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ABSTRACT

DESIGN AND SYNTHESIS STUDIES OF NEW S-ADENOSYL-L-METHIONINE ANALOGUES

**by
Shanshan Wu**

S-Adenosyl-L-Methionine (SAM), which is involved in methyl group transfers, is the second most abundant coenzyme in the human body. It is made from adenosinetriphosphate (ATP) and methionine catalyzed by adenosyltransferase. Methyltransferases (MTs) transfer the activated methyl group from the sulfur center to a specific position in a variety of substrates, e.g., DNA, RNA, proteins, and secondary metabolites. Methyltransferases have been linked to various diseases, including cancer.

In recent years, more and more SAM analogues have been reported. These analogues show activities in biological target labeling. The purpose of this thesis is to design and synthesize new SAM analogues to inhibit cytosine DNA Methyltransferases in tumor cells. A series of target compounds was designed and synthesized in several steps from the starting material adenosine. The synthesis route has been divided into three parts: introduction of the nitrogen at the 5' position, synthesis of aromatic aldehyde, and connection of these two compounds. More compounds with the same core structure will be designed and synthesized. The inhibition activities will be tested in future studies.

**DESIGN AND SYNTHESIS STUDIES OF NEW
S-ADENOSYL-L-METHIONINE ANALOGUES**

**by
Shanshan Wu**

**A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Pharmaceutical Chemistry**

Department of Chemistry and Environmental Science

May 2012

APPROVAL PAGE

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S-ADENOSYL-L-METHIONINE ANALOGUES
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This thesis is dedicated to my parents and my brother for their love, support and encouragement

谨以此文，献给给予我无限爱与鼓励的家人

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TABLE OF CONTENTS

Chapter	Page
1 BACKGROUND.....	1
1.1 Definition	1
1.2 SAM Cycle	4
1.3 Medical Aspect	5
1.4 SAM Analogues	7
1.5 DNMT Inhibitors.....	9
2 PURPOSE AND DESIGN CONCEPT.....	12
2.1 Purpose	12
2.2 Crystal Structure Study and Design Concept.....	13
3 EXPERIMENTAL	21
3.1 Synthesis Part1.....	22
3.2 Synthesis Part2.....	25
3.3 Synthesis Part3.....	28
4 EXPERIMENTAL INFORMATION.....	30
5 CONCLUSIONS AND FUTURE STUDIES.....	42
REFERENCES.....	43

LIST OF FIGURES

Figure	Page
1.1 The structure of SAM.	1
1.2 The structure of SAH.	2
1.3 The mechanism of DNA methyl transfer.....	3
1.4 The SAM regeneration cycle.....	5
1.5 The structures of two SAM analogues.....	8
1.6 The structures of 5'-N-chloroethylamino-5'-deoxyadenosines	8
1.7 The structure of an azide-bearing N-mustard SAM analogue	9
1.8 The structure of Vidaza® (azacitidine) and Dacogen® (decitabine).....	10
1.9 The mechanism of 5-azanucleoside analogues... ..	10
1.10 The structure of a potent DNMT1 inhibitor	11
2.1 A cartoon for the function of DNMT1 inhibitors	13
2.2 Interaction between SAH and DNMT1	15
2.3 Superposition	15
2.4 The crystal structure of SAH and Human DNMT1-DNA complex.....	16
2.5 Cytosine stabilized by surrounding residues in the active site of the enzyme...	17
2.6 Design of a transition state inhibitor of DNMT	18
2.7 Structure of compounds B , C , D and E	19
2.8 The electrostatic potential maps	20
3.1 Retrosynthetic analysis of target compound	21
3.2 The byproduct of reaction e	25

LIST OF FIGURES
(Continued)

Figure		Page
3.3	The structure of aromatic molecules	27

LIST OF SCHEMES

Scheme	Page
3.1 Synthesis route of compound 5	22
3.2 Supposed ethyl 4-aminobutanoate hydrochloride –reaction	23
3.3 Protect of the 6-amino group of adenosine	24
3.4 Supposed synthesis route of compound 8	25
3.5 Swern and PCC oxidation of 4-nitrophenylethanol	28
3.6 Synthesis route of Part 3.....	29

LIST OF SYMBOLS

Ac ₂ O	Acetic anhydride
ATP	Adenosine triphosphate
DCM	Dichloromethane or methylene chloride
DMF	Dimethylformamide
DNMT	DNA methyltransferase inhibitors
dSAM	decarboxylated SAM, S-adenosylmethioninamine
Me-THF	methyltetrahydrofolate
MsCl	Methanesulfonyl chloride
MTs	Methyltransferases
PCC	Pyridinium chlorochromate
Pi	Phosphate
PPi	Pyrophosphate
p-TsOH	p-Toluenesulfonic acid
SAM	S-Adenosyl-L-Methionine
SAH	S-adenosylhomocysteine
THF	Tetrahydrofuran
PRMT1	Protein methyltransferases 1

CHAPTER 1

BACKGROUND

1.1 Definitions

S-Adenosyl-L-Methionine (Figure 1.1) (SAM) is the second most abundant coenzyme in the human body. SAM is involved in methyl group transfer reactions catalyzed by a variety of methyltransferases. SAM was first discovered in Italy by G. L. Cantoni in 1952 as an active methyl donor in nature. ^[1] It is formed enzymatically from L-methionine and adenosine triphosphate (Eq. 1.1).



In vivo, the inorganic pyrophosphate (PPi) formed concomitantly with S-adenosylmethionine. PPi is hydrolyzed by pyrophosphatase to two equivalents of phosphates (Pi). Consequently, the synthesis of SAM is metabolically costly owing to consumption of all three phosphoryl groups of ATP. The enzyme that catalyzes the formation of SAM is S-adenosylmethionine synthetase (adenosyl transferase, MAT).

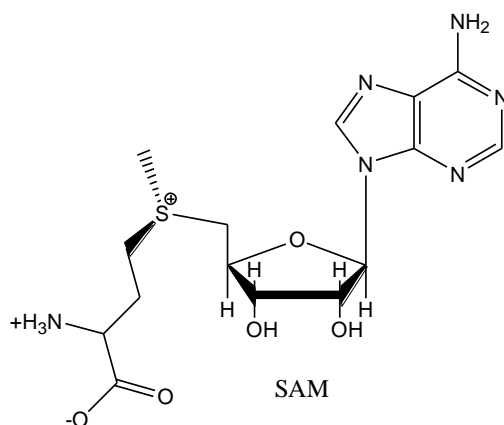


Figure 1.1. The structure of SAM.
(2S)-2-Amino-4-[[[(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl-methylsulfonio]butanoate.

The stereochemical configuration at the sulfur in enzymatically formed S-adenosylmethionine is (S); however, S-adenosylmethionine spontaneously racemizes to the R isomer over a period of a few days. The carbons attached to the positively charged sulfur are electrophilic and readily transferred.

The methyl group attached to the methionine sulfur atom in SAM is chemically reactive. This allows donation of this active methyl group from the sulfur center to a specific position in a variety of substrates, e.g., DNA, RNA, proteins, and secondary metabolites catalyzed by methyltransferases (MTs) (Eq. 1.2). More than 40 metabolic reactions use this mechanism, and the product is S-adenosylhomocysteine (Figure 1.2) (SAH), which is an inhibitor of most methylases.

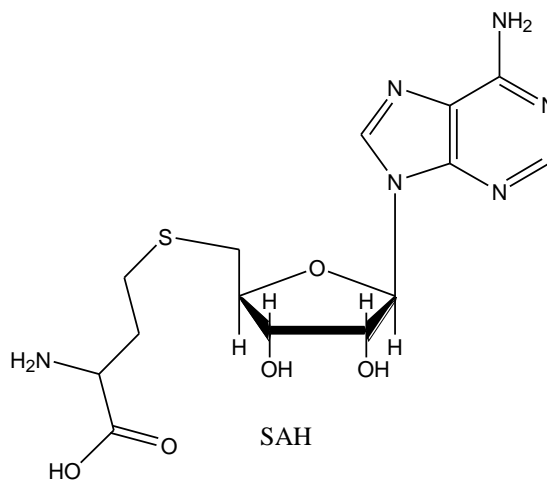
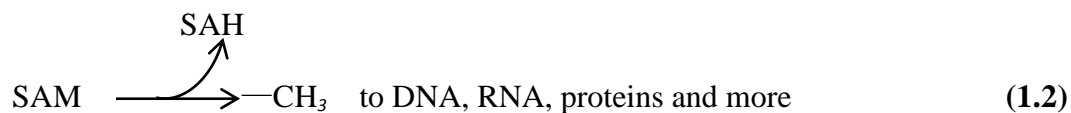


Figure 1.2. The structure of SAH. S-(5'-Deoxyadenos-5'-yl)-L-homocysteine.



The intracellular SAM/SAH ratio is considered to be a regulator of the activity of these enzymes. The less the SAM/SAH ratio, the more tendency SAH binds and inhibits MTs. A dynamic balance exists between free SAH and bound SAH.

This thesis is specifically concerned with DNA methyltransferases (DNMTs). All the known DNA methyltransferases use SAM as the methyl donor.

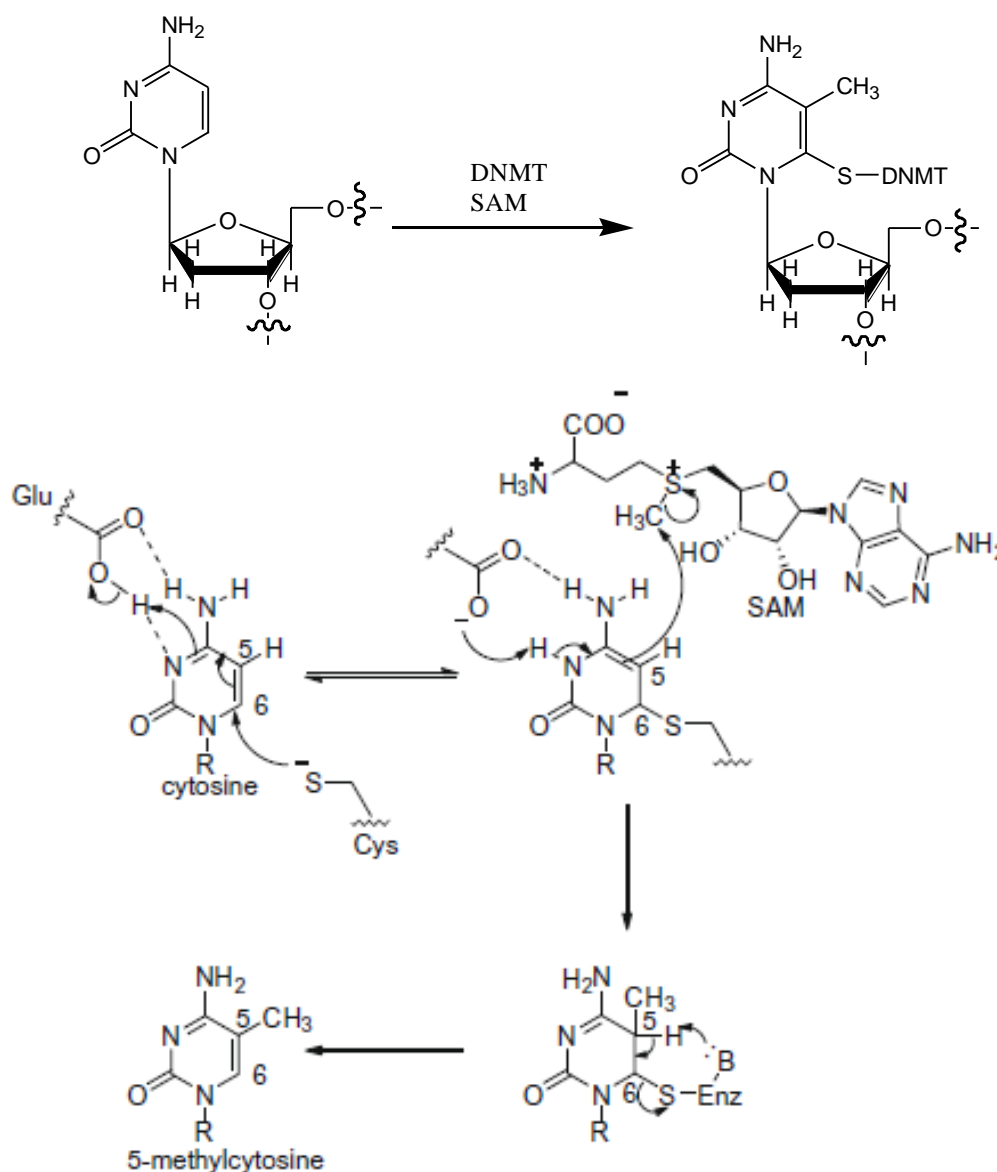


Figure 1.3. The process and mechanism for methylation of cytosine-5.

There are five different DNMTs known in mammals: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L. DNMT1 (DNA cytosine-5-methyltransferase 1) is responsible for the maintenance of methylated DNA by copying DNA methylation patterns from the parental strand to the newly synthesized daughter strand during the DNA replication process. It helps regulate gene expression, genome imprinting, and x-chromosome inactivation.

The catalytic mechanism for the methylation of cytosine-5 has been studied extensively, and is shown as Figure 1.3. The thiol group of a cysteine residue in the active site of DNMTs serves as a nucleophile that attacks the 6-position of cytosine to generate a covalent DNA-protein intermediate that possesses nucleophilic properties at the 5-position. This reactive intermediate accepts a methyl group from SAM to form the 5-methyl covalent adduct and SAH. Following the methyl transfer, the proton at the 5-position is abstracted by a basic residue in the active site of the enzyme that is later removed from the 6-position by β -elimination to generate the methylated cytosine and the free enzyme.^[2]

1.2 SAM Cycle

One of the essential metabolic functions of the body is active methyl donation. The reactions that produce, consume, and regenerate SAM are called the SAM cycle, Figure 1.4.

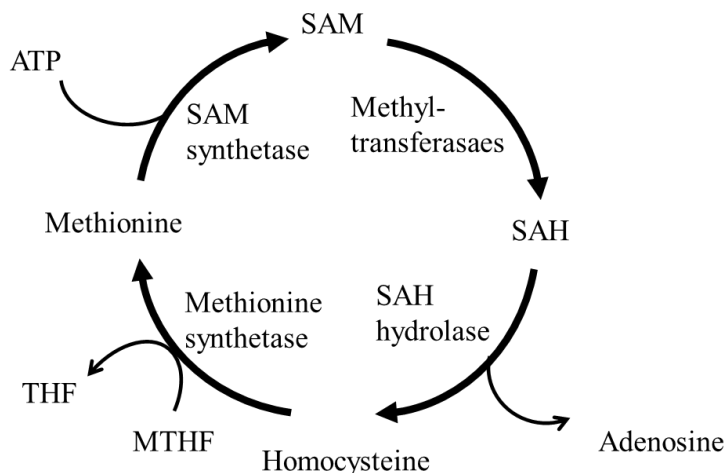


Figure 1.4. The SAM regeneration cycle.

In the first step of this cycle, SAM is produced from methionine by the addition of ATP. After the methyl group has been donated, homocysteine remains. Because homocysteine has pro-oxidant properties (elevated levels are associated with cardiovascular disease), it must be detoxified. Two separate mechanisms exist, both of which are serine dependent. In the first mechanism, homocysteine is re-methylated by methyltetrahydrofolate (Me-THF, or “activated” folic acid) back to methionine, and the SAM cycle is closed. Under most circumstances, this should be the dominant pathway. In the second mechanism, homocysteine is combined with serine to form cystathionine which is split back apart (slightly differently) to yield cysteine and homoserine. This reaction depends on the enzyme cystathionine beta-synthase which opens up the SAM cycle and results in loss of methionine (and accumulation of cysteine).^[3]

1.3 Medical Aspect

SAM is a naturally occurring compound that is found in almost every tissue and fluid in the body. It is involved in many important processes. Humans synthesize approximately 7

grams of SAM per day, primarily in the liver. SAM plays a role in the immune system, maintains cell membranes, and helps produce and break down brain chemicals, such as serotonin, melatonin, and dopamine. It works with vitamin B12 and folate (vitamin B6). Being deficient in either vitamin B12 or folate may reduce levels of SAM in the body.

In the United States and Canada, SAM is sold as a nutritional supplement under the marketing name SAM-e (also spelled SAME or SAME). Some research, including multiple clinical trials, has indicated taking SAM on a regular basis may help fight depression,^[4] liver disease, and the pain of osteoarthritis.^[5]

Formation of SAM is a major pathway in the metabolism of dietary methionine, which is a requirement for the growth of many tumour cells. Inhibitors of the polyamine biosynthetic enzyme S-adenosylmethionine decarboxylase have been investigated clinically in the contexts of anticancer and antiparasitic therapies.^[6]

Epigenetic regulation involving SAM includes methylation at the 5' position of cytosine in CpG dinucleotides, histones, and several nonhistone transcriptional regulators, such as the tumor suppressor protein p53 and the general transcriptional factor TAF10.^[7] It is a pivotal mechanism for the epigenetic regulation of gene expression. DNA methylation represses transcription and is essential for the maintenance of chromatin structure and genomic stability. Alterations of the promoter methylation have been associated with changes of transcription profiles of cancers. Research has shown that improper DNA methylation can either activate or inactivate a gene. If certain genes are activated when they are supposed to be inactive, or inactivated when they are supposed to be active, cancer can result.

Furthermore, methylation of homocysteine re-generates methionine. The methyl donor in this case is either betaine, which is derived from choline, or 5-methyl-tetrahydrofolate. Thus, the SAM/SAH ratio may also be tied to metabolism of phospholipids and folic acid. How the status of SAM/SAH, phospholipids, and folate may affect the life span, and whether such an effect contributes to epigenetic control are interesting questions, and they may turn out to be highly relevant to human health.

Although the roles of SAM in cancer cells are not completely understood currently, studies have demonstrated that DNMTs are highly expressed in cancer cells. Inhibition of DNMTs has resulted in anti-cancer activities. More details will be discussed in section 1.5.

1.4 SAM Analogues

In recent years, many SAM analogues have been developed to label and identify biological targets, e.g., the SAM riboswitch aptamer and protein arginine methyltransferases, or to probe methylation patterns.^{[8][9]} These compounds contain reactive functional groups that can be conjugated with reporters such as fluorophores (Figure 1.5). The compound on the left in Figure 1.5 demonstrated 2-fold higher binding over the original metabolite SAM toward the SAM riboswitch aptamer (the K_d of this compound is 3.1 μM , while that of SAM is 6.0 μM). This compound may constitute a novel antibiotic. In the second example, the researchers combined SAM analogues (the compounds on the right in Figure 1.5) with a protein-engineering approach to label and identify PRMT1 target in the context of a complex cellular mixture. The SAM-binding pocket of PRMT was modified to utilize a clickable SAM analogue for substrate labeling.

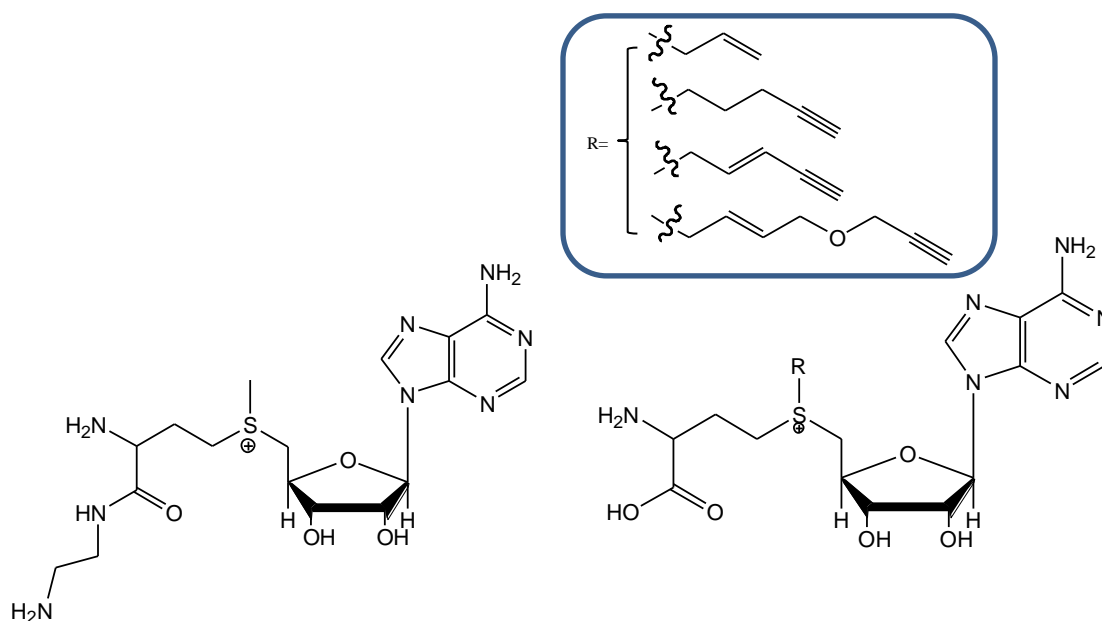


Figure 1.5. The structures of two new SAM analogues which have been reported, left: a new analogue targeting SAM riboswitch aptamer; right: SAM analogues labeling substrates of protein arginine methyltransferase.

Another type of SAM analogues is N-chloromustard-substituted adenosines which were expected to react with the proteins that the analogues bind (Figure 1.6).^[10] The reason to replace simple alkyl N-iodomustards by the chlorine analogues is the concern of the high reactivity of alkyl N-iodomustards. Although these nitrogen-containing SAM analogues did not react with the protein targets as expected, they were used for a detailed investigation of the reaction kinetics of aziridine formation.^[11]

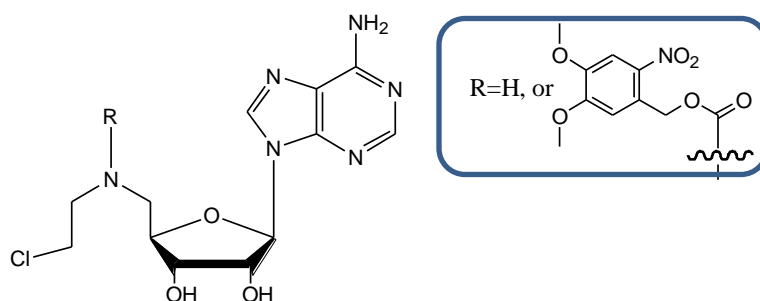


Figure 1.6. The structures of 5'-N-chloroethylamino-5'-deoxyadenosines.

Recently, a new advanced N-iodomustard SAM analogue has been reported. This SAM analogue (Figure 1.7) bears an azide group, which would be capable of undergoing the Staudinger ligation or copper-catalyzed azide–alkyne cycloaddition (CuAAC or Click) chemistry. This bifunctional molecule was designed to covalently label DNA and protein in a methyltransferase-dependent fashion. It was successfully synthesized and shown to be transferred to both oligonucleotides and plasmid DNA using a variety of DNA methyltransferases.^[12]

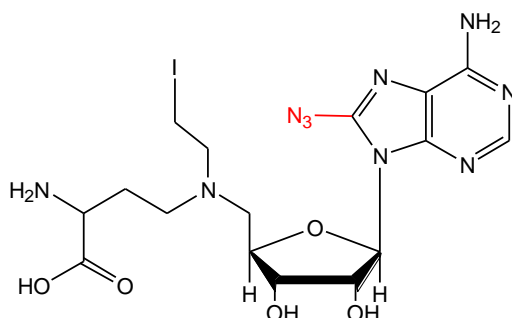


Figure 1.7. The structure of an azide-bearing N-mustard SAM analogue.

1.5 DNMT1 Inhibitors

DNMT1 has been linked to various diseases including cancer. DNMT1 inhibitors are regarded as potential anticancer agents. Several DNMT1 inhibitors for the treatment of neoplastic diseases have been developed. Vidaza[®] (azacitidine) and Dacogen[®] (decitabine), which are irreversible, are among the new agents approved by the FDA for the treatment of myelodysplastic syndrome.^[13] The structures of these two drugs are shown as Figure 1.8. These 5-azanucleoside analogues can be incorporated into DNA to replace cytidine and the absence of a hydrogen atom at the C5 position hinders DNMT release by β -elimination, with consequent DNMT trapping and degradation (Figure 1.9).

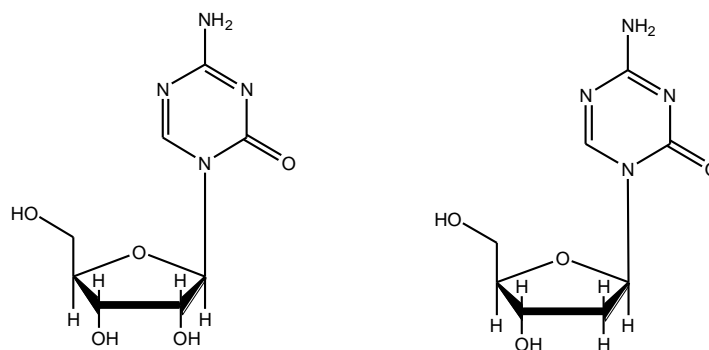


Figure 1.8. The structure of Vidaza® (azacitidine) and Dacogen® (decitabine) which are DNMT inhibitors approved by FDA for the treatment of myelodysplastic syndrome.

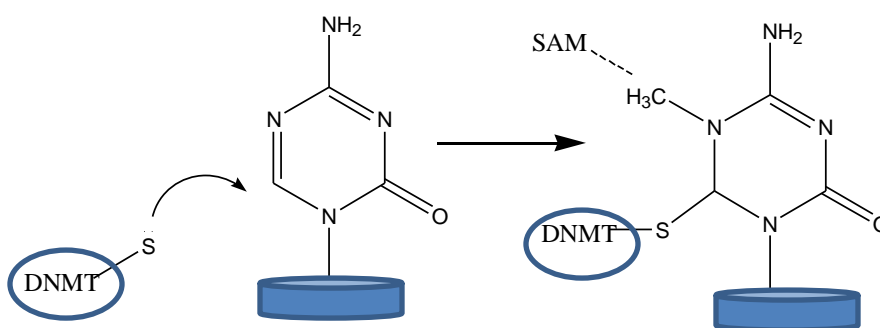


Figure 1.9. The mechanism of 5-azanucleoside analogues induced degradation of DNMTs.

Although many similar suicide inhibitors of DNMT1 have been developed, few reports on selective reversible DNMT1 inhibitors can be found in the literature. Recently, a series of Δ^2 -isoxazoline constrained analogues of procaine were prepared and their inhibitory activity against DNMT1 was tested by S. Castellano and her colleagues. Among these reversible inhibitors, derivative a (Figure 1.10) is the most potent drug in vitro ($IC_{50} = 150 \mu M$) and it has been a novel lead compound for the development of non-nucleoside DNMT inhibitors.^[14] The binding mode of derivative a with the enzyme was investigated by means of a simple competition assay as well as by docking simulations on to a crystallographic structure of human DNMT1.^[14] The nitro group was shown to interact with Val1580, Leu1153 and Gly1150 of the enzyme. There is also a hydrophobic

interaction between the phenyl ring of compound a and the DNMT1 binding pocket. This work demonstrated that it is possible to develop reversible inhibitors to specifically target DNMTs.

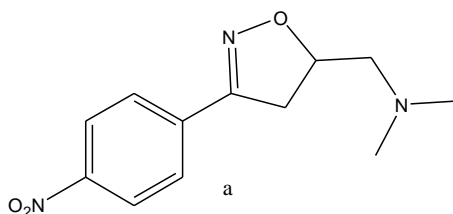


Figure 1.10. The structure of a potent DNMT1 inhibitor developed by S. Castellano and her colleagues. ^[14]

CHAPTER 2

PURPOSE AND DESIGN CONCEPT

1.1 Purpose

Cancer, known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million) and the rates are still rising.^[16] More and more anticancer drugs have been developed over the last three decades, such as Generic Cytosar (Cytarabine), Generic Eloxatin (Oxaliplatin). Many of the anticancer drugs are irreversible, which means they form covalent adducts with the molecular targets and the inhibition cannot be reversed. This irreversibility may result in high cellular toxicity. Therefore, reversible inhibition of targets in cancer cells is highly desirable.

There is a lack of reports on the inhibition effect of SAM analogues on DNA methyltransferase. SAH is a known strong inhibitor of DNA methyltransferases at the SAM binding site. However, SAH-binding proteins are widely found in cells and it is unlikely to use SAH to selectively inhibit DNMTs. The purpose of this thesis is to design and synthesize a series of new SAM analogues to work as DNMT1 inhibitors. These analogues should fill the following requirements. First, they can specifically inhibit cytosine DNA methyltransferases, and have less activities on other similar targets, such as protein methyltransferases. The crystallographic structure of human DNMT1 is a very important tool to guide the design. Second, as mentioned before, reversible inhibition needs to be developed. The interactions between the ligands and the target may be H-bonding and hydrophobic effects instead of covalent bonding.

2.1 Crystal Structure Study and Design Concept

A combination of mechanism-based design (transition state inhibitor) and structural modifications was the basic design concept in this thesis. The crystal structures of DNA methyltransferases made a contribution to the understanding of the enzyme binding pocket. Based on the well-understood reaction mechanism, a lead SAM analogue was designed to mimic the reaction transition state while occupying the binding pocket. Then, a series of SAM analogues was also designed in succession by structural modifications.

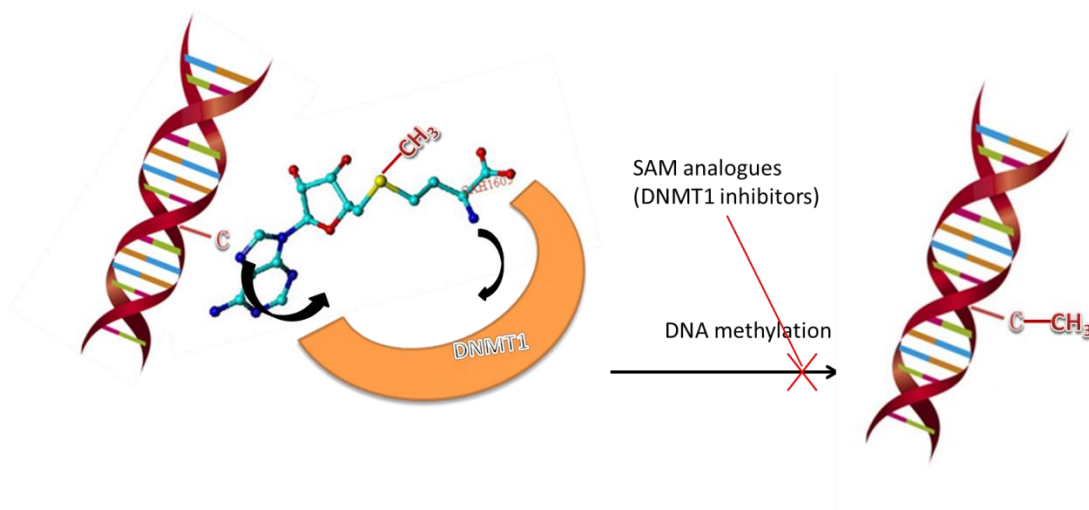


Figure 2.1. A cartoon of cytosine DNA methyltransfer process and the function of DNMT1 inhibitors.

In the process of cytosine DNA methyl transfer, DNA binds to the DNMT1 pocket first through hydrogen-bonding and electrostatic interactions.^[17] The enzyme-DNA complex helps form the correct binding pocket for the free SAM. Then, the active enzyme can transfer the methyl group from the sulfur center to the 5' position of cytosine. While it is difficult for a small molecule to interrupt the entire binding between DNA to DNMT, it

is likely for a small molecule (such as SAH) to enter the SAM binding site and restrict or slow the reaction, (Figure 2.1).

There is more than one binding site within DNMT1. The one for SAM, and the one for the extrahelical cytosine of the duplex DNA are close to each other. It is possible to design an SAH analogue to occupy both pockets at the same time. In the process of DNA methylation, DNA binds to the enzyme first. There is a concern whether the SAH analogues would bind to the enzyme in its active conformation in the absence of the DNA which contains an extrahelical cytosine. Therefore, a superposition study of a bound SAH in free mDNMT1 and the productive covalent mDNMT1-DNA complex (Figure 2.3) is needed to evaluate the significance of the change. Crystal structures of DNMT co-crystallized with SAH shows that SAH undergoes a slight positional shift from the DNA-free state to its productive covalent complex with DNA. This slight positional shift is in response to the covalent link between Cys1229 and the flipped-out cytosine in a duplex DNA, and helps accommodate the newly added 5-methyl group. However, the overall structural change is so small that the SAH analogue would bind the protein active conformation whether the DNA is in the presence or not. Interactions between SAH and DNMT1 with and without DNA were (PDB: 3PT6 and 3PT9) shown as Figure 2.2. ^[18]

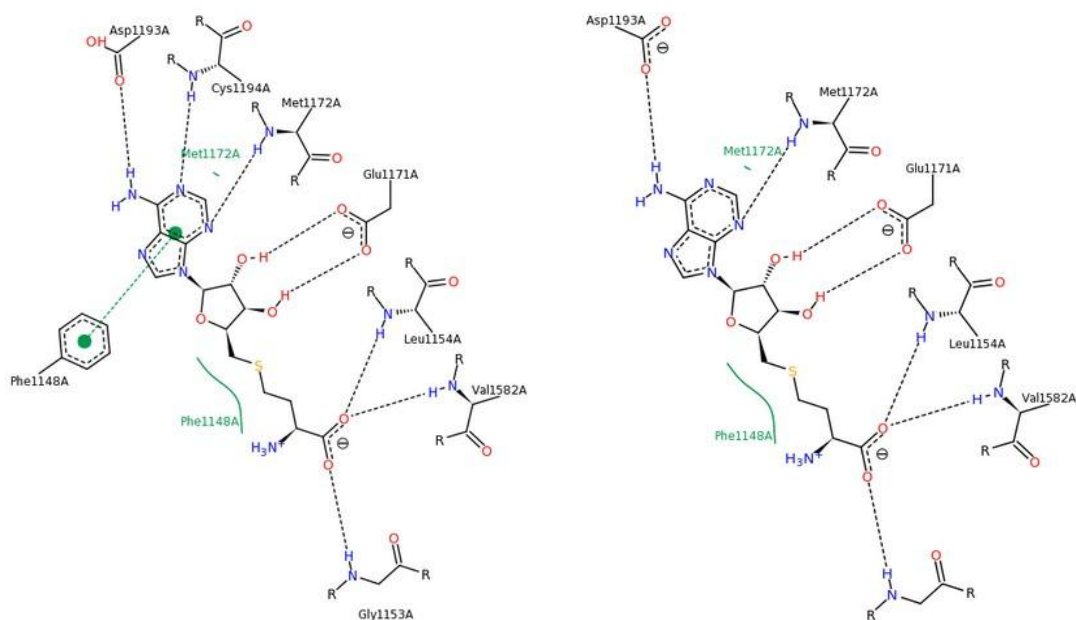


Figure 2.2. Left: Interactions between SAH and binding pocket residues in free mDNMT1 (PDB: 3PT9). Right: Bound SAH in the productive covalent mDNMT1-DNA complex is stabilized. Hydrogen bonds: black dashed lines; hydrophobic interactions: green dashed lines. ^[18]



Figure 2.3. Superposition of bound SAH in free mDNMT1 (blue color) and the productive covalent mDNMT1-DNA complex (pink color), using Alchemy. Superposed atoms are the sulfur atom and all the atoms of adenosine.

A crystal structure containing a hemimethylated DNA trapped with DNMT and SAH was examined to facilitate the design. The unmethylated cytosine adopts an extra helical position to insert into a cytosine binding site that is in close proximity to the sulfur of SAH (Figure 2.4) The distance between the carbon at 5 position of cytosine and the sulfur atom of SAH is 4.5 Å. The flipped-out cytosine was stabilized by surrounding residues in the active site of the enzyme. (Figure 2.5) It was shown that the carbonyl of the

cytosine forms two hydrogen bonds with Arg 1313 and Arg 1315, and the 3-nitrogen forms hydrogen bond with Glu 1269. The 4-amino group forms hydrogen bond with Pro 1227. This result indicates that the carbonyl group, the 3-position nitrogen and the 4-position amino group of cytosine may play vital roles in binding.

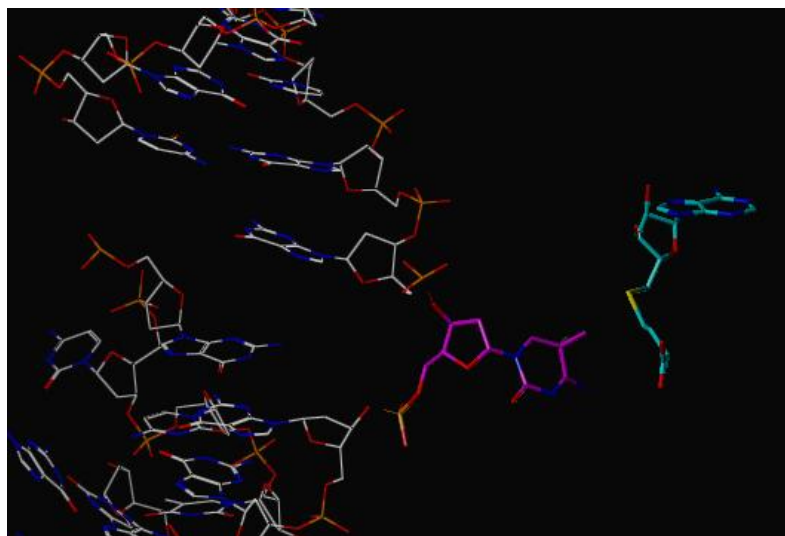


Figure 2.4. The crystal structure of SAH and Human DNMT1-DNA complex (PDB: 4DA4), using SYBYL-X1.3. SAH1701 in chain A, Cytosine4918 in chain D, and parts of the DNA sequences in chain D were selected and shown only.

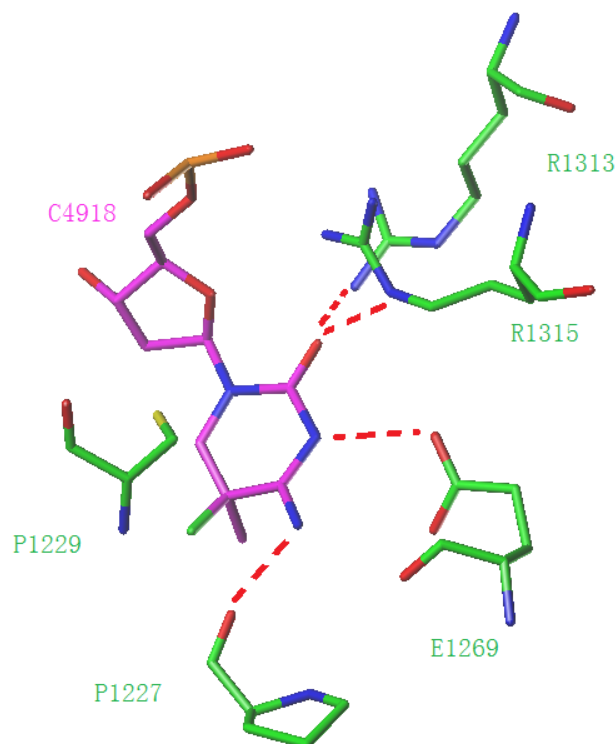


Figure 2.5. The flipped-out cytosine is stabilized by surrounding residues in the active site of the enzyme. ^[18]

A transition state analogue was designed to occupy the SAM binding site and the cytosine binding site simultaneously and force the cytosine to adopt an intrahelical position. After the crystal structure studies, it was decided that part of the SAM core structure was to be kept and part to be deleted.

First, adenosine and the long chain should be kept. As Figure 2.2 shows, SAH can form several hydrogen bonds with the amino acid residues at the binding site of DNMT1, such as Glu 1171, Met 1172, Leu 1154, Val 1582, Gly 1153. Therefore, to maintain these interactions between DNMT1 and SAM analogues, the adenosine and carboxyl group with the long chain should be kept. Second, to be a good inhibitor, extra binding interactions other than the ones involving SAH are required. The process of DNA cytosine methylation involves the addition of a methyl group to cytosine at the C5 position. Therefore,

introducing a cytosine to the SAM analogue may enhance binding by taking advantages of the interactions between cytosine and cytosine-binding side chains. To simplify the synthesis, it was decided to use isocytosine to take the place of the cytosine which is connected to the sulfur atom. The hydrogen bonding sites on the cytosine base may be necessary to enhance binding, despite still being a hypothesis. Other functionalized aromatic rings (benzene or heterocyclic rings) may also be used to play the same role as the cytosine. Third, the sulfur atom with positive charge can be changed to a nitrogen atom, which is expected to be protonated under physiological conditions. Fourth, the α -amino group of SAM can be removed because it is not involved in any interaction with the protein, and it may affect the protonation state of the adjacent tertiary amine.

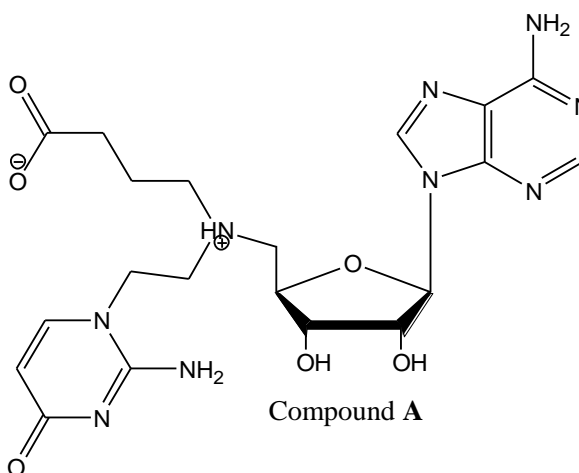


Figure 2.6. Design of a transition state inhibitor of DNMT.

In summary, the design of SAM analogues was inspired by the catalytic mechanism of DNMT and the classic DNMT inhibitors like Vidaza® (azacitidine) and Dacogen® (decitabine). In addition, the study of the crystal structure of DNMT/DNA/SAM complex made a contribution. The first target compound (compound A) is shown as Figure 2.6. Based on this first SAM analogue, a series of compounds was designed by changing the

substitutes of the 5'-amine, (Figure 2.7). The electrostatic potential maps of these compounds are shown as Figure 2.8.

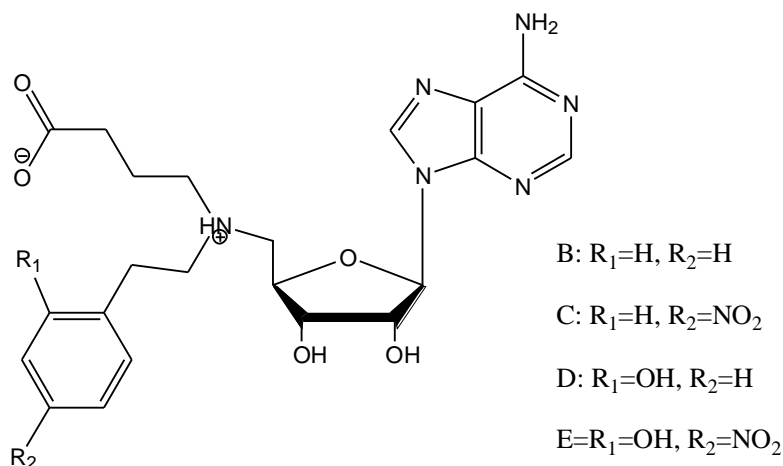


Figure 2.7. Structures of compounds **B**, **C**, **D**, and **E**.

The equilibrium geometry was calculated with Hartree-Fock 6-31G*. Compare to the isocytosine, the electrostatic map of 2-Methyl-5-nitro-phenol is the most similar one among the others. The carbonyl group of isocytosine and the nitro group of 2-Methyl-5-nitro-phenol are both negative (-210-- -150 kcal). Moreover, the hydrogen on the hydroxyl group and the one on the amino group of isocytosine are both within the range of 150--332 kcal. That indicates that both hydroxyl group and nitro group may have the same interactions with the enzyme binding sites as the carbonyl group and the amino group of isocytosine. The surface areas of these four compounds are 166.5 Å, 161.2 Å, 140.1 Å, 141.1Å, respectively. The surface area of ortho-creasol is the closest to isocytosine. The role of the substituted groups of the benzyl ring and isocytosine needs to be verified in future experimental studies.

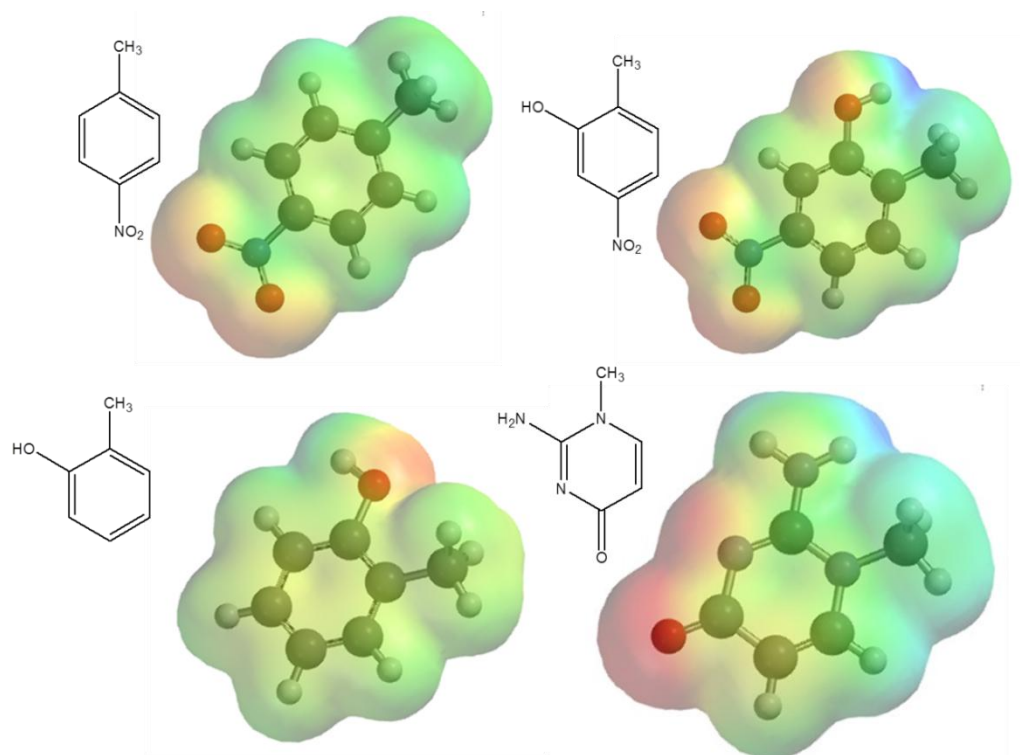


Figure 2.8. The electrostatic potential maps of A, C, D, and E (calculated using Spartan'10). Calculated equilibrium geometry with Hartree-Fock 6-31G*. Color code: red (-210— -150 kcal), orange (-150— 80 kcal), yellow (-80— -20 kcal), green (-20—150 kcal), blue (150—332 kcal).

CHAPTER 3
EXPERIMENTAL

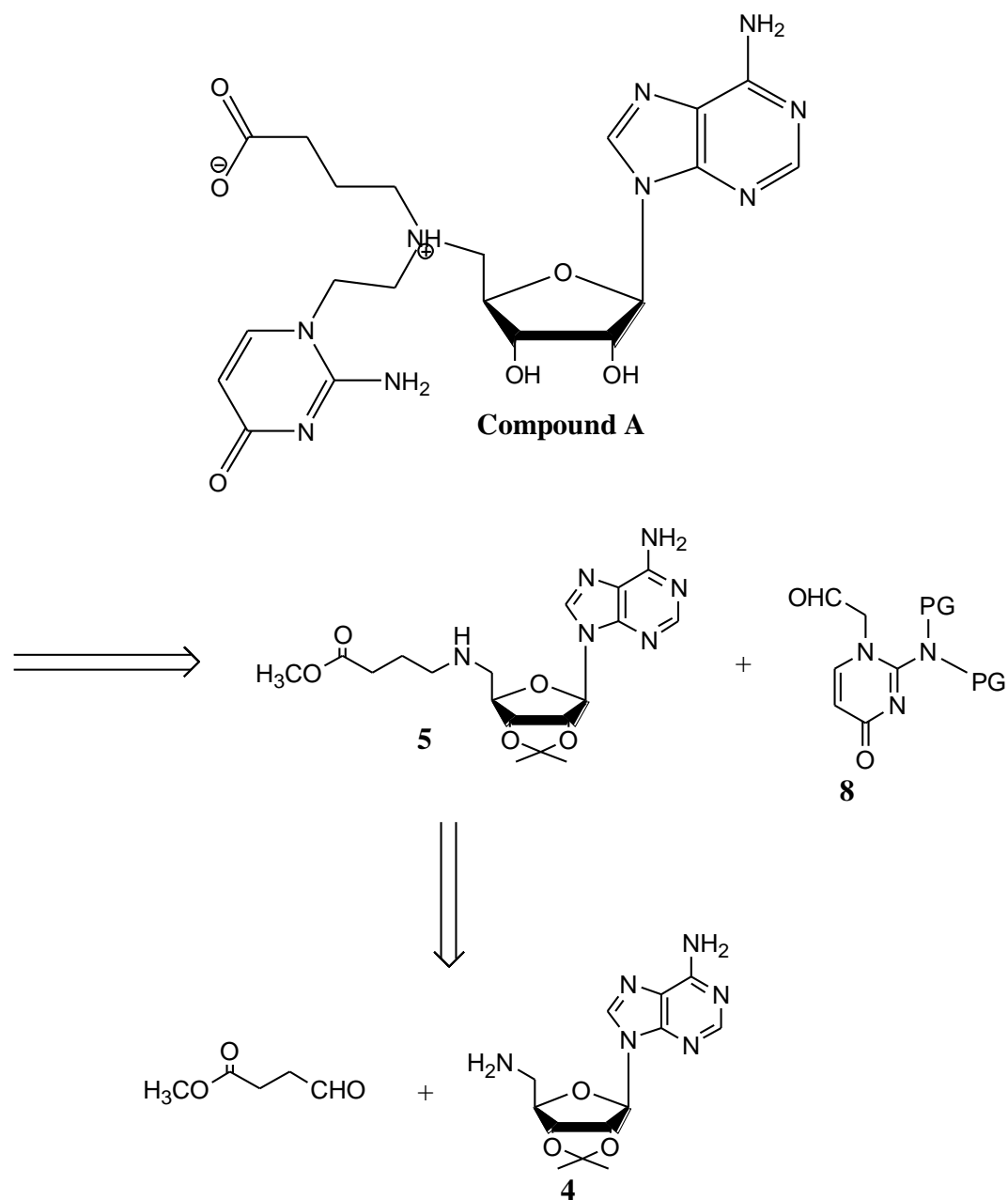
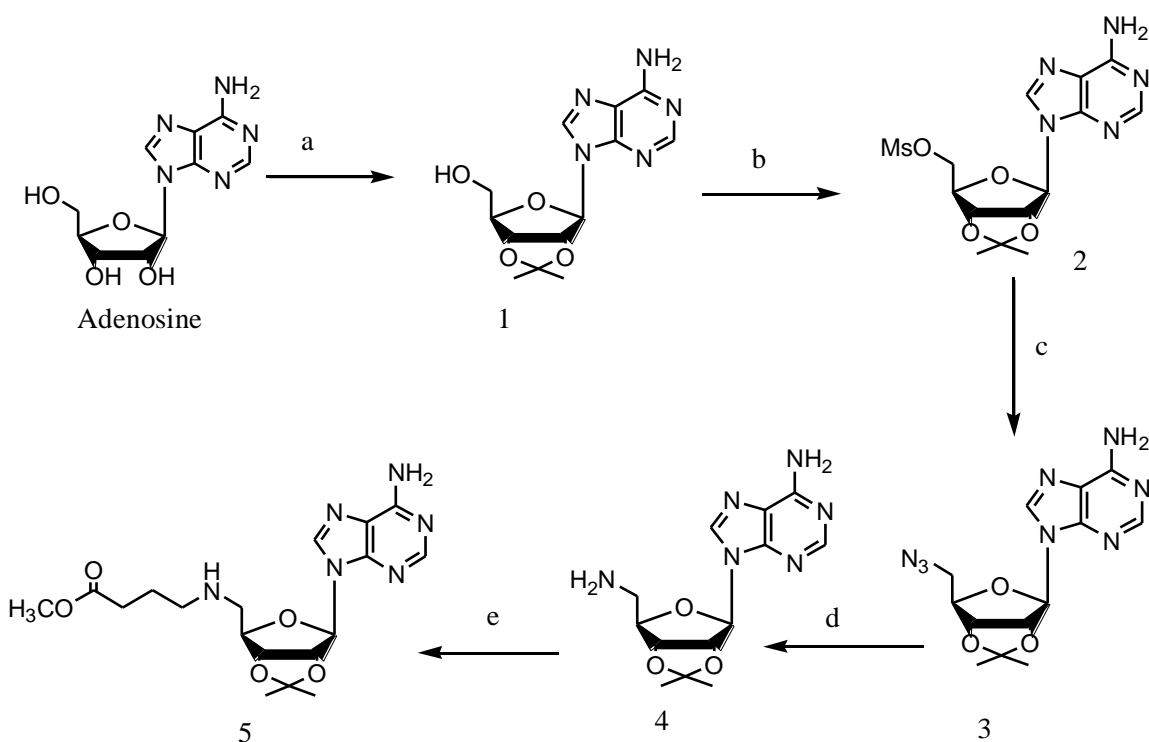


Figure 3.1. Retrosynthetic analysis of target compound, which was divided into three parts: starting material adenosine, introduction of amine to adenosine, and isocytosine part.

The synthesis route of compound **A** has been divided into three parts using retrosynthetic analysis (Figure 3.1): introduction of the nitrogen at the 5' position (compound **5**), synthesis of protected isocytosine acetaldehyde (compound **8**) from iso-cytosine, and connection of compound **5** and **8**. Compound **5** was made from methyl-4-oxobutanoate and amine **4**. The synthesis routes are shown as Scheme 3.1, 3.4 and 3.6, respectively.

3.1 Synthesis Part1

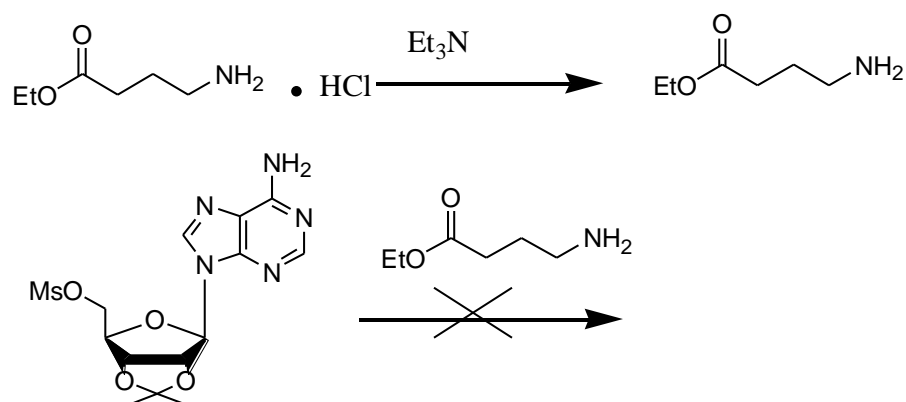


Scheme 3.1. Synthesis route of compound **5** (a) Acetone, p-TsOH, RT., overnight, 51%. (b) CH₂Cl₂, EtN₃, MsCl, RT. 1hr, 81%. (c) NaN₃, DMF, 70 ° C, 48hr, 68%. (d) H₂, 10% Pd/C, EtOH, RT.,overnight, 89%. (e) NaBH₃CN, AcOH, methyl-4-oxobutanoate, RT. 1.5 hr, 23%.

Introduction of the amino group is the key reaction in the first part of the synthesis. Based on some reported reactions, reductive amination was chosen to introduce the complete N-substituent on the 5'-position.^[10] Starting from commercially available adenosine, the 2'-and 3'-hydroxyl groups were protected well in nearly 60% yield to yield

the corresponding acetonide. Acetone was used as both the solvent and the reactant. It was found that maintaining acetone under anhydrous condition was critical to the reaction yields. There are several kinds of desiccants used in the lab, namely, potassium carbonate, magnesium sulfate, and Drierite® (calcium sulfate). Potassium carbonate was used to dry acetone in the first attempt. However, it was found that potassium carbonate absorbed moisture from the air too fast, which as a result gave very poor yield (less than 20%). Finally, anhydrous acetone was successfully prepared by distillation over Drierite®.

After the acetonide was installed, a leaving group was introduced by transforming the free 5'-hydroxy group of the 2', 3'-O-isopropylideneadenosine (**1**) into a mesylate ester (**2**) using MsCl in the presence of triethylamine. Subsequent displacement could not be achieved by reacting with ethyl 4-aminobutanoate hydrochloride, as planned at the beginning- (Scheme 3.2).

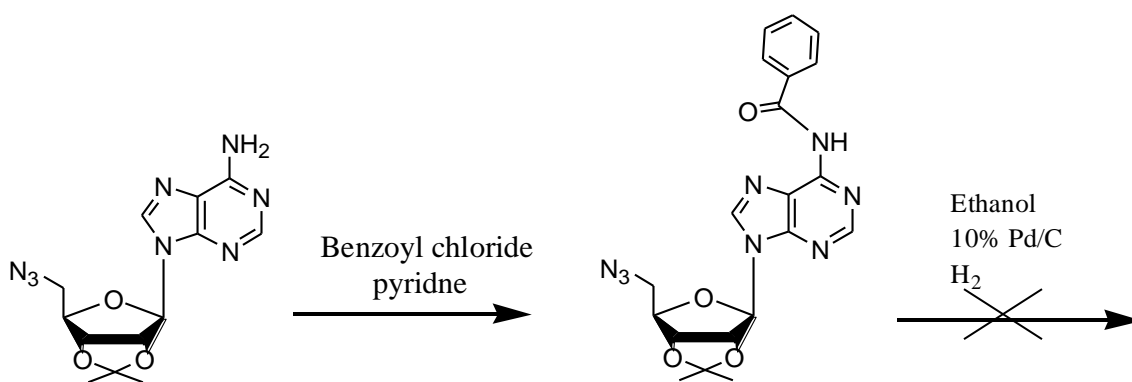


Scheme 3.2. Supposed reaction which did not work using ethyl 4-aminobutanoate hydrochloride, DCM, 24 hr.

TLC analysis of the reaction indicated too many products that would be difficult separate. It was possible that the mesylate was not reactive enough. As a result, a large

amount of mesylate coexists with the secondary amine product, which could further react to form the tertiary amine.

Instead, the 5' nitrogen was introduced using sodium azide in DMF under reflux condition for 48 hr, followed by reduction to give amine **4** in 49.7% yield over three steps. At first, the 6-amino group of adenosine was thought to require protection. But this method was discarded due to its low yield (less than 20%) and the difficulty in the subsequent reduction (Scheme 3.3).



Scheme 3.3. Protection of the 6-amino group of adenosine.

However, amine **4** could be subjected to a direct condensation with methyl-4-oxobutanoate. The exocyclic amino group of adenosine is much less reactive than the primary aliphatic amine. The aliphatic amine easily reacted with aldehyde, although a problem was found in controlling the quantity of methyl-4-oxobutanoate. As a result, a mixture of the desired product (**5**) and the 2 equiv. condensation byproduct (Figure 3.2) was found.

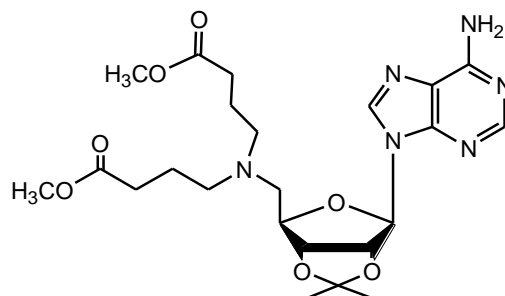
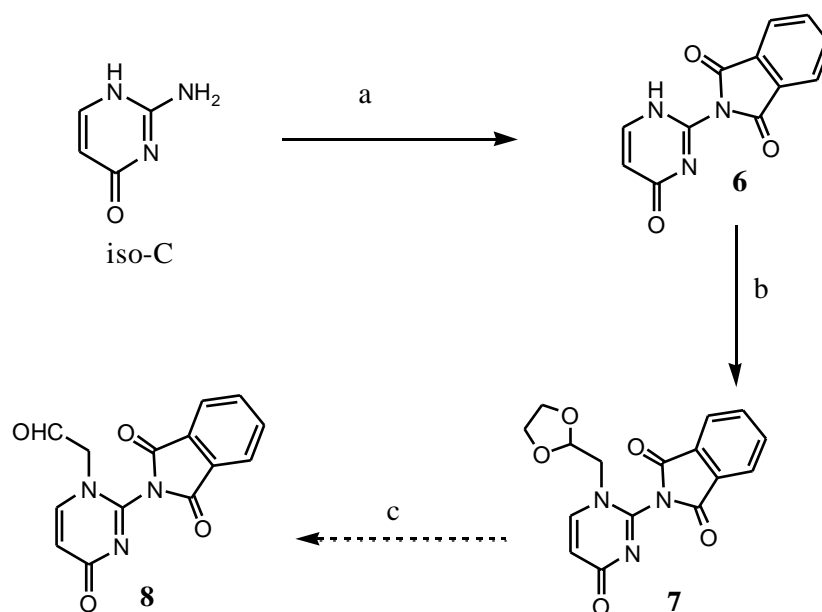


Figure 3.2. The byproduct of reaction e.

In sum, for the first part of the synthesis, compound **5** was made from adenosine in five steps (Scheme 1): protection of the 2'-and the 3'-hydroxyl groups, introduction of a leaving group to the 5'-hydroxy group, introduction of the 5' nitrogen using sodium azide, followed by reduction to give amine, and reaction with aldehyde.

3.2 Synthesis Part2



Scheme 3.4. Supposed synthesis route of compound **8** (a) phthalic anhydride, pyridine, reflux, 24hr , 46% (b)NaH, 2-bromomethyl-1,3-dioxalane, pyridine, reflux, 24 hr, 64% (c) MeOH, p-TsOH, RT., overnight, unexpected product.

It was more difficult to carry out the second part synthesis due to the following reasons. First, isoC can dissolve in water, but it has very low solubility in organic solvents.

Several solvents were tried, such as DMF, pyridine, and THF. The results showed that pyridine was better than the others, but still required a high temperature (140 °C) to completely dissolve isoC.

Second, the amino group of isoC needs to be protected to avoid intramolecular condensation after the aldehyde is introduced. Ac₂O was tried at the beginning using pyridine as a solvent. However, it was difficult to control the quantity of the acetate groups to avoid insufficient N-acetylation or overreaction on the oxygen of isoC. Then, phthalic anhydride was chosen to be the protecting group, and the reaction conditions were shown in Scheme 3.4.

Third, instead of forming the aldehyde, the dioxalane may have spontaneously cyclized with the adjacent phthalimide over a period of a few days or less than one day under the acid conditions in the presence of MeOH, (**8**)

Therefore, this synthesis route of compound **8** was abandoned due to the incompatibility between the amino group and the aldehyde group. As the alternatives, phenylacetaldehyde and 4-nitrophenylacetaldehyde were used to replace **8** to connect with compound **5**. The structures of phenylacetaldehyde, 4-nitrophenylethanol and other similar compounds are shown in Figure 3.3.

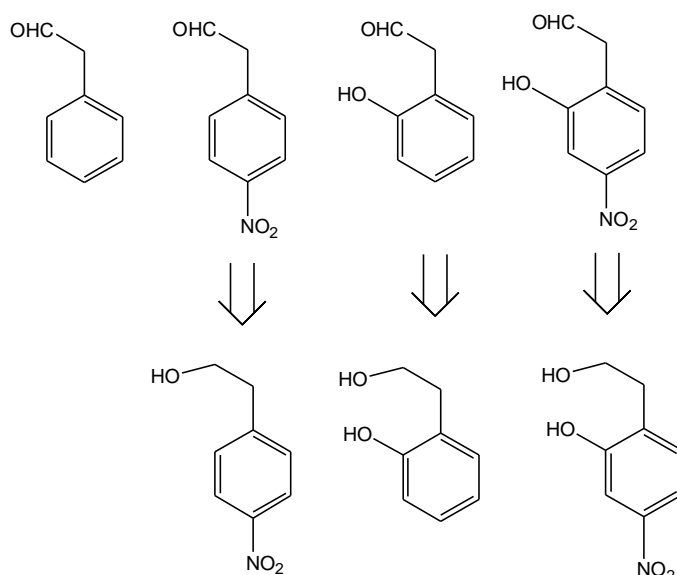
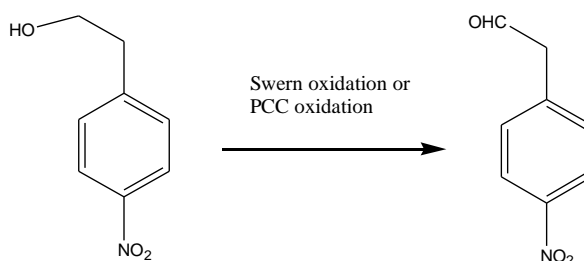


Figure 3.3. Aromatic molecules used to replace isoC part. Left to right: phenylacetaldehyde; 4-nitrophenylacetaldehyde, which can be synthesized from 4-nitrophenylethanol; 2-(2-hydroxyphenyl) acetaldehyde, which can be synthesized from 2-hydroxyphenethyl alcohol; 2-(2-hydroxyphenyl, 4-nitro)-acetaldehyde which can be synthesized from 2-(2-hydroxyphenyl, 4-nitro)-alcohol.

Phenylacetaldehyde is commercially available, and it can be directly conjugated to compound **5**, while 4-nitrophenylacetaldehyde needs to be synthesized by oxidation from 4-nitrophenylethanol, shown as Scheme 3.5. The original PCC oxidation was tried at the beginning. Two products were obtained after this reaction, which could not be identified. NMR showed that one product had no aldehyde peak at around 9-10 ppm, and the other did have one peak at around 10 ppm. However, the latter compound was lack of the expected CH₂ peaks at around 3-4 ppm but showed an extra CH₂ peak at around 2 ppm which implies that the aromatic ring may have undergone some unknown transformations.

Swern oxidation was also attempted using oxalyl chloride in DMSO. This reaction did not afford 4-nitrophenylacetaldehyde either. Then, PCC oxidation with silica gel successfully afforded the right compound. The reason was unclear, but it might be

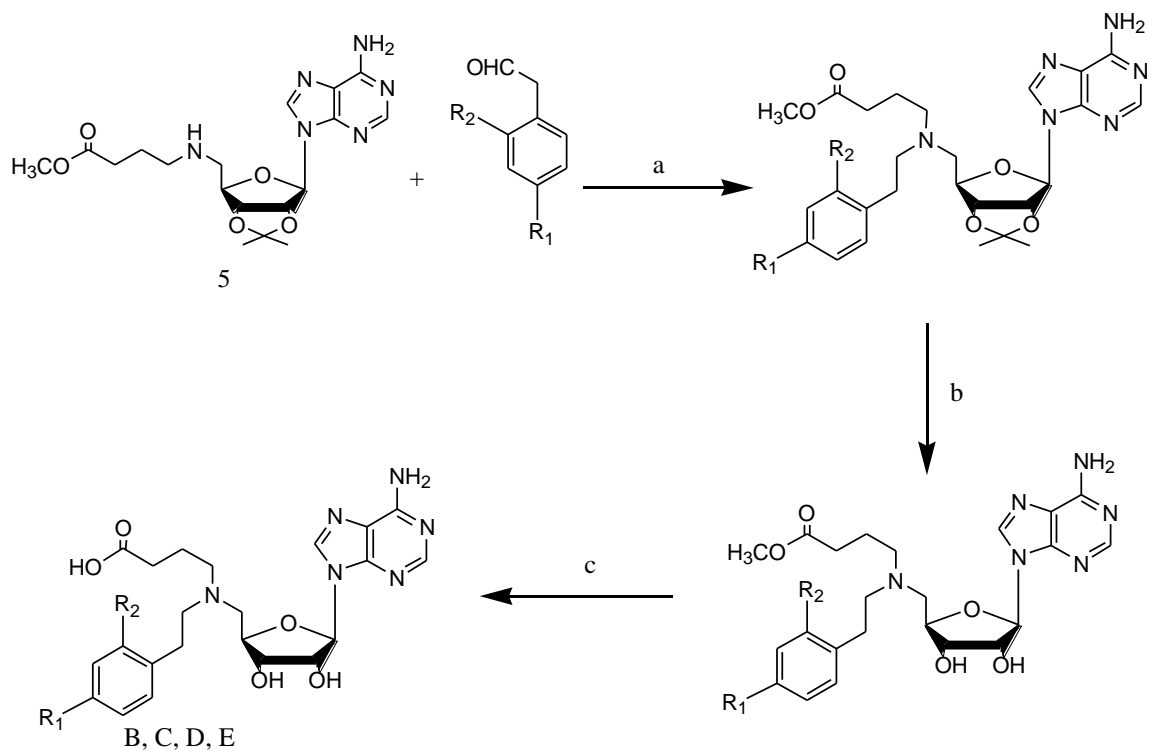
attribute to the fact that silica gel can absorb water from the reaction, or it helps the reaction get off from the byproduct based on the mechanism of PCC oxidation.



Scheme 3.5. Swern and PCC oxidation of 4-nitrophenylethanol. PCC oxidation: DCM, PCC (2 equiv.), RT., 3 hr. Swern oxidation: DMSO, DCM, TEA, oxalyl chloride (1.2 equiv.), -78°C to R.T., 2 hr.

3.3 Synthesis Part3

Phenylacetaldehyde easily reacted with compound **5** via the same procedure as the reductive amination in part1, affording a yield of 92%. After 2 steps of deprotection by acid and base, one after another, the final compound **B** was obtained, shown as Scheme 3.6. The synthesis of compounds **C**, **D** and **E** are under way following the same procedure as was used for the synthesis of compound **B**.



B: $R_1=H, R_2=H$

C: $R_1=H, R_2=NO_2$

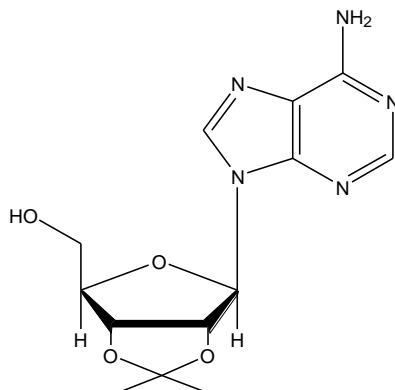
D: $R_1=OH, R_2=H$

E: $R_1=OH, R_2=NO_2$

Scheme 3.6. Synthesis route of part 3. (a) $NaBH_3CN$, AcOH, RT. 1.5hr (b) $p-TsOH \cdot H_2O$ (2equiv.), MeOH, RT., overnight (c) KOH, MeOH, RT. overnight

CHAPTER 4
EXPERIMENTAL INFORMATION

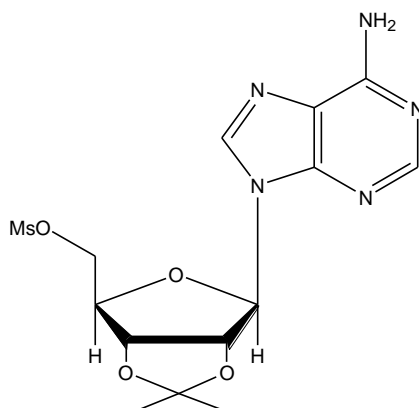
(1) Synthesis of 2',3'-O-Isopropylideneadenosine [1]:



To a suspension of adenosine (1.0 g, 3.74 mmol) in dry acetone (200 ml) was added 41.14mmol of dry p-TsOH (7.1 g, 11 equiv.) in one portion. The mixture was stirred under an atmosphere of Ar at room temperature overnight. The mixture was concentrated under reduced pressure. The residues were stirred with 250 ml Sat. NaHCO₃ solution for 6 hr and then extracted with ethyl acetate three times. The organic layer was dried over MgSO₄ and then filtered. The solvent was evaporated and the crude product was purified by column chromatography (dichloromethane/methanol 20:1, silica). After drying under vacuum, a white solid was obtained (0.582g, 51%): ¹H NMR (CDCl₃) δ8.31(s, 1H), 7.84(s, 1H), 6.40(m, 1H), 5.84(m, 1H), 5.72(m, 1H), 5.20(m, 1H), 5.12(m, 1H), 4.53(m, 1H), 3.94(m, 1H), 1.63(s,3H), 1.36(s, 3H)

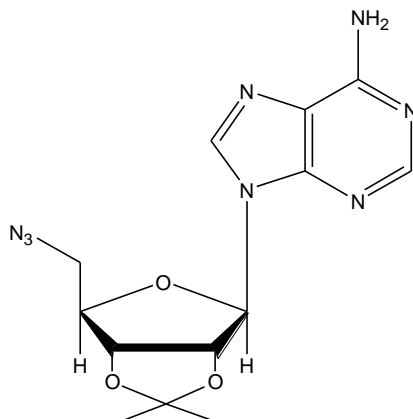
(2) Synthesis of

**6-(6-Amino-9H-purin-9-yl)-2,2-dimethyl-tetrahydrofuro[3,4-d],[1,3]dioxol-4-yl)
methyl methanesulfonate.[2]**



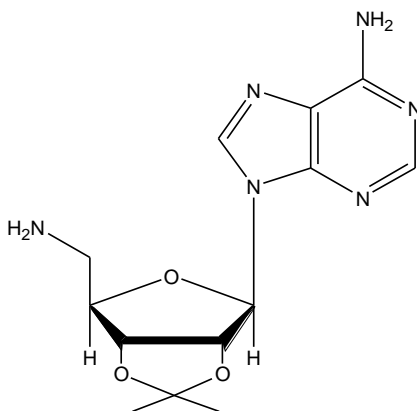
A solution of 0.53 mmol of 2',3'-O-isopropylideneadenosine (162 mg 1.0 equiv.) in 7.5 ml of dichloromethane was flushed with Ar before 162 μ L of triethylamine (1.17 mmol, 2.4 equiv.) and 50 μ L mesylchloride (0.64 mmol, 1.2 equiv.) were added.^[12] Solution was stirred at room temperature 1hr and then 7.5 mL of saturated NaHCO₃ solution was added. The layers were separated and the organic layer was washed two times with 7.5 mL NaCl. The combined aqueous layers were re-extracted with CH₂Cl₂ (2 x 5ml) and the combined organic layers were dried over MgSO₄. The solids were filtered off and the solvent was evaporated. The crude product weight 167 mg of the mesylate (0.43 mmol, 81 %) as a colourless foam. The product was directly used for the next reaction without purification and NMR identification.

(3) 9-((3aR,4R,6R,6aR)-6-Azidomethyl-2,2-dimethyl-tetrahydro-furo[3,4-d]-1,3-dioxol-4-yl)-9H-purin-6-ylamine [3]



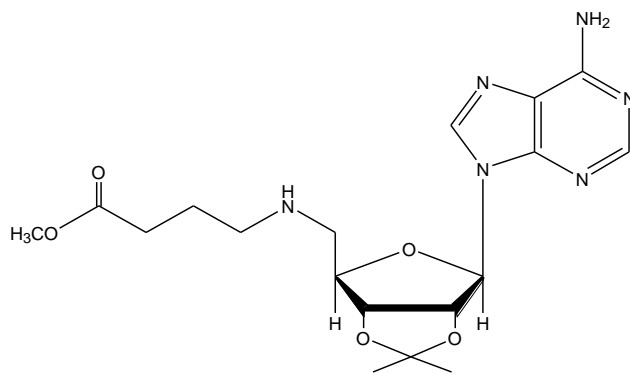
A mixture of **2**, (167 mg, 0.397 mmol) and sodium azide (260 mg, 3.97 mmol, 10 equiv.) was suspended in anhydrous DMF (6 ml). The reaction was heated at 70 °C for 48 hr. ^[19] The mixture was filtered, and evaporated under reduced pressure. DMF was removed in vacuo. The residue was purified by silica gel chromatography, (dichloromethane/ methanol 20:1). Light yellow syrup (90 mg, 68.2%) was obtained. ¹H NMR (CDCl₃) δ8.25(s, 1H), 7.92(s, 1H), 6.12(s, 1H), 5.95(s, 1H), 5.50(m, 1H), 5.12(m, 1H), 4.38(m, 1H), 3.60(m, 1H), 1.60(s, 3H), 1.25(s, 3H).

(4) 9-((3aR,4R,6R,6aR)-6-Aminomethyl-2,2-dimethyl-tetrahydro-furo[3,4-d]-1,3-dioxol-4-yl)-9H-purin-6-ylamine [4]



Compound **3** (40 mg) was dissolved in 5 ml anhydrous ethanol, 15 mg of 10% Pd/C was added. The reaction mixture was placed under vacuum and then under an atmosphere of H₂ at room temperature overnight. The mixture was filtered, and evaporated under reduced pressure. The residue was purified by silica gel chromatography, (dichloromethane/methanol 10:1). Compound **4** (33 mg) of was obtained in 89.2% yield. ¹H NMR (CDCl₃) δ8.36(s, 1H), 8.15(s, 1H), 6.15(s, 1H), 5.50(m, 1H), 5.05(m, 1H), 4.20(m, 1H), 3.30(m, 1H), 2.90(m, 1H), 2.45(d, 2H), 1.50(s, 3H), 1.25(s, 3H).

(5)



NaBH₃CN 6.8 mg (0.108 mmol, 1equiv.) and AcOH 5.57 μl (0.097 mmol, 0.9 equiv.) were added to a solution of 33 mg of compound **4** (0.108 mmol, 1 equiv.) and 0.01 mL of methyl-4-oxobutanoate (0.097 mmol, 0.9 equiv.) in 1 mL MeOH. The reaction mixture was stirred at room temperature for 1.5 hr. After diluting the reaction with ethyl acetate and

sat. NaHCO_3 (aq.), the organic layer was washed with NaHCO_3 , dried over MgSO_4 , and then evaporated under reduced pressure. ^[12] The residue was purified by silica gel chromatography, (dichloromethane/ methanol 50:1). Compound **5** (10 mg) was obtained in 22.8% yield. ^1H NMR (CDCl_3) δ 8.25(s, 1H), 7.80(s, 1H), 5.90(s, 1H), 5.64(s, 1H), 5.38(m, 1H), 4.95(m, 1H), 4.26(m, 1H), 3.58(m, 3H), 2.80(m, 2H), 2.50(m, 2H), 2.20(m, 2H), 1.78(m, 2H), 1.54(s, 3H), 1.26(s, 3H). ^{13}C NMR (CDCl_3) δ : 173.2, 155.5, 153.0, 146.5, 114.7, 90.6, 83.6, 82.4, 53.3, 48.5, 33.1, 31.5, 29.7, 27.2, 25.4.

Window Display Report

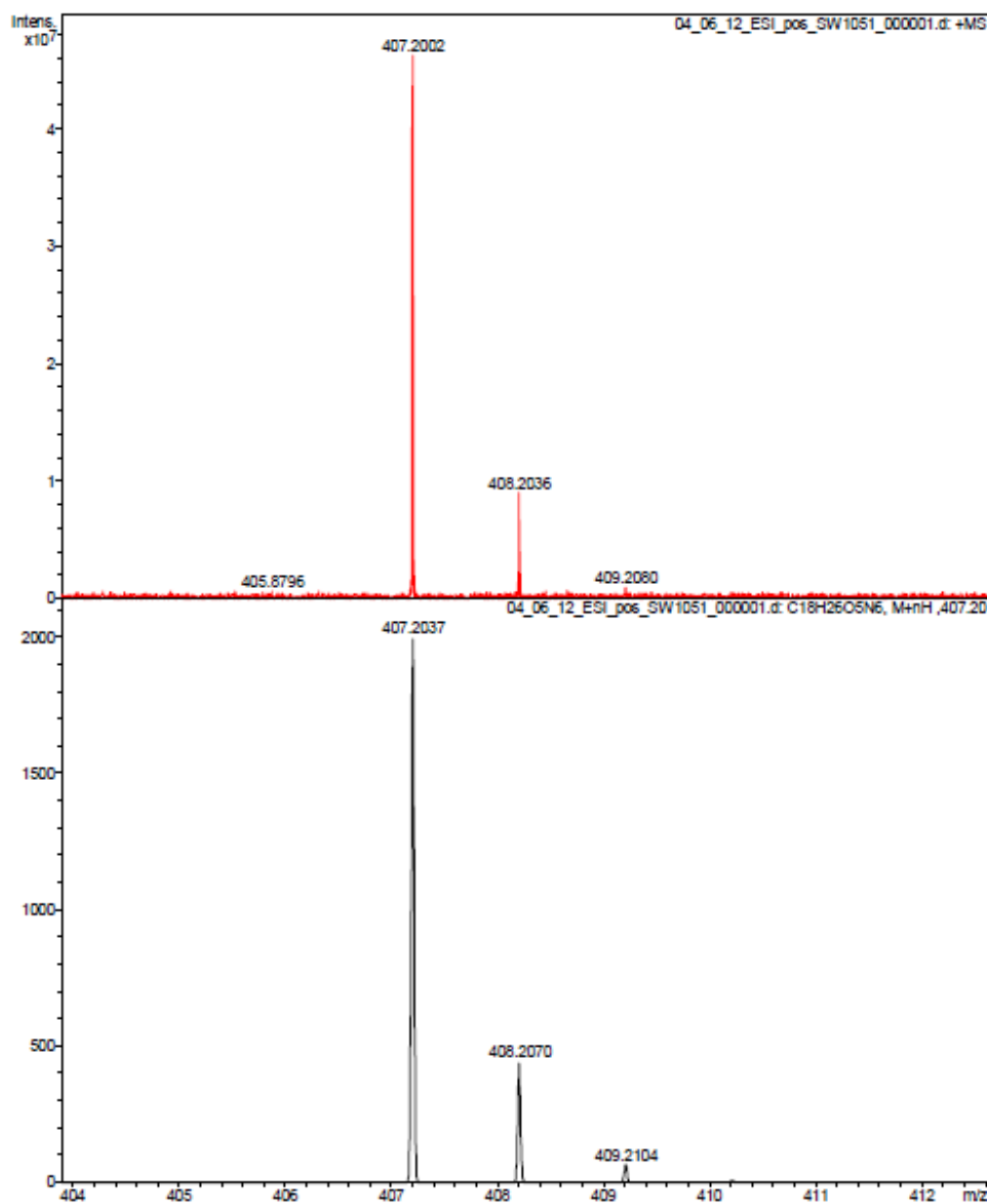
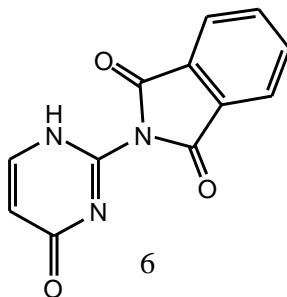


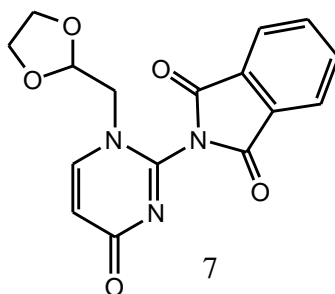
Figure 4.1. Mass spectrometry of compound 5.

(6)

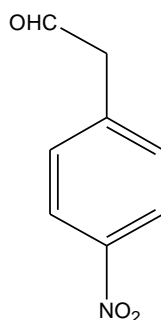


A mixture of iso-cytosine, (111 mg, 1 mmol) and phthalic anhydride (148 mg, 1 mmol, 1equiv.) was suspended in anhydrous pyridine (15 ml). The reaction was heated at 140 °C for 24 hr. Pyridine was removed in vacuo reduced pressure. The residue was purified by silica gel chromatography, (dichloromethane/ methanol 50:1). White foam (110 mg, 45.9%) was obtained. $^1\text{H NMR}$ (CDCl_3) δ 8.18 (s, 1H), 7.72 (m, 2H), 7.54 (m, 1H), 6.50 (s, 1H), 5.85 (s, 1H), 3.95 (s, 2H).

(7)



Compound **6** (110 mg, 0.458 mmol) was dissolved in 5 ml dry pyridine was added sodium hydride (10 mg, 60% in oil), and the mixture was stirred for 1 hr at room temperature. The reaction mixture was heated to 110 °C and a solution of 2-bromomethyl-1,3-dioxalane (70 μL) was added in several portions over 24 hr. ^[20] Pyridine was removed under reduced pressure. The residue was purified by silica gel chromatography, (dichloromethane/ methanol 100:1). Colorless syrup (78 mg, 64%) was obtained. $^1\text{H NMR}$ (CDCl_3) δ 8.55 (s, 1H), 8.02 (m, 2H), 7.78 (m, 2H), 6.88 (s, 1H), 5.33 (s, 1H) 4.42 (s, 2H), 3.98 (m, 4H) .

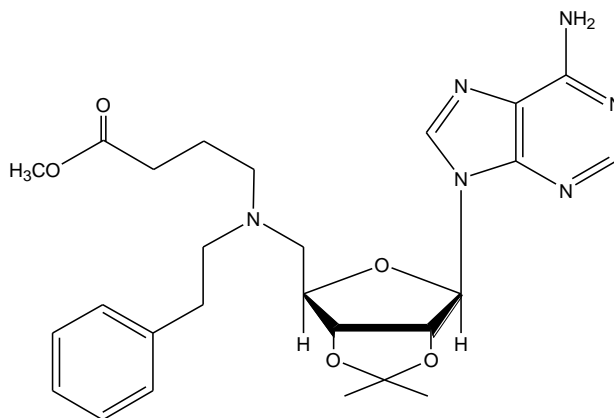
(8) (4-nitro-phenyl)-acetaldehyde

PCC oxidation: 4-nitrophenylethanol (100 mg, 0.6 mmol), was dissolved in 10 ml MeOH. PCC (156 mg, 1.2 equiv.) was added at first, and the mixture was stirred at RT. for 1.5 hr. After checked TLC, another 104 mg of PCC was added, and the reaction was continued for another 1.5 hr. The residues were diluted with 20 ml of dichloromethane and washed with 3x10 ml of sat. NaHCO₃ solution. The organic layer was dried over MgSO₄ and then filtered. The solvent was evaporated and the crude product was purified by column chromatography (Hexane/ EA= 4:1, silica). After drying under vacuum, a white solid compound was obtained.

Swern oxidation: A solution of anhydrous DMSO (3 mL) in CH₂Cl₂ (3 mL) was added slowly dropwise to stirred solution of oxalyl chloride (1.218 mmol, 150 μL) in CH₂Cl₂ (3 mL) at -78 °C. After the addition was complete, the solution was stirred for 5 min, and then, 4-nitrophenylethanol (200 mg, 1.2 mmol) in CH₂Cl₂ (3 mL) was added (15 min). The solution was stirred for 20 min. Anhydrous NEt₃ (4 mL) was slowly added dropwise. The reaction was let warm to R.T. for 2hr, and then, poured into 50 mL of water. It was washed with 3x30 mL CH₂Cl₂, and then the organic layer was dried over MgSO₄, and then filtered. The solvent was evaporated and the crude product was purified by

column chromatography (Hexane/ EA= 2:1, silica). After drying under vacuum, a red syrup compound was obtained (80 mg, 40%).

(9)



NaBH₃CN 2.5 mg (0.04 mmol, 1 equiv.) and AcOH 2.30 μ l (0.04 mmol, 1 equiv.) were added to a solution of 20 mg of compound **5** (0.04 mmol, 1 equiv.) and extra Phenyl acetaldehyde (14 μ l, 3 equiv.) in 5 ml MeOH. The reaction mixture was stirred at room temperature 1.5 hr. After diluting the reaction mixture with ethyl acetate and sat. NaHCO₃ (aq.), the organic layer was washed with NaHCO₃, dried over MgSO₄, and then evaporated under reduced pressure. The residue was purified by silica gel chromatography, (dichloromethane/ methanol 50:1). A colorless syrup compound **9** (22 mg, 92%) was obtained. ¹H NMR (CDCl₃) δ 8.35(s, 1H), 7.92(s, 1H), 7.15(m, 5H), 6.06(s, 1H), 5.76(s, 1H), 5.54(s, 1H), 4.98(s, 1H), 4.30(s, 1H), 3.65(m, 3H), 2.76(m, 6H), 2.55(m, 2H), 2.28(m, 2H), 1.84(m, 2H), 1.56(s, 3H), 1.30(s, 3H). ¹³C NMR (CDCl₃) δ : 174.1, 155.5, 153.0, 146.1, 140.0, 128.7, 128.4, 125.9, 114.3, 91.1, 83.7, 83.2, 56.2, 55.8, 53.3, 51.5, 33.1, 31.5, 29.7, 27.2, 25.4.

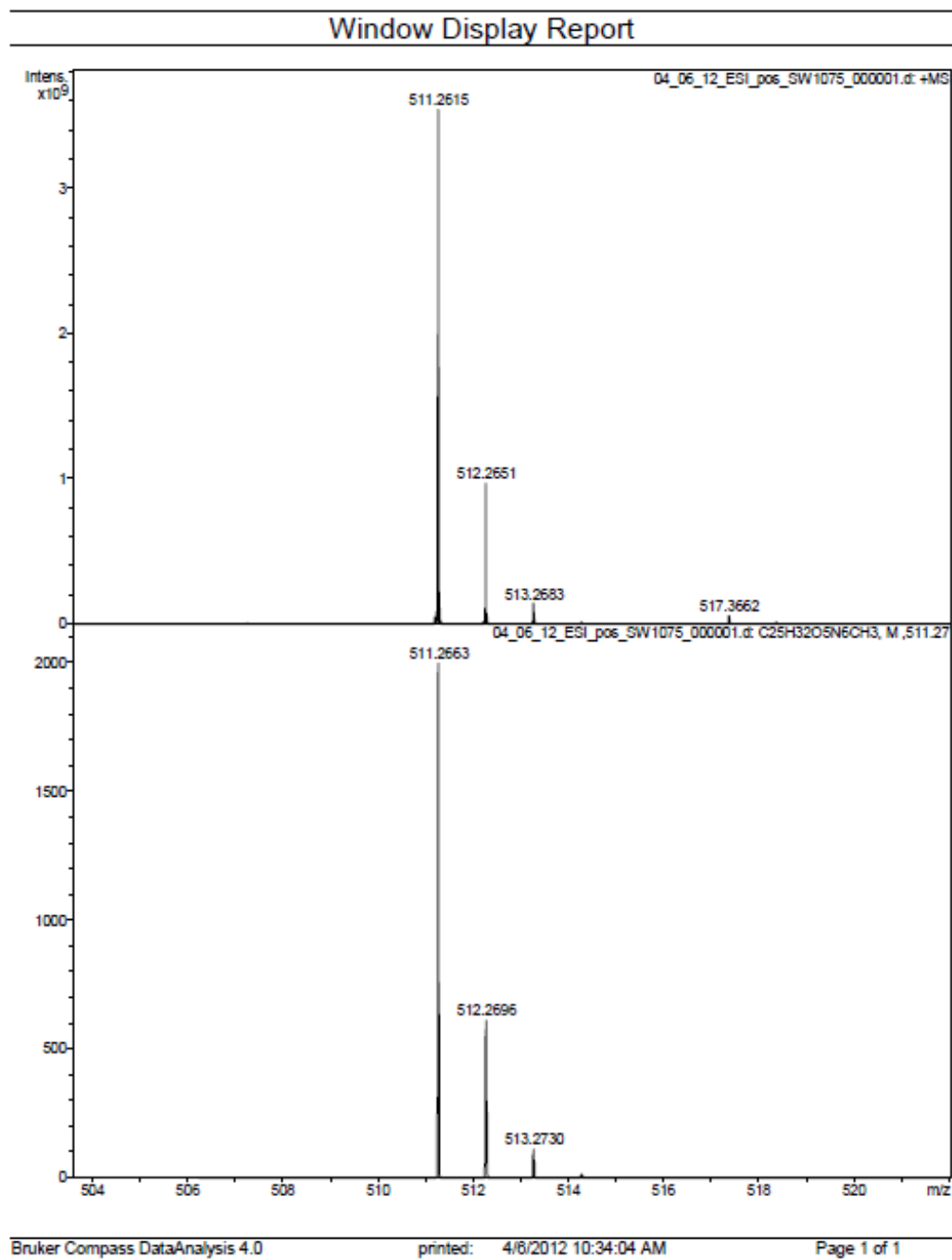
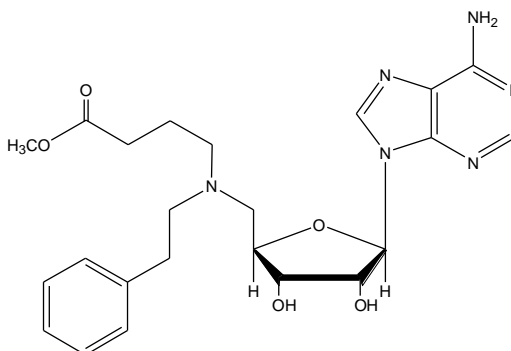


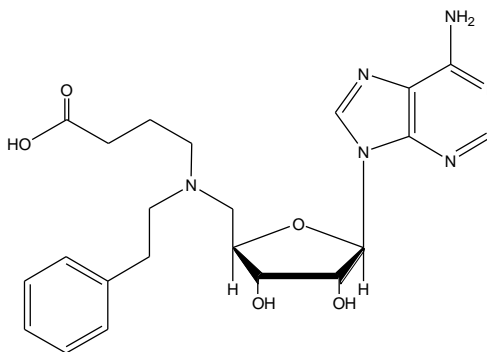
Figure 4.2. Mass spectrometry of compound **9**.

(10)



To a suspension of compound **9** (18 mg), in a mixture of MeOH/ H₂O= (10:1, 3 mL: 0.3 mL), was added 40 mg of p-TsOH in one portion. The mixture was stirred at room temperature overnight, and then concentrated under reduced pressure. The residues were washed with 3x40 mL sat. NaHCO₃ solution. The organic layer was dried over MgSO₄ and then filtered. A colorless syrup (10 mg) was obtained, yield in 71%. The product was directly used for the next reaction without purification and NMR identification.

(11)



To a suspension of compound **9** (10 mg), in a mixture of MeOH/H₂O= (10:1, 3 mL: 0.3 mL), was added 30 mg of KOH in one portion. The mixture was stirred at room temperature overnight, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography, (dichloromethane/ methanol 1:1). A colorless syrup (4 mg, 40%) was obtained. ¹H NMR (MeOH) δ8.35(s, 1H), 8.22(s, 1H), 7.15(m, 5H),

6.01(s, 1H), 4.75(s, 1H), 4.35(s, 1H), 4.15(s, 1H), 3.18(m, 2H), 2.89(m, 2H), 2.76(m, 5H),
2.22(m, 2H), 1.98(m, 2H), 1.76(m, 2H).

CHAPTER 5

CONCLUSIONS AND FUTURE STUDIES

In summary, a series of SAM analogues was designed using a combination of mechanism-based design (transition state inhibitor) and structural modifications. The analogues were expected to inhibit cytosine DNA Methyltransferases. Among these analogues, compound B was synthesized successfully in eight steps from the starting material adenosine. The synthesis of other compounds is under way following the same procedure as for the synthesis of compound B.

The inhibition activities will be tested in vitro using human DNMT1 in future studies.

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