

Purine Carbocyclic Nucleoside Analogues as Antiviral Agents

by

Qi Chen

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
December 13, 2010

Copyright 2010 by Qi Chen

Approved by

Stewart W. Schneller, Chair, Professor of Chemistry and Biochemistry
Edward J. Parish, Professor of Chemistry and Biochemistry
Susanne Striegler, Associate Professor of Chemistry and Biochemistry
Evert C. Duin, Associate Professor of Chemistry and Biochemistry

Abstract

The carbocyclic nucleosides aristeromycin and neplanocin A are two of the most potent inhibitors of S-adenosylhomocysteine hydrolase, an enzyme that plays a critical role in methyl transfer reactions during a viral replication cycle. As a consequence, these two carbocyclic nucleosides have been found to have significant broad-spectrum antiviral activities. However, their clinical applications are limited due to the toxicity associated with their 5'-hydroxyl metabolites. To develop new antiviral agents that retain the antiviral activities of aristeromycin and neplanocin A while avoiding their toxicities, homoaristeromycin (**1**) has been synthesized. Also, in order to detect the relationship between the *syn-anti* conformations of nucleoside analogues and antiviral activities, a series of 8-methyl carbocyclic nucleosides derivatives have been prepared. Among the analogues in this series 8-methylhomoaristeromycin (**2**) displayed a significant activity towards human cytomegalovirus (HCMV) and moderate activity against yellow fever virus.

3-Deazapurine carbocyclic nucleosides (that is 3-deazaaristeromycin and 3-deazaneplanocin A) have been discovered to possess remarkable antiviral activities, especially towards Ebola virus, with limited toxicity. Therefore, in this dissertation research, the 3-deaza structural element was introduced to supplement the library of 8-methylcarbocyclic nucleoside analogues that included 8-methyl-3-deaza-5'-

norneplanocin A (**3**), 8-methyl-3-deazaneplanocin A (**4**), 8-methyl-3-deaza-5'-homoneplanocin A (**5**) and 8-methyl-3-deazaaristeromycin (**6**).

Aristeromycin analogues modified at C-4' (4'-*iso*aristeromycin **8a**, 3-deaza-4'-*iso*aristeromycin **8b**, 4'-*isohomo*aristeromycin **9a**, 3-deaza-4'-*isohomo*aristeromycin **9b**) have been also investigated as part of a program to further understand the structure-activity relationships in the adenine derived purine carbocyclic nucleosides. Although analogues **4**, **9a** and **9b** were found to be inactive towards most viruses, the antiviral data still provide valuable information for structure-activity relationships.

A final feature of this dissertation arose from considering S-adenosyl-L-methionine (AdoMet) dependant methyl transferations. AdoMet plays a pivotal role in viral mRNA capping process and inhibition of this process offers another pathway to antiviral drug design. Sinefungin is one of the most potent inhibitors of AdoMet dependant methyltransferases and has shown potential in antiviral, antifungal, antiparasitic drug discovery. However, its toxicity due to inhibition of cellular methyltransferases limits its usefulness. In this direction, deamino-carbocyclic sinefungin (**10**) has been prepared and targeted for antiviral analysis.

The bioassay data for compounds **3**, **5**, **6**, **10** will be forthcoming and under study in our laboratories.

Acknowledgments

It is a pleasure to thank those who made this dissertation research possible. Though only my name appears on the cover, a great many people have contributed to its production. First, I owe my deepest gratitude to my advisor Dr. Stewart W. Schneller. It has been a great fortune to have an advisor who encouraged me not only grow as an experimentalist but also as an independent thinker. His guidance and support helped me overcome many difficult situations and complete this dissertation. I sincerely thank you, Dr. Schneller, for all you have done for me. I am also grateful to Drs. Edward Parish, Susanne Striegler, Evert Duin and Peter Livant for their constructive suggestions and lectures on related topics that helped me improve my knowledge in the area. I like to thank Drs. Wei Ye, Xueqiang Yin, Yan Zhang, Haisheng Wang, Mingzhu He and other members in my research group for their inspiring discussion, advice and friendship. I like to thank Drs. Michael Meadows and Yonnie Wu for their assistance in obtaining Nuclear Magnetic Resonance (NMR) spectroscopic and Mass Spectrometric (MS) data. Gratitude is also extended to the Department of Chemistry and Biochemistry and the National Institutes of Health for their financial support.

Finally, and most importantly, I would like to thank my husband, Chong Liu, for his unending encouragement, support and patience.

Table of Contents

Abstract.....	ii
Acknowledgments.....	iv
List of Tables	vii
List of Figures	ix
List of Schemes	xi
Introduction.....	1
Introduction of Viruses	1
Perspective for Antiviral Drug Design	2
Nucleoside Analogues	6
Adenosylhomocysteine(AdoHcy) Hydrolase as A Key Enzyme for Antiviral Activity	9
The Inhibitors of AdcHcy Hydrolase as Antiviral Agents.....	15
Target Design Based on Inhibition of AdoHcy Hydrolase	21
Target Design Based on Direct Inhibition of Methyltransferase	27
Chapter 1 Synthesis of Important Precursors.....	30
Chapter 2 Synthesis of 5'-Homoaristeromycin and 8-Methyl-5'-Homoaristeromycin.....	36
Chapter 3 Synthesis of 8-Methyl-3-Deazaneplanocin Analogues	45
Chapter 4 Synthesis of 8-Methyl-3-Deazaaristeromycin Analogues.....	67
Chapter 5 Synthesis of 4'- <i>Iso</i> aristeromycin and Its 3-Deaza Analogues	81

Chapter 6 Synthesis of Deamino-Carbocyclic Sinefungin	87
Biological Results	96
Conclusion	111
Experimental Section	113
References	158

List of Tables

Table 1 Viruses affected by AdoHcy hydrolase inhibitors.....	13
Table 2 Mitsunobu reaction between 3-deaza-6-chloropurine and cyclopentanols.....	51
Table 3 Mitsunobu reaction for coupling (<i>Part A</i>)	79
Table 3 Mitsunobu reaction for coupling (<i>Part B</i>)	80
Table 4 The spectrum of viruses to be assayed.....	96
Table 5 Antiviral activity of compound 2 , 4 against HSV-1, HSV-2 and HCMV, based on cytopathogenic effect (CPE) inhibition assay.....	98
Table 6 Antiviral activity of compound 2 , 4 and 9a against adenovirus, influenza A (H1N1), influenza A (H3N2), influenza B inhibition assay	99
Table 7 Antiviral activity of compounds 2 , 4 and 9a against measles, PIV, rhinovirus, rift valley fever inhibition assay	100
Table 8 Antiviral activity of compounds 2 , 4 and 9a against yellow fever, RSVA, and inhibition assay	101
Table 9 Antiviral activity of compounds 2 , 4 and 9a against dengue, tacaribe, VEE and WNV inhibition assay.....	102
Table 10 Antiviral activity of compounds 2 , 4 and 9a against EBV virus based on DNA hybridization inhibition assay	103
Table 11 Antiviral activity of compounds 2 , 4 and 9a against vaccinia virus and cowpox virus based on cytopathogenic effect (CPE) inhibition assay.....	104
Table 12 Antiviral activity of compounds 2 , 9a in vero cell cultures.....	105
Table 13 Antiviral activity of compounds 2 , 9a in HEL cell cultures.....	106
Table 14 Antiviral activity of compounds 2 , 9a against cytomegalovirus in Human Embryonic Lung (HEL) cells.....	107

Table 15 Antiviral activity of compounds 2 , and 9a against varicella-zoster in Human Embryonic Lung (HEL) Cells.....	108
Table 16 Cytotoxicity and antiviral activity of compounds 2 and 9a in hela cell cultures....	109
Table 17 Antiviral activity of compounds 2 , 4 and 9a against HCV and HBV	110

List of Figures

Figure 1 Examples of NRTIs and NNRTIs.....	5
Figure 2 Naturally occurring nucleosides	6
Figure 3 Structure of IDU and Brivudin	7
Figure 4 Aristeromycin and neplanocin A	8
Figure 5 Structure of mRNA 5'-terminal cap	10
Figure 6 S-Adenosylmethionine (AdoMet)	11
Figure 7 Metabolic pathway of AdoMet	14
Figure 8 Metabolites of Ari	17
Figure 9 Phosphate derivatives of Ari and Npc A	18
Figure 10 3-Deazaaristeromycin and 3-deazaneplanocin	19
Figure 11 Structure of DHCeA, DHCaA and their 3-deaza analogues	20
Figure 12 5'-Deoxyaristeromycin	20
Figure 13 5'-Homoanalogues of Ari and Npc A	21
Figure 14 <i>syn</i> and <i>anti</i> conformation for nucleosides	22
Figure 15 8-Methyl-5'-noraristeromycin	23
Figure 16 8-Methyl-5'-homoaristeromycin	23
Figure 17 Analogues of 3-deazaAri and 3-deazaNcp A	24
Figure 18 3-Deaza-5'-norAri and its 8-methyl analogues	25
Figure 19 8-Methyl analogue of C ³ -DHCeA	25

Figure 20 D, L-like nucleosides	26
Figure 21 5'-Methyneplanocin A	27
Figure 22 Target compounds for 4'- <i>iso</i> Ari analogues	27
Figure 23 Analogues of AdoHcy	28
Figure 24 Deamino-carbocyclic sinfungine	29
Figure 25 Precursors for carbocyclic nucleosides synthesis	30
Figure 26 First generation of Grubbs catalysts	34
Figure 27 X-ray crystal structure of compound 43	49
Figure 28 NOESY analysis for two isomers of 94	78
Figure 29 NOESY analysis for 100	86

List of Schemes

Scheme 1 Acyclic Nucleosides	7
Scheme 2 Stability of carbocyclic nucleosides	8
Scheme 3 AdoMet-dependent methyl-transferation	12
Scheme 4 Mechanism of AdoHcy hydrolase.....	15
Scheme 5 Metabolic pathways of Npc A.....	16
Scheme 6 Enzymatic hydrolysis of 16	31
Scheme 7 Synthesis of diacetate 16	31
Scheme 8 Synthesis of enone 12 from monoacetate 11	32
Scheme 9 Synthesis of enone 12 from D-ribose	33
Scheme 10 Mechanism of ring closure metathesis.....	35
Scheme 11 Jones method for homoaristeromycin synthesis.....	37
Scheme 12 Synthesis of homoaristeromycin from enone.....	38
Scheme 13 Retrosynthesis for homoaristeromycin and 8-methyl-homoaristeromycin.....	39
Scheme 14 Synthesis of the intermediate 27	40
Scheme 15 Synthesis of (-)-homoAri	41
Scheme 16 Hydroboration reaction for compound 29	42
Scheme 17 Synthesis of compound 34	42
Scheme 18 Synthesis of 2 from intermediate 27	43
Scheme 19 Strategy for target compounds 3, 4, 5 from nucleobase 38	46

Scheme 20 Retrosynthetic analysis of 38	47
Scheme 21 Synthesis of 6-chloro-3-deazapurine.....	48
Scheme 22 Mechanism for the ring closing reaction of 42	49
Scheme 23 <i>di</i> -Boc protected 3-deazaadenine as nucleophile in the Mitsunobu reaction ..	52
Scheme 24 Synthesis of 8-methyl-3-deazaadenine.....	53
Scheme 25 Protection for 8-methyl-3-deazaadenine	54
Scheme 26 Selective deprotection of compound 46	54
Scheme 27 Postulated mechanism for Boc-deprotection by TABF	55
Scheme 28 Retrosynthesis of 8-methyl-3-deaza-5'-norneplanocin A (3).....	56
Scheme 29 Synthesis of 8-methyl-3-deaza-5'-norneplanocin A.....	57
Scheme 30 Retrosynthesis of 8-methyl-3-deaza-neplanocin 4	58
Scheme 31 Previous synthesis for cyclopentenone 49	59
Scheme 32 New strategy for synthesis for cyclopentenone 49	60
Scheme 33 Synthesis of cyclopentenol 49a	61
Scheme 34 Synthesis of 8-methyl-3-deazaneplanocin	62
Scheme 35 Retrosynthesis of 8-methyl-3-deaza-homoneplanocin 5	62
Scheme 36 Shtuo's method for synthesis of cyclopentenol 72	63
Scheme 37 Synthesis of compound 75	64
Scheme 38 Proposed mechanism for the reaction of 75 with 38	65
Scheme 39 Synthesis of precursor 72	66
Scheme 40 Synthesis of compound 5	66
Scheme 41 Retrosynthesis of compound 6, 7	68
Scheme 42 Mitsunobu reaction of nucleobase with compound 27	69

Scheme 43 Reaction and general mechanism for Hendrickson reagent	70
Scheme 44 S _N 2 reaction for the compound 27	71
Scheme 45 Reaction and mechanism for the palladium catalyzed substitution	72
Scheme 46 Mitsunobu reaction for compound 11 with 3-deaza-8-methyl nucleobase	73
Scheme 47 Proposed mechanism for Mitsunobu reaction	74
Scheme 48 Synthesis and Mitsunobu reaction for 85	75
Scheme 49 Synthesis and Mitsunobu reaction for the isomer of 85	76
Scheme 50 Synthesis of Grignard reagent 90	77
Scheme 51 Synthesis of 3-deaza-8-methylaristeromycin	78
Scheme 52 Retrosynthetic analysis of 4'-isoaristeromycin and 3-deaza-4'- isoaristeromycin	82
Scheme 53 Retrosynthetic analysis of 4'-isohomoaristeromycin and 3-deaza-4'- isohomoaristeromycin	83
Scheme 54 Old method for synthesis of compound 8 and 10	84
Scheme 55 Synthesis of <i>bis</i> -Boc protected adenine and 3-deazaadenine	85
Scheme 56 Synthesis of 8 and 9	86
Scheme 57 Retrosynthetic analysis of deamino-carbocyclic sinfungine 10	88
Scheme 58 Synthesis of phosphonium salt 12	89
Scheme 59 First attempt for synthesis of aldehyde 127	90
Scheme 60 Revised synthesis for target compound 10	91
Scheme 61 Synthesis of amino acid olefin 140	92
Scheme 62 First attempt of metathesis	94
Scheme 63 Second attempt of metathesis	95

Introduction

There are about 2,200 viral species, which have been recognized since the discovery of the tobacco mosaic virus in 1892, and the list is continuing to grow.¹ These highly infectious intracellular parasites comprising either DNA or RNA genomic material sometimes can lead to life-threatening diseases including smallpox, yellow fever, poliomyelitis, influenza and measles,²⁻⁵ which could cause damages in the central nervous system and various vital organs, such as lung, liver and intestine. Approximately 20% of the human cancers are due to viral infections. Viruses also can cross species boundaries. The acquired immune deficiency syndrome (AIDS),⁶ Ebola hemorrhagic fever,⁷ severe acute respiratory syndrome (SARS),⁸ and more recently H5N1 avian and H1N1 swine influenza⁹⁻¹¹ are examples of cross-species (zoonotic) infections between humans and other hosts.¹²

Introduction of Viruses

Viruses are unique particles. They are extremely small, often made up of nothing but more than a nucleic acid molecule within a protein shell; they are ultimate, intracellular parasites, must depend on a living host for reproduction. All viral genomes are synthesized by cellular machinery and packed into progeny infectious particles called virions, which are ready to start the next infectious cycle.¹³

There are four characteristics which can be used for classification of all viruses: 1. The nature of the nucleic acid in the virion (DNA or RNA); 2. The symmetry of the protein shell (capsid); 3. The presence or absence of a lipid membrane (envelope); 4. Dimensions of the virion and capsid. Since the nucleic acids of viruses carry all the information for virus propagation, no doubts they are considered as the most important property. The current classification system, which is based on the genome type of the virus and the replication pathway, was established by David Baltimore, a Nobel prize-winning biologist. All viruses fall into one of the following seven groups:¹⁴

1. Double-stranded DNA viruses;
2. Single-stranded DNA viruses;
3. Double-stranded RNA viruses;
4. Positive-sense single-stranded RNA viruses;
5. Negative-sense single-stranded RNA viruses;
6. Positive-sense single-stranded RNA viruses that use reverse transcription;
7. Circular double-stranded DNA viruses that use reverse transcription.

Viruses attach to and enter host cells to start the infectious cycle. Several crucial steps like translation of viral mRNA by the host ribosome, replication of viral genome, assembly and release of the progeny virions from the host cell are involved in all viruses life cycle. Each stage in the viral life cycle has been studied extensively as potential targets for antiviral drug design.¹⁵

Perspective for Antiviral Drug Design

Vaccines and drugs are two effective tools for fighting virus infections. Many commercially successful vaccines have been used to diminish viral infections, and in a few cases lead to eradication of some viral diseases, like smallpox,¹⁶ polio,¹⁷ measles,¹⁸ mumps and rubella.¹⁹ However, some other diseases especially caused by human immunodeficiency (HIV), hepatitis C (HCV), Ebola and SARS virus still remain untouched by vaccine approaches.²⁰⁻²³ Side effects are often observed with many vaccines especially when applied to immunosuppressed individuals. Live-attenuated vaccines can inadvertently cause the spread of viruses to vaccinees and may also be able to infect unvaccinated individuals.²⁴⁻²⁷ Finally, since vaccine act as a prophylactic measure, the development of antiviral drugs to treat people who are already infected is also needed.

Viruses are infectious particles with high replication and mutation rates. The mutation rate for RNA viruses is one mutation per genome per replication compared to DNA viruses' one mutation per genome per 1000 replications.²⁸ Because the latter use the host cell's DNA polymerase for viral replication, which has proofreading capability, they are often larger and evolve much more slowly than RNA viruses.²⁹ The high mutation rates along with using the host cell's molecular machinery makes viruses more difficult to fight with drugs than bacteria and other infections.

It has been 50 years since 5-iodo-2'-deoxyuridine (IDU) was described as the first antiviral drug (and still) marketed.³⁰ Now, after five decades, 50 compounds are licensed for clinical use to treat viral infections, including 25 compounds as anti-HIV drugs and others approved for treating herpes simplex virus (HSV), varicella-zoster virus (VZV),

cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV), and influenza virus infections.³¹

Antiviral drug design could target any stage of the viral replicative circle. Taking retroviruses (HIV) as an example, infusion to the host cell is the first step for its replication. The HIV virus employs chemokine receptors as co-receptors for cellular entry, such as CCR5 and CXCR4, which are naturally expressed on the surfaces of immune cells promoting their activation or migration by chemokines.^{32,33} UK-427857 (Maraviroc)³⁴⁻³⁶ is a potent inhibitor of CCR5 that prevents HIV entry into T-cells, and thus, possesses good pharmacological properties. Other compounds like AMD3100,^{37,38} work as antagonists of CXCR4 with similar results.

The replication of viral RNA can be prevented by reverse transcriptase (RT) inhibitors, including NRTIs (nucleoside reverse transcriptase inhibitors)³⁹ like AZT (azidothymidine, Retrovir[®])⁴⁰ ddI (Didanosine, videx[®])⁴¹ or (-)-3TC (Lamivudine, Epivir[®])⁴² (Figure 1) and NNRTIs (Non-nucleoside reverse transcriptase inhibitors)⁴³ like nevirapine (Viramune[®])⁴⁴⁻⁴⁶ or efavirenz (Sustiva[®]) (Figure 1).⁴⁷⁻⁴⁹ The former type acts as alternative substrates of natural nucleosides, which, when incorporated into viral DNA strands, results in termination of chain elongation.^{39,50-53} The latter NNRTIs specifically bind to a non-substrate binding (i.e., allosteric) site of HIV-1 RT, which results in a change of enzyme conformation from catalytically active into fixed inactive form.^{54,55}

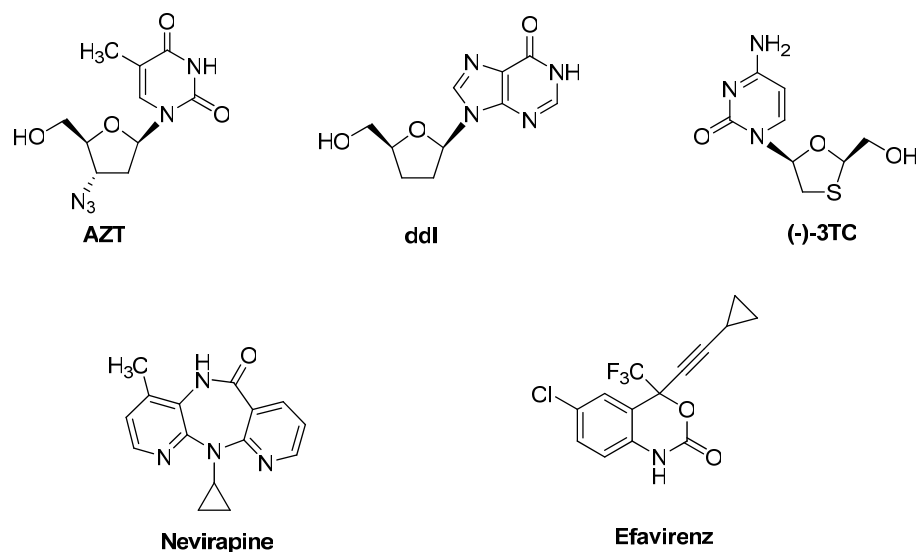


Figure 1. Examples of NRTIs and NNRTIs

The cleavage of newly expressed viral protein precursor into smaller and mature pieces and assembly with viral RNA into newly formed viruses can be inhibited by protease inhibitors⁵⁶⁻⁵⁹ like ritonavir (Norvir[®]) or/and lopinavir (Kaletra[®]).⁶⁰ These drugs were rationally designed containing a peptidomimetic hydroxyethylene bond instead of the normal peptide linkage to block the action of viral protease.⁶¹ Lastly, the release of progeny viruses from host cells can be halted by the administration of neuraminidase inhibitors.⁶²⁻⁶⁴ A well known example is oseltamivir (Tamiflu[®]), which is widely used for the treatment of influenza A and B viral infections,⁶⁵ including avian flu.⁶⁶

Nucleoside Analogues

Nucleosides are naturally occurring biological molecules which are fundamental building blocks of DNA and RNA (Figure 2). They also participate as essential intermediates in virtually all aspects of cellular metabolism.

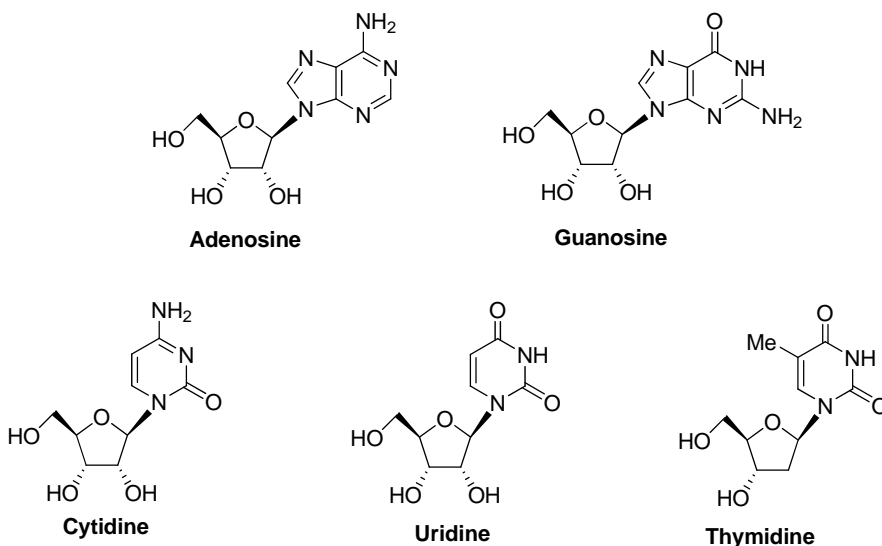


Figure 2. Naturally Occuring Nucleosides

For example, adenosine is a major component of adenosine triphosphate (ATP), coenzyme A and nicotinamide adenosine dinucleotide (NAD⁺) and is implicated in sleep regulation.^{67,68} Because of their pervasiveness, nucleoside analogues produce a variety of biological effects,⁶⁹ including functioning as non-toxic, selective inhibitors of viral kinases and polymerases.⁷⁰⁻⁷³ IDU and its 5 position modified analogue Brivudin (Figure 3) were first recognized as selective antiviral agents against HSV and VZV.⁷⁴ Later on, acyclic guanosine analogs acyclovir, penciclovir, ganciclovir and their oral prodrug forms valaciclovir, famciclovir and valganciclovir (Scheme 1) were approved for the treatment of herpesvirus (HSV, VZV and/or CMV) infections.^{75,76} All of these compounds target the viral DNA polymerase and, consequently, interrupt viral DNA synthesis. As a result, they only effect DNA-containing viruses and leave the RNA viruses untouched.⁷⁷ On the other hand, many 2', 3'-dideoxynucleoside analogues, such as azidothymidine (AZT), have been licensed as anti-HIV agents. After triphosphorylation by cellular kinases, they

are incorporated by the HIV reverse transcriptase into the viral DNA chain and result in chain termination since they lack the 3' hydroxyl group for further chain elongation.⁷⁸

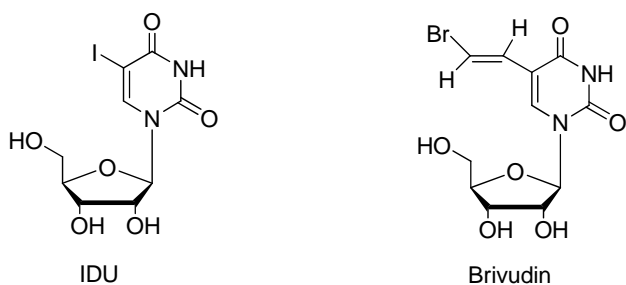
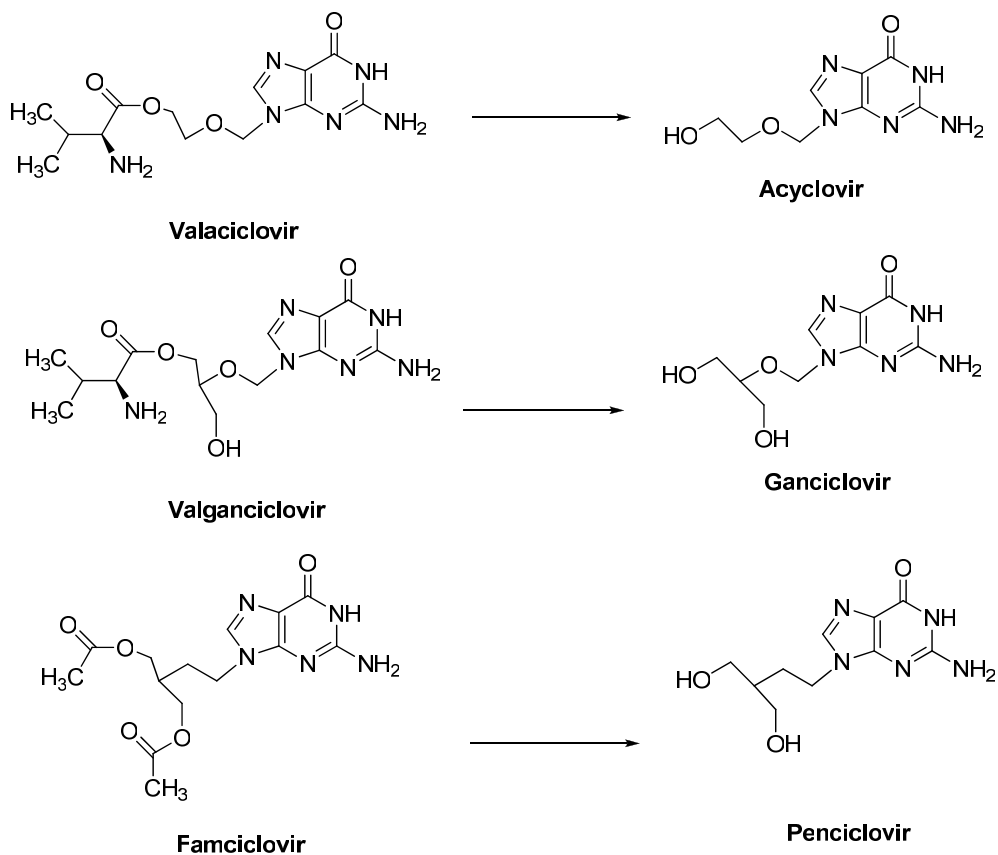


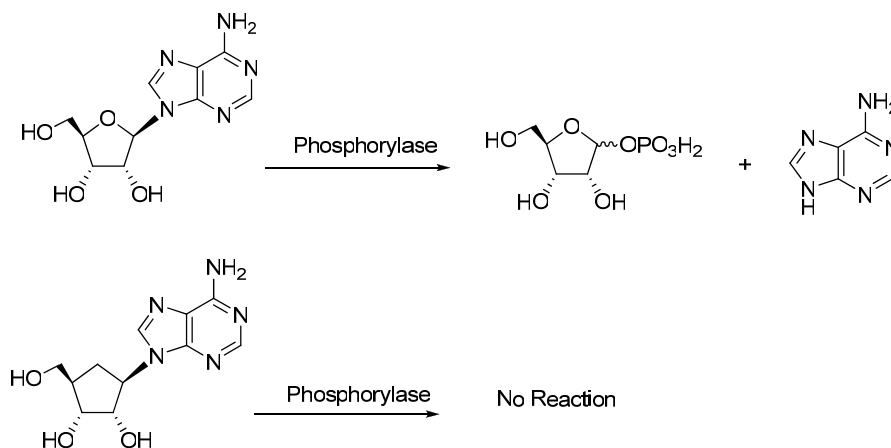
Figure 3. Structure of IDU and Brivudin



Scheme 1. Acyclic Nucleosides

Among the modified nucleosides, one of the most important classes is the carbocyclic nucleoside in which the oxygen in the sugar portion of traditional nucleosides is replaced

by a methylene unit.^{79,80} This characteristic makes the carbocyclic nucleoside more resistant to phosphorylases due to the absence of the glycosidic bond (Scheme 2).^{70,71,81,82}



Scheme 2. Stability of Carbocyclic Nucleosides

Two of the most important carbocyclic nucleosides are aristeromycin (Ari) and Neplanocin A (NpcA) (Figure 4), which show prominent broad-spectrum antiviral activity. Both compounds are naturally occurring carbocyclic analogues of adenosine. Ari was first synthesized by Shealy⁸³ in 1966 before it was isolated from a metabolite of *Streptomyces cirtricolor*⁸⁴ in 1968, while NpcA was isolated from the culture broth of *Ampullariella regulars* in 1981.^{85,86} Their antiviral potency has been proven to be due to the inhibition of AdoMet dependant biomethyltransferation, a pivotal step in viral replication.^{87,88}

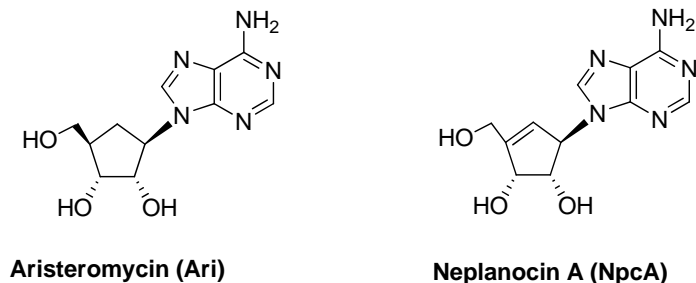


Figure 4. Aristeromycin and Neplanocin A

Adenosylhomocysteine (AdoHcy) Hydrolase as a Key Enzyme for Antiviral Activity

Generally speaking, there are two strategies for anti-viral drug design: one, is to target on the viral proteins. This approach could develop many very specific, low toxic antiviral agents with high selectivity but, will more likely, lead to high drug-resistance by viral adaptation. Another option is to target host proteins. This approach opens the door for broad spectrum antiviral compound discovery with a low chance of drug-resistance development but high toxicity.⁷⁷ IMP dehydrogenase is an example of the latter sposed, which is a host target enzyme that is responsible for the NAD-dependent oxidation of IMP to xanthosine 5'-monophosphate (XMP).⁸⁹ Inhibition of this cellular enzyme upon viral metabolic demand could have anti-viral effectiveness preferentially since there is an increasing demand for RNA and/or DNA synthesis in virus-infected cells. A well known anti-viral agent acting as an IMP dehydrogenase inhibitor is ribavirin,⁹⁰ which is clinically used for the treatment of rous sarcoma virus (RSV) infections.⁹¹⁻⁹³

In our laboratories we have been focusing on methylation of the capped structure of viral m-RNA as the subject for searching anti-viral agents. A relevant enzyme in modulating the process is AdoHcy hydrolase, which is a cellular enzyme that is also

employed for viral protein translation and has been studied as a potential target responsible for the inhibition of this capping process.

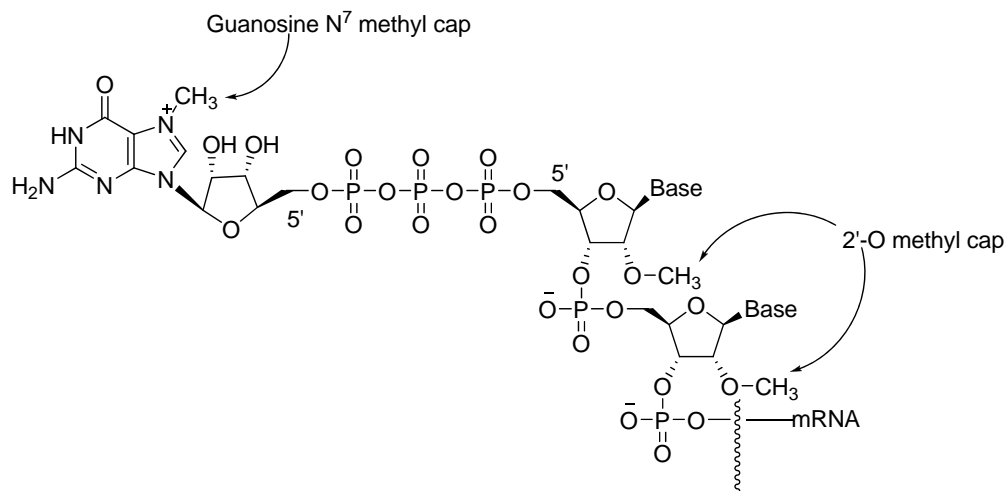


Figure 5. Structure of mRNA 5'-Terminal Cap

Viruses and eukaryotic cells both require capping structures at the 5'-end of m-RNA for successful ribosomal translation (Figure 5). These capping structures consist of a 7-methylguanosine linked to the 5'-end of m-RNA by a 5'-5' triphosphate bridge.⁹⁴ Most 5'-capped RNA structures are also methylated at the 2'-hydroxyl site of one or more of the penultimate nucleotides. The importance of the capped structures in mRNA synthesis has been shown to be manifold:^{95,96} 1) facilitating post transcriptional processing, nucleocytoplasmic transport; 2) recognition of mature mRNA by the translation machinery;⁹⁷⁻⁹⁹ 3) improving affinity for binding to the ribosome in the translational initiation complex;¹⁰⁰ and, 4) preventing the mRNA degradation by 5'-end nuclease.¹⁰¹

The capping process consists of three enzymatic reactions. First, the 5' triphosphate end of primary RNA is cleaved to a diphosphate terminus by RNA triphosphatase. Then,

capping with a GMP group by RNA guanylyltransferase follows. Finally, RNA (guanine-7)methyltransferase catalyzes the methylation reaction at the N7 position of guanine.¹⁰²

- (i) $\text{pppN(pN)}_n \rightarrow \text{ppN(pN)}_n + \text{P}_i$
- (ii) $\text{ppN(pN)}_n + \text{pppG} \rightleftharpoons \text{G(5')pppN(pN)}_n + \text{PP}_i$
- (iii) $\text{G(5')pppN(pN)}_n + \text{AdoMet} \rightarrow \text{m}^7\text{G(5')pppN(pN)}_n + \text{AdoHcy}$

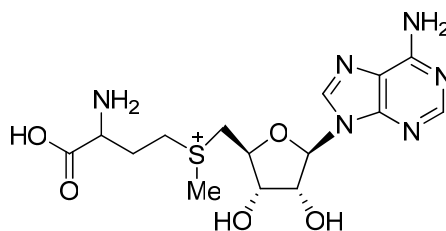
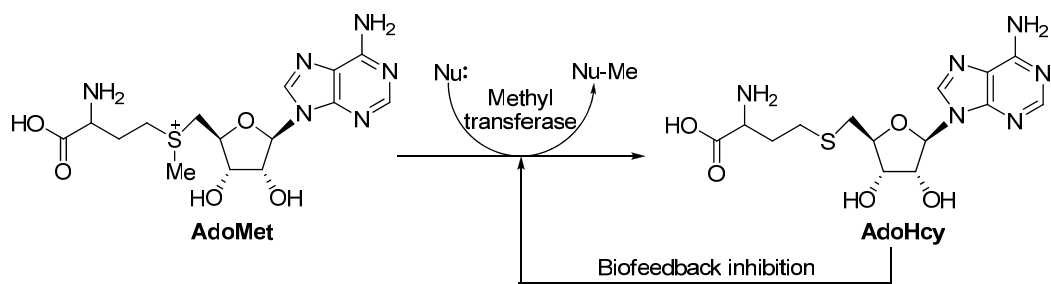


Figure 6. S-Adenosylmethionine (AdoMet)

AdoMet (S-adenosyl-L-methionine) (Figure 6) serves as the methyl donor in step (iii) of the capped mRNA. AdoMet is also a participant in many other biological methylation processes such as methylation of histamine,¹⁰³ serotonin,¹⁰⁴ membrane phospholipids¹⁰⁵ and DNA,¹⁰⁶⁻¹⁰⁸ RNA or protein.^{109,110} Besides acting as a universal methyl donor, AdoMet is also involved in many other group transfer reactions, such as, (1) after decarboxylation, an aminopropyl donor in the biosynthesis of polyamines spermidine and spermine,¹¹¹ (2) a 3-amino-3-carboxypropyl donor in tRNA modification¹¹² and (3) an adenosyl donor in the modification of the enzyme pyruvate formate-lyase.¹¹³



Scheme 3. AdoMet-dependent Methyl-Transferation

However, in the methyl transfer pathways, AdoMet is converted into adenosylhomocysteine (AdoHcy) by different methyltransferases.¹¹⁴⁻¹¹⁶ It is to be noted that the metabolite AdoHcy is a potent biofeed back inhibitor of AdoMet-dependent methyltransferases *in vivo* (Scheme 3).¹¹⁷⁻¹²² The methylase enzyme affinity for AdoHcy is the same or even higher than for AdoMet.¹²³ This inhibition is relieved by hydrolysis of AdoHcy to adenosine and L-homocysteine (Figure 7). This reversible reaction is catalyzed by the enzyme AdoHcy hydrolase, which makes this enzyme an interesting target for antiviral drug design.^{124,125} By inhibiting this enzyme, the concentration of AdoHcy builds up, and increases the intracellular ratio of AdoHcy/AdoMet, which will lead to the suppression of the viral mRNA methylation and, consequently, preventing the capping process from occurring. Viruses, like vesicular stomatitis virus (VZV), that rely on the mRNA capping process for their replication, are particularly sensitive to inhibition by AdoHcy hydrolase inhibitors.¹²⁶ Many other negative-sense single-stranded RNA viruses, double strand RNA viruses or even some of DNA viruses are also affected by AdoHcy hydrolase inhibitors (Table 1).

Virus type	Virus classification	specific example
(-) RNA	Rhabdovirus	Rabies
(-) RNA	Filovirus	Ebola, Marburg
(-) RNA	Arenavirus	Junin, Tacarobe
ds RNA	Reovirus	Rota
(-) RNA	Paramyxovirus	Parainfluenza, Respiratory Syncytial virus
(+) RNA (RT)	Retrovirus	HIV
ds DNA	Herpesvirus	Cytomegalovirus
ds DNA	Poxvirus	Vaccinia, Monkeypox

(-) RNA: Negative-sense single-stranded RNA virus

ds RNA: Double-stranded RNA virus

(+) RNA (RT): Positive-sense single-stranded RNA virus that use reverse transcription

ds DNA: Double-stranded DNA virus

Table 1. Viruses affected by AdoHcy hydrolase inhibitors

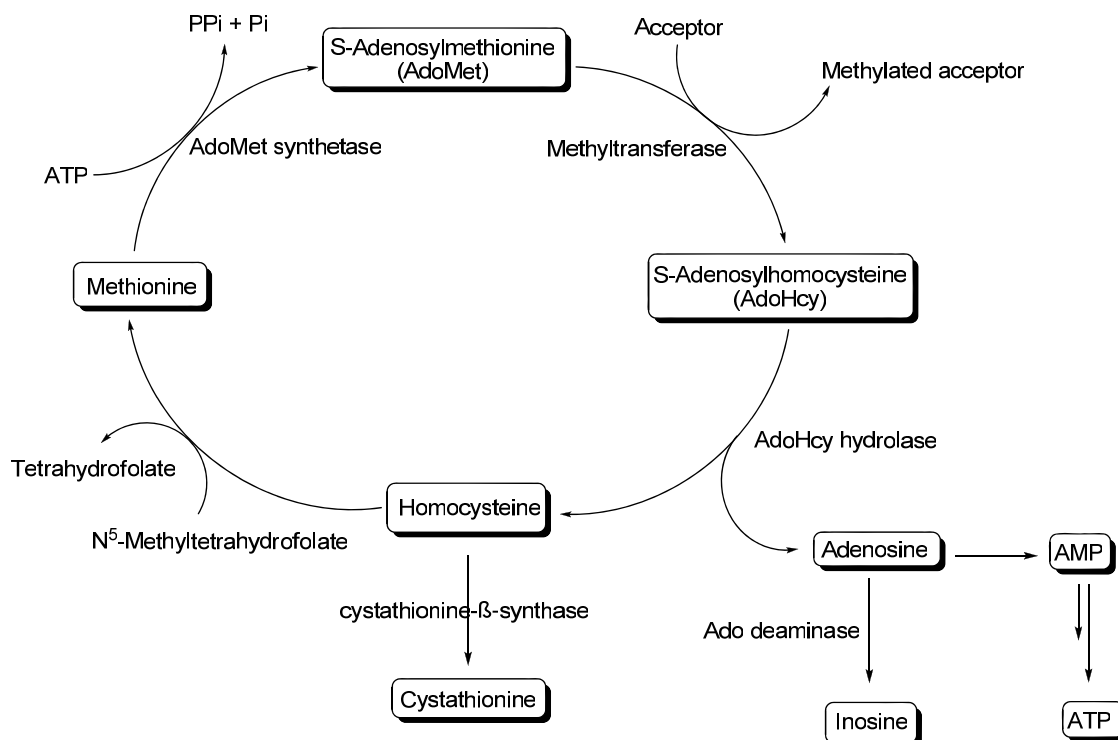
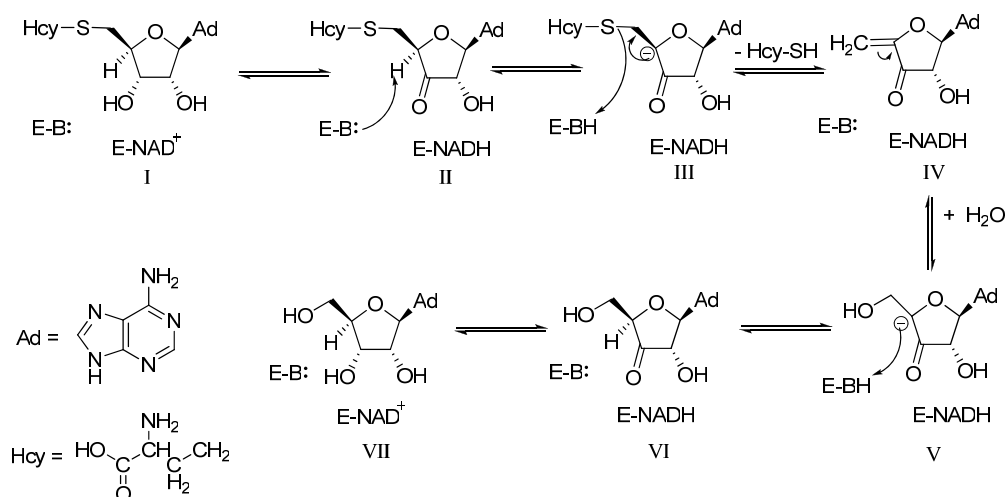


Figure 7. Metabolic pathways of AdoMet

The mechanism of AdoHcy hydrolysis has first been proposed by Palmer and Abeles in 1976.¹²⁷ They used enzyme purified from beef liver, which contains tightly bound NAD^+ , to oxidize the 3'-hydroxyl group to a ketone form in first step (Scheme 4). This change increases the acidity of 4' hydrogen and makes it labile and easily removed by enzymatic base (II→III). Following proton removal, L-homocysteine is eliminated (III→IV) and 3'- ketoadenosine is produced by adding a water molecule through a Michael addition (IV→V→VI). Finally the catalytic cycle is completed by forming the adenosine via reduction on the C-3' keto group (VI→VII).^{127,128}



Scheme 4. Mechanism of AdoHcy Hydrolase

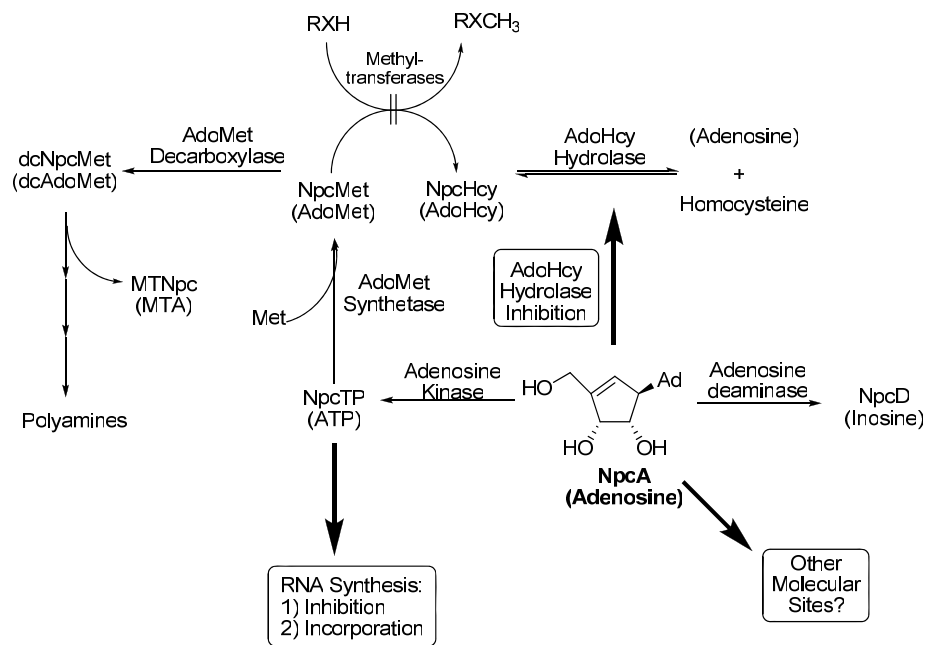
The reaction favors the synthetic direction (VII→VI) with an equilibrium constant of 10^{-6} M, quick removal of adenosine and homocysteine in the cell facilitates the reaction towards the hydrolytic direction (I→VII). Therefore, adenosine analogues capable of serving as substrates for the AdcHcy hydrolase become attractive target compounds for inhibition of this enzyme.¹²⁹

The Inhibitors of AdcHcy Hydrolase as Antiviral Agents

Ari^{130,131} and NpcA^{85,132,133} are considered as the first generation inhibitors of AdoHcy hydrolase. Both compounds show antiviral activity against, for example, poxvirus, reovirus, and smallpox virus ect.. Despite the high potency, the cytotoxicity of these compounds precluded them as clinical antiviral agents. This toxicity has been attributed to formation of their corresponding 5'-triphosphates (Figure 9) by adenosine kinase, followed by adenylate kinase and subsequently, nucleoside diphosphokinase. These nucleotides can interfere with a number of fundamental cellular processes.¹³⁴⁻¹³⁷

5'-Triphosphate NpcA (NpcTP) also could serve as a substrate of AdoMet synthetase forming the AdoMet counterpart of NpcA that is NpcMet^{135,136,138-140} which may be responsible for the antiviral activity and/or cytotoxicity of NpcA by direct inhibition of viral or cellular methyltransferase.^{141,142} NpcA's effect could be reduced by adenosine deaminase and converted to its inactive form: neplanocin D, a carbocyclic analog of inosine (Scheme 5).

The mechanism for NpcA inhibition of AdoHcy hydrolase has been classified as an “enzyme specific cofactor depletion mechanism”.^{143,144} The study shows that NpcA will be oxidized to its 3'-keto form by NAD⁺ and no further transformation occurs because this latter product lacks the C-4' proton. The effect of this oxidation is depletion of NAD⁺ for the I→II step of Scheme 4. Hence, the concentration of AdoHcy remains high and biofeedback inhibition of the methyltransferases occurs.^{132,145}



Scheme 5. Metabolic pathways of NpcA

A possible reason for the toxicity of Ari is due to the metabolite 5'-monophosphated Ari, which is transformed into the inosine monophosphate (IMP) analogue by adenosine monophosphate (AMP) deaminase^{146,147} followed by conversion to the carbocyclic analog of guanosine monophosphate (GMP) (Figure 8). This latter metabolite is a good inhibitor of hypoxanthine (guanine) phosphoribosyltransferase,¹⁴⁶ an important enzyme in the normal cell purine salvage pathway.⁸⁸

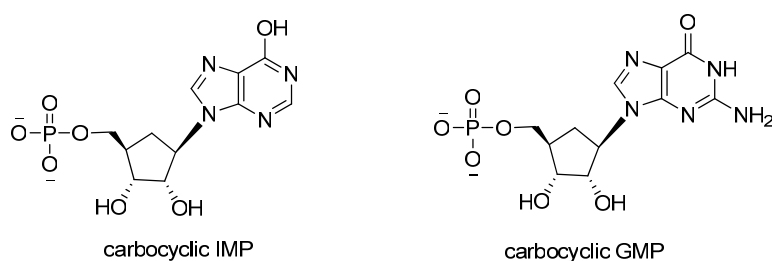


Figure 8. Metabolites of Ari

Therefore, it is apparent that the 5'-phosphorylation of Ari and NpcA (Figure 9) is, for most part, the cause of the unwanted cytotoxicity. Thus, a great number of Ari and NpcA analogues have been designed and synthesized by several groups, including the Schneller laboratory, to avoid the undesired nucleotide metabolism process while retaining the promising antiviral activity.

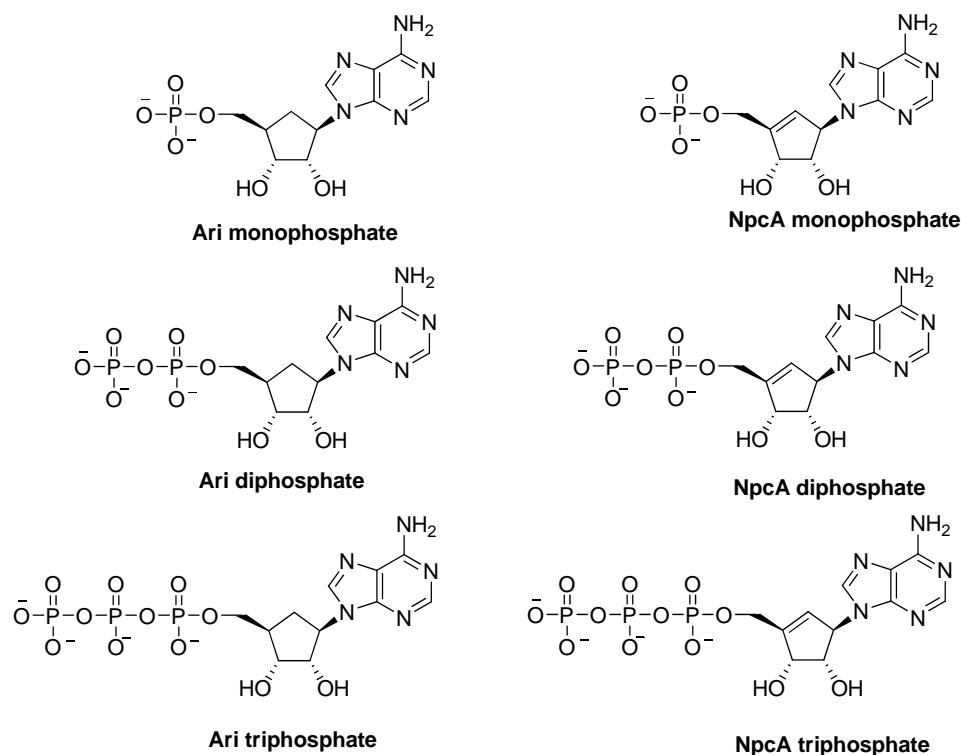


Figure 9. Phosphate Derivatives of Ari and NpcA

Among various modifications, 3-deazaaristerimycin (3-deazaAri) and 3-deazaneplanocin A (3-deazaNpcA) (Figure 10) have drawn considerable attention. Evidences showed that both 3-deazaAri and 3-deazaNpcA could fully protect mice against a lethal challenge with Ebola virus.¹⁴⁸ In fact, when mice were treated with 3-deazaNpcA, increased amounts of interferon α were found in Ebola infected mice, but not in the uninfected mices.¹⁴⁹ It was suggested that 3-deazaNpcA restrained the methylation of viral mRNA through inhibiting the AdoHcy hydrolase, hereby preventing the mature mRNA from the (-) RNA template. This results in the accumulation of double-stranded RNA, which is an excellent inducer for interferon.¹⁵⁰ Filovirus including Ebola and Marburg virus are highly infectious and have no vaccine or antiviral drug available in clinical use.¹⁵¹ Thus, an AdoHcy hydrolase inhibitor based on 3-deazaAri and 3-

deazaNpcA, may provide the most promising chemotherapeutic strategy for treating this dreadful viral disease. Besides the higher activity of 3-deazaNcpA and 3-dezazAri, they also possess less cytotoxicity than their parent compounds.¹⁵²

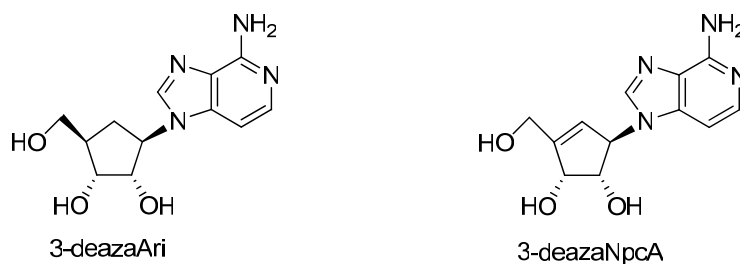


Figure 10. 3-Deazazaristeromycin and 3-Deazaneplanocin

Based on the fact that neither of these two compounds are substrates for adenosine kinase and adenosine deaminase,^{153,154} additional adenine carbocyclic nucleosides were designed and synthesized. For example, since it was clear that the 5'-hydroxyl group was essential for substrate activity of adenosine kinase and deaminase,¹⁵⁵ Borchardt et al. designed and synthesized the 5'-truncated analogues of NpcA, 3-deazaNcpA (DHCeA and C³-DHCeA) (Figure 11).¹⁵⁶ They also found that both compounds had much lower cytotoxicity than their parent compounds while still retaining antiviral activity^{157,158} (DHCeA is 34 times less cytotoxic comparing with NpcA, measured as the ID₅₀ value).⁸⁸ Similar results have been found for their Ari counterparts, DHCaA and C³-DHCaA (Figure 11).¹⁵⁹

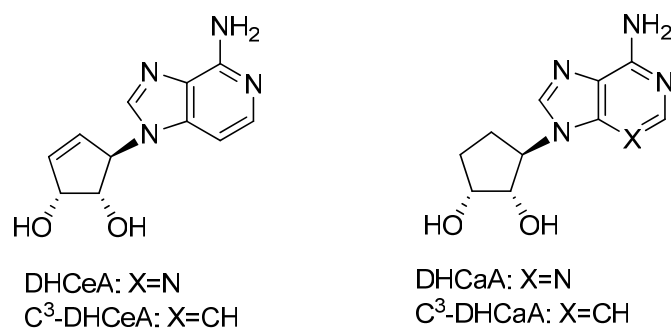


Figure 11. Structure of DHCeA , DHCaA and their 3-deaza analogues

Similarly 5'-deoxyAri (Figure 12) was designed and synthesized by Schneller and coworkers.¹⁶⁰ Without the hydroxyl group at C-5' position, this compound displayed moderate antiviral activity against vaccinia virus (VV) and vesicular stomatitis virus VSV with little toxicity.¹⁶¹

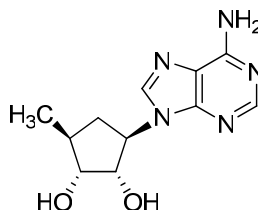


Figure 12. 5'-Deoxyaristeromycin

Another reasonable strategy for diminishing 5'-hydroxyl interference via its phosphorylation was extension of the C-5' side chain, rendering the 5'-hydroxyl group displaced to a site possibly less susceptible to phosphorylation by adenosine kinase. In that direction, 5'-homoaristeromycin (5'-homoAri) (Figure 13) was synthesized by Schneller and other research groups.¹⁶²⁻¹⁶⁴ An improved stereoselective synthesis approach was reported in 2005 from the Schneller laboratories and notable antiviral

activities toward vaccinia, cowpox and monkeypox viruses were found with low cytotoxicity ($CC_{50} > 100 \mu\text{g/mL}$).¹⁶⁵ A 5'-Homoneplanocin A (5'-homoNpcA) (Figure 13) analog was first described by Shuto in 1996,¹⁶⁶ and synthesized later by the Schneller group via a more efficient and practical route. 5'-HomoNpcA also showed activities against CMV, vaccinia virus, parainfluenza virus, VSV, arenaviruses, HBV and HCV with no interfering toxicity.¹⁶⁷

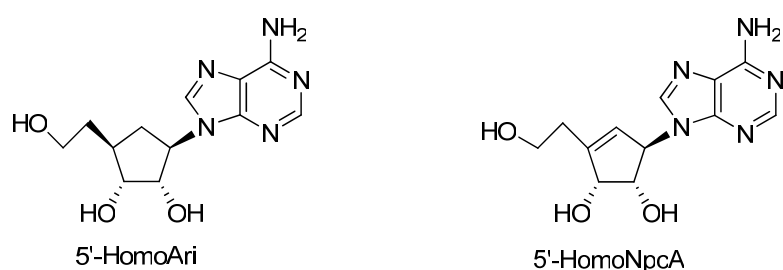


Figure 13. 5'-Homoanalogs of Ari and NpcA

Target Design Based on Inhibition of AdoHcy Hydrolase

Since the discovery of AdoHcy hydrolase as a potent target for antiviral drugs discovery, many adenosine analogues, including acyclic, carbocyclic or adenine base modified, have been designed, synthesized and tested for their activities. Due to their unique stability against phosphorylase metabolism, the aforementioned carbocyclic nucleosides arose as a prominent group of compounds.

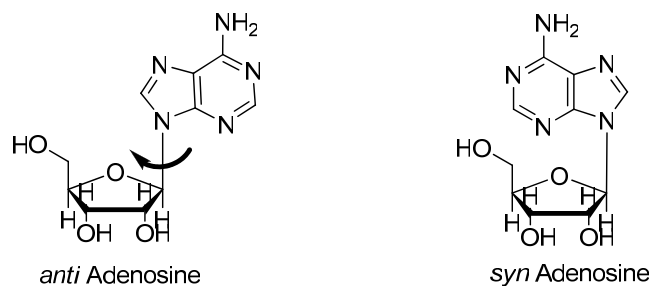


Figure 14. *syn* and *anti* conformation for nucleosides

In considering structural variations for adenine derived antiviral candidates, nucleoside conformation offers an option. In the case of purine nucleosides rotation around the glycosidic bond results in two conformational extremes: a *syn* or an *anti* conformation (Figure 14).¹⁶⁸ Concerning nucleoside-enzyme interactions, *syn-anti* conformation is one of the most important conformational aspects.¹⁶⁹ Adding a bulky substituent on a nucleobase or a sugar portion are both feasible ways for fixing the nucleoside conformation. But like *syn/anti* conformation around the glycosyl bond, the sugar pucker is also an important conformational aspect in nucleoside-enzyme interactions.^{170,171} Since that, introducing substituent only on a nucleobase is sufficient to control the *syn/anti* conformation without greatly affecting its sugar puckering. For example, 8-bromoadenosine and 8-bromoguanosine have been found to adopt a *syn* conformations in the solid state as well as in solution. The steric repulsion between the 8-bromo group and the sugar portion is the only reasonable explanation for this. Therefore the 8-bromoguanosine 5'-triphosphate does not interact with Q β replicase or *Escherichia coli* transcriptase which prefers an *anti* rotational isomer either during or after the polymerization reaction.^{172,173} Modification of the purine base on carbonucleosides by adding a methyl group at the C-8 position favor of the *syn* conformation. In fact, previous

research in our laboratory showed 8-methyl-5'-noraristeromycin (Figure 15) possesses moderate inhibitory activity towards AdoHcy hydrolase.¹⁷⁴ In this dissertation, a series of 8-methyl carbocyclic adenosine analogs as probes for the conformational role in AdoHcy hydrolase inhibitory effect will be pursued.

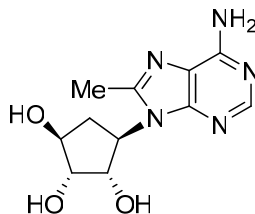


Figure 15. 8-Methyl-5'-noraristeromycin

To use the 8-alkylated aristeromycin framework for drug design must mention the high cytotoxicity^{131,137} that is associated with aristeromycin. Recognizing that this toxicity is due to C-5' phosphorylations, perhaps by adding a methylene at this center (hence, 5'-homoaristeromycin) (Figure 16) would decrease this possibility.

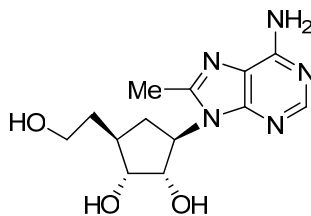


Figure 16. 8-Methyl-5'-homoaristeromycin

As mentioned above, 3-deazaAri and 3-deazaNpcA are two of the most potent inhibitors for AdoHcy hydrolase, with a $K_i=2 \times 10^{-9}$ M and 5×10^{-11} M, respectively (5×10^{-9} M for Ari).^{130,141} The 3-deazaAri prototype has good antiviral activity against

vaccinia, vesicular stomatitis, measles, parainfluenza, and reo viruses,¹⁵³ while 3-dezazNpcA showed excellent activity against VSV, parainfluenza, vaccinia and yellow fever viruses.¹⁵⁴ In addition to their antiviral activities, both compounds display much lower cytotoxicity than the parent compounds Ari and NpcA.⁸⁸ Research shows that 3-deazaAri, unlike Ari, is not deaminated by adenosine deaminase nor phosphorylated by adenosine kinase. These properties are also seen with 3-deazaadenosine.¹⁷⁵ However 3-deazaAri undergoes further anabolism to the corresponding S-AdoHcy analogues, which may cause some unwanted side effects.^{137,153,175} 3-deazaNpcA is neither phosphorylated nor converted to its S-AdoHcy analogue metabolites, which makes its cytotoxicity much lower.^{141,176} So another project in this dissertation is to modify 3-dezazAri and 3-deazaNpcA with a methyl group at the C-8 position with and without extension of the 5'-hydroxyl group by a methylene group (Figure 17) rendering it less cytotoxic.

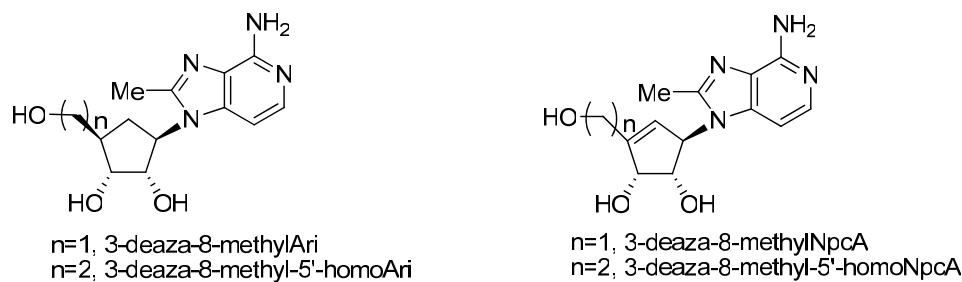


Figure 17. Analogues of 3-deazaAri and 3-deazaNcp A

In our laboratories, 3-deaza-5'-noraristeromycin (Figure 18) has been prepared and proven to possess antiviral activities against a series of DNA and RNA viruses.¹⁷⁷ Based on that, the 8-methyl analogue of 3-deaza-5'-norAri (Figure 18) was set as a target agent.

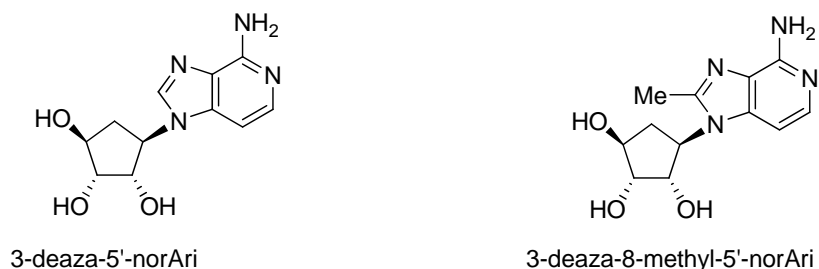


Figure 18. 3-Deaza-5'-norAri and its 8-methyl analogue

Additionally, c^3 -DHCeA is known to have potent antiviral activity with less associated toxicity,¹⁵⁶ so the 8-methyl analogue of c^3 -DHCeA (Figure 19) is another valuable target for 8-methyl modification.

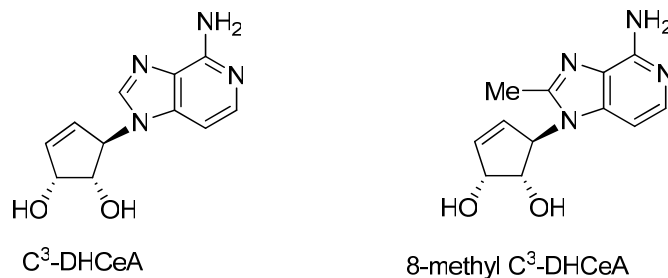


Figure 19. 8-Methyl analogue of C^3 -DHCeA

Stereochemistry is also considered in the design of the target compounds for this dissertation. Natural nucleosides are D-nucleosides (being derived from D-ribose) in which the heterocyclic base connects with the sugar ring in the β configuration via an N-glycosidic bond. To facilitate correlations with natural nucleosides the D-designation has carried over to carbocyclic nucleosides where stereo similar structures of D-nucleosides

are referred to as D-like carbocyclic nucleosides and their enantiomers are recognized as L-like carbocyclic nucleosides (Figure 20).

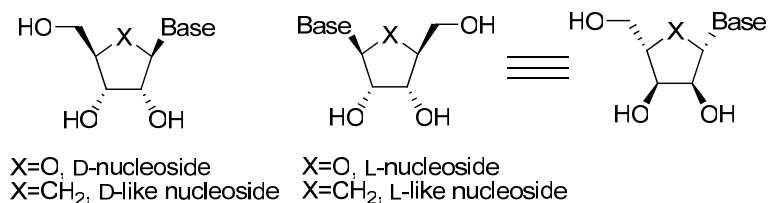


Figure 20. D, L -like Nucleosides

The real world is asymmetric, where, for example, two enantiomers will likely have different or even opposite activity. For example, (-)-5'-norAri (the D-like enantiomer) is 100-fold more potent against cytomegalovirus (CMV) than (+)-5'-norAri (the L-like enantiomer); however, the latter is more active against HBV.^{161,178} Therefore, pharmacologic drug design often requires compounds to be optically pure (only one enantiomer).

Not only do enantiomers have different pharmaceutical properties, changing a stereocenter in a chiral molecule (to a diastereomer) may affect the biological activity as well. A pronounced example of this is between two diastereoisomers of 5'-methylneplancin A (Figure 21). In this case only the 5'*R* isomer was found to be an excellent AdoHcy hydrolase inhibitor ($K_i=0.086\mu\text{M}$ for murine L929 cell) and to display significant activity against a variety of DNA and RNA viruses, whereas the 5'*S* isomer lacked hydrolase activity ($K_i=55.7\mu\text{M}$) and antiviral effects.^{179,180}

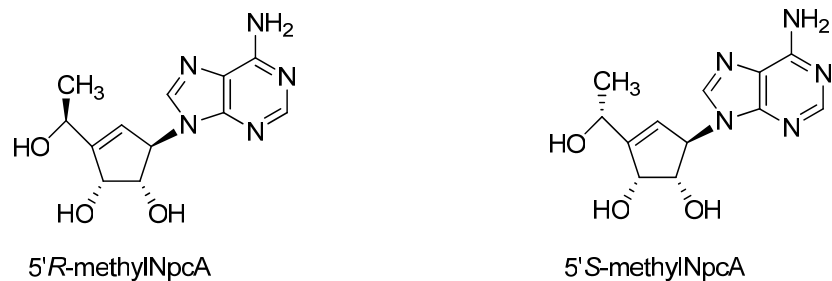


Figure 21. 5'-Methylneplanocin A

A study showed that modification at the C-4' position of Ari and NpcA offered a possibility for interesting biological activities.¹⁵⁹ Since the 5'-hydroxyl group is necessary for phosphorylation and deamination, development of C-4' modified analogues may lead to nucleotides or inosine analogues, which may cause biological inactivity and/or cytotoxicity.¹⁵⁵ As a consequence, a change of the configuration at the 4'-position was investigated for impact on biological effects and toxicity. In this direction, this dissertation project sought inverting C-4' stereocenter of Ari, homoAri and their 3-deaza analogues (Figure 22. designated as iso-analogues).

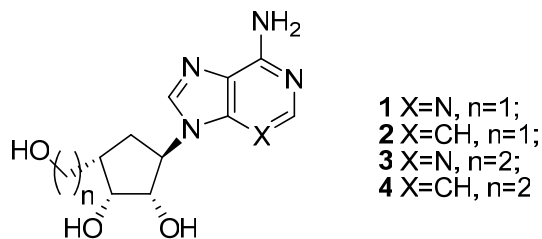


Figure 22. Target compounds for 4'-isoAri analogues

Target Design Based on Direct Inhibition of Methyltransferase

As mentioned, AdoHcy is the metabolite of AdoMet following methyl transfer in the capping of mRNA and is a potent feedback inhibitor for the transmethylation reaction.

However, AdoHcy itself is not a long lasting inhibitor of the AdoMet process due to its rapid catabolic breakdown.^{119,181,182} Moreover, extracellular AdoHcy can not cross the cellular membrane as an intact molecule,¹⁸³ It is with this in mind that many AdoHcy analogues have been synthesized with the modification in the sugar ring, base portion (C⁷ AdoHcy, Figure 23), or even amino acid part (SIBA, Figure 23) to overcome the disadvantages of AdoHcy.¹⁸⁴⁻¹⁹³

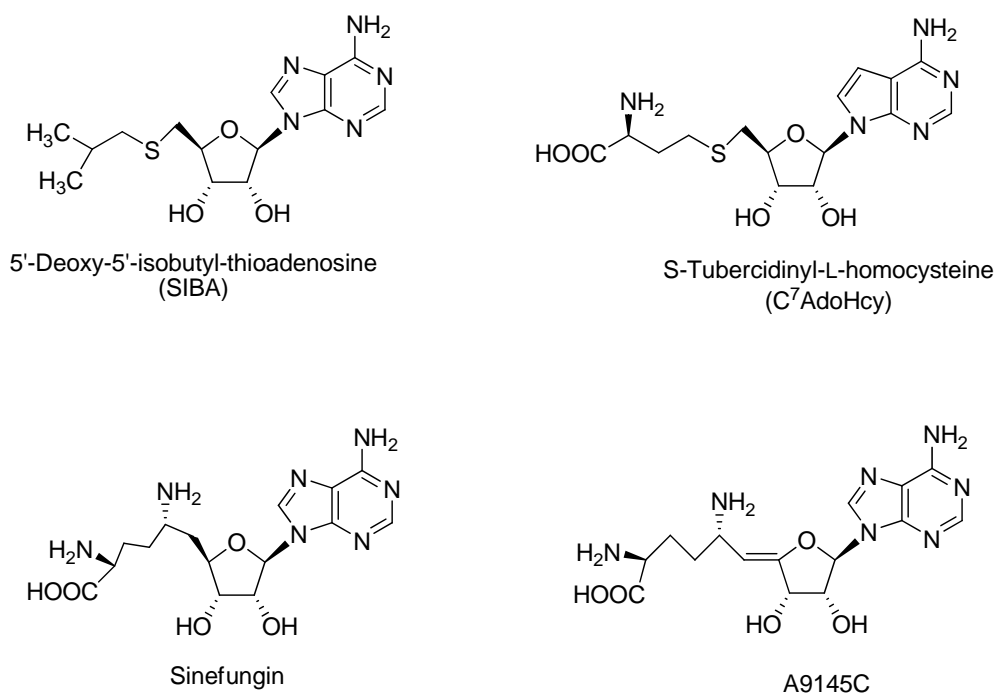


Figure 23. Analogues of AdoHcy

In the area of AdoHcy analogue discovery, sinefungin (Figure 23), which was first isolated from *Streptomyces griseolus* at Lilly Research Laboratories,¹⁹⁴ was found to possess antifungal and antiparasitic activities.¹⁹⁵ These biological effects have been attributed to the inhibition of many AdoMet depended methylation reactions. The structure of sinefungin can envisioned as a sulfurless analogue of AdoHcy.¹⁹⁶⁻²⁰⁰ Both

sinefungin and its related metabolite, A9145C (Figure 23) are extremely active as inhibitors of vaccinia virion mRNA (guanine-7-)methyltransferase and nucleoside-2'-methyltransferase.^{198,199} For example, the inhibition constant K_i of A9145C for mRNA (guanine-7-)methyltransferase is 1.34 nM compared to AdoHcy ($K_i=1040$ nM).^{199,201} Unfortunately, both compounds possess significant toxicity for host cells at concentrations higher than 10 μ M.¹⁹⁹ This is due to the associated potent inhibition of cellular methyltransferase, including, for example, tRNA methyltransferase or protein methylase, by sinefungin and A9145C.^{196,197}

However, biological potential of sinefungin has stimulated many research groups, including the Schneller group, to pursue the synthesis of sinefungin and related analogues where modification at the side chain, heterocyclic base and ribofuranose unit is considered.^{193,202,203} In that regard, this dissertation research sought the synthesis of the deamino- carbocyclic analogue of sinefungin (Figure 24). It was believed that this target compound would have improved activity by removing the side chain amino, while simultaneously making the compound more lipophilic to easily penetrate through the cellular membrane.

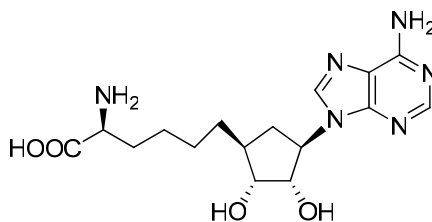


Figure 24. Deamino-carbocyclic sinefungin

Chapter 1. Synthesis of Important Precursors

A variety of compounds have been recognized as versatile precursors for the carbocyclic nucleosides synthesis. Among them (1*R*, 4*S*)-4-hydroxy-2-cyclopentyl monoacetate ((+)-monoacetate, **11**) and (-)-(4*R*,5*R*)-4,5-*O*-isopropylidene-2-cyclopentenone (**12**) (Figure 25) are considered as most important intermediates for the D-like configuration carbocyclic nucleosides in this research.

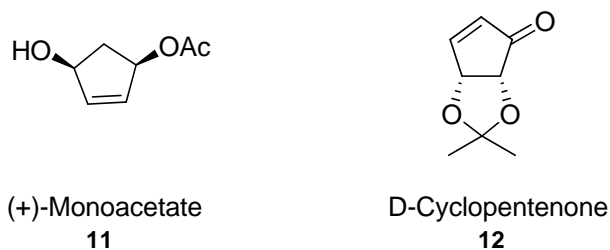
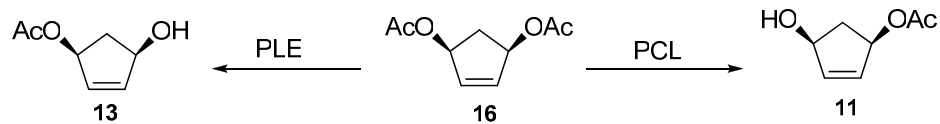


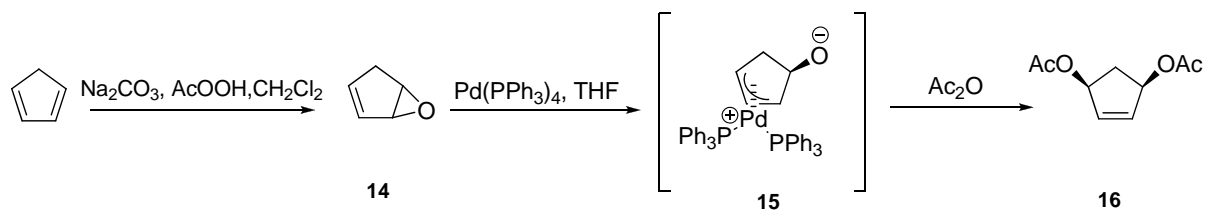
Figure 25. Precursors for carbocyclic nucleosides synthesis

In this direction, the past decades have seen enzymatic- and microbial- catalysts become powerful tools for organic synthesis, especially for enantioselective synthesis. These catalysts not only provide a simple and highly efficient approach for organic chemists but also are economical and environmentally friendly. Relevant here is the transformation of allylic diacetate **16** to the allylic monoacetate **11** and its enantiomer **13** by choosing different enzymatic conditions, *pseudomonas cepacia* lipase (PCL) to **11** or pig liver esterase (PLE) to **13** respectively (Scheme 6).^{160,204,205}



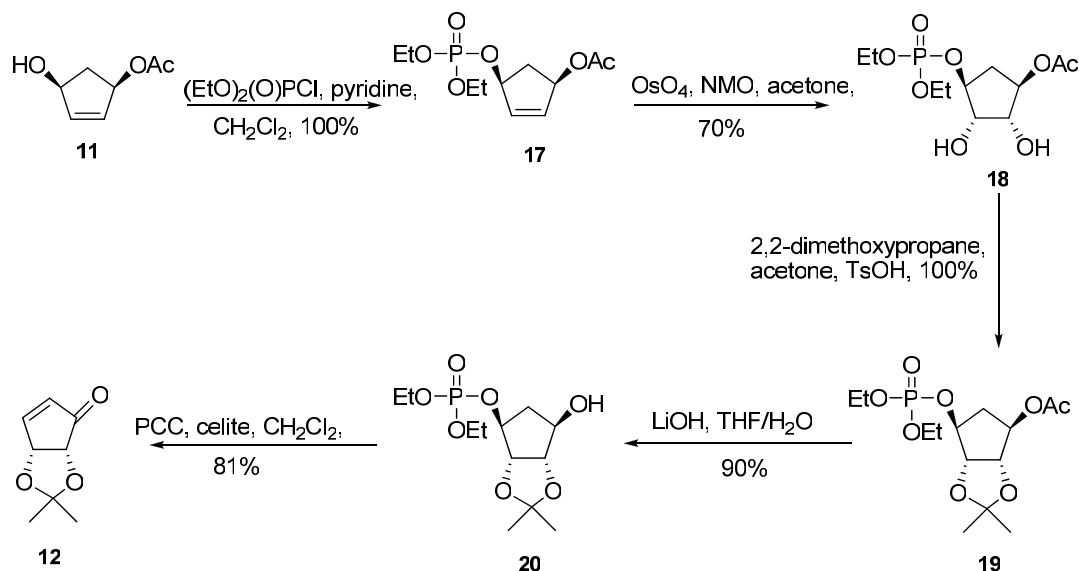
Scheme 6. Enzymatic hydrolysis of 16

To achieve **11** and **13** in this way, *meso*-diacetate **16** was synthesized in 3 steps from cyclopentadiene following a literature procedure (Scheme 7).²⁰⁶ First, epoxidation of freshly cracked cyclopentadiene by peroxidic acid, the Pd-catalyst *tetrakis*-(triphenylphosphine)palladium(0) was used to open the oxidation product vinyl epoxide **14**. The π -allylpalladium complex **15** that formed and is used for construction of various stereo- and regiospecific substituted cyclopentanoids. In this case, acetic anhydride produced the *cis*-1,4-addition product **16**. The reaction with acetic anhydride is presumed to be initiated by a nucleophilic attack of the basic oxygen anion in **15** on acetic anhydride. The resultant acetoxy anion subsequently *trans* attacked the *distal* end of the π -allylpalladium complex through the less hindered face to realize the *cis* stereochemical product **16**.



Scheme 7. Synthesis of diacetate 16

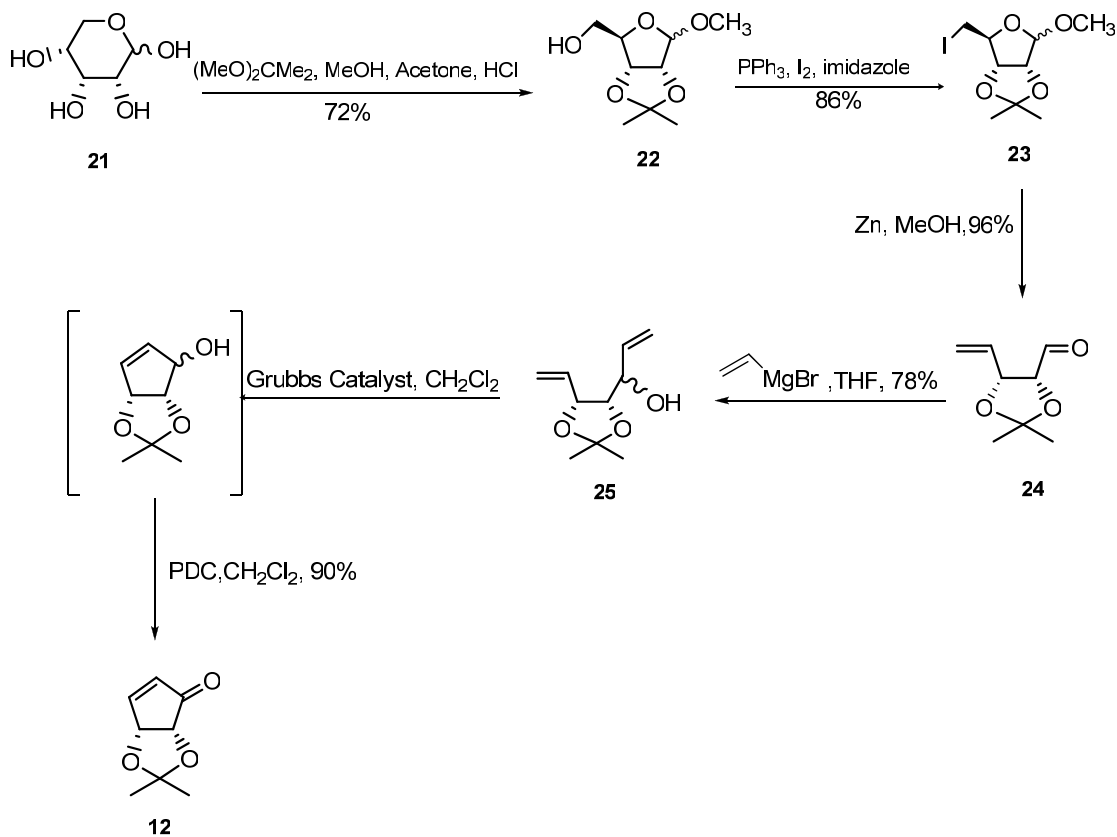
These results are relevant to the centrality of cyclopentenone **12**, as well as its different protecting group analogues, as a versatile synthon in carbocyclic nucleosides synthesis. There are several stereoselective procedures available to **12**,¹⁵⁶ one of which is used in our laboratory from (+)-monoacetate **11** (Scheme 8).²⁰⁷ This synthesis begins with the conversion of **11** into its phosphate derivative **17**, followed by dihydroxylation with osmium tetroxide to give diol **18**. After protection of **18** with 2,2-dimethoxypropane, the acetate group was removed by treating with lithium hydroxide. The synthesis was completed to **12** by an oxidative elimination of **20** with pyridinium chlorochromate (PCC).



Scheme 8. Synthesis of enone 12 from monoacetate 11

Another preparative route to **12** was developed in our laboratory in 2004 (Scheme 9).²⁰⁸ This approach is considered more economical, by including the inexpensive natural

sugar D-ribose as the starting material, and being more efficient (5 steps start from D-ribose with 42% yield).



Scheme 9. Synthesis of enone 12 from D-Ribose

This synthesis started with the protection of D-ribose with 2,2-dimethoxypropane and simultaneous methylation of the anomeric hydroxyl group. The primary hydroxyl group in **22** was transformed into an iodo substituent (compound **23**) by treating with triphenylphosphine and iodine.²⁰⁹ Reductive cleavage of **23** with activated zinc powder in hot methanol afforded aldehyde **24**.²¹⁰ Reaction of **24** with the Grignard reagent vinyl magnesium bromide provided diene **25**, which was followed by the crucial ring closing step utilizing a 1st generation Grubbs catalyst (Figure 26), for a ring closure metathesis

(RCM) process that was completed easily. After the oxidation of the intermediate allylic alcohol by pyridinium dichromate **12** was obtained in high yield.

Metathesis is one of the most important methods available for ring formation. The extraordinary functional group tolerance and high catalytic reactivity of the ruthenium based Grubbs catalysts enable various efficient inter- or intramolecular metathesis.²¹¹

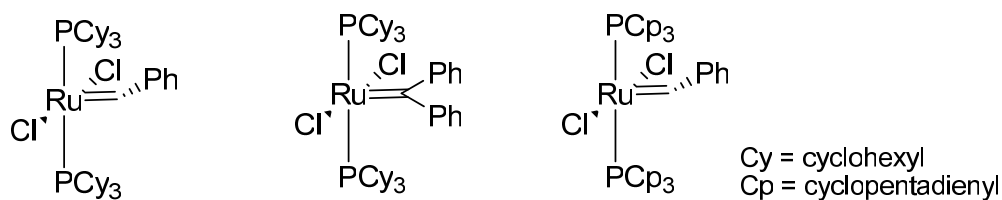
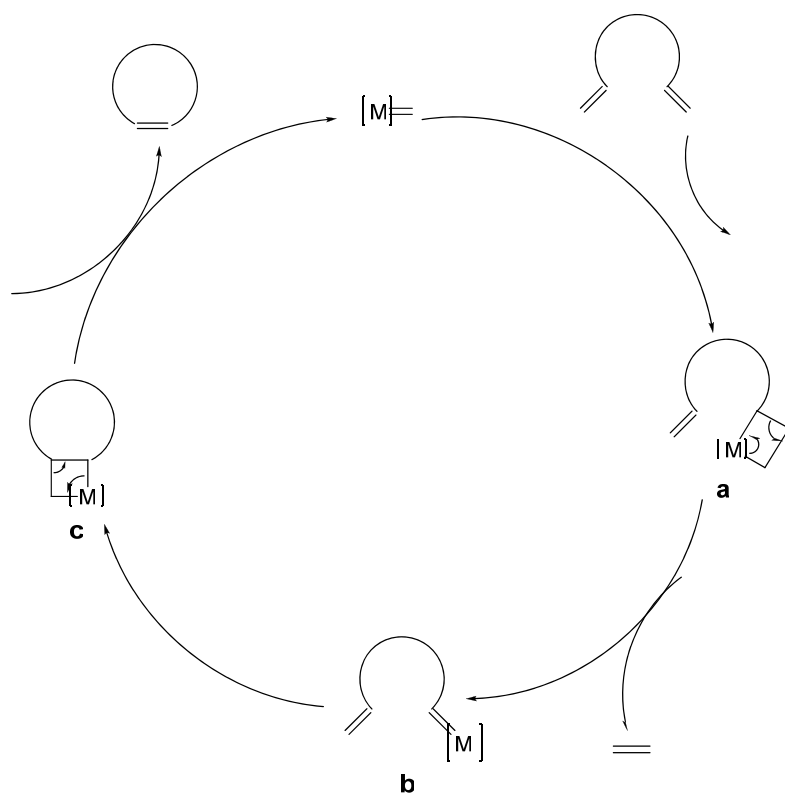


Figure 26. First Generation of Grubbs Catalysts

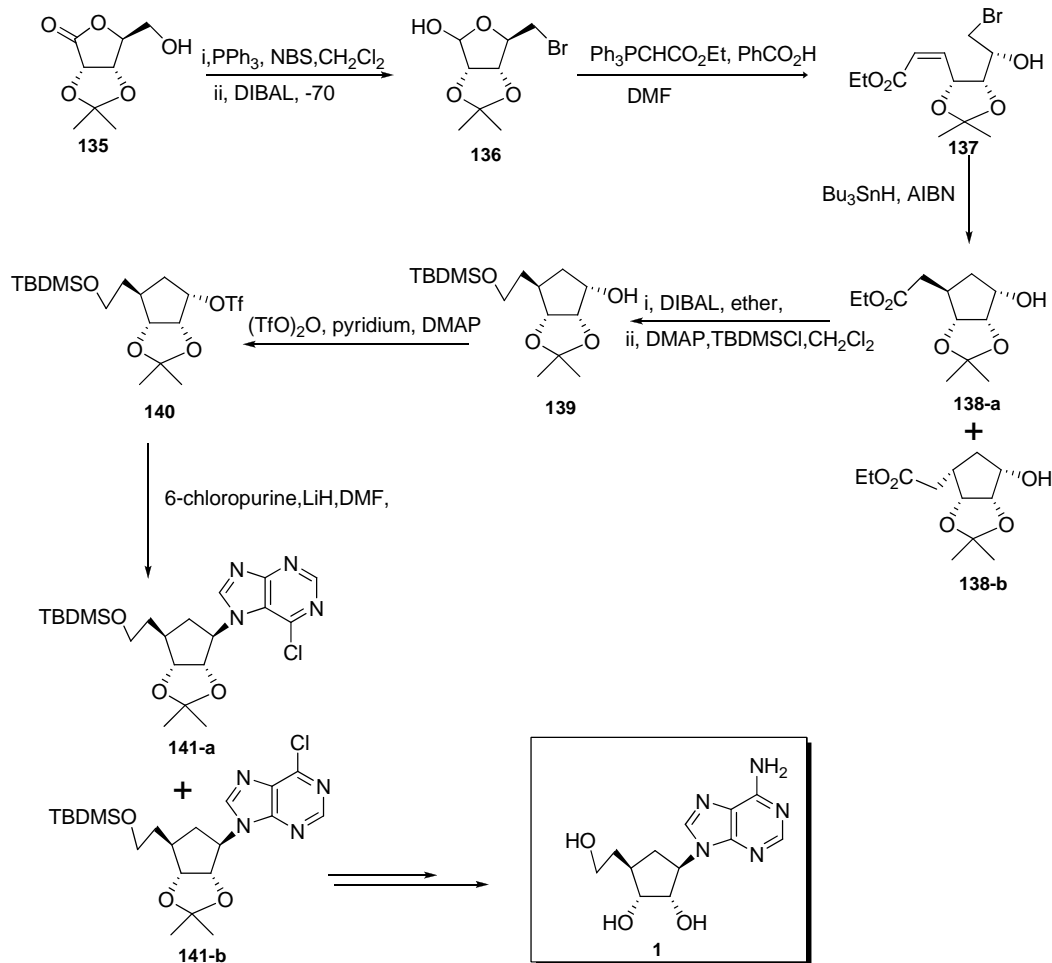
The mechanism of the Grubbs reaction was first proposed by Chauvin^{212,213} (Scheme 10) with initiation at one of the olefins coordinated to the metal center. This intermediate then undergoes a [2+2] cycloaddition to afford a metallocyclobutane **a**. Followed by cleavage of the cyclobutane to release an olefin, a metal carbene **b** is formed, poised to react with the other side olefin. After a second [2+2] cycloaddition and cleavage of cyclobutane **c**, a new carbon-carbon bond is formed and the catalyst is restored for another cycle.



Scheme 10. Mechanism of Ring Closure Metathesis

Chapter 2. Synthesis of 5'-Homoaristeromycin and 8-Methyl-5'- Homoaristeromycin

As an inhibitor of S-adenosyl-L-homocysteine hydrolase, the carbocyclic nucleoside aristeromycin, shows broad-spectrum antiviral activity. However, significant toxicity excludes it from clinic use.^{87,147} One useful strategy to lower its toxicity is extension of the C-5' hydroxymethyl side chain by a methylene group to provide 5'-homoaristeromycin.¹⁶⁶ A previously reported method¹⁶³ leading to 5'-homoaristeromycin involved a radical cyclization of an α , β -unsaturated ester derived from a halo sugar (see **137** in scheme 11).



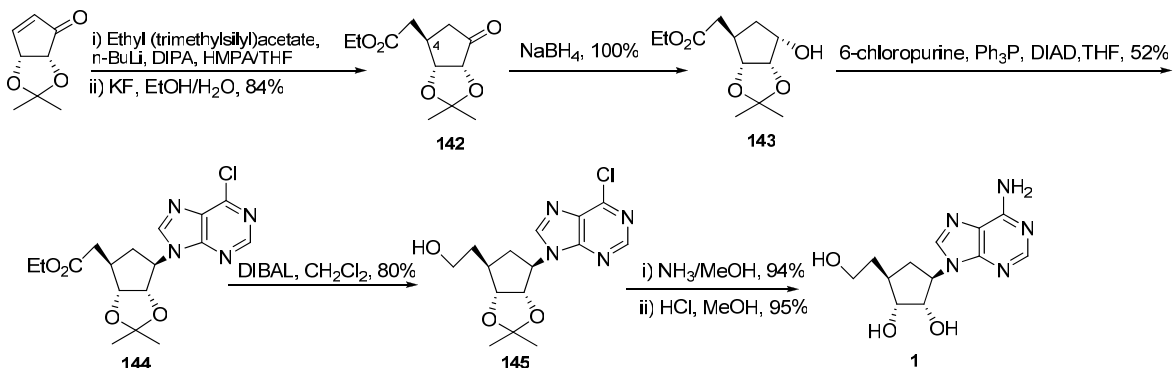
Scheme 11. Jones Method for Homoaristeromycin synthesis

A problem associated with the radical cyclization (**137**→**138**) is that two isomers result in the ratio of 6:1. A further difficulty is in the $\text{S}_{\text{N}}2$ reaction for coupling of the purine base with the carbocyclic ring produces two products (that is, the N9 and N7 isomers). Furthermore, this route suffers from too many steps, limited scale-up and low yields.

Thus, a new stereoselective synthesis for 5'-homoaristeromycin was developed in our lab.¹⁶⁵ This began with the 1,4-addition of ethyl trimethylsilylacetate to enone **12** followed by in situ cleavage of the trimethylsilyl group that established the ethylacetate

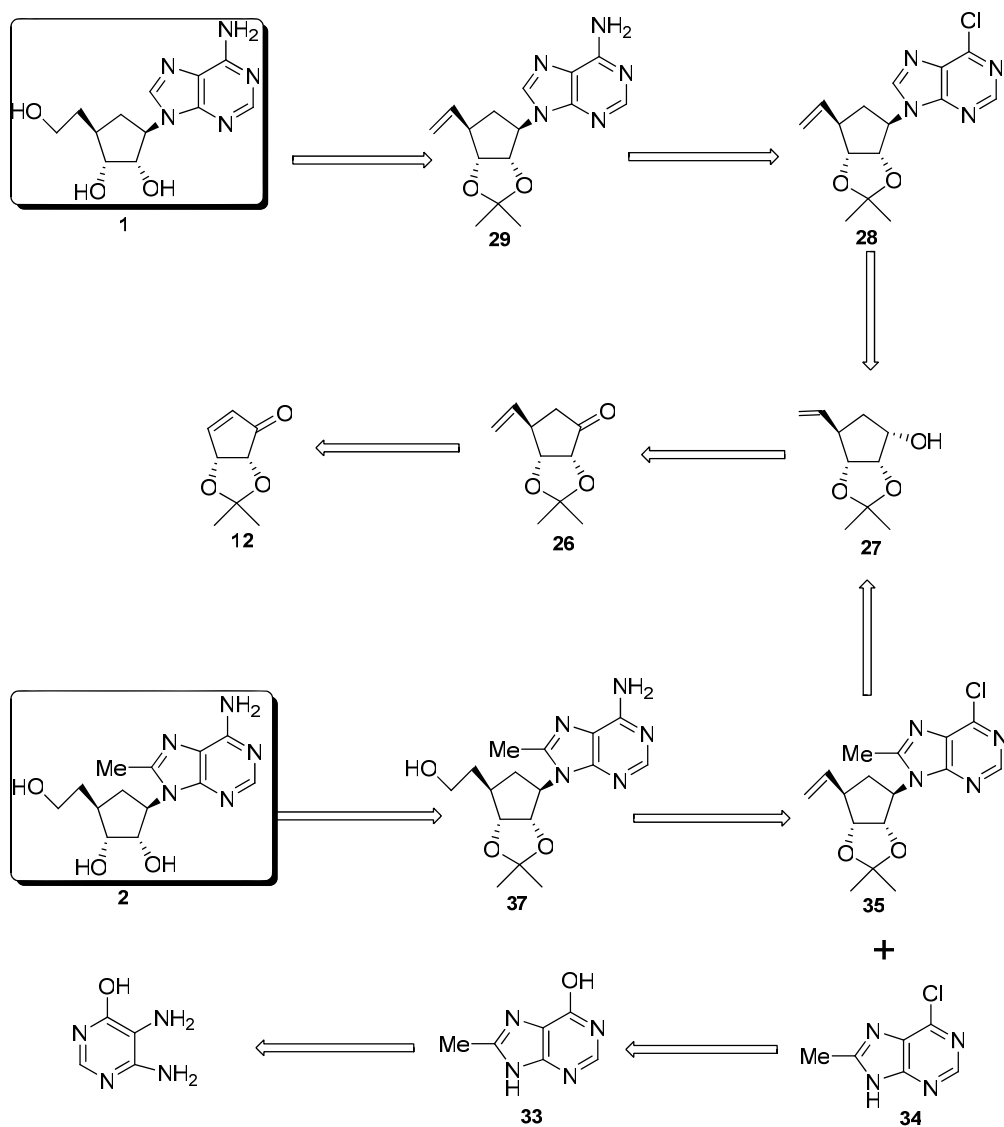
group at the C4 position. The β (up) side chain ester was proven to be the only product.²¹⁴

A selective reduction of the ketone group of **142** afforded the precursor for coupling with 6-chloropurine. The reduction and amination was followed to achieve the target compound **1**. (Scheme 12)



Scheme 12. Synthesis of Homoaristeromycin from enone

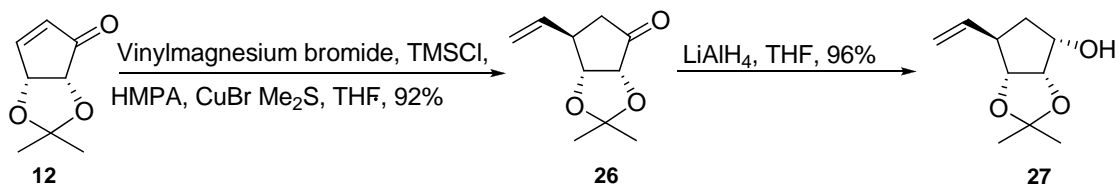
An alternative method for introducing the homo side chain at the C4 position is via the 1,4-addition of a vinyl organocuprate reagent to enone **12**. This reaction occurs with good stereochemical selectivity. Based on this, the synthetic strategy for 5'-homoaristeromycin (**1**) and 8-methyl-5'-homoaristeromycin (**2**) is shown below. (Scheme 13)



Scheme 13. Retrosynthesis for Homoaristeromycin and 8-methyl-homoaristeromycin

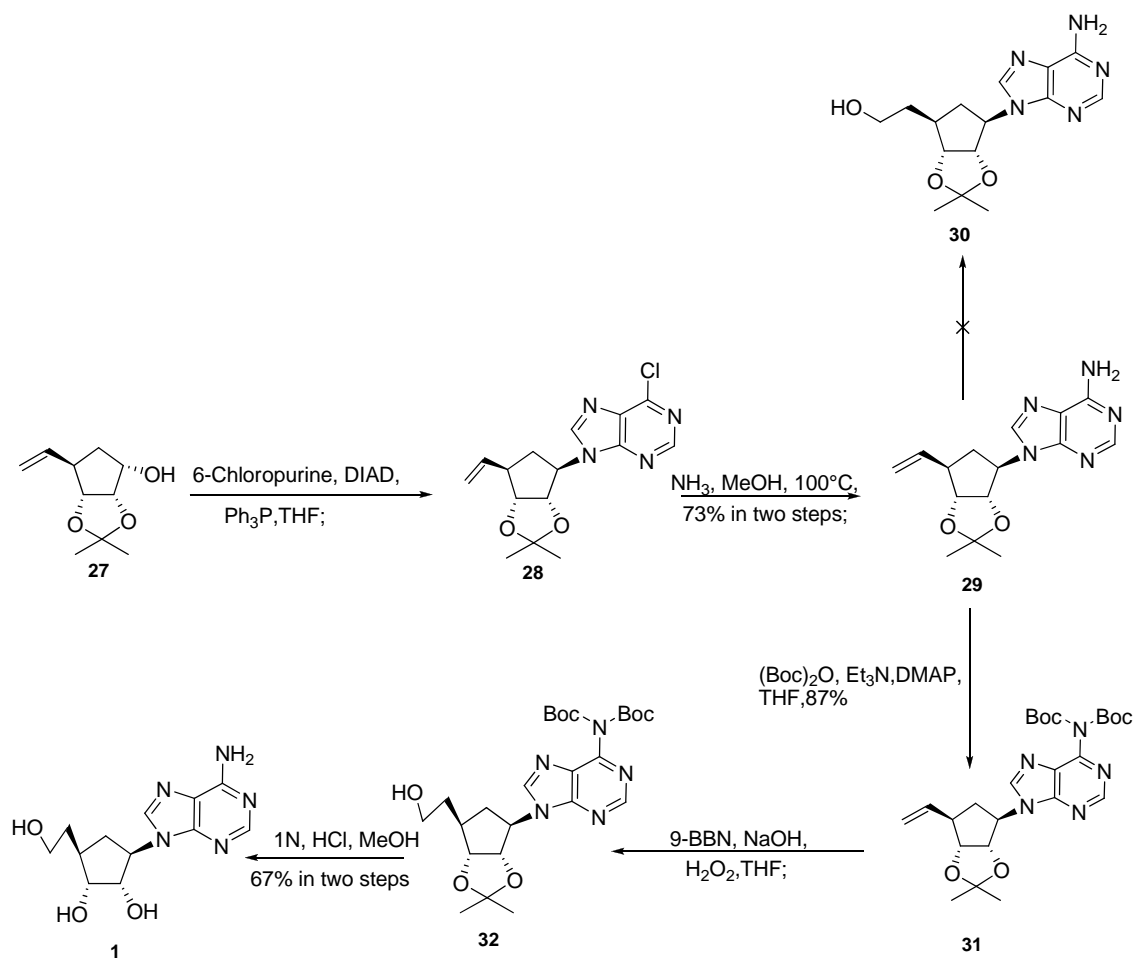
Both compounds were synthesized through the same intermediate **27**. The Mitsunobu reaction was considered for coupling of cyclopentyl moiety with the 6-chloropurine base portion in both pathways. The requisite 5'-homohydroxyl unit was to be achieved by

hydroboration of the vinyl group introduced early in the scheme 14 via the Gilman reagent.



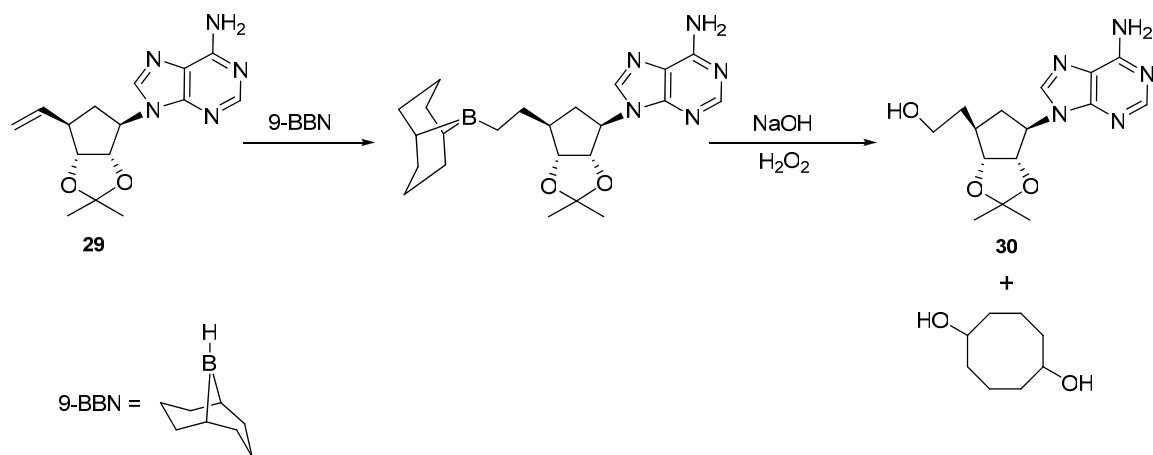
Scheme 14. Synthesis of the intermediate 27

In this research a successful synthesis of **27** involves two steps by starting with the incorporation of a vinyl group into compound **12** by the 1,4-enone addition to give the vinyl ketone **26** in 92% yield. Reduction of **26** with lithium aluminum hydride provided alcohol **27** in 96% yield. (Scheme 14)



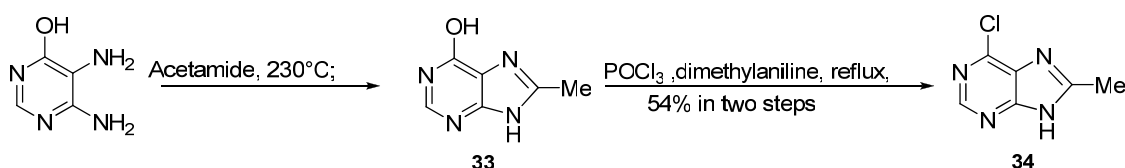
Scheme 15. Synthesis of (-)-HomoAri

The cyclopentyl alcohol **27** was coupled with 6-chloropurine under Mitsunobu conditions to give **28**, which, treated with methanolic ammonia at 100 °C overnight, afforded **29** as a single isomer with some inseparable DIAD derived side-products (Scheme 15). The reaction of crude olefin **29** with 9-BBN generated not only the desired target alcohol **30**, but also 1, 5-cyclooctanediol (Scheme 16). Since both compounds have very similar polarity, the separation of the desired alcohol from the diol byproduct was extremely difficult.



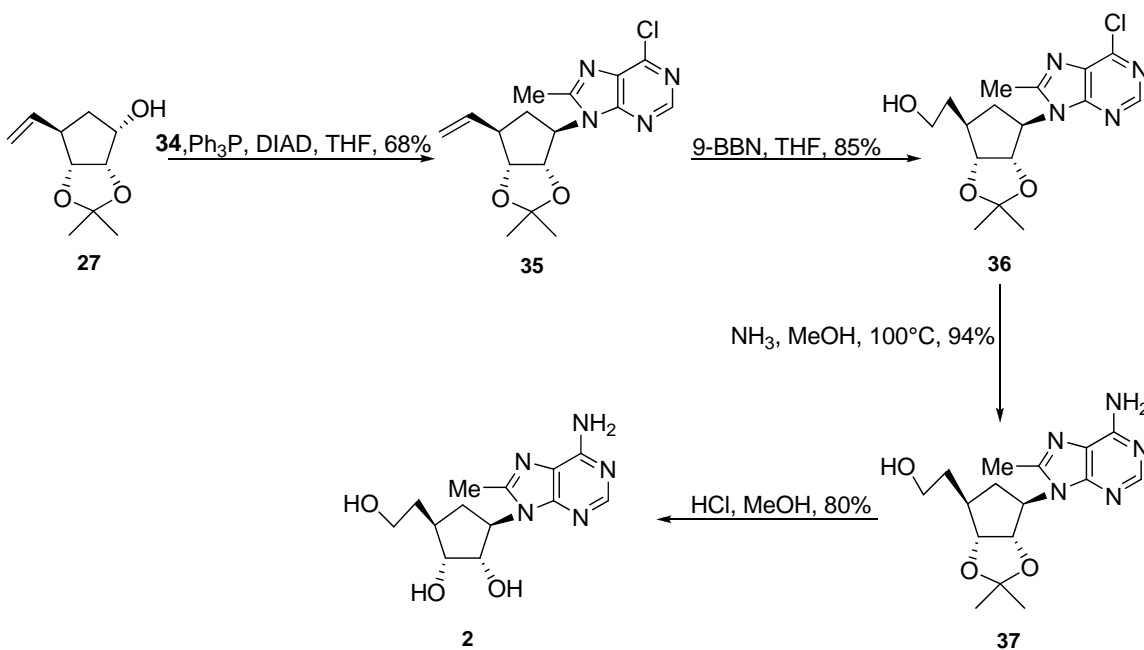
Scheme 16. Hydroboration Reaction for Compound 29

To overcome this problem, we switched to the *bis*-Boc protected compound **31**, which was obtained by treatment of compound **29** with *tert*-butyl dicarbonate and a catalytic amount of 4-(dimethylamino)pyridine, for another attempt at the hydroboration-oxidation sequence. By adding two Boc protecting groups, the molecular weight and hydrophobic character of the desired alcohol product were increased, rendering any products from it much easier to separate by column chromatography. Deprotection of **32** with hydrochloric acid led to the target compound **1** (Scheme 15).



Scheme 17. Synthesis of Compound 34

The synthesis of the 8-methyl-5'-homoaristeromycine (**2**) started with the preparation of the 8-methyl modified 6-chloropurine base, which was obtained by following a reported procedure.²¹⁵ Commercially available 4, 5-diamino-6-hydroxypyrimidine hemisulfate was treated with acetoamide to furnish the bicyclic heterocyclic ring **33**. The hydroxyl functional group of **33** was converted to chloro by refluxing **33** in phosphorus oxychloride to afford **34** (Scheme 17).



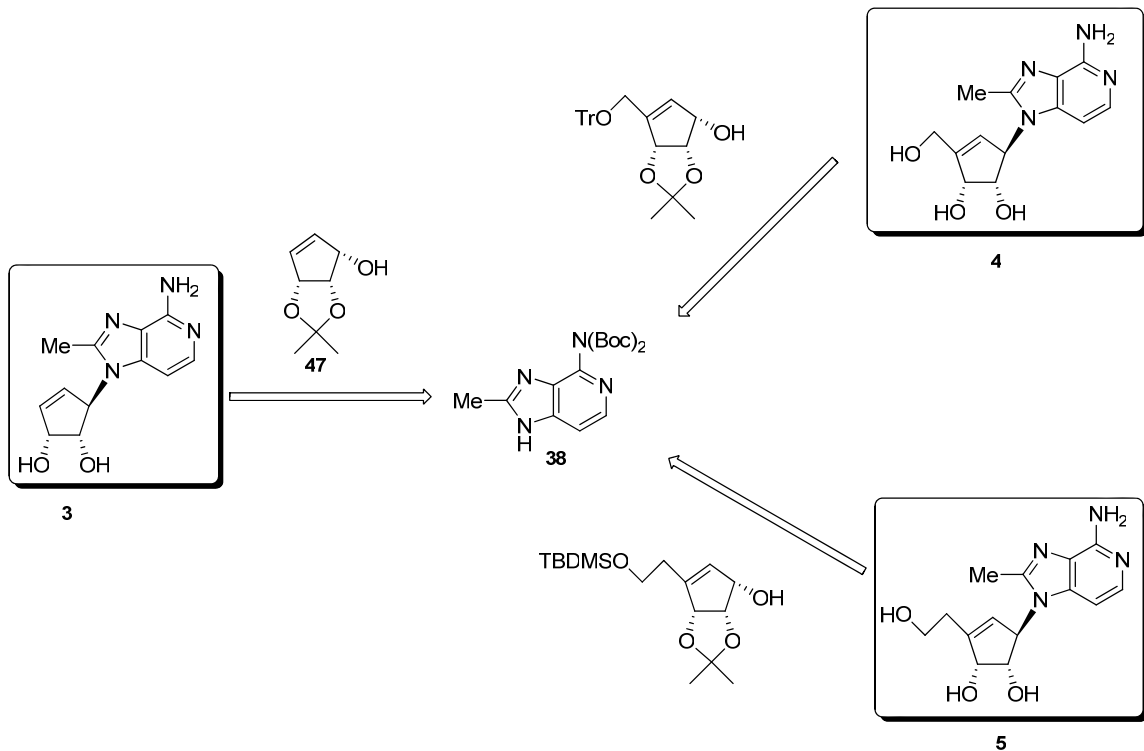
Scheme 18. Synthesis of 2 from intermediate 27

Drawing from the hydroboration difficulties described previously (Scheme 16) a different procedure was used for the synthesis of 8-methyl-5'-homoaristeromycin (**2**). In this regard the synthesis was carried out by starting with a Mitsunobu reaction coupling of **34** with **27**. The product **35** was subjected to hydroboration and followed by oxidation

with hydrogen peroxide to give the desired alcohol **36**. Ammonation of **36** by treating with methanolic ammonia provided compound **37**, which was easily converted to the final product **2** after a deprotection step under an acidic conditions (Scheme 18).

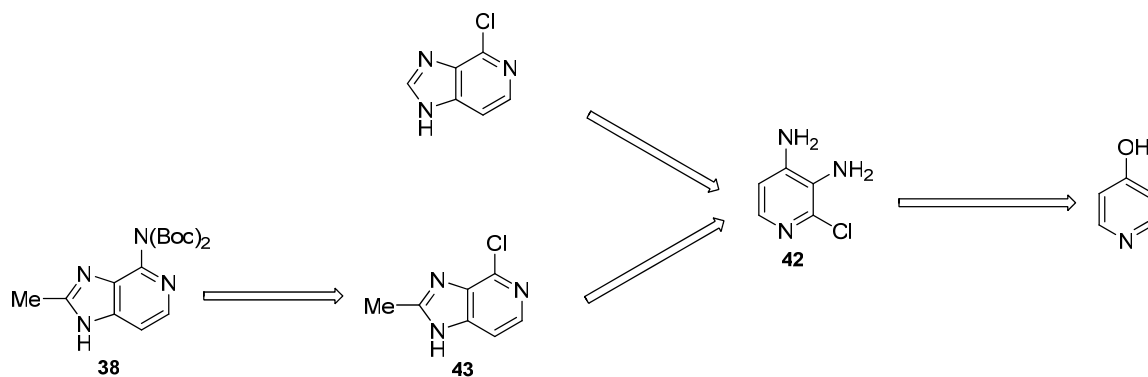
Chapter 3. Synthesis of 8-Methyl-3-Deazaneplanocin Analogues

Due to the efficiency and apparent versatility of the previously described synthetic schemes to carbocyclic nucleoside that demonstrated that the convergent synthetic strategy is preferred over a linear one, 8-methyl-3-deaza carbocyclic nucleoside analogues were sought with this in mind. In this chapter neplanocin A was chosen as the framework for 8-methyl-3-deaza nucleobase series. Compounds **3**, **4**, **5**, were set as target compounds. All three were envisioned as being assembled by coupling the same nucleobase, *bis*-Boc protected 8-methyl-3-deazaadenine (**38**) with the cyclopentyl portions of neplanocin A, 5'-norneplanocin A, and 5'-homoneplanocin A, respectively (Scheme 19).



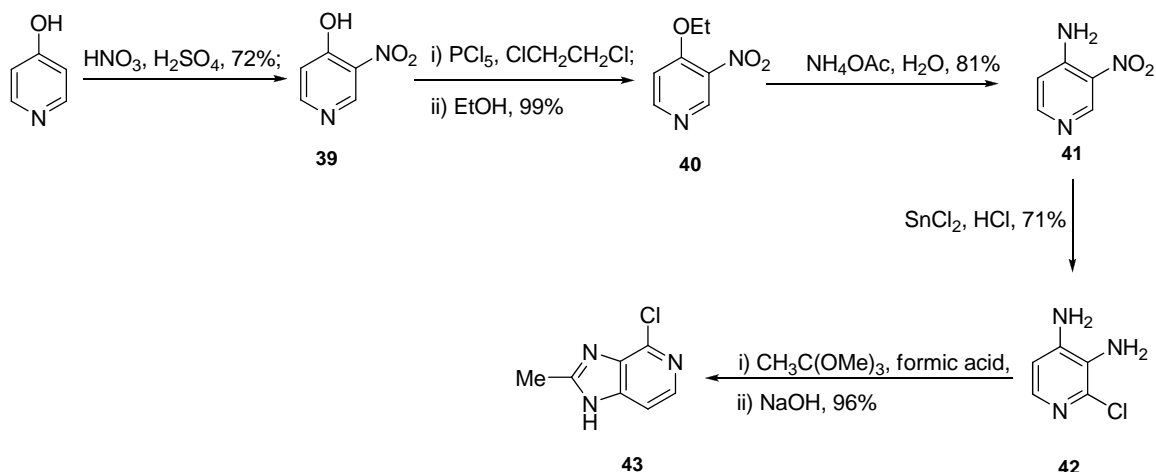
Scheme 19. Strategy for target compounds 3, 4, 5 from nucleobase 38

The direct synthesis of nucleobase *bis*-Boc protected 8-methyl-3-deazaadenine is not available in published literature. As a consequence, a practical and efficient procedure leading to 6-chloro-3-deazapurine was developed for this research. After careful analysis, it was determined to subject **42** with methyl possessing ring closing it would be possible to realize the 6-chloro-8-methyl-3deazapurine, needed for the nucleobase **38** in few steps (Scheme 20).



Scheme 20. Retrosynthetic analysis of 38

Thus, 4-hydroxypyridine, which is commercially available and inexpensive, was chosen as the starting material for the synthesis. After nitration (Scheme 21), a nitro group was installed adjacent to the hydroxyl group (**39**). The nitration product **39** was treated with phosphorus pentachloride and then, without purification, ethanol, whereby hydroxyl group of **39** was substituted by an ethoxyl group through a chloride intermediate. By introducing the ethoxy leaving group, compound **40** was converted to **41** through an ammonia nucleophilic substitution process. Reduction of the nitro group of **41** with tin chloride in concentrated hydrochloric acid was accompanied by a chloration on the 2 position to give **42**.



Scheme 21. Synthesis of 6-chloro-3-deazapurine

Ring closure reaction of **42** at 100°C with trimethyl orthoacetate and added formic acid as catalyst failed to conveniently give the 8-methyl derivative in contrast to previously described 3-deaza-6-chloropurine. Instead of getting the desired ring closure product **43**, a di-imino substituted intermediate **44** was separated from the reaction. Treating the intermediate **44** with 1.5N sodium hydroxide and refluxing for one more day, **43** was isolated and its structure proven by X-ray crystallography (Figure 27). Acid condition also promoted the ring closing reaction of **44** and proved to be equally effective. Below is the proposed mechanism for this ring closing reaction process (Scheme 22).

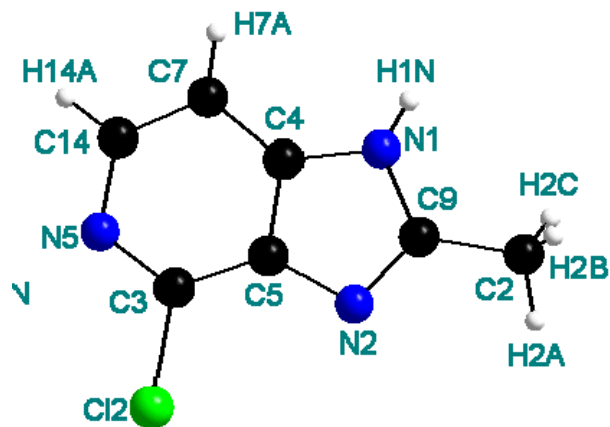
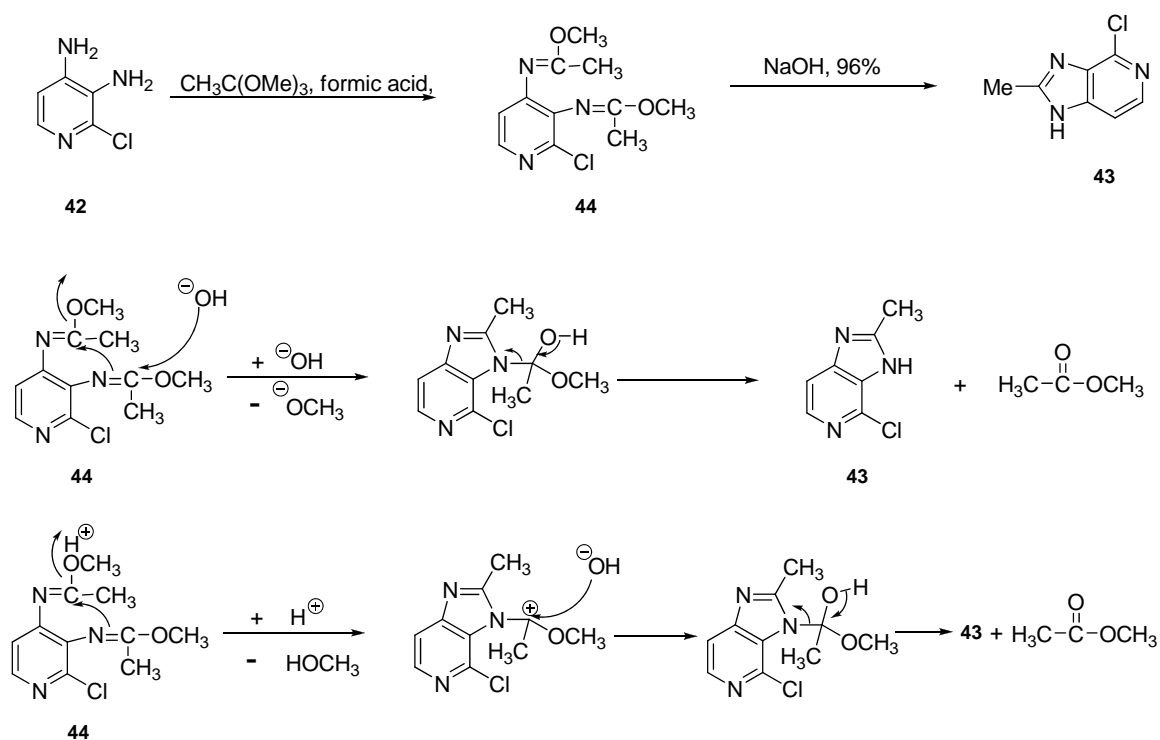


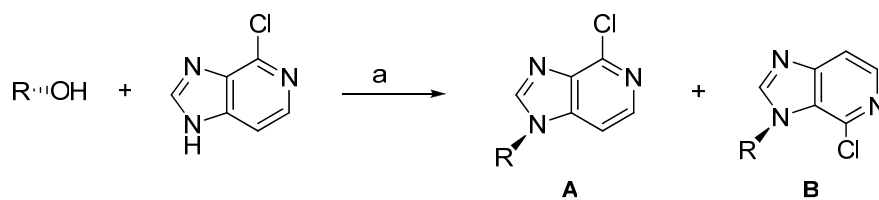
Figure 27. X-ray crystal structure of compound 43



Scheme 22. Mechanism for the ring closing reaction of 42

With 43 in hand its use in the Mitsunobu reaction, which has been studied in our laboratories for 3-deaza carbocyclic nucleosides, was considered.²¹⁶ As background, in our laboratory we have shown that the 3-deaza-6-chloropurine reacted with saturated

cyclopentanol under Mitsunobu conditions, and only one isomer, N-1 (purine N-9), was obtained. However, if the substrates were more reactive allylic alcohols, two regioisomers were observed, with the N-3 (purine N-7) isomer as the major product along with the minor N-1 (purine N-9) isomer. (See table 2)



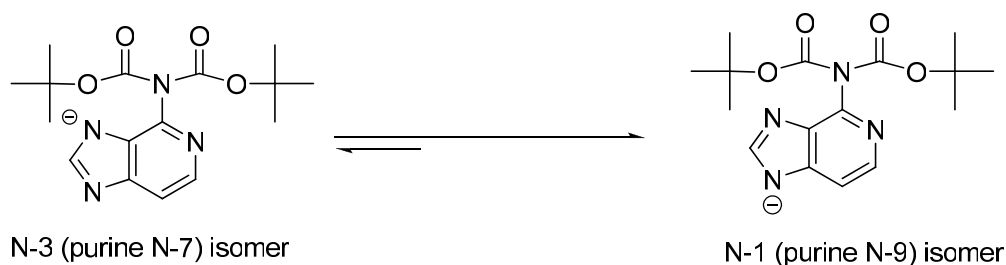
a. DIAD, PPh₃, THF

Entry	R-OH	Products (%)	
		A	B
a		86	0
b		70	0
c		42	53
d		38	57
e		32	43

Table 2. Mitsunobu reaction between 3-deaza-6-chloropurine and cyclopentanols

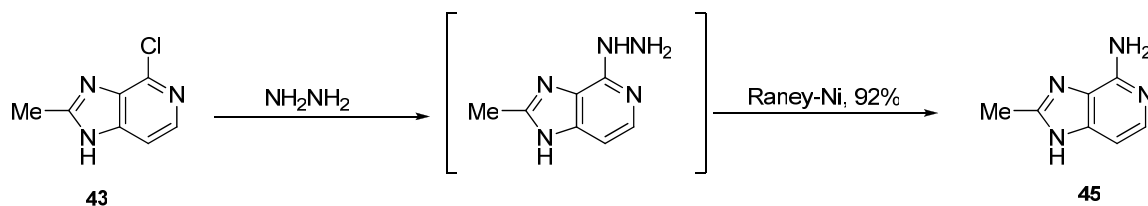
Based on this, an improved method was developed to overcome this unwanted N-3 (purine N-7) coupled by-product. In this regard, *tert*-butoxycarbonyl protected 3-

deazaadenine derivative was chosen as the substrate for the Mitsunobu coupling reaction and found no N-3 isomer. The high regioselectivity (see Scheme 23) may be due to the steric hindrance from two bulky Boc protecting groups on the 4-nitrogen, which blocked the nucleophilic substitution at the N-3 position. In addition to steric control, the reaction occurred faster and was achieved in higher yield than 6-chloro-3-deazaadenine, due to improvement of the solubility for the *tert*-butoxycarbonyl protected 3-deazaadenine. Therefore, the di-Boc protected 8-methyl-3-deazaadenine **38** was considered as a better candidate for the desired Mitsunobu coupling reactions than 6-chloro derivative **43**.



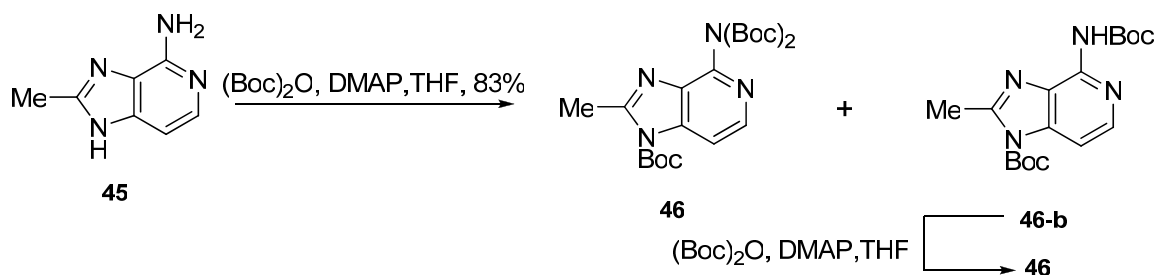
Scheme 23. di-Boc protected 3-deazaadenine as nucleophile in Mitsunobu reaction

The desired precursor to **38** was **45**. The literature^{153,177} suggested that standard conditions (ammonia/methanol) would not be successful to displace the chloro substrate of **43**. Therefore, a procedure using anhydrous hydrazine, followed by treatment with Raney nickel to the resultant hydrazino derivative was chosen and afforded 8-methyl-3-deazaadenosine (**45**) (Scheme 24).



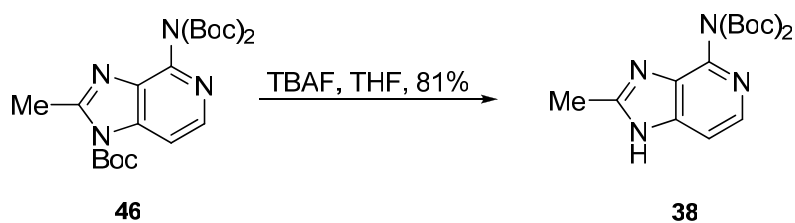
Scheme 24. Synthesis of 8-methyl-3-deazaadenine

Compound **45** was next treated with excess *tert*-butyl dicarbonate and a catalytic amount of 4-(dimethylamino)pyridine in tetrahydrofuran at room temperature for 2 days and the *tris*-Boc-protected **46** along with a by-product bearing high polarity observed in TLC (Scheme 25). After the separation and NMR spectroscopic analysis, the by-product **46-b** was determined to be another *bis*-Boc-protected compound isomeric to but different from **38**. The $^1\text{H-NMR}$ data of **46-b** showed two different peaks at $\delta=1.71$ and 1.55 respectively, for *tert*-butyl groups of the Boc protecting moiety, which indicated that the two Boc groups were installed in different chemical environment. Based on that, it was suggested that the N-6 amino group was deactivated by the first attached Boc group, along with, perhaps, increased steric crowding at the newly formed NHBoc center, reduced the N-9 in the heterocyclic center more accessible, this suggested the *bis*-Boc-protected **38** could not be obtained by controlling the amount of *tert*-butyl dicarbonate. A more successful approach began by forming the *tris*-Boc-protection followed with selectively deprotecting of N-9 site. Thus, after recovery of **46-b**, it was treated with *tert*-butyl dicarbonate and a catalytic amount of 4-(dimethylamino)pyridine for one day, to provide **46** in high yield. (Scheme 25)



Scheme 25. Protection for 8-methyl-3-deazaadenine

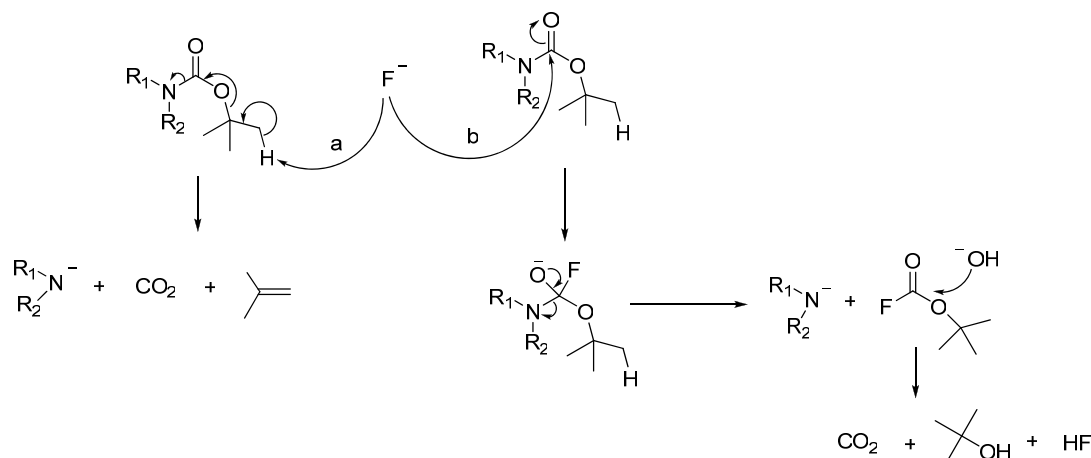
Attempts to remove the N-9 Boc group were seen as possible by hydrogenolysis, or under acidic or basic conditions. But none of these were effective without also removing the N-6 amino Boc groups. A recent study²¹⁷ has shown that tetra-*n*-butylammonium fluoride (TBAF) was a mild and selective deprotection approach, which had been known for the removal of a silyl ether group. Applying TBAF to compound **46** for two days, the N-6 *bis*-Boc protected 8-methyl-3-deazaadenine **38** was obtained as a single isomer. (Scheme 26)



Scheme 26. Selective deprotection of Compound 46

The mechanism for this selective reaction can be envisioned as proceeding in two different ways (Scheme 27). In the first case^{218,219} (a), the fluoride can act as a base and promote a β -elimination to result in the free amino group or (b) the fluoride is acting as a

nucleophile and directly attacks the Boc carbonyl group leading to an amino anion and Boc-F, which, upon hydrolysis, becomes *tert*-butanol, carbon dioxide and hydrofluoric acid.²²⁰ Studies have shown that the reaction more likely follows pathway b, since there were several substrates without a susceptible proton that still underwent the reaction.²¹⁷

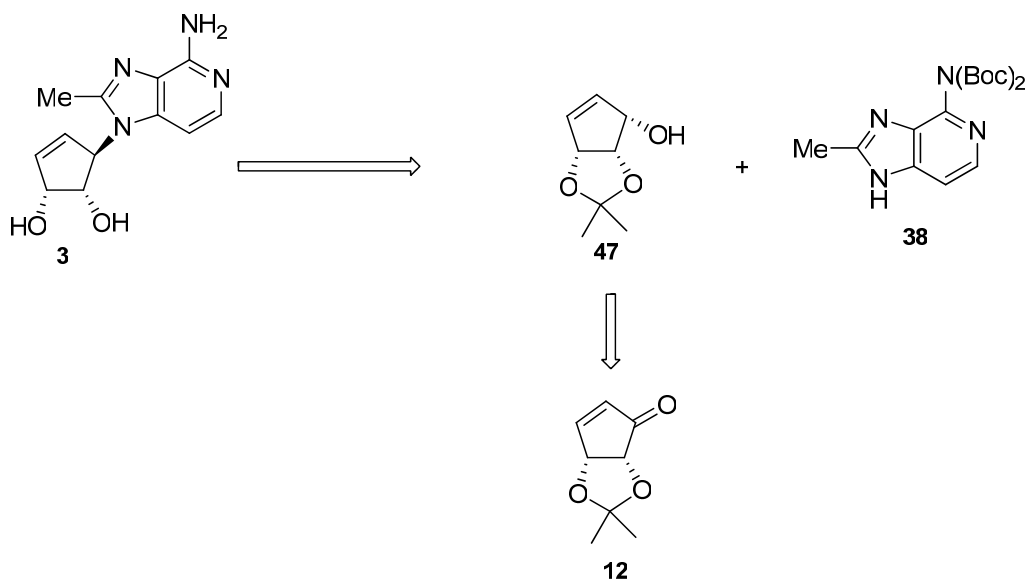


Scheme 27. Postulated mechanism for Boc-deprotection by TABF

The selective deprotection of the N-9 Boc group of compound **46** can be considered as another piece of evidence to support the theory. The selectivity for the leaving amide anion generally followed the order: aromatic > benzylic > aliphatic. In this case, the leaving group was hetero aromatic amide anion comparing with the alternative *tert*-butoxycarbonyl amide anion from C-6. The activities for those two were quite close. If the reaction occurred through pathway a, the likelihood for cleavage at the N-6 Boc group would increase, since there are more hydrogen atoms available at that center. The results indicated that the fluoride anion acted as a nucleophile attacking the less crowded N-9 carbonyl group, by pathway b, and giving the N-9 deprotected product **38**.

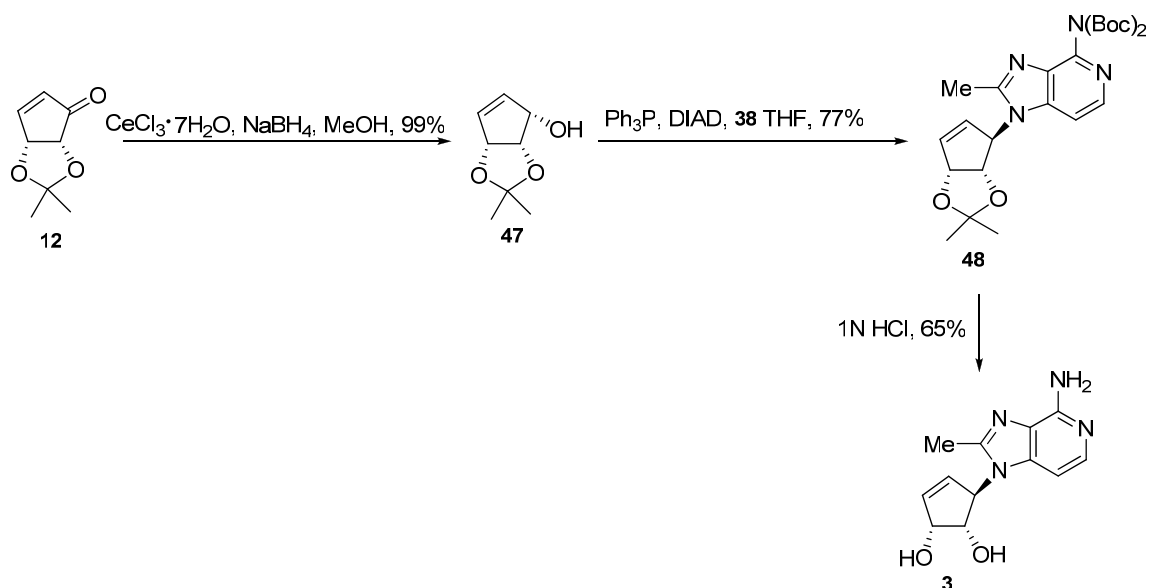
Synthesis of 8-Methyl-3-Deaza-5'-Norneplanocin A

With the important building block **38** in hand, the synthesis of 8-methyl-3-deaza-5'-norneplanocin A (**3**) calling on the retrosynthetic designed below. Thus, target compound **3** was to be assembled through a coupling reaction of **38** with **47**, which was accessible from the crucial precursor, cyclopentenone **12** (Scheme 28).



Scheme 28. Retrosynthesis of 8-methyl-3-deaza-5'-norneplanocin A (3)

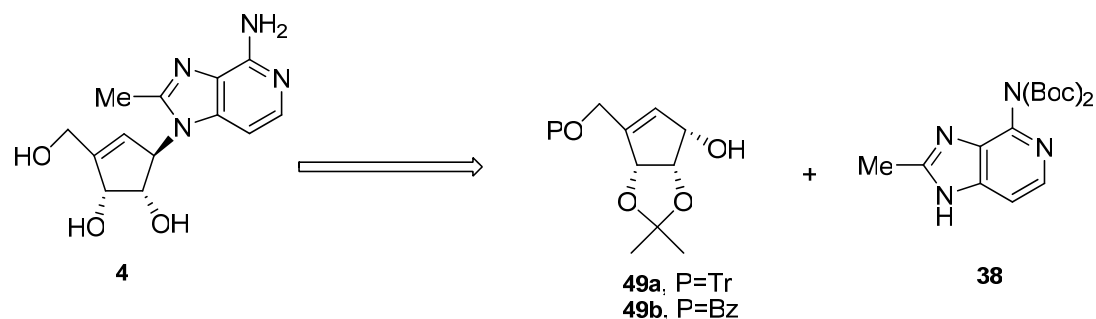
Beginning with conversion of cyclopentenone **12** to allylic alcohol **47** called on a highly stereospecific 1, 2 -reduction under Luche conditions of sodium borohydride and cerium (III) chloride heptahydrate (Scheme 29),²²¹ this was followed by a Mitsunobu coupling reaction to join **47** with nucleobase **38**. After cleavage of the protecting groups under acidic conditions, 8-methyl-3-deaza-5'-norneplanocin A (**3**) was afforded in good yield.



Scheme 29. The synthesis of 8-methyl-3-deaza-5'-norneplanocin A

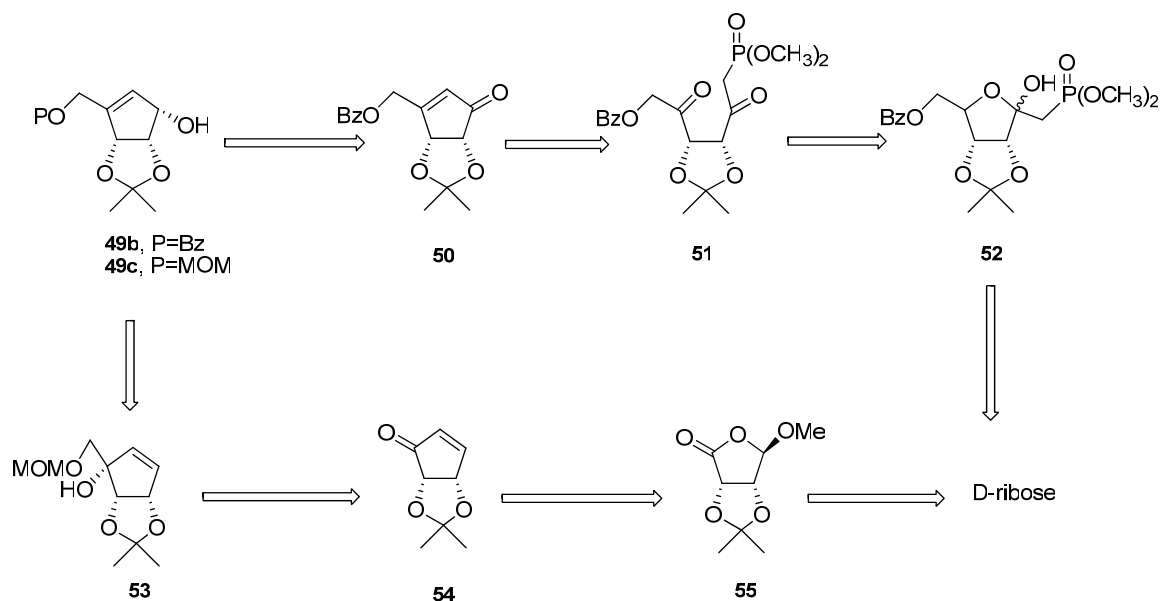
Synthesis of 8-Methyl-3-Deaza-Neplanocin A

A straightforward approach to compound **4** is outlined in Scheme 30 by coupling the allylic cyclopentenol **49** with **38**. Chiral cyclopentenol **49** with various protecting groups for example, trityl for compound **49a**, benzoyl for **49b** has been used as a key intermediate for the synthesis of various neplanocin A related compounds. The first synthesis for **49** was in 1983 by Marquez.²²² Since then, many new or modified synthetic routes have been developed. The following is a brief review and analysis of diverse synthesis methods for this pivotal molecule.



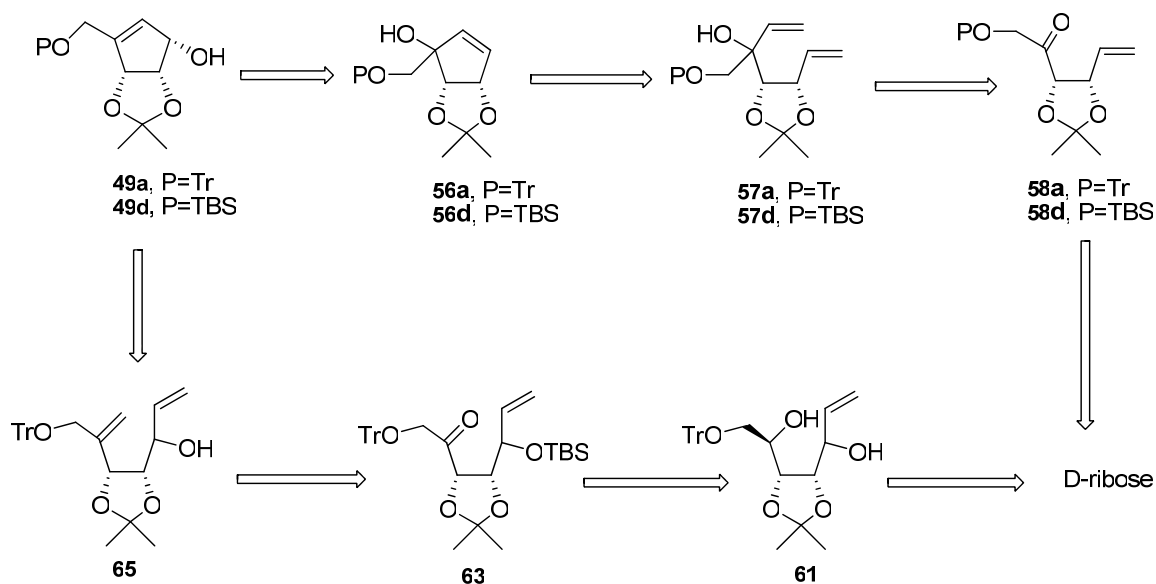
Scheme 30. Retrosynthesis of 8-methyl-3-deaza-neplanocin 4

The synthesis of the desired cyclopentenol rings start with D-ribose. In the early investigations, the strategy was based on a Wittig reaction to afford a cyclopentenone **54** (**55** to **54**)²²³ or substituted cyclopentenone **50**.²²⁴ The key step for building up two important carbocyclic intermediates (**50** and **54**) was realized by employing the Wittig type reaction after the installation of the methyl phosphonate either on C-1 (route on top for **50**) or C-4 (route on bottom for **54**) of D-ribose (Scheme 31). Compound **50** was selectively reduced to the benzoyl group protected **49b** and **53** was transferred to the methoxy methyl group protected **49c** by a palladium catalyzed migration of tertiary allylic alcohol. This old methodology usually suffered from long synthetic steps, and low yield.



Scheme 31. Previous synthesis for cyclopentenone 49

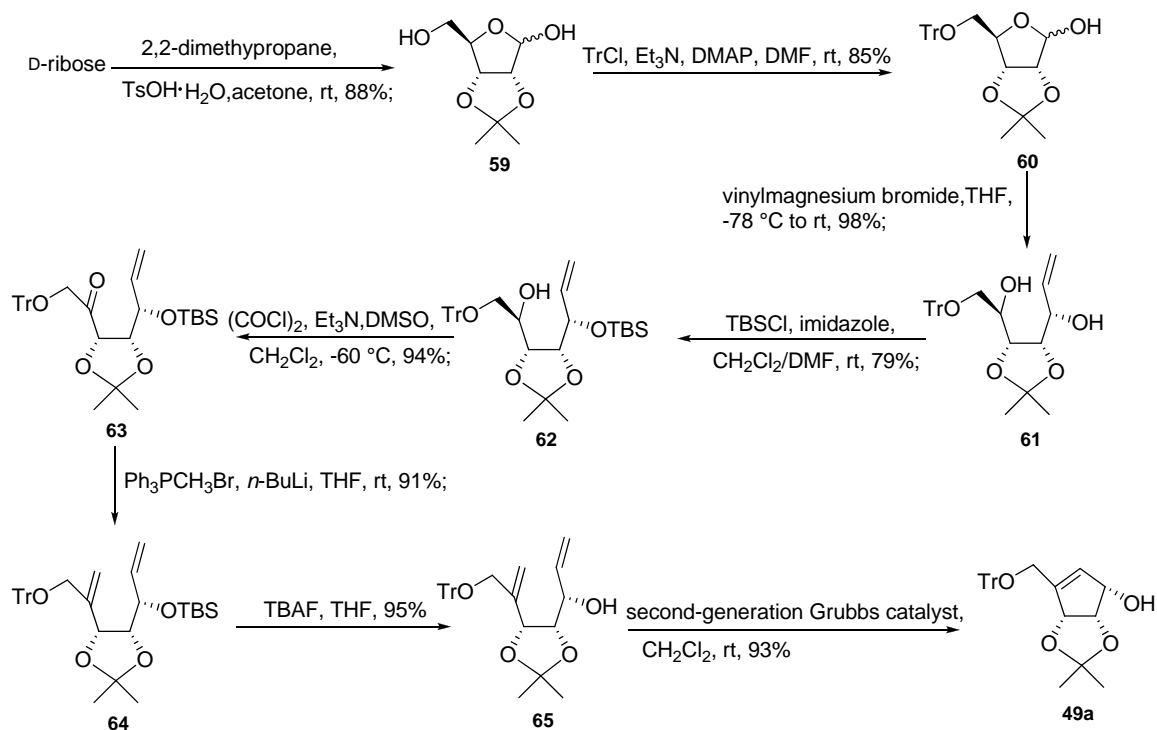
New synthetic approaches to **49** have been developed by utilizing a ring closing metathesis (RCM) to build the carbocyclic ring of **49**. Based on this, in 2005, Schneller et al. discovered a new synthetic route to **49** (Scheme 32 on the top) by applying a Wittig reaction at the C-4 position of D-ribose and later a Grignard reaction to install a vinyl group at C-1 to realize the compound **57**, the precursor for RCM.²²⁵ After the RCM ring closure, an oxidative rearrangement of tertiary allylic alcohol of **56** was employed to complete the process to cyclopentenone **49**. Two years later, Chu's group used a similar strategy to obtain diene **65** for the RCM reaction (Scheme 32 on the bottom).²²⁶



Scheme 32. New strategy for synthesis for cyclopentenone 49

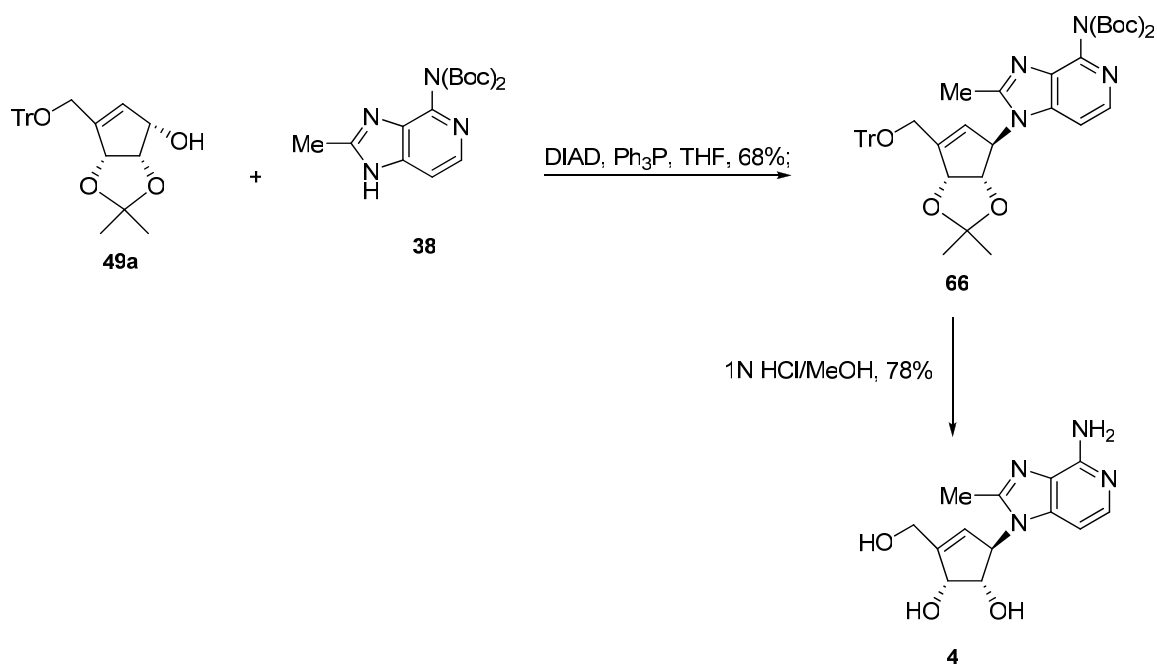
Since the efficiency and relatively high yield, Chu's approach was chosen for the synthesis of **49** for this research (Scheme 33). Thus, D-Ribose was transferred to the isopropylidene protected **59** by treatment with 2, 2-dimethylpropane in the presence of a catalytic amount of *p*-toluenesulfonic acid. This was followed by selective protection of the primary hydroxyl group by triphenylmethyl chloride gave **60**. Reaction of **60** with vinylmagnesium bromide opened the furanose ring of **60** to provide the single diastereoisomer **61**. The newly produced allylic hydroxyl group of **61** was protected with a *tert*-butyldimethylsilyl (TBDMS) group. The unprotected secondary hydroxyl group of **62** was oxidized under Swern condition to afford the ketone **63**, which in turn underwent a Wittig reaction with methyltriphenylphosphonium bromide and butyllithium (*n*-BuLi) in tetrahydrofuran to result in the diene **64**. Subjecting on **64** to the RCM reaction, with either first or second generation Grubbs of catalysts failed. This may be due to steric

interference from the bulky protecting groups of **64**. To circumvent this, the TBDMS protecting group was removed by TBAF to provide dienol **65**. The RCM reaction was then successfully carried out on **65** with a second-generation of Grubbs catalyst to form **49a**.



Scheme 33. Synthesis of cyclopentenol 49a

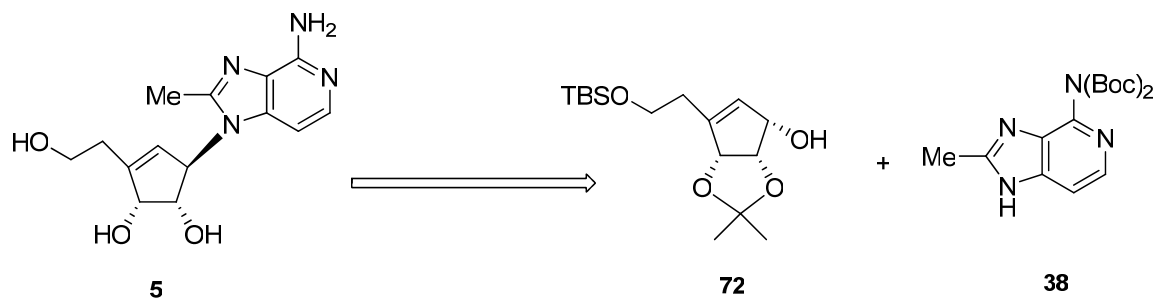
After finishing the synthesis of **49a**, a Mitsunobu reaction was conducted with nucleobase **38** (Scheme 34). The coupling product **66** was achieved in good yield. Hydrochloric acid was used to remove the protecting groups to give the final product 8-methyl-3-deazaneplanocin A (**4**).



Scheme 34. Synthesis of 8-methyl-3-deazaneplanocin

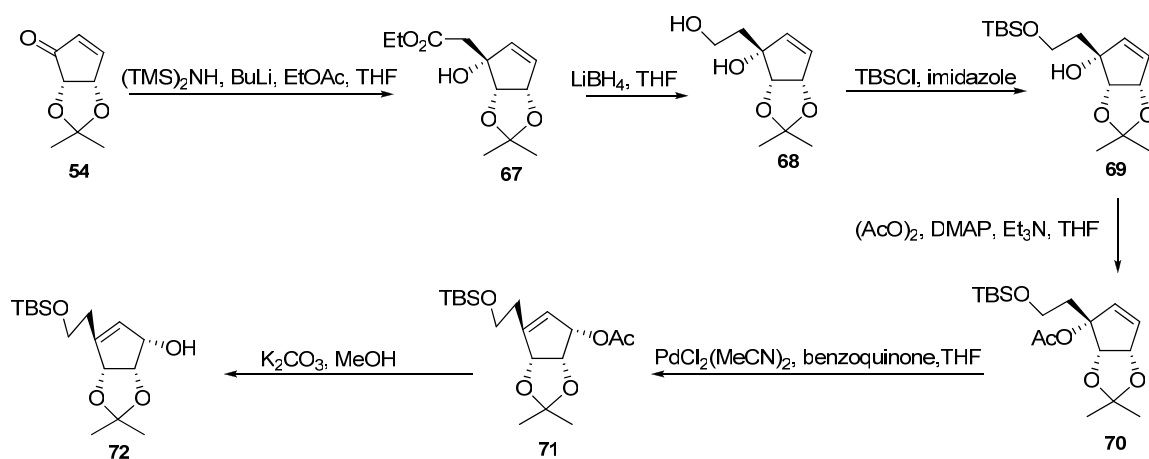
Synthesis of 8-Methyl-3-Deaza-Homoneplanocin A

Based on the success of synthesis of the previously described 8-methyl-3-deazaneplanocin analogues, a similar synthetic route for 8-methyl-3-deaza-homoneplanocin (5) was designed as illustrated below by coupling of cyclopentenol 72 with nucleobase 38.



Scheme 35. Retrosynthesis of 8-methyl-3-deaza-homoneplanocin 5

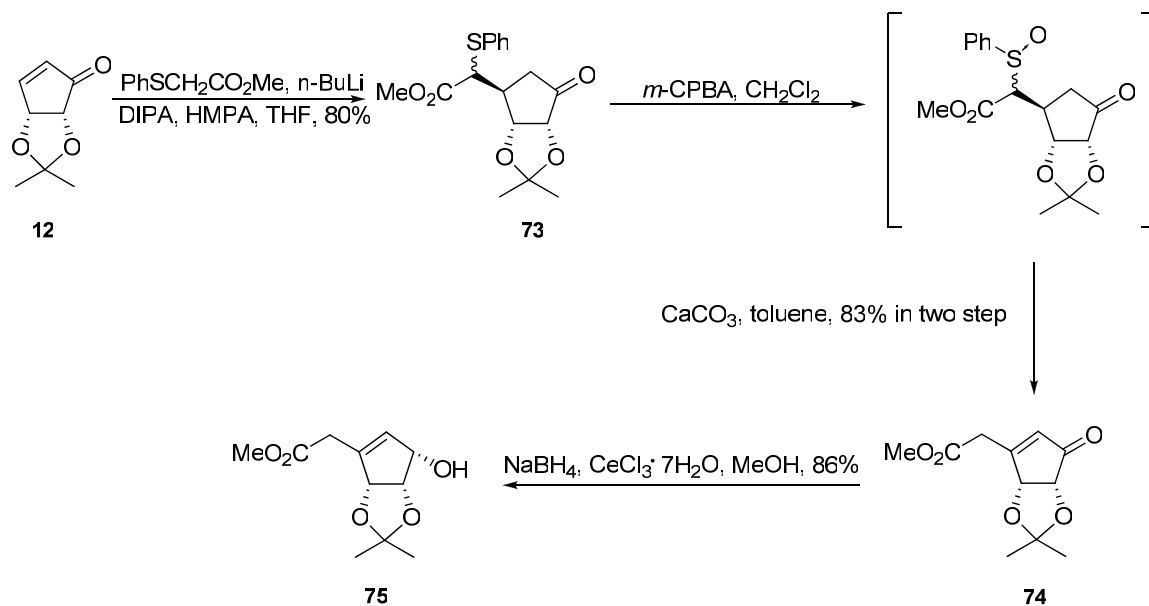
To provide access to the homoneplanocin cyclopentyl portion **72** the 1996 method of Shuto's research¹⁶⁶ was considered (Scheme 36). The approach was derived from the synthesis of the neplanocin building block **49c** reported by Hill (Scheme 31).²²³ From the same intermediate **54**, a 1,2-addition of the carbanion of ethyl acetate was used instead of methoxymethyl for **49c** in Scheme 31. After the ester reduction and two different subsequent protection steps for the primary and tertiary alcohols, the same palladium-catalysed allylic rearrangement was employed. A selective deprotection was followed to this important intermediate **72**.



Scheme 36. Shuto's method for synthesis of cyclopentenol 72

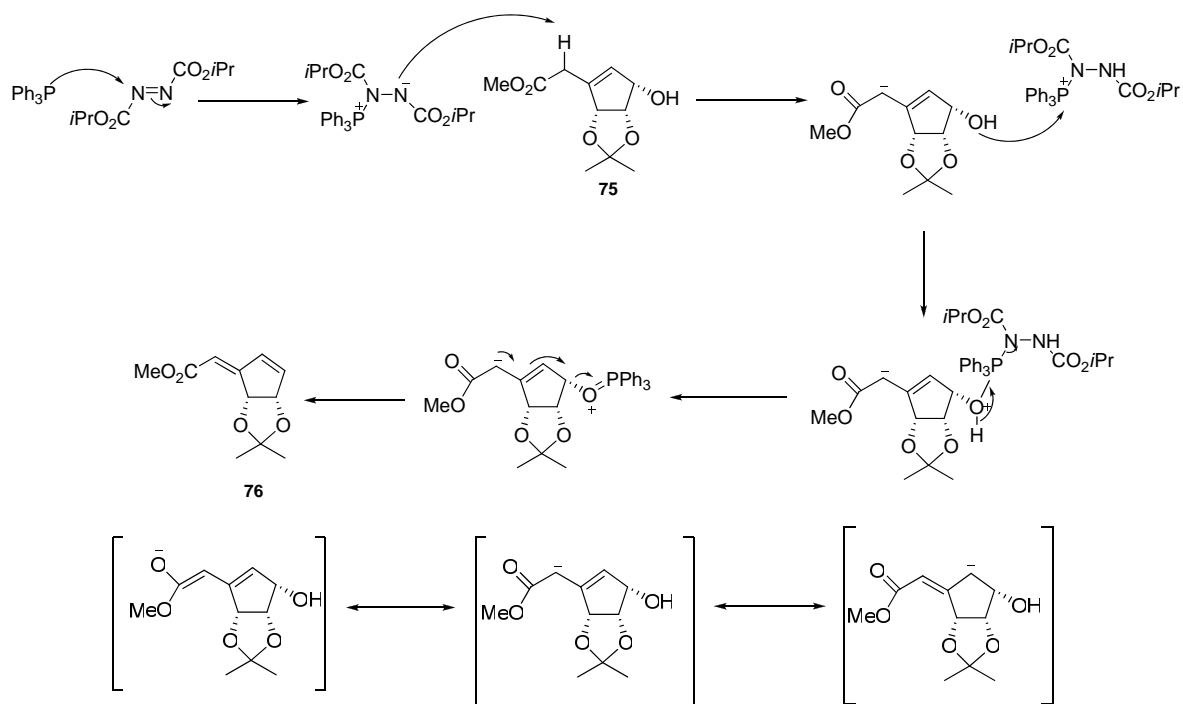
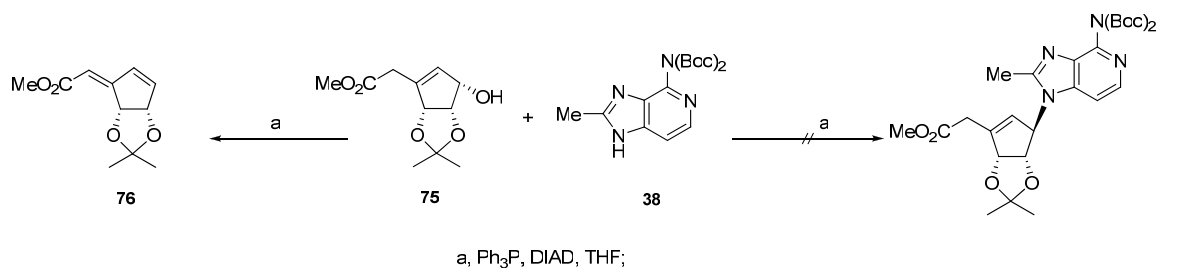
A few years later, a more efficient approach to cyclopentenol **72** was discovered in our laboratories (Scheme 37).¹⁶⁷ This route started with the important precursor cyclopentenone **12**, which is the enantiomer of **54**. The synthesis began with a regio and stereoselective 1,4-addition on **12** with methyl phenylthioacetate to yield **73**. Oxidation of the thioether with *m*-chloroperoxybenzoic acid (*m*-CPBA) to the sulfoxide was followed

by dehydrosulfenylation providing a new double bond that quickly rearranged to the thermodynamically more stable conjugated system **74**. After a Luche reduction on **74**, **75** was cleanly afforded in high yield.



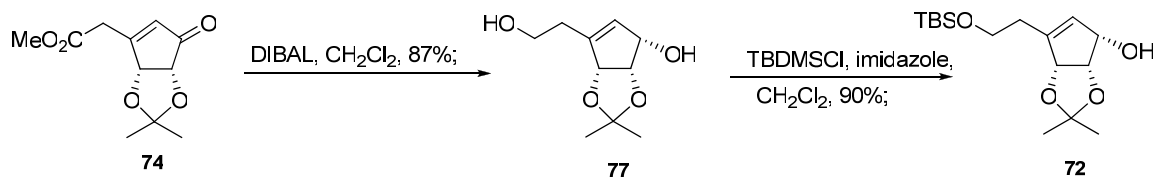
Scheme 37. The Synthesis of compound 75

The initial plan was to use the compound **75** as a substrate for the Mitsunubo reaction, but, instead of getting the desired coupling product, **75** underwent an elimination reaction to give the product **76**. A Mechanism for this outcome is given in Scheme 38. Appearance of by-product **76** was believed to be due to the deprotonation of cyclopentenol **75** (instead of **38**) by the triphenylphosphine-diisopropyl azodicarboxylate (TPP-DIAD) complex. The driving force for this carbanion formation from **75** was large delocalized conjugated system as a consequence.



Scheme 38. The proposed mechanism for the reaction of 75 with 38

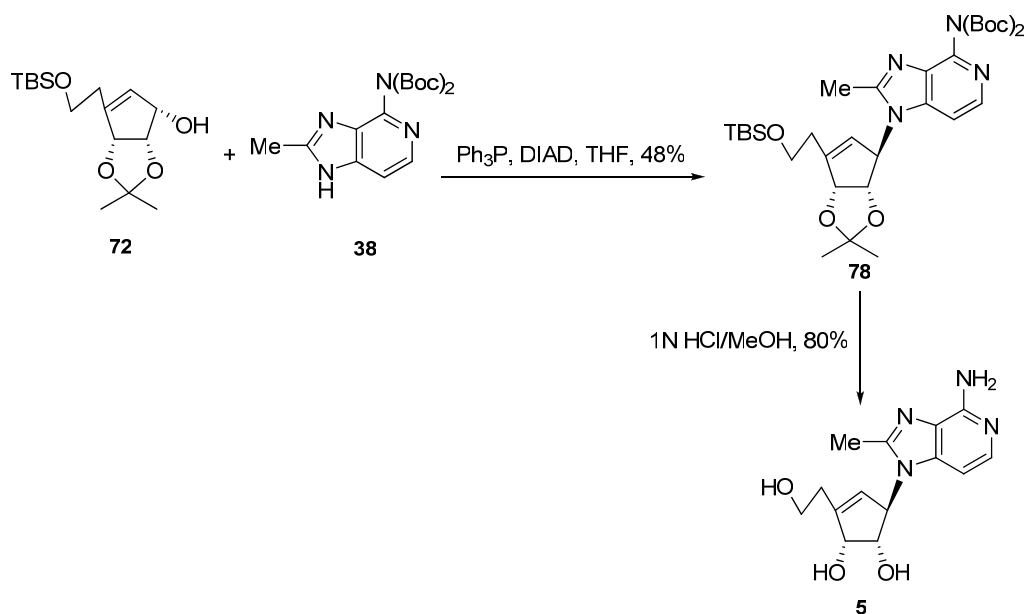
A solution for avoiding this was reduction of both ester and ketone functionalities in **74** by diisobutylaluminum hydride (DIBAL). The coupling precursor **72** was obtained after selective protection of the primary alcohol of **77**. The large protecting reagent *tert*-butyldimethylsilyl chloride (TBDMSCl) was used to assure the primary alcohol selectivity, since the activities for secondary allylic alcohol and primary alcohol would have been competitive (Scheme 39).



Scheme 39. The synthesis of precursor 72

This synthesis method for **72** was preferred from the previous one (Scheme 36) for 3 reasons: 1) fewer steps from a similar intermediate (**12**); 2) higher overall yield (27% versus 9.5%) and 3) less expensive reagents particularly with palladium reagent of the old method.¹⁶⁷

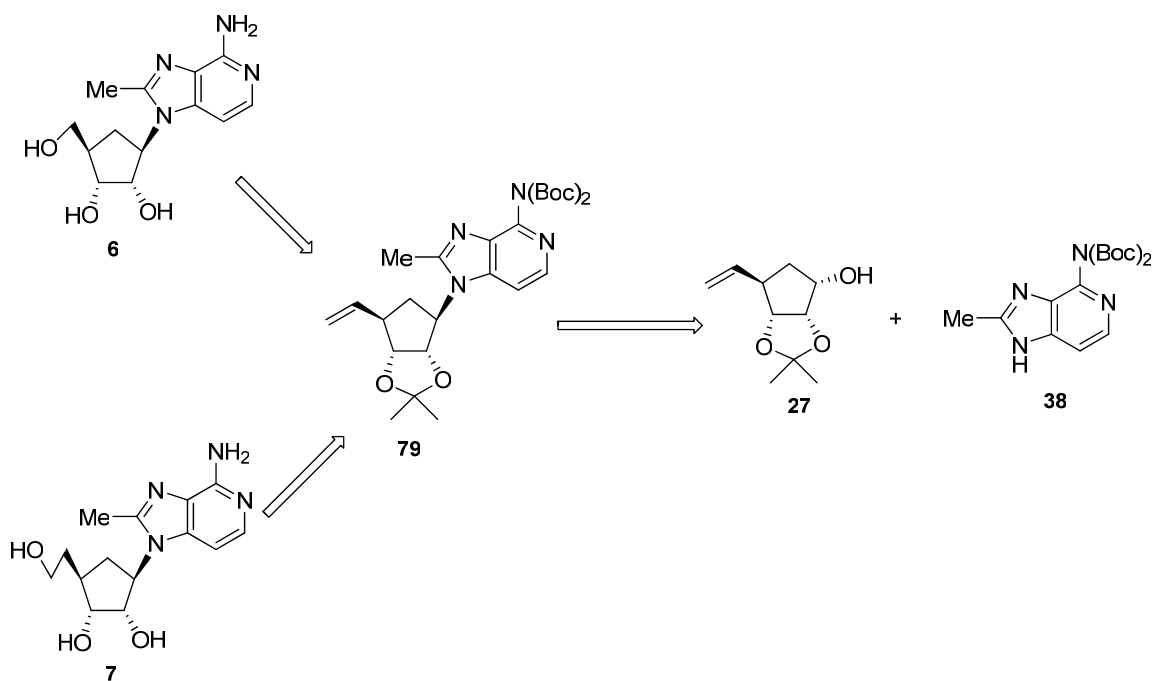
Compound **72** was subjected to Mitsunobu reaction conditions with nucleobase **38** to give compound **78**, which was treated with hydrochloric acid to remove the *isopropylidene* and TBDMS simultaneously to obtain the final product 8-methyl-3-deaza-homoneplanocin (**5**) (Scheme 40).



Scheme 40. The synthesis of compound 5

Chapter 4. Synthesis of 8-Methyl-3-Deazaaristeromycin Analogues

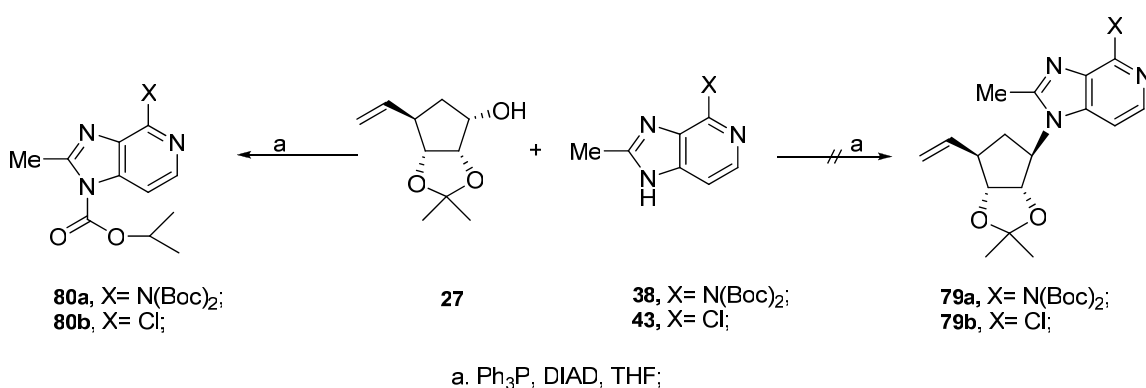
The initial plan for synthesis of 8-methyl-3-deaza aristeromycin analogues was based upon the success of the synthesis of neplanocin analogues with the same nucleobase. Thus, the retrosynthetic analysis was shown in Scheme 41 was established. Compound **27** was chosen as the coupling component for the cyclopentyl portion, since the terminal alkene provided a versatile framework for various C-5' aristeromycin modifications.



Scheme 41. Retrosynthesis of compound 6, 7

The bis-Boc protected nucleobase **38** was reacted with cyclopentanol **27** under Mitsunobu condition. In that direction, alcohol **27**, nucleobase **38** and triphenylphosphine (TPP) were suspended at 0 °C for 10 minutes and diisopropyl azodicarboxylate (DIAD) was added dropwise over 20 minutes. After 12 hours at room temperature, no reaction occurred. Upon continuing the reaction for another 12 hours at 50 °C, an unknown product was detected by thin layer chromatography (TLC). Instead of the expected **79**, the compound confirmed by NMR spectral analysis was **80a**, a product of nucleophilic substitution of nucleobase **38** to DIAD (Scheme 42). This result may be due to the slow formation of the desired alkoxyphosphonium salt between alcohol **27** and TPP or perhaps, the sluggishness of the reaction of the deprotonated nucleobase with this salt. Based on these suggestions, we switched the nucleobase **38** to **43** and modified the conditions to improve the formation of phosphonium salt before other side reactions occurred. Two

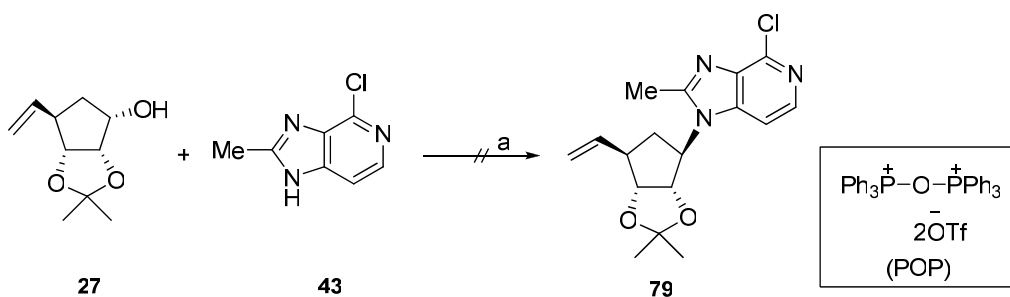
conditions were tried: first, TPP and **27** were dissolved in tetrahydrofuran (THF) at -40 °C. After stirring at this temperature for 20 minutes, a solution of DIAD in THF was added over an hour. The unexpected side product **80b**, as before, was obtained. In the second approach, the TPP-DIAD complex was preformed first in THF at 0 °C, followed by the addition of a mixture of **27** with **43** in THF at -20 °C. No reaction occurred even after heating the reaction mixture to 70 °C for more than 12 hours.



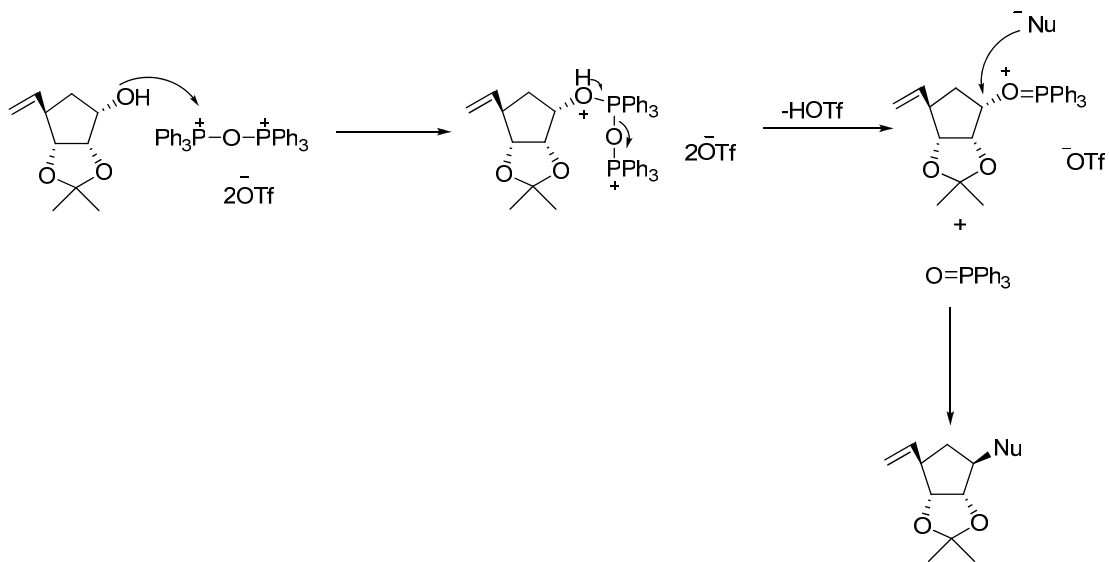
Scheme 42. Mitsunobu reaction of nucleobase with compound **27**

The consistent appearance of side products was intriguing us to seek coupling reagents other than DIAD. Recently, a study showed an alternative protocol by using the Herdickson “POP” (triphenylphosphonium anhydride trifluoromethanesulfonate) reagent for the Mitsunobu reaction.²²⁷ In the reported case, the POP reagent was used for dehydration in the formation of esters, amides and other similar compounds in a manner similar to the Mitsunobu reaction. A key intermediate, an alkoxyphosphonium salt, was apparent in this procedure. Since azodicarboxylates are not involved in the POP process, it was believed this would avoid the steps leading to **80** (Scheme 43). Thus, the reaction

was carried out by addition of triflic anhydride to an ice-chilled solution of triphenylphosphine oxide in dry dichloromethane (DCM). To this **27**, **43**, and diisopropylethylamine (DIEA) were added in succession. Unfortunately, no reaction occurred except for recovery the starting materials, which indicated that use of the Hendrickson reagent for secondary alcohols (as in **27**) was not amenable to success, perhaps, due to steric interference.



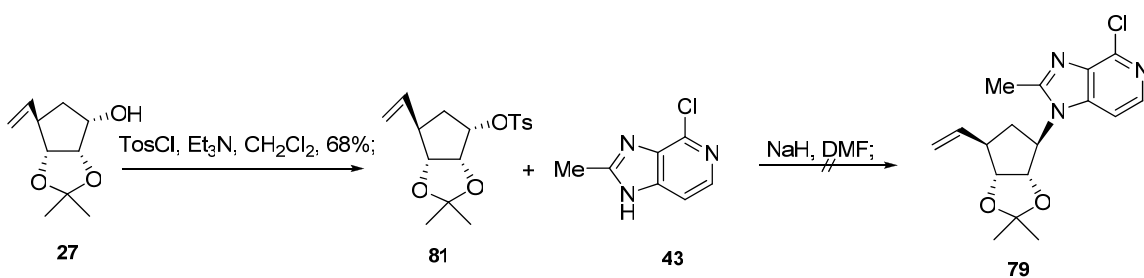
a. Tf_2O , triphenylphosphine oxide, DIEA, DCM;



Scheme 43. The reaction and general mechanism for Hendrickson reagent

Because of the failure of the Mitsunobu type reactions, a direct $\text{S}_{\text{N}}2$ reaction seemed to be a reasonable alternative for the enantioselective coupling reaction. Similar conditions

have been called on before by treating an appropriate nucleobase under basic circumstance, with the corresponding cyclopentyl tosylate²²⁴ or epoxide²²⁸ for the desired carbocyclic nucleosides synthesis. In this regard, compound **27** was readily transferred to tosylate **81** by treating with *p*-toluenesulfonyl chloride. However, no product could be found after the addition of the sodium salt of **43** to a solution of **81** in *N,N*-dimethylformamide (DMF) for over 24 hours (Scheme 44).

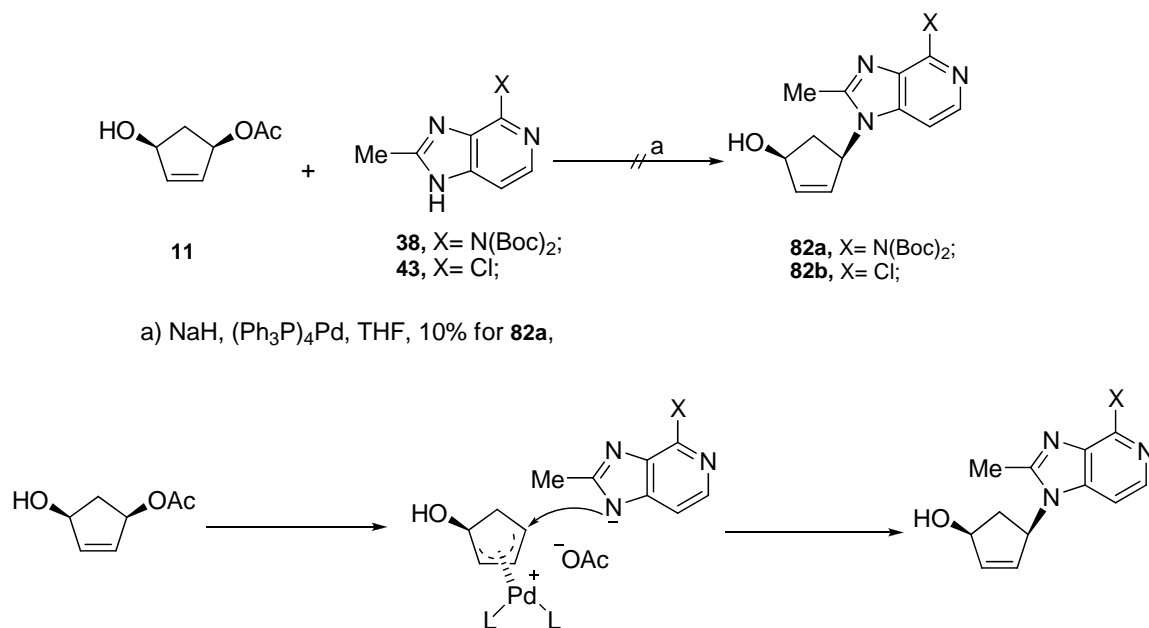


Scheme 44. The S_N2 reaction for the compound 27

Even though similar reactions met no difficulty for cyclopentenol **47**, **49a** and **72**, attempts at coupling cyclopentanol **27** to the 8-methyl-3-deazapurine base did not succeed. Previous experiences caused us to replace **27** with an unsaturated cyclopentene ring for the coupling reaction. For that purpose, monoacetate **11** became an option for several reasons: it was amenable to scale up; it provided the simplest template for the coupling reaction and itself also could serve as the precursor for noraristeromycin analogues. From studies that have shown that an enantioselective substitution promoted by tetrakis(triphenylphosphine)palladium catalyst was very effective for building up both the 3-deaza¹⁷⁷ and 8-methyl-noraristeromycin.¹⁷⁴ Therefore, it was reasonable to believe that the similar outcome would occur with a base combining two synthetic elements that

is, 3-deaza, and 8-methyl. The stereochemistry was insured by the nucleophilic attack to the less congested β -face of the early formatted carbocation palladium complex.²²⁹

(Scheme 45)

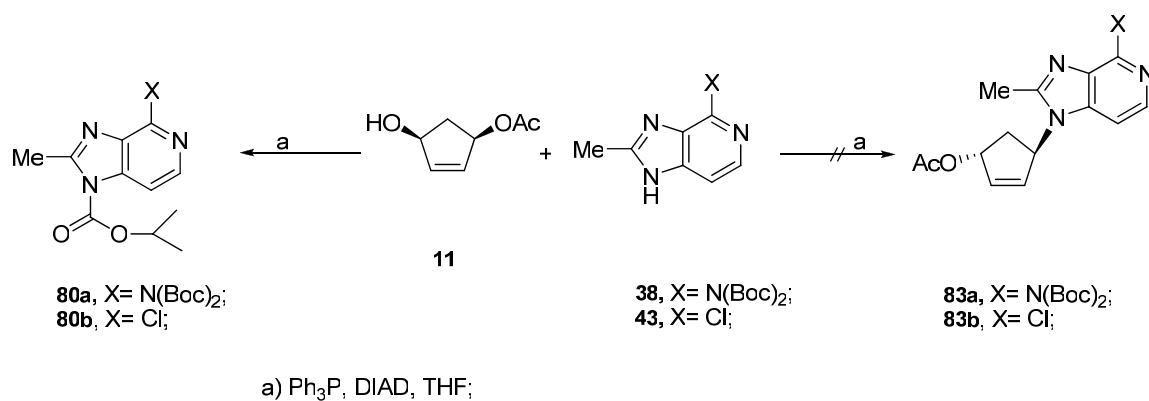


Scheme 45. Reaction and mechanism for the palladium catalyzed substitution

Both 3-deaza-8-methyl-6-chloropurine **43** and *bis*-Boc protected **38** were used. After reacted with sodium hydride, the nucleobases were treated with a solution of tetrakis(triphenylphosphine) palladium, TPP and **11**. The reaction produced only a trace amount of **82a** (less than 10%) after 24 hours at 55 °C. This result was due to the nucleophilic activity for acetate anion competing with the deprotonated nucleobase **43** or **38** for the palladium complex, this consequence led to a return to further investigation of the Mistunobu reaction.

Study of the Mitsunobu Reaction

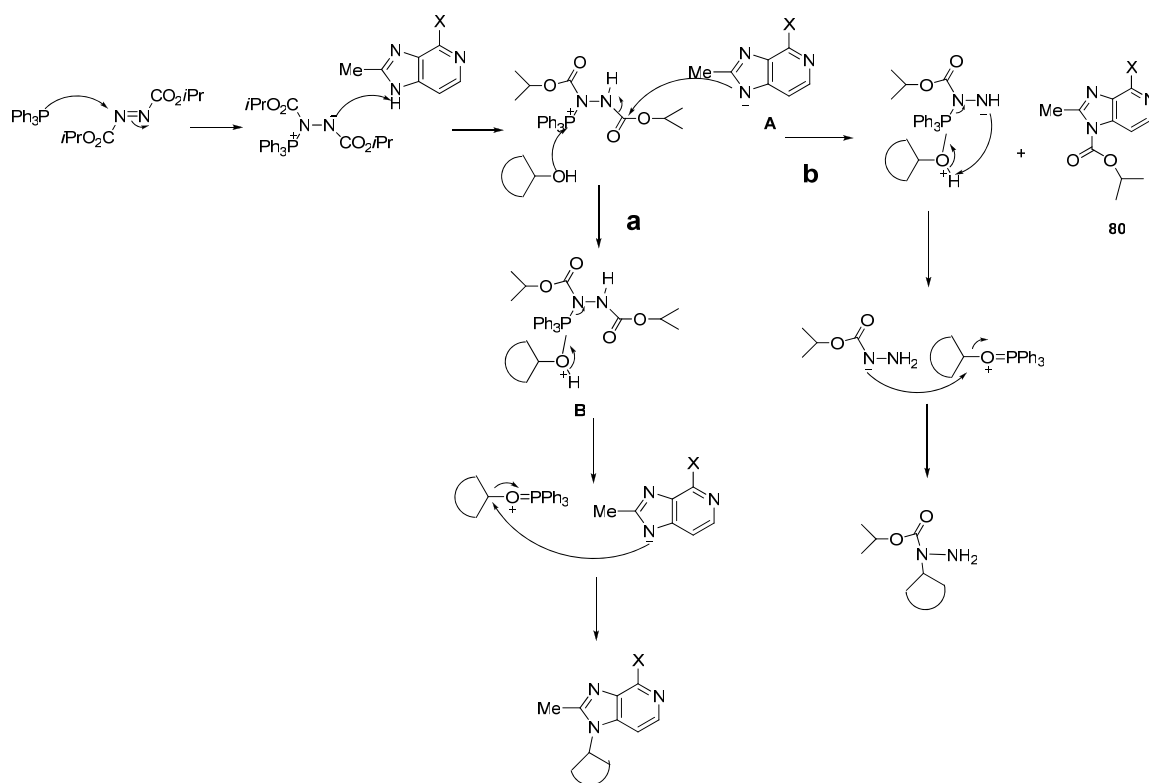
Based on the experience with the secondary allylic alcohols (**47**, **49a**, **72**) as good substrates for the Mitsunobu reaction with 3-deaza-8-methyl purine bases (Table 3 entry 1~3), monoacetylated **11** was subjected to the Mitsunobu reaction with nucleobase **43** and **38**. However, byproduct **80** was obtained rather than the desired coupling product **82** (Scheme 46).



Scheme 46. Mitsunobu reaction for compound **11** with 3-deaza-8-methyl nucleobase

The Mitsunobu reactions conducted with various cyclopentanol or cyclopentenol and a 3-deaza-8-methyl nucleobase are summarized in Table 3. Based on the trend of reactivity towards alcohol substrates and the accompanying complicating materials (triphenylphosphine oxide and compound **80**), a hypothetical mechanism is presented here (Scheme 47). The first two steps are the same as the conventional Mitsunobu reaction: that is, deprotonation of the nucleobase after a TPP-DIAD complex is formed. The steps that follow proceed in two directions. A competition arises between the modified purine anion (A in Scheme 47), which is more active than adenine due to the

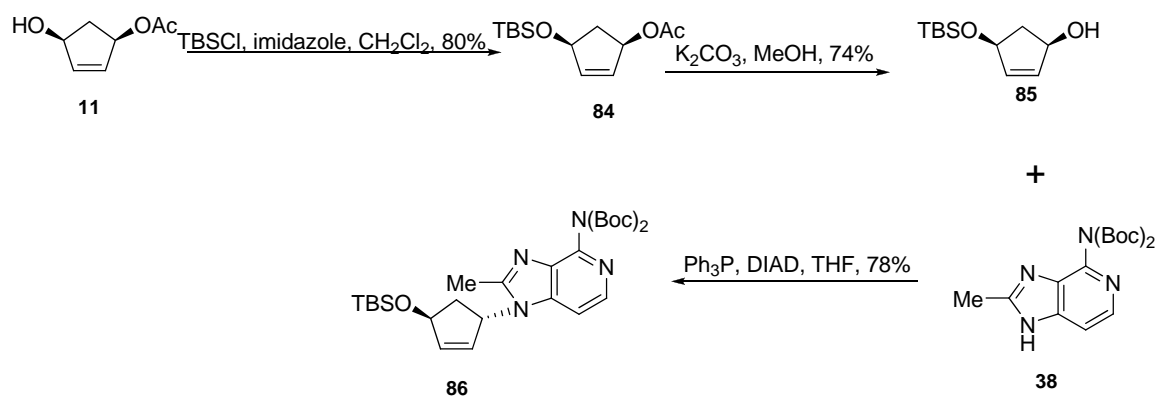
electron-donating methyl group on C-8 position and the absence of the electron-withdrawing N-3 leads to reaction in the complex at the *isobutyl* carbonyl (path b), this occurs before formation of more common alkoxyphosphonium salt (B). The kinetic controlled compound **80** is afforded as well as triphenylphosphine oxide. Increasing the reactivity of the cyclopentyl, allylic substrate **47**, **49a** and **72** for example, facilitates the reaction towards the conventional Mitsunobu reaction direction (pathway a).



Scheme 47. Proposed mechanism for Mitsunobu reaction

To test this hypothesis, monoacetate **11** bearing an allylic alcohol functional group was subjected to the Mitsunobu reaction but also yielded **80**. It was believed that the electron-withdrawing acetate group adjacent to the double bond in **11**, which could lower

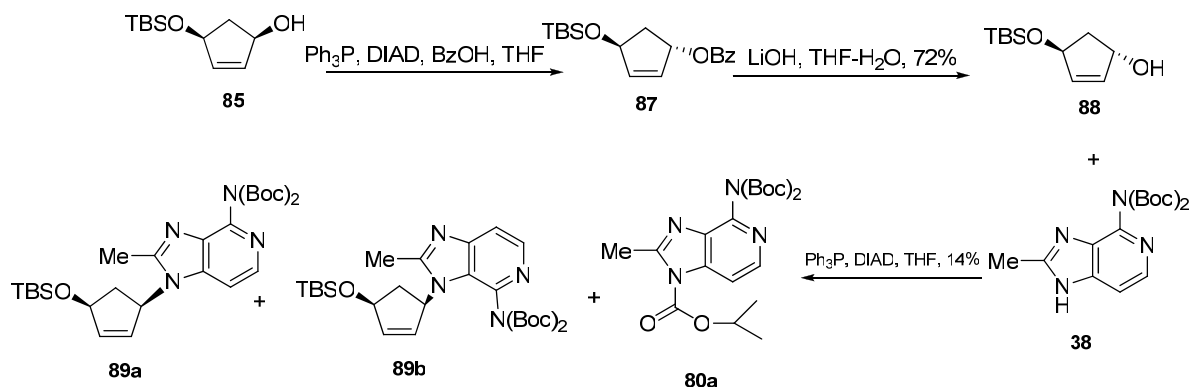
the activity of the allylic alcohol acting as the Mitsunobu nucleophile, could be the reason for this result. To verify this assumption, the Mitsunobu reaction was tested with the TBDMS protected compound **85**, which was easily obtained from **11** in two steps: protection of the C-4 hydroxyl of **11** with *tert*-butyldimethylsilyl chloride followed by selective removal of the acetate under basic conditions. This time Mitsunobu reaction of **38** with **85** produced **86**, this opened an effective pathway to many based modified aristeromycin analogues synthesis (Scheme 48).



Scheme 48. Synthesis and Mitsunobu reaction for 85

Due to the success in realizing compound **85**, the most straightforward scheme to the preferred β-isomer of **86** would be to manipulate the hydroxyl group in **85** to an α-configuration prior to Mitsunobu conditions (Scheme 49). Thus, the conversion of the hydroxyl stereocenter of **85** was accomplished by applying a Mitsunobu reaction with benzoic acid to afford an α-benzoyl group that was removed in strong basic conditions to give **88**. This diastereomer of compound **85** was subjected to the Mitsunobu conditions, but the result was complicated by an inseparable mixture: **89a** (N-9 isomer) and **89b** (N-

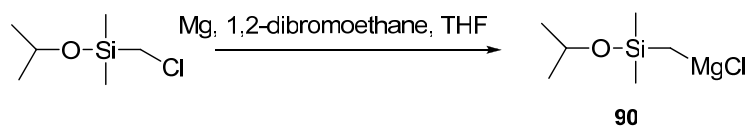
7). The ratio of these two products was 1:1 by TLC in a combined yield of less than 14%. Disappointingly **80a** was also separated from the reaction mixture, this suggested that the bulky TBS protecting group on the same side of the cyclopentyl moiety as the incoming nucleobase was affecting the reaction. To evaluate this assumption, compound **93** (See Table 3) with a methylene group between the cyclopentenol ring and the TBS protected primary alcohol was sought with the hope the longer side chain of **93** would alleviate the congestion in the transition state.



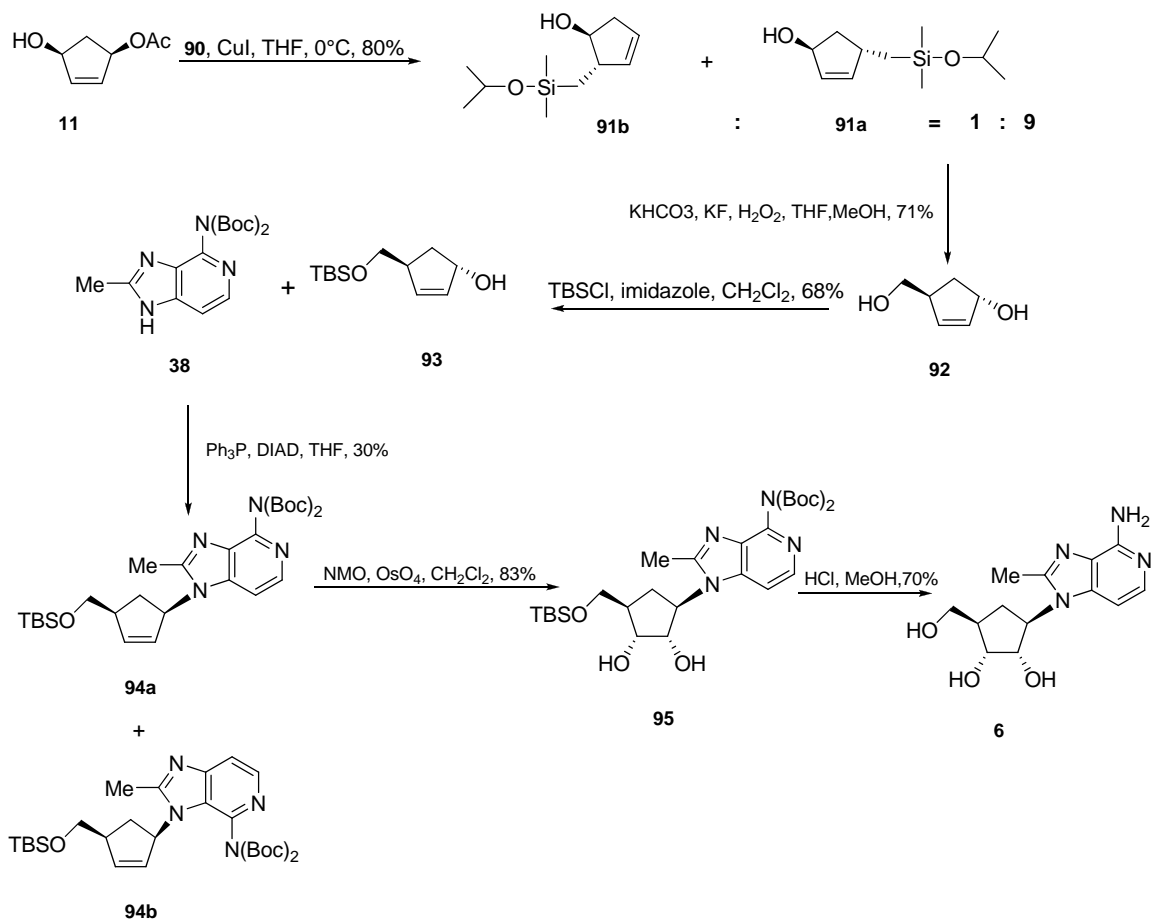
Scheme 49. Synthesis and Mitsunobu reaction for the isomer of **85**

Compound **93** was achieved from the monoacetate **11** (Scheme 51) by adapting a published procedure.^{230,231} Thus, five equivalents of freshly made (*isopropoxydimethylsilyl*)methyl Grignard reagent (**90**) (Scheme 50) was added slowly to a solution of compound **11** in the presence of copper(I) iodide in THF to afford **91a** in high regioselectivity (**91a** : **91b**= 9:1). Conversion **91a** to the useful intermediate **92** was carried out by the Tamao oxidation conditions to **92**. After the selective protection of the primary alcohol of **92**, the desired coupling precursor **93** was obtained in good yield. The

Mitsunobu reaction with **93** processed nicely to give the N-9 isomer **94a** in 48% yield. The N-7 isomer **94b** was also found and separated from **94a** by chromatography column. The ratio of two isomers (**94a:94b**) was around 4:1. The structures of the two isomers were distinguished by a NOESY spectral analysis (Figure 28). The methyl group on the C-8 position of **94a** can be expected adapt to a *syn* conformation while the N-7 isomer **94b** would be in the more preferred *anti* conformation due to the bulky Boc protecting group on the C-6 position. A consequence of these conformations would be a strong NOESY relationship between hydrogen at C-3 with the C-6' hydrogens in **94a**, and a NOESY consequence between the C-8 methyl group and the C-6' protons of **94b**. After these predictions were observed, the dihydroxylation of **94b** by osmium tetroxide, followed desilylation under acid conditions and removal the Boc protecting groups, compound **94** was smoothly transferred to the final product **6**, 3-deaza-8-methylaristeromycin.



Scheme 50. Synthesis of Grignard reagent 90



Scheme 51. Synthesis of 3-deaza-8-methylaristeromycin

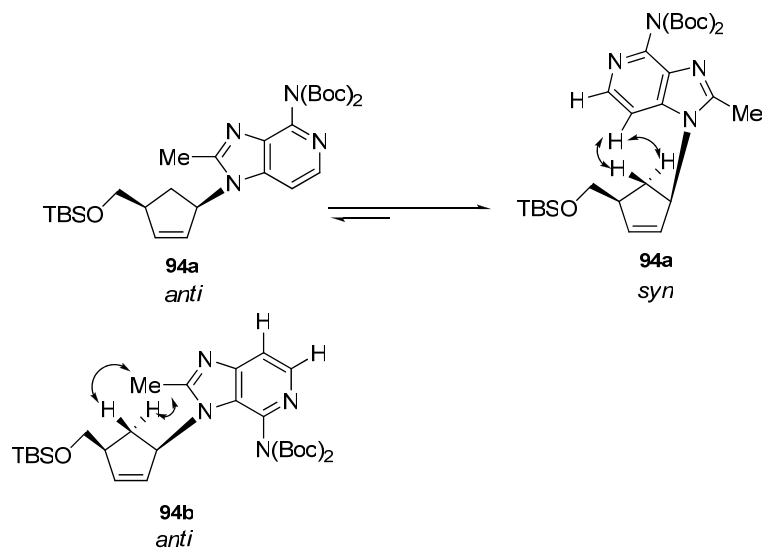


Figure 28. NOESY analysis for two isomers of 94

Table 3. Mitsunobu reaction for coupling (Part A)

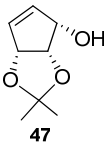
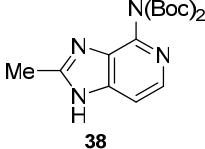
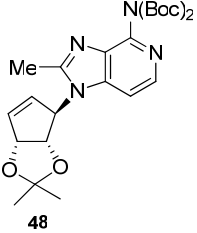
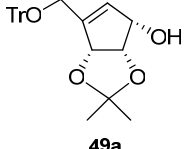
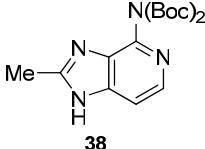
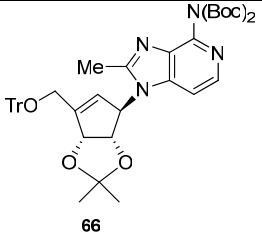
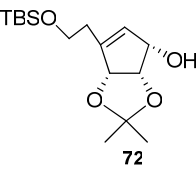
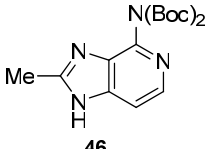
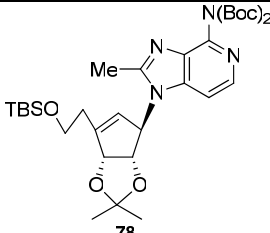
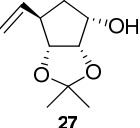
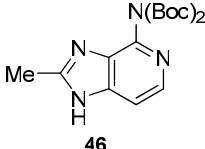
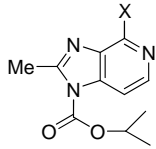
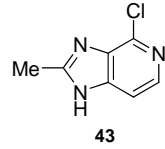
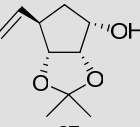
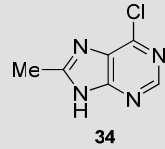
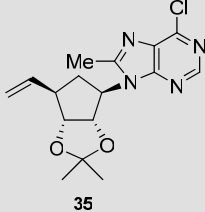
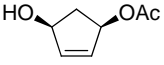
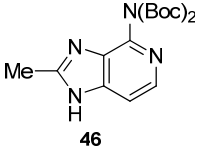
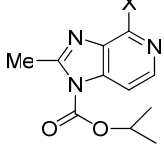
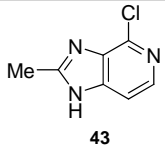
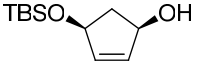
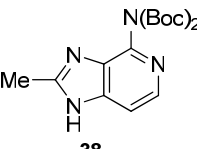
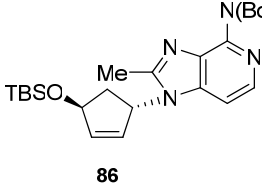
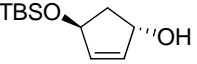
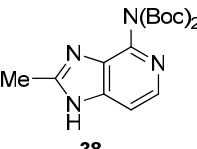
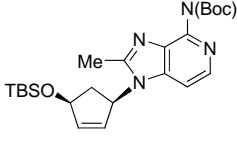
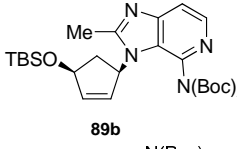
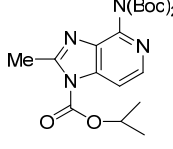
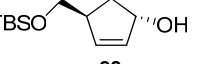
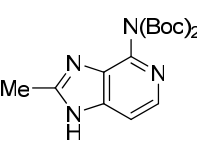
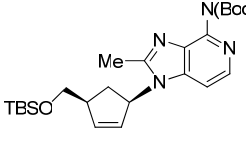
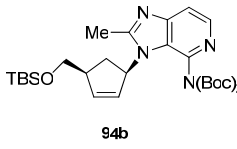
<i>Entry</i>	<i>Alcohol</i>	<i>Purine Base</i>	<i>Product</i>
1)	 47	 38	 48
2)	 49a	 38	 66
3)	 72	 46	 78
4)	 27	 46	 80a, X= N(Boc) ₂ ; 80b, X= Cl;
		 43	
5)	 27	 34	 35

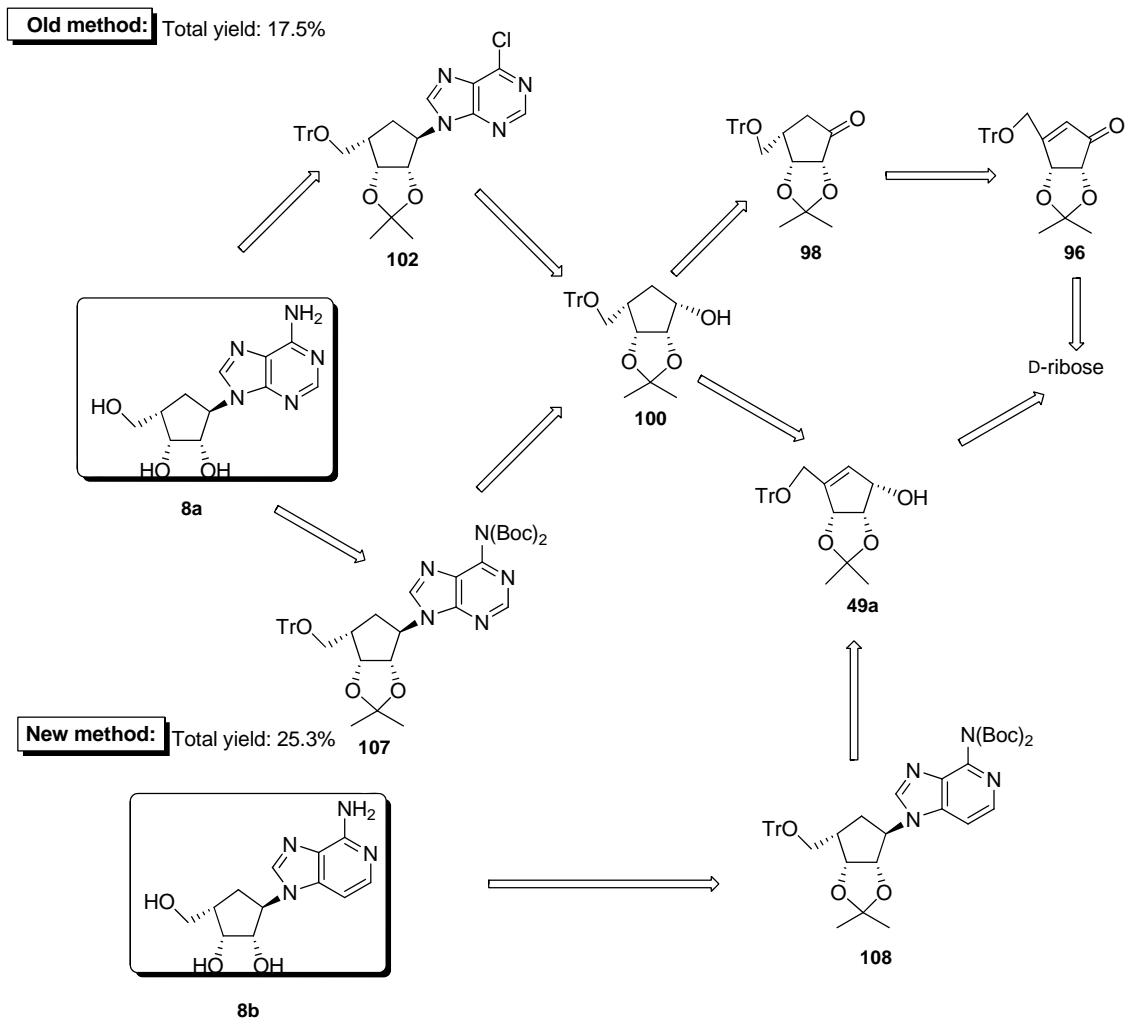
Table 3. Mitsunobu reaction for coupling (Part B)

<i>Entry</i>	<i>Alcohol</i>	<i>Purine Base</i>	<i>Product</i>
6)	 <p>11</p>	 <p>46</p>	 <p>80a, X= N(Boc)₂; 80b, X= Cl;</p>
		 <p>43</p>	
7)	 <p>85</p>	 <p>38</p>	 <p>86</p>
8)	 <p>88</p>	 <p>38</p>	 <p>89a</p>  <p>89b</p>  <p>80a</p>
9)	 <p>93</p>	 <p>38</p>	 <p>94a</p>  <p>94b</p>

Chapter 5. Synthesis of 4'-*Isoaristeromycin* and Its 3-Deaza Analogues

The 4'-*isoaristeromycin* (**8a**) and its 5'-homo analogue (**9a**) have been studied and synthesized before in our laboratories, but the synthetic routes suffered from long steps, and low yields. Investigations in this project produced two important cyclopentyl building blocks **49a** and **72** as good scaffolds for isomeric carbocyclic nucleoside analogues including 4'-*isoaristeromycin* and its 5'-congener. Therefore, new synthetic procedures were explored for 4'-*isoaristeromycin* (**8a**) and 4'-*isohomoaristeromycin* (**9a**) as well as their 3-deaza analogues (**8b** and **9b**, respectively).

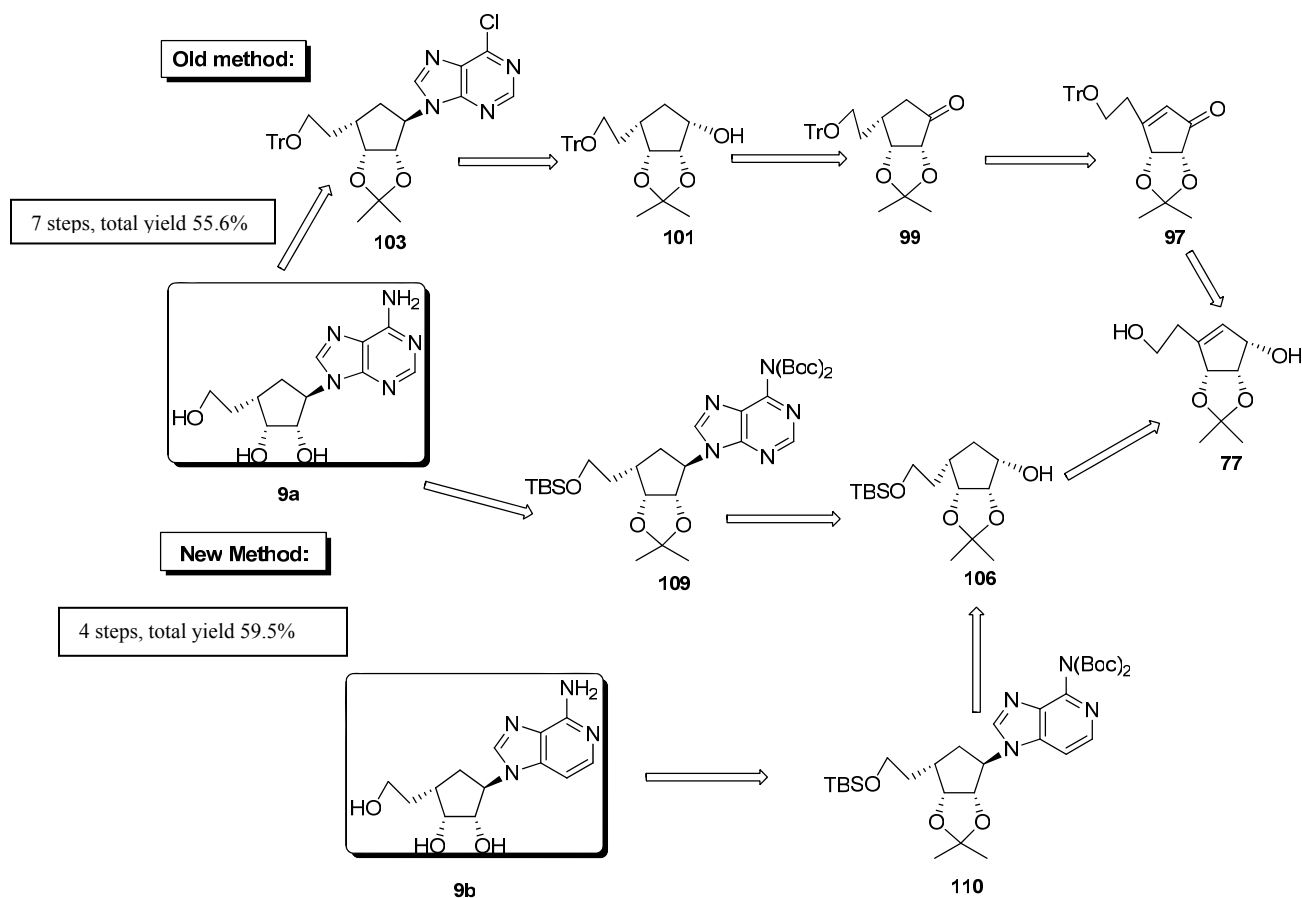
For the 4'-*isoaristeromycin*, two synthetic approaches were analyzed (Scheme 52). Both methods shared the same intermediate **100** as the precursor for a Mitsunobu reaction. Compound **100** coupling with the 6-chloropurine was the old method; use of *bis*-Boc protected adenine in the coupling presented a new approach. The intermediate **100** was achieved in different pathways derived from the same starting material, D-ribose.^{225,226} Total synthetic steps for **8a** were same (11steps) for two procedures, but the yield was improved significantly for the new one (25.3% Vs 17.5%).



Scheme 52. Retrosynthetic analysis of 4'-isovaristeromycin and 3-deaza-4'-isovaristeromycin.

The two pathways envisioned for the 4'-isohomoaristeromycin (**9a**) started with the intermediate **77**, which was the precursor of allylic alcohol **72**. The way to **9a** in old method was involved a tedious oxidation then reduction of C-1' hydroxyl group (**77**→**97**→**99**→**101**). However, the new synthetic route was designed to be more efficient

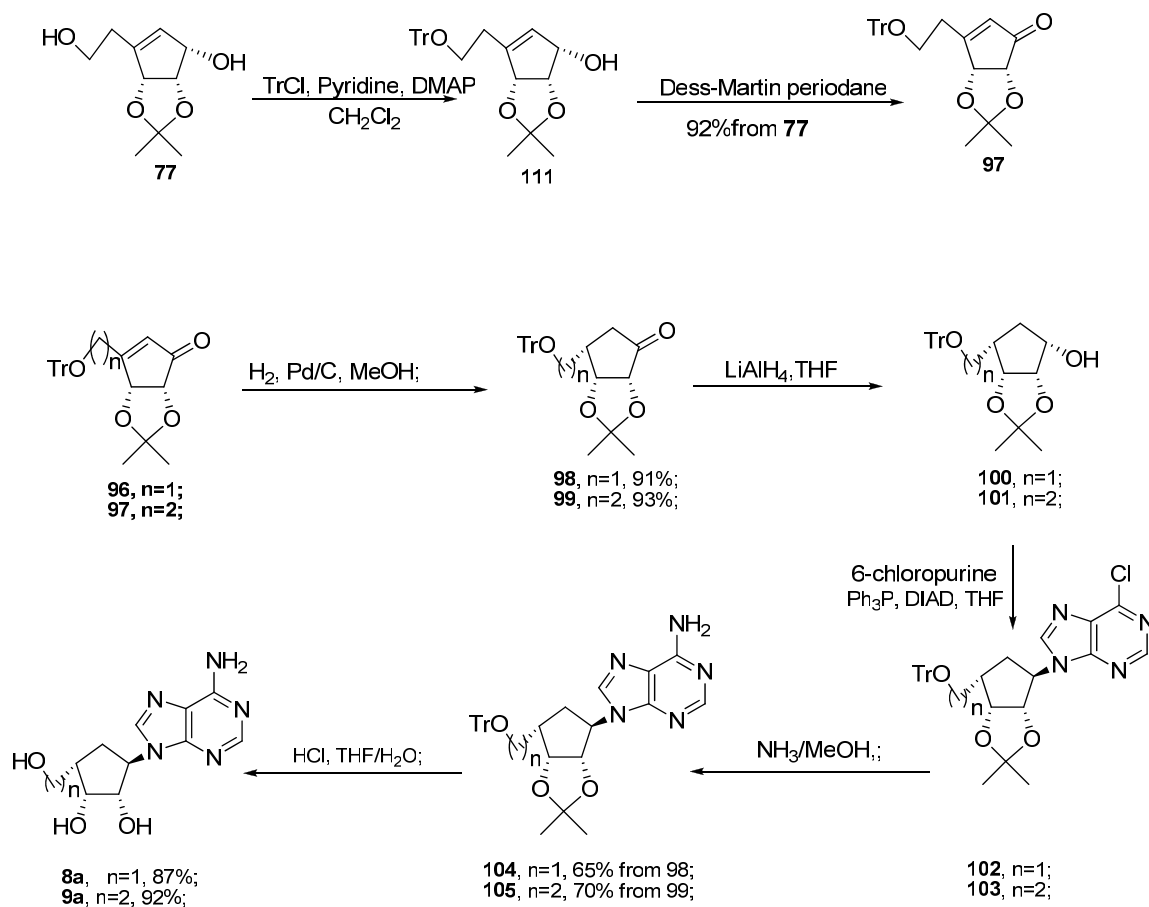
by eliminating unnecessary steps. This resulted in 3 fewer steps and an improved yield. Moreover, these routes were amenable to the 3-deaza analogues (Scheme 53).



Scheme 53. Retrosynthetic analysis of 4'-isohomoaristeromycin and 3-deaza-4'-isohomoaristeromycin.

The old synthetic routes for **8a** and **9a** started with the cyclopentenone **96** and **97** (Scheme 54). The former one was derived directly from the D-ribose (Scheme 32 on the top)²²⁵ and the later from diol **77** (Scheme 54). For **97**, after selectively protecting the primary alcohol of **77**, compound **111** was subjected to a Dess-Martin oxidation to provide **97**. Hydrogenation to the top face was applied to both **96** and **97** to ensure the α -

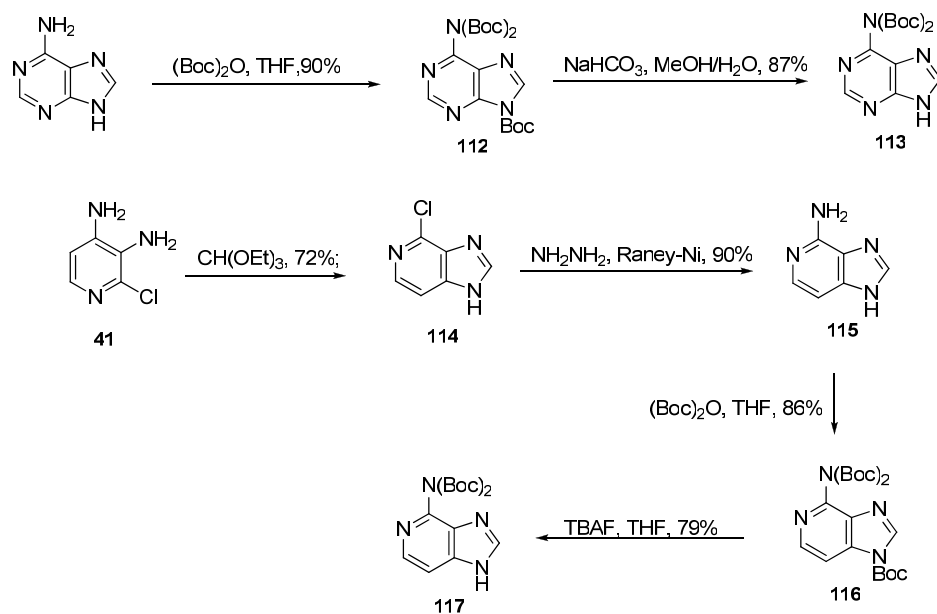
stereochemistry of the C-4 position (structure proof vide infra for Figure 29). This was followed by a stereospecific reduction of the ketone functional group of **98** and **99** to furnish the coupling precursors **100** and **101**. Both compounds were treated with 6-chloropurine, TPP and DIAD to afford **102** and **103**, which went through the ammonolysis and deprotection to give final products **8a** and **9a**, respectively.



Scheme 54. Old method for synthesis of compound 8 and 10

Before discussing the new synthetic approach developed here for the 4'-*isoaristeromycin* analogues, the synthesis of two requisite nucleobases, *bis*-Boc protected

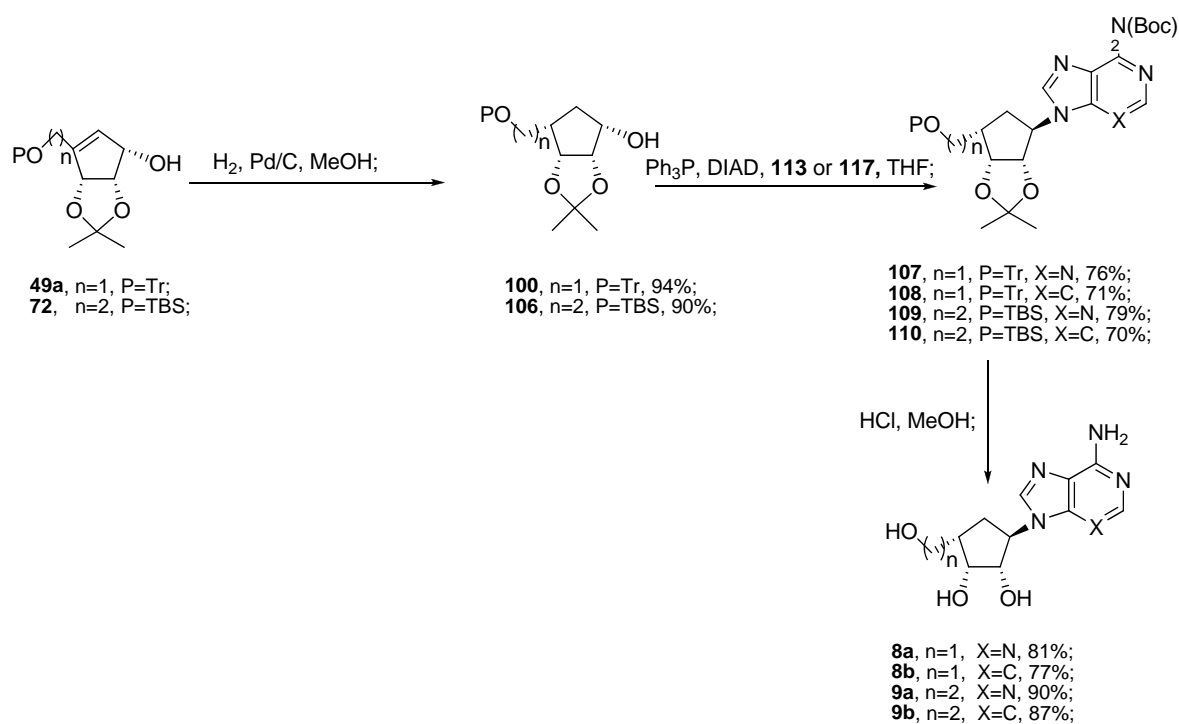
adenine and 3-deazaadenine is described (Scheme 55). Protected adenine **113** was accomplished by generating the *tris*-Boc protected adenine **112** then selectively removing N-9 Boc group with aqueous sodium bicarbonate (NaHCO₃). For the synthesis of the 3-deazaadenine derivatives (**115**), 2-chloro-3, 4-diaminopyridine (**41**) was used as a starting material, the same reagent used for the synthesis of 3-deaza-8-methyladenine and triethyl orthoformate to give **114**. Displacement of the chloro substituent in **114** was carried out by treatment with hydrazine followed by Raney nickel reduction. The newly formed amine group in 3-deazaadenine **115** was then protected with Boc groups in a two-step sequence: *tris*-Boc protection by *tert*-butyl dicarbonate; followed by selective deprotection of the N-9 Boc group (Scheme 55).



Scheme 55. The synthesis of *bis*-Boc protected adenine and 3-deazaadenine

For the cyclopentyl portion, a new chiral center on the C-4 position was established by employing a catalytic hydrogenation to cyclopentenone **49a** or **72**. The stereochemistry of

the products **100** and **106** were determined by a NOESY spectral analysis (Figure 29). The strong NOESY relationship between C-4 with C-2, C-3 and one of the C-6 protons indicates the α -configuration at C-4 position. With these two coupling precursors available, Mitsunobu reactions with both nucleobase **113** and **117** were conducted. After removing protecting groups for all coupling products by hydrochloric acid, final products **8a**, **8b**, **9a**, and **9b** were achieved in high yields (Scheme 56).



Scheme 56. The synthesis for 8 and 9

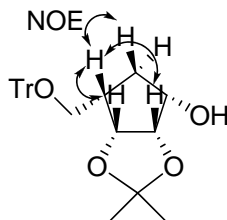
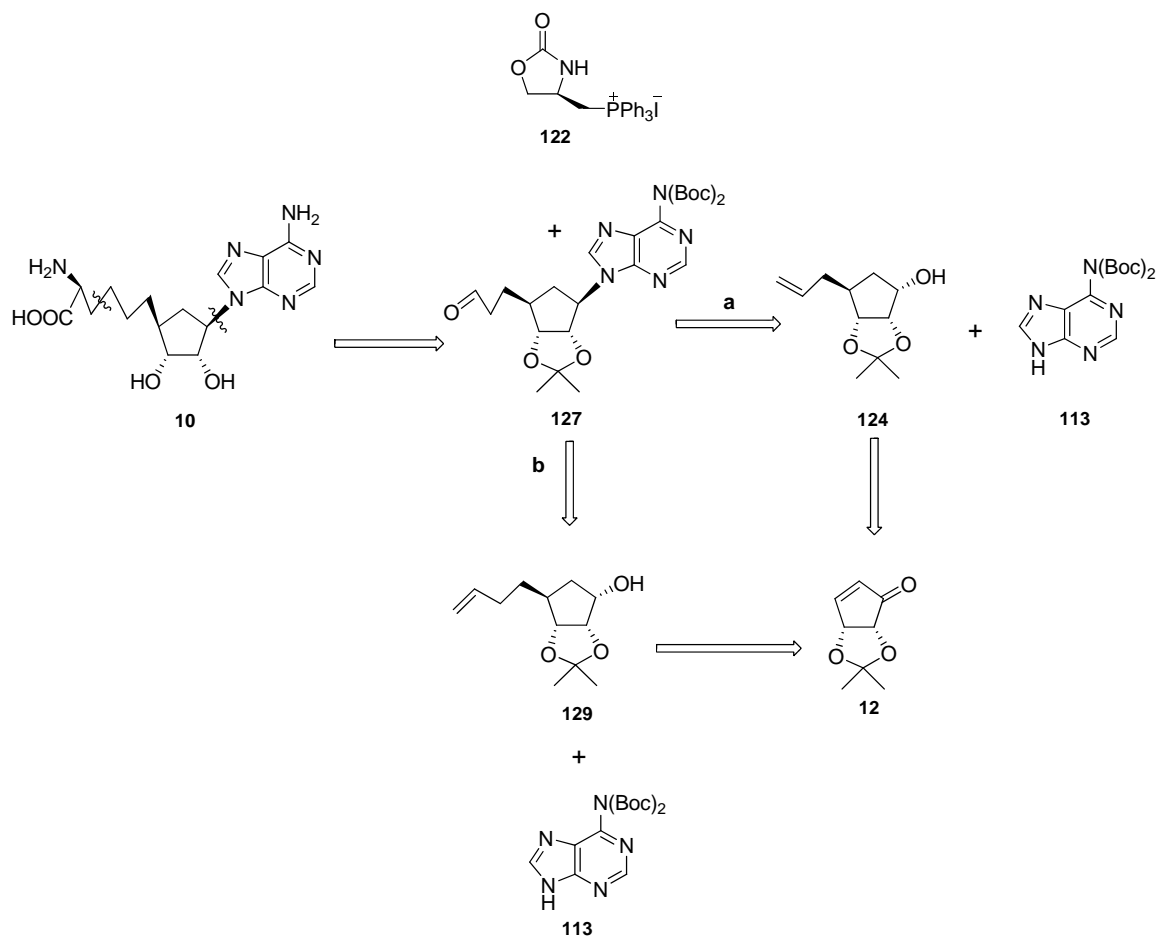


Figure 29. NOSEY for 100

Chapter 6. Synthesis of Deamino-Carbocyclic Sinefungin

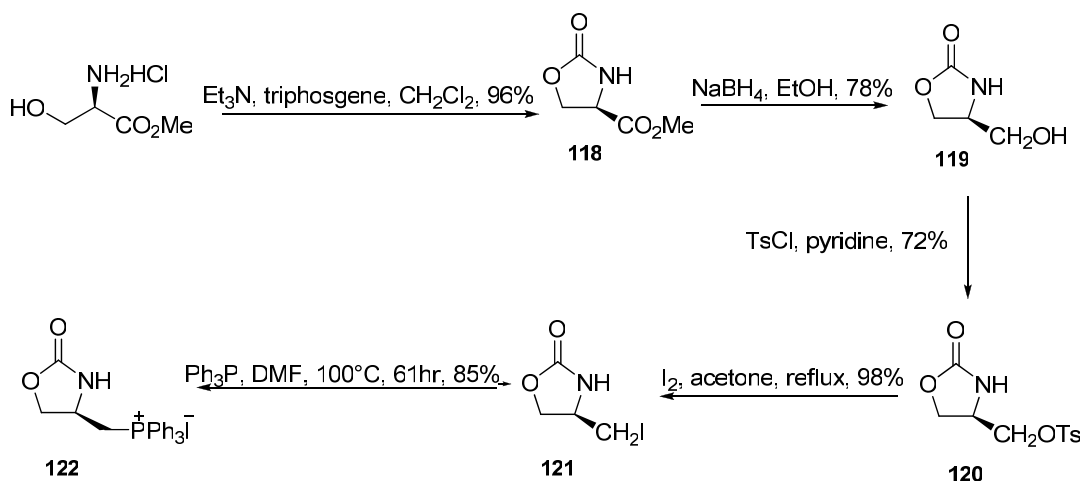
After careful analysis, a practical convergent synthetic strategy was designed for deamino-carbocyclic sinefungin **10**. It was envisioned the target **10** could be prepared from an alaninol synthon (**122**) and an appropriate carbocyclic nucleoside, the latter being accessed by utilizing a Mitsunobu reaction to connect a functionalized cyclopentenol with a protected adenine. An initial attempt (pathway a in Scheme 57) to the carbocyclic nucleoside aldehyde **127** following conversion of the coupled product of **124** with **113** was not encouraging. Thus, an alternative pathway was explored by using the *n*-butenyl side chain of **129** to achieve the aldehyde functional group (pathway b in Scheme 57).



Scheme 57. Retrosynthetic analysis of deamino-carbocyclic sinfungine **10**

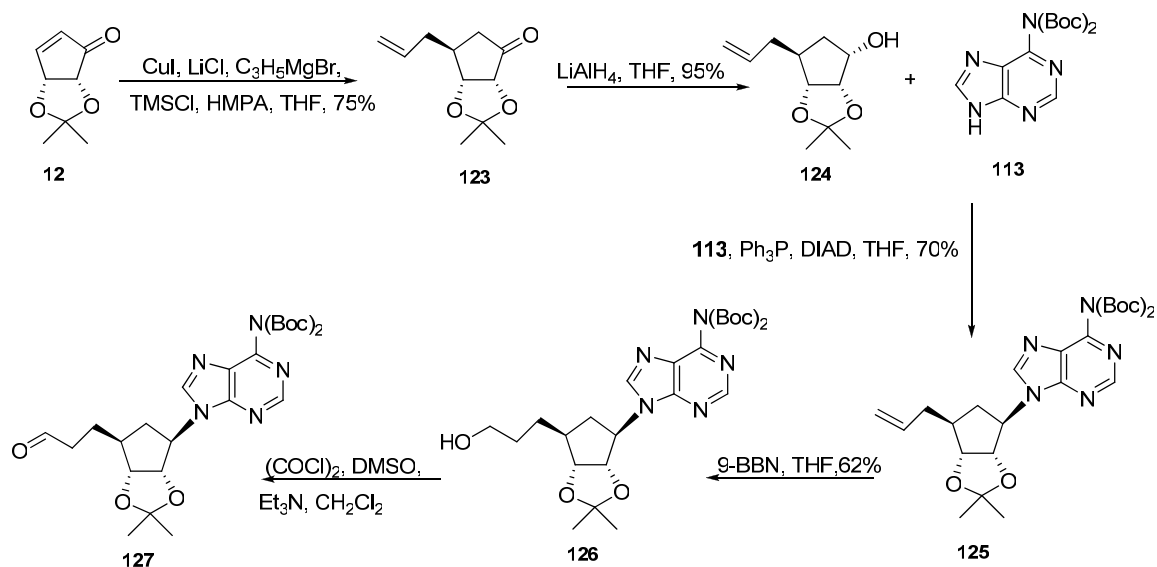
In that direction, naturally occurring D-serine bearing a chiral center was seen as a good source for building a nucleophilic alanine synthon. Therefore, a precursor of this species **122** (actually a Wittig precursor) was prepared from L-serine methyl ester hydrochloride following a methodology developed by Sibi and coworkers.²³² The synthesis commenced with the conversion of starting reagent to the oxazolidinone **118** by treating with triphosgene and triethylamine. Reduction of the ester functionality with sodium borohydride provided a highly water-soluble primary alcohol **119**, which was converted to a corresponding iodo compound **121** in two steps: tosylation of the primary

alcohol, followed by refluxing with sodium iodide. The phosphonium salt **122** was then achieved by stirring the iodo compound **121** with excess TPP in DMF for over 24 hours (Scheme 58).



Scheme 58. The Synthesis of phosphonium salt 12

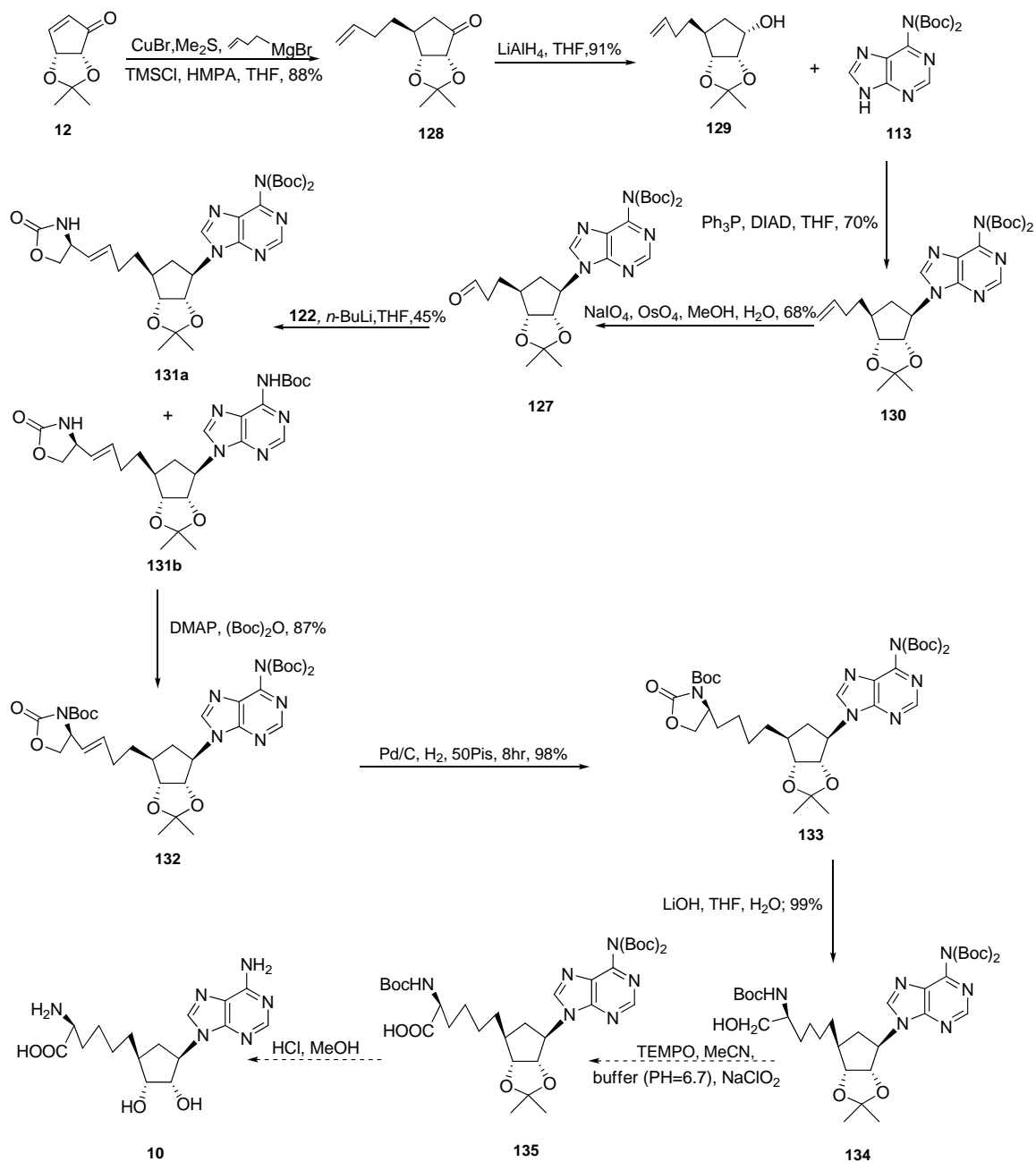
The first attempt for the synthesis of the carbocyclic nucleoside aldehyde **127** started with a 1, 4-Michael addition to cyclopentenol **12** by applying a copper salt along with Grignard reagent and allylic magnesiumbromide to **123**. An enantioselective reduction with lithium aluminum hydride generated the substrate **124** for the Mitsunobu reaction. The resulting coupled product **125** was then subjected to hydroboration conditions, but the purification for the product **126** was difficult, due to a formation of the byproduct derived from 9-BBN and the apparent loss of the Boc protecting groups under the reaction condition or during purification. An attempt to carry-out a Swern oxidation in this mixture was inconsistent most likely due to complexity of the mixture including **126** but ^1H NMR spectrum does show some evidences for existence of **127** (Scheme 59).



Scheme 59. First attempt for synthesis of aldehyde 127

Based on that, the synthetic route was revised by changing the Grignard reagent from allylic magnesiumbromide to the 3-butenylmagnesium bromide in the copper catalytic Michael addition to provide **128**. After reduction of ketone **128** to the secondary alcohol **129** and coupling with the protected adenine **113**, the resultant **130** was directly converted to the aldehyde **127** through an oxidative cleavage of the terminal alkene. In comparing to the previous approach, the yield by this method was improved and the reaction steps were shortened as well. The alaninol synthon **122** was then introduced by a Wittig reaction to afford **131a** along with **131b**, which was an unexpected product arising by losing one Boc protecting group. Its reprotection formed **132**. Traditional hydrogenation of **132** produced **133** in high yield. By treating **133** with strong base, the oxazolidinone was readily converted to the amino alcohol **134**. With **134** in hand, it will be necessary to conduct the proper oxidation and deprotection to restore the amino acid

and other functional groups. The final compound and its biological activity results are expected in the future study of our labs (Scheme 60).

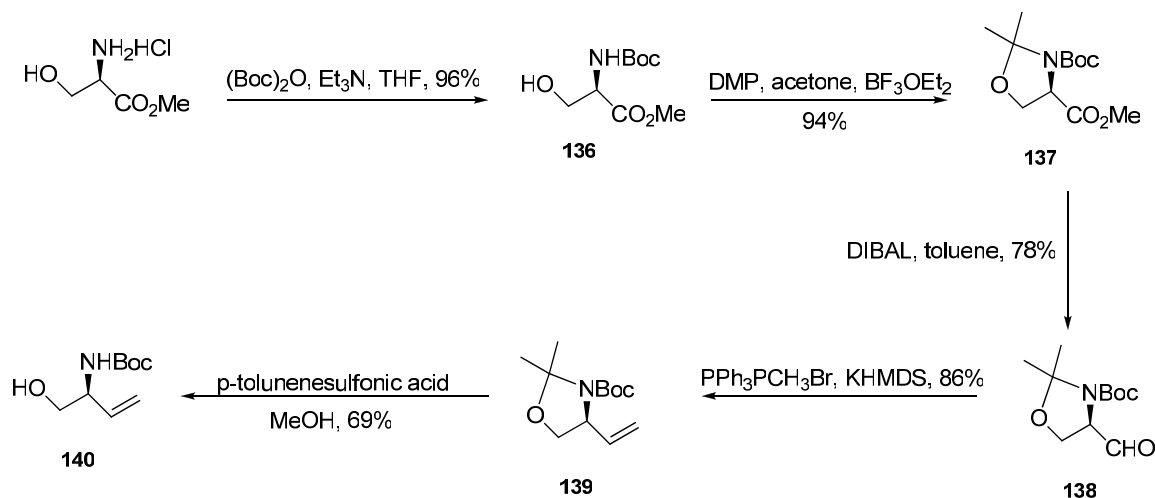


Scheme 60. The revised synthesis for target compound 10

New Synthetic Approach for Deamino-Carbocyclic Sinefungin

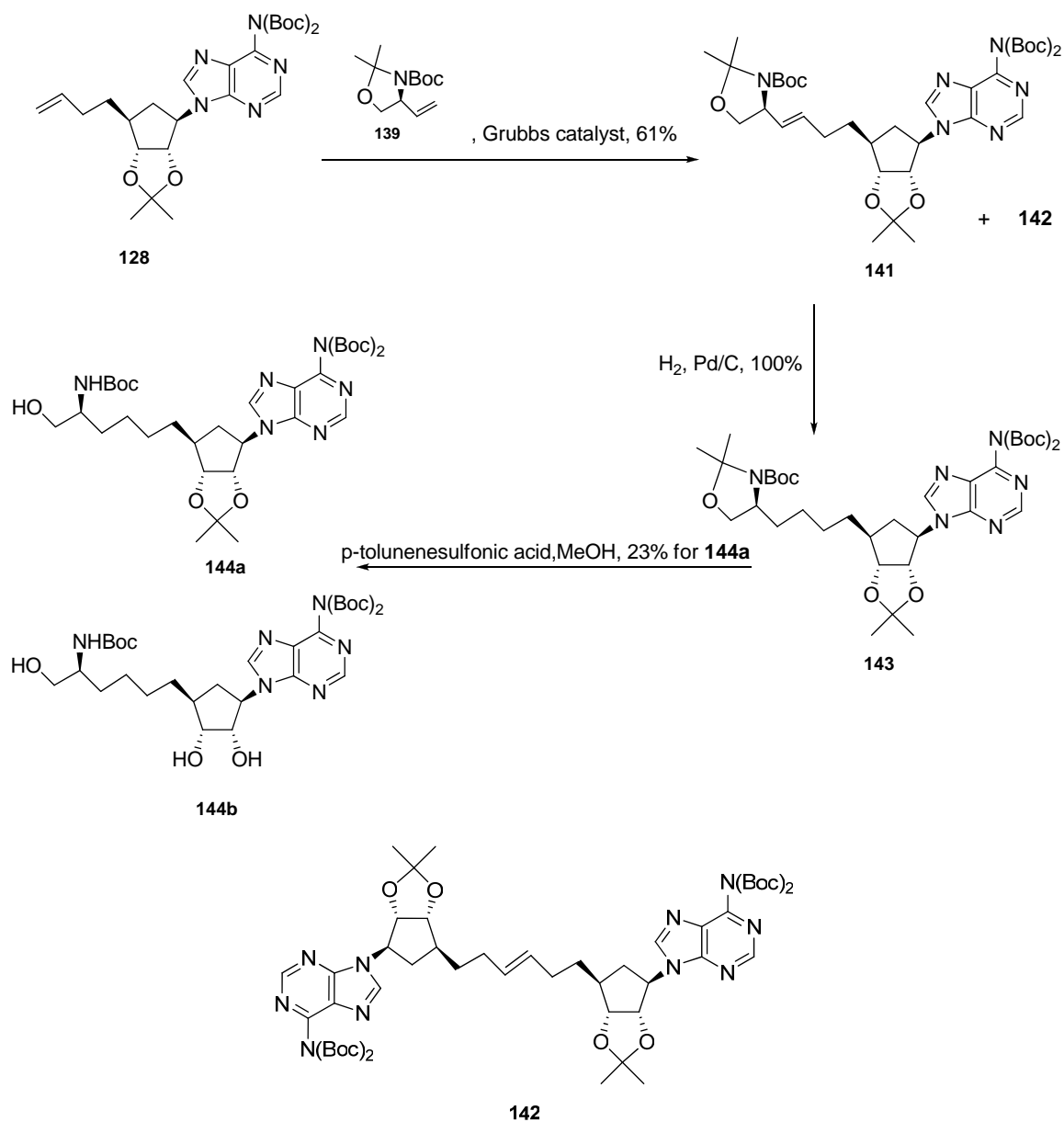
Since previous methods for the synthesis of compound **10** suffered from harsh reaction conditions (toxicity for synthesis of compound **118** and **127**; losing protecting group caused by strong basic condition for coupling of **122** with **127**), long reaction time (synthesis of phosphonium salt **122**) and a low yield for the Wittig reaction (**127** to **131**), a new synthetic approach was also explored based on the idea of coupling amino acid block with carbonucleoside piece by exerting olefin metathesis. Advantages of this method are including mild reaction conditions, easily prepared precursors and normally stable and high reaction yields.

The amino acid olefin portion began with D-serine methyl ester.²³³ After protecting of the amino group by using di-*tert*-butyl dicarbonate, the oxazolidine **137** was then obtained by using dimethoxypropane (DMP) with trifluoride etherate as catalyst. Reduction of ester **137** to aldehyde **138** was realized by using diisobutylaluminum hydride and the aldehyde function group was smoothly transformed to the terminal olefin by utilizing a Wittig methylenation reaction (Scheme 61).



Scheme 61. Synthesis of amino acid olefin 140

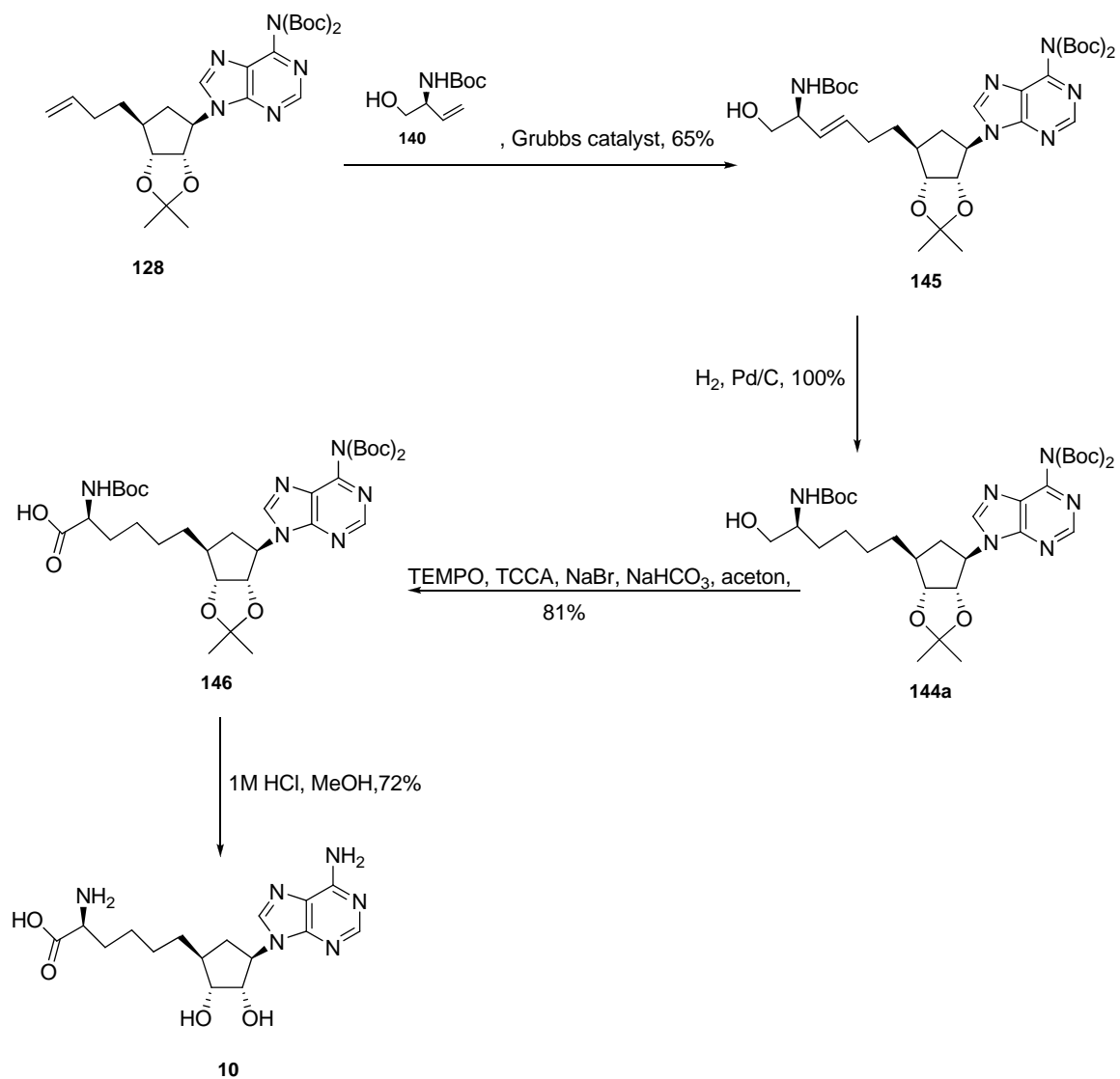
The first attempt of olefin metathesis was carried out between **139** and **128**. The reaction gave a good yield of coupling product **141** along with some **128** self-coupling byproduct **142**. The ^1H NMR coupling constant ($J_{\text{H-H}}$) of hydrogen installed on the double bond is 15.5, ensure the *trans* configuration of **141**. After catalytic hydrogenation, **143** was treated with acid for the selective hydrolysis of the acetonide, but unfortunately the fully deprotected product **144b** was also produced, which significantly lowered the yield of **144a** (Scheme 62).



Scheme 62. First attempt of metathesis

A better way to avoid this inconvenient situation is to use the deprotected **140** as the metathesis precursor. The free hydroxyl group doesn't affect the reaction yield at all and by using 1.5 equivalents of **140** no self-coupling by-product **142** was found. The ^1H NMR coupling constant ($J_{\text{H-H}} = 15.5$) of double bond hydrogens confirmed the E conformation

of **145**. After going through the catalytic hydrogenation and oxidation **146** was obtained in high yield. Cleavage of all the protection groups in **146** gave the final product **10** (Scheme 63).



Scheme 63. Second attempt of metathesis

Biological Results

Target compounds were evaluated against a wide variety of DNA viruses and RNA viruses. The spectrum of viruses used is shown in Table 4.

Table 4. The spectrum of viruses to be assayed

Virus family	Individual viruses
Adenoviridae	Adenovirus
Arenaviridae	Pichinde virus
Bunyaviridae	Punta toro virus
Coronaviridae	Human coronavirus, Severe acute respiratory syndrome (SARS)
Flaviviridae	Hepatitis C virus (HCV), West Nile virus, Yellow fever virus
Hepadnaviridae	Hepatitis B virus (HBV)
Herpesviridae	Epstein-Barr virus (EBV), Human Cytomegalovirus (HCMV), Varicella-Zoster virus (VZV), Herpes simplex virus (HSV)
Orthomyxoviridae	Influenza A virus, Influenza B virus
Paramyxoviridae	Parainfluenza virus, Measles virus, Respiratory syncytial virus (RSV)
Picornaviridae	Rhinovirus
Poxviridae	Cowpox virus, Vaccinia virus (VV)
Reoviridae	Reovirus
Rhabdoviridae	Vesicular stomatitis virus (VSV)
Togaviridae	Venezuelan Equine Encephalitis virus (VEE), Sindbis virus

Compound **2** showed significant activity against human cytomegalovirus (HCMV) and moderate activity against yellow fever virus. Compound **2** also showed slight activity against Flu A (H1N1) virus. Compound **4**, **9a** and **9b** were found to be not active against most of the test viruses. The bioassay data for compounds **3**, **5**, **6**, **10** will be forthcoming and under study in our laboratories.

Antiviral Activity Assays¹⁶¹: The antiviral assays were based on an inhibition of virus induced cytopathicity in either HeLa, Vero, MDCK, or HEL cell cultures, following previously established procedures²³⁴⁻²³⁶. Briefly, confluent cell cultures in microtiter trays were inoculated with 100 CCID₅₀ of virus, 1 CCID₅₀ being the virus dose required to infect 50% of the cell cultures. After a 1h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... µg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus infected cell cultures.

Cytostatic Activity Assays¹⁶¹: The cytostatic assays were performed as previously described²³⁷. Briefly, 100-µL aliquots of the cell suspensions (5 X 10⁵ murine leukemia L1210 or 7.5 X 10⁵ human T-lymphocyte Molt-4 or CEM cells/mL) were added to the wells of a microtiter plate containing 100 µL of varying concentrations of the test compounds. After a 2-day (L1210) or 3-day (Molt-4 and CEM) incubation period at 37 °C in a humidified CO₂-controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the compound concentration that reduced the number of viable cells by 50% (CC₅₀). The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (HeLa, Vero, and MDCK) or inhibition of normal cell growth (HEL), as previously described.

Table 5. Antiviral Activity of Compounds 2, 4 against HSV-1, HSV-2 and HCMV, Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		2	4
HSV-1 ^d	EC50 ^a	>300	>300
	EC90 ^b	>300	>300
	CC50 ^c	>300	>300
HSV-2 ^d	EC50	>300	>300
	EC90	>300	>300
	CC50	>300	>300
HCMV ^d	EC50	0.27	
	EC90	0.42	
	CC50	>300	

^aEffective concentration (μM) required to reduce virus plaque formation by 50%.

^bEffective concentration (μM) required to reduce virus plaque formation by 90%.

^cCytotoxic concentration (μM) required to reduce cell growth by 50%.

^dTested on human foreskin fibroblasts (HFF) cells.

Table 6. Antiviral Activity of Compounds 2, 4 and 9a against Adenovirus, Influenza A (H1N1), Influenza A (H3N2), Influenza B Inhibition Assay

Virus	Compound	Assay	Cell Line	EC50	IC50	SI
Adeno	4	Neutral Red	A-549	>100	>100	0
		Visual	A-549	>100	>100	0
	9a	Neutral Red	A-549	>100	>100	0
		Visual	A-549	>100	>100	0
	9b	Neutral Red	A-549	>100	>100	0
		Visual	A-549	>100	>100	0
Flu A (H1N1)	2	Neutral Red	MDCK	33	>100	>3
		Visual	MDCK	32	>100	>3.1
	4	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	9a	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
Flu A (H3N2)	2	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	4	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	9a	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
Flu A (H5N1)	2	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	4	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	9a	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
Flu B	2	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	4	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
	9a	Neutral Red	MDCK	>100	>100	0
		Visual	MDCK	>100	>100	0
9b	Neutral Red	MDCK	71	>100	>1.4	
	Visual	MDCK	32	>100	>3.1	

Units = μM

Table 7. Antiviral Activity of Compounds 2, 4 and 9a against measles, PIV, Rhinovirus, Rift Valley Fever Inhibition Assay

Virus	Compound	Assay	Cell Line	EC50	IC50	SI
measles	4	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	>100	>100	0
	9a	Neutral Red	CV-1	>100	>100	0
		Visual	CV-1	>100	>100	0
	9b	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	>100	>100	0
PIV	2	Neutral Red				
		Visual				
	4	Neutral Red	MA-104	>100	>100	0
		Visual	MA-104	>100	>100	0
	9b	Neutral Red	MA-104	>1.8	1.8	0
		Visual	MA-104	32	32	1
Rhinovirus	2	Neutral Red				
		Visual				
	4	Neutral Red	Hela Ohio-1	61	>100	>1.6
		Visual	Hela Ohio-1	>100	>100	0
	9a	Neutral Red	Hela Ohio-1	>100	100	0
		Visual	Hela Ohio-1	>100	24	0
	9b	Neutral Red	Hela Ohio-1	>100	>100	0
		Visual	Hela Ohio-1	>100	>100	0
Rift Valley Fever	2	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	90	>100	>1.1
	4	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	>100	>100	0
		Neutral Red				
		Visual				

^aMeasles was tested on Vero 76 cells.

^bPIV was tested MA-104 cells.

^cRhinovirus was tested on Hela ohio-1 cells.

^dRift Valley Virus was test on Vero 76 cells.

Table 8. Antiviral Activity of Compounds 2, 4 and 9a Against Yellow Fever, RSVA, and Inhibition Assay

Virus	Compound	Assay	Cell Line	EC50	IC50	SI
RSVA	2	Neutral Red				
		Visual				
	4	Neutral Red	MA-104	>100	>100	0
		Visual	MA-104	>100	>100	0
	9a	Neutral Red	MA-104	>100	>100	0
		Visual	MA-104	>100	>100	0
SARS	2	Neutral Red				
		Visual				
	4	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	>100	>100	0
	9a	Neutral Red	vero	>100	15	0
		Visual				
Yellow Fever	2	Neutral Red	vero	6.2	>100	>16
		Visual	vero	100	>100	>1
	4	Neutral Red				
		Visual				
	9a	Neutral Red	vero	>100	>100	0
		Visual	vero	>100	>100	0

Units = μ M

RSVA was tested on MA-104 cells.

SARS was tested on vero 76 cells.

Yellow Fever was tested on vero cells.

Table 9. Antiviral Activity of Compounds 2, 4 and 9a against Dengue, Tacaribe, VEE and WNV Inhibition Assay

Virus	Compound	Assay	Cell Line	EC50	IC50	SI
Dengue	2	Neutral Red	vero	77	>100	>1.3
		Visual				
	4	Neutral Red	vero 76	>100	>100	0
		Visual				
	9a	Neutral Red	vero	>100	>100	0
		Visual	vero	>100	>100	0
Tacaribe	2	Neutral Red	vero 76	>100	>100	0
		Visual	vero 76	>100	>100	0
	4	Neutral Red				
		Visual				
	9a	Neutral Red				
		Visual				
VEE	2	Neutral Red	vero	81	>100	>1.2
		Visual	vero	>100	>100	0
	4	Neutral Red	vero	>100	>100	0
		Visual	vero	>100	>100	0
	9a	Neutral Red	vero	>100	>100	0
		Visual	vero			
WNV	2	Neutral Red	vero	>100	>100	0
		Visual	vero	>100	>100	0
	4	Neutral Red				
		Visual				
	9a	Neutral Red	vero	>100	>100	0
		Visual	vero	>100	>100	0

Units = μM

Dengue was tested on Vero cells.

Tacaribe was tested on Vero 76 cells.

VEE was tested on Vero cells

WNV was tested on Vero cells.

Table 10. Antiviral Activity of Compounds 2, 4 against EBV virus based on DNA hybridization Inhibition Assay

Compound	Virus	Cell Line	EC50	E90	CC50	SI
2	EBV	Akata Cells	>20	>20	53.7	<2.7
4	EBV	Akata Cells	>20	>20	76.9	3.8

Table 11. Antiviral Activity of Compounds 2, 4 and 9a Against Vaccinia Virus and Cowpox Virus Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		2	4	9a
Vaccinia Virus ^a	EC50	>300	>300	237
	CC50	>300	>300	>300
Cowpox Virus ^b	EC50	>300	>300	293
	CC50	>300	>300	>300

Units = μM

^aVaccinia Virus was tested on HFF cells.

^bCowpox Virus was tested on HFF cells.

Table 12. Antiviral Activity of Compounds 2, 9a in Vero Cell Cultures

Compound	Minimum cytotoxic concentration ^a (μM)	Minimum inhibitory concentration ^b (μM)				
		Para-Influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
2	>100	>100	>100	>100	>100	>100
9a	>200	>200	>200	>200	>200	>200
DS-5000 (μg/mL)	>100	>100	>100	20	>100	20
(S)-DHPA	>250	250	>250	>250	>250	>250
Ribavirin	>250	50	146	250	>250	50

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50%.

Table 13. Antiviral Activity of Compounds 2, 9a in HEL Cell Cultures

Compound	Minimum cytotoxic concentration ^a (μM)	Minimum inhibitory concentration ^b (μM)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 (TK ⁻ KOS ACV)
2	>100	>100	>100	>100	>100	>100
9a	>200	>200	>200	>200	>200	>200
Brivudin	>250	0.02	29	4	>250	50
Ribavirin	>250	>250	250	112	125	>250
Cidofovir	>250	2	1	20	>250	1
Ganciclovir	>100	0.02	0.2	>100	>100	9

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50%.

Table 14. Antiviral Activity of Compounds 2, 9a against Cytomegalovirus in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity EC ₅₀ (μM) ^a		Cytotoxicity (μM)	
	AD-169 strain	Davis strain	Cell morphology (MCC) ^b	Cell growth (CC ₅₀) ^c
2	>100	>100	>100	>100
9a	>400	>400	>400	200
Ganciclovir	12.6	8.3	1575	175.6 209
Cidofovir	1.5	1.2	1270	87.3 122

^aEffective concentration (μM) required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).

^bMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

^ccytotoxic concentration required to reduce cell growth by 50%.

^eNo complete inhibition at higher drug concentration.

Table 15. Antiviral Activity of Compounds 2 and 9a against Varicella-zoster in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity		Cytotoxicity	
	EC ₅₀ (μM) ^a		(μM)	
	TK ⁺ VZV	TK ⁻ VZV	Cell morphology (MCC) ^b	Cell growth (CC ₅₀) ^c
	OKA strain	07/1 strain		
2	>100	>100	>100	>100
9a	>400	>400	>400	200
Acyclovir	3.4	42	>1778	356
brivudin	0.013	>240	1200	452

^aEffective concentration (μM) required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).

^bMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

^cCytotoxic concentration required to reduce cell growth by 50%.

Table 16. Cytotoxicity and Antiviral Activity of Compounds 2 and 9a in Hela Cell Cultures

Compound	Minimum cytotoxic concentration ^a (μM)	Minimum inhibitory concentration ^b (μM)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
2	>100	>100	>100	>100
9a	>200	>200	>200	>200
DS-5000 (μg/mL)	>100	20	9	0.8
(S)-DHPA	>250	>250	>250	>250
Ribavirin	>250	22	50	10

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50%.

Table 17. Antiviral Activity of Compounds 2, 4 and 9a against HCV and HBV

Compound		2	4	9a	9b
		(μ M)	(μ M)	(μ M)	(μ M)
HCV	EC50			59.7	>100
	EC90			>100	>100
	CC50			>100	>100
	SI			1.7	0
HBV	EC50	>100	>100	>100	>100
	EC90	>100	>100	>100	>100
	CC50	>100	>100	>100	>100
	Sib	>100	>100	>100	>100

^aSI = CC50/EC90

Conclusion

S-Adenosyl-L-homocysteine (AdoHcy) hydrolase is a pivotal enzyme involved in a viral replication cycle, which makes it a unique target for antiviral drug design. The consequence by inhibition of AdoHcy hydrolase is elevation of intracellular level of AdoHcy, which acts as a feedback inhibitor of S-adenosylmethionine (AdoMet) dependent methyltransferase. AdoMet dependent biomethylation reaction is an essential step for viral m-RNA 5'-capping process. So AdoHcy hydrolase inhibitors can lead to significant antiviral activities. Aristeromycin (Ari) and neplanocin A (NpcA) are two of the most potent inhibitors of AdoHcy hydrolase.

Besides showing remarkable antiviral properties, Ari and NpcA are also associated with high toxicity relevant to their 5'-nucleoside metabolites. Compound **1** was synthesized based on the modification of the 5'-hydroxyl group by adding a methylene group. 8-Methyl group was also introduced in order to examine the relationship between the anti-viral activities with *syn-anti* conformations. Compound **2** was prepared following this direction, which showed significant activity against human cytomegalovirus (HCMV), moderate activity against yellow fever virus and slight activity against Flu A (H1N1) virus. Since 3-deaza has been identified as another valuable structural feature, compound **3**, **4**, **5**, **6** have been synthesized as derivatives of Ari and Npc A. The configuration of 4'-stereocenter also provides an interesting aspect to explore new antiviral agents, since these changes may affect the phosphorylation and

deamination of 5'-hydroxyl group, which is essential for biological activity and/or cytotoxicity. Compound **8a**, **8b**, **9a**, **9b** were designed and prepared following this lead. Even though compounds **4**, **9a** and **9b** show no antiviral activities, they still provide valuable structure-activity relationship information for future design of antiviral agents.

At the end, for direct inhibition of methyltransferase, sinefungin provides a great model to study with. Its biological effects were significant, but cytotoxicity was associated as well. The relationship between the inhibitions of methyltransferase and its biological activities intrigued the study for design and synthesis of deamino-carbocyclic sinfungine **10**. Two different synthetic approaches have been developed and evaluated. The antiviral data will be forthcoming as part of a ongoing study.

Experimental Section

General

Melting points were recorded on a Meltemp II melting point apparatus and the values are uncorrected. Elemental analyses were performed at Atlantic Microlab, Norcross, GA. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AC 250 spectrometer (250 MHz for proton and 62.9 MHz for carbon) or a Bruker AC 400 spectrometer (400 MHz for proton and 101 MHz for carbon). All ^1H chemical shifts are referenced to internal tetramethylsilane (TMS) at 0.0ppm. ^{13}C chemical shifts are reported in δ relative to CDCl_3 (center of triplet, δ 77.2), or relative to DMSO-d_6 (center of septet, δ 39.5). The spin multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). The reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F254 precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine. Column chromatography was performed on Whatman silica, 230–400 mesh and 60 Å using elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous material.

6-Oxabicyclo[3.1.0]hex-2-ene (14). Dicyclopentadiene was freshly cracked to cyclopentadiene (181 g, 2.75 mol) and immediately dissolved in 1500 mL CH₂Cl₂. Sodium carbonate (350 g, 3.31 mol) was then added portionwise at -10 °C and the suspension then treated with a solution of sodium acetate (14 g, 0.21 mol) in 350 mL of peracetic acid (39% in acetic acid) dropwise. The temperature was maintained -10 °C to -5 °C during the addition. After the addition, resulting mixture was stirred at rt for 12 hrs. The mixture was filtered and the filtrate was evaporated to give a pale yellow liquid. The crude monoepoxide **14** was used directly in the next step.¹⁶²

(Z)-Cyclopentene-3,5-diol diacetate (16). *Tetrakis*(triphenylphosphine)palladium (0) was prepared at first. PdCl₂ (1.69 g, 9.53 mmol) and triphenylphosphine (12.67 g, 48.33 mmol) were added to 113 mL anhydrous dimethyl sulfoxide. The mixture was heated to about 160 °C under a nitrogen atmosphere until complete solution occurred. The heat was taken away and stirring continued for 5 min. Hydrazine hydrate (1.35 g, 27.0 mmol) was added dropwise in 1 min with rapid stirring. The solution was cooled to rt with water bath and yellow crystals appeared. Solid was collected by filtration and washed with 2 × 10 mL ethanol and 2 × 10 mL ether. A light yellow solid (10.1 g) was dried up and kept under nitrogen, whose ¹H and ¹³C NMR spectral data agreed with literature.²⁰⁶

A solution of crude **14** from last step in 100 mL THF was added dropwise to a dry ice acetone cooled solution of *tetrakis*(triphenylphosphine)palladium(0) (4.0 g) in 300 mL dry THF and acetic anhydride (225 g, 2.20 mol) at 0 °C to -5 °C. After addition, resulting mixture was stirred at room temperature overnight. Filtration of the resulting mixture was passed through a pad of silica gel to remove the catalyst. Ethyl ether (2 × 50 mL) was used to wash it. Evaporation of the solvent and fractional distillation afforded

18 (120 g) as a pale yellow oil, whose ^1H and ^{13}C NMR spectral data agreed with literature.¹⁶²

(+)-(1R, 4S)-4-Hydroxy-2-cyclopenten-1-yl acetate ((+)-11). Compound **16** (120 g, 0.761 mol) was added to 0.1 M phosphate buffer (400 mL). The pH of the resulting suspension was adjusted to 7 by addition of 6 N NaOH dropwise. *Pseudomonas cepacia* lipase (12 g, Amano International Enzyme Corporation) was added to the mixture. The mixture was stirred and the pH of the mixture was kept constant around 7 during the hydrolysis by the continuous addition of 1 N NaOH. After the addition of 760 mL of NaOH solution, the reaction mixture was filtered through a celite pad. The filtrate was extracted with 3 × 400 mL ethyl acetate. The combined organic phases were dried over anhydrous MgSO_4 . Evaporation of the solvent under reduced pressure afforded yellow oil. The residual oil was fractionally distilled under reduced pressure to give **(+)-11** (85.2g, 78.8%) as a light yellow solid, whose ^1H and ^{13}C NMR spectral data agreed with literature.²³⁸

Methyl-2,3-O-isopropylidene- β -D-ribofuranoside (22). Concentrated hydrochloric acid (10 mL) was added to a suspension of D-ribose (100 g, 0.666 mol) in acetone (420 mL), methanol (420 mL) and 2,2-dimethoxypropane (200 mL) at rt. The mixture was refluxed for 6 hr and then cooled to room temperature. After neutralized with pyridine, the reaction mixture was concentrated and followed by addition of water (1000 mL) and ether (300 mL). The separated aqueous phase was extracted with ether (2 × 200 mL) and ethyl acetate (2 × 200 mL). Then the organic layer was washed with water, and brine. The combined organic extracts were dried over anhydrous sodium sulfate overnight. The

solvent was evaporated and the residue was distilled to give 100.5 g (73.9%) of **22** as colorless oil. The NMR spectral data agreed with literature.²⁰⁹

Methyl-5-deoxy-5-iodo-2,3-O-isopropylidene-β-D-ribofuranoside (23). Iodine (150.7 g, 0.590 mol) was treated portionwise to a solution of **22** (100.5 g, 0.490 mol), imidazole (50.7 g, 0.738 mol), and triphenylphosphine (156.8 g, 0.598 mol) in toluene (2.0 L) and acetonitrile (502 mL). After addition the reaction mixture was refluxed for 30 min, and then cooled to rt. Diluted with ether and repeated washing of the organic extracts sequentially with 10% sodium thiosulfate solution, water, and brine, the solution was dried over anhydrous MgSO₄ and concentrated in vacuo to give a residue which was filtered through a short plug of silica gel by flushing with hexanes/EtOAc = 15:1 to give **96** (132.4 g, 86.1%) as a colorless oil of the mixture of anomers. The NMR spectral data agreed with literature.²⁰⁹

(2R,4R)-2-Dimethyl-5-vinyl-1,3-dioxolane-4-carboxaldehyde (24). Activated zinc powder (Aldrich, dust, <10 micron, 5.50 g, 84.6 mmol) was added in portion to a stirred solution of iodide **96** (5.00 g, 15.9 mmol) in methanol (35 mL) at room temperature. The reaction mixture was heated to reflux for 2 h and then cooled to room temperature, filtered through a short plug of celite, and washed with a 200 mL of a 1:1 mixture of THF/pentane. The filtrate was concentrated by evaporation around 20 °C ~30 °C, under reduced pressure to provide a colorless oil, which was purified by silica gel column chromatography (hexanes/EtOAc = 2:1) to afford the product **97** (2.41 g, 95.7%) as a colorless oil. The NMR spectral data agreed with literature.²¹⁰

(4S,5R)-1-(2,2-Dimethyl-5-vinyl-[1,3]dioxolan-4-yl)-prop-2-en-1-ol (25). To a solution of **24** (45.6 g, 0.290 mol) in anhydrous THF (1000 mL) was added dropwise a

solution of vinylmagnesium bromide (1.0 M in THF, 377 mL, 377 mmol) at -30 °C. The reaction was allowed to warm to 0 °C over 1 h and remained in this temperature for 2 h. the reaction was quenched with saturated NH₄Cl (150 mL). The organic layer was separated, washed with brine, and dried by anhydrous MgSO₄. The solvent was removed by evaporation under reduced pressure and the residue purified by silica gel column chromatography (EtOAc/hexanes = 1:4) to afford **25** as a mixture of two isomers (42.0 g, 78.3%) as a colorless oil. The NMR spectral data agreed with literature.²⁰⁸

(3aR,6aR)-2,2-Dimethyl-3a,6a-dihydrocyclopenta[1,3]-dioxol-4-one ((4R,5R)-4,5-O-isopropylidene-2-cyclopentenone) (12). To a solution of the diene **25** (31.26 g, 168 mmol) in anhydrous CH₂Cl₂ (600 mL), the Grubbs' catalyst benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (1.50 g, 1.81 mmol, flushed with N₂ three times) was added. After stirred at room temperature for 4h, 4 Å molecular sieve (50 g) and pyridinium dichromate (63.5 g, 291 mmol) were added to the resulting dark brown mixture. The reaction was kept at the same temperature for 12 h and filtered over a silica gel pad with EtOAc. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (EtOAc/hexanes = 1:9), giving compound **12** (23.0 g, 90.2%) as a white crystal. The NMR spectral data agreed with literature.²³⁹

(3aR,6R,6aR)-2,2-Dimethyl-6-vinyltetrahydrocyclopenta[1,3]dioxol-4-one (26). To a suspension of CuBr·Me₂S (0.12 g, 0.58 mmol) in THF (40 mL) at -78 °C was added vinylmagnesium bromide (8.14 mL, 8.14 mmol) in dropwise and the mixture was stirred for 10 min before a solution of **12** (1.00 g, 6.58 mmol), TMSCl (1.70 mL, 13.3 mmol), and HMPA (3.00 mL, 17.1 mmol) in THF (10 mL) was added in dropwise. After stirring at -78 °C for 3 h, the reaction mixture was warmed to 0 °C followed by adding a

saturated NH₄Cl (10 mL) and the resulting mixture stirred for 30 min. EtOAc (100 mL) was then added afterwards. The organic layer was separated and washed with brine (15 mL), then dried (MgSO₄). The solvent was removed under reduced pressure and the residue purified by silica gel column chromatography (EtOAc/hexanes = 1:4) to give **26** as a colorless liquid (1.10 g, 91.8%). The NMR spectral data agreed with literature.²⁴⁰

(3a*S*,4*S*,6*R*,6a*R*)-2,2-Dimethyl-6-vinyltetrahydrocyclopenta[1,3]dioxol-4-ol (27).

To a suspension of LiAlH₄ (0.6 g, 15.36 mmol) in dry THF (30 mL) at 0 °C was added dropwise a solution of **26** (1.62 g, 8.88 mmol) in THF (10 mL). The reaction mixture was then stirred at room temperature for 3 h before it was quenched sequentially with H₂O (0.6 mL), aqueous NaOH (15%, 0.6 mL), and H₂O (1.8 mL). The resulting solid was removed by filtration and the filtrate evaporated in vacuo to afford sufficiently pure **27** as a colorless liquid (1.58 g, 95.7%) The NMR spectral data agreed with literature.²⁰⁸

6-Chloro-9-[(3a*S*,4*R*,6*R*,6a*R*)-2,2-dimethyl-6-vinyltetrahydro-cyclopenta[1,3]dioxol-4-yl]-purine (28). To a solution of 6-chloropurine (1.69 g, 10.83 mmol), Ph₃P (4.03 g, 15.4 mmol), and **27** (1.41 g, 7.66 mmol) in dry THF (50 mL) at 0 °C, DIAD (3.18 mL, 15.4 mmol) was added dropwise. The mixture was then stirred at the same temperature for 30 min and warmed to room temperature. After stirring at room temperature for 12 h, the reaction was heated to 50 °C and stirred for another 8 h. The solvent was removed under the reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexanes = 1:4) to afford **28** contaminated with the azadicarboxylate byproduct.

6-Amine-9-[(3a*S*,4*R*,6*R*,6a*R*)-2,2-dimethyl-6-vinyltetrahydro-cyclopenta[1,3]dioxol-4-yl]-purin (29). A solution of crude **28** (2.30 g, 7.17mmol) in MeOH (100 mL)

was cooled to 0 °C for 30 min before saturated with NH₃ at same temperature for 1 hr. The solution was heated to 100 °C for 24 hr in a Parr stainless steel, sealed reaction vessel. The solvent was removed under reduced pressure, and residue was purified by column chromatography (EtOAc/Methanol = 10:1) to give **29** as a white solid (1.68 g, 73.1% in two steps). ¹H NMR (250 MHz, CDCl₃) δ 8.33 (s, 1H), 7.84 (s, 1H), 6.10 (s, 2H), 5.95 (ddd, *J* = 17.1, 10.2, 6.8 Hz, 1H), 5.23-5.5.07 (m, 3H), 4.77 (m, 1H), 4.65 (t, *J* = 6.8Hz, 1H), 2.79 (m, 1H), 2.60-2.44 (m, 2H), 1.57 (s, 3H), 1.31 (s, 3H); ¹³C NMR (62.9 MHz, CDCl₃) δ 155.9, 153.0, 150.1, 140.0, 137.8, 120.6, 116.3, 114.3, 84.0, 83.6, 61.8, 48.3, 36.8, 27.6, 25.3.

Compound 31. Triethylamine (99.5%, 3.00 mL, 21.5 mmol) was added to a solution of **29** (1.86 g, 7.17 mmol) in THF (100 mL) at room temperature, followed by addition of tert-butyl carbamate (6.25 g, 28.7 mmol) and DMAP (0.122 g, 1.00 mmol). The reaction mixture was stirred for 8 hr at same temperature under N₂ atmosphere. The solvent was removed under reduced pressure. The crude product was purified by chromatography (EtOAc/Hexane = 1:4) on silica gel to give **31** (3.13 g, 87.1%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 8.86 (s, 1H), 8.10 (s, 1H), 5.96 (ddd, *J* = 17.2, 10.3, 6.9 Hz, 1H), 5.24-5.14 (m, 2H), 5.11 (dd, *J* = 7.4, 5.1, 1H), 4.85 (ddd, *J* = 11.5, 6.9, 5.1 Hz, 1H), 4.67 (t, *J* = 6.8 Hz, 1H), 2.84 (m, 1H), 2.61-2.48 (m, 2H), 1.59 (s, 3H), 1.48 (s, 18H), 1.32 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 151.9, 150.7, 144.2, 137.4, 116.3, 114.2, 83.9, 83.4, 61.9, 60.4, 48.1, 36.4, 27.9, 27.5, 25.1, 21.1, 14.2.

Compound 32. To a solution of **31** (0.95 g, 1.90 mmol) in THF (200 mL) at 0 °C under N₂ atmosphere was added 9-BBN-H (0.5 M in THF, 7.60 mL, 3.80 mmol). The resultant mixture was brought up to room temperature and stirred at same temperature for

3 hr. NaOH aqueous solution (1M, 3.60 mL) was added, followed by addition of H₂O₂ solution (50% in H₂O, 2.4 mL) and the stirring continued an addition 30 min. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and washed with saturated NaHCO₃ solution (3 x 10 mL). The organic layer was dried (MgSO₄) and concentrated later in vacuo to give the crude product which was purified by chromatography (EtOAc/Hexane = 1:5) to afford **32** as white foam combined with 9-BBN hydrolyzed byproduct.

(1R,2S,3R)-3-(6-amino-purin-9-yl)-5-(2-hydroxyethyl)cyclopentane-1,2-diol (1).

Crude compound **32** was dissolved in a mixture of 1N HCl (18 mL) and MeOH (18 mL). The reaction mixture was stirred at room temperature for 3 hr. The solution was then neutralized with weekly basic exchange resin (Amberlite IRA-67). After filtration and evaporation of the solvent, the crude product was purified by chromatography (EtOAc/MeOH/NH₃·H₂O = 8:2:1) to give **1** (0.359, 67.6%) as white solid.

¹H NMR (400 MHz, MeOD) δ 8.19 (s, 1H), 8.18 (s, 1H), 4.76 (td, *J* = 9.4, 7.6 Hz, 1H), 4.50 (dd, *J* = 7.8, 6.4 Hz, 1H), 3.93 (t, *J* = 6.0 Hz, 1H), 3.69-3.63 (m, 2H), 2.42 (m, 1H), 2.14 (m, 1H), 1.92-1.82 (m, 2H), 1.71 (m, 1H); ¹³C NMR (100 MHz, MeOD) δ 157.4, 153.5, 151.1, 142.2, 120.8, 76.8, 76.2, 62.6, 61.6, 41.9, 38.3, 33.7.

6-Hydroxy-8-methylpurine (33). 4,5-Diamino-6-hydroxypyrimidine hemisulfate salt (5.00 g, 14.3mmol) was refluxed in acetoamide (60.0 g, 1.02 mol) at 230 °C. After a clear yellow solution resulted, the reaction mixture was continued to reflex for 4hr. The excess acetoamide was distilled under reduced pressure. The residue was diluted with ice water (10 mL) and filtrated. The filtrate was washed with another ice water (10 mL). After second time filtration, the residue was dried in the oven to give a grey solid **33**.

8-Methyl-6-chloropurine (34).²¹⁵ To a solution of phosphorus oxychloride (30 mL), containing N, N-dimethylaniline (3 mL) was added crude compound 33 portion wise and slowly. The mixture is heated to reflux for 7 hr. The excess phosphorus oxychloride was evaporated under reduced pressure. The residue was adjusted to strongly basic (PH > 10) by adding NH₃·H₂O (30%, 100mL). After extracted by EtOAc (2 x 100 mL), the mixture was continued to extract by Et₂O. The water phase was evaporated and adjusted the PH value around 7 by diluted with water. The aqueous solution was extracted with EtOAc (5 x 100 mL) and the combined organic layer was dried (Na₂SO₄). After filtration and evaporation of the solvent, 34 was obtained as yellow solide 1.30, 54.0%). ¹H NMR (400 MHz, MeOD) δ 8.61 (s, 1H), 2.66 (s, 3H).

6-Chloro-9-[(3a*S*,4*R*,6*R*,6a*R*)-2,2-dimethyl-6-vinyltetrahydro-cyclopenta[1,3]dioxol-4-yl]-8-methyl-purine (35). To a solution of **34** (0.66 g, 3.9 mmol), Ph₃P (1.60 g, 6.10 mmol), and **27** (0.60g, 3.2 mmol) in dry THF (50 mL) at 0 °C, DIAD (1.33 mL, 6.40 mmol) was added dropwise. The mixture was then stirred at the same temperature for 30 min and warmed to room temperature. After stirred at room temperature for overnight, the reaction was heated to 50 °C and stirred for another 8 h. The solvent was evaporated under the reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexanes = 1:2) to afford **35** (0.73 g) contaminated with the azadicarboxylate byproduct. ¹H NMR (400 MHz, CDCl₃) δ 8.62 (s, 1H), 5.95 (ddd, *J* = 17.2, 10.3, 6.8 Hz, 1H), 5.20-5.10 (m, 3H), 4.70-4.6 (m, 2H), 2.90-2.74 (m, 2H), 2.71 (s, 3H), 2.26 (dt, *J* = 6.1, 12.2 Hz, 1H), 1.54 (s, 3H), 1.26 (s, 3H).

2-[(3a*R*,4*R*,6*R*,6a*S*)-6-(6-chloro-8-methyl-9H-purin-9-yl)-2,2-dimethyltetrahydro-3-cyclopenta[1,3]dioxol-4-yl]ethanol (36). To a solution of **35** (0.54 g, 1.61 mmol) in

THF (200 mL) at 0 °C under N₂ atmosphere was added 9-BBN-H (0.5 M in THF, 6.44 mL, 3.22 mmol). The resultant mixture was brought up to room temperature and stirred at same temperature for 12 hr. a NaOH aqueous solution (1M, 3.0 mL) was added, followed by H₂O₂ solution (50% in H₂O, 3.22 mL) and the stirring continued an addition 30 min. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and washed with saturated NaHCO₃ solution (3 x 10 mL). The organic layer was dried (MgSO₄) and concentrated later in vacuo to give the crude product which was purified by chromatography (EtOAc/Hexanes = 1:2) to afford **36** (0.48 g, 84.7%) as white foam. ¹H NMR (400 MHz, MeOD) δ 8.63 (s, 1H), 5.21 (dd, *J* = 7.4, 5.5 Hz, 1H), 4.82 (m, 1H), 4.56 (m, 1H), 3.66 (t, *J* = 6.8 Hz, 2H), 2.73 (s, 3H), 2.68 (q, *J* = 12.0 Hz, 1H), 2.38 (m, 1H), 2.29 (m, 1H), 1.88 (dt, *J* = 13.6, 6.8 Hz, 1H), 1.74 (dt, *J* = 13.6, 6.8 Hz, 1H), 1.54 (s, 3H), 1.28 (s, 3H); ¹³C NMR (100 MHz, MeOD) δ 158.3, 154.3, 152.0, 149.6, 132.0, 115.2, 86.3, 84.4, 63.6, 61.5, 37.6, 36.5, 28.0, 25.6, 14.8.

2-[(3a*R*,4*R*,6*R*,6a*S*)-6-(6-amino-8-methyl-9*H*-purin-9-yl)-2,2-dimethyltetrahydro-3-cyclopenta[1,3]dioxol-4-yl]ethanol (37). A solution of **36** (0.48 g, 1.4 mmol) in MeOH (100 mL) was cooled to 0 °C for 30 min before saturated with NH₃ at same temperature for 1 hr. The solution was heated to 100 °C for 24 hr in a Parr stainless steel, sealed reaction vessel. The solvent was removed under reduced pressure, and residue was purified by column chromatography (EtOAc/Methanol = 7:1) to give **37** (0.43 g, 94%) as a white solid. ¹H NMR (400 MHz, MeOD) δ 8.10 (s, 1H), 5.22 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.74 (m, 1H), 4.58 (m, 1H), 3.68 (t, *J* = 6.8 Hz, 2H), 2.91 (m, 1H), 2.64 (s, 3H), 2.38-2.23 (m, 2H), 1.91 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.75 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.55 (s, 3H), 1.30 (s, 3H); ¹³C NMR (100 MHz, MeOD) δ 156.4, 152.8, 151.8, 151.7, 119.4, 115.1, 86.3,

84.4, 63.1, 61.6, 42.5, 37.6, 36.8, 28.0, 25.6, 14.5. Anal. Calcd for $C_{16}H_{23}N_5O_3 \cdot 0.2H_2O$: C, 57.03; H, 7.00; N, 20.78. Found: C, 57.28; H, 7.15; N, 20.48.

(1*R*,2*S*,3*R*,5*R*)-3-(6-amino-8-methyl-9*H*-purin-9-yl)-5-(2-hydroxyethyl) cyclopentane-1,2-diol (2). Compound **37** (0.43 g, 1.3 mmol) was dissolved in a mixture of 1N HCl (20 mL) and MeOH (20 mL). The reaction mixture was stirred at room temperature for 3 hr. After distilled the solvent, the residue was redissolved in distilled H₂O (20 mL) and MeOH (20 mL). The solution was then neutralized with weakly basic exchange resin (Amberlite IRA-67). After filtration and evaporation of the solvent, the crude product was purified by chromatography (EtOAc/ MeOH/NH₃·H₂O = 8:2:1) to give **2** (0.30 g, 80%) as white solid. ¹H NMR (400 MHz, MeOD) δ 8.00 (s, 1H), 4.78 (dd, *J* = 7.2, 6.0 Hz, 1H), 4.58 (m, 1H), 3.96 (dd, *J* = 5.6, 5.2 Hz, 1H), 3.65-3.61 (m, 2H), 2.55 (s, 3H), 2.20-2.07 (m, 3H), 1.89 (m, 1H), 1.72 (m, 1H); ¹³C NMR (100 MHz, MeOD) δ 156.1, 152.5, 152.3, 151.9, 119.3, 76.9, 74.9, 63.1, 61.6, 41.9, 38.1, 31.8, 14.5. Anal. Calcd for $C_{12}H_{16}N_4O_3 \cdot 0.3H_2O$: C, 52.27; H, 6.61; N, 23.44. Found: C, 52.36; H, 6.41; N, 23.48.

4-Hydroxy-3-nitropyridine (39). To mechanically stirred concentrated HNO₃ (122 g) in 0 °C was added fuming H₂SO₄ (140 g, *d* = 1.94) slowly. 4-Hydroxypyridine (30 g, 95%, 0.30 mol) was then added portion-wise in 20 min afterwards. The temperature was warmed up to around 30 °C during the addition. The reaction mixture was brought to reflux for 1hr. The reaction was cooled to room temperature with water bath and then pouring the solution over ice slowly with continuously stirring. Treatment of the resulting suspension with saturated NH₄OH and then aqueous Na₂CO₃ (30%) adjusted the pH to 7. Precipitate was collected by filtration and washed with H₂O (60 mL × 3) to afford

compound **39** (30.2 g, 72.0%) as a pale yellow solid after drying in oven at 100 °C under vacuum. The NMR spectral data agreed with literature.^{241,242}

4-Ethoxy-3-nitropyridine (40). Compound **39** (30.2 g, 22.6 mmol) and PCl₅ (56.6 g, 95%, 2.58 mmol) were added to ClCH₂CH₂Cl (200 mL) sequentially. The resulting suspension was heated to reflux for about 3.5 h until slurry turned into clear solution. Temperature was lowered to 15 °C by ice-water bath. Absolute ethanol (130 mL) was added to the reaction dropwise and the temperature was controlled below 50 °C. After the addition, the mixture was brought to reflux for 1hr. After removal of heating, the reaction mixture was cooled to 10 °C and stood for 1 hr to form precipitate. The solid was collected by filtration and washed with ethanol (2 × 150 mL). Compound **40** (43.6 g, 99.0%) was obtained as white solid after drying in oven at 35 °C under vacuum: mp 46-48 °C (lit.²⁴³ mp 46.5-48 °C)

4-Amino-3-nitropyridine (41). To 100 mL of water was added Compound **40** (43.6 g, 31.2 mmol) and ammonium acetate (65.6 g, 85.0 mmol). The resulting slurry was heated to reflux for 6 hours. After TLC showing the disappearance of the starting material **40**, the heating was removed and the reaction mixture was cooled to room temperature. After adjusted the pH to 8 by approximately 35 mL of concentrated aq ammonium hydroxide , the slurry was chilled by an ice-water bath for 1 h to form precipitate. The solid was collected by filtration and washed with cold water (30 mL × 2). Compound **41** (23.8 g, 81.2%) was afforded as a yellow solid after drying in oven at 100 °C under vacuum. mp 198-200 °C (lit.²⁴⁴ mp 200 °C)

2-Chloro-3,4-diaminepyridine (42). SnCl₂ (58.0 g, 30.5 mmol) was added to 240 mL HCl and the resulting suspension was heated to 60-70 °C. The reaction mixture

became clear solution. Compound **41** (23.8 g, 17.2 mmol) was then added portionwise slowly at this temperature. After the addition, the reaction mixture was brought to reflux and allowed to react for another 5 h. After TLC showed the disappearance of the starting material **41**, the heating was removed and the reaction was cooled to rt with an ice-water bath. The cooled mixture was poured over 250 g of crushed ice. 10 M NaOH and then saturated ammonium hydroxide solution were added to adjust the acidic solution to pH 8. The neutralized solution was extracted with ethyl acetate (160 mL × 4). The combined organic layers were dried over anhydrous Na₂SO₄. Evaporation of the solvent afforded **42** (17.6 g, 71.2%) as a yellow solid. ¹H NMR (250 MHz, DMSO-d₆) δ 7.29 (d, *J* = 5.3 Hz, 1H), 6.43 (d, *J* = 5.3 Hz, 1H), 5.78 (br, 2H), 4.67 (br, 1H); ¹³C NMR (62.9 MHz, DMSO-d₆) δ 142.9, 137.6, 135.1, 126.2, 108.3.

(1E,1'E)-Dimethyl N',N''-2-chloropyridine-3,4-diylldiacetimidate (44). **42** (10.0 g, 69.6 mmol) was added to trimethyl orthoacetate (161 mL) and the mixture was heated to 80 °C. After the solution was mostly clear, formic acid (4.2 mL) was added dropwise and the solution was continued to heat to 100 °C for 8 hr. Removal the heat before the solvent was evaporated and the crude product was purified by chromatography (EtOAc/Hexane = 1:1) to give **44** as white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.87 (d, *J* = 5.2 Hz, 1H), 6.56 (d, *J* = 5.2 Hz, 1H), 3.71 (s, 3H), 3.64 (s, 3H), 1.74 (s, 3H), 1.70 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 164.0, 162.2, 148.9, 143.7, 133.8, 116.4, 53.8, 53.7, 17.3, 17.0.

4-Chloro-2-methyl-1H-imidazo[4,5-c]pyridine (43). **44**, obtained from the last step was dissolved in aqueous NaOH solution (1.5 N, 110 mL) and the reaction mixture was heated to reflux for 10 min. After cooled down to the room temperature the solution was extracted by EtOAc (4 × 100 mL). The combined organic phase was dried by Na₂SO₄.

The solvent was evaporated afterwards and the crude product was purified by chromatography (EtOAc/MeOH = 8:1) to give **43** (11.2 g, 96.0%) as white solid. ¹H NMR (400 MHz, MeOD) δ 8.07 (d, *J* = 5.6 Hz, 1H), 7.49 (d, *J* = 5.6 Hz, 1H), 2.64 (s, 3H).

2-Methyl-1H-imidazo[4,5-c]pyridin-4-amine (45). **44** (2.42 g, 14.4 mmol) was dissolved to a mixture of anhydrous hydrazine (99%, 18.8 mL) and propan-1-ol (14.0 mL). The solution was brought to reflux overnight. After the reaction mixture was cooled to rt the residual hydrazine and propan-1-ol was evaporated under reduced pressure. Water (38 mL) was added to dissolve the residues, followed by addition of Raney nickel (1.91 g) portionwisely. The mixture was heated to reflux for 1 and the reaction mixture was filtered through a Celite pad. The filtrate was evaporated under reduced pressure to afford **45** (1.96 g, 91.6%). ¹H NMR (250 MHz, MeOD) δ 8.00 (d, *J* = 5.8 Hz, 1H), 7.42 (d, *J* = 5.8 Hz, 1H), 2.57 (s, 3H).

Tert-butyl-4-(bis(tert-butoxycarbonyl)amino)-2-methyl-1H-imidazo[4,5-c]pyridine-1-carboxylate (46). Compound **45** (1.7 g, 11.3 mmol) and 4-(dimethylamino)pyridine DMAP (0.134 g, 1.1 mmol) were dissolved in dry THF(50 mL) at room temperature, followed by addition of Boc₂O (10 mL, 44 mmol) in the same temperature. The resulting suspension was stirred for 2 days under a nitrogen atmosphere. TLC analysis (EtOAc) showed **46** was obtained (*R_f* = 0.9) with appearance of a little **46b** (*R_f* = 0.6). The ratio was about 9:1 (detected by ¹H NMR). After evaporation of the solvent, the mixture was separated by chromatography (EtOAc/Hexane = 2:1) to give **46** (4.22 g, 83.0%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 8.37 (d, *J* = 5.6 Hz, 1H),

7.75 (d, $J = 5.6$ Hz, 1H), 2.84 (s, 3H), 1.73 (s, 9H), 1.42 (s, 18H); ^{13}C NMR (100 MHz, CDCl_3) δ 154.6, 151.4, 146.4, 142.9, 140.4, 110.2, 86.8, 83.1, 77.4, 28.3, 28.1, 18.6, 0.21. Byproduct **46b**: ^1H NMR (250 MHz, CDCl_3) δ 8.29 (d, $J = 6.0$ Hz, 1H), 7.43 (d, $J = 6.0$ Hz, 1H), 2.82 (s, 3H), 1.71 (s, 9H), 1.55 (s, 9H).

4-(bis(*tert*-butoxycarbonyl)amino)-2-methyl-1*H*-imidazo[4,5-*c*]pyridine-1-carboxylate (38). Compound **46** (2.84 g, 6.34 mmol) was dissolved under argon in 100 mL of dry THF. A 1 M solution of Bu_4NF (1M in THF, 19.7 mL, 19.7 mmol) in THF was added and the reaction mixture was stirred for 12 h. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography (hexanes/AcOEt = 1/2) on silica gel to afford **38** (1.79 g, 81.1%) as a white foam. ^1H NMR (250 MHz, MeOD) δ 8.16 (d, $J = 5.8$ Hz, 1H), 7.54 (d, $J = 5.8$ Hz, 1H), 2.64 (s, 3H), 1.33 (s, 18H).

(+)-2,3-(Isopropylidenedioxy)-4-cyclopenten-1-ol (47). To a stirred solution of cyclopentenone **12** (1.40 g, 9.08 mmol) and $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (3.40 g, 9.12 mmol) in MeOH (60 mL) at 0 °C was added NaBH_4 (0.68 g, 18.1 mmol) in small portions. After stirring at rt for 1 h the mixture was neutralized with conc. HCl, reduced to 2/3 volume, extracted with brine and ether, and the organic layers combined, dried (MgSO_4), and concentrated to give **47** as a colorless syrup (1.40 g, 99%) which was used directly in the next step. ^1H NMR (250 MHz, CDCl_3) δ 5.89 (s, 2H), 5.02 (m, 1H), 4.74 (m, 1H), 4.57 (m, 1H), 3.14 (br, 1H), 1.43 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (62.9 MHz, CDCl_3) δ 135.9, 131.5, 111.9, 83.1, 76.7, 73.7, 27.2, 26.1.

Compound 48. To a solution of **38** (2.24 g, 6.44 mmol), Ph_3P (1.48 g, 5.66 mmol), and **47** (0.81 g, 5.19 mmol) in dry THF (100 mL) at 0 °C, DIAD (1.14 mL, 5.65 mmol)

was added dropwise. The mixture was then stirred at the same temperature for 30 min and warmed to room temperature for 12 hr. The solvent was removed under the reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexanes = 1:2) to afford **48** (1.94 g, 77%). ¹H NMR (400 MHz, CDCl₃) δ 8.12 (d, *J* = 5.6 Hz, 1H), 7.04 (d, *J* = 5.6 Hz, 1H), 6.29 (dt, *J* = 5.6, 2.0 Hz, 1H), 5.95 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.50-5.48 (m, 2H), 4.64 (dd, *J* = 6.0, 1.2 Hz, 1H), 2.63 (s, 3H), 1.48 (s, 3H), 1.39 (s, 18H), 1.31 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 153.4, 151.4, 143.4, 140.9, 140.1, 136.1, 135.7, 131.0, 112.6, 106.0, 84.4, 84.0, 82.6, 68.2, 27.8, 27.0, 25.1, 14.7.

(1*S*,2*R*,5*R*)-5-(4-amino-2-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopent-3-ene-1,2-diol (3). Compound **48** (1.95 g, 4.00 mmol) was dissolved in a mixture of MeOH (42 mL) and 1N HCl (42 mL). The resulting solution was stirred at rt. for 12 hr and brought to reflux for another 3 hr. Basic resin (Amberlite IR67) was added to neutralize the solution, followed by filtration. The filtrate was removed under vacuum and residue was purified by chromatography (EtOAc/ MeOH/NH₃·H₂O = 8:2:1) to give **3** (0.64 g, 65%) as white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.44 (d, *J* = 6.0 Hz, 1H), 6.61 (d, *J* = 6.0 Hz, 1H), 6.17 (td, *J* = 2.8, 6.4 Hz, 1H), 6.09 (dd, *J* = 1.6, 6.4 Hz, 1H), 5.34 (m, 1H), 4.49 (ddd, *J* = 1.2, 3.0, 6.0 Hz, 1H), 4.21 (dd, *J* = 5.6, 6.4 Hz, 1H), 2.52 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 153.2, 152.5, 140.5, 140.3, 136.4, 135.2, 126.8, 99.9, 78.1, 74.0, 67.5, 14.3; Anal. Calcd for C₁₂H₁₄N₄O₂: C, 58.53; H, 5.73; N, 22.75. Found: C, 58.37; H, 5.83; N, 22.52.

6-Hydroxymethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (59).²²⁶ To a solution of d-ribose (25.0 g, 0.17 mol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (TsOH·H₂O, 0.80 g, 0.5 mmol) in 500 mL of acetone was added 2,2

dimethoxypropane (19.1 g, 0.18 mol) at 0 °C. The suspension was stirred for 1 hr at rt. until a clear solution was achieved. The solution was then treated with NaHCO₃ (0.05 g, 0.60 mmol) and was stirred for an additional 30 min at rt. The solid was filtered and the filtrate purified by silica gel column chromatography (hexane/EtOAc = 3:1) to give compound **59** as a mixture of β- and α-isomers (28.4 g, 88%). The NMR spectral data agreed with literature.²²⁶

2,2-Dimethyl-6-(trityloxymethyl)tetrahydrofuro[3,4-*d*][1,3]-dioxol-4-ol (60).²²⁶ To a solution of compound **5** (28.4 g, 0.15 mol) in 200 mL of anhydrous DMF (372 mL), a catalytic amount of DMAP (0.56 g, 4.48 mmol), trityl chloride (50.1 g, 0.180 mol) and Et₃N (22.7 g, 0.223 mol) were added at rt. under nitrogen atmosphere. The resulting solution was stirred for 48 h at room temperature and poured into ice water (200 mL). The organic layer was extracted with CH₂Cl₂ (300 mL × 3), washed with saturated aqueous NH₄Cl (200 mL × 2) and water (200 mL), and then dried over Na₂SO₄ anhydrous. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1 to 2:1) to give compound **60** (55.1 g, 84.9%) as a mixture of β- and α-isomers. The NMR spectral data agreed with literature.²²⁶

1-[5-(1-Hydroxy-2-trityloxyethyl)-2,2-dimethyl-[1,3]dioxolan-4-yl]prop-2-en-1-ol (61).²²⁶ To a solution of compound **60** (47.5 g, 0.110 mol) in 400 mL of anhydrous THF was added 330 mL of vinylmagnesium bromide (0.330 mol, 1.0 M in THF) dropwise at -78 °C under nitrogen atmosphere. After stirred at same temperature for 1 hr, the reaction mixture was brought up to rt. and was stirred for an additional 6 hr. NH₄Cl aqueous solution (200 mL) was added slowly to quench the reaction at 0 °C, and the resulting

solution was poured into ice (200 mL). The organic layer was separated and the aqueous layer was washed with ether (200 mL × 2). The combined organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc = 10:1 to 3:1) to give compound **61** (49.6 g, 98%). The NMR spectral data agreed with literature.²²⁶

1-{5-[1-((*tert*-Butyldimethylsilyl)oxy)allyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(trityloxy)ethanol (62).²²⁶ To a solution of compound **61** (37.9 g, 82.3 mmol) in anhydrous CH₂Cl₂ (400 mL) and DMF (50 mL) were added imidazole (16.8 g, 24.8 mmol) and TBDMSCl (15.5 g, 0.103 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 8 h at room temperature and then poured into 600 mL of ether-water solution (1:1). The organic layer was separated and the aqueous layer was washed with ether (150 mL × 2). The combined organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 30:1) to give compound **62** (37.4 g, 79%). The NMR spectral data agreed with literature.²²⁶

1-{5-[1-((*tert*-Butyldimethylsilyl)oxy)allyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(trityloxy)ethanone (63).²²⁶ To a solution of oxalyl chloride (4.67 g, 53.3 mmol) in 100 mL of anhydrous CH₂Cl₂ was added DMSO (7.57 g, 106 mmol) at -60 °C under nitrogen atmosphere, and then the resulting solution was stirred for 10 min. A solution of compound **62** (24.7 g, 42.7 mmol) in 250 mL of anhydrous CH₂Cl₂ was added to the reaction mixture dropwise over 20 min at -60 °C. After 30 min, Et₃N (21.6 g, 0.213 mol) was added dropwise over 20 min to the reaction mixture at -60 °C. The mixture was stirred for 1 h at -60 °C and then stirred for 30 min at room temperature. The reaction

mixture was treated with water (200 mL) dropwise at 0 °C. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (200 mL × 3). The combined organic layer was washed with brine (200 mL × 2), dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 20:1 to 10:1) to give compound **63** (28.7 g, 94%). The NMR spectral data agreed with literature.²²⁶

***tert*-Butyl-{1-[2,2-dimethyl-5-(1-(trityloxymethyl)vinyl)-[1,3]-dioxolan-4-yl]allyloxy}dimethylsilane (64).**²²⁶ To a suspension of Ph₃PCH₃Br (12.8 g, 73.7 mmol) in 50 mL of THF was added 45 mL of *n*-BuLi (70.0 mmol, 1.6 M in hexane) at 0 °C under N₂ atmosphere. After 30 min, a solution of compound **63** (8.50 g, 14.7 mmol) in 100 mL of THF was added to the reaction mixture at 0 °C. The resulting mixture was stirred for 12 h at room temperature, treated with 25 mL of MeOH and 50 mL of water, and then poured into 200 mL of ether-water solution (2:1). The organic layer was separated and the aqueous layer was extracted with ether (100 mL × 2). The combined organic layer was washed with brine (10 mL × 2), dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 50:1 to 10:1) to give compound **64** (7.64 g, 91.0%). The NMR spectral data agreed with literature.²²⁶

1-[2,2-Dimethyl-5-(1-(trityloxymethyl)vinyl)-[1,3]dioxolan-4-yl]prop-2-en-1-ol (65).²²⁶ A solution of compound **64** (7.68 g, 13.4 mmol) in 50 mL of THF was treated with 35 mL of TBAF (16.8 mmol, 1.0 M in THF) at room temperature. After being stirred for 2 h, the reaction mixture was adsorbed on silica gel and purified on a silica gel

column (hexane/EtOAc = 30:1) to give compound **65** (5.81 g, 95%). The NMR spectral data agreed with literature.²²⁶

2,2-Dimethyl-6-trityloxymethyl-4,6a-dihydro-3aH-cyclopenta-[1,3]dioxol-4-ol (49a).²²⁶ To a solution of compound **65** (5.81 g, 12.7 mmol) in 200 mL of anhydrous CH₂Cl₂ was added second-generation Grubbs catalyst (0.23 g, 0.25 mmol) at room temperature under argon atmosphere. After being stirred for 24 h, the reaction mixture was adsorbed on silica gel and purified on a silica gel column (hexane/EtOAc = 10:1 to 5:1) to give compound **49a** (5.06 g, 93%). The NMR spectral data agreed with literature.²²⁶

Compound 66. To a solution of **49a** (0.80 g, 1.86 mmol), Ph₃P (0.53 g, 2.02 mmol), and **38** (0.80 g, 2.30 mmol) in dry THF (40 mL) at 0 °C was added dropwise a solution of DIAD (0.41 mL, 2.02 mmol) in THF (5 mL). The mixture was then stirred at the same temperature for 30 min and warmed to room temperature for 12 hr. The solvent was removed under the reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexanes = 1:4) to afford **66** (0.96 g, 68%). ¹H NMR (250 MHz, CDCl₃) δ 8.19 (d, *J* = 5.7 Hz, 1H), 7.48 (d, *J* = 7.0, 6H), 7.31-7.15 (m, 10H), 6.08 (s, 1H), 5.48 (s, 1H), 5.31 (d, *J* = 6.0 Hz, 1H), 4.69 (d, *J* = 6.0 Hz, 1H), 3.97 (s, 2H), 2.69 (s, 3H), 1.45 (s, 18H), 1.30 (s, 3H), 1.24 (s, 3H); ¹³C NMR (62.9 MHz, CDCl₃) δ 170.6, 151.3, 147.8, 143.4, 140.8, 140.0, 135.7, 128.2, 127.7, 127.1, 123.6, 112.6, 106.2, 87.1, 84.5, 83.8, 82.4, 61.1, 61.0, 60.0, 27.7, 20.7, 14.6. Anal. Calcd for C₄₅H₅₀N₄O₇ · 0.6H₂O: C, 70.49; H, 6.84; N, 7.15. Found: C, 70.47; H, 6.75; N, 7.20.

(1S,2R,5R)-5-(4-amino-2-methyl-1H-imidazo[4,5-c]pyridin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (4). Compound **66** (1.31 g, 2.34 mmol) was

dissolved in a mixture of MeOH (23.4 mL) and 1 N HCl (23.4 mL) and the resulting solution was stirred at rt. for 5 hr. After addition of basic resin (Amberlite IR67) for neutralization of the solution and filtration, the solvent was removed under vacuum and the residue was purified by column chromatography to give **4** (0.50 g, 78%) as white solid. ¹H NMR (400 MHz, MeOD) δ 7.57 (d, *J* = 6.0, 1H), 6.80 (d, *J* = 6.0, 1H), 5.98 (dd, *J* = 3.3, 1.5 Hz, 1H), 5.44 (d, *J* = 5.4 Hz, 1H), 4.54 (d, *J* = 5.8, 1H), 4.34-4.31 (m, 3H), 2.65 (s, 3H). ¹³C NMR (62.9 MHz, MeOD) δ 153.1, 150.2, 140.6, 140.2, 126.8, 100.2, 78.4, 73.6, 67.5, 60.4, 14.3. Anal. Calcd for C₁₃H₁₆N₄O₃: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.07; H, 5.82; N, 19.94.

Methyl 2-((3a*R*,6*S*,6a*R*)-Tetrahydro-2,2-dimethyl-4-oxo-3a*H*-cyclopenta[*d*][1,3]dioxol-6-yl)-2-(phenylthio)-acetate (73).¹⁶⁷ To a solution of diisopropylamine (4.25 mL, 30.5 mmol) in THF (75 mL) was added dropwise *n*-BuLi (13 mL, 2.5 M in hexanes, 32.5 mmol) at 0 °C. The solution was stirred at the same temperature for 30 min before it was cooled to -70 °C. Methyl phenylthioacetate (5.00 g, 27.5 mmol) was then added dropwise. After the resulting pale-yellow solution was stirred at the same temperature for 1 h, hexamethylphosphoramide (HMPA) (15.0 mL) was added followed by the dropwise addition of **12** (3.85 g, 25.0 mmol) in THF (25.0 mL). This mixture was kept at -70 °C for 1 h, and saturated NH₄Cl solution (50 mL) was added to quench the reaction. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 150 mL). The combined organic phase was dried (anhydrous MgSO₄), and the solvent was removed. The residue was purified by column chromatography to afford **73** as a colorless liquid mixture of two diastereoisomers (6.73g, 80%). The NMR spectral data agreed with literature.¹⁶⁷

Methyl 2-((3a*R*,6a*R*)-4,6a-Dihydro-2,2-dimethyl-4-oxo-3a*H*-cyclopenta[*d*][1,3]dioxol-6-yl)acetate (74).¹⁶⁷ To a solution of **73** (2.24 g, 6.72 mmol) in CH₂Cl₂ (70 mL) at -78 °C was added *m*-CPBA (1.75 g, 77%, 7.77 mmol). The mixture was gradually warmed to -40 °C and stirred at this temperature for 2.5 h. A solution of NaHSO₃ (0.52 g in 21 mL of H₂O) was added to quench the reaction. The organic layer was separated, washed with saturated Na₂CO₃ (2 × 30 mL), and dried (anhydrous Na₂SO₄). The crude product was purified by chromatography (EtOAc/Hexane = 1:2) to give the intermediate as white foam.

This white foam was dissolved in toluene (84 mL), and calcium carbonate (0.70 g, 7 mmol) was added. The mixture was brought to reflux for 12 h. The solvent was removed and the residue was purified by column chromatography (hexane/EtOAc = 4:1 to 2:1) to afford **74** as colorless liquid (1.26 g, 83%). ¹H NMR (400 MHz, CDCl₃) δ 6.14 (s, 1H), 5.26 (d, *J* = 5.6 Hz, 1H), 4.50 (d, *J* = 5.6 Hz, 1H), 3.75 (s, 3H), 3.67-3.53 (m, 2H), 1.40 (s, 1H), 1.36 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 201.7, 168.9, 168.5, 131.7, 115.6, 80.0, 77.7, 52.5, 35.6, 27.5, 26.4.

Methyl 2-((3a*S*,4*S*,6a*R*)-4,6a-Dihydro-4-hydroxy-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]dioxol-6-yl)acetate (75). To a stirred solution of **74** (0.23 g, 1.0 mmol) and CeCl₃·7H₂O (0.40 g, 1.0 mmol) in MeOH (10 mL) at 0 °C was added in small portions of NaBH₄ (0.15 g, 3.9 mmol). The mixture was stirred at the same temperature for 1.5 h before the reaction was quenched with H₂O (10 mL). CH₂Cl₂ (30 mL) was added to the mixture, and the organic phase was separated. The aqueous phase was extracted with CH₂Cl₂ (2 × 15 mL). The combined organic phases were washed with brine and dried (anhydrous Na₂SO₄). Evaporation of the solvent afforded **75** as a clean product (0.20 g,

86%). ¹H NMR (400 MHz, CDCl₃) δ 5.65 (s, 1H), 4.97 (d, *J* = 5.5 Hz, 1H), 4.72 (t, *J* = 5.5 Hz, 1H), 4.53 (br, 1H), 3.65 (s, 3H), 3.19 (s, 2H), 2.77 (d, *J* = 10 Hz), 1.36 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 138.3, 133.3, 112.6, 84.3, 78.0, 73.5, 52.1, 33.4, 27.7, 26.8.

(2Z)-Methyl 2-((3a*S*,6a*R*)-2,2-Dimethyl-3a*H*-cyclopenta-*d*][1,3]dioxol-6(6a*H*)-ylidene)acetate (76). To a suspension of **75** (0.14 g, 0.70 mmol), Ph₃P (0.27 g, 1.05 mmol), and **38** (0.36 g, 1.05 mmol) in THF (5 mL) at 0 °C was added a solution of DIAD (0.21 g, 1.05 mmol). The mixture was warmed to room temperature and then stirred for 2 h. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hexane = 1:4) to afford **76** as a colorless liquid. ¹H NMR (400 MHz, CDCl₃) δ 7.35 (d, *J* = 5.6 Hz, 1H), 6.5 (m, 1H), 5.95 (m, 1H), 5.14 (dd, *J* = 5.6, 2.0 Hz, 1H), 5.00 (d, *J* = 5.6 Hz, 1H), 3.13 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.7, 158.2, 143.6, 132.9, 114.0, 112.8, 81.9, 79.8, 51.4, 27.5, 26.3.

(3a*S*,4*S*,6a*R*)-4,6a-Dihydro-6-(2-hydroxyethyl)-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (77).¹⁶⁷ To a solution of **74** (1.00 g, 4.40 mmol) in anhydrous CH₂Cl₂ (43 mL) at -50 °C was added diisobutylaluminum hydride (DIBAL) (17.2 mL, 1 M in CH₂Cl₂, 17.2 mmol) dropwise. The mixture was stirred at the same temperature for 4 h before the reaction was quenched by saturated solution of NH₄Cl (10 mL), and the mixture was warmed to room temperature and then stirred for 1 h. The organic phase was separated, and the aqueous solution was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic phases were dried (anhydrous Na₂SO₄). The solvent was removed under vacuum to cleanly afford **77** as a colorless liquid (0.77 g, 87%). The NMR spectral data agreed with literature.¹⁶⁷

(3a*S*,4*S*,6a*R*)-6-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-4,6a-dihydro-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (72).¹⁶⁷ To an ice-chilled solution of **77** (0.66 g, 3.30 mmol) in CH₂Cl₂ (40 mL) was added imidazole (0.36 g, 5.3 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl) (0.54 g, 3.4 mmol). The mixture was stirred at room temperature for 12 h. The resulting precipitate was removed by filtration, and the filtrate was evaporated. The residue was purified by column chromatography (EtOAc/hexanes = 1:4) to afford **72** as a colorless oil (0.93 g, 90%). The NMR spectral data agreed with literature.¹⁶⁷

Compound 78. To a suspension of **72** (0.97 g, 3.1 mmol), Ph₃P (0.98 g, 3.72 mmol), and **38** (1.29 g, 3.72 mmol) in THF (40 mL) at 0 °C was added a solution of DIAD (0.75 g, 3.72 mmol). The mixture was warmed to room temperature and then stirred for 2 h. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hexane = 1:4) to afford **76** (0.99 g, 48%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 8.11 (d, *J* = 5.6 Hz, 1H), 7.13 (d, *J* = 6.0 Hz, 1H), 5.62 (s, 1H), 5.34 (s, 1H), 5.29 (d, *J* = 6.0 Hz, 1H), 4.56 (d, *J* = 6.0 Hz, 1H), 3.97-3.84 (m, 2H), 2.68 (s, 3H), 2.51 (t, *J* = 5.6 Hz, 2H), 1.44 (s, 3H), 1.38 (s, 18H), 1.30 (s, 3H), 0.85 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 153.5, 151.5, 148.4, 143.5, 141.2, 140.2, 135.8, 124.1, 112.7, 106.3, 85.8, 84.5, 82.7, 67.7, 60.9, 32.3, 28.0, 27.3, 26.0, 25.9, 15.0, -5.2.

(1*S*,2*R*,5*R*)-5-(4-amino-2-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-3-(2-hydroxyethyl)cyclopent-3-ene-1,2-diol (5). Compound **78** (0.43 g, 0.65 mmol) was dissolved in a mixture of MeOH (6.5 mL) and 1 N HCl (6.5 mL) and the resulting solution was stirred at rt. for 5 hr. After addition of basic resin (Amberlite IR67) for neutralization of the solution and filtration, the solvent was removed under vacuum and

the residue was purified by column chromatography to give **5** (0.15 g, 80%) as white solid. ^1H NMR (400 MHz, MeOD) δ 7.47 (d, $J = 6.4$ Hz, 1H), 6.78 (d, $J = 6.4$ Hz, 1H), 5.78 (d, $J = 1.6$ Hz, 1H), 5.38 (dd, $J = 6.0, 1.2$ Hz, 1H), 4.46 (d, $J = 5.6$ Hz, 1H), 4.26 (t, $J = 6.0$ Hz, 1H), 3.78 (td, $J = 6.2, 1.6$ Hz, 2H), 2.57 (s, 3H), 2.50-2.47 (m, 2H); ^{13}C NMR (100 MHz, MeOD) δ 153.4, 152.1, 148.0, 140.7, 138.9, 128.5, 126.8, 100.3, 78.4, 75.9, 68.0, 61.0, 34.0, 14.4. Anal. Calcd for $\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3 \cdot 0.57\text{HCl}$: C, 53.74; H, 6.00; N, 17.90. Found: C, 53.83; H, 6.32; N, 17.83.

Isopropyl 4-(bis(tert-butoxycarbonyl)amino)-2-methyl-1H-imidazo[4,5-c]pyridine-1-carboxylate (80a). ^1H NMR (400 MHz, CDCl_3) δ 8.37 (d, $J = 5.6$ Hz, 1H), 7.78 (d, $J = 5.6$ Hz, 1H), 5.35 (sep, $J = 6.4$ Hz, 1H), 2.86 (s, 3H), 1.54 (d, $J = 6.4$ Hz, 6H), 1.42 (s, 18H); ^{13}C NMR (100 MHz, CDCl_3) δ 154.5, 151.3, 149.5, 143.9, 143.0, 140.3, 135.1, 110.2, 83.1, 73.8, 28.1, 22.1, 18.4.

(1R,4S)-(+)-4-tert-Butyldimethylsilyloxy-2-cyclopentenyl acetate (84). To a solution of **11** (9.10 g, 63.5 mmol) in anhydrous CH_2Cl_2 (200 mL) was treated with imidazole (11.2 g, 0.164 mol) and TBDMSCl (12.0 g, 80.0 mmol). The reaction mixture was stirred at rt. for 6 hr, followed by adding ice water (120 mL) and ether (120 mL). The organic layer was separated and washed with brine (3×100 mL), dried with NaSO_4 . The solvent was evaporated under reduced pressure. The residue was purified by chromatography (EtOAc/Hexane = 1:4) to give **84** (13.0 g, 80%) as a colorless liquid. The NMR spectral data agreed with literature.^{245,246}

(1R,4S)-4-(tert-butyldimethylsilyloxy)cyclopent-2-enol (85). To a suspension of K_2CO_3 (6.44 g, 46.0 mmol) in MeOH (250 mL) was added **84** (11.6 g, 45.2 mmol). The resulting mixture was stirred at rt. for 30 min. The solvent was removed under reduced

pressure and the residue was dissolved in ether (100 mL). After washed with brine (100 mL), the combined organic layers were dried with Na₂SO₄. The solvent was evaporated and residue was purified by chromatography (EtOAc/Hexane = 1:4) to give **85** (7.17 g, 74%) as a colorless liquid. The NMR spectral data agreed with literature.^{245,246}

Compound 86. To a suspension of **85** (0.08 g, 0.37 mmol), Ph₃P (0.147 g, 0.56 mmol), and **38** (0.129 g, 0.372 mmol) in THF (5 mL) at 0 °C was added a solution of DIAD (0.113 g, 0.560 mmol). The mixture was warmed to room temperature and then stirred for 2 h. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hexane = 1:4) to afford **86** (0.16 g, 78%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, *J* = 5.6 Hz, 1H), 7.14 (d, *J* = 5.6 Hz, 1H), 6.24-6.21 (m, 1H), 6.07-6.05 (m, 1H), 5.78-5.74 (m, 1H), 5.22- 5.20 (m, 1H), 2.64 (s, 3H), 2.39-2.26 (m, 2H), 1.45 (s, 18H), 0.93 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 153.4, 151.8, 143.6, 140.8, 140.1, 138.5, 136.1, 132.9, 106.6, 82.9, 76.4, 61.2, 40.6, 28.0, 26.0, 18.4, 14.9, 4.5.

(1S,4S)-4-(tert-butyldimethylsilyloxy)cyclopent-2-enyl benzoate (87). To a solution of Ph₃P (1.18 g, 9.93 mmol) and DIAD (2.05 g, 9.93 mmol) in anhydrous THF (40 mL) were added benzoic acid (1.18 g, 9.98 mmol) and a solution of **85** (1.42 g, 6.62 mmol) in THF (20 mL) at 0 °C under nitrogen atmosphere. Then the reaction was brought to rt. and stirred at same temperature for 12 hr. The solvent was evaporated under reduced pressure and resulting residue was purified by chromatography (EtOAc/Hexane = 1:4 to 2:1) to give **87** contaminated with the azadicarboxylate byproduct. ¹H NMR (400 MHz, CDCl₃) δ 8.02-7.99 (m, 2H), 7.53 (tt, *J* = 7.6, 1.6 Hz, 1H), 7.41 (tt, *J* = 7.6, 1.6 Hz, 2H), 6.10-6.06 (m, 2H), 6.03 (m, 1H), 5.12 (m, 1H), 2.32 (ddd, *J* = 14.5, 6.8, 2.4 Hz, 1H), 2.18 (ddd,

$J = 14.5, 7.0, 4 \text{ Hz}, 1\text{H}$), 0.91 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.6, 141.1, 133.0, 131.6, 130.5, 129.7, 128.4, 79.8, 76.5, 41.3, 21.7, 18.3, -4.54.

(1S,4S)-4-(tert-butyltrimethylsilyloxy)cyclopent-2-enol (88). The crude compound **87** was treated with LiOH (0.87 g, 19.9 mmol) in THF- H_2O solution (100 mL) for 12 hr at rt.. The product was extracted by EtOAc (100 mL \times 3). The combined organic layer was washed with brine (100 mL) and dried by MgSO_4 . After removal of solvent, the residue was purified by chromatography (EtOAc/Hexane = 1:2) to give the clean **88** (1.02 g, 72%) as colorless liquid. The NMR spectral data agreed with literature.²⁴⁷

Compound 94a and 94b. To a suspension of **93**²³¹ (0.32 g, 1.40 mmol), Ph_3P (0.55 g, 2.10 mmol), and **38** (0.57 g, 1.64 mmol) in THF (40 mL) at 0 °C was added a solution of DIAD (0.42 g, 2.10 mmol). The mixture was warmed to room temperature and then stirred for 2 h. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hexane = 1:4) to afford **94a** (0.37g, 48%) and **94b** (93.9 mg, 12%) as white foams. **94a**: ^1H NMR (400 MHz, CDCl_3) δ 8.17 (d, $J = 5.6 \text{ Hz}, 1\text{H}$), 7.23 (d, $J = 5.6 \text{ Hz}, 1\text{H}$), 6.15 (m, 1H), 5.74 (dt, $J = 5.6, 2.0 \text{ Hz}, 1\text{H}$), 5.51 (dt, $J = 6.0, 2.4$), 3.72 (dd, $J = 10, 4.4 \text{ Hz}, 1\text{H}$), 3.63 (dd, $J = 10, 6.0$), 2.80 (m, 1H), 2.68 (s, 3H), 2.64 (m, 1H), 2.38 (m, 1H), 1.44 (s, 18H), 0.88 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 153.7, 151.8, 143.5, 141.4, 139.9, 136.2, 135.2, 129.0, 106.9, 82.8, 64.1, 63.7, 47.0, 34.9, 28.1, 26.1, 18.5, 14.9, -5.1, -5.3. **94b**: ^1H NMR (400 MHz, CDCl_3) δ 8.19 (d, $J = 5.6 \text{ Hz}, 1\text{H}$), 7.28 (d, $J = 5.6 \text{ Hz}, 1\text{H}$), 6.19 (td, $J = 5.6, 2.4 \text{ Hz}, 1\text{H}$), 5.92 (td, $J = 5.6, 2 \text{ Hz}, 1\text{H}$), 5.60 (m, 1H), 3.69 (d, $J = 5.2, 1.2 \text{ Hz}, 2\text{H}$), 3.28 (m, 1H), 2.65 (s, 3H), 2.37 (m, 1H), 2.13 (m, 1H), 1.45 (s, 18H), 0.95 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C

NMR (100 MHz, CDCl₃) δ 153.3, 151.8, 143.5, 141.0, 139.9, 137.9, 136.1, 130.6, 106.9, 82.8, 65.9, 62.2, 48.3, 33.6, 28.1, 26.0, 22.1, 14.9, -5.2.

Compound 95. 4-methyl morpholine *N*-oxide NMO (31.7 mg, 0.27 mmol) was added to a solution of **94** (50.0 mg, 0.089 mmol) in CH₂Cl₂ (5 mL) which contained a small amount of H₂O (0.05 mL). After the solution was cooled to 0 °C, a catalytic amount of solid OsO₄ (0.9 mg, 0.003 mmol) was added and the solution was stirred for 4 hr at rt. The reaction was quenched by addition of NaHSO₃. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hexane = 1:2) to give the **95** (43.8 mg, 83%) as white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.94 (d, *J* = 5.6 Hz, 1H), 7.09 (d, *J* = 5.6 Hz, 1H), 4.60 (m, 1H), 4.50 (m, 1H), 4.17 (m, 1H), 3.60 (dd, *J* = 10.0, 4.8 Hz, 1H), 3.52 (dd, *J* = 10, 4.0 Hz, 1H), 3.48 (m, 1H), 2.62 (m, 1H), 2.49 (s, 3H), 2.38 (m, 1H), 2.00 (br, 1H), 1.80 (m, 1H), 1.47 (s, 18H), 0.87 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 155.2, 152.1, 151.9, 143.4, 139.8, 106.4, 83.32, 83.28, 75.9, 71.1, 64.4, 63.5, 40.1, 32.5, 28.1, 26.0, 18.5, 14.8, -5.27, -5.34.

(1R,2S,3R,5R)-3-(4-amino-2-methyl-1H-imidazo[4,5-c]pyridin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (6). Compound **95** (40.0 mg, 0.089 mmol) was dissolved in a mixture of MeOH (5.0 mL) and 1 N HCl (5.0 mL) and the resulting solution was stirred at rt. for 5 hr. After addition of basic resin (Amberlite IR67) for neutralization of the solution and filtration, the solvent was removed under vacuum and the residue was purified by column chromatography to give **6** (17.3 mg, 70.0%) as white solid. ¹H NMR (400 MHz, MeOD) δ 7.62 (d, *J* = 6.0 Hz, 1H), 6.86 (d, *J* = 6.0 Hz, 1H), 4.62 (m, 1H), 4.55 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.15 (m, 1H), 3.63-3.55 (m, 2H), 2.69 (m, 1H), 2.64 (s, 3H), 2.40 (m, 1H), 1.63 (ddd, *J* = 14.8, 4, 1.6 Hz, 1H); ¹³C NMR (100 MHz,

MeOD) δ 153.9, 152.8, 140.0, 139.7, 127.0, 99.9, 77.3, 72.6, 66.2, 65.9, 41.2, 33.4, 14.5.

EI-MS calcd for C₁₃H₁₈N₄O₃: [(M + H)⁺]: 279.1412, found: 279.1574.

(3*S*,4*S*,6*R*)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]ioxol-4-ol (100). Similar procedure as **106**, white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.47-7.44 (m, 6H), 7.31-7.20 (m, 9H), 4.66 (t, *J* = 4.8 Hz, 1H), 4.44 (t, *J* = 5.6 Hz, 1H), 3.84 (s, 1H), 3.37 (dd, *J* = 8.8, 7.6 Hz, 1H), 3.09 (dd, *J* = 8.8, 6.4 Hz, 1H), 2.36 (s, 1H), 1.96-1.82 (m, 2H), 1.38 (s, 3H), 1.35 (s, 3H), 1.30-1.24 (m, 1H); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 144.3, 128.8, 127.7, 126.9, 79.4, 78.70, 72.19, 62.1, 39.3, 33.6, 25.7, 24.3.

(3*S*,4*S*,6*R*)-6-(2-(tert-butyldimethylsilyloxy)ethyl)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (106). A mixture of **72** (2.52 g, 8.01 mmol) and 10% Pd/C (0.86 g, 0.80 mmol) in 20 mL of anhydrous ethyl alcohol was hydrogenated at 50 psi in a Parr hydrogenation apparatus for 2 h when TLC showed that the starting material had disappeared and a new spot was detected (hexanes/EtOAc, 4:1), The catalyst was removed by filtration with celite and washed carefully with ethyl alcohol. The filtrate and washings were combined and evaporated in vacuo to give 2.28 g (90.0%) of product as an off-white solid. ¹H NMR (400 MHz, CDCl₃) δ 4.44 (t, *J* = 4.8 Hz, 1H), 4.38 (t, *J* = 5.6 Hz, 1H), 3.80-3.70 (m, 1H), 3.64-3.58 (m, 2H), 1.87-1.83 (m, 1H), 1.69-1.75 (m, 2H), 1.57-1.53 (m, 1H), 1.47 (s, 3H) 1.30 (s, 3H), 1.50-1.24 (m, 2H), 0.79 (s, 9H), 0.05(s, 6H); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 110.1, 80.6, 78.77, 72.2, 61.6, 35.6, 35.0, 31.4, 25.9, 25.6, 24.1, 18.2, -5.4. EI-MS calcd for C₁₆H₃₃O₄Si: [(M + H)⁺]:317.2143, found:317.2148.

Compound 107. Similar procedure as **109**, white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.88 (s, 1H), 7.98 (s, 1H), 7.48 -7.45 (m, 6H), 7.30-7.20 (m, 9H), 4.99-4.92 (m, 2H), 4.87

(d, $J = 6.4$ Hz, 1H), 3.57 (dd, $J = 9.2, 7.2$ Hz, 1H), 3.23 (dd, $J = 9.2, 7.2$ Hz, 1H), 2.56 (m, 1H), 2.22- 2.07 (m, 2H), 1.48 (s, 18H), 1.44 (s, 3H), 1.33 (s, 3H);

Compound 109. To a suspension of Ph_3P (0.95 g, 3.8 mmol), **106** (0.79 g, 2.5 mmol), and **113** (1.0 g, 3.0 mmol) in 50 mL of anhydrous THF was added dropwise a solution of DIAD (0.76 g, 3.8 mmol) in THF (10 mL) in 0 °C. After stirred at room temperature for 12 hr, the mixture was heated to 60 °C for another 12 hr. The solvent was removed and residue was purified by column chromatography (EtOAc/hexanes = 1:4) to give **109** as a white foam. (1.25 g, 79.0%) ^1H NMR (400 MHz, CDCl_3) δ 8.76 (s, 1H), 7.86 (s, 1H), 4.97 (d, $J = 5.2$, 1H), 4.87-4.81 (m, 2H), 3.77-3.67 (m, 2H), 2.65-2.60 (m, 1H), 2.28-2.19 (m, 1H), 2.13-2.08 (m, 1H), 1.94-1.88 (m, 1H), 1.71-1.68 (m, 1H), 1.56 (s, 18H), 1.52 (s, 3H), 1.35 (s, 3H), 0.86 (s, 9H), 0.037 (s, 3H), 0.027 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 153.2, 151.2, 150.0, 159.9, 122.1, 111.0, 85.6, 82.4, 82.1, 61.9, 61.7, 39.8, 34.7, 31.9, 28.3, 26.5, 26.1, 24.3, 21.3, 18.4, -5.1.

Compound 108. Similar procedure as **109**, white solid: ^1H NMR (400 MHz, CDCl_3) δ 8.36 (d, $J = 5.6$ Hz, 1H), 7.82 (s, 1H), 7.48-7.45 (m, 6H), 7.39 (d, $J = 5.6$ Hz, 1H), 7.33-7.23 (m, 9H), 4.78 (m, 1H), 4.68 (d, $J = 5.2$ Hz, 1H), 4.60 (m, 1H), 3.59 (dd, $J = 9.6, 6.8$ Hz, 1H), 3.25 (dd, $J = 9.6, 7.2$ Hz, 1H), 2.39 (m, 1H), 2.22-2.18 (m, 2H), 1.45 (s, 18 H), 1.44 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 151.6, 144.2, 141.3, 140.7, 137.0, 128.9, 128.0, 127.3, 111.5, 106.3, 87.0, 85.7, 83.2, 80.4, 62.4, 61.8, 43.7, 31.4, 26.3, 24.3.

Compound 110. To a suspension of Ph_3P (1.77 g, 6.75 mmol), **106** (1.42 g, 4.50 mmol), and **117** (1.5 g, 4.5 mmol) in 30 mL of anhydrous THF was added dropwise DIAD (1.37 g, 6.75 mmol) in THF (10 mL) at 0 °C. Stirred at room temperature for 12 hr,

and then the mixture were heated to 50 °C for 8 hr. The solvent was removed and residue was purified by column chromatography (EtOAc/hexanes = 1:4) to afford **110** as a white foam. (2.0 g, 70%). ¹H NMR (400 MHz, CDCl₃) δ 8.29 (d, *J* = 5.6 Hz, 1H), 7.84 (s, 1H), 7.37 (d, *J* = 5.6 Hz, 1H), 4.67-4.63 (m, 2H), 4.60-4.58 (m, 1H), 3.73-3.63 (m, 2H), 2.45-2.41 (m, 1H), 2.24-2.20 (m, 2H), 1.91-1.83 (m, 1H), 1.72-1.63 (m, 1H), 1.47 (s, 3H), 1.37 (s, 18H), 1.28 (s, 3H), 0.83 (s, 9H), 0.05 (s, 6H); ¹³C NMR (100.6 MHz, DMSO-d₆) δ 151.4, 144.6, 141.1, 141.0, 140.6, 136.7, 111.0, 106.0, 85.5, 82.8, 81.4, 61.8, 61.4, 39.7, 33.1, 31.6, 27.9, 27.8, 26.2, 25.9, 24.0, 22.0, 18.2, -5.3.

(1R,2S,3R,5S)-3-(6-amino-9H-purin-9-yl)-5-(2-hydroxyethyl)cyclopentane-1,2-diol (9a). Compound **109** (0.58 g, 1.09 mmol) was treated with a mixture of 1N HCl (5 mL), MeOH (10 mL). The reaction mixture was stirred at room temperature overnight and then brought to reflux for 1 hr. The solution was neutralized with basic ion exchange resin (Amberlite IRA-67). After filtration, the solvent was evaporated under reduced pressure. Purification of the residue by column chromatography afforded **1** as white solid. (0.27 g, 90%). ¹H NMR (400 MHz, DMSO-d₆) δ 8.16 (s, 1H), 8.11 (s, 1H), 7.15 (s, 2H), 4.97 (d, *J* = 6.4 Hz, 1H), 4.73-4.69 (m, 1H), 4.66 (d, *J* = 3.2 Hz, 1H), 4.51-4.49 (m, 1H), 4.39 (t, *J* = 5.2 Hz, 1H), 3.84-3.83 (m, 1H), 3.45-3.40 (m, 2H), 2.45-2.41 (m, 1H), 2.10-2.01 (m, 1H), 1.95-1.87 (m, 1H), 1.69 (td, *J* = 13.6, 6.4 Hz, 1H), 1.45 (td, *J* = 13.6, 6.4 Hz, 1H); ¹³C NMR (100.6 MHz, DMSO-d₆) δ 156.0, 152.1, 149.5, 140.6, 119.5, 77.2, 73.0, 59.5, 59.1, 35.2, 33.5, 33.0. Anal. Calcd for C₁₂H₁₇N₅O₃: C, 51.60; H, 6.14; N, 25.08. Found: C, 51.43; H, 6.22; N, 24.83. EI-MS calcd for C₁₂H₁₇N₅O₃: [(M + H)⁺]: 280.1410, found: 280.1427.

(1R,2S,3R,5S)-3-(4-amino-1H-imidazo[4,5-c]pyridin-1-yl)-5-(2-hydroxyethyl)cyclopentane-1,2-diol (9b). ¹H NMR (400 MHz, MeOD) δ 8.17 (s, 1H), 7.66 (d, *J* = 6.0 Hz, 1H), 6.89 (d, *J* = 6.0 Hz, 1H), 4.82-4.75 (m, 1H), 4.39 (dd, *J* = 12.0, 3.6 Hz, 1H), 4.03 (t, *J* = 3.6 Hz, 1H), 3.67-3.58 (m, 2H), 2.55-2.46 (m, 1H), 2.19-2.14 (m, 2H), 1.86 (td, *J* = 13.6, 6.4 Hz, 1H), 1.64 (td, *J* = 13.6, 6.4 Hz, 1H); ¹³C NMR (100.6 MHz, MeOD) δ 153.5, 142.7, 140.0, 128.4, 99.3, 79.9, 75.4, 62.6, 61.3, 50.0, 37.4, 34.3, 34.0. Anal. Calcd for C₁₃H₁₈N₄O₃ · 0.1H₂O: C, 55.74; H, 6.55; N, 20.00. Found: C, 55.67; H, 6.62; N, 19.63. EI-MS calcd for C₁₃H₁₈N₄O₃: [(M + H)⁺]: 279.1457, found: 279.1456.

(1R,2S,3R,5S)-3-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (8a). White solid. ¹H NMR (400 MHz, DMSO-d₆) δ 8.17 (s, 1H), 8.11 (s, 1H), 7.17 (s, 2H), 5.00 (d, 1H, *J* = 6.4 Hz, 1H), 4.73-4.68 (m, 2H), 4.50-4.45 (m, 1H), 4.36 (t, *J* = 4.2 Hz, 1H), 3.97 (q, *J* = 3.6 Hz, 1H), 3.64-3.58 (m, 1H), 3.40-3.29 (m, 1H), 2.47-2.44 (m, 1H), 2.00-1.93 (m, 2H); ¹³C NMR (100.6 MHz, DMSO-d₆) δ 156.0, 152.0, 149.5, 140.7, 119.6, 77.1, 72.0, 60.8, 59.4, 41.4, 30.3. Anal. Calcd for C₁₁H₁₅N₅O₃ · 0.6H₂O: C, 47.86; H, 5.91; N, 25.37. Found: C, 47.94; H, 5.91; N, 25.15. EI-MS calcd for C₁₁H₁₅N₅O₃: [(M + H)⁺]: 266.1253, found: 266.1246.

(1R,2S,3R,5S)-3-(4-amino-1H-imidazo[4,5-c]pyridin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (8b). White solid. ¹H NMR (400 MHz, DMSO-d₆) δ 8.16 (s, 1H), 7.64 (d, *J* = 6.0 Hz, 1H), 6.76 (d, *J* = 6.0 Hz, 1H), 6.17 (s, 2H), 5.08 (d, *J* = 6.8 Hz, 1H), 4.76 (d, *J* = 4.4 Hz, 1H), 4.65-4.60 (m, 1H), 4.40 (t, *J* = 4.8 Hz, 1H), 4.29-4.24 (m, 1H), 3.96 (q, *J* = 3.6 Hz, 1H), 3.64-3.60 (m, 1H), 3.42-3.36 (m, 1H), 2.45-2.41 (m, 1H), 2.03-1.90 (m, 2H); ¹³C NMR (100.6 MHz, DMSO-d₆) δ 152.4, 140.9, 139.7, 137.8, 127.1, 97.2, 77.7, 72.0, 60.9, 60.0, 41.2, 30.0. Anal. Calcd for C₁₂H₁₆N₄O₃ · 0.6H₂O: C, 52.39; H, 6.30;

N, 20.37. Found: C, 52.20; H, 6.25; N, 20.26. EI-MS calcd for C₁₂H₁₆N₄O₃: [(M + H)⁺]: 265.1301, found: 265.1304.

(R)-(-)-4-Carbomethoxy-2-oxazolidinone (118). D-serine methyl ester hydrochloride (25.0 g, 0.162 mol) was in charge with CH₂Cl₂ (380 mL). The suspension was cooled to 0 °C before triethylamine (68 mL, 0.48 mol) was added dropwise. The reaction was stirred for 15 min. A solution of triphosgene (16.2 g, 54.0 mmol) in CH₂Cl₂ (165 mL) was transferred into the addition funnel and added to the reaction mixture over 5 hr. The reaction was stirred for 0.5 hr. Dry ether (500 mL) was added and the reaction was cooled to -78 °C to precipitate triethylamine hydrochloride. After filtration and wash the precipitate by EtOAc (2 X 150 mL), the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (hexane:EtOAc = 1:9) to give **118** (22.5 g, 95.7%) as a pale yellow solid. The NMR spectral data agreed with literature.²³²

(S)-(+)-4-Hydroxymethyl-2-oxazolidinone (119). To a solution of **118** (4.25 g, 29.3 mmol) in dry ethanol (70 mL) at 0 °C was added sodium borohydride (1.23 g, 35.7 mmol) in portions. The reaction was stirred for 3 hr and then treated with saturated NH₄Cl (5.8 mL). The reaction mixture was stirred for additional 30 min and white solids were filtered through a pad of celide. The filter cake was washed with dry ethanol and the filtrate was concentrated. The residue was purified through column chromatography (EtOAc:MeOH = 9:1) to give **119** (2.67 g, 77.5%) as white solid. The NMR spectral data agreed with literature.²³²

(R)-(+)-4-(4'-Toluenesulfonyloxymethyl)-2-oxazolidinone (120). To a solution of **119** (16.5 g, 0.141 mol) in dry pyridine (53.7 mL), pure TsCl (42.5g, 0.223 mol) was added at 0 °C. The reaction was stirred at rt. for 6 hr. Pyridine was removed under

reduced pressure. The residue was dissolved in CH₂Cl₂ (300 mL). The organic layer was washed with 2.0 M HCl (3 X 50 mL). The acid layer was back extracted with CH₂Cl₂ two times and combined organic layer was dried by MgSO₄. The solvent was removed and crude product was purified through column chromatography (hexane:EtOAc = 1:1) to give **120** (27.5g, 71.9%). The NMR spectral data agreed with literature.²³²

(R)-(-)-4-Iodomethyl-2-oxazolidinone (121). A mixture of **120** (2.60 g, 9.60 mmol) and sodium iodide (7.28 g, 44.8 mmol) in 60 mL of freshly distilled dry acetone was refluxed under dry nitrogen for 5 hr. The reaction was cooled to room temperature, the excess sodium iodide was filtered off, and the solids were washed with ethyl acetate. The combined filtrate was concentrated. The crude yellow solid was dissolved in EtOAc and washed with a saturated sodium sulfite solution until the layers became colorless. The aqueous layer was extracted with EtOAc (5 X 50 mL). The combined organic layers were dried over anhydrous MgSO₄ and filtered, and the solvent was removed under reduced pressure. The crude product was purified through column chromatography (EtOAc:hexanes = 3:1) to give **121** (2.13 g, 97.7%). The NMR spectral data agreed with literature.²³²

(R)-(+)-4-(2-Oxazolidonyl)-methyltriphenylphosphonyl Iodide (122). A solution of the iodide **121** (2.39 g, 10.53 mmol) and triphenylphosphine (28.0 g, 106.9 mmol) in 30 mL of dry DMF was stirred at 100 °C for 61 h. The DMF was removed under vacuum, and the resulting residue was triturated with dry THF to remove excess triphenylphosphine, followed by repeated washings with ether to afford a yellow solid. This solid was crystallized from MeOH/EtOAc and dried in an Abderhalden, giving 4.40 g (85%) of a white solid. The NMR spectral data agreed with literature.²³²

(3aR,6S,6aR)-6-allyl-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (123). A solution of CuI (8.17 g, 42.9 mmol), LiCl (1.81 g, 42.2 mmol) in THF (60 mL) was stirred for 5 min in rt before cooled down to -78 °C. A solution of allylic magnesium bromide (1 M in THF, 40.87 mL, 40.87 mmol) was added dropwise. After stirred additional 30 min in the same temperature, TMSCl (5.56 mL) and HMPA (7.9 mL) was added, followed by addition of **12** (2.4 g, 15.6 mol) dropwise. After stirred at same temperature for 2 hr, the reaction mixture was warmed up to 0 °C and quenched by saturated NH₄Cl (10 mL). The solvent was evaporated under reduced pressure and the residue was purified by chromatography (EtOAc/Hexane = 1:4) to give **123** (2.30 g, 75.1%) as a colorless liquid. ¹H NMR (400 MHz, CDCl₃) δ 5.71 (m, 1H), 5.12-5.06 (m, 2H), 4.55 (d, *J* = 5.2 Hz, 1H), 4.20 (d, *J* = 5.2 Hz, 1H), 2.74 (m, 1H), 2.50 (m, 1H), 2.22-2.00 (m, 3H), 1.42 (s, 3H), 1.33 (s, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ 214.2, 134.7, 118.2, 112.2, 81.6, 78.3, 39.5, 37.8, 36.2, 27.0, 25.0.

(3aS,4S,6S,6aR)-6-allyl-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (124). To a suspension of LiAlH₄ (0.345 g, 8.83 mmol) in dry THF (20 mL) at 0 °C was added dropwise a solution of **123** (1.00 g, 5.10 mmol) in THF (10 mL). After stirred at rt. for 3 hr, the reaction was quenched sequentially with H₂O (0.30 mL), aqueous NaOH (15%, 0.30 mL) and H₂O (1.00 mL). The solvent was evaporated to afford **124** (0.96 g, 95%) as colorless liquid.

Compound 125. To a suspension of Ph₃P (2.72 g, 10.7 mmol), **124** (1.04 g, 5.24 mmol), and **113** (2.55 g, 7.60 mmol) in 50 mL of anhydrous THF was added dropwise a solution of DIAD (2.21 mL, 10.7 mmol) in THF (10 mL) in 0 °C. After stirred at room temperature for 12 hr, the mixture was heated to 60 °C for another 12 hr. The solvent was

removed and residue was purified by column chromatography (EtOAc/hexanes = 1:4) to give **125** as a white foam. (1.89 g, 70.0%) ^1H NMR (400 MHz, CDCl_3) δ 8.82 (s, 1H), 8.08 (s, 1H), 5.82 (m, 1H), 5.13-5.04 (m, 3H), 4.77 (m, 1H), 4.51 (dd, $J = 7.3, 4.6$ Hz, 1H), 2.49-2.41 (m, 2H), 2.30-2.22 (m, 3H), 1.55 (s, 3H), 1.44 (s, 18H), 1.29 (s, 3H).

Compound 126. To a solution of **125** (1.50 g, 2.90 mmol) in THF (50 mL) at 0 °C under N_2 , was added 9-BBN-H (0.5M in THF, 12 mL, 6 mmol) and resulting mixture stirred for 3hr at rt. NaOH solution (1M, 5.00 mL) was added and followed by adding H_2O_2 (50% in H_2O , 3.30 mL). The reaction mixture was stirred for another 2 hr and solvent was removed under reduced pressure. The residue was purified by chromatography (EtOAc/Hexane = 1:2) to give **126** (0.96 g, 62%) as a white foam. ^1H NMR (400 MHz, CDCl_3) δ 8.75 (s, 1H), 7.96 (s, 1H), 5.81 (m, 1H), 5.68 (m, 1H), 5.18-5.08 (m, 2H), 4.98 (dd, $J = 18.6, 8.6$ Hz, 1H), 2.53-2.46 (m, 2H), 2.43-2.39 (m, 2H), 2.29-2.11 (m, 2H), 1.56 (s, 9H), 1.51 (s, 9H), 1.46 (m, 1H), 1.39 (s, 6H).

(3aR,6S,6aR)-6-(but-3-enyl)-2,2-dimethyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (128). To a suspension of $\text{CuBr}\cdot\text{Me}_2\text{S}$ (0.18 g, 0.87 mmol) in THF (40 mL) was added dropwise a solution of 3-butenyl magnesium bromide (1 M in THF, 26.00 mL, 13.00 mmol) at 78 °C. After stirred additional 30 min in the same temperature, TMSCl (2.60 mL, 20.3 mmol) and HMPA (4.5 mL, 25.7 mmol) was added, followed by addition of **12** (1.54 g, 10.0 mol) dropwise. After stirred at same temperature for 2 hr, the reaction mixture was warmed up to 0 °C and quenched by saturated NH_4Cl (10 mL). The solvent was evaporated under reduced pressure and the residue was purified by chromatography (EtOAc/Hexane = 1:4) to give **128** (1.85 g, 88.0%) as a colorless liquid. ^1H NMR (400 MHz, CDCl_3) δ 5.78 (m, 1H), 5.07-4.99 (m, 2H), 4.55 (d, $J = 5.2$ Hz, 1H), 4.23 (d, $J = 5.2$

Hz, 1H), 2.76 (m, 1H), 2.40 (m, 1H), 2.16-2.05 (m, 3H), 1.55 (m, 1H), 1.43 (s, 3H), 1.33 (m, 1H), 1.35 (s, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 213.9, 137.3, 115.7, 112.2, 82.0, 78.2, 40.0, 36.4, 32.8, 31.4, 26.9, 25.0. Anal. Calcd for $\text{C}_{12}\text{H}_{18}\text{O}_3 \cdot 0.1\text{H}_2\text{O}$: C, 67.96; H, 8.65; Found: C, 67.96; H, 8.65. EI-MS calcd for $\text{C}_{12}\text{H}_{18}\text{O}_3$: $[(\text{M} + \text{H})^+]$: 211.1289, found: 211.1271.

(3a*S*,4*S*,6*S*,6a*R*)-6-(but-3-enyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (129). Similar produce as **124**; ^1H NMR (400 MHz, CDCl_3) δ 5.73 (m, 1H), 5.00-4.90 (m, 2H), 4.43 (t, $J = 5.6$ Hz, 1H), 4.27 (dd, $J = 6.2, 2$ Hz, 1H), 4.01 (m, 1H), 2.51 (d, $J = 7.6$ Hz, 1H), 2.08-1.99 (m, 3H), 1.84 (m, 1H), 1.61 (m, 1H), 1.45 (s, 3H), 1.30 (s, 3H), 1.38-1.82 (m, 2H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 138.1, 115.0, 111.7, 85.1, 79.3, 71.2, 41.0, 36.8, 32.2, 31.7, 26.2, 24.4. Anal. Calcd for $\text{C}_{12}\text{H}_{20}\text{O}_3$: C, 67.89; H, 9.50; Found: C, 67.70; H, 9.58. EI-MS calcd for $\text{C}_{12}\text{H}_{20}\text{O}_3$: $[(\text{M} + \text{H})^+]$: 213.1446, found: 213.1403.

Compound 130. Similar procedure as **125**; ^1H NMR (400 MHz, CDCl_3) δ 8.83 (s, 1H), 8.10 (s, 1H), 5.83 (m, 1H), 5.10-4.93 (m, 3H), 4.78 (m, 1H), 4.51 (t, $J = 6.4$ Hz, 1H), 2.48 (m, 1H), 2.29 (q, $J = 13.3$ Hz, 1H), 2.23-2.14 (m, 3H), 1.76 (m, 1H), 1.62 (m, 1H), 1.56 (s, 3H), 1.46 (s, 18H), 1.31 (s, 3H).

Compound 127. Compound **130** (4.21 g, 7.95 mmol) was dissolved in MeOH (21.0 mL) and H_2O (10 mL), and NaIO_4 (2.54 g, 12 mmol) was added slowly. After the reaction mixture was cooled to 0 °C, OsO_4 (17.5 mg) was added. The reaction was stirred at the same temperature for 1 hr and then warmed to room temperature for 2 hr. The white solid that resulted was removed by filtration and the filtrate was concentrated in vacuo. CH_2Cl_2 (117 mL) was added to the residue and washed with H_2O (17.5 mL) and brine

(17.5 mL), and dried with MgSO₄. The solvent was removed under reduced pressure to give **127** (2.87 g, 68%) as white foam. ¹H NMR (400 MHz, CDCl₃) δ 9.84 (d, *J* = 1.6 Hz, 1H), 8.84 (d, *J* = 1.6 Hz, 1H), 8.09 (s, 1H), 5.10 (m, 1H), 4.77 (m, 1H), 4.51 (m, 1H), 2.65 (td, *J* = 7.4, 1.2 Hz, 2H), 2.48 (m, 1H), 2.37 (q, *J* = 12.6 Hz, 1H), 2.18 (m, 1H), 1.91 (q, *J* = 7.2 Hz, 2H), 1.55 (s, 3H), 1.48 (s, 18 H), 1.31 (s, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ 201.7, 153.3, 152.1, 150.8, 144.4, 129.8, 114.4, 84.6, 84.1, 83.6, 62.1, 43.8, 42.2, 37.1, 28.0, 27.6, 25.6, 25.3.

Compound 132. A solution of **122**²³² (2.23 g, 4.61 mmol) in THF (70 mL) was cooled down to -78 °C, before the n-BuLi (5.69 mL, 13.7 mmol) was added over a 10 min period. The resulting orange-color solution was stirred at same temperature for 1 hr, followed by adding a solution of **127** (2.30 g, 4.33 mmol) in THF (20 mL) slowly. After stirred at same temperature for 3 hr, the reaction was brought up to rt.. A saturated NH₄Cl solution was added and stirred for 10 min. The THF was removed under reduced pressure. The aqueous solution was extracted with EtOAc (3 x 60 mL) and dried with MgSO₄. After evaporated the solvent, the resulting residue was purified by chromatography (EtOAc/Hexane = 1:4) to give mixture of **131a** and **131b** (1.01 g, 45.3%).

The mixture of **131** was dissolved in THF (1.01 g, 1.96 mmol), followed by addition of DMAP (0.024 g, 0.2 mmol), Boc₂O (1.38 mL, 6.00 mmol) at room temperature. The reaction mixture was stirred at same temperature for 8 hr. After removal of solvent, the crude product was purified by chromatography (EtOAc/Hexane = 1:2) to afford **132** (1.18 g, 86.9%) as white solid.

Compound 133. A parr hydrogenation apparatus was charged with **132** (0.79 g, 1.1 mmol), 10% Pd/C and 45 psi hydrogen in MeOH (20 mL) for 5 hr. The catalyst was

removed by filtration and washed with EtOH. Evaporated the combined solvent to give **143** (0.77 g, 97.6%) as a white foam.

Compound 134. To a solution of **133** (0.74 g, 1.03 mmol) in THF:H₂O (1:1) was added LiOH (0.12 g, 5.20 mmol) at room temperature. The reaction mixture was stirred at same temperature for 2hr. The solvent was evaporated under reduced pressure and the crude residue was purified by columgraphy (EtOAc: Hexane = 1:1) to give **134** (0.70 g, 98.5%) as white solid.

(R)-methyl 2-(tert-butoxycarbonylamino)-3-hydroxypropanoate (136). Et₃N (14.0 g, 138 mmol) was added to a suspended serine methyl ester hydrochloride (10 g, 64.3 mmol) in THF (200 mL). After the resulting white suspension was cooled to 0 °C, a solution of (Boc)₂O (14.3 g, 97%, 63.6 mmol) in THF (100 mL) was added dropwise over 45 min. The mixture was allowed to warm to room temperature and stirred for 6 hr, then warmed to 50 °C for another 2hr. The solvent was removed in vacuo and the residue partitioned between Et₂O (200 mL) and H₂O (200 mL). The aqueous phase was extracted with Et₂O (2 x 160 mL). The combined organic layer was washed with 3% HCl (100 mL), 5% NaHCO₃ (100 mL) and brine (200 mL). After drying by MgSO₄, the solvent was evaporated to afford **136** (13.53 g, 96.0%) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 5.49 (d, *J* = 5.6 Hz, 1H), 4.34 (d, *J* = 3.2 Hz, 1H), 3.95-3.83 (m, 2H), 3.75 (s, 3H), 2.69 (m, 2.69, 1H), 1.42 (s, 9H); ¹³C NMR (100.6 MHz, CDCl₃) δ 171.6, 156.0, 80.5, 63.7, 55.9, 52.8, 28.5.

(R)-3-tert-butyl 4-methyl 2,2-dimethyloxazolidine-3,4-dicarboxylate (137). To a solution of **136** (13.5 g, 61.7 mmol) in acetone (200 mL) and 2, 2-dimethyloxylpropane (66.7 mL) was added BF₃ · OEt₂ (0.4 mL). The resulting solution was stirred at room

temperature for 2 hr. The solvent was removed under pressure and the residual was dissolved in CH₂Cl₂ (200 mL) and washed with a mixture of saturated NaHCO₃ and H₂O (1:1, 133 mL), then brine (133 mL). The solvent was evaporated in vacuo after dried by MgSO₄ to give **137** (15.02 g, 93.9%) as a pale yellow oil. ¹H NMR (400 MHz, DMSO, 70 °C) δ 4.40 (dd, *J* = 7.2, 2.8 Hz, 1H), 4.16 (dd, *J* = 9.2, 7.2 Hz, 1H), 3.94 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.69 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H), 1.40 (s, 9H);

(*R*)-tert-butyl 4-formyl-2,2-dimethylloxazolidine-3-carboxylate (138). A solution of compound **137** (15.12 g, 58.3 mmol) in anhydrous toluene (120 mL) was cooled down to -78 °C followed by addition of DIBALH (100 mL, 1M in hexane, 100 mmol) dropwise. Stirring was continued for 2 hr before careful addition of anhydrous MeOH (23 mL) and then the reaction mixture was warmed to room temperature. The mixture was poured into a solution of potassium sodium tartrate (100.8 g) in H₂O (280 mL). The biphasic mixture stirred vigorously for 2hr and then separated into two phase. The aqueous layer extracted with Et₂O (2 x 150 mL) and combined organic extracts was dried by MgSO₄. Evaporated the solvent in vacuo to give **138** (10.5 g, 78.2%) as colorless oil.

(*S*)-tert-butyl 2,2-dimethyl-4-vinylloxazolidine-3-carboxylate (139). Methyltriphenylphosphonium bromide (26.2 g, 73.6 mmol) was suspended in THF (300 mL) under N₂ at room temperature and KHMDS (0.5 M in toluene, 140.8 mL, 70.4 mmol) was added. The resulting yellow suspension was stirred at room temperature for 60 min, then cooled to -78 °C and a solution of the **138** (9.61 g, 41.9 mmol) in THF (128 mL) was added dropwise. The cooling bath was removed and the mixture allowed to reach room temperature over 2 hr. The reaction was quenched with MeOH (60 mL) and the resulting mixture was poured into a mixture of saturated potassium sodium tartrate and

H₂O (1:1, 200 mL). Extraction with ether (2 x 100 mL), drying with MgSO₄ and evaporation of the solvent. The residue was purified by column chromatography (4:1 = Hexane : EtOAc) to give **139** (8.18 g, 85.9%) as colorless liquid. ¹H NMR (400 MHz, CDCl₃) δ 5.81 (m, 1H), 5.25-5.12 (m, 2H), 4.33 (m, 1H), 4.04 (dd, *J* = 6.4, 8.8 Hz, 1H), 3.73 (dd, *J* = 2.0, 8.8 Hz, 1H), 1.60 (s, 3H), 1.51-1.43 (m, 12H); ¹³C NMR (100.6 MHz, CDCl₃) δ 151.7, 137.3, 115.5, 93.7, 79.3, 67.9, 59.5, 28.0, 26.4, 23.5.

(S)-tert-butyl 1-hydroxybut-3-en-2-ylcarbamate (140). To a solution of **139** (6.65 g, 29.2 mmol) in MeOH (200 mL) was added p-toluenesulfonic acid monohydrate (2.78 g, 14.6 mmol) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 12 hr. The solvent was evaporated and the residue was diluted with EtOAc (88 mL). The organic layer was washed with saturated sodium bicarbonate solution (3 x 50 mL). The organic phase was combined and dried over MgSO₄. Evaporating the solvent to give the desired **140** (3.75 g, 68.6%) as a pale yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 5.82 (ddd, *J* = 5.2, 10.6, 16.8 Hz, 1H), 5.33 (d, *J* = 8.0 Hz, 1H), 5.27-5.18 (m, 2H), 4.20 (br, 1H), 3.69-3.57 (m, 3H), 1.45 (s, 9H).

(S)-tert-butyl 4-((E)-4-((3aR,4S,6R,6aS)-6-(6-(bis(*tert*-butoxycarbonyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)but-1-enyl)-2,2-dimethyloxazolidine-3-carboxylate (141). To a solution of **128** (3.08 g, 5.81 mmol) and **139** (1.32 g, 5.81 mmol) in dry CH₂Cl₂ (150 mL) was added 2nd generation of Grubbs catalyst (0.1g, 0.12 mmol) at room temperature under N₂ atmosphere. The reaction mixture was stirred 8 hr at room temperature and heated to reflux for overnight. The mixture was filtrated through celite and the solvent was removed under reduced pressure. The residue was purified by column chromatography (2:1 = Hexane : EtOAc) to give

141 (2.58 g, 60.9%) as white foam with **142** (1.11 g, 36.8%) as byproduct. **141** ¹H NMR (400 MHz, CDCl₃) δ 8.78 (s, 1H), 8.04 (s, 1H), 5.52 (m, 1H), 5.43 (dd, *J* = 7.2, 15.4 Hz, 1H), 5.03 (t, *J* = 6.0 Hz, 1H), 4.71 (td, *J* = 11.6, 6.0 Hz, 1H), 4.45 (t, *J* = 6.4 Hz, 1H), 4.24 (m, 1H), 3.96 (dd, *J* = 6, 8.8 Hz, 1H), 3.66 (dd, *J* = 2.0, 8.8 Hz, 1H), 2.42 (m, 1H), 2.25 (m, 1H), 2.15-2.08 (m, 3H), 1.69 (m, 1H), 1.53 (m, 1H), 1.49 (s, 3H), 1.44 (s, 3H), 1.406 (s, 30 H), 1.25 (s, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ 171.2, 153.3, 152.0, 151.9, 150.7, 150.5, 144.4, 131.5, 129.7, 114.1, 93.9, 84.6, 83.9, 83.4, 79.4, 68.6, 65.9, 62.3, 60.5, 59.2, 37.0, 30.4, 28.6, 27.9, 27.6, 25.2, 21.2, 14.3; EI-MS calcd for C₃₇H₅₆N₆O₉: [(M + H)⁺]: 729.4174, found: 729.4180.

142 ¹H NMR (400 MHz, CDCl₃) δ 8.79 (s, 1H), 8.06 (s, 1H), 5.42 (m, 1H), 5.04 (t, *J* = 6.4 Hz, 1H), 4.73 (td, *J* = 11.6, 6.0 Hz, 1H), 4.45 (t, *J* = 6.4 Hz, 1H), 2.44 (m, 1H), 2.24 (m, 1H), 2.14-2.06 (m, 4H), 1.68 (m, 1H), 1.51 (s, 3H), 1.41 (s, 18H), 1.26 (s, 3H); EI-MS calcd for C₅₂H₇₄N₁₀O₁₂: [(M + H)⁺]: 1031.2497, found: 1031.2487.

(S)-tert-butyl 4-(4-((3aR,4S,6R,6aS)-6-(6-(bis(*tert*-butoxycarbonyl)amino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)butyl)-2,2-dimethyloxazolidine-3-carboxylate (143). A parr hydrogenation apparatus was charged with **141** (1.15 g, 1.58 mmol), 10% Pd/C and 45 psi hydrogen in EtOH (20 mL) for 3 hr. The catalyst was removed by filtration and washed with EtOH. Evaporated the combined solvent to give **143** (1.15 g, 99.7%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 8.77 (s, 1H), 8.05 (s, 1H), 5.02 (br, 1H), 4.71 (td, *J* = 6.0, 11.6 Hz, 1H), 4.41 (t, *J* = 6.0 Hz, 1H), 3.86-3.65 (m, 3H), 2.41 (m, 1H), 2.21 (td, *J* = 11.6, 22.8 Hz, 1H), 2.08 (m, 1H), 1.59-1.46 (m, 7H), 1.49 (s, 3H), 1.39 (s, 33H), 1.27 (m, 1H), 1.23 (s, 3H); ¹³C NMR (100.6 MHz, CDCl₃) δ 153.3, 151.9, 150.7, 150.5, 144.3, 129.7, 114.0, 93.4, 84.6, 83.8,

83.4, 79.7, 66.9, 62.3, 57.5, 43.9, 37.1, 33.7, 32.8, 28.6, 27.9, 27.8, 27.6, 26.9, 26.4, 25.2, 24.6, 23.3; EI-MS calcd for $C_{37}H_{58}N_6O_9$: $[(M + H)^+]$: 731.4344, found: 731.4352.

Compound 144a and 144b. To a solution of **143** (1.15 g, 1.58 mmol) in MeOH (50 mL) was added p-toluenesulfonic acid monohydrate (0.15 g, 0.79 mmol) for 6 hr at room temperature. A basic resin (Amberlite IR67) was added to neutralize the reaction mixture. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography (Hexane: EtOAc =1:1) to give **144a** (0.25 g, 23.0%) and **144b** (0.23 g, 22.5%).

144a 1H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.06 (s, 1H), 5.02 (dd, $J = 6.0, 7.2$ Hz, 1H), 4.74-4.68 (m, 2H), 4.41 (dd, $J = 6.0, 7.4$ Hz, 1H), 3.55-3.47 (m, 3H), 2.96 (br, 1H), 2.41 (td, $J = 6.4, 12.4$ Hz, 1H), 2.08 (td, $J = 6.0, 12.4$ Hz, 1H), 1.49 (s, 3H), 1.43-1.29 (m, 9H), 1.39 (s, 18H), 1.37 (s, 9H), 1.24 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 156.5, 153.3, 151.9, 150.7, 150.5, 144.4, 129.7, 114.0, 84.7, 83.9, 83.4, 65.9, 62.3, 43.8, 37.0, 33.6, 31.5, 28.5, 27.9, 27.8, 27.6, 26.1, 25.2, 15.4; EI-MS calcd for $C_{34}H_{54}N_6O_9$: $[(M + H)^+]$: 691.4031, found: 691.4033.

144b 1H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.06 (s, 1H), 5.11 (d, $J = 4.0$ Hz, 1H), 4.84 (d, $J = 7.2$ Hz, 1H), 4.68 (m, 1H), 4.41 (m, 1H), 3.89 (br, 1H), 3.76 (br, 1H), 3.57-3.47 (m, 3H), 3.18 (br, 1H), 2.49 (m, 1H), 2.376 (m, 1H), 2.04 (m, 1H), 1.57 (m, 1H), 1.50 (m, 1H), 1.45-1.31 (m, 4H), 1.41 (s, 18H), 1.38 (s, 9H), 1.25 (m, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 156.7, 153.6, 151.6, 150.8, 150.5, 144.6, 129.6, 84.7, 75.3, 75.2, 65.6, 62.0, 43.5, 34.3, 32.6, 31.5, 28.5, 28.3, 28.0, 27.6, 26.0, 25.2; EI-MS calcd for $C_{31}H_{50}N_6O_9$: $[(M + H)^+]$: 651.3718, found: 651.3719.

Compound 145. To a solution of **128** (1.49 g, 2.82 mmol) and **140** (0.79 g, 4.23 mmol) in dry CH₂Cl₂ (100 mL) was added 2nd generation of Grubbs catalyst (0.1g, 0.12 mmol) at room temperature under N₂ atmosphere. The reaction mixture was stirred 8 hr at room temperature and heated to reflux for overnight. The mixture was filtrated through celite and the solvent was removed under reduced pressure. The residue was purified by columatography (1:1 = Hexane : EtOAc) to give **145** (1.26 g, 64.8%) as white foam. ¹H NMR (250 MHz, CDCl₃) δ 8.70 (s, 1H), 8.04 (s, 1H), 5.56 9(dt, *J* = 6.50, 15.5 Hz, 1H), 5.35 (dd, *J* = 5.75, 15.5 Hz, 1H), 5.07-4.95 (m, 2H), 4.67 (m, 1H), 4.38 (m, 1H), 4.02 (br, 1H), 3.52-3.46 (m, 2H), 3.24 (m, 1H), 2.34 (m, 1H), 2.23-2.02 (m, 4H), 1.58-1.46 (m, 2H), 1.43 (s, 3H), 1.32 (s, 18H), 1.30 (s, 9H), 1.18 (s, 3H); ¹³C NMR (62.5 MHz, CDCl₃) δ 155.9, 153.2, 151.7, 150.5, 150.3, 144.4, 131.5, 129.5, 128.3, 113.8, 84.4, 83.7, 83.3, 79.4, 65.2, 62.1, 43.1, 36.8, 32.8, 30.4, 28.4, 27.7, 27.4, 25.1; EI-MS calcd for C₃₄H₅₂N₆O₉: [(M + H)⁺]: 689.3874, found: 689.3876.

Compound 144a. A parr hydrogenation apparatus was charged with **145** (0.83 g, 1.20 mmol), 10% Pd/C and 45 psi hydrogen in EtOH (20 mL) for 3 hr. The catalyst was removed by filtration and washed with EtOH. Evaporated the combined solvent to give **143** (0.81 g, 97.5%) as a white foam.

(S)-6-((3aR,4S,6R,6aS)-6-(6-(bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2-(tert-butoxycarbonylamino)hexanoic acid (146). To a solution of **144a** (0.70 g, 1.01 mmol) in acetone (25 mL), an aqueous 15% solution of NaHCO₃ (3.5 mL) was added. The mixture was stirred at 0 °C, followed by adding NaBr (0.03 g, 0.29 mmol), TEMPO (4.0 mg, 0.03 mmol) into solution, then trichloroisocyanuric acid (0.50 g, 2.1 mmol) was

slowly added within 20 min. After addition, the mixture was warmed to rt. and stirred for overnight. Then 2-propanol (1 mL) was added. The mixture was filtered on celite and water was added. The mixture was extracted twice with EtOAc (20 mL X 2). The organic layers were dried with Na₂SO₄. The solvent was evaporated and the residue was purified by column (EtOAc: Methanol = 10:1) to gave a white solid (0.58 g, 81%). ¹H NMR (400 MHz, MeOD) δ 8.78 (s, 1H), 8.55 (s, 1H), 5.03 (dd, *J* = 6.0, 7.2 Hz, 1H), 4.95-4.89 (m, 2H), 4.44 (dd, *J* = 5.6, 7.2 Hz, 1H), 2.42 (dt, *J* = 6.4, 12.4 Hz, 1H), 2.26-2.09 (m, 2H), 1.94-1.84 (m, 2H), 1.50-1.35 (m, 3H), 1.34-1.22 (m, 3H), 1.47 (s, 3H), 1.39 (s, 9H), 1.30 (s, 18H), 1.17 (s, 3H); ¹³C NMR (100.6 MHz, MeOD) δ 173.2, 157.1, 154.9, 152.9, 151.7, 151.6, 151.1, 147.5, 130.7, 115.4, 86.1, 85.6, 85.1, 84.9, 63.3, 45.0, 38.1, 34.6, 29.8, 28.4, 28.2, 28.1, 27.9, 27.0, 25.5; EI-MS calcd for C₁₅₉H₂₄₇N₃₀O₄₃: [(M + H)⁺]: 705.3778, found: 705.3820.

(*S*)-2-amino-6-((1*S*,2*R*,3*S*,4*R*)-4-(6-amino-9*H*-purin-9-yl)-2,3-dihydroxycyclopentyl)hexanoic acid (10). To a solution of **146** (0.58 g, 0.83 mmol) in MeOH (10 mL) added 1 N HCl (5 mL). The mixture stirred at rt. for overnight. The solution was then neutralized with weekly basic exchange resin (Amberlite IRA-67). After filtration and evaporation of the solvent, the crude product was purified by chromatography (EtOAc/ MeOH/NH₃·H₂O = 3:1:1) to give **10** (0.22, 72%) as white solid. ¹H NMR (400 MHz, D₂O) δ 8.22 (s, 1H), 8.18 (s, 1H), 4.75 (m, 1H), 4.47 (dd, *J* = 6.0, 9.0 Hz, 1H), 3.95 (dd, *J* = 4.0, 6.0 Hz, 1H), 3.74 (t, *J* = 6.0 Hz, 1H), 2.48 (dt, *J* = 7.8, 12.9 Hz, 1H), 2.03 (m, 1H), 1.92-1.87 (m, 2H), 1.70-1.61 (m, 1H), 1.45-1.44 (m, 5H); ¹³C NMR (100.6 MHz, D₂O) δ 174.9, 154.7, 151.2, 149.0, 140.8, 118.6, 75.0, 74.6, 59.6, 54.8,

42.5, 33.2, 32.2, 30.3, 26.5, 24.2; Anal. Calcd for $C_{16}H_{24}N_6O_4 \cdot HCl \cdot 0.5H_2O$: C, 46.89; H, 6.39; N, 20.50. Found: C, 46.89; H, 6.66; N, 20.58.

References

- (1) Lustig, A.; Levine, A. J. *J. Virol.* **1992**, *66*, 4629.
- (2) Puissant, B.; Combadiere, B. *Cell. Mol. Life Sci.* **2006**, *63*, 2249.
- (3) Hovi, T.; Roivainen, M.; Blomqvist, S. *Perspect. Med. Virol.* **2007**, *17*, 69.
- (4) Lowen, A. C.; Palese, P. *Infect. Disord.: Drug Targets* **2007**, *7*, 318.
- (5) Roukens, A. H.; Visser, L. G. *Expert Opin. Biol. Ther.* **2008**, *8*, 1787.
- (6) Butler, I. F.; Pandrea, I.; Marx, P. A.; Apetrei, C. *Curr. HIV Res.* **2007**, *5*, 23.
- (7) Stroehrer, U.; Feldmann, H. *Expert Opin. Invest. Drugs* **2006**, *15*, 1523.
- (8) Haagmans, B. L.; Osterhaus, A. D. M. E. *Antiviral Res.* **2006**, *71*, 397.
- (9) Aamir, U. B.; Naeem, K.; Ahmed, Z.; Obert, C. A.; Franks, J.; Krauss, S.; Seiler, P.; Webster, R. G. *Virology* **2009**, *390*, 212.
- (10) Jones-Engel, L.; May, C. C.; Engel, G. A.; Steinkraus, K. A.; Schillaci, M. A.; Fuentes, A.; Rompis, A.; Chalise, M. K.; Aggimarangsee, N.; Feeroz, M. M.; Grant, R.; Allan, J. S.; Putra, A.; Wandia, I. N.; Watanabe, R.; Kuller, L.; Thongsawat, S.; Chaiwarith, R.; Kyes, R. C.; Linial, M. L. *Emerging Infect. Dis.* **2008**, *14*, 1200.
- (11) Malik Peiris, J. S.; Poon, L. L. M.; Guan, Y. *J. Clin. Virol.* **2009**, *45*, 169.
- (12) Flint, S. J. E., L. W.; Racaniello, V. R.; Skalka, A. M. *Principles of Virology*; ASM Press, 2009.
- (13) Dimmock, N. J. E., A. J.; Leppard, K. *Introduction to Modern Virology*; Blackwell, 2007.

- (14) Baltimore, D. *Bacteriol. Rev.* **1971**, *35*, 235.
- (15) Acheson, N. H. *Fundamentals of Molecular Virology*; John Wiley & Sons, 2006.
- (16) Henderson, D. A. *Emerg Infect Dis* **1999**, *5*, 537.
- (17) Dove, A. W.; Racaniello, V. R. *Science* **1997**, *277*, 779.
- (18) Moss, W. J.; Griffin, D. E. *Nat. Rev. Microbiol.* **2006**, *4*, 900.
- (19) Amanna, I. J.; Slifka, M. K. *Antiviral Res.* **2009**, *84*, 119.
- (20) Burnett, J. C.; Henschel, E. A.; Schmaljohn, A. L.; Bavari, S. *Nat. Rev. Drug Discovery* **2005**, *4*, 281.
- (21) Singh, M. *Virol J* **2006**, *3*, 60.
- (22) Carrat, F.; Flahault, A. *Vaccine* **2007**, *25*, 6852.
- (23) Duerr, A.; Wasserheit, J. N.; Corey, L. *Clin. Infect. Dis.* **2006**, *43*, 500.
- (24) Welsh, R. M.; Fujinami, R. S. *Nat. Rev. Microbiol.* **2007**, *5*, 555.
- (25) Allred Norma, J.; Shaw Kate, M.; Santibanez Tammy, A.; Rickert Donna, L.; Santoli Jeanne, M. *Am J Prev Med* **2005**, *28*, 221.
- (26) Gangarosa, E. J.; Galazka, A. M.; Wolfe, C. R.; Phillips, L. M.; Gangarosa, R. E.; Miller, E.; Chen, R. T. *Lancet* **1998**, *351*, 356.
- (27) Freed Gary, L.; Clark Sarah, J.; Hibbs Beth, F.; Santoli Jeanne, M. *Am J Prev Med* **2004**, *26*, 11.
- (28) Drake, J. W.; Holland, J. J. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 13910.
- (29) Holmes, E. C. *Trends Microbiol.* **2003**, *11*, 543.
- (30) Prusoff, W. H. *Biochim. Biophys. Acta* **1959**, *32*, 295.
- (31) De Clercq, E. *Adv. Virus Res.* **2009**, *73*, 1.
- (32) Castagna, A.; Biswas, P.; Beretta, A.; Lazzarin, A. *Drugs* **2005**, *65*, 879.

- (33) Citterio, P.; Rusconi, S. *Expert Opin. Invest. Drugs* **2007**, *16*, 11.
- (34) Ginesta, J. B.; Castaner, J.; Bozzo, J.; Bayes, M. *Drugs Future* **2005**, *30*, 469.
- (35) Lederman, M. M.; Penn-Nicholson, A.; Cho, M.; Mosier, D. *JAMA, J. Am. Med. Assoc.* **2006**, *296*, 815.
- (36) Overton, E. T.; Powderly, W. G. *Future Virol.* **2006**, *1*, 605.
- (37) Schols, D.; Struyf, S.; Van Damme, J.; Este, J. A.; Henson, G.; De Clercq, E. *J. Exp. Med.* **1997**, *186*, 1383.
- (38) De Clercq, E. *Mol. Pharmacol.* **2000**, *57*, 833.
- (39) Menendez-Arias, L.; Matamoros, T.; Deval, J.; Canard, B. *Drug Des. Rev.--Online* **2005**, *2*, 101.
- (40) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 7096.
- (41) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 1911.
- (42) Gao, W. Y.; Agbaria, R.; Driscoll, J. S.; Mitsuya, H. *J Biol Chem* **1994**, *269*, 12633.
- (43) Waters, L.; John, L.; Nelson, M. *Int. J. Clin. Pract.* **2006**, *61*, 105.
- (44) Wang, J.; Smerdon, S. J.; Jaeger, J.; Kohlstaedt, L. A.; Rice, P. A.; Friedman, J. M.; Steitz, T. A. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 7242.
- (45) Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; et al. *Nat. Struct. Biol.* **1995**, *2*, 293.
- (46) Grozinger, K.; Proudfoot, J.; Hargrave, K. *Drug Discovery Dev.* **2006**, *1*, 353.

- (47) Ren, J.; Milton, J.; Weaver, K. L.; Short, S. A.; Stuart, D. I.; Stammers, D. K. *Structure (London)* **2000**, *8*, 1089.
- (48) Frampton, J. E.; Croom, K. F. *Drugs* **2006**, *66*, 1501.
- (49) Vrouenraets, S. M. E.; Wit, F. W. N. M.; van Tongeren, J.; Lange, J. M. A. *Expert Opin. Pharmacother.* **2007**, *8*, 851.
- (50) Jeffries, D. J. *J. Antimicrob. Chemother.* **1989**, *23*, 29.
- (51) Alfano, M.; Poli, G. *Drug Des. Rev.--Online* **2004**, *1*, 83.
- (52) De Clercq, E. *Expert Opin. Emerging Drugs* **2005**, *10*, 241.
- (53) Uckun, F. M.; D'Cruz, O. J. *Expert Opin. Ther. Pat.* **2006**, *16*, 265.
- (54) De Clercq, E.; Field, H. J. *Antiviral Chem. Chemother.* **2008**, *19*, 75.
- (55) Ren, J.; Nichols, C.; Bird, L.; Chamberlain, P.; Weaver, K.; Short, S.; Stuart, D. I.; Stammers, D. K. *J. Mol. Biol.* **2001**, *312*, 795.
- (56) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H. *Science (Washington, D. C., 1883-)* **1989**, *245*, 616.
- (57) Stoll, V.; Qin, W.; Stewart, K. D.; Jakob, C.; Park, C.; Walter, K.; Simmer, R. L.; Helfrich, R.; Bussiere, D.; Kao, J.; Kempf, D.; Sham, H. L.; Norbeck, D. W. *Bioorg. Med. Chem.* **2002**, *10*, 2803.
- (58) Byrd, C. M.; Hruby, D. E. *Drug Dev. Res.* **2006**, *67*, 501.
- (59) Mastrolorenzo, A.; Rusconi, S.; Scozzafava, A.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2006**, *16*, 1067.
- (60) Tan, D.; Walmsley, S. *Expert Rev. Anti-Infect. Ther.* **2007**, *5*, 13.

- (61) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; et al. *Science (Washington, D. C., 1883-)* **1990**, *248*, 358.
- (62) Kim, C. U.; Lew, W.; Williams, M. A.; Zhang, L.; Liu, H.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Tai, C. Y.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681.
- (63) Colman, P. *Struct.-Based Drug Discovery* **2006**, 193.
- (64) von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan Tho, V.; Smythe, M. L.; White, H. F.; et al. *Nature (London)* **1993**, *363*, 418.
- (65) Laver, G. *Future Virol.* **2006**, *1*, 577.
- (66) Russell, R. J.; Haire, L. F.; Stevens, D. J.; Collins, P. J.; Lin, Y. P.; Blackburn, G. M.; Hay, A. J.; Gamblin, S. J.; Skehel, J. J. *Nature (London, U. K.)* **2006**, *443*, 45.
- (67) Porkka-Heiskanen, T.; Strecker, R. E.; Thakkar, M.; Bjorkum, A. A.; Greene, R. W.; McCarley, R. W. *Science (Washington, D. C.)* **1997**, *276*, 1265.
- (68) Isono, K. *J. Antibiot.* **1988**, *41*, 1711.
- (69) De Clercq, E. *Nucleosides Nucleotides* **1994**, *13*, 1271.
- (70) Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 517.
- (71) Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, S. R.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611.
- (72) Huryn, D. M.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745.
- (73) Marquez, V. E.; Lim, M. I. *Med. Res. Rev.* **1986**, *6*, 1.
- (74) Schabel, F. M., Jr.; Montgomery, J. A. *Chemotherapy of virus diseases*; Pergamon Press, Oxford, 1972.

- (75) De Clercq, E. *Clin. Microbiol. Rev.* **2001**, *14*, 382.
- (76) De Clercq, E.; Field, H. J. *Br. J. Pharmacol.* **2006**, *147*, 1.
- (77) De Clercq, E. *Nat. Rev. Drug Discovery* **2002**, *1*, 13.
- (78) De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115.
- (79) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229.
- (80) Zhu, X.-F. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 651.
- (81) Marquez, V. E. *Adv. Antiviral Drug Des.* **1996**, *2*, 89.
- (82) Rodriguez, J. B.; Comin, M. J. *Mini-Rev. Med. Chem.* **2003**, *3*, 95.
- (83) Fulmer Shealy, Y.; Clayton, J. D. *J. Am. Chem. Soc.* **1966**, *88*, 3885.
- (84) Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. *J. Antibiot. (Tokyo)* **1968**, *21*, 255.
- (85) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359.
- (86) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675.
- (87) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. *Adv. Antiviral Drug Des.* **1996**, *2*, 41.
- (88) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521.
- (89) Leyssen, P.; De Clercq, E.; Neyts, J. *Antiviral Res.* **2008**, *78*, 9.
- (90) Isaacs, A.; Lindenmann, J. *Proc R Soc Lond B Biol Sci* **1957**, *147*, 258.
- (91) Manns, M. P.; Foster, G. R.; Rockstroh, J. K.; Zeuzem, S.; Zoulim, F.; Houghton, M. *Nat. Rev. Drug Discovery* **2007**, *6*, 991.
- (92) Feld, J. J.; Hoofnagle, J. H. *Nature (London, U. K.)* **2005**, *436*, 967.
- (93) Hoofnagle, J. H.; Seeff, L. B. *N. Engl. J. Med.* **2006**, *355*, 2444.

- (94) Banerjee, A. K. *Microbiol. Rev.* **1980**, *44*, 175.
- (95) Moss, B.; Gershowitz, A.; Wei, C.-M.; Boone, R. *Virology* **1976**, *72*, 341.
- (96) Furuichi, Y.; Muthukrishnan, S.; Tomasz, J.; Shatkin, A. J. *J. Biol. Chem.* **1976**, *251*, 5043.
- (97) Banerjee, A. D.; Abraham, G.; Colonno, R. J. *J Gen Virol* **1977**, *34*, 1.
- (98) Krug, R. M.; Broni, B. A.; Bouloy, M. *Cell (Cambridge, Mass.)* **1979**, *18*, 329.
- (99) Jones, P. A.; Takai, D. *Science (Washington, DC, U. S.)* **2001**, *293*, 1068.
- (100) Both, G. W.; Furuichi, Y.; Muthukrishnan, S.; Shatkin, A. J. *Cell* **1975**, *6*, 185.
- (101) Furuichi, Y.; LaFiandra, A.; Shatkin, A. J. *Nature (London)* **1977**, *266*, 235.
- (102) Shuman, S. *Prog. Nucleic Acid Res. Mol. Biol.* **1995**, *50*, 101.
- (103) Eiden, L. E., Borchardt, R. T. & Rutledge, C. O *Transmethylation* Elsevier, Amsterdam, 1979.
- (104) Mandell, A. J.; Morgan, M. *Nature (London), New Biol.* **1971**, *230*, 85.
- (105) Hirata, F.; Axelrod, J. *Science (Washington, D. C.)* **1980**, *209*, 1082.
- (106) Wyatt, G. R. *Biochem. J.* **1951**, *48*, 584.
- (107) Drahovsky, D.; Boehm, T. L. *J. Int. J. Biochem.* **1980**, *12*, 523.
- (108) Simon, D.; Grunert, F.; Von Acken, U.; Doering, H. P.; Kroeger, H. *Nucleic Acids Res.* **1978**, *5*, 2153.
- (109) F. Salvatore, E. B., V. Zappia, H.G. Williams-Ashman, and F. Schlenk *The Biochemistry of adenosylmethionine* Columbia University Press, 1977.
- (110) V. Zappia, E. U., F. Salvatore *Biochemical and Pharmacological Roles of Adenosylmethionine and the central Nervous System*; Pergamon Press, Oxford, 1979.
- (111) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1976**, *45*, 285.

- (112) Ohashi, Z.; Maeda, M.; McCloskey, J. A.; Nishimura, S. *Biochemistry* **1974**, *13*, 2620.
- (113) Knappe, J.; Schmitt, T. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 1110.
- (114) Cantoni, G. L.; Scarano, E. *J. Am. Chem. Soc.* **1954**, *76*, 4744.
- (115) Salvatore, F. B., E.; Zappia, V.; Willams-Ashman, H. G.; Schlenk, F.; *The biochemistry of S-Adenosylmethionine* Columbia University Press, New York, 1977.
- (116) Borchardt, R. T. C., C. R.; Ueland, P. M.; *Biological Methylation and Drug Design*; Humana Press: Clifton, NJ., 1986.
- (117) Gibson, K. D.; Wilson, J. D.; Udenfriend, S. *J. Biol. Chem.* **1961**, *236*, 673.
- (118) Coward, J. K.; Slisz, E. P.; Wu, F. Y. H. *Biochemistry* **1973**, *12*, 2291.
- (119) Cox, R.; Prescott, C.; Irving, C. C. *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.* **1977**, *474*, 493.
- (120) Deguchi, T.; Barchas, J. *J. Biol. Chem.* **1971**, *246*, 3175.
- (121) Glick, J. M.; Ross, S.; Leboy, P. S. *Nucleic Acids Res.* **1975**, *2*, 1639.
- (122) Hoffman, D. R.; Haning, J. A.; Cornatzer, W. E. *Lipids* **1981**, *16*, 561.
- (123) Ueland, P. M. *Pharmacol. Rev.* **1982**, *34*, 223.
- (124) Liu, S.; Wolfe, M. S.; Borchardt, R. T. *Antiviral Res.* **1992**, *19*, 247.
- (125) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. *Cell Biochem. Biophys.* **2000**, *33*, 101.
- (126) De Clercq, E.; Cools, M. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 306.
- (127) Palmer, J. L.; Abeles, R. H. *J Biol Chem* **1976**, *251*, 5817.
- (128) Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1979**, *254*, 1217.
- (129) De la Haba, G.; Cantoni, G. L.; Richards, H. H. *J. Biol. Chem.* **1959**, *234*, 603.

- (130) Guranowski, A.; Montgomery, J. A.; Cantoni, G. L.; Chiang, P. K. *Biochemistry* **1981**, *20*, 110.
- (131) Hill, D. L.; Straight, S.; Allan, P. W.; Bennett, L. L., Jr. *Mol. Pharmacol.* **1971**, *7*, 375.
- (132) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. *J. Biol. Chem.* **1984**, *259*, 4353.
- (133) De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84.
- (134) Saunders, P. P.; Tan, M. T.; Robins, R. K. *Biochem. Pharmacol.* **1985**, *34*, 2749.
- (135) Glazer, R. I.; Knode, M. C. *J. Biol. Chem.* **1984**, *259*, 12964.
- (136) Whaun, J. M.; Miura, G. A.; Brown, N. D.; Gordon, R. K.; Chiang, P. K. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 277.
- (137) Bennett, L. L., Jr.; Allan, P. W.; Hill, D. L. *Mol. Pharmacol.* **1968**, *4*, 208.
- (138) Linevsky, J.; Cohen, M. B.; Hartman, K. D.; Knode, M. C.; Glazer, R. I. *Mol. Pharmacol.* **1985**, *28*, 45.
- (139) Keller, B. T.; Borchardt, R. T. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 131.
- (140) Ramakrishnan, V.; Borchardt, R. T. *Neurochem. Int.* **1987**, *10*, 423.
- (141) Glazer, R. I.; Knode, M. C.; Tseng, C. K. H.; Haines, D. R.; Marquez, V. E. *Biochem. Pharmacol.* **1986**, *35*, 4523.
- (142) Inaba, M.; Nagashima, K.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1986**, *46*, 1063.
- (143) Paisley, S. D.; Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1989**, *32*, 1415.
- (144) Matuszewska, B.; Borchardt, R. T. *J. Biol. Chem.* **1987**, *262*, 265.
- (145) Yin, D.; Yang, X.; Hu, Y.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T.; Squier, T. C. *Biochemistry* **2000**, *39*, 9811.

- (146) Bennett, L. L., Jr.; Brockman, R. W.; Rose, L. M.; Allan, P. W.; Shaddix, S. C.; Shealy, Y. F.; Clayton, J. D. *Mol. Pharmacol.* **1985**, *27*, 666.
- (147) Bennett, L. L., Jr.; Allan, P. W.; Rose, L. M.; Comber, R. N.; Secrist, J. A., III *Mol. Pharmacol.* **1986**, *29*, 383.
- (148) Bray, M.; Driscoll, J.; Huggins, J. W. *Antiviral Res.* **2000**, *45*, 135.
- (149) Bray, M.; Raymond, J. L.; Geisbert, T.; Baker, R. O. *Antiviral Res.* **2002**, *55*, 151.
- (150) Carter, W. A.; De Clercq, E. *Science* **1974**, *186*, 1172.
- (151) Bray, M. *Antiviral Res.* **2003**, *57*, 53.
- (152) De Clercq, E. *Nat. Rev. Microbiol.* **2004**, *2*, 704.
- (153) Montgomery, J. A.; Clayton, S. J.; Thomas, H. J.; Shannon, W. M.; Arnett, G.; Bodner, A. J.; Kion, I. K.; Cantoni, G. L.; Chiang, P. K. *J. Med. Chem.* **1982**, *25*, 626.
- (154) Tseng, C. K. H.; Marquez, V. E.; Fuller, R. W.; Goldstein, B. M.; Haines, D. R.; McPherson, H.; Parsons, J. L.; Shannon, W. M.; Arnett, G.; et al. *J. Med. Chem.* **1989**, *32*, 1442.
- (155) Bloch, A.; Robins, M. J.; McCarthy, J. R., Jr. *J. Med. Chem.* **1967**, *10*, 908.
- (156) Borcharding, D. R.; Scholtz, S. A.; Borchardt, R. T. *J. Org. Chem.* **1987**, *52*, 5457.
- (157) De Clercq, E.; Cools, M.; Balzarini, J.; Marquez, V. E.; Borcharding, D. R.; Borchardt, R. T.; Drach, J. C.; Kitaoka, S.; Konno, T. *Antimicrob. Agents Chemother.* **1989**, *33*, 1291.
- (158) Hasobe, M.; McKee, J. G.; Borcharding, D. R.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1987**, *31*, 1849.
- (159) Wolfe, M. S.; Lee, Y.; Bartlett, W. J.; Borcharding, D. R.; Borchardt, R. T. *J. Med. Chem.* **1992**, *35*, 1782.

- (160) Siddiqi, S. M.; Schneller, S. W. *Nucleosides Nucleotides* **1993**, *12*, 185.
- (161) Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 551.
- (162) Siddiqi, S. M.; Chen, X.; Schneller, S. W. *Nucleosides Nucleotides* **1993**, *12*, 267.
- (163) Jones, M. F.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1 (1972-1999)* **1988**, 2927.
- (164) Kapeller, H.; Baumgartner, H.; Griengl, H. *Monatsh. Chem.* **1997**, *128*, 191.
- (165) Yang, M.; Schneller, S. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 149.
- (166) Shuto, S.; Obara, T.; Saito, Y.; Andrei, G.; Snoeck, R.; De Clercq, E.; Matsuda, A. *J. Med. Chem.* **1996**, *39*, 2392.
- (167) Yang, M.; Schneller, S. W.; Korba, B. *J. Med. Chem.* **2005**, *48*, 5043.
- (168) Thibaudeau, C.; Kumar, A.; Bekiroglu, S.; Matsuda, A.; Marquez, V. E.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 5447.
- (169) Aoyagi, M.; Minakawa, N.; Matsuda, A. *Tetrahedron Lett.* **1993**, *34*, 103.
- (170) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739.
- (171) Rodriguez, J. B.; Marquez, V. E.; Nicklaus, M. C.; Mitsuya, H.; Barchi, J. J., Jr. *J. Med. Chem.* **1994**, *37*, 3389.
- (172) Tavale, S. S.; Sobell, H. M. *J Mol Biol* **1970**, *48*, 109.
- (173) Minakawa, N.; Kojima, N.; Matsuda, A. *J. Org. Chem.* **1999**, *64*, 7158.
- (174) Kitade, Y.; Kozaki, A.; Miwa, T.; Nakanishi, M. *Tetrahedron* **2002**, *58*, 1271.
- (175) Chiang, P. K.; Richards, H. H.; Cantoni, G. L. *Mol. Pharmacol.* **1977**, *13*, 939.

- (176) Glazer, R. I.; Hartman, K. D.; Knode, M. C.; Richard, M. M.; Chiang, P. K.; Tseng, C. K. H.; Marquez, V. E. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 688.
- (177) Siddiqi, S. M.; Chen, X.; Rao, J.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1995**, *38*, 1035.
- (178) Seley, K. L.; Schneller, S. W.; Korba, B. *Nucleosides Nucleotides* **1997**, *16*, 2095.
- (179) Shuto, S.; Minakawa, N.; Niizuma, S.; Kim, H.-S.; Wataya, Y.; Matsuda, A. *J. Med. Chem.* **2002**, *45*, 748.
- (180) Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 324.
- (181) Michelot, R. J.; Lesko, N.; Stout, R. W.; Coward, J. K. *Mol. Pharmacol.* **1977**, *13*, 368.
- (182) Robert-Gero, M.; Lawrence, F.; Farrugia, G.; Berneman, A.; Blanchard, P.; Vigier, P.; Lederer, E. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 1242.
- (183) Duerre, J. A.; Miller, C. H.; Reams, G. G. *J. Biol. Chem.* **1969**, *244*, 107.
- (184) Borchardt, R. T. *J. Med. Chem.* **1980**, *23*, 347.
- (185) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Org. Chem.* **1976**, *41*, 565.
- (186) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. *J. Med. Chem.* **1976**, *19*, 1094.
- (187) Borchardt, R. T.; Wu, Y. S. *J. Med. Chem.* **1974**, *17*, 862.
- (188) Borchardt, R. T.; Wu, Y. S.; Wu, B. S. *J. Med. Chem.* **1978**, *21*, 1307.
- (189) Chang, C.-D.; Coward, J. K. *J. Med. Chem.* **1976**, *19*, 684.
- (190) Coward, J. K.; Bussolotti, D. L.; Chang, C.-D. *J. Med. Chem.* **1974**, *17*, 1286.
- (191) Coward, J. K.; Slisz, E. P. *J. Med. Chem.* **1973**, *16*, 460.
- (192) Coward, J. K.; Sweet, W. D. *J. Med. Chem.* **1972**, *15*, 381.

- (193) Vuilhorgne, M.; Blanchard, P.; Hedgecock, C. J. R.; Lawrence, F.; Robert-Gero, M.; Lederer, E. *Heterocycles* **1978**, *11*, 495.
- (194) Hamill, R. L.; Hoehn, M. M. *J. Antibiot.* **1973**, *26*, 463.
- (195) Gordee, R. S.; Butler, T. F. *J. Antibiot.* **1973**, *26*, 466.
- (196) Vedel, M.; Lawrence, F.; Robert-Gero, M.; Lederer, E. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 371.
- (197) Vedel, M.; Robert-Gero, M. *FEBS Lett.* **1981**, *128*, 87.
- (198) Pugh, C. S. G.; Borchardt, R. T. *Biochemistry* **1982**, *21*, 1535.
- (199) Pugh, C. S. G.; Borchardt, R. T.; Stone, H. O. *J. Biol. Chem.* **1978**, *253*, 4075.
- (200) Fuller, R. W.; Nagarajan, R. *Biochem. Pharmacol.* **1978**, *27*, 1981.
- (201) Martin, S. A.; Moss, B. *J. Biol. Chem.* **1975**, *250*, 9330.
- (202) Yin, X.; Zhao, G.; Schneller, S. W. *Tetrahedron Lett.* **2007**, *48*, 4809.
- (203) Jiang May, X.-W.; Jin, B.; Gage Jennifer, L.; Priour, A.; Savela, G.; Miller Marvin, J. *J Org Chem* **2006**, *71*, 4164.
- (204) Johnson, C. R.; Bis, S. J. *Tetrahedron Lett.* **1992**, *33*, 7287.
- (205) Deardorff, D. R.; Matthews, A. J.; McMeekin, D. S.; Craney, C. L. *Tetrahedron Lett.* **1986**, *27*, 1255.
- (206) Deardorff, D. R.; Myles, D. C.; MacFerrin, K. D. *Tetrahedron Lett.* **1985**, *26*, 5615.
- (207) Yin, X.-Q.; Rajappan, V. P.; Roy, A.; Schneller, S. W. *Synth. Commun.* **2003**, *33*, 1477.
- (208) Yang, M.; Ye, W.; Schneller, S. W. *J. Org. Chem.* **2004**, *69*, 3993.
- (209) Palmer, A. M.; Jager, V. *Eur. J. Org. Chem.* **2001**, 1293.

- (210) Paquette, L. A.; Bailey, S. *J. Org. Chem.* **1995**, *60*, 7849.
- (211) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.
- (212) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606.
- (213) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100.
- (214) Wolfe, M. S.; Anderson, B. L.; Borcharding, D. R.; Borchardt, R. T. *J. Org. Chem.* **1990**, *55*, 4712.
- (215) Koppel, H. C.; Robins, R. K. *J. Org. Chem.* **1958**, *23*, 1457.
- (216) Yang, M.; Zhou, J.; Schneller, S. W. *Tetrahedron* **2006**, *62*, 1295.
- (217) Jacquemard, U.; Beneteau, V.; Lefoix, M.; Routier, S.; Merour, J.-Y.; Coudert, G. *Tetrahedron* **2004**, *60*, 10039.
- (218) Coutant, C.; Jacquinet, J.-C. *J. Chem. Soc., Perkin Transactions I: Organic and Bio-Organic Chemistry* **1995**, 1573.
- (219) Routier, S.; Sauge, L.; Ayerbe, N.; Coudert, G.; Merour, J.-Y. *Tetrahedron Letters* **2002**, *43*, 589.
- (220) Dang Vu, A.; Olofson, R. A. *J. Org. Chem.* **1990**, *55*, 1851.
- (221) Seley, K. L.; Mosley, S. L.; Zeng, F. *Organic Letters* **2003**, *5*, 4401.
- (222) Lim, M. I.; Marquez, V. E. *Tetrahedron Letters* **1983**, *24*, 5559.
- (223) Hill, J. M.; Hutchinson, E. J.; LeGrand, D. M.; Roberts, S. M.; Thorpe, A. J.; Turner, N. J. *J. Chem. Soc., Perkin Transactions I: Organic and Bio-Organic Chemistry* **1994**, 1483.
- (224) Marquez, V. E.; Lim, M. I.; Tseng, C. K. H.; Markovac, A.; Priest, M. A.; Khan, M. S.; Kaskar, B. *J. Org. Chem.* **1988**, *53*, 5709.

- (225) Torrence, P. F. *Antiviral Drug Discovery for Emerging Diseases and Bioterrorism Threats*; John Wiley & Sons, Inc.: New Jersey, 2005.
- (226) Cho, J. H.; Bernard, D. L.; Sidwell, R. W.; Kern, E. R.; Chu, C. K. *J. Med. Chem.* **2006**, *49*, 1140.
- (227) Elson, K. E.; Jenkins, I. D.; Loughlin, W. A. *Org. & Biomol.Chem.* **2003**, *1*, 2958.
- (228) Yin, X.-q.; Schneller, S. W. *Tetrahedron* **2005**, *61*, 1839.
- (229) Merlo, V.; Reece, F. J.; Roberts, S. M.; Gregson, M.; Storer, R. *J. Chem. So., Perkin Transactions 1: Organic and Bio-Organic Chemistry* **1993**, 1717.
- (230) Yin, X.-q.; Schneller, S. W. *Tetrahedron Letters* **2005**, *46*, 7535.
- (231) Matsuumi, M.; Ito, M.; Kobayashi, Y. *Synlett* **2002**, 1508.
- (232) Sibi, M. P.; Rutherford, D.; Renhowe, P. A.; Li, B. *J. Am. Chem. Soc.* **1999**, *121*, 7509.
- (233) McKillop, A.; Taylor, R. J. K.; Watson, R. J.; Lewis, N. *Synthesis* **1994**, 31.
- (234) Schols, D.; De Clercq, E.; Balzarini, J.; Baba, M.; Witvrouw, M.; Hosoya, M.; Andrei, G.; Snoeck, R.; Neyts, J.; et al. *Antiviral Chemistry & Chemotherapy* **1990**, *1*, 233.
- (235) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature (London, United Kingdom)* **1986**, *323*, 464.
- (236) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dise.* **1980**, *141*, 563.
- (237) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Molecular Pharmacology* **1981**, *19*, 321.

- (238) Laumen, K.; Schneider, M. *Tetrahedron Letters* **1984**, *25*, 5875.
- (239) Jin, Y. H.; Liu, P.; Wang, J.; Baker, R.; Huggins, J.; Chu, C. K. *J. Org. Chem.* **2003**, *68*, 9012.
- (240) Johnson, C. R.; Chen, Y. F. *J. Org. Chem.* **1991**, *56*, 3344.
- (241) Reich, M. F.; Fabio, P. F.; Lee, V. J.; Kuck, N. A.; Testa, R. T. *J. Med. Chem.* **1989**, *32*, 2474.
- (242) Kruger, S.; Mann, F. G. *J. Chem. Soc.* **1955**, 2755.
- (243) Houston, D. M.; Dolence, E. K.; Keller, B. T.; Patel-Thombre, U.; Borchardt, R. *J. Med. Chem.* **1985**, *28*, 467.
- (244) Bakke, J. M.; Riha, J. *J. Hetero. Chem.* **1999**, *36*, 1143.
- (245) Danishefsky, S. J.; Paz Cabal, M.; Chow, K. *J. Am. Chem. Soc.* **1989**, *111*, 3456.
- (246) Curran, T. T.; Hay, D. A.; Koegel, C. P. *Tetrahedron* **1997**, *53*, 1983.
- (247) Bhuniya, D.; DattaGupta, A.; Singh, V. K. *J. Org. Chem.* **1996**, *61*, 6108.