)	116.5(2)
O(7)-S(2)-C(28)	103.0(2)
O(6)-S(2)-C(28)	102.7(2)
O(5)-S(2)-C(28)	103.4(3)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (Å^2× 10^3) for **233**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	41(2)	28(2)	31(2)	1(1)	2(1)	0(2)
C(2)	83(3)	52(3)	44(2)	-13(2)	14(2)	-19(3)
C(3)	104(4)	53(3)	59(3)	25(3)	-25(3)	-25(3)
C(4)	40(2)	64(3)	100(4)	-34(3)	-1(2)	-5(2)
C(5)	36(2)	52(3)	68(3)	-13(2)	13(2)	9(2)
C(6)	57(2)	32(2)	53(2)	5(2)	13(2)	1(2)
C(7)	25(2)	39(2)	35(2)	-6(2)	0(1)	3(1)
C(8)	26(2)	26(2)	33(2)	1(1)	-1(1)	3(1)
C(9)	28(2)	35(2)	36(2)	-2(2)	-4(1)	1(1)
C(10)	39(2)	37(2)	29(2)	-1(2)	-1(1)	-3(2)
C(11)	24(1)	21(1)	28(1)	2(1)	10(1)	-2(1)
C(12)	40(2)	58(3)	40(2)	-20(2)	13(2)	1(2)
C(13)	51(2)	44(2)	58(2)	-12(2)	21(2)	6(2)
C(14)	34(2)	52(2)	38(2)	1(2)	14(1)	12(2)
C(15)	20(1)	28(2)	27(1)	0(1)	6(1)	1(1)
C(16)	18(1)	32(2)	29(1)	0(1)	6(1)	1(1)
C(17)	22(1)	31(2)	30(1)	-2(1)	6(1)	-3(1)
C(18)	25(2)	25(2)	37(2)	1(1)	6(1)	1(1)
C(19)	20(1)	32(2)	36(2)	-1(1)	5(1)	3(1)
C(20)	19(1)	33(2)	30(2)	-5(1)	4(1)	0(1)
C(21)	15(1)	42(2)	44(2)	10(2)	3(1)	-2(1)
C(22)	25(2)	36(2)	42(2)	-1(2)	4(1)	-6(2)
C(23)	19(1)	34(2)	50(2)	-2(2)	-2(1)	0(1)
C(24)	27(2)	33(2)	73(3)	3(2)	3(2)	6(2)
C(25)	32(2)	33(2)	52(2)	2(2)	9(2)	9(2)
C(26)	46(2)	48(3)	52(2)	5(2)	8(2)	8(2)
C(27)	48(2)	55(3)	56(2)	-4(2)	11(2)	12(2)
C(28)	50(2)	26(2)	87(3)	-5(2)	30(2)	-7(2)
N(1)	23(1)	23(1)	33(1)	-5(1)	0(1)	4(1)
N(2)	32(1)	28(1)	28(1)	-2(1)	7(1)	-2(1)
N(3)	24(1)	34(2)	34(1)	1(1)	9(1)	6(1)
O(1)	34(1)	37(2)	37(1)	-7(1)	4(1)	0(1)

O(2)	31(1)	37(1)	31(1)	-9(1)	2(1)	10(1)
O(3)	24(1)	28(1)	59(2)	1(1)	-2(1)	-3(1)
O(4)	29(1)	26(1)	67(2)	1(1)	8(1)	3(1)
O(5)	48(2)	91(3)	96(3)	62(3)	-6(2)	-11(2)
O(6)	92(3)	74(3)	52(2)	5(2)	-4(2)	-42(2)
O(7)	53(2)	69(3)	72(2)	-8(2)	8(2)	23(2)
Si(1)	30(1)	28(1)	35(1)	-3(1)	7(1)	4(1)
S(1)	20(1)	26(1)	33(1)	-2(1)	2(1)	2(1)
S(2)	40(1)	41(1)	44(1)	11(1)	-6(1)	-7(1)
F(1)	58(2)	74(2)	188(5)	4(3)	63(2)	6(2)
F(2)	89(2)	74(2)	46(1)	-2(2)	8(1)	-24(2)
F(3)	107(3)	59(2)	119(3)	-47(2)	53(2)	-42(2)

Table 5. Hydrogen coordinates (× 10^4) and isotropic displacement parameters (Å^2× 10^3) for **233**.

	x	y	z	U(eq)
H(2A)	4860	3310	15490	89
H(2B)	3652	4078	15037	89
H(2C)	5487	4305	15207	89
H(3A)	3678	4538	13256	112
H(3B)	4995	4106	12647	112
H(3C)	5485	4784	13551	112
H(4A)	7323	3551	14203	103
H(4B)	6702	2850	13346	103
H(4C)	6669	2558	14471	103
H(5A)	1115	3478	13272	77
H(5B)	1236	3119	14382	77
H(5C)	628	2440	13501	77
H(6A)	2981	1016	14103	70
H(6B)	3702	1609	15028	70
H(6C)	4816	1245	14244	70
H(7A)	1708	1990	11607	40
H(7B)	2628	1140	12157	40
H(8)	3741	2186	10594	34
H(9A)	2627	316	10537	40
H(9B)	1916	1149	9856	40
H(10A)	3572	230	8978	42
H(10B)	4277	1253	9118	42
H(12A)	6901	92	8903	54
H(12B)	5700	-739	9012	54
H(13A)	7185	-1285	10411	60
H(13B)	8380	-1124	9599	60
H(14A)	9396	-443	11104	49
H(14B)	9054	341	10281	49
H(21A)	4815	-985	11622	50
H(21B)	5109	-267	12511	50
H(21C)	4229	-1223	12664	50
H(22A)	5373	-3554	12821	51

H(22B)	4548	-2809	12073	51
H(22C)	4437	-2704	13227	51
H(23A)	11853	-1331	13574	52
H(23B)	11016	-369	13763	52
H(23C)	11409	-634	12681	52
H(24A)	11113	-2857	14258	53
H(24B)	11560	-2930	13146	53
H(26A)	9796	-4131	14921	73
H(26B)	11185	-4670	14473	73
H(26C)	9421	-5036	14267	73
H(27A)	9599	-4972	12455	79
H(27B)	11338	-4557	12585	79
H(27C)	9960	-4008	11954	79
H(1)	5663	1487	11843	32