Synthesis of Protein Arginine N-Methyltransferase 6 Inhibitors

by

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Abstract

Protein arginine N-methyltransferases (PRMTs) are pertinent targets for drug discovery as their dysfunction is associated with a number of diseases such as cancers, cardiovascular diseases and viral pathogenesis. The precise role of PRMTs in the initiation, development, or progression of diseases is not known yet.

Due to association of PRMT1 and 4 with transcriptional activation, the main focus of inhibitor discovery has been on these two enzymes. On the other hand, the goal of this study is to find a PRMT6 specific inhibitor. PRMT6 methylates DNA polymerase β, histones H3 and H4 and HIV proteins: Rev and Tat.

PRMT6 uses S-adenosyl-L-methionine (AdoMet) as the “methyl group” source. AdoMet fits into a distinct conserved binding site in the enzyme, which is located adjacent to the protein substrate/catalytic site such that its S⁺-Me motif is correctly positioned with respect to the substrate arginine nitrogen atom that undergoes methylation.

Based on crystallography data for PRMT1, the purine C8 center in AdoMet is in close proximity to the methionine sulfur atom (M166 in PRMT6). As shown by Frankel et al. (Faculty of Pharmaceutical Sciences, UBC), the M166C PRMT6 mutant displays activity. Based upon this observation, we hypothesize that Ado-Met analogues with reactive substituents (e.g., CHO) at C8 position of adenine ring will form a covalent bond with the proximal Cys SH group in M166C PRMT6. This validates our further hypothesize that in appropriately designed analogues, it will be possible to subsequently detach the sugar and amino acid components of Ado-Met to leave the adenine ring component alone bound to the enzyme. This provides a unique opportunity to explore the “fragment based approach in drug discovery” to design PRMT6 specific inhibitors.
Table of contents

Abstract ........................................................................................................................................... ii

Table of contents ......................................................................................................................... iii

List of tables .................................................................................................................................. vi

List of figures .................................................................................................................................. vii

List of schemes .............................................................................................................................. xi

List of abbreviations ...................................................................................................................... xii

Acknowledgements ..................................................................................................................... xvi

Dedication ...................................................................................................................................... xvii

1 Introduction ................................................................................................................................ 1

1.1 An overview for protein arginine methyltransferases (PRMTs) ............................................. 1

1.2 The common structure of PRMTs .......................................................................................... 3

1.3 Mechanism of methylation .................................................................................................... 4

1.4 PRMTs and diseases ............................................................................................................... 6

  1.4.1 Cancer ................................................................................................................................ 6

  1.4.2 Cardiovascular and chronic kidney diseases ........................................................................ 7

  1.4.3 Viral pathogenesis .............................................................................................................. 8

1.5 PRMT inhibitors ..................................................................................................................... 9

  1.5.1 PRMT inhibition: targeting arginine binding pocket .......................................................... 9

  1.5.2 PRMT inhibition: targeting the arginine and the AdoMet binding pockets ...................... 12

  1.5.3 PRMT inhibition: library screening .................................................................................... 13

1.6 Fragment approach: achieving specificity within the PRMT family ..................................... 17
1.7 Identification of a potential linker site in PRMT6 ................................................................. 19
1.8 Research hypothesis: .............................................................................................................. 20

2 Synthetic strategy and inherent problems for the synthesis of the AdoMet Analogue 1A ............................................................................................................................................. 23

2.1 Synthesis of the AdoMet Analogue 1A ...................................................................................... 27
  2.1.1 Approach 1: synthesis of the AdoMet Analogue 1A via glycosylation of C5’ homoserine substituted D-ribose .................................................................................................................. 28
  2.1.2 Approach 2: synthesis of the AdoMet Analogue 1A via selective ester to ether reduction of C5’ ester substituted adenosine derivatives ........................................................................................................... 30
  2.1.3 Approach 3: synthesis of the AdoMet Analogue 1A via Williamson etherification of C8 CHO substituted adenosine ...................................................................................................................... 33

3 Switching from AdoMet Analogue 1A to AdoMet Analogue 1B ........................................... 36

4 Strategies for the synthesis of the AdoMet Analogue 1B....................................................... 37
  4.1 Approach 1: synthesis of the AdoMet Analogue 1B via addition of the disodium salt of L-homocysteine to the C5’ activated C8-bromoadenosine ......................................................................................................................... 39
    4.1.1 Step 1: C8 bromination of adenosine .................................................................................. 40
    4.1.2 Step 2: C5’ activation of adenosine .................................................................................. 40
    4.1.2.1 C5’ chlorination of C8-bromoadenosine and its addition to L-cysteine ...................... 41
    4.1.2.2 C5’ iodination of C8-bromoadenosine and its addition to L-cysteine ...................... 46
    4.1.2.3 C5’ triflation of C8-bromoadenosine and its addition to L-cysteine ...................... 48
  4.2 Approach 2: synthesis of the AdoMet Analogue 1B from S-adenosylhomocysteine... 51
    4.2.1 Step 1: L-homocysteine synthesis and its addition to 39 ............................................. 52
    4.2.2 Step 2: C8 bromination of S-adenosylhomocysteine, 32 .......................................... 54

5 Conclusion .................................................................................................................................. 56
6 Experimental section ........................................................................................................................................... 58

6.1 Materials and methods: ........................................................................................................................................ 58

6.2 Experiments ......................................................................................................................................................... 59

6.3 NMR data ............................................................................................................................................................ 78

References .............................................................................................................................................................. 104
List of tables

Table 1. The percent amino acid identities between the catalytic core region of PRMT6 and PRMT1-9 are listed in this table. BLAST 2 sequence program was used to calculate the sequence identity for these PRMTs.
List of figures

Figure 1. Both AdoMet (cofactor) and an arginine rich protein (arginine is only shown here) bind to the active site of PRMTs. PRMTs catalyze transfer of a methyl group from AdoMet to the arginine to give monomethylated arginine (MMA). Type I PRMTs transfer a second methyl group from another AdoMet to the same nitrogen to give an asymmetric dimethylated arginine (aDMA) while type II PRMTs transfer the second methyl group to another guanidine nitrogen to give a symmetric dimethylated arginine (sDMA). ................................................................. 2

Figure 2. A proposed structure of the active site showing all the PRMT6 amino acid residues that are involved in the catalysis.............................................................................................................. 5

Figure 3. PRMT inhibitors................................................................................................................................. 10

Figure 4. Structure of C21........................................................................................................................................ 11

Figure 5. Fragment based approach in a general form: (a) enzyme has two binding pockets. (b) The first fragment (circle) forms a covalent bond with the active site of the enzyme and occupies one of the binding pockets. (C) The enzyme in complex with the first fragment is incubated with a library of second fragments one of which is complementary to the second binding pocket of the enzyme. (d) The first and second fragments react together to form a 1,4-disubstituted 1,2,3-triazole ring (shown in red)................................................................................................... 17

Figure 6. In click chemistry, one fragment has a terminal alkyne while the second fragment has an azido function. Via a 1,3 dipolar cycloaddition, the first fragment (circle) and second fragment (square) is connected by 1,4-trisubstituted 1,2,3-triazole ring. .............................................................................................................. 18

Figure 7. The crystal structure of the AdoMet binding site for PRMT1: the corresponding amino acid residues for PRMT6 are in parenthesis. The adenine C8 center of AdoHcy is in close proximity to the methionine sulfur atom of the Met 166. .......................................................................................................................... 19

Figure 8. The hypothesized reaction of the AdoMet analogue 1A and 1B with the M166C PRMT6 mutant............ 20

Figure 9. The hypothesized reaction of the AdoMet analogue 1C with the M166C PRMT6 mutant: Using the AdoMet analogue 1C, it would be possible to shed the sugar and the amino acid components to leave the adenine ring component alone bound in the binding pocket of the enzyme. This adenine ring will be used as the starting point/template for the fragment based approach for the synthesis of the PRMT6 specific inhibitors. ...................... 21

Figure 10. The structure of the AdoMet Analogue 1A, 1B, 2A and 2B ........................................................................ 22

Figure 11. Structural differences between the AdoMet and the AdoMet Analogue 1A.......................... 23

Figure 12. Williamson ether synthesis at the C5’ position of adenosine: a) Reaction of adenosine with the halogenated homoserine motif. Schneller et al. has already reported the synthesis of this halogenated amino acid. b) Reaction of the C5’ halogenated adenosine and the Schöllkopf’s bis-lactim ether form of homoserine. (R= Hydroxyl protecting group) .......................................................................................... 24

Figure 13. Multialkylation problem: the alkylation takes place at the 5’-OH position of the adenosine as well as at the N1, N3, N6 or N7 position of the adenine ring. (R= Hydroxyl protecting group) ................................................................. 25
Figure 14. Intramolecular reaction: The halogenated adenosine undergoes intramolecular reaction under the basic condition of Williamson reaction. (R= Hydroxyl protecting group)

Figure 15. Two model reactions for reduction of ester to ether: (a) 27 undergoes complete reductive deoxygenation in 90 minutes. (b) Reduction of 27 is impaired in the presence of amide, 29.

Figure 16. The aldehyde function at the C8 position of the adenosine withdraw electrons away from the N6 position so under Williamson etherification reaction, Schöllkopf's bis-lactim homoserine, 12, can be added to the C5' position of the C8 forylated adenosine without any intramolecular reaction.

Figure 17. Structural differences between the AdoMet and the AdoMet Analogue 1B.

Figure 18. There are 3 steps for the synthesis of the AdoMet Analogue 1B: i) C8 bromination of adenosine; ii) C5' addition of homocysteine to adenosine; iii) reductive carbonylation of the C8 position.

Figure 19. Synthesis of S-adenosylhomocysteine, 32, from the coupling of 2', 3'-O-isopropylidene-5'-tosyladenosine, 30, with disodium salt of homocysteine, 31.

Figure 20. Treatment of 33 with thionyl chloride and pyridine in anhydrous DMF give 34b instead of 34a due to the halogen exchange at the C8 position of 33.

Figure 21. Treatment of 34b with 3.5 equivalents of the disodium salt of L-cysteine gives 37 instead of 36. No nucleophilic substitution (SN2) reaction at the C5' position of 37 was observed even after stirring the reaction for another 5 days at the room temperature.

Figure 22. The nucleophilic substitution (SN2) reaction of the disodium salt of L-cysteine with 39 took 24 h at room temperature to give 40.

Figure 23. The nucleophilic aromatic substitution (SNAr) reaction at the C8 position of 41 took an hour. This reaction was much faster than the SNAr reaction at the C8 position of 34b and the nucleophilic substitution (SN2) reaction at the C5' position of 39.

Figure 24. The nucleophilic aromatic substitution (SNAr) reactions at the C8 position of 33 and 34b are much faster than the nucleophilic substitution (SN2) reaction at the C5' position of 39.

Figure 25. The nucleophilic substitution (SN2) reaction at the C5' position of 43 is much faster than 39. Neither nucleophilic aromatic substitution (SNAr) nor nucleophilic substitution (SN2) was observed for 44.

Figure 26. The C8-bromoadenosine, 33, has syn conformation while 47 has anti conformation.

Figure 27. Synthesis of racemized Homocysteine: a) Reduction of L-homocystine with sodium in liquid ammonia; b) Opening the L-homocysteine thiolactone ring under alkaline condition; C) Refluxing L-methionine in sulfuric acid or hydriodic acid.

Figure 28. Adamczyk, M. et. al. report a 4-step procedure for asymmetric synthesis of L-homocysteine using Schöllkopf reagent: a) i) n-BuLi, THF, DMEU, - 78 °C, 30 mins; ii) 2-bromoethyltriphenylmethyl sulfide, - 78 °C, 20 h b) i) 0.25 M HCl, ii) 0.25 M LiOH; c) Na/NH3; d) Air e) HI.
Figure 29. L-homocysteine was synthesized by modifying the procedure by Shiraiwa, T. et. al.\textsuperscript{76-78}; a) Dichloroacetic acid, concentrated hydrochloric acid, reflux, overnight; b) Ethanolic hydroxylamine hydrochloride, triethylamine, reflux, 1h.

Figure 30. The mechanism for rapid oxidation of sulfides with bromine in aqueous media

Figure 31. $^1$H NMR spectra for compound 14

Figure 32. $^1$H NMR spectra for compound 15

Figure 33. $^1$H NMR spectra for compound 16

Figure 34. $^1$H NMR spectra for compound 17

Figure 35. $^1$H NMR spectra for compound 18

Figure 36. $^1$H NMR spectra for compound 20a

Figure 37. $^1$H NMR spectra for compound 20b

Figure 38. $^1$H NMR spectra for compound 20c

Figure 39. $^1$H NMR spectra for compound 20d

Figure 40. $^1$H NMR spectra for compound 20e

Figure 41. $^1$H NMR spectra for compound 21a

Figure 42. $^1$H NMR spectra for compound 21b

Figure 43. $^1$H NMR spectra for compound 23a

Figure 44. $^1$H NMR spectra for compound 32

Figure 45. $^1$H NMR spectra for compound 33

Figure 46. $^1$H NMR spectra for compound 34b

Figure 47. $^1$H NMR spectra for compound 37

Figure 48. $^1$H NMR spectra for compound 39

Figure 49. $^1$H NMR spectra for compound 40
Figure 50. $^1$H NMR spectra for compound 41........................................................................................................ 97

Figure 51. $^1$H NMR spectra for compound 43........................................................................................................ 98

Figure 52. $^1$H NMR spectra for compound 44........................................................................................................ 99

Figure 53. $^1$H NMR spectra for compound 46........................................................................................................ 100

Figure 54. $^1$H NMR spectra for compound 47........................................................................................................ 101

Figure 55. $^1$H NMR spectra for compound 49........................................................................................................ 102

Figure 56. $^1$H NMR spectra for compound 50........................................................................................................ 103
List of schemes

Reaction Scheme 1. Approach 1: a) COMe₂, MeOH, HCl; b) NaH, allyl bromide, THF; c) RuCl₃, NaIO₄, CH₃CN: H₂O (6:1); d) DBU, DCM......................................................................................................................... 29

Reaction Scheme 2. Approach 2: a) TBSCl, Imidazole, DMF; b) TFA-H₂O-THF (1:1:4); C) DMAP, propanoic acid, DCC, DMF; d) BzCl, pyridine........................................................................................................................................ 30

Reaction Scheme 3. Approach 3: a) TFA-H₂O-THF (1:1:4); b) BzCl, pyridine; C)LDA, THF, DMF/methyl formate ......................................................................................................................................................... 34

Reaction Scheme 4. Nucleophilic substitution reaction of the disodium salt of L-homocysteine to the C5' activated adenosine: a) Br₂, sodium acetate buffer (pH 4); b) SOCl₂, pyridine, CH₃CN (to get 34b from 33); PPh₃, pyridine (to get 34c from 33) ............................................................... 39

Reaction Scheme 5. A synthetic scheme showing the steps for the synthesis of the AdoMet Analogue 1B by reacting the C5' triflated adenosine, 48, with the disodium salt of L-cysteine. a) TBDMSCl, Imidazole, DMF, 24 h, r.t.; b) TFA/H₂O/THF (1:1:4), 0 °C, 4h. ........................................................................................................ 49

Reaction Scheme 6. Second approach: synthesis of AdoMet Analogue 1B from S-adenosylhomocysteine. a) NaH, DMF......................................................................................................................................................... 51
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>0.1N methanolic HCl</td>
<td>0.1 normal methanolic hydrogen chloride</td>
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<tr>
<td>1H NMR</td>
<td>proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>AAI</td>
<td>5’-(diaminobutyric acid)-N-iodoethyl-5’-deoxyadenosine ammonium hydrochloride</td>
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<tr>
<td>aDMA</td>
<td>asymmetric dimethylated arginine</td>
</tr>
<tr>
<td>AdoHcy</td>
<td>S-adenosylhomocysteine</td>
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<td>human protein arginine methyltransferase 1</td>
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<tr>
<td>LTR</td>
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<td>N-TBS</td>
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<tr>
<td>sDMA</td>
<td>symmetric dimethylated arginine</td>
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<td>small interfering ribonucleic acid</td>
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<td>omega nitrogen 2 of guanidine</td>
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Acknowledgements

Foremost, I would like to express my sincere gratitude to my supervisor, Professor David Grierson, for his guidance, patience and encouragement. It was a great honor to work under supervision of a great mentor who is truly passionate about science.

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Last but not least, my deepest gratitude goes to my family for their unflagging love and support. Thank you for always believing in me.
To my Family

Rafat, Javad, Mojtaba and Mostafa Zamiri
1 Introduction

1.1 An overview for protein arginine methyltransferases (PRMTs)

Protein arginine methyltransferases (PRMTs) are a family of enzymes that catalyze the post-translational modification of proteins. Having a common cofactor, S-adenosylmethionine (AdoMet), as their methyl source, they transfer two methyl groups from AdoMet to the arginine residue of the substrate in a consecutive manner.\(^1\) Though the charge of the arginine remains unchanged, these modifications cause steric hindrance and hydrogen bonding disruption.\(^2,3\) The consequence is a change in how the methylated protein interacts with other intracellular molecules.

Currently, 10 different mammalian PRMTs have been discovered.\(^1\) They are categorized into 2 different types: Type I and Type II.\(^1,4,6\) As shown in Figure 1, both types first synthesize monomethylated arginine (MMA) by transferring one methyl group from AdoMet to one of the guanidine nitrogen of arginine. In Type I PRMTs, the second methyl group is transferred to the same guanidine nitrogen to make an asymmetric dimethylated arginine (aDMA). In Type II PRMTs, the second methyl group is transferred to the other guanidine terminal nitrogen to produce a symmetric dimethylated arginine (sDMA).
Figure 1. Both AdoMet (cofactor) and an arginine rich protein (arginine is only shown here) bind to the active site of PRMTs. PRMTs catalyze transfer of a methyl group from AdoMet to the arginine to give monomethylated arginine (MMA). Type I PRMTs transfer a second methyl group from another AdoMet to the same nitrogen to give an asymmetric dimethylated arginine (aDMA) while type II PRMTs transfer the second methyl group to another guanidine nitrogen to give a symmetric dimethylated arginine (sDMA).
1.2 The common structure of PRMTs

The methyltransferase domain (active site) in PRMTs, contains the AdoMet binding site and the catalytic site. Considering that the mammalian PRMTs use AdoMet as the methyl source, their methyltransferase domains contain a number of conserved amino acid residues. In fact, based on the three available crystal structures (rat PRMT, rat PRMT3 catalytic core, and yeast RMT1/HMT1) it is known that PRMTs display a common architecture, which can be divided into three domains: i) the methyltransferase domain (including the AdoMet binding site), ii) the β-barrel domain, and iii) the dimerization arm domain. Further, a comparison of the catalytic core region of PRMT6 to the nine other mammalian PRMTs shows that there is a 30 to 40% sequence identity (Table 1). To emphasize the similarity between the PRMTs, a superposition of the conserved core structure of PRMT1 and PRMT3 shows that there is less than 1 Å of root-mean-square deviation between them.

<table>
<thead>
<tr>
<th></th>
<th>PRMT1</th>
<th>PRMT2</th>
<th>PRMT3</th>
<th>PRMT4</th>
<th>PRMT5</th>
<th>PRMT6</th>
<th>PRMT7</th>
<th>PRMT8</th>
<th>PRMT9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRMT6</td>
<td>33</td>
<td>38</td>
<td>35</td>
<td>38</td>
<td>29</td>
<td>100</td>
<td>26</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 1. The percent amino acid identities between the catalytic core region of PRMT6 and PRMT1-9 are listed in this table. BLAST 2 sequence program was used to calculate the sequence identity for these PRMTs.
1.3 Mechanism of methylation

The catalytic reaction of PRMT3 has been shown to involve a classical nucleophilic substitution (SN2) mechanism.\textsuperscript{7,8} Using the available crystal structure of PRMT1 and PRMT3, the conserved catalytic core for PRMT6 is modeled and shown in Figure 2. By analogy to the reported mechanism for PRMT3, the following mechanism for Arg methylation is proposed for PRMT6. The interactions of substrate arginine with Glu164 in the catalytic active site redistribute the positive charge of guanidine on one of its $\omega$-nitrogen ($N_{\eta 1}$). This makes the other $\omega$-nitrogen of guanidine ($N_{\eta 2}$) more nucleophilic toward the methyl group of an AdoMet. The negative charge on the side chain of Glu164 stabilizes the positive charge of $N_{\eta 1}$. Arg166 neutralizes the negative charge on Glu155 so that its carboxylate oxygen is accessible for hydrogen bonding with $N_{\eta 2}$. This hydrogen bonding aligns the $N_{\eta 2}$, methyl and sulfur atom of the AdoMet together such that $N_{\eta 2}$ can engage a nucleophilic attack on the methyl group of AdoMet. Through hydrogen bonding with Asp63, His317 is at the right position to accept the proton from methylated $N_{\eta 2}$ and transfer it to Asp63 and eventually to solvent.
Figure 2. A proposed structure of the active site showing all the PRMT6 amino acid residues that are involved in the catalysis

PRMT methylations are implicated in a variety of intracellular activities such as: RNA processing and metabolism, transcriptional regulation and co-activation, signal transduction, DNA and protein repair, protein protein interaction, stress response, aging, T-cell activation, nuclear transport, and neuronal differentiation.\textsuperscript{1-6}
1.4 PRMTs and diseases

PRMTs are a pertinent target for drug discovery as their dysfunction is associated with a number of diseases. However, their precise role in the initiation, development, or progression of diseases is not known. The following gives a few examples linking PRMTs to cancers, cardiovascular and chronic kidney diseases and viral pathogenesis.\(^9\)

1.4.1 Cancer

PRMT1 and PRMT4 (also known as CARM1) are involved in the activation of estrogen and androgen receptors and therefore they may provide a new way for treatment of hormone dependent cancers.\(^1,9,10\) For example, prostate and breast cancers are usually hormone dependent and studies have shown that knockout or silencing of PRMT4 disrupts the growth of breast cancer tumors. Moreover, PRMT4 modulation effects the transcription of prostate cancer cells.\(^9,11\) It also has been shown that elevated PRMT4 is linked with development of prostate carcinoma and the progression of androgen independent prostate cancer.\(^9,11-13\)
1.4.2 **Cardiovascular and chronic kidney diseases**

Nitric oxide synthase (NOS) synthesizes nitric oxide which is essential in cardiovascular system.\(^9\) MMA and aDMA are the negative feedback inhibitors of NOS\(^{14}\) and dimethylarginine dimethylaminohydrolase regulates the concentration of free MMA and aDMA.\(^9\) Over expression or malfunction of Type I PRMTs can result in the build up of aDMA, which inhibits NOS and cause cardiovascular problems. At the same time, studies have shown that high concentration of aDMA cause endothelial dysfunction and generation of vascular and organ diseases such as chronic kidney disease.\(^{15}\)
1.4.3 Viral pathogenesis

Tat is the key trans-activator protein of Human Immunodeficiency Virus (HIV). It enhances elongation efficiency of RNA Polymerase II (RNAP II) that initiates RNA synthesis from the 5’ long terminal repeat (LTR).\textsuperscript{2,6} Tat binds to cyclin T1 (CycT1) in the cyclin-dependent kinase 9 (CDK9), a lysine methyltransferase (Set7/9), and then to the transactivation response region (TAR) at the 5’ terminus of the nascent HIV RNA transcript.\textsuperscript{16} Upon this association, CDK9 phosphorylates the negative elongation factors and the C terminus of large subunit of RNAP II which is then capable of transcribing the entire proviral genome.

Tat activity can be deregulated by methylation of its RNA binding domain by PRMT6.\textsuperscript{2,16,17} In vivo and vitro studies have shown that the Arg52 and Arg53 of Tats’ arginine-rich transactivation motif (ARM), which is within the Tat-TAR binding site, is the target of PRMT6.\textsuperscript{2} Studies have shown that over expression of PRMT6 inhibits HIV-1 transcription while down regulation of PRMT6 by siRNA elevates the transcription.\textsuperscript{2} It has been shown that dimethylated Tat shows low Tat-TAR binding affinity.\textsuperscript{2} In this way, the virus enters the latency stage in which it hides in the host cells and becomes undetectable to Highly Active Antiretroviral Therapy (HAART). Upon stopping treatment or by following irregular treatment patterns, the virus becomes active again and the infection typically progresses quicker to AIDS.
1.5 PRMT inhibitors

As therapeutic targets, PRMTs open new avenues for drug discovery. By making specific inhibitors for these enzymes, their structural characteristics and their exact roles in diseases can be investigated. PRMT inhibitors are discovered by synthesizing arginine analogue that targets the arginine containing peptide binding site or by synthesizing AdoMet and arginine analogues (bisubstrate analogue) that target the AdoMet and substrate binding pockets. The potential PRMT specific inhibitors can be discovered after screening a library of compounds. The following gives current progresses and challenges for each of these classes of inhibitors.

1.5.1 PRMT inhibition: targeting arginine binding pocket

The active sites of PRMTs have two binding pockets. One is for the cofactor binding site (AdoMet) and the other one is for the arginine residue of the substrate. Chloroacetamidine and Nη-substituted arginine (1 and 2 in Figure 3) are designed to fit in the arginine-binding pocket. By targeting the arginine rather than the AdoMet binding pocket, selectivity for arginine methyltransferases over other methyltransferase enzymes can be achieved.
Figure 3. PRMT inhibitors
Enzymatic studies have shown that chloroacetamidine (1 in Figure 3) inhibits PRMT1 with an IC\textsubscript{50} of 1.8±0.1 µM when 1 is incorporated into a 21-residue peptide (Figure 4) which resembles the N-terminus of histone 4, a PRMT1 substrate.\textsuperscript{18} This inhibitor shows no affinity for PRMT3 but its IC\textsubscript{50} for PRMT6 is 8.8±0.5 µM indicating a 5-fold preference for PRMT1 over PRMT6.

Figure 4. Structure of C21

- N\textsubscript{η}-substituted arginine

In this class of inhibitors, an arginine with a fluorinated ethyl group at one of its guanidine nitrogen, N\textsubscript{η}, (2 in Figure 3) is incorporated in a 12mer peptide which is based on a PRMT substrate, fibrillarin.\textsuperscript{19} These peptides show strong affinity for PRMT1 and 6 but not 4. Increasing the number of fluorine atoms enhances the affinity of the peptide for PRMT6.

This class of inhibitors shows poor specificity within the PRMT family and they have no drug like characteristics.\textsuperscript{19}
1.5.2 **PRMT inhibition: targeting the arginine and the AdoMet binding pockets.**

AdoMet analogues cannot be used for designing PRMT inhibitors as they can interfere with many AdoMet dependent biological pathways. Instead, in this class of PRMT inhibitors, bi-substrate analogues can be used to target both arginine and the AdoMet binding pockets.

- **AdoMet mustard congener**

AdoMet mustard congener (3 in Figure 3) inhibits PRMT1 when it is incorporated in a peptide which resembles the N-terminus of histone 4, a PRMT1 substrate.\(^\text{20}\) This bi-substrate was synthesized in situ and it shows 5.5-fold preference for PRMT1 over PRMT4 at 50 µM concentration. Osborne et al. used AAI as an AdoMet analogue and they observed that the presence of S-adenosylhomocysteine (AdoHcy) or sinefungin blocks this inhibition indicating that AAI sits in the AdoMet binding pocket.

- **AzaAdoMet analogue 15**

AzaAdoMet shows no selectivity within the methyltransferase enzyme but AzaAdoMet analogue 15 (4 in Figure 3) which bears an arginine residue at N6’ position shows improved selectivity.\(^\text{21}\) The IC\(_{50}\) of 4 for PRMT1 is 6.2±3.9 µM while the IC\(_{50}\) for SET7 is greater than 100 µM. Specificity of 4 within PRMTs has not been investigated yet.

Clearly, bi-substrate inhibitors have more affinity for PRMTs than other methyltransferase enzymes by targeting both arginine and the AdoMet binding pockets, which are unique to PRMTs, but no significant specificity within the PRMTs was obtained.
1.5.3 PRMT inhibition: library screening

High throughput screening of a library of compounds can be performed to see which molecule gives significant inhibitory effect with a low micromolar concentration.

- AMI-1

Without competing for the AdoMet binding site, AMI-1 (5 in Figure 3) inhibits arginine and not lysine methylation.\textsuperscript{22} In terms of structure, the sulfonated urea like structure and hydrophobic naphthalene ring of AMI-1 resemble guanidino group and alkyl chain of arginine respectively. Nevertheless, its compatibility for arginine pocket is not known. Its sulfonated urea like structure also resembles pleiotropic drugs that shows no specificity within their target proteins.\textsuperscript{23} It inhibits PRMT1, 3, 4 and 6 so it has no specificity within PRMT family and high concentration has to be used for cellular effects.
- Allantodapson and its analogue

Using a 21 amino acids long peptide (H-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Leu-Gly-Lys-Gly-Gly-Ala-Lys-Arg-His-Arg-Lys-Val-OH) which represent the N-terminus of histone 4, Spannhoff et al. have shown that allantodapson (6 in Figure 3) inhibits the PRMT1 in human with an IC$_{50}$ of 1.7±3.0 µM.$^{24}$ Nonetheless, much greater concentration was necessary for cellular effects. The poor cellular activity of 6 may be caused by its poor cell permeability, instability or its affinity for other PRMTs. Also, a complete inhibition of arginine methylation may be required in order to see a significant effect on transcription.

Via virtual screening, Bissinger et al. discovered an allantodapson analogue (7 in Figure 3), which shows improved cellular activity with respect to 6.$^{23}$ The IC$_{50}$ of enzymatic studies, MCF7A breast cancer cells and LNCap prostate cancer cells are 1 µM, 1.97±0.14 µM, and 4.49±0.14 µM, respectively. Though 7 shows inhibition in micromolar concentrations, it can not be used as a drug candidate as it shows significant cytotoxic activity.
- Benzoimidazole[d]imidazole 17b

When AdoMet and histone 3 were used as cofactor and substrate respectively, 8a (Figure 3) shows an IC$_{50}$ of 0.07 µM for PRMT4 while it gives an IC$_{50}$ of greater than 25 µM for PRMT1 and 3.\textsuperscript{25} This is greater than a 350-fold difference.

- (1-(benzo[d][1,2,3]thiadiazol-6-yl)-3-(2-cyclohexenylethyl) urea

(1-(benzo[d][1,2,3]thiadiazol-6-yl)-3-(2-cyclohexenylethyl) urea (8b in Figure 3) inhibits PRMT3 with an IC$_{50}$ of 2.5 µM when H4 (1-24) peptide (H-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu-Gly-Lys-Gly-Ala-Lys-Arg-His-Arg-Lys-Val-OH) is used as substrate. Combination of crystal structure and enzyme kinetic assays has been demonstrated that 8b inhibits PRMT3 through allosteric inhibition. In addition, 8b does not cause significant inhibition of PRMT1, 4, 5, 8 or lysine methyltransferases.\textsuperscript{26}
- Thiophene 7a and Pyrazole 7f

Using histone 3 as a substrate, Allan et al. have shown that Thiophene 7a (9 in Figure 3) inhibits PRMT4 with an IC$_{50}$ of 0.06 µM. The specificity of 9 within the PRMT family has not been investigated but when it was tested on estrogen-dependent transcription breast cancer growth and androgen independent prostate cancer growth, it showed no cell activity. This could be because of low cellular permeability for 9. By replacing one of the amides with an amide surrogate (eg. 1,3,4-oxadiazole and 1,2,4-oxadiazole), Hyunn et al. improved the permeability of 9. Their hit compound, pyrazole 7f (10 in Figure 3) shows an IC$_{50}$ of 0.04 µM for PRMT4 and an IC$_{50}$ of greater than 25 µM for PRMT 1, 3, and 4.

All these inhibitors that have been discussed so far were discovered after screening numerous chemical structures but almost all of them do not give good selectivity or cell activity so they cannot be used as drug candidates. Significant selectivity can be obtained by allowing the enzyme to choose its own inhibitor. The fragment approach, which is discussed fully in the next section, is based on this notion.
1.6 Fragment approach: achieving specificity within the PRMT family

The fragment approach to drug design is based on the idea that the requisite components in a library of small drug-like substructures will be able to simultaneously accumulate/occupy the active site of an enzyme. This approach, which is shown in a general form in Figure 5, comprises a unique combination of random screening and structure based design.

![Figure 5](image-url)

Figure 5. Fragment based approach in a general form: (a) enzyme has two binding pockets. (b) The first fragment (circle) forms a covalent bond with the active site of the enzyme and occupies one of the binding pockets. (C) The enzyme in complex with the first fragment is incubated with a library of second fragments one of which is complementary to the second binding pocket of the enzyme. (d) The first and second fragments react together to form a 1,4-disubstituted 1,2,3-triazole ring (shown in red).

The first step in fragment approach is to design a fragment which can be covalently bound in the active site of the enzyme. The covalent complex is then incubated with a library of other fragments with which it can react through click chemistry. Because the inhibitors are built up from small subunits that are “accepted” in the active site of the enzyme, the resultant construct will have a specific affinity for the PRMT active site.

Click chemistry, which is shown in a general form in Figure 6, is a powerful technique to connect fragments together. In this reaction, the terminal alkyne motif in one fragment reacts with the azido function in another fragment via a 1,3 dipolar cycloaddition to produce a 1,4-disubstituted 1,2,3-triazole ring which connects the first fragment to the second one. The close proximity of the two reacting components in the active site provides a low energy pathway to product formation.
Figure 6. In click chemistry, one fragment has a terminal alkyne while the second fragment has an azido function. Via a 1,3 dipolar cycloaddition, the first fragment (circle) and second fragment (square) is connected by 1,4-trisubstituted 1,2,3-triazole ring.
1.7 Identification of a potential linker site in PRMT6

Analysis of a homology model for PRMT6 (Figure 7) indicates that the methionine 166 residue in the catalytic site is situated in close proximity to the C8 carbon of the adenine ring in AdoMet (unpublished data). This suggests that by a suitable point mutation of this residue in PRMT6, a reactive (and active) mutant PRMT6 enzyme could be generated that will react with a C8 activated AdoMet analogue with a covalent bond formation. The AdoMet analogue component in the resultant small molecule enzyme conjugate could thus serve as the anchoring and starting point for our envisaged fragment approach to the construction of PRMT6 specific inhibitors.

![Figure 7](image.png)

Figure 7. The crystal structure of the AdoMet binding site for PRMT1: the corresponding amino acid residues for PRMT6 are in parenthesis. The adenine C8 center of AdoHcy is in close proximity to the methionine sulfur atom of the Met 166.

The M166C/S/K/A mutant forms of PRMT6 were constructed in the Dr. Frankel laboratory. The M166C mutant, which displays weak but significant arginine methylation activity, was chosen for our work. The idea is that an electrophilic center at the C8 position of the adenine ring in an AdoMet analogue will react with the proximal nucleophilic sulfur atom in the M166C mutant.
1.8 Research hypothesis:

We hypothesize that the aldehyde functionality of the AdoMet Analogue 1A (X= O) and 1B (X= S) and the proximal cysteine sulfur atom in the active M166C PRMT6 mutant will undergo a nucleophilic addition reaction to form a covalent bond between them (Figure 8). This reaction will be favored because the adenine ring in the AdoMet analogue 1A and 1B is held tightly in the AdoMet binding pocket.

This study, using the AdoMet Analogues 1A and 1B will validate our further hypothesis that with an appropriately designed molecule, such as AdoMet Analogues 1C (Figure 9), it will be possible to subsequently shed the sugar and the amino acid components to leave the adenine ring component alone bound in the binding pocket of the enzyme. This feature is an obligatory condition for the use of a tethered adenine ring as the starting point/template for fragment based synthesis of PRMT6 specific inhibitors. (Figure 9)

![Figure 8](image_url)

**Figure 8.** The hypothesized reaction of the AdoMet analogue 1A and 1B with the M166C PRMT6 mutant.
Figure 9. The hypothesized reaction of the AdoMet analogue 1C with the M166C PRMT6 mutant: Using the AdoMet analogue 1C, it would be possible to shed the sugar and the amino acid components to leave the adenine ring component alone bound in the binding pocket of the enzyme. This adenine ring will be used as the starting point/template for the fragment based approach for the synthesis of the PRMT6 specific inhibitors.
While the synthesis of S-adenosyl-L-homocysteine (AdoMet Analogue 2B in Figure 10) has been well documented in the literature,\textsuperscript{31-33} the synthesis of S-Adenosyl-L-homoserine (AdoMet Analogue 2A) and the CHO containing S-Adenosyl-L-homoserine (AdoMet Analogue 1A) or S-Adenosyl-L-homocysteine (AdoMet Analogue 1B) have not been reported previously. (Figure 10) The focus of this research project is on the synthesis of the AdoMet Analogue 1A and 1B.

\[
\begin{align*}
\text{AdoMet Analogue 1A} & \\
\text{AdoMet Analogue 1B} & \\
\text{AdoMet Analogue 2A} & \\
\text{AdoMet Analogue 2B} &
\end{align*}
\]

\textbf{Figure 10. The structure of the AdoMet Analogue 1A, 1B, 2A and 2B}
2 Synthetic strategy and inherent problems for the synthesis of the AdoMet Analogue 1A

With respect to the AdoMet, the AdoMet Analogue 1A is substituted by homoserine instead of methionine at the C5’ position and it has an aldehyde function at the C8 position. The synthesis of this molecule is divided into two tasks: i) introduction of a homoserine component at the C5’ position and ii) creation of an aldehyde motif at the C8 position. (Figure 11)

![Figure 11. Structural differences between the AdoMet and the AdoMet Analogue 1A](image)

The crucial carbon oxygen bond at the 5’-oxygen that connects the amino acid motif to the ribose ring can be formed via a Williamson ether synthesis approach, involving the substitution reaction of an alkyl halide by the anion of an alcohol under basic conditions.

As shown in Figure 12, in the context of the synthesis, two scenarios exist for the Williamson etherification reaction: i) the 5’-OH in the nucleoside component reacts with a suitable alkyl halide corresponding to the amino acid motif (note that this synthon has been developed by Schneller et al.\textsuperscript{34}, 11 in Figure 12); ii) the alkyl halide motif at the C5’ of the nucleoside reacts with the hydroxyl group in the Schöllkopf bis-lactim ether equivalent of homoserine\textsuperscript{35,36},
(compound 12 in Figure 12). Note that in both cases, the amino acid part is in a protected/masked form in order to permit introduction of the C-8 aldehyde and to prevent racemization during this transformation.

Figure 12. Williamson ether synthesis at the C5' position of adenosine: a) Reaction of adenosine with the halogenated homoserine motif. Schneller et al. has already reported the synthesis of this halogenated amino acid. b) Reaction of the C5' halogenated adenosine and the Schöllkopf's bis-lactim ether form of homoserine. (R= Hydroxyl protecting group)

An inherent problem to both approaches for the formation of the ether bond under Williamson reaction conditions is the susceptibility of the adenine ring to undergo competing N-alkylation or intramolecular cyclization. This can occur by intermolecular reaction of the N-1, 3, 6 or 7 nitrogens of the adenine ring with 11 (Figure 13) or by the known intramolecular reaction of the adenine N-3 nitrogen with the C5' halogenated adenosine (Figure 14).
Figure 13. Multialkylation problem: the alkylation takes place at the 5’-OH position of the adenosine as well as at the N1, N3, N6 or N7 position of the adenine ring. (R= Hydroxyl protecting group)

Figure 14. Intramolecular reaction: The halogenated adenosine undergoes intramolecular reaction under the basic condition of Williamson reaction. (R= Hydroxyl protecting group)
Importantly, the intramolecular alkylation process can be suppressed by temporary attachment of an electron withdrawing group onto the C6 amino nitrogen atom, as in N-benzoyladenosine\textsuperscript{37,38} or by carrying out the synthesis using 6-chloropurine as the base, and introducing the amino group in one of the final steps. Unfortunately, both alternatives add extra steps to the synthesis.

For the AdoMet Analogue 1A, the intrinsic electron attracting properties of the C8 aldehyde function can be used to diminish the electron density on the adenine ring. This should result in a decreased reactivity of the N-3 nitrogen. In this way, the intramolecular reaction of the C5’ halogenated adenosine (Figure 14) possibly would be avoided.
2.1 Synthesis of the AdoMet Analogue 1A

The synthesis of the AdoMet Analogue 1A was studied using 3 different approaches. The approaches 1 and 2 deal with introducing substituents onto the 5’-OH as a means to construct the homoserine motif while the third approach deals with the formylation of adenosine prior to the addition of homoserine to the C5’ position.
2.1.1 Approach 1: synthesis of the AdoMet Analogue 1A via glycosylation of C5’ homoserine substituted D-ribose

In the first approach, the N-alkylation of adenosine under Williamson reaction condition was avoided by incorporating the adenine ring after assembling a dehydrohomoserine at the C5’ position of D-ribose. The attractive feature of using a dehydrohomoserine is that it can be reduced to a L-homoserine enantioselectively in the last step of the reaction scheme so there would be no concern about its racemization during the glycosylation steps.

As shown in the Reaction Scheme 1, the commercially available D-ribose, 13, was subjected to concomitant protection of the anomeric hydroxyl group and the two secondary alcohols in an acidic solution of acetone and methanol (Yield: 50%). Subsequent Williamson reaction of 14 with allyl bromide in presence of NaH 39 afforded 15 in 86% yield. Oxidative cleavage of the terminal alkene using RuCl₃-NaIO₄ in CH₃CN-H₂O (6:1) 40 gave 16 in a 26% yield. Varying the reaction time and the amount of oxidizing agent, NaIO₄, and catalyst, RuCl₃, did not improve the yield.
**Reaction Scheme 1.** Approach 1: a) COMe₂, MeOH, HCl; b) NaH, allyl bromide, THF; c) RuCl₃, NaIO₄, CH₃CN: H₂O (6:1); d) DBU, DCM

In a Horner–Wadsworth–Emmons reaction, the aldehyde 16 was coupled with the phosphonate carbanion formed upon treatment of 17 with DBU in dry DCM, to afford 18 in a high yield of 70%.

The challenging part of this reaction scheme is the glycosylation step. Since the C8 formylated adenine is deactivated toward the glycosylation, this ring has to be assembled at the C1’ position of 18. This could be very difficult as the glycosylation reaction lacks both regioselectivity and good yield.
2.1.2 Approach 2: synthesis of the AdoMet Analogue 1A via selective ester to ether reduction of C5’ ester substituted adenosine derivatives.

In the second approach, again we attempted to build up homoserine but this time at the C5’ position of adenosine instead of D-ribose. In this approach, the plan was to connect the homoserine to the adenosine first through an ester bond which would be then reduced to an ether using a novel method which uses InBr3/Et3SiH for ester to ether reduction. Through this method, the multialkylation problem of adenosine (Figure 13) would be avoided.

Reaction Scheme 2. Approach 2: a) TBSCl, Imidazole, DMF; b) TFA-H2O-THF (1:1:4); C) DMAP, propanoic acid, DCC, DMF; d) BzCl, pyridine.

The 2’, 3’, and 5’ hydroxyl groups in adenosine were protected as their tert-butyldimethylsilyl ethers. When an excess of TBSCI is used for this reaction, two products can be isolated, one major 20a (90%; less polar) and one minor 20b (10%; more polar). The 1H NMR and mass spec of the minor component corresponds to our expected trisilylated product, 20b. The 1HNMR and mass spectrometry of the major product, 20a, indicates that there are 4 TBS instead of 3 TBS groups present in its structure. This suggests that the exocyclic amino group at C6 of the adenine ring is also silylated. This comes as a surprise because it was anticipated that N-silylated adenosine would not be stable under work up conditions. After investigating the NMR data in a
number of literature papers, it was determined that in most instances the NMR for 2’, 3’, 5’-tris(tertbutyldimethylsilyl)adenosine (20b) actually corresponds to N6, 2’,3’, 5’-tetra(tertbutyldimethylsilyl)adenosine (20a).

Selective 5’ monodeprotection of 20b and removal of both the N-TBS and 5’ TBS groups in 20a under the conditions developed by Scott et al. 42 provided compound 20c (MS and crude ¹HNMR).

Acylation of 20c with succinic anhydride 43 turned out to be very slow and low yielding. In contrast, the requisite ester linkage between 20c and propanoic acid was made in high yield using DCC and DMAP as reported by Shen et al. 44 In this reaction, high regioselectivity at the C5’ position was observed, as the NMR of 21a indicates no acylation at the N6 position of the adenosine.

Stirring 21a, InBr₃, and Et₃SiH in DCM at 60 °C failed to give 22a while a total conversion of 27 to 28 was obtained under the same conditions (Figure 15a). Assuming that primary amines interfere with this reduction, the N6 amine was benzoylated but this change did not make a difference, as the reduction of 21b to 22b would not occur using InBr₃/Et₃SiH. Using a model study (Figure 15b) it was shown that this reduction does not work in the presence of an amide functionality (such as 29) suggesting that the benzoyl protecting group at the N6 position is interfering with this ester to ether reduction. It seems that the reduction of an ester to ether using InBr₃/Et₃SiH is not compatible with nitrogen containing compounds.
Figure 15. Two model reactions for reduction of ester to ether: (a) 27 undergoes complete reductive deoxygenation in 90 minutes. (b) Reduction of 27 is impaired in the presence of amide, 29.
2.1.3 Approach 3: synthesis of the AdoMet Analogue 1A via Williamson etherification of C8 CHO substituted adenosine

In contrast to the first and the second approaches, in the third approach, the C8 position of the adenine ring was formylated prior to the homoserine addition. It was anticipated that by placing an electron-withdrawing group (EWG) at the C8 position, the intramolecular reaction of the 5’ halogenated adenosine (Figure 14) under Williamson reaction condition would be circumvented. As shown in Figure 16, the aldehyde function at the C8 position will withdraw electrons away from the N6 position so there will not be a chain of electron movement from N6 to C5’ (i.e., there would be no ring closure similar to the one shown in Figure 14). It is thus anticipated that the desired product will be obtained in the Williamson reaction using Schöllkopf’s bis-lactim homoserine. (Figure 16)

![Figure 16](image)

Figure 16. The aldehyde function at the C8 position of the adenosine withdraw electrons away from the N6 position so under Williamson etherification reaction, Schöllkopf's bis-lactim homoserine, 12, can be added to the C5’ position of the C8 formylated adenosine without any intramolecular reaction.
As shown in Reaction Scheme 3, formylation of 20a using either methyl formate or DMF as an aldehyde source was successful affording 23a in 40-50% yield. As expected, the presence of aldehyde function on the adenine ring caused a bathochromic shift in the UV spectra. The formylated adenosine, 23a, shows an absorption at 270 nm while 20a and 20b have an absorption at 275 nm. When the aldehyde is hydrated, a hypsochromic shift from 275 nm to 273 nm is observed.

Reaction Scheme 3. Approach 3: a) TFA-H$_2$O-THF (1:1:4); b) BzCl, pyridine; C)LDA, THF, DMF/methyl formate

Interestingly, when the 2’, 3’-disilylated compound 20c, prepared by selective 5’-monodeprotection of 20b or by removal of both the N-TBS and 5’ TBS groups in 20a under the conditions developed by Scott et al. $^{42}$, was reacted under the formylation conditions, a mixture of products was obtained, resulting from formylation at N6 or C8 in addition to formylation at C5’.

In the event that the C-8 CHO group does not sufficiently deactivate the 6-amino nitrogen with respect to its participation in the undesired intramolecular alkylation reaction (Figure 14), the N-benzoxy derivative was prepared through reaction of 20a with benzoyl chloride. In fact,
depending on whether the crude product is treated with NH₄OH or not, the mono or dibenzoylated product 20d or 20e was obtained (yield: 79-90%). Subsequent reaction of these compounds under the formylation conditions did not lead to clean conversion to the expected product. In each case, a mixture of compounds was formed, from which the presence of 20d or 20e could be detected by mass spectrometry.

Surprisingly, many attempts for complete deprotection of 23a using 0.1N methanolic HCl, TBAF in THF, and with 80% acetic acid turned out to be unsuccessful. 23a underwent either decomposition or gave a mixture of its mono- and di-deprotected nucleoside.

Treatment of 23a with TFA and H₂O (9:1) in DCM for 45 minutes or 90 minutes at 25°C or 0 °C were also not successful. In order to moderate the acidic condition of silyl ether hydrolysis, HF pyridine was used as fluoride source which also failed to yield the target molecule. This suggests that the C8 CHO substituted adenosine is labile due to the presence of an electron-withdrawing group (CHO) which weakens the glycoside bond. This means that the formylation of the C8 position of adenosine makes the molecule labile so it cannot be used as a way to deactivate the N6 amine group with respect to its participation in the undesired intramolecular reaction.
3 Switching from AdoMet Analogue 1A to AdoMet Analogue 1B

Since the hydroxyl group of homoserine is weakly acidic and has poor nucleophilicity, strong basic conditions are required for its alkoxide formation and often elevated temperature is necessary for its nucleophilic substitution reaction under Williamson conditions. This condition unfortunately also favors the intramolecular reaction of the C8 halogenated adenosine as shown in Figure 14. In the approach 1 and 3, we tried to get around this problem by deactivating N6 by placing an EWG on the adenine ring or by adding this ring after homoserine addition. As described in the previous section both of these strategies failed to give us the target molecule.

Another way to diminish this intramolecular reaction is by using a stronger nucleophile than alkoxide. In contrast to homoserine, homocysteine is more acidic, easier to deprotonate and its anion is even stable in aqueous conditions meaning a milder condition is required for its anion formation. More importantly, with respect to the alkoxide, the sulfur anion is more nucleophilic because of its higher polarizability and lower electronegativity, and smaller solvation energy. Another attractive fact about homocysteine is that its addition to adenosine will not require using protecting groups for adenosine or homocysteine. Consequently, it is assumed that by switching to the AdoMet Analogue 1B, all the inherent problems present in the AdoMet Analogue 1A would be eliminated.
4 Strategies for the synthesis of the AdoMet Analogue 1B

With respect to the AdoMet, the AdoMet Analogue 1B has homocysteine instead of methionine at the C5’ position and it has an aldehyde function at the C8 position.

![Chemical structures](image)

Figure 17. Structural differences between the AdoMet and the AdoMet Analogue 1B

The synthesis of this molecule is divided into 3 steps: i) C8 bromination of adenosine; ii) C5’ addition of homocysteine to adenosine; iii) reductive carbonylation of the C8 position (Figure 18).

![Synthesis steps](image)

Figure 18. There are 3 steps for the synthesis of the AdoMet Analogue 1B: i) C8 bromination of adenosine; ii) C5’ addition of homocysteine to adenosine; iii) reductive carbonylation of the C8 position

In contrast to the AdoMet Analogue 1A, synthesis of the AdoMet Analogue 1B is deemed to be less challenging since the addition of cysteine or homocysteine to the C5’ position of adenosine is well reported in the literature. Three different ways have been documented for the coupling of these two molecules. The coupling of 2’, 3’-O-isopropylidene-5’-tosyladenosine, with disodium salt of homocysteine, is the oldest method (Figure 19). The difficulties of
this synthetic route are: the nucleoside 30 is unstable and the yield of this reaction is low (5 – 20%).

![Chemical structure](image)

**Figure 19.** Synthesis of S-adenosylhomocysteine, 32, from the coupling of 2’, 3’-O-isopropylidene-5’-tosyladenosine, 30, with disodium salt of homocysteine, 31.

Mitsunobu reaction is another method for the addition of homocysteine to the C5’ position of adenosine\(^{49,50}\) but the low reactivity of the aliphatic thiol in the Mitsunobu reaction put a challenge on this pathway.

The third and most widely used method for the coupling of L-homocysteine to the C5’ position of adenosine is the nucleophilic substitution of disodium salt of homocysteine to the C5’ activated adenosine\(^{31-33}\). This method has been explored for the synthesis of the AdoMet Analogue 1B.
4.1 Approach 1: synthesis of the AdoMet Analogue 1B via addition of the disodium salt of L-homocysteine to the C5’ activated C8-bromoadenosine.

It is envisioned that the disodium salt of homocysteine can be added to the C5’ activated adenosine in a nucleophilic substitution reaction as shown in Reaction Scheme 4.

![Reaction Scheme 4](image)

Reaction Scheme 4. Nucleophilic substitution reaction of the disodium salt of L-homocysteine to the C5’ activated adenosine: a) Br₂, sodium acetate buffer (pH 4); b) SOCl₂, pyridine, CH₃CN (to get 34b from 33); PPh₃, pyridine (to get 34c from 33)
4.1.1 Step 1: C8 bromination of adenosine

As shown in the Reaction Scheme 4, direct bromination of the C8 position of adenosine was achieved using bromine in sodium acetate buffer (pH 4). The $^1$H NMR of the isolated product confirmed formation of 33, as the characteristic peak of the C8 proton at 8.35 ppm is not present in the isolated product. Furthermore, the mass spectroscopy of 33 shows the presence of the characteristic bromine isotopes with the right molecular mass (346 [M$^+$], 348 [M$^{13}$]) reaffirming formation of 33. When less toxic and more easily to handle N-bromoacetamide was used as the bromine source, the reaction was slow and the product was difficult to purify.

4.1.2 Step 2: C5’ activation of adenosine

The C5’ position of 33 was activated using chlorine, iodine and triflate as shown in the Reaction Scheme 4. Their leaving group strength in nucleophilic substitution reaction was studied using the disodium salt of L-cysteine. L-cysteine instead of L-homocysteine was used in this part of our studies because L-homocysteine is expensive and its synthesis is challenging while L-cysteine is commercially available and is inexpensive. The plan is to switch to L-homocysteine once we have a better understanding of the chemistry of the C5’ and the C8 position of adenosine.
4.1.2.1 C5’ chlorination of C8-bromoadenosine and its addition to L-cysteine

Chlorination of the C5’ position of adenosine has been achieved by stirring the unprotected nucleoside 19 and thionyl chloride in HMPA for 15 h at room temperature.\textsuperscript{31-33,55-58} Because HMPA is toxic and has a high boiling point, the synthesis of 34a can be achieved alternatively by treatment of 33 with thionyl chloride and pyridine in anhydrous DMF.\textsuperscript{46,47,59-63} Surprisingly, under this condition, 34b instead of 34a formed due to the halogen exchange at the C8 position (Figure 20). The mass spectrum of the isolated product shows no characteristic bromine isotopes but instead the parent ion ($m/z = 320 [M^+1], 322 [M^+3], 324 [M^+5]$) shows 9:6:1 isotopic ratio, which is a feature of dichlorinated molecules. Considering that the C8 position can be re-brominated using halogen exchange after the L-cysteine addition, in the next step, 34b was reacted with the disodium salt of L-cysteine.

![Figure 20](image.png)

Figure 20. Treatment of 33 with thionyl chloride and pyridine in anhydrous DMF give 34b instead of 34a due to the halogen exchange at the C8 position of 33.

Under anhydrous conditions, the disodium salt of L-cysteine was formed by stirring L-cysteine and NaH in DMF for 12 h at 40 °C. The C5’ activated nucleoside, 34b, was then added dropwise to this disodium salt of L-cysteine at room temperature. Monitoring the reaction by TLC shows that after two hours of stirring at room temperature, all the starting nucleoside was consumed. After purifying the product by HPLC, UV measurement shows that this molecule
gives a strong absorption at 281 nm which could be only caused by the presence of a cysteine at the C8 position of the adenine ring. As shown in Figure 21, this suggests that 37 instead of 36 is formed in this reaction. The NMR spectrum of the purified product also reaffirms the formation of 37 as there is no change in the chemical shift of the C5’ hydrogen with respect to 34b.

Figure 21. Treatment of 34b with 3.5 equivalents of the disodium salt of L-cysteine gives 37 instead of 36. No nucleophilic substitution (SN2) reaction at the C5’ position of 37 was observed even after stirring the reaction for another 5 days at the room temperature.

Nucleophilic substitution reaction at the C5’ position of 37 did not occur even after stirring the reaction for 5 days at room temperature. This could be due to steric effect caused by the C8 cysteine. In order to avoid this steric effect while studying the reactivity of the C5’ position of adenosine, the previous experiment (Figure 21) was repeated with 5’-chloro-5’-deoxyadenosine, 39, instead of 34b as shown in Figure 22.
Figure 22. The nucleophilic substitution (SN2) reaction of the disodium salt of L-cysteine with 39 took 24 h at room temperature to give 40.

Nucleophilic substitution reaction of the disodium salt of L-cysteine with the C5’ position of 39 took 24 h. This result suggests that in the case of 34b, the C5’ position of adenosine is far less reactive than its C8 position.

Next, we studied the reactivity of the C8 halogenated adenosine with respect to nucleophilic aromatic substitution reaction (SNAr). In terms of halogens, fluorine is the best leaving group in a SNAr reaction. The order of the leaving group strength for bromine, iodine, and chlorine is dependent on the nature of the aromatic rings.\textsuperscript{65} In order to see whether bromine or chlorine is a better leaving group for a SNAr reaction at the C8 position of adenosine, a model molecule, 33, was reacted with 2 equivalents of the disodium salt of L-cysteine (Figure 23). This reaction took an hour for a total conversion suggesting that bromine is a better leaving group than chlorine for SNAr reaction at the C8 position. Figure 24 summarizes all these findings.
Figure 23. The nucleophilic aromatic substitution (SNAr) reaction at the C8 position of 41 took an hour. This reaction was much faster than the SNAr reaction at the C8 position of 34b and the nucleophilic substitution (SN2) reaction at the C5’ position of 39.
Figure 24. The nucleophilic aromatic substitution (SNAr) reactions at the C8 position of 33 and 34b are much faster than the nucleophilic substitution (SN2) reaction at the C5’ position of 39.

These results suggest that the SNAr reaction for both C8 brominated and chlorinated adenosine occur more rapidly than the nucleophilic substitution reaction (SN2) at the C5’ position of adenosine so in order to have a better chemoselectivity for the C5’ position of adenosine, the C5’ chlorine has to be substituted with a better leaving group.
4.1.2.2 C5’ iodination of C8-bromoadenosine and its addition to L-cysteine

In terms of SN2, the trend of leaving group strength is in the order of F < Cl < Br < I as fluorine is the weakest and iodine is the strongest. A total conversion was achieved within 6 h by stirring 5’-iodo-5’-deoxy-adenosine, 43, with 3.3 equivalents of disodium salt of L-cysteine, 42, at 0 °C (Figure 25). This result looked very promising as SNAr reaction at the C8 position of adenosine does not occur below the room temperature. This suggests that the chemoselectivity for the C5’ position can be achieved by substituting the C5’ chlorine with iodine and running the experiment below room temperature. Surprisingly, the starting material remained intact after stirring 44 and 42 at 0 °C for 3 h and then at room temperature for 24 h. Heating the mixture for half an hour at 100 °C resulted in the decomposition of the nucleoside.
Figure 25. The nucleophilic substitution (SN2) reaction at the C5' position of 43 is much faster than 39. Neither nucleophilic aromatic substitution (SNAr) nor nucleophilic substitution (SN2) was observed for 44.
4.1.2.3 C5’ triflation of C8-bromoadenosine and its addition to L-cysteine

The reactivity of the C5’ position of adenosine was explored further using triflate which is a much better leaving group than chlorine and iodine. Triflation of adenosine at the C5’ position of adenosine, 47, is not reported but the triflation of the primary alcohols in the presence of secondary alcohols has been done in general. 67-71 However, in order to avoid any minor intramolecular reaction under basic conditions 72 and enhance the solubility of 33 in aprotic and non-nucleophilic solvents, as shown in Reaction Scheme 5, the 2’, 3’ and the 5’ position of 33 was silylated by stirring 33, imidazole, and tert-butylimethylsilyl chloride in DMF for 24 h to give 46 in a high yield of 93%. 73 Selective 5’ mono-deprotection was done by stirring 46 in ice cold solution of TFA/H₂O/THF (1:1:4) for 4 h to provide compound 47 in 72% yield. 74
Reaction Scheme 5. A synthetic scheme showing the steps for the synthesis of the AdoMet Analogue 1B by reacting the C5’ triflated adenosine, 48, with the disodium salt of L-cysteine. a) TBDMSCl, Imidazole, DMF, 24 h, r.t.; b) TFA/H₂O/THF (1:1:4), 0 °C, 4h.

The C5’ position of 47 was then triflated using triflic anhydride and pyridine in DCM. Monitoring the reaction by TLC indicated that a UV active product forms in a steady rate at -32 °C. Removing the dry ice bath for 20 minutes and allowing the reaction mixture to reach the room temperature accelerated this conversion. After removing the solvent under a reduced pressure, 48 was added to the disodium salt of L-cysteine. Monitoring the reaction by TLC shows no sign formation of new product after stirring the reaction for a few hours at room temperature. This suggests either triflation was unsuccessful or pyridine is acting as a nucleophile for the C5’ position of 48. Switching pyridine to 2,6-lutidine did not make a difference.
Next, benzylamide was added to 48 to see whether the conversion of 47 to 48 was successful or not. No new product formed upon stirring benzylamide and 48 at room temperature overnight. This suggests that failing to successfully triflate the C5’ position of 47 is the main reason why no 45 formed.

The most striking fact is that the reactivity of the C8 position of 33 and 47 are greatly different. No SNAr reaction was observed for 47 even after stirring the reaction overnight at room temperature while 33 only took an hour to give 41. This could be caused by steric hindrance from two silyl ether groups at the 2’ and 3’ position of 47.

A more valid reasoning for this observation would be that 33 and 47 have syn$^{52}$ and anti conformation respectively. As shown in Figure 26, the bromine of 33 is not hindered with ribose ring and it is more accessible for the nucleophilic addition of the disodium salt of L-cysteine.

Figure 26. The C8-bromoadenosine, 33, has syn conformation while 47 has anti conformation.
4.2 Approach 2: synthesis of the AdoMet Analogue 1B from S-adenosylhomocysteine.

In the first approach for the synthesis of the AdoMet Analogue 1B, it was learned that the SNAr reaction of the C8 brominated and chlorinated adenosine proceed much faster than the SN2 reaction of the C5’ chlorinated adenosine (Figure 24). It was assumed that by placing a good leaving group at the C5’ position of 33, a better chemoselectivity for the C5’ position would be achieved. We failed to test this hypothesis due to difficulties synthesizing the C5’ iodinated and triflated form of C8-bromoadenosine, 44 and 48 respectively. In the second approach for the synthesis of AdoMet Analogue 1B, the competing reaction between SN2 and SNAr reaction at the C5’ and C8 position of 34 was eliminated by brominating the C8 position of S-adenosylhomocysteine, 32, as it is outlined in the Reaction Scheme 6 which is divided into two steps: i) addition of the disodium salt of L-homocysteine to the C5’ position of 39; ii) C8 bromination of S-adenosylhomocysteine, 32.

Reaction Scheme 6. Second approach: synthesis of AdoMet Analogue 1B from S-adenosylhomocysteine. a) NaH, DMF
4.2.1 Step 1: L-homocysteine synthesis and its addition to 39

As shown in Figure 27, homocysteine can be synthesized by reducing the homocysteine with sodium in liquid ammonia, opening the homocysteine thiolactone ring under alkaline condition, or refluxing L-methionine in sulfuric acid or hydriodic acid.\textsuperscript{76-78} Nevertheless, all these synthetic routes end up in partial racemization. There are only two reported procedures in the literature for the asymmetric synthesis of L-homocysteine.\textsuperscript{76-79}

![Synthesis of racemized Homocysteine](image)

**Figure 27. Synthesis of racemized Homocysteine:** a) Reduction of L-homocystine with sodium in liquid ammonia; b) Opening the L-homocysteine thiolactone ring under alkaline condition; C) Refluxing L-methionine in sulfuric acid or hydriodic acid.

Adamczyk, M. et al. report a 4-step procedure for asymmetric synthesis of L-homocysteine using Schöllkopf reagent.\textsuperscript{79} As it is outlined in Figure 28, first, lithiated Schöllkopf reagent is alkylated with 2-bromoethyltriphenylmethyl sulfide. Next, the pyrazine ring is hydrolyzed under acidic condition to give S-triphenylethyl-L-homocysteine methyl ester. This is followed by removal of methyl ester and triphenylmethylene by lithium hydroxide and sodium in liquid ammonia, respectively. Finally, treating disodium L-homocysteine with degassed hydriodic acid gives L-homocysteine with an overall yield of 20%. The major problem with this procedure is that the acidic condition necessary for transforming the disodium salt of L-homocysteine to L-
homocysteine causes major oxidation of L-homocysteine to L-homocystine. To avoid this oxidation, the disodium salt of L-homocysteine has to be used in situ. However, since the reducing condition provided by sodium in liquid ammonia causes dehalogenation of C8-bromoadenosine, 33, this procedure cannot be used for the synthesis of L-homocysteine.

Figure 28. Adamczyk, M. et. al. report a 4-step procedure for asymmetric synthesis of L-homocysteine using Schöllkopf reagent: a) i) n-BuLi, THF, DMEU, -78 °C, 30 mins; ii) 2-bromoethyltriphenylmethyl sulfide, -78 °C, 20 h b) i) 0.25 M HCl, ii) 0.25 M LiOH; c) Na/NH3; d) Air  e) HI

Instead L-homocysteine was synthesized by modifying the procedure by Shiraiwa, T. et. al. As shown in Figure 29, refluxing L-methionine with four equivalents of dichloroacetic acid in concentrated hydrochloric acid gives 49 through intramolecular condensation of (2S)-ACM.HCl. After collecting and washing 49 with THF, 49 was refluxed with ethanolic hydroxylamine hydrochloride at pH 7 to open the ring. The white precipitate was then collected and washed.
with ethanol to give L-homocysteine with 15% yield. The $^1$H NMR of the isolated product corresponds to that found in the literature$^{76-78}$ but its optical activity still has to be determined.

![Chemical Structure](image)

Figure 29. L-homocysteine was synthesized by modifying the procedure by Shiraiwa, T. et. al.$^{76-78}$: a) Dichloroacetic acid, concentrated hydrochloric acid, reflux, overnight; b) Ethanolic hydroxylamine hydrochloride, triethylamine, reflux, 1h.

For formation of disodium salt of L-homocysteine, 2.6 rather than usual 1.8 equivalents of NaH was necessary as the synthesized L-homocysteine is in HCl salt form. The addition of the disodium salt of L-homocysteine to the C5’position of 39 took 24 hours at room temperature and $^1$H NMR of the isolated product matches the literature values confirming formation of 32.

### 4.2.2 Step2: C8 bromination of S-adenosylhomocysteine, 32

Treatment of 32 with bromine in sodium acetate buffer (pH 4) for half an hour gives adenosine, 19 as the major product based on $^1$H NMR analysis. This is caused by rapid oxidation of sulfides with bromine in aqueous media.$^{80}$ Figure 30 shows the mechanism for the formation of 19 from 32. This suggests that synthesis of the AdoMet Analogue 1B via this route is not feasible.
Figure 30. The mechanism for rapid oxidation of sulfides with bromine in aqueous media
5 Conclusion

Five different approaches for the synthesis of C8 activated AdoMet analogue have been unsuccessful so far. The whole purpose of synthesizing AdoMet analogues was to see if the C8 activated analogues undergo nucleophilic addition reaction with the proximal cysteine sulfur atom in the active M166C PRMT6 mutant to form a covalent bond between them. Though synthesis of the C8 activated adenine is far less challenging, it cannot be used to validate this hypothesis. The covalent bond formation between the C8 activated AdoMet analogue and the active M166C PRMT6 is favored when the two are in close proximity and this only can occur after the AdoMet analogue sits in the AdoMet binding pocket. Furthermore, this feature is an obligatory condition for the use of a tethered adenine ring as the starting point/template for the fragment based synthesis of PRMT6 specific inhibitors. By detaching sugar and amino acid component after AdoMet analogue sits in the AdoMet binding pocket, the adenine ring will be left in the binding pocket so the additional fragments will be added to adenine in the AdoMet binding pocket.
Synthesis of C8 activated AdoMet analogues proved to be much more challenging than expected. Though synthesis of both AdoMet Analogue 1A and 1B were unsuccessful in my studies, some valuable facts were acquired about the chemistry of adenosine.

1. It is easy to assemble homoserine at the C5’ position of D-ribose.
2. The esterification of the C5’ adenosine is easy but the reductive deoxygenation of this ester bond using InBr₃ and Et₃SiH is not possible.
3. Placing CHO at the C8 position of the adenosine ring gives a labile molecule.
4. SNAr reaction at the C8 position of adenosine ring is faster than the SN2 reaction at the C5’ position of adenosine.
5. C8 bromination of AdoHcy is not possible due to rapid oxidation of sulfide with bromine under aqueous condition.
6 Experimental section

6.1 Materials and methods:

All chemicals were purchased from Sigma Aldrich and used without purification unless mentioned. All solvents were dried and kept under N₂. All syntheses were carried out under N₂ using standard Schlenk techniques. Mass spectra were obtained with Waters Acquity Ultra Performance Liquid Chromatograph connected to the Waters Quattro Premier XE triple quadruple (UPLC/MS/MS). Flash column chromatography was performed using silica gel (Manufacturer: Silicycle, Siliaflash® F60, 40-63µm, 230-400 mesh) or on a Biotage Isolera Four System (Manufacturer: PartnerTech Åtvidaberg AB) with pre-packed silica gel columns (Manufacturer: Biotage, part no. FSKO-1107-0010, FSKO-1107-0025, or FSKO-1107-0050). ¹H NMR spectra were recorded on a Bruker AV400 at 400.19 MHz. NMR solvents were from Cambridge Isotope Laboratories. UV spectra were obtained with Varian, Cary 100 Bio.
6.2 Experiments

Preparation of \((3aR,4R,6aR)-6\text{-methoxy-2,2-dimethyltetrahydrofuro}[3,4-\text{d}][1,3]\text{dioxol-4-yl})\text{methanol (14)}\): Acetone (25 mL) and methanol (25 mL) were added to a 100 mL round bottom flask containing D-ribose (5 g, 33.3 mmol). After addition of concentrated HCl (0.5 mL), the mixture refluxed for 1 h during which it turned to a pale yellow solution. Then, the solution was neutralized with pyridine and was partitioned between water and diethyl ether. The aqueous layer was washed with diethyl ether and ethyl acetate and the combined organic layers was washed with saturated copper sulfate solution, water, and brine and was dried over Na\(_2\)SO\(_4\). The crude product was purified over silica gel using 10% hexane in diethyl ether to afford 14 in 50% yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)): 1.35 (s, 3H), 1.52 (s, 3H), 3.26 (s, 1H), 3.47 (s, 3H), 3.64 (m, 1H), 3.73 (dd, 1H, J=2.2Hz, J=12.6Hz)
**Preparation of (3aR,6R,6aR)-4-methoxy-2,2-dimethyl-6-(pent-4-en-1-yl)tetrahydrofuro[3,4-d][1,3]dioxole (15):** A solution of 14 (300 mg, 1.46 mmol) in THF (2 mL) was added dropwise to a mixture of NaH (67 mg, 2.8 mmol) in THF (10 mL). After refluxing the mixture for 4 h, it turned from ivory to orange. Allyl bromide (0.21 ml, 2.4 mmol) was then added dropwise to the mixture at room temperature. After refluxing the solution for another 43 h, triethylamine (5 mL) was added to react with the excess allyl bromide. After 2 h of stirring at room temperature, sodium bicarbonate was added to the mixture and the aqueous layer was washed with hexane. The organic layer was washed with water and brine and dried over Na₂SO₄. The crude product was purified with column chromatography using 20% EtOAc/Hex. Yield: 86%; ¹H NMR (400 MHz, CDCl₃): 5.30 (m, 1H), 5.20 (ddd, 1H, J=1.3Hz, J=2.8Hz, J=10.4Hz), 4.98 (s, 1H), 4.69 (d, 1H, J=6.0Hz), 4.59 (d, 1H, J=6.0Hz), 4.35 (m, 1H), 4.03 (ddd, 2H, J=1.4Hz, J=2.8Hz, J=5.6Hz), 3.47 (ddd, 2H, J=7.4Hz, J=9.7Hz, J=18.0Hz), 3.34 (s, 3H), 1.50 (s, 3H), 1.34 (m, 3H)
Preparation of 2-(((3aR,4R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)acetaldehyde (16): Upon addition of Ruthenium(III) chloride monohydrate (3.7 mg, 0.016 mmol) to a solution of 15 (100 mg, 0.42 mmol) in CH$_3$CN/H$_2$O (6:1), the solution turned from pale yellow to dark brown and then to a dark green. Sodium periodate (180 mg, 0.84 mmol) was added in small portions to obtain a yellow solution with green precipitate. After 30 minutes of stirring at room temperature, the reaction was quenched with saturated solution of sodium thiosulfate. The aqueous layer was washed with ethyl acetate and the combined organic layer was washed with water and brine. It was then dried over Na$_2$SO$_4$ and concentrated under vacuum. The crude product was purified with column chromatography using 100% EtOAc.

Yield: 26%; $^1$H NMR (400 MHz, CDCl$_3$): 9.76 (s, 1H), 4.99 (s, 1H), 4.74 (d, 1H, $J$=6.0Hz), 4.61 (d, 1H, $J$=6.0Hz), 4.39 (m, 1H), 4.15 (m, 1H), 3.61 (m, 2H), 3.36 (m, 3H), 1.58 (s, 1H), 1.51 (s, 3H), 1.35 (s, 3H)
Preparation of methyl 2-(((benzyloxy)carbonyl)amino)-2-(dimethoxyphosphoryl)acetate (17): Glyoxylic acid monohydrate (500 mg, 5.4 mmol) and tert-butyl carbamate (631 mg, 5.4 mmol) were dissolved in anhydrous diethyl ether (8 mL). After 16 h of stirring at room temperature, the solvent was removed under vacuum. It was then dissolved in anhydrous methanol (12.5 mL) and sulfuric acid (0.17 mL) and it was stirred for 2 days at room temperature. The reaction mixture was then poured over ice cold saturated solution of sodium bicarbonate. The aqueous layer was washed with ethyl acetate, dried over sodium sulfate and concentrated under vacuum. The isolated oil was dissolved in ethyl acetate and passed through a neutral alumina column. The isolated solution was concentrated under vacuum and was dissolved in toluene (5 mL). Phosphorus trichloride was then added in one portion and the solution stirred for 18 h at 70 °C. Then, trimethyl phosphite was added and solution was stirred for another 2 h at 70 °C. After reaching the room temperature, the solution was concentrated and was dissolved in ethyl acetate and it was washed with sodium bicarbonate and dried over Na₂SO₄. The organic solution was concentrated and was used in the next step without any farther purification. Yield: 34%; ¹H NMR (400 MHz, CDCl₃): 5.37 (d, 1H, J=8.2Hz), 4.89 (dd, 1H, J=9.3Hz, J=22.6Hz), 3.84 (dd, 8H, J=4.1Hz, J=10.8Hz), 1.47 (s, 9H)
Preparation of \((E)\)-methyl 2-(((benzyloxy)carbonyl)amino)-4-(((3aR,4R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-\(d\)][1,3]dioxol-4-yl)methoxy)but-2-enoate (18): Upon addition of DBU (0.09 mL, 0.61 mmol) to a solution of 17 (182 mg, 0.61 mmol) in anhydrous methylene chloride (4 mL), the solution turned from pale to darker yellow. After 30 minutes of stirring at room temperature, 16 (130 mg, 0.53 mmol) in methylene chloride (1 mL) was added. After 30 h of stirring at room temperature, the solvent was removed under vacuum and it was purified with column chromatography using 30% Hex/EtOAc. Yield: 60%; \(^1\)H NMR (400 MHz, CDCl\(_3\)): 6.53 (t, 1H, \(J=5.7\)Hz), 6.49 (s, 1H), 4.99 (m, 1H), 4.68 (d, 1H, \(J=5.9\)Hz), 4.60 (d, 1H, \(J=6.0\)Hz), 4.34 (m, 1H), 4.21 (d, 2H, \(J=5.7\)Hz), 3.82 (s, 3H), 3.35 (s, 3H), 1.56 (s, 3H), 1.49 (m, 15H), 1.34 (s, 4H)
Preparation of **9-**((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-N-(tert-butyldimethylsilyl)-9H-purin-6-amine (20a)**; **9-**((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9H-purin-6-amine (20b): A mixture of adenosine (1 g, 3.74 mmol), TBSCl (3 g, 20 mmol), imidazole (2.24 g, 33 mmol) in DMF (4 mL) was stirred for 48 h at room temperature. It was then poured over a 20 mL saturated solution of NaCl. The aqueous layer was washed with ethyl acetate and the organic layer was washed with brine and dried over Na$_2$SO$_4$. The crude product was then purified with column chromatography using 10-75% EtOAc/Hex. **(20a)**: Yield: 68%; ESI-MS m/z 724.6 [M$^+$]; $^1$H NMR (400 MHz, CDCl$_3$): 8.35 (s, 1H), 8.09 (s, 1H), 6.01 (d, 1H, J=5.2Hz), 5.24 (s, 1H), 4.75 (t, 1H, J=4.8Hz), 4.36 (t, 1H, J=3.9Hz), 4.14 (dd, 1H, J=3.6Hz, J=7.3Hz), 4.06 (dd, 1H, J=4.4Hz, J=11.2Hz), 3.79 (dd, 1H, J=2.9Hz, J=11.3Hz), 1.03 (s, 9H), 0.96 (d, 1H, J=5.9Hz), 0.82 (s, 9H), 0.39 (s, 6H), 0.13 (dd, 1H, J=2.3Hz, J=7.9Hz), -0.02 (s, 3H), -0.19 (s, 3H); **(20b)**: Yield: 12%; ESI-MS m/z 608.4 [M$^+$]. $^1$H NMR (400 MHz, CDCl$_3$): 8.37 (s, 1H), 8.17 (s, 1H), 6.05 (d, 1H, J=5.2Hz), 5.53 (s, 1H), 4.71 (t, 1H, J=4.7Hz), 4.34 (t, 1H, J=3.8Hz), 4.15 (dd, 1H, J=3.3Hz, J=6.4Hz), 4.05 (dd, 1H, J=4.2Hz, J=11.3Hz), 3.81 (dd, 1H, J=2.7Hz, J=11.3Hz), 1.66 (s, 1H), 1.27 (s, 1H), 0.97 (d, 1H, J=8.7Hz), 0.82 (s, 1H), 0.14 (dd, 1H, J=2.5Hz, J=12.0Hz), -0.02 (s, 1H), -0.21 (s, 1H)
Preparation of (2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-bis((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (20c): To an ice-cold solution of 20b (237 mg, 0.33 mmol) in THF (4 ml) was added 2 mL solution of ice-cold TFA/H₂O (1:1) dropwise at 0 °C. After 3 h of stirring at 0 °C, the reaction was quenched by addition of ice-cold sodium bicarbonate. The aqueous layer was washed with ethyl acetate and the organic layer was washed with H₂O and brine and dried over Na₂SO₄. The organic layer was concentrated and purified with column chromatography using 94% EtOAc/Hex. Yield: 78%; ESI-MS m/z 496.6 [M⁺]; ¹H NMR (400 MHz, CDCl₃): 8.37 (s, 1H), 7.89 (s, 1H), 5.81 (d, 1H, J=8.0Hz), 5.02 (dd, 1H, J=4.7Hz, J=7.8Hz), 4.34 (d, 1H, J=4.5Hz), 4.18 (s, 1H), 3.96 (d, 1H, J=12.5Hz), 3.73 (d, 9sH, J=13.7Hz), -0.62 (s, 9H), -0.11 (s, 6H), 0.14 (d, 3H, J=5.5Hz), 0.76 (s, 1H), 0.96 (s, 3H)
Preparation of $N$-(9-((2R,3R,4R,5R)-3,4-bis((tert-butylidimethylsilyl)oxy)-5-(((tert-butylidimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (20d); $N$-benzoyl-$N$-(9-((2R,3R,4R,5R)-3,4-bis((tert-butylidimethylsilyl)oxy)-5-(((tert-butylidimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (20e)  To a solution of 20a (216 mg, 292 mmol) in pyridine (1.5 mL) was added benzoyl chloride (0.05 mL, 0.435 mmol) at 0 °C. After stirring the solution for 5 h at room temperature, a solution of K$_2$HPO$_4$ (0.6 g in 3 mL H$_2$O) was added at 0 °C. The solution was then diluted by addition of 10 mL DCM. The organic layer was washed with brine and dried over Na$_2$SO$_4$. After removing the solvent under vacuum, the crude product was purified with column chromatography using 50% Hex/EtOAc to afford 90% 20e. $^1$H NMR (400 MHz, CDCl$_3$): 8.67 (m, 1H), 8.35 (s, 1H), 7.88 (d, 4H, J=7.3Hz), 7.49 (t, 2H, J=7.4Hz), 7.36 (t, 4H, J=7.7Hz), 6.08 (d, 1H, J=5.7Hz), 4.71 (m, 1H), 4.32 (m, 1H), 4.14 (m, 2H), 4.01 (dd, 1H, J=4.4Hz, J=11.3Hz) 3.81 (dd, 1H, J=3.1Hz, J=11.3Hz), 1.28 (m, 9H), 0.96 (d, 21H, J=), 0.78 (s, 8H), 0.13 (s, 12H), -0.06 (s, 3H), -0.35 (s, 3H). To obtain 20d from 20e, 50 mg of 20e was dissolved in pyridine (0.6 mL) and NH$_4$OH (0.2 mL). After 30 minutes of stirring at room temperature, the reaction was stopped and the work up and purification procedure for 20e was repeated to afford 79% 20d. $^1$H NMR (400 MHz, CDCl$_3$): δ 9.010 (s, 1H), 8.841 (s, 1H), 8.38 (s, 1H), 6.15 (d, 1H, J= 5.26), 4.72 (t, 1H, J= 4.78), 4.34 (t, 1H, J= 3.84), 4.18 (q, 1H, J= 3.34, J= 6.49 ), 4.06 (dd, 1H, J= 3.95, J=11.38), 3.85 (dd, 1H, J= 2.74, J= 11.38), 0.98 (d, 18H, J=), 0.82 (s, 9H), 0.17 (d, 6H, J= 3.99), 0.13 (s, 6H), -0.02 (s, 3H), -0.23 (s, 3H)
Preparation of \( ((2R,3R,4R,5R)-5-(6\text{-amino-9H-purin-9-yl})-3,4\text{-bis((tert-butyldimethylsilyloxy)tetrahydrofuran-2-yl})methyl propionate}\) (21a): DMAP (9.5 mg, 0.08 mmol) and 20a (190 mg, 0.26 mmol) were dried under high vacuum overnight. Propanoic acid (58.5 mg, 0.79 mmol) in DMF (2 mL) was then added to the reaction flask. This was followed by dropwise addition of DCC (163 mg, 0.79 mmol) in DMF (2 mL) at 0 °C. After 7 h of stirring at 0 °C, anhydrous EtOH (2 mL) was added to the mixture and it was stirred overnight. The solvent was then removed under vacuum. It was then dissolved in ethyl acetate and the white solid was removed by filtration. The filtrate was washed with saturated solution of ammonium chloride and brine. It was then dried over Na\(_2\)SO\(_4\) and concentrated under vacuum. The crude product was purified with column chromatography using 90% Hex/EtOAc. Yield: 72%; ESI-MS \( m/z \) 552.3 \([M^+\])\; \(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.36 (s, 1H), 8.01 (s, 1H), 5.92 (d, 1H, \( J=4.0 \)Hz), 5.49 (m, 2H), 4.90 (t, 1H, \( J=4.1 \)Hz), 4.53 (dt, 1H, \( J=2.2 \)Hz, \( J=5.4 \)Hz), 4.34 (m, 3H), 2.40 (dq, 2H, \( J=2.0 \)Hz, \( J=7.5 \)Hz), 1.18 (t, 3H, \( J=7.6 \)Hz), 0.95 (s, 9H), 0.87 (s, 9H), 0.11 (d, 6H, \( J=4.8 \)Hz), 0.03 (s, 3H), -0.09 (s, 3H)

Preparation of \( ((2R,3R,4R,5R)-5-(6\text{-N-benzoylbenzamido-9H-purin-9-yl})-3,4\text{-bis((tert-butyldimethylsilyloxy)tetrahydrofuran-2-yl})methyl propionate}\) (21b): The procedure for 20e was repeated here. Yield: 48%; ESI-MS \( m/z \) 760.5 \([M^+\])\; \(^1\)H NMR (400 MHz, CDCl\(_3\)): -0.16 (s, 3H), 0.01 (s, 3H), 0.11 (d, 6H, \( J=6.6 \)Hz), 0.85 (s, 9H), 0.95 (s, 9H), 1.16 (t, 3H, \( J=7.5 \)Hz), 1.28 (dd, 2H, \( J=6.2 \)Hz, \( J=8.1 \)Hz), 2.39 (m, 2H), 4.35 (dd, 3H, \( J=3.1 \)Hz, \( J=9.3 \)Hz), 4.50 (m, 1H), 4.82 (t, 1H, \( J=3.7 \)Hz), 6.00 (d, 1H, \( J=4.1 \)Hz), 7.37 (t, 4H, \( J=7.7 \)Hz), 7.50 (t, 2H, \( J=7.4 \)Hz), 7.87 (m, 4H), 8.26 (s, 1H), 8.67 (s, 1H)
Preparation of 6-amino-9-((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-9H-purine-8-carbaldehyde (23a):

After stirring the solution of LDA (1.6 M in C₆H₆, 4 mL, 6 mmol) in THF (10 mL) at -78 °C for 30 minutes, 20b (723.44 mg, 1mmol) in THF (10 mL) was added dropwise to it at -78 °C. After 1.5 h of stirring at -78 °C, methylformate (0.4 mL, 6 mmol) was added to the solution in one portion. After 1 h of stirring at -78 °C, the reaction was quenched by addition of ammonium chloride. The organic layer was washed with ammonium chloride and brine and dried over Na₂SO₄. The crude product was purified with column chromatography using 90% Hex/EtOAc to afford 36% of 23a. ESI-MS m/z 752.4 [M⁺]; UV λ_max (EtOAc): 266.67 nm; ¹H NMR (400 MHz, CDCl₃): 9.99 (s, 1H), 8.40 (s, 1H), 6.80 (d, 1H, J=5.0Hz), 5.51 (s, 1H), 5.43 (t, 1H, J=4.7Hz), 4.70 (t, 1H, J=3.9Hz), 4.08 (m, 2H), 3.71 (dd, 1H, J=2.2Hz, J=9.4Hz), 1.05 (s, 1H), 0.99 (s, 10H), 0.81 (d, 1H, J=3.1Hz), 0.42 (s, 6H), 0.18 (d, 6H, J=0.9Hz), 0.01 (s, 3H), -0.04 (s, 3H), -0.06 (s, 3H), -0.31 (s, 3H)
Preparation of (S)-2-amino-4-(((2S,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)thio)butanoic acid (32): A mixture of 50 (95 mg, 0.70 mmol) and NaH (45 mg, 1.88 mmol) in DMF (0.7 mL) was stirred overnight at 40 °C. The solution of 39 (60 mg, 0.21 mmol) in DMF (0.9 mL) was then added dropwise at room temperature. The resulting yellow solution was stirred for 24 h at room temperature. After addition of H₂O (2.0 mL), and neutralizing the pH with 5% HCl in H₂O, the mixture was concentrated and purified with HPLC (solvent A = 0.1% TFA in H₂O; solvent B = 35% MeOH, 0.1% TFA in H₂O). ¹H NMR (400 MHz, D₂O): 8.45 (s, 1H), 8.38 (s, 1H), 6.14 (t, 1H, J=36.3Hz), 4.80 (t, 1H, J=5.1Hz), 4.38 (t, 1H, J=5.1Hz), 4.28 (td, 1H, J=4.9Hz, J=6.8Hz), 4.02 (t, 1H, J=6.4Hz), 2.98 (dq, 2H, J=5.9Hz, J=14.2Hz), 2.67 (t, 2H, J=7.5Hz), 2.12 (tttd, 2H, J=7.5Hz, J=14.7Hz, J=36.5Hz)
Preparation of (2R,3R,4S,5R)-2-(6-amino-8-bromo-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (33): Bromine (0.6 mL, 23.4 mmol) was dissolved in 0.5 M aqueous sodium acetate buffer (40 ml, pH 4) and it was then added dropwise to a mixture of adenosine (2 g, 7.48 mmol) in 0.5 M aqueous sodium acetate buffer (80 ml, pH 4) at room temperature. After stirring the orange solution for 24 hours at room temperature, the color of solution was discharged by addition of solid sodium disulfite and the pH of the solution was adjusted to 6-7 with 50% sodium hydroxide solution (w/w). After cooling the solution in ice bath for few hours, the orange precipitate was filtered off and washed with plenty of water and acetone and dried under high vacuum to yield 2.6 g (69%) of the pure product. \(^1\)H NMR (400 MHz, DMSO): 8.12 (s, 1H), 7.58 (s, 2H), 5.83 (d, 1H, J=6.8Hz), 5.48 (m, 2H), 5.23 (d, 1H, J=3.3Hz), 5.09 (dd, 1H, J=6.1Hz, J=11.7Hz), 4.19 (s, 1H), 3.98 (dd, 1H, J=3.9Hz, J=6.3Hz), 3.68 (d, 1H, J=12.5Hz), 3.51 (m, 1H)
Preparation of (2R,3R,4S,5S)-2-(6-amino-8-chloro-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (34b): Thionyl chloride (0.04 mL, 0.54 mmol) was added to a mixture of 33 (60 mg, 0.18 mmol) in anhydrous acetonitrile (0.5 ml) at 0 °C. Pyridine (0.03 ml, 0.36 mmol) was then added and the resulting orange solution was stirred for 4 h at 0 °C and for overnight at room temperature. After cooling the mixture in ice bath, H₂O (7 mL) was added and the pH of the solution was adjusted to 6-7 by addition of solid NaHCO₃. The mixture was extracted with EtOAc and the combined organic layers was washed with saturated NaHCO₃, H₂O, and brine, and dried over Na₂SO₄. The organic layer was concentrated and the resulting yellow solid was dispersed in MeOH (12 mL) and H₂O (2.4 mL). NH₄OH (1.2 mL) was then added to this mixture at 0 °C and the resulting solution was stirred for 4 h at room temperature. The solution was then extracted with EtOAc and washed many times with water. The organic layer was concentrated to give 19 mg (33%) of pure product. ¹H NMR (400 MHz, DMSO): 8.20 (s, 1H), 7.51 (s, 2H), 5.90 (d, 1H, J=5.5Hz), 5.63 (d, 1H, J=5.9Hz), 5.27 (qd, 2H, J=5.5Hz, J=11.1Hz), 4.38 (dd, 1H, J=5.1Hz, J=9.2Hz), 3.97 (dd, 1H, J=5.2Hz, J=11.5Hz), 3.85 (dd, 2H, J=6.7Hz, J=11.5Hz).
Preparation of \((R)-2\text{-amino-3-}((6\text{-amino-9-}((2R,3R,4S,5S)\text{-5-} (\text{chloromethyl)}\text{-3,4-}}\text{dihydroxytetrahydrofuran-2-yl)-9H-purin-8-yl})\text{thio)propanoic acid (37):}\) A mixture of L-cysteine (174 mg, 1.44 mmol) and NaH (60 mg, 2.5 mmol) in DMF (1.2 mL) was stirred overnight at 40 °C. A solution of 34b (128 mg, 0.4 mmol) in DMF (1.7 mL) was added dropwise to this white mixture. The resulting yellow mixture was stirred for 2 h at room temperature. After addition of H\(_2\)O (2 mL), and neutralizing the pH with 5% HCl in H\(_2\)O, the solution was extracted with EtOAc to remove unreacted starting material. The aqueous layer was concentrated and purified with HPLC (solvent A = 0.1% TFA in H\(_2\)O; solvent B = 35% MeOH, 0.1% TFA in H\(_2\)O). \(^1\)H NMR (400 MHz, D\(_2\)O): 8.29 (s, 1H), 6.01 (d, 1H, J=5.4Hz), 5.13 (t, 1H, J=5.5Hz), 4.54 (dd, 1H, J=4.9Hz, J=5.4Hz), 4.42 (dd, 1H, J=3.9Hz, J=7.8Hz), 4.28 (td, 1H, J=4.7Hz, J=6.2Hz), 4.07 (dd, 1H, J=3.9Hz, J=15.2Hz), 3.88 (m, 2H), 3.72 (dd, 1H, J=7.8Hz, J=15.2Hz)
Preparation of (2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (39): Thionyl chloride (0.8 ml, 11.22 mmol) was added to a mixture of adenosine (1 g, 3.74 mmol) in anhydrous acetonitrile (3.8 ml) at 0 °C. Pyridine (0.6 ml, 7.48 mmol) was then added to this mixture and the resulting yellow solution was stirred for 3 h at 0 °C and then 15 h at room temperature. After cooling the mixture in ice bath, the reaction was quenched by addition of H₂O (12 ml) and then the pH of the solution was adjusted to 6-7 by addition of solid NaHCO₃. The solution was extracted with EtOAc and the combined organic layers was washed with saturated NaHCO₃, H₂O, and brine and dried over Na₂SO₄. The organic layer was concentrated and the resulting white solid was dispersed in MeOH (20 mL) and H₂O (4 mL). NH₄OH (1.6 mL) was then added to this mixture at 0 °C and the resulting solution was stirred for 5 h at room temperature. After few minutes of stirring, lots of white precipitates formed which was collected by filtration and was washed with ice-cold MeOH and a small volume of diethyl ether to afford 56% of pure 39. UV λ_max (H₂O): 205.00, 258.33 nm; ¹H NMR (400 MHz, DMSO): 8.34 (s, 1H), 8.16 (m, 1H), 7.31 (s, 2H), 5.93 (d, 1H, J=5.7Hz), 5.59 (d, 1H, J=6.0Hz), 5.45 (d, 1H, J=5.2Hz), 4.76 (dd, 1H, J=5.6Hz, J=11.2Hz), 4.23 (dd, 1H, J=5.1Hz, J=9.0Hz), 4.09 (dd, 1H, J=5.4Hz, J=9.7Hz), 3.90 (ddd, 2H, J=5.7Hz, J=11.6Hz, J=17.9Hz).
Preparation of $(R)$-2-amino-3-(((2S,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)thio)propanoic acid (40): A mixture of L-cysteine (264 mg, 2.18 mmol) and NaH (95 mg, 4 mmol) in DMF (2.5 mL) was stirred overnight at 40 °C. The solution of 43 (250 mg, 0.66 mmol) in DMF (3.5 mL) was then added dropwise at room temperature. The resulting yellow solution was stirred for 6.5 h at 0 °C. After addition of H$_2$O (2.5 mL), and neutralizing the pH with 5% HCl in H$_2$O, the mixture was concentrated and purified with HPLC (solvent A = 0.1% TFA in H$_2$O; solvent B = 35% MeOH, 0.1% TFA in H$_2$O) to give 98 mg (40%) of pure product. $^1$H NMR (400 MHz, D$_2$O): 8.44 (s, 1H), 8.38 (s, 1H), 6.07 (d, 1H, J=4.8Hz), 4.82 (t, 1H, J=5.1Hz), 4.38 (t, 1H, J=5.1Hz), 4.29 (td, 1H, J=4.8Hz, J=7.1Hz), 4.08 (dd, 1H, J=4.3Hz, J=7.7Hz), 3.15 (dd, 1H, J=4.3Hz, J=14.9Hz), 3.00 (m, 3H)

Preparation of $(R)$-2-amino-3-((6-amino-9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9H-purin-8-yl)thio)propanoic acid (41): A mixture of L-cysteine (363 mg, 3 mmol) and NaH (108 mg, 4.5 mmol) in DMF (4.5 mL) was stirred overnight at 40 °C. A solution of 33 (517 mg, 1.5 mmol) in DMF (6.0 mL) was added dropwise to this white mixture and the resulting yellow mixture was stirred for 15 mins at 0 °C and 1 h at room temperature. After addition of H$_2$O (4 mL), and neutralizing the pH with 5% HCl in H$_2$O, the mixture was concentrated and purified with HPLC (solvent A = 0.1% TFA in H$_2$O; solvent B = 35% MeOH, 0.1% TFA in H$_2$O). $^1$H NMR (400 MHz, D$_2$O): 8.31 (s, 1H), 5.99 (d, 1H, J=6.2Hz), 4.97 (t, 1H, J=5.9Hz), 4.41 (m, 2H), 4.18 (td, 1H, J=3.6Hz, J=7.3Hz), 4.08 (dd, 1H, J=3.9Hz, J=15.2Hz), 3.82 (dq, 2H, J=4.3Hz, J=12.6Hz), 3.72 (dd, 1H, J=7.9Hz, J=15.2Hz)
Preparation of (2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-5-(iodomethyl)tetrahydrofuran-3,4-diol (43): A solution of adenosine (1 g, 3.75 mmol), iodine (1.5 g, 5.72 mmol), and triphenylphosphine (1.14 g, 5.72 mmol) in pyridine (8 mL) was stirred at room temperature for 2 h. Excess iodine was quenched by addition of saturated aqueous solution of Na$_2$S$_2$O$_3$.5H$_2$O. The solution was then extracted with EtOAc and dried over Na$_2$SO$_4$. The crude product was purified using isolera (9:1 CHCl$_3$/MeOH) to give 18% 43. $^1$H NMR (400 MHz, DMSO): 8.39 (s, 1H), 8.17 (s, 1H), 7.32 (s, 2H), 5.94 (d, 1H, J=5.8Hz), 5.59 (d, 1H, J=6.1Hz), 5.48 (d, 1H, J=5.1Hz), 4.83 (dd, 1H, J=5.7Hz, J=11.2Hz), 4.19 (dd, 1H, J=4.9Hz, J=8.7Hz), 4.03 (m, 1H), 3.63 (dd, 1H, J=5.9Hz, J=10.4Hz), 3.49 (dd, 1H, J=6.9Hz, J=10.4Hz)

Preparation of (2R,3R,4S,5S)-2-(6-amino-8-bromo-9H-purin-9-yl)-5-(iodomethyl)tetrahydrofuran-3,4-diol (44): A solution of 33 (1 g, 2.9 mmol), iodine (1.14 g, 4.35 mmol), and triphenylphosphine (1.14 g, 4.35 mmol) in pyridine (6.5 mL) was stirred at room temperature for 2 h. Excess iodine was quenched by addition of saturated aqueous solution of Na$_2$S$_2$O$_3$.5H$_2$O. The solution was then extracted with EtOAc and dried over Na$_2$SO$_4$. The crude product was purified using isolera (9:1 DCM/MeOH). Yield: 18%; $^1$H NMR (400 MHz, DMSO): 8.17 (s, 1H), 7.51 (s, 2H), 5.87 (d, 1H, J=5.6Hz), 5.77 (s, 1H), 5.56 (dd, 1H, J=5.6Hz, J=37.6Hz), 5.38 (dd, 1H, J=5.6Hz, J=11.1Hz), 4.34 (dd, 1H, J=5.0Hz, J=8.7Hz), 4.03 (m, 1H), 3.56 (ddd, 2H, J=6.6Hz, J=10.4Hz, J=17.7Hz)
Preparation of 9-((2R,3R,4R,5R)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-8-bromo-9H-purin-6-amine (46):
Tert-butyldimethylsilyl chloride (180 mg, 1.16 mmol) and imidazole (160 mg, 2.32 mmol) were added to a mixture of 33 (100 mg, 0.29 mmol) in DMF (1,2 mL) and stirred for 24 h at room temperature. The orange solution was then quenched by addition of saturated aqueous solution of NH₄Cl and extracted with EtOAc. The organic layer was washed with H₂O and brine, and dried over Na₂SO₄. The after concentrating the organic layer, it was purified with isolera (1:1 Hex/EtOAc) to give 185 mg (93%) of the pure product. ¹H NMR (400 MHz, CDCl₃): 8.29 (s, 1H), 5.99 (d, 1H, J=5.8Hz), 5.72 (s, 2H), 5.54 (dd, 1H, J=4.4Hz, J=5.7Hz), 4.61 (dd, 1H, J=4.6Hz, J=8.0Hz), 4.11 (m, 2H), 3.75 (q, 1H, J=7.7Hz), 1.00 (s, 9H), 0.85 (d, 18H, J=16.8Hz), 0.19 (d, 6H, J=1.8Hz), 0.02 (m, 9H), -0.30 (s, 3H)

Preparation of ((2R,3R,4R,5R)-5-(6-amino-8-bromo-9H-purin-9-yl)-3,4-bis((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methanol (47): To an ice-cold solution of 46 (2 g, 2.91 mmol) in THF (35 mL) was added an ice-cold solution of TFA:H₂O (8.75 mL: 8.75 mL) in a dropwise manner. After 4 h of stirring at 0 °C, the reaction was quenched by addition of ice-cold saturated solution of sodium bicarbonate. The aqueous layer was washed with ethyl acetate and organic layer was washed with H₂O and brine and dried over Na₂SO₄. The organic layer was then concentrated and purified with isolera to give 72% yield. ¹H NMR (400 MHz, CDCl₃): 8.34 (m, 1H), 6.51 (d, 1H, J=11.5Hz), 6.09 (d, 1H, J=8.0Hz), 5.83 (s, 1H), 5.13 (dd, 1H, J=4.6Hz, J=8.0Hz), 4.36 (d, 1H, J=4.6Hz), 4.18 (s, 1H), 3.95 (dd, 1H, J=1.1Hz, J=13.0Hz), 3.73 (t, 1H, J=11.6Hz), 0.99 (m, 9H), 0.81 (s, 9H), 0.15 (m, 6H), -0.09 (s, 3H), -0.54 (s, 3H)
Preparation of (4S)-2,4-dicarboxy-1,3-thiazinan-3-ium chloride (49): DCA (4.2 mL, 100 mmol) was added to a mixture of methionine (3.75g, 25mmol) in concentrated HCl (50 mL) and the resulting pale yellow solution was refluxed overnight during which lots of precipitate formed. These white precipitates were collected using suction filtration and was washed with THF to give 20% pure 49. $^1$H NMR (400 MHz, D$_2$O): 1.95 (dtd, 1H, J=3.6Hz, J=12.6Hz, J=14.9Hz), 2.58 (m, 1H), 2.91 (m, 1H), 3.09 (ddd, 1H, J=2.7Hz, J=12.5Hz, J=15.0Hz), 3.97 (dd, 1H, J=3.1Hz, J=12.9Hz), 5.03 (s, 1H).

Preparation of (S)-1-carboxy-3-mercaptopropan-1-aminium chloride (50): The pH of 49 (100 mg, 0.44 mmol) in ethanol (2.2 mL) was adjusted to 7 by NEt$_3$. Then 0.5 M hydroxyamine hydrochloride in ethanol (0.45 mL) was added to the mixture at reflux and the pH was quickly readjusted to 7 using NEt$_3$. After refluxing for 25 minutes, another portion of 0.5 M hydroxyamine hydrochloride in ethanol (0.45mL) was added to the solution and again the pH was quickly readjusted to 7 using NEt$_3$. After refluxing the mixture for 1h, the white solid was collected by suction filtration to afford 15% pure 50. $^1$H NMR (400 MHz, D$_2$O): 3.81 (dd, 1H, J=5.7Hz, J=7.1Hz), 2.58 (m, 2H), 2.08 (m, 2H)
6.3 NMR data

**Figure 31.** $^1$H NMR spectra for compound 14
Figure 32. $^1$H NMR spectra for compound 15
Figure 33. $^1$H NMR spectra for compound 16
Figure 34. $^1$H NMR spectra for compound 17
Figure 35. $^1$H NMR spectra for compound 18
Figure 36. \( ^1 \)H NMR spectra for compound 20a
Figure 37. $^1$H NMR spectra for compound 20b
Figure 38. $^1$H NMR spectra for compound 20c
Figure 39. $^1$H NMR spectra for compound 20d
Figure 40. $^1$H NMR spectra for compound 20e
Figure 41. $^1$H NMR spectra for compound 21a
Figure 42. $^1$H NMR spectra for compound 21b
Figure 43. $^1$H NMR spectra for compound 23a
Figure 44. $^1$H NMR spectra for compound 32
Figure 45. $^1$H NMR spectra for compound 33
Figure 46. $^1$H NMR spectra for compound 34b
Figure 47. $^1$H NMR spectra for compound 37
Figure 48. $^1$H NMR spectra for compound 39
Figure 49. $^1$H NMR spectra for compound 40
Figure 50. $^1$H NMR spectra for compound 41
Figure 51. $^1$H NMR spectra for compound 43
Figure 52. $^1$H NMR spectra for compound 44
Figure 53. $^1$H NMR spectra for compound 46
Figure 54. ¹H NMR spectra for compound 47
Figure 55. $^1$H NMR spectra for compound 49
Figure 56. $^1$H NMR spectra for compound 50
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