DESIGN AND SYNTHESIS OF ADOHCY HYDROLASE INHIBITORS AS A

SOURCE OF ANTIVIRAL AGENTS

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DESIGN AND SYNTHESIS OF ADOHCY HYDROLASE INHIBITORS AS A SOURCE OF ANTIVIRAL AGENTS

Chong Liu

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DESIGN AND SYNTHESIS OF ADOHCY HYDROLASE INHIBITOR AS A SOURCE OF ANTIVIRAL AGENTS

Chong Liu

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VITA

Chong Liu, son of Maorong Liu and Kexiu Wang, was born in Mengyin county, Shandong province, China, on July 14, 1974. After graduating from high school, he began his studies at Jiangsu Institute of Chemical Technology in September 1993 and received a Bachelor degree in applied chemistry in July 1997. He attended East China University of Science and Technology in September 1997 and obtained a Master degree in applied chemistry in July 2000. He worked as a research chemist at Shanghai Institute of Organic Chemistry and then FMC (Shanghai) from August 2000 to July 2003. He began his Ph.D studies in chemistry under the direction of Prof. S. W. Schneller at Auburn University in August 2003. He is married to Qi Chen, daughter of Yongkang Chen and Xiaokui Mao.

DISSERTATION ABSTRACT

DESIGN AND SYNTHESIS OF ADOHCY HYDROLASE INHIBITOR AS A SOURCE OF ANTIVIRAL AGENTS

Chong Liu

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The significant antiviral properties of the carbocyclic nucleosides aristeromycin and neplanocin A have been attributed to inhibition of AdoHcy hydrolase, which in turn affects viral mRNA capping methylation. However, their antiviral potential is limited due to toxicity, for most part, from phosphorylation of the primary hydroxyl group at the 5′ position. 5′-Noraristeromycin and 3-deazapurine carbocyclic nucleosides (3-deazaneplanocin A and 3-deazaaristeromycin) have been found to have retained antiviral activity with significant reduction of toxicity as a result of their inability to undergo phosphorylation. To further exploit the 5′-nor and 3-deaza carbocyclic nucleoside

platform as a source for new antiviral candidates, modifications at the C-3 position have been recognized as important means to promising compounds. 3-Deaza-5′-noraristeromycin derivatives possessing a halo atom (**1**-**3**) at the C-3 position have been synthesized and evaluated. 3-Chloro-3-deaza-5'-noraristeromycin (**1**) exhibits activity against heptatitis C virus (HCV). Meanwhile, 3-bromo-3-deaza-5'-noraristeromycin (**2**) and 3-iodo-3-deaza-5'-noraristeromycin (**3**) display marked activity against heptatitis B virus (HBV). Compound **1**, **2**, and **3** were also found to have a wide variety of other biological properties. As a logical extension of the 3-halo derivatives, 3-methy-3-deaza-5'-noraristeromycin (**4**) has been identified as an important target and prepared. Compound **4** only showed good activity against vesicular stomatitis virus (VSV) and vaccinia virus (VV), and affected none of the other viruses assayed. Derivatives of 3-deazaneplancin A possessing bromo (**5**) or methyl (**6**) groups at the C-3 position were sought as important targets. A convergent synthesis of this series of compounds employing Mitsunobu coupling was studied. Precedent suggested the derivative (**7**) of 3-deazaneplancin A which lacks the C-4' hydroxymethyl, would also be relevant to this study. Compounds **5**, **6**, and **7** were prepared and their bioassay is under study.

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INTRODUCTION

Virus infections have caused serious public health problems worldwide during the last few decades. The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) have reported continuous emergence of new and reemerging infectious diseases. In the recent 35 years, more than 50 new pathogens, which caused human diseases, have been identified. [1-3]

Table 1: Emerging Viruses in Humans[4]

1973: Rotavirus
1975: Parvovirus B19
1977: Ebola virus, Hantavirus
1980: Human T-lymphotrophic virus type I (HTLV-I)
1982: Human T-lymphotrophic virus type II (HTLV-II)
1983: Human immunodeficiency virus (HIV)
1988: Human herpesvirus-6 (HHV-6), hepatitis E virus
1989: Hepatitis C virus (HCV)
1991: Guanarito virus
1993: Sin Nombre virus
1994: Sabia virus, Hendra virus
1995: Human Herpesvirus 8 (HHV-8)

1997: Avian influenza virus (H5N1)

1999: Nipah virus, West Nile virus (in U.S.A.)

2001: Human metapneumovirus

2003: Severe acute respiratory syndrome coronavirus (SARS)

Despite the great effort and progress in vaccine development to protect against the effects of virus infection, a number of issues must be resolved. The first one is the accompanying side effects of some existing vaccines such as the hepatitis B virus (HBV) vaccine.[5] The other issue is that there are no vaccines available for combating viruses such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), and Ebola and Marburg viruses but they are urgently needed. [4-10]

Because of the possible undesirable effects of vaccines, difficulties in widespread distribution, and lack of vaccine candidates in some cases, the need for developing new antiviral drug candidates is essential. Within that group nucleoside derivatives have been set as one of the major areas of pursuit.

To grasp an appreciation for nucleoside derivatives as antiviral agents, it is useful to start with comments on the structure and function of naturally occurring nucleosides, namely adenosine, guanosine, cytidine, uridine, and thymidine (Figure 1). The phosphate esters of nucleosides, which are called nucleotides are the monomeric units degenerated from nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It's known that DNA and RNA are the molecules that preserve hereditary information (DNA) and that transcribe and translate (RNA) it in a way that the synthesis of all various proteins of cells can be achieved. Besides the importance of working as building blocks

of nucleic acids, natural nucleosides also play important roles in fundamental metabolism. For example, as a key component of ATP, NAD^+ (nicotinamide adenosine dinucleotide) and coenzyme A, adenosine is necessary for essential biological process.[11,12]

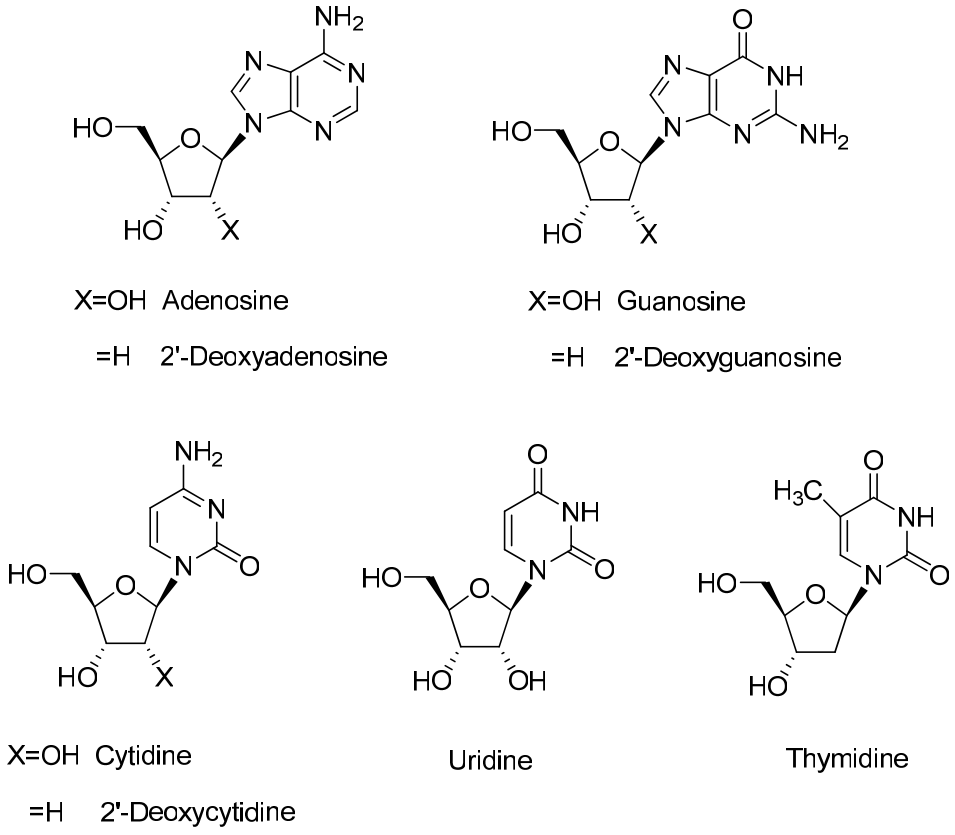


Figure 1. Naturally occurring nucleosides

Traditional nucleoside derivatives

Because of the importance of nucleosides for virus replication, structure modification has been pursued as an important way for developing compounds possessing a variety of biological effects.[13,14] Natural nucleosides all contain D-ribose or 2'-deoxy-D-ribose as their sugar components and either adenine, guanine, cytosine, uracil or thymine as their

heterocyclic base (Figure 1). The structure modification to find new nucleosides with potential antiviral activity can either occur on the heterocyclic base or the sugar moiety.[15] Since 5-iodo-2'-deoxyuridine (Figure 2) was discovered to have antiherpes activity in the 1950's,[16] a great number of nucleoside derivatives have been synthesized and tested for antiviral activities. Below are some important examples among which 5-iodo-2'-deoxyuridine, dideoxyinosine, acyclovir, and ribavirin have been formally approved and thus are currently available for clinical use. (Figure 2)

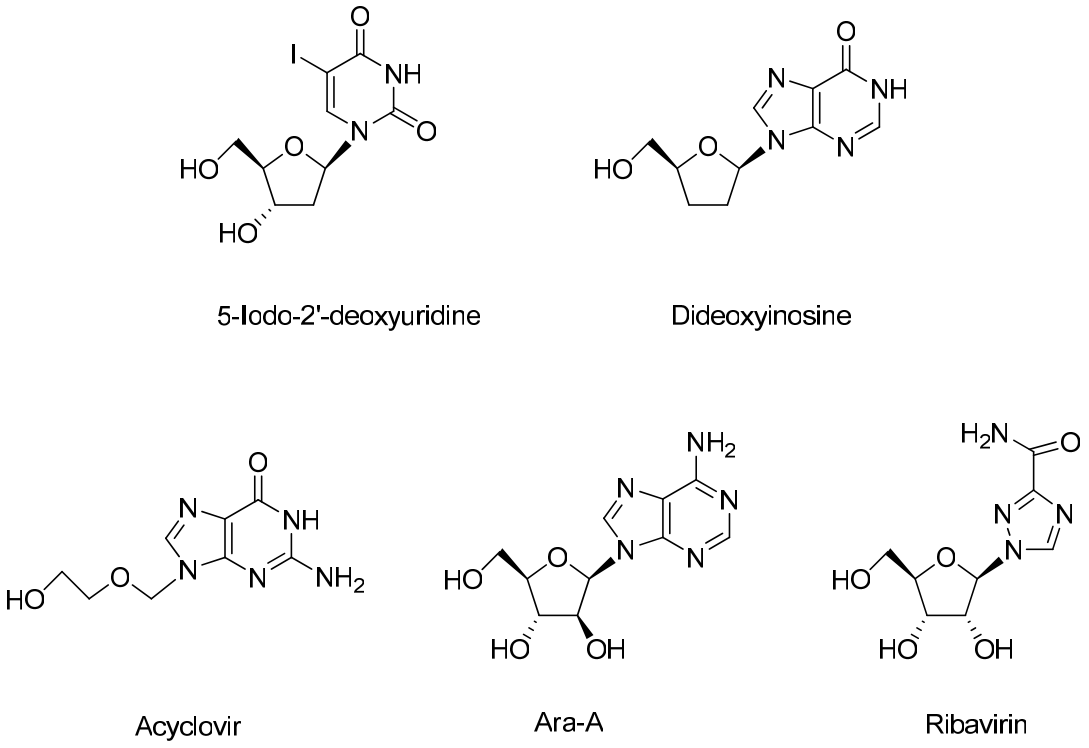
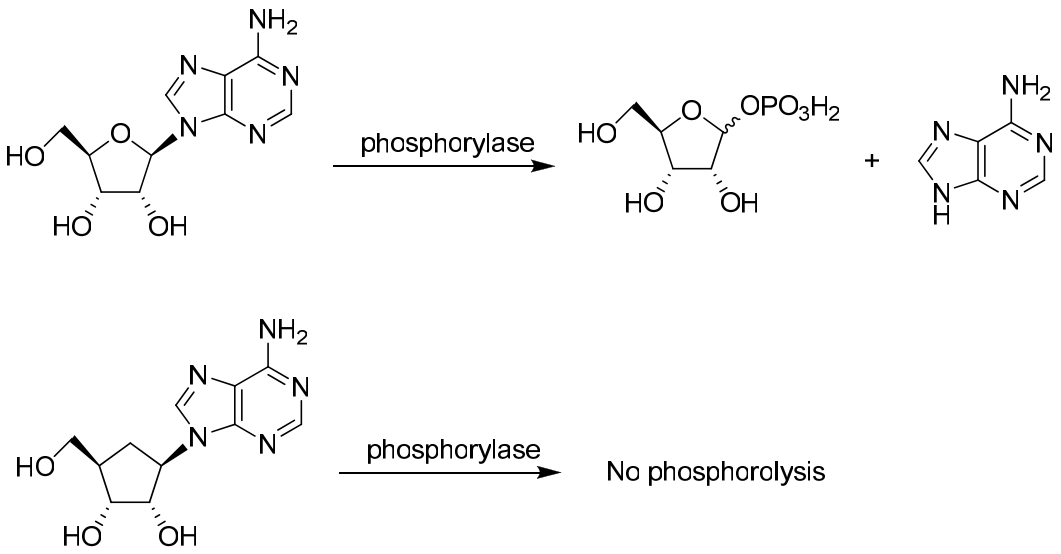


Figure 2. Nucleosides with antiviral activity

Dideoxyinosine has been approved as drug against AIDS.[17] 5-Iodo-2'-deoxyuridine and acyclovir are used for the treatment of herpes virus infections.[18,19] Ara-A showed efficacy in the treatment of vaccinia.[20] Finally ribavirin is another approved drug whose broad-spectrum antiviral activity includes the orthopox viruses[21] and hepatitis C virus (HCV).[22]

Development of carbocyclic nucleosides

However, further investigation showed apparent accompanying side-effects such as toxicity and drug-resistance for traditional nucleoside derivatives during medical use.[23] Among following modifications on the structure, an important series has arisen: carbocyclic nucleosides in which the endocyclic oxygen atom of traditional nucleosides is replaced by a methylene group. This modification is relevant because of the instability of the N-glycosidic bond of the more common nucleosides to phosphorylases that cleave the heterocyclic base to the sugar moiety linkage. Phosphorolysis produces 1'-phosphoribose and the heterocyclic base, which makes it difficult for active nucleosides to be delivered intact to the viral target (Scheme 1).[23] Carbocyclic nucleosides provide a means to avoid the undesired phosphorolysis due to their much more stable C-N bond towards phosphorylases.[24,25] Another advantage of carbocyclic nucleosides is their higher lipophilicity, which is important for oral uptake and cellular penetration.[26,27] In addition to these properties, the similarity between the cyclopentane and the tetrahydrofuran ring makes carbocyclic nucleosides recognizable by enzymes that customarily call on natural nucleosides as substrates.[28]



Scheme 1. Samples of stability of nucleosides and carbocyclic nucleosides against phosphorylase

With the optimistic interest in carbocyclic nucleosides, various derivatives were isolated from nature or synthesized in the lab over the last few decades. Many of the compounds were found to have broad-spectrum or specific antiviral activity. Figure 3 presents examples of carbocyclic nucleosides which have been found to have therapeutic potential such as abacavir and carbovir (anti-HIV activity),[29,30] entecavir (anti-HBV activity),[31-33] and carboxentanocin G (anti-HIV activity).[34]

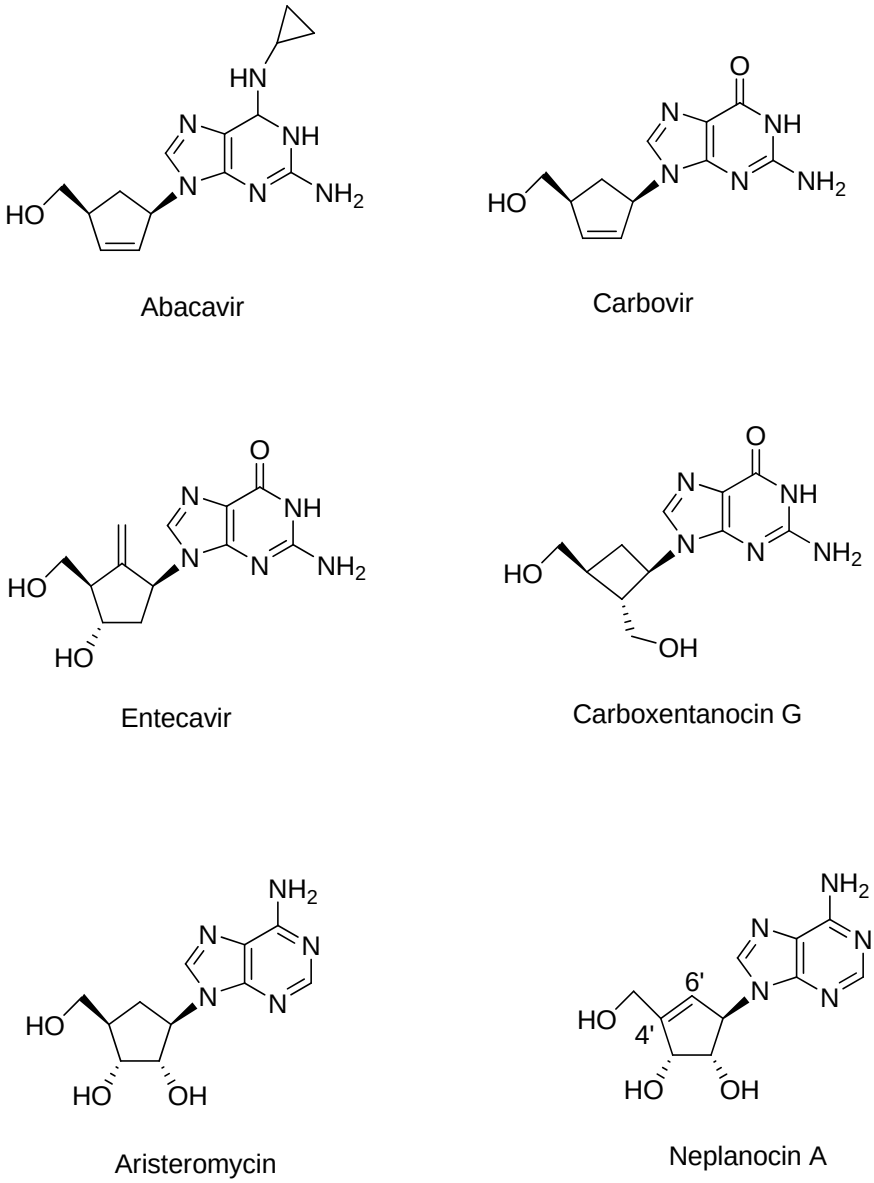


Figure 3. Carbocyclic nucleosides with antiviral activity

Two of the early and most important carbocyclic nucleosides are aristeromycin (Ari) and neplanocin A (NpcA), which showed meaningful broad-spectrum antiviral activity. These two derivatives are carbocyclic analogues of adenosine with the only difference being the presence of a double bond between the C-4′ and C-6′ on the cyclopentyl ring of

neplanocin A. They were first found in nature. Aristeromycin was isolated from a metabolite of *Steptomyces citricolor* in 1968,[35] while neplanocin A was obtained from the culture broth of *Ampullariella regularis* in 1981.[36] Both of the compounds showed effective antiviral activity against, for example, poxvirus, reovirus, smallpox virus.[35-37] Their antiviral effects have been attributed to inhibition of *S*-adenosyl-L-homocysteine (SAH) hydrolase, which in turn affects viral mRNA capping methylation.[38-40] Although NpcA and Ari are two of the most potent AdoHcy hydrolase inhibitors with broad-spectrum antiviral activity, they are cytotoxic to host cells. That renders them unacceptable for antiviral therapeutic development.[41-43] The toxicity of Ari and NpcA derivatives, for the most part, from phosphorylation of the primary hydroxyl group at their 5′-position by adenosine kinase with subsequent metabolism by cellular enzymes. Using Ari as an example, adenosine kinase metabolizes it to Ari monophosphate. Following this phosphorylation, Ari monophosphate is acted upon by adenylate kinase and nucleoside diphosphokinase to yield Ari diphosphate and then Ari triphosphate (Figure 4), respectively, which can interfere with the metabolic processes involving ATP utilization. This arises because of its resemblance to the structure of ATP. This represents the unexpected toxicity.[41,44-47] A similar procedure occurs with NpcA (Figure 4).[40-43,48] Ari and NpcA are also rapidly deaminated by Ado deaminase to a chemotherapeutically inactive inosine congener,[49] which may account for the reduced therapeutic potency of NpcA, especially *in vivo*.[48]

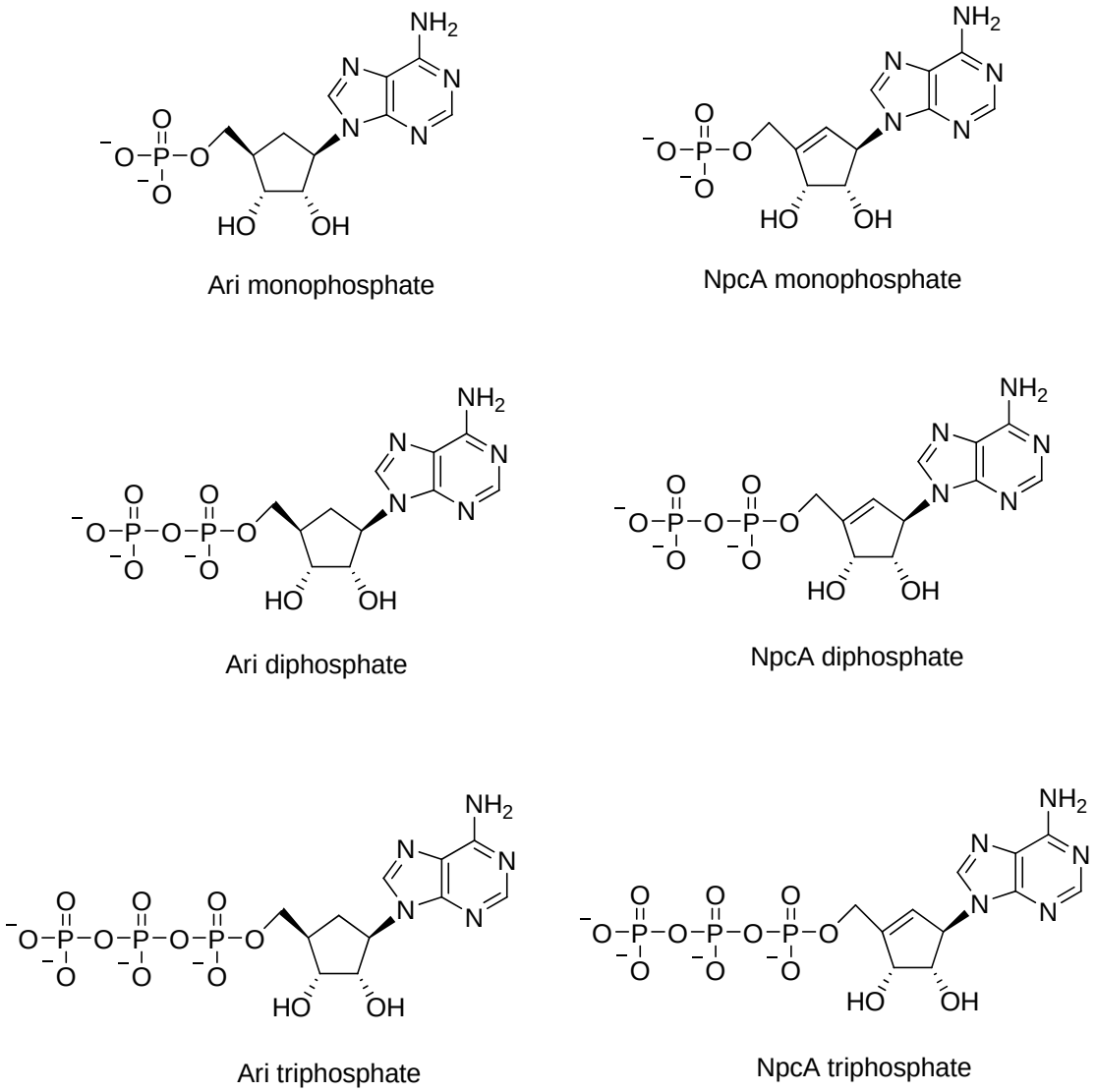


Figure 4. Phosphate derivatives of Ari and NpcA

On the basis of the above results, chemical modifications of Ari and NpcA have been studied to develop efficient antiviral agents that eliminate the undesired phosphorylation yet retain the promising antiviral activity. In this direction, effort by researchers, including Dr. Schneller's group, took two different approaches. One approach is to

modify the cyclopentane or cyclopentene moiety of Ari and NpcA, respectively, to reduce the possibility of the phosphorylation at the 5′ positon. In this direction, the change can be either making the chain length at the 5′-C longer or shorter or replacing the OH group at 5′-C with H, NH_2 etc (Figure 5).

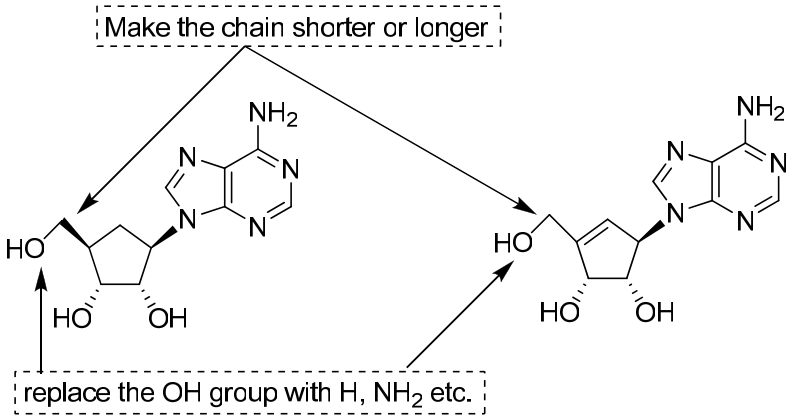


Figure 5. Modification of Ari and NpcA

Truncated analogues of Ari and NpcA (DHCaA and DHCeA) (Figure 6), which lack the 4′-hydroxymethyl group, were synthesized by Borchardt's group and found to retain

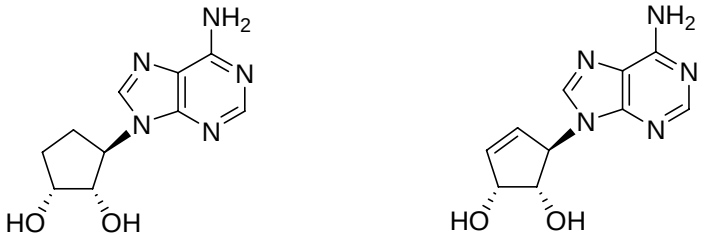


Figure 6. Truncated analogues of Ari and NpcA

the potent antiviral activity against vesicular stomatitis virus, vaccinia virus, parainfluenza virus, reovirus, and rotavirus etc. with less associated toxicity.[50-53]

The 5′-deoxy analogue of Ari (Figure 7), which was synthesized by Schneller and coworkers,[54] is another example that cannot be phosphorylated due to the absence of the 5′-hydroxyl group. The subsequent antiviral test showed this compound displayed moderate antiviral activity against vaccinia virus (VV), vesicular stomatitis virus (VSV) with little toxicity.[55,56]

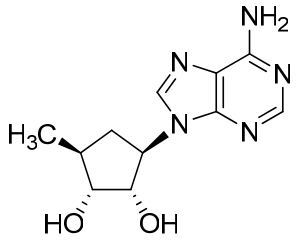
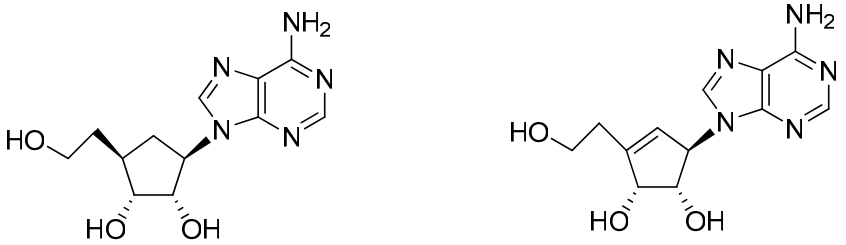


Figure 7. 5′-Deoxyaristeromycin

An approach explored by extension of the C-5′ hydroxymethyl side chain by a methylene group to provide the C-5′ homoanologues of Ari and NpcA (Figure 8) with the aim of reducing phosphate-based toxicity yielded meaningful agents. These analogues can be expected to have displaced the phosphate-susceptible hydroxyl from the phosphate-transfer zone where the kinases act on Ari and NpcA. Early synthesis[54,57,58] of 5′-homoaristeromycin suffered from too many steps, limited scale-up, low yields and, in one case, a racemic product. Efficient and stereoselective synthesis of Ari was reported by the Schneller group in 2005.[59] The first design and synthesis of 5′-homoneplanocin A was described by De Clercq and coworkers in 1996.[48] A more convenient and practical route was provided by the Schneller group in 2005.[60]

5′-Homoaristeromycin and 5′-homoneplanocin A were evaluated against a wide variety of both DNA viruses and RNA viruses. From this, very significant effects were seen by the Dr. Schneller's group for 5′-homoaristeromycin toward vaccinia, cowpox, and monkeypox viruses, all in Vero 76 cells with CC_{50} > 100 μg/mL.[59] 5′-Homoneplanocin A was also found to display antiviral activity against human cytomegalovirus, vaccinia virus, parainfluenza virus, vesicular stomatitis virus, arenaviruses, hepatitis B (HBV), and hepatitis C (HCV). with no interfering cytotoxicity.[48,60]



5′-Homoaristeromycin 5′-Homoneplanocin A

Figure 8. 5′-Homo anologues of Ari and NpcA

Another approach to prevent the 5′-phosphorylation led to 5′-noraristeromycin, which is the chain-shortened analog of Ari (Figure 9). 5′-Noraristeromycin was developed in Dr. Schneller's group in 1993.[55] This compound has shown very marked antiviral activity towards vaccinia virus (VV), vesicular stomatitis virus (VSV), parainfluenza-3 virus, and reovirus-1.[56] It also displayed very potent activity against human cytomegalovirus (HCMV), hepatitis B virus (HBV), measles, and influenza along with the considerably low cytotoxity probably due to its shortened chain at the 4′-C position of Ari giving a

secondary alcohol that is less reactive to phosphorylation than the 5′-C primary alcohol of Ari.[56,61,62]

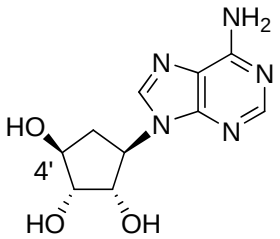
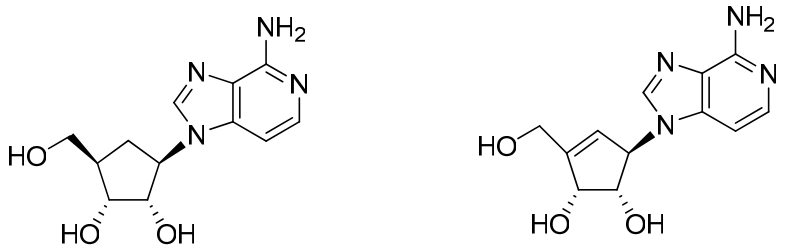


Figure 9. 5′-Noraristeromycin

Besides modification of the cyclopentyl moiety, novel analogues of Ari and NpcA have been based on the fact that 3-deazaadenosine is not phosphorylated, nor is it a substrate for adenosine deaminase.[63] 3-Deazaaristeromycin and 3-deazaneplanocin A (Figure 10) have resulted in excellent activity against several viral types. 3-Deazaaristeromycin was synthesized by Montgomery and coworkers in 1982[64] and found to have antiviral activity in cell culture against herpes simplex virus type 1, vaccinia virus, reo, measles, parainfluenza and vesicular stomatitis. In fact it has more potent antiviral properties than 3-deazaadenosine. At the same time, it shows considerably low cytotoxicity to the host at effective antiviral concentrations and is not subject to deamination or phosphorylation. [64-66]

The NpcA analogue, 3-deazaneplanocin A, was synthesized by Marquez et al. in 1989.[20] It displays excellent antiviral activity in cell culture against vesicular stomatitis, parainfluenza type 3, yellow fever, and vaccinia viruses. In 2000 Bray and coworkers reported that 3-deazaneplanocin together with 3-deazaaristeromycin showed significant

activity towards Ebola.[67] As Marquez pointed out the significantly lower cytotoxicity of 3-deazaneplanocin A may be due to its lack of conversion to its 5'-triphosphate and S-adenosylmethionine metabolites.[20] The test results for both compounds indicated that their bioproperties were consistent with their potent inhibition of S-adenosylhomocysteine (SAH) hydrolase.



3-Deazaaristeromycin 3-Deazaneplanocin A

Figure 10. 3-Deaza analogues of Ari and NpcA

Mechanism of Antiviral Action by SAH Hydrolase Inhibitors

SAH hydrolase is an intracellular enzyme that regulates biological transmethylations in various biochemical processes. One such process is the methylation of the capped structures at the 5'-terminal end of mRNA. SAH hydrolase plays a regulatory role in methylation processes (Figure 11) that are necessary for viral protein translation and replication. As a consequence, inhibition of SAH hydrolase has been set as a suitable antiviral target.[20,53,68,69]

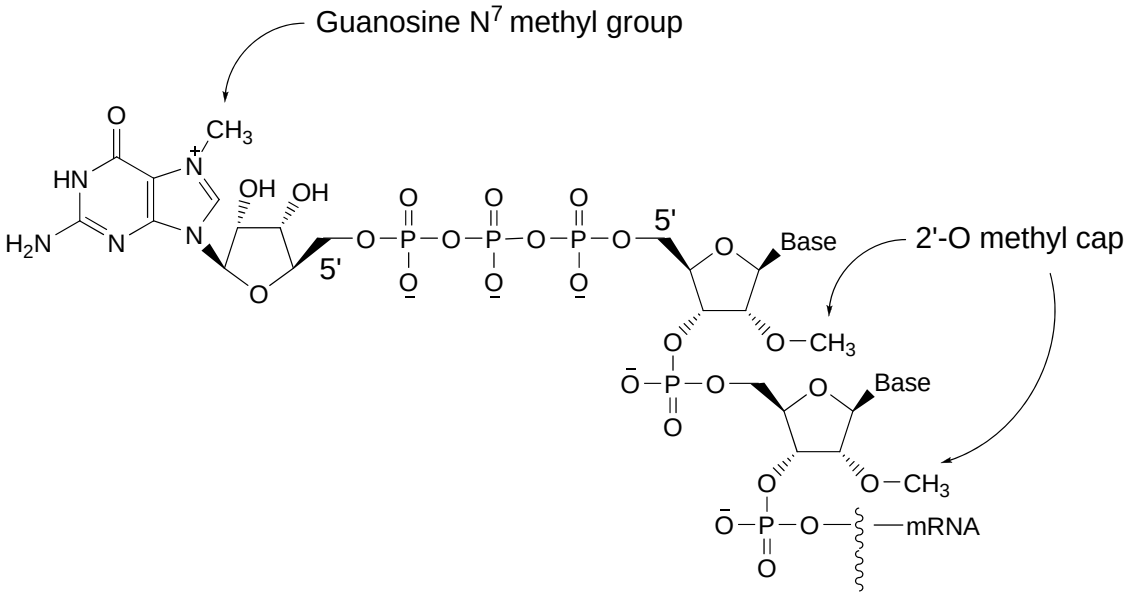


Figure 11. Structure of mRNA 5'-terminal cap

These unique cap structures consist of an N-7 methylguanosine residue linked at the 5'-hydroxyl group by a triphosphate to the 5'-end of the mRNA strand. A methyl substituent also resides on the 2'-hydroxyl of the penultimate nucleoside. The capped structures play an important role in many aspects of mRNA metabolism including RNA processing, RNA nuclear transport, and translation initiation.[70,71] It provides resistance to 5'-3' exonucleases,[70] and contributes to a variety of cellular processes including polyadenylation,[72] pre-mRNA splicing,[73-75] RNA nuclear export,[76,77] and mRNA translation.[71,78] There are several reasons reported that capped structures can play privotal roles in mRNA metabolism. At first they have improved affinity for binding to the ribosome in the translational ignition complex.[79] Secondly, they enhanced the stability of mRNA in the cytoplasm against 5'-end nuclease digestion.[70] Thirdly, they have effective transcriptional processing, nucleocytoplastic transport and recognition of mature mRNA

by translational machinery.[80-82] The capping process consists of three enzymatic reactions. First, initial 5'-triphosphate terminus is cleaved by RNA triphosphatase to a diphosphate terminated RNA. Then, a capping with GMP promoted by RNA guanyltransferase follows. Finally, the structure is methylated by methyltransferase. The methylation leading to a fully functional mRNA is catalyzed by N-7 methyltransferases and nucleoside 2'-methyltransferases, which use S-adenosylmethionine (AdoMet) as the co-factor.[82]

(i) $pppN(pN)_n \rightarrow ppN(pN)_n + Pi$

(ii) $ppN(pN)_n + pppG \rightarrow G(5')pppN(pN)_n + PPi$

(iii) $G(5')pppN(pN)_n + AdoMet \rightarrow m\ G(5')pppN(pN)_n + AdoHcy$

S-Adenosylmethionine (AdoMet) (Figure 12) is one of nature's most versatile molecules.[83] Since it was discovered in 1952 by Cantoni,[84] it has been found to serve directly as the methyl donor for numerous methyltransferases.[85] Besides being a methyl donor, AdoMet can also work as a 3-amino-3-carboxypropyl donor in tRNA modification,[86] as an adenosyl donor in the modification of the enzyme pyruvate formate-lyase[87] and as a precursor to decarboxylated AdoMet, which is the aminopropyl donor for the aminopropyltransferases involved in the biosynthesis of the polyamines spermidine and spermine.[88-90]

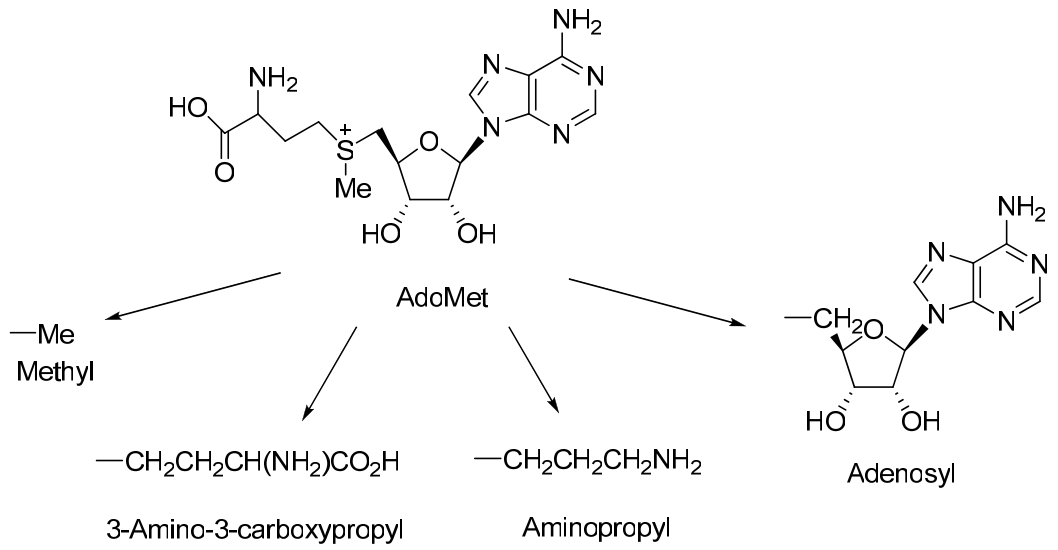


Figure 12. Structure of AdoMet and its potential transfer groups

Among these group transfer reactions of AdoMet, arguably the most important and widely occurring one in nature is the methyl transfer. A nucleophilic displacement catalyzed by methyltransferase can take place between AdoMet and an electron rich group (OH, NH, SH or double bond) as methyl acceptors. The nucleophilic attack occurs via positions on the 7-N of the guanosine and 2'-O of the sugar moiety of the capped mRNA structure (Figure 13). The S-methyl group is transfered to the capped structure from AdoMet and AdoMet is converted to AdoHcy (adenosyl-homocysteine) at the same time.

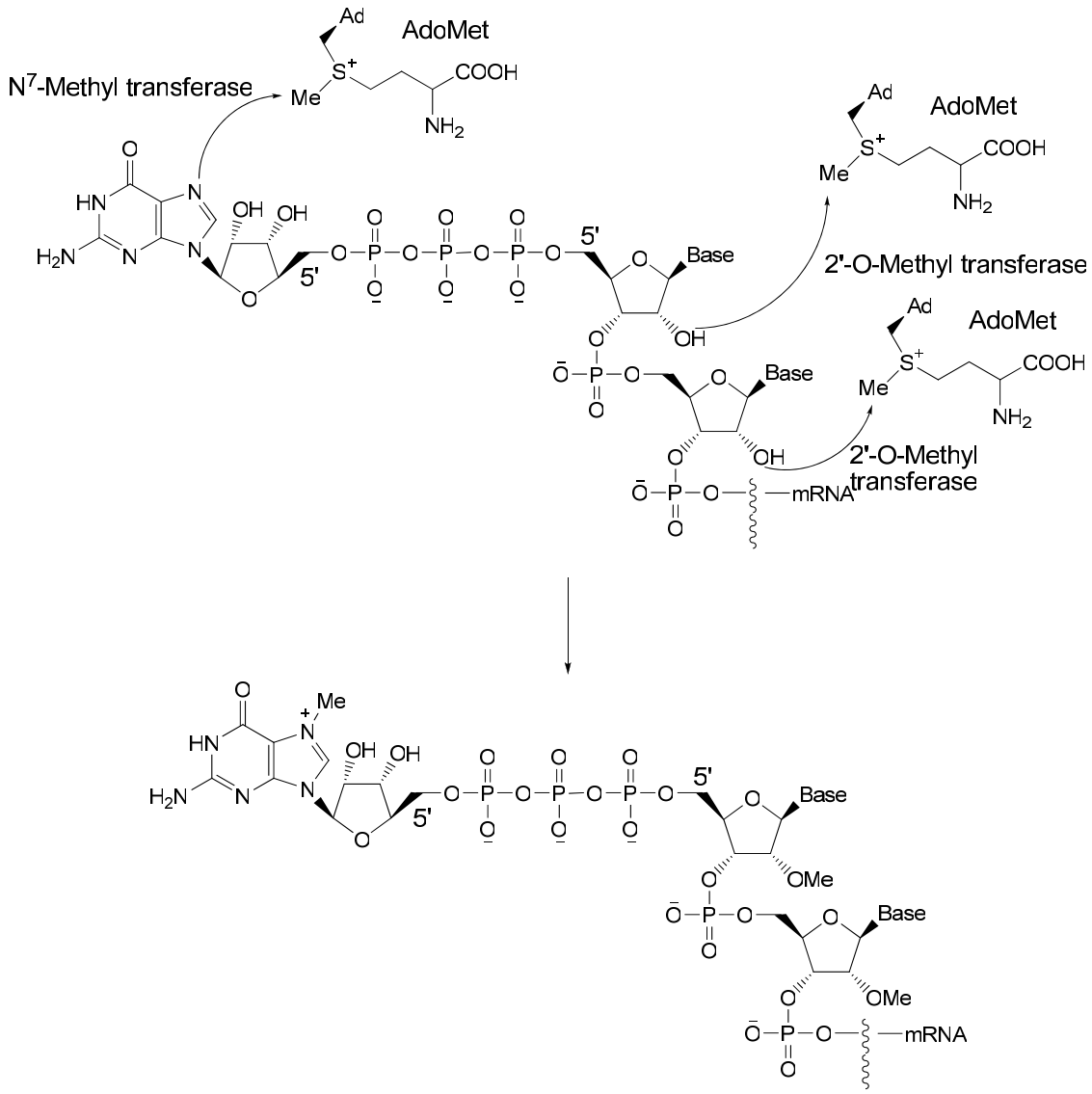


Figure 13. Methylation leading to the formation of 5'-terminal capped mRNA

The full metabolic cycle consists of four basic steps (Figure 14). First, SAH forms following methylation of the capped structure of mRNA as described above. It is a strong feed-back inhibitor of the methyl transferase and must be metabolized rapidly.[91,92] Thus, a reversible hydrolysis catalyzed by SAH hydrolase follows to give

adenosine and homocysteine.[92] The mechanism of this reaction was first proposed by Palmer and Abeles in 1979[93] and has been widely studied.[94,95] Adenosine can be transformed to inosine by adenosine deaminase or it can be converted to ATP through series of

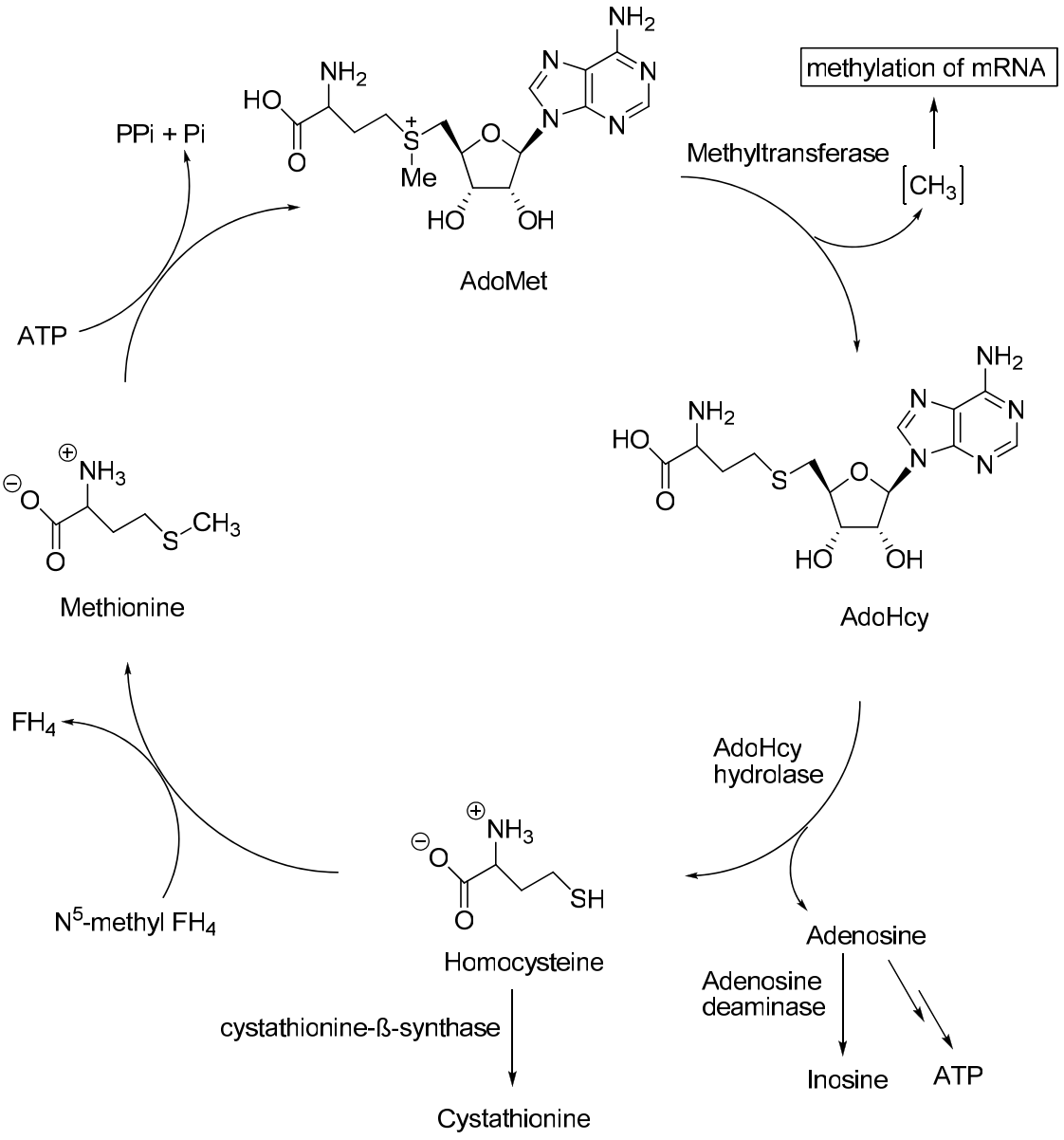


Figure 14. AdoMet metabolic cycle

phosphorylations.[96] Homocysteine, which is the other hydrolase product, can be remethylated to methionine or metabolized to cystathionine. The remethylation is catalyzed by methionine synthase with the donation of the methyl group from N^5-methyltetrahydrofolate (THF).[97] Finally, the biosynthesis of AdoMet is promoted by adenosyltransferase from ATP and methionine.[98]

SAH hydrolase has been long recognized as a potential target for antiviral drug

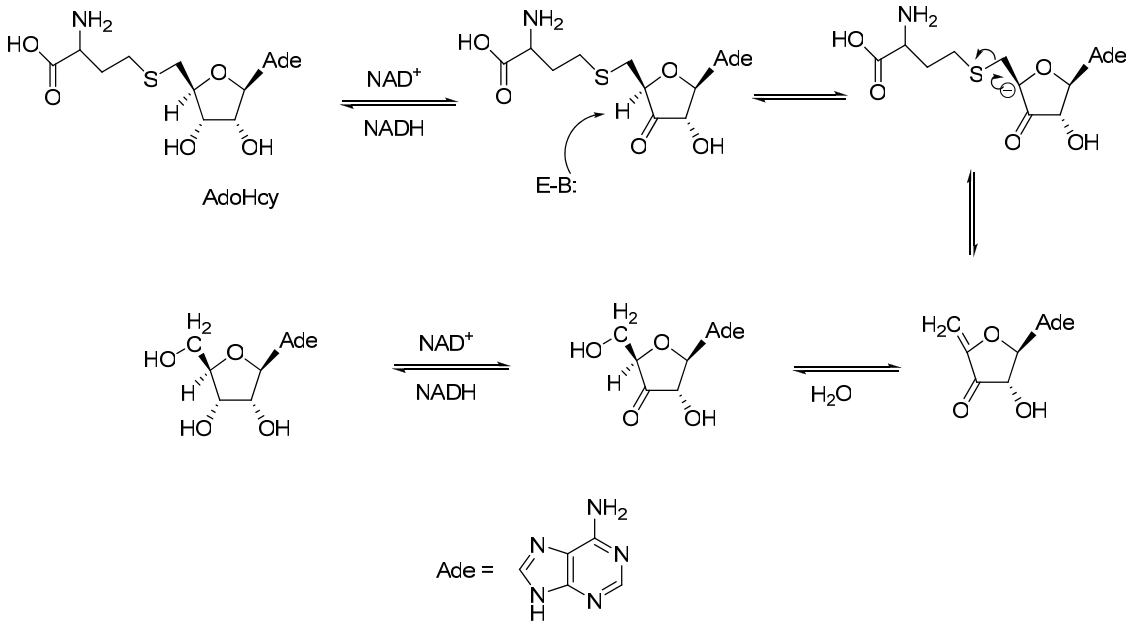


Figure 15. Proposed mechanism of AdoHcy Hydrolase

design. The mechanism of SAH hydrolase was studied thoroughly in the last few decades (Figure 15).[93,94,99-101] It begins with selective oxidation by NAD^+ at the 3' position forming 3'-ketoAdoHcy. The enzyme-bound NAD^+ is converted to NADH. As a result of the oxidation, the acidity at the 4' position is improved and the proton is easily removed by an active site enzymatic base. In the following steps, the homocysteine group at the 5'

position is eliminated and water is added in a Michael fashion. Finally, adenosine is obtained with the 3'-keto being reduced to hydroxyl group by NADH.

SAH is both a product and a feedback inhibitor of methyl transferase in the AdoMet-dependent methylation reaction.The action of the hydrolase controls the concentration

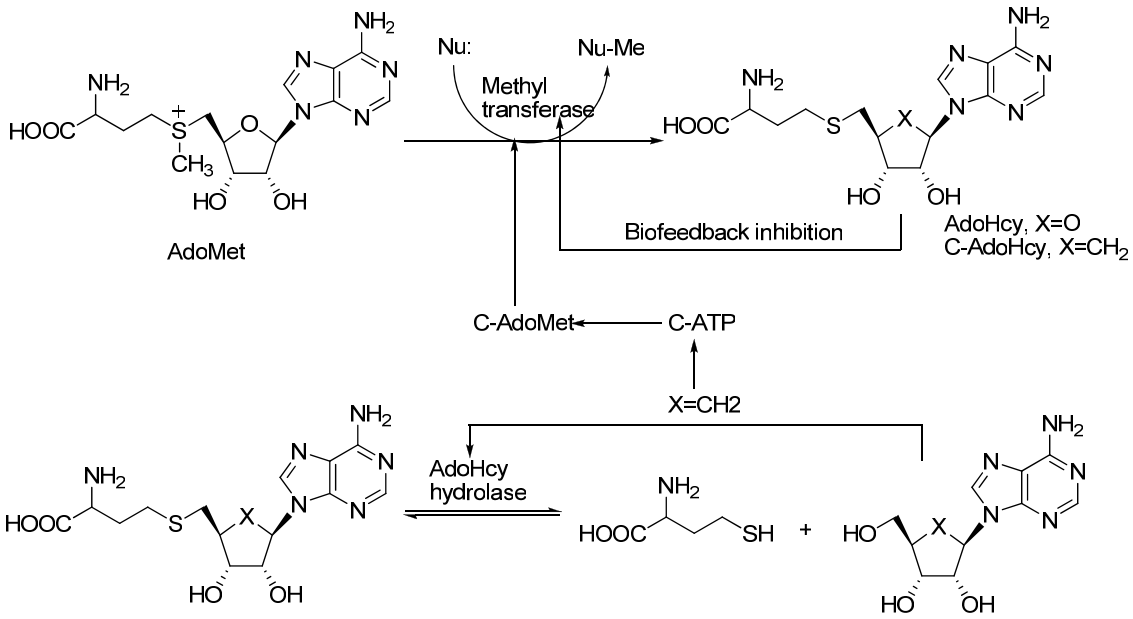


Figure 16. Inhibition mechanism on AdoHcy hydrolase

of SAH and, hence, the methylations, on the other hand, by blocking SAH hydrolase, the concentration of SAH builds up and the methylation reaction that follows from AdoMet to SAH whose rate is regulated by intracellular ratio of AdoMet/AdoHcy, will be suppressed.[40,83,93] As described above, AdoMet is essential as a methyl donor for the methylation of viral mRNAs. This means that inhibitors of AdoHcy hydrolase will be expected to affect maturation of viral mRNAs and, in turn, the production of progency virus particles.[40,102]

Research has shown some viruses such as vaccinia virus encode their own AdoMet dependent enzymes, to catalyze methylation of the capped mRNA structure (i.e., guanine-7-methyltransferase and 2'-O-nucleoside methyltransferase)[38,103,104]and are susceptible to inhibition by SAH.[105,106] There are other viral pathogens whose SAH hydrolase inhibition can be expected to have an effect: poxviruses (e.g., vaccinia, variola, monkeypox), filoviruses (e.g., Ebola, Marburg), rhabsoviruses (e.g., vesicular stomatittis, rabies), arenaviruses (e.g., Junin, Tacaribe), reoviruses (e.g., rota), paramyxoviruses [e.g., parainfluenza, mumps, measles, respiratory syncytial virus (RSV)], retroviruses (e.g., HIV), and herpesviruses [e.g., cytomegalovirus (CMV)].[107]

With all of this in mind, SAH hydrolase inhibitors as a target for the design of antiviral agents has drawn wide attention because (i) a methylated cap structure at the 5'-terminus of mRNA is required for most plant and animal viruses for development of functional mRNA for viral rep1ication, (ii) viral methyltransferases that are involved in the formation of this methylated cap structure are affected by the inhibition of Adohcy hydrolase, and (iii) inhibition of SAH hydrolase, which leads to suppression of methylation of the viral mRNA cap structure, can be correlated with antiviral activity.

Target design based on the SAH hydrolase inhibition

Ever since the SAH hydrolase was recognized a suitable target for antiviral agents,[64] a large numbers of adenosine, acyclic adenosine, and carbocyclic adenosine analogues have been synthesized and their mechanism of inhibition as well as their antivial activity studied. Among the most promising anviral agents based on inhibition of SAH hydrolase are carbocyclic nucleosides in which the cyclopentane ring improves their stability as

potential antiviral agents by rendering the analogues resistant to phosphorylases (*vide infra*). Also, conformational changes and stereoelectronic perturbations that occur with replacing the ribofuranose unit with a cyclopentyl ring bring about the unique biological properties of carbocyclic nucleosides.[108] According to De Clercq,[109] the compounds that combine potent SAH hydrolase inhibition with antiviral effects are 3-deazaAri, NpcA, 3-deazaNpcA, and 5'-nor derivatives of Ari.

Another concern in nucleoside drug design is stereochemistry. In this regard, chirality is relevant since the two enantiomers may have different activity.

Natural nucleosides are D-nucleosides [Those monosaccharides whose highest-numbered stereocenter, (i.e., the one farthest from the aldehyde or keto group) has the same absolute configuration as that of D-(+)-glyceraldehyde are the labled D; those with the opposite configuration at that stereocenter are named L] in which the heterocyclic base is attached through an N-glycosidic linkage to C1' of the ribose or the deoxyribose unit and the linkage is always β (α and β are designated according to the stereochemistry of the anomeric carbon. If that configuration is S, it is labled α; when it is R, it is called β.). This designation has been extended to carbocyclic nucleosides where stereosimilar structures of D-nucleosides are referred to as D-like carbocyclic nucleosides and their enantiomers are called L-like carbocyclic nucleosides.

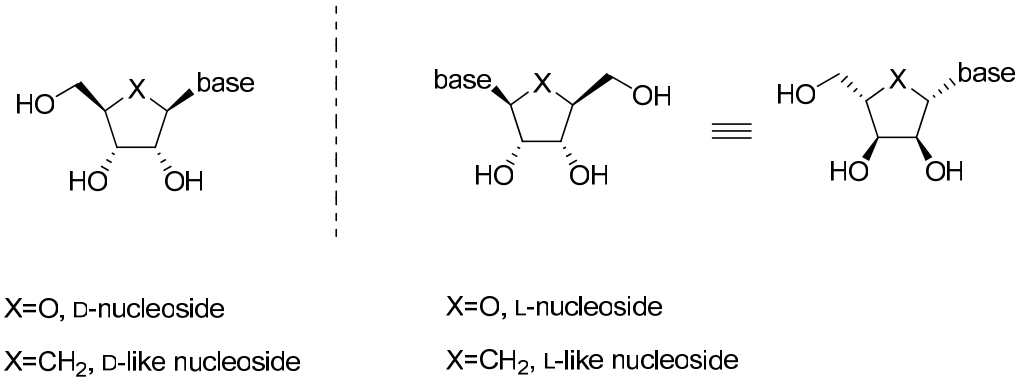


Figure 17. D, L-Nucleosides

Generally speaking, most D-like carbocyclic nucleosides exert greater antiviral activity and AdoHcy hydrolase inhibitory effects compared to the L-like enantiomer. For example, (-)-5'-norAri (the D-like enantiomer) is 100-fold more potent towards cytomegalovirus (CMV) than (+)-5'-norAri (the L-like enantiomer).[56,62] This dissertation research will focus on the D-like carbocyclic nucleosides as target compounds.

In our laboratory, 5'-noraristeromycin (Figure 9) has been found to possess significant antiviral properties against a series of viruses, which is likely due to its inhibition of SAH hydrolase.[56,63] At the same time, as mentioned previously, 3-deazapurine nucleosides have shown potential benefits in antiviral agent design and biochemical investigations. In efforts to further exploit the 5'-noraristeromycin platform as a source for new antiviral candidates, the 3-deaza carbocyclic nucleoside analogs have displayed particular promise.

3-Deaza-5'-noraristeromycin (Figure 18) is a noteworthy example in this category.[110] Antiviral study showed 3-deaza-5'-noraristeromycin produced an activity pattern similar to 5'-noraristeromycin but less potent, and the observation supports the mechanism that the 5'-noraristeromycin class of adenosine analogues inhibits the AdoHcy hydrolase.[110]

Modeling studies by Borchardt indicate that all nitrogens of purines are needed for interacting with AdoHcy hydrolase. So, it is not surprising that 3-deaza-5'-noraristeromycin is less inhibitory on the AdoHcy hydrolase than 5'-noraristeromycin.

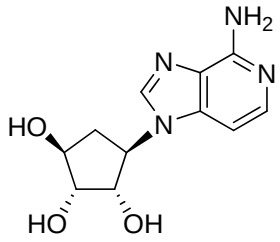
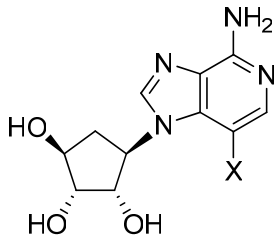


Figure 18. 3-Deaza-5'-noraristeromycin

To improve upon the antiviral scope, structure modifications in 3-deaza-5'-noraristeromycin were exploited. Substituents such as halo groups at the C-3 position (Figure 19) were considered by us as important targets that would mimic the electronic environment of the nitrogen of the parent 5'-nor series.



1 X=Cl
2 X=Br
3 X=I

Figure 19. 3-Halo-3-deaza-5'-noraristeromycin

A logical extension of the 3-halo derivatives would be the 3-deaza-5'-noraristeromycin derivative possessing a methyl group at C-3 position (**4**) (Figure 20).

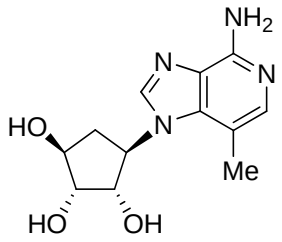


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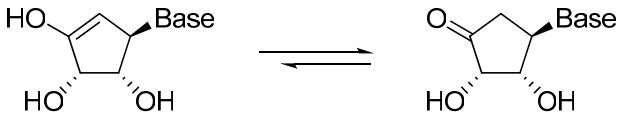
Figure 20. 3-Methyl-3-deaza-5'-noraristeromycin

To extend these studies to a 5'-nor cyclopentene series is not possible due to an enol/keto tautomeric situation. Thus, 3-deazaneplanocin A became the structural



framework. The compound considered was 3-bromo-3-deazaNpcA (**5**, Figure 21).

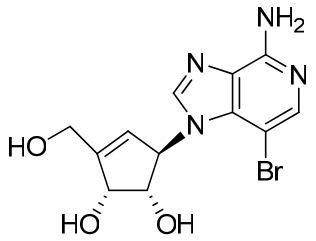


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Figure 21. 3-Bromo-3-deazaNpcA

3-Methyl-3-deazaNpcA (**6**, Figure 22) was also part of this plan.

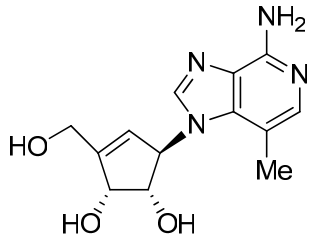

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Figure 22. 3-Methyl-3-deazaNpcA

It should be pointed out that the NpcA derivative, 9-(*trans*-2', *trans*-3'-dihydroxycyclopent-4'-enyl)-3-deazaadenine (DHCDA), is effective against vesicular stomatitis virus (VSV), vaccinia virus (VV), parainfluenza virus, reovirus, and rotavirus. Its selectivity was greater than that of neplanocin A, particularly against vesicular stomatitis virus and rotavirus.[53] This activity has been attributed to the inhibitory effects of DHCDA towards SAH hydrolase.[111]

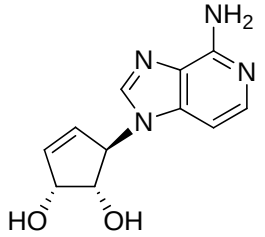

Figure 23. DHCDA

Thus, the DHCDA derivative **7** (Figure 24), which has a bromo atom at the C-3 position, arose as a worthy candidate for our 3-deazapurine carbocyclic nucleoside series.

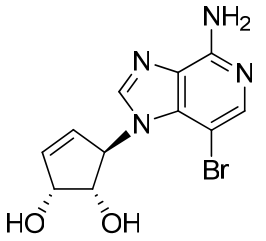

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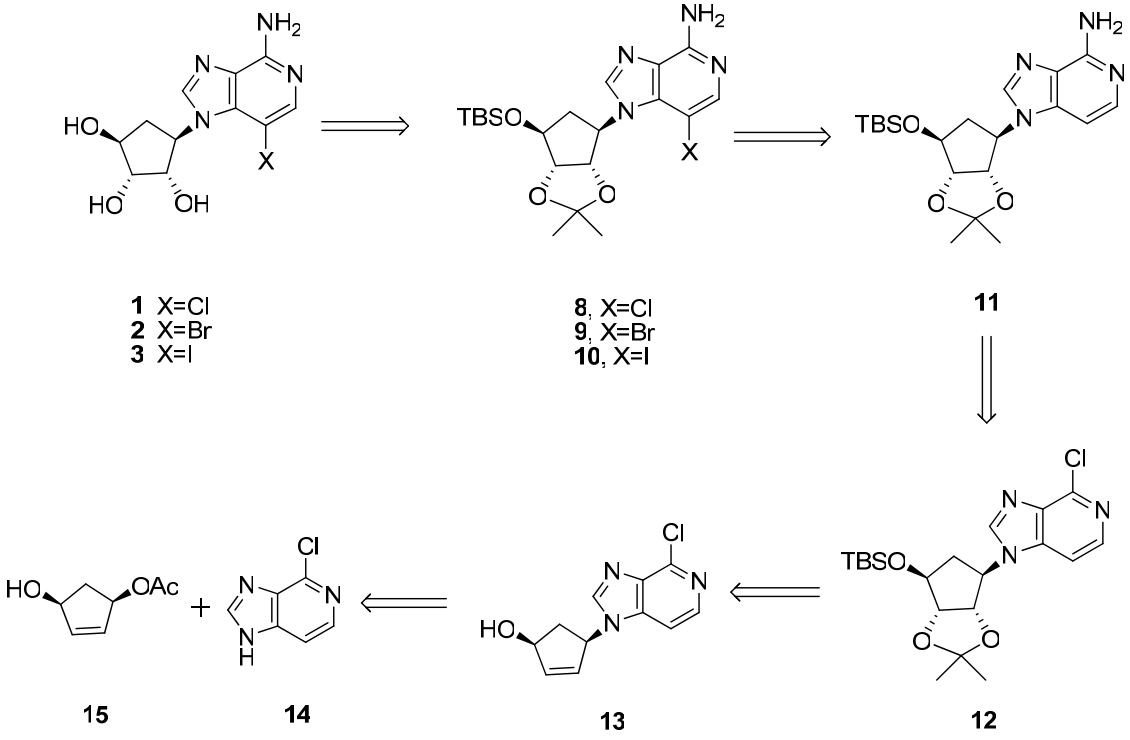
Figure 24. 3-BromoDHCDA

CHAPTER 1. SYNTHESIS OF 3-HALO-3-DEAZA-5'-NORARISTEROMYCIN

Retrosynthetic approach toward 3-halo-3-deaza-5'-noraristeromycin

The retrosynthetic analysis (Scheme 2) of the target compounds **1**, **2**, and **3** suggested that they could be synthesized from protected forms of 3-deaza-5'-noraristeromycin (for example, **12**). The allylic monoacetate (**15**) and 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (6-

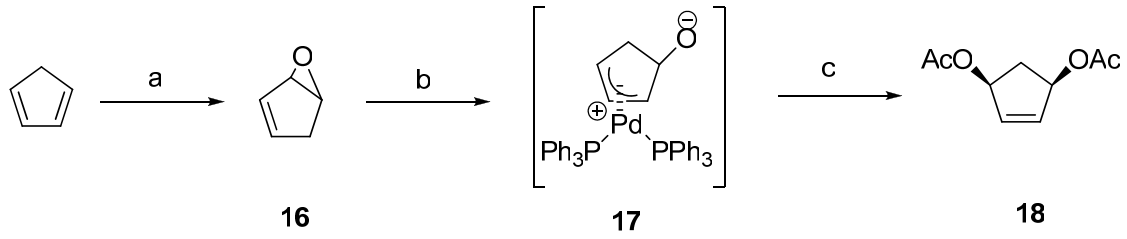


Scheme 2. Retrosynthetic analysis of 3-halo-3-deaza-5'-noraristeromycin

chloro-3-deazapurine, **14**) could serve as convenient precursors, which is sought in large scale and provide entry to the requisite D-like configuration of the target compounds.

Synthesis of important precursors

To synthesize allylic monoacetate **15**, allylic diacetate **18** was obtained in 3 steps, starting with the epoxidation of freshly cracked cyclopentadiene following a literature procedure.[112] In that regard, opening of the vinyl epoxide **16** with the palladium(0) catalyst *tetrakis*-(triphenylphosphine)palladium in the presence of acetic anhydride cleanly afforded *cis*-1,4-addition product **18**. The π-allylpalladium complex **17** derived from cyclopentadiene monoepoxide serves as an ideal synthon for the stereo- and regiospecific construction of substituted cyclopentanoids. It was believed[112,113] that the reaction is presumably initiated through a nucleophilic attack by the basic oxygen atom in **17** on acetic anhydride. A subsequent *trans* attack by the freshly liberated acetoxy anion on the *distal* end of the π -allylpalladium complex insures the *cis* stereochemistry.[112]

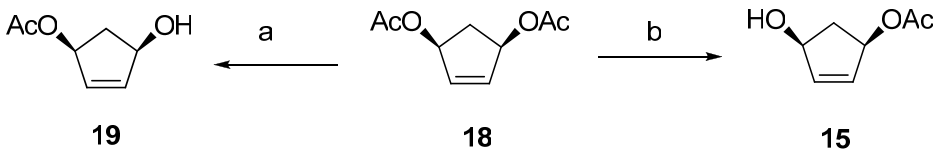


16 **17** **18**

a. Na_2CO_3, CH_3CO_3H, CH_2Cl_2; b. $Pd(PPh_3)_4$, THF; c. Ac_2O

Scheme 3. Synthesis of diacetate 18

Depending on the enzyme used, hydrolysis of **18** would lead to different products. The allylic monoacetate **15** and its enantiomer **19** can be selectively obtained via enzymatic hydrolysis with pseudomonas cepacia lipase (PCL) and porcine liver esterase (PLE) respectively.[54,113-115]



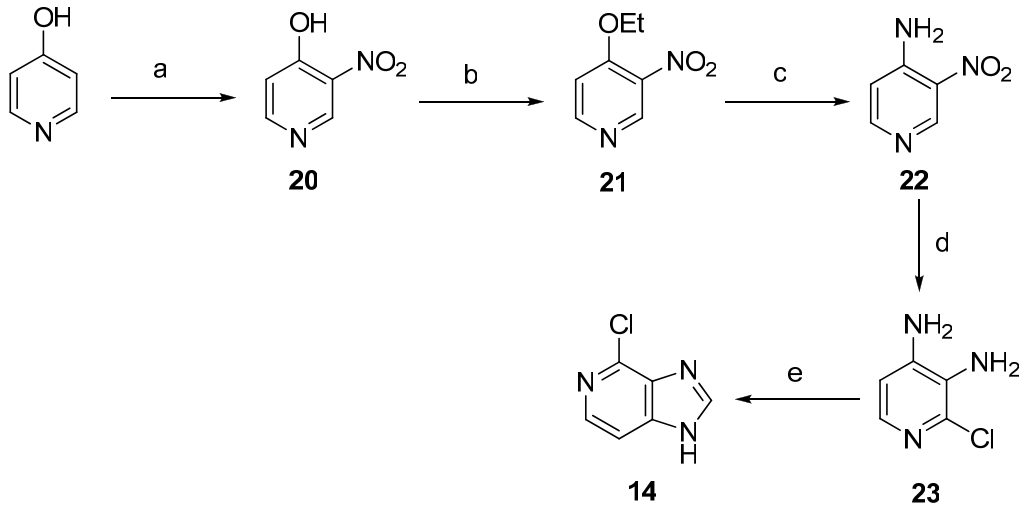
a. Porcine liver esterase (PLE)

b. Pseudomonas cepacia lipase (PCL)

Scheme 4. Enzymatic hydrolysis of 18

The heterocyclic base unit, 6-chloro-3-deazapurine (**14**) is the other important synthon in this project. After carefully searching the literature, the existing procedures to **14** were found to be few in number, low yielding, and not amenable to safe scale-up. By combining the most practical and efficient steps to **14** in the literature, a convenient pathway was developed in our laboratory to this important heterocyclic base. This synthesis started with the nitration of 4-hydroxypyridine, which is commercially available and inexpensive. The nitration product **20** (Scheme 5) was treated with phosphorus pentachloride and followed by ethanol to afford compound **21** in high yield. Transformation of **21** to **22** was achieved with an aqueous solution of ammonium acetate. Reduction of the nitro group of **22** by tin chloride in concentrated hydrochloric acid was

accompanied by a chloriation reaction occurring at the 2 position to give **23**. Compound **23** was treated with ethyl orthoformate leading to the cyclization product **14**.



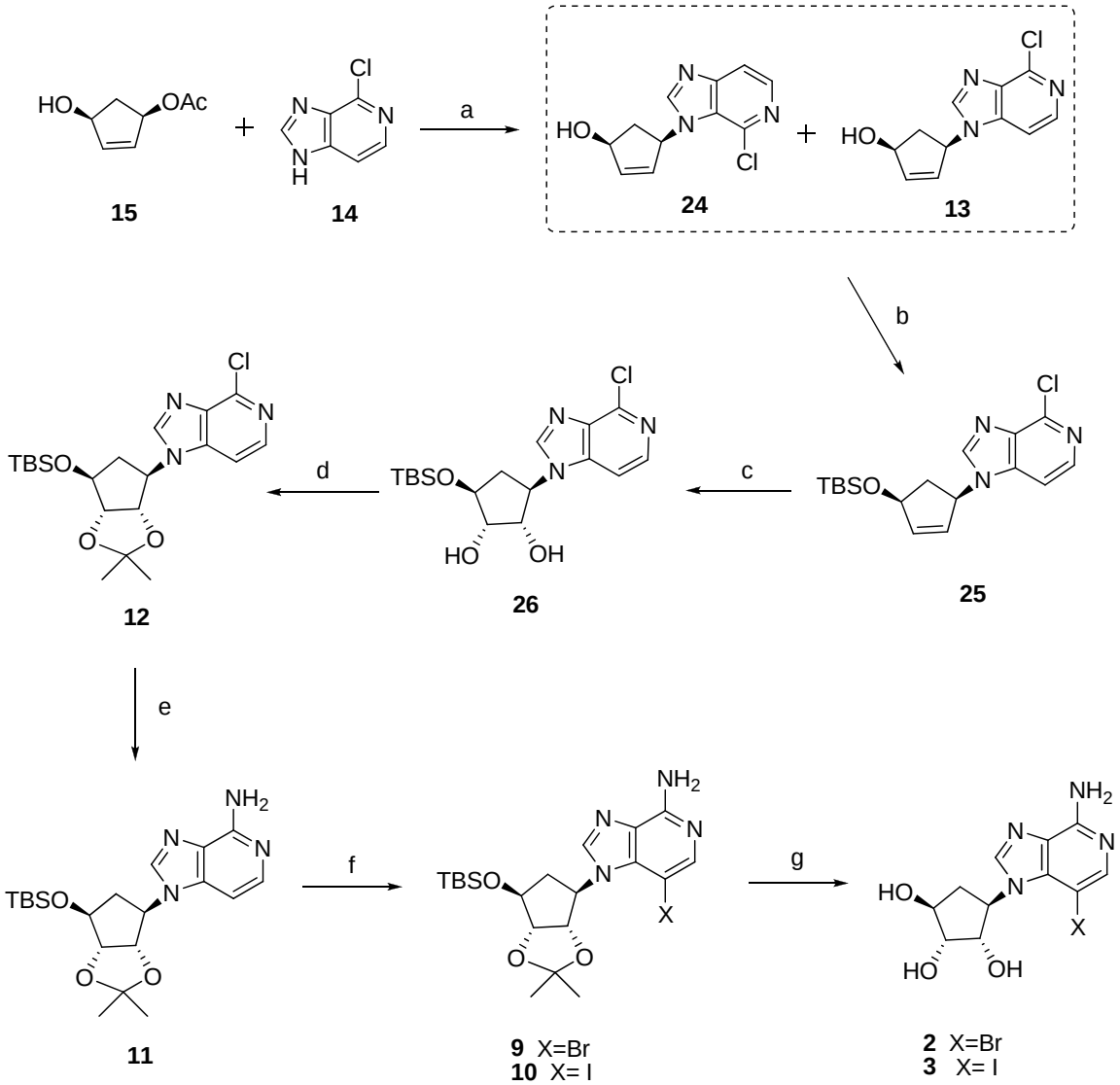
a. HNO_3, H_2SO_4, 72%; b.1) PCl_5, $ClCH_2CH_2Cl$; 2) EtOH, 99%; c. NH_4OAc, H_2O, 81%; d. $SnCl_2$, HCl, 71%; e. $CH(OEt)_3$, 72%.

Scheme 5. Synthesis of 6-chloro-3-deazapurine

Synthesis of target compounds 2 and 3

A Pd (0) mediated coupling reaction between allylic acetate **15** and the sodium salt of 6-chloro-3-deazapurine (**14**) (Scheme 2) gave N-1 (purine N-9, **13**) and N-3 (purine N-7, **24**) coupling products as a mixture as indicated by NMR spectroscopy. The mixture was treated with *tert*-butyldimethylsilyl chloride and imidazole, to produce a mixture of silyl protected hydroxyl products. Flash chromatagraphy column was applied to this mixture to produce the pure **25** in 34% yield for two steps. The ^{13}C-NMR data with a characteristic peak at $\delta = \sim 106$ ppm is consistant with N-1 coupling products for 3-

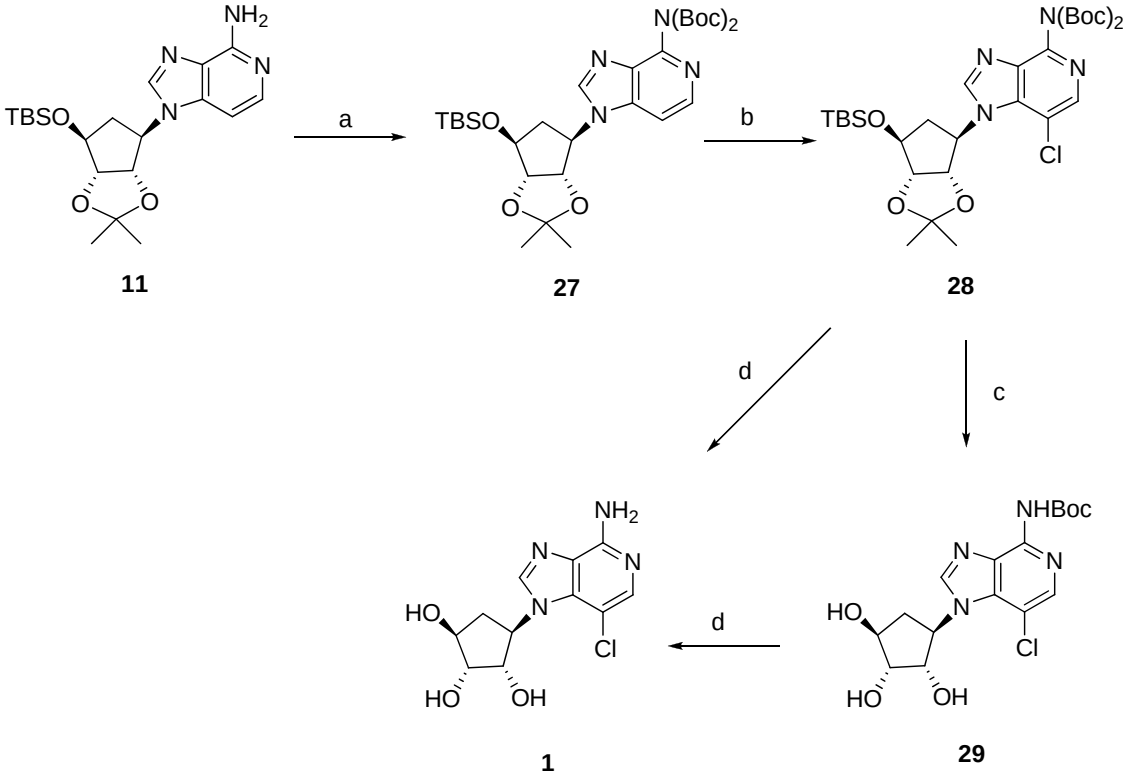
deaza-6-chloro compounds.[116] The dihydroxylation of **25** with osmium tetroxide and N-methylmorpholine-N-oxide provided diol **26**. Treatment of **26** with 2, 2-dimethoxypropane in acetone gave **12**. Literature[64,110] indicated that the standard



a. $Pd(Ph_3P)_4$, Ph_3P, NaH; b. TBSCl, Imidazole, CH_2Cl_2, 34% for 2 steps; c. OsO_4, NMO, 81%; d. 2, 2-dimethoxypropane, Acetone, 99%; e. 1) NH_2NH_2, 2) Raney Ni, MeOH, 62%; f. NBS, 87% or NIS, 61%; g. HCl/MeOH, 84% for X=Br; 70% for X=I.

Scheme 6. Synthesis of 2 and 3

conditions (ammonia/methanol) for conversion of a chloro substituent into an amino group at the 6-position would not be successful for the transformation of **12** into **11**. Therefore, **12** was subjected to anhydrous hydrazine followed by treatment of the resultant hydrazino derivative with Raney nickel. Bromination and iodination of **11** with *N*-bromosuccinimide (NBS) and *N*-iodosuccinimide (NIS) were achieved as described in Scheme 6 to give **9** and **10** in yields of 84% and 70%, respectively. Deprotection of **9** and **10** with dilute hydrochloric acid gave target compounds **2** and **3**.



a.$(Boc)_2O$, DMAP, THF, rt., 84%; b. NCS, CH_2Cl_2, rt., 92%; c. HCl/MeOH, rt. d. HCl/MeOH, reflux, 2h, 80%.

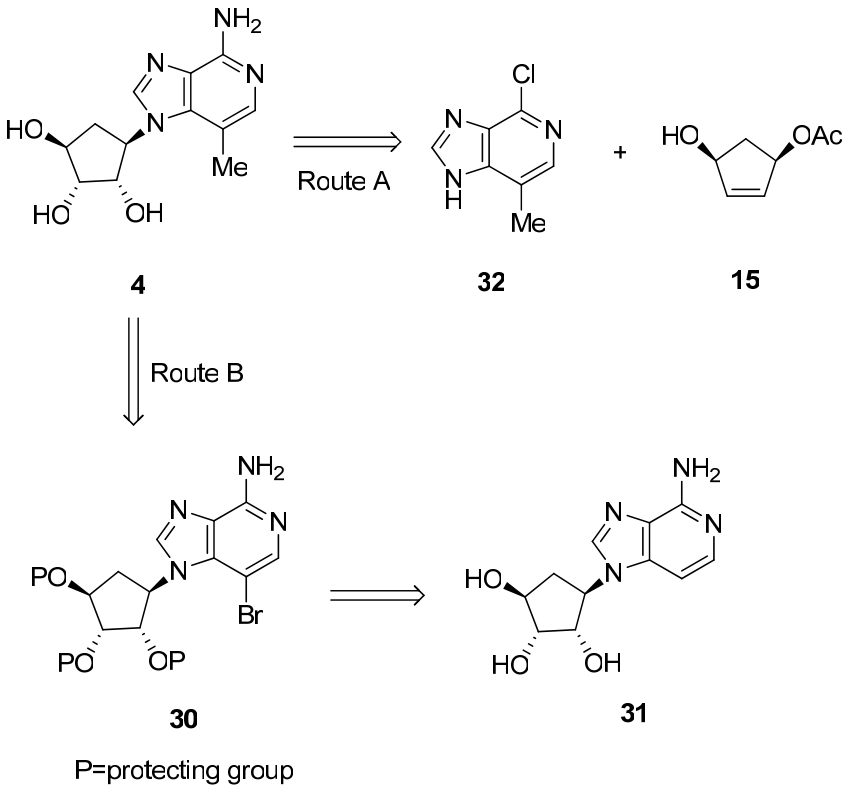
Scheme 7. Synthesis of 1

Treatment of **11** with *N*-chlorosuccinimide (NCS) gave a complex mixture including a product chlorinated at the 3-positon in low yield. To reduce the reactivity of **11** towards *N*-chlorosuccinimide, *tert*-butyloxycarbonyl (Boc) groups were added to protect the exocyclic amino group prior to the chlorination (**27**, Scheme 7). Chlorination of **27** was conducted with excess *N*-chlorosuccinimide at room temperature. The desired product **28** was smoothly obtained in 92% yield. Reaction of **28** with dilute hydrochloric acid at room temperature produced compound **29**, which retained one Boc group. Refluxing conditions (2 hours) resulted in complete protecting group removal to produce 3-chloro-3-deaza-5'-noraristeromycin (**1**).

CHAPTER 2. SYNTHESIS OF 3-METHYL-3-DEAZA-5'-NORARISTEROMYCIN

Retrosynthetic approach toward 3-methyl-3-deaza-5'-noraristeromycin

Introduction of various carbon chains onto the ring of the naturally occurring purine nucleosides has been extensively investigated for preparing biologically active analogs.[117] By carefully searching the literature, a retrosynthetic analysis revealed that **4** could be prepared through two routes.



Scheme 8. Retrosynthetic analysis of 3-methyl-3-deaza-5'-noraristeromycin

Route A called for a coupling reaction between the precursor 3-methyl purine base **32** and allylic monoacetate **15**. In route B, a methylation reaction of protected 3-bromo-3-deaza-5'-noraristeromycin (**30**) with a suitable methylating agent was the key step. Since **30** was envisioned as being accessible from 3-deaza-5'-noraristeromycin (**31**), an efficient and practical synthesis of an appropriate derivative of **31** would be sought in route B.

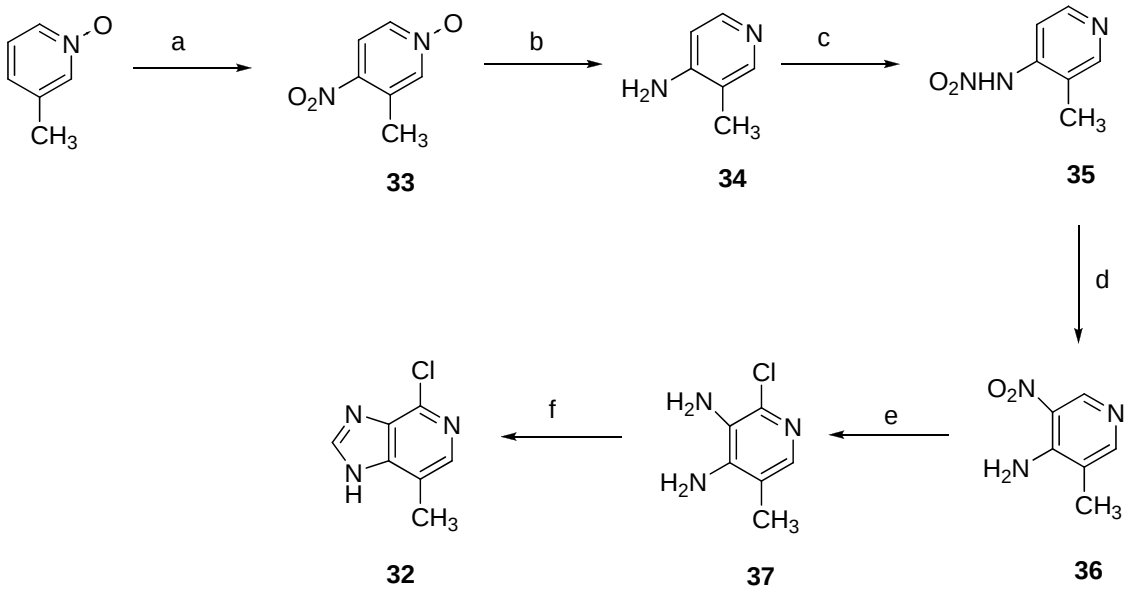
Synthesis of target compound 4

Route A

Synthesis of precursor 32

Following a reported method for **32**,[118] its synthesis was undertaken (Scheme 9) starting with 3-picoline N-oxide. Regioselective nitration of 3-picoline N-oxide with fuming nitric acid and concentrated sulfuric acid yielded 4-nitro-3-picoline N-oxide (**33**). Reduction of compound **33** to 4-amino-3-methylpyridine (**34**) in the presence of tin (II) chloride and concentrated hydrochloric acid had been reported[118] to cause only reduction of the nitro group and with N-oxide remaining. To find a convenient and practical synthesis for **34**, other reduction conditions (such as iron powder in hydrochloric acid or glacial acetic acid, hydrogen catalyzed by palladium on activated carbon, etc.) were attempted. All these reduction reactions generated a mixture of partially reduced compounds along with compound **34**. Returning to the literature for guidance,[118] reduction with Raney nickel under 65 psi of hydrogen in a Parr hydrogenator on a small scale gave **34** as the only product in 95% yield. Nitration of **34** in the presence of concentrated nitric acid and concentrated sulfuric acid in an ice bath yielded 3-methyl-4-nitraminopyridine (**35**) in 84% yield, as described previously.[119] Treatment of **35** with

concentrated sulfuric acid overnight at room temperature led to 4-amino-3-methyl-5-nitropyridine (**36**) resulting from nitro group migration from the amine to the ring with a 54% yield.[119] Reduction of **36** in the presence of tin (II) chloride and hydrochloric acid was a key step in the synthesis, because, in addition to converting the nitro group to a ring amino, it led to regioselective introduction of a chloro group to afford 6-chloro-4,5-diamino-3-methylpyridine (**37**). The yield for this step was 36%, which is lower than the previously reported yield of 58% by Mizuno in 1964,[120] but higher than the yield of 29% described by Irani in 2002.[118]



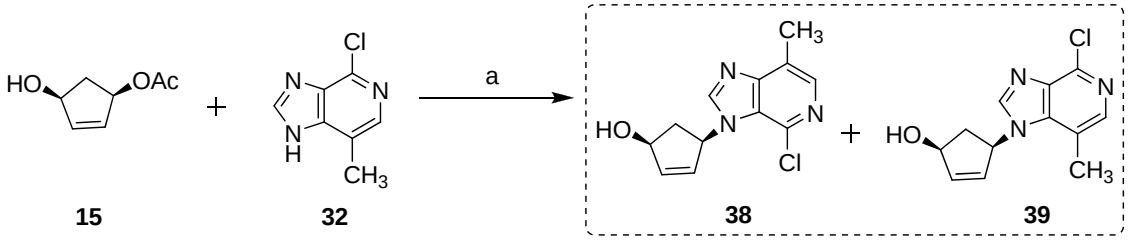
a. 90% fuming HNO_3/ H_2SO_4, 63%; b. Raney Ni, H_2, 95%; c. HNO_3/H_2SO_4, 84%; d. conc. H_2SO_4, 53%; e.$SnCl_2$, HCl, 36%; f. $HC(OEt)_3$, 71%.

Scheme 9. Synthesis of 4-chloro-7-methyl-1*H*-imidazo[4,5-*c*]pyridine

The desired 4-chloro-7-methyl-1*H*-imidazo[4,5-*c*]pyridine (**32**) was obtained by ring closure of **37** by refluxing of **37** in triethylorthoformate with an improved yield of 71%.

Attempted coupling reaction between 32 and 15

With precursors **32** and **15** in hand, a Pd (0) mediated coupling reaction was attempted. A mixture (by NMR) of the N-3 (purine N-7, **38**) and N-1 (purine N-9, **39**) coupling products were obtained. Efforts to separate these two compounds at this point or after the mixture was treated with *tert*-butyldimethylsilyl chloride and imidazole to give compounds with silyl protection on the hydroxyl group failed to avail pure compound **39** or its silyl derivative.



15 **32** **38** **39**

a.$Pd(Ph_3P)_4$, Ph_3P, NaH

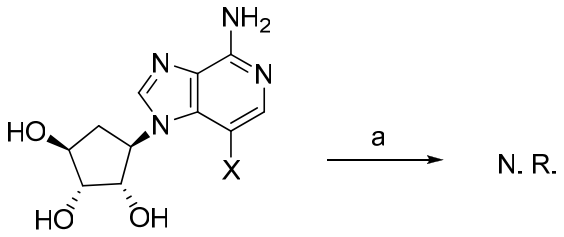
Scheme 10. Attempted reaction to 38

Thus, attempts through route A were not satisfying. The synthesis of the precursor of compound **32** suffered from dramatic conditions, low yields and requisite small scale procedure to **34** in a Parr hydrogenator. Furthermore, the Pd (0) mediated coupling reaction (Scheme 10) was not fruitful due to the low selectivity at N-1 and N-3 positions and difficulties in separating the products. Attention then turned to route B.

Route B

The literature contains ample precedent for the preparation of 2- and 8-alkylpurine nucleosides. These methods for the direct introduction of alkyl groups depend mainly on the application of C-lithiation[121,122] and radical reaction conditions[123,124] and are not always satisfactory with respect to regioselectively, yield, and/or the scope of reactions. Although the cross-coupling of Grignard reagents with aryl halides has achieved great success in the field of synthetic organic chemistry application of such reactions to nucleoside derivatives is far from satisfactory because of its inefficiency.[125] On the other hand, studies[126,127] have shown that the palladium-catalyzed cross-coupling reaction is efficient with value in the application on nucleosides. The palladium-catalyzed cross-coupling reaction using trialkylaluminums[126] or organotin reagents[127] is of general use for the formation of C-C bonds in the 2-, 6-, and 8-positions of purine nucleosides. These coupling reactions are normally achieved in the presence of Pd(0) catalysts. Alkenyl and alkynyl groups are introduced with little difficulty; introduction of an alkyl group, however, is somewhat more difficult.

The first attempt in this dissertation research was the cross-coupling reaction of 3-halo-5'-nor-3-deazaaristeromycin with trimethylaluminum in the presence of palladium (0) catalyst (Scheme 11). These reactions resulted in the recovery of the starting material.

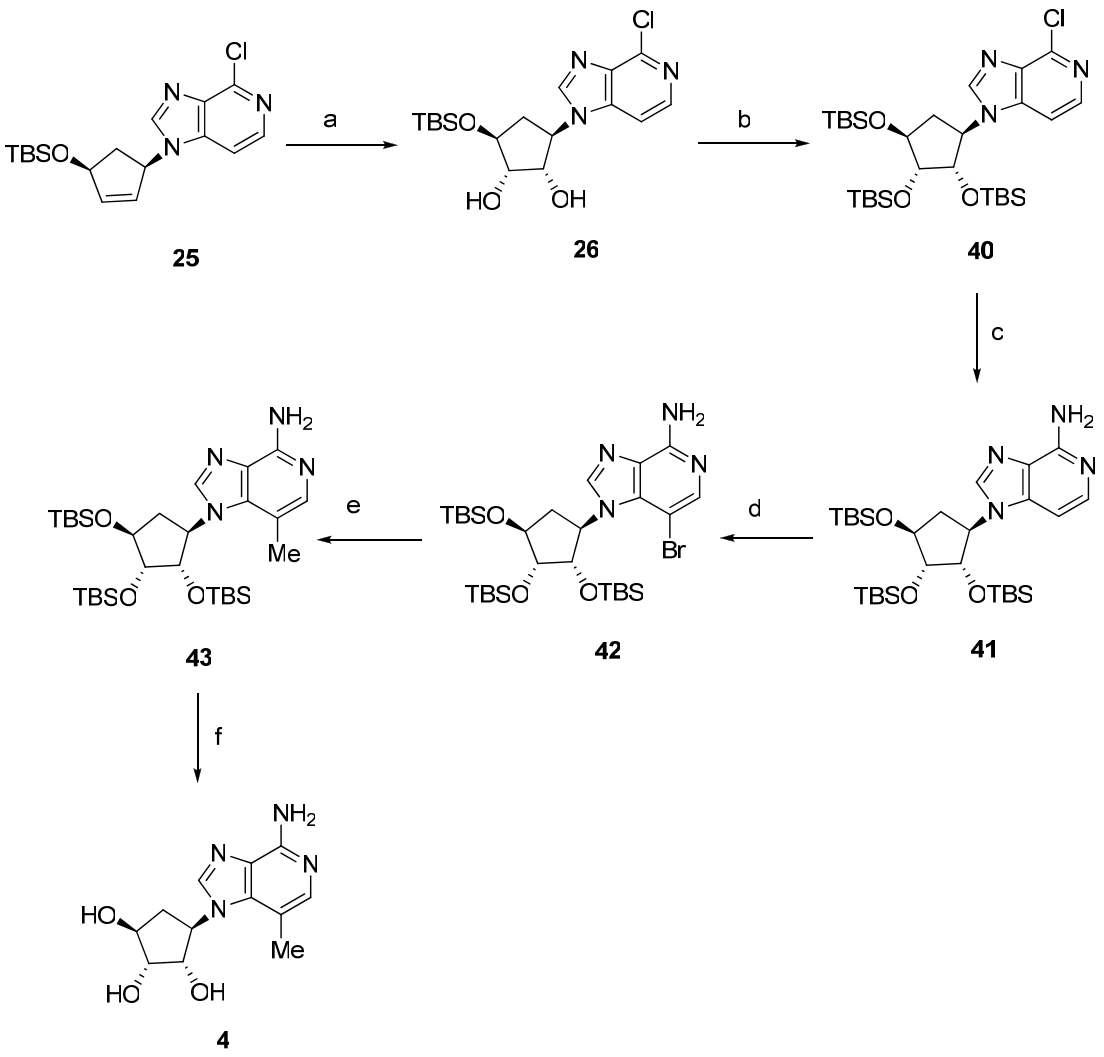


X = Cl, Br, I

a. $AlMe_3$, $Pd(PPh_3)_4$, THF

Scheme 11. Attempted methylation to 4

Attention then turned to using protected 3-halo-5'-nor-3-deazaaristeromycins as the reactant. In this direction, the preparation of **4** began with the dihydroxylation of **25**, (Scheme 12) which was obtained by a procedure modified from a pathway reported in the Schneller lab,[110] using osmium tetroxide/N-methylmorpholine N–oxide to give **26**. Use of the common *iso*propylidene 2', 3'-diol protecting group with **26** for the subsequent steps was not employed due to concerns that its subsequent acidic deprotection would fail to provide the final product in its free base form. Thus, **26** was protected as the *tert*-butyldimethylsilyloxy derivative **40**. Conversion of **40** into **41** followed a standard procedure for 3-deazapurines by reacting **40** with hydrazine followed by treatment of the resultant hydrazino derivative with Raney nickel. Bromination of **41** with *N*-bromosuccinimide was achieved to give **42** in yield of 85%. A palladium-catalyzed cross-coupling reaction of **42** with trimethylaluminium succeeded to convert **42** into **43** in high yield. Finally, deprotection of **43** with tetra-*n*-butylammonium fluoride (TBAF), was clean, easy to work-up, and led to the target compound **4**.



a. OsO_4 , NMO, 84%; b. TBSCl, imidazole,CH_2Cl_2, 92%; c. 1) Hydrazine; 2) Raney-Ni, 68%; d. NBS, CH_2Cl_2, 85%; e. $Al(Me)_3$, $Pd(PPh_3)_4$, 92%; f. TBAF, THF, 90%.

Scheme 12. Synthesis of 4

In addition to NMR data, satisfactory microanalytical results were also obtained for **4**. The structure of **4** was confirmed by X-ray crystallography (Figure 25). Natural DNA nucleosides can adopt either the *syn* or the *anti* conformation about the glycosidic dihedral angle. Several nucleosides have been synthesized where the nucleoside is locked

in either the *syn* or the *anti* conformation.[128-130] X-ray studies on **4** indicated that steric hindrance from the extra methyl group was adequate to hinder the rotation of the purine analog around the glycosidic bond and bring about an *anti* arrangement.

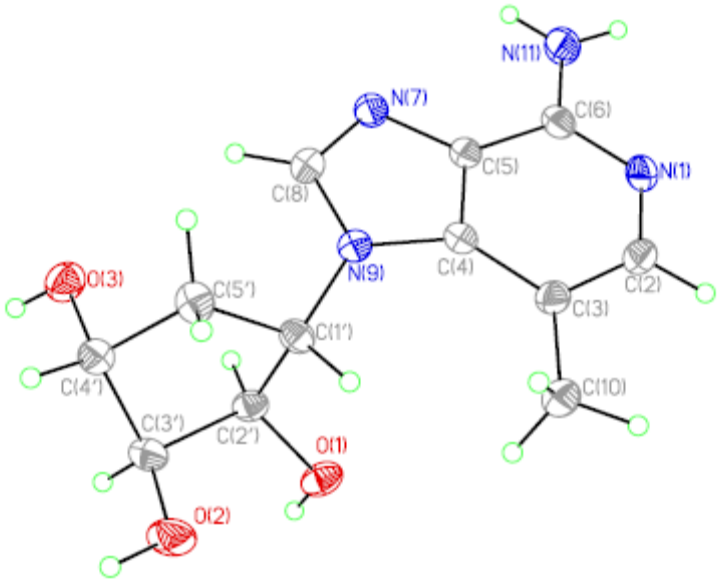


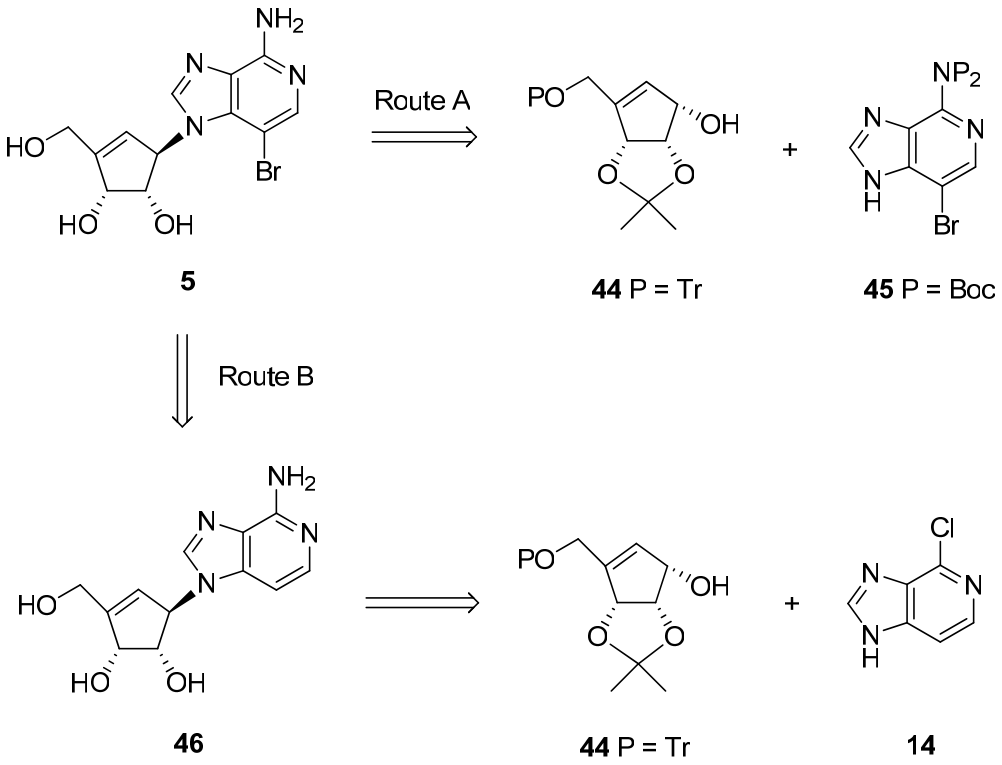
Figure 25. X-ray crystal structure of 4

The present studies on the palladium-catalyzed cross-coupling reaction with trialkylaluminums found that trialkylaluminums smoothly coupled with 3-halo-3-deazapurine nucleosides. The research also uncovered a convenient method for the preparation of C-alkylated 3-deazapurine nucleosides leading an efficient synthesis of a 3-methyl-3-deaza-5'-noraristeromycin. This procedure can serve as an effective pathway to introduce a series of alkyl groups at the C-3 position of 5'-nor-3-deazaaristeromycin using different trialkylaluminiums in the alkylating step.

CHAPTER 3. SYNTHESIS OF 3-BROMO-3-DEAZANEPLANOCIN A

Retrosynthetic approach toward 3-bromo-3-deazaneplanocin A

The success in obtaining of 3-halo-5'-nor-3-deazaaristeromycin compounds suggested a similar approach to 3-bromo-3-deazaneplanocin A (**5**). Thus, a retrosynthetic analysis to **5** was designed. Route A gave a convergent strategy through a coupling reaction between



Scheme 13. Retrosynthesis of 3-bromo-3-deazaneplanocin A

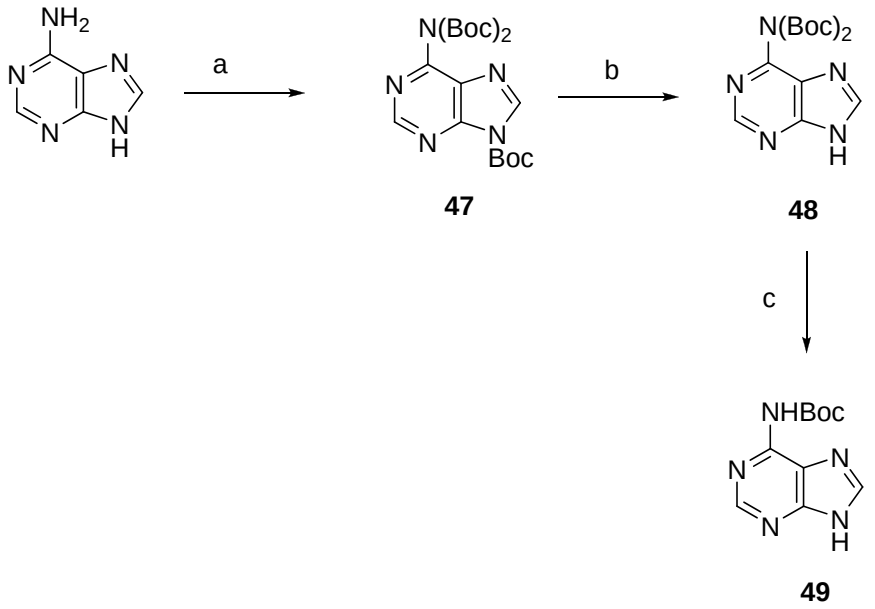
a protected cyclopentyl moiety **44** and a purine base with a bromo atom **45**. The second option (route B) considered bromination of the protected 3-deazaneplanocin A **46**. Compound **46** could be obtained through a coupling reaction similar to route A between **44** and the previously constructed purine base **14.**

Considering the possible steric interference of the trityl protecting group and electronic effects of the double bond in **44** in the bromination reaction of route B, route A was preferred for the synthesis of **5**. Furthermore, working as a convergent strategy, route A was projected to be more efficient than route B.

Synthesis of precursor 45

The N-*tert*-butoxycarbonyl protecting group (N-Boc), which is frequently used in peptide and nucleoside syntheses as well as in heterocyclic chemistry (for example, Scheme 7), was considered appropriate. For this, several methods for N-*tert*-butoxycarbonyl protection (for **45**, Scheme 13) as well as deprotection have been introduced.[131,132] It has been reported by Dey and Garner [133] that adenine can be protected by the *tert*-butoxycarbonyl group and then deprotected selectively under basic conditions. Study[133] has shown that treating adenine with *tert*-butyl dicarbonate ($(Boc)_2O$) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) for Boc-protected adenine was unsuccessful in polar solvents such as dimethyl sulfoxide (DMSO) and N, N-dimethylformamide (DMF). Although these solvents dissolve adenine very well, the reaction resulted in mono-, *bis*-, and *tris*-Boc protected adenines, along with a major amount of recovered adenine. Significantly, the ratio of these products remained constant over time. Changing reaction conditions such as warming the reaction mixture led to a more complicated reaction mixture. After evaluating different reaction conditions, it was

observed that use of tetrahydrofuran (THF) as solvent, excess *tert*-butyl dicarbonate, a catalytic amount of 4-(dimethylamino)pyridine at room temperature gave *tris*-Boc-protected adenine **47** as a single product in high yield over a long period due to the low solubility of adenine in tetrahydrofuran. To avail the desired **48**, acidic conditions were to

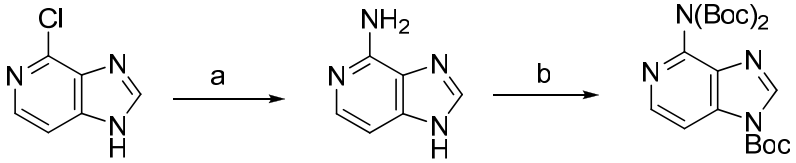


a. $(Boc)_2O$, DMAP, THF, 90%; b. $NaHCO_3$, $MeOH/H_2O$, 87%; c. NaOH/EtOH, 77%.

Scheme 14. Protection and deprotection of adenine

be avoided.[134] Thus, conversion of **47** to **48** was carried out by treatment with aqueous sodium bicarbonate ($NaHCO_3$). In turn, compound **48** was converted to the mono-Boc derivative **49** in very good yield by treatment with sodium hydroxide for 3 days at room temperature.[133]

Based on the above research on adenine, a similar procedure was applied to 3-deazaadenine. The synthesis of *tris*-Boc-protected 3-deazaadenine **51** began with 6-chloro-3-deazapurine (**14**). Following the method reported by Crey-Desbiolles and Kotera,[135] conversion of compound **14** to 3-deazaadenine **50** was achieved by hydrazine treatment followed by Raney nickel hydrogenolysis. Treatment of compound **50** with excess *tert*-butyl dicarbonate and a catalytic amount of 4-(dimethylamino)pyridine in tetrahydrofuran at room temperature for 3 days afforded *tris*-Boc-protected compound **51**.

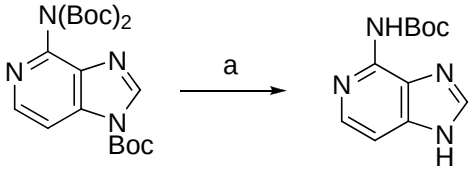


14 **50** **51**

a. i) NH_2NH_2; ii) Raney-Ni, 90%; b.$(Boc)_2O$, THF, 86%.

Scheme 15. Synthesis of *tris*-Boc protected 3-deazaadenine

With compound **51** in hand, treatment with aqueous sodium bicarbonate at room



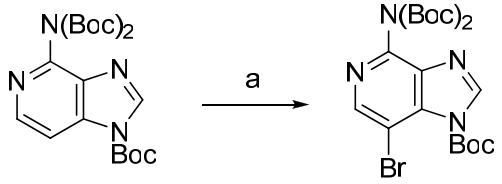
51 **52**

a. $NaHCO_3$, $MeOH/H_2O$, r.t. 81%.

Scheme 16. Deprotection compound 51 with $NaHCO_3$

temperature did not give the expected *bis*-Boc protected compound analogous to **48**. Analysis by NMR showed that the resulting compound was the mono-Boc derivative **52**. Changing the concentration of aqueous sodium bicarbonate and reaction conditions did not change the result of the reaction.

Efforts were undertaken for the bromination of **51**. Following our general procedure by treating compound **51** with *N*-bromosuccinimide in different solvents resulted in low conversation of reactant and a complex mixture of products.

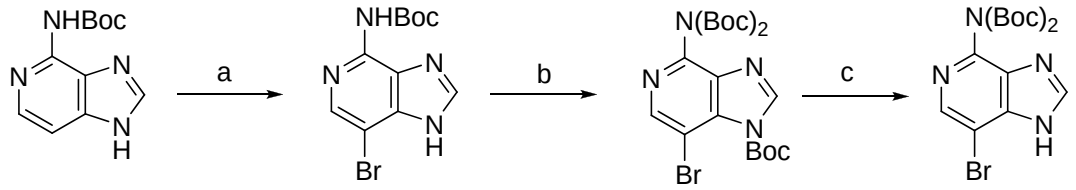


51 **53**

a. NBS, THF or CH_2Cl_2

Scheme 17. Bromination of compound 51

Re-evaluation of the plan resulted in the following research, which showed that bromination of compound **52** resulted in **54** as the only product. Repeating the protecting step with excess *tert*-butyl dicarbonate and a catalytic amount of 4-(dimethylamino)pyridine in tetrahydrofuran at room temperature in 3 days afforded *tris*-Boc-protected **55**. Treatment with aqueous sodium bicarbonate at room temperature did give the expected *bis*-Boc protected compound **45** as the only product.



52 **54** **55** **45**

a. NBS, CH_2Cl_2, 80%; b. $(Boc)_2O$, DMAP, THF, 88%; c. $NaHCO_3$, $MeOH/H_2O$, r.t., 85%

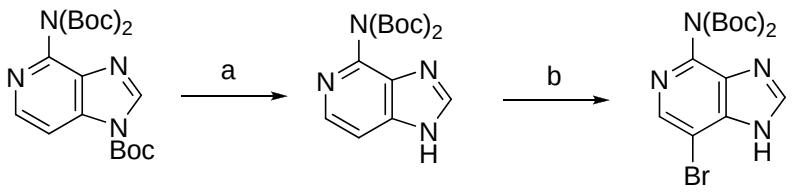
Scheme 18. Synthesis of compound 45 (Path 1)

Although compound **45** was finally obtained, it became obvious that the route (Schemes 15, 16 and 18) were providing a lengthy and somewhat inefficient path. Furthermore, the repeated Boc protection and deprotection steps made this route uneconomical. Thus, other synthetic approaches to **45** were given attention.

Use of tetra-*n*-butylammonium fluoride for the removal of silyl ether group protections is well known[134]; however it has rarely been used for the cleavage of other acid-sensitive groups. A recent study has shown it could be employed for selective deprotection of substrates containing both aromatic and aliphatic *N*-Boc groups.[119,136] It has been observed that the leaving amide anion generally followed the order: aromatic > benzylic > aliphatic.[136] In this direction, using this reagent, this research investigated use of tetra-*n*-butylammonium fluoride to remove *N*-Boc protective groups selectively from a heteroaromatic position.

Treatment of *tris*-Boc protected 3-deazaadenine (**51**) with tetra-*n*-butylammonium fluoride in THF at room temperature overnight afforded the complete conversion to di-

Boc-protected **56**. Bromination of **50** with *N*-bromosuccinimide gave the desired product **45**.



51 **56** **45**

a. TBAF, THF, 79%; b.NBS, CH_2Cl_2, 77%

Scheme 19. Synthesis of compound 45 (Path 2)

In addition to NMR data, the structure of **45** was confirmed by X-ray crystallography (Figure 26), which showed the bromine atom at the 3-position and the location of the two Boc protecting groups.

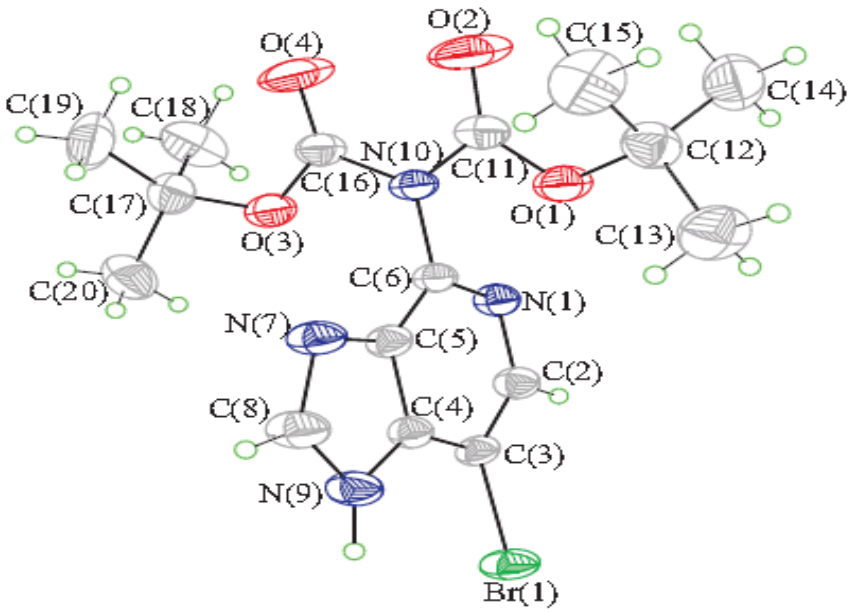
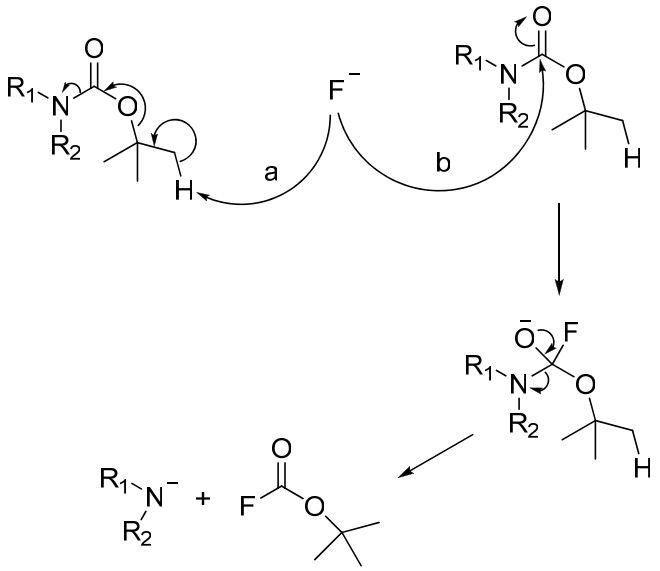


Figure 26. X-ray crystal structure of compound 45

Mechanism for deprotection of Boc with tetra-*n*-butylammonium fluoride

Two possible proposals could explain the mechanism of fluoride (as a nucleophile)[137,138] promoted Boc deprotection (Scheme 20). The first possibility (a) was the β-elimination, which gave the amide, *iso*butene, carbon dioxide and hydrofluoric acid.[139,140] The other possibility (b) was fluoride acting directly on the carbonyl group leading to the amino anion and Boc-F.[141] *tert*-Butanol, carbon dioxide and hydrofluoric acid would then follow upon hydrolysis. In both cases, hydrofluoric acid was neutralized by the released amide.[140] Studies[136] have shown that several substrates (such as CH_3OCO-), which underwent this reaction, but do not contain a proton susceptible for elimination (path a). Thus, the first proposal (a) could be rejected. Proposal (b) appears to be most appropriate to explain the mechanism of deprotection of *tert*-butyl ester derivatives by tetra-*n*-butylammonium fluoride.



Scheme 20. Postulated mechanism for deprotection of Boc

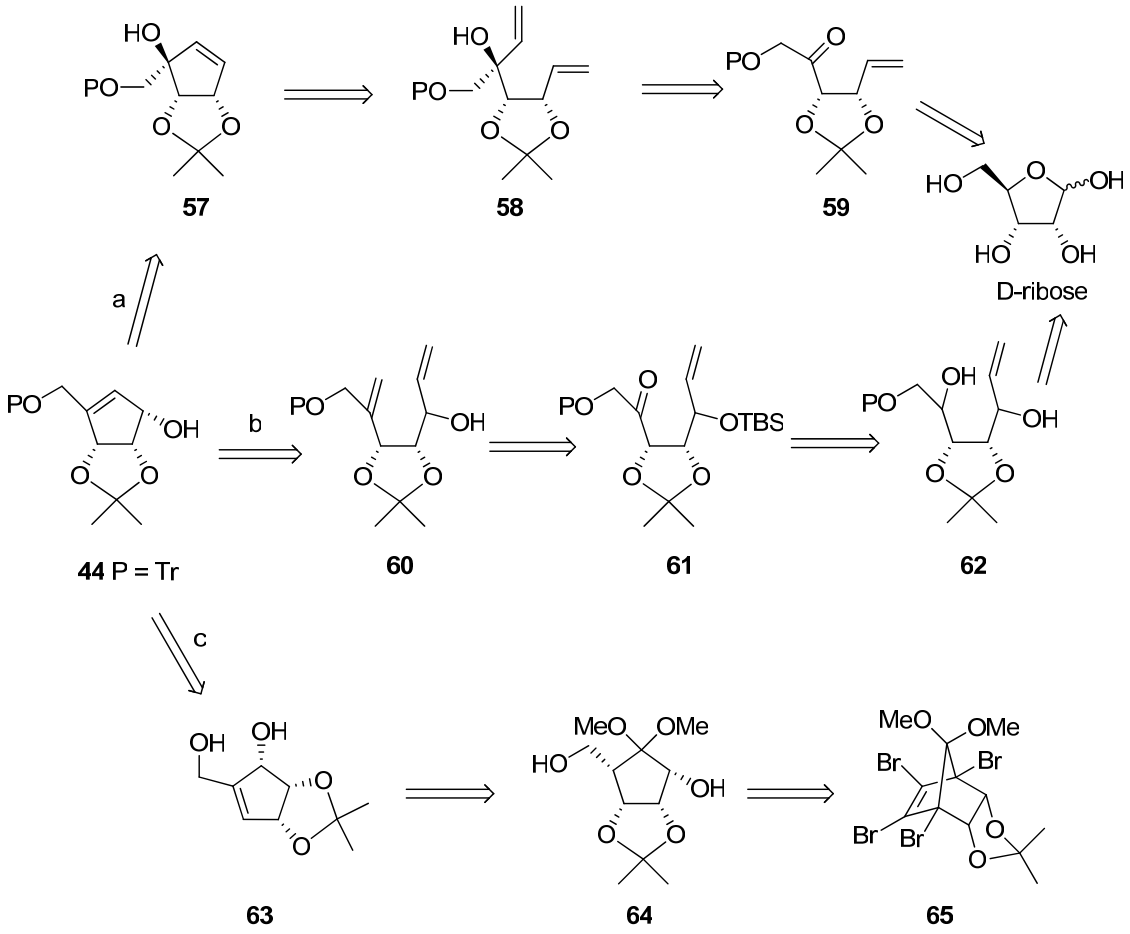
Synthesis of precursor 44

Retrosynthetic approach toward 44

Chiral cyclopentenol **44** has been recognized[142] as a key intermediate in the synthesis of 3-deazaNpc A derivatives. Thus a retrosynthetic analysis to **44** was designed (Scheme 21). The analysis envisioned that **44** could be obtained from either diene **58** (route a) or **60** (route b) employing the ring-closing metathesis (RCM) reaction, one of the most powerful methods for the formation of small-sized rings via C-C double bonds.[143] This strategy has been set as a focus in the synthesis of **44** in recent years.[142,144,145] Several syntheses have been reported. One of the practical routes applying Grubbs catalyst to **58** is route a, which was reported by Jeong and coworkers in 2004.[144,145] Compond **44** was envisioned as accessible from the product of the RCM reaction, that is, tertiary allylic alcohol **57**, upon oxidative rearrangement. Compound **58** could be approached by applying Grignard conditions on **59**, which, in turn, would be obtainable from ribose. The alternative route b approach to **44** would also employ the RCM procedure as the key step. Among the approaches using compound **60** as precursor for the RCM reaction, Chu's pathway in 2006 appeared to be more efficient.[142] Compound **60** was anticipated to be accessible from **61** via a Wittig reaction and deprotection. In turn, **61** could be approached by protecting the allylic alcohol of **62** followed by oxidation of the secondary alcohol. Compound **62** would be available from ribose, much like **59** from pathway a.

A recent access to **44** was reported by Khan and Rout in 2007 (route c).[146] They developed a process whereby the acetonide group in **63** could be shuffled to afford **44**. Compound **63** was envisioned to be accessible from **64** by shifting the acetonide group and utilizing a reduction reaction. In turn, compound **64** was obtainable from the

somewhat complex **65**.[147] This method also results in questionable stereochemical outcomes.



Scheme 21. Three retrosynthetic approaches toward 44

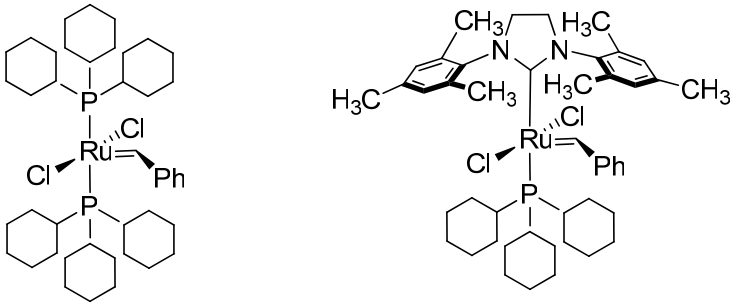
Grubbs catalysts for RCM

Since the Grubbs catalyzed ring closure metathesis is central to the preparation of the key intermediate (that is, chiral cyclopentenol **44**), a comment about this is in order.

The olefin metathesis reaction catalyzed by the Grubbs catalyst (Figure 27) is now well-developed as a synthetic method for the redistribution of a carbon-carbon double bond.[143] Ring closure between terminal vinyl groups, cross metathesis of terminal vinyl

groups and ring opening of strained alkenes reflect the diversity of olefin metathesis reactions. When molecules with terminal vinyl groups are used, the equilibrium can be driven by the ready removal of the product ethene from the reaction mixture. Ring opening metathesis can employ an excess of a second alkene (for example, ethene), but can also be conducted as a homo- or co-polymerization reaction. The driving force in that case is the loss of ring strain.

The ring-closing metathesis reaction[143,148-151] is one of the most powerful methods for the formation of small-sized rings via C-C double bonds. Recently, it has been employed



1st generation 2nd generation

Figure 27. Grubbs catalysts

for the synthesis of disubstituted cyclopentenols.[144,152-154] The literature also provides a few examples of the use of the Grubbs catalyzed RCM reaction as a key synthetic step for the trisubstituted cyclopentenol derivatives (as with **44**).[155-157]

Grubbs catalysts show high catalytic reactivity, great functional tolerance, and moderate air and moisture stability. The second generation Grubbs catalysts are even more stable and more active than the original versions.[143,158-161]

The mechanism has been investigated thoroughly both experimentally[162,163] as well as theoretically.[164-166] A mechanism proposed by Chauvin[167] is generally accepted. The mechanism is based on the facile [2 + 2] cycloaddition of an alkene and a 14 valence electron ruthenium carbene intermediate to a ruthenacyclobutane complex and its cycloreversion.

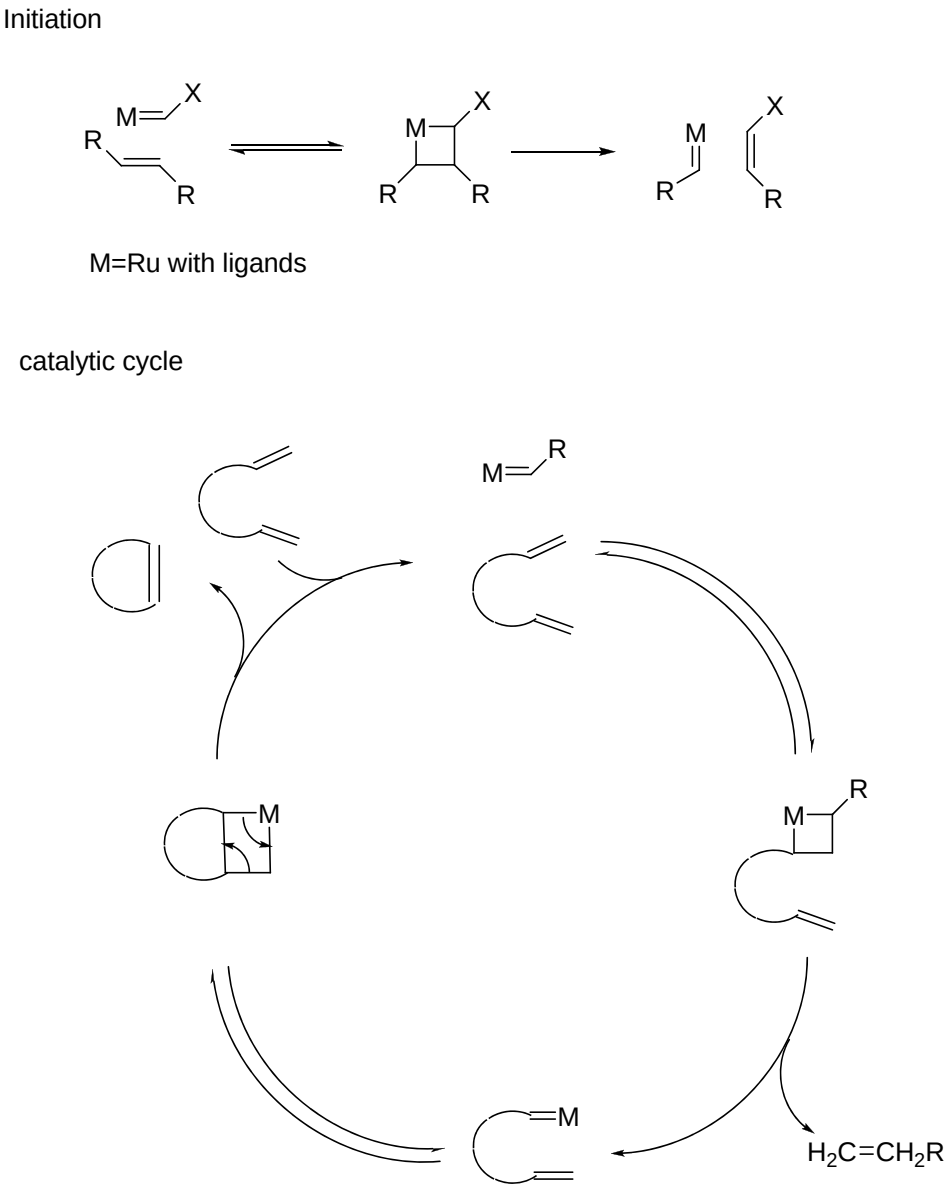
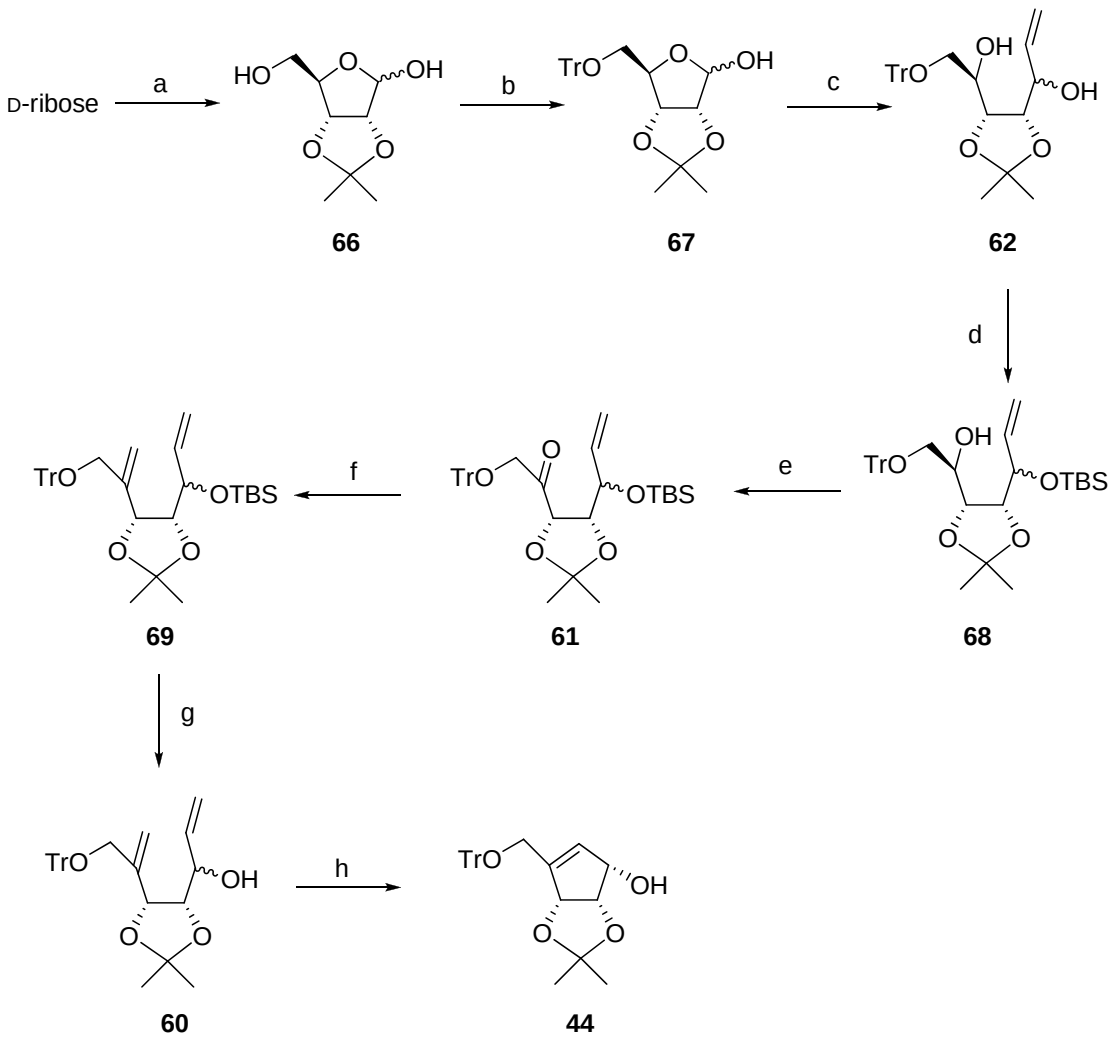


Figure 28. Mechanism of RCM

Route b to 44

The synthesis of **62** followed a reported procedure, which has been well studied.[142,168-170] In that direction, 2,2-dimethoxypropane was applied to the starting D-ribose in the presence of a catalytic amount of *p*-toluenesulfonic acid to give the *iso*propylidene protected compound **66** (Scheme 22). Protection of the primary alcohol of **66** with triphenylmethyl chloride (TrCl) provided **67**. Application of the Grignard reagent vinylmagnesium bromide to **67** gave diol **62**, which was observed as two configurations in the NMR spectrum. Compound **62** was subsequently protected with a *tert*-butyldimethylsilyl group (TBDMS) that occurred only at the allylic hydroxyl position to afford diastereomeric silyldienol **68**.[171] Swern oxidation of the protected secondary alcohol **68** gave the ketone **61**. Wittig reaction with methyltriphenylphosphonium bromide and butyllithium (*n*-BuLi) in tetrahydrofuran was applied to **61** to introduce the requisite double bond for the RCM reaction. The resulting product of Wittig reaction, diene **69**, was subjected to the RCM reaction in the presence of first- or second-generation Grubbs catalysts. The reaction failed to give the RCM product **44**. The reason for the result may be due to the significant steric hindrance inflicted by the bulky group at the terminal double bond, which is a major obstacle of the RCM reaction.[172-174] To reduce the steric hindrance, removing the silyl group from diene **69** with tetrabutylammonium fluoride provided dienol **60**. This less sterically hindered **60** was successfully subjected to RCM reaction with first or second-generation Grubbs catalyst. Only 2.0 mol % of the second-generation catalyst was needed (compared to 40.0 mol % first-generation catalyst needed to get the similar result).[142] Finally, cyclopentenol (+)-**44** was in hand in a multigram-scale in 8 steps from ribose.



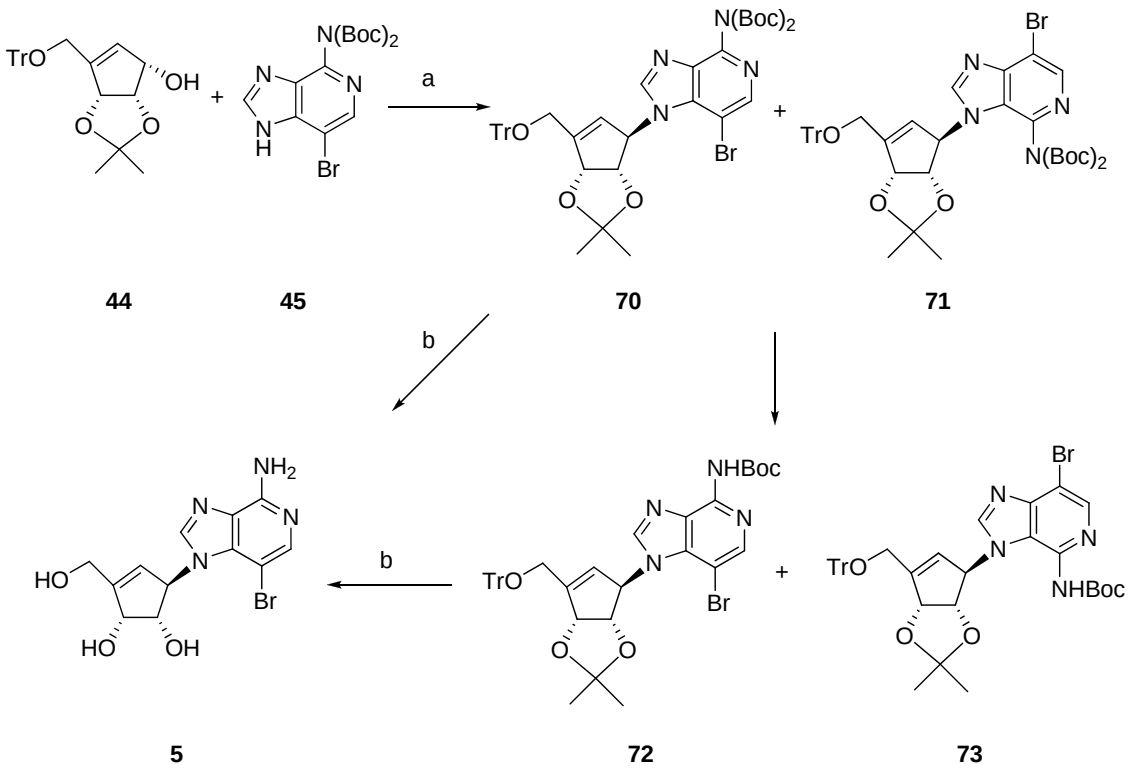
a. 2,2-dimethoxypropane, $TsOH{\cdot}H_2O$, acetone, rt, 89%; b.TrCl, Et_3N, DMAP, DMF, rt, 88%; c. vinylmagnesium bromide,THF, -78 °C to rt, 98%; d. TBSCl, imidazole, CH_2Cl_2/DMF, rt, 78%; e. $(COCl)_2$, Et_3N, CH_2Cl_2, -60 °C, 97%; f. Ph_3PCH_3Br, *n*-BuLi, THF, rt, 90%; g. TBAF, THF, rt, 95%; h. second-generation Grubbs catalyst, CH_2Cl_2, rt, 95%.

Scheme 22. Synthesis of cyclopentenol 44

Synthesis of 3-bromo-3-deazaneplanocin A

With **44** and **45** available, the synthesis of 3-bromo-3-deazaneplanocin A (**5**) was carried out (Scheme 23) starting with a Mitsunobu reaction between these two precursors.

NMR spectra of the resulting product indicated that N-1 (purine N-9, **70**) was the major product with N-3 (purine N-7, **71**) as a minor product. It was observed that one Boc



a. DIAD, PPh_3, THF, 70%; b. 1N HCl/MeOH, 75% from crude **70**.

Scheme 23. Synthesis of compound 5

was easily removed resulting in **72** and **73** during workup of step a of Scheme 23. Thus flash chromatography was applied to expeditiously purify **70** and **71** to minimize loss of one Boc group. The determination of the N-7 and N-9 product of Mitsunobu reaction was done via the X-ray of the product in the next step. Hydrochloric acid was used on the product of Mitsunobu reaction to remove all protecting groups. The crude product from

this reaction was applied to flash chromatagraphy and then recrystallized from methanol to give 3-bromo-3-deazaneplanocin A (**5**).

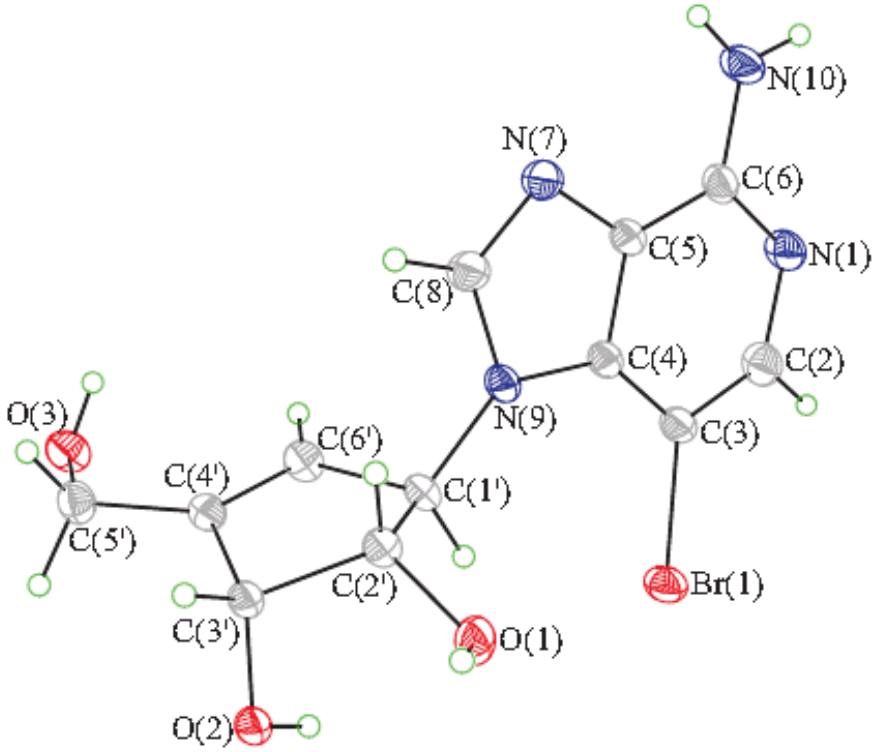


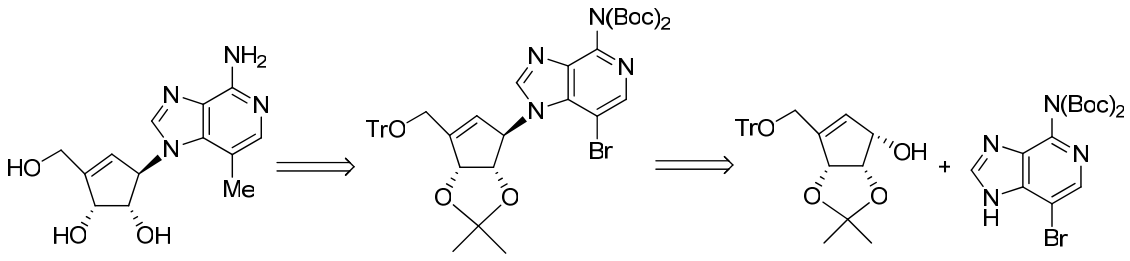
Figure 29. X-ray crystal structure of 5

In addition to NMR and microanalysis data, the structure of **5** was confirmed by X-ray crystallography, which showed the linkage between N-7 and C-1' and the bromine atom at the 3-position.

CHAPTER 4. SYNTHESIS OF 3-METHYL-3-DEAZANEPLANOCIN A

Retrosynthetic approach toward 3-methyl-3-deazaneplanocin A (6)

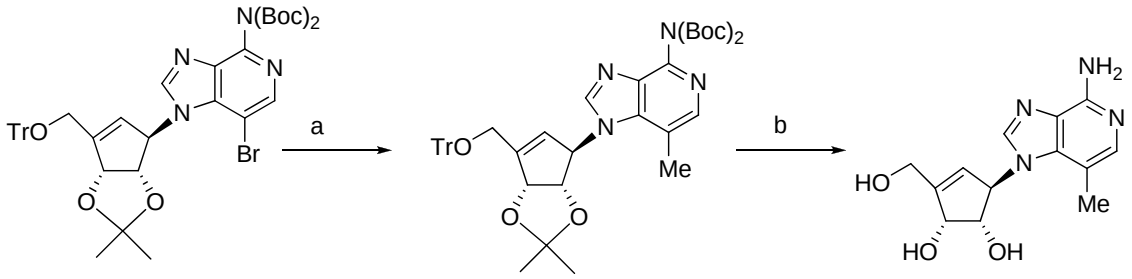
Success in the synthesis of 3-methyl-5'-nor-3-deazaaristeromycin suggested a similar approach to 3-deaza-3-methylneplanocin A (**6**). Thus, a retrosynthetic analysis to **6** was designed. This route provided a strategy such that the methyl group at the 3 position could be incorporated from a protected form of 3-bromo-3-deazaneplanocin A through an alkylation reaction. The previous section reported that 3-bromo-3-deazaneplanocin A derivatives were accessible from a cyclopentenyl moiety **44** and a purine base **45** through a coupling reaction.



Scheme 24. Retrosynthesis of 3-methyl-3-deazaneplanocin A

Synthesis of 3-methyl-3-deazaneplanocin A (6)

A palladium-catalyzed cross-coupling reaction of **70** with trimethylaluminum succeeded in converting **70** into **74** in high yield. Deprotection of **74** with hydrochloric acid followed by flash chromatography and recrystallization, led to the target compound **6**.



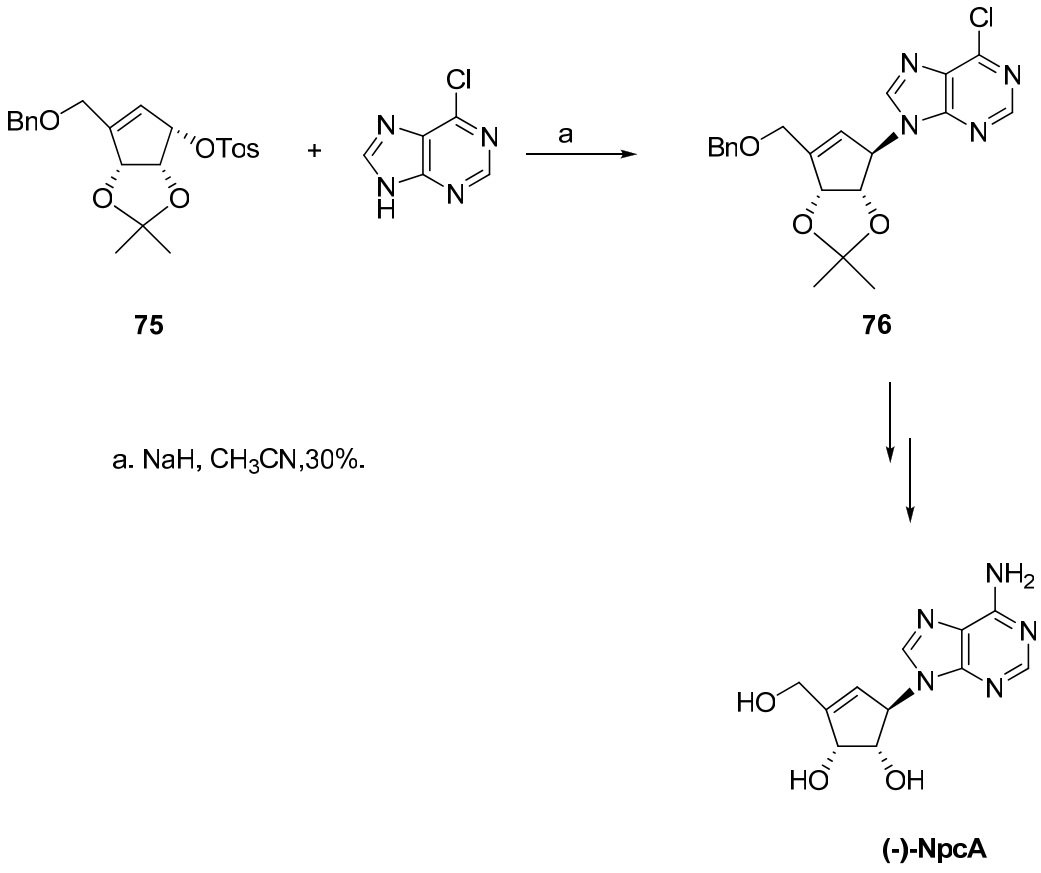
70 **74** **6**

a. $AlMe_3$, $Pd(Ph_3)_4$, THF, 78%; b. 1N HCl/MeOH, 67%.

Scheme 25. Synthesis of 6

CHAPTER 5. AN EFFICIENT SYNTHESIS OF 3-DEAZANEPLANOCIN A EMPLOYING MITSUNOBU REACTION

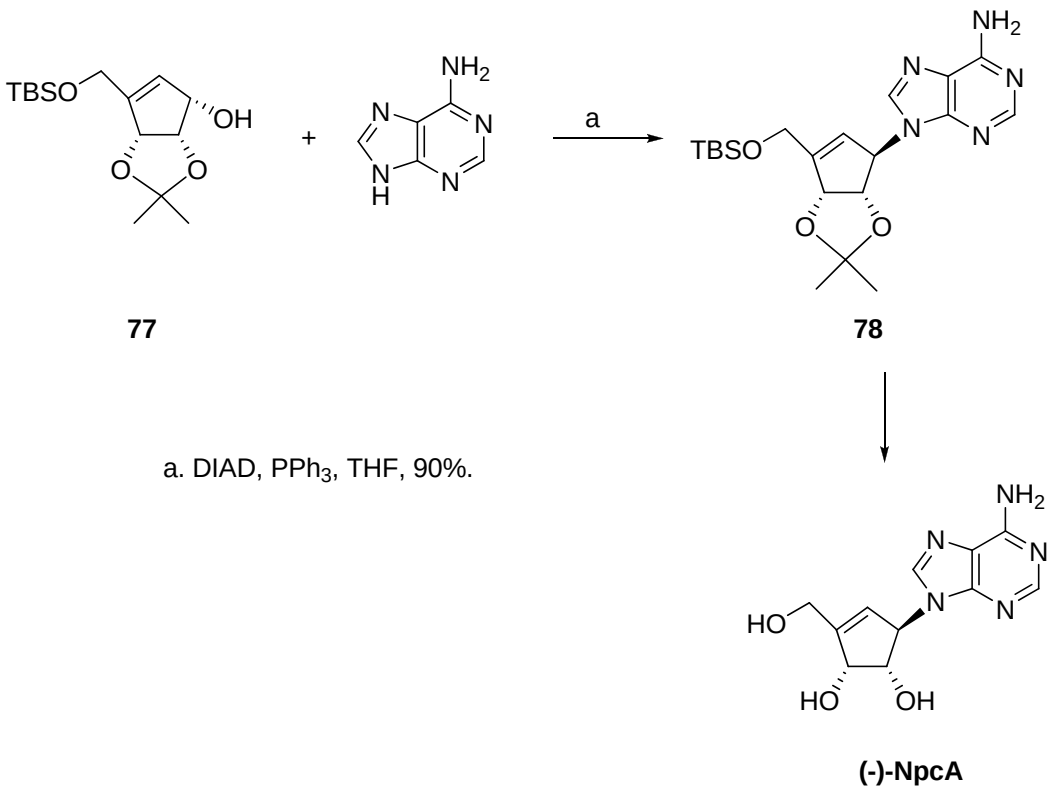
The early enantioselective synthesis of NpcA by Marquez and co-workers [175] employed a direct S_N2 displacement process by involving the sodium salt of 6-chloropurine and the cyclopentyl tosylate **75**. This reaction afforded a 30% yield of the



Scheme 26. Synthesis of NpcA through S_N2 reaction[175]

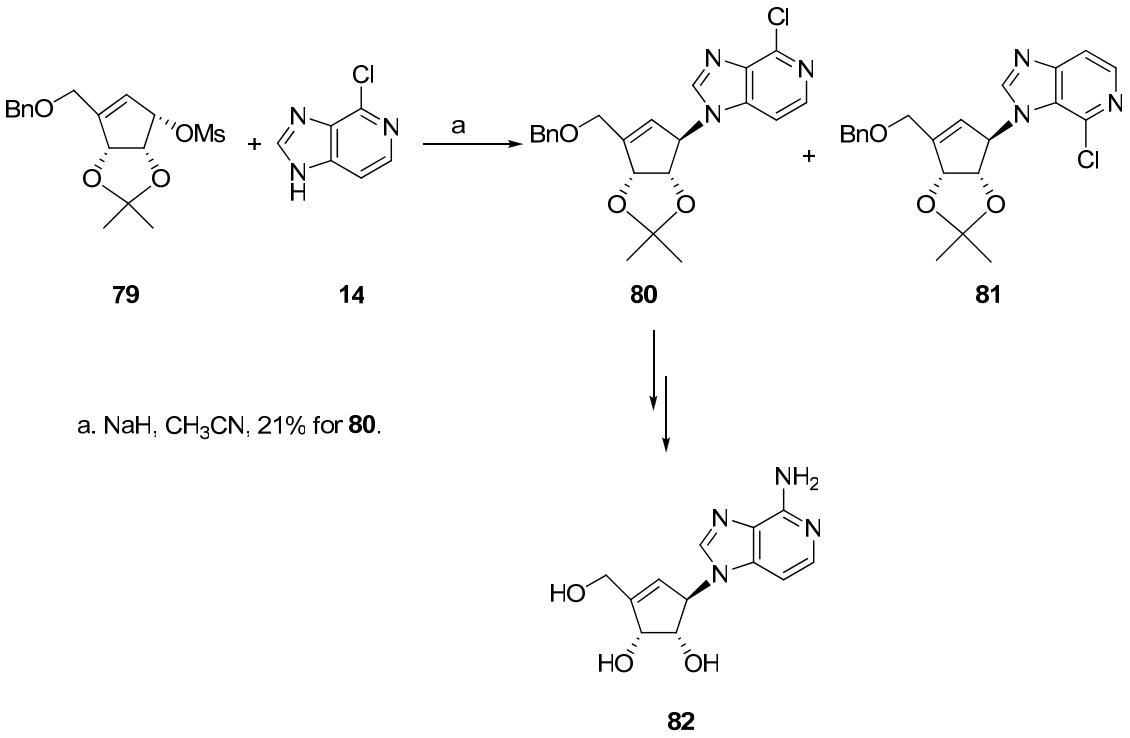
N-9 isomer **76**. Small amounts of either unreacted or degraded tosylate were also found in the product mixture. The amount of the N-7 isomer detected (by TLC) was insignificant. Treatment of the product of this reaction with methanolic ammonia and deprotection of the resulting product with boron trichloride afforded neplanocin A.

Application of the Mitsunobu coupling with the appropriate cyclopentenyl moiety and heterocyclic base significantly improved the efficacy of the synthesis of NpcA. Employing the Mitsunobu reaction the protected cyclopentenyl α-hydroxyl compound **77** was coupled with adenine with inversion to give the penultimate derivative of NpcA, **78**, which was treated with aqueous acid to give neplanocin A. The yield of this key coupling reaction was reported from moderate to high (63% - 90%).[176-178]



Scheme 27. Synthesis of NpcA employing Mitsunobu reaction

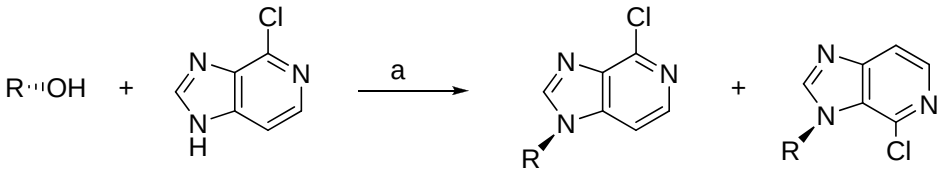
For the preparation of 3-deazaneplanocin A, Tseng and coworkers[20] utilized an approach similar to the S_N2 process of Scheme 26. In this regards, the cyclopentenyl mesylate **79** was reacted with the sodium salt of 6-chloro-3-deazapurine (**14**) to give a mixture of the N-3 isomer **81** and the N-1 isomer **80** in 24% yield. The major product was later identified as the desired N-1 isomer **80** and was obtained in 21% yield after purification by column chromatography. The simultaneous removal of both benzyl and *iso*propylidene moieties from the product afforded the corresponding chloro-3-deazapurine, which was subsequently reacted with anhydrous hydrazine to give the intermediate hydrazino compound. This compound was immediately reduced with Raney nickel, and the resulting target compound, 3-deazaneplanocin A (**82**) was obtained.



Scheme 28. Synthesis of 3-deazaNpcA through S_N2 reaction

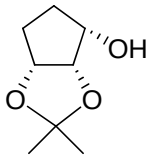
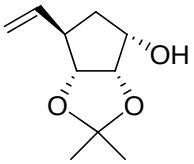
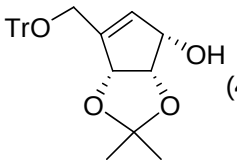
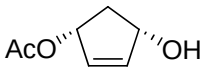
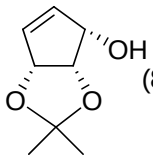
In efforts in the Schneller group to further exploit the 3-deazapurine carbocyclic nucleoside platform as a source for new antiviral candidates, a more efficient synthetic means for 3-deazaNpcA and 3-deazaAri were required, which could lead to a number of structural variations. In the hope that the Mitsunobu reaction would serve our purposes, our group began to investigate this reaction, which has recently been successfully employed to produce traditional carbocyclic nucleosides in the 3-deazapurine *genera*.[116] Following standard Mitsunobu conditions (that is triphenylphosphine and di*iso*propyl azodicarboxylate in tetrahydrofuran), the reaction of 4-chloro-1*H*-imidazo-[4,5-*c*]pyridine **(14)** with various cyclopentanols (**83**, **84**, **44**, **85**, **86**[179]) gave the results presented in the Table 2. Structural assignments for the N-1 and N-3 isomers were carried out by comparing the ^{1}H NMR and the ^{13}C NMR of the isomers. The proton on the cyclopentyl carbon bearing the heterocyclic ring in the N-3 product is downfield in the ^{1}H NMR spectrum compared to the N-1 product. A characteristic ^{13}C NMR peak at 106 ppm was observed for the carbon (possibly C-2) in the heterocyclic ring of all N-1 products, while the peak moves to 115 ppm in all N-3 products. Supporting these NMR assignments for N-3 product is **88d**, whose structure was confirmed by X-ray crystallography and whose NMR spectrum fit the diagnostic peaks used for isomer distinction.[116] The reaction in the first two entries cleanly gave N-1 compounds (**87a**, **b**) as the only regioisomers. This led to the Ari series of compounds. The more reactive allylic alcohols (entries c-e), which were the cyclopentenyl moieties of the NpcA series of compounds, however, yielded the N-1 products (**87c**, **d**, **e**) along with the N-3 isomers (**88c**, **d**, **e**) as the major isomer.

Table 2. Mitsunobu reaction between 14 and sugar moieties[116]

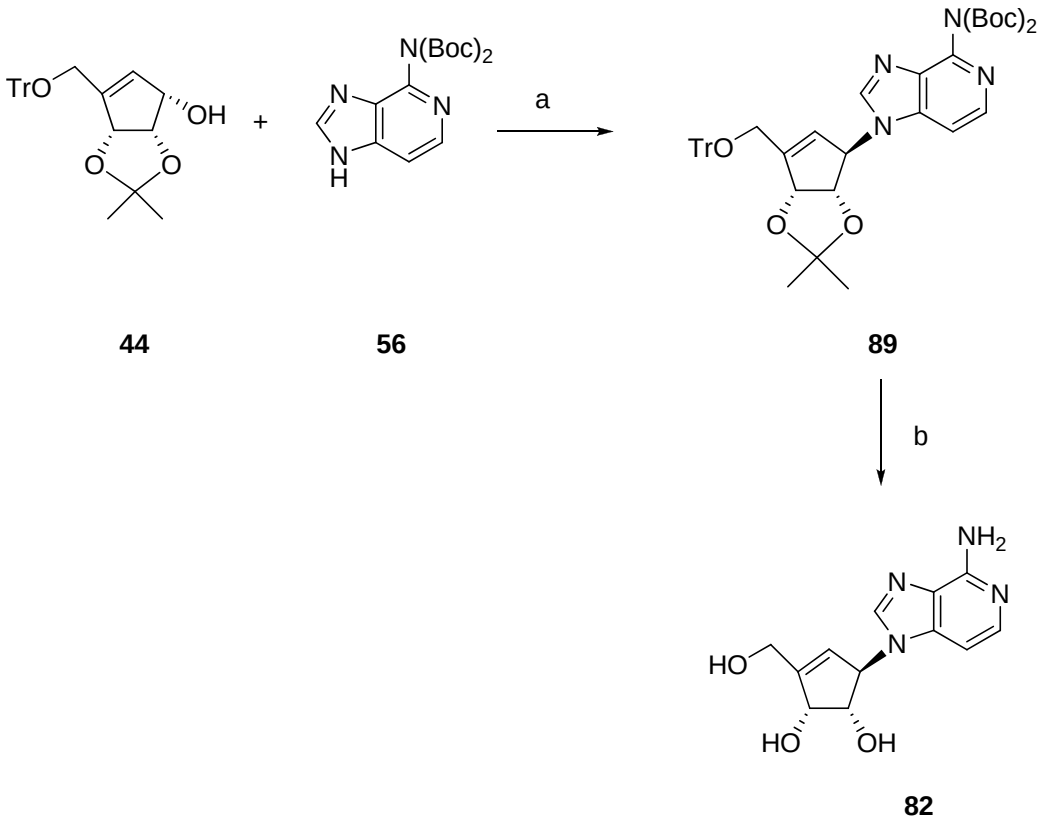


14 **87a-e** **88a-e**

a. DIAD, PPh_3, THF

Entry	R···OH	Products (%)	
		87	**88**
a	 (**83**)	86	0
b	 (**84**)	70	0
c	 (**44**)	42	53
d	 (**85**)	38	57
e	 (**86**)	32	43

The *tert*-butoxycarbonyl protected 3-deazaadenine derivative **56** was considered to be a worthy candidate to subject to the Mitsunobu reaction with **44** as the start of a convergent pathway leading to NpcA. Compound **56** (Scheme 29) proved to have overwhelming advantages compared with 6-chloro-3-deazapurine used in Table 2 in the Mitsunobu reaction. One is the significantly accelerated reaction rate due to the improved solubility of **56** in THF compared to 6-chloro-3-deazapurine. Also, the reaction could be achieved at room temperature within 2 h rather than overnight. Another advantage was the exclusive regioselectivity for the N-1 isomer **89** in a yield of 71% with no N-3 isomer being observed. Exclusive formation of **89** may be due to the steric hindrance by the two



44 **56** **89** **82**

a. DIAD, PPh_3, THF, 71%; b. 1N HCl/MeOH,72%.

Scheme 29. Synthesis of 3-deazaNpcA employing Mitsunobu reaction

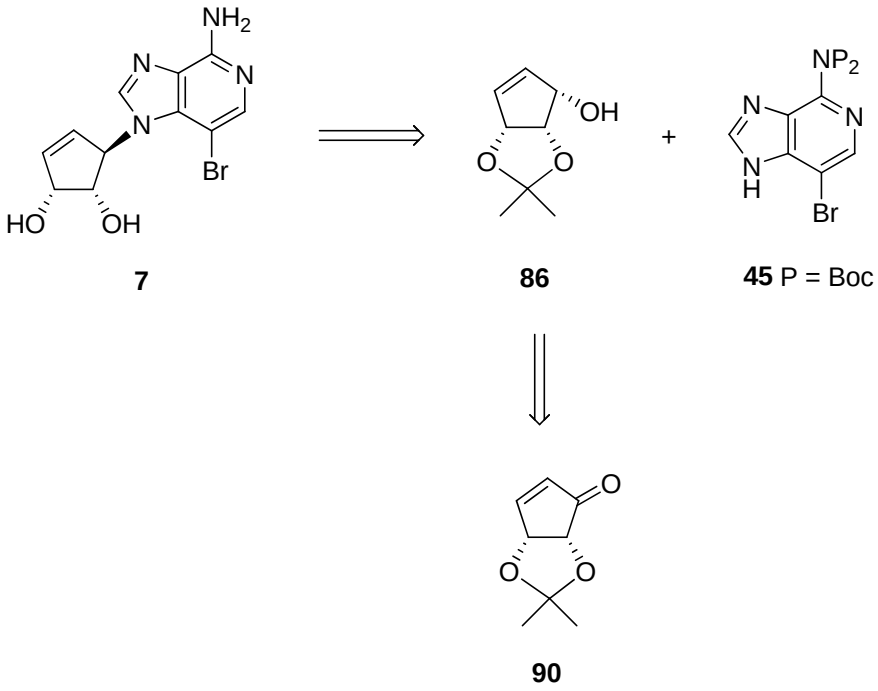
bulky Boc groups on the 4-nitrogen, which blocked the nucleophilic attack by the N-3 position.

Thus, a new convergent pathway to 3-deazaNpc A has been established by employing the Mitsunobu reaction between *tert*-butoxycarbonyl protected 3-deazaadenine **56** and allylic alcohol **44**, followed by a deprotection step under acidic conditions.

CHAPTER 6. SYNTHESIS OF 3-BROMO-3-DEAZA-5'-NORNEPLANOCIN A

Retrosynthetic approach toward 3-bromo-3-deaza-5'-norneplanocin A

Success with the synthesis of 3-bromo-3-deazaneplanocin A suggested similar approach to 3-bromo-3-deaza-5'-norneplanocin A (**7**). Thus, a retrosynthetic analysis to **7** was designed. Compound **7** was envisioned as being assembled by coupling the readily available **45** with cyclopentenol **86**, which would be accessible from an important precursor, cyclopentenone **90**.



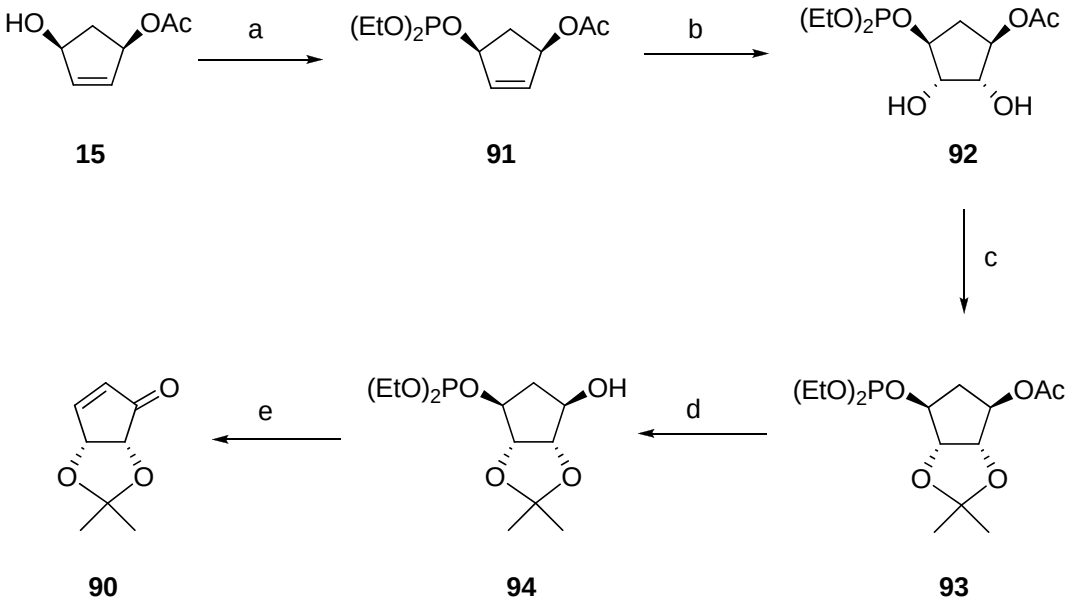
7 **86** **45** P = Boc

90

Scheme 30. Retrosynthetic approach towards 3-bromo-3-deaza-5'-norNpcA

Synthesis of precursor 90

With compound **15** in hand, preparation of cyclopentenone **90** followed the pathway developed in our laboratories[54,56,180] beginning with the conversion of **15** to the monophosphate **91** with diethyl chlorophosphate. Glycolization of **91** to diol **92** was achieved by using a standard procedure with N-methylmorpholine N-oxide and a catalytic amount of osmium tetroxide. Protection of **92** as an *iso*propylidene derivative by applying 2, 2-dimethoxypropane afforded **93**. The removal of the acetate group with lithium hydroxide gave **94**. The desired **90** was obtained by treatment of **94** with pyridinium chlorochromate via an oxidative elimination.[54,181,182] Counting the three steps

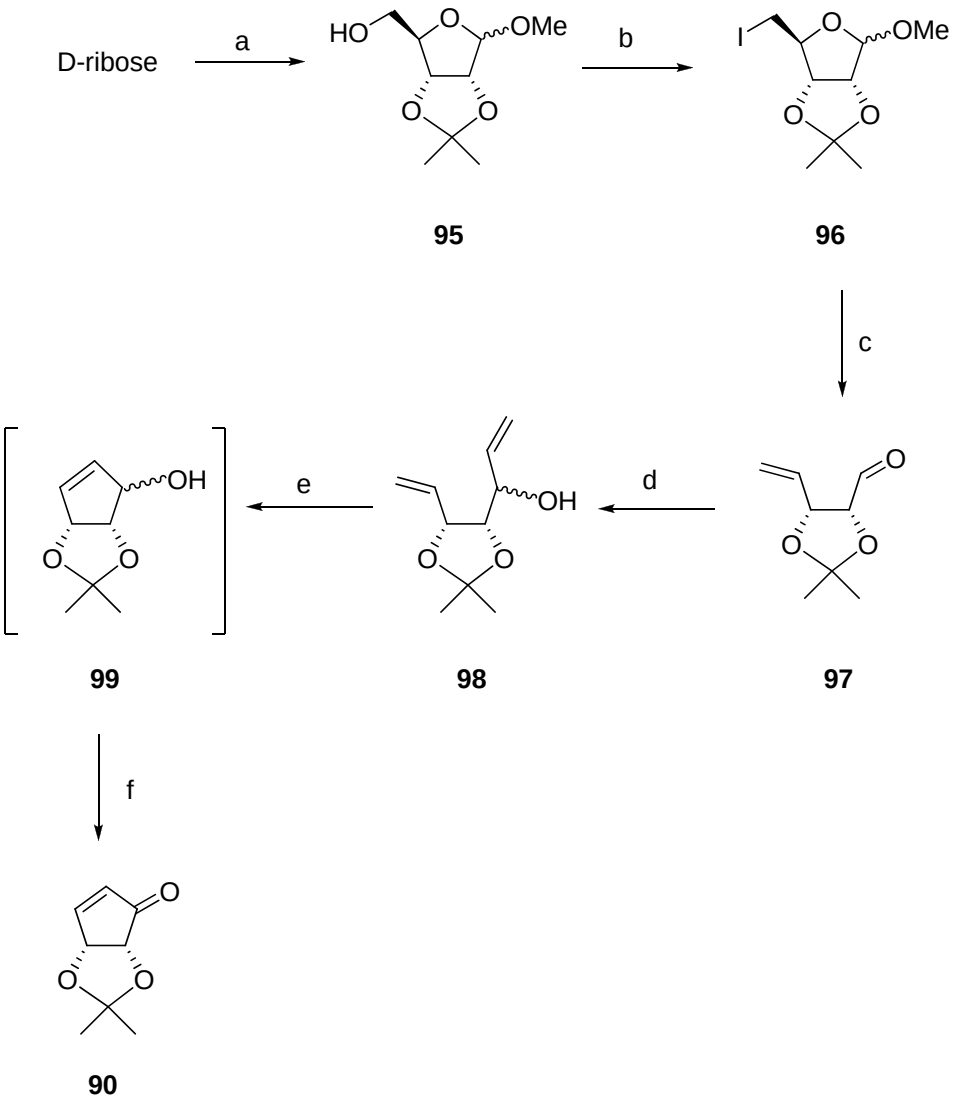


a. $(EtO)_2POCl$, pyridine, CH_2Cl_2, 100%; b. OsO_4, NMO, acetone, 70%; c. 2, 2-dimethoxypropane, acetone, TsOH, r.t., 100%; d. LiOH, THF/H_2O, 90%; e. PCC, celite, CH_2Cl_2, 81%.

Scheme 31. Synthesis of precursor 90 from monoacetate 15

for the preparation of **15**, the synthesis of **90** was achieved in eight total steps.

Another more efficient pathway was developed to achieve **90** in our laboratories by combining and optimizing steps in the literature starting from D-ribose.[169,183-186] As



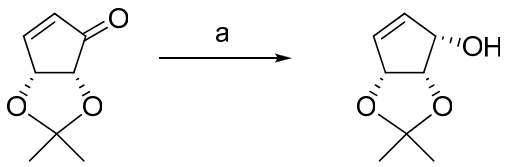
a. $(MeO)_2CMe_2$, MeOH, 78%; b. Ph_3P, I_2, imidazole, 99%; c. Zn, MeOH, 85%; d. Vinylmagnesium bromide, CH_2Cl_2, 80%; e. Grubbs catalyst, CH_2Cl_2; f. PCC, CH_2Cl_2, 93% for two steps.

Scheme 32. Synthesis of precursor 90 from D-ribose

shown in Scheme 32, treatment of D-ribose with 2, 2-dimethoxypropane and methanolic hydrochloric acid afforded **95** with diol protection as the isopropylidene and methylation at the anomeric hydroxyl group. Triphenylphosphine and iodine transformed the primary hydroxyl group of **95** to iodide **96**. Aldehyde **97**, which was quite volatile, was obtained after the reductive cleavage of **96** with powdered zinc in hot methanol. A Grignard 1, 2-addition with vinylmagnesium bromide to **97** provided **98**. Diene **98** was subjected to ring closure metathesis with the Grubbs 1st generation catalyst. The unisolated **99** was treated with pyridinium chlorochromate to give **90**. This pathway proved to be efficient and practical in the lab, providing **90** in multigram quantity in five steps from ribose.

Synthesis of 86 via reduction of 90

The cyclopentenone **90** to allylic alcohol **86** conversion was carried out using Luche conditions (sodium borohydride and cerium(III) chloride heptahydrate) following a literature procedure.[187-189] The general utility of the Luche conditions gives a highly diastereoselective 1, 2-reduction.[188,189]



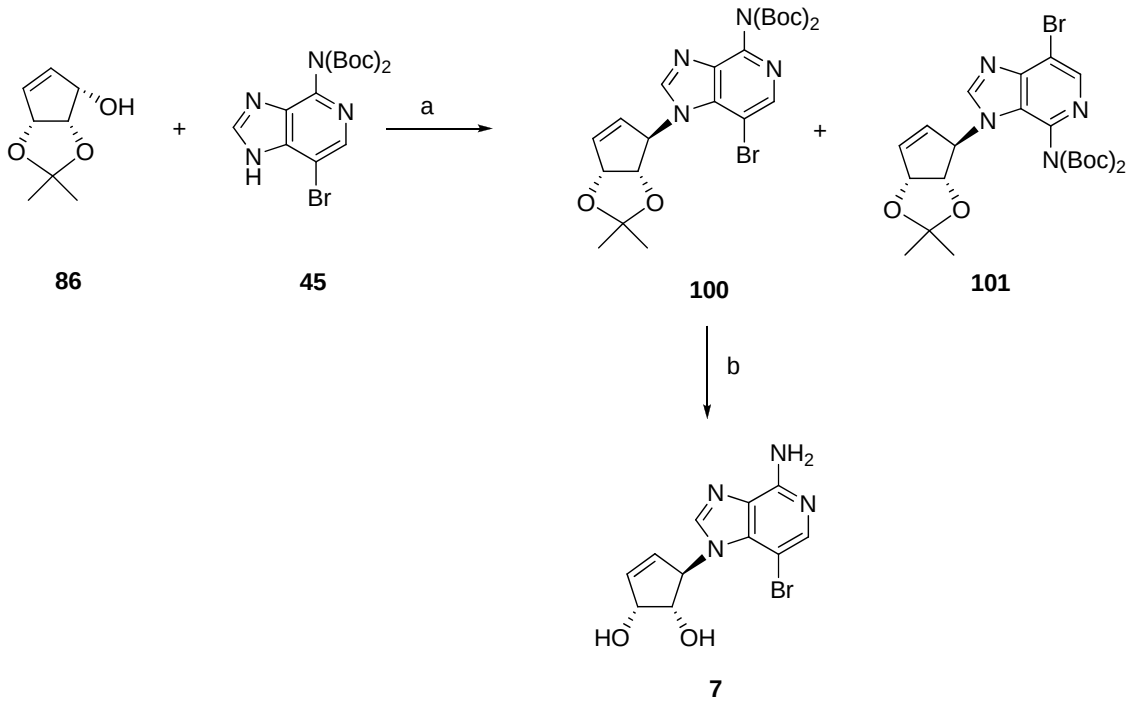
90 **86**

a. $CeCl_3 \cdot 7H_2O$, $NaBH_4$, MeOH, 99%.

Scheme 33. Synthesis of compound 86

Synthesis of 3-bromo-3-deaza-5'-norneplanocin A

With the appropriate two building blocks of **88** and **45** available, the synthesis of 3-bromo-3-deaza-5'-norneplanocin A (**7**) was carried out starting from a Mitsunobu reaction between these two precursors. NMR spectra for the resulting product indicated that N-1 (purine N-9, **100**) was the major product and N-3 (purine N-7, **101**) was a minor product. Hydrochloric acid was used to remove all protecting groups. The crude product was applied to flash chromatography column purification and the resultant product recrystallized from methanol to give 3-bromo-3-deaza-5'-norneplanocin A (**7**).



86 **45** **100** **101** **7**

a. DIAD, PPh_3, THF, 71% for **100**.; b. 2N HCl, reflux, 67%.

Scheme 34. Synthesis of compound 7

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 3.

Table 3. 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)
Filoviridae	Ebola virus
Flavivridae	Hepatitis C virus (HCV), West Nile virus, Yellow fever virus
Hepadnaviridae	Hepatitis B virus (HBV)
Heroesviridae	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

One of the most noteworthy observations from these analyses was that **2** and **3** showed significant activity against HBV, and **1** against HCV. Besides these, **1** showed activity against VSV, VV, para-influenza-3 virus, reovirus-1, Punta toro virus, cytomegalovirus, and measles virus. Compound **2** exhibited activity against VSV, VV, Varicella-zoster virus, cytomegalovirus, HCMV, parainluenza-3, and reovirus-1. Compound **3** was found to be highly active against Flu B and slightly active against Flu A. It also has activity against VSV, VV, reovirus-1, measles virus, RSVA, parainflurenza, and adeno virus. Compound **4** only showed good activity against VSV and VV as well as **1**, **2**, **3** and affected none of the other viruses assayed. The assay so far revealed some of the activities of target compounds. It also offers a basis for analogue development of agents to treat virus infections. These possibilities are under intense pursuit in our laboratories. Other bioassay data for target compounds will be forthcoming and be under study in our laboratories.

Table 4. Antiviral Activity of Compounds 1, 2, 3 against HSV-1, HSV-2, HCMV, VZV and EBV Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		**1**	**2**	**3**
HSV-1[d]	EC50[a]	>60	152	>60
	EC90[b]	>60	254.3	>60
	CC50[c]	205	300	235
HSV-2[d]	EC50	>60	222.2	>60
	EC90	>60	>300	>60
	CC50	>60	>300	235
HCMV[d]	EC50		1.7	1.2
	EC90		3.6	>60
	CC50		>300	219
VZV[d]	EC50	>300	0.11	>60
	EC90	>300		>60
	CC50	>300	>300	201
EBV[e]	EC50		>50	
	EC90		>50	
	CC50		>50	

[a]Effective concentration (μM) required to reduce virus plaque formation by 50%.

[b]Effective concentration (μM) required to reduce virus plaque formation by 90%.

[c]Cytotoxic concentration (μM) required to reduce cell growth by 50%.

[d]Tested on human foreskin fibroblasts (HFF) cells.

[e]Tested on Daudi cells. The data for EBV was based on viral capsid antigen enzyme linked immunsorbent assay (VCA Elisa assay).

Table 5. Antiviral Activity of Compounds 1, 2, 3, 4 against Punta Toro A, Adenovirus, Measles, West Nile and VEE Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		**1**	**2**	**3**	**4**
Punta Toro A[a]	EC50	1.6		>20	>100
	CC50	>200		100	>100
Adenovirus[b]	EC50	>100	>100	>100	>43
	CC50	N.A.	N.A.	N.A.	N.A.
Measles[c]	EC50	0.09	5	2	
	CC50	>100	N.A.	24	
West Nile[d]	EC50	>10		>100	>100
	CC50	N.A.		N.A.	N.A.
VEE[e]	EC50	>100			
	CC50	N.A.			

Units = μM

[a]Punta Toro A was tested on vero cells.

[b]Adenovirus was tested on A549 cells.

[c]Measles was tested on CV-1 cells.

[d]West Nile was tested vero cells.

[e]VEE was tested on vero cells.

Table 6. Antiviral Activity of Compounds 1, 2, 3, 4 Against Pinchinde, Yellow Fever, RSV, Parainfluenza and SARS CoV Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		**1**	**2**	**3**	**4**
Yellow Fever[a]	EC50	>100		>100	
	CC50	N.A.		N.A.	
RSV[b]	EC50	>100	>100	>100	>100
	CC50	N.A	N.A.	N.A.	N.A.
Parainfluenza[c]	EC50	>100	>100	>100	>100
	CC50	N.A	N.A	N.A	N.A
SARS CoV[d]	EC50			32	
	CC50			N.A	

Units = μM

[a]Yellow Fever was tested on vero cells.

[b]RSV was tested on MA-104 cells.

[c]Parainfluenza was tested on MA-104 cells.

[d]SARS CoV was tested on vero cells.

Table 7. Antiviral Activity of Compounds 1, 2, 3, 4 against Rhinovirus, Influenza A (H1N1), Influenza A (H3N2), Influenza B and Human Coronavirus Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		**1**	**2**	**3**	**4**
Rhinovirus[a]	EC50	>100	>100	>100	>100
	CC50	N. A.	N. A.	N. A.	N. A.
Influenza A (H1N1) [b]	EC50	>100	>100	45	N. A.
	CC50	N. A.	N. A.	N. A.	4
Influenza A (H3N2) [c]	EC50	>100	>100	>100	N. A.
	CC50	N. A.	N. A.	N. A.	4
Influenza B[d]	EC50	>100	10	1	N. A.
	CC50	N. A.	N. A.	N. A.	4
Human Coronavirus[e]	EC50	93±10		>100	
	CC50	>100		>100	

Units = μM

[a]Rhinovirus was tested on Hela cells.

[b] Influenza A (H1N1) was tested on MDCK cells.

[c] Influenza A (H3N2) was tested on MDCK cells

[d]Influenza B was tested on MDCK cells.

[e]Human Coronavirus was tested on B-SC-1 cells

Table 8. Antiviral Activity of Compounds 1, 3, 4 Against Vaccinia Virus and Cowpox Virus Based on Cytopathogenic Effect (CPE) Inhibition Assay

Compound		**1**	**3**	**4**
Vaccinia Virus[a]	EC50	197	248	>300
	CC50	>300	>300	>300
Cowpox Virus[b]	EC50	230	>300	>300
	CC50	>300	>300	>300

Units = μM

[a]Vaccinia Virus was tested on HFF cells.

[b]Cowpox Virus was tested on HFF cells.

Table 9. Antiviral Activity of Compounds 1, 2, 3, 4 in Vero Cell Cultures

Compound	Minimum cytotoxic concen-tration[a] (μM)	Minimum inhibitory concentration[b] (μM)				
		Para-Influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	>200	1.6	1.6	>200	>200	24
2	≥8	8	1.6	>8	>8	>8
3	100	20	4	>20	>20	≥20
4	>100	>100	>100	>100	>100	>100
Brivudin	>250	>250	>250	>250	>250	>250
(S)-DHPA	>250	50	250	>250	>250	>250
Ribavirin	>250	150	250	>250	>250	150

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 10. Antiviral Activity of Compounds 1, 2, 3, 4 in HEL Cell Cultures

Compound	Minimum cytotoxic concentr-ation[a] (μM)	Minimum inhibitory concentration[b] (μM)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 (TK^- KOS ACV)
1	>200	>200	>200	120	>200	>200
2	>200	>200	>200	24	>200	>200
3	>100	60	60	4	4	60
4	>100	>100	>100	>100	20	>100
Brivudin	>250	0.08	0.8	6	>250	250
Ribavirin	>250	250	250	50	150	250
Acyclovir	>250	0.4	0.16	>250	>250	150
Ganciclovir	>100	0.032	0.096	>100	>100	4

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 11. Antiviral Activity of Compounds 1 and 2 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]
1	34	N. D.[d]	>100	<0.16
2	8[e]	16[e]	400	18.7
Ganciclovir	7.1	9.0	>400	138
Cidofovir	1.3	3.2	>400	89

[a]Effective concentration (μM) required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).

[b]Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

[c]cytotoxic concentration required to reduce cell growth by 50%.

[d]Not determined.

[e]No complete inhibition at higher drug concentration.

Table 12. Antiviral Activity of Compounds 1 and 2 against Varicella-zoster in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity EC_{50} (μM)[a]		Cytotoxicity (μM)	
	TK^+ VZV	TK^- VZV	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
	OKA strain	07/1 strain		
1	>100	>100	>100	<0.16
2	40	36	≥80	18.7
Acyclovir	3.4	42	>1778	356
brivudin	0.013	>240	1200	452

[a]Effective concentration (μM) required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).

[b]Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

[c]Cytotoxic concentration required to reduce cell growth by 50%.

Table 13. Cytoxity and Antiviral Activity of Compounds 1, 2 and 4 in Hela Cell Cultures

Compound	Minimum cytotoxic concentration[a] (μM)	Minimum inhibitory concentration[b] (μM)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	>200	8	>200	200
2	>200	40	>200	>200
4	>100	20	>100	
Brivudin	>250	6	>250	250
(S)-DHPA	>250	>250	>250	>250
Ribavirin	>250	10	30	6

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table 14. Antiviral Activity of Compounds 1, 2, 3 against HBV

Compound			**1**	**2**	**3**
HBV	Assay: VIR[a]	EC50 (3TC)	>10 (0.048)		0.808 (0.045)
		EC90 (3TC)	>10 (0.142)		3 (0.139)
		CC50 (3TC)	>300 (2395)		>300 (2290)
		SI[c] (3TC)	(16127)		>100 (16475)
	Assay: HBV 2.2.15[b]	EC50 (3TC)		0.022 (0.0021)	0.6115 (0.026)
		IC50		>10	>20
		SI		454	>32.7

Units = μM

[a]VIR data are based on extracellular virion HBV DNA.

[b]Cell line is Hep G2 2.2.15.

[c]SI = CC50/EC90

Table 15. Activity of Compounds 1, 3 against HCV Assay Summary[a]

compound		**1** (μM)	**3** (μM)	Control: alFNB2 (μM)	Control: 2'CmeCyt (μM)
HCV	EC50	6.9	>10	2.1	1.8
	EC90	22	>10	8.6	6.5
	CC50	>100	>100	>10000	>300
	SI	>14		>4761	>167

[a]Geno-type: 1B. Assay type: Primary.

Table 16. Antiviral Activity of Compounds 1, 2, 3 and 4 against HCV in Huh7 ET Cells

compound	High test concentration (μM)[b]	Assay[a]		
		Activity (% inhibition virus control)	Cytotoxity (% cell control)	SI[c] value
1	20	56.5	24.7	<1
2	20	0.0	46.9	<1
3	20	29.2	51.3	<1
4	20	0.0	65.9	<1
Control: alpha-IFN	2	97.4	113.9	>1
Control: alpha-IFN	2	97	92.0	>1

[a]Assay on HCV RNA replicon.

[b]Assay type is single dose (primary).

[c]SI = Selectivity index.

CONCLUSIONS

S-Adenosyl-L-homocysteine (AdoHcy) hydrolase is an important target for antiviral agent design. Carbocyclic nucleosides represent a prominent class of compounds whose antiviral properties were attributed to their potent inhibition of AdoHcy hydrolase, which in turn affects viral mRNA capping methylation. Within this category, aristeromycin and neplanocin A are at the center of these investigations. But their promise is limited by a toxicity arising from 5′-phosphate formation. Structural modifications with the aim of reducing phosphate-based toxicity have yielded promising candidates, such as 5′-noraristeromycin and 3-deaza carbocyclic nucleosides.

In the rational design of derivatives of 3-deaza-5′-noraristeromycin, as means to improve upon its antiviral scope, substituents at the C-3 position have been identified as important targets. Derivatives of 3-deaza-5′-noraristeromycin possessing chloro (**1**), bromo (**2**), iodo (**3**) or methyl (**4**), at the C-3 position were prepared and tested for antiviral activity. 3-Chloro-3-deaza-5'-noraristeromycin (**1**) exhibits activity against heptatitis C virus (HCV) while 3-bromo-3-deaza-5'-noraristeromycin (**2**) and 3-iodo-3-deaza-5'-noraristeromycin (**3**) display marked activity against heptatitis B virus (HBV). Compound **1**, **2**, and **3** were also found to have a wide variety of other biological properties. 3-Methyl-3-deaza-5'-noraristeromycin (**4**) shows good activity only against Vesicular stomatitis virus (VSV) and Vaccinia virus (VV), and affects none of the other viruses assayed. As part of the program investigating 3-deazaneplanocin A derivatives, 3-

bromo-3-deazaneplanocin A (**5**), 3-methyl-3-deazaneplanocin A (**6**) and 3-bromoDHCDA (**7**) were synthesized and their antiviral data will be forthcoming as part of ongoing study in the Schneller lab.

EXPERIMENTAL SECTION

General

Melting points were recorded on a Meltemp II melting point apparatus and the values are uncorrected. The combustion analyses were performed at Atlantic Microlab, Norcross, GA. ^{1}H 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 100.6 MHz for carbon). All ^{1}H chemical shifts are referenced to internal tetramethylsilane (TMS) at 0.0 ppm. ^{13}C chemical shift are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.23), or relative to DMSO-d_6 (center of septet, δ 39.51). 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. 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 (^{1}H and ^{13}C NMR) homogeneous material.

6-Oxabicyclo[3.1.0]hex-2-ene (16).[190] Dicyclopentadiene was cracked by distillation maintaining the distillation head at around 40 °C by controlling the temperature of heating mantle at 160~180 °C. Cyclopentadiene (363 g, 5.49 mol) was obtained which was immediately dissolved in 3000 mL CH_2Cl_2. Sodium carbonate (1400 g, 13.21 mol) was then added portionwise and a suspension was obtained. The suspension was cooled down to -10 °C and then treated with a solution of sodium acetate (28 g, 0.42 mol) in 700 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 overnight. The mixture was filtered and the filtrate was evaporated to give a pale yellow liquid which was the crude monoepoxide **16**. This crude product was used directly in the next step.[54]

(Z)-Cyclopentene-3,5-diol diacetate (18).[54] Fresh *tetra*kis(triphenylphosphine)palladium (0)[191] was prepared at first. $PdCl_2$ (5.07 g, 28.6 mmol) and triphenylphosphine (38.01 g, 145.0 mmol) were added to 340 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 (4.05 g, 81.0 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 × 30 mL ethanol and 2 × 30 mL ether. Product as a light yellow solid (30.5 g) was dried and kept under nitrogen, whose ^1H and ^{13}C NMR spectral data agreed with literature.[112]

A solution of crude **16** from last step in 200 mL THF was added dropwise to a dry ice acetone cooled solution of *tetra*kis(triphenylphosphine)palladium(0) (8.0 g) in 600 mL

dry THF and acetic anhydride (450 g, 4.41 mol) at 0 °C to 5 °C. After addition, resulting mixture was stirred at room temperature overnight. Filtration of the resulting mixture through a pad of silica gel removed the catalyst. Ethyl ether (2 × 100 mL) was used to wash it. Evaporation of the solvent afforded a dark residue. Fractional distillation afforded **18** (256 g) as a pale yellow oil, whose ^{1}H and ^{13}C NMR spectral data agreed with literature.[54]

(+)-(1*R*, 4*S*)-4-Hydroxy-2-cyclopenten-1-yl acetate ((+)-15).[192] Compound **18** (256 g, 1.62 mol) was added to 0.1 M phosphate buffer (850 mL). The pH of the resulting suspension was adjusted to 7 by addition of 6 N NaOH dropwise. *Pseudomonas cepacia* lipase (25 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 1.62 L of NaOH solution, the reaction mixture was filtered through a celite pad. The filtrate was extracted with 3 × 800 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 **(+)-15** (184 g, 80.2%) as a light yellow solid, whose ^{1}H and ^{13}C NMR spectral data agreed with literature.[115]

4-Hydroxy-3-nitropyridine (20). To mechanically stirred ice-water cold concentrated HNO_3 (609 g) was added fuming H_2SO_4 (698 g, d = 1.94) slowly. 4-Hydroxypyridine (150 g, 95%, 1.50 mol) was added portion-wise in 20 min. The temperature was kept around 30 °C during the addition. The reaction was brought to reflux for 1h. This was followed by cooling the reaction to room temperature with water bath and then pouring

the solution over ice slowly with continuously stirring. Treatment of the resulting suspension with saturated aq. NH_4OH and then aq. Na_2CO_3 (30%) adjusted the pH to 7. Precipitate was collected by filtration and washed with 300 mL × 3 water. Compound **20** (151 g, 72.1%) was obtained as a pale yellow solid after drying in oven at 100 °C under vacuum: mp 278-279 °C (lit.[192,193] mp 278-279 °C).

4-Ethoxy-3-nitropyridine (21). Compound **20** (151 g, 1.08 mol) and PCl_5 (283 g, 95%, 1.29 mol) were added to $ClCH_2CH_2Cl$ (1000 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 hath. Absolute ethanol (650 mL) was added to the reaction dropwise below 50 °C. After the addition, the mixture was brought to reflux for 1h. Heating was removed and the reaction was cooled to 10 °C by an ice-water bath. The mixture stood for 1 h to form precipitate. The solid was collected by filtration and washed with ethanol (2 × 600 mL). Compound **21** (218 g, 99.1%) was obtained as white solid after drying in oven at 35 °C under vacuum: mp 46-48 °C (lit.[194,195] mp 46.5-48 °C)

4-Amino-3-nitropyridine (22). Compound **21** (217 g, 1.56 mol) and ammonium acetate (328 g, 4.25 mol) was added to 510 mL of water. The resulting slurry was heated to reflux for about 6 hours. TLC was used to monitor the reaction progress. After TLC showed the disappearance of the starting material **21**, the heating was removed and the reaction was cooled to rt by an ice-water bath. Addition of approximately 170 mL of concentrated aq. ammonium hydroxide to the solution adjusted the pH to 8. The slurry stood in an ice-water bath for 1 h to form precipitate. The solid was collected by filtration and was washed with cold water (150 mL × 2). Compound **22** (119 g, 81.0%) was

obtained as a yellow solid after drying in oven at 100 °C under vacuum. mp 198-200 °C (lit.[196] mp 200 °C)

2-Chloro-3,4-diaminepyridine (23). $SnCl_2$ (145 g, 0.762 mol) was added to 600 mL HCl and the resulting suspension was heated to 60-70 °C. The reaction mixture became clear solution. Compound **22** (60 g, 0.43 mol) 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 **22**, the heating was removed and the reaction was cooled to rt with an ice-water bath. The cooled mixture was poured over 600 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 (400 mL × 4). The combined organic layers were dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded **23** (44 g, 71%) as a yellow solid. ^{1}H NMR (250 MHz, DMSO-d_6): 7.29 (d, J = 5.3 Hz, 1H), 6.43 (d, J = 5.3 Hz, 1H), 5.78 (br, 2H), 4.67 (br, 1H). ^{13}C NMR (62.9 MHz, DMSO-d_6): 142.9, 137.6, 135.1, 126.2, 108.3.

4-Chloro-1*H*-imidazo[4,5-*c*]pyridine (14). Under a nitrogen atmosphere, compound **23** (30.0 g, 0.209 mol) was added to 420 mL of anhydrous trimethyl orthoformate. The solution was heated to reflux at about 100 °C to afford a clear solution. The solution was allowed to cool to 90 °C. Then 11 mL of formic acid was added dropwise at 90 °C. The reaction was brought to reflux again and solid began to appear in the solution. Reflux was allowed for another 2 h. After TLC showed the disappearance of the starting material **23**, the heat was removed and the reaction was cooled to 10 °C with an ice-water bath. The mixture stood for another 1 h in the bath to form a precipitate completely. The mixture

was filtered and the precipitate was washed with 2 × 50 mL of cold ether. Compound **14** (23 g, 72%) was obtained as a light yellow solid after drying in oven at 100 °C under vacuum. ^{1}H NMR (250 MHz, DMSO-d_6): 8.52 (s, 1H), 8.11 (d, *J* = 5.5 Hz, 1H), 7.63 (d, *J* = 5.5 Hz, 1H). ^{13}C NMR (62.9 MHz, DMSO-d_6): 144.7, 141.1, 140.7, 139.5, 135.4, 108.8.

(*1R,4S*)-1-[4-(*tert*-Butyldimethylsilanyloxy)-cyclopent-2-enyl]-4-chloro-1*H*-imidazo[4,5-*c*]pyridine (25). NaH (1.75 g, 69.0 mmol) was added to a solution of **14** (10.0 g, 65.1 mmol) in dry DMF (90 mL). Reaction mixture was stirred at rt for 3 h, followed by the addition of tetrakis(triphenylphosphine)palladium(0) (3.7 g, 3.2 mmol), triphenylphosphine (2.5 g, 9.5 mmol), and a solution of monoacetate **15** (11.0 g, 77.5 mmol) in dry THF (90 mL). This mixture was stirred at 55 °C for 24 h under a nitrogen atmosphere. TLC was used to monitor the reaction progress. Solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/MeOH = 10:1) to afford 17.08 g solid as a mixture of N-7 (**24**) and N-9 (**13**) coupling products indicated by NMR spectra and used in the next step without further separation.

To a solution of the above mixture in CH_2Cl_2 (150 mL) containing imidazole (5.22 g, 76.8 mmol) was added *tert*-butyldimethysilyl chloride (7.99 g, 51.2 mmol) under N_2 atmosphere. Reaction was stirred at room temperature for 24 h. TLC was used to monitor the reaction progress. Water (80 mL) was added. The organic layer was separated, washed with brine and dried with $MgSO_4$. Solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/hexanes = 2:1) to afford **25** as a white solid (7.8 g, 34% for two steps), mp 91-92 °C.^{1}H NMR (400 MHz, $CDCl_3$): 8.16 (d, J = 5.6 Hz, 1H), 8.14 (s, 1H), 7.55 (d, *J* = 5.6 Hz, 1H), 6.23 (m, 1H), 5.95 (m, 1H), 5.34

(m, 1H), 4.93 (m, 1H), 2.99 (ddd, *J* = 14.6, 8.6, 7.3 Hz, 1H), 1.95 (dt, *J* = 14.6, 3.5 Hz, 1H), 0.92 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 144.0, 143.1, 141.3, 140.0, 139.3, 138.5, 130.3, 106.3, 75.4, 60.3, 41.4, 25.9, 18.2 –4.5, -4.6. Anal. Calcd for $C_{17}H_{24}ClN_3OSi$: C, 58.35; H, 6.91; N, 12.01. Found: C, 58.57; H, 6.95; N, 12.03.

(1*S*,2*R*,3*S*,5*R*)-3-(*tert*-Butyldimethylsilyloxy-5'-(4-chloro-imidazo[4,5-*c*]pyridin-1-yl)cyclopentane-1,2-diol (26). N-Methylmorpholine-N-oxide (1.70 g, 46.6 mmol) was added to a solution of **9** (3.50 g, 10.0 mmol) in CH_2Cl_2 (50 mL) containing a small amount of H_2O (0.8 mL). After the solution was cooled to 0 °C, a catalytic amount of solid osmium tetroxide (30 mg, 0.12 mmol) was added and the solution was stirred for 12 h at rt. TLC (EtOAc/hexanes = 2:1) was used to monitor the reaction progress. The reaction mixture was quenched by addition of sodium bisulfite. Solvent was removed by evaporation under reduced pressure and the residue was purified by flash column chromatography on silica gel (EtOAc/hexanes = 3:1) to afford **26** as a white solid (3.1 g, 81%), mp 202-203 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.00 (s, 1H), 7.92 (d, *J* = 5.6 Hz, 1H), 7.68 (d, *J* = 5.6 Hz, 1H), 5.76 (s, 1H), 5.10 (s, 1H), 4.75 (m, 1H), 4.31 (d, *J* = 6.4 Hz, 1H), 4.13 (d, *J* = 3.6 Hz, 1H), 1.99 (dd, *J* = 6.4, 17.2 Hz, 1H), 0.99 (s, 9H), 0.20 (s, 3H), 0.17 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 144.7, 141.9, 141.2, 138.6, 137.4, 106.8, 78.4, 76.6, 74.7, 62.7, 37.3, 26.0, 18.3, -4.6, -4.7. Anal. Calcd for $C_{17}H_{26}ClN_3O_3Si \cdot 0.1H_2O$: C, 52.89; H, 6.79; N, 10.89. Found: C, 52.54; H, 6.83; N, 10.79.

1-((3a*S*,4*R*,6*S*,6a*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-4-chloro-1*H*-imidazo[4,5-*c*]pyridine (12). To a solution of **26** (2.7 g, 7.0 mmol) and 2, 2-dimethoxypropane (10 mL) in dry acetone (15 mL) was

added a catalytic amount of p-toluenesulforic acid (80 mg, 0.5 mmol). The reaction was stirred at room temperature overnight under a nitrogen atmosphere. After TLC showed the disappearance of the starting material **26**, solvent was removed by evaporation under reduced pressure and the residue was dissolved in CH_2Cl_2 (40 mL) and washed with saturated sodium bicarbonate, water and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to afford **12** as a white foam (2.9 g, 99%). This material was used without further purification in the next step. ^{1}H NMR (250 MHz, $CDCl_3$): 8.35 (s, 1H), 8.22 (d, *J* = 5.6 Hz, 1H), 7.42 (d, *J* = 5.6 Hz, 1H), 4.77 (d, *J* = 1.6 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 1H), 4.58 (d, *J* = 5.6 Hz, 1H), 4.49 (d, *J* = 4.5 Hz, 1H), 2.82 (m, 1H), 2.24 (m, 1H), 1.54 (s, 3H), 1.32 (s, 3H), 0.81 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 143.8, 143.1, 141.6, 140.3, 138.0, 112.0, 105.6, 87.4, 86.3, 77.7, 63.1, 37.2, 26.9, 26.0, 24.4, 18.4, -4.7, -4.8. Anal. Calcd for $C_{20}H_{30}ClN_3O_3Si$: C, 56.65; H, 7.13; N, 9.91. Found: C, 56.94; H, 7.26; N, 9.77.

1-((3a*S*,4*R*,6*S*,6a*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (11). A solution of **12** (2.12 g, 5.00 mmol) in anhydrous hydrazine (20 mL) and THF (10 mL) was brought to reflux for 2 h under a nitrogen atmosphere. After cooling to rt, the solution was concentrated by evaporation under reduced pressure. The residue was dissolved in MeOH (40 mL) and water (20 mL), and W2-Raney Ni (20 g) was added to it portionwise. Reaction was heated to reflux for 1 h. The hot reaction mixture was filtered and washed with hot MeOH (3 ×15 mL). The combined filtrates were evaporated to dryness. The residue was purified via column chromatography on silica gel (EtOAc/MeOH = 10:1) to afford **11** as a white solid (1.3 g, 62%), mp 140-141 °C. ^{1}H NMR (400 MHz, $CDCl_3$):

8.10 (s, 1H), 7.89 (d, *J* = 5.6 Hz, 1H), 6.86 (d, *J* = 5.6 Hz, 1H), 5.18 (br, 2H), 4.80 (d, *J* = 5.6 Hz, 1H), 4.70 (m, 1H), 4.57 (d, *J* = 5.6 Hz, 1H), 4.50 (d, *J* = 4.4 Hz, 1H), 2.78 (m, 1H), 2.22 (d, *J* = 14.8 Hz, 1H), 1.54 (s, 3H), 1.32 (s, 3H), 0.91(s, 9H), 0.14 (s, 3H), 0.11(s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 151.9, 141.1, 140.5, 139.2, 127.5, 111.8, 98.0, 87.6, 86.3, 77.8, 62.5, 37.4, 26.9, 26.1, 24.5, 18.4, -4.6, -4.7. Anal. Calcd for $C_{20}H_{32}N_4O_3Si$: C, 59.37; H, 7.97; N, 13.85. Found: C, 59.16; H, 8.06; N, 13.69.

7-Bromo-1-((3a*S*,4*R*,6*S*,6a*S*)-6-(*tert*-butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (9). A solution of **11** (1.62 g, 4.00 mmol) in dry CH_2Cl_2 (80 mL) was cooled to –15 °C. NBS (1.06 g, 6.00 mmol) was added to the solution portionwise, and the resulting mixture was stirred for about 30 min under a nitrogen atmosphere. TLC (EtOAc/hexanes = 3:1) was used to monitor the reaction progress. The mixture was then concentrated by evaporation under reduced pressure and the residue was purified by flash column chromatography on silica gel (EtOAc/hexanes = 2:1) to afford bromide **9** (1.68 g, 87.3%) as a yellow solid, which was used in the next step immediately.

1-((3a*S*,4*R*,6*S*,6a*S*)-6-(tert-butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-7-iodo-1*H*-imidazo[4,5-*c*]pyridin-4-amine (10). Compound **10** was prepared in a similar manner as that above. A solution of **11** (881 mg, 2.18 mmol) in dry DMF (30 mL) was cooled to –15 °C. NIS (590 mg, 2.62 mmol) was added to the solution portionwise, and the resulting mixture was stirred for about 30 minutes under a nitrogen atmosphere. TLC (EtOAc/hexanes = 3:1) was used to monitor the reaction progress. The mixture was then concentrated by evaporation under reduced pressure and the residue was purified by flash column chromatography on silica gel

(EtOAc/hexanes = 2:1) to afford iodide **10** (705 mg, 61.5%) as a yellow solid, which was used in the next step immediately.

(1*S*,2*R*,3*S*,4*R*)-4-(4-Amino-7-bromo-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopentane-1,2,3-triol (2). Compound **9** (798 mg, 1.65 mmol) was dissolved in a mixture of 2N HCl (20 mL) and MeOH (20 mL). This reaction mixture was stirred at room temperature for 3 h. TLC (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) analysis was used to monitor the reaction process. The reaction mixture was neuturalized with basic ion-change resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) to yield **2** as a light yellow solid (507 mg, 84.5%), mp 206-208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.58 (s, 1H), 7.88 (s, 1H), 7.75 (br, 2H), 5.41 (m, 1H), 5.26 (d, *J* = 2.8 Hz, 1H), 5.18 (s, 1H), 5.05 (s, 1H), 4.60 (t, *J* = 4.0 Hz, 1H), 3.92 (s, 1H), 3.79 (d, *J* = 4.4 Hz, 1H), 2.76 (m, 1H), 1.59 (m, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): 149.7, 142.6, 136.1, 134.7, 127.6, 89.3, 76.5, 76.0, 73.1, 59.3, 39.9. HRMS calcd for $C_{11}H_{13}BrN_4O_3$ 328.0170, found 328.0171.

(1*S*,2*R*,3*S*,4*R*)-4-(4-Amino-7-iodo-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopentane-1,2,3-triol (3). Compound **10** (479 mg, 0.903 mmol) was dissolved in a mixture of 2N HCl (10 mL) and MeOH (10 mL). This reaction mixture was stirred at room temperature for 3 h. TLC analysis was used to monitor the reaction progress. The reaction mixture was neuturalized with basic ion-change resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) to yield **2** as a light yellow solid (260 mg, 76.6%), mp 233-234 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.32 (s, 1H), 7.87 (s, 1H),

6.48 (br, 1H), 5.44 (m, 1H), 5.21 (d, *J* = 3.9 Hz, 1H), 5.09 (d, *J* = 6.7 Hz, 1H), 4.98 (d, *J* = 3.9 Hz, 1H), 4.66 (m, 1H), 3.89 (s, 1H), 3.78 (s, 1H), 2.75 (m, 1H), 1.44 (m, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): 149.4, 143.9, 138.8, 138.1, 127.6, 76.9, 76.2, 73.6, 59.2, 58.8, 40.0. Calcd for $C_{11}H_{13}IN_4O_3$·HCl: C, 32.02; H, 3.42; N, 13.58. Found: C, 31.99; H, 3.32; N, 13.28.

1-((3a*S*,4*R*,6*S*,6a*S*)-6-(*tert*-Butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-1*H*-imidazo[4,5-*c*]pyridin-*bis*-boc-4-amine (27). To 100 mL flask containing **11** (405 mg, 10.0 mmol) and DMAP (25 mg, 0.21 mmol) was added 25 mL of THF. Then 0.7 mL (3 mmol) of $(Boc)_2O$ was added under a N_2 atmosphere. The reaction mixture was stirred for 8 h at rt under N_2 atmosphere. TLC (EtOAc/hexanes = 2:3) analysis indicated the disappearance of the starting material and the presence of a single product. The excess THF was removed by evaporation under reduced pressure to give yellow oil. The crude product was purified by flash chromatography on silica gel (EtOAc/hexanes = 1:1) on silica gel to give **27** as a white foam (505 mg, 84.5%), mp 73-74 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.35 (d, *J* = 5.75 Hz, 1H), 8.26 (s, 1H), 7.44 (d, *J* = 5.75 Hz, 1H), 4.77 (m, 2H), 4.56 (d, *J* = 6.25 Hz, 1H), 4.47 (d, *J* = 3.75 Hz, 1H), 2.80 (m, 1H), 2.2 (d, *J* = 15 Hz, 1H), 1.63 (s, 3H), 1.41 (s, 18H), 1.33 (s, 3H), 0.90 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 151.5, 144.7, 143.5, 141.1, 136.9, 116.4, 106.1, 87.5, 86.3, 82.9 ,77.4, 62.8, 37.2, 28.1, 26.9, 26.1, 24.5, 18.4, -4.7. Anal. Calcd for $C_{30}H_{48}N_4O_7Si$: C, 59.58; H, 8.00; N, 9.26. Found: C, 59.63; H, 8.02; N, 9.18.

7-Chloro-1-((3a*S*,4*R*,6*S*,6a*S*)-6-(*tert*-butyldimethylsilyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[d][1,3]dioxol-4-yl)-1*H*-imidazo[4,5-*c*]pyridin-*bis*-boc-4-

amine (28). A solution of **27** (480 mg, 0.79 mmol) in dry CH_2Cl_2 (50 mL) was cooled to –30 °C. NCS (211 mg, 1.58 mmol) was added to the solution portionwise, and the resulting mixture was stirred for 8 h at rt under a N_2 atmosphere. TLC analysis (EtOAc/Hexanes = 2:3) indicated the disappearance of the starting material. Then the mixture was then concentrated in vacuo and the residue was purified by column chromatography (EtOAc/Hexanes = 1:2) on silica gel to afford chloride **28** (466 mg, 92.4%) as a white solid, mp 74-76 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.28 (s, 1H), 8.24 (s, 1H), 5.62 (d, *J* = 7.8 Hz, 1H), 5.00 (d, *J* = 5.8 Hz, 1H), 4.62 (d, *J* = 5.8 Hz, 1H), 4.43 (d, *J* = 4.5 Hz, 1H), 2.79 (m, 1H), 2.11 (d, *J* = 15.3 Hz, 1H), 1.52 (s, 3H), 1.41 (s, 18H), 1.34 (s, 3H), 0.86 (s, 9H), 0.11 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 151.3, 145.1, 143.6, 140.8, 138.1, 136.4, 114.3, 111.5, 87.9, 86.7, 83.2, 78.1, 77.4, 63.3, 39.1, 28.1, 26.8, 26.1, 24.4, 18.3, -4.7, -4.8. Anal. Calcd for $C_{30}H_{47}ClN_4O_7Si$: C, 56.37; H, 7.41; N, 8.76. Found: C, 56.49; H, 7.43; N, 8.70.

(1*S*,2*R*,3*S*,4*R*)-4-(4-Amino-7-chloro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopentane-1,2,3-triol (1). Compound **28** (403 mg, 0.630 mmol) was dissolved in a mixture of 2N HCl (10 mL) and MeOH (10 mL). This reaction mixture was stirred at rt for 3 h under a N_2 atmosphere. TLC analysis (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) indicated disappearance of the starting material and formation of two products. The reaction mixture was neuturalized with basic ion-change resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 32/2/1). NMR of obtained products indicated one was the desired compound **1** and the other is compound **29** which possesses a Boc protecting group. ^{1}H NMR (400 MHz, DMSO-d_6): 9.48 (br., 1H), 8.63 (s,1H), 8.12 (s, 1H), 5.30 (d,

J = 3.6 Hz, 1H), 5.24 (d, *J* = 6.8 Hz, 1H), 5.09 (d, *J* = 3.6 Hz, 1H), 4.61 (m,1H), 3.98 (s, 1H), 3.85 (s, 1H), 2.82 (m, 1H), 1.65 (m, 1H), 1.66 (s, 9H). ^{13}C NMR (62.9 MHz, DMSO-d_6): 152.6, 143.7, 143.6, 139.7, 136.2, 134.8, 110.5, 79.66, 77.1, 76.6, 73.7, 60.1, 31.8, 28.5.

Compound **29** was treated with 2N HCl and MeOH again under the reflux condition. After 1h, TLC (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) indicated the completion of the conversion from **29** to **1**. The reaction mixture was neuturalized with basic ion-change resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) to yield **1** (combined with **1** obtained before) as a slightly yellow solid (162 mg, 80.4%), mp 209-211 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.32 (s,1H), 7.61 (s, 1H), 6.41 (br, 2H), 5.25 (m, 1H), 5.19 (d, *J* = 3.75 Hz, 1H), 5.10 (d, *J* = 6.75 Hz, 1H), 4.98 (d, *J* = 3.75 Hz, 1H), 4.52 (m,1H), 3.90 (s, 1H), 3.76 (s, 1H), 2.73 (m, 1H), 1.56 (m, 1H). ^{13}C NMR (62.9 MHz, DMSO-d_6): 152.2, 140.8, 140.0, 134.6, 128.4, 102.5, 77.1, 76.5, 73.7, 59.7, 39.8. Anal. Calcd for $C_{11}H_{13}ClN_4O_3 \cdot HCl \cdot 0.8H_2O$: C, 39.37; H, 4.69; N, 16.70. Found: C, 39.32; H, 4.48; N, 16.50. HRMS calcd for $C_{11}H_{13}ClN_4O_3$ 284.0672, found 284.0676.

4-Nitro-3-picoline N-oxide (33).[118] 3-Picoline N-oxide (15 g, 0.14 mol) was dissolved in 75 mL of concentrated H_2SO_4. 30 mL of 90% fuming HNO_3 was added dropwise below 60 °C. The reaction temperature was raised to 80 °C for 2 h. Then the mixture was cooled to rt and poured onto 300 g crushed ice. The cooled mixture was adjusted to pH = 7 with concentrated NH_4OH. The precipitate was filtered and dried in a vacuum oven at 100 °C to afford **33** as a yellow solid (13 g, 63%). The NMR spectral data agreed with literature.[118]

4-Amino-3-methylpyridine (34).[118] To a mixture of 4 grams of wet Raney Ni and 40 mL of methanol in a Parr hydrogenator was added compound **33** (3.0 g, 19 mmol). Hydrogen was introduced to 65 psi for 12 h with vigorous shaking. The mixture was filtered through celite and the filtrate was concentrated under reduced pressure. After drying in a vacuum oven at 100 °C, compound **34** was obtained as a yellow solid (2.0 g, 95%). The NMR spectral data agreed with literature.[118]

3-Methyl- 4-nitraminopyridine (35).[118] To 25 mL of concentrated H_2SO_4 was added compound **34** (1.80 g, 16.7 mmol). The resulting mixture was cooled in ice-water bath. Concentrated HNO_3 (10mL) was added dropwise maintaining the reaction temperature below 10 °C. After 1 h, the reaction mixture was poured onto 80 g crushed ice. The cooled mixture was neutralized to pH = 7 with concentrated NH_4OH. The creamy mixture was filtered and the precipitate was dried in a vacuum oven at 100 °C. Compound **35** was obtained as a white solid (2.15 g, 84.1%). The NMR spectral data agreed with literature.[118]

4-Amino-3-methyl-5-nitropyridine (36).[118] To 15 mL of concentrated H_2SO_4 was added **35** (2.1 g, 14 mmol) slowly. The mixture was stirred at rt overnight and the reaction was quenched by pouring over 50 g of crushed ice. The cooled mixture was neutralized to pH = 7 with concentrated NH_4OH. The precipitate was filtered and dried in vacuum oven at 100 °C to afford **36** as a yellow solid (1.1 g, 53%). The NMR spectral data agreed with literature.[118]

6-Chloro-4,5-diamino-3-methylpyridine (37).[118] To 50 mL of concentrated HCl was added $SnCl_2$ (2 g, 10 mmol). The mixture was heated to reflux for 1 h and the temperature was cooled to around 80 °C. Compound **36** (1.0 g, 6.5 mmol) was added and

the mxture heated to reflux for another hour. The mixture was cooled to rt with an ice-water bath. The mixture was poured over 100 g of crushed ice and 7 M NaOH was added to pH = 8-9. The solution was extracted with ethyl acetate (3 ×100 mL). The organic layer was combined and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform/methanol = 8/2) on silica gel to afford **37** (0.37 g, 36%) as a yellow solid. The NMR spectral data agreed with literature.[118]

4-Chloro-7-methyl-1*H*-imidazo[4,5-*c*]pyridine (32).[118] To 10 mL of anhydrous triethyl orthoformate was added **37** (0.29 g, 1.8 mmol). The mixture was brought to reflux for 8 h. The reaction was cooled to rt with an ice-water bath. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (chloroform/methanol = 8/2) on silica gel to afford **32** (0.22 g, 71%) as a slightly yellow solid. The NMR spectral data agreed with literature.[118]

4-Chloro-1-((1*R*,2*S*,3*R*,4*S*)-2,3,4-tris(*tert*-butyldimethylsilyloxy)cyclopentyl)-1*H*-imidazo[4,5-*c*]pyridine (40). To a solution of **26** (1.64 g, 4.27 mmol) in dry CH_2Cl_2 (50 mL) was added TBSCl (1.5 g, 9.6 mmol) and then imidazole (2.07 g, 13.8 mmol). The reaction mixture was stirred at rt overnight under a N_2 atmosphere. TLC analysis (EtOAc/Hexanes = 3:2) indicated the disappearance of the starting material. Then water (20 ml) was added. The organic layer was separated, washed with brine and dried with anhydrous $MgSO_4$. Solvent was removed and the residue was purified by column chromatography (EtOAc/Hexanes = 1:1) on silica gel to afford **40** as a white solid (2.4 g, 92%), mp 102-104 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 8.15 (d, J = 5.5 Hz, 1H), 8.09 (s, 1H), 7.76 (d, J = 5.5 Hz, 1H), 4.67-4.76 (m, 2H), 4.07 (dd, J = 5.5, 1.3 Hz, 1H), 3.84 (d, J = 1.0 Hz, 1H), 2.88 (m, 1H), 1.97 (m, 1H), 0.99 (s, 9H), 0.95 (s, 9H), 0.71 (s, 9H), 0.17 (s,

3H), 0.16 (s, 3H), 0.15 (s, 3H), 0.13 (s, 3H), -0.26 (s, 3H), -0.68 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 144.8, 143.3, 140.9 139.3, 138.4 106.5 78.74, 77.9, 75.0, 60.8, 36.8 25.8 25.7 25.7, 18.0, 17.8, -4.5, -4.6, -4.7, -4.8, -5.0, -5.8. Anal. Calcd for $C_{29}H_{54}ClN_3O_3Si_3$: C, 56.87; H, 8.89; N, 6.86. Found: C, 56.80; H, 8.89; N, 6.75.

1-((1*R*,2*S*,3*R*,4*S*)-2,3,4-*tris*(*tert*-Butyldimethylsilyloxy)cyclopentyl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (41). In the similar manner as described for amination process**,** a solution of **40** (2.19 g, 3.58 mmol) in anhydrous hydrazine (20 mL) and THF (10 mL) was brought to reflux for 2 h. After cooling to rt, the solution was concentrated. The residue was dissolved in MeOH (40 mL), and W2-Raney Ni (20 g) was added to it. Reaction was heated to reflux for 1 h. The hot reaction mixture was filtered and washed with hot MeOH (3 ×15 mL). The combined filtrates were evaporated to dryness. The residue was purified via column chromatography (EtOAc/MeOH = 10:1) to afford **41** as a white solid (1.44 g, 68.0%), mp 140-142 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 7.83 (s, 1H), 7.79 (d, J = 5.8 Hz, 1H), 7.17 (d, J = 5.8 Hz, 1H), 5.17 (br, 2H), 4.61-4.73 (m, 2H), 4.04 (dd, J = 5.5, 1.3 Hz, 1H), 3.82 (d, J = 1.3 Hz, 1H), 2.81 (m, 1H), 1.98 (m, 1H), 0.98 (s, 9H), 0.94 (s, 9H), 0.72 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), 0.12 (s, 3H), 0.11 (s, 3H), -0.26 (s, 3H), -0.63 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 151.9, 141.5, 140.3, 138.1, 128.1, 99.0, 78.9, 74.9, 60.3, 36.6, 25.8, 18.0, 18.0, 17.8, -4.5, -4.7, -4.8, -5.0, -5.8. Anal. Calcd for $C_{29}H_{56}N_4O_3Si_3 \cdot 0.2H_2O$: C, 58.38; H, 9.53; N, 9.39. Found: C, 58.17; H, 9.47; N, 9.18. HRMS calcd for $C_{29}H_{56}N_4O_3Si_3$ 592.3662, found 592.3660.

7-Bromo-1-((1*R*,2*S*,3*R*,4*S*)-2,3,4-tris(tert-butyldimethylsilyloxy)cyclopentyl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (42). A solution of **41** (886 mg, 1.49 mmol) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C. NBS (399 mg, 2.24 mmol) was added to the

solution portionwise, and the resulting mixture was stirred for about 2 h under a nitrogen atmosphere. TLC (EtOAc/MeOH = 10:1) was used to monitor the reaction progress. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes = 1:1) on silica gel to afford bromide **42** (850 mg, 85%) as a white solid, mp 116-117 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 8.08 (s, 1H), 7.86 (s, 1H), 5.91 (m, 1H), 5.39 (br, 2H), 4.60 (dd, *J* = 8.0, 3.0 Hz, 1H), 3.98 (d, *J* = 5.0 Hz, 1H), 3.80 (s, 1H), 2.96 (m, 1H), 1.67 (dd, *J* = 14.5, 4.5 Hz, 1H), 0.94 (s, 9H), 0.92 (s, 9H), 0.70 (s, 9H), 0.12 (s, 6H), 0.11 (s, 6H), -0.24 (s, 3H), -0.51 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 151.5, 143.3, 140.8, 136.2, 128.6, 90.80, 81.6, 79.0, 74.9, 57.8, 40.6, 25.9, 25.8, 18.1, 18.1, 18.0, -4.3, -4.5, -4.6, -4.7, -4.9, -5.5. Anal. Calcd for $C_{29}H_{55}BrN_4O_3Si_3$: C, 51.84; H, 8.25; N, 8.34. Found: C, 52.10; H, 8.28; N, 8.24.

7-Methyl-1-((1*R*,2*S*,3*R*,4*S*)-2,3,4-tris(tert-butyldimethylsilyloxy)cyclopentyl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (43). To a solution of **42** (660 mg, 0.98 mmol) in dry THF (50 mL) was added $Pd(Ph_3P)_4$ (0.1 mg, 0.09 mmol). Then $AlMe_3$ (1.96 ml, 3.92 mmol) was added to this mixture dropwise at rt under a nitrogen atmosphere. The reaction mixture was stirred at rt for 1 h and was heated to reflux for 2 h. TLC (EtOAc/hexanes = 1:1) was used to monitor the reaction progress. The reaction was cooled to rt by using an ice-water bath. Evaporation of the solvent under reduced pressure gave a solid residue. This residue was subjected to flash silica gel chromatography (EtOAc/MeOH = 12:1) to yield a white solid **43** (510 mg, 92%) which can be used to the next step without further purification. ^{1}H NMR (400 MHz, CD_3OD): 8.15 (s, 1H), 7.42 (s, 1H), 5.31 (m, 1H), 4.53 (dd, *J* = 8.4, 3.2 Hz, 1H), 4.11 (d, *J* = 5.6 Hz, 1H), 3.90 (d, *J* = 1.6 Hz, 1H), 3.02 (m, 1H), 2.52 (s, 3H), 1.92 (dd, *J* = 14.8, 5.2 Hz, 1H), 1.00 (s, 9H),

0.96 (s, 9H), 0.68 (s, 9H), 0.20 (s, 6H), 0.16 (s, 6H), -0.21 (s, 3H), -0.51 (s, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): 152.3, 141.5, 141.0, 140.3, 127.5, 109.3, 83.5, 80.2, 75.9, 59.9, 40.3, 26.3, 26.2, 18.9, 18.8, 18.7, 16.1, -4.0, -4.4, -4.6, -4.7, -4.8, -5.6.

(1*S*,2*R*,3*S*,4*R*)-4-(4-Amino-7-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopentane-1,2,3-triol (4). TBAF (tetrabutylammonium fluoride, 3.2 ml, 1M in THF, 3.2 mmol) was added to a solution of **43** (488 mg, 0.804 mmol) in THF (80 ml). Reaction mixture was stirred at the room temperature for 6 h. TLC analysis (EtOAc/MeOH = 12:1) was used to monitor the reaction progress. Solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography (EtOAc/MeOH = 10:1) to afford **4** (191 mg, 89.9%) as a white solid. mp 297-299 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.21 (s, 1H), 7.37 (s, 1H), 5.89 (br, 2H), 5.22 (d, J = 3.6 Hz, 1H), 5.08 (d, J = 6.8 Hz, 1H), 4.82 ~ 4.95 (m, 2H), 4.32 (m, 1H), 3.93 (m, 1H), 3.76 (s, 1H), 2.73 (m, 1H), 2.43 (s, 3H), 1.68 (m, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): 151.2, 140.4, 139.5, 137.9, 126.6, 106.5, 77.8, 76.8, 73.4, 59.2, 38.9, 15.2. Anal. Calcd for $C_{12}H_{16}N_4O_3$: C, 54.54; H, 6.10; N, 21.20. Found: C, 54.22; H, 6.21; N, 20.95.

1*H*-imidazo[4,5-*c*]pyridin-4-amine (50). To a mixture of anhydrous hydrazine (99%, 160 mL) and propan-1-ol (110 mL) was added **14** (10.0 g, 65.1 mmol). The solution was brought to reflux for 8 h. The reaction was cooled to rt and the residual hydrazine and propan-1-ol was evaporated under reduced pressure. Water (300 mL) was added to dissolve the residue. Raney nickel (18 g) was added portionwise. The mixture was heated to reflux for 1 h. After the reaction had completed, the reaction mixture was filtered through a Celite pad and the filtrate was evaporated under reduced pressure to afford **50** (7.9 g, 90%). The NMR spectral data agreed with literature.[135]

***tert*-Butyl 4-(*bis*(*tert*-butoxycarbonyl)amino)-1*H*-imidazo[4,5-*c*]pyridine-1-carboxylate (51).** To **50** (7.70 g, 57.4 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.70 g, 5.7 mmol) was added 500 mL of dry THF. To the resulting suspension was added 52.8 mL (230 mmol) of $(Boc)_2O$. The reaction mixture was stirred for 2 days at room temperature under a nitrogen atmosphere. TLC analysis (EtOAc/hexanes = 3/2) was used to monitor the reaction progress. Solvent was removed by evaporation under reduced pressure to give a yellow oil. The crude product was purified by flash chromatography on silica gel with EtOAc/hexanes = 1/1 to give **51** (21.4 g, 86.4%) as a white foam. ^{1}H NMR (250 MHz, $CDCl_3$): 8.47 (s, 1H), 8.46 (d, J = 5.5 Hz, 1H), 7.88 (d, J = 5.5 Hz, 1H), 1.73 (s, 9H), 1.40 (s, 18H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 150.9, 147.1, 144.7, 143.5, 142.7, 138.4, 136.7, 109.67, 9.10, 83.1, 28.0, 27.8. Anal. Calcd for $C_{21}H_{30}N_4O_6$: C, 58.05; H, 6.96; N, 12.89. Found: C, 57.95; H, 6.95; N, 12.75.

***tert*-Butyl 1*H*-imidazo[4,5-*c*]pyridin-4-ylcarbamate (52).** Tris-Boc 3-deazaadenine **51** (4.35 g, 10.0 mmol) was dissolved in 100 mL of MeOH, to which 45 mL of saturated aq. $NaHCO_3$ was added. The turbid solution was stirred at room temperature for 3 h. TLC analysis (EtOAc/hexanes = 5:1) was used to monitor the reaction progress. When it was indicated that the **51** disappeared, a clean conversion to one product was observed by TLC. After evaporation of solvent by rotary evaporation, 200 mL of water was added to the suspension and the aqueous layers were extracted with EtOAc (3 ×100 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent gave a white solid. It was purified by column chromatography (EtOAc/hexanes = 3:1) to afford **52** (1.9 g, 81%) as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$): 11.77 (br, 1H), 9.16 (br, 1H), 8.13 (s, 1H), 8.11 (d, J = 5.75 Hz, 1H), 7.48 (d, J =

5.75 Hz, 1H), 1.58 (s, 9H). Anal. Calcd for $C_{11}H_{14}N_4O_2$: C, 56.40; H, 6.02; N, 23.92. Found: C, 56.47; H, 6.36; N, 23.34.

***tert*-Butyl 7-bromo-1*H*-imidazo[4,5-*c*]pyridin-4-ylcarbamate (54).** A solution of **52** (1.4 g, 6.0 mmol) in dry CH_3CN (100 mL) was cooled to –15 °C. NBS (1.6 g, 9.0 mmol) was added to the solution portionwise, and the resulting mixture was brought to reflux for 5 h. TLC analysis (EtOAc/hexanes = 3:1) was used to monitor the reaction progress. The mixture was cooled to rt and 1 mL cyclohexene was added dropwise. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by flash column chromatography (EtOAc/hexanes = 3:1) on silica gel to afford bromide **54** (1.5 g, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 12.08 (br., 1H), 10.41 (br., 1H), 8.32 (s, 1H), 8.19 (s, 1H), 1.62 (s, 9H).

***tert*-Butyl 4-(bis(*tert*-butoxycarbonyl)amino)-7-bromo-1*H*-imidazo[4,5-*c*]pyridine-1-carboxylate (55**). To **54** (0.90 g, 2.9 mmol) and DMAP (35 mg, 0.29 mmol) was added 25 mL of THF. To the resulting suspension was added 2.64 mL (11.5 mmol) of $(Boc)_2O$. The reaction mixture was stirred for 8 h at rt under a nitrogen atmosphere. TLC analysis (EtOAc/hexanes = 1/1) was used to monitor the reaction progress. Solvent was removed by evaporation to give yellow oil. The crude product was purified by flash chromatography on silica gel with EtOAc/hexanes = 1/2 to give **55** (1.3 g, 88%) as a white foam. ^{1}H NMR NMR (250 MHz, $CDCl_3$): 8.56 (s, 1H), 8.55 (s, 1H), 1.66 (s, 9H), 1.40 (s, 18H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 150.7, 146.2, 145.6, 143.8, 137.8, 126.2, 111.8, 87.6, 83.1, 28.0, 27.9.

4-(*bis*(*tert*-Butoxycarbonyl)amino)-1*H*-imidazo[4,5-*c*]pyridine-1-carboxylate (56). Compound **51** (9.40 g, 21.6 mmol) was dissolved under argon in 250 mL of dry THF. A

1 M solution of Bu_4NF (43.3 mL, 43.3 mmol) in THF was added and the reaction mixture was stirred for 12 h. TLC analysis was used to monitor the reaction progress. Water (200 mL) was added. After extraction with AcOEt (2 × 100 mL), the combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (hexanes/AcOEt = 1/3) on silica gel to afford **56** (5.7 g, 79%) as a white solid. ^{1}H NMR NMR (250 MHz, $CDCl_3$): 8.31 (d, J = 5.5 Hz, 1H), 8.30 (s, 1H), 7.61 (d, J = 5.5 Hz, 1H), 1.35 (s, 18H). Anal. Calcd for $C_{16}H_{22}N_4O_4$: C, 57.47; H, 6.63; N, 16.76. Found: C, 57.55; H, 6.66; N, 16.93.

4-(*bis*(*tert*-butoxycarbonyl)amino)-7-bromo-1*H*-imidazo[4,5-*c*]pyridine-1-carboxylate (45). Pathway A: Compound **55** (0.84 g, 1.6 mmol) was dissolved in 30 mL of MeOH, to which 15 mL of saturated aq. $NaHCO_3$ was added. The turbid solution was stirred at rt for 3 h. TLC analysis (EtOAc/Hexanes = 1:1) was used to monitor the reaction progress. When **55** had disappeared, a clean conversion to one product was observed by TLC. After evaporation of solvent by rotary evaporation, 100 mL of water was added to the suspension and the aqueous layer was extracted with EtOAc (3 × 80 mL). The organic layer was combined and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent gave a white solid. It was purified by column chromatography (EtOAc/Hexanes = 2:1) on silica gel to afford **45** (0.58 g, 81%) as a white solid. ^{1}H NMR NMR (250 MHz, $CDCl_3$): 12.40 (br, 1H), 8.40 (s, 1H), 8.34 (s, 1H), 1.37 (s, 18H).

Pathway B: A solution of **56** (3.0 g, 6.0 mmol) in dry CH_3CN (150 mL) was cooled to –15 °C. NBS (1.75 g, 6.58 mmol) was added to the solution portionwise, and the resulting mixture was brought to reflux for 6 h. TLC analysis (EtOAc/hexanes = 5:1) was used to

monitor the reaction progress.The mixture was cooled to rt and 1 mL cyclohexene was added dropwise. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography (EtOAc/hexanes = 2:1) to afford bromide **45** (1.9 g, 77%) as a white solid. The NMR data are consistent with that of the product of path **A**. Anal. Calcd for $C_{16}H_{21}BrN_4O_4 \cdot 0.5H_2O$: C, 45.51; H, 5.25; N, 13.27. Found: C, 45.51; H, 5.21; N, 13.19.

6-Hydroxymethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (66).[142] To 500 mL of acetone was added D-ribose (60 g, 0.4 mol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$, 1.9 g, 1.0 mmol) to obtain a clear solution. 2,2-Dimethoxypropane (45 g, 0.37 mol) was added dropwise at 0 °C. The resulting suspension was stirred for 1 h at rt until a clear solution was achieved. $NaHCO_3$ (0.10 g, 1.2 mmol) was added to this solution to neutralize the excess acid. Additional 30 min was needed to stir the reaction at rt. The solid was filtered and the filtrate was evaporated under reduced pressure. Purification of the residue by silica gel column chromatography (hexane/EtOAc =3:1 to 1:1) gave compound **66** as a colorless oil as a mixture of α- and β-isomers (68 g, 89%). The NMR spectral data agreed with literature.[142]

2,2-Dimethyl-6-(trityloxymethyl)tetrahydrofuro[3,4-*d*][1,3]-dioxol-4-ol (67).[142] To a solution of compound **66** (30.6 g, 161 mmol) in 400 mL of anhydrous *N*,*N*-dimethylformamide (DMF) was added a catalytic amount of 4-(dimethylamino)pyridine (DMAP, 0.60 g, 4.8 mmol), trityl chloride (53.8 g, 197 mmol) and triethylamine (24 g, 0.24 mol) at rt under a nitrogen atmosphere. The resulting solution was stirred for 24 h at rt and TLC analysis (hexane/EtOAc = 1:1) was used to monitor the reaction progress. After the completion of the reaction, it was poured into ice water (200 mL). The organic

layer was extracted with CH_2Cl_2 (3 × 300 mL), washed with water (300 mL), and saturated NaCl (200 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1 to 2:1) to give compound **67** as a colorless oil as a mixture of α- and β-isomers (52 g, 88%). The NMR spectral data agreed with literature.[142]

1-[5-(1-Hydroxy-2-trityloxyethyl)-2,2-dimethyl-[1,3]dioxolan-4-yl]prop-2-en-1-ol (62).[142] To a solution of compound **67** (40.0 g, 92.5 mmol) in 400 mL of anhydrous THF was added 165 mL of vinylmagnesium bromide (278 mmol, 1.0 M of THF solution) at -78 °C under a nitrogen atmosphere. The reaction stood for 1 h at -78 °C. Then the temperature was raised to rt and the reaction mixture was stirred for an additional 4 h. TLC analysis (hexane/EtOAc = 2:1) was used to monitor the reaction process. After the disappearance of the starting material, the reaction was quenched with saturated NH_4Cl solution (200 mL) dropwise in a water-ice bath. The resulting solution was poured into 300 g ice. The organic layer was separated and the aqueous layer was washed with ether (2 × 200 mL). The combined organic layer was washed with saturated NaCl solution and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 10:1 to 3:1) to give compound **62** as a white solid (42 g, 98%). The NMR spectral data agreed with literature.[142]

1-{5-[1-((*tert*-Butyldimethylsilanyl)oxyl)allyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(trityloxy)ethanol (68).[142] To a solution of compound **62** (41 g, 89 mmol) in 500 mL anhydrous CH_2Cl_2 and 50 mL DMF solution were added imidazole (18.2 g, 268 mmol) and TBDMSCl (16.7 g, 111 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred at rt for 4 h and TLC analysis (hexane/EtOAc = 10:1) was used to

monitor the reaction progress. The reaction was poured into 350 mL of water and 350 mL ether was added. The organic layer was separated and the aqueous layer was washed with ether (2 ×150 mL). The combined organic layer was washed with saturated NaCl solution and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 30:1) to give compound **68** as a mixture of two conformers (40 g, 78%) as a white foam. Minor 2TBS product could be obtained. The NMR spectral data **68** of agreed with literature.[142]

1-{5-[1-((*tert*-Butyldimethylsilanyl)oxyl)allyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(trityloxy)ethanone (61).[142] To a solution of oxalyl chloride (3.02 g, 34.5 mmol) in 80 mL of anhydrous CH_2Cl_2 was added DMSO (4.9 g, 69 mmol) at -60 °C under a nitrogen atmosphere. After the resulting solution was stirred for 10 min, a solution of compound **68** (16.0 g, 27.8 mmol) in 200 mL of anhydrous CH_2Cl_2 was added to the reaction mixture dropwise for 30 min at -60 °C. After the reaction was stirred for another 30 min at -60 °C, triethyl amine(14.0 g, 138 mmol) was added dropwise over 20 min to the reaction mixture at -60 °C. The mixture was stirred for another 1 h at -60 °C and then warmed to rt. TLC analysis (hexane/EtOAc = 10:1) was used to monitor the reaction progress. The reaction mixture was treated with 200 mL of water dropwise at 0 °C. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 ×200 mL). The combined organic layers were washed with saturated NaCl solution (200 mL), then dried over $MgSO_4$. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 20:1 to 10:1) to give compound **61** as a colorless oil (15.3 g, 96.6%). The NMR spectral data agreed with literature.[142]

***tert*-Butyl-{1-[2,2-dimethyl-5-(1-(trityloxymethyl)vinyl)-[1,3]-dioxolan-4-yl]allyloxy}dimethylsilane (69).**[142] To a suspension of Ph_3PCH_3Br (43.3 g, 121mmol) in 100 mL of THF was added 75 mL of *n*-BuLi (120 mmol, 1.6 M in hexane) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 30 min. Then a solution of compound **61** (14.0 g, 24.3 mmol) in 200 mL of THF was added to the reaction mixture at 0 °C. The resulting mixture was stirred overnight at room temperature under a nitrogen atmosphere. Then 50 mL of MeOH and 100 mL of water were added dropwise. The reaction mixture was poured into 200 mL of water. The organic layer was separated and the aqueous layer was extracted with ether (2 × 200 mL). The combined organic layers were washed with brine (50 mL), then dried over anhydrous $MgSO_4$. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified on a silica gel column (hexane/EtOAc = 50:1 to 10:1) to give compound **69** as a white solid (12.5 g, 90.2%). The NMR spectral data agreed with literature.[142]

1-[2,2-Dimethyl-5-(1-(trityloxymethyl)vinyl)-[1,3]dioxolan-4-yl]prop-2-en-1-ol (60).[142] To a solution of compound **69** (12.0 g, 21.0 mmol) in 100 mL of THF was added 26 mL of TBAF (26.0 mmol, 1.0 M in THF) at rt. The reaction mixture was stirred at rt for 2 h. TLC analysis 9hexane/EtOAc = 10:1) was used to monitor the reaction progress. Solvent was removed under reduced pressure and the residue was purified by a silica gel column chromatography (hexane/EtOAc = 30:1) to give compound **60** (9.1 g, 95%) as a white solid. The NMR spectral data agreed with literature.[142]

2,2-Dimethyl-6-trityloxymethyl-4,6a-dihydro-3a*H*-cyclopenta-[1,3]dioxol-4-ol (44).[142] To a solution of compound **60** (9.0 g, 20 mmol) in 400 mL of anhydrous CH_2Cl_2 was added second-generation Grubbs catalyst (1,3-Bis(2,4,6-trimethylphenyl)-2-

imidazolidinylidene)dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium (0.36 g, 0.40 mmol) at rt under under a nitrogen atmosphere. TLC analysis (hexane/EtOAc = 5:1) was used to monitor the reaction progress. After being stirred for 3 h, the reaction mixture was evaporated to dryness and the residue was purified on a silica gel column (hexane/EtOAc = 10:1 to 5:1) to give compound **44** (8.0 g, 95%) as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$): 7.46 (m, 6H), 7.33-7.20 (m, 9H), 6.00 (s, 1H), 4.88 (d, *J* = 5.5 Hz), 4.75 (t, *J* = 5.5 Hz, 1H), 4.59 (m, 1H), 3.88 (d, *J* = 14.25 Hz, 1H), 3.65 (d, *J* = 14.25 Hz), 2.74 (d, *J* = 10 Hz, 1H), 1.37 (s, 3H), 1.36 (s, 1H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 144.1, 143.6, 130.0, 128.8, 128.1, 127.3, 112.7, 87.1, 83.5, 78.0, 73.6, 61.1, 28.0, 27.0.

Compounds 70 and 72. To a solution of cyclopentanol **44** (1.28 g, 3.00 mmol) and triphenylphosphine (1.18 g, 4.50 mmol) in THF (20 mL) was added **45** (1.26 g, 2.98 mmol). This suspension was cooled by ice to 0 °C and Diisopropyl azodicarboxylate (DIAD, 0.91 g, 4.5 mmol) was added dropwise. After completion of the addition, the reaction mixture was warmed to rt and stirred at this temperature for 12 h. TLC analysis (Hexanes/EtOAc = 4:1) was used to monitor the reaction progress. The solvent was removed under reduced pressure and the residue purified by column chromatography (hexanes/EtOAc = 6:1 to 4:1) on silica gel to afford the coupled product **70** (1.71 g, 70.0%) contaminated with minor **71** as a white foam. This mixture was used for the next step without further separation. Data for **70**: ^{1}H NMR (400 MHz, $CDCl_3$): 8.43 (s, 1H), 7.81 (s, 1H), 7.48 (m, 6H), 7.35-7.24 (m, 9H), 6.38 (s, 1H), 6.14 (s, 1H), 5.15 (d, *J* = 5.6 Hz, 1H), 4.64 (d, *J* = 5.6 Hz, 1H), 4.07 (d, *J* = 15.6 Hz, 1H), 3.90 (d, *J* = 15.6 Hz, 1H), 1.44 (s, 9H), 1.43 (s, 9H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 151.4, 151.3 144.2 143.8 143.7, 143.5, 143.2 138.6, 137.61, 128.7 128.1, 127.4, 121.6

113.1, 112.9, 101.3, 87.5 85.0, 83.9, 83.7, 83.7, 3.3, 70.1, 65.6, 61.3, 28.1, 28.1, 27.7, 26.3, 22.1.

During the work-up, a Boc protection group was easy to lose on the column which resulted in compounds **72**, with a minor amount of **73**. The fraction was collected and evaporated to dryness. Compound **72** was obtained as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): 8.34 (s, 1H), 8.14 (s, 1H), 7.68 (s, 1H), 7.49 (m, 6H), 7.35-7.24 (m, 9H), 6.38 (s, 1H), 6.14 (s, 1H), 5.15 (d, *J* = 5.6 Hz, 1H), 4.63 (d, *J* = 5.6 Hz, 1H), 4.11 (d, *J* = 15.4 Hz, 1H), 4.88 (d, *J* = 15.4 Hz, 1H), 1.54 (s, 9H), 1.44 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 151.4, 150.7, 144.3, 143.7, 143.6, 141.1, 136.0, 131.6, 128.6, 128.1, 127.4, 121.5, 112.9, 95.6, 87.5, 85.1, 83.6, 81.4, 65.5, 61.3, 28.4, 28.4, 27.7, 26.3. Anal. Calcd for $C_{39}H_{39}BrN_4O_5 \cdot 2.1H_2O$: C, 61.41; H, 5.72; N, 7.36. Found: C, 61.03.16; H, 5.33; N, 7.76.

(1*S*,2*R*,5*R*)-5-(4-Amino-7-bromo-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (5). Compound **70** (830 mg, 1.2 mmol) was treated with a mixture of 2N HCl (20 mL, 40 mmol) and MeOH (20 mL). This reaction mixture was stirred at rt overnight and then brought to reflux for 1 h. TLC analysis (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) was used to monitor the reaction progress.After having been cooled to rt, the reaction mixture was neuturalized with basic ion-change resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) and then recrytallized from methanol to yield **5** as a white solid (293 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.08 (s, 1H), 7.73 (s, 1H), 6.40 (s, 2H), 6.02 (s, 1H), 5.74 (s, 1H), 5.10 (d, *J* = 6.8 Hz, 1H), 5.03(d, *J* = 5.6 Hz, 1H), 4.91 (t, *J* = 5.6 Hz, 1H), 4.46 (t, *J* = 5.6

Hz, 1H), 4.22 (m, 1H), 4.13 (m, 2H). ^{13}C NMR (100.6 MHz, DMSO-d_6): 152.1, 150.9, 142.3, 140.3, 134.8, 128.4, 123.5, 88.2, 77.6, 72.3, 64.9, 58.5. Anal. Calcd for $C_{12}H_{13}BrN_4O_3 \cdot 0.2H_2O$: C, 41.80; H, 3.92; N, 16.25. Found: C, 41.80; H, 3.94; N, 16.00.

Compound 74. To a solution of **70** (1.03 g, 1.25 mmol) in dry THF (50 mL) was added $Pd(Ph_3P)_4$ (100 mg, 0.087 mmol). Then $AlMe_3$ (1.25 ml, 2.0 M in THF, 2.5 mmol) was added to this mixture dropwise at rt. The reaction mixture was stirred at rt for 1 h and heated to reflux for 12 h. The reaction was allowed to cool to rt. The solvent was evaporated *in vacuo*. The residue was subjected to flash chromatography (hexanes/EtOAc = 4:1) to yield **74** (740 mg, 78.2%) as a white solid which can be used to the next step without further purification.

(1*S*,2*R*,5*R*)-5-(4-amino-7-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6). Compound **74** (0.72 g, 0.95 mmol) was treated with a mixture of 2N HCl (10 mL, 20 mmol) and MeOH (10 mL). This reaction mixture was stirred at rt for 1 h and then brought to reflux for 3 h. TLC analysis (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) was used to monitor the reaction progress. After having been cooled to rt, the reaction mixture was neuturalized with basic ion exchange resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 32/2/1) to yield **6** as a white solid (176 mg, 67.0%). ^{1}H NMR (400 MHz, CD_3OD): 8.04 (s, 1H), 7.44 (s, 1H), 5.95 (dd, J = 2.0 Hz, 3.6 Hz, 1H), 5.77 (m, 1H), 4.62 (dd, J = 0.8 Hz, 5.6 Hz, 1H), 4.34 (dd, J = 2.0 Hz, 4.0 Hz, 2H), 4.12 (dd, J = 4.8 Hz, 5.6 Hz, 1H), 2.58 (s, 3H).

Compound 89. To a solution of cyclopentanol **44** (429 mg, 1.00 mmol) and triphenylphosphine (393 mg, 1.50 mmol) in THF (10 mL) was added **56** (334 mg, 1.00

mmol). This suspension was cooled by ice to 0 °C and DIAD (303 mg, 1.50 mmol) was added dropwise. After completion of the addition, the reaction mixture was warmed to rt and stirred at this temperature for 2 h. TLC analysis (hexanes/EtOAc = 3:2) was used to monitor the reaction progress. The solvent was removed under reduced pressure and the residue purified by silica gel column chromatography (hexanes/EtOAc = 2:1) to afford the coupled product **89** (529 mg, 71.0%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): 8.35 (d, *J* = 5.6 Hz, 1H), 7.88 (s, 1H), 7.42 (d, *J* = 5.6 Hz, 1H), 6.14 (s, 1H), 5.46 (s, 1H), 5.20 (d, *J* = 5.6 Hz, 1H), 4.58 (d, *J* = 5.6 Hz, 1H), 4.06 (d, *J* = 15.6 Hz, 1H), 3.93 (d, *J* = 15.6 Hz, 1H), 1.46 (s, 3H), 1.44 (s, 18H), 1.32 (s, 3H). ^{13}C NMR(62.9 MHz, $CDCl_3$): 151.4, 150.4, 144.7, 143.6, 142.4 141.1, 140.1, 137.3, 128.5, 128.0, 127.3, 121.6, 113.0, 106.1, 87.4, 84.5, 83.8, 82.9, 77.7, 77.2, 76.7, 66.6, 61.3, 28.0, 27.3, 25.8.

(1*S*,2*R*,5*R*)-5-(4-amino-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (82). Compound **89** (410 mg, 0.55 mmol) was treated with a mixture of 2N HCl (10 mL, 20 mmol) and MeOH (10 mL). This reaction mixture was stirred at rt for 1 h and brought to reflux for 2 h. TLC analysis (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 8/2/1) was used to monitor the reaction process. After having been cooled to rt, the reaction mixture was neuturalized with basic ion exchange resin (Amberlite IRA-67). After filtration, the solvent was evaporated under reduced pressure. The residue was applied to column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) to yield **82** as a white solid (104 mg, 72%). The NMR spectral data agreed with literature.[175]

Methyl-2,3-*O*-*iso*propylidene-β-D-ribofuranoside (95). Concentrated hydrochloric acid (5 mL) was added to a suspension of D-ribose (50 g, 0.33 mmol) in acetone (140 mL)

and methanol (140 mL) at rt. The mixture was refluxed for 1 h. The reaction was cooled to rt, neutralized with pyridine, and partitioned between water (350 mL) and ether (100 mL). The separated aqueous phase was extracted with ether (2 x 100 mL) and ethyl acetate (3 x 100 mL), and the combined organic phases were washed with saturated copper sulfate solution, water, and brine prior to drying and solvent evaporation. The residue was distilled to give 37 g (78%) of **95** as a colorless oil as a mixture of anomers. The NMR spectral data agreed with literature.[184]

Methyl-5-deoxy-5-iodo-2,3-*O*-*iso*propylidene-β-D-ribofuranoside (96). A solution of these epimers (26.1 g, 128 mmol), imidazole (13.1 g, 192 mmol), and triphenylphosphine (40.5 g, 154 mmol) in toluene (500 mL) and acetonitrile (100 mL) was treated portionwise with iodine (39.0 g, 154 mmol), refluxed for 5 min, and cooled to rt. Additional iodine was introduced in approximately 100 mg portions until the reaction mixture remained dark-brown in color. After dilution with ether and repeated washing of the organic extracts sequentially with 10% sodium thiosulfate solution, water, and brine, the solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo to leave a residue which was filtered through a short plug of silica gel which was eluted with hexanes/EtOAc = 95:5 to give **96** (39.7 g, 99.0%) as a colorless oil of the mixture of anomers. The NMR spectral data agreed with literature.[184]

(2*R*,4*R*)-2-Dimethyl-5-vinyl-1,3-dioxolane-4-carboxaldehyde (97). To a stirred solution of iodide **96** (3.70 g, 11.7 mmol) in THF (25 mL) and EtOH (95%, 3.7 mL) at room temperature was added zinc powder (Aldrich, dust, <10 micron, 3.8 g, 59 mmol) in one batch, followed by addition of acetic acid (0.37 mL, 6.5 mmol) in one portion via syringe. The reaction mixture was heated to reflux for 5 h. The reaction was cooled to rt,

filtered through a short plug of celite, and washed with a 100 mL of a 1:1 mixture of THF/pentane. The filtrate was concentrated by evaporation 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** (1.55 g, 85.1%) as a colorless oil. The NMR spectral data agreed with literature.[185]

(4*S*,5*R*)-1-(2,2-Dimethyl-5-vinyl-[1,3]dioxolan-4-yl))-prop-2-en-1-ol (98). To a solution of **97** (8.50 g, 5.50 mmol) in anhydrous CH_2Cl_2 (150 mL) was added dropwise a solution of vinylmagnesium bromide (1.0 M in THF, 6.5 mL, 6.5mmol) at -40 °C. The reaction was allowed to warm to 0 °C over 1 h and then stirred at this temperature for 2 h. Saturated NH_4Cl (20 mL) was added to quench the reaction. The organic layer was separated, washed with brine, and dried ($MgSO_4$). The solvent was removed by evaporation under reduced pressure and the residue purified by silica gel column chromatography (EtOAc/hexanes = 1:5) to afford **98** as a mixture of two isomers with the ratio of 4.5:1 (8.0 g, 80%) as a colorless oil. The NMR spectral data agreed with literature.[183]

(3a*R*,6a*R*)-2,2-Dimethyl-3a,6a-dihydrocyclopenta[1,3]-dioxol-4-one ((4*R*,5*R*)-4,5-*O-iso*propylidene-2-cyclopentenone) (90). To a 500 mL round-bottom flask filled with the Grubbs' catalyst benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (670 mg, 0.81 mmol, flushed with N_2 three times) was added a solution of the diene **7** (15.1 g, 81.3 mmol) in anhydrous CH_2Cl_2 (300 mL). After being stirred at 24 °C for 4h, 4 Å molecular sieve (30 g), pyridinium dichromate (35.3 g, 162 mmol), and acetic acid (0.23 mL, 3.8 mmol) were added to the resulting dark brown mixture. The reaction mixture was stirred at the same temperature for 12 h and filtered through 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 = 1:10), giving compound **90** (11.4 g, 93.0%) as a white crystal.[169]

(+)-2,3-(Isopropylidenedioxy)-4-cyclopenten-1-ol (86). To a stirred solution of cyclopentenone **90** (2.31g, 15.0 mmol) and $CeCl_3 \cdot 7H_2O$ (5.59 g, 15.0 mmol) in MeOH (70 mL) at 0 °C was added $NaBH_4$ (1.13 g, 30.0 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 **4** as a colorless syrup (2.3 g, 99%) which was used directly in the next step. ^{1}H NMR (250 MHz, $CDCl_3$): 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, $CDCl_3$): 135.9, 131.5, 111.9, 83.1, 76.7, 73.7, 27.2, 26.1.

Compound 100. To a solution of cyclopentanol **88** (156 mg, 1.00 mmol) and triphenylphosphine (393 mg, 1.50 mmol) in THF (10 mL) was added **45** (413 mg, 1.00 mmol). This suspension was cooled by ice to 0 °C and DIAD (303 mg, 1.50 mmol) was added dropwise. After completion of the addition, the reaction mixture was warmed to rt and stirred at this temperature for 2 h. TLC analysis (hexanes/EtOAc = 1:1) was used to monitor the reaction progress. The solvent was removed by evaporation under reduced pressure and the residue purified by silica gel column chromatography (hexanes/EtOAc = 2:1) to afford the coupled product **100** (529 mg, 71.0%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (s, 1H), 7.80 (s, 1H), 6.41 (m, 2H), 6.07 (m, 1H), 5.38 (m, 1H), 4.65 (dd, J = 0.4, 5.6 Hz, 1H), 1.50 (s, 3H), 1.44 (s, 18H), 1.36 (s, 1H). ^{13}C NMR (100.6 MHz,

$CDCl_3$): 151.3, 143.6, 142.8, 139.6, 138.6,137.6, 129.2, 112.7, 101.4, 84.5, 84.2, 83.4, 66.6, 28.1, 28.09, 27.6, 26.1.

(1*S*,2*R*,5*R*)-5-(4-amino-7-bromo-1*H*-imidazo[4,5-*c*]pyridin-1-yl)cyclopent-3-ene-1,2-diol (7). Compound **100** (0.72 g, 0.95 mmol) was treated with a mixture of 2N HCl (10 mL) and MeOH (10 mL). This reaction mixture was stirred at rt for 1 h and then brought to reflux for 3 h. TLC analysis (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 16/2/1) was used to monitor the reaction progress. After having been cooled to rt, the reaction mixture was neuturalized with basic ion exchange resin (Amberlite IRA-67). After filtration, solvent was evaporated under reduced pressure. The residue was applied to silica gel column chromatography (EtOAc/MeOH/$NH_3 \cdot H_2O$ = 32/2/1) and then recrystllized from methanol to yield **7** as a white solid (176 mg, 67%). ^{1}H NMR (400 MHz, CD_3OD): 8.09 (s, 1H), 7.77 (s, 1H), 6.36 (m, 1H), 6.28 (m, 1H), 6.12 (m, 1H), 4.71 (m, 1H), 4.27 (t, J = 5.6 Hz, 1H). ^{13}C NMR (100.6 MHz, CD_3OD): 153.1, 143.5, 142.0, 138.4, 137.2, 133.4, 129.6, 90.9, 79.6, 74.8, 67.1. Anal. Calcd for $C_{11}H_{11}BrN_4O_2$: C, 42.46; H, 3.56; N, 18.01. Found: C, 42.73; H, 3.60; N, 17.94.

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