
Access from the University of Nottingham repository:
http://eprints.nottingham.ac.uk/12835/1/537640.pdf

Copyright and reuse:

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the University of Nottingham End User licence and may be reused according to the conditions of the licence. For more details see:
http://eprints.nottingham.ac.uk/end_user_agreement.pdf

For more information, please contact eprints@nottingham.ac.uk
Design and Synthesis of Protein Arginine Methyltransferase Inhibitors

Wei Hong

School of Chemistry
University of Nottingham

GEORGE GREEN LIBRARY OF
SCIENCE AND ENGINEERING

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

July 2009
Abstract

Biological methylation is defined as the transfer of a methyl group from \( S\)-adenosyl-L-methionine (SAM) to one of a wide range of potential acceptors such as DNA, RNA, protein, hormones and neurotransmitters. Protein arginine methylation is a common post-translational modification facilitated by protein arginine methyltransferases (e.g. PRMT1). The roles of these enzymes *in vivo* are currently poorly understood.

![Chemical structures](image)

The focus of the project is design and synthesis of PRMT inhibitors with the ultimate goal of evaluating their activities in cells. Preliminary work toward the synthesis of \( S\)-adenosyl-trifluoromethyl-L-homocysteine 69 and adenosyl 5’-[2-(tert-butoxycarbonylamino)ethyl-trifluoromethyl] thiophenium 70 is described. The ternary crystal
structure of PRMT1 in complex with S-adenosyl-L-homocysteine (SAH) and an arginine containing peptide (PBD 1OR8) was used to design a series of potential bisubstrate inhibitors of PRMT1. The prototypical SAM analogues bearing guanidine group were sought to replace the reactive sulfonium centre with nitrogen. Analogue synthesis proceeded via successive reductive amination of 5'-amino-5'-deoxyadenosine and deprotection in good overall yields. An alkyne SAM analogue, 5'-[(S-3-amino-3-carboxypropyl)-propargylamino]-5'-deoxyadenosine 77 was prepared, which underwent efficient Cu (I) catalysed Huisgen reaction to yield a triazole derived SAM analogue 5'-[(S-3-amino-3-carboxypropyl)-[1-(2-guanidinoethyl)-1H-1,2,3-triazol-4-yl]methyl-amino]-5'-deoxyadenosine 78. Preliminary biological evaluation of the compounds by collaborators Professor Steve Ward and Dr Richard Parry at the University of Bath, confirmed that 5'-[(S-3-amino-3-carboxypropyl)-3-guanidinopropyl-amino]-5'-deoxyadenosine 74a and 5'-[(S-3-amino-3-carboxypropyl)-5-guanidinopentyl-amino]-5'-deoxyadenosine 74c are potent inhibitors of PRMT1 but not the lysine methyltransferase SET7. A related N-6 modified SAM analogue 5'-[(S-3-amino-3-carboxypropyl)-3-guanidinopropyl-amino]-5'-deoxy-N6-(11-azido-3,6,9-trioxaundecane)-amino adenosine 79 bearing an azide tether was developed with the aim of allowing facile introduction of biotin or fluorescent dyes, using either Staudinger ligation, or Cu (I) catalysed Huisgen reaction to provide compounds that can be used for affinity purification of the target protein or study of its localisation in cells respectively.

Finally, progress toward a novel, rapid and enantioselective synthesis of the natural
product (+)-sinefungin is reported. Key dihydropyridazine intermediates were generated from adenosyl 5'-propaldehyde, commercially available azodicarboxylate derivatives and ester substituted vinyltriphenylphosphonium salt by successful extension of methodology first reported by Ley and co-workers. Deprotection and ring opening of dihydropyridazine compounds was attempted, and unfortunately we were not able to generate (+)-sinefungin, although it is hoped that this route can be developed to achieve this in the future.
Acknowledgments

I would like to thank Dr. James Dowden for providing the opportunity for me to undertake this project, his supervision and patience throughout the project, and to the University of Nottingham for funding this project.

At the same time, I would like to thank Professor Steve Ward and Dr. Richard Parry for performing the biological work; and to Dr. Charles Laughton and Dr. Hao Wang for providing the Gold software and helping me complete the computational docking work.

I am grateful to Dr. Sara Rossi for the help in my project and proofreading of my thesis. I would also like to acknowledge the rest of Dowden group for their assistance during problem classes and for general experimental suggestion.

Finally, I would like to thank the analytical technical staff in the School of Chemistry for the help of collecting experimental data.
Contents

Abstract ........................................................................................................................ 2
Acknowledgments ........................................................................................................ 5
Abbreviations ............................................................................................................... 9
Introduction ................................................................................................................ 12
  1.1. Biological methylation and S-adenosyl-L-methionine (SAM) ..................... 12
  1.2. Protein arginine methyltransferases (PRMTs) and protein arginine methylation ............................................................ 15
  1.3. Crystal structure of PRMT1 ......................................................................... 20
  1.4. Inhibitors of PRMTs ..................................................................................... 24
  1.5. Chemical synthesis and stability of SAM .................................................... 29
  1.6. Stable nitrogen analogues of SAM .............................................................. 32
  1.7. The natural (+)-sinefungin as a potent inhibitor of methyltransferases ...... 36
      1.7.1. Biological activity of (+)-sinefungin .................................................. 36
      1.7.2. Background of (+)-sinefungin total synthesis .................................... 36
      1.7.3. Structural modifications of (+)-sinefungin ........................................ 45
Project Aims and Objectives .................................................................................... 48
  2.1 Design and synthesis of trifluoromethyl SAM analogue toward general methyltransferases inhibition .......................................................... 48
  2.2. Design and synthesis of specific inhibitors of PRMT1 ............................. 49
      2.2.1. Guanidine containing SAM analogues with different carbon linkers 49
2.2.2. Guanidine containing SAM analogue with triazole linker................. 52

2.3. Design and synthesis of $N$-6 azide side chain SAM analogue ............... 53

2.4. Total synthesis of (+)-sinefungin ................................................................. 54

Results and Discussion............................................................................................... 55

3.1 Preparation of trifluoromethyl SAM analogue ............................................. 55

3.1.1. The synthetic strategy ........................................................................ 55

3.1.2. Synthesis of the model compound ......................................................... 56

3.2. Preparation of putative bisubstrate inhibitors: SAM analogues containing
   guanidine with carbon linkers ............................................................................. 62

3.2.1. Guanidine containing SAM analogue.................................................. 62

3.2.2. Nitrogen SAM analogues containing guanidine .................................. 73

3.3. Preparation of triazole linker SAM analogue with click chemistry .......... 81

3.4. Preparation of $N$-6 azide side chain SAM analogue .................................. 88

3.5. Attempted synthesis of (+)-sinefungin ....................................................... 93

3.5.1. The original synthetic strategy ............................................................... 93

3.5.1.1. Preparation of the key diene by C-6 and C-7 bond formation . 94

3.5.1.2. Preparation of the key diene by C-5 and C-6 bond formation . 96

3.5.2. An alternative route to dihydropyridazines........................................... 103

3.5.2.1. Preparation of the dihydropyridazine precursor........................... 105

3.5.2.2. Deprotection and ring opening of the precursor .......................... 111

3.5.2.3. Modification of the second synthetic strategy ............................... 117

Computational Docking and Biological Results ...................................................... 123
4.1. Molecular docking ..................................................................................... 123

4.2. Biological Results ...................................................................................... 131

4.2.1. *In vitro* evaluation of PRMT1 inhibition.......................................... 131

4.2.2. Evaluation of selectivity versus lysine methyltransferase SET7 ..... 132

Summary and Future Works............................................................................ 135

Experimental ........................................................................................................ 139

References.......................................................................................................... 218
Abbreviations

ADMA asymmetric dimethylarginine
AIBN azobisisobutyronitrile
ATP adenosine triphosphate
BAIB \((bis\text{(acetoxy)}\text{-}\text{iodo})\text{benzene}\)
Boc tert-butoxycarbonyl
Bz benzoyl
BzCl benzoyl chloride
CARM1 coactivator associated arginine methyltransferase 1
Cbz benzyloxycarbonyl
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC \(N, N'\text{-dicyclohexyl-carbodiimide}\)
DEAD diethyl azodicarboxylate
DIAD diisopropyl azodicarboxylate
DIBAL diisobutylaluminium hydride
DIPEA \(N, N'\text{-diisopropylethylamine}\)
DMF dimethylformamide
DMSO dimethylsulfoxide
DMAP 4-dimethylaminopyridine
DNA deoxyribonucleic acid
DPPA diphenyl phosphorazide
EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

Fmoc 9H-fluoren-9-ylmethyl carbonyl

hnRNP heterogeneous nuclear ribonucleoprotein

HIV human immunodeficiency virus

HOBt hydroxybenzotriazole

LDA lithium diisopropylamide

MES morpholinomethanesulfonic acid

MetJ methionine repressor protein

MMA monomethylarginine

NBS N-bromosuccinimide

PKMTs protein lysine methyltransferases

PRMTs protein arginine methyltransferases

Py pyridine

PyBOP benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate

rpS2 ribosome protein S2

RNA ribonucleic acid

SAH \(\delta\)-adenosyl-L-homocysteine

SAM \(\delta\)-adenosyl-L-methionine

SDMA symmetric dimethylarginine

snRNP small nuclear ribonucleoprotein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-1-oxy</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMSBr</td>
<td>bromotrimethylsilane</td>
</tr>
<tr>
<td>TMSCI</td>
<td>trimethylsilylchloride</td>
</tr>
<tr>
<td>TsCl</td>
<td>p-toluenesulfonyl chloride</td>
</tr>
<tr>
<td>TsOH</td>
<td>p-toluenesulfonate acid</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1. Biological methylation and $S$-adenosyl-$L$-methionine (SAM)

Biological methylation refers to the transfer of one or more methyl groups from a donor to a substrate in living organisms, usually under the control of enzymes called methyltransferases.\(^1\) Biomethylation is an important step in the regulation of a number of cellular processes in both prokaryotes and eukaryotes, providing the signal for processes, such as control of interactions with other macromolecules.\(^2\)

$S$-adenosyl-$L$-methionine (SAM, Figure 1) was discovered in 1952 by Cantoni and emerged as the major methyl group carrier in cells.\(^3\) SAM is claimed to be the second most abundant co-substrate in cells after ATP\(^4\) with an intracellular concentration of approximately 0.04mM\(^5\) and is responsible for a wide range of biological transformations in addition to methylation.\(^6\) The biosynthesis of SAM involves reaction between ATP and $L$-methionine catalysed by ATP: $L$-methionine $S$-adenosyltransferase (EC 2.5.1.6) (Figure 1).\(^6\)

SAM dependent methyltransferases catalyse the transfer of a methyl group from the sulfonium centre at SAM to a wide range of substrates, including DNA, RNA, proteins, hormones and neurotransmitters.\(^7\) The transfer of the methyl group from SAM to a nucleophilic atom of the substrate presumably occurs via $S_N2$ nucleophilic
displacement, leading to the by-product $S$-adenosyl-$L$-homocysteine (SAH). SAH is actually a strong inhibitor of methyltransferases and must then be displaced from the binding site before being hydrolysed into adenosine and $L$-homocysteine by the enzyme SAH hydrolase (EC 3.3.1.1) (Figure 1).$^6$ Each SAM dependent methyltransferase is necessarily adapted to display a substrate specific binding site in order to achieve selectivity for individual methyl acceptors.$^6$

![Figure 1: Biosynthesis of SAM, methyltransfer, and SAH's metabolism](image)

Methylation is a common post-translational modification of proteins, and any potentially nucleophilic atom including nitrogen, oxygen, sulfur and carbon can be transformed, leading to new physical properties that may be recognised by other proteins potentially allowing signal transfer.$^8$ The most common post-translational
methylations occur at oxygen or nitrogen functional groups. The transformation of side chain carboxylates of glutamates and aspartates into methyl esters is a common example of the former methyl transformation\(^9\)\(^{10}\) that has an obvious impact on charge as well as the size of the functional group.\(^9\) Obviously, such changes can be easily reversed by hydrolysis, thus offering modulation of target proteins.\(^11\)

Methylation at nitrogen atoms, such as the ε-amino group of lysine, the guanidine moiety of arginine, the imidazole ring of histidine, or the amide side chain of glutamine and asparagine is generally less easily reversed.\(^8\) Methylation of nitrogen atoms can occur up to three times (mono-, di- and trimethylation) in the ε-amino group of lysine, leading to a substantial increase in the size and hydrophobicity at that position, potentially disrupting protein-protein interactions, and the monomethylation of lysine can lead to a increase in the pK\(_a\) value,\(^12\) yet the overall charge may remain unaltered.\(^13\) In addition, it is not surprising that protein methylation is as important for regulating key cellular events, including regulation of transcription, stress response, ageing and protein repair, T-cell activation, nuclear transport, neuronal differentiation, ion channel function and cytokine signaling as other better-known post-translational modifications, such as phosphorylation.\(^14\)

The primary focus of this thesis is on protein arginine methyltransferases (PRMTs), which catalyse methylation of nitrogen atoms on the guanidine moiety of arginine residues of protein substrates.
1.2. Protein arginine methyltransferases (PRMTs) and protein arginine methylation

Protein arginine methyltransferases (PRMTs, EC 2.1.1.125) catalyse the methylation of arginine residues of a variety of proteins involved in cell signaling, RNA splicing and gene regulation such as histones H2A, H3 and H4. In common with the general pathway outlined above, all PRMTs utilise SAM as the methyl group donor. Cheng and co-workers described a possible catalytic mechanism of PRMT1 and PRMT3 based on the structural features of the active sites of these two enzymes. Two invariant glutamate residues (E144 and E153 of PRMT1 and E326 and E335 of PRMT3) located at the substrate arginine binding site are essential for methylation to occur. There is an electrostatic interaction between the substrate guanidine and E153 of PRMT1 (or E335 of PRMT3), while another glutamate residue E144 of PRMT1 (or E326 of PRMT3) acts as a general base, thus liberating a lone pair of electrons on the guanidine group that undergoes S_N2 reaction with SAM to generate the methylated arginine (Figure 2). Similar pairs of glutamate residue are conserved among all PRMTs, suggesting the common mechanism for PRMT catalysis.
Figure 2: The possible catalysed mechanism of PRMTs and their classification based on product type: Type I generating MMA and ADMA, and Type II generating MMA and SDMA, Type III only generating MMA and Type IV generating ß-N-MMA.

In general, PRMTs are categorised as Type I, Type II, Type III and Type IV enzymes (Figure 2). Type I, II and III enzymes catalyse the formation of monomethylarginine (MMA) at the terminal guanidine nitrogen atom. Type I and Type II enzymes may promote further methylation of MMA substrates, either at the previously methylated nitrogen to generate asymmetric dimethylarginine (ADMA) or the unsubstituted amine leading to symmetric dimethylarginine (SDMA) respectively. In addition, Type IV enzyme catalyse the monomethylation at the internal (or ß) guanidine nitrogen atom to generate ß-N-monomethylarginine. At present, 9 mammalian PRMTs have been identified, of which PRMT1, 3, 4, 6 and 8 belong to Type I enzymes, PRMT5, 7 are Type II enzymes, while PRMT2, and 9 are not classified because their exact enzymatic activity has not been demonstrated.
FBXO11 and FBXO10, a second family of putative enzymes related to the F box-only proteins have been suggested to be PRMTs, but their biochemical characterisation remains to be verified.\textsuperscript{20}

In general, PRMTs influence a number of important cellular processes including cellular growth, nuclear or cytoplasmic protein shuttling, differentiation and embryogenesis, RNA splicing and transport, and post-transcriptional gene regulation.\textsuperscript{21} The following discussion provides a very brief outline of some known biological roles for specific PRMTs, although this information continues to emerge.

PRMT1, a predominant Type I PRMT in mammalian cells, is one of the best studied PRMTs at the cellular level. The central role of PRMT1 is to provide regulation of protein function, for example methylation of heterogeneous nuclear ribonucleoprotein (hnRNP) molecules leads to shuttling of these proteins between the cytoplasm and the nucleus.\textsuperscript{22} PRMT1 can also methylate histone H4, providing modification as a transcriptional activation mark that may regulate gene expression, and thus contribute to what is called the “histone code”.\textsuperscript{23,24} Although PRMT2 does not seem to have enzymatic activity, it does appear to function as a coactivator for the estrogen receptor.\textsuperscript{25} PRMT3’s unique character is that it is located exclusively in the cytoplasm and harbors a zinc-finger domain at its N-terminal, which acts as a substrate recognition module.\textsuperscript{26} In fact, PRMT3 seems to be associated with ribosome protein S2 (rpS2), because in the absence of PRMT3, rpS2 is hypomethylated.\textsuperscript{27} The modification of rpS2 with PRMT3 may therefore play a role in the regulation of protein synthesis.\textsuperscript{27} PRMT4 binds with the steroid coactivators
and has apparent transcriptional co-activity itself, and is consequently also named as
coactivator associated arginine methyltransferase 1 (CARM1). PRMT4 is a
coactivator for nuclear receptors and its over-expression is correlated with human
prostate and breast cancers. PRMT5, the major Type II enzyme, methylates histone
H2A, H3 and H4 and localises to both the cytoplasm and nucleus. In the cytoplasm,
methylation of Sm protein by PRMT5 is implicated in small nuclear
ribonucleoprotein (snRNP) biogenesis. Nuclear PRMT5 associates with regulators
of transcriptional elongation and can also complex with the hSWI/SNF
ATP-dependent chromatin remodelling proteins, presumably acting as a
transcriptional coactivator. PRMT6 is a nuclear enzyme and seems to have the
ability to methylate itself. PRMT6 specific substrates include the nuclear scaffold
protein, histone H3 and H4, DNA polymerase β and HIV proteins. PRMT7 has
been described as having Type II activity to form SDMA and Type III activity to
form MMA on different substrates. PRMT8, is mainly limited to the brain and is
the only PRMT known to be membrane associated, being attached to the plasma
membrane via N-terminal myristoylation. PRMT9 (4q31) was identified based on
homology with other family members as a product of a gene on human chromosome
4q31. It has not been biochemically characterised to date and its N-terminal has a
TPR repeat, which may modulate protein-protein interactions. FBXO11, referred to
as PRMT9, was identified from a “forced” alignment of its amino acid sequence to
the PRMT family. Its enzymic activity is not clear, because FLAG-tagged
hFBXO11 has been reported to have Type II activity, but HA-tagged hFBXO11 and
its C. elegans orthologue have no PRMT activity. FBXO10 has been temporarily identified as PRMT11, but no biological characterisation has been reported to date.

Besides their important roles in maintaining normal cellular function, PRMT activities are dysregulated in some human diseases, such as cardiovascular diseases and cancer. PRMT1 has recently emerged as a potentially new target for the development of a novel therapeutic for heart disease, due to its overexpression in patients with coronary heart disease. Moreover, PRMT1 appears to be responsible for generating the majority of the ADMA in cells, whose plasma levels are apparently directly related to heart diseases. Raised plasma levels of free ADMA is indeed detected in patients with atherosclerosis, hypercholesterolemia and heart failure. Therefore, an efficient therapy for cardiovascular disease may be targeted toward the inhibition of PRMT1. Additionally, PRMT4/CARM1 targeted therapeutics may also provide a novel treatment for a variety of human cancers, because this enzyme acts as a transcriptional co-activator for multiple nuclear receptor family members including androgen and estrogen receptors and dysregulation of nuclear receptor signalling, which are features of hormone dependent cancers, such as prostate and breast cancer.

The possible relationship between dysregulated PRMT functions with human diseases has stimulated interest in the design and synthesis of selective inhibitors for PRMTs. PRMT1 was identified as the predominant Type I PRMT in mammalian cells, consequently it is one of the best studied PRMTs at the cellular level and its
x-ray crystal structure is available,\textsuperscript{16} for this reason our initial efforts are focused on developing specific inhibitors for this protein.

1.3. Crystal structure of PRMT1

The X-ray crystal structure of rat PRMT1 in complex with SAH, the by-product of SAM, and a 19 residues peptide substrate containing three arginines can be accessed from the Protein Data Bank (PDB 1OR8).\textsuperscript{16} The overall structure of PRMT1 can be divided into four parts: N-terminal, SAM binding domain, $\beta$ barrel domain and dimerisation arm (Figure 3). The SAM binding domain resembles other methyltransferases, whereas, the $\beta$ barrel domain is unique to the PRMT family. In fact, PRMTs have a hydrophobic interface that enables dimerisation and even though the precise crystal structure of the dimer is not currently known, it is apparent that this dimer interface is formed between the dimerisation arm and the outer surface of SAM binding site. Furthermore, dimerisation is believed to be essential for methylation activity and the formation of the asymmetric dimethylarginine product.\textsuperscript{16}

The crystal structure reveals that the cofactor product, SAH is surrounded by amino acid residues that are conserved throughout the PRMT family (Figure 3).\textsuperscript{16} Various electrostatic and hydrogen bonding interactions between SAH and PRMT1 include: the homocysteine carboxylate group and R54; the ribose dihydroxyl group and E100; one of the ribose hydroxyl group and H45; the adenine ring amino group and E129; the adenine ring and V128 (Figure 4).\textsuperscript{16}
Figure 3: Overall monomeric structure of PRMT1 in complex with SAH (tube) and substrate arginine peptide (ARG, ball and stick) was visualised by software Visual Molecular Dynamics (VMD).  

Figure 4: SAH (black) bound with PRMT1 residues (blue) by hydrogen bonds. Dashed lines indicate hydrogen bonds.

Most PRMT1 substrates contain glycine- and arginine-rich sequences, and bind with the substrate binding site which is expected to be acidic (Figure 5) and located...
in a deep pocket between the SAM binding domain and the β barrel domain (Figure 3). The residues that make up the substrate binding site are also conserved in the PRMT family, and include two glutamates E144 and E153 which form two hydrogen bonds with the substrate arginine and stabilise the substrate arginine in the binding pocket via electrostatic interactions, thus, are important for the catalysed methylation (Figure 5).  

![Figure 5: Active site of PRMT1 (solid electrostatic surface) in complex with SAH (yellow tube) and substrate arginine (ball and stick). Key glutarnates residues E144 and 153 are the red areas surrounding the target arginine.](image)

However, the major protein methylation sites within cells are not only on arginine residues but also on the ε-amino group of lysine. Protein lysine methyltransferases (PKMTs, EC 2.1.1.43) catalyse the methyl group transfer from SAM to protein lysine residues, including K4, K9, K27, K36 and K79 in histone H3, K20, K59 in histone H4, K26 in histone H1B. Almost all PKMTs contain a SET domain with the
DOT1 family as one known exception. In fact, the active site of PKMTs differ from PRMTs mainly in SET domain by comparing the X-ray crystal structure of human SET7/9 in complex with SAH (SAM cofactor) and mono-methylated lysine 4 on histone H3 (PDB 1O9S) with X-ray crystal structure of PRMT1 (PDB 1OR8), which has been described above.

**Figure 6:** Active site of SET7/9 (solid electrostatic surface) in complex with SAH (yellow tube) and substrate lysine (ball and stick). There is a narrow channel passing through the enzyme that connects the substrate lysine and SAH binding sites.

The crystal structure of human SET7/9 revealed that the peptide substrate and SAH are located on opposite sides of the SET domain and there is a narrow channel passing through the enzyme that connects the peptide and cofactor binding surfaces. The target lysine residue of the substrate accesses the methyl group donor, SAM by inserting its side chain into this narrow channel (Figure 6). At the lysine binding
site, the channel surface is formed by hydrophobic residues that engage in van der Waals interactions with the lysine side chain. In addition, two tyrosine residues (Tyr 245 and Tyr 305) at the lysine binding site hydrogen bond to the lysine ε-amino group, aligning it for reaction with SAM.\textsuperscript{38}

The X-ray crystal structure of PRMT1 confirms that its substrate arginine binding surface is hydrophilic, which reinforces the possibility of discovering specific inhibitors that might bind with PRMT1 selectively, but not with PKMTs.

1.4. Inhibitors of PRMTs

Even though PRMTs are known to play physiologically important roles in numerous cellular processes, their general function \textit{in vivo} is still not well understood. Global inhibitors of methyltransferases that have so far been used for the study of biological role of protein arginine methylation \textit{in vivo} fall into two general types: the first are analogues of SAM that compete at the binding site, such as SAH, methylthioadenosine 1 and (+)-sinefungin 2 (Figure 7); the second are small molecules such as adenosine dialdehyde 3 that inhibit SAH hydrolase, thus causing intracellular accumulation of SAH and feedback inhibition of most methylation reactions (Figure 7).\textsuperscript{39} However, use of such broad-spectrum inhibitors is problematic, because PRMTs are inhibited along with lysine methyltransferases, DNA methyltransferases and other SAM-dependent enzymes, thus it is difficult to assign specific cellular functions to a distinct methylation event.\textsuperscript{40,41}
Development of specific inhibitors of PRMTs will therefore be very attractive for the study of PRMT biological activities in vivo. Chemical interference offers exquisite control over biological pathways since various concentrations of inhibitors can be added, or withdrawn at any time in a biological process. There is resurgent interest in protein arginine methylation, but there are relatively few chemical probes with which to dissect the biological significance of this pathway, creating strong demand for such compounds. Development of novel therapeutics is a related but distant ambition when compared to the attempt to address this initial demand for such chemical probes. The design of specific PRMT inhibitors faces a number of common, but important challenges: 1) selective inhibition of PRMTs in the presence of a wide...
range of SAM utilising enzymes and 2) competition with the high intracellular concentration of SAM (0.04mM).

The first specific inhibitor of PRMTs, AMI-1 4 (Figure 7) was discovered to selectively inhibit arginine methylation, but not lysine methylation in vitro in 2004 by Bedford and co-workers through the screening of a random compound library. Furthermore, AMI-1 is cell permeable and can prevent protein arginine methylation in cells and has been shown to act as a brake on the action of certain hormones in vivo. For example, AMI-1 can modulate nuclear receptor regulated transcription from estrogens and androgen response elements. This molecule does not compete at the SAM/SAH binding site, and is presumed to insert into the arginine binding pocket, although this remains to be established. If true, then this might explain the reason that this compound displayed the suggested specificity for PRMTs. Although there is no crystal structural data available to prove this hypothesis, it is possible that the urea functionality of AMI-1 resembles the guanidine group, while the hydrophobic naphthalene ring system is of similar length to the alkyl chain of arginine. On the other hand, AMI-1 is a dye and resembles sulfonated ureas of the Suramin type which have been reported to be pleiotropic drugs that target many proteins, so it is not an attractive candidate for the therapeutics and the possibility that AMI-1 targets other proteins required further investigation. Nonetheless, AMI-1 provides the first indications that selective chemical modulation of PRMTs over other methyltransferases is possible and how such probes might be used to give an indication of biological processes controlled by PRMTs. The longer term goal of
developing compounds that are able to selectively modulate individual PRMTs requires compounds that are rationally designed to exploit individual variations between the binding sites of individual PRMTs.

Subsequently, a target-based approach for the rational design of PRMT inhibitors was described by Jung and co-workers.\textsuperscript{42,43} Virtual screening of a compound library lead to the identification of three compounds, stilbamidine 5, allantodapsone 6 and 7 (Figure 7), and importantly compound 7 showed no inhibitory effect on lysine methyltransferase SET7/9.\textsuperscript{43} These inhibitors were later found to cause cellular hypomethylation and a block of estrogen receptor activation, offering a potential drug strategy for the treatment of hormone dependent cancer, such as breast cancer.\textsuperscript{43}

In addition, a “bump and hole” approach has been developed in an attempt to resolve the problem of improving selectivity of potential chemical probes. This strategy creates an ligand-mutant receptor pair in which the SAM binding site is genetically modified to feature a “hole” that complements the shape of a “bumped” ligand that is too large to bind with the wild-type enzyme (Figure 8).\textsuperscript{44} The “bump and hole” approach was firstly used by Schreiber and co-workers to describe an engineered cyclosporin and cyclophilin interface\textsuperscript{45} and then successfully implemented with kinases, nuclear hormone receptors and human growth hormone receptors.\textsuperscript{46} In fact, the most important function of the “bump and hole” approach is generating a chemical probe to selectively regulate transcription, apoptosis, genetic recombination, signal transduction for the manipulation and study of biological systems.\textsuperscript{44}
Figure 8: Explanation of "bump and hole" approach. The nature ligand (inhibitor or substrate) can bind with protein (wide-type enzyme) and bumped ligand with mutant protein respectively.

Therefore, Gray and co-workers attempted to deliver substrates for specific PRMT mutants using the "bump and hole" strategy in which a mutation introduced to yeast arginine methyltransferase (Rmt1) binding site is designed to complement a chemically modified version of the co-substrate, in this case SAM. During these studies, benzyl-SAH 8 and naphthylmethyl-SAH 9 analogues (Figure 9) were identified to selectively inhibit mutant Rmt1 and not the wild-type enzyme in vitro due to their structure being complementary to the expanded SAM binding site. SAM analogue 10 (Figure 9) was found to be preferentially utilised by the mutant Rmt1 as methyl group donor and not by the wide-type enzyme. Such target-selective substrates or inhibitors may be useful as a chemical tool to study the function and perhaps identify the substrates of specific PRMTs in vivo. Even though the "bump
and hole” derivatives can inhibit the mutant protein arginine methyltransferases, they
do not inhibit the wild type PRMTs and they require significant manipulation of the
model organism.

Figure 9: Chemical structure of “bump and hole” derivatives, SAH analogues (8 and
9) as selective inhibitors and SAM analogues (10) as selective substrate to the mutant
yeast Rmt1 respectively.

This thesis concentrates on the rational design of a series of potential inhibitors of
PRMT1 which hopefully can be used for the study of PRMT function in vivo and as
prototype compounds that would ideally lead to novel therapeutic strategies as well.

1.5. Chemical synthesis and stability of SAM

There are a number of important factors that need to be taken into account when
developing SAM analogues. In particular, the sulfonium centre of SAM is chiral and it has been demonstrated that the (-)-isomer is required for biomethylation. Unfortunately, the sulfonium centre undergoes slow pyramidal inversion, leading to the inactive (+)-isomer (Scheme 1). Moreover, the sulfonium centre can act as an efficient leaving group and degradation may occur by a range of routes, for example at high pH the nucleophilic carboxylate may displace the sulfonium centre, leading to the formation of methylthioadenosine 1 and lactone 11 (Scheme 1). Wong reported studies that revealed that large counteranions, such as sulfates are able to stabilise the sulfonium centre of SAM against epimerisation and decomposition over a pH range of 3.5 to 5.5 to some extent.47

Scheme 1

In addition, SAM is very sensitive to mild alkaline conditions resulting in the cleavage of the glycosidic bond. Borchardt reported the mechanism of this alkaline hydrolysis of SAM as outlined in Scheme 2.48 In the first stage, cleavage of the glycosidic bond occurs with base catalysed elimination between C-4 and C-5,
leading to removal of purine ring at C-1 to form the alkene 12 and purine 13. Next, nucleophilic addition to the aldehyde at C-1 proceeds with conjugate addition to the vinyl sulfonium to form sugar 14 with loss of stereochemistry at C-4.

Scheme 2

Scheme 3

Reagents and conditions:

a) CH$_3$I, formic acid, 80%.\textsuperscript{49}

Even though the sulfonium centre of SAM is unstable, Borchardt reported a chemical synthesis of SAM (Scheme 3).\textsuperscript{49} Treatment of SAH with methyl iodide and formic acid in the dark for 5 days delivered SAM as a mixture of diastereoisomers in 80% yield after purification by Amberlite IRC-50 ion exchange resin.
Recently an industrial manufacture of SAM leading to enriched (S,S)-isomer has been reported (Scheme 4). Treatment of adenosine 15 with thionyl chloride and pyridine in acetonitrile gave 5'-chloro adenosine 16 in 94% yield. L-Homocysteine sodium salt was produced by treatment of commercially available L-methionine with sodium metal in the presence of liquid ammonia. SAH was then obtained by condensing 5'-chloro adenosine 16 and L-homocysteine sodium salt in the presence of potassium iodide and water in 75% yield, and SAH was finally subject to methylation using Meerwein's salt 17 in the presence of trifluoroacetic acid to afford SAM with enrichment of (S,S)-isomer in the yield of 60-65%.

1.6. Stable nitrogen analogues of SAM

Because of the relative instability of SAM and its analogues, it is attractive to replace
the sulfonium centre with another more stable functional group, which would allow for a more robust inhibitor analogue. A series of nitrogen analogues of SAM was reported by Nelson and Eeclick recently.\textsuperscript{51,52} Nitrogen analogues of SAM, named AzaAdoMet 18 and MeAzaAdoMet 19, in which the sulfonium moiety is replaced by a tertiary amine or quaternary ammonium centre respectively have been reported originally by Blackburn and co-workers (Figure 10).\textsuperscript{53}

![Structure of SAM nitrogen analogues: AzaAdoMet 18 and MeAzaAdoMet 19.](image.png)

Figure 10: Structure of SAM nitrogen analogues: AzaAdoMet 18 and MeAzaAdoMet 19.

The tertiary amine at the 5'-position of adenosine was generated by initial alkylation of methylamine with adenosyl 5'-tosylate 20 in 80% yield, then subsequently alkylated with either the oxazolidinone protected iodide (S isomer) 22 or the racemic $\text{N}$-Boc and O-methylester protected bromide 23 to generate the protected precursors 24 and 25 respectively (Scheme 5).\textsuperscript{53}
Scheme 5

Reagents and conditions:

a) CH₃NH₂, 80%; b) DIPEA, CH₃CN, 51% (33%); c) BF₃·Et₂O, C₂H₅SH, CH₂Cl₂, 68%. ⁵³

Unfortunately, this second alkylation to introduce the side chain needed long reaction times (2-4 days) and produced the desired products in a disappointing yield (51% and 31% respectively). If strong base, such as sodium hydride was used in the second alkylation, the yield presumably would be improved, but the deprotonation of the secondary amine occurred at the same time as the purinyl amine in adenosine leading to alkylation on the N-6 position as by-product.⁵³ Moreover, deprotection of either precursor 24 with BF₃·EtSH complex, or precursor 25 with bromotrimethylsilane (TMSBr) proved to be problematic and resulted in partial deprotection in both.
cases. This problematic and quite lengthy synthesis highlights the fact that development of a new, more efficient synthetic route toward bisubstrate inhibitors based on this tertiary amine motif would be highly desirable.

Blackburn reported that AzoAdoMet 18 had a relatively low pK$_a$ ~ 7.08, which meant that switching 18 into its protonated form requires a pH below 6.5, which may be unsuitable for some proteins. So MeAzoAdoMet 19, a quaternary dimethyl-ammonium analogue was developed, which bears a permanent positive charge at 5'-position that is independent of pH.\textsuperscript{53}

In fact, a racemic form of AzoAdoMet 18 was previously reported by Kenyon to potentially inhibit \textit{E.coli} transfer RNA uracil-5-methyltransferase and \textit{E.coli} B methionine synthase.\textsuperscript{54} Consequently, Blackburn reported that the protonated form of homochiral AzoAdoMet 18 was a potent inhibitor of the \textit{E. coli} methionine repressor protein (MetJ), binding more tightly than SAM and indeed the crystal structure of the resulting MetJ-AzoAdoMet 18 complex was reported.\textsuperscript{55} Moreover, the quaternary ammonium compound, MeAzaAdoMet 19 was competitive with SAM in the inhibition of DNA methyltransferase Hhal at nanomolar concentrations. Therefore, these highly promising biological results promoted us to design and prepare modified nitrogen SAM analogues that might be expected to act as specific bisubstrate inhibitors of PRMT1.
1.7. The natural (+)-sinefungin as a potent inhibitor of methyltransferases

1.7.1. Biological activity of (+)-sinefungin

The natural product (+)-sinefungin 2 (Figure 7) was isolated in 1971 from a strain of Streptomyces grisoleus at Lilly Research Laboratories\textsuperscript{56} as a nucleoside antibiotic with broad biological activities,\textsuperscript{57} such as anti-fungal activity especially against Candida albicans, anti-viral activity including strong activity against HIV,\textsuperscript{58} anti-parasitic activity particularly against several species of Leishmania and anti-tumor activity. The biological properties of (+)-sinefungin all derive from inhibition of SAM dependent proteins because of its structural similarity with SAM, in that it preserves all of the structure of the co-substrate, but replaces the sulfonium centre with an amine side-chain that is presumably protonated at physiological pH thus supplying a positive charge at a similar location to the sulfonium centre. Unfortunately, clinical use of (+)-sinefungin is limited due to its known in vivo toxicity, which is not surprising given its general activity. The total synthesis and structural modifications of (+)-sinefungin have been attractive to many researchers.

1.7.2. Background of (+)-sinefungin total synthesis

A number of total synthetic routes to (+)-sinefungin have been published.\textsuperscript{56,57,59-63}
Four of the synthetic procedures produce a mixture of epimers at the C-6 position, while other routes generate the correct stereochemistry at C-6, but over many steps. Of these approaches Barton's route using radical chemistry is perhaps the most elegant (Scheme 6). 57

**Scheme 6**

![Scheme 6 diagram]

Reagents and conditions:

a) N-methylmorpholine, isobutyl chloroformate, N-hydroxyl-2-thiopyridone, then olefin 28, 45%; b) Bu3SnH, AIBN, 36% (30) and 38% (31); c) (CF3CO2)2IC6H5, Py, DMF/H2O; ii) (BOC)2O, Et3N, DMF, 66%; d) i) H2, Pd/C; ii) NH3/MeOH; iii) TFA/H2O, 80%. 57

The N-hydroxyl-2-thiopyridyl ester 27 was synthesised from adenosyl 5'-carboxylic
acid 26, then treated with initiator, resulting in the generation of the radical at C-4 position which then reacted with electron deficient olefin 28 and the resulting C-6 radical quenched by further reaction with thiocarbonyl compound to provide the sinefungin precursor 29 as a mixture of diastereoisomers in 45% yield. Reduction of precursor 29 with tributyltin hydride furnished amides 30 and 31 which were isolated separately as R and S isomers at C-6 position in a yield of 36% and 38% respectively. Finally, precursor 31 was transformed into (+)-sinefungin by Hoffmann rearrangement and deprotection in 80% yield. Although very concise, the synthesis lacks efficiency as the yields for the key reactions are poor and there is no control over the C-6 stereocenter.

Other routes to sinefungin have also been reported. Fourrey and co-workers reported a synthesis of (+)-sinefungin by forming the C-6 and C-7 carbon bond via Horner-Emmons reaction (Scheme 7).56 The key Horner-Emmons condensation of phosphonate 32 and aldehyde 33 produced a 1:1 mixture of unsaturated nitriles 34 and N-6-debenzoylated derivative in a total yield of 72%. Reduction of the alkene 34 using magnesium in methanol afforded the nitriles 35 as a diastereoisomeric mixture at C-6 in 84% yield. Hydration of the nitriles 35 with hydrogen peroxide afforded the corresponding amides in a disappointing yield of 22% for the two epimers 36 and 37, and the single diastereoisomer was isolated by HPLC. Consequently, sinefungin was synthesised from the key precursor 37 by Hoffmann rearrangement and deprotection. Although short, Fourrey’s procedure is limited due to the poor yielding nitrile hydration and the use of HPLC which make large scale synthesis unattractive.
Scheme 7

Reagent and conditions:

a) Mg(OCH₃)₂, aldehyde 33, CH₃OH, 72%; b) Mg, CH₃OH, 84%; c) H₂O₂, NaOH, CH₃OH, 22% as two epimers (36 and 37). ⁵⁶

Moffatt and co-workers reported successive nitroaldol reactions to form the C-5 and C-6 bond, C-6 and C-7 bond of sinefungin (Scheme 8). ⁵⁹ The adenosyl 5'-aldehyde 38 was reacted with nitromethane and potassium tert-butoxide to generate 5'-hydroxyl compound 39 in 91% yield, this was deoxygenated by acetylation and reduction to afford the nitro compound 40 in 55% yield. Next, the second nitroaldol reaction and deoxygenation were carried out with nitro compound 40 and aldehyde 41 using the previous procedures to afford the nitro precursor 43 as a mixture of epimers. Deprotection and reduction produced (+)-sinefungin from the precursor 43 in 22% yield, which was purified by preparative reverse HPLC.
Scheme 8

Reagents and conditions:
a) CH₃NO₂, t-BuOK, t-BuOH, 91%; b) i. Ac₂O, HClO₄, ii. NaBH₄, EtOH/THF, 55%;
c) t-BuOK, t-BuOH, aldehyde 41, 69%; d) i. Ac₂O, DMAP, THF, ii. NaBH₄, EtOH/THF, 77%;
e) i. 90%TFA, ii. H₂, Pd/C, MeOH, PtO₂, 22%.59

Another approach depending on the nitroaldol reaction was reported by Buchanan
which provides sinefungin in fewer steps (Scheme 9).63 Key to this route is the
formation of C-5 and C-6 bond using a single nitroaldol reaction conditions. The
adenosyl 5'-aldehyde 38 and lysine derived nitro 44 were combined in the presence
of tetrabutylammonium fluoride (TBAF) as base to generate the 5'-hydroxyl
intermediate, which was deoxygenated using standard conditions to furnish the nitro
compound 45 as a 1:1 mixture of two C-6 epimers in 70% over three steps.
Subsequently, deprotection and reduction of nitro with Raney Ni gave (+)-sinefungin
and its C-6 epimer 46 in 32% yield. Although this route is short, they did not report the synthesis of stereochemically pure sinefungin.

Scheme 9

Reagents and conditions:

a) i. TBAF, THF, ii. 

Ac2O, DMAP, iii. NaBH4, EtOH, 70% (1:1 C6-epimers); b) i. ZnBr2, MeOH, ii. 80% TFA, iii. Raney Ni, MeOH, 32%.

The four approaches to synthesise (+)-sinefungin as described above cannot generate C-6 (S) epimer selectively, but Rapoport and co-workers reported a stereoselective route to generate the C-6 (S) epimer again using a nitroaldol reaction (Scheme 10). The ribose derived 5'-aldehyde 47 was treated with nitro side chain 48 and potassium fluoride to generate the nitro alcohol, and then dehydrated with dicyclohexyl-carbodiimide (DCC) and copper chloride to give nitro alkene 49 as a cis and trans mixture in a yield of 83% over two steps. Reduction with zinc and acetic acid produced the oxime 50 in 84% yield, which was then oxidatively hydrolysed with ceric ammonium nitrate (CAN) to afford the ketone 51 in 81% yield.
Selective reduction of the ketone 51 using L-selectride gave the desired alcohol in 80% yield as the major diastereoisomer (92% de), which was subsequently treated with toluenesulfonic acid anhydride to furnish the tosylate 52 as a single diastereoisomer after recrystallisation in 63% yield. The azide group was introduced into the C-6 position by S_N2 nucleophilic displacement with obvious inversion of stereochemistry to produce the key precursor 53 in quantitative yield. Finally, (+)-sinefungin was synthesised by installing the adenine ring, reduction of azide and deprotection.
Ghosh and co-workers published another enantioselective synthesis of (+)-sinefungin at C-6 and C-9 positions in many steps (Scheme 11). The key diastereoselective alkylation at the C-6 position was achieved using the chiral auxiliary 55 which was generated by following Evan’s protocol. Oxazolidinone 55 was deprotonated with n-butyllithium and then treated with the anhydride formed from the D-ribose derived acid 54 and \textit{tert}-butyl chloroformate in the presence of triethylamine to give imide 56 in 70\% yield. Next, imide 56 was converted to the lithium enolate with lithium hexamethyldisilazane ((TMS)$_2$NLi) and then treated with allyl iodide to afford the allylation product 57 as a single diastereoisomer in 78\% yield. Hydrolysis of the chiral auxiliary was achieved using lithium hydroperoxide to provide the chiral acid 58 in near quantitative yield.

Scheme 11

\[
\begin{align*}
\text{HO} & \quad \text{OMe} \\
\text{54} & \quad \text{a)} \quad \text{a)} \quad \text{55} \quad \text{b)} \quad \text{56} \\
\text{H} & \quad \text{N} \\
\text{H} & \quad \text{Me} \\
\text{H} & \quad \text{OMe} \\
\text{57} & \quad \text{c)} \quad \text{58} \\
\end{align*}
\]

Reagent and conditions:

a) CICOOr-Bu, Et$_3$N, THF, then 55, n-BuLi, 70\%; b) (TMS)$_2$NLi, THF, then allyl iodide, 78\%; c) LiOOH, THF/H$_2$O, 98\%.61
Scheme 12

Reagent and conditions:

a) i. DPPA, Et₃N, toluene, then PhCH₂OH, 79%, ii. NaH, PhCH₂Br, n-Bu₄N⁺T (cat), THF/DMF, 87%; b) i. O₃, CH₂Cl₂/CH₃OH, then (CH₃)₂S, ii. t-BuOK, Wittig reagent 60, CH₂Cl₂, 74%; c) H₂, [Rh(COD)(R,R-DIPAMP)₂]⁺BF₄⁻, 50psi, CH₃OH, 95%.

Subsequently, stereochemical control at the C-9 position was achieved by asymmetric hydrogenation using a chiral rhodium catalyst (Scheme 12). Chiral acid 58 was converted to the protected amine derivative 59 by Curtius rearrangement using diphenyl phosphorazide (DPPA), triethylamine and benzyl alcohol in refluxing toluene, then benzylation. Introduction of C-9 amino acid species was achieved by oxidation and Horner-Emmons reaction of protected amine derivative 59. Ozonolysis
of alkene 59, followed by reductive workup with dimethyl sulfide produced the intermediate aldehyde, which was reacted with phosphonate 60 and potassium tert-butoxide to produce a 1:5 mixture of E and Z enamides 61 and 62 in 74% yield. Asymmetric hydrogenation of the mixture of enamides 61 and 62 in the presence of the rhodium catalyst \([\text{Rh(COD)}(R,R\text{-DIPAMP})_2]^:+\text{BF}_4^-\) generated 63 selectively as S isomer in C-9 position with good yield.

The diastereoselective allylation and asymmetric hydrogenation successfully installed the correct stereochemistry at C-6 and C-9 position. The remaining total synthesis of (+)-sinefungin involved the installation of the adenine ring and deprotection over several steps. Even though Ghosh’s synthesis is enantioselective, the control of the stereoselectivity at C-6 and C-9 position is achieved through a quite lengthy route from the starting material. Therefore, we were interested in developing an efficient, flexible, rapid and stereoselective synthesis of (+)-sinefungin.

1.7.3. Structural modifications of (+)-sinefungin

Although sinefungin shows strong bioactivity against fungi, viruses, parasites and tumors, when tested with larger mammals, it caused fatalities, probably due to its in vivo toxic side effects. Therefore, development of new therapeutic agents maintaining sinefungin’s bioactivity without toxic side effects to humans continues to be of considerable interest. Barton reported the sinefungin analogues 64 and 65
(Figure 11) bearing uracil or dihydrouracil groups instead of the adenine fragment and Robertgero published the sinefungin analogues 66 and 67 (Figure 11), lacking the amino moiety at C-9 and the terminal carboxylate respectively. The biological tests as anti-parasite showed that all of the analogues 64-67 have a lower affinity with protein carboxylmethyltransferase of leishmanial promastigotes than sinefungin leading to decreased inhibition of growth of Leishmania donovani promastigotes in vitro. As a result of these studies it can be concluded that the adenine fragment and the C-9 terminal amino acid group are important for binding to carboxylmethyltransferase and for the growth inhibitory effect. Moreover, inspired by the potent antiviral activity of natural carbocyclic nucleosides, Miller recently reported the synthesis of a carbocyclic analogue 68 (Figure 11), which contains an isosteric replacement of the ribosyl oxygen for a methylene unit. However, there are no reported biological results for carbocyclic analogue 68 in detail.

In addition, the structure-activity relationships studied of (+)-sinefungin and its derivatives have shown that some functional groups are necessary to retain methyltransferases inhibition including the L configuration of the terminal amino acid group, the three-carbon linker between the S carbon atom and the terminal amino acid group, and either the 2' or 3' hydroxyl groups on the ribosyl ring.
Figure 11: Chemical structure of sinefungin analogues: uracil or dihydrouracil analogues (64, 65), deamino at C-9 analogue (66), decarboxylate analogue (67), carbocyclic analogue (68).
Chapter 2

Project Aims and Objectives

This project is broadly focused on improving on the very small number of chemical tools available for the evaluation of protein arginine methylation in cells and can be defined in four aims: 1) development of general inhibitors of methyltransferases; 2) design and synthesis of specific inhibitors of protein arginine methyltransferases (such as PRMT1); 3) synthesis of a N-6 modified S-adenosyl-L-methionine (SAM) analogue for possible affinity purification of the target protein; 4) development of a novel, rapid and enantioselective synthesis of natural product (+)-sinefungin and its analogues.

2.1 Design and synthesis of trifluoromethyl SAM analogue toward general methyltransferases inhibition

Altering the electronic nature of the methyl group might be expected to influence the kinetics of biomethylation. Consequently, simple replacement of the methyl group of SAM with a trifluoromethyl group as in analogue 69 was envisaged (Figure 12); model compound 70 was useful for evaluating the reaction conditions required for installation of the trifluoromethylsulfonium centre. If SAM analogue 69 can compete with SAM at the receptor binding site of a methyltransferases then the electron withdrawing nature of the three fluorines might be expected to slow down the
methylation reaction and thereby influence the progress of the biological reaction, so
providing a preliminary point for the development of a new inhibitor. On the other
hand, trifluoromethyl-SAM 69 might act as a substrate, allowing potentially specific
transfer of a trifluoromethyl group to biological targets. Trifluoromethylation of
proteins and DNA might be attractive because $^{19}$F has spin $\frac{3}{2}$ and 100% natural
abundance making it a powerful probe for NMR, whereas the addition of 54 mass
units compared to the native methyl group might be useful for target identification by
mass spectrometry.

\[ \text{Figure 12: Chemical structure of trifluoromethyl SAM analogue 70 and model}
\text{compound 71.} \]

2.2. Design and synthesis of specific inhibitors of PRMT1

2.2.1. Guanidine containing SAM analogues with different carbon linkers

SAM is claimed to be the second most widely used substrate in enzymatic reactions
after ATP, so it is a significant challenge to achieve selective inhibition between
PRMTs and other SAM utilising proteins. We chose to explore the potential of
bisubstrate inhibitors that might achieve greatest binding by targeting both SAM and
arginine substrate binding sites, while potentially being excluded from the substrate
of other proteins due to poor fit arising from the extra functionality compared to SAM. The crystal structure of PRMT1 complexed with the product of methylation, S-adenosyl-L-homocysteine (SAH) and a substrate arginine bearing peptide (PDB 1OR8)\textsuperscript{16} shows that the arginine binding site includes two negatively charged glutamate residues (E144 and E153) which are believed to directly bind to arginine and be essential for catalysis, being a conserved feature across the known PRMTs.\textsuperscript{16} Therefore, the selective inhibition for PRMTs might be achieved by incorporating a guanidine group into the intended SAM analogues so that they could bind at both SAM binding site and the glutamate residues that form part of the arginine substrate binding site (Figure 13). Presumably the bisubstrate inhibitors could inhibit PRMT1 selectively as individual chemical tools to probe the process of protein arginine methylation or candidate drugs for the therapeutic of some diseases, such as cancer.

Figure 13: General concept for the design of bisubstrate inhibitors (black) for PRMT1, which presumably could interact with SAM binding site (blue) and two glutamates residues E144 and E153 (red).
SAM analogues 71, 74(a-c) and 75 (Figure 14) were designed to include functionality that mimics the guanidine portion of the arginine substrate side chain joined by short linkers that bridge between the SAM and substrate arginine binding sites. Improved potency and selectivity is expected to be achieved by further rational improvement of the functionalities appended to the rest of the nucleoside core. Additionally, SAH analogues 72 and 73 bearing guanidine moieties derived from the precursor of SAM analogue 71 were designed as a tool to investigate further the inhibition for PRMT1.

**Figure 14:** Chemical structure of bisubstrate inhibitors: guanidine containing SAM analogue 71, nitrogen SAM analogues 74 (a-c) and 75, SAH analogue 72 and 73.
2.2.2. Guanidine containing SAM analogue with triazole linker

Cu(I) catalysed [3+2] Huisgen reaction between azides and terminal alkynes is an exceedingly facile and high yielding reaction that is a popular method for assembling architectures from simple precursors. Occasionally, protein binding sites have been used to accelerate the reaction instead of copper, leading to a template induced synthesis of potential inhibitors. Azide SAM analogue 76 and alkyne SAM derivative 77 were designed as potential inhibitor fragments with the additional aim of attempting PRMT-templated synthesis (Figure 15).

Figure 15: Chemical structure of azide SAM analogue 76 and alkyne SAM analogue 77 and triazole linker SAM analogue 78.

In principle, only the best-fit combination of azide SAM analogue 76 with alkyne derivatives that target the arginine binding site, or alkyne SAM analogue 77 with corresponding azides will be selected from an array of potential coupling partners to undergo Huisgen reaction at the target binding site, thus revealing inhibitors that may
be detected at typical experimental concentration using HPLC and mass spectrometry. Such an experiment is an attractive long term goal that also requires expression and purification of substantial quantities of protein. In the near term, SAM derived bisubstrate inhibitor, containing triazole linkers, such as 78, can easily be generated using the Cu (I) catalysed [3+2] Huisgen reaction and then tested as a selective inhibitor of PRMT1 (Figure 15).

2.3. Design and synthesis of N-6 azide side chain SAM analogue

The function of PRMTs is currently poorly understood, thus modification of inhibitors to facilitate labeling of proteins, or affinity purification of their targets and associated proteins are desirable. Introduction of an azide side chain at the N-6 adenosine position of the SAM analogue 79 was expected to retain the inhibition for PRMTs by extending into space away from the binding pocket, yet allow easy attachment of biotin or fluorescent dyes via Staudinger ligation, or Cu (I) catalysed [3+2] Huisgen reaction as described (Figure 16)\textsuperscript{69}.

![Chemical structure of N-6 modified SAM analogue 79](image)

\textbf{Figure 16: Chemical structure of N-6 modified SAM analogue 79}
2.4. Total synthesis of (+)-sinefungin

The natural product (+)-sinefungin 2 (Figure 17) displays a wide variety of biological activities, being potent against various fungi, viruses, parasites and tumors as a result of its very potent, but not specific inhibition for methyltransferases.\textsuperscript{57} This potency arises from its close similarity of the structure of sinefungin and SAM. Presumably, the primary amine connected to C-6 of sinefungin is protonated at physiological pH and resembles the sulfonium centre of SAM to achieve strong binding, but not turnover at target methyltransferases. We were attracted by the possibility of using sinefungin as an alternative scaffold for inhibitor development and sought a novel and rapid synthetic method for the total synthesis of (+)-sinefungin. In addition, we aimed to design and synthesise sinefungin analogue 80 (Figure 17), bearing a guanidine group which is connected to the C-6 amino group by a carbon linker, as bisubstrate inhibitor toward the selective inhibition for PRMTI, perhaps without cytotoxicity.

![Figure 17: Chemical structure of (+)-sinefungin 2 and its analogue 80](image-url)
Chapter 3

Results and Discussion

3.1 Preparation of trifluoromethyl SAM analogue

Synthesis of the trifluoromethyl SAM analogue 69 (Figure 12) was attempted with the aim of exploring how the highly electron withdrawing fluorine atoms might affect the properties of SAM. The compound 69 might compete with SAM at the binding site, but would be expected to turn over slowly, therefore providing a preliminary point for the development of new general inhibitors of methyltransferases. Alternatively, if compound 69 was able to act as a substrate, the transfer of trifluoromethyl group to some biological targets might be attractive for either NMR spectroscopy due to the strong signal arising from $^{19}$F, which is present in 100% abundance, or mass spectrometry studies where trifluoromethyl groups can be identified at 54 Da higher than equivalent methylated substrates.

3.1.1. The synthetic strategy

As illustrated in Scheme 13, the sulfonium centre could be formed by three routes: 1) trifluoromethylation of thioether 81 using trifluoromethylation reagent (route a); 2) nucleophilic displacement of 5'-halogen modified adenosine 83 (or other leaving groups) with a thioether 82 containing a trifluoromethyl group (route b); 3)
alkylation of 5'-trifluoromethylthioadenosine 85 with an appropriate electrophile 84 (route c). We initially explored this trifluoromethylation chemistry using the readily available model compound 70 (Figure 12).

Scheme 13

3.1.2. Synthesis of the model compound

In the beginning, we explored direct trifluoromethylation of thioether 81 utilising trifluoromethylation reagent 86 (Scheme 14). It was recognised that there is a fine energetic balance between the target sulfonium 70 and the reagent 86, but we were enticed by the simplicity of this potential route. Following a known protocol, the 5'-chloro-5'-deoxyadenosine 16 was generated by the treatment of adenosine 15 with thionyl chloride and pyridine in acetonitrile in quantitative yield, which was purified
by ion exchange resin chromatography on Dowex 1 x 2-100 (OH'). Subsequently, 
N-protection of 2-aminoethanethiol with Boc anhydride was attempted in the 
presence of base, sodium hydride or triethylamine. In fact, the sodium hydride 
method\textsuperscript{71} provided a mixture of the desired compound 88 and a large amount of 
disulfide impurity. Although the disulfide can be reduced by some reagents, such as 
tributylphosphine,\textsuperscript{72} it was most practical to employ a milder base, such as 
triethylamine,\textsuperscript{73} which gave protected amino thiol 88 in a satisfactory isolated yield. 
Nucleophilic displacement of the 5'-chloro-5'-deoxyadenosine 16 with thiolate 
generated by protected amino thiol 88 and sodium hydroxide\textsuperscript{7} furnished the desired 
thioether 81 in 56% yield.

**Scheme 14**

![Scheme 14](image)

Reagents and conditions:

a) SOCl\textsubscript{2}, pyridine, CH\textsubscript{3}CN, 0°C-r.t., quantitative yield; b) compound 88, NaOH, 
60°C, 54%; c) trifluoromethylation reagent 86, DMF, r.t.; d) (Boc)\textsubscript{2}O, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 
r.t., 84%.
The electrophilic trifluoromethylation reagent 86 has been developed by Urnemoto.\textsuperscript{70} Alkanethiolate 90 was trifluoromethylated by the reagent 86 to give the trifluoromethyl alkane thioether 91 (Scheme 15). The possible reaction mechanism is bimolecular ionic substitution by the kinetic study, which refers a side-on attack to the S-CF$_3$ bond, not the conventional S$_{N2}$ attack mechanism, since the CF$_3$ carbon atom is covered by three electron withdrawing fluorine atoms of larger size than hydrogen atoms (scheme 16).\textsuperscript{74}

Scheme 15

\[
\begin{align*}
n-C_{12}H_{25}SH & \xrightarrow{a) i. NaH, THF; ii. trifluoromethylation reagent 86, rt, 47\%} n-C_{12}H_{25}SCF_3 \\
n-C_{12}H_{25}S & \xrightarrow{86} n-C_{12}H_{25}S^+ \\
& \xrightarrow{\text{OTf}^-} n-C_{12}H_{25}SCF_3
\end{align*}
\]

Reagents and conditions:

a) i. NaH, THF; ii. trifluoromethylation reagent 86, rt, 47\%.

Scheme 16

---

58
Unfortunately, all attempts to carry out electrophilic trifluoromethylation of the model thioether 81 in DMF were unsuccessful (Scheme 14). Presumably the sulfur atom in thioether 81 is less nucleophilic than thiolate 90, so the displacement of trifluoromethyl group from the reagent 86 could be difficult.

We next sought to generate the sulfonium centre by alkylation of a trifluoromethyl thioether precursor (Scheme 17). Deprotonation of thiol 88 with sodium hydride and treatment with the trifluoromethylation reagent 86 in THF produced the side chain 82 in poor yield of 42%, alongside significant amounts of the dimer. Unfortunately, attempts to subsequently alkylate the trifluoromethyl thioether 82 by displacement at 5'-chloro-5'-deoxyadenosine 16 in acetonitrile using a combination of sodium iodide and silver perchloride\textsuperscript{75} for in situ generation of the more reactive iodide did not deliver the desired product.

Scheme 17

![Scheme 17](image)

Reagents and conditions:

a) compound 82, NaI, AgClO₄, CH₃CN, r.t.; b) trifluoromethylation reagent 86, NaH, THF, 0°C-r.t., 42%.
The polarity of the sulfonium product means that its purification is as much of an issue as the reactions to generate it. Therefore, sulfonium centre formation was attempted with the protected 5'-iodo-5'-deoxyadenosine 93 with the intention that the product could be more easily purified by silica column chromatography (Scheme 18). 2',3'-Isopropylidene adenosine 92 was treated with methylphenoxyphosphonium iodide in dry CH₂Cl₂ to furnish protected 5'-iodo-5'-deoxyadenosine 93 in 48% yield. Reaction of the resulting iodide 93 with the trifluoromethyl thioether 82 in the presence of silver perchloride in acetonitrile, did result in consumption of starting material as monitored by TLC. However, purification from the complex reaction mixture proved difficult, so the desired product 94 could not be obtained.

Scheme 18

Reagents and conditions:

a) methylphenoxyphosphonium iodide, CH₂Cl₂, -70³C-r.t., 48%; b) compound 82, AgClO₄, CH₃CN, r.t.

In summary, the approaches to generate the desired trifluoromethyl sulfonium centre
for the model compound 70 failed. In fact, it may be possible to find conditions to effect this transformation in the future, such as alkylation of 5'-trifluoromethyl-thioadenosine 85, but we decided to turn our attention to a more readily achievable strategy for the exploration of the bisubstrate inhibitors concept.
3.2. Preparation of putative bisubstrate inhibitors: SAM analogues containing guanidine with carbon linkers

SAM is the second most abundant co-substrate after ATP, so it is a significant challenge to achieve selective inhibition of PRMTs over all other SAM utilising proteins. We sought to explore whether this PRMT selective inhibition might be achieved by linking guanidine functionality to SAM analogues with the idea that such selective binding for PRMTs but not with other methyltransferases might be achieved.

3.2.1. Guanidine containing SAM analogue

The failed synthesis of trifluoromethyl sulfonium centre for the model compound 70 lead us to examine a different route toward generation of the sulfonium centre involving alkylation at 5'-thio-5'-deoxy-adenosine 96. Additionally, the sulfonium centre can result in decomposition via attack of carboxylate at high pH environment, as described in Chapter 1. Therefore, the synthetic strategy of guanidine containing SAM analogue 71 would include the protection of the amino acid side chain 97 (or 98) prior to the generation of sulfonium centre, and then the stable amino acid might be obtained by deprotection in carefully controlled pH environment (Scheme 19).
Initially, we sought to make the protected guanidine containing thioether 96. Generally, the classical method of preparing protected guanidine derivatives is the treatment of amines with various guanidinylating reagents, such as protected thiourea or S-methylisothiourea. Consequently, two approaches were explored: one involved reaction of a guanidine containing side chain with 5'-chloro-5'-deoxyadenosine 16, or the related iodide 93 (Scheme 20); the other involved late stage guanidinylation of a modified adenosine 81 (Scheme 21). Protected guanidine containing compounds were sought as they would be sufficiently hydrophobic to allow normal purification until a final, ideally global deprotection step to liberate the highly polar target compounds.

As illustrated in Scheme 20, thiourea 99 was reacted with Boc anhydride and sodium hydride to produce the protected guanidinylating agent 100 in 59% yield. Subsequent reaction with cysteamine hydrochloride in the presence of triethylamine in DMF proceeded to give the side chain 101 in a disappointing yield of 34%. Of course, the reactivity of protected thiourea 100 could be increased with the utility of
mercury (II) chloride, but the cysteamine hydrochloride's thiol group might also be affected by such reaction conditions, thus we accepted the poor yield at this stage.

Scheme 20

Reagents and conditions:
- a) (Boc)$_2$O, NaH, dry THF, 0°C-r.t., 59%;
- b) cysteamine hydrochloride, Et$_3$N, DMF, r.t., 34%;
- c) compound 101, NaOH or t-BuOK, DMF, r.t.-40°C;
- d) compound 101, Cs$_2$CO$_3$ or DBU, DMF, r.t.

The subsequent deprotonation of thiol 101 using either sodium hydroxide or potassium tert-butoxide, and reaction with 5'-chloro-5'-deoxyadenosine 16 unfortunately failed. Similarly, attempts to react thiol 101 with the more hydrophobic acetonide protected 5'-ido-5'-deoxyadenosine 93 in the presence of bases, such as...
caesium carbonate and DBU also failed. Instead the mass spectrometry analysis which showed a major peak at 289 indicated that presumably the alternative product was the elimination compound 103 bearing an alkene at the C-4 and C-5 position.

Scheme 21

![Scheme 21](image)

Reagents and conditions:

a) i. TFA, CH₂Cl₂, 0°C-r.t., ii. compound 100, Et₃N, HgCl₂, DMF, r.t., 56%; b) TFA, CH₂Cl₂, 0°C-r.t., 78%.

Ultimately, the guanidine containing thioether 96 was generated by late stage guanidine formation using the cysteamine modified adenosine 81 (Scheme 21). Removal of the Boc protecting groups with TFA, followed by direct treatment of the resulting salt with guanidinylating reagent 100 in the presence of triethylamine and mercury (II) chloride gave the desired compound 96 in 56% yield, which was easily purified by silica gel column chromatography. This reaction was also attempted in the absence of mercury (II) chloride, but the rate of reaction was quite slow. Therefore, the key intermediate, guanidine containing thioether 96 was synthesised through three potentially simple steps, including the formation of alkyl chloride,
alkylation with thiolate and guanidinylation, which may be scaled up to larger quantities if required. In addition, this intermediate 96 can be used to evaluate subsequent formation of the sulfonium centre of the target bisubstrate SAM analogue 71, leading to a guanidine containing SAH analogue 72 via deprotection as a potential inhibitor of PRMTs.

Next, the Boc protected guanidine containing compound 96 was treated with TFA, and the reaction mixture adjusted to pH 9 with ammonia solution to get the target compound 72 as the free base in a good yield of 78%, which was then purified using ion exchange resin chromatography on Dowex 1 x 2-100 (OH-) in order to remove residual trifluoroacetic acid ammonium salt. We subsequently uncovered that compound 72 was claimed in a patent as a poor inhibitor for DNA methyltransferases (IC50 ~ 40μM). While it was a little disappointing to find that the compound has been previously described, it might have potential activity of the inhibitor for PRMTs and suggests that the planned synthesis can be applied to the synthesis of other guanidine containing analogues.

We next sought to adapt this route to generate more hydrophobic analogues, such as those containing a cycloethyl guanidine (Scheme 22). Originally, late formation of the cycloethyl guanidine was attempted following the procedure described above. Thus, Boc deprotection of thioether 81 and treatment of the TFA salt with 2-methyl-thio-2-imidazolium iodide in aqueous sodium hydroxide solution, was attempted but unfortunately this did not lead to the formation of desired compound 73.
Scheme 22

Reagents and conditions:
a) i. 4M HCl/dioxane, ii. 2-methylthio-2-imidazolium iodide, NaOH, r.t.-85°C.

Scheme 23

Reagents and conditions:
a) i. FmocCl, DIPEA, DMF, 0°C, ii. Bu3P, MeOH, r.t., 88%; b) compound 105, Cs2CO3 or DIPEA, DMF, r.t.

In fact, purification of the highly polar target compound 73 presented a significant problem. Therefore, a route incorporating two protecting groups, the acid sensitive acetonide and the base labile Fmoc group was explored (Scheme 23). Thus cystamine dihydrochloride 104 was reacted with Fmoc chloride and Hünig’s base in
DMF to give the protected disulfide in quantitative yield, which could be used without further purification. Subsequent reduction with tributylphosphine\textsuperscript{72} in methanol gave Fmoc protected thiol \textbf{105} in a good yield of 88\%. Unfortunately, the alkylation of thiol \textbf{105} with protected 5'-iodo-5'-deoxyadenosine \textbf{93} in the presence of either caesium carbonate or Hünig's base did not deliver the precursor \textbf{106}, and it was difficult to find a suitable base that could affect thiol alkylation without causing cleavage of Fmoc group (as detected by TLC and mass spectrometry).

We next sought to explore guanidinylation with protected thiomethyl-2-imidazoline \textbf{111} or imidazolidine-2-thione \textbf{113} (Scheme 24). Firstly, the two guanidinylating reagents \textbf{111} and \textbf{113} were prepared following known procedures.\textsuperscript{82,83} Thus, the protected thiomethyl-2-imidazoline \textbf{111} produced by treatment of methylthio-2-imidazolium iodide \textbf{110} with Boc anhydride and triethylamine in quantitative yield\textsuperscript{83} and protected 2-imidazolidinethione \textbf{113} was generated from 2-imidazolidinethione \textbf{112} with Boc anhydride and sodium hydride in 57\% yield.\textsuperscript{82} Consequently, the two guanidinylating agents were progressed toward formation of desired cycloethyl guanidine. Deprotection of compound \textbf{81} with TFA and direct treatment with guanidinylating reagents, either \textbf{111} or \textbf{113} in the presence of triethylamine and mercury (II) chloride in DMF produced only a trace of the cycloethyl guanidine \textbf{108} or \textbf{109} which were detected by mass spectrometry at peak 494 and 594, and much of the starting amine remained. Therefore, these reactions did not work well on scale up, but it indicates the possibility of forming cycloethyl guanidine containing analogues \textbf{73}. Essentially, we had to improve the overall yields for this route, so we turned our
attention to investigate the key bromoethane or iodoethane side chain 97 (or 98),
required for the formation of the guanidine containing SAM analogue 71.

**Scheme 24**

Reagents and conditions:

a) i. TFA, CH₂Cl₂, 0°C-r.t., ii. compound 111, Et₃N, HgCl₂, DMF, 80°C; b) i. TFA,
CH₂Cl₂, 0°C-r.t.; ii. compound 113, Et₃N, HgCl₂, DMF, 80°C; c) (Boc)₂O, Et₃N,
CH₂Cl₂, 0°C-r.t., 98%; d) (Boc)₂O, NaH, THF, 0°C-r.t., 57%.

Initially, we attempted to synthesise the bromide 97 from racemic methionine
(Scheme 25). The key lactone 115 was prepared as the hydrobromide salt in one step
from racemic methionine 114 by treatment with bromoacetic acid in water,
isopropanol and acetic acid in 65% yield.⁴⁴ Alternatively, the equivalent
hydrochloride salt 117 was synthesised in two steps from methionine 114, by
treatment with methyl iodide in aqueous methanol to provide the sulfonium salt 116
in 87% yield, which was then cyclised in the presence of aqueous sodium hydrogen
carbonate to give the lactone 117 in 60% yield. Obviously, the former approach for the formation of lactone hydrobromide 115 was more direct than the latter route involving discrete methylation and cyclisation to lactone hydrochloride 117.

Scheme 25

Reagents and conditions:
a) BrCH₂COOH, H₂O/isopropanol/CH₃COOH, 50-85°C, 65%; b) HBr/CH₃COOH; c) CH₃I, MeOH/H₂O, 35°C, 87%; d) NaHCO₃, H₂O, reflux, 60%; e) HBr/CH₃COOH; f) CH₃COOBr-Bu, H₂SO₄, CH₃COOH, and then (Boc)₂O, NaHCO₃, EtOAc/H₂O.

Opening of lactone 115 (or 117) using hydrobromic acid in acetic acid under a variety of conditions was attempted, as described in table 1, but it proved difficult to isolate the pure desired product 118 due to contamination of the product with the lactone starting material as observed by ¹H NMR spectroscopy. Unfortunately, attempts to prepare a protected version of the bromo amino acid 98 by treating the crude mixture with tert-butyl acetate and sulphuric acid in acetic acid and then Boc anhydride and sodium hydrogen carbonate also failed.
Table 1

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>Crude Yield(^a) (%)</th>
<th>Purity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100°C, 5.5h; then r.t. overnight</td>
<td>53</td>
<td>Mixture (Lactone and product)</td>
</tr>
<tr>
<td>2</td>
<td>50°C, 20h</td>
<td>84</td>
<td>Mixture (Lactone and product)</td>
</tr>
<tr>
<td>3</td>
<td>Sealed tube, 65°C, 6h; then r.t. overnight</td>
<td>42</td>
<td>Mixture (Lactone and product)</td>
</tr>
</tbody>
</table>

\(^a\)Yield before the purification; \(^b\)Estimated purity based on \(^1\)HNMR spectroscopy.

An alternative approach to generate the iodoethane derived amino acid 98 via L-aspartic acid was successful (Scheme 26). Treatment of L-aspartic acid 119 with thionyl chloride in dry methanol provided selective esterification of the \(\gamma\)-L-aspartate carboxylic acid 120 in 68% yield.\(^8\) Installation of the Boc group at the amine and the remaining tert-butyl ester protecting groups using two approaches was investigated. In the first, the Boc group was installed at the amine using standard conditions to provide compound 121 in 56% yield,\(^8\) then the tert-butyl ester was subsequently formed with the general method of dry tert-butanol, 4-dimethylaminopyridine (DMAP), \(N,N^\prime\)-dicyclohexyl-carbodiimide (DCC) to afford the desired protected aspartate 122 in 53% yield.\(^8\) However, an alternative one pot protocol gave superior overall yields, thus treatment of compound 120 with tert-butyl acetate in perchloric acid,\(^9\) then Boc anhydride and sodium hydrogen carbonate directly gave the protected aspartate 122 in 61% yield. Saponification of this fully protected aspartate derivative 122 provided \(\gamma\)-carboxylic acid 123 which was then isolated in 88% yield after recrystallisation.\(^9\) The target iodide 98 was then obtained using a one pot
procedure which proceeded in 55% overall yield from the carboxylic acid 123, thus treatment with ethyl chloroformate and triethylamine provided the corresponding anhydride which was reduced in situ with sodium borohydride to generate an intermediate alcohol\(^\text{89}\) that was finally converted into the iodide 98 by treatment with iodine, triphenylphosphine and imidazole.\(^\text{91}\)

**Scheme 26**

![Scheme 26](image)

Reagents and conditions:

- a) SOCl\(_2\), dry MeOH, \(-10^\circ\text{C}\), 68%;
- b) HClO\(_4\), CH\(_3\)COO\(-\)Bu, 0\(^\circ\)C-rt, and then (Boc\(_2\))O, NaHCO\(_3\), H\(_2\)O/THF, 0\(^\circ\)C-rt, 61%;
- c) 1M NaOH, acetone/H\(_2\)O, 0\(^\circ\)C, 88%;
- d) ClCOOC\(_2\)H\(_5\), Et\(_3\)N, dry THF, -5\(^\circ\)C-rt, NaBH\(_4\)/H\(_2\)O, 0\(^\circ\)C-rt, and then I\(_2\), Ph\(_3\)P, imidazole, CH\(_2\)Cl\(_2\), 0\(^\circ\)C, 55%;
- e) (Boc\(_2\))O, Na\(_2\)CO\(_3\), dioxane/H\(_2\)O, 0\(^\circ\)C-rt, 56%;
- f) t-BuOH, DMAP, DCC, dry CH\(_2\)Cl\(_2\), 0\(^\circ\)C-rt, 53%.

Reliable access to the iodoethane side chain 98 permitted investigation into alkylation of the thioether 96 that would ultimately lead to the desired sulfonium compound 95 (Scheme 27). Unfortunately, all attempts to alkylate the thioether

72
intermediate 96 failed, such as performing the reaction in the presence of silver perchlorate or silver tetrafluoroborate. As indicated in section 3.1, formation of the trifluoromethyl substituted sulfonium centre was also difficult to access and represents a significant barrier to the formation of sulfonium centre based SAM analogues.

Scheme 27

Reagents and conditions:
a) iodide compound 98, AgClO₄, CH₃CN, 0°C-rt; b) iodide compound 98, AgBF₄, CH₃CN, 0°C-rt.

3.2.2. Nitrogen SAM analogues containing guanidine

Access to the guanidine containing SAM analogue 71 (Figure 14) was unsuccessful, and formation of the desired sulfonium centre still faces a number of problems, including decomposition pathways, such as intramolecular nucleophilic displacement by the amino acid carboxylate group or slow pyramidal inversion of the sulfonium centre under physiological conditions. Wong reported that the inversion of the sulfonium centre can be slowed by maintaining pH between 3.5 and 5.5 and using an excess of large, non-nucleophilic counter ions such as sulfate, but it is still likely to
be difficult to maintain this sulfonium centre throughout the planned deprotection steps at the end of the synthesis. In light of these problems, our attention turned to the more accessible nitrogen containing SAM analogues 74(a-c) and 75 (Figure 14). The target compounds feature a nitrogen atom at the 5'-adenosine position which, like the sulfonium centre can support a positive charge, moreover they were expected to be much easier to handle and manipulate.

Initially, introduction of nitrogen at the 5'-position of adenosine was explored by nucleophilic displacement of 5'-iodo-5'-deoxyadenosine 93 with an amine 126 carrying the protected guanidine prepared using the standard guanidinylation conditions (Scheme 28). Reaction of ethylenediamine 124 and protected S-methylisothiourea 125 in the presence of mercury (II) chloride and triethylamine produced the desired amine in 48% yield. Alternatively the reaction between ethylenediamine 124 and protected S-methylisothiourea 125 was performed in THF and water (20/1) solvent to give the desired amine 126 in a better yield of 56%. Attempts to alkylate the resulting amine 126 with 5'-iodo-5'-deoxyadenosine 93 in the presence of N,N'-diisopropylethylamine (DIPEA) in DMF did not provide the target compound 127, instead presumably leading to the elimination product 103, as detected by mass spectrometry which showed the peak at 289. Generally, alkylation of amines is complicated by mixtures due to the increased nucleophilicity of the product secondary amine. A synthesis involving successive reductive aminations would be expected to proceed in higher overall yields.
Consequently, installation of nitrogen to 5'-position adenosine and introduction of subsequent fragments using such a reductive amination strategy was envisaged. This route required preparation of 5'-amino-5'-deoxy adenosine and two aldehydes 129 and 132(a-c) bearing amino acid and protected guanidine side chains respectively. As shown in Scheme 29, amino acid derived aldehyde 129 was generated as a single enantiomer by oxidation of alcohol 128, which itself could be efficiently prepared by reduction of γ-carboxylic acid 123 as described earlier. Two oxidation reactions were examined for generation of aldehyde 129, thus Swern oxidation provided superior yield of 82% for this transformation, compared to chromium oxide and pyridine (57%).
Meanwhile, the guanidine containing aldehydes 132(a-c) were synthesised using the standard guanidinylation reaction followed by Swern oxidation. Thus, amino alcohols 130(a-c) were treated with protected S-methylisothiourea 125, triethylamine and mercury (II) chloride to give the corresponding alcohols 131(a-c) in a satisfactory yield (92-95%). Subsequent oxidation of the corresponding alcohols 131(a-c) using Swern conditions proceeded smoothly to provide aldehydes 132(a-c) in moderate to good yields (Scheme 30). 93

Reagents and conditions:
a) compound 125, Et₃N, HgCl₂, DMF, rt, 92-95%; b) oxalyl chloride, dry DMSO, Et₃N, dry CH₂Cl₂, 40-71%;
5'-Amino-5'-deoxy adenosine 133 was generated following the Gabriel synthesis described by Townsend (Scheme 31). A Mitsunobu reaction was used to convert the protected adenosine 92 into the phthalimide, which could be directly obtained from the reaction mixture by filtration then treated with hydrazine hydrate in ethanol to afford the protected 5'-amino-5'-deoxy adenosine 133 in 60% overall yield. Aldehydes 129 and 132(a-c) were then incorporated by sequential reductive amination to generate the respective precursors 135(a-c). Reductive alkylation of protected 5'-amino-5'-deoxy adenosine 133 with aldehyde 129 using sodium
triacetoxyborohydride in 1,2-dichloroethane smoothly provided compound 134 in 73% yield, which was immediately treated with the respective guanidine containing aldehydes 132(a-e) using the same conditions to provide the protected tertiary amines 135(a-c) in yields of 34-89%. Finally, global deprotection of the precursors using 90% TFA in water generated the target compounds 74(a-c) which were purified with Amberlite IRA 400 (CI) ion-exchange resin as hygroscopic hydrochloride salts in yields that ranged between 67-90%.

Therefore, a series of guanidine containing SAM analogues 74(a-c), bearing the different carbon linkers, was produced in four concise steps, including an one-pot Mitsunobu-Gabriel synthesis, two successive reductive aminations and deprotection in satisfactory overall yields. Blackburn’s original synthesis of AzoAdoMet 18 relied on an alkylation strategy, that proved inefficient for the second alkylation and problematic in the deprotection step.\(^{53}\) We are able to access a protected form of AzaAdoMet 18 using a more efficient and robust route, and then access guanidine containing SAM analogues 74(a-c) using a subsequent reductive amination and an efficient final global deprotection. Moreover, the intermediate, second amine 134 can be easily produced on a large scale, which can be used for the production of other bisubstrate inhibitors bearing different functionalities, such as cycloethyl guanidine for the development of further specific PRMT inhibitors.

Subsequently, further guanidine derivatives featuring potentially hydrophobic substituents, cycloethyl compound 75, were sought with the idea of exploring
potential binding at the arginine binding site and synthesising more bisubstrate inhibitors for PRMTs. Following with the successful route for the synthesis of guanidine containing SAM analogues 74(a-e), it became necessary to prepare key aldehydes 138 or 139 from their respective alcohols 136 or 137. Consequently, two approaches to oxidise 3-amino propanol were attempted (Scheme 32). In fact, treatment of 3-amino propanol with guanidinylating agent 113 in the presence of triethylamine and mercury (II) chloride afforded compound 136 in a very poor 5% yield. An alternative approach involving treatment of 3-aminopropanol with methylthio-2-imidazoline 110 in ethanol, then Boc protection also furnished compound 137 in an impractical yield of 8%. There is clearly room for improving synthesis of the substituted guanidiniums which can be explored in the future.

Scheme 32

Reagents and conditions:

a) compound 113, Et3N, HgCl2, DMF, 0°C-rt, 5%; b) methylthio-2-imidazoline 110, C2H5OH, reflux, and then (Boc)2O, THF/H2O, 0°C-rt, 8%.

In summary, although the guanidine containing SAM analogue 71 could not be obtained because of the challenges of forming the sulfonium center, the guanidine
containing compound 72 was generated. In addition, the cycloethyl guanidine containing compound 73 was difficult to obtain in realistic yields.

It is important that a series of nitrogen SAM analogues 74(a-c) were achieved in a high yielding four steps synthesis. This reductive amination strategy offers a significant improvement to the overall yields obtained by Blackburn's original synthesis of AzoAdoMet 18, and while we have not carried out deprotection of intermediate 134, our experience of the later analogues justified this claim. Production of the key amine intermediate 134 is convenient and can be produced on large scale that is amenable to the generation of a range of potential bisubstrate inhibitors. The results of biological evaluation of nitrogen SAM analogues 74(a-c) for inhibition PRMT1, which were carried out by Professor Steve Ward and Dr Richard Parry at the University of Bath will be described in Chapter 4 in this thesis. Unfortunately, it was difficult to generate the more hydrophobic cycloethyl guanidine analogues 75 because of the impractical yields of the key intermediates 136 and 137. So the investigation of exploring the conditions for the synthesis of cycloethyl guanidine is necessary for the compound 75, and improved routes to these compounds must be explored in the future.
3.3. Preparation of triazole linker SAM analogue with click chemistry

Huisgen cycloaddition between azides and terminal alkynes is particularly attractive for fragment based inhibitor design because it offers very fast and efficient condensation reaction while the triazole products may gain positive interaction with their biological targets through hydrogen bonding and dipole interactions.\(^6^8\) As illustrated in Scheme 33, a mixture of both regioisomers, \textit{syn} and \textit{anti} isomers can be obtained by thermal cycloaddition, alternatively the regioisomerically pure 1,4-disubstituted triazole, \textit{anti} isomer can be prepared by the Cu (I) catalysis.\(^9^7\)

Scheme 33

\[
R_1-N_3 + \equiv-R_2 \xrightarrow{\text{Cu (I)}} R_1^\text{anti}-N-N-R_2 \xrightarrow{\text{neat, 80°C}} R_1^\text{anti}-N=N-R_2 + R_1-N=N-R_2\]

In fact, Sharpless reported that the binding site of some proteins can be used to promote Huisgen cycloaddition in the absence of Cu (I) due to the proximity of the reactive components (positive template) and therefore promote the reaction to form the pure 1,4-triazole regioisomer.\(^9^8\) For instance, some inhibitors of HIV-1 protease have been developed using this method (Scheme 34).\(^9^8\) The building blocks, alkyne 140 and azide 141 were incubated in the presence of HIV-1 protease, SF-2-Pr, in a morpholinomethanesulfonic acid (MES) and sodium chloride buffer solution at 23°C.
for 24 hours, and only the anti triazole 142 was detected by HPLC and mass spectrometry.

Scheme 34

In principle, only the best-fit combination of the azide SAM analogue 76 with other alkynes or the alkyne SAM analogue 77 with other azides could be selected from an array of potential coupling partners to undergo Huisgen reaction at the target site, thus revealing inhibitors as detected by HPLC and mass spectrometry. A SAM analogue featuring either azide or alkyne functionality attached to nitrogen atom capable of mimicking the positively charged sulfonium centre might be useful for the rapid synthesis of bisubstrate inhibitors for a range of methyl transferases, including PRMTs. Theoretically, such compounds could also be used to explore PRMT templated synthesis, for example alkyne derived SAM analogue might bind to PRMT and only react with azides bearing guanidiniums with an appropriately sized linker. Therefore, SAM analogues bearing an azide 76 and alkyne 77 (Figure 15) were envisaged.
The azide containing SAM analogue 76 could, in principle, be generated from known 1-azidopropanal and secondary amine 134 by reductive amination, but it is likely to be difficult to handle the potentially explosive low molecular weight 1-azidopropanal. Instead, the azide functionality could be installed at a much later stage with the view that higher molecular weight azide should be more stable (Scheme 35). To this end, 3-propanediol 143 was treated with toluenesulfonyl chloride in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) to give tosylate 144 in 73% yield, however attempts to oxidise the remaining alcohol to aldehyde 145 using Swern conditions failed and this route had to be abandoned for the time being.

Scheme 35

Reagents and conditions:

a) TsCl, Et₃N, DMAP, rt, 73%; b) oxalyl chloride, dry DMSO, Et₃N, CH₂Cl₂.

In the meantime, the synthesis of alkyne bearing SAM analogue 77 was also pursued.
It was envisaged that reductive amination of the secondary amine 134 with 2-propynal might provide the appropriate precursor with the minimum distance to the tertiary amine that might mimic the sulfonium centre of SAM. Unfortunately, a literature method to oxidise commercially available propargyl alcohol (Scheme 36), was unsuccessful because it was difficult to get pure 2-propynal by distillation. However, an alternative method involving simple alkylation of secondary amine 134 with commercially available propargyl bromide in the presence of potassium carbonate proceeded smoothly to produce the alkyne precursor 148 in 71% yield, which could be deprotected with 98% TFA to give the alkyne bearing SAM analogue 77 after purification with Dowex 50WX4-400 ion-exchange resin in 91% yield. This alkyne derived SAM analogue will be used to explore PRMT or other methyltransferase templated synthesis toward identification of new inhibitors.

Scheme 36

Reagents and conditions:

a) CH≡CCH₂Br, K₂CO₃, DMF, rt, 71%; b) 98%TFA, rt, 91%.
We next attempted Cu(I) catalysed synthesis of the related triazole containing SAM analogue 78 from the respective alkyne 148 and guanidine containing azide 154. Initially, synthesis of the azide side chain 154 was envisaged to proceed by Mitsunobu reaction from the alcohol 150 generated by guanidinylation of 2-amino ethanol 149 (Scheme 37). Unfortunately, treatment of alcohol 150 with diphenylphosphoryl azide (DPPA), triphenylphosphine and diisopropyl azodicarboxylate (DIAD) did not provide the corresponding azide 154. Alternatively, conversion of the alcohol 150 to its corresponding tosylate was attempted using toluenesulfonyl chloride, triethylamine and 4-dimethylaminopyridine (DMAP), but no tosylate 151 was observed.

Scheme 37

Reagents and conditions:

a) compound 125, Et₃N, HgCl₂, DMF, rt., 99%; b) DPPA, Ph₃P, DIAD, dry THF; c) TsCl, Et₃N, DMAP, dry CH₂Cl₂, rt; d) i. TsCl, Et₃N, DMAP, dry CH₂Cl₂, rt, ii. NaN₃, DMF, 60°C, 71%; e) i. TFA, CH₂Cl₂, ii. compound 125, Et₃N, HgCl₂, DMF, rt, 78%.

Subsequently, the Boc protected ethanolamine 152 was first converted into the tosylate and replaced by nucleophilic displacement to give the azide derivative 153.
in 71% overall yield. TFA in CH₂Cl₂ was used to remove the remaining Boc protecting group and the resulting amine was subject to the standard guanidinylation conditions to provide the target protected guanidine containing azide side chain 154 in a yield of 78%.

Scheme 38

Reagents and conditions:
a) azide compound 154, CuSO₄·5H₂O, C₆H₇O₆Na, MeOH/H₂O, rt, 70%; b) 98% TFA, rt, 95%.

Afterwards, synthesis of the triazole ring was performed using Huisgen cycloaddition catalysed by Cu (I) generated in situ from copper sulfate and sodium ascorbate according to a known protocol (Scheme 38). The treatment of the protected alkyne 148 and the azide side chain 154 with copper sulfate and sodium ascorbate in acetonitrile and water afforded the precursor 155 in a yield of 70% with formation of
the *anti* isomer only. Global deprotection of this precursor 155 using the same synthetic approach as the amine compounds 74(a-c) achieved the target triazole 78 as a hygroscopic hydrochloride salt with 95% yield.

Briefly, the azide SAM analogue 76 could not be generated because of difficulty with the formation of the side chain 145, but the alkyne SAM analogue 77 was synthesised successfully from the protected adenosine in four simple steps. It is expected that the alkyne SAM analogue 77 can be used in the future to explore templated synthesis of potential inhibitors of PRMTs or other methyltransferases. The subsequent triazole derived SAM analogue 78 was efficiently realised using the Cu (I) catalysed Huisgen reaction to deliver a potential bisubstrate inhibitor of PRMTs. Both of these compounds were evaluated for PRMT inhibition by collaborators, Professor Steve Ward and Dr Richard Parry at the University of Bath, and the results will be described in Chapter 4 in this thesis.
3.4. Preparation of N-6 azide side chain SAM analogue

Cu (I) catalysed Huisgen reaction between azide and terminal alkynes is so efficient that it has been exploited for the labeling of biomolecules using fluorescent dyes or biotin for the purpose of either localisation or affinity purification of target proteins because the resulting triazole is small and easily produced in protein compatible conditions.\(^6^9\) Unfortunately, the copper promoted Huisgen reaction is not compatible with live cells due to the toxicity of copper. An alternative, biologically compatible reaction for azides has been developed by Bertozzi and co-workers who have optimised the reaction condition for the phosphine promoted conversion of azides into amides, now known as Staudinger ligation.\(^6^9\) This transformation has been used to effectively manipulate exogenously applied azides within live cells, confirming the relatively benign nature of the reagents and their potential utility for synthetic manipulation of compounds within a relative biological setting.\(^6^9\)

Ideally, any reactive functionality presented on any inhibitor should be remote from its binding site so that it does not interfere with binding site. The crystal structure of PRMT1 in complex with SAH (PDB 1OR8, Figure 5) shows that the N-6 position of the adenine ring is relatively exposed in a shallow binding site for this co-enzyme. Therefore, N-6 modification of adenosine to incorporate azide functionality \textit{via} a long tether was sought in order to explore the potential of such SAM analogues \(^7^5\)(a-c). It is anticipated that the flexible linker might ensure that the reactive azide functionality is presented in a remote position away from the protein, thus offering
maximum opportunity for Huisgen reaction or Staudinger ligation.

The azide linker 159 was formed following the approach shown in Scheme 39. Tetraethylene glycol 156 was converted to the bis-tosylate 157 using tosylate chloride in the presence of sodium hydroxide as base in THF and water in 71% yield.\textsuperscript{104} Displacement of both esters with sodium azide in dry DMF provided the bis-azide 158 in an excellent yield of 96%.\textsuperscript{102} Desymmetrisation of bis-azide 158 was achieved in high yield using a biphasic variation of the Staudinger reaction,\textsuperscript{102} thus treatment with triphenylphosphine in ethyl acetate/1M aqueous HCl (5/1) afforded the monoamine 159 in 75% yield after purification by silica gel chromatography. Exclusive reduction of only one of the azide groups was achieved because the water soluble ammonium hydrochloride formed after the first reduction was transported to the aqueous water layer, thus removing the remaining azide from further triphenylphosphine which is only soluble in the organic layer.

Scheme 39

\begin{equation}
\begin{split}
156 & \xrightarrow{\text{a)} \text{TsCl, NaOH, THF/H}_2\text{O, 0°C, 71%}} 157 \\
& \xrightarrow{\text{b)} \text{NaN}_3, \text{dry DMF, 75°C, 96%}} 158 \\
& \xrightarrow{\text{c)} \text{Ph}_3\text{P, EtOAc/1M HCl, rt, 75%}} 159
\end{split}
\end{equation}

Reagents and conditions:

a) TsCl, NaOH, THF/H\textsubscript{2}O, 0°C, 71%; b) NaN\textsubscript{3}, dry DMF, 75°C, 96%; c) Ph\textsubscript{3}P, EtOAc/1M HCl, rt, 75%.
Installing the azide tether 159 at the N-6 position of adenosine was attempted using two approaches shown in Scheme 40. Ribofuranose tetraacetate 160 was treated with 6-chloropurine in the presence of Lewis acid, tin tetrachloride, in acetonitrile to give the protected 6-chloride adenosine analogue 161 in 78% yield.\textsuperscript{105} The purinyl chloride was then displaced with the amine tether 159 in the presence of triethylamine in dry DMF to form the desired compound 162 in a disappointing yield of 43%. Finally, sodium methoxide in dry methanol was used to cleave the acetate protecting groups, leading to a polar intermediate, which was directly reacted with 2,2-dimethoxy propane in the presence of toluenesulfonic acid and acetone to give the acetonide protected compound 165 in 51% yield.

**Scheme 40**

![Scheme 40](image)

Reagents and conditions:

a) SnCl\(_4\), 6-chloropurine, CH\(_3\)CN, rt, 78%; b) compound 159, Et\(_3\)N, dry DMF, rt-60°C, 43%; c) NaOCH\(_3\), dry MeOH, rt, and then TsOH, 2,2-dimethoxy propane, acetone, rt, 51%; d) TsOH, 2,2-dimethoxy propane, acetone, rt, 63%; e) compound 159, PyBOP, DIPEA, dry DMF, rt, 75%.
Alternatively, compound 165 could be synthesised much more efficiently from inosine 163, thus familiar conditions furnished the acetonide protected inosine 164 in 63% yield. The azide-tethered amine 159 was installed by activation of using benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) in the presence of N, N'-diisopropylethylamine (DIPEA) in dry DMF and subsequent displacement with amine 159 to furnish the compound 165 in 75% yield. Obviously, the second route, which contains two steps reactions in satisfactory yields, was much more efficient than the first route and easy to scale up.

Scheme 41

Reagents and conditions:

a) phthalimide, DIAD, Ph₃P, dry THF, rt, 76%;
b) Hydrazine hydrate, C₂H₅OH, reflux, 82%;
c) compound 129, NaBH(OAc)₃, DCE, rt, 75%;
d) compound 132a, NaBH(OAc)₃, DCE, rt, 99%;
e) 98% TFA, rt, 87%.
With the modified N-6 azide side chain installed, the adenosine was converted into the SAM analogue 79 using the familiar route, including Mitsunobu reaction, Gabriel synthesis, twice reductive amination and deprotection (Scheme 41). Notably, there was a little difference in the Mitsunobu reaction to form azide-bearing 5'-amino-5'-deoxyadenosine 165, compared with that of 5'-amino-5'-deoxyadenosine 133 (Scheme 31). In this instance optimum yields were obtained using 3.5 equivalents of phthalimide, diisopropyl azodicarboxylate (DIAD) and triphenylphosphine respectively, while those three reagents were used at 1.0 equivalent in the synthesis of 5'-amino-5'-deoxyadenosine 133.

Briefly, the key modified adenosine 165 was prepared over two steps in a yield of 47%, which is better than the previous method prepared over three steps in 17% yield. The azide-bearing SAM analogue 79 was generated successfully, following the preparation of nitrogen analogues 74(a-c), which will be used in the localisation and purification of the target protein with alkynes or Staudinger ligation in the future.
3.5. Attempted synthesis of (+)-sinefungin

3.5.1. The original synthetic strategy

The synthesis of sinefungin has been reported by numerous approaches, but some of them produce a mixture of epimers at the C-6 position, while enantioselective routes to (+)-sinefungin are quite long winded requiring numerous steps. We envisaged a novel, rapid and efficient synthesis of (+)-sinefungin (Scheme 42), which focused on preparation of the dihydropyridazine 170 and ideally, subsequent one-pot deprotection and ring opening to generate the target compound. It was initially conceived that the central dihydropyridazine could be generated by a Diels-Alder reaction, although inspection of the relative stereochemistry of sinefungin shows that a *trans* stereochemical relationship across the dihydropyridazine would be required. Fortunately this can be obtained by epimerisation adjacent to the ester functionality.

Scheme 42

\[ (+)\text{-sinefungin} \rightarrow \text{Dihydropyridazine} 170 \]
3.5.1.1. Preparation of the key diene by C-6 and C-7 bond formation

In the forward direction, synthesis of the target diene 174 for the proposed Diels-Alder reaction by Wittig olefination to form the C-6 and C-7 alkene was anticipated, therefore, demanding the adenosyl 5'-ethylaldehyde 175 and commercially available triethyl 4-phosphonocrotonate (Scheme 43). We chose to ignore the geometry of the diene in the first instance, being most concerned with evaluation of the general strategy. The target aldehyde required one-carbon homologation of adenosine, which can be achieved via the protected nitrile compound 173. Protected adenosine 92 was converted to nitrile 173 by Mitsunobu reaction with acetone cyanohydrin, diisopropyl azodicarboxylate (DIAD) and triphenylphosphine in 73% yield.108

Scheme 43

Reagents and conditions:

a) acetone cyanohydrin, Ph3P, DIAD, dry THF, rt, 73%; b) i. DIBAL, THF, ii. triethyl 4-phosphonocrotonate, NaH, THF; c) DIBAL, THF.
Initially, a one-pot reduction and Wittig olefination to form diene 174 was attempted involving treatment of nitrile 173 and diisobutylaluminium hydride (DIBAL) in THF to afford the crude aldehyde, which was then subject to triethyl 4-phosphonocrotonate and sodium hydride. Unfortunately, this protocol did not generate the diene 174. Verification of the intermediate, adenosyl 5'-ethylaldehyde 175 was sought by performing the reduction and isolation. Unfortunately, the adenosyl 5'-ethylaldehyde 175 was unstable, because the crude $^1$H NMR spectrum revealed two aldehyde containing compounds, presumably arising from a ring opening, ring closing sequence (Scheme 44).

Scheme 44

This mixture formation can be envisaged to start with enol formation 176 and subsequent retro-Michael to open the ribosyl ring which may close again to scramble the stereochemistry at C-4. Further decomposition can occur by elimination of the purine leading to an aldehyde 178 that may then undergo subsequent nucleophilic addition by methanol or water and subsequent intramolecular Michael addition to
give the sugar 179. This route was abandoned for the time being and alternative methods to connect C-5 and C-6 were investigated as an alternative strategy.

3.5.1.2. Preparation of the key diene by C-5 and C-6 bond formation

In order to form the C-5 and C-6 bond, three synthetic routes were examined: 1) SN2 nucleophilic displacement of adenosyl 5'-tosylate 188 with an appropriate Grignard reagent or related cuprate (Scheme 48); 2) addition of alkynyllithium anions to the aldehyde modified adenosine at 5'-position (Scheme 51); 3) addition of alkynyllithium anions to the Weinreb amide modified adenosine at 5'-position 194 (Scheme 52).

Firstly, it was necessary to explore the formation of the target vinyl bromide 183 or vinyl iodide 186, ultimately required to form the respective Grignard or cuprate that will displace adenosyl 5'-tosylate. The strategy for the formation of the vinyl bromide 183 starting from propargyl alcohol 180 was attempted (Scheme 45). Thus, propargyl alcohol 180 was converted to alkyne derived alkene compound 181 via in situ manganese dioxide oxidation and subsequent Wittig olefination with (carbethoxymethylene)triphenylphosphorane, exclusively as the C-2 and C-3 trans isomer in 63% yield. Consequently, bromination of the alkyne derived alkene compound 181 was achieved by treatment with N-bromosuccinimide (NBS) and silver nitrate in acetone to afford the bromide 182 in 83% yield. Attempts to reduce the alkyne bromide 182 by hydrogenation catalysed with Lindlar’s catalyst, or
hydroboration with dicyclohexylborane followed by protonolysis with acetic acid unfortunately failed to deliver vinyl bromide 183.

Scheme 45

Reagents and conditions:

a) MnO₂, EtOOCCH=PPh₃, dry CH₂Cl₂, rt, 63%; b) NBS, AgNO₃, acetone, rt, 83%;
c) Lindlar’s catalyst, H₂ or BH₃·THF, cyclohexene, pentane, then CH₃COOH

Scheme 46

Reagents and conditions:

a) NaI, CH₃COOH, 70°C, quantitative yield; b) i. DIBAL, dry CH₂Cl₂, ii. n-BuLi, triethyl phosphonoacetate, dry THF, -78°C, 62%.

An alternative preparative route was explored as outlined in Scheme 46. Treatment of ethyl propynoate 184 with sodium iodide in refluxing acetic acid proceeded to give the vinyl ester 185 exclusively as the Z isomer in quantitative yield, subsequent reduction of the ester 185 using diisobutylaluminium hydride (DIBAL) in dry CH₂Cl₂ gave the crude aldehyde which was immediately treated with triethyl
phosphonoacetate and \( n \)-butyllithium to give the vinyl iodide 186 with the desired configuration in a promising yield of 62\%.\textsuperscript{114}

Next, the model reaction was attempted in Scheme 47, with the aim of exploring the C-5 and C-6 bond formation with \( S_N2 \) nucleophilic displacement. Starting from 2',3'-O-isopropylideneadenosine 92, the benzoyl protecting group was installed using a known procedure, thus treatment with benzoyl chloride in pyridine generated \( N \)-6 benzoyl-isopropylideneadenosine 187 in 88\% yield.\textsuperscript{76} The tosylate leaving group was then installed at the remaining 5'-hydroxyl using toluenesulfonyl chloride in pyridine to afford the adenosyl 5'-tosylate 188 in 80\% yield.\textsuperscript{115}

Scheme 47

\[
\begin{align*}
\text{92} & \xrightarrow{\text{a)}} \text{187} & \text{b)} \xrightarrow{\text{TsCl, Py, } 0^\circ\text{C}} \text{188} & \xrightarrow{\text{c)}} \text{189}
\end{align*}
\]

Reagents and conditions:

a) BzCl, Py, rt, 88\%; b) TsCl, Py, \( 0^\circ\text{C} \), 80\%; c) vinyl magnesium bromide, THF.

Unfortunately, treatment of adenosyl 5'-tosylate 188 with neither vinyl magnesium bromide, nor the corresponding cuprate arising from \textit{in situ} treatment of the Grignard with copper chloride, generated the model compound 189. Even though the model
reaction for exploring the formation of C-5 and C-6 bond with nucleophilic
displacement failed, we were keen to investigate this reaction further using model
nucleophile 188.

The attempt to form the C-5 and C-6 bond relied on SN2 nucleophilic displacement at
the adenosyl 5'-tosylate 188 using alkenylmagnesates prepared from vinyl iodide 186
following Oshima's iodine-magnesium exchange procedure (Scheme 48). In this
procedure 'Pr'Bu2MgLi was prepared by mixing the isopropyl magnesium chloride
and n-butyllithium which could then exchange with the vinyl iodide to generate the
alkenylmagnesate with retention of the double bond configuration and the otherwise
potentially reactive ester functionality. For our studies, 'Pr'Bu2MgLi was first
prepared by the treatment of isopropyl magnesium chloride and n-butyllithium in a
1:2 molar ratio in THF at 0°C. Vinyl iodide 186 was then treated with fresh
'Pr'Bu2MgLi at -78°C to furnish the desired alkenylmagnesate which was reacted
with adenosyl 5'-tosylate 188 in the presence of a catalytic amount of CuCN-2LiCl.
Unfortunately, this procedure did not generate the desired diene 171.

Scheme 48

Reagents and conditions:

a) i. n-BuLi, i-PrMgCl, vinyl iodide 186, ii. CuCN-2LiCl (cat), THF, -78°C.
Subsequently, we decided to examine a model reaction using \(^{t}PrBu_2MgLi\) in order to find out why the reaction did not occur using adenosyl 5'-tosylate 188 (Scheme 49). Reaction between benzylaldehyde and the vinyl iodide 186 prepared using the same procedure as described above was attempted, but no corresponding alcohol 190 was observed. This model reaction showed that it was difficult to generate alkenylmagnesate from the vinyl iodide 186 using the iodine-magnesium exchange procedure. Therefore, the C-5 and C-6 bond formation could not be generated from the vinyl iodide 186 and adenosyl 5'-tosylate 188.

Scheme 49

![Scheme 49](image)

Reagents and conditions:

a) \(n\)-BuLi, \(i\)-PrMgCl, PhCHO.

The unsuccessful displacement of the adenosyl 5'-tosylate 188 lead to consideration of an alternative route involving reaction of the alkynyllithium with the adenosyl 5'-aldehyde to generate alcohol 193 (Scheme 51), which could be subject to dehydroxylation and reduction to afford the target diene 171 (scheme 42). A model reaction was first attempted involving deprotonation of the alkyne 181 with \(n\)-butyllithium and subsequent reaction with benzylaldehyde at -78°C to furnish the model compound 191 in 38% yield (Scheme 50).
Scheme 50

Reagents and conditions:

a) \( n\)-BuLi, PhCHO, THF, -78°C, 38%

Subsequently, the model reaction was applied to the formation of C-5 and C-6 bond of the key diene 171 and preparation of the adenosyl 5'-aldehyde was required for the target synthesis (Scheme 51). According to Moffatt's original procedure,\(^{117}\) the adenosyl 5'-aldehyde was not stable and instead stored as 1,3-diphenylimidazolidine derivative 192. The \( N\)-6-benzoyl-2',3'-O-isopropylideneadenosine 187 was oxidised under Moffatt’s conditions of dimethyl sulfoxide (DMSO) and dicyclohexyl-carbodiimide (DCC) in the presence of dichloroacetic acid to afford the crude aldehyde. Immediately, the resulting protected aldehyde was treated with diphenylethlenediamine and isolated as 1,3-diphenylimidazolidine derivative 192 in 67% yield. When required, the aldehyde functionality could be restored by treating the protected compound 192 with Dowex 50WX4-400 (H\(^+\)) in aqueous THF to generate the hydrated aldehyde which could be converted to the analytically pure aldehyde upon refluxing in benzene with Dean-Stark apparatus. The alkyne compound 181 was then deprotonated with \( n\)-butyllithium and reacted with the adenosyl 5'-aldehyde, but unfortunately no desired compound 193 was produced using these reaction conditions.
Another synthetic route focused on installing a Weinreb amide in the 5'-position of adenosine and reaction with alkynyllithium 181 to give a ketone that may later be reduced to the desired methylene (Scheme 52). To this end, the adenosyl 5'-carboxylic acid 26 was generated by oxidation of the protected adenosine 187 with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and (bis(acetoxy)-iodo)benzene (BAIB) system in acetonitrile and water as solvent in 86% yield. The resulting carboxylic acid was converted to the Weinreb amide 194 by reaction with N,O-dimethylhydroxylamine hydrochloride, triethylamine and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) in 71% yield. The alkynyllithium compound was generated as before, and then reacted with Weinreb amide 194, but unfortunately this reaction did not furnish the target ketone 195.
Reagents and conditions:
a) BAIB, TEMPO, CH₃CN/H₂O, rt, 86%; b) CH₃ONHCH₃·HCl, PyBOP, DMF, 71%;
c) compound 181, n-BuLi, THF.

In brief, some synthetic approaches were attempted to generate the key diene 171, which was essential for the Diels-Alder reaction to synthesise the target dihydropyridazine 170, including C-6 and C-7 bond formation by Wittig olefination form adenosyl 5'-ethylaldehyde 175 and C-5 and C-6 bond formation by nucleophilic displacement of adenosyl 5'-tosylate 188 with an appropriate Grignard reagent or related cuprate, addition of alkynyllithium anions to the adenosyl 5'-aldehyde or adenosyl 5'-Weinreb amide 194. Unfortunately, all of them failed, so an alternative synthetic route was designed.

3.5.2. An alternative route to dihydropyridazines

The initial Diels-Alder strategy failed because generation of the key diene
intermediate 171 was difficult (Scheme 42). We were intrigued by Ley’s description of an organocatalytic procedure to access dihydropyridazines.\textsuperscript{120} This one-pot three component coupling occurs between an enolisable aldehyde 196, azodicarboxylates 197 and vinyltriphenylphosphonium salt 201 to concisely generate dihydropyridazines 203 (Scheme 53). Ley reported optimised reaction conditions that control the stereogenic amination using catalytic amounts of (S)-pyrrolidinyl-tetrazole 198 to provide the intermediate hydrazinocarbonyl compound 199 which then undergoes base promoted conjugate addition to the vinyltriphenylphosphonium salt 201, and the resulting intermediate ylide 202 then undergoes intramolecular Wittig reaction to generate chiral dihydropyridazines 203.

**Scheme 53**

$$\begin{align*}
\text{196} + \text{197} &\xrightarrow{\text{a)} (S)-\text{pyrrolidinyl-tetrazole 198 (cat), CH}_2\text{Cl}_2, \text{rt}, \text{b)} \text{NaH, vinylphosphonium bromide 201, THF, 0°C}} \longrightarrow \text{199} \rightarrow \text{200} \rightarrow \text{203}
\end{align*}$$

Reagents and conditions:

a) (S)-pyrrolidinyl-tetrazole 198 (cat), CH$_2$Cl$_2$, rt, b) NaH, vinylphosphonium bromide 201, THF, 0°C.
We set out to exploit and further develop this reaction toward a new strategy toward a very concise synthesis of sinefungin (Scheme 54). The planned route focuses on generation of a key dihydropyridazine precursor 170 from adenosyl 5'-propaldehyde 204, commercially available azodicarboxylate derivatives 172 and an ester substituted vinyltriphenylphosphonium salt 205.

Scheme 54

3.5.2.1. Preparation of the dihydropyridazine precursor

We first set out to prepare the intermediates, adenosyl 5'-propaldehyde 204 and ester substituted vinyltriphenyl phosphonium bromide 205 and organocatalyst, (S)-pyrrolidinyl-tetrazole 198. The adenosyl 5'-propaldehyde 204 could be obtained by Moffatt oxidation of the protected adenosine 187, subsequent one-pot Wittig reaction according to Wnuk’s procedure121 and then hydrogenation of the alkene (Scheme 55). Thus, protected adenosine was subject to Moffatt oxidation as before to give the crude aldehyde, which was then directly reacted with Wittig reagent,
(triphenylphosphoranylidene)acetaldehyde to provide the unsaturated aldehyde 206 in 57% yield. The alkene group of the unsaturated aldehyde 206 was reduced using Pd/C and a hydrogen atmosphere in ethyl acetate to provide the target intermediate aldehyde 204 in a yield of 71%.

Scheme 55

Reagents and conditions:
a) i. DCC, Cl\_2CHCOOH, dry DMSO, ii. CHOCH=PPh\_3, 57%; b) H\_2, Pd/C, EtOAc, 71%.

The ester substituted vinyltriphenylphosphonium bromide 205 was generated using a procedure first described by Pattenden (Scheme 56).\(^{122}\) Ethyl propynoate 184 was treated with lithium bromide in refluxing acetic acid to give the Z-vinyl bromide. Subsequent reaction with triphenylphosphine in toluene at room temperature provided the Z-vinyltriphenylphosphonium bromide 205 in 69% yield over the two steps.
The organocatalyst, (S)-pyrrolidinyl-tetrazole 198, not commercially available, was prepared according to Ley’s procedure (Scheme 57).\textsuperscript{123} L-Proline 207 was converted to the amide 208 using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), hydroxybenzotriazole (HOBt), and ammonia aqueous solution in 75% yield. Subsequently, the amide 208 was converted to the nitrile 209 using toluenesulfonyl chloride and pyridine in 72% yield. The target tetrazole 198 was finally obtained in an overall 46% yield by [3+2] cycloaddition of nitrile 209 with sodium azide in the presence of ammonium chloride in DMF and subsequent hydrogenolysis catalysed by Pd/C in acetic acid and water.\textsuperscript{123}

Scheme 56

\[
\begin{align*}
\text{184} & \xrightarrow{\text{a)}} \text{205} \\
\end{align*}
\]

Reagents and conditions:
a) i. LiBr, CH\(_3\)COOH, 70°C, ii. PPh\(_3\), toluene, rt, 69%.

Scheme 57

\[
\begin{align*}
\text{207} & \xrightarrow{\text{a)}} \text{208} & \text{208} & \xrightarrow{\text{b)}} \text{209} & \text{209} & \xrightarrow{\text{c)}} \text{198} \\
\end{align*}
\]

Reagents and conditions:
a) HOBt, EDCI, THF, then NH\(_3\)-H\(_2\)O, rt, 75%; b) i. TsCl, Py, CH\(_2\)Cl\(_2\), rt, 72%; c) NaN\(_3\), NH\(_4\)Cl, DMF, ii. H\(_2\), Pd/C, CH\(_3\)COOH/H\(_2\)O, 46%.
Next, a series of dihydropyridazine compounds 170 were generated via Ley's method by using adenosyl 5'-propaldehyde 204, ester substituted vinyltriphenyl-phosphonium salt 205 and variant azodicarboxylate derivatives 172, such as diethyl azodicarboxylate (DEAD), dibenzyl azodicarboxylate, di-tert-butyl azodicarboxylate and di-trichloroethyl azodicarboxylate, with the aim of selecting a good precursor for the deprotection and ring opening step (Scheme 59). In principle, the stereochemistry of the amination at C-6 can be controlled by the organocatalyst, (S)-pyrrolidinyl-tetrazole 198, although the chemistry at C-9 will not be directly controlled during base promoted conjugate addition to the ester substituted vinyltriphenyl-phosphonium bromide 205. Presumably the major diastereoisomer will feature trans relative stereochemistry arising from facile epimerisation at C-9.

In fact, Ley reported that the methyl substituted vinyltriphenylphosphonium salt 211 was involved in the formation of 1,2-oxazines 212a and 212b with a diastereoisomeric ratio 6:1 (Scheme 58). Ley did not report the formation of dihydropyridazine compounds with an ester substituted vinyltriphenylphosphonium salt 205, thus we sought to develop a novel application of Ley's procedure to synthesise the key intermediate 170.
Scheme 58

\[
\begin{array}{ccc}
\text{Scheme 58} & \\
\begin{array}{c}
\text{H} \\
\text{210}
\end{array}
\quad \xrightarrow{\text{a)}}
\quad \begin{array}{c}
\text{NPh} \\
\text{198}
\end{array}
\quad \xrightarrow{\text{b)}}
\quad \begin{array}{c}
\text{NPh} \\
\text{212a}
\end{array}
\quad + \\
\quad \begin{array}{c}
\text{NPh} \\
\text{212b}
\end{array}
\quad \text{6:1}
\end{array}
\]

Reagents and conditions:

a) PhNO, (S)-pyrrolidinyl-tetrazole 198, CH₂Cl₂, rt; b) phosphonium salt 211, NaH, THF, 0°C, 59% (for two epimers).

Scheme 59

\[
\begin{array}{ccc}
\text{Scheme 59} & \\
\begin{array}{c}
\text{O} \\
\text{204}
\end{array}
\quad \xrightarrow{\text{a)}}
\quad \begin{array}{c}
\text{C₂H₅O} \\
\text{170a \quad R=C₂H₅} \\
\text{170b \quad R=Br} \\
\text{170c \quad R=t-Bu} \\
\text{170d \quad R=CH₂CCl₃}
\end{array}
\quad \begin{array}{c}
\text{O} \\
\text{213}
\end{array}
\quad \xrightarrow{\text{NaH}}
\quad \begin{array}{c}
\text{O} \\
\text{214}
\end{array}
\quad \text{Ph₃P}
\end{array}
\]

Reagents and conditions:

a) i. (S)-pyrrolidinyl-tetrazole 198 (cat), ROOC=NCOOR 172, CH₂Cl₂, rt; ii, phosphonium salt 205, NaH, THF, 0°C.
The yield for the isolated single diastereoisomer is 33% and the diastereomeric mixture is 22%.

TLC analysis revealed that all of the commercially available azodicarboxylate derivatives appeared to react with the adenosyl 5'-propaldehyde 204, but subsequent addition of the ester substituted vinyltriphenylphosphonium salt 205 appeared to proceed to form the dihydropyridazine for all except the di-tert-butyl azodicarboxylate, which may be too sterically hindered to allow the intramolecular cyclisation (Entry 3, Table 2). Generally, purification of the dihydropyridazine compounds 170(a, b and d) suffered from a number of issues including difficulty in removing the triphenylphosphine oxide and problem achieving separation of the diastereoisomers by silica gel chromatography, which ultimately complicated the $^1$H NMR spectra, thus only crude yields are reported in Table 2. In fact, the purification the dihydropyridazine compound with ethyl carbamate 170a (Entry 1) was better than the related benzyl carbamate compound 170b or trichloroethyl carbamate compound 170d (Entry 2 and 4). One apparent diastereoisomer of diethyl carbamate was isolated in a yield of 33%, while a further 22% yield of material was obtained as
a mixture of two diastereoisomers after silica gel chromatography using CH$_2$Cl$_2$/MeOH (60/1) as the eluent. Unfortunately, the relative stereochemistry of the single diastereoisomer of diethyl carbamate compound 170a could not be confirmed using advanced NMR techniques, such as ROESY correlation spectroscopy, because the protons of C-6 and C-9 could not be unambiguously identified due to overlapping signals. Unfortunately, the apparent single diastereoisomer form of compound 170a proved difficult to recrystallise, which precluded X-ray crystallography.

Nevertheless, the deprotection and ring opening conditions were investigated with the diastereomeric mixture of the dihydropyridazine compounds 170 with the aim of evaluating the overall route to sinefungin on the basis that we could return to the issue of stereochemistry once the entire route had been established.

3.5.2.2. Deprotection and ring opening of the precursor

According to Arakawa’s method,$^{124}$ the N-N bond on the cyclic hydrazine 215 could be cleaved by platinum oxide under hydrogenation (Scheme 60).

Scheme 60

Reagents and conditions:

a) H$_2$, PtO$_2$, 2M HCl, 99%.$^{124}$
Initially, base promoted cleavage of the diethyl carbamate 170a was attempted with the knowledge that both the ethyl ester and benzoyl protecting group are sensitive to the base (Scheme 61). The main reason for examining this reaction first was the superior quality of the $^1$H NMR spectrum for the starting material. Therefore, the ethyl carbamate compound 170a was treated with potassium hydroxide in 95% ethanol at room temperature overnight. Analysis of the reaction mixture by electrospray mass spectrometry suggested cleavage of the ethyl ester and benzoyl amide groups to form pyridazine 217 due to the major peak at 561 compared with the staring material parent ion at 693, but no cleavage of the carbamates was observed. The same deprotection reaction was carried at reflux, but again no cyclic hydrazine 218 was observed.

**Scheme 61**

![Scheme 61](image)

Reagents and conditions:

a) KOH, 95% C$_2$H$_5$OH, rt. or reflux.
Subsequently, deprotection of the dibenzylcarbamate protected dihydropyridazine 170b was examined with the aim of achieving deprotection, ring opening and reduction in one-pot reaction (Scheme 62). We were interested in reports from Micouin's and co-workers that described one-pot transformation of the cyclic dibenzylcarbamate 219 by carbamate deprotection and ring cleavage using hydrogenolysis over platinum oxide in acetic acid under a hydrogen atmosphere pressure in quantitative yield.125

Scheme 62

Reagents and conditions:
a) PtO₂, H₂, CH₃COOH, 96%.125

Hydrogenolysis of dibenzylcarbamate protected dihydropyridazine 170b over platinum oxide under one atmosphere of hydrogen in acetic acid (Scheme 63) was analysed by mass spectrometry which showed a major peak at 819 that suggested the C-7 and C-8 double bond had been reduced compared with the starting material parent ion at 817, but no ring opening had occurred. Consequently, the hydrogen pressure was increased to 10 bar, and this time analysis by mass spectrometry showed a large peak at 685 that suggested reduction of the C-7 and C-8 double bond and removal of one Cbz groups, but no ring opening was apparent.
Scheme 63

Reagents and conditions:

a) PtO₂, H₂, CH₃COOH.

Table 3

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(OH)₂/C, cyclohexene, MeOH, reflux</td>
<td>[Chemical structures with product images and labels]</td>
</tr>
<tr>
<td>2</td>
<td>Pd/C, H₂, THF, rt</td>
<td>[Chemical structures with product images and labels]</td>
</tr>
<tr>
<td>3</td>
<td>Pd/C, ammonium formate, MeOH, reflux</td>
<td>[Chemical structures with product images and labels and uncharacterised compound]</td>
</tr>
<tr>
<td>4</td>
<td>Pd/C, H₂, MeOH, rt</td>
<td>one major uncharacterised compound</td>
</tr>
</tbody>
</table>

These results were promising in that the alkene and carbamate protecting groups
could be cleaved, so we set out to explore formation of cyclohydrazine 222 using a range of conditions (Table 3) with the aim to explore the ring cleavage at a later stage. Attempts at transfer hydrogenolysis using Pd(OH)$_2$/C and cyclohexene in refluxing methanol (Entry 1, Table 3), were analysed by mass spectrometry which showed a large peak at 545 and a small peak at 549, indicating a mixture compounds comprised of a major pyridazine 223 and a minor compound 224 where the double bond may have migrated, but the individual components could not be separated using silica gel chromatography. The possible reason for the formation of major pyridazine 223 is that the hydrogen is transferred from intermediate 226 to cyclohexene promoted by conjugation with ester group at C-9 to form the aromatic ring (Scheme 64).

Scheme 64

Next, hydrogenation of the dibenzylcarbamate-protected dihydropyridazine 170b
with Pd/C in THF at atmosphere pressure of hydrogen over two nights yielded the double bond migration compound 224 which could be isolated in 28% yield (Entry 2, Table 3). The presumed mechanism is shown in Scheme 65, in which deprotonation of the intermediate 226 leads to the enolate 227, which then undergoes tautomerisation to give enamine 228 that may further tautomerise to give the isolated imine compound 224. It is likely that the ester group at C-9 of the dihydropyridazine helps to stabilise the product of double bond migration.

Scheme 65

Examination of other transfer hydrogenolysis conditions using Pd/C and ammonium formate in refluxing methanol (Entry 2, Table 3), resulted in two compounds that
could be separated, one of which arose from cleavage of Bz group compound 225 and another of which could not be characterised by mass spectrometry or \(^1\)H NMR spectroscopy. Finally, hydrogenation of Cbz protected compound 170b with Pd/C in methanol at atmosphere pressure was tried, the resulting compound was analysed by mass spectrometry and \(^1\)H NMR, but the resulting peak at 563, could not be matched with the possible cyclohydrazine 223 (peak at 551) or double bond migration compound 224 (peak at 549), meanwhile its \(^1\)H NMR spectrum provide difficult to interpret (Entry 4, Table 3).

Clearly, many challenges for the deprotection and ring opening of the dihydropyridazines were encountered: 1) it was not possible to remove the two ethylcarbamates from the compound 170a using base hydrolysis; 2) one-pot deprotection, reduction and ring opening with platinum oxide did not give the target compound 221; 3) transfer hydrogenolysis generated the pyridazine oxidation compound 223; 4) palladium catalysed hydrogenolysis apparently furnished the double bond migration compound 224. The cleavage of the N-N bond could not be achieved in order to access sinefungin.

**3.5.2.3. Modification of the second synthetic strategy**

The difficulty in cleaving the cyclohydrazine lead us to consider a modified synthetic strategy, generating the acyclic precursors 229 as outlined in Scheme 66. Briefly the same aldehyde precursor 204 could be used to perform a stereoselective amination
using commercial azodicarboxylate derivatives 172 which this time could be isolated as the aldehyde and then transformed by olefination using an appropriate phosphonopropanoate 230. Cleavage of these hydrazines should be easier than their cyclic equivalents 170. In principle, the stereochemistry at C-9 can be controlled independently of the C-6 centre by making it before the olefination step. It was therefore necessary to synthesise the substituted phosphonopropanoate 230 for the proposed route (Scheme 67).

Scheme 66

As shown in Scheme 67, the substituted phosphonopropanoates 233(a-b) were synthesised with either Cbz or Boc, protecting group at nitrogen, both of which could be easily removed in the later stages of synthesis. Following Vederas's procedure,126 the stereochemically pure β-lactones 232(a-b) were synthesised from the commercially available N-protected L-serine derivatives 231(a-b) by intramolecular Mitsunobu reaction. Thus the serine derivatives 231(a-b) were treated with triphenylphosphine and diethyl azodicarboxylate (DEAD) at -78°C in THF to afford
the Cbz protected β-lactones 232a and Boc protected β-lactones 232b in the yields of 33% and 32% respectively. Subsequently, the nucleophilic addition of triethylphosphine to the protected β-lactones 232(a-b) at 70°C generated the substituted phosphonopropanoates 233(a-b) as the stereoisomerically pure isomers in the yields of 81% and 62% respectively according to Smith's method. 127

Scheme 67

\[
\begin{align*}
\text{Reagent and Conditions:} \\
a) & \text{PPh}_3, \text{DEAD, dry THF, } -78^\circ\text{C, 33\% (Cbz) and 32\% (Boc);} \\
b) & \text{P(OCH}_2\text{H}_3\text{), 70^\circ\text{C, 81\% (Cbz) and 62\% (Boc).}
\end{align*}
\]

Model reactions were performed to evaluate the ability of the substituted phosphonopropanoates 233(a-b) to undergo olefination (Table 4). Various bases were used to deprotonate the phosphonopropanoates 233(a-b), such as sodium hydride, potassium tert-butoxide, n-butyllithium and lithium diisopropylamide (LDA), but subsequent attempted reaction with benzylaldehyde or 4-methoxy benzylaldehyde did not generate the expected alkene 234. Presumably deprotonation next to the phosphonopropanoates 233(a-b) was difficult due to deprotonation of the adjacent α-proton to the carbonyl. Unfortunately, we could not resolve this problem, undermining the value of this strategy.
Table 4

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>aldehyde</th>
<th>Base</th>
<th>Solvent</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz</td>
<td>PhCHO</td>
<td>NaH</td>
<td>CH₂Cl₂</td>
<td>No alkene observed</td>
</tr>
<tr>
<td>2</td>
<td>Cbz</td>
<td>PhCHO</td>
<td>t-BuOK</td>
<td>CH₂Cl₂</td>
<td>No alkene observed</td>
</tr>
<tr>
<td>3</td>
<td>Cbz</td>
<td>PhCHO</td>
<td>n-BuLi</td>
<td>THF</td>
<td>No alkene observed</td>
</tr>
<tr>
<td>4</td>
<td>Boc</td>
<td>4-MeOPhCHO</td>
<td>LDA</td>
<td>THF</td>
<td>No alkene observed</td>
</tr>
</tbody>
</table>

An alternative approach to link the C-7 and C-8 carbon bond by the addition of a zincate 236 to the aldehyde arising from the amination step was envisaged (Scheme 68).

Scheme 68

Following Jackson’s procedure, the nucleophilic α-amino acid organozinc reagent
236 was generated from iodoalanine 237, in which the ester group and stereocentre were compatible for the zinc reagent (Scheme 69). Boc-L-serine 231b was treated with potassium and methyl iodide in dry DMF to afford the crude protected serine, which was converted to the iodide 237 by reaction with triphenylphosphine, iodine and imidazole in THF in 28% yield over two steps. Treatment of this iodide with zinc dust, dibromoethane and trimethylsilylchloride in dry DMF was meant to generate the organozinc reagent 236, which was then added to benzyl aldehyde as a model reaction, but unfortunately no reaction occurred and insufficient time remained for further investigation of this approach.

**Scheme 69**

\[
\begin{align*}
\text{HO}_2\text{C} & \quad \text{NH}_{\text{Boc}} \quad \text{a) } \quad \text{MeO} \quad \text{NH}_{\text{Boc}} \quad \text{b) } \quad \text{MeO} \quad \text{NH}_{\text{Boc}} \\
231b & \quad \text{237} & \quad 236
\end{align*}
\]

Reagents and conditions:

- a) i. K₂CO₃, dry DMF, CH₃I, rt, ii. PPh₃, I₂, imidazole, dry THF, 0°C, 28%; b) Zn dust, BrCH₂CH₂Br, TMSCl, dry DMF, then PhCHO.

In summary, our route to (+)-sinefungin relied on the rapid generation of dihydropyridazine compounds 170 and subsequent deprotection and ring opening leading to the target compound. Our efforts to extend Ley’s methodology by employing the ester derived vinyltriphenylphosphonium salt 205 were promising,
although spectroscopic characterisation of the resulting dihydropyridazines was hindered by difficulty in separating the individual diastereoisomers. Clearly, more work to solve these key problems will be valuable for establishing the level of stereochemical control in the reaction and deciding whether it is a viable strategy to sinefungin. Unfortunately, we were not able to establish a set of conditions to fulfill our ambitious strategy of simultaneously cleaving all protecting groups as well as the opening of cyclohydrazine. There are promising indications that the benzyl carbamate protecting groups and the alkene can be manipulated under reducing conditions although the formation of the product where the double bond seems to have migrated into conjugation with the ester carbonyl appears to be a common problem that will need to be addressed in the future. Our failure to cleave the cyclohydrazine is obviously a significant barrier to the utility of this route and this encouraged us to explore alternative strategies that lead to acyclic precursor 229 that should be more easily deprotected and allow independent control of the stereochemistry at C-6 and C-9. Unfortunately, our initial studies into these strategies were frustrated by inherent problems associated with the precursors required for this synthetic strategy and a lack of time. Further time would allow more thorough investigation of new method of connecting the precursor’s C-7 and C-8 carbon bond, such as Grubbs cross-metathesis.
Chapter 4

Computational Docking and Biological Results

4.1. Molecular docking

Computational docking was used to visualise possible orientations of the bisubstrate inhibitors which have been synthesised and perhaps rationalise any inhibition. The crystal structure of PRMT1 in complex with the cofactor product SAH (PDB 1OR8)\(^6\) was taken as a starting point for molecular docking. Any results from these experiments should be treated with caution because PRMT1 is believed to operate as a dimer, but the published crystal structure used for modelling is of the monomeric protein. The atoms of SAH were used as a reference to indicate the binding site for the bisubstrate inhibitors, and then the coordinates for SAH could be discarded. The receptor coordinates which remained were then taken as the input for docking software along with the definition of the desired binding site. The structures of the bisubstrate inhibitors, 74(a-c), 77 and 78 (Figure 18) were built by commercially available software InsightII created by Accelrys Software Inc.\(^{130}\) Docking software Gold 3.2 (Genetic Optimization for Ligand Docking)\(^{131}\) was used to dock all bisubstrate inhibitors into the binding site of PRMT1. For each inhibitor, Gold docks the ligand 10 times starting each time from a different random population of ligand orientations.\(^{132}\)
Gold docking results gave 10 possible conformations with which each bisubstrate inhibitor bound to PRMT1. For compounds 74(a-c), there are conformations which can be overlapped with SAH and bind with SAM binding sites, whereas, none of the conformations of alkyne SAM analogue 77 or triazole derived SAM analogue 78 (Figure 19-23) can bind with SAM binding site residues. In Figure 19, compound 74a mainly binds to the PRMT1 active site by forming hydrogen bonds with E129, E100, H45, R54 and essentially the guanidine group binds to the key glutamates residue E153, and additionally to the residues W145 and M146. Compound 74b can bind with SAM binding site residues (E129, E128, E100) and glutamate residue (E153) from the Figure 20. Interestingly, the compound 74c appears to bind at the SAM binding site residue (V128, E100, H45), but does not reach either glutamate residue E153 or E144, but instead appears to bind Y148 (Figure 21). In fact, the five
carbon linker can rotate about the alkane bonds, so it is possible that the guanidine
group could bind with E153 after rotation. Although compounds 74(a-c) can
potentially bind with one glutamate residue E153, not with E144, these experiments
suggest that there is a good chance that the prototype bisubstrate inhibitors 74(a-c)
might inhibit PRMT1.

Moreover, as illustrated in Figure 22, the alkyne SAM analogue 77 can not dock with
either SAM binding site or two key glutamate residues (E144 or E153). The docking
conformation of triazole derived SAM analogue 78 is quite different from SAH in
Figure 23, in which the arginine side chain of compound 78 is overlapped with the
amino acid side chain of SAH. Presumably, the triazole derived guanidine side chain
is too long, so there is not enough space for it to dock with E153, therefore, it has to
overlap with the amino acid side chain of SAH. The docking results suggest that
compounds 77 and 78 will not inhibit PRMT1. Of course, the computational docking
just provides a graphical interpretation that may predict the relative activity of
compounds 74(a-c), 77 and 78, and the exact biological results have to be
demonstrated by the biological essays.
Figure 19: Binding conformation of bisubstrate inhibitor 74a into rat PRMT1 (PDB 1OR8) using the Gold algorithm. The tested compound 74a (tube, carbon atoms as cyan) were overlapped with SAH (yellow tube) and PRMT1 residue were shown as ball and stick (carbon atoms as silver).
Figure 20: Binding conformation of bisubstrate inhibitors 74b into rat PRMT1 (PDB 1OR8) using the Gold algorithm. The tested compound 74b (tube, carbon atoms as cyan) were overlapped with SAH (yellow tube) and PRMT1 residue were shown as ball and stick (carbon atoms as silver).
Figure 21: Binding conformation of bisubstrate inhibitor 74e into rat PRMT1 (PDB 1OR8) using the Gold algorithm. The tested compound 74e (tube, carbon atoms as cyan) were overlapped with SAH (yellow tube) and PRMT1 residue were shown as ball and stick (carbon atoms as silver).
Figure 22: Binding conformation of bisubstrate inhibitor 77 into rat PRMT1 (PDB 1OR8) using the Gold algorithm. The tested compound 77 (tube, carbon atoms as cyan) were overlapped with SAH (yellow tube) and PRMT1 residue were shown as ball and stick (carbon atoms as silver).
Figure 23: Binding conformation of bisubstrate inhibitor 78 into rat PRMT1 (PDB 1OR8) using the Gold algorithm. The tested compound 78 (tube, carbon atoms as cyan) were overlapped with SAH (yellow tube) and PRMT1 residue were shown as ball and stick (carbon atoms as silver).
4.2. Biological Results

PRMTs have been known to play physiologically important roles in numerous cellular processes, but their function in vivo is still not clear to understand, so it is necessary to develop specific inhibitors of PRMTs which can be used in the study of PRMT biological activities in vivo. In fact, only one specific inhibitor of PRMTs, AMI-1, was reported recently, but it is a dye and resembles sulfonated ureas of the Suramin type which have been reported to be pleiotropic and target many proteins. Therefore, we designed and synthesised a series of bisubstrate inhibitors and their biological evaluation was carried out by Professor Steve Ward and Dr Richard Parry in the University of Bath.

4.2.1. In vitro evaluation of PRMT1 inhibition

The in vitro screen of known inhibitor, (+)-sinefungin 2 and bisubstrate inhibitors (74(a-c), 77, 78) was carried using human recombinant PRMT1, [\(S-^{3}H_{3}C\)]-SAM and substrate recombinant Sam68 and the methyl transfer measured by scintillation counting. The substrate Sam68 encodes glycine- and arginine-rich sequences and is known to be an in vivo substrate for PRMT 1. In the P3 region (GRGVGPPR) of Sam68, the arginine 304 can be asymmetrically dimethylated. As shown in Table 5, the known compound, sinefungin as a reference showed good inhibition for PRMT1 with an approximate IC\textsubscript{50} value of 2.0\(\mu\)M. Additionally, the guanidine containing SAM analogues 74(a-c) appear to inhibit PRMT1 with approximate IC\textsubscript{50} values in
the range 2.7 to 3.3μM. Therefore, the PRMT1 inhibition activity of compounds 74(a-c) appears to be of similar potency to sinefungin under these assay conditions. However, both the alkyne SAM analogue 77 and the triazole linker SAM analogue 78 exhibited relatively weak inhibitions for PRMT1 at 100μM. Interestingly, these biological assay results are broadly in line with those predicted by the computational docking study.

4.2.2. Evaluation of selectivity versus lysine methyltransferase SET7

The ε-amino lysine residues are another significant target for protein methylation within cells. Lysine methyltransferases bearing SET domains can catalyse mono-, di- and trimethylation of lysine and are well known for their role in histone modification which is involved in a wide range of cellular processes, such as transcription regulation, chromosome inactivation and heterochromatin formation. The SAM binding site of SET domains is structurally different from that of PRMTs, thus it is possible to identify specific inhibitors that distinguish between these two types of binding site. Therefore, sinefungin and novel compounds 74(a-c), 77, 78 were compared for their inhibition activity between PRMT1 and SET7 (Table 5). The alkyne SAM analogue 77 and triazole linker SAM analogue 78 are poor inhibitors of either PRMT1 or SET7 (43 and 27% inhibition at 100μM respectively). As expected sinefungin is a potent inhibitor of both PRMT1 (IC50 ~ 2.0μM) and SET7 (IC50 ~ 2.7μM) respectively and contains no specificity element. SAM analogue 74b also appears to display a lack of specificity with no more than a 5-fold reduction in
potency against SET7 (IC$_{50}$ $\sim$ 11$\mu$M). Interestingly, SAM analogues 74a and 74c show much better selectivity for PRMT1 (IC$_{50}$ $\sim$ 3$\mu$M) than SET7 (both $\sim$ 40% inhibition at 100$\mu$M). Therefore, compounds 74a and 74c were identified as the potential selective inhibitors of PRMT1 compared to the other compounds, presumably because these two compounds can bind with SAM binding site residues and key glutamate residue, E153 (Figure 19 and 21). In fact, compound 74b can bind with SAM binding site residues and E153 (Figure 20) as well, but it exhibits poor selectivity for SET7. We have not performed computational docking of compounds 74(a-c) at the SET7 binding site, so it is not possible to visualise any potential reason for this difference in selectivity. Ultimately, further structural binding studies by crystallography or NMR spectroscopy are required in order to uncover a structural rationale for this selectivity observed for compounds 74a and 74c.

Table 5. In vitro inhibition of PRMT1 and SET7

<table>
<thead>
<tr>
<th>Compounds</th>
<th>PRMT1 IC$_{50}$ ($\mu$M)</th>
<th>SET7 IC$_{50}$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sinefungin</td>
<td>2.0</td>
<td>2.7</td>
</tr>
<tr>
<td>75a</td>
<td>2.7</td>
<td>40% at 100$\mu$M</td>
</tr>
<tr>
<td>75b</td>
<td>3.3</td>
<td>11</td>
</tr>
<tr>
<td>75c</td>
<td>3.3</td>
<td>36% at 100$\mu$M</td>
</tr>
<tr>
<td>78</td>
<td>29.5% at 100$\mu$M</td>
<td>43% at 100$\mu$M</td>
</tr>
<tr>
<td>79</td>
<td>25% at 100$\mu$M</td>
<td>24% at 100$\mu$M</td>
</tr>
</tbody>
</table>

a. IC$_{50}$: the concentration of compound required to reduce the enzyme activity by 50%.
However, from the biological evaluation and computational docking results, it is clear that compared to the alkyne analogue 77, accommodation of a guanidine group in the side chain of the analogues provides a substantial increase in potency against PRMT1 and in the case of two of the analogues, provides the basis for selectivity over the lysine methyltransferase SET7. Furthermore, the relatively poor potency of the much larger and less flexible triazole based inhibitor is suggestive of structure-based selectivity that can be better explored and optimised in the future. Clearly more analogues containing guanidine isosteres will be explored using the synthetic route developed in this thesis in order to develop a rationale for the discovery of more efficient and specific inhibitors of PRMT1.
Chapter 5

Summary and Future Work

This thesis focused on improving on the very small number of chemical tools available for the evaluation of protein arginine methylation in cells and can be defined in four aims:

1. Development of general inhibitors of methyltransferases.
2. Design and synthesis of specific inhibitors of protein arginine methyltransferases (such as PRMT1).
3. Synthesis of a N-6 modified SAM analogue for possible affinity purification of the target protein.

For the first aim, trifluoromethyl SAM analogue 69 was explored as general methyltransferases inhibitor based on the model compound 70 which was used as evaluation the reaction condition of forming trifluoromethyl sulfonium centre. However, it was difficult to generate the desired trifluoromethyl sulfonium containing model compound 70 either by trifluoromethylation of thioether 81 using trifluoromethylation reagent 86, or alkylation of a trifluoromethyl thioether 82 with 5'-halogen adenosine 16 or 93. This objective was quickly abandoned in order to
concentrate on synthesis of more practical analogues.

For the second aim, some guanidine containing bisubstrate SAM analogues were investigated with the aim of developing specific inhibitors of PRMT1. A series of nitrogen SAM analogues 74(a-c) were achieved in a high yield using an efficient four step synthesis. The reductive amination strategy offers a significant improvement to the overall yields obtained by Blackburn's original synthesis of AzoAdoMet 18 which relied on an alkylation strategy. This route offers much potential for the development of inhibitors of other SAM utilizing enzymes. Alkyne derived SAM analogue 77 was also successfully synthesised using this route and provides the opportunity for future exploration of protein templated synthesis of inhibitors of methyltransferases. The subsequent triazole derived SAM analogue 78 was efficiently prepared using the Cu (I) catalysed Huisgen reaction to deliver a potential bisubstrate inhibitor of PRMTs.

However, synthesis of the related sulfonium-tethered guanidine SAM analogue 71 could not be obtained successfully because of the difficulty of forming the sulfonium centre, but the guanidine containing thioether analogue 72 was made, although it was claimed in a patent to be a poor inhibitor of DNA methyltransferase. In addition, the cycloethyl guanidine containing compounds 73 and 75 were difficult to achieve because of the formation of cycloethyl guanidine in an unfeasible yield. So the investigation of exploring the conditions for the synthesis of cycloethyl guanidine is necessary for the compounds 73 and 75, and improved routes to these compounds
must be explored in the future.

The molecular docking results predicted that bisubstrate inhibitors 74(a-c) might inhibit PRMT1 and that compound 77 and 78 have no inhibition for PRMT1. Subsequently, the bisubstrate inhibitors (74(a-c), 77, 78) were evaluated for PRMT1 inhibition by collaborators, Professor Steve Ward and Dr Richard Parry at the University of Bath. The biological results showed that compound 74a and 74c can selectively inhibit PRMT1 not SET7 at IC_{50} values 2.7μM and 3.3μM respectively, compound 74b can inhibit both PRMT1 and SET7 without much selectivity. It is very satisfying that two prototype bisubstrate inhibitors were discovered to be selective PRMT inhibitors 74a and 74c. It is hoped that they may prove to be useful for the study of arginine methylation or perhaps the development of candidate drugs for the therapeutic of some diseases, such as cancer. These preliminary results encourage the design of more analogues with guanidine-like functional group in order to discover more potent and specific inhibitors of PRMTs in the future. Eventually, we would like to be able to develop inhibitors that select between the various classes of PRMT.

For the third aim, the azide bearing SAM analogue 79 was generated successfully, which may be used in the localisation and purification of the target protein with alkynes or Staudinger ligation in the future. It remains to be seen whether this compound retains potency for inhibition of PRMT1, which would clarify whether the location of the tether is indeed well placed to avoid repulsive steric interactions with
rest of the target protein.

For the fourth aim, a novel, rapid and enantioselective route for the total synthesis of natural product (+)-sinefungin was investigated. The dihydropyridazine compound 170 was successfully generated from adenosyl 5'-propaldehyde 204, commercially available azodicarboxylate derivatives 172 and ester substituted vinyltriphenyl-phosphonium salt 205 by extending Ley's methodology. Deprotection and ring opening of dihydropyridazine compounds 170b was then attempted, but this concise route seems difficult to generate (+)-sinefungin. The modified strategy seeking to generate acyclic precursor 229 was explored, but it was frustrated by inherent problems associated with the precursors required for this synthetic strategy and a lack of time. Further time would allow more thorough investigation of new method of connecting the precursor's C-7 and C-8 carbon bond, such as olefin cross metathesis. In addition, sinefungin analogue 80 will be explored as bisubstrate inhibitor of PRMT1 in the future.
Experimental

All chemicals were purchased from commercial sources and used as supplied unless stated, dry CH$_2$Cl$_2$ was distilled over calcium hydride and dry THF was distilled over sodium benzophenone. TLC was carried out using silica gel 60 pre-coated aluminium plates (0.20mm thickness) from Macherey-Nagel, with visualisation by UV light (254nm) or exposure to potassium permanganate solution. Flash chromatography was performed on silica gel (particle size 40-63µm) from Fischer Chemicals. $^1$H, $^{13}$C, $^{19}$F and $^{31}$P NMR spectra were obtained from JEOL EX270, Bruker AV400, DPX400 and AV500 spectrometers. The chemical shifts, given as δ values, are quoted in parts per million (ppm); $^1$H and $^{13}$C NMR chemical shifts were measured relative to internal tetramethylsilane; $^{19}$F NMR chemical shifts were measured relative to neat CFCl$_3$; $^{31}$P NMR chemical shifts are positive downfield from external 85% H$_3$PO$_4$. Apparent coupling constants (absolute values), $J$, are measured in Hertz and multiplicities quoted as singlet (s), doublet (d), triplet (t), quartet (q) or combinations thereof as appropriate. Mass spectra were obtained from the School of Chemistry mass spectrometry service using an LC-TOF, running in an open-access mode. IR spectra were obtained from the solid phase using a thermo AVATAR 320 FT-IR or from solution samples using a Perkin Elmer FTIR 1600 and Bruker Tensor 27 spectrometer. Optical rotations were obtained at ambient
temperature using a Jasco DIP-370 digital polarimeter and ADP 440 polarimeter. Melting points were determined using a SMP3 melting point measurement and are uncorrected.
Thionyl chloride (667 mg, 5.61 mmol) was added to a solution of adenosine (500 mg, 1.87 mmol) in CH₃CN (5 mL) at 0 °C. Pyridine (296 mg, 3.74 mmol) was added to this mixture and stirring continued at 0 °C for 4 hr, then at room temperature overnight. MeOH (5 mL), aqueous ammonia solution (35%, 5 mL) and H₂O (5 mL) were added to the yellow solution at room temperature, and stirring continued for 1 hr. The solution was then applied to Dowex 1 x 2 -100 (OH⁻) ion-exchange resin, eluting using H₂O/MeOH (1/1, 100 mL) and MeOH to give chloride 16 as a white solid (550 mg, quantitative yield), m.p. 113-114 °C (lit., 110-111 °C);¹³⁵¹H NMR (400 MHz, DMSO-d₆) δH 8.35 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 5.93 (d, J = 5.1, 1H, 1'-H), 4.76 (app. t, 1H, J = 5.1, 2'-H), 4.22 (app. t, 1H, J = 5.1, 3'-H), 4.09 (m, 1H, 4'-H), 3.95 (dd, 1H, J₁ = 11.6, J₂ = 5.2, 5'-CH₂H₆b), 3.84 (dd, 1H, J₁ = 11.6, J₂ = 6.4, 5'-H₆H₆b);¹³C NMR (100 MHz, DMSO-d₆) δC 156.1 (C), 152.7 (CH), 149.4 (C), 139.7 (CH), 119.1 (C), 87.4 (CH), 83.6 (CH), 72.6 (CH), 71.2 (CH), 44.8 (CH₂); ES-MS 286.1 (M + H⁺); HRMS calcd for C₁₀H₁₂ClN₃O₃⁺ 286.0662, found 286.0687.
$N_6$-Benzoyl-2',3'-O-isopropylidene-adenosyl-5'-carboxylic acid 26

Bis(acetoxy)iodo benzene (689 mg, 2.14 mmol), TEMPO (30 mg, 0.20 mmol) and protected adenosine 187 (400 mg, 0.97 mmol) were combined in a reaction flask and a solution of CH$_3$CN/H$_2$O (1/1, 1.2 mL) was added to the mixture. The reaction solution was stirred at room temperature for 3 hr. The precipitate was filtered and washed with successive portions of ether and acetone to give protected adenosyl 5'-carboxylic acid 26 as a white solid (356 mg, 86%), m.p. 230-232 °C (dec) (lit., m.p. 208-209 °C);$^{118}$ $^1$H NMR (400 MHz, DMSO) $\delta$ 11.19 (s, 1H, NHBz), 8.70 (s, 1H, Ar-H), 8.61 (s, 1H, Ar-H), 8.06 (m, 2H, BzH), 7.64 (m, 1H, BzH), 7.57 (m, 2H, BzH), 6.49 (m, 1H, 1'-H), 5.60 (m, 2H, 2'-H and 3'-H), 4.78 (m, 1H, 4'-H), 1.56 (s, 3H, CH$_3$), 1.39 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO) $\delta$ 170.7 (C), 165.5 (C), 152.0 (C), 151.3 (CH), 150.2 (C), 144.1 (CH), 133.4 (CH), 132.4 (CH), 128.46 (CH), 128.45 (CH), 125.4 (C), 112.7 (C), 89.8 (CH), 85.7 (CH), 83.8 (CH), 83.4 (CH), 26.4 (CH$_3$), 24.9 (CH$_3$); ESI-MS 426.1 (M + H$^+$); HRMS calcd for C$_{20}$H$_{20}$N$_5$O$_6^+$ 426.1408, found 426.1402.
5'-({2-Guanidinoethylthio})-5'-deoxyadenosine 72

TFA (5 mL) was added to a solution of protected guanidine derived thioether 96 (240 mg, 0.42 mmol) in CH₂Cl₂ (5 mL) at 0 °C and stirring continued at room temperature for 1.5 hr. The reaction mixture was concentrated and the residue adjusted to pH 9 with aqueous ammonia. The resulting solution was applied to Dowex 1 x 2 -100 (OH⁻) ion exchange resin and elution using H₂O/CH₃OH (1/1, 100 mL) gave guanidine containing compound 72 as a pale yellow solid (120 mg, 77.9%), m.p. 145-147 °C; ν max/ cm⁻¹ 3172 (OH), 1641 (C=C, aromatic), 1036 (C-O); ¹H NMR δ H (270 MHz, D₂O) 8.07 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 5.77 (d, J = 5.0, 1H, 1'-H), 4.60 (app. t, J = 5.0, 1H, 2'-H), 4.18 (app. t, J = 5.0, 1H, 3'-H), 4.12 (m, 1H, 4'-H), 3.10 (t, J = 6.6, 2H, CH₂S), 2.87 (dd, 1H, J₁ = 14.4, J₂ = 4.4, 5'-CH₃H₃), 2.76 (dd, 1H, J₁ = 14.4, J₂ = 6.7, 5'-CH₃H₃), 2.54 (t, J = 6.6, 2H, CH₂NH); ¹³C NMR (100 MHz, D₂O) δC 165.8 (C), 155.0 (C), 152.5 (CH), 148.4 (C), 139.7 (CH), 118.2 (C), 87.4 (CH), 83.3 (CH), 73.2 (CH), 72.2 (CH), 40.5 (CH₂), 33.7 (CH₂), 31.0 (CH₂); ES-MS 369.1(M + H⁺); HRMS calcd for C₁₃H₂₀N₆O₃S⁺ 369.1349, found 369.1338.
5'-[(S-3-Amino-3-carboxypropyl)-3-guanidinopropyl-amino]-5'-deoxyadenosine

74a

Protected nitrogen SAM analogue 135a (0.11 g, 0.13 mmol) was dissolved in neat TFA (5.4 mL) at room temperature and stirred for 3 hr, H₂O (0.60 mL) was then added and stirring continued for 1 hr. The reaction mixture was concentrated and dissolved in H₂O (10 mL) and extracted with EtOAc (2 x 5 mL). The aqueous layer was condensed and dried in freezer-dryer overnight. The resulting TFA salt was applied to Amberlite IRA 400 (Cl⁻) ion-exchange resin, eluting with H₂O to give nitrogen SAM analogue 74a as a light yellow solid (0.06 g, 82%), m. p. 134 °C (dec); [α]²⁵D 22.75 (c 1.06 in MeOH); νmax cm⁻¹ 1683 (C=O), 1506 (C=C, aromatic), 1049 (C-O); ¹H NMR (400 MHz, D₂O) δH 8.37 (s, 2H, Ar-H), 6.07 (d, J = 3.6, 1H, 1'-H), 4.69 (m, 1H, 2'-H), 4.42 (m, 2H, 3'-H and 4'-H), 3.92 (m, 1H, CHCH₂), 3.75 (dd, J₁ = 14.0, J₂ = 9.6, 1H, 5'-CH₉H₅), 3.64 (d, J = 14.0, 1H, 5'-CH₉H₅), 3.43 (m, 2H, CH₂NH), 3.27 (m, 2H, CHCH₂CH₂N), 3.12 (m, 2H, CH₂CH₂CH₂N), 2.30 (m, 1H, CHCH₃H₅), 2.16 (m, 1H, CHCH₂H₅), 1.90 (m, 2H, CH₂CH₂CH₂); ¹³C NMR (100 MHz, D₂O) δC 171.5 (C), 156.6 (C), 149.9 (C), 148.0 (C), 144.4 (CH), 143.5, (CH), 119.3 (C), 90.1 (CH), 78.2 (CH), 73.0 (CH), 71.5 (CH), 54.9 (CH₂), 51.4 (CH, 2x CH₂), 38.0 (CH₂), 24.5 (CH₂), 22.5 (CH₂); ESI-MS 467.2 (M + H⁺); HRMS calcd for C₁₈H₃₁N₁₀O₅⁺ 467.2479, found 467.2474.
5'-[(S-3-Amino-3-carboxypropyl)-4-guanidinobutyl-amino]-5'-deoxyadenosine

Nitrogen SAM analogue 74b was prepared by the same procedure of nitrogen SAM analogue 74a as a lightly yellow solid (0.12 g, 90%), m. p. 160 °C (dec); [α]$^20_D$ 25.97 (c 1.06 in H$_2$O); ν max/ cm$^{-1}$ 1684 (C=O), 1506 (C=C, aromatic), 1047 (C-O);
$^1$H NMR (400 MHz, D$_2$O) δH 8.41 (s, 2H, Ar-H), 6.11 (d, J = 4.0, 1H, 1'-H), 4.75 (m, 1H, 2'-H), 4.44 (m, 2H, 3'-H and 4'-H), 3.88 (m, 1H, CHCH$_2$), 3.62 (m, 2H, 5'-CH$_2$), 3.44 (m, 2H, CH$_2$NH), 3.26 (m, 2H, CHCH$_2$CH$_2$N), 3.08 (m, 2H, CH$_2$CH$_2$CH$_2$N), 2.26 (m, 1H, CHCH$_2$H$_b$), 2.06 (m, 1H, CHCH$_2$H$_b$), 1.68 (m, 2H, NCH$_2$CH$_2$CH$_2$CH$_2$NH), 1.54 (m, 2H, NCH$_2$CH$_2$CH$_2$CH$_2$NH); $^{13}$C NMR (100 MHz, D$_2$O) δC 172.0 (C), 156.7 (C), 150.0 (C), 148.1 (C), 144.5 (CH), 143.4, (CH), 119.2 (C), 90.0 (CH), 78.2 (CH), 73.0 (CH), 71.6 (CH), 55.0 (CH$_2$), 51.9 (CH, 2x CH$_2$), 40.0 (CH$_2$), 24.9 (CH$_2$), 24.6 (CH$_2$), 20.3 (CH$_2$); ESI-MS 481.3 (M + H$^+$); HRMS calcd C$_{18}$H$_{31}$N$_5$O$_5$+ 481.2635, found 481.2645.
5'-[(S-3-Amino-3-carboxypropyl)-5-guanidinopentyl-amino]-5'-deoxyadenosine

74c

Nitrogen SAM analogue 74c was prepared by the same procedure of nitrogen SAM analogue 74a as a lightly yellow solid (0.09 g, 67%), m.p. 131 °C (dec); [α]_D^20 23.30 (c 1.09 in H_2O); ν_max/cm⁻¹ 1685 (C=O), 1510 (C=C, aromatic), 1056 (C-O); ^1H NMR (400 MHz, D_2O) δ_H 8.41 (s, 2H, Ar-H), 6.11 (d, J = 4.0, 1H, 1'-H), 4.75 (m, 1H, 2'-H), 4.45 (m, 2H, 3'-H and 4'-H), 3.96 (m, 1H, CHCHAIN), 3.70 (m, 2H, 5'-CH_2), 3.44 (m, 2H, CH_2NH, 3.23 (m, 2H, CHCHAIN), 3.02 (m, 2H, CHCHAIN), 2.32 (m, 1H, CHCHAIN_H_b), 2.20 (m, 1H, CHCHAIN_H_b), 1.66 (m, 2H, NCHAINCHAINCHAINCHAINCHAIN), 1.45 (m, 2H, NCHAINCHAINCHAINCHAINCHAIN), 1.29 (m, 2H, NCHAINCHAINCHAINCHAINCHAIN); ^13C NMR (100 MHz, D_2O) δ_C 171.7 (C), 156.6 (C), 150.0 (C), 148.1 (C), 144.5 (CH), 143.5, (CH), 119.2 (C), 90.0 (CH), 78.3 (CH), 73.0 (CH), 71.6 (CH), 54.9 (CH), 52.6 (CH), 51.6 (CH), 50.7 (CH), 40.7 (CH), 27.3 (CH), 24.6 (CH), 22.8 (CH), 22.5 (CH); ESI-MS 495.3 (M + H^+); HRMS calcd for C_{18}H_{31}N_{10}O_{5}^+ 495.2792, found 495.2772.
5'-[(S-3-Amino-3-carboxypropyl)-propargylamino]-5'-deoxyadenosine 77

Protected alkyne derived SAM analogue 148 (0.18 g, 0.30 mmol) was dissolved in TFA (4 mL) at 0 °C and stirring continued at room temperature overnight. The reaction mixture was concentrated and dissolved in H2O (5 mL) and extracted with EtOAc (2 x 5 mL). The aqueous layer was condensed and dried in freezer-dryer overnight. The resulting TFA salt was applied to Dowex 50WX4-400 (NH4+) ion-exchange resin and the column was washed with H2O, and then eluted with a gradient NH4HCO3 (0-1.2M). Fractions from the major UV-active peak were collected and evaporated and then co-evaporated with water. The residue was lyophilized to give alkyne derived SAM analogue 77 (0.11 g, 91%), m.p. 169 °C (dec); [α]D22 9.11 (c 1.01 in H2O); νmax/ cm⁻¹ 2360 (C=O), 1604 (C=C, aromatic); 1H NMR (400 MHz, D2O) δH 8.17 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 5.92 (d, J = 4.8, 1H, 1'-H), 4.64 (app. t, J = 4.8, 1H, 2'-H), 4.24 (m, 1H, 3'-H), 4.16 (m, 1H, 4'-H), 3.70 (m, 1H, CHCH2), 3.47 (dd, J1 = 17.4, J2 = 2.2, 1H, CH2HbC=CH), 3.40 (dd, J1 = 17.4, J2 = 2.2, 1H, CHaHbC=CH), 2.86 (m, 2H, 5'-CHaHb), 2.73 (m, 2H, CH2N), 2.54 (t, J = 2.2, 1H, HC=C), 1.97 (m, 1H, CHCH2Hb), 1.86 (m, 1H, CHCH2Hb); 13C NMR (100 MHz, D2O) δC 174.1 (C), 155.4 (C), 152.6 (CH), 148.5 (C), 139.9 (CH), 118.7 (C), 88.1 (CH), 81.3 (CH), 77.7 (CH), 75.0 (C), 73.3 (CH), 72.0 (CH), 55.7 (CH2), 54.6 (CH), 50.7 (CH2), 41.9 (CH2), 26.6 (CH2); ESI-MS 404.2 (M - H+); HRMS calcld for C17H22N7O5+ 404.1682, found 404.1694.
5'-[(S-3-Amino-3-carboxypropyl)-1-(2-guanidinoethyl)-1H-1,2,3-triazol-4-yl]-methyl-amino]-5'-deoxyadenosine 78

Triazole derived SAM analogue 78 was prepared by the same procedure of nitrogen SAM analogue 74a as a yellow solid which is hygroscopic (88 mg, 92%), m.p. 114-115 °C (dec); [α]24D 15.79 (c 1.36 in MeOH), 1H NMR (400 MHz, D2O) δH 8.36 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 8.19 (s, 1H, triazole-H), 6.06 (d, J = 4.0, 1H, 1'-H), 4.71 (m, 1H, 2'-H), 4.56 (m, 2H, CH2CH2NHC=N), 4.52 (m, 2H, NCH2-triazole), 4.47-4.40 (m, 2H, 3'-H and 4'-H), 3.99 (m, 1H, CHCH2CH2), 3.75-3.55 (m, 4H, CH2NHC=N and 5'-CH2), 3.49-3.35 (m, CHCH2CH2), 2.46-2.36 (m, 1H, CHCH2H2b), 2.30-2.24 (m, 1H, CHCH2H2b); 13C NMR (100 MHz, D2O) δC 170.4 (C), 156.8 (C), 149.8 (C), 148.0 (C), 144.3 (CH), 143.5 (CH), 135.6 (C), 128.4 (CH), 119.3 (C), 90.0 (CH), 78.0 (CH), 73.1 (CH), 71.5 (CH), 55.1 (CH2), 50.7 (CH), 50.2 (CH2), 49.4 (CH2), 47.2 (CH2), 40.7 (CH2), 24.3 (CH2); ESI-MS 534.3 (M + H+); HRMS calcd for C20H32N13O5+ 534.2649, found 534.2609.
$5'$-[(S-3-Amino-3-carboxypropyl)-3-guanidinopropyl-aminol-5'-deoxy-N6-(11-azido-3,6,9-trioxaundecane)-amino adenosine 79

\[
\text{HO} \quad \text{NH}_2 \\
\text{HN} \quad \text{H}_2 \text{N} \quad \text{NH}
\]

$N$-6 Azide tethered SAM analogue 79 was prepared by the same procedure of nitrogen SAM analogue 74a as a slightly yellow solid which is hygroscopic (0.13 g, 87%); $[\alpha]_{23}^{20}$ 20.59 (c 1.02 in MeOH); $\nu_{\text{max}}$ cm$^{-1}$: 2108 (N=N), 1737 (C=O), 1670 (C=C, aromatic); $^1$H NMR (400 MHz, D$_2$O) $\delta$H 8.35 (s, 2H, ArH), 6.07 (d, $J = 3.2$, 1H, 1'-H), 4.71 (m, 1H, 2'-H), 4.39 (m, 2H, 3'-H and 4'-H), 4.02 (m, 1H, CHCH$_2$), 3.75 (m, 2H, 5'-CH$_2$), 3.72 (m, 2H, adenosine-NHCH$_2$), 3.65-3.37 (m, 14H, OCH$_2$ x 6 and CH$_2$N$_3$), 3.33 (m, 2H, CH$_2$NH), 3.27 (m, 2H, CHCH$_2$CH$_2$N), 3.12 (m, 2H, CH$_2$CH$_2$CH$_2$N), 2.33 (m, 1H, CHCH$_3$H$_b$), 2.21 (m, 1H, CHCH$_3$H$_b$), 1.89 (m, 2H, CH$_2$CH$_2$CH$_2$); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$C 170.6 (C), 156.6 (C), 149.0 (C), 146.7 (C), 144.2 (CH), 143.1, (CH), 119.7 (C), 90.1 (CH), 78.0 (CH), 73.0 (CH), 71.5 (CH), 70.4-68.2 (CH x 5), 65.1 (CH$_2$), 54.9 (CH$_2$ x 2), 51.0 (CH$_2$), 50.4 (CH), 50.1 (CH$_2$), 42.4 (CH$_2$), 38.0 (CH$_2$), 24.3 (CH$_2$), 22.6 (CH$_2$); ESI-MS 668.4 (M + H$^+$); HRMS calcd for C$_{26}$H$_{46}$N$_3$O$_8^+$ 668.3587, found 668.3611.
Protected amino thiol 88 (220 mg, 1.24 mmol) was added to a 1 M aqueous NaOH solution (2.1 mL) at room temperature under nitrogen and stirring continued for 30 min. Chloride 16 (100 mg, 0.35 mmol) was then added to the solution and the mixture heated at 60 °C overnight. The reaction solution was cooled and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated, and the residue subject to column chromatography on silica gel using CH₂Cl₂/MeOH (10/1, v/v) as eluent to give thioether 81 as a white solid (80 mg, 54%), m.p. 90-94 °C; [α]D²⁴ -5.67 (c 1.23 in MeOH); νmax/ cm⁻¹ 3355 (OH), 1687 (C=O), 1606 (C=C, aromatic), 1530 (C=C, aromatic), 1049 (C-O); ¹H NMR (270 MHz, CD₃OD) δ 8.33 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 6.02 (d, 1H, J = 5.0, 1'-H), 4.80 (app. t, J = 5.0, 1H, 2'-H), 4.35 (app. t, J = 5.0, 1H, 3'-H), 4.23 (m, 1H, 4'-H), 3.21 (t, J = 6.8, 2H, CH₂S), 3.04 (dd, J₁ = 14.1, J₂ = 5.6, 1H, 5'-CH₉H₉β), 2.95 (dd, J₁ = 14.1, J₂ = 6.3, 1H, 5'-CH₉H₉β), 2.65 (t, J = 6.8, 2H, CH₂NH), 1.42 (s, 9H, C(CH₃)₃); ¹³C NMR δC (68 MHz, CD₃OD) 157.0 (C), 155.9 (C), 152.6 (CH), 149.3 (C), 140.1 (CH), 119.2 (C), 88.7 (CH), 84.4 (CH), 78.8 (C), 73.6 (CH), 72.6 (CH), 40.0 (CH₂), 33.9 (CH₂), 32.3 (CH₂), 27.4 (CH₃); ES-MS 427.2 (M + H⁺); HRMS calcd for C₁₇H₂₇N₆O₅S⁺ 427.1719, found 427.1799.
2-(tert-Butoxycarbonylamino)ethyl-trifluoromethyl-sulphide 82\textsuperscript{70}

\[
\text{F}_3\text{C} - \text{S} \xrightarrow{\text{NHBoc}}
\]

Sodium hydride (60%, 24 mg, 0.59 mmol) was slowly added in portions to a solution of protected amino thiol 88 (105 mg, 0.59 mmol) in dry THF (10 mL) at 0 °C under argon and stirred for 10 min. \( S\)-(trifluoromethyl)dibenzothiophenium tetrafluoroborate (200 mg, 0.59 mmol) was added to the solution at 0 °C and the mixture warmed to room temperature and stirring continued for 30 min. The reaction solution was quenched by \( \text{H}_2\text{O} \) (10 mL) and extracted with EtOAc (3 x 10 mL), and the combined organic layers dried with \( \text{Na}_2\text{SO}_4 \), filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/petroleum ether (1/10, v/v) as eluent to give trifluoromethyl thioether 82 as colourless oil (60 mg, 42%), \( ^1\text{H NMR} \) (270 MHz, CDCl\(_3\)) \( \delta \) 3.42 (app. q, \( J = 6.3, 2\text{H}, \text{CH}_2\text{NH} \)), 3.30 (\( J = 6.3, 2\text{H}, \text{CH}_2\text{S} \)), 1.47 (s, 9H, C(CH\(_3\))\(_3\)); \( ^{13}\text{C NMR} \) (68 MHz, CDCl\(_3\)) \( \delta \) 133.2 (C), 128.7 (C), 79.9 (C), 39.8 (CH\(_2\)), 30.3 (CH\(_2\)), 28.4 (CH\(_3\)); \( ^{19}\text{F NMR} \) (282 MHz, CDCl\(_3\)) -41.08 (s).

2-(tert-Butoxycarbonylamino)ethanethiol 88\textsuperscript{73}

\[
\text{HS} \xrightarrow{\text{NHBoc}}
\]

Triethylamine (889 mg, 8.8 mmol) was added to a solution of 2-aminoethanethiol hydrochloride (500 mg, 4.4 mmol) in CH\(_2\)Cl\(_2\) (25 mL) at room temperature under nitrogen. Stirring was continued for 30 min at room temperature, and then di-tert-butyl dicarbonate (961 mg, 4.4 mmol) added to the mixture and stirring...
continued for 1.5 hr at room temperature. The reaction mixture was quenched with H2O (5 mL) and extracted with Et2O (3 x 50 mL). The combined organic layers were washed with 0.5 M aqueous HCl, dried with Na2SO4, filtered and evaporated to give protected amino thiol 88 as colourless oil (660 mg, 84%). 1H NMR (270 MHz, CDCl3) δH 3.30 (t, J = 6.3, 2H, CH2SH), 2.65 (m, 2H, CH2NH), 1.45 (s, 9H, C(CH3)3); 13C NMR (68 MHz, CDCl3) δC 155.8 (C), 79.6 (C), 43.7 (CH2), 28.4 (CH3), 25.1 (CH2).

5'-Iodo-5'-deoxy-2', 3'-O-isopropylideneadenosine 93

![Structure](image)

Methylphenoxyphosphonium iodide (441 mg, 0.98 mmol) was added to a solution of 2', 3'-isopropylideneadenosine (200 mg, 0.65 mmol) in dry CH2Cl2 (20 mL) at -70 °C under argon. The reaction mixture was warmed to room temperature and stirred for 2 hr, then diluted with CH2Cl2 (20 mL), washed with aqueous Na2S2O3 solution and H2O. The organic layer was dried with Na2SO4, filtered, concentrated, and the residue purified by column chromatography on silica gel using CH2Cl2/MeOH (1/50, v/v) as eluent to give iodide 93 as a white solid (130 mg, 48%), m.p. 208-210 °C (lit., 220-225 °C); [α]D25 -56.4 (c 1.0 in CHCl3); 1H NMR δH (270 MHz, CDCl3) 8.35 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 6.15 (s, 2H, NH2), 6.13 (d, J = 2.0, 1H, 1'-H), 5.53 (dd, J1 = 6.3, J2 = 2.0, 1H, 2'-H), 5.13 (dd, J1 = 6.3, J2 =
2.9, 1H, 3'-H), 4.44 (ddd, J1 = 8.4, J2 = 5.4, J3 = 2.9, 1H, 4'-H), 3.49 (dd, J1 = 10.2, J2 = 8.4, 1H, 5'-H), 3.27 (dd, J1 = 10.2, J2 = 5.4, 1H, 5'-Hb), 1.62 (s, 3H, CH3), 1.41 (s, 3H, CH3); 13C NMR (100 MHz, CDCl3) δc 155.6 (C), 153.2 (CH), 149.1 (C), 140.2 (CH), 120.3 (C), 114.6 (C), 91.2 (CH), 87.2 (CH), 84.4 (CH), 84.3 (CH), 27.0 (CH3), 25.3 (CH3), 5.4 (CH2); ES-MS 418.0(M + H+); HRMS calcd for C13H16lN5O3+ 418.0268, found 418.0180.

5'-[2-[2, 3-bis-(tert-Butoxycarbonyl)guanidino]ethylthio]-5'-deoxyadenosine 96

TFA (2 mL) was added to a solution of thioether 81 (62 mg, 0.14 mmol) in CH2Cl2 (2 mL) at 0 °C and stirring continued at room temperature for 2 hr. The reaction solution was concentrated and the residue dissolved in DMF (3 mL), triethylamine (0.3 mL), protected thiourea 100 (40 mg, 0.14 mmol), mercury chloride (38 mg, 0.14 mmol) were then added to the DMF solution and stirring continued at room temperature for 2 hr, then EtOAc (20 mL) was added to the reaction mixture and the resultant solid removed by filtration. The filtrate was washed with H2O (2 x 10mL) and the organic layer was dried with Na2SO4, filtered and concentrated, and the residue purified by column chromatography on silica gel using CH2Cl2/MeOH (10/1, v/v) as eluent to give protected guanidine derived thioether 96 as a white solid (45mg, 56%), m.p. 110-112 °C; [α]25D -9.5 (c 1.14 in CHCl3); νmax/ cm⁻¹ 3411 (NH2), 3326 (OH), 1723 (C=O), 1644 (C=C, aromatic), 1059 (C-O); 1H NMR (270 MHz, CDCl3)
$\delta$H 11.46 (bs, 1H, NHBoc), 8.61 (m, 1H, CH$_2$NH), 8.13 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 6.00 (d, $J$ = 4.8, 1H, 1'-H), 4.76 (m, 1H, 2'-H), 4.42 (m, 1H, 3'-H), 4.34 (m, 1H, 4'-H), 3.60 (m, 2H, CH$_2$S), 2.94 (m, 2H, 5'-CH$_2$), 2.76 (m, 2H, CH$_2$NH), 1.47 (s, 18H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 163.3 (C), 156.2 (C), 155.3 (C), 153.1 (CH), 152.4 (C), 149.0 (C), 139.4 (CH), 119.5 (C), 89.3 (CH), 84.4 (CH), 83.3 (C), 79.5 (C), 74.4 (CH), 72.8 (CH), 40.0 (CH$_2$), 34.2 (CH$_2$), 32.1 (CH$_2$), 28.3 (CH$_3$), 28.1 (CH$_3$); ES-MS 569.2 (M + H$^+$); HRMS calcd for C$_{23}$H$_{36}$N$_8$O$_7$S$^+$ 569.2398, found 569.2343.

$S$-$1$-$t$-Bun 2-($t$-bun$-b$oxyccarbonylamino)-4-iodobutyrat 98$^{89,91}$

\[ \text{Ot-Bu} \]

\[ \text{NH} \]

Ethyl chloroformate (0.22 mL, 2.26 mmol) was added to a mixture of protected aspartate 123 (0.65 g, 2.26 mmol) and triethylamine (0.32 mL, 2.26 mmol) in dry THF (10 mL) at -5°C and the reaction mixture stirred at room temperature for 30 min. The reaction mixture was filtered and the white solid washed with THF (5 mL). The filtrate was added to a solution of sodium borohydride (0.17 g, 4.52 mmol) in H$_2$O (2 mL) at 0°C and the reaction mixture stirred at room temperature for 2.5 hr. The reaction mixture was acidified to pH 5 with 0.5 M aqueous HCl and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered and concentrated, and the residue applied to the next step directly. Iodine (1.15 g, 4.52 mmol) was added to a solution of triphenylphosphine (1.19 g, 4.52
mmol) in CH₂Cl₂ (10 mL) at 0 °C and stirred for 20 min and the mixture solution of the residue from the above procedure and imidazole (0.31 g, 4.52 mmol) in CH₂Cl₂ (10 mL) added slowly at 0 °C, then stirred 2.5 hr. The reaction mixture was then filtered and the filtrate washed with Na₂S₂O₃ solution (5 mL) and brine (5 mL). The CH₂Cl₂ solution was dried with Na₂SO₄, filtered and concentrated, and the residue was purified by column chromatography on silica gel using Et₂O/Petroleum ether (1/10, v/v) as eluent to give iodide 98 as yellow oil (0.48 g, 55%), [α]₃₀D 18.14 (c 2.15 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 5.10 (d, J = 6.4, 1H, NHBOc), 4.20 (m, 1H, CH), 3.20 (m, 2H, ICH₂), 2.39 (m, 1H, CHCH₂Hb), 2.18 (m, 1H, CHCH₂Hb), 1.49 (s, 9H, C(CH₃)-), 1.47 (s, 9H, C(CH₃)₂), 13C NMR (100 MHz, CDCl₃) δC 171.1 (C), 155.8 (C), 83.0 (C), 80.5 (C), 55.5 (CH), 38.2 (CH₂), 28.8 (CH₃), 28.5 (CH₃), 0.0 (CH₂).

N,N'-bis-tert-Butoxycarbonylthiourea 100⁷⁸

Sodium hydride (60%, 1.2 g, 29.6 mmol) was added slowly to a solution of thiourea (0.5 g, 6.57 mmol) in dry THF (100 mL) at 0 °C under nitrogen and stirred for 5 min and then at room temperature for 10 min. Di-tert-butyl dicarbonate (3.2 g, 14.5 mmol) was added at 0 °C and the ice bath was removed after 20 min and stirring continued for 1.5 hr. Saturated aqueous NaHCO₃ (10 mL) was then added to the resulting slurry solution and the mixture poured into H₂O (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated.
The residue was purified by column chromatography on silica gel using Et₂O/Hexane (1/3, v/v) as eluent to give protected thiourea 100 as a white solid (1.07 g, 59%), m.p. 128-129 °C (lit., 124-127 °C); ¹H NMR δ_H (270 MHz, CDCl₃) 1.51 (s, 9H, C(CH₃)₃); ¹³C NMR (68 MHz, CDCl₃) δ_C 178.0 (C), 150.4 (C), 84.1 (C), 28.0 (CH₃); ES-MS 277.1 (M + H⁺); HRMS calcd for C₁₁H₂₀N₂O₄S⁺ 277.1177, found 277.1245.

2-[2, 3-bis-(tert-Butoxycarbonyl)guanidino]ethanethiol 101

Protected thiourea 100 (121 mg, 0.44 mmol) was added to a mixture of cysteamine hydrochloride (50 mg, 0.44 mmol) and triethylamine (0.18 mg, 1.32 mmol) in DMF (2 mL) at room temperature and stirring continued overnight. H₂O (10 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/hexane (1/3, v/v) as eluent to give guanidine derived thiol 101 as a white solid (48 mg, 34%), ¹H NMR (270 MHz, CDCl₃) δ_H 11.47 (bs, 1H, NHBoc), 8.64 (m, 1H, CH₂NH), 3.77 (app. q, J=6.2, 2H, CH₂NH), 2.86 (t, J = 6.2, 2H, CH₂SH), 1.49 (s, 9H, C(CH₃)₃); ¹³C NMR (68 MHz, CDCl₃) δ_C 163.5 (C), 156.1 (C), 153.1 (C), 83.3 (C), 79.5 (C), 39.2 (CH₂), 37.1 (CH₃), 28.4 (CH₃)₃), 28.1 (CH₃); ES-MS 320.1 (M + H⁺); HRMS calcd for C₁₃H₂₅N₃O₄S⁺ 320.1599, found 320.1581.
2-(9-Fluorenlymethoxycarbonylamino)ethanethiol 105

N,N'-Diisopropylethylamine (0.68 mL, 3.8 mmol) was added to a solution of cystamine dihydrochloride (87 mg, 0.38 mmol) in DMF (5 mL) at room temperature and stirred for 30 min. The reaction mixture was cooled to 0 °C and 9-fluorenlymethoxycarbonyl chloride (200 mg, 0.77 mmol) added, and stirring continued for 30 min. The reaction mixture was poured to H2O (30 mL) and the solution extracted with CHCl3 (2 x 20 mL). The combined organic layers were washed with H2O (10 mL), dried with Na2SO4, filtered and concentrated. To the residue was added methanol (20 mL) and tributylphosphine (0.95 mL, 3.8 mmol) at room temperature and stirred for 2 hr. The reaction solution was concentrated and the residue purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/2, v/v) as eluent to give thiol 105 as a white solid (200 mg, 88%), m.p. 125-127 °C; νmax/ cm−1 1721 (C=O), 1513 (C=C, aromatic); 1H NMR (400 MHz, CDCl3) δH 7.79 (d, J = 7.4, 2H, Ar-H), 7.60 (d, J = 7.4, 2H, Ar-H), 7.43 (t, J = 7.4, 2H, Ar-H), 7.35 (ddd, J₁ = 7.4, J₂ = 7.4, J₃ = 1.2, 2H, Ar-H), 4.43 (d, J = 6.5, 2H, CHCH₂), 4.25 (t, J = 6.5, 1H, CHCH₂), 3.40 (app. q, J = 6.3, 2H, CH₂SH), 2.67 (app. q, J = 6.3, 2H, CH₂NH); 13C NMR (100 MHz, CDCl₃) δC 156.3 (C), 143.9 (C), 141.4 (C), 127.6 (CH), 127.1 (CH), 125.0 (CH), 120.0 (CH), 66.7 (CH₂), 47.3 (CH), 44.0 (CH₂), 24.9 (CH₂); ES-MS 300.1 (M + H⁺); HRMS calcd for C₁₇H₁₇NO₂S+ 300.1014, found 300.1110.
**N-tert-Butoxycarbonyl-2-thiomethyl-2-imidazoline 111**

Di-tert-butyl dicarbonate (0.89 g, 4.1 mmol) was added to a mixture of 2-methylthio-2-imidazoline hydroiodide (1.0 g, 4.1 mmol) and triethylamine (1.1 mL, 8.2 mmol) in \( \text{CH}_2\text{Cl}_2 \) (50 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirring continued overnight. The reaction solution was washed with \( \text{H}_2\text{O} \) (2 x 10 mL) and dried with \( \text{Na}_2\text{SO}_4 \), filtered and concentrated, and the residue purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/2, v/v) as eluent to give protected 2-methylthio-2-imidazoline 111 as a white waxy solid (260 mg, 98%). \(^1\text{H NMR} \delta (270 \text{ MHz, CDCl}_3) 3.81 \text{ (m, 4H, CH}_2\text{CH}_2\text{), 2.36 (s, 3H, CH}_3\text{), 1.48 (9H, s, C(CH}_3\text{)}_3);} \(^{13}\text{C NMR} \delta (68 \text{ MHz, CDCl}_3) \delta \text{ c} 159.6 \text{ (C), 150.9 (C), 82.6 (C), 53.4 (CH}_2\text{), 47.6 (CH}_2\text{), 28.2 (CH}_3\text{), 15.1 (CH}_3\text{);} \) ES-MS 217.1 (M + H\(^+\)); HRMS calcd for C\(_9\)H\(_{16}\)N\(_2\)O\(_2\)S\(^+\) 217.0966, found 217.1055.

**N, N'-bis-tert-Butoxycarbonylimidazolidine-2-thione 113**

Sodium hydride (60%, 176.2 mg, 4.4 mmol) was added slowly to a solution of 2-imidazolidinethione (100 mg, 1.0 mmol) in dry THF (40 mL) at 0 °C under nitrogen and stirred for 5 min. The reaction mixture was warmed to room temperature and stirring continued for 10 min. The reaction mixture was cooled to 0 °C. Di-tert-butyl dicarbonate (470 mg, 2.2 mmol) was added and stirring continued.
for 20 min, then warmed to room temperature and stirring continued for 2 hr. Saturated aqueous NaHCO₃ (5 mL) was added to the reaction mixture and the solution extracted with EtOAc (3 x 5 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/hexane (1/3, v/v) as eluent to give protected imidazolidine-2-thione 113 as a yellow solid (170 mg, 57%), m.p. 108-110 °C (lit., 117-119 °C); ¹H NMR (270 MHz, CDCl₃) δH 3.88 (m, 4H, CH₂CH₂), 1.54 (s, 9H, C(CH₃)₃); ¹³C NMR (68 MHz, CDCl₃) δC 175.6 (C), 150.3 (C), 84.0 (C), 44.6 (CH₂), 28.1 ((CH₃)₃); ES-MS 303.1 (M + H⁺); HRMS calcd for C₁₃H₂₂N₂O₄S⁺ 303.1334, found 303.1393.

α-Amino-γ-butyrolactone hydrobromide 115

Bromoacetic acid (0.93 g, 6.70 mmol) was added to a solution of methionine (1.00 g, 6.70 mmol) in deionised water (27 mL), isopropanol (27 mL) and acetic acid (11 mL) at room temperature under nitrogen and then stirred at 50 °C for 2 hr and at 82-85 °C for 5 hr. The reaction solution was concentrated and the residue was heated at 90 °C in vacuum for 2 hr. 4 M HCl in dioxane (13 mL) was added to the residue and stirring continued at room temperature for 3 hr. Filtration of this reaction produced a yellow solid which was suspended in isopropanol (10 mL) and stirred for 1.5 hr at room temperature. Filtration gave lactone hydrobromide 115 as lightly yellow solid.
(0.78 g, 65%), m.p. 209 °C; \( v_{\text{max}} \) cm\(^{-1} \) 1771 (C=O); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) \( H \) 4.55 (m, 1H, CH), 4.39 (m, 2H, OCH\(_2\)), 2.74 (m, 1H, \( CH_aH_bCH \)), 2.38 (m, 1H, \( CH_aH_bCH \)); \(^{13}\)C NMR (100 MHz, D\(_2\)O) \( \delta \) \( C \) 174.5 (C), 67.3 (CH\(_2\)), 48.5 (CH), 26.7 (CH\(_2\)).

**Methionine methylsulfonium iodide 116** \(^{85}\)

\[ \text{CH}_3\text{C}^+\text{S}^+\text{NH}_2\text{I}^- \]

Iodomethane (5.10 mL, 81.10 mmol) was added to a solution of methionine (5.00 g, 33.50 mmol) in H\(_2\)O and MeOH (93 mL/13mL) and the reaction mixture heated to 35 °C with stirring overnight. This solution was concentrated and the resulting solid dissolved in H\(_2\)O (minimum quantity) and EtOH added until precipitation of a white solid. This mixture was then allowed to stand at room temperature overnight, and filtration gave methylsulfonium iodide 116 as a white solid (8.40 g, 87%), m.p. 147 °C (lit., 149-150 °C); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) \( H \) 3.79 (t, \( J = 6.4 \), 1H, CH), 3.38 (m, 2H, SCH\(_2\)), 2.86 (s, 6H, CH\(_3\) \( x2 \)), 2.28 (m, 2H, CHCH\(_2\)); \(^{13}\)C NMR \( \delta \) \( C \) (100 MHz, D\(_2\)O) 172.5 (C), 52.7 (CH), 39.4 (CH\(_2\)), 25.0 (CH\(_2\)), 24.7 (CH\(_3\)).

**\( \alpha\)-Amino-\( \gamma\)-butyrolactone hydrochloride 117** \(^{86}\)

\[ \text{NH}_2\text{HCl} \]

NaHCO\(_3\) (0.58 g, 6.90 mmol) was added to a solution of methylsulfonium iodide 116 (2.00 g, 6.90 mmol) in H\(_2\)O (30 mL) and the reaction mixture heated at reflux.
overnight. The reaction solution was concentrated, then 6 M aqueous HCl (30 mL) added and 30% aqueous H₂O₂ (0.30 mL) added in order to remove iodide. The resulting mixture was then extracted with EtOAc (3 x 20 mL) and the remaining acidic aqueous layer refluxed for 1 hr. The aqueous solution was then concentrated and EtOH added to the resulting residue. The resulting white solid was removed by filtration and the filtrate concentrated. 6 M aqueous HCl (30 mL) was added to the residue and the solution refluxed for 1 hr. The aqueous solution was concentrated and a yellow solid precipitated which was collected by filtration. The crude product was then stirred in isopropanol (10 mL) for 1 hr. Filtration gave lactone hydrochloride 117 as a off-white solid (0.56 g, 60%), m.p. 218 °C (lit., 220-221 °C); ν max cm⁻¹ 1780 (C=O); ¹H NMR (400 MHz, D₂O) δH 4.55 (m, 1H, CH), 4.39 (m, 2H, OCH₂), 2.74 (m, 1H, CH₃CH₃CH₂), 2.38 (m, 1H, CH₃CH₂CH₂); ¹³C NMR δC (100 MHz, D₂O) 174.5 (C), 67.4 (CH₂), 48.5 (CH), 26.7 (CH₂).

S-4-Methyl aspartate hydrochloride 120

H₂CO  \text{HCO} \quad \text{COOH} \quad \text{NH₂HCl}

Thionyl chloride (2.30 mL, 31.50 mmol) was added dropwise to a solution of L-aspartic acid (3.00 g, 22.50 mmol) in anhydrous MeOH (15.60 mL) at -10 °C and then the reaction solution warmed slowly to room temperature and stirring continued for 25 min. Et₂O (45 mL) was added to the reaction solution under cooling. Immediate filtration gave γ-methyl aspartate 120 as a white solid (2.82 g, 68%), m.p. 193-194 °C (dec) (lit., 190-192 °C (dec)); [α]²³D 12.87 (c 1.30 in MeOH); ¹H NMR
(400 MHz, D$_2$O) $\delta$H 4.29 (t, $J$ = 5.4, 1H, CH), 3.68 (s, 3H, OCH$_3$), 3.05 (m, 2H, CH$_2$CH); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$C 171.9 (C), 170.9 (C), 52.8 (CH$_3$), 49.3 (CH), 33.8 (CH$_2$).

**S-4-Methyl N-(tert-butyloxycarbonyl) aspartate 121**

![Chemical Structure](image)

$\gamma$-Methyl aspartate 120 (2.00 g, 10.90 mmol) was added to a solution of Na$_2$CO$_3$ (1.15 g, 10.90 mmol) in dioxane and H$_2$O (2/1, 33 mL) at 0 °C, and when the evolution of carbon dioxide had ceased, Na$_2$CO$_3$ (1.15 g, 10.90 mmol) was added and followed by di-tert-butyl dicarbonate (2.60 g, 12.00 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hr and then at room temperature overnight. The reaction mixture was concentrated and the residue poured into ice water, and the solution extracted with EtOAc (2 x 10 mL). The aqueous solution was acidified to pH 3 with 0.5 M aqueous HCl and extracted with EtOAc (3 x 20 mL). The organic layers were dried with Na$_2$SO$_4$, filtered and concentrated, and the residue purified by column chromatography on silica gel using EtOAc as eluent to give Boc protected $\gamma$-methyl aspartate 121 as colourless oil (1.50 g, 56%), $\lbrack\alpha\rbrack_{D}^{20} 28.48$ (c 2.50 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 5.60 (d, $J$ = 8.0, 1H, NHBoc), 4.63 (m, 1H, CH), 3.72 (s, 3H, OCH$_3$), 3.06 (dd, $J_1$ = 17.1, $J_2$ = 4.2, 1H, CH$_3$H$_2$CH), 2.85 (dd, $J_1$ = 17.1, $J_2$ = 4.8, 1H, CH$_3$H$_2$CH), 1.45 (s, 9H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 175.6 (C), 171.6 (C), 155.7 (C), 80.5 (C), 52.1 (CH$_3$), 49.8 (CH), 36.4 (CH$_2$), 28.3 (CH$_3$).
Perchloric acid (60%, 0.06 mL, 0.54 mmol) was added to a solution of γ-methyl aspartate 120 (0.10 g, 0.54 mmol) in tert-butyl acetate (5 mL) at 0 °C and stirring continued at room temperature overnight. 1 M aqueous NaHCO₃ (1.08 mL, 1.08 mmol) was added at 0 °C and followed by a solution of di-tert-butyl dicarbonate (1.17 g, 0.54 mmol) in THF (5 mL), and the stirring continued at room temperature overnight. The reaction mixture was extracted with EtOAc (2 x 20 mL) and the combined organic layers dried with Na₂SO₄, filtered and concentrated, and the residue was purified by column chromatography on silica gel using Et₂O/hexane (1/5, v/v) as eluent to give protected methyl aspartate 122 as colourless oil (0.10 g, 61%), [α]₂⁵D 22.55 (c 1.41 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 1.54 (d, J = 7.2, 1H, NHBoc), 4.43 (m, 1H, CH), 3.67 (s, 3H, OCH₃), 2.91 (dd, J₁ = 16.5, J₂ = 4.4, 1H, CH₆CH₆CH), 2.75 (dd, J₁ = 16.5, J₂ = 5.2, 1H, CH₆CH₆CH), 1.44 (s, 9H, C(CH₃)₃), 1.43 (s, 9H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δC 171.3 (C), 170.0 (C), 155.4 (C), 82.3 (C), 79.8 (C), 51.8 (CH₃), 50.5 (CH), 36.9 (CH₂), 29.7 (CH₃), 28.3 (CH₃).

1 M aqueous NaOH (4.60 mL, 4.60 mmol) was added dropwise to a solution of
protected γ-methyl aspartate 122 (1.07 g, 3.50 mmol) in acetone (17.50 mL) and H₂O (3 mL) at 0 °C, whilst maintaining the pH between 8 and 9. The reaction mixture was stirred at room temperature for 1 hr and the acetone removed under vacuum and the residue diluted with H₂O (20 mL). The aqueous solution was washed with Et₂O (2 x 10 mL), then adjusted to pH 6 using 1 M aqueous HCl and extracted with Et₂O (3 x 20 mL). The combined ether layers were dried with Na₂SO₄, filtered and concentrated. The crude product was recrystallised from hexane to give protected aspartate 123 as a white solid (0.90 g, 88%), m.p. 106 °C (lit., 105-106 °C)⁹⁹; [α]₂⁵ D 4.94 (c 1.22 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 5.49 (d, J = 8.0, 1H, NHBOc), 4.47 (m, 1H, CH), 3.03 (dd, J₁ = 17.2, J₂ = 3.6, 1H, CH₃H₅CH), 2.82 (dd, J₁ = 17.2, J₂ = 4.4, 1H, CH₃H₅CH), 1.46 (s, 18H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δC 176.5 (C), 169.8 (C), 155.5 (C), 82.6 (C), 80.1 (C), 51.7 (CH), 36.9 (CH₂), 28.3 (CH₃), 27.8 (CH₃).

N, N'-bis(tert-Butoxycarbonyl)-S-methylisothiourea 125¹³⁶

Saturated aqueous Na₂CO₃ (50 mL) was added to a solution of S-methylisothiourea sulfate (2.00 g, 7.20 mmol) in CH₂Cl₂ (50 mL) at room temperature and followed by di-tert-butyl dicarbonate (6.30 g, 28.80 mmol) and the reaction mixture vigorously stirred overnight. Layers were separated and the aqueous phase extracted with CH₂Cl₂ (2 x 20mL). The combined organic solution was dried with Na₂SO₄, filtered and concentrated, and the residue purified by column chromatography on silica gel
using Et$_2$O/hexane (1/20, v/v) as eluent to give protected methylisothiourea 125 as a white solid (2.59 g, 62%), m. p. 122 °C (lit., 122-123 °C); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 2.41 (s, 3H, SCH$_3$), 1.54 (s, 9H, C(CH$_3$)$_3$), 1.52 (s, 9H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 171.4 (C), 160.7 (C), 150.8 (C), 83.2 (C), 81.0 (C), 28.0 (CH$_3$), 14.4 (CH$_3$); ESI-MS 313.1 (M + Na$^+$); HRMS calcd for C$_{12}$H$_{22}$N$_2$O$_4$SNa$^+$ 313.1198, found 313.1177.

2-[2, 3-bis-(tert-Butoxycarbonyl)guanidino]ethylamine 126

A solution of protected methylisothiourea 125 (0.19 g, 0.65 mmol) in THF (1 mL) was added dropwise to a solution of ethylenediamine (0.13 mL, 1.94 mmol) in THF/H$_2$O (25/1, 2.60 mL) at room temperature. The reaction solution was stirred at 50 °C for 1 hr, then extracted with EtOAc (5 mL) and washed with 10% aqueous NaHCO$_3$, dried with Na$_2$SO$_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (4/1, v/v) with 2% triethylamine as eluent to give amine 126 as a white solid (0.11 g, 56%), m. p. 190°C (dec); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 8.66 (s, 1H, CH$_2$NH=CH), 3.50 (app. q, J = 5.8, 2H, CH$_2$NH), 2.90 (t, J = 5.8, 2H, CH$_2$NH$_2$), 1.47 (s, 18H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 163.6 (C), 156.4 (C), 153.2 (C), 83.2 (C), 79.2 (C), 43.4 (CH$_2$), 41.0 (CH$_2$), 28.3 (CH$_3$), 28.2 (CH$_3$); ESI-MS 303.2 (M + H$^+$); HRMS calcd for C$_{13}$H$_{27}$N$_4$O$_4$$^+$ 303.2032, found 303.2009.
Ethyl chloroformate (0.18 mL, 1.90 mmol) was added to a mixture of protected aspartate 123 (0.50 g, 1.73 mmol) and triethylamine (0.30 mL, 2.08 mmol) in dry THF (5 mL) at -5 °C and the reaction mixture stirred at room temperature for 30 min. The reaction mixture was filtered and the white solid washed with THF (5 mL). The combined filtrate was added to a solution of sodium borohydride (0.13 g, 3.46 mmol) in H2O (1 mL) at 0 °C and the reaction solution stirred at room temperature for 2.5 hr. The reaction mixture was then adjusted to pH 5 using 0.5 M aqueous HCl and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried with Na2SO4, filtered and concentrated, and the residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/3, v/v) as eluent to give alcohol 128 as colourless oil (0.40 g, 84%), [α]20 D 13.92 (c 1.25 in CHCl3); 1H NMR (400 MHz, CDCl3) δH 5.37 (d, J = 5.2, 1H, NHBoc), 4.36 (m, 1H, CHCH2), 3.67 (m, 2H, CH2OH), 2.14 (m, 1H, CHCH3Hb), 1.58 (m, 1H, CHCH2Hb), 1.48 (s, 9H, C(CH3)3), 1.46 (s, 9H, C(CH3)3); 13C NMR (100 MHz, CDCl3) δc 172.0 (C), 156.6 (C), 82.3 (C), 80.3 (C), 58.2 (CH2), 50.9 (CH), 36.6 (CH2), 28.3 (CH3), 28.0 (CH3).
**S-1-tert-Butyl-2-(tert-butyloxycarbonylamino)-4-oxobutanoate 129**

Dry DMSO (1.20 mL, 17.20 mmol) was added to a solution of oxalyl chloride (0.75 mL, 8.60 mmol) in dry CH₂Cl₂ (25 mL) at -78 °C and stirred for 30 min. A solution of alcohol 128 (1.99 g, 7.20 mmol) in dry CH₂Cl₂ (25 mL) was added to the mixture at -78 °C and stirred for 30 min. Triethylamine (5.0 mL, 36.00 mmol) was added to the reaction solution at -78 °C, stirred for 30 min and then warmed to room temperature. The reaction solution was quenched with H₂O (10 mL), separated and the H₂O layer was washed with CH₂Cl₂ (2 x 10 mL). The combined CH₂Cl₂ layers were dried with Na₂SO₄, filtered and condensed, and the residue purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/4, v/v) as eluent to give aldehyde 129 as colourless oil (1.61 g, 82%), [α]²³D 22.64 (c 1.48 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 9.74 (s, 1H, CHO), 5.37 (d, J = 6.8, 1H, NHBoc), 4.48 (m, 1H, CH), 3.04 (dd, J₁ = 17.8, J₂ = 4.8, 1H, CH₂CH₂CH), 2.96 (dd, J₁ = 17.8, J₂ = 4.8, 1H, CH₃CH₂CH), 1.46 (s, 9H, C(CH₃)₃), 1.45 (s, 9H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δC 198.4 (CHO), 168.9 (C), 154.4 (C), 81.7 (C), 79.0 (C), 48.3 (CH), 45.4 (CH₂), 27.3 (CH₃), 27.0 (CH₃).

**3-[2,3-bis-(tert-Butyloxycarbonyl)guanidino]propanol 131a**

Protected methylisothiourea 125 (0.60 mg, 2.06 mmol) was added to a mixture of
3-amino-propanol (0.16 mL, 2.06 mmol) and triethylamine (0.58 mL, 4.12 mmol) in DMF (6 mL) at room temperature. Mercury chloride (0.56 mg, 2.06 mmol) was then added to the reaction mixture and stirring continued for 90 min. The reaction mixture was diluted with EtOAc (10 mL) and filtered. The filtrate was washed with brine (3 x 10 mL), dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/1, v/v) as eluent to give alcohol 131a as a white solid (0.65 g, 99%), m. p. 110 °C; ¹H NMR (400 MHz, CDCl₃) δH 8.46 (m, 1H, CH₂NHC=N), 4.74 (s, 1H, CH₂OH), 3.58 (m, 4H, CH₂OH and CH₂NH), 1.71 (m, 2H, CH₂CH₂CH₂), 1.50 (s, 9H, C(CH₃)₃), 1.48 (s, 9H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δC 162.9 (C), 157.2 (C), 153.2 (C), 83.4 (C), 79.5 (C), 57.7 (CH₂), 36.8 (CH₂), 32.8 (CH₂), 28.4 (CH₃), 28.0 (CH₃); ESI-MS 318.2 (M + H⁺); HRMS calcd for C₁₄H₂₈N₃O₅⁺ 318.2029, found 318.2016.

4-[2,3-bis-(tert-Butoxycarbonyl)guanidinobutanol 131b]

Alcohol 131b was prepared by the same procedure of alcohol 131a as a white solid (2.10 g, 92%), m. p. 120 °C; ¹H NMR (400 MHz, CDCl₃) δH 8.39 (m, 1H, CH₂NHC=N), 3.70 (t, 2H, J = 6.0, HOCH₂), 3.45 (app. q, J = 7.0, 2H, CH₂NH), 2.20 (s, 1H, CH₂OH), 1.65 (m, 4H, CH₂CH₂CH₂CH₂NH), 1.50 (s, 18H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δC 163.5 (C), 156.2 (C), 153.3 (C), 83.1 (C), 79.3 (C), 62.1 (CH₂), 40.4 (CH₂), 29.5 (CH₃), 28.5 (CH₃), 28.3 (CH₃), 25.6 (CH₂); ESI-MS 332.2 (M + H⁺); HRMS calcd C₁₄H₂₈N₃O₅⁺ 332.2186, found 332.2174.
5-[2,3-bis-(tert-Butoxycarbonyl)guanidino]pentanol 131c

Alcohol 131c was prepared by the same procedure of alcohol 131a as a white solid (2.10 g, 95%), m.p. 91 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.33 (m, 1H, CH₂NHC=CN), 3.66 (t, J=6.4, 2H, HOCH₂), 3.43 (app. q, J=7.2, 2H, CH₂NH), 1.65 (m, 4H, CH₂CH₂CH₂CH₂CH₂), 1.51 (s, 9H, C(CH₃)₃), 1.50 (s, 9H, C(CH₃)₃), 1.46 (m, 2H, CH₂CH₂CH₂CH₂CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 163.6 (C), 156.2 (C), 153.3 (C), 83.1 (C), 79.3 (C), 62.6 (CH₂), 40.8 (CH₂), 32.3 (CH₂), 28.8 (CH₂), 28.3 (CH₃), 28.1 (CH₃), 23.0 (CH₂); ESI-MS 346.2 (M + H⁺); HRMS calcd for C₁₄H₂₈N₃O₅⁺ 346.2342, found 346.2338.

3-[2,3-bis-(tert-Butoxycarbonyl)guanidino]propanal 132a

Dry DMSO (0.25 mL, 3.47 mmol) was added to a solution of oxalyl chloride (0.15 mL, 1.74 mmol) in dry CH₂Cl₂ (10 mL) at -78 °C and stirred for 30 min under nitrogen and a solution of alcohol 131a (0.25 g, 0.79 mmol) in dry CH₂Cl₂ (10 mL) was added and stirred for 30 min. Triethylamine (0.88 mL, 6.31 mmol) was added to the reaction mixture at -78 °C and the resulting mixture stirred for 30 min. The reaction mixture was warmed slowly to room temperature, then washed with brine (2 x 10 mL), dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/3, v/v) as
eluent to give aldehyde 132a as colourless oil (0.11 g, 44%), $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 9.84 (s, 1H, CHO), 8.60 (m, 1H, CH$_2$NHC=N), 3.74 (app. q, $J = 6.0$, 2H, CH$_2$NH), 2.80 (t, $J = 6.0$, 2H, CH$_2$CHO), 1.51 (s, 9H, C(CH$_3$)$_3$), 1.50 (s, 9H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 200.7 (CHO), 163.5 (C), 156.2 (C), 153.0 (C), 83.2 (C), 79.4 (C), 43.5 (CH$_2$), 34.1 (CH$_3$), 28.3 (CH$_3$), 28.1 (CH$_3$).

4-[2,3-bis-(tert-Butoxycarbonyl)guanidino]butanal 132b

Aldehyde 132b was prepared by the same procedure of aldehyde 132a as colourless oil (0.78 g, 40%), $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 9.80 (s, 1H, CHO), 8.37 (m, 1H, CH$_2$NHC=N), 3.45 (app. q, $J = 7.1$, 2H, CH$_2$NH), 2.54 (t, $J = 7.1$, 2H, CH$_2$CHO), 1.92 (app. pent, $J = 7.1$, 2H, CH$_2$CH$_2$CH$_2$), 1.51 (s, 18H, C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 201.2 (CHO), 163.5 (C), 156.3 (C), 153.3 (C), 83.2 (C), 79.3 (C), 41.1 (CH$_2$), 40.0 (CH$_2$), 28.3 (CH$_3$), 28.2 (CH$_3$), 21.7 (CH$_2$).

5-[2,3-bis-(tert-Butoxy carbonyl)guanidino]pentanal 132c

Aldehyde 132c was prepared by the same procedure of aldehyde 132a as a white solid (1.30 g, 71%), m.p. 105 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 9.78 (t, $J = 1.4$, 1H, CHO), 8.34 (m, 1H, CH$_2$NHC=N), 3.45 (app. q, $J_1 = 7.0$, 2H, CH$_2$NH), 2.50 (dt, $J_1 = 7.2$, $J_2 = 1.4$, 2H, CH$_2$CHO), 1.66 (m, 4H, CH$_2$CH$_2$CH$_2$CH$_2$), 1.51 (s, 9H, C(CH$_3$)$_3$),
1.50 (s, 9H, C(CH₃)); ¹³C NMR (100 MHz, CDCl₃) δc 202.0 (CHO), 163.6 (C), 156.2 (C), 153.3 (C), 83.1 (C), 79.3 (C), 43.4 (CH₂), 40.4 (CH₂), 28.5 (CH₂), 28.3 (CH₂), 28.1 (CH₂), 19.3 (CH₂).

5'-Amino-5'-deoxy-2', 3'-O-isopropylideneadenosine 133

![Chemical structure of 5'-Amino-5'-deoxy-2', 3'-O-isopropylideneadenosine](image)

Diisopropylazodicarboxylate (0.32 mL, 1.63 mmol) was added dropwise to a suspended solution of 2', 3'-isopropylideneadenosine (0.50 g, 1.63 mmol), phthalimide (0.25 g, 1.68 mmol) and triphenylphosphine (0.43 g, 1.63 mmol) in dry THF (6 mL) at room temperature. The reaction mixture was stirred for 2 hr and a precipitate appeared. The suspension was filtered and the solid washed with Et₂O which was used in the next step directly. Hydrazine hydrate (1.29 mL, 26.08 mmol) was added to a suspended solution of solid prepared from the above step in EtOll (30 mL) at room temperature and the reaction mixture refluxed for 2 hr, then cooled to room temperature. The reaction mixture was filtered, the filtrate concentrated, and then the resulting residue purified by column chromatography on silica gel using MeOH/CH₂Cl₂ (15/85, v/v) as eluent to give amine 133 as a white solid (0.30 g, 60%), m.p. 203-204 °C (lit., 204-205 °C);⁹⁵ [α]₂⁴D -35.02 (c 1.13 in MeOH); ¹H NMR (400 MHz, CDCl₃) δH 8.35 (s, 1H, Ar-H₁), 7.94 (s, 1H, Ar-H₂), 6.04 (d, J = 3.0, 1H, 1'H), 5.89 (s, 2H, adenosine-NH₂), 5.49 (dd, J₁ = 6.4, J₂ = 3.0, 1H, 2'-H), 5.03
(dd, J₁ = 6.4, J₂ = 3.6, 1H, 3'-H), 4.27 (m, 1H, 4'-H), 3.05 (dd, J₁ = 13.4, J₂ = 4.4, 1H, 5'-CH₃H₉), 2.97 (dd, J₁ = 13.4, J₂ = 5.8, 1H, 5'-CH₂H₉), 1.64 (s, 3H, CH₃), 1.41 (s, 3H, CH₃); ^13C NMR (100 MHz, CDCl₃) δC 155.6 (C), 153.2 (CH), 149.4 (C), 140.0 (CH), 120.4 (C), 114.6 (C), 90.7 (CH), 87.5 (CH), 83.6 (CH), 81.8 (CH), 43.8 (CH₂), 27.3 (CH₃), 25.4 (CH₃); ESI-MS 307.1 (M + H⁺); HRMS calcd C₁₃H₁₉N₆O₃^⁺ 307.1518, found 307.1496.

5'-[3'-tert-Butyloxycarbonyl-3-(tert-butyloxycarbonylamino)-propylaminol-5'-deoxy-2', 3'-O-isopropylideneadenosine

A solution of aldehyde 129 (0.06 g, 0.21 mmol) in 1,2-dichloroethane (2 mL) was added to a solution of amine 133 (0.08 g, 0.25 mmol) in 1,2-dichloroethane (2 mL) at room temperature and the mixture heated slightly until complete dissolution occurred, then cooled to room temperature. Sodium triacetoxyborohydride (0.06 g, 0.29 mmol) was added portionwise to the reaction mixture and stirred at room temperature for 2 hr. The reaction solution was then quenched with saturated aqueous Na₂CO₃ (2 mL), extracted with CH₂Cl₂ (2 x 5 mL) and dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (25/1, v/v) as eluent to give secondary amine 134 as a white solid (0.09 g, 73%), m.p. 88 °C; ν max/ cm⁻¹ 3414 (NH₂), 1707 (C=O), 1632 (C=C,
aromatic), 1589 (C=C, aromatic), 1077 (C-O); \([\alpha]^{23}_{D}\) -34.57 (c 1.70 in CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta \) 8.33 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 6.06 (s, 2H, adenosine-NH₂), 6.00 (d, \(J = 8.4\), 1H, NHBoc), 5.98 (d, \(J = 3.6\), 1H, \(1'\)-H), 5.50 (dd, \(J_1 = 6.2\), \(J_2 = 3.6\), 1H, \(2'\)-H), 5.08 (dd, \(J_1 = 6.2\), \(J_2 = 3.2\), 1H, \(3'\)-H), 4.37 (m, 1H, \(4'\)-H), 4.30 (m, 1H, CHCH₂), 2.96 (dd, \(J_1 = 12.7\), \(J_2 = 3.8\), 1H, 5'-CH₃H₆b), 2.80 (dd, \(J_1 = 12.7\), \(J_2 = 5.2\), 1H, 5'-CH₃H₆b), 2.75 (m, 1H, NHCH₃H₆b), 2.63 (m, 1H, NHCH₃H₆b), 1.94 (m, 1H, CHCH₃CH₆b), 1.84 (m, 1H, CHCH₃CH₆b), 1.62 (s, 3H, CH₃), 1.51 (s, 9H, C(CH₃)₃), 1.40 (s, 3H, CH₃), 1.38 (s, 9H, C(CH₃)₃); \(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta \) C 171.9 (C), 155.7 (C), 155.6 (C), 153.1 (CH), 149.5 (C), 139.9 (CH), 120.4 (C), 114.6 (C), 91.1 (CH), 85.0 (CH), 83.0 (CH), 82.2 (CH), 81.7 (C), 79.4 (C), 53.0 (CH), 51.4 (CH₂), 46.3 (CH₂), 32.3 (CH₂), 28.3 (CH₃), 28.0 (CH₃), 27.4 (CH₃), 25.5 (CH₃); ESI-MS 564.3 (M + H⁺); HRMS calcd for C₂₆H₄₂N₇O₇⁺ 564.3146, found 564.3111; Anal. Calcd for C₂₆H₄₁N₇O₇·0.5H₂O: C 54.53, H 7.39, N 17.12. Found C 54.96, H 7.33, N 16.94.

5'-[[3-S-tert-Butyloxycarbonyl-3-(tert-butyloxycarbonylamino)-propyl]-3-[2,3-bis-(tert-butoxycarbonyl)guanidinopropyl-amino]-5'-deoxy-2',3'-O-isopropylide ne-adenosine 135a

Aldehyde 132a (0.06 mg, 0.19 mmol) was added to a solution of secondary amine
134 (0.07 g, 0.13 mmol) in 1,2-dichloroethane (2 mL) at room temperature and stirred for 15 min. Sodium triacetoxyborohydride (0.04 g, 0.18 mmol) was added portionwise and the mixture stirred at room temperature for 2 hr. The reaction solution was quenched with saturated aqueous Na₂CO₃ (2 mL) and extracted with CH₂Cl₂ (2 x 5 mL) and dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (50/1, v/v) as eluent to give protected nitrogen SAM analogue 135a as a white solid (0.10 g, 89%), m.p. 116 °C; [α]²⁴° -33.27 (c 1.10 in CH₃OH); ν max/cm⁻¹ 3413 (NH₂), 1711 (C=O), 1632 (C=C, aromatic), 1095 (C-O), 1058 (C-N); ¹H NMR (400 MHz, DMSO) δ H 8.37 (t, J= 5.2, 1H, CH₂NH), 8.32 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 7.33 (s, 2H, adenosine-NH₂), 7.09 (d, J= 8.0, 1H, CHNHBOc), 6.15 (d, J = 2.1, 1H, 1'-H), 5.48 (d, J₁ = 6.3, J₂ = 2.1, 1H, 2'-H), 5.00 (dd, J₁ = 6.3, J₂ = 2.8, 1H, 3'-H), 4.22 (m, 1H, 4'-H), 3.93 (m, 1H, CHCH₂), 3.28 (m, 2H, 5'-CH₂), 2.80 (m, 2H, CH₂NH), 2.33 (m, 4H, CHCH₂CH₂N and CH₂CH₂CH₂N), 1.85 (m, 1H, CHCH₄H₆b), 1.64 (m, 1H, CHCH₄H₆b), 1.59 (m, 2H, NCH₂CH₂CH₂NH), 1.53 (s, 3H, CH₃), 1.48 (s, 9H, C(CH₃)₃), 1.39 (s, 9H, C(CH₃)₃), 1.38 (s, 9H, C(CH₃)₃), 1.37 (s, 9H, C(CH₃)₃), 1.33(s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ C 171.8 (C), 163.6 (C), 156.1 (C), 155.6 (C), 155.5 (C), 153.13 (CH), 153.11 (C), 149.2 (C), 140.2 (CH), 120.3 (C), 114.4 (C), 90.9 (CH), 85.5 (CH), 83.7 (CH), 83.4 (CH), 82.9 (C), 81.7 (C), 79.5 (C), 79.2 (C), 56.1 (CH₂), 52.8 (CH), 52.1 (CH₂), 50.6 (CH₂), 39.3 (CH₂), 29.1 (CH₂), 28.4 (CH₃), 28.3 (CH₃), 28.1 (CH₂), 28.0 (CH₃), 27.1 (CH₃), 26.3 (CH₂), 25.4 (CH₃); ESI-MS 863.5 (M + H⁺); HRMS calcd for C₄₀H₆₇N₁₀O₁₁⁺ 863.4991, found 863.4990; Anal.
Calcd for C_{40}H_{66}N_{16}O_{11}: 0.5H_{2}O: C 55.09, H 7.74, N 16.06. Found C 55.02, H 7.69, N 15.76.

5'\-[3-\textit{S-tert-Butyloxycarbonyl}-3-(\textit{tert-butyloxycarbonylamino)-propyl]-4-[2,3-bis-(\textit{tert-butoxycarbonyl})guanidino]butyl-amino]-5'-deoxy-2',3'-O-isopropyliden e-adenosine 135b

Protected nitrogen SAM analogue 135b was prepared by the same procedure of protected nitrogen SAM analogue 135a as a white solid (0.64 g, 82%), m.p. 109 °C; [\alpha]_{D}^{21} -24.65 (c 1.23 in MeOH); \nu_{\text{max}}/ \text{cm}^{-1} 3413 (\text{NH}_{2}), 1713 (\text{C}=\text{O}), 1632 (\text{C}=\text{C}, aromatic), 1094 (\text{C}-\text{O}), 1053 (\text{C-N}); ^{1}H \text{NMR (400 MHz, DMSO)} \delta_{\text{H}} 8.32 (s, 1H, Ar-H), 8.24 (t, J = 5.4, 1H, CH_{2}NH), 8.14 (s, 1H, Ar-H), 7.33 (s, 2H, adenosine-NH_{2}), 7.09 (d, J = 7.6, 1H, CH_{2}NH\text{Boc}), 6.15 (d, J = 2.1, 1H, 1'\text{-H}), 5.51 (dd, J_{1} = 6.4, J_{2} = 1', 1H, 1'\text{-H}), 5.00 (dd, J_{1} = 6.4, J_{2} = 2.4, 1H, 3'\text{-H}), 4.21 (m, 1H, 4'\text{-H}), 3.95 (m, 1H, CHCH_{2}), 3.23 (m, 2H, 5'-CH_{2}), 2.76 (m, 2H, CH_{2}NH), 2.36 (m, 2H, CHCH_{2}CH_{2}N), 2.30 (m, 2H, CH_{2}CH_{2}CH_{2}N), 1.82 (m, 1H, CHCH_{2}H_{b}), 1.62 (m, 1H, CHCH_{2}H_{b}), 1.53 (s, 3H, CH_{3}), 1.47 (s, 9H, C(CH_{3})_{3}), 1.43 (m, 2H, NCH_{2}CH_{2}CH_{2}CH_{2}NH), 1.39 (s, 9H, C(CH_{3})_{3}), 1.38 (s, 18H, C(CH_{3})_{3}), 1.34 (s, 3H, CH_{3}), 1.29 (m, 2H, NCH_{2}CH_{2}CH_{2}CH_{2}NH); ^{13}C \text{NMR (100 MHz, CDCl}_{3}) \delta_{C} 171.8 (C), 163.6 (C), 156.0
Protected nitrogen SAM analogue 135c was prepared by the same procedure of protected nitrogen SAM analogue 135a as a white solid (0.27 g, 34%), m.p. 109 °C; [α]_D^21 -20.55 (c 1.08 in MeOH); v max/ cm⁻¹ 3413 (NH₂), 1714 (C=O), 1633 (C=C, aromatic), 1054 (C-N); ^1H NMR (400 MHz, DMSO) δ 8.31 (s, 1H, Ar-H), 8.25 (t, J = 5.2, 1H, CH₂NH), 8.14 (s, 1H, Ar-H), 7.33 (s, 2H, adenosine-NH₂), 7.08 (d, J = 7.6, 1H, CHNH/Boc), 6.15 (d, J = 2.1, 1H, 1'-H), 5.52 (dd, J₁ = 6.2, J₂ = 2.6, 1H, 2'-H), 4.99 (dd, J₁ = 6.2, J₂ = 2.6, 1H, 3'-H), 4.19 (m, 1H, 4'-H), 3.94 (m, 1H, CHCH₂),
3.23 (m, 2H, 5'-CH₂), 2.73 (m, 2H, CH₂NH), 2.37 (m, 2H, CHCH₂CH₂N), 2.28 (m, 2H, CH₂CH₂CH₂N), 1.81 (m, 1H, CHCH₃H₆), 1.58 (m, 1H, CHCH₆H₆), 1.53 (s, 3H, CH₃), 1.47 (s, 9H, C(CH₃)₃), 1.44 (m, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂NH), 1.39 (s, 9H, C(CH₃)₃), 1.38 (s, 18H, C(CH₃)-i), 1.34 (s, 3H, CH₃), 1.26 (m, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂NH), 1.19 (m, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂NH); ¹³C NMR (100 MHz, CDCl₃) δC 171.8 (C), 163.6 (C), 156.1 (C), 155.8 (C), 155.5 (C), 153.3 (CH), 153.1 (C), 149.2 (C), 140.3 (CH), 120.3 (C), 114.2 (C), 91.0 (CH), 85.9 (CH), 83.8 (CH), 83.5 (CH), 83.0 (C), 81.5 (C), 79.3 (C), 79.2 (C), 55.9 (CH₂), 54.3 (CH₂), 53.0 (CH), 50.7 (CH₂), 40.9 (CH₂), 29.3 (CH₂), 28.9 (CH₂), 28.4 (CH₃), 28.3 (CH₃), 28.1 (CH₃), 28.0 (CH₃), 27.1 (CH₃), 26.3 (CH₂), 25.4 (CH₃), 24.6 (CH₂); ESI-MS 891.5 (M + H); HRMS calcd for C₄₂H₇₀N₁₀O₁₁⁺ 891.5303, found 891.5248; Anal. Calcd for C₄₂H₇₀N₁₀O₁₁·0.5H₂O: C 56.05, H 7.95, N 15.56. Found C 55.86, H 7.81, N 15.48.

3-[1,3-bis-(tert-Butyloxycarbonyl)-2-imidazolidinylimino] propanol 136

Protected imidazolidine-2-thione 113 (0.30 g, 0.99 mmol) was added to a mixture of 3-amino-1-propanol (0.07 mL, 0.90 mmol) and triethylamine (0.25 mL, 1.80 mmol) in DMF (2 mL). Mercury chloride (0.27 g, 0.99 mmol) was then added at 0 °C and the reaction mixture stirred for 30 min and at room temperature overnight. The reaction mixture was diluted with EtOAc (10 mL) and filtered. The filtrate was
washed with brine (3 x 10 mL), dried with Na₂SO₄, filtered and concentrated, and then the residue purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/2, v/v) as eluent to give cycloethyl guanidine derived alcohol 136 as colourless oil (0.02 g, 5.3%), ¹H NMR (400 MHz, CDCl₃) δ 5.04 (s, 1H, HOCH₂), 4.50 (t, J = 6.0, 2H, HOCH₂), 3.88 (app. q, J = 5.8, 2H, NCH₂CH₂N), 3.72 (t, J = 6.0, 2H, CH₂CH₂N=C), 3.45 (app. q, J = 5.8, 2H, NCH₂CH₂N), 1.89 (app. pent, J = 6.0, 2H, CH₂CH₂CH₂), 1.57 (s, 9H, C(CH₃)₃), 1.44 (s, 9H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δ C 184.3 (C), 156.0 (C), 154.9 (C), 85.1 (C), 79.2 (C), 59.5 (CH₂), 48.4 (CH₂), 43.5 (CH₂), 39.8 (CH₂), 31.6 (CH₂), 28.4 (CH₃), 28.0 (CH₃).

3-[3-tert-Butoxycarbonyl-2-imidazoline-2-tert-butoxycarbonylamino] propanol 137

HO
\[\text{N} \quad \text{Boc} \quad \text{N} \quad \text{Boc}\]

2-Methylthio-2-imidazoline (0.50 g, 2.00 mmol) was added to a solution of 3-amino-1-propanol (0.14 mL, 1.78 mmol) in ethanol (10 mL) and the mixture refluxed for 16 hr. The reaction solution was concentrated and the residue dissolved in H₂O/THF (1/1, 20 mL). Di-tert-butyl dicarbonate (0.80 g, 2.20 mmol) was added to the solution at 0 °C and stirred at room temperature overnight. The reaction solution was extracted with EtOAc (2 x 10 mL) and the organic layers dried with Na₂SO₄, filtered and concentrated, and the residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/1, v/v) as eluent to
give cycloethyl guanidine derived alcohol 137 as a white wax solid (0.05 g, 8.3%), m.p. 96 °C; \( \nu_{\text{max}} \, \text{cm}^{-1} \) 1713 (C=O), 1240 (C-O), 1150 (C-OH); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 5.32 (s, 1H, HOCH\(_2\)), 3.88 (t, \( J = 5.8, 2H, \text{HOCH}_2 \)), 3.67 (t, \( J = 5.8, 2H, \text{CH}_2\text{CH}_2\text{CH}_2\text{N} \)), 3.45 (app. q, \( J = 5.8, 2H, \text{BocNCH}_2\text{CH}_2\text{N} \)), 3.33 (m, 2H, BocNCH\(_2\text{CH}_2\text{N} \)), 1.75 (app. pent, \( J = 5.8, 2H, \text{CH}_2\text{CH}_2\text{CH}_2\text{N} \)), 1.55 (s, 9H, C(CH\(_3\))\(_3\)), 1.51 (s, 9H, C(CH\(_3\))\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 156.6 (C), 156.0 (C), 154.9 (C), 83.8 (C), 79.1 (C), 59.0 (CH\(_2\)), 43.4 (CH\(_2\)), 40.2 (CH\(_2\)), 36.7 (CH\(_2\)), 32.7 (CH\(_2\)), 28.4 (CH\(_3\)), 28.0 (CH\(_3\))

3-Hydroxypropyl tosylate 144

\[\text{HO-} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \quad \begin{array}{c} \text{O} \\ \text{C} \end{array} \]

p-Toluenesulfonyl chloride (1.00 g, 5.20 mmol) was added to a solution of triethylamine (0.76 mL, 5.46 mmol) and DMAP (0.02 mg, 0.16 mmol) in 1,3-propanediol (3.00 mL, 41.60 mmol) at room temperature and stirred for 2 hr. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (20 mL) and washed with 0.8 M aqueous HCl (30 mL), and then H\(_2\)O (20 mL). The CH\(_2\)Cl\(_2\) solution was dried with Na\(_2\)SO\(_4\), filtered and concentrated, and the residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/1, v/v) as eluent to give hydroxypropyl tosylate 144 as colourless oil (0.88 g, 73%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.8 (d, \( J = 8.0, 2H, \text{Ar-H} \)), 7.36 (d, \( J = 8.0, 2H, \text{Ar-H} \)), 4.19 (t, \( J = 6.0, 2H, \text{HOCH}_2 \)), 3.71 (t, \( J = 6.0, 2H, \text{CH}_2\text{Ots} \)), 2.46 (s, 3H, CH\(_3\)), 1.89 (app. pent, \( J = 6.0, 2H, \text{CH}_2\text{CH}_2\text{CH}_2 \)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 144.9 (C), 132.9 (C), 129.9 (CH), 127.9 (CH), 67.5

179
(CH₂), 58.3 (CH₂), 31.6 (CH₂), 21.6 (CH₃); ESI-MS 253.0 (M + Na); HRMS calcd for C₁₀H₁₄NaO₄S+: 253.0505, found 253.0494.

5'-[(3-S-tert-Butyloxy carbonyl-3-(tert-butyloxy carbonylamino)propyl]-propargy 11]-5'-deoxy-2',3'-O-isopropylidene-adenosine 148

Propargyl bromide (80% in toluene, 0.22 mL, 1.96 mmol) was added to a solution of secondary amine 134 (1.00 g, 1.78 mmol) and K₂CO₃ (0.27 g, 1.96 mmol) in DMF (10 mL) at room temperature and stirring continued for 3 hr. The reaction mixture was diluted with EtOAc (50 mL) and washed with brine (3 x 20 mL), dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using CH₂Cl₂/MeOH (30/1, v/v) as eluent to give protected alkyne derived SAM analogue 148 as a white solid (0.76 g, 71%), m.p. 90 °C; [α]₂³D -21.67 (c 1.02 in MeOH); ν max/ cm⁻¹ 3413 (NH₂), 3304 (C=C, aromatic), 1709 (C=O), 1632 (C=C, aromatic), 1587 (C=C, aromatic), 1078 (C-O); ¹H NMR (400 MHz, CDCl₃) δH 8.34 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 6.20 (s, 2H, adenosine-NH₂), 6.07 (d, J = 1.6, 1H, 1'-H), 5.62 (d, J = 8.0, 1H, NHBoc), 5.49 (m, 1H, 2'-H), 4.99 (m, 1H, 3'-H), 4.35 (m, 1H, 4'-H), 4.22 (m, 1H, CHCH₂), 3.42 (m, 2H, HC=CH₂N), 2.80 (dd, J₁ = 13.4, J₂ = 6.0, 1H, 5'-CH₃H₆), 2.71 (dd, J₁ = 13.4, J₂ = 6.8, 1H, 5'-CH₃H₆), 2.57 (t, J = 6.6, 2H, CH₂CH₂N), 2.11 (m, 1H, HC=C), 1.93 (m, 1H,
CH\textsubscript{2}H\textsubscript{3}), 1.78 (m, 1H, CH\textsubscript{2}H\textsubscript{3}), 1.61 (s, 3H, CH\textsubscript{3}), 1.43 (s, 18H, C(CH\textsubscript{3})\textsubscript{3}), 1.39 (s, 3H, CH\textsubscript{3}); $^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) $\delta$C 171.7 (C), 155.8 (C), 155.5 (C), 153.0 (CH), 149.2 (C), 140.0 (CH), 120.3 (C), 114.4 (C), 90.8 (CH), 85.5 (CH), 83.9 (CH), 83.1 (CH), 81.7 (C), 79.5 (C), 77.9 (C), 73.4 (CH), 55.3 (CH\textsubscript{2}), 52.6 (CH), 50.2 (CH\textsubscript{2}), 42.6 (CH\textsubscript{2}), 29.8 (CH\textsubscript{2}), 28.4 (CH\textsubscript{3}), 28.0 (CH\textsubscript{3}), 27.1 (CH\textsubscript{3}), 25.4 (CH\textsubscript{3}); ESI-MS 602.3 (M + H\textsuperscript{+}); HRMS calcd for C\textsubscript{29}H\textsubscript{44}N\textsubscript{7}O\textsubscript{7}\textsuperscript{+} 602.3296, found 602.3317.

2-[2,3-bis-(tert-Butoxycarbonyl)guanidino]ethanol 150\textsuperscript{77}

Protected methylisothiourea 125 (300 mg, 1.03 mmol) was added to a mixture of aminoethanol (0.06 mL, 1.03 mmol) and triethylamine (0.29 mL, 2.06 mmol) in DMF (3 mL) and followed by mercury chloride (280 mg, 1.03 mmol) at room temperature and stirring continued for 1.5 hr. The reaction mixture was diluted with EtOAc (10 mL) and filtered. The filtrate was washed with brine (3 x 10 mL), dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/1, v/v) as eluent to give alcohol 150 as a white solid (310 mg, 99%), m.p. 118-119 °C, $^1$H NMR (400 MHz, CDCl\textsubscript{3}) $\delta$H 8.70 (m, 1H, CH\textsubscript{2}NHC=N), 4.56 (s, 1H, CH\textsubscript{2}OH), 3.78 (t, $J$ = 5.0, 2H, CH\textsubscript{2}OH), 3.58 (app. q, $J$ = 5.0, 2H, CH\textsubscript{2}NH), 1.51 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 1.49 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}); $^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) $\delta$C 162.8 (C), 157.4(C), 153.1 (C), 83.5 (C), 79.5 (C), 63.1 (CH\textsubscript{2}), 44.4 (CH\textsubscript{3}), 28.2 (CH\textsubscript{3}), 28.0 (CH\textsubscript{3}); ESI-MS 304.2 (M + H\textsuperscript{+}); HRMS calcd for C\textsubscript{13}H\textsubscript{26}N\textsubscript{3}O\textsubscript{5}\textsuperscript{+} 304.1872, found 304.1849.
2-(tert-Butyloxycarbonyl)amino-ethyl azide 153

\[
\text{BocHN} \quad \text{N}_3
\]

p-Toluenesulfonyl chloride (3.90 g, 20.4 mmol) was added to a solution of Boc-amino ethanol (3.00 g, 18.6 mmol) and triethylamine (5.20 mL, 37.2 mmol) and DMAP (0.23 g, 1.86 mmol) in dry CH₂Cl₂ (50 mL) and stirring continued for 3 hr. The reaction solution was washed with H₂O (20 mL), adjusted to pH 4 with 1M aqueous HCl and washed with H₂O (2 x 20 mL), dried with Na₂SO₄, filtered and concentrated. The residue was dissolved in dry DMF (10 mL) and then NaN₃ (3.60 g, 55.8 mmol) was added. The reaction solution was stirred at 60 °C for 4 hr, then diluted with H₂O (50 mL) and extracted with Et₂O (30 mL). The ether layer was washed with brine (3 x 20 mL) and dried with Na₂SO₄, filtered and concentrated to give azide 153 as colourless oil (2.46 g, 71%); \(^1\)H NMR (400 MHz, CHCl₃) δ 4.92 (s, 1H, NH), 3.42 (t, J = 5.4, 2H, N₃CH₂), 3.31 (m, 2H, NHCH₂), 1.46 (s, 9H, CH₃); \(^13\)C NMR (100 MHz, CHCl₃) δC 156.8 (C), 79.8 (C), 51.2 (CH₂), 40.1 (CH₂), 28.4 (CH₃).

2-[2,3-bis-(tert-Butyloxycarbonyl)guanidino]ethyl azide 154

\[
\text{BocHN} \quad \text{N}_3
\]

TFA (1 mL) was added to a solution of the azide 153 (133 mg, 0.72 mmol) in CH₂Cl₂ (1 mL) at 0 °C and stirring continued at room temperature for 1 hr. The reaction solution was concentrated, the residue dissolved in DMF (2 mL), triethylamine (0.20 mL, 1.43 mmol), protected methylisothiourea (207 mg, 0.72 mmol), mercury
chloride (194 mg, 0.72 mmol) were added to this solution and stirring continued at room temperature for 1 hr, then EtOAc (20 mL) added to the reaction mixture and the resultant solid removed by filtration. The filtrate was washed with water and the organic layer was dried with Na₂SO₄, filtered, concentrated, and the residue was purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/10, v/v) as eluent to give guanidine derived azide 154 as a white solid (183 mg, 78%), m.p. 82-83 ºC; ν max/ cm⁻¹ 2106 (N₃), 1721 (C=O); ¹H NMR (400 MHz, CDCl₃) δ H 8.61 (m, 1H, NHBoc), 3.63 (app. q, J = 5.6, 2H, NHCH₂), 3.54 (t, J = 5.6, 2H, CH₂N₃), 1.52 (s, 18H, C(CH₃)₃); ¹³C NMR (100 MHz, CDCl₃) δ C 163.4 (C), 156.3 (C), 153.2 (C), 83.4 (C), 79.5 (C), 50.5 (CH₂), 40.0 (CH₂), 28.3 (CH₃), 28.1 (CH₃); ESI-MS 329.2 (M + H⁺); HRMS calcd for C₁₃H₂₅N₆O₄⁺ 329.1932, found 329.1927.

5’-[[3-S-tert-Butyloxycarbonyl-3-(tert-butyloxycarbonylamino)-propyl][1-2-[2,3-bis-(tert-Butyloxycarbonyl)guanidine]ethyl]-1H-1,2,3-triazol-4-yl]methylamino]-5’-deoxy-2’,3’-O-isopropylidene-adenosine 155

Copper sulphate (41 mg, 0.16 mmol) and sodium ascorbate (163 mg, 0.82 mmol) were added sequentially to a suspended solution of protected alkyne derived SAM analogue 148 (493 mg, 0.82 mmol) and guanidine derived azide 154 (269 mg, 0.82
mmol) in MeOH/H2O (8 mL, 3/1) at room temperature and stirring continued overnight. The reaction mixture was diluted with CH2Cl2 (10 mL) and H2O (10 mL), separated, water phase extracted with CH2Cl2 (2 x 10 mL). The combined organic phase was dried with Na2SO4, filtered and evaporated, and the residue purified by column chromatography on silica gel using CH2Cl2/MeOH (30/1, v/v) as eluent to give protected triazole derived SAM analogue 154 as a white solid (530 mg, 70%), m.p. 105-107 °C (dec); [a]23D -2.38 (c 1.30 in CHCl3), νmax/cm⁻¹ 3413 (NH2), 1722 (C=O), 1631 (C=C, aromatic), 1500 (C=C, aromatic), 1096 (C-O); 1H NMR (400 MHz, C6D6) δH 8.67 (s, 1H, Ar-H), 8.48 (t, J = 5.6, 1H, CH2NHC=N), 7.75 (s, 1H, Ar-H), 6.84 (s, 1H, triazole-H), 6.33 (s, 2H, adenosine-NH2), 6.16 (d, J = 8.0, CHNHBoc), 6.12 (d, J = 0.8, 1H, 1'-H), 5.54 (m, 1H, 2'-H), 5.13 (m, 1H, 3'-H), 4.69-4.63 (m, 2H, 4'-H and CHCH2CH2), 4.09 (m, 2H, CH2CH2NHCH=N), 3.87 (m, 2H, NCH2-triazole), 3.64 (m, 2H, CH2NHCH=N), 2.87-2.52 (m, 4H, 5'-CH2 and CHCH2CH2), 2.12 (m, 1H, CHCH2Hb), 1.92 (m, 1H, CHCH2Hb), 1.70 (m, 3H, CH3), 1.63 (s, 9H, C(CH3)3), 1.59 (s, 9H, C(CH3)3), 1.48 (s, 9H, C(CH3)3), 1.38 (s, 3H, CH3), 1.28 (s, 9H, C(CH3)3); 13C NMR (125 MHz, C6D6) δC 171.9 (C), 163.9 (C), 156.4 (C), 155.6 (C x 2), 153.2 (CH), 152.8 (C), 149.3 (C), 143.4 (C), 140.3 (CH1), 123.0 (CH), 120.6 (C), 113.6 (C), 90.7 (CH), 86.2 (CH), 84.0 (CH), 83.5 (CH), 82.5 (C), 80.7 (C), 78.7 (C), 78.7 (C), 55.5 (CH2), 53.2 (CH), 50.3 (CH2), 48.4 (CH2 x 2), 40.3 (CH2), 29.2 (CH2), 28.2 (CH3), 28.1 (CH3), 27.7 (CH3), 27.4 (CH3), 27.0 (CH3), 25.2 (CH3); ESI-MS 930.5 (M + H⁺); HRMS calcd for C42H68N13O11⁺ 930.5156, found 930.5185.
Tetra(ethylene glycol) ditosylate 157

A solution of tetraethylene glycol (10.00 mL, 57.87 mmol) in THF (100 mL) was added to an aqueous solution of NaOH (8.10 g, 202.50 mmol) in H₂O (100 mL) at 0 °C. A solution of p-toluenesulfonyl chloride (24.00 g, 127.31 mmol) in THF (150 mL) was added to the stirred mixture at 0 °C dropwise for 45 min and stirring continued for 2 hr at 0 °C. The reaction mixture was poured to 10% aqueous HCl (50 mL) at 0 °C and extracted with EtOAc (200 mL). The organic layer was washed with aqueous dilute NaHCO₃ solution (100 mL) and dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc as eluent to give ditosylate 157 as colourless oil (20.70 g, 71%); ¹H NMR (400 MHz, CDCl₃) δH 7.78 (d, J = 8.0, 4H, Ar-H), 7.33 (d, J = 8.0, 4H, Ar-H), 4.15 (t, J = 4.7, 4H, CH₂ x 2), 3.67 (t, J = 4.7, 4H, CH₂ x 2), 3.55 (s, 8H, CH₂ x 4), 2.44 (s, 6H, CH₃ x 2); ¹³C NMR (100 MHz, CDCl₃) δC 144.9 (C x 2), 132.9 (C x 2), 130.2 (CH x 4), 128.2 (CH x 4), 70.7 (CH₂ x 2), 70.5(CH₂ x 2), 69.3 (CH₂ x 2), 68.6 (CH₂ x 2), 21.6 (CH₃ x 2); ESI-MS 503.1 (M + H⁺) [Found 503.1391 C₂₂H₃₁O₉S₂ requires 503.1404].

Tetraethylene bis-azide 158

Sodium azide (6.30 g, 97.50 mmol) was added to a solution of ditosylate 157 (19.60 g, 39.00 mmol) in dry DMF (20 mL) at room temperature and stirring continued at
75 °C for 6 hr. The reaction mixture was diluted with EtOAc (100 mL) and washed with H₂O (3 x 50 mL). The organic layer was dried with NaSO₄, filtered and concentrated, and the residue purified by column chromatography on silica gel using EtOAc/Petroleum ether (1/1, v/v) as eluent to give bis-azide 158 as lightly yellow oil (9.10 g, 96%), ¹H NMR (400 MHz, CDCl₃) δ H 3.67 (m, 12H, CH₂O × 6), 3.82 (t, J = 5.2, 4H, CH₂N₃ × 2); ¹³C NMR (100 MHz, CDCl₃) δ C 70.7 (CH₂ x 4), 70.0 (CH₂ x 2), 50.7 (CH₂N₃ x 2).

1-Amino-11-azido-3,6,9-trioxaundecane 159

Triphenylphosphine (6.84 g, 26.10 mmol) was added to a solution of bis-azide 158 (6.06 g, 24.80 mmol) in EtOAc (50 mL) and 1M aqueous HCl (50 mL) at room temperature and stirred overnight. The reaction solution was separated, and then the aqueous layer washed with EtOAc (2 x 20 mL), adjusted to pH 12 and concentrated. The residue was extracted with CH₂Cl₂ (100 mL), and the organic layer washed with 4M aqueous NaOH (2 x 30 mL), dried with NaSO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (4/1, v/v) with 2% triethylamine as eluent to give monoamine 159 as yellow oil (4.00 g, 75%), ν max/cm⁻¹ 2108 (N₃); ¹H NMR (400 MHz, CDCl₃) δ H 3.67-3.59 (m, 10H, CH₂O x 5), 3.49 (t, J = 5.2, 2H, CH₂CH₂NH₂), 3.37 (t, J = 5.2, 2H, CH₂N₃), 2.85 (t, J = 5.2, 2H, CH₂NH₂); ¹³C NMR (100 MHz, CDCl₃) δ C 73.2 (CH₂), 70.7 (CH₂), 70.63 (CH₂), 70.61 (CH₂), 70.3 (CH₂), 70.0 (CH₂), 50.7 (CH₂N₃), 41.7 (CH₂NH₂).
2′,3′,5′-Tri-O-acetyl-6-chlorinosine 161

Tin tetrachloride (1.00 M in CH₂Cl₂, 40 mL, 40.00 mmol) was added to a solution of β-D-ribofuranose 1,2,3,5-tri-O-acetate (6.40 g, 20.00 mmol) and 6-chloropurine (3.80 g, 24.60 mmol) in CH₃CN (100 mL) at room temperature and stirring continued overnight. The reaction solution was condensed and dissolved in EtOAc (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 50 mL) and H₂O (50 mL). EtOAc was removed and the residue purified by column chromatography on silica gel using EtOAc as eluent to give protected 6-chloride adenosine 161 as colourless oil (6.42 g, 78%), [α]²⁰D -15.54 (c 1.21 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 8.77 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 6.23 (d, J = 5.2, 1H, 1′-H), 5.95 (app. t, J = 5.2, 1H, 2′-H), 5.64 (app. t, J = 5.2, 1H, 3′-H), 4.48-4.36 (m, 3H, 4′-H and 5′-CH₂), 2.15 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.08 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 170.2 (C), 169.5 (C), 169.3 (C), 152.3 (CH), 151.6 (C), 151.2 (C), 143.6 (CH), 132.3 (C), 86.9 (CH), 80.5 (CH), 73.1 (CH), 70.5 (CH), 62.9 (CH₂), 20.7 (CH₃), 20.5 (CH₃), 20.4 (CH₃); ESI-MS 435.1 (M + Na); HRMS calcd for C₁₆H₁₇ClN₄NaO₇⁺ 435.0678, found 435.0679.
2',3',5'-Tri-O-acetyl-N6-(11-azido-3,6,9-trioxaundecane)-amino adenosine 162

Monoamine 159 (2.20 g, 10.08 mmol) was added to a mixture of protected 6-chloride adenosine 161 (2.10 g, 5.04 mmol) and triethylamine (1.37 mL, 10.08 mmol) in dry DMF (10 mL) at room temperature and the reaction solution stirred at 60 °C for 2 hr. The reaction mixture was diluted with EtOAc (30 mL) and washed with H2O (2 x 10 mL) and dried with Na2SO4, filtered and concentrated, and the residue purified by column chromatography on silica gel using EtOAc/MeOH (50/1, v/v) as eluent to give protected N-6 azide tethered adenosine 162 (1.20 g, 43%) as yellow oil, [α]23° -27.12 (c 1.04 in CHCl3); ν max/cm⁻¹ 3427 (NH), 2109 (N₃), 1747 (C=O), 1620 (C=C, aromatic), 1097 (C-O); ¹H NMR (400 MHz, CDCl₃) δH 8.37 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.30 (s, 1H, adenosine-NH), 6.18 (d, J= 5.3, 1H, 1'-H), 5.93 (app. t, J= 5.3, 1H, 2'-H), 5.68 (app. t, J= 5.3, 1H, 3'-H), 4.47-4.37 (m, 3H, 4'-H and 5'-CH₂), 3.86 (m, 2H, adenosine-NHCH₂), 3.74 (t, J= 5.2, 2H, CH₂O), 3.70-3.66 (m, 10H, CH₂O x 5), 3.39 (t, J= 5.2, 2H, CH₂N₃), 2.15 (s, 3H, CH₃), 2.13 (s, 3H, CH₃), 2.08 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 170.3 (C), 169.6 (C), 169.4 (C), 154.8 (C), 153.3 (CH), 148.9 (C), 138.1 (CH), 120.3 (C), 86.1 (CH), 80.1 (CH), 73.1 (CH), 70.7-69.8 (CH, CH₂ x 6), 63.1 (CH₃), 50.6 (CH₂), 40.3 (CH₂), 20.7 (CH₃), 20.5 (CH₃), 20.4 (CH₃); ESI-MS 595.2 (M + H⁺); HRMS calcd for C₂₄H₃₃N₆O₁₀⁺ 595.2471, found 595.2486.
2',3'-Isopropylideneinosine 164\textsuperscript{106}

\begin{center}
\includegraphics[width=0.3\textwidth]{2pN164.png}
\end{center}

p-Toluenesulfonic acid monohydrate (3.50 g, 18.66 mmol) was added to the solution of inosine (5.00 g, 18.66 mmol) and 2,2-dimethoxyl propane (11.50 mL, 93.28 mmol) in acetone (200 mL) at room temperature and stirring continued overnight. NaHCO\textsubscript{3} aqueous solution (1.57 g in 40 mL H\textsubscript{2}O) was added to the suspension and stirred for 15 min. The solution was concentrated and the product was recrystallised form the residual water. Filtration gave isopropylideneinosine 164 as a white solid (3.60 g, 63\%), m.p. 263-265 °C (dec) (lit., 266-268 °C\textsuperscript{106}; [\(\alpha\)]\textsubscript{D}\textsuperscript{21} -62.81 (c 0.61 in MeOH);

\textsuperscript{1}H NMR (400 MHz, DMSO) \(\delta\)\textsubscript{H} 8.31 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 6.10 (d, \(J = 1.9\), 1H, 1'-H), 5.27 (dd, \(J_1 = 5.6\), \(J_2 = 1.9\), 1H, 2'-H), 4.93 (m, 1H, 3'-H), 4.23 (m, 1H, 4'-H), 3.54 (m, 2H, 5'-CH\textsubscript{2}), 1.54 (s, 3H, CH\textsubscript{3}), 1.32 (s, 3H, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, DMSO) \(\delta\)\textsubscript{C} 156.6 (C), 147.8 (C), 146.1 (CH), 138.7 (CH), 124.4 (C), 113.1 (C), 89.6 (CH), 86.6 (CH), 83.8 (CH), 81.2 (CH), 61.4 (CH\textsubscript{2}), 27.0 (CH\textsubscript{3}), 25.1 (CH\textsubscript{3});

ESI-MS 309.1 (M + H\textsuperscript{+}); HRMS calcd for C\textsubscript{13}H\textsubscript{17}N\textsubscript{4}O\textsubscript{5}\textsuperscript{+} 309.1194, found 309.1196.

2',3'-Isopropylidene-N\textsubscript{6}-(11-azido-3,6,9-trioxaundecane)-amino adenosine 165\textsuperscript{107}

\begin{center}
\includegraphics[width=0.3\textwidth]{2pN165.png}
\end{center}

\textsuperscript{189}
Monoamine 159 (1.59 g, 7.31 mmol) was added to a solution of isopropylideneinosine 164 (1.50 g, 4.87 mmol), PyBOP (3.00 g, 5.84 mmol), and N,N-diisopropylethylamine (1.27 mL, 7.31 mmol) in dry DMF (20 mL) at room temperature under nitrogen and stirring continued overnight. EtOAc (50 mL) was added to the reaction solution and washed with brine (3 x 20 mL), and the organic layer dried with Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel using CH₂Cl₂/MeOH (50/1, v/v) as eluent to give protected N-6 azide tethered adenosine 165 (1.85 g, 75%) as colourless oil, [α]₂³D -81.56 (c 1.28 in CHCl₃); ν max/cm⁻¹ 3423 (NH), 3206 (OH), 2108 (N₃), 1626 (C=C, aromatic), 1586 (C=C, aromatic), 1083 (C-O); ¹H NMR (400 MHz, CDCl₃) δH 8.30 (s, 1H, Ar-H), 7.80 (s, 1H, Ar-H), 6.54 (s, 1H, adenosine-NH), 5.84 (d, J = 5.3, 1H, 1'-H), 5.20 (app. t, J = 5.3, 1H, 2'-H), 4.52 (m, 1H, 3'-H), 3.98 (dd, J₁ = 12.8, J₂ = 1.6, 1H, 5'-CH₃Hₙ), 3.80 (m, 2H, adenosine-NHCH₂), 3.72 (t, J = 5.0, 2H, CH₂O), 3.68-3.60 (m, 10H, CH₂O x 5), 3.37 (t, J = 5.0, 2H, CH₂N₃), 1.64 (s, 3H, CH₃), 1.37 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 155.2 (C), 152.6 (CH), 147.3 (C), 139.6 (CH), 121.4 (C), 113.9 (C), 94.3 (CH), 86.0 (CH), 83.0 (CH), 81.7 (CH), 70.7-69.7 (CH₂ x 6), 63.4 (CH₂), 50.7 (CH₂), 40.4 (CH₂), 27.7 (CH₃), 25.2 (CH₃); ESI-MS 509.2 (M + H⁺); HRMS calcd for C₂₁H₃₃N₆O₇⁺ 509.2472, found 509.2509.
5'-N-Phthaloyl-2',3'-O-isopropylidene-N6-(11-azido-3,6,9-trioxaundecane)amino adenosine 166

Diisopropylazodicarboxylate (2.00 mL, 10.33 mmol) was added to a solution of protected N-6 azide tethered adenosine 165 (1.50 g, 2.95 mmol) and triphenylphosphine (2.70 g, 10.33 mmol) in dry THF (50 mL) at room temperature and stirring continued for 30 min. Phthalimide (1.50 g, 10.33 mmol) was added to the reaction solution at room temperature. After 2 hr, the reaction mixture was concentrated and the residue purified by column chromatography on silica gel using EtOAc/MeOH (80/1, v/v) as eluent to give protected phthaloyl N-6 azide tethered adenosine 166 as yellow oil (1.42 g, 76%), [α]$_D^{22}$ 5.53 (c 1.99 in CHCl$_3$); ν$_{max}$/ cm$^{-1}$ 3427 (NH), 2108 (N$_3$), 1716 (C=O), 1620 (C=C, aromatic), 1584 (C=C, aromatic), 1097 (C-O); $^1$H NMR (400 MHz, CDCl$_3$) δ$_H$ 8.11 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.78 (m, 2H, Ar-H), 7.69 (m, 2H, Ar-H), 6.37 (s, 1H, adenosine-NH), 6.03 (m, 1H, 1'-H), 5.54 (m, 1H, 2'-H), 5.25 (dd, $J_1 = 5.8$, $J_2 = 3.4$, 1H, 3'-H), 4.54 (m, 1H, 4'-H), 4.05 (dd, $J_1 = 14.0$, $J_2 = 6.2$, 1H, 5'-CH$_2$H$_b$), 3.98 (dd, $J_1 = 14.0$, $J_2 = 6.2$, 1H, 5'-CH$_2$H$_b$), 3.84 (m, 2H, adenosine-NHCH$_2$), 3.73 (t, $J = 4.4$, 2H, CH$_2$O), 3.68-3.60 (m, 10H, CH$_2$O x 5), 3.38 (t, $J = 4.4$, 2H, CH$_2$N$_3$), 1.58 (s, 3H, CH$_3$), 1.38 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) δ$_C$ 168.0 (C x 2), 154.8 (C), 152.9 (CH), 148.3 (C), 139.7 (CH), 133.9 (CH x 2), 131.8 (C x 2), 123.2 (CH x 2), 120.4 (C), 114.3 (C),
90.5 (CH), 85.0 (CH), 84.1 (CH), 82.5 (CH), 70.6-69.4 (CH₂ x 6), 50.6 (CH₂), 40.5 (CH₂), 39.5 (CH₂), 27.1 (CH₃), 25.4 (CH₃); ESI-MS 638.3 (M + H⁺); HRMS calcd for C₂₉H₃₆N₉O₈⁺ 638.2681, found 638.2686.

5'-Amino-5'-deoxy-2',3'-O-isopropylidene-N6-(11-azido-3,6,9-trioxaundecane)-amino adenosine 167

Hydrazine hydrate (1.46 mL, 30.14 mmol) was added to a solution of protected phthaloyl N-6 azide tethered adenosine 166 in EtOH (50 mL) at room temperature and the reaction mixture refluxed for 2 hr and cooled to room temperature. The reaction mixture was filtered and the filtrate concentrated, and the residue was purified by column chromatography on silica gel using MeOH/CH₂Cl₂ (15/85, v/v) as eluent to give protected N-6 azide tethered adenosyl amine 167 as colourless oil (0.18 g, 82%), [α]₂²°D -16.38 (c 1.16 in MeOH); ν max/cm⁻¹ 3426 (NH₂), 2107 (N₃), 1620 (C=C, aromatic), 1583 (C=C, aromatic), 1085 (C-O); ¹H NMR (400 MHz, CD₃OD) δH 8.27 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 6.15 (d, J = 2.9, 1H, 1'-H), 5.48 (dd, J₁ = 6.2, J₂ = 2.9, 1H, 2'-H), 5.03 (dd, J₁ = 6.2, J₂ = 3.0, 1H, 3'-H), 4.26 (m, 1H, 4'-H), 3.80 (m, 2H, adenosine-NHCH₂), 3.75 (m, CH₃O), 3.64 (m, 10H, CH₂O x 5), 3.33 (m, 2H, CH₂N₃), 2.93 (m, 2H, 5'-CH₂), 1.62 (s, 3H, CH₃), 1.40 (s, 3H, CH₃); ¹³C NMR (100 MHz, CD₃OD) δC 156.3 (C), 154.0 (CH), 149.5 (C), 141.5 (CH), 121.2
Protected N-6 azide tethered secondary amine 168 was prepared by the same procedure of secondary amine 134 as colourless oil (0.17 g, 75%), [α]$_{D}^{20}$ = -25.50 ($c$ 1.09 in CHCl$_3$); ν$_{max}$/ cm$^{-1}$ 3428 (NH), 2107 (N$_3$), 1704 (C=O), 1620 (C=C, aromatic), 1583 (C=C, aromatic), 1088 (C-O); $^1$H NMR (400 MHz, CDCl$_3$) δ$_H$ 8.29 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 6.51 (s, 1H, adenosine-NH), 6.00 (d, $J$ = 8.0, 1H, NHBoc), 5.91 (d, $J$ = 2.4, 1H, 1'-H), 5.46 (m, 1H, 2'-H), 5.04 (m, 1H, 3'-H), 4.31 (m, 1H, 4'-H), 4.23 (m, 1H, CHCH$_2$), 3.80 (m, 2H, adenosine-NHCH$_2$), 3.68 (t, $J$ = 4.4, 2H, CH$_2$O), 3.63 (m, 10H, CH$_2$O x 5), 3.31 (t, $J$ = 4.4, 2H, CH$_2$N$_3$), 2.90 (dd, $J_1$ = 12.4, $J_2$ = 2.4, 1H, 5'-CH$_2$H$_b$), 2.75 (dd, $J_1$ = 12.4, $J_2$ = 4.6, 5'-CH$_2$H$_b$), 2.70 (m, 1H, CH$_2$H$_b$N$_3$), 2.58 (m, 1H, CH$_2$H$_b$NH), 1.88 (m, 1H, CHCH$_2$H$_b$), 1.80 (m, 1H, CHCH$_2$H$_b$), 1.56 (s, 3H, CH$_3$), 1.41 (s, 9H, C(CH$_3$)$_3$), 1.34 (s, 12H, CH$_3$ and C(CH$_3$)$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$) δ$_C$ 171.8 (C), 155.5 (C), 154.9 (C), 153.1

\[
\text{5'-[3-S-tert-Butyloxycarbonyl-3-(tert-butyloxycarbonylamino)-propylamino]-5'-deoxy-2',3'-O-isopropylidene-N6-(11-azido-3,6,9-trioxaundecane)amino adenosine 168$^{80}$}
\]
(CH), 148.6 (C), 139.2 (CH), 120.6 (C), 114.5 (C), 91.0 (CH), 84.9 (CH), 83.0 (CH),
82.1 (CH), 81.5 (C), 79.2 (C), 70.6-69.8 (CH2 x 6), 53.0 (CH), 51.4 (CH2), 50.6
(CH2), 46.3 (CH2), 40.3 (CH2), 32.2 (CH2), 28.3 (CH3), 28.0 (CH3), 27.3 (CH3), 25.4
(CH3); ESI-MS 765.4 (M + H+); HRMS calcd for C34H57NiO10+ 765.4254, found
765.4289; Anal. Calcd for C34H56NiO10·0.5H2O: C 52.77, H 7.42, N 18.10. Found C
53.05, H 7.34, N 17.75.

5′-[[3-S-tert-Butyloxycarbonyl-3-(tert-butyloxycarbonylamino)propyl]-3-[2,3-bis
-(tert-butoxycarbonyl)guanidino]propyl-amino]-5′-deoxy-2′,3′-O-isopropylidene
-N6-(11-azido-3,6,9-trioxaundecane)-amino adenosine 169

Protected N-6 azide tethered SAM analogue 169 was prepared by the same procedure
of protected nitrogen SAM analogue 135a as colourless oil (0.23 g, 99%), [α]20D
-14.77 (c 1.07 in CHCl3); νmax/cm−1 3428 (NH), 2107 (N3), 1713 (C=O), 1633 (C=C,
aromatic), 1586 (C=C, aromatic), 1093 (C-O); 1H NMR (400 MHz, DMSO) δH
8.41(m, 1H, CH2NH), 8.32 (s, 1H, Ar-H), 8. (s, 1H, Ar-H), 7.74 (s, 1H,
adenosine-NH), 7.01 (d, J = 4.3, 1H, 1′-H), 6.21 (m, 1H, CHNHBoc), 5.48 (m, 1H,
2′-H), 5.01 (m, 1H, 3′-H), 4.35 (m, 1H, 4′-H), 4.04 (m, 1H, CHCH2), 3.60 (m, 2H,
adenosine-NHCCH2), 3.58 (t, J = 4.8, 2H, CH2O), 3.52 (m, 10H, CH2O x 5), 3.37 (t, J
= 4.8, 2H, CH2N3), 3.27 (m, 2H, 5'-CH2), 2.83-2.30 (m, 6H, NHCH2CH2CH2N and CHCH2CH2N), 1.82 (m, 1H, CHCH2H3), 1.64 (m, 1H, CHCH3H3), 1.62 (m, 2H, CH2CH2CH2), 1.53 (s, 3H, CH3), 1.48-1.38 (m, 36H, C(CH3)2 x 4), 1.33 (s, 3H, CH3); 13C NMR (100 MHz, CDCl3) δC 171.7 (C), 163.6 (C), 156.0 (C), 155.4 (C), 154.8 (C), 153.0 (CH and C), 148.2 (C), 139.5 (CH), 120.5 (C), 114.5 (C), 90.7 (CH), 85.5 (CH), 83.7 (CH), 83.4 (CH), 82.8 (C), 81.5 (C), 79.3 (C), 79.0 (C), 70.6-69.8 (CH2 x 6), 56.1 (CH2), 52.8 (CH), 52.1 (CH2), 50.6 (CH2 x 2), 40.3 (CH2), 39.2 (CH2), 29.0 (CH2), 28.3 (CH3), 28.2 (CH3), 28.0 (CH3), 27.9 (CH3), 27.1 (CH3), 26.3 (CH2), 25.4 (CH3); ESI-MS 1064.6 (M + H+); HRMS calcd for C48H82N13O14+ 1064.6099, found 1064.6140; Anal. Calcd for C48H81N13O14·H2O: C 53.27, H 7.73, N 16.82, Found C 53.20, H 7.55, N 16.99.

N6-Benzoyl-5'-deoxy-2',3'-O-isopropylidene-5'-[6-(1,2,3-tricarboxethoxy-3,4,5,6-tetrahydropyridazine)]adenosine 170a²

(2S)-5-Pyrrolidin-2-yl-1H-tetrazole (12.60 mg, 0.09 mmol) was added to a solution of the adenosyl 7'-aldehyde 204 (475 mg, 1.09 mmol) and diethyl azodicarboxylate (0.14 mL, 91 mmol) in dry CH2Cl2 (1 mL) at room temperature and stirring continued for 1 hr. The mixture was cooled to 0 °C and dry THF added and then triphenylphosphonium bromide (600 mg, 1.36 mmol) added, followed by sodium
hydride (60%, 91 mg, 2.28 mmol). The reaction solution was stirred at 0 °C for 45 min, and quenched with saturated aqueous NH₄Cl (1 mL) and extracted with EtOAc (2 x 5 mL). The combined organic layers were dried with Na₂SO₄, filtered and concentrated, and the residue purified with column chromatography on silica gel using CH₂Cl₂/MeOH (60/1, v/v) as eluent to give the single diastereoisomer 204 (210 mg, 33%) and the diastereomeric mixture (136 mg, 22%), total yield 55%, the pure compound ¹H NMR (400 MHz, DMSO) δH 11.25 (s, 1H, NH₂), 8.79 (s, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 8.06-8.05 (m, 2H, BzH), 7.66-7.55 (m, 3H, BzH), 6.32 (d, J = 2.4, 1H, 1'-H), 6.05 (m, 1H, 7'-H), 5.63 (m, 1H, 8'-H), 5.55 (m, 1H, 2'-H), 5.05 (m, 1H, 3'-H), 4.60 (m, 1H, 9'-H), 4.50 (m, 1H, 6'-H), 4.43 (m, 1H, 4'-H), 4.14 (m, 6H, CH₂CH₃ x 3), 2.15-1.90 (m, 2H, 5'-CH₂), 1.57 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.18 (m, 9H, CH₂CH₃ x 3); ¹³C NMR (100 MHz, CDCl₃) δC 168.5 (C), 164.8 (C), 156.9 (C), 155.8 (C), 152.6 (CH), 151.1 (C), 149.8 (C), 142.8 (CH), 133.6 (C), 132.9 (CH), 132.2 (CH), 128.9 (CH), 127.9 (CH), 123.8 (C), 121.5 (CH), 114.4 (C), 91.5 (CH), 84.6 (CH), 84.3 (CH), 83.9 (CH), 63.1 (CH₂), 62.6 (CH₂), 61.9 (CH₂), 57.8 (CH), 52.6 (CH), 36.0 (CH₂), 27.1 (CH₃), 25.3 (CH₃), 14.4 (CH₃ x 2), 14.0 (CH₃); ESI-MS 694.3 (M + H⁺); HRMS calcd for C₃₃H₄ON₇O₁₀⁺ 694.2831, found 694.2808.

5'-Nitrile-2',3'-O-isopropylidene-adenosine 173

![5'-Nitrile-2',3'-O-isopropylidene-adenosine 173](image)
Acetone cyanohydrin (0.06 mL, 0.67 mmol) was added to a suspended solution of 2', 3'-isopropylideneadenosine (59 mg, 0.19 mmol), and triphenylphosphine (176 mg, 0.67 mmol) in dry THF (5 mL) at room temperature. The reaction mixture was stirred for 5 min, diisopropylazodicarboylate (0.13 mL, 0.67 mmol) was added at 0 °C and stirring continued at room temperature overnight. The reaction solution was concentrated and the residue purified by column chromatography on silica gel using CH₂Cl₂/MeOH (25/1, v/v) as eluent to give nitrile 173 as a off white solid (44 mg, 73%), m.p. 206-208 °C (dec); [α]⁺ D = -4.41 (c 1.02 in CH₃OH); ¹H NMR (400 MHz, CDCl₃) δH 8.35 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.11 (d, J = 1.9, 1H, 1'-H), 5.99 (s, 2H, adenosine-NH₂), 5.49 (dd, J₁ = 6.3, J₂ = 1.9, 1H, 2'-H), 5.15 (dd, J₁ = 6.3, J₂ = 3.2, 1H, 3'-H), 4.54 (dd, J₁ = 7.1, J₂ = 6.5, J₃ = 3.2, 1H, 4'-H), 3.02 (dd, J₁ = 16.6, J₂ = 7.1, 1H, 5'-CH₃H₂), 2.90 (dd, J₁ = 16.6, J₂ = 7.1, 1H, 5'-CH₃H₂), 1.64 (s, 3H, CH₃), 1.41 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 155.8 (C), 153.2 (CH), 148.9 (C), 140.2 (CH), 120.3 (C), 116.7 (C), 114.9 (C), 91.1 (CH), 84.3 (CH), 83.9 (CH), 83.0 (CH), 27.0 (CH₂), 25.2 (CH₃), 22.1 (CH₂); ESI-MS 317.1 (M + H⁺); HRMS calcd for C₁₄H₁₇N₆O₃ 317.1357, found 317.1353.

Ethyl 2(E)-pent-4-ynoate 181

Manganese dioxide (11.80 g, 135.40 mmol) was added to a mixture of propargyl alcohol (0.80 mL, 13.54 mmol) and (carbethoxymethylene)triphenylphosphorane (5.66 g, 16.25 mmol) in dry CH₂Cl₂ (100 mL) at room temperature and stirring.
continued 2 days. The manganese dioxide was removed by filtration through Celite
and washed with CH₂Cl₂ and the filtrated concentrated. The residue was purified by
column chromatography on silica gel using Et₂O/Petroleum ether (1/3, v/v) as eluent
to give alkyne derived alkene compound 181 as yellow oil (0.80 g, 63%), ¹H NMR
(400 MHz, CDCl₃) δH 6.74 (dd, J₁ = 16.0, J₂ = 2.3, 1H, EtOOCCH), 6.31 (dd, J₁ =
16.0, J₂ = 0.7, 1H, CHC≡CH), 4.22 (q, J = 7.2, 2H, CH₂CH₃), 3.36 (dd, J₁ = 2.3, J₂ =
0.7, 1H, C≡CH), 1.31 (t, J = 7.2, 3H, CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δC
165.5 (C), 132.5 (CH), 123.9 (CH), 85.9 (C), 80.2 (C), 60.9 (CH₂), 14.2 (CH₃).

**Ethyl 2(E)-penten-5-bromo-4-ynoate 182**

Silver nitrate (38.40 mg, 0.23 mmol) was added to a solution of alkyne derived
alkene compound 181 and NBS (470 mg, 2.64 mmol) in acetone (10 mL) at room
temperature and stirring continued for 2 hr. The reaction solution was evaporated and
the residue purified by column chromatography on silica gel using Et₂O/Hexane (1/3,
v/v) as eluent to give alkyne bromide 182 as a white solid (381 mg, 83%), ¹H NMR
(400 MHz, CDCl₃) δH 6.76 (d, J = 15.8, 1H, EtOOCCH), 6.31 (d, J = 15.8, 1H,
CHC≡CBr), 4.22 (q, J = 7.1, 2H, CH₂CH₃), 1.31 (t, J = 7.1, 3H, CH₂CH₃); ¹³C NMR
(100 MHz, CDCl₃) δC 165.5 (C), 132.1 (CH), 122.4 (CH), 60.9 (CH₂), 59.5 (C), 14.2
(CH₃).
Ethyl (Z)-3-iodo-propenoate 185 \(^{113}\)

\[
\begin{align*}
\text{H}_3\text{CH}_2\text{CO} & \quad \equiv \\
& \quad 1
\end{align*}
\]

Dry sodium iodide (220 mg, 1.47 mmol) was added to a solution of ethyl propynoate (0.10 mL, 0.98 mmol) in acetic acid (1 mL) and stirring continued at 70 °C overnight. The reaction solution was diluted with H\(_2\)O (10 mL) and neutralized with K\(_2\)CO\(_3\) until no CO\(_2\) was evolved and extracted with Et\(_2\)O (3 x 10 mL). The combined organic layer was washed with 10% Na\(_2\)S\(_2\)O\(_3\) (10 mL) and brine (10 mL), dried with Na\(_2\)SO\(_4\), filtered and concentrated to give vinyl iodide 185 as colorless oil in quantitative yield, \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) \(7.45\) (d, \(J = 8.8\), 1H, EtOOCCH), 6.89 (d, \(J = 8.8\), 1H, CH=CHI), 4.26 (q, \(J = 7.1\), 2H, CH\(_2\)CH\(_3\)), 1.33 (t, \(J = 7.1\), 3H, CH\(_2\)CH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) \(164.6\) (C), 129.9 (CH), 94.6 (CH), 60.8 (CH\(_2\)), 14.2 (CH\(_3\)).

Ethyl 5-iodopenta-2E, 4Z-dienoate 186 \(^{114}\)

\[
\begin{align*}
\text{H}_3\text{CH}_2\text{CO} & \quad \equiv \\
& \quad 1
\end{align*}
\]

DIBAL (1M in hexanes, 1.75 mL, 1.75 mmol) was added to a solution of vinyl iodide 185 (0.36 g, 1.59 mmol) in dry CH\(_2\)Cl\(_2\) (5 mL) at -78 °C dropwise and stirring continued for 15 mins. The reaction mixture was quenched with MeOH (0.30 mL), followed by 15% aqueous K/Na tartrate (6.90 mL), warmed to room temperature, then diluted with Et\(_2\)O (4 mL), stirred for 1 hr while protected from light. The reaction solution was separated and extracted with Et\(_2\)O (3 x 4 mL), dried with
Na₂SO₄, filtered and concentrated. n-BuLi (1.6M in hexane, 1.04 mL, 1.67 mmol) was added to a solution of triethyl phosphonoacetate (0.33 mL, 1.67 mmol) in dry THF (5 mL) at -78 °C and stirring continued for 30 min. The crude aldehyde in dry THF (5 mL) was added to the reaction solution at -78 °C. The reaction mixture was warmed to room temperature over 2 hr, stirred at room temperature for 1 hr, diluted with Et₂O (10 mL) and H₂O (10 mL) and separated. The aqueous phase was extracted with Et₂O (3 x 10 mL) and the combined organic extracts dried with Na₂SO₄, filtered and concentrated. The residue was purified with column chromatography on silica gel using Et₂O/Hexane (1/20, v/v) as eluent to give vinyl iodide 186 as yellow oil (0.25 g, 62%).¹H NMR (400 MHz, CDCl₃) δH 7.42 (ddd, J₁ = 15.3, J₂ = 10.3, J₃ = 0.9, 1H, C₃-H), 6.92 (ddd, J₁ = 10.3, J₂ = 7.8, J₃ = 0.9, 1H, C₄-H), 6.84 (d, J = 7.8, 1H, C₅-H), 6.14 (d, J = 15.3, 1H, C₂-H), 4.25 (q, J = 7.0, 2H, CH₂CH₃), 1.34 (t, J = 7.0, 3H, CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 166.5 (C), 143.0 (CH), 136.6 (CH), 125.8 (CH), 91.9 (CH), 60.7 (CH₂), 14.3 (CH₃).

N₆-Benzylation-2', 3'-O-isopropylidene-adenosine 187

Benzoyl chloride (0.76 mL, 6.52 mmol) was added dropwise to a solution of 2', 3'-O-isopropylideneadenosine (0.50 g, 1.63 mmol) in pyridine (5 mL) at 0 °C and the mixture was warmed to room temperature and stirring continued for 4 hr. The
reaction mixture was quenched by ice-water and evaporated. The residue was dissolved in CH₂Cl₂ (100 mL) and washed with H₂O (50 mL), saturated aqueous NaHCO₃ (50 mL) and concentrated. The residue was dissolved in pyridine (4.50 mL) and a solution of KOH (0.65 g) in H₂O (4.50 mL) was added and stirred vigorously for 20 min. Acetic acid (0.60 mL) was added to the cooled mixture and evaporation removed the solvents. The residue was dissolved in CH₂Cl₂ (100 mL) and washed with H₂O (50 mL), saturated aqueous NaHCO₃ (50 mL) and dried with Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel using CH₂Cl₂/MeOH (50/1, v/v) as eluent to give protected adenosine 187 as a white solid (0.53 g, 80%), m.p. 143-145 °C (lit., 146-148 °C)⁷⁶; [α]²⁴ D -90.83 (c 1.02 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 9.30 (s, 1H, NHBz), 8.79 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 8.05-8.03 (m, 2H, BzH), 7.65-7.52 (m, 3H, BzH), 5.98 (d, J = 4.4, 1H, 1'-H), 5.25 (m, 1H, 2'-H), 5.13 (dd, J₁ = 6.0, J₂ = 1.2, 1H, 3'-H), 4.56 (m, 1H, 4'-H), 4.00 (dd, J₁ = 12.9, J₂ = 1.6, 1H, 5'-CH₃H), 3.81 (m, 1H, 5'-CH₃H), 1.67 (s, 3H, CH₃), 1.40 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 164.6 (C), 152.4 (CH), 150.5 (C), 150.4 (C), 142.6 (CH), 133.4 (C), 132.9 (CH), 128.9 (CH), 128.0 (CH), 124.3 (C), 114.3 (C), 94.2 (CH), 86.3 (CH), 83.2 (CH), 81.6 (CH), 63.3 (CH₂), 27.6 (CH₃), 25.2 (CH₃); ESI-MS 412.2 (M + H⁺); HRMS calcd for C₂₀H₂₂N₅O₅⁺ 412.1616, found 412.1605.
p-Toluenesulfonyl chloride (526 mg, 2.76 mmol) was added to a solution of protected adenosine 187 (453 mg, 1.10 mmol) in dry pyridine (6 mL) at 0 °C and stirring continued for 3 hr and then at room temperature overnight. The reaction solution was concentrated and dissolved in EtOAc (10 mL) and washed with H2O (2 x 10 mL) and dried with Na2SO4. EtOAc was removed and the residue purified by column chromatography on silica gel using CH2Cl2/MeOH (80/1, v/v) as eluent to give adenosyl tosylate 188 as a white solid (494 mg, 80%), m. p. 119-120 °C; [α]23D 20.54 (c 0.87 in CHCl3); 1H NMR (400 MHz, CDCl3) δH 9.27 (s, 1H, NHBz), 8.71 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 8.06-8.04 (m, 2H, BzH), 7.66-7.60 (m, 3H, BzH), 7.55-7.52 (m, 2H, PhH), 7.24-7.22 (m, 2H, PhH), 6.16 (d, J = 2.1, 1H, 1'-H), 5.38 (dd, J1 = 6.2, J2 = 2.1, 1H, 2'-H), 5.06 (dd, J1 = 6.2, J2 = 3.0, 1H, 3'-H), 4.52 (m, 1H, 4'-H), 4.29 (dd, J1 = 10.6, J2 = 4.2, 1H, 5'-CH3Hb), 4.24 (dd, J1 = 10.6, J2 = 5.8, 1H, 5'-CH3Hb), 2.40 (s, 3H, CH3), 1.61 (s, 3H, CH3), 1.40 (s, 3H, CH3); 13C NMR (100 MHz, CDCl3) δC 164.7 (C), 152.7 (CH), 150.9 (C), 149.8 (C), 145.3 (C), 142.1 (CH), 133.5 (C), 132.9 (C), 132.2 (CH), 129.8 (CH), 128.9 (CH), 127.9 (CH), 127.8 (CH), 123.5 (C), 114.8 (C), 91.1 (CH), 84.7 (CH), 84.2 (CH), 81.4 (CH), 68.9 (CH2), 27.1 (CH3), 25.3 (CH3), 21.6 (CH3); ESI-MS 566.2 (M + H+); HRMS calcd for C27H28N5O7S+ 566.1704, found 566.1678.
Ethyl 6-hydroxy-6-phenyl-2(E)-hexen-4-yonoate 191

\[
\text{\includegraphics{ethyl_6-hydroxy-6-phenyl-2(E)-hexen-4-yonoate_191.png}}
\]

\(n\)-BuLi (1.6 M in hexane, 1.5 mL, 2.42 mmol) was added to a solution of alkyne derived alkene compound 181 (300 mg, 2.42 mmol) in dry THF (10 mL) at -78 °C and then benzaldehyde (0.20 mL, 1.94 mmol) added, the stirring continued at -78 °C for 1 hr. The reaction solution was quenched with saturated aqueous NH₄Cl and extracted with EtOAc (2 x 10 mL) and dried with Na₂SO₄, filtered and evaporated. The residue was purified with column chromatography on silica gel using Et₂O/Petroleum ether (1/3, v/v) as eluent to give benzalcohol 191 as yellow oil (170 mg, 38%), \(v \text{ cm}^{-1}\ 3598 (\text{OH}), 1712 (\text{C=O}), 1622 (\text{C=C, aromatic})\); \(^1\)H NMR (400 MHz, CDCl₃) \(\delta_{\text{H}} 7.53 (\text{m}, 2\text{H}, \text{Ph-H}), 7.43-7.41 (\text{m}, 3\text{H}, \text{Ph-H}), 6.85 (\text{dd}, J_1 = 15.9, J_2 = 1.7, 1\text{H}, \text{C=C-CH=CH}), 6.26 (\text{d}, J = 15.9, 1\text{H}, \text{CH=CHCOOEt}), 5.63 (\text{d}, J = 1.7, 1\text{H}, \text{PhCH}), 4.21 (\text{q}, J = 7.2, 2\text{H}, \text{CH₂CH₃}), 1.31 (\text{t}, J = 7.2, 3\text{H}, \text{CH₂CH₃}); \(^{13}\)C NMR (100 MHz, CDCl₃) \(\delta_{\text{C}} 165.8 (\text{C}), 140.0 (\text{C}), 131.0 (\text{CH}), 128.7 (\text{CH}), 128.6 (\text{CH}), 126.7 (\text{CH}), 124.5 (\text{CH}), 98.0 (\text{C}), 83.0 (\text{C}), 64.9 (\text{CH}), 60.9 (\text{CH₂}), 14.7 (\text{CH₃}).\)
Dichloroacetic acid (0.05 mL, 0.64 mmol) was added slowly to a solution of protected adenosine 187 (0.52 g, 1.27 mmol) and DCC (0.78 g, 3.80 mmol) in dry DMSO (3 mL) with ice cooling and the mixture stirred at room temperature for 1.5 hr. A solution of oxalic acid dihydrate (0.32 g, 2.54 mmol) in MeOH (3 mL) was added slowly to the mixture and stirring continued at room temperature for 30 min. The mixture was filtered and the white solid washed with cold MeOH. N,N'-Diphenylethylenediamine (0.31 g, 1.46 mmol) was added to the combined filtrate and stirring continued at room temperature for 1 hr. The reaction mixture was diluted with EtOAc (20 mL) and washed with brine (2 x 5 mL) and dried with Na2SO4. filtered and concentrated. The residue was recrystallised from EtOH to give protected adenosyl 5'-aldehyde 192 as off white solid (0.51 g, 67%), m.p. 134 °C (lit., 132-135 °C); \([\alpha]_{D}^{24} = 13.96 (c 0.73 \text{ in CHCl}_3); \) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta_{1} 9.25\) (s, 1H, NHBz), 8.73 (s, 1H, Ar-H), 8.05-8.03 (m, 2H, BzH), 7.83 (s, 1H, Ar-H), 7.63-7.52 (m, 3H, BzH), 7.28-7.16 (m, 4H, PhH), 6.83-6.73 (m, 6H, PhH), 6.18 (d, \(J = 2.4, 1H, 1'-H\)), 5.76 (m, 1H, 2'-H), 5.23 (m, 1H, 3'-H), 5.18 (dd, \(J_1 = 6.4, J_2 = 2.4, 1H, 4'-H\)), 4.65 (dd, \(J_1 = 4.8, J_2 = 2.4, 2H, 5'-H\)), 3.77-3.58 (m, 4H, NCH\(_2\)CH\(_3\)N), 1.51 (s, 3H, CH\(_3\)), 1.35 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta_{C} 164.8\) (C), 204
152.8 (CH), 151.3 (C), 149.6 (C), 146.5 (C × 2), 141.8 (CH), 133.6 (C), 132.8
(CH), 129.4 (CH), 129.2 (CH), 128.8 (CH), 128.0 (CH), 123.1 (C), 118.4 (CH),
118.3 (CH), 115.0 (C), 113.7 (CH), 113.5 (CH), 88.4 (CH), 87.0 (CH), 83.8 (CH),
80.1 (CH), 73.3 (CH), 47.7 (CH₂), 46.9 (CH₂), 27.4 (CH₃), 25.8 (CH₃); ESI-MS
604.3 (M + H⁺); HRMS calcd for C₃₄H₃₃N₇O₄⁺ 604.2667, found 604.2650.

N6-Benzoyl-2',3'-O-isopropylidene-adenosyl-5'-carboxamide,N-methoxy,N-
methyl 194

PyBOP (72 mg, 0.14 mmol) was added to a solution of protected adenosyl
5'-carboxylic acid 26 (54 mg, 0.13 mmol) and N,O-dimethylhydroxylamine
hydrochloride (15 mg, 0.15 mmol) in dry DMF (2 mL) and stirring continued at
room temperature for 1.5 hr. The reaction solution was diluted with EtOAc (10 mL)
and washed with 5% aqueous KHSO₄ (5 mL), saturated aqueous NaHCO₃ (5 mL),
brine (5 mL) and dried with Na₂SO₄, filtered and concentrated. The residue was
purified with column chromatography on silica gel using CH₂Cl₂/MeOH (70/1, v/v)
as eluent to give protected adenosyl 5'-amide 194 as a white solid (178 mg, 71%),
m.p. 73-75 °C; [α]D²¹ -48.39 (c 1.12, CHCl₃); ν max/cm⁻¹ 3408 (NH), 1708 (C=O),
1612(C=C, aromatic), 1588 (C=C, aromatic), 1087 (C-O); ¹H NMR (400 MHz,
CDCl₃) δH 9.12 (s, 1H, NHBz), 8.84 (s, 1H, Ar-H), 8.47 (s, 1H, Ar-H), 8.05-8.03 (m,
2H, BzH), 7.65-7.61 (m, 1H, BzH), 7.56-7.52 (m, 2H, BzH), 6.49 (d, J = 1.7, 1H, 1'-H), 5.48 (dd, J1 = 5.6, J2 = 1.7, 1H, 2'-H), 5.32 (m, 2H, 3'-H and 4'-H), 3.76 (s, 3H, OCH3), 3.17 (s, 3H, NCH3), 1.69 (s, 3H, CH3), 1.46 (s, 3H, CH3); 13C NMR (100 MHz, CDCl3) δC 169.5 (C), 164.6 (C), 152.8 (CH), 151.8 (C), 149.5 (C), 142.3 (CH), 133.8 (C), 132.8 (CH), 128.9 (CH), 127.8 (CH), 122.8 (C), 114.2 (C), 91.8 (CH), 85.1 (CH), 83.8 (CH), 83.3 (CH), 61.9 (CH3), 32.3 (CH3), 27.0 (CH3), 25.3 (CH3); ESI-MS 469.2 (M + H+); HRMS calcd for C22H25N6O6+ 469.1830, found 469.1824.

(2S)-5-Pyrrolidin-2-yl-1H-tetrazole 198

Sodium azide (0.34 g, 5.2 mmol) and NH4Cl (0.29 g, 5.5 mmol) were added to a solution of nitrile 209 (1.15 g, 5.0 mmol) in DMF (10 mL) and the reaction mixture stirred at 90 °C for 8 hr. After cooling down, the reaction solution was acidified to pH 2 with 1 M aqueous HCl and extracted with CHCl3 (3 x 30 mL), and the combined organic layers were washed with brine (50 mL) and dried with Na2SO4, filtered and concentrated to give crude product as slightly yellow oil. The crude oil and Pd/C (10%, 0.27 g) in acetic acid/H2O (9:1, 75 mL) were stirred under an atmosphere of H2 at room temperature for 4 hr. The reaction mixture was filtered and the filtrate concentrated, the residue was recrystallised with MeOH/Et2O to give tetrazole 198 as a off-white solid (0.32 g, 46%), m. p. 267-268 °C (lit., 269-271 °C); [α]D20 -23.41 (c 0.62 in MeOH), 1H NMR δH (400 MHz, CD3OD) 4.99 (t, J = 7.8, 1H, 206
NHCH), 3.52 (m, 2H, CH₂NH), 2.60-2.29 (m, 4H, CH₂CH₂CH); ¹³C NMR δC (100 MHz, CD₃OD) 159.6 (C), 56.3 (CH), 46.6 (CH₂), 31.1 (CH₂), 24.8 (CH₂).

_N6-Benzoyl-2',3'-O-isopropylideneadenosyl-5'-propanal 204_

\[
\text{O} \quad \text{N} \quad \text{N} \\
\text{O} \quad \text{N} \quad \text{N}
\]

Pd/C (2.7 g) was added to a solution of unsaturated adenosyl 7'-aldehyde 206 (5.40 g, 12.40 mmol) in EtOAc (500 mL) and stirred under H₂ (balloon) for 5 hr. The reaction solution was filtered and the filtrate concentrated, then the residue was purified with column chromatography on silica gel using CHCl₃/MeOH (70/1, v/v) as eluent to give adenosyl 5'-propaldehyde 204 as slightly yellow solid (3.85 g, 71%), m.p. 70-71°C; [α]²⁰D⁻1.40 (c 1.21 in CHCl₃), ν max/cm⁻¹ 3407 (NH), 1711 (C=O), 1612 (C=C, aromatic), 1587 (C=C, aromatic), 1091 (C-O); ¹H NMR (400 MHz, CDCl₃) δH 9.70 (t, J=0.9, 1H, CHO), 9.25 (s, 1H, NHBz), 8.80 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.03 (m, 2H, BzH), 7.62 (m, 1H, BzH), 7.52 (m, 2H, BzH), 6.10 (d, J = 2.4, 1H, 1'-H), 5.50 (dd, J₁ = 6.5, J₂ = 2.4, 1H, 2'-H), 4.92 (dd, J₁ = 6.5, J₂ = 4.2, 1H, 3'-H), 4.23 (ddd, J₁ = 10.2, J₂ = 6.2, J₃ = 4.2, 1H, 4'-H), 2.56 (dt, J₁ = 7.1, J₂ = 0.9, 2H, 6'-CH₂), 2.08 (m, 2H, 5'-CH₂), 1.62 (s, 3H, CH₃), 1.41 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 200.9 (CHO), 164.8 (C), 152.7 (CH), 151.3 (C), 149.9 (C), 142.4 (CH), 133.6 (C), 132.8 (CH), 128.9 (CH), 128.0 (CH), 123.8 (C), 115.0 (C), 90.3 (CH), 86.0 (CH), 84.0 (CH), 83.9 (CH), 39.8 (CH₂), 27.2 (CH₃), 25.6 (CH₂).
25.4 (CH₃); ESI-MS 438.2 (M + H⁺); HRMS calcd for C₂₂H₂₄N₅O₅⁺ 438.1772, found 438.1768; Anal. Calcd for C₂₂H₂₃N₅O₅·0.5H₂O: C 59.19, H 5.42, N 15.69. Found C 59.07, H 5.25, N 15.32.

Cis-2-Ethoxycarbonylvinyltriphenylphosphonium bromide 205

Lithium bromide (2.50 g, 29 mmol) was added to a solution of ethyl propynoate (2.00 mL, 19.6 mmol) in acetic acid (20 mL) and stirring continued at 70 °C overnight. The reaction solution was diluted with H₂O (100 mL) and neutralized with K₂CO₃ and extracted with Et₂O (3 x 50 mL). The combined organic layer was washed with brine (50 mL), dried with NaSO₄, filtered and concentrated. The residue was dissolved in toluene (60 mL), and triphenylphosphine (6.10 g, 23.50 mmol) was added at room temperature and stirring continued for 2 days. The precipitate was filtered and the filtrate was concentrated. The residue was washed by ether to give phosphonium bromide 205 as white powder (5.90 g, 69%), m.p. 106-108 °C; ¹H NMR (400 MHz, CDCl₃) δH 8.29 (dd, J₁H-P= 19.6, J₂ = 16.8, 1H, CH=CHCOOEt), 7.96-7.90 (m, 3H, Ph-H), 7.85-7.80 (m, 6H, Ph-H), 7.75-7.69 (m, 6H, Ph-H), 6.57 (dd, J₁H-P = 20.4, J₂ = 16.8, 1H, CH=CHCOOEt), 4.36 (q, J = 7.2, 2H, CH₂CH₃).
N6-Benzoyl-2',3'-O-isopropylideneadenosyl-5'-(E)-propenal 206

Dichloroacetic acid (0.52 mL, 6.25 mmol) was added to a solution of protected adenosine 187 (5.14 g, 12.50 mmol) and DCC (7.70 g, 37.50 mmol) in dry DMSO (29 mL) at -5 °C under argon and stirring continued at room for 1.5 hr. The (triphenylphosphoranylidene)acetaldehyde (4.00 g, 13.0 mmol) was added and the resulting solution stirred at room temperature for 5 hr. The reaction solution was filtered and the filtrate diluted with EtOAc (60 mL) and washed with brine (2 x 30 mL) and dried with Na₂SO₄, filtered and concentrated. The residue was purified with column chromatography on silica gel using CHCl₃/MeOH (90/1, v/v) as eluent to give unsaturated aldehyde 206 as a yellow solid (5.98 g, 57%), m.p. 98-100 °C; [α]²³D 32.22 (c 1.17 in CHCl₃), ν max/cm⁻¹ 3408 (NH), 1696 (CHO), 1612 (C=C, aromatic), 1589 (C=C, aromatic), 1089 (C=O); ¹H NMR (400 MHz, CDCl₃) δ₁H 9.40 (d, J = 7.6, 1H, CHO), 8.69 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 8.00 (m, 2H, BzH), 7.56 (m, 1H, BzH), 7.46 (m, 2H, BzH), 6.81 (dd, J₁ = 15.8, J₂ = 5.4, 1H, 5'-H), 6.22 (d, J = 1.3, 1H, 1'-H), 6.07 (ddd, J₁ = 15.8, J₂ = 7.6, J₃ = 1.4, 2H, 6'-H), 5.54 (dd, J₁ = 6.3, J₂ = 1.3, 1H, 2'-H), 5.23 (dd, J₁ = 6.3, J₂ = 3.8, 1H, 3'-H), 4.91 (m, 1H, 4'-H), 1.62 (s, 3H, CH₃), 1.39 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 192.5 (C), 165.0 (C), 152.6 (CH), 151.5 (CH), 151.1 (C), 150.0 (C), 142.7 (CH), 133.9 (C), 132.8 (CH), 132.2 (CH), 128.8 (CH), 128.0 (CH), 123.8 (C), 115.0 (C), 90.6 (CH),
86.5 (CH), 84.4 (CH), 84.1 (CH), 27.1 (CH₃), 25.4 (CH₃); ESI-MS 436.2 (M + H⁺);
HRMS calcd for C₂₂H₂₄N₅O₅⁺ 436.1615, found 436.1612.

(2S)-2-Carbamoyl-pyrrolidine-1-carboxylic acid benzyl ester 208

1-Hydroxybenzotriazole (8.00 g, 52 mmol) and EDCI (6.70 g, 35 mmol) were added
to a solution of Cbz-L-proline (8.64 g, 35 mmol) in THF (200 mL) at room
temperature and stirring continued for 30 min. The aqueous ammonia (23 mL) was
added slowly and the reaction mixture was stirred for 24 hr. The saturated aqueous
NH₄Cl (200 mL) was added and extracted with EtOAc (3 x 100 mL). The combined
organic layers were dried with Na₂SO₄, filtered and concentrated, and the residue
purified with column chromatography on silica gel using EtOAc/MeOH (100/1, v/v)
as eluent to give amide 208 as colourless oil (6.48 g, 75%), [α]²⁹D -88.08 (c 1.06,
CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 7.37 (m, 5H, Ph-H), 6.74-5.84 (m, 2H,
NH₂), 5.19 (d, J = 12.6, 1H, PhCH₂Hb), 5.15 (d, J = 12.6, 1H, PhCH₂Hb), 4.37 (m,
1H, NH₂), 3.56-3.48 (m, 2H, NCH₂), 2.34-1.90 (m, 4H, CH₂CH₂CH₂CH₂COCH₂N)
¹³C NMR (100 MHz, CDCl₃, as a mixture of rotamers) δC 175.3/174.4 (C), 156.1/155.1 (C),
136.4 (C), 128.6 (CH x 2), 128.2 (CH), 127.9 (CH x 2), 67.4 (CH₂), 60.7/60.2 (CH),
47.5/47.1 (CH₃), 31.1/28.5 (CH₂), 24.6/23.7 (CH₂).
(2S)-2-Cyano-pyrrolidine-1-carboxylic acid benzyl ester 209

\[
\begin{align*}
&\text{p-Toluenesulfonyl chloride (9.20 g, 48 mmol) was added to a solution of amide 208} \\
&(6.00 g, 24 mmol) and pyridine (9.78 mL, 120 mmol) in CH}_2\text{Cl}_2 (60 mL). \\
&\text{The reaction mixture was stirred at room temperature for 72 hr, and then saturated} \\
&\text{aqueous NH}_4\text{Cl (45 mL) was added. The aqueous solution was extracted with EtOAc} \\
&(3 x 100 mL) and combined organic layers dried with Na}_2\text{SO}_4, \text{filtered and} \\
&\text{concentrated. The residue was purified with column chromatography on silica gel} \\
&\text{using EtOAc/Petroleum ether (1/1, v/v) as eluent to give nitrile 209 as yellow oil} \\
&(4.00 g, 72%), [\alpha]^{21}_D -74.27 (c 2.25 in CHCl}_3); \\
&^1\text{H NMR (400 MHz, CDCl}_3) \delta \text{H} \\
&(7.45-7.33 (m, 5H, Ph-H), 5.26-5.16 (m, 2H, PhCH}_2), 4.65-4.57 (m, 1H, CHCN), \\
&3.66-3.58 (m, 1H, NCH}_2H}_2b), 3.50-3.40 (m, 1H, NCH}_2H}_2b), 2.29-2.07 (m, 4H, \\
&CH}_2CH}_2CH}_2); \\
&^{13}\text{C NMR (100 MHz, CDCl}_3, \text{as a mixture of rotamers}) \delta \text{C} 154.3/153.7 \\
&(C), 136.1/136.0 (C), 128.6 (CH x 2), 128.3 (CH), 128.2 (CH x 2), 118.9/118.8 (C), \\
&67.8/67.7 (CH}_2), 47.6/47.0 (CH), 46.4/46.0 (CH}_2), 31.8/30.8 (CH}_2), 24.7/23.8 (CH}_2). \\
\end{align*}
\]

\[\text{N6-Benzoyl-5'-deoxy-2',3'-O-isopropylidene-5'-[6-(3-carbethoxy-1,4,5,6-tetra-} \\
&\text{hydropyridazine)]adenosine 224}
\]

Pd/C (10%, 53 mg) was added to a solution of crude Cbz protected dihydro-
pyridazine 170b (106 mg) in THF (5 mL) and stirred under H₂ (balloon) over 2 nights. The reaction solution was filtered and the filtrate concentrated, the residue purified with column chromatography on silica gel using CH₂Cl₂/MeOH (70/1, v/v) as eluent to give tetrahydropyridazine 224 as colourless oil (20 mg, 28%), ¹H NMR (400 MHz, CDCl₃) δH 9.17 (s, 1H, NHBz), 8.82 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.06-8.04 (m, 2H, BzH), 7.65-7.52 (m, 3H, BzH), 6.60 (s, 1H, NHCH), 6.09 (d, J = 2.4, 1H, 1'-H), 5.50 (dd, J₁ = 6.6, J₂ = 2.4, 1H, 2'-H), 5.00 (dd, J₁ = 6.6, J₂ = 4.8, 1H, 3'-H), 4.33-4.25 (m, 3H, 4'-H and CH₂CH₃), 3.37 (m, 1H, 6'-H), 2.46 (t, J = 6.8, 2H, 8'-CH₂), 1.99-1.87 (m, 3H, 5'-CH₂ and 7'-CH₂H₆), 1.64 (s, 3H, CH₃), 1.60 (m, 1H, 7'-CH₂H₆), 1.41 (m, 3H, CH₃), 1.34 (t, J = 7.2, 3H, CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δC 165.7 (C), 165.3 (C), 153.5 (CH), 151.8 (C), 150.6 (C), 143.0 (CH), 134.2 (C), 133.5 (C), 133.2 (CH), 129.6 (CH), 128.6 (CH), 124.4 (C), 116.0 (C), 90.7 (CH), 84.8 (CH), 84.6 (CH), 84.5 (CH), 61.5 (CH₂), 48.8 (CH), 37.9 (CH₂), 27.9 (CH₃), 26.1 (CH₃), 24.2 (CH₂), 20.9 (CH₂), 15.1 (CH₃); ESI-MS 550.2 (M + H⁺) [Found 550.2407 C₂₇H₃₂N₇O₆ requires 550.2409].

N-(Benzyloxycarbonyl)-L-serine β-lactone 232a

DEAD (0.66 mL, 4.18 mmol) was added dropwise to a solution of triphenylphosphine (1.1 g, 4.18 mmol) in dry THF (10 mL) at -78 °C under argon. After 10 min a solution of Cbz-L-serine (1.0 g, 4.18 mmol) in dry THF (30 mL) was added dropwise over 10 min at -78 °C, and stirring continued for 20 min and at room
temperature for 2.5 hr. The reaction solution was concentrated and the residue purified with column chromatography on silica gel using EtOAc/Petroleum ether (1/2, v/v) as eluent to give β-lactone 232a as a white solid (303 mg, 33%), m.p. 133-134 °C (lit., 133-134 °C); \([\alpha]_{D}^{24} -4.47 (c 1.3 \text{ in CHCl}_3)\); \(^1\)H NMR (400 MHz, CD\(_3\)CN) \(\delta_{H} 7.40 (m, 5 \text{ H, Ph-H}), 6.41 (s, 1 \text{ H, NH}), 5.15 (s, 2 \text{ H, PhCH}_2), 5.10 (m, 1 \text{ H, CHCH}_2), 4.43 (d, J = 6.4, 2 \text{ H, CHCH}_2)\); \(^{13}\)C NMR (100 MHz, CD\(_3\)CN) \(\delta_{C} 169.6 (\text{ C}), 155.2 (\text{ C}), 136.3 (\text{ C}), 128.2 (\text{ CH}), 127.9 (\text{ CH}), 127.7 (\text{ CH}), 66.5 (\text{ CH}_2), 65.5 (\text{ CH}_2), 59.1 (\text{ CH})\).

\(N-(\text{tert-Butoxycarbonyl})-\text{L-serine β-lactone 232b}\)\(^{126}\)

![BocHN](image)

β-Lactone 232b was prepared by the same procedure of β-lactone 232a as a white solid (290 mg, 32%), m.p. 119-120 °C (dec) (lit., 119.5-120.5 °C (dec)); \([\alpha]_{D}^{24} -5.02 (c 0.69 \text{ in CHCl}_3)\); \(^1\)H NMR (400 MHz, CD\(_3\)CN) \(\delta_{H} 6.05 (s, 1 \text{ H, NH}), 5.02 \text{ (app. q, } J = 5.9, 1 \text{ H, CHCH}_2), 4.39 (d, } J = 5.9, 2 \text{ H, CHCH}_2), 1.45 (s, 9 \text{ H, C(CH}_3)_3)\); \(^{13}\)C NMR (100 MHz, CD\(_3\)CN) \(\delta_{C} 170.3 (\text{ C}), 154.9 (\text{ C}), 80.2 (\text{ C}), 65.9 (\text{ CH}_2), 59.2 (\text{ CH}), 27.4 (\text{ CH}_3)\).

\((S)-\text{Ethyl-N-(benzyloxycarbonyl)-2-amino-3-(diethylphosphono)propanoate 233a}\)\(^{127}\)

![Cbz](image)
Triethyl phosphite (1.7 mL, 9.6 mmol) and β-lactone 232a (212 mg, 0.96 mmol) were combined and heated at 70 °C under argon for 2 days. The reaction solution was concentrated and the residue was purified with column chromatography on silica gel using CH₂Cl₂/CH₃OH (70/1, v/v) as eluent to give phosphonopropanoates 233a as colourless oil (300 mg, 81%), [α]²⁴D 8.43 (c 0.45 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 7.35 (m, 5H, Ph-H), 5.96 (d, J = 7.6, 1H, NH), 5.15 (s, 2H, PhCH₂), 4.62 (m, CH₂CH₂), 4.26-4.06 (m, 6H, CH₂CH₃ x 3), 2.38 (m, 2H, CHCH₂), 1.35-1.29 (m, 9H, CH₂CH₃ x 3); ¹³C NMR (100 MHz, CDCl₃) δC 170.6 (d, Jᵖ⁻ᶜ = 8.7, C=O), 155.8 (C=O), 136.3 (C), 128.5 (CH), 128.1 (CH), 128.1 (CH), 67.0 (CH₂), 62.2 (d, Jᶜ⁻ᵖ = 7.5, CH₂), 62.0 (d, Jᶜ⁻ᵖ = 13.1, CH₂), 49.6 (d, Jᶜ⁻ᵖ = 6.5, CH), 28.5 (d, Jᶜ⁻ᵖ = 141.3, CH₂), 16.4 (CH₃), 16.3 (CH₃), 14.1 (CH₃); ³¹P NMR (162 MHz, CDCl₃) δP 26.60.

(S)-Ethyl-N-(tert-butoxycarbonyl)-2-amino-3-(diethylphosphono)propanoate

Phosphonopropanoates 233b was prepared by the same procedure of phosphonopropanoates 233a as colourless oil (210 mg, 62%), [α]²⁴D 8.35 (c 1.02 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δH 5.69 (d, J = 7.6, 1H, NH), 4.54 (m, 1H, CHCH₂), 4.25-4.11 (m, 6H, CH₂CH₃ x 3), 2.36 (m, 2H, CHCH₂), 1.47 (s, 9H, C(CH₃)₃), 1.37-1.30 (m, 9H, CH₂CH₃ x 3); ¹³C NMR (100 MHz, CDCl₃) δC 171.0 (d, Jᶜ⁻ᵖ = 10.0, C=O), 155.2 (C=O), 80.0 (C), 62.1 (d, Jᶜ⁻ᵖ = 5.0, CH₂), 62.0 (d, Jᶜ⁻ᵖ =
6.0, (CH₂), 61.7 (CH₂), 49.2 (d, \( J^{C_6} = 6.0, \text{CH} \)), 28.5 (d, \( J^{C_6} = 142.0, \text{CH₂} \)), 28.3 (CH₃), 16.4 (d, \( J^{C_6} = 2.0, \text{CH₃} \)), 16.3 (d, \( J^{C_6} = 2.0, \text{CH₃} \)), 14.1 (CH₃); \(^{31}\text{P} \text{NMR} (162 \text{MHz, CDCl}_3) \delta_p 26.90.

\[ S-1-\text{Methyl-2-(tert-butyloxycarbonylamino)-3-iodopropylamino} 237\]^{129}

K₂CO₃ (0.74 g, 5.36 mmol) was added to a solution of Boc-L-serine (1.0 g, 4.87 mmol) in dry DMF (5 mL) at 0 °C and stirring continued for 10 min. Methyl iodide (0.61 mL, 9.74 mmol) was added to this mixture at 0 °C and stirring continued for 30 min and then at room temperature for 2 hr. The reaction solution was filtered and the filtrate diluted with EtOAc (50 mL) and washed with brine (3 x 20 mL). The organic layer was dried with Na₂SO₄, filtered and concentrated. Iodine (2.47 g, 9.74 mmol) was added to a solution of triphenylphosphine (2.55 g, 9.74 mmol) in dry CH₂Cl₂ (10 mL) at 0°C and stirring continued for 20 min. A mixed solution of the crude serine and imidazole (0.66 g, 9.74 mmol) was added to the iodine solution at 0 °C and stirring continued for 3 hr. The reaction solution was diluted with Et₂O (20 mL) and washed with saturated aqueous Na₂S₂O₃ (10 mL) and H₂O (10 mL), dried with Na₂SO₄, filtered and concentrated. The residue was purified with column chromatography on silica gel using EtOAc/Petroleum ether (1/10, v/v) as eluent to give iodide 237 (439 mg, 28%) as yellow oil, \([\alpha]^{21}_D 41.28 (c 1.02 \text{ in CHCl}_3)\); \(^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta_H 5.38 (d, J = 6.4, 1H, NHBoc), 4.55 (m, 1H, CHCH₂), 3.83 (s, 3H, CH₃), 3.60 (m, 2H, CHCH₂), 1.48 (s, 9H, CH₃); \(^{13}\text{C} \text{NMR} (100 \text{ MHz,}...
CDC13) δC 170.1 (C), 154.9 (C), 80.5 (C), 53.7 (CH), 53.0 (CH₃), 28.3 (CH₃), 7.9 (CH₂).

**Biological assays**

Determination of IC₅₀ values were carried out by Dr Richard Parry at the Department of Pharmacy and Pharmacology, University of Bath.

*In vitro* methylation reactions were carried out as detailed by Cheng and co-workers. All methylation reactions were carried out in the presence of 0.5 μM S-adenosyl-L-[S³H₃C] methionine (85 Ci/mmol from a 12.6 μM stock solution; Amersham Biosciences) and a final volume of 30 μL of PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4). The reactions contained 0.1-0.2 μg of recombinant PRMT1 or SET7 and 0.5-1.0 μg of substrate (recombinant Sam68 and histone H3 (Sigma Aldrich) respectively) with various concentrations of inhibitor (e.g. 74a). The reactions were incubated at 30 °C for 90 min and terminated by addition of 20 μL SDS-PAGE sample buffer and boiling for 5 min. Proteins were separated by SDS/PAGE, transferred to nitrocellulose membrane, and stained by Ponceau S. The visualised bands of substrate protein were excised and counted by liquid scintillation.

**Computational docking**

Computational docking was carried out by Dr Hao Wang at the School of Pharmacy, University of Nottingham.
The structures of the bisubstrate inhibitors, 74(a-c), 77 and 78 were built by commercially available software Insight II created by Accelrys Software Inc. The docking software Gold 3.2 (Genetic Optimization for Ligand Docking) was used to dock all bisubstrate inhibitors into the binding site of PRMT1. During the Gold docking process, the crystal structure of PRMT1 in complex with the cofactor product SAH (PDB 1OR8) was taken as a starting point for molecular docking. The atoms of SAH were used as a reference to indicate the binding site (all protein atoms within 5 Å of SAH) for the bisubstrate inhibitors, and then the coordinates for SAH could be discarded during the docking process. The coordinates that remained were then taken as the input protein receptor for the docking along with the definition of the desired binding site.
References


(21) Osborne, T. C.; Obianyo, O.; Zhang, X.; Cheng, X.; Thompson, P. R. Biochemistry 2007, 46, 13370-13381.


(29) Friesen, W. J.; Paushkin, S.; Wyce, A.; Massenet, S.; Pesiridis, G. S.; Van Duyne,


(42) Spannhoff, A.; Heinke, R.; Bauer, I.; Trojer, P.; Metzger, E.; Gust, R.; Schule, R.;


(50) Deshpande, P. B.; Senthilkumar, U. P.; Padmanabhan, R. US 20020188116 A1


(58) Pugh, C. S. G.; Borchardt, R. T. Biochemistry 1982, 21, 1535-1541.


(80) Wahhab, A.; Besterman, J. M. WO2006078752


(84) Box, D.; Colclough, D. WO0250021

(85) Boyle, P. H.; Davis, A. P.; Dempsey, K. J.; Hosken, G. D.

(87) Shah, V. J.; Kenyon, G. L. WO9604000


(97) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem. Int. Ed. 2002, 41, 2596-2599.


(130) http://accelrys.com/ 24/04/2009


