INVESTIGATIONS INTO 5'-EXTENDED 3-DEAZAARISTEROMYCIN DERIVATIVES

by

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Abstract

Nucleosides have provided a plentiful source in structural leads in antiviral drug discovery. Included in this class of compounds are the carbocyclic nucleosides, which because of the presence of a cyclopentyl moiety, offer sites for modification not possible with the typical ribofuranosyl analogues. Naturally occurring carbocyclic nucleosides aristeromycin and neplanocin A exhibit a broad-spectrum antiviral activity attributed to the inhibition of *S*-adenosylhomocysteine (AdoHcy) hydrolase. This, in turn, affects viral mRNA capping methylation, which is vital for the reproduction of viruses.

While application of aristeromycin and neplanocin A is limited by their toxicity due to 5′-nucleotide formation, synthetic analogues thereof designed in our lab have shown significant antiviral activity without associated toxicity.

The Ebola virus, for instance, is an emerging pathogen causing severe hemorrhagic fevers with a mortality rate as high as 90%. At this time, no treatment exists for Ebola, which is stimulating the search for new therapeutic agents. In this direction our lab has found that carbocyclic nucleosides offer a source for these agents.

Herein is a reported the convergent and stereospecific syntheses of several 5'-extended 3-deazaaristeromycin analogues starting from D-ribose with potential to be effective anti-Ebola agents.

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Table of Contents

List of Figures

List of Schemes

List of Tables

I. INTRODUCTION

Bacteria, fungi, parasites and viruses are among pathogenic microorganisms that can cause infectious diseases in host organisms.[1] The different classes of these pathogens employ different mechanisms to enter their host and to avoid imminent elimination by the host's immune system. The outcome of these mechanisms does not always give rise to illness. Bacteria, for example, are in most cases not pathogenic and can coexist symbiotically with their host, with both organisms mutually benefitting from this relationship. Bacteria that do act as pathogens contain specific virulence genes that encode for proteins, which interact with the host cell. This results in cell toxicity while simultaneously promoting replication and spread of the pathogen. Because fundamental metabolisms differ vastly in eubacteria, it is possible to design antibiotic drugs which target and inhibit these specific processes, while leaving the eukaryotic host unharmed.[1,2]

In contrast, pathogenic fungi, protozoa, and parasites are eukaryotic like the host. As a result of this, antifungal and antiparasitic drugs are often less effective and associated with higher toxicity than antibiotics. Even so, eukaryotic pathogens have their own cell machinery that ensures replication and provides metabolic energy. This enables the design of drugs which specifically target these processes albeit with an expected amount of toxicity due to the similarities in host and pathogen metabolism.[1,3,4]

Unlike any of the aforementioned pathogenic classes, viruses do not have a metabolism of their own and lack the intrinsic ability to produce the proteins encoded by their DNA or RNA genomes. They infiltrate the host's cell and use the host's cell processes to synthesize the proteins needed for replication. The intense involvement of

the host cell in viral replication makes the development of viable antiviral drugs extremely problematic, since targeting the virus essentially means targeting the host.[1,5,6]

The emergence and reemergence of virus diseases over the past few decades in form of a yet unrecognized infection or in an expanded version of a previously known infection has caused unexpected threat to the health of humans, wild animals and livestock. Management of epidemics consists of identifying and controlling these diseases. Epidemics can be economically devastating, especially in poverty-stricken third world countries where many of these viral infections have their origins. The recent emergence of viral outbreaks can be attributed to urbanization, ease of travel, climate change and agricultural practices. Vaccination has been the primary means of assault on these infections.[7,8]

Mathematical models have been developed to simulate the spread of virus outbreaks,[9] the results of which have shown that it is extremely challenging to control virus outbreaks by vaccination alone. Presuming the existence of a given vaccine, the immunization of a large percent of the population would be required to ensure containment of a virus. While there have been extensive efforts to develop new vaccines, there are many factors making vaccines problematic.[10] Distribution and dispensing thereof can be difficult and expensive and raises ethical questions of public good versus individual autonomy i.e. the right of individuals to deny vaccination. In addition, the immunization of high-risk populations, such as immunocompromised people, is not easily accomplished, since a compromised immune systems lessens the protection afforded by the vaccine.[10,11]

The use of attenuated (live) vaccines, for example, the smallpox vaccine, is also

generally contraindicated for persons with altered immunocompetence. This is due to a higher risk of actually inducing infection with the virus upon administration. Moreover, there is a risk that attenuated vaccine viruses may mutate towards increased virulence and cause infection even in healthy patients.[11] Elderly persons usually have a lower protective immune response following a vaccination, which can cause a lower effectiveness of vaccinations in this group. Vaccination of pregnant women is also usually contraindicated due to the possibility of causing birth defects to the fetus. Furthermore, in spite of the urgency, vaccines could not be developed to date for many emerging virus diseases such as the Ebola and Marburg viruses as well as human immunodeficiency virus (HIV) and the hepatitis C virus (HCV). This calls to forefront the development of novel antiviral agents for chemotherapy. Nucleosides have a permanent place in this therapeutic regimen, however, some of the premium leads in this category provide synthetic challenges. This dissertation focuses on the synthetic development for new drugs in this nucleoside category.

II. BACKGROUND

A. Natural nucleoside structure and function

Effective therapeutic candidates for anti-viral drugs are often nucleoside analogues that are derived from naturally occurring nucleosides[12] of which there are many. The most notable are adenosine, guanosine, cytidine, uridine and thymidine. These consist of one of five heterocyclic nucleobases, namely adenine, guanine, cytosine, uracil or thymine, connected via a glycosidic bond between a ring nitrogen in the heterocyclic base and the sugar 2'-deoxy-D-ribose or D-ribose. In their natural function they are transformed into nucleotides that are the phosphate esters of nucleosides and constitute the basic building block of nucleic acids, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Figure 1). It is well understood that DNA and RNA are the molecules that ensure the preservation of hereditary information.[13] DNA transcribes into mRNA, which, in turn, is translated by the assembly of amino-acids carried by t-RNA into a plethora of biologically necessary cell proteins. This is known as the central dogma of molecular biology[14] (Figure 2).

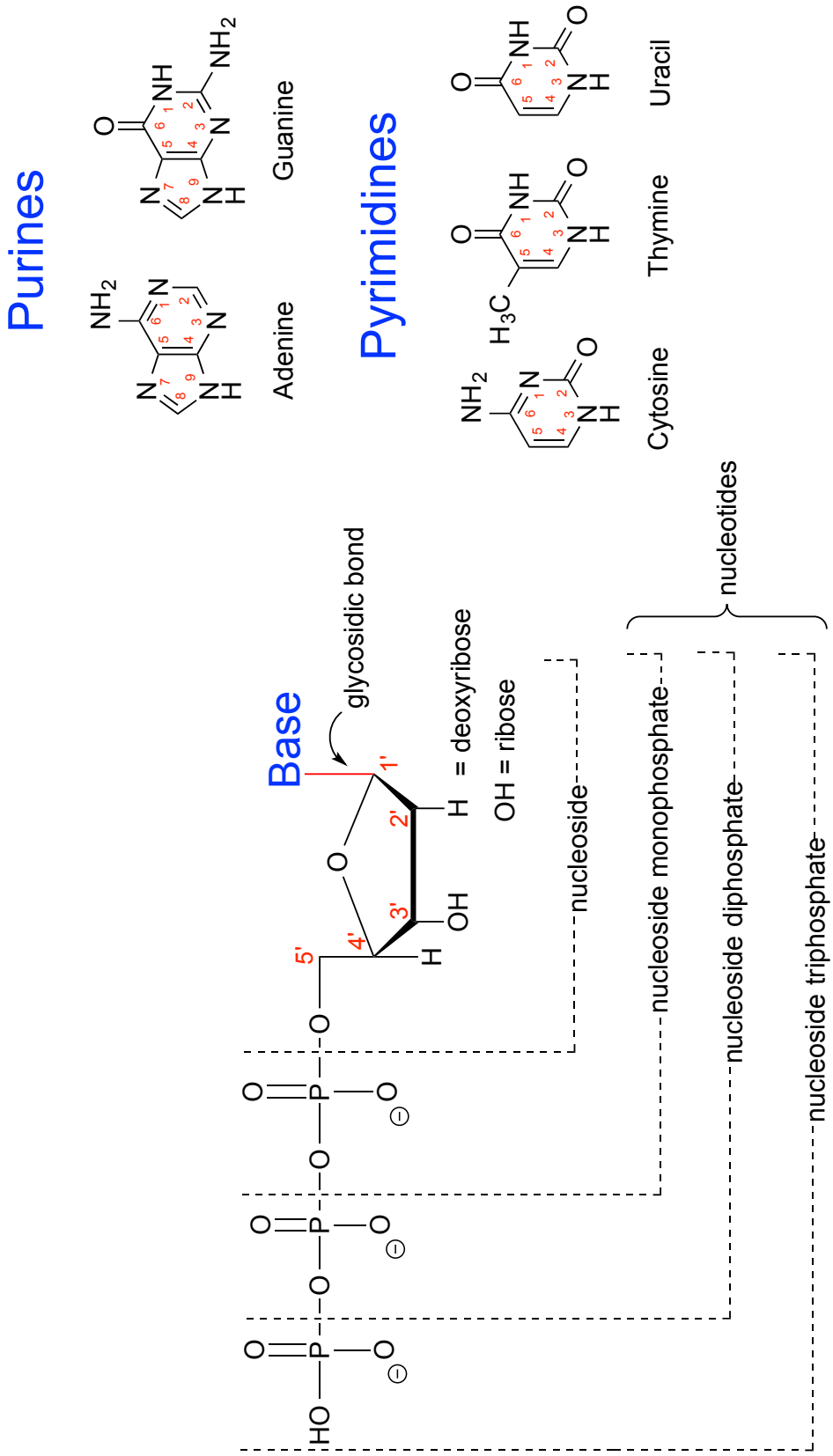


Figure 1: Building blocks of nucleic acids

Figure 2: The Central Dogma of Molecular Biology

Additionally, naturally occurring nucleosides are vastly involved as regulatory factors in fundamental cellular metabolism and, as such, represent the most important biomolecules discovered to date. In this regard, for example, adenosine is a component of ATP, coenzyme A and nicotinamide adenosine dinucleotide (NAD^+), which are vital in all parts of metabolism.[13] Various biological effects may therefore be achieved from modifying the structure of nucleosides.

B. Viral structure and classification

Viruses are small infectious agents, which contain either an RNA or DNA genome encapsuled by a protective, protein coat or capsid. This capsid is symmetrical in form and its shape serves as the basis for morphological distinction.[5] Associated basic proteins are often also inside of the capsid, such as, proteases, integrases or reverse-transcriptases. Proteases are enzymes responsible for proteolysis, which is the initiation of protein catabolism via hydrolysis of peptide bonds. Integrases, on the other hand, are enzymes, which are associated with a retrovirus and which enable the viral genome to be integrated into the DNA of the host. Finally, reverse-transcriptases, which are also

associated with retroviruses, are enzymes, which enable the formation of DNA from an RNA template in a process referred to as reverse transcription. The viral genome, the capsid and these basic viral enzymes make up the complete virus particle and are called a virion.

The function of the virion is to deliver the viral DNA or RNA genome into the host cell. There, the genome can be expressed by the host cell. Some of the proteins inside the capsid are bound to the viral nucleic-acids and are called nucleoproteins, which together with the genome, form the so-called nucleocapsid. Some viruses form envelopes, which are derived from the host's cell membrane. In this case the capsid is surrounded by a lipid bilayer membrane and studded with an outer layer of virus envelope glycoproteins, which are coded for either by the virus or by the host genome. The lipid bilayer and any present carbohydrates are coded for entirely by the host's genome.

The Ebola virus, for instance, is an emerging pathogen causing severe hemorrhagic fevers. It was first described in 1976, when a major breakout occurred in Central Africa. With a mortality rate as high as 90%, and ease of transmittance from host to host, the Ebola virus is an imminent threat. At this time, no treatment exists for Ebola, which is stimulating the search for new therapeutic agents.

Recently, a 3D model was developed for the structure of the Ebola Virus.[15] This model was developed using virology data and a variety of methods including X-ray analysis, NMR spectroscopy and molecular modeling. Figure 3 shows Ebola inserting into its host cell. In this Figure pink refers to the Ebola virus and grey refers to structures originating from human cells (lipid membrane).

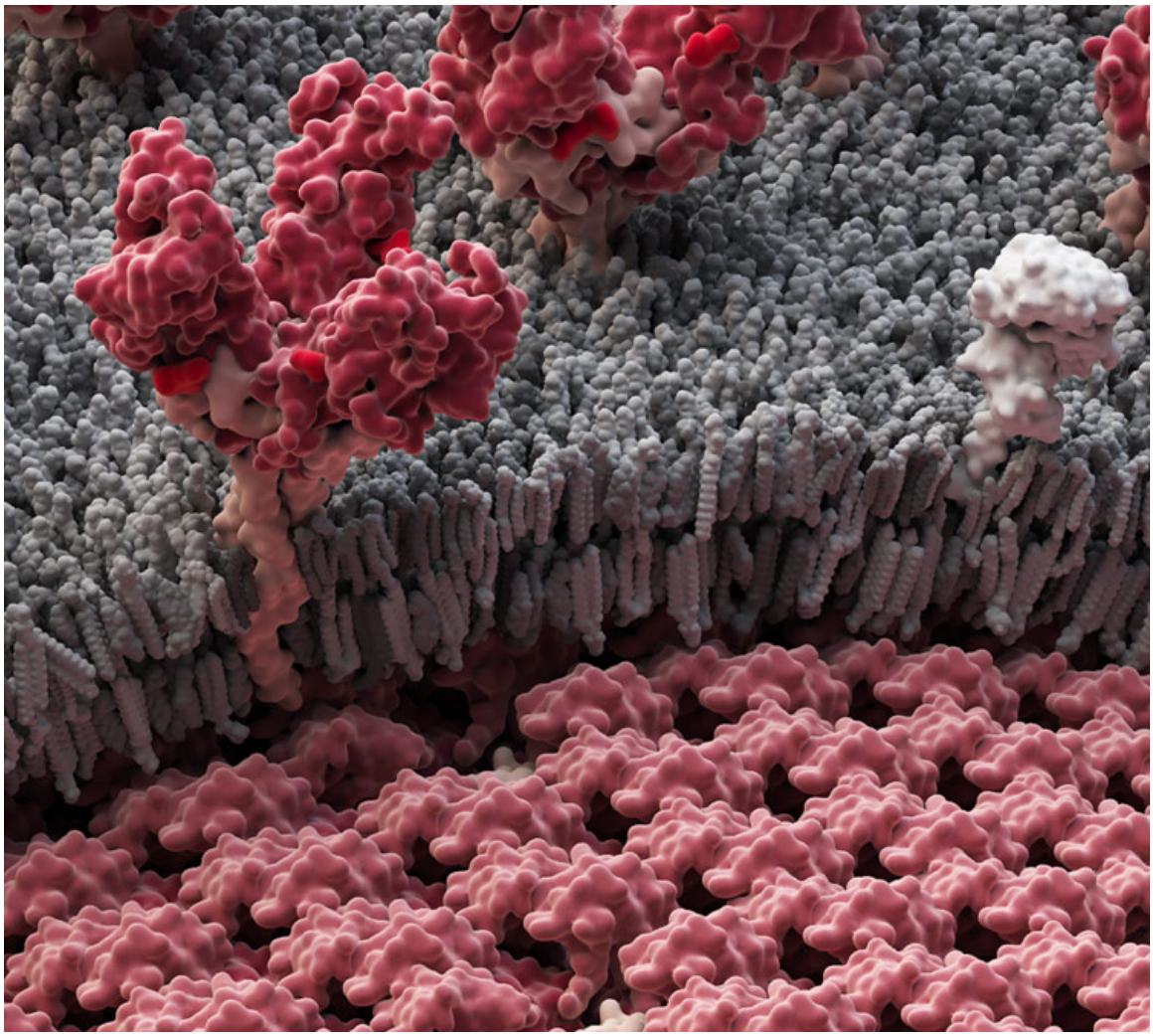


Figure 3: 3D model of the Ebola virus[15]

Two systems exist for viral classification: The Hierarchical Virus Classification System and the Baltimore Classification System.[16] The former system uses information on viral morphology, such as, symmetry of the capsid, the existence of an envelope or the dimensions of the virion together with information on the nature of the viral nucleic acid (RNA or DNA) in order to classify viruses into different families. The latter system classifies viruses solely on their mechanisms of viral genome replication.

Central to the idea of the Baltimore Classification is that no matter in what

nucleic-acid form a virus enters a host cell, in the end the genome must be present in form of mRNA, in order to code for the proteins necessary for replication. This allows for a break-up into seven fundamentally different groups of viruses, which takes into consideration whether the original genome of the virus is DNA, RNA, single stranded (ss) or double stranded (ds), + sense or – sense (Figure 3). It is convention that the top strand of the coding DNA written in the 5' - 3' direction is called the + sense and that the mRNA sequence is also called + sense.

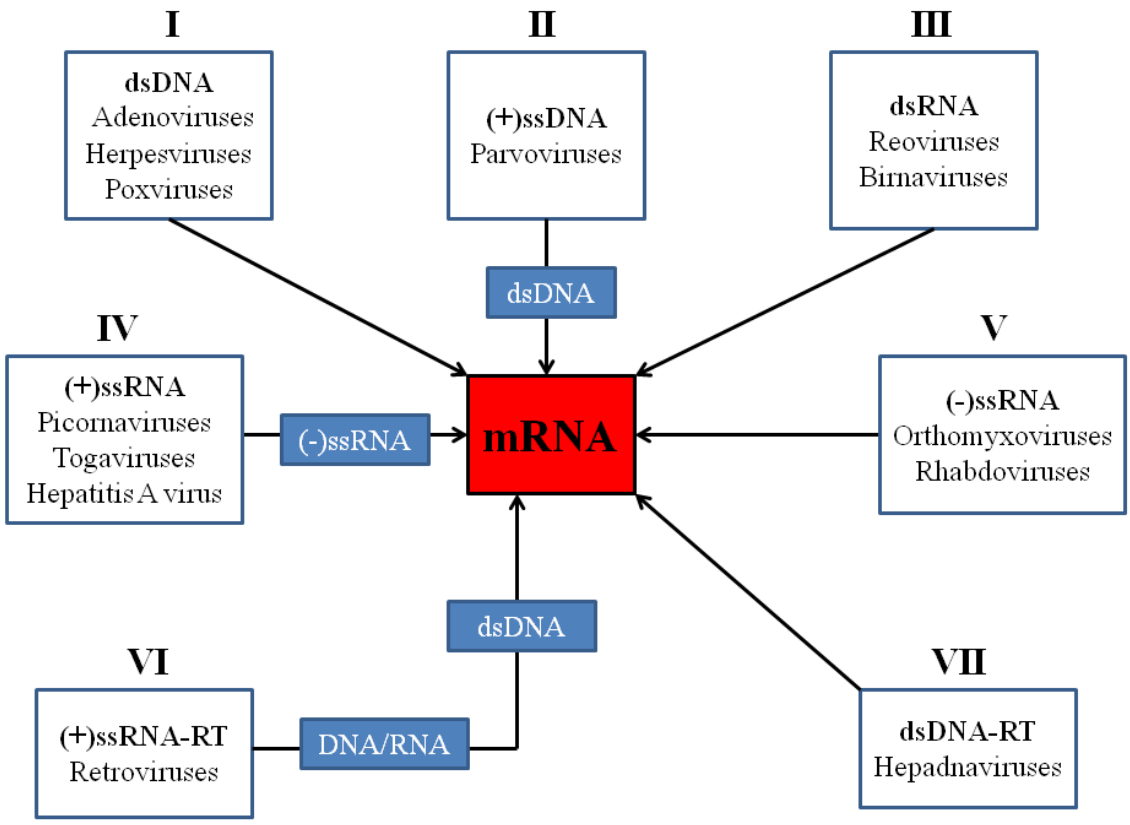


Figure 4: Baltimore Classification of Viruses

(RT = reverse transcriptase)

C. Traditional nucleoside derivatives

Nucleosides play an important role in viral replication. Modifications in the structure of nucleosides have resulted in anti-viral drugs responsible for a variety of biological consequences. Essentially, modification of the nucleoside can take place either at the nucleobase or at the sugar moiety. The first such nucleoside derivative that was discovered to possess anti-viral activity was 5'-iodo-2'deoxyuridine (Figure 5), which was shown in the 1950's to have activity against the herpes virus.[17]

Since then numerous modified nucleosides have been discovered and investigated. Modifications of the sugar moiety encompass functionalization of the hydroxyl groups and the incorporation of amines, azides, halides, sulfur-containing groups etc. as new functional groups. In some cases the sugar was replaced by an acyclic structure.[18] Modifications of the base moiety are less prominent but have included the functionalization of the base scaffold with groups such as halogens, amides, nitriles etc. as well as the replacement of the heterocyclic base nitrogens with carbon.[19,20] The latter are referred to as deaza-purines and -pyrimidines. Some of these proved to be potent anti-viral agents, which were clinically approved and are currently on the market as therapeutic drugs. A few important examples are shown below in Figure 5.

In addition to 5'-iodo-2'-deoxyuridine, acyclic guanosine derivatives, such as acyclovir have also proven to be potent anti-herpetic drugs.[21] Another example is ribavirin, which exhibits a broad-spectrum anti-viral activity, i.e. activity is displayed against more than one virus, including activity against respiratory syncyntial virus, hepatitis C virus (HCV) and many viral hemorrhagic fevers.[22] [Viral hemorrhagic fevers are a group of diseases caused by a family of RNA viruses. Multiple organ systems of the

body are affected resulting in possibly fatal loss of self-regulation of the body as well as severe hemorrhage (bleeding).] Finally, dideoxyinosine, Zidovudine (Retrovir, AZT) and Lamivudine (3TC) are highly potent anti-retroviral agents used for the treatment of HIV infection in combination with protease inhibitors.[23] [Protease inhibitors selectively bind to viral proteases and hinder the catabolism of protein precursors necessary for the formation of functional protein products, which are involved in the production of infectious viral particles.] A parallel timeline in Table 1 shows chronological nucleosidic drug development versus emerging and re-emerging viral diseases.

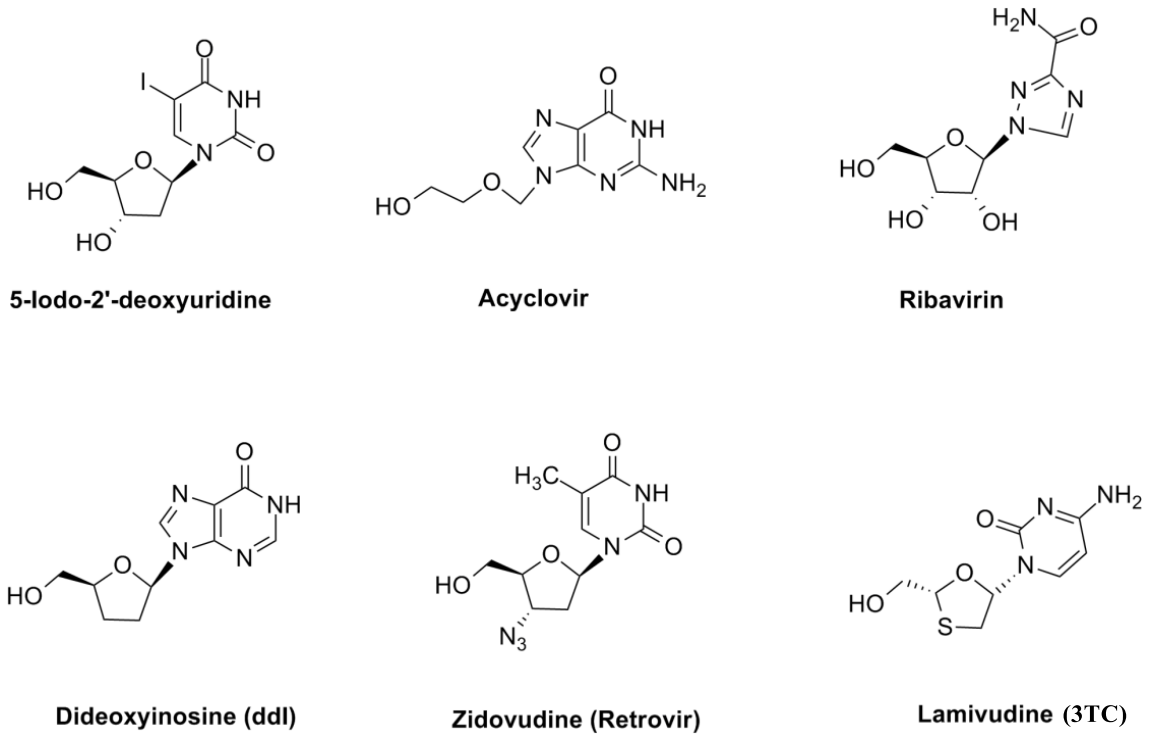


Figure 5: FDA-approved Modified Nucleosides

Frequently, nucleoside derived anti-viral drugs inhibit viral replication by selective interaction of their respective 5'-triphosphates (NTP) with the viral nucleic acid

polymerases[24]. The initial nucleoside derivative is, therefore, a prodrug, which is converted into its active NTP metabolite by either cellular or viral kinases. In cases when therapeutic nucleosides are preferentially phosphorylated by viral kinases, the NTPs only accumulate in infected cells. This is the case with acyclovir, which is transformed into its active NTP by the herpesvirus kinase. This, in turn, allows for a very high activity and selectivity against the herpes virus.[21,22,24]

1953	Discovery of DNA double helix by Watson and Crick
1959	Discovery of 5'-iodo-2'deoxyuridine, first marketed anti-viral nucleoside drug
1960s	Discovery of antiviral/antibiotic activity of nucleoside natural products
1964	First synthesis of Zidovudine
1970s	Discovery of Acyclovir, Ribavirin and Abacavir; emergence of Rotavirus, Parvovirus B19, Ebola virus and Hantavirus
1980s	Approval of Acyclovir as drug; emergence and identification of HIV as cause of AIDS
1985	Zidovudine's anti-retroviral activity discovered
1987	Approval of Ziduvudine as a drug for HIV treatment
1989	Emergence of Hepatitis C
1990s	Emergence of Guanarito virus, Sin Nombre virus, Sabia virus, Hendra virus, Human Herpesvirus 8, Avian Influenza virus (bird flu, H5N1), West Nile Virus (in USA)
1998	Approval of Ribavirin for combination treatment of Hepatitis C; approval of Abacavir for anti-HIV treatment
1990s+	Numerous nucleosides approved as anti-viral, anti-cancer, anti-protozoan drugs
2000s	Emergence of severe acute respiratory syndrome coronavirus (SARS), Swine Influenza (H1N1)

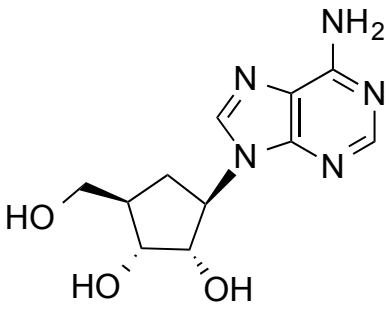
Table 1: Selected timeline for nucleoside drugs and emerging viral diseases

Unfortunately, there are not only success stories. The substrate specificity of the NTPs for viral or host polymerases is the deciding factor between activity or toxicity. Thus, the utility of nucleoside drugs is oftentimes compromised by the toxicity resulting from incorporation of the NTP into the host's nucleic acids or by the full inhibition of the host's enzymes.

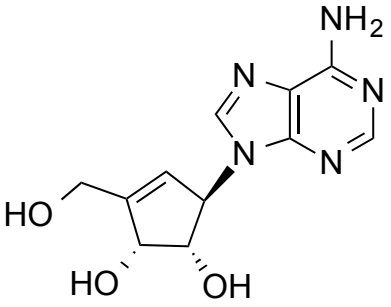
D. Carbocyclic nucleosides

It is possible for nucleoside analogs to exhibit different mechanistic motifs other than polymerase inhibition by the NTPs.[24] In recent years a number of such nucleosides have been discovered, an important group of which are the carbocyclic nucleosides (carbanucleosides). The structures of a few important compounds in this class are shown below (Figure 6).

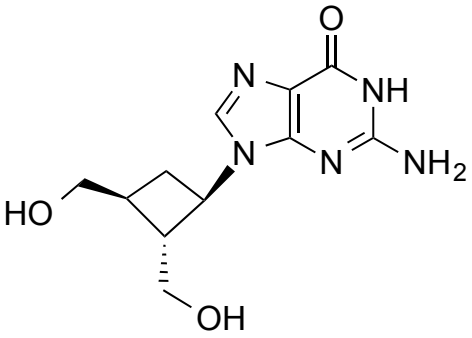
Carbocyclic nucleosides can be naturally occurring. In fact, adenosine derivatives aristeromycin (Ari) and neplanocin A (NpcA), two of the early as well as most important compounds in this class, have their roots in nature. In 1968 aristeromycin[25] was isolated from *Streptomyces citricolor* and in 1981 neplanocin A[26] was isolated from the culture filtrate of *Ampullariella regularis*. Both compounds were later synthesized and display high broad-spectrum anti-viral activity against various viruses including poxvirus, reovirus and smallpox virus, however both have exhibited such high cytotoxicity that they are unsuitable for therapeutic use.



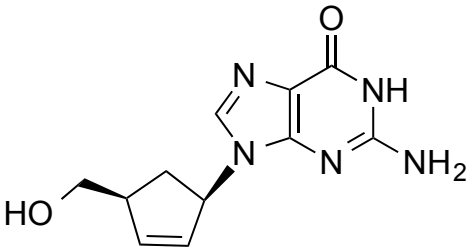
Aristeromycin (Ari)



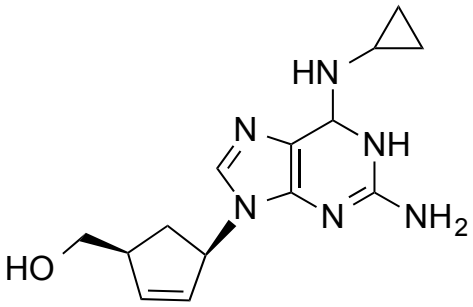
Neplanocin A (NpcA)



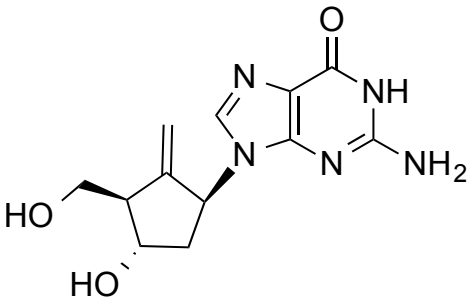
Carboxetanocin G



Carbovir



Abacavir

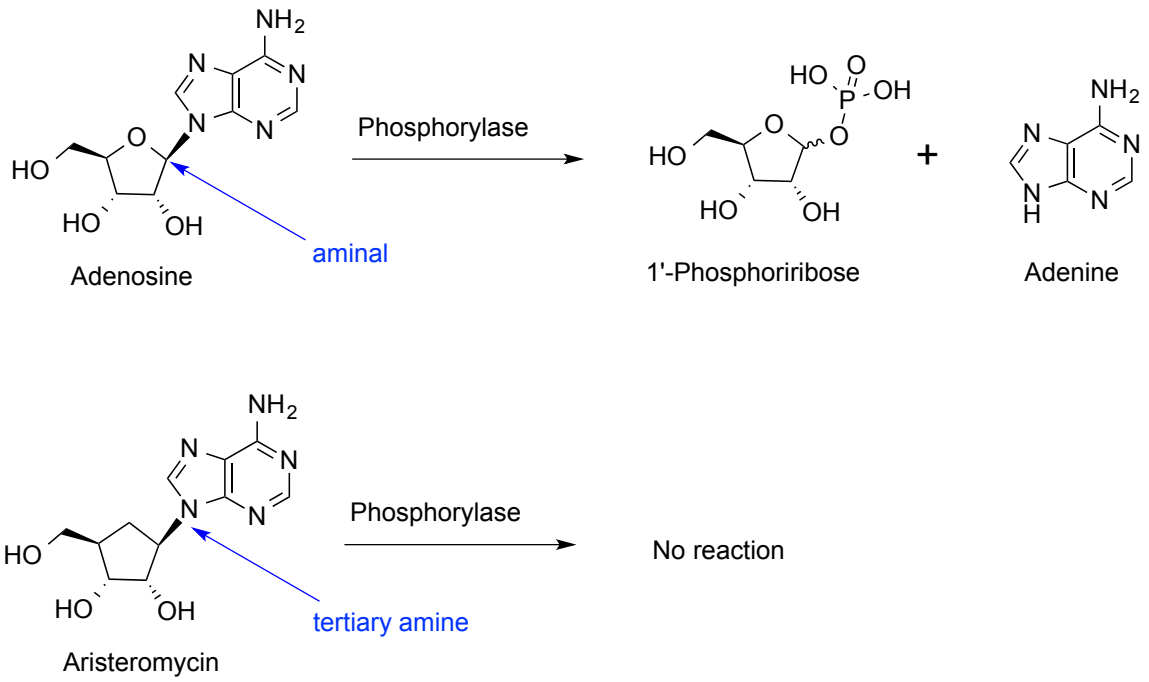


Entecavir

Figure 6: Carbocyclic nucleosides with high anti-viral activity

The structural difference between this class of compounds and more common nucleosides is that the endocyclic ribose oxygen of the latter has been replaced by a

methylene moiety thereby changing the furanose ring to a cyclopentyl group. This modification dramatically changes the stability of nucleosides toward phosphorolysis. Traditional nucleosides have an N-glycosidic bond connecting the sugar to the base. This aminal-like bond is fairly unstable and easily cleaved by phosphorylases. The products of this phosphorolysis are 1'-phosphoribose and the heterocyclic base. Because of this metabolism, delivery of the intact ribose-based nucleoside to interact with the viral target is difficult. In carbocyclic nucleosides however, the C-N bond in question constitutes a tertiary amine with much higher stability and, consequently does not easily undergo cleavage by phosphorolysis. These properties are illustrated below for adenosine versus aristeromycin in Scheme 1.



Scheme 1: Different stabilities toward phosphorolysis of regular versus carbocyclic nucleosides

In addition to increased stability, carbocyclic nucleosides exhibit higher lipophilicity, which allows for improved oral bioavailability and cellular uptake.

Carboxetanocin G,[27] a promising anti-herpes and anti-hepatitis B agent, and carbovir, an anti-HIV agent, received FDA approval for treatment in clinical trials, which were later halted due to their extensive long-term side-effects. More recently, carbocyclic nucleosides have found greater therapeutic application with Abacavir, which was FDA approved in 1998 for use as an anti-retroviral (HIV) agent and entecavir, which was approved to treat chronic hepatitis B infection in 2005. Their structures can be found in Figure 6.

E. SAH Hydrolase Inhibitors

The mechanism of action for both Ari and NpcA is the inhibition of S-adenosyl-L-homocysteine (SAH) hydrolase. This enzyme plays a vital role in mRNA capping methylation. In order to ensure stability against phosphatases and ribonucleases, it is imperative that eukaryotic mRNA possesses a methylated 5'-cap structure. Most viruses are unable to replicate without a methylated cap structure on their mRNA. The donor of the methyl group is one of the most versatile biomolecules, S-adenosyl-L-methionine (AdoMet, also known as SAM or SAMe) shown below in Figure 7. AdoMet is biosynthesized from L-methionine and ATP in a process catalyzed by adenosyltransferase that was first discovered in 1953 by Cantoni.[28] It is not only the most important biological methylating reagent but is also involved in many other key metabolic reactions and, in this role, crucial for the existence of life.[29]

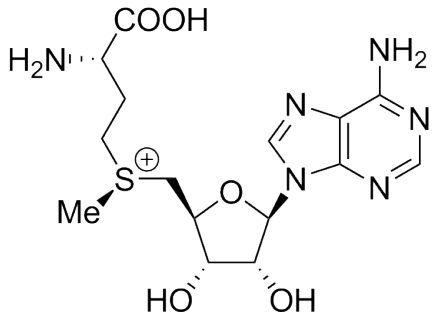
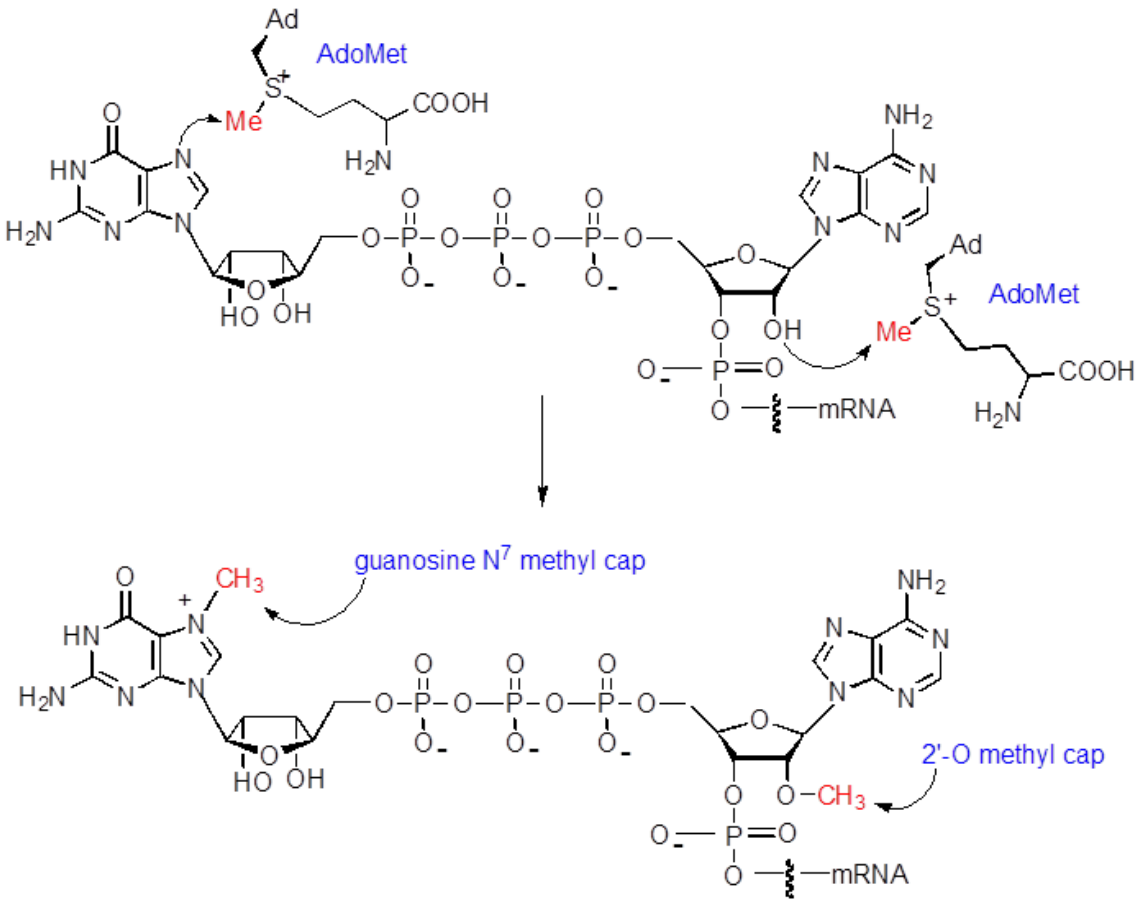


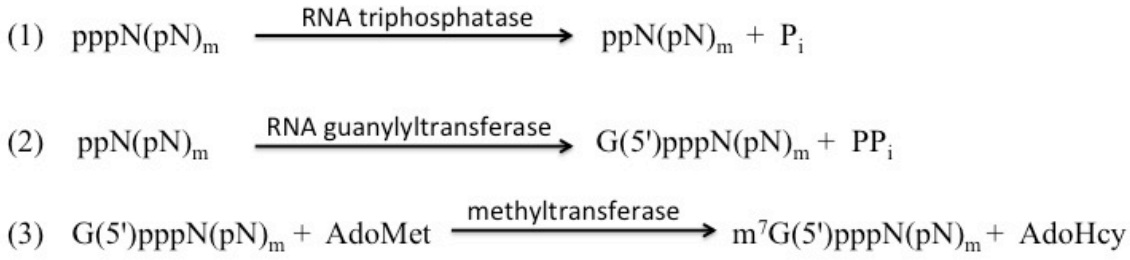
Figure 7: S-adenosyl-L-methionine (AdoMet)

As stated, AdoMet plays a key role in the maturation of mRNA. Each nascent mRNA has at its terminus a guanosine residue connected with its 5'-hydroxyl group via a triphosphate bridge to the 5'-end of the mRNA chain via an unusual 5' to 5' linkage. This is referred to as the mRNA cap. In further processing of this cap a methyl group is transferred from AdoMet to the N-7 of the terminal guanosine residue. In most cases, the capping process also involves the methylation of the 2'-hydroxyl group of the penultimate nucleotide (Scheme 2).



Scheme 2: The 5'-terminus of mRNA and capping methylation

The overall capping process consists of three enzymatic reactions. In the first step, the initial (uncapped) mRNA 5'-triphosphate terminus is cleaved to a diphosphate catalyzed by RNA triphosphatase. In the second step, guanosine monophosphate (GMP) is transferred onto this diphosphate in a 5' to 5' linkage catalyzed by RNA guanylyltransferase. In the third and final step, the methylation of the guanosine as well as the penultimate residues takes place catalyzed by AdoMet-dependent methyltransferase (Scheme 3).



Scheme 3: Overall capping process of mRNA

The capped structure enables mRNA to take part in RNA processing, nuclear transport and initiation of translation. In addition, it protects the mRNA from digestion by exonucleases [enzymes that break nucleotides off one by one from the end (exo) of a nucleic acid by hydrolysis of phosphodiester bonds], thus adding stability. Capped mRNA structures also have improved binding affinity to the ribosome, the site at which translation takes place. Therefore, the translational machinery does not effectively recognize uncapped mRNA, but only mature mRNA that has been capped and methylated as described above. As a result of this, interference with the capping methylation process will lead to incomplete viral mRNA, which is unable to translate into the proteins needed for assembly of the virus, and ultimately lead to the inhibition of viral replication.[29,30]

Cellular and viral methyltransferases catalyze the methylation process (Scheme 4). The compound S-adenosyl-L-homocysteine (AdoHcy, SAH) shown in Figure 8 is the product of the methylation reaction as well as the biofeedback-inhibitor of the methyltransferases.

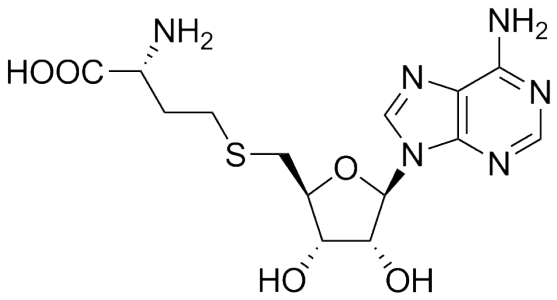
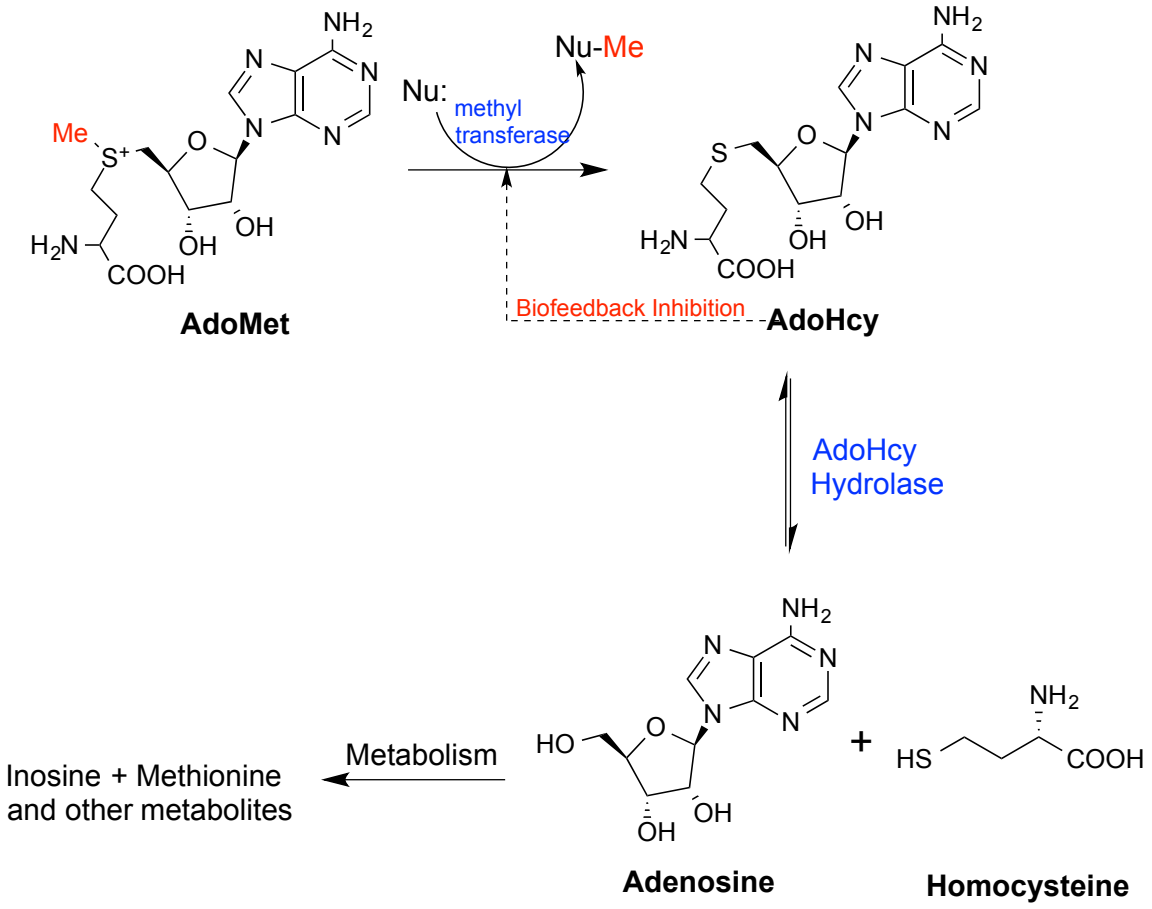


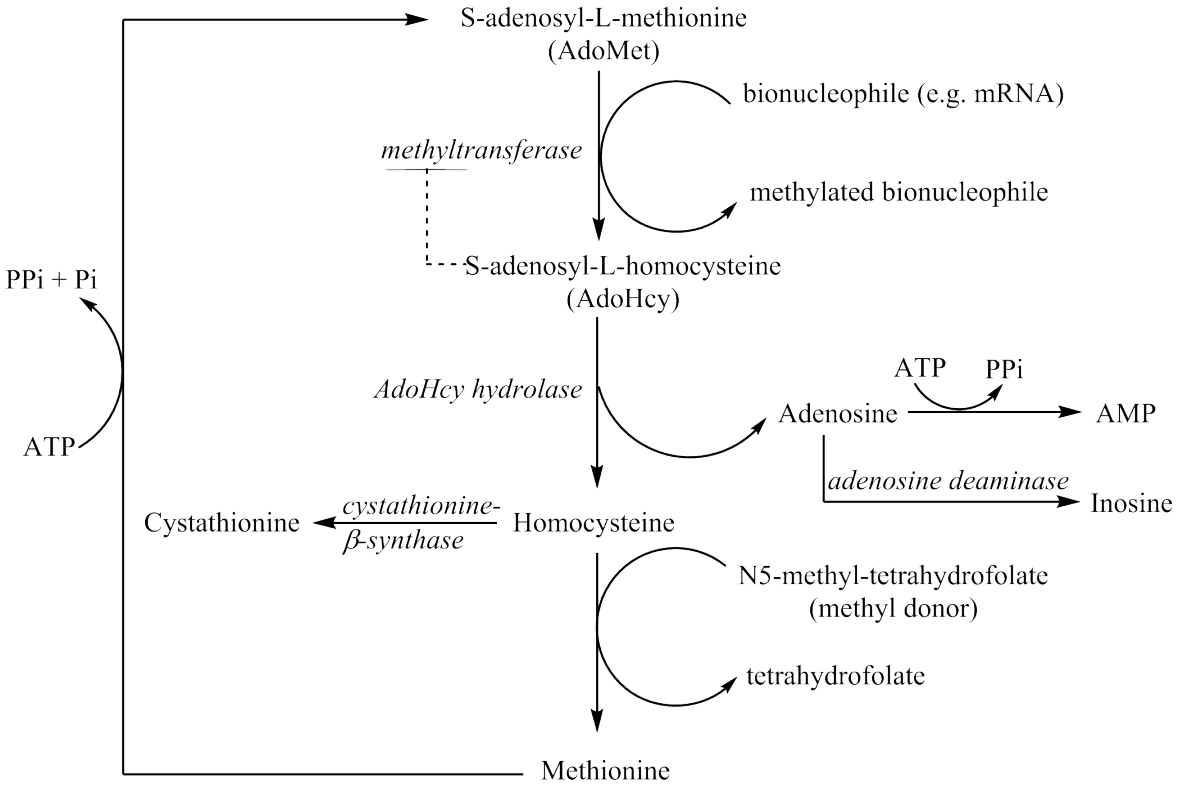
Figure 8: S-adenosyl-L-homocysteine (AdoHcy)

Methylation is discontinued as long as high levels of SAH are present. SAH is broken down by the enzyme S-adenosyl-L-homocysteine-hydrolase (AdoHcy hydrolase, SAH hydrolase) into adenosine and homocysteine.



Scheme 4: AdoMet induced methylation of mRNA

This SAH hydrolase catalyzed reaction takes place under a chemical equilibrium, which is shifted toward the products' side. This is a result of further metabolism of adenosine (e.g., to ATP and inosine) and homocysteine (e.g., to methionine and cystathionine), which causes a shift of the rate toward the products according to LeChatelier's principle. This ensures the reaction in the hydrolytic direction (i.e. the direction of SAH hydrolase catalyzed decomposition), thus driving the metabolism of SAH. Biofeedback-inhibition of the methyltransferase is seized as SAH is removed from the cell. As a result of this, mRNA capping and, with it, protein synthesis can eventually resume. The larger picture of the biosynthesis and metabolism of SAH is illustrated in Scheme 5 below.



Scheme 5: Biosynthesis and metabolism of AdoMet

Carbocyclic adenosine analogs mimic adenosine but, unlike adenosine, are not readily catabolized and removed from the equilibrium. Consequently, when they are present in high concentration the equilibrium of the process that is catalyzed by SAH hydrolase is shifted toward the reactants. This elevates the intracellular levels of SAH, thereby inactivating the methyltransferase via bio-feedback.

It is noteworthy that the simultaneous decrease of homocysteine levels is not responsible for the antiviral activity and exogenous addition of homocysteine does not counteract the antiviral activity but potentiates it. Increased cellular levels of SAH, however, have been shown to correlate with antiviral activity.[30] The consequence of inhibition of the biomethylation process is the inhibition of mRNA 5'-capping methylation, as a result of which viral mRNA is rendered incomplete and hence degraded by nucleases. Without active mRNA, viral protein synthesis is inhibited and viral replication comes to a halt. Inhibitors of SAH hydrolase therefore are important targets for broad-spectrum antiviral agents and are the focus of this dissertation research.

The SAH hydrolase pathway constitutes the only known mechanism for SAH catabolism in eukaryotes.[30] No known viral genomes code for SAH hydrolase, however many viruses that are affected by its inhibition express their own 5'-cap mRNA methyltransferases (e.g. vaccinia, reovirus).[30] This allows for possibly selective inhibition of viral methyltransferases, while leaving cellular mRNA unaffected. Even in the scenario that cellular methyltransferases are also inhibited, which would lead to a general suppression of protein synthesis, this would be a lot more detrimental for the virus than for the host. Virus infected cells have an increased rate of protein synthesis and are more sensitive to changes in SAH levels. Moreover, the host cell has a large supply of proteins

already synthesized and is, unlike the virus, not immediately affected by a shortage of proteins.[30]

While Ari is known to exert antiviral activity only via AdoHcy hydrolase inhibition, NpcA is thought to act in one additional way.[31] NpcA is transformed into S-neplanocylmethionine (NpcMet), which is believed to inhibit viral methyltransferases. The toxicity that is exerted by both Ari and NpcA arises because, like adenosine, they are phosphorylated on their 5'-positions.[30] Adenosine kinase, adenylate kinase and nucleoside diphosphate kinase, respectively, transform them to their corresponding monophosphate, diphosphate and triphosphates (Figure 9). Due to the close resemblance of these triphosphates to the structure of adenosine triphosphate (ATP), carbocyclic ATP-like metabolites get involved in numerous biological processes in which ATP plays a role, and they may even be incorporated into RNA. The results can be detrimental for the cell.

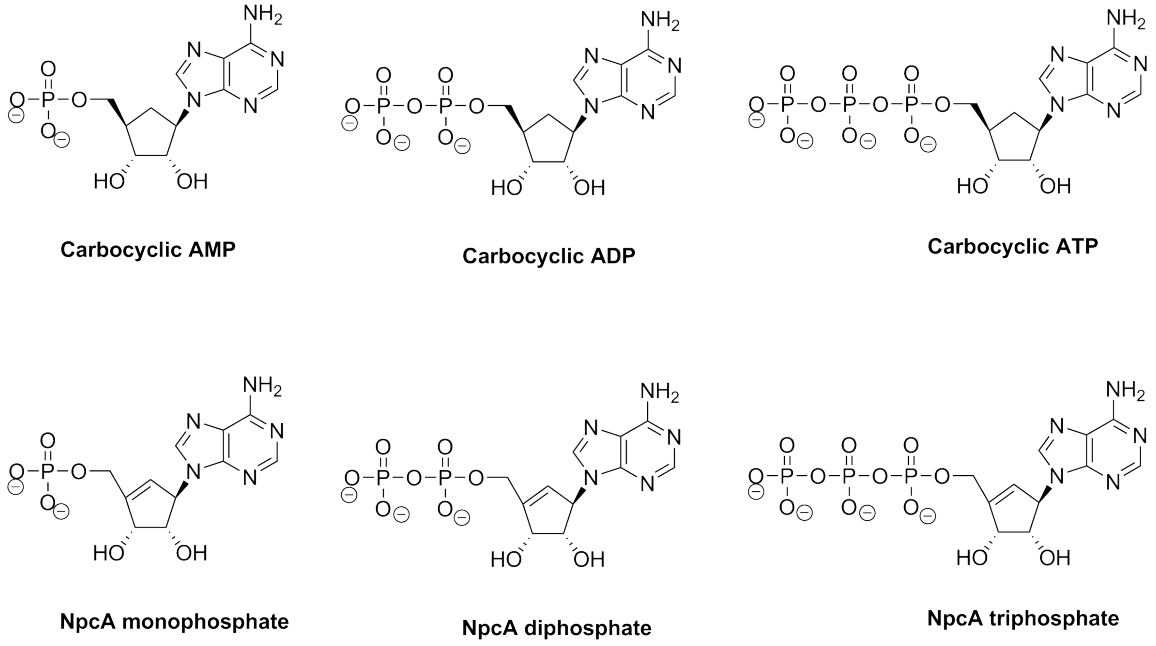
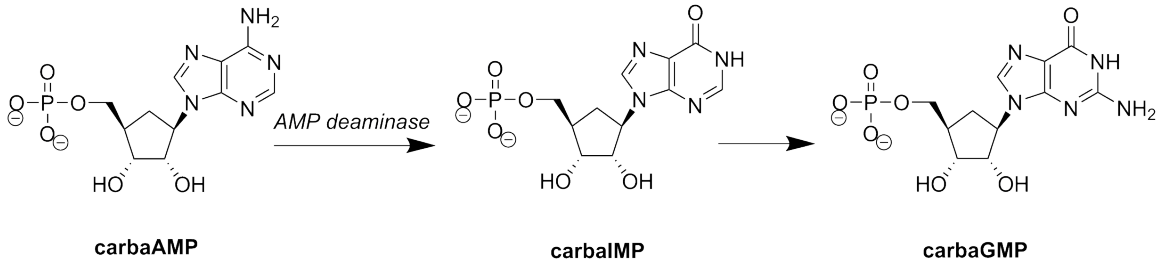


Figure 9: Phosphate derivatives of carbocyclic adenosine derivatives Ari and NpcA

The carbocyclic adenosine monophosphate (AMP)-like metabolites may also add to cell toxicity. AMP deaminase can show affinity toward the monophosphate of Ari, which is carbocyclic AMP (carbaAMP), and convert it to the corresponding inosine monophosphate (carbaIMP) and subsequently into the carbocyclic guanosine monophosphate (carbaGMP) in amounts that compare to the natural GTP pool (Scheme 6).



Scheme 6: Monophosphate metabolites of Ari

Carba GMP acts as an inhibitor of hypoxanthine-guanine-phosphoribosyltransferase (HGPRTase), an enzyme which plays a role in the purine salvage pathway. Deficiencies in HGPRTase cause gout, a painful condition resulting from the deposition of uric acid in the joints.

In comparison, no conversion of NpcA into the corresponding carbocyclic analogs of guanine nucleotides has been detected, which means that the monophosphate of NpcA is a poor substrate for AMP deaminase.[31]

F. Target Design and Reduction of Toxicity

It is desirable to design Ari and NpcA analogues which retain their antiviral activity but with reduced toxicity compared to the parent compounds. Because the main cause for toxicity is 5'-phosphorylation by adenosine kinase, it is necessary to design targets, which are not easily accessed at the 5'-position by cellular kinases.

It is known that 3-deaza-adenosine is neither phosphorylated nor is it a substrate of adenosine deaminase. With this in mind 3-deazaneplanocin A (Figure 10) was synthesized in 1989 by Marquez et al.[32] It was found that this target had antiviral activity against several viruses including vesicular stomatitis virus (VSV), parainfluenza virus type 3, yellow fever and vaccinia virus while displaying reduced toxicity compared to Npc A. It is not fully understood why the removal of the heterocyclic ring hydrogen at the 3- position prevents phosphorylation.[30]

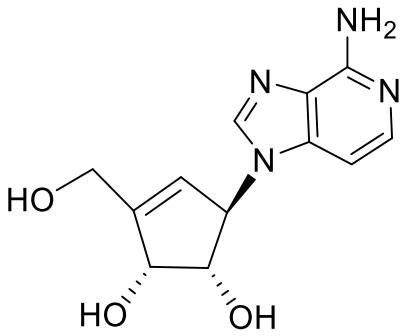


Figure 10: 3-Deazaneplanocin A[32]

Other approaches to reducing toxicity have been in form of truncated Ari and NpcA derivatives. For example, the removal of the 4'-hydroxymethyl group (compounds DHCaA and DHCeA) as well as their 3-deaza analogues (compounds c^3-DHCaA and c^3-

DHCeA), as synthesized by Borchard et al.[100-103] or the 5'-deoxy analogue of Ari as well as the exo-chain shortened derivative lacking the methylene group at the 4'-position (5'-noraristeromycin) synthesized by Schneller et al.[104] (Figure 11) have shown promise in this regard. These compounds were all shown to retain their antiviral activity, while displaying less associated toxicity.[33]

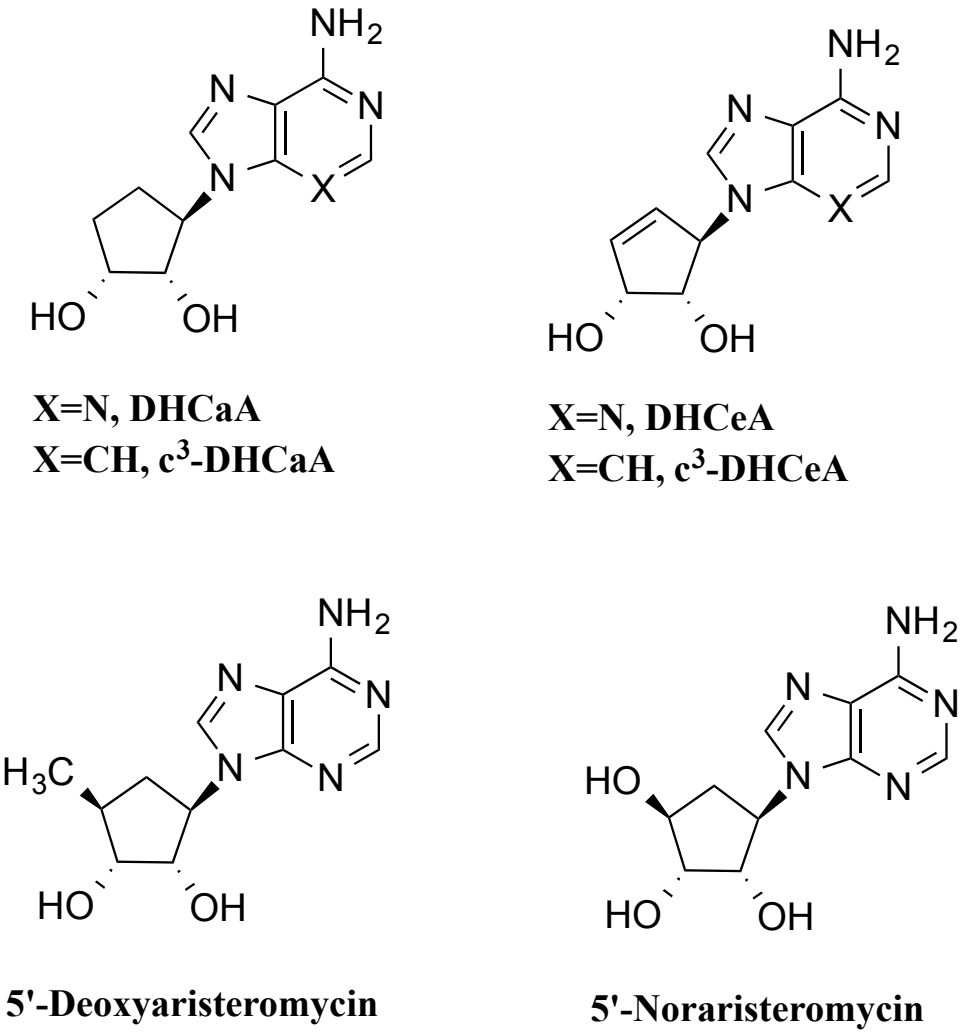
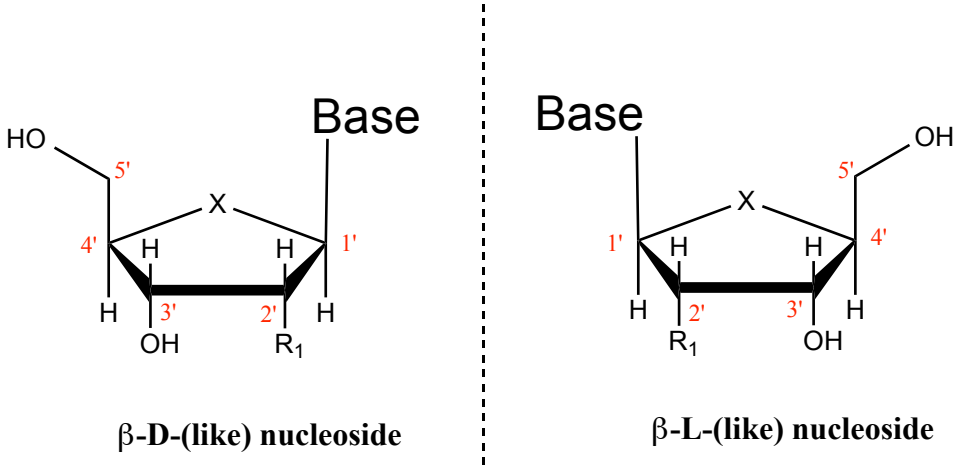


Figure 11: Truncated Ari and NpcA derivatives

G. Significance of Stereochemistry in Target Design

Stereochemistry is a very important concept in drug design. The natural world is three dimensional in the macroscopic sense as much as at the molecular level. The enzymes that the drug targets interact with are stereoselective toward their substrates and binding affinities can differ vastly for diastereomers or for enantiomers.[34,35] The differences can be so dramatic that one stereoisomer might not only be less active as a drug but even toxic to the host.[35] It is therefore very important to consider stereochemistry when designing small molecules to affect these enzymes. The stereochemistry of nucleosides is illustrated in Figure 12.



β-D-(like) nucleoside **β-L-(like) nucleoside**

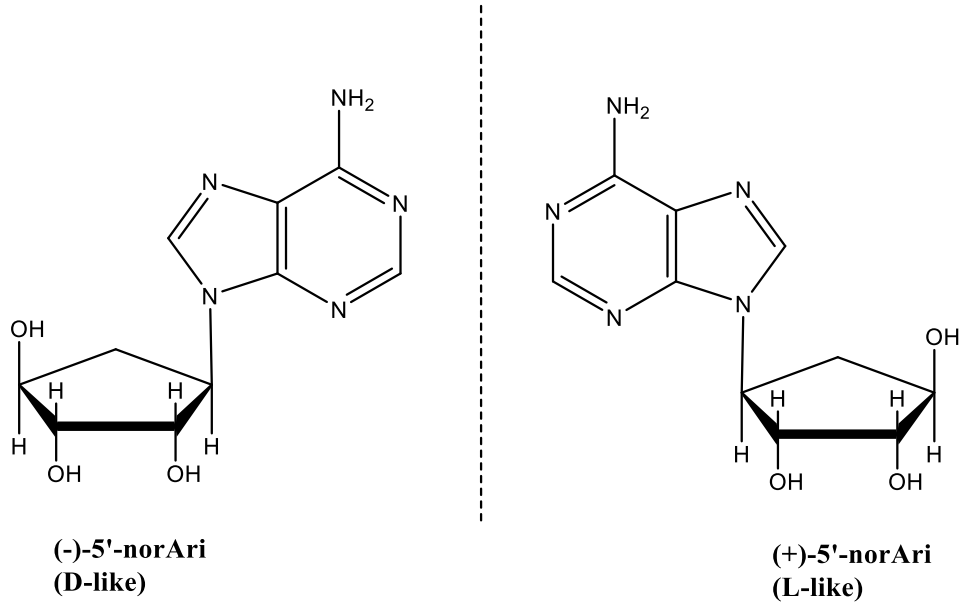
X = O for Ribose-based nucleosides
X = C for carbocyclic nucleosides
R_1 = H for DNA nucleosides
R_1 = OH for RNA nucleosides

Figure 12: Stereochemistry of nucleosides

In nature, nucleosides, because they contain D-carbohydrates, occur in the D-form and only very rarely are found in the L-form. In the D-form the glycosidic bond, which is located on the 1'-position and binds the sugar to the base, is designated to be in the β-configuration and the heterocyclic base is in a *cis*-relationship to the hydroxymethyl group of the sugar on the 4'- position. The enantiomers are called L-nucleosides. Carbocyclic nuleosides, which do not contain carbohydrates, are referred to as either D-like or L-like.

Contrary to long-standing belief that only nucleoside analogues possessing the natural D-configuration could interact with enzymes in living systems, possession of natural stereochemistry is not an excluding criterion for the exhibition of antiviral activity.[36] 3TC, mentioned previously and shown in Figure 5, is an L-nucleoside and yet one of the most potent anti HIV and anti-Hepatitis B drugs at this time.[37,38] Since the discovery of 3TC, many other L-nucleosides exhibiting high antiviral activities have been found, indicating that L-nucleoside analogues may possess antiviral activity comparable to or even greater than their D-counterparts, may have lower toxicity and a greater metabolic stability.[36]

An example from the realm of carbocyclic nucleosides are the D-like and L-like enantiomers of 5'-noraristeromycin, (-)-5'-norAri (D-like) and (+)-5'-norAri (L-like) respectively (Figure 13). When comparing their antiviral activities, it was found that, while the (+)-enantiomer shows selective activity against the hepatitis-B virus, the (-)-enantiomer is inactive.[39] On the other hand, the (-)-enantiomer possessed a 100-fold higher activity versus cytomegalosvirus (CMV) and is on average 10-fold more potent against an array of different viruses.[40]

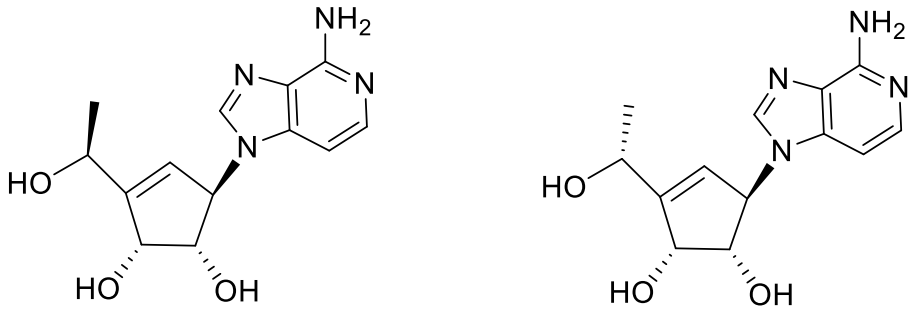


(-)-5'-norAri (D-like)

(+)-5'-norAri (L-like)

Figure 13: D- and L-like enantiomers of 5'-Noraristeromycin

The introduction of new stereocenters is another area of concern for nuclceosidic design as it may dramatically influence the interaction of a drug with an enzyme. For example (5'*R*)- and (5'*S*)-5'-methylneplanocin A, which, with the introduction of a new stereocenter at the 5'-position, can be considered conformationally restricted analogues of neplanocin A.



(5'*S*)-5'-methylneplanocin A **(5'*R*)-5'-methylneplanocin A**

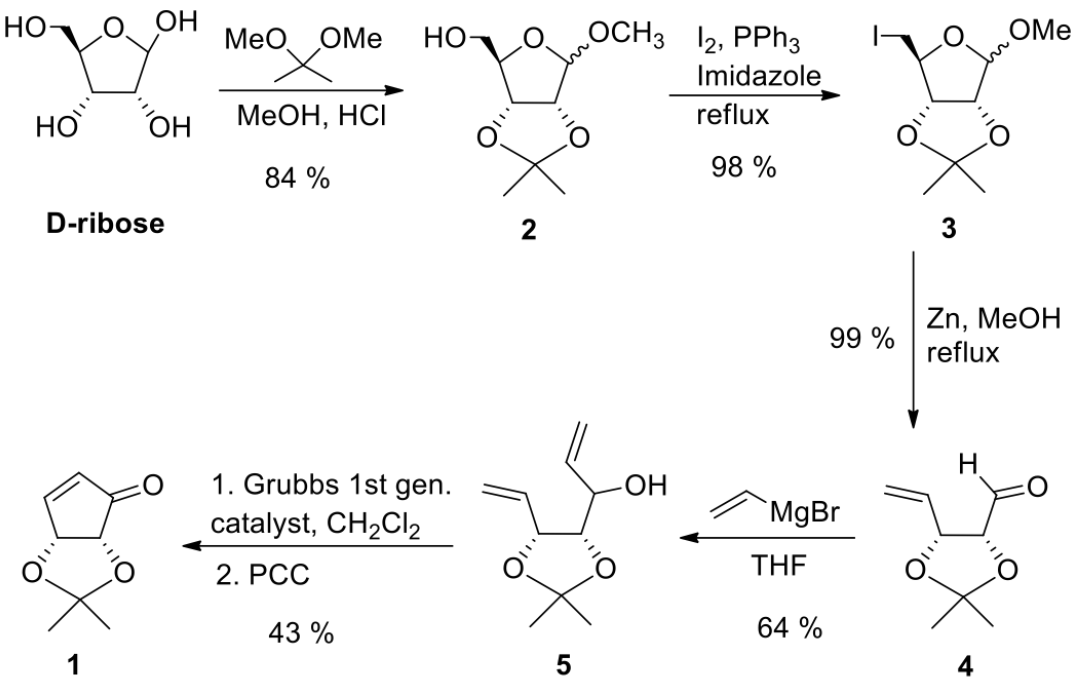
Figure 14: 5'-Methyl analogues of neplanocin A

The methyl group at the C-5' affects the interaction of these compounds with the enzymes SAH-hydrolase, adenosine deaminase and adenosine kinase. In fact, the *R*-isomer was found to be a promising broad-spectrum antiviral drug candidate with activity versus pox-, paramyxo-, arena-, rhabdo-, reo-, and cytomegalovirus, while the *S*-isomer was inactive.[41]

CHAPTER 1

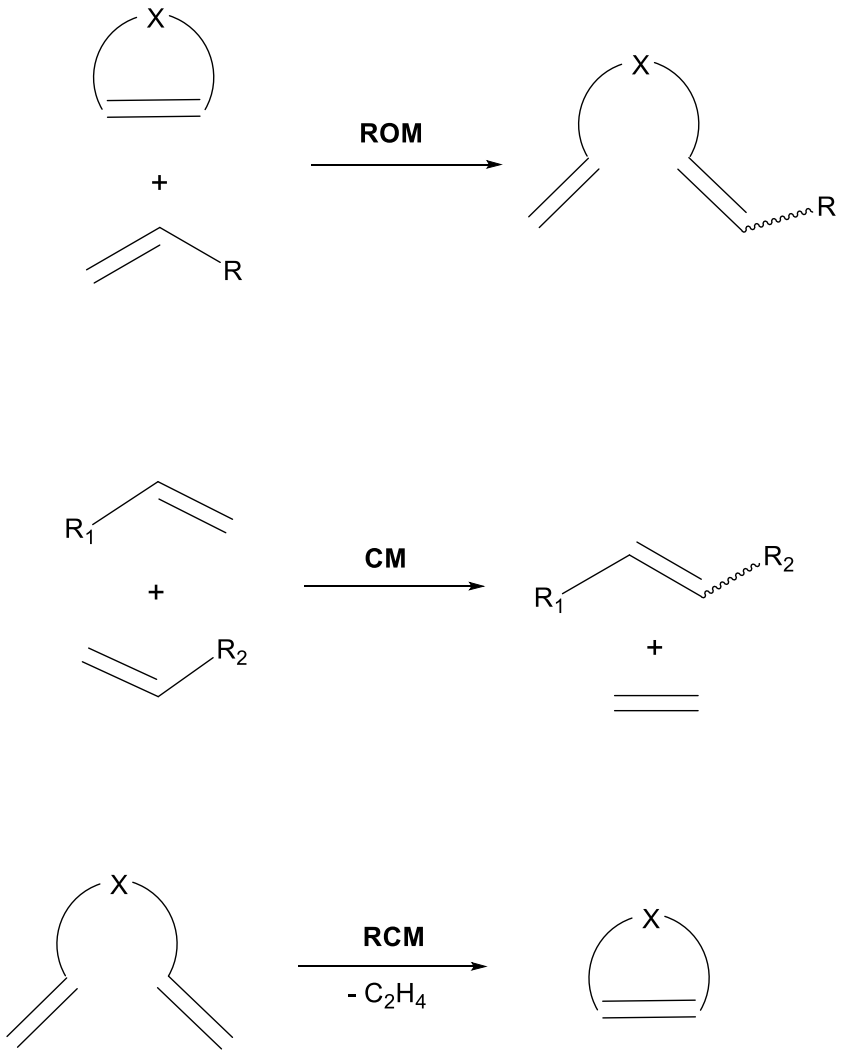
SYNTHESIS OF IMPORTANT PRECURSORS

A very important precursor for the synthesis of aristeromycin analogs is the cyclopentenone **1** shown below in Scheme 7. It has recently become accessible in a short and optimized 5-step synthesis[42] starting from the inexpensive and readily available D-ribose, which already contains the correct stereochemistry at the 2'- and 3'- positions of eventual carbocyclic nucleosides. The free hydroxyl groups at those positions were protected as an acetonide, by reaction of D-ribose with 2,2-dimethoxypropane in methanolic hydrochloric acid. The anomeric hydroxyl group at the C-1-position was methylated at the same time to afford isopropylidine ketal derivative **2**. In a variation of the Appel reaction,[43-45] molecular iodine and triphenylphosphine transformed the primary hydroxyl group of **2** into iodide **3**. Subsequent transformation into the relatively volatile aldehyde **4** was achieved by Boord elimination (Boord olefin synthesis) in a reductive cleavage induced by the reaction with zinc powder in methanol under reflux.[46-48] Due to its volatility **4** was immediately treated with vinylmagnesium bromide via a 1,2-addition of the vinyl group to afford diene **5**. A ring-closing metathesis using Grubbs 1st generation catalyst resulted in the formation of a cyclopentenol intermediate, which was not isolated but directly converted into cyclopentenone derivative **1** by pyridinium chlorochromate (PCC) oxidation. This important precursor was synthesized in an overall 22% yield with the only limiting factor being the cost of the Grubbs catalyst. Prior to 2013 compound **1** was prepared in our laboratory. Now it is commercially available.



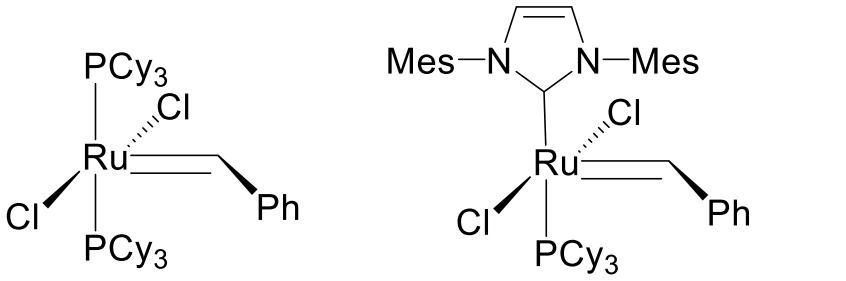
Scheme 7: Synthesis of cyclopentenone 1

The employment of the Grubbs catalyst is of great importance for the success of the synthesis of precursor **1** and a few comments about the catalyst as well as the reaction mechanism are in order. Olefin metathesis is the metal-catalyzed redistribution of carbon-carbon double bonds and was first defined in 1967.[49] There are a variety of applications including but not limited to ring-opening metathesis (ROM), crossmetathesis (CM or XMET) and ring-closing metathesis (RCM).[50] These different reaction varieties are shown in Scheme 8. While many different metal catalysts have been investigated in the decades following the discovery of these reactions, ruthenium-based catalysts have been of particular interest due to their high activities and stabilities as well as their broad functional group tolerance.[50] Compared to other metal catalysts, such as titanium, molybdenum, or tungsten, it has been shown that ruthenium-based Grubbs' catalysts have the highest specificities towards reaction with olefins.[51]



Scheme 8: Select varieties of Olefin Metathesis reactions

Two generations of the Grubbs' catalyst exist (Figure 15). The 1st generation catalyst predates the 2nd generation catalyst and is less active and also less stable towards air and moisture. Both are commercially available ruthenium carbene complexes but with different ligands. The 2nd generation carries N-heterocyclic carbene (NHC) ligands in place of one of the phosphine ligands. NHC ligands are phosphine mimics that are more electron-donating than most phosphines and are responsible for the higher activity of the 2nd generation catalyst and analogs thereof.[52]



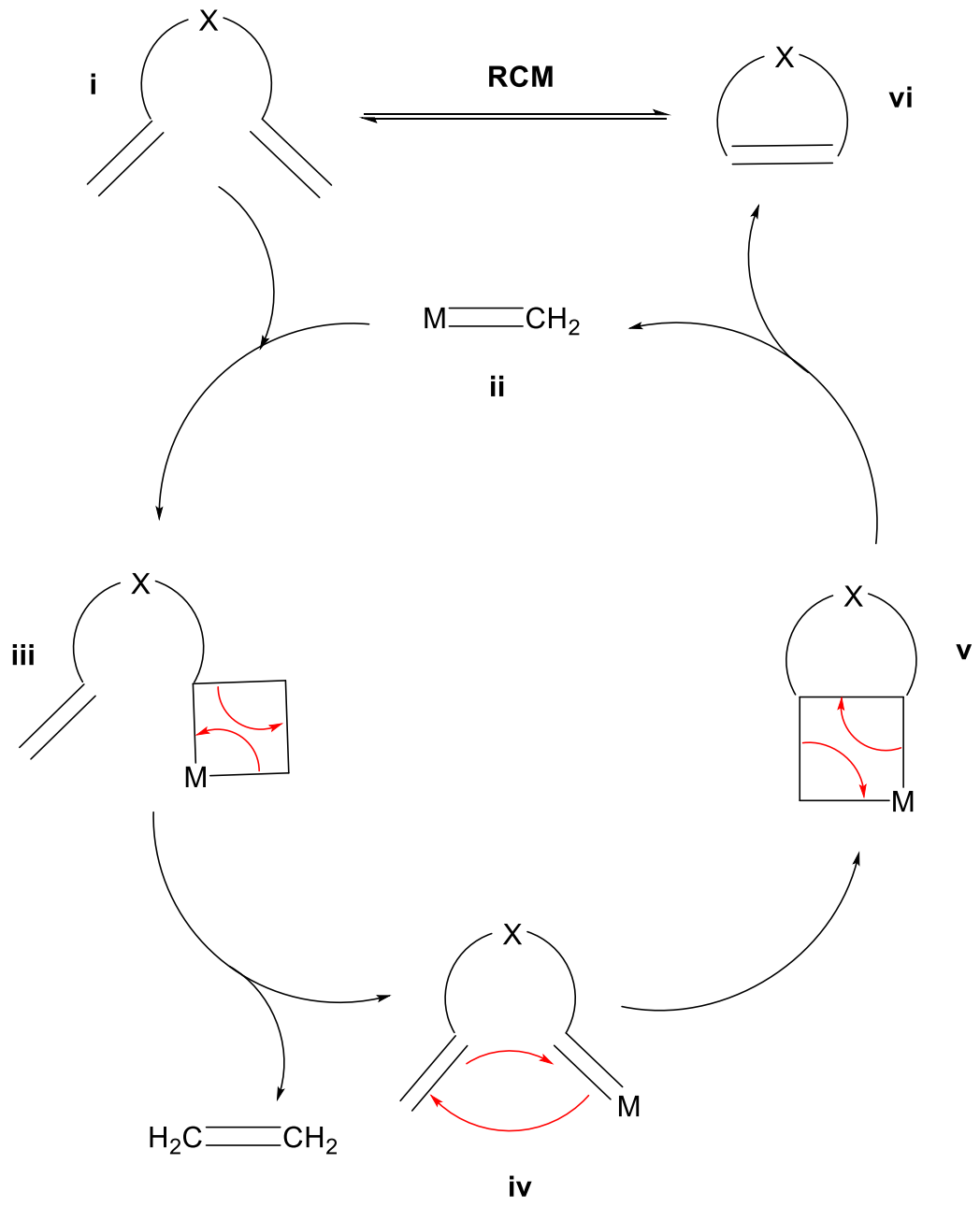
1st generation

2nd generation

Cy = cyclohexyl
Mes = mesityl ($C_6H_2Me_3$)

Figure 15: Structure of Grubbs Catalysts

The conversion of diene **5** to cyclopentenone **1** involves a RCM reaction. Many different reaction mechanisms have been proposed for the olefin metathesis reactions, but the one that has been most consistent with experimental evidence has been the one developed by Chauvin.[53,54] This mechanism, generalized for different metals, is shown in Scheme 9 for the case of an intramolecular diene forming a cyclic olefin as a reaction product. The starting point of the mechanism is the [2+2] cycloaddition of a metal carbene (ii) (for example the Grubbs catalyst) with a diene (i) resulting in the formation of a metallocyclobutane intermediate (iii). This is followed by a retro [2+2] cycloaddition, during which olefin is released and a metal carbene (iv) is formed. Subsequently, a second [2+2] cycloaddition takes place forming a second metallocyclobutane (v). Finally, a second [2+2] retro cycloaddition results in the formation of the new olefin product (vi) and the release of the recycled metal carbene catalyst (ii).

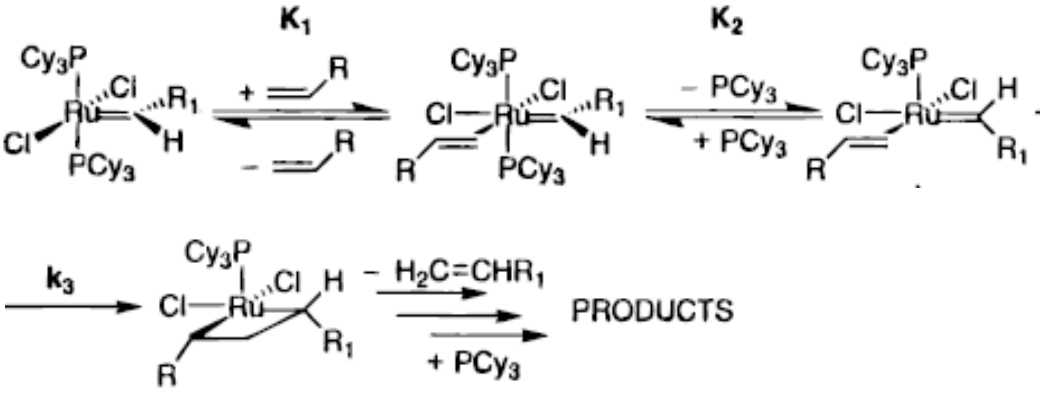


Scheme 9: RCM mechanism according to Chauvin[53]

Specifically for the case of the ruthenium based Grubbs catalyst mechanistic studies have been conducted and have shown that predominately a dissociative mechanism is at work, whereupon binding of the olefin, a phosphine ligand is displaced

from the metal center to form a 14-electron olefin complex. Metathesis then yields the (cyclic) olefin product and the catalyst is reformed upon recoordination of the phosphine ligand (Scheme 10).[52,55] In the course of the mechanism the carbene olefin complex is transformed into a metallacycle, which involves the oxidation of the metal center. It was found that the more basic the second phosphine ligand (the one that doesn't get displaced), the higher the activity of the catalyst as a result of better stabilization of the metallacycle-intermediate.[52] Moreover, the tricyclohexyl-phosphine ligand was shown to be the best suited phosphine ligand, because less bulky phosphines coordinated too strongly and could not be displaced to initiate the reaction, while phosphines with larger groups than cyclohexyl, were shown to be too labile to form stable complexes.[56] The substitution of one of the phosphine ligands with an N-heterocyclic carbene ligand, as is the case in the 2nd generation Grubbs catalyst, improves on activity and stability of the catalyst. [57,58]

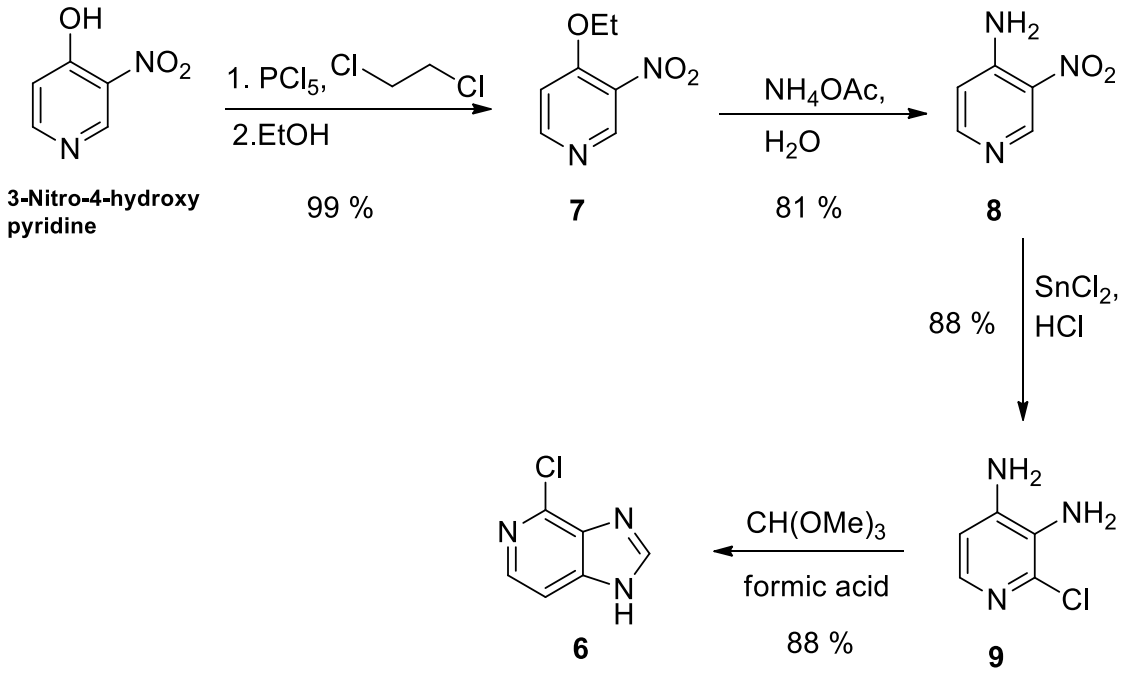
The NHC provides a strong electron donor, which stabilizes the metallacycle more efficiently than most phosphine donors. The remaining labile phosphine ligand provides the leaving group to form the required 14-electron intermediate species.[56]



Scheme 10: Mechanism of ruthenium based olefin metathesis[52]

The second important precursor for this dissertation research is the heterocyclic base **6**. This compound is not commercially available and only a few synthetic routes are available in the literature, which are overall low in yield. For overcoming this an effective synthesis was developed by combining the most effective literature synthetic steps.[59] This is illustrated in Scheme 11.

The synthesis began with the reaction of commercially available 3-nitro-4-hydroxypyridine with phosphorus pentachloride followed by reaction with ethanol in a nucleophilic hetero-aromatic substitution reaction to give compound **7** in quantitative yield. Reaction with aqueous ammonium acetate resulted in a second nucleophilic aromatic substitution in which the ethoxy group was replaced by an amino function to afford **8**. Treating **8** with stannous chloride in hot hydrochloric acid afforded reduction of the nitro group to an amino substituent and, at the same time, introduced a chlorine at the 2-position of the pyridine ring to produce **9**.[60] Finally, treatment of **9** with trimethyl orthoformate and formic acid resulted in a cyclization reaction to give the precursor 6-chloro-3-deazapurine (**6**).[61]

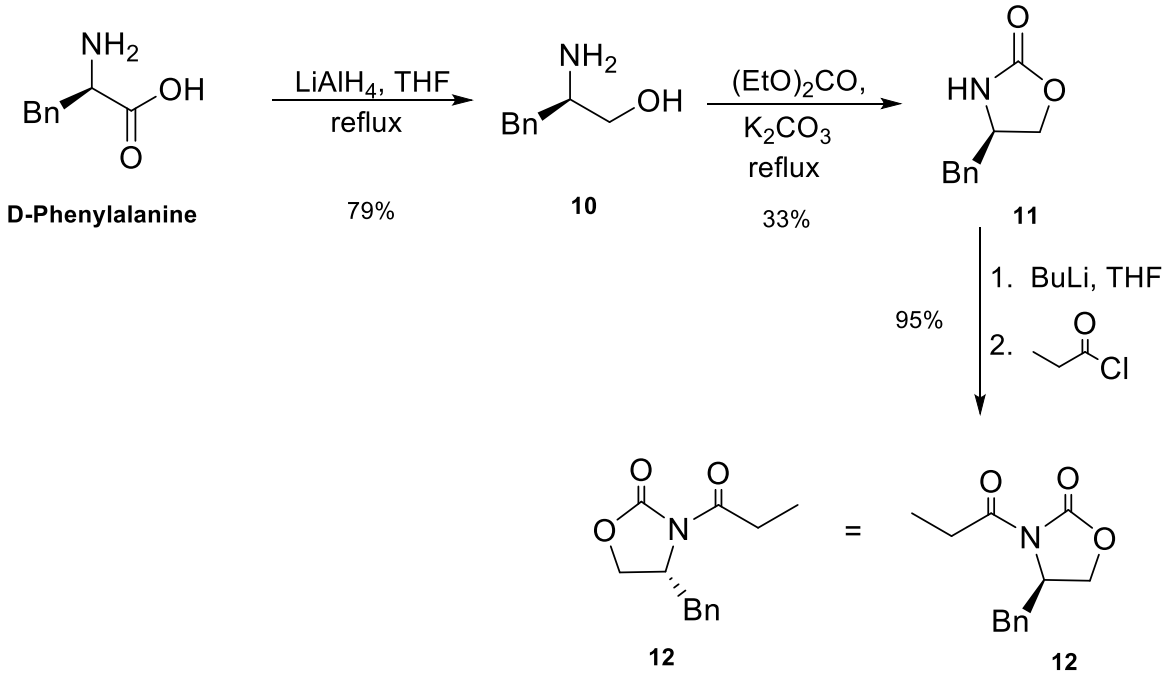


Scheme 11: Synthesis of 6-chloro-3-deazapurine

Another important precursor for this project was an oxazolidinone-based chiral Evans auxiliary such as **12** (Scheme 12). This type of auxiliary is employed in asymmetric syntheses for its exceptional control in the stereoselective formation of carbon-carbon bonds. Auxiliaries such as **12** are used for many synthetic reactions requiring stereoselectivity, including alkylation reactions,[62] aldol reactions,[63] Michael additions[64,65] (conjugate additions) and pericyclic reactions.[66]

The synthesis of this auxiliary **12** began with the reduction of the natural amino acid D-phenylalanine into D-phenylalaninol (**10**) by means of lithium aluminum hydride (LAH).[67] Compound **10** was then submitted to a cyclization reaction with diethyl carbonate under reflux to yield oxazolidinone **11**.[68,69] Subsequent reaction with

n-butyllithium (n-BuLi) at low temperature formed the N-lithiated **11**, which was trapped by subsequent treatment with propionyl chloride, resulting in the finished auxillary **12**.[70]

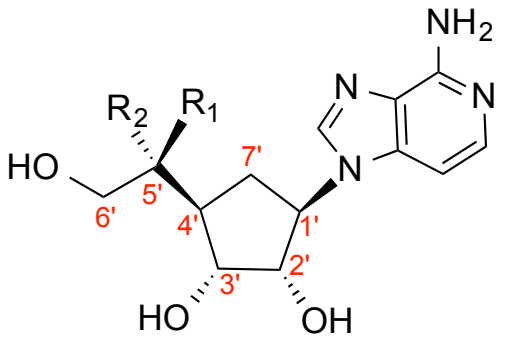


Scheme 12: Synthesis of chiral Evans auxiliary 12

CHAPTER 2

ATTEMPTED SYNTHESIS OF (5'*S*)-5'-METHYL-3-DEAZAHOMOARISTEROMYCIN

The design of an antiviral drug with reduced toxicity led to the structure of two 5'-methyl substituted diastereomers, (5'*S*)- 5'-methyl-3-deazahomoaristeromycin (**13**) and (5'*R*)- 5'-methyl-3-deazahomoaristeromycin (**14**) shown below in Figure 16.

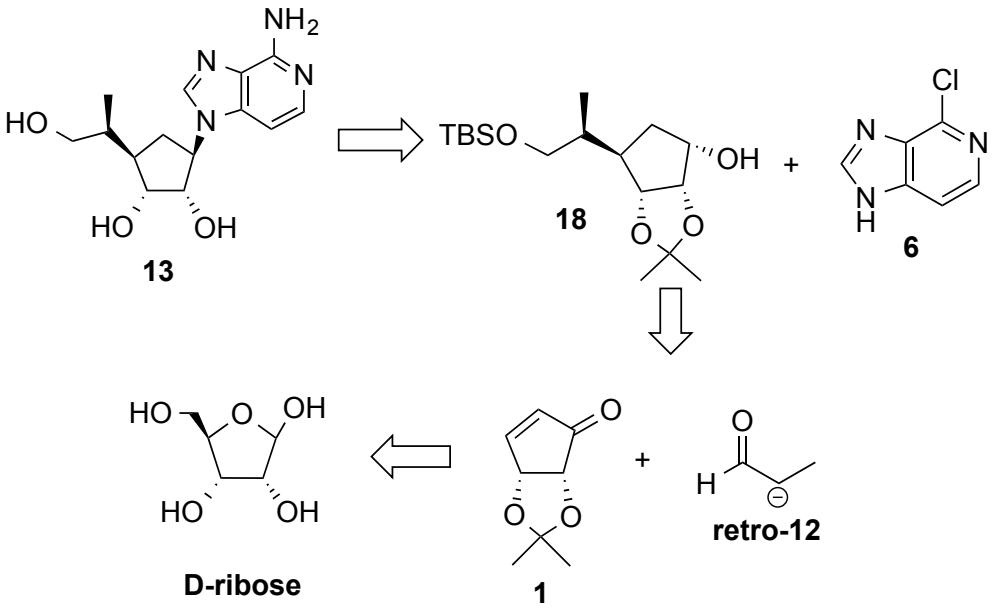


13 R_1=Me; R_2=H
14 R_1=H; R_2=Me

Figure 16: Structure of two epimeric 5'-methyl substituted target compounds

This structure has three features which could result in a reduction of toxicity. First, the extension of the OH group to the 6'-position, has been shown[71,72] to reduce toxicity as a result of being less recognizable to the undesirable cellular kinases. Second, the inclusion of a methyl group on the 5'-position, should enhance the first feature by adding steric hindrance at the docking site of the kinases. Third, the incorporation of a 3-deaza purine base is expected[12] to reduce toxicity. This chapter deals with the synthesis of the *S*-diastereomer **13**; the next chapter will present the synthesis of the *R*-diastereomer **14**.

Retrosynthetically, the target compound can be disconnected at the bond connecting the heterocyclic base to the cyclopentyl moiety (Scheme 13). From there, the resulting cyclopentyl structure can be derived from a conjugate addition of a synthon propionaldehyde to cyclopentenone **1** that was described in the previous chapter.



Scheme 13: Retrosynthesis of the *S*-diastereomer 13

In the forward direction of the synthesis, the conjugate addition was foreseen as calling on a chiral auxiliary in place of propionaldehyde to ensure that the stereogenic centers created as a result of this addition are controlled. Chiral auxiliaries have played a key role in asymmetric synthesis, enabling the stereoselective formation of stereocenters.[73-75]

In 1991 Evans[64] reported the stereoselective Michael reaction between chlorotitanium enolates that are part of chiral N-acyl-oxazolidinones (for an example see **12**) with α,β-unsaturated carbonyl compounds. These reactions were often highly

diastereoselective and took place with high yields. But when attempting such a Michael reaction with cyclohexenone, it was found that the reaction took place without stereoselectivity, likely due to the presence of two prochiral carbons.[64] On the other hand, for cyclopentenones, such as **1**, it was discovered that the conjugate addition afforded high diastereoselectivity when the reaction occurred with Z-titanium enolates from the oxazolidinones. The acetonide with **1** forms a bicyclic structure that sterically hinders the attack of the enolate nucleophile from one side.[64] Thus, the Michael reaction between cyclopentenone **1** and the chiral Evans auxiliary **12** resulted in the creation of two new stereogenic centers in the addition product **15**, as shown in Figure 17.

In stereoselective syntheses, such as this, the creation of new stereogenic centers is achieved by transformation of a prochiral group into a chiral one. There are two general ways of controlling the stereochemistry of a stereocenter that is formed in the course of a reaction: (1) by means of a chiral reagent or auxiliary, which is referred to as reagent control of stereoselectivity or (2) by means of asymmetric induction by stereocenters already present in the substrate, which is referred to as substrate control of diastereoselectivity.[76]

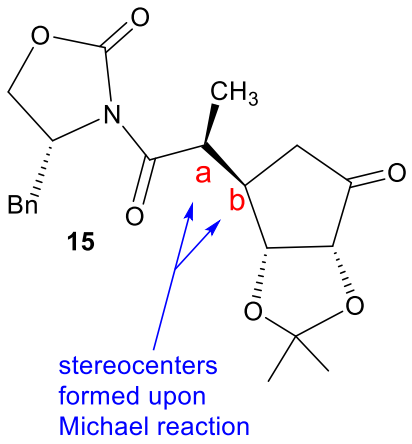
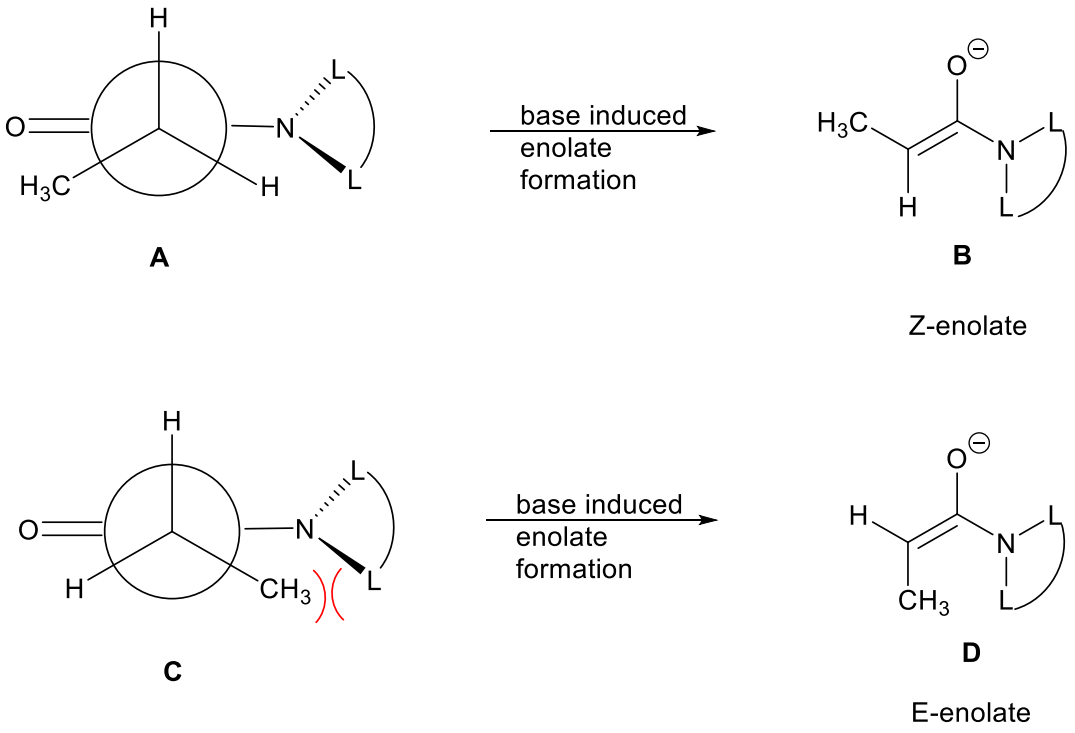


Figure 17: Stereocenters formed during Michael reaction of 1 with 12

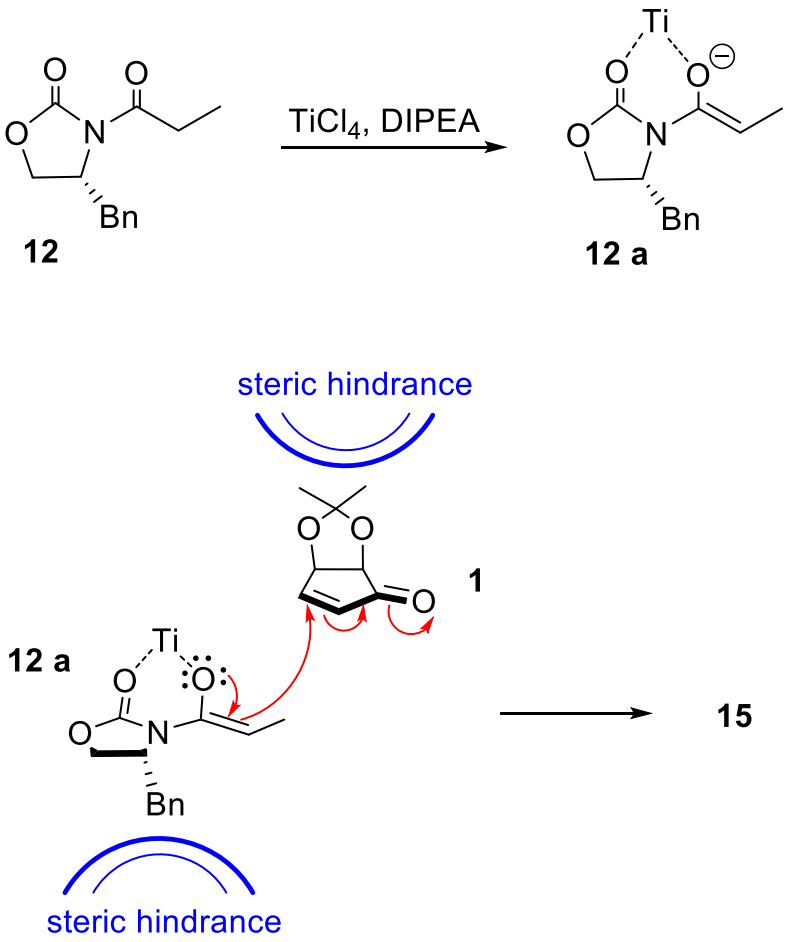
For the formation of compound **15** the stereochemistry of the stereocenter assigned as "a" in Figure 17 is controlled by the type of enolate that is formed (*E* vs *Z*) by the Michael donor and therefore reagent controlled. The other stereocenter assigned as "b" is controlled by the steric hindrance of the acetonide moiety on the Michael acceptor and is therefore substrate controlled.

The reaction initially involves the formation of a *Z*-titanium-enolate. The preference of the *Z*-enolate over the *E*-enolate is driven by 1,3- allylic-strain[76] and illustrated in Scheme 14. Conformer A, which doesn't have 1,3- allylic strain forms the *Z*-enolate (B) upon reaction with base. Conformer C, on the other hand, does have 1,3-allylic strain between the methyl group and alkyl-side-chain of the auxilliary. The resulting *E*-enolate upon reaction with base is therefore unfavored. The Z-preference is further reinforced by the presence of titanium, which prevents the rotation of the amide carbon-nitrogen bond by chelation to the oxyanion.



Scheme 14: Z-Enolate preference as a result of allylic 1,3 strain

A side-view of approach of the Michael donor to the Michael acceptor is illustrated in Scheme 15 (*vide infra*) as a proposed explanation of diastereoselectivity for the formation of **15.**

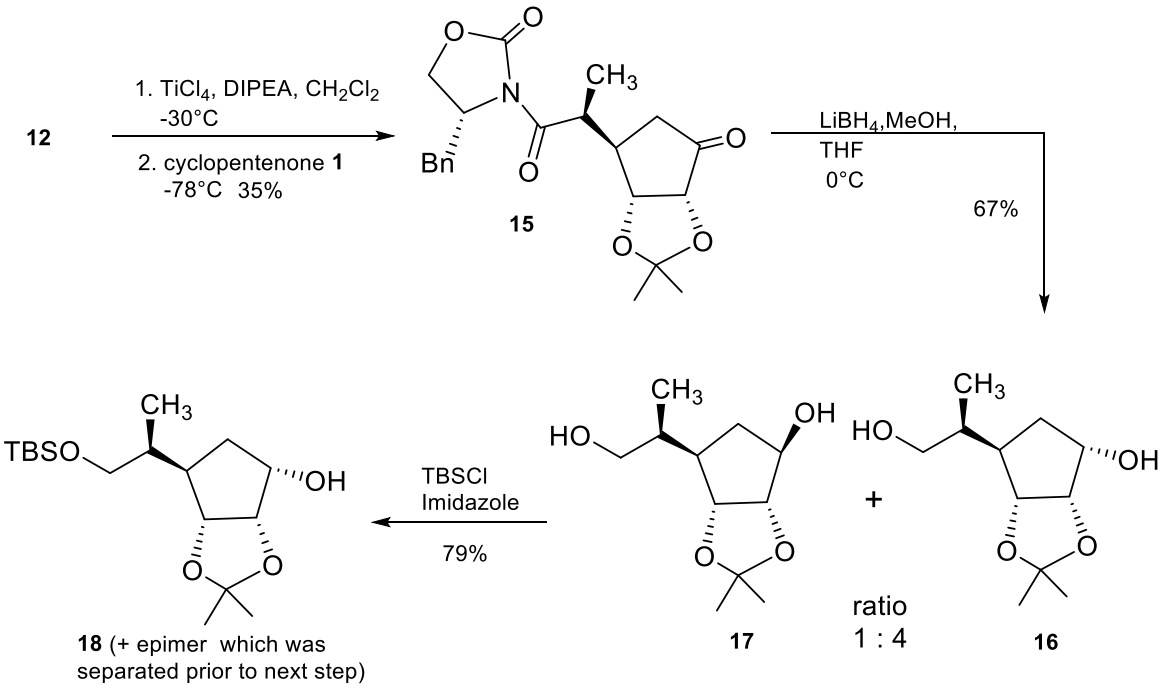


Scheme 15: Proposed explanation of diastereoselectivity for the formation of 15

Thus, when the Michael reaction was conducted, as shown below in Scheme 16, compound **15** was formed and was separated as a pure fraction in 35% yield. The yield for this same reaction had previously been reported at 45%.[77] However formation of epimer **27** was also observed. The ratio between **15** and **27** was determined to be 1:5 by separation, determination of mass and comparison of the molar yield ratios.

In the following step, the reduction of the amide bond of the acyl-oxazolidinone was carried out with an unusual reducing agent, lithium borohydride, which had been shown by my coworkers in the Schneller laboratory to be more efficient than

di*iso*butylaluminum hydride (DIBAL) or lithium aluminum hydride (LAH) for the same reaction.[77] The ketone of the cyclopentenone was also reduced in this step to the secondary alcohol. Nucleophilic attack of hydride was sterically blocked by the acetonide on the bottom face of **15**, causing the hydride to attack preferentially from the top (the Re-face). After reduction diols **16** and **17** were formed in a ratio of 1:4. This was determined by separation followed by determination of mass and comparison of the molar yield ratios. These compounds were difficult, but not impossible to separate chromatographically. This was overcome by carrying the epimers forward in the next step in the synthesis and separating them thereafter. Thus, the mixture of **16** and **17** was reacted with silylating agent *tert*-butyldimethylsilyl chloride (TBSCl), which afforded selective protection of the primary alcohol over the secondary. The TBS protected compound **18** could now be isolated from its epimer with more ease.

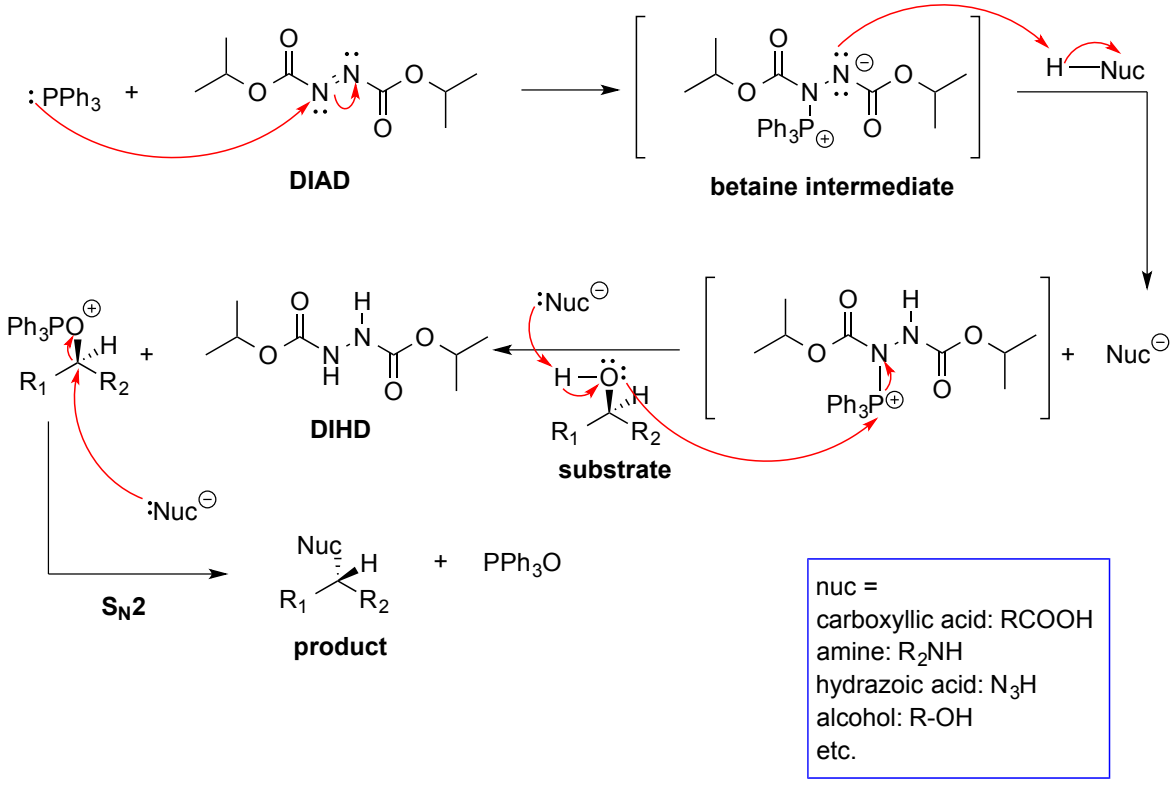


Scheme 16: Michael reaction and following conversions to compound 18

The next step in the synthesis involved the coupling of cyclopentanol derivative **18** with the purine derivative **6**, which were synthesized in separate convergent routes as previously described. In considering how this might be done, direct S_N2 displacements for this type of coupling, involving the sodium salt of the purine base and a cyclopentyl tosylate derivative, have been shown in the literature to be low yielding. On the other hand, the Mitsunobu reaction has been shown to significantly improve the efficiency for this reaction, with the use of an appropriate cyclopentyl derivative and heterocyclic base.

In 1967, Oyo Mitsunobu reported the preparation of esters of carboxylic acids *via* quaternary phosphonium salts. It was found that carboxylic acids reacted with triphenyl phosphine and diethyl azodicarboxylate in the presence of an alcohol to form the corresponding esters of the carboxylic acid as well as triphenylphosphine oxide, and diethyl hydrazodicarboxylate as byproducts.[78] Subsequently the Mitsunobu reaction was found to be a highly stereoselective S_N2-like reaction, which can be carried out under mild conditions, resulting in the inversion of the stereocenter of a secondary alcohol component. A primary or secondary alcohol can be converted into many different functional groups including esters, ethers or amines by the Mitsunobu process. The reaction is very versatile as a wide range of functional groups are tolerated by the reaction and many different nucleophiles can be employed. A drawback is that the removal of the triphenylphosphine oxide byproduct can sometimes be problematic. The complexity of the reaction mechanism and the formation and role of the intermediates has been subject to debate.[78-82] The course of the generally accepted reaction mechanism for a secondary alcohol substrate is shown in Scheme 17.

Diethylazodicarboxylate (DEAD) or the more stable analogue di*iso*propylazodicarboxylate (DIAD) readily react with triphenylphosphine to generate a phosphonium intermediate called betaine. The nucleophile of choice is then deprotonated by the betaine intermediate and, in turn, deprotonates the alcohol of the substrate into an activated alkoxide. Making use of the affinity of phosphorus for oxygen, this alkoxide intermediate binds to the triphenylphosphine group of the betaine, thereby converting it into a good leaving group. The nucleophile can then displace the triphenylphosphine oxide with a back-side attack and inversion of stereochemistry. Byproducts of the reaction are triphenylphosphine oxide and, when DIAD is used, di*iso*propyl hydrazodicarboxylate (DIHD).



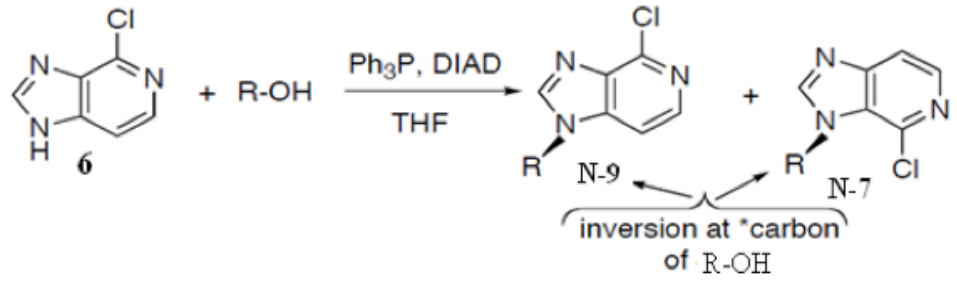
Scheme 17: General Mitsunobu mechanism for a secondary alcohol substrate[82]

The Mitsunobu reaction of 6-chloro-3-deazapurine (**6**), which is much less reactive and also less soluble than 6-chloroadenine for the Mitsunobu reaction, can be expected to need longer reaction times and have much lower yields. The Mitsunobu reaction for **6** has been refined in the Schneller lab for reaction with various different cyclopentanols (Table 2).[83] The heterocyclic purine base has two ring nitrogens, N-7 and N-9, which are capable of acting as a nucleophile, with the N-9 being the desired regioisomer for this project. For these different substrates the ratio between N-9 and N-7 products were determined, with the structural assignments done by comparing the ^{1}H- and ^{13}C-NMR of the regioisomers to a prototype X-ray structure. Important for these structural assignments via ^{1}H-NMR is the signal of the proton on the carbon of the cyclopentyl moiety, which carries the heterocyclic ring. As a general trend, it was found that this proton signal was further downfield for the N-7 product compared to the N-9 product. Comparing the ^{13}C-NMRs it was found that a peak at 106 ppm is present for all N-9 products. This peak has not been assigned with full certainty but it is believed to belong to the C-8 of the heterocyclic ring (the carbon located between N-7 and N-9). In the respective N-7 product this peak disappears and instead appears shifted downfield to 115 ppm.[83]

For the planned synthesis of this project, there had been no previous investigations into the Mitsunobu reaction of cyclopentanol **18** with **6**. However, when **17** was reacted with the more common (non-deaza) 6-chloroadenine it was found that N-9 was the only product, and the formation of the N-7 product was not observed.[77]

The Mitsunobu reaction of **18** and **6** was conducted with triphenylphosphine and DIAD under reflux over a period of 5 days (Scheme 18). The product was formed only in

22% yield and X-ray structure at a later step in the synthesis (*vide infra*) showed that the N-7 isomer **19** had been formed. No trace of the N-9 isomer could be detected.



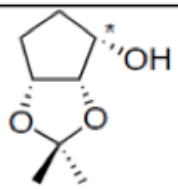
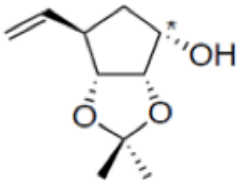
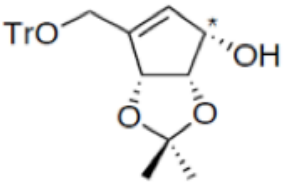
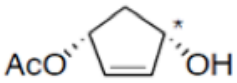
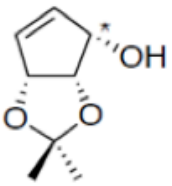
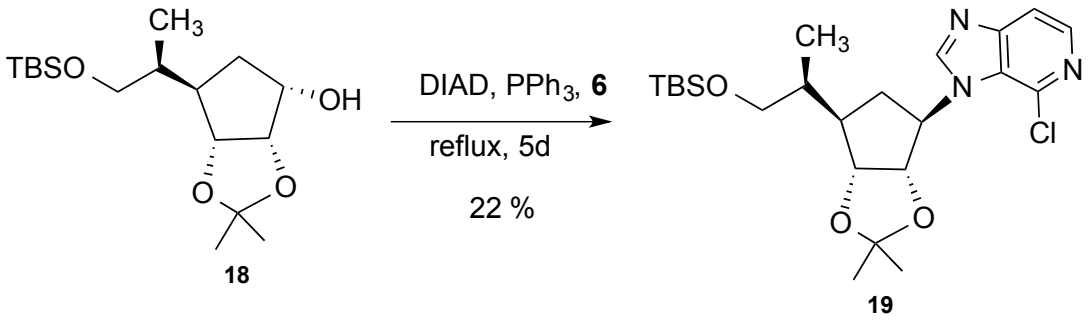
Entry	R-OH		Products (%)	
			N-9	N-7
1		**a**	86	0
2		**b**	70	0
3		**c**	42	53
4		**d**	38	57
5		**e**	32	43

Table 2: Regioselectivity in the Mitsunobu reaction of 6-Chloro-3-deazapurine (6)[83]



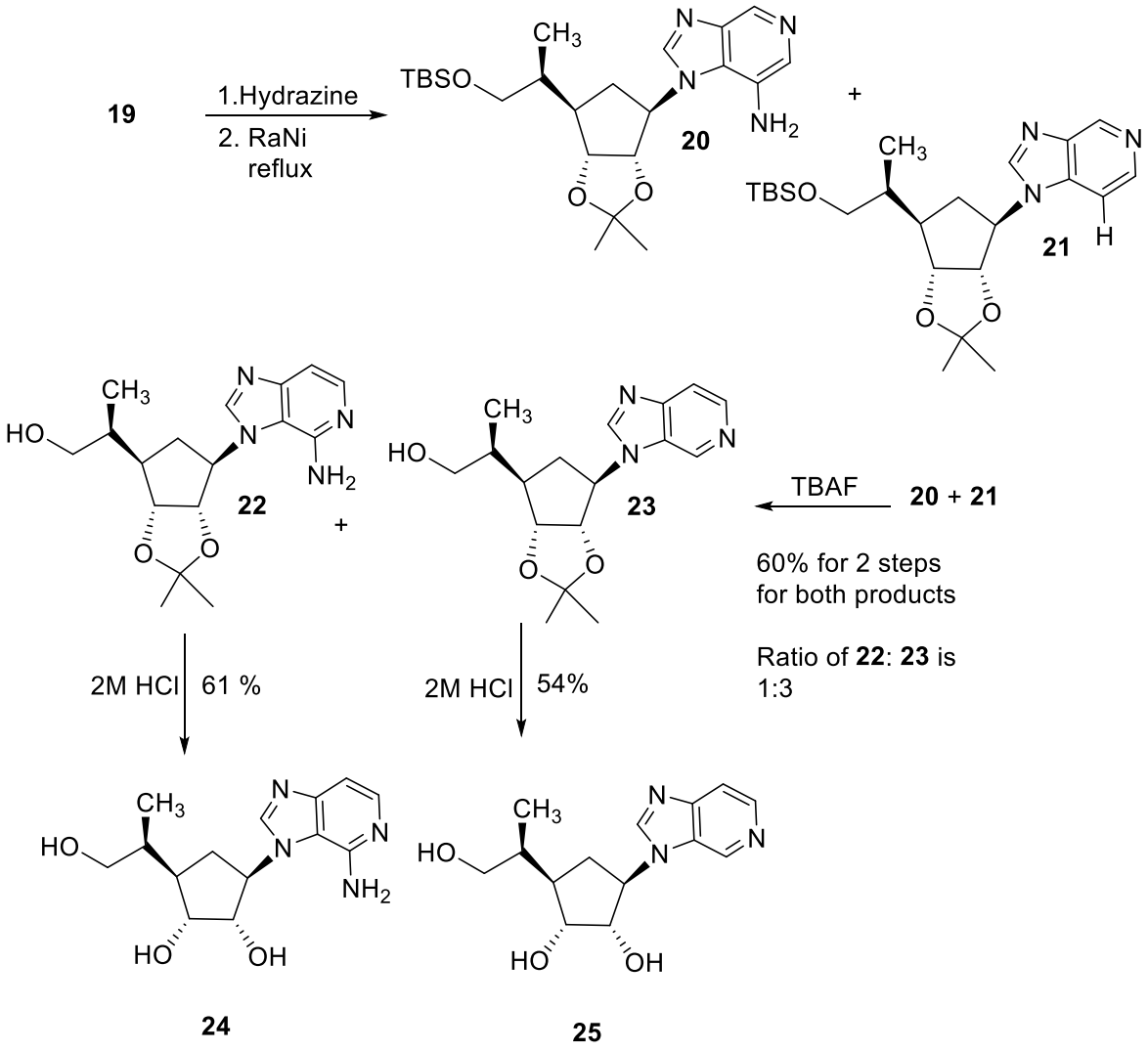
Scheme 18: Mitsunobu reaction of 18 and 6 to afford the N-7 product

The next step in the synthesis utilized compound **19**, which at the time was believed to be the N-9 isomer, in a two-step reaction with hydrazine followed by Raney nickel. Hydrazine was intended to act as a nucleophile to displace the chlorine followed by reduction to an amine with Raney nickel. This procedure was chosen because ammonia was not suitable for chlorine displacement for a N-3-deazapurines, which are more electron-rich than regular bases and, thus, less susceptible to nucleophilic attack.[84,85] Interestingly, it was found that the intended product **20** was only formed in small quantity, while the major product of the reaction was compound **21**, in which the chlorine was reductively removed. The two products were not easily separable. Consequently they were silyl-deprotected as a mixture by means of *tert*-butylammonium fluoride (TBAF) to afford the two primary alcohols **22** and **23**. The ratio of **22** to **23** was determined to be 1:3 by separation, determination of mass and comparison of the molar yield ratios.

Compound **23** is a very crystalline substance that allowed for the growth of crystals suitable for X-ray analysis. The X-ray structure is shown below in Figure 18. (Note that the numbering in the X-ray drawing does not follow purine numbering.) The position of the N-1 nitrogen (N-4 in the X-ray drawing), the nitrogen in the 6-membered

heterocyclic ring, in relation to the nitrogen to which the cyclopentyl moiety is bound indicated that, in fact, the N-7 product had been previously formed during the Mitsunobu reaction (**19**, Scheme 18).

Following separation of **22** and **23,** they were individually deprotected under acidic conditions to **24** and **25**, respectively (Scheme 19).



Scheme 19: Synthesis of compound 24 and 25

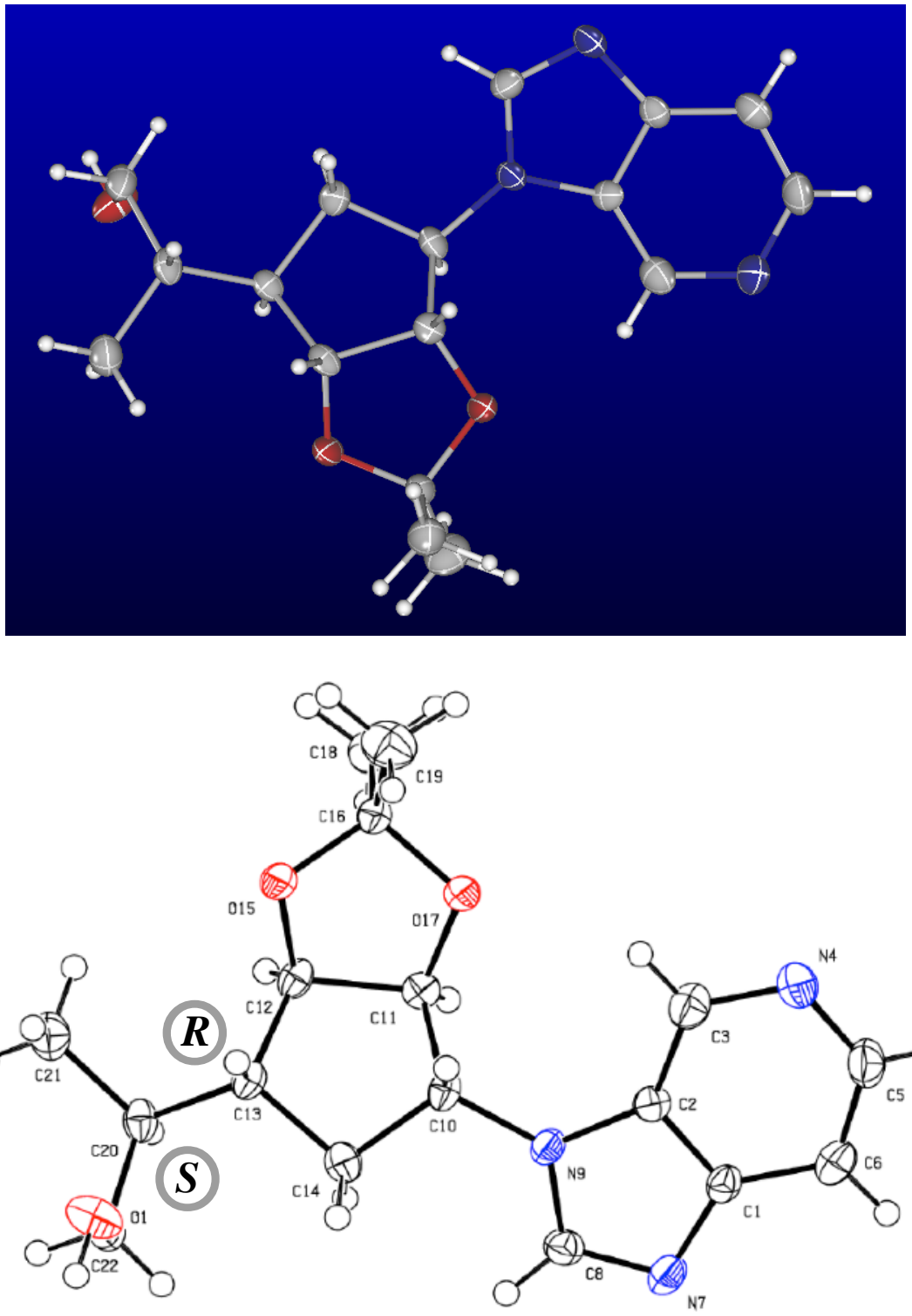


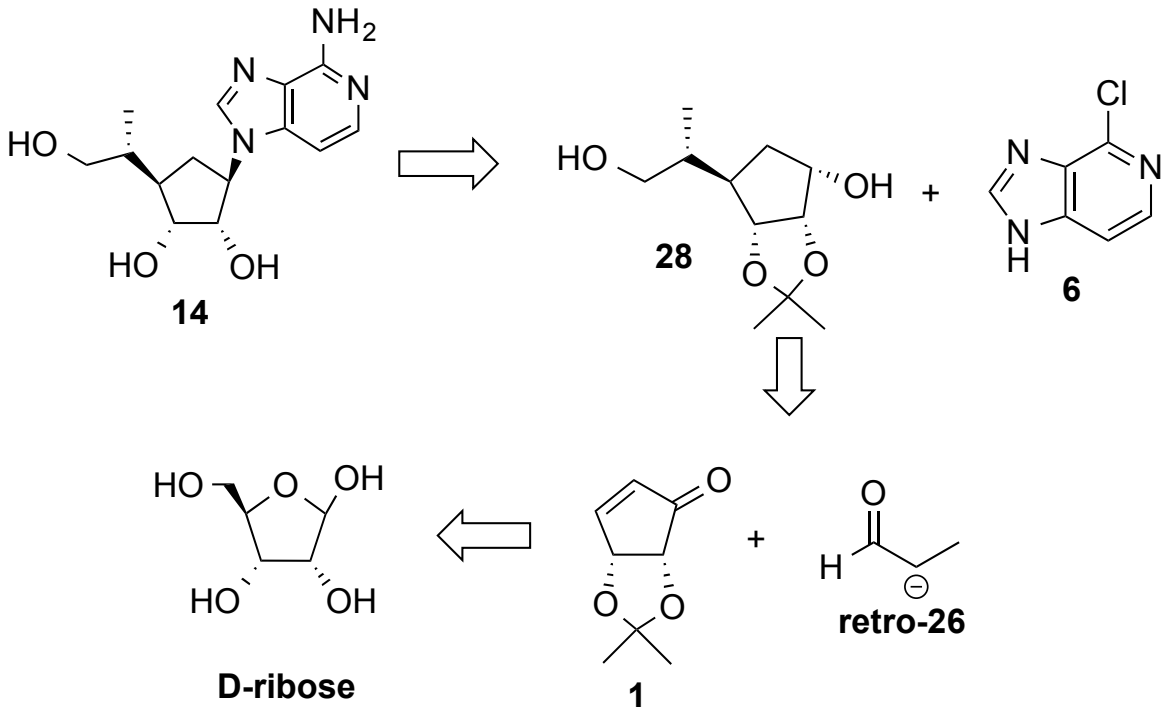
Figure 18: X-ray structures of 23

(numbering in this drawing does not follow purine numbering**)**

CHAPTER 3

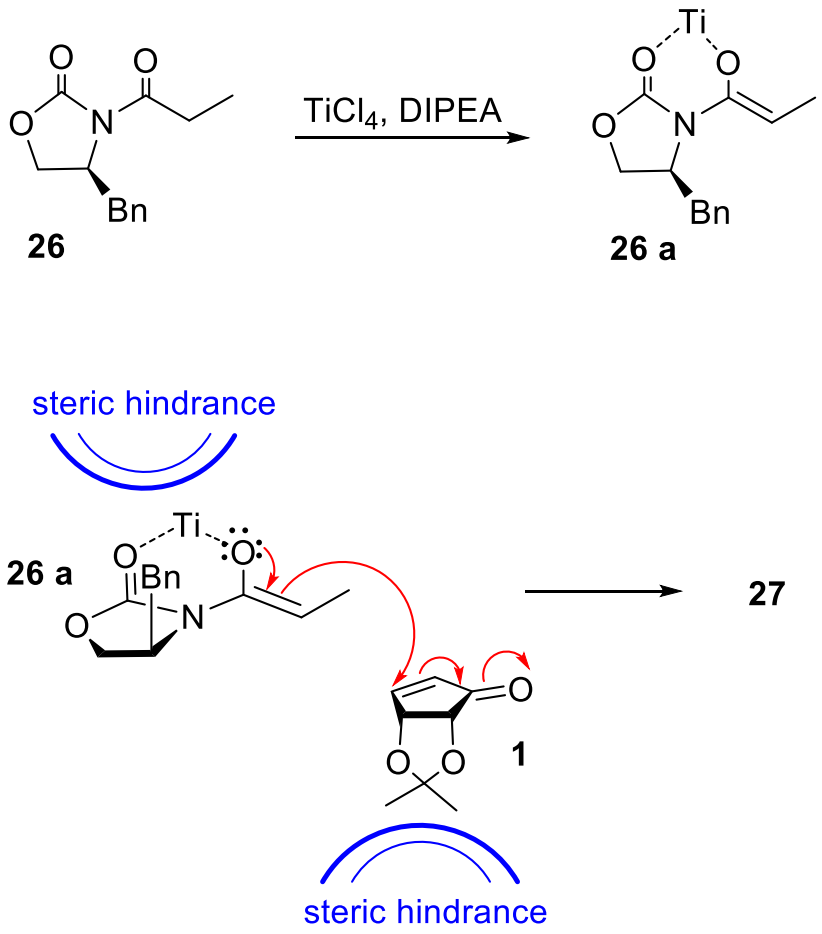
SYNTHESIS OF (5'*R*)- 5'-METHYL-3-DEAZAHOMOARISTEROMYCIN

(5'*R*)-5'-Methyl-3-deazahomoaristeromycin (**14**) is an epimer of the attempted target compound in the previous chapter (**13**). Therefore the synthetic route was identical except in the choice of the chiral Evans auxiliary, which is the enantiomer of the one used in the previous chapter (**12** in Scheme 15). Below in Scheme 20 is a retrosynthesis of **14**, which features propionaldehyde as the requisite synthon for the chiral auxiliary, which was planned to be used in the actual synthetic direction. The inversion in the chirality of the auxiliary would ensure the correct side-chain stereochemistry in the target. The Evans auxiliary **26** available from L-phenylalanine was available in our lab.



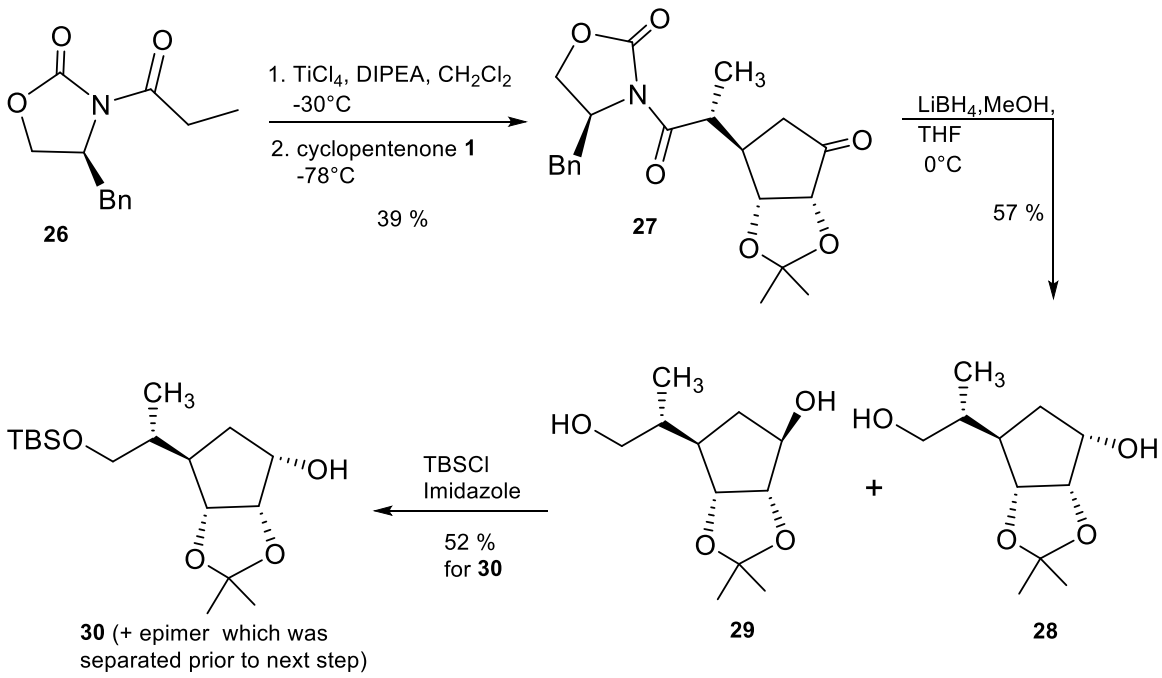
Scheme 20: Retrosynthesis of the *R*-diastereomer 14

N-acyl-oxazolidinone **26** was reacted with cyclopentenone **1** in a titanium tetrachloride aided Michael addition to afford **27** (Scheme 22) in a slightly higher yield than for the epimer **15** (35% vs 39%.). Again, the formation of the Z-titanium-enolate is preferred, but due to the benzyl group on the auxiliary having the opposite orientation, the attack of the Michael donor will occur from the opposite face, resulting in the opposite stereochemistry (R) at the C-5 bearing the side-chain methyl group. The stereochemistry at the C-4 of the cyclopentyl moiety is directed by the acetonide moiety on the cyclopentanone. Thus, the chirality at that center will be the same as for compound **15** from the previous chapter. The proposed diastereoselectivity is illustrated in Scheme 21.



Scheme 21: Side-view of approach of Michael donor to the Michael acceptor - proposed explanation of diastereoselectivity for the formation of 27

The auxiliary was cleaved reductively, by means of lithium borohydride accompanied by simultaneous reduction of the ketone to the secondary alcohol. The two resulting alcohols **28** and **29** were obtained in a 3:1 ratio as was determined by separation followed by determination of mass and comparison of the molar yield ratios. As previously, these two epimers were not readily separable. It proved more efficient to react the mixture of **28** and **29** with TBSCl and then isolate silyl-protected **30** (Scheme 22).

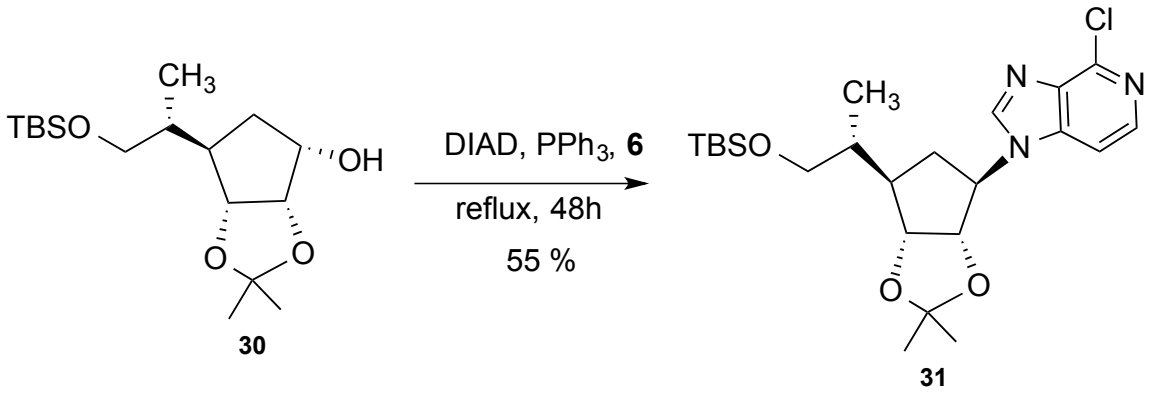


Scheme 22: Michael reaction and following conversions to compound 30

The Mitsunobu reaction between **30** and **6** was conducted as previously with DIAD and triphenylphospine except the reaction was allowed to react for only 2 days. The reaction was much more successful this time with a 55% yield and the correct N-9 regioisomer was formed as determined by NMR (Scheme 23).

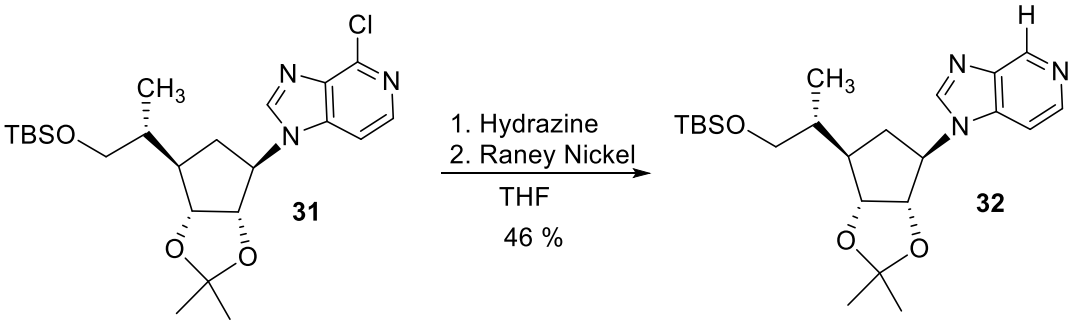
It seems that the reaction time in the Mitsunobu reaction plays a role in the formation of one regioisomer (N-7) over another (N-9). It is hypothesized that the shorter reaction time results in the formation of the kinetic N-9 product and the longer reaction time results in rearrangement of the N-9 product to the thermodynamic N-7 product. For **31,** three peaks were found near the aromatic region of the ^{1}H-NMR belonging to the hydrogens on the heterocyclic base: δ 8.22 (d, *J*=5.7 Hz, Ar-H, 1H), 8.03 (s, 1H), 7.61 (d, *J*=5.7 Hz, Ar-H, 1H), whereas for regioisomer **19**, which had its structure verified by X-ray (Figure 18), shows the following peaks near the aromatic region: 8.26 (s, Ar-H,

1H), 8.22 (d, *J*=5.6 Hz, Ar-H, 1H), 7.66 (d, *J*=5.6 Hz, Ar-H,1H), 5.54-5.47 (m, 1H), The order of singlet doublet, doublet in the N-7 isomer changes to doublet, singlet doublet in the N-9 isomer. Moreover, the peak belonging to the proton of the carbon (C1') bearing the glycosidic linkage was found at δ 5.54-5.47 (m, 1H) for the N-7 isomer and at δ 4.62-4.57 (m, 1H) for the N-9 isomer. Previously conducted studies (Table 2) suggested that this peak can be expected to be found upfield for the N-7 isomer compared to the N-9 isomer.[83] The same study suggested a characteristic peak of the C-8 of the heterocyclic ring in the ^{13}C-NMR that can be found in the region 115 ppm for the N7 isomer but around 106 ppm for the N9 isomer. This trend is also found when comparing the ^{13}C-NMR of **19** and **31**.The remaining parts of the spectra were very similar for both isomers.



Scheme 23: Mitsunobu reaction of 30 and 6 to afford the N-9 product

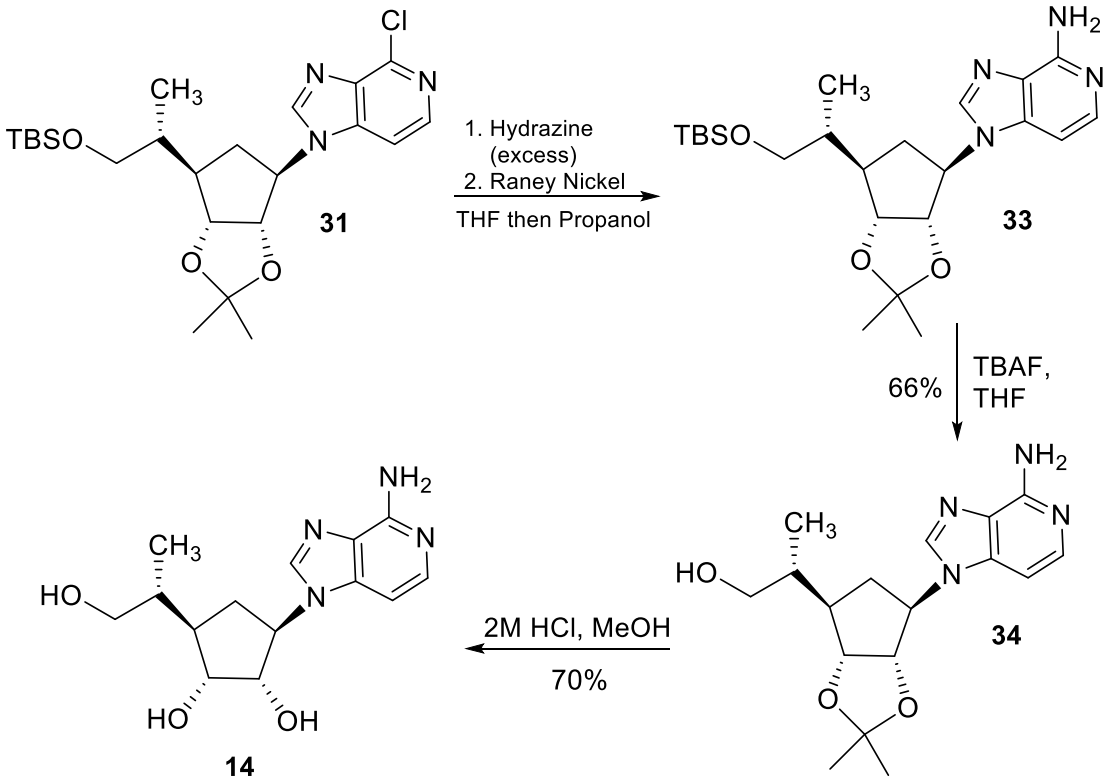
The Mitsunobu adduct **31** was converted into **33** by successful replacement of the chlorine by an amino using the hydrazine/ Raney-Ni protocol. In an initial trial of this reaction again a hydrogen replaced the chlorine to form **32** as shown in Scheme 24.



Scheme 24: Formation of 32 when amount of hydrazine was not optimized

Optimization of the amount of hydrazine that was employed as well as the use of extremely dry and fresh THF and 1-propanol resulted in the successful conversion to **33** (Scheme 25). It should be noted that anhydrous hydrazine must be used in a very large excess in a solvolysis-like reaction otherwise the reaction will not proceed with amination but with reduction of the 6-position to hydrogen.

Compound **33** was isolated and identified; however, its purification was difficult and some of the impure fractions were used in the next step. Conversion to **34** via silyl-deprotection with TBAF was accomplished in an overall yield of 66% for two steps. This was followed by full deprotection with methanolic hydrochloric acid to desired target nucleoside **14**.



Scheme 25: Final steps in the synthesis of 14

CHAPTER 4

ATTEMPTED SYNTHESIS OF 6'-METHYL-3-DEAZAHOMOARISTEROMYCIN

The synthesis of 6'(*R*)-6'-Methyl-homoaristeromycin (**35**) and 6'(*S*)-6'-Methyl-homoaristeromycin (**36**) was done previously in our lab,[86] and their promising antiviral activity toward vaccinia, cowpox and vesicular stomatitis viruses as well as lack of toxicity prompted the search for the 3-deaza analogues of these compounds: 6'(*R*)-6'-methyl-3-deazahomoaristeromycin (**37**) and 6'(*S*)-6'-methyl-3-deazahomoaristeromycin (**38**). A diastereomeric pair may display similar antiviral activities toward certain viruses but the activities can just as well differ vastly. For example, with **35** and **36** the *S*-isomer was much more potent against vaccinia virus than the *R*-isomer, whereas their activities toward other viruses were more similar.

With that in mind, it was important to design a stereoselective synthesis for **37** and **38** (Figure 19).

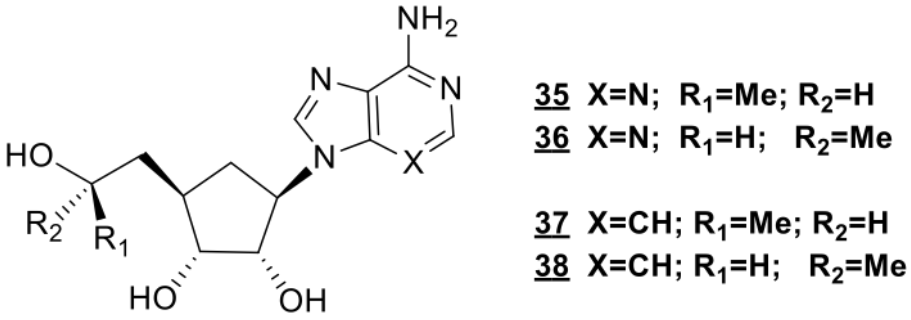
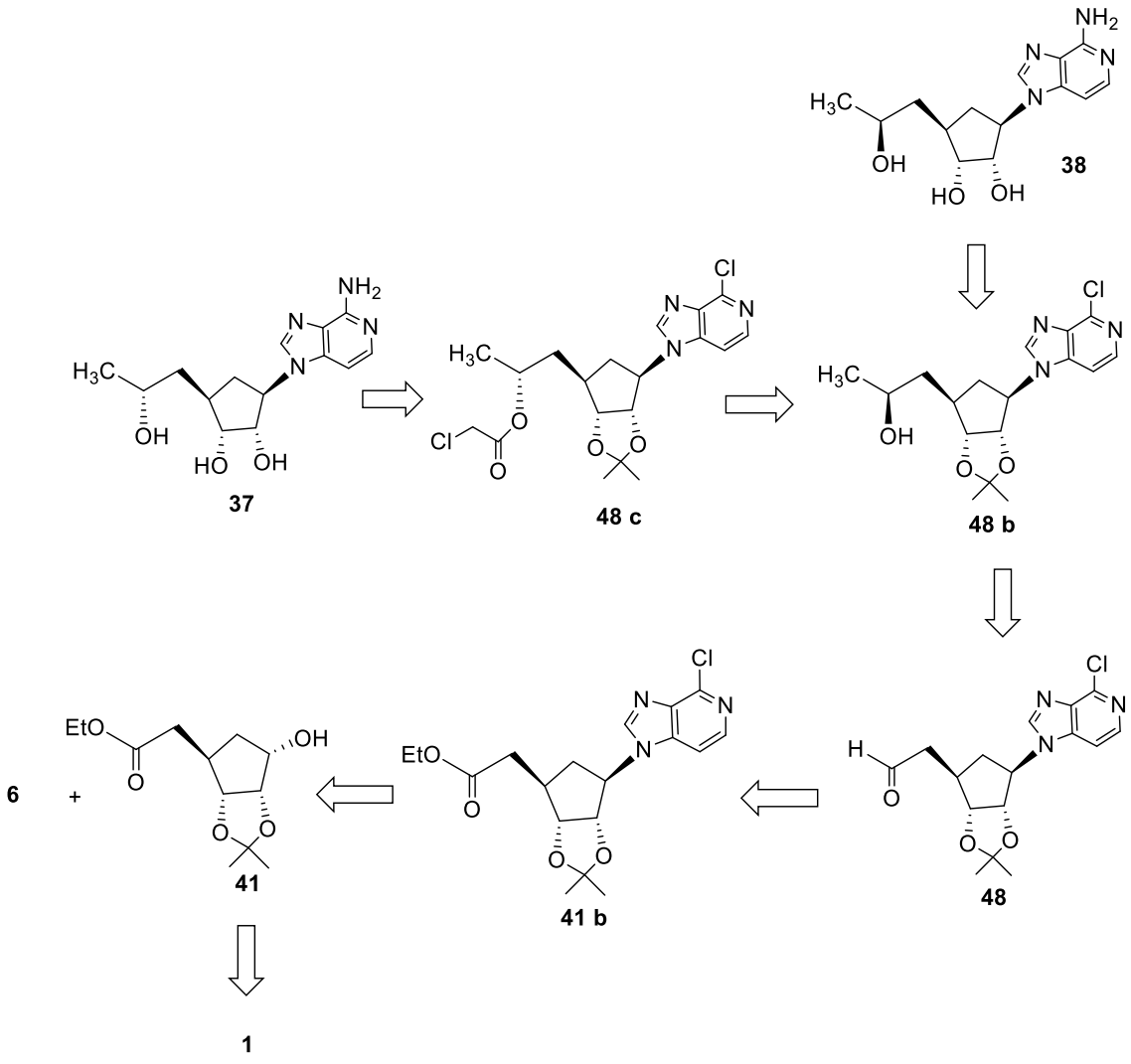


Figure 19: Structures of 6'-Methyl-homoaristeromycin compounds (35, 36) and their 3-deaza derivatives (37, 38)

A retrosynthesis for **37** and **38** is outlined in Scheme 26.

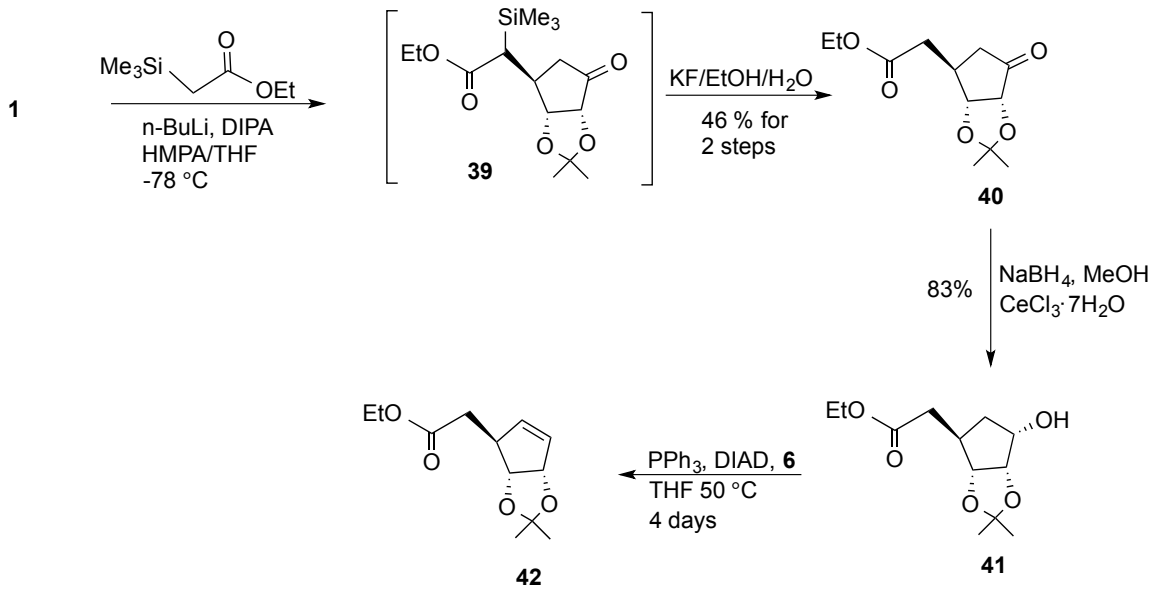


Scheme 26: Retrosynthesis for (6'*R*)- and (6'*S*)-6'-Methyl-3-deaza-homoaristeromycin (37 and 38)

We envisioned the Mitsunobu coupling of a cyclopentyl-ester derivative (for example **41**), which could be reduced to a key aldehyde intermediate (for example **48**) that would then undergo stereoselective methylation with an organo-zinc reagent and a TADDOL-based chiral catalyst.

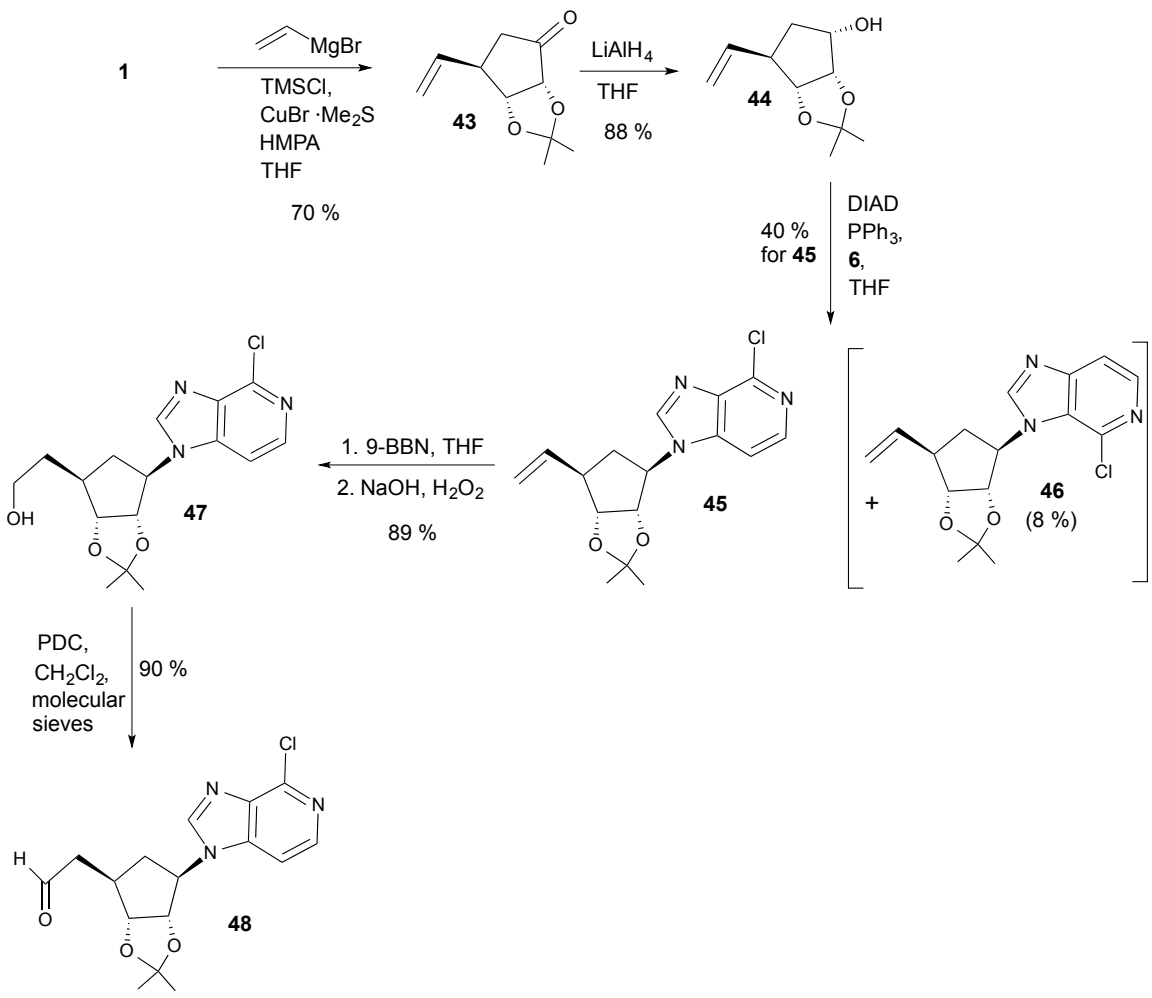
The starting point of the synthesis, as shown in Scheme 27 (*vide infra*), was the 1,4-addition of ethyl trimethylsilylacetate to cyclopentenone **1** to form **39**. The trimethylsilyl group was then cleaved *in situ* with potassium fluoride, which stereoselectively afforded ketone ester **40**. As in previous syntheses, the stereochemistry and concave structure of the acetonide group directs the addition of the carbon nucleophile from the top (Re) face to the C-4 position.[86] Reduction of **40** with sodium borohydride resulted in the stereoselective formation of **41**, where again the acetonide group directed the hydride attack from the top face, resulting in a β-hydroxyl group (pointing down).

The subsequent Mitsunobu coupling between **41** and 6-chloro-3-deazapurine **6** failed to give the desired nucleoside, even though many attempts were made, with variations in reaction time and addition order of the reagents. Instead, after a period of 4 days, only the elimination product **42** was observed as well as unreacted starting material. This route was abandoned. Interestingly, it should be noted that coupling of **41** with 6-chloropurine was previously reported as successful with a 50% yield.[71,86] The 3-deazapurine analogue is, however, much less soluble, and less reactive under Mitsunobu conditions, which may account for the failure of this reaction.



Scheme 27: Unsuccessful Mitsunobu reaction of 41 with chloro-deazapurine 6

A different direction was taken to achieve the synthesis of the key intermediate aldehyde **48** (Scheme 28). Again, starting with cylcopentenone **1**, the copper promoted conjugate addition of vinyl magnesiumbromide stereoselectively afforded vinyl cyclopentenone **43**. Reduction with lithium aluminum hydride gave rise to the vinyl-cyclopentenol **44** as one stereoisomer. The presence of another epimer was not observed on TLC or in the NMR (and the later obtained X-ray structure of **45** (Figure 20) proves the formation of a D-like nucleoside, which could have only have arisen from **44**). Mitsunobu coupling of **44** with **6** was successful and afforded the N-9 product **45** in 40% yield. The formation of N-7 product **46** was also observed at 8%, contrary to what was expected. The yields were determined by chromatographic separation followed by determination of mass and molar yield values. Variations in the addition order of the reagents as well as the amount of reaction time beyond 24 hours in the **44** to **45**/ **46** process had no impact on the regioselectivity of the reaction.



Scheme 28: New route to key intermediate aldehyde 48

The stereochemistry of **45** was confirmed via X-ray as shown in Figure 20.

Hydroboration of **45** with 9-BBN and subsequent cleavage of the boroalkyl intermediate with hydrogen peroxide under basic conditions afforded the anti-Markovnikov primary alcohol **47**. Subsequent oxidation with pyridinium dichromate (PDC) afforded the important precursor aldehyde **48**.

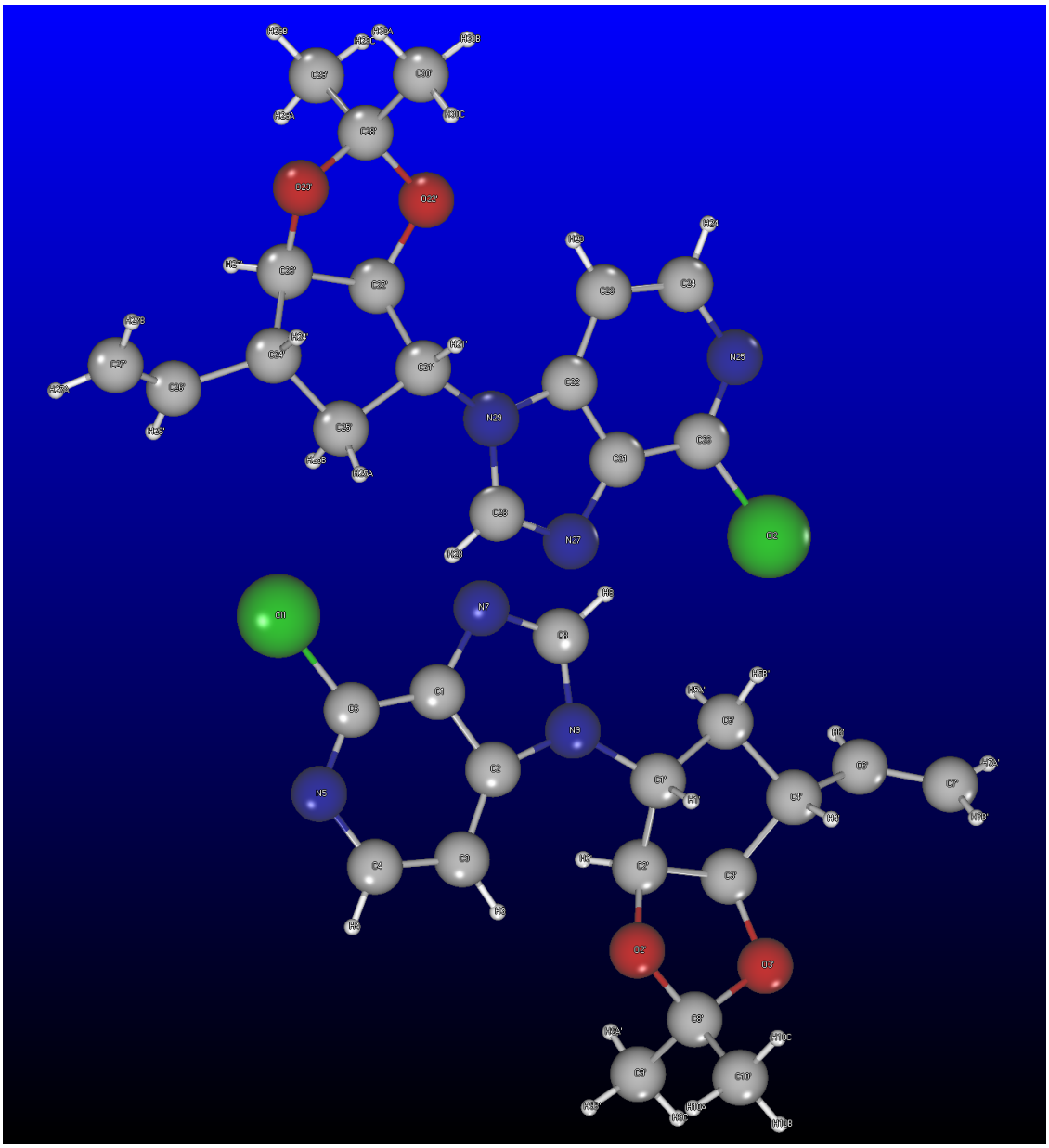


Figure 20: X-ray structure of 45

With the important precursor **48** in hand an attempt was undertaken into stereoselective methylation of the carbonyl carbon. Previous synthetic research with the 6-chloropurine derivative of **48** had shown that use of various organometallic reagents including methylmagnesium Grignard did not afford a stereoselective methylation, but

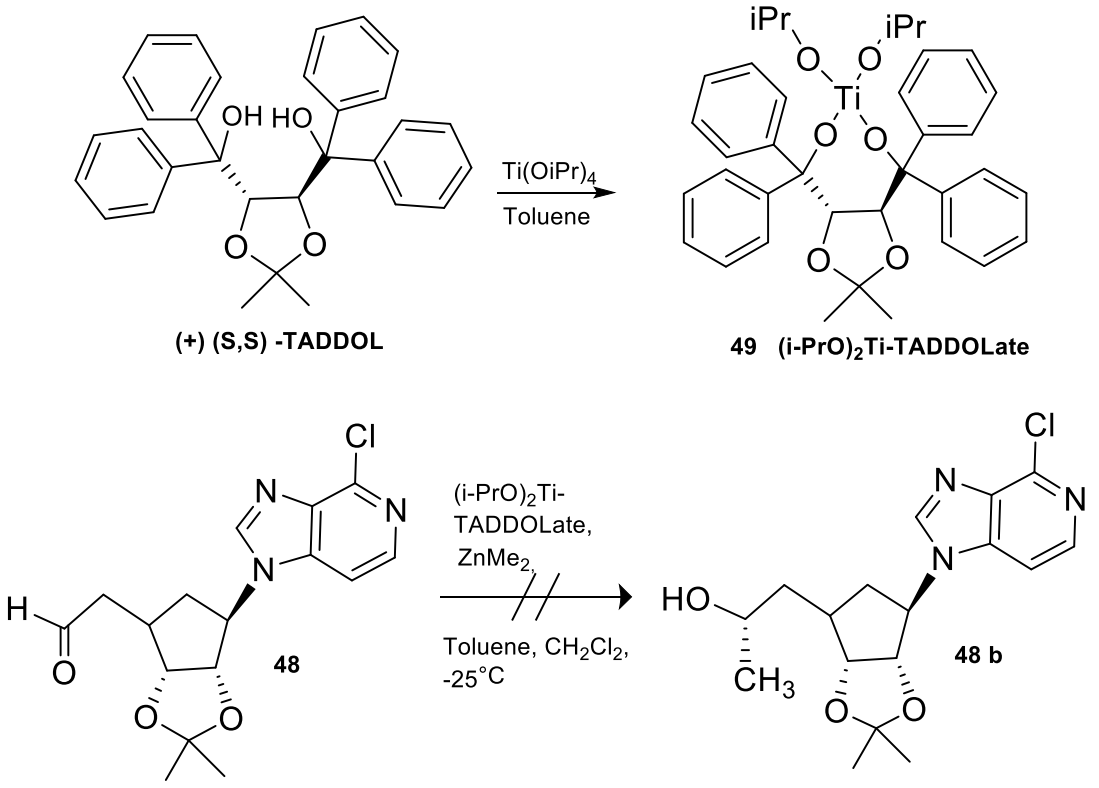
resulted in mixture of the two diastereomers. On the other hand, a (+)-(4S,5S)-TADDOL directed addition of dimethylzinc to the purine-derived aldehyde resulted in a highly stereoselective addition affording a diastereomeric mixture with a ratio of 92:8.[86] It has been proven for other aldehydes that nucleophilic addition in this system employing (+)-(4S,5S)-TADDOL as the chiral auxiliary occurs from the Re-face of the carbonyl center.[87]

TADDOLs, which are also known as Seebach reagents, have been used as chiral auxiliaries since the 1980s.[88] They are commercially available and can also be prepared from acetals or ketals of enantiomerically pure tartrate esters by reacting them with aromatic Grignard reagents.[89] Thus far, several hundred different TADDOLs and derivatives have been described. Structurally, TADDOLs contain two adjacent diarylhydroxymethyl groups, which are in a *trans* relationship to each other and located on a 1,3-dioxolane ring.[89] Enantioselective nucleophilic additions reactions to aldehydes are mediated by titanium TADDOLates, which are prepared by treatment of TADDOL with $Ti(OiPr)_4$.[89,90] Relative to Li and Mg derivatives, organotitanium derivatives have been shown to react with similar functional groups, such as aldehydes and other carbonyl groups, while being more selective.[89] It has been found that reactions of $(OiPr)_2$Ti-TADDOLates with organozinc reagents are highly enantioselective and result in the addition of alkyl groups to aldehydes.[89,90]

The mechanism responsible for the stereoselectivity has not been fully established in most cases. Two mechanistic pathways are possible. In the one case, the nucleophile could be transferred directly from a Nuc-TiX-TADDOLate species to the electrophilic center. In the other pathway the titanium TADDOLate could coordinate to the

electrophile (for the purpose of this dissertation research to the carbonyl oxygen of aldehyde **48**), thereby activating it like a chiral Lewis acid, followed by an attack of a nonchiral nucleophile.[89]

With this in mind, the reaction of titanium tetra*iso*propoxide with (+)-(4*S*,5*S*)-TADDOL was carried out, resulting in the formation of the activated chiral titanium-TADDOLate, which was used *in situ* with the dimethylzinc and the aldehyde substrate. However, it was observed that no reaction with **48** took place; the unreacted aldehyde was recovered quantitatively (Scheme 29).



Scheme 29: Attempted stereoselective methylation with TADDOLate and $ZnMe_2$

The lack of reaction may suggest that the organozinc methylating reagent had dissociated, even though it was a fresh sample.

CHAPTER 5

SYNTHESIS OF 6'-SUBSTITUTED 3-DEAZAHOMOARISTEROMYCIN DERIVATIVES

The successful Mitsunobu coupling of vinyl-cyclopentanol **44** and deazapurine **6** gave an accessible means to nucleoside **45** and opened a means to investigate substitutions of various functional groups at the 6'-position. The hydroboration product of **45**, primary alcohol **47**, was deemed an important precursor in this regard.

The first such derivative to seek was the 6'-amine-substituted 3-deaza-homoaristeromycin analogue (**50**). This compound is a homologue of 5'-amino-3-deaza-homoaristeromycin (**50a**) previously synthesized by Montgomery and Secrist, which was shown to possess moderate activity against vaccinia virus.[91] Both compounds are shown in Figure 21.

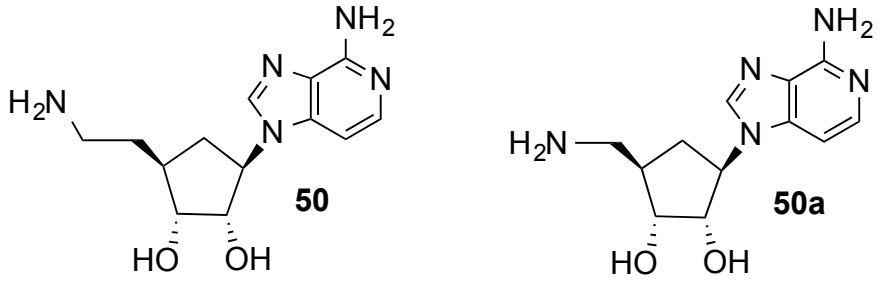
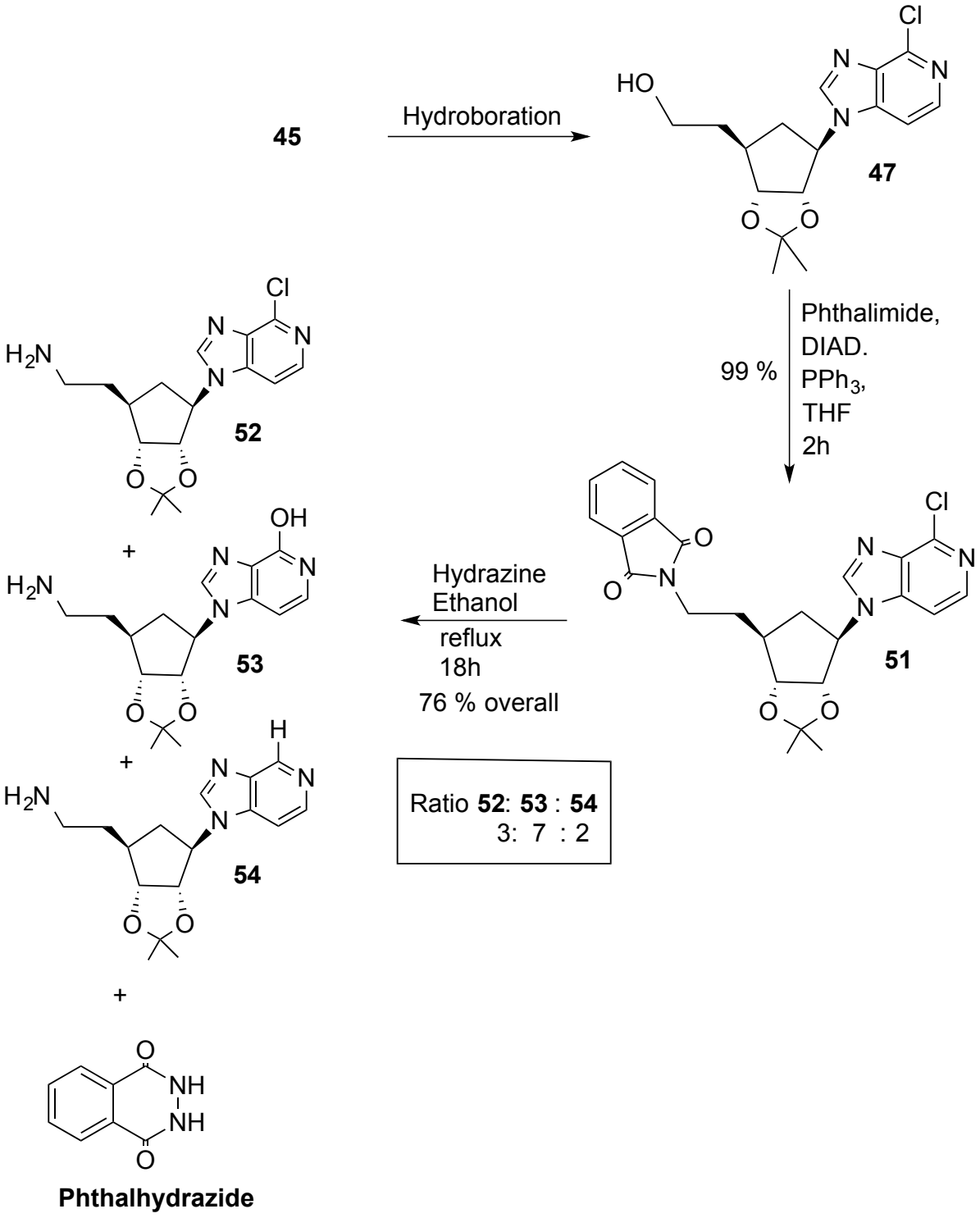


Figure 21: 6'-amino-3-deazahomoaristeromycin and 5'-aminoaristeromycin

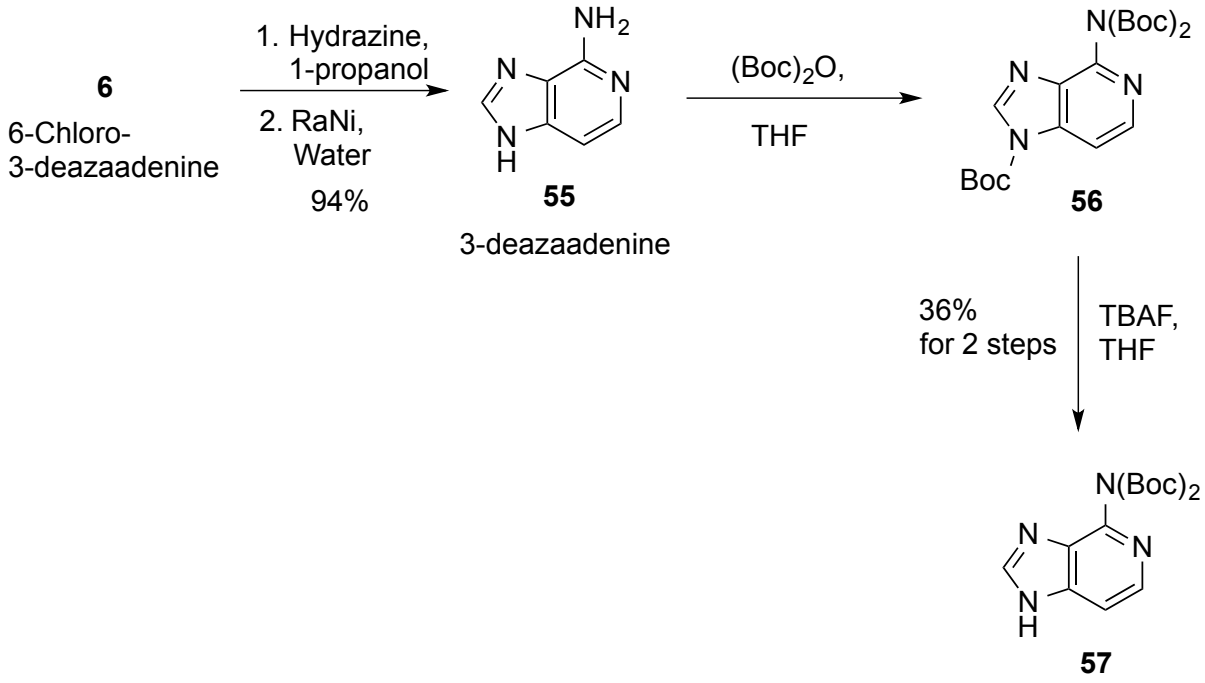
The starting point for the synthesis of **50** was the reaction of compound **47** with phthalimide under Mitsunobu conditions to give the phthalimide derivative **51** in very good yield (Scheme 30). In the next step, conversion of the phthalimido group into an

amino was attempted using hydrazine in ethanol. It was found that many by-products were formed in this reaction, in addition to the expected phthalhydrazide and the predicted aminated product **52**. Interestingly, the major product was **53**, in which a hydroxyl-group had replaced the ring chlorine. A small amount of **54** was also detected, in which a hydrogen had replaced the halogen. The structures were determined by chromatographic separation, followed by NMR and MS analyses.



Scheme 30: Synthesis of intermediate 52

To avoid complications with this reaction, an alternate halogen-free deazapurine base was synthesized, in which the requisite amino group at the 6 position was present in a protected form (two Boc-groups). Scheme 31 shows how this protected base was synthesized starting from 6-chloro-3-deazapurine **6**. Treatment with anhydrous hydrazine in a quasi-solvolysis reaction with a small amount of 1-propanol followed by reduction with Raney nickel gave 3-deazaadenine (**55**) in high yield.



Scheme 31: Synthesis of di-Boc-protected 3-Deazaadenine (57)

Treatment with 2 equivalents of di-*tert*-butyl dicarbonate ($(Boc)_2O$) has been shown to result in the formation of a mixture of the various possible Boc-protected products, including, mono-, di- and tri- substituted product shown in Figure 22.[59,92] Therefore, a direct conversion of **55** to **57** was not possible. The two different di-Boc protected products (**57** and **58**) are not easily separated or distinguished by NMR. Instead,

55 was treated with 4 equivalents of di-*tert*-butyl dicarbonate, resulting in the tri-Boc substituted product **56** as the major product. Isolation of **56** and subsequent treatment with TBAF resulted in compound **57**, due to an apparent greater affinity of the fluoride anion to react at the aromatic nitrogen position rather than at the exocyclic amino nitrogen.[59,92]

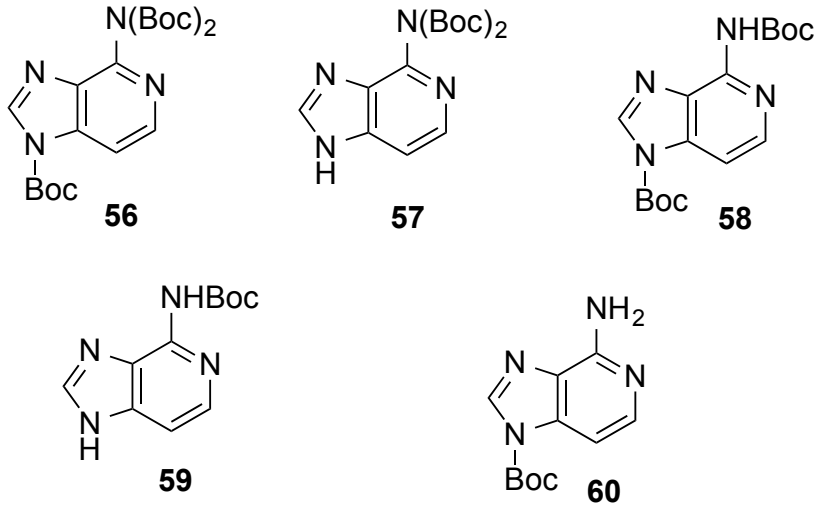
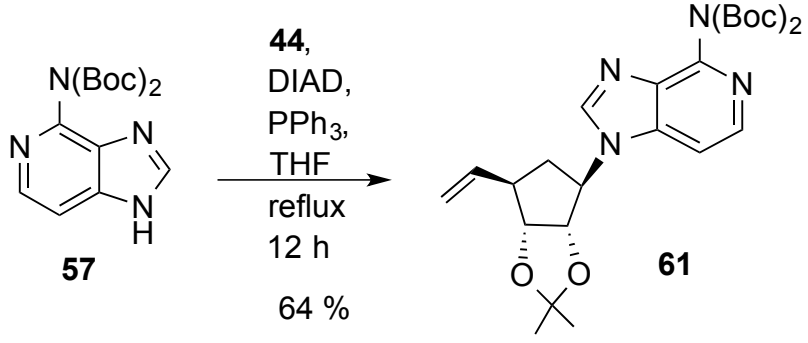


Figure 22: Mono-, di- and tri-Boc-protected products

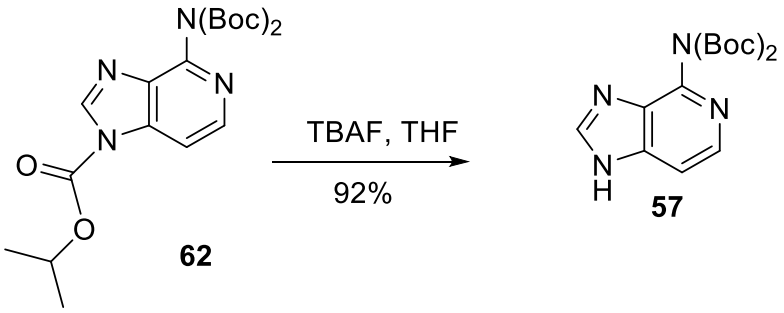
Di-Boc-protected deazabase **57** was then converted via a Mitsunobu coupling with vinyl-cyclopentanol **44** to afford compound **61** as shown in Scheme 32.



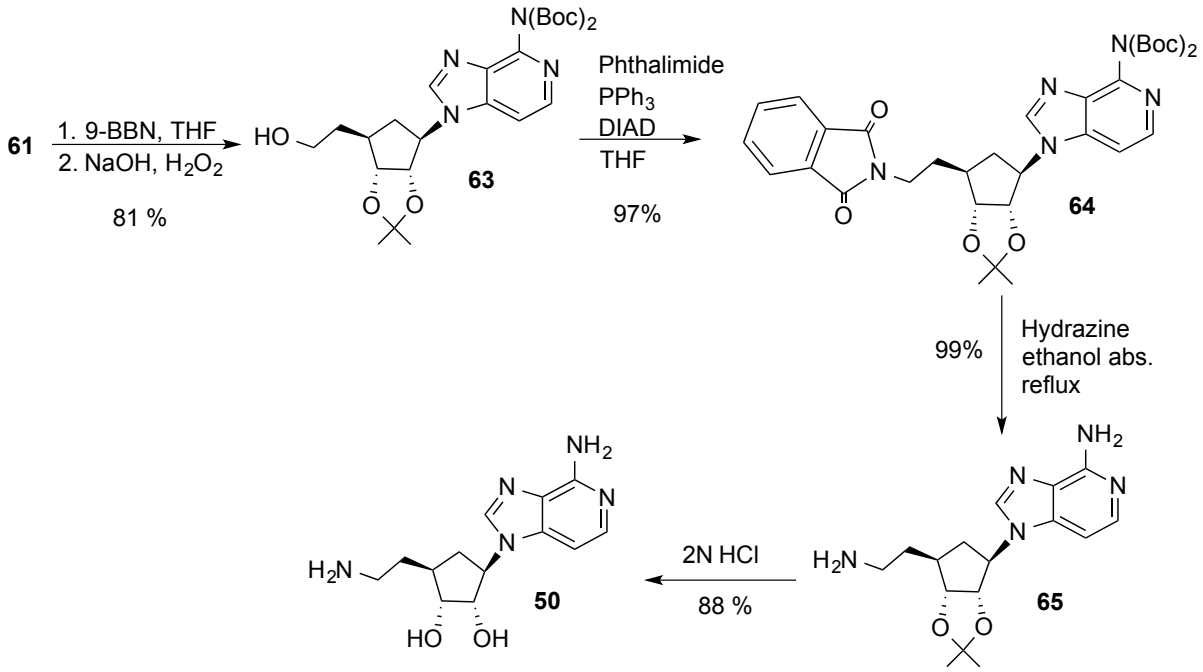
Scheme 32: Mitsunobu coupling of 57 with 44

In comparison to **6** the di-Boc-protected derivative **57** showed improved solubility, which resulted in better reactivity, a much shortened reaction time and a much higher yield in the analogous Mitsunobu reaction of Scheme 28. Due to the steric effect of the Boc-groups of **57**, interference by the N-7 nitrogen was highly reduced and the Mitsonobu coupled product was isolated as a single N-9-regioisomer. No trace of the N-7 isomer could be detected.

A byproduct (**62**) was formed during the Mitsunobu reaction as a result of reaction of the 3-deaza base **6** with DIAD. This byproduct could be recycled back into the di-boc protected base **57** by reaction with TBAF as shown in Scheme 33.



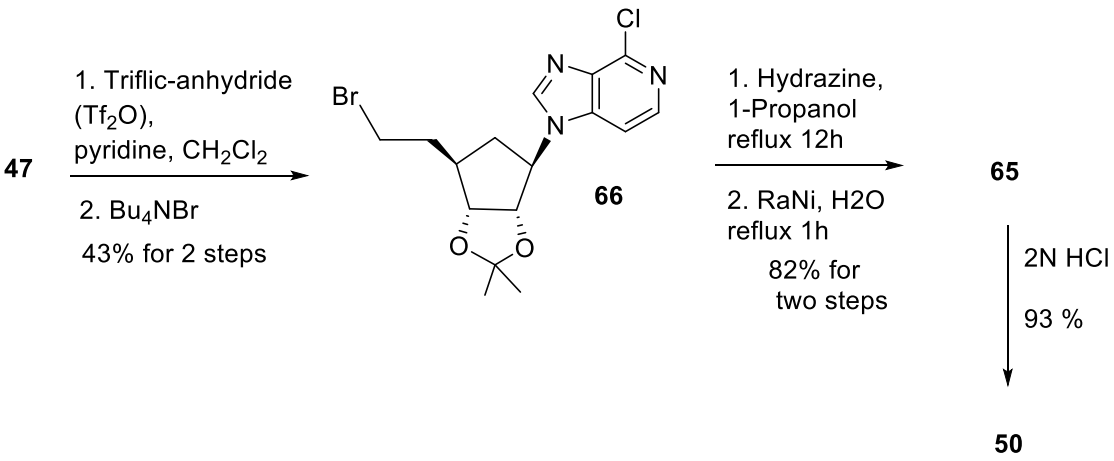
Scheme 33: Reformation of 57 from byproduct 62



Scheme 34: Synthesis of 6'-amino substituted 3-deaza-homoaristeromycin derivative 50

Following the Mitsunobu coupling reaction, **61** was subjected to hydroboration using 9-BBN. Subsequent oxidation by hydrogen peroxide resulted in primary alcohol **63** in good yield. Substitution of the alcohol with phthalimide under Mitsunobu conditions afforded compound **64** in great yield. Conversion of the phthalimide functionality into an amino group was achieved quantitatively by reaction with hydrazine in ethanol (compound **65**). No other byproducts were observed (other than the expected phthalhydrazide) in significant contrast to the analogous conversion of the chloro-deazapurine **51**. The boc-protection was removed during work-up of this reaction. Finally, treatment of **65** with acidic conditions, resulted in the removal of the acetal protecting group and yielded fully deprotected 6'-amino-3-deaza-homoaristeromycin **50** in 88% yield (Scheme 34).

In an alternate route, compound **50** was also synthesized according to Scheme 35 below:



Scheme 35: Alternative synthesis of amine 50

While in the previous route the product had to be carefully separated from the phthalhydrazide byproduct, this new route proved to be less tedious with easier work-up and afforded the product with higher purity, but lower overall yield.

Another target compound that can be derived from alcohol **43** is 6'-bromo-3-deazahomoaristeromycin (**67**), a homolog of 5'-bromo-3-deazahomoaristeromycin (**67a**), which was like its amino analogue (**50a**) also previously synthesized by Montgomery and Secrist and shown to possess moderate activity against vaccinia virus.[91] **67** and **67a** are shown below in Figure 23.

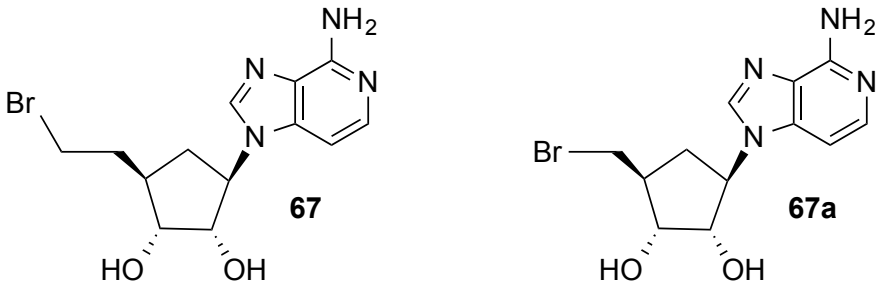
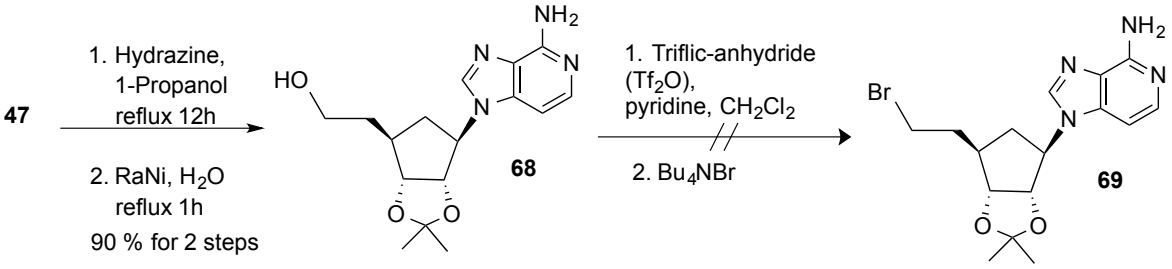


Figure 23: Structure of 6'- and 5'-Bromo derivatives 67 and 67a

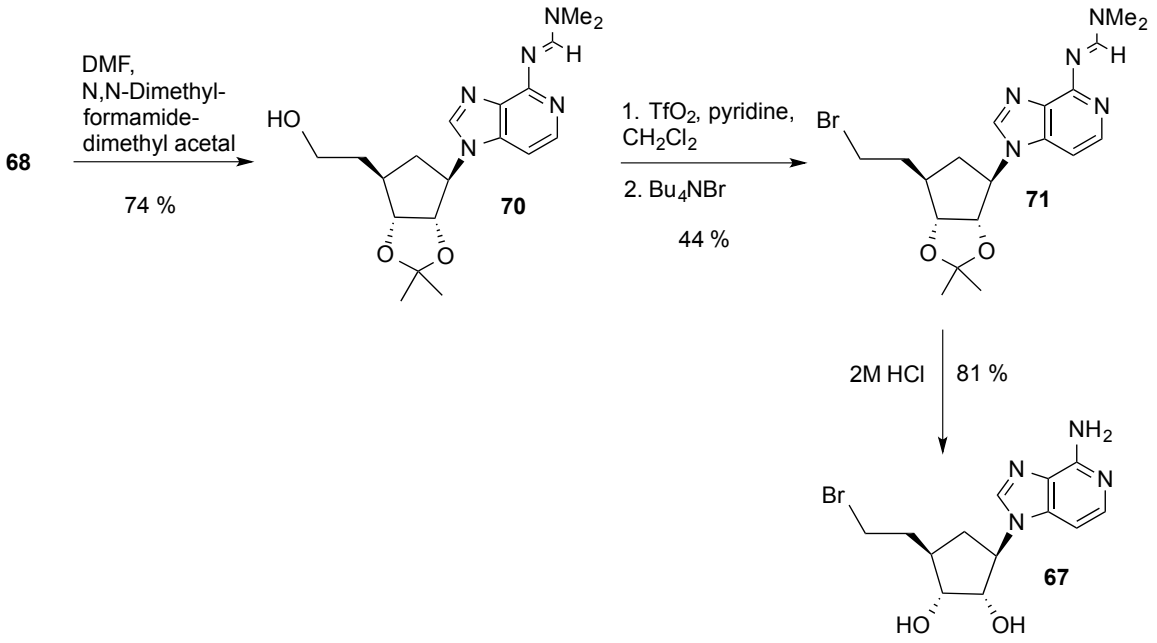
In a first attempt, the starting point for the synthesis of **67** was compound **47**. However, after the successful amination of **47** into compound **68** the direct conversion into bromide **69** proved unsuccessful using literature conditions. Because bromination was previously successful (conversion of **47** to **66** in Scheme 36) it was hypothesized that the amino group on the heterocyclic base interferes with the halogenation of the attempted **63** to **64** process.



Scheme 36: Unsuccessful bromination of 68 to 69

To circumvent this possibility, the amino of **68** was protected in form of an imine by reacting it with N,N-dimethylformamide dimethylacetal and DMF as shown in Scheme 37. The hydroxyl group was then transformed into a triflate by reaction of **70** with triflic anhydride, followed by reaction with tetrabutylammonium bromide, which

resulted in the successful displacement of the triflate by bromide to give **71**. Treatment of **71** with methanolic hydrochloric acid resulted in fully deprotected target compound **67**.



Scheme 37: Conversion into bromide 67 via an imine-protected intermediate

Finally the synthesis of a mercaptan derived compound 6'-*iso*butylthio-3-deazahomoaristeromycin (**72**) was envisioned. Compound **72** is a homologue of 5'-*iso*butylthio-3-deazaaristeromycin (**72a**), which was previously synthesized by Pankaskie et al.[93] Both structures are shown below in Figure 24.

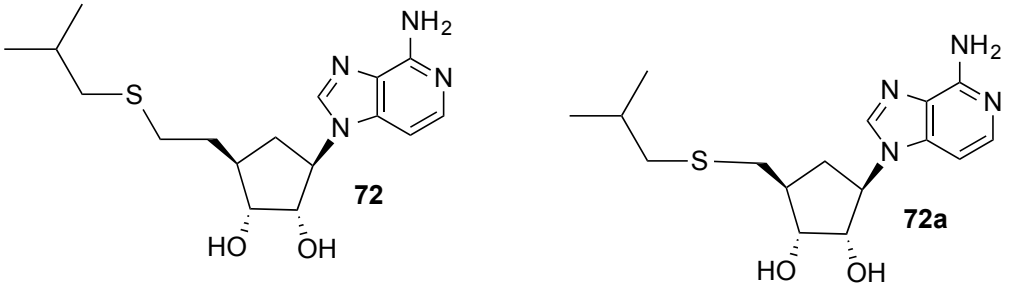
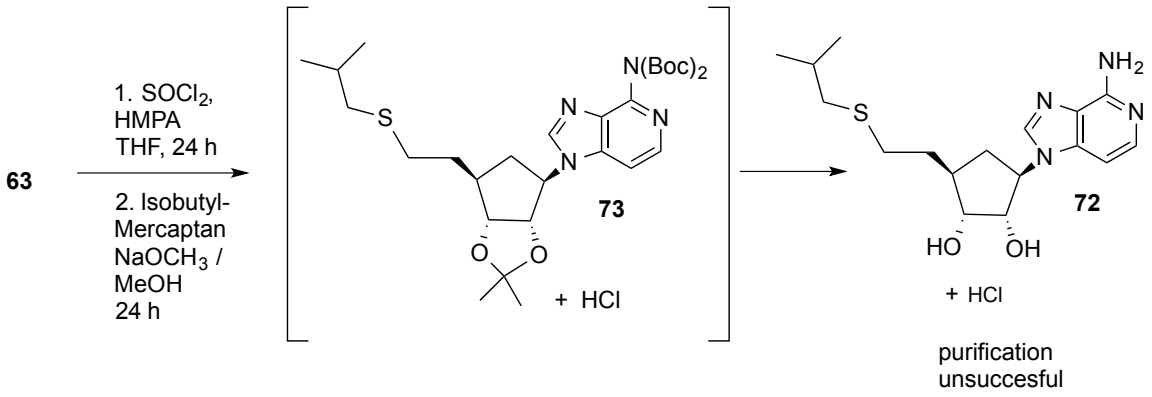


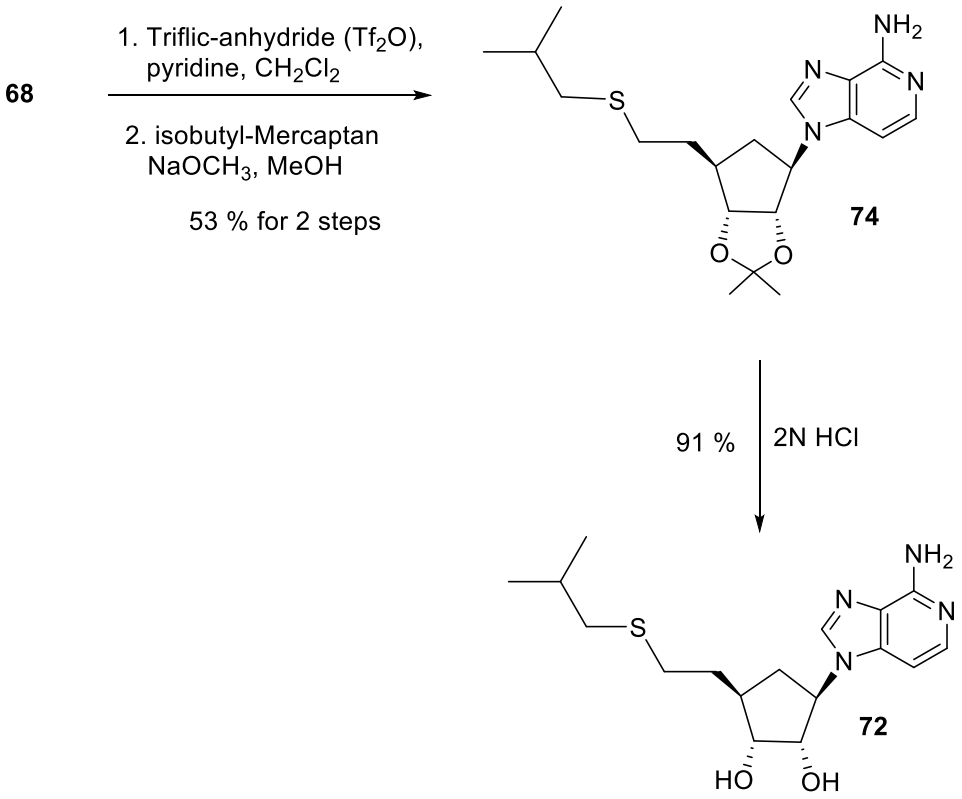
Figure 24: Sulfide derivatives 72 and 72a

The first attempt was starting from boc-protected **63**. Reaction with thionyl chloride resulted in the conversion of the hydroxyl group to a chlorine as a leaving group. In the next step *iso*butyl mercaptan was called for a nucleophillic substitution reaction with HMPA acting as a proton scavenger for the HCl, which would be formed as a byproduct in this reaction. However, the reaction conditions were sufficiently acidic that not only the boc-protecting group but also the acetal protecting group was prematurely removed (Scheme 38). The resulting product could not be purified.



Scheme 38: Acidic conditions prevent the isolation of 72

In order to avoid these unwanted acidic conditions, the synthesis was attempted with a different leaving group. Thus, triflic anhydride converted the alcohol **68** into a triflate, which was then displaced by sulfur nucleophile *isobutyl* mercaptan to yield **74**. Purification and full deprotection resulted in successful conversion into target compound **72** (Scheme 39)



Scheme 39: Synthesis of sulfide 72

CONCLUSION

Carbocyclic nucleoside derivatives are potent inhibitors of S-adenosyl-L-homocysteine (AdoHcy) hydrolase. This class of compound interferes with viral replication, because viral mRNA is rendered incomplete without AdoHcy-hydrolase-dependent capping methylation. Clinical application of many existing carbocyclic nucleosides, such as aristeromycin, is limited due to their associated high toxicities, which arise from 5'-phosphate formation.

In this direction, 3-deaza-homoaristeromycin derivatives **13**, **14, 37**, **38**, **50**, **67** and **72** were sought out as promising targets. The 3-deaza feature, the extension of the OH-group to the 6'-position, as well as inclusion of a methyl substituent on the 5'-position (of **13** and **14**) or the 6'-position (of **37** and **38**) was foreseen to result in reduced toxicity compared to the parent compound aristeromycin as a result of being less recognizable to cellular kinases, the source of toxicity. In the case of **13** and **14**, interesting discoveries were made with the formation of the N-7 versus N-9 regioisomer during the requisite Mitsunobu reaction. On the other hand, during the attempted synthesis of **13**, the N-7 isomer was formed with a longer reaction time, while the correct N-9 isomer resulted with a shorter reaction time in the synthesis of its epimer and a successful synthesis leading to **14** was described.

The synthesis of compounds **37** and **38** was aborted, when the attempted stereoselective methylation reaction of the precursor aldehyde **48** in a key step of the synthesis proved unsuccessful.

The 6'-extended derivatives, **50**, **67** and **72** were successfully synthesized by a convenient method and this is reported.

EXPERIMENTAL SECTION

General

^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AC 400 spectrometer (400 MHz for proton and 100.6 MHz for carbon) or a Bruker AC 600 spectrometer (600 MHz for proton and 150 MHz for carbon). All ^{1}H chemical shifts are referenced to internal tetramethylsilane (TMS) at 0.0 ppm. ^{13}C chemical shifts 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 following 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 systems. Yields refer to chromatographically and spectroscopically (^{1}H- and ^{13}C- NMR) homogeneous material.

Reagent-grade tetrahydrofuran for solvent use was freshly distilled from sodium metal and benzophenone. Reagent-grade dichloromethane for solvent use was dried for at least 24 hr over 4-Å molecular sieves before use.

Methyl-2,3-*O-iso*propylidene-β-D-ribofuranoside (2). To D-ribose (33.3 g, 0.222 mol) were added acetone (140 mL), methanol (140 mL) and 2,2-dimethoxy propane (66.6 mL). Concentrated sulfuric acid (2.00 mL) was added slowly. The reaction mixture was stirred overnight. Reaction progress was monitored via TLC using hexanes/EtOAc = 1:1. After completion, the reaction was neutralized to pH 6.5 using 6.5 mL pyridine. The solvent volume was reduced on the rotary evaporator. Water (300 mL) and ether (100 mL) were added. The layers were separated and the aqueous layer was washed with ether (3x 100 mL) and EtOAc (3x 300 mL). The combined organic layers were washed with saturated $CuSO_4$, water and brine, the solution was dried over $NaSO_4$ and concentrated in vacuo. Purification was done via silica gel chromatography using hexanes/EtOAc and a solvent gradient (25:1, 9:1 then 4:1) to yield 31.83 g (70.3%) of product as a mix of anomers as a colorless oil. Alternatively purification may be done via *in-vacuo* distillation at 149 °C with a higher yield (83.5%). The NMR spectral data agreed with literature.[43]

Methyl-5-deoxy-5-iodo-2,3-*O-iso*propylidene-β-D-ribofuranoside (3). A solution of **2** (120 g, 557 mmol), imidazole (57.6 g, 846 mmol) and PPh_3 (178 g, 678 mmol) in toluene (1.5 L) and acetonitrile (300 mL) was treated with iodine (45.0 g, 177 mmol) portionwise. The reaction mixture was stirred overnight. The solvent was decanted off and thereafter diluted with ether. The yellow precipitate in the original reaction flask was washed with ether until fully white. The combined ether phases were filtered to get rid of any further residue. Then this organic phase, which is orange/yellow in color, was washed with 10% sodium thiosulfate solution. At this point the solution turns colorless, indicating the remaining iodine had reacted with sodium thiosulfate. The

organic phase was extracted with 2x 200 mL sodium thiosulfate, 2x 150 mL water and 2x 150 mL brine. The aqueous phase was extracted with 3x 150 mL ether. The combined organic phases were dried over $NaSO_4$. Solvent was reduced *in vacuo* and the crude product, which includes white PPh_3O crystals, was added to silica gel and column chromatography was performed (hexanes/EtOAc = 15:1) to yield the product (170.5 g, 97.5%) as a colorless oil. The NMR spectral data agreed with literature.[43]

(2*R*,4*R*)-2-Dimethyl-5-vinyl-1,3-dioxolane-4-carboxaldehyde (4). Zinc (19.0 g, 0.291 mol) was activated by adding 150 mL of acetic acid and allowing it to stand for 1h, followed by filtration and washing with methanol. The zinc was then added to a flask containing compound **3** (10.0 g, 31.8 mmol) and 100 mL methanol. The reaction mixture was stirred under N_2 for 3.5 hours. The zinc was filtered off from the reaction mixture and the solvent was carefully evaporated *in vacuo* not exceeding 25 °C due to the instability of the aldehyde product. Purification was done by silica gel column chromatography (hexane/EtOAc = 2:1) to afford the product (99.0%, 4.91 g) as a colorless oil. The NMR spectral data agreed with literature.[46]

(4*S*,5*R*)-1-(2,2-Dimethyl-5-vinyl-[1,3]dioxolan-4-yl))-prop-2-en-1-ol (5). To a solution of **4** (33.3 g, 213 mmol) in anhydrous THF (200 mL) under N_2 was added dropwise a solution of fresh vinylmagnesium bromide (1.0 M in THF, 150 mL, 150 mmol) at -30 °C. The reaction was allowed to warm to rt and stirred overnight. Saturated NH_4Cl (200 mL) was added to quench the reaction. After separation of layers, the aqueous phase was extracted 3x with 100 mL EtOAc. The combined organic phases were washed with 2x 200 mL brine, and dried over $MgSO_4$. After filtration, the solvent was removed by evaporation under reduced pressure and the residue purified by silica gel

column chromatography (hexanes/EtOAc = 2:1) to afford **5** as a mixture of two epimers (11.2 g, 63.9%) as a colorless oil. The NMR spectral data agreed with literature.[42]

(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) (1). A solution of diene **5** (20.9 g, 0.114 mol) in dry CH_2Cl_2 was flushed with N_2 for 20 minutes. Grubbs catalyst (700 mg) was added at this time. The reaction was monitored via TLC (hexanes/EtOAc 2:1). After 24 h PDC (85.8 g, 0.228 mol) was added and the reaction mixture was allowed to stir at rt for another 24 hours. Purification of the product was done via silica gel column chromatography (Hexanes/EtOAc = 25:1 gradient to 9:1) followed by recrystallization. For this, the crude crystals were dissolved in a small amount of EtOAc, heated and allowed to cool to rt. When no more visible crystal growth occurred (approximately 2 minutes) the crystals were washed with a small amount of hexane to yield high-purity product crystals (7.55 g, 43% for two steps). The NMR spectral data agreed with literature.[94]

4-Ethoxy-3-nitropyridine (7). 4-Hydroxy-3-nitropyridine (50.0 g, 0.357 mol) was added to $ClCH_2CH_2Cl$ (250 mL) followed by addition of PCl_5 (95.0 g, 0.456 mmol). The reaction mixture was refluxed at 95 °C for 3h, which is when the solution turned from yellow to colorless. The reaction flask was placed into an ice-bath and very carefully absolute ethanol (200 mL) was added dropwise. 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-bath and left there for 1h for full crystallization. The formed crystals were collected by filtration and washed with ethanol (2 × 300 mL). Compound **7** (59.9 g,

99.9%) was obtained as white solid after drying in oven at 35 °C under vacuum. The melting point was in accordance with the literature with an mp of 47-48 °C. [95,96]

4-Amino-3-nitropyridine (8). To a flask containing compound **7** (59.9 g, 0.357 mol) and ammonium acetate (77.1 g, 1.00 mol) was added 300 ml of water. The clear solution was refluxed for 6 h at which point the solution had turned yellow and a precipitate had formed. The reaction slurry was cooled with an ice-bath and adjusted to pH 8 by addition of ammonium hydroxide. This was accompanied by further precipitation for about 1 hour. The yellow crystals were filtered and washed with ice water. The product (40.0 g, 81.0%) was obtained as a yellow solid after drying in the oven at 100 °C under vacuum. The melting point was in accordance with the literature with an mp of 201-202.5 °C.[97]

3,4-Diamino-2-chloropyridine (9). $SnCl_2$ (30.0 g, 0.158 mol) was added to HCl (250 mL) and the resulting clear suspension was heated to 60-70 °C. At this temperature compound **8** (13.9 g, 0.100 mol) was added portionwise and the resulting suspension refluxed overnight. The reaction slurry was poured over 200 g crushed ice and neutralized using first 10M NaOH and then conc. NH_4OH. The resulting solution was extracted 6x with 400 mL EtOAc and dried over $MgSO_4$. Filtration and evaporation of solvent under reduced pressure yielded the product as a yellow solid (12.6 g, 87.5%). ^{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. The melting point was in accordance with the literature with a mp of 218-220 °C.[60]

6-Chloro-3-deazaadenine (or 4-Chloro-1H-imidazo[4,5-c]pyridine) (6). Trimethyl orthoformate (250 mL) was added to compound **9** (12.6 g, 87.8 mmol) under

N_2 atmosphere. The reaction mixture was refluxed to 100 °C for 30 minutes at which point the solution has turned clear. Heat was reduced to 90 °C and formic acid (6 mL) was added. Reflux was resumed at 100 °C overnight and reaction progress was monitored via TLC (CH_2Cl_2/Methanol/ NH_4OH = 9 :1: 0.1).

After completion of the reaction, the heat was removed and the reaction was cooled with an ice-water bath for 1 hour to fully precipitate. The crystals were collected by filtration and washed with 2x 30 ml cold diethyl ether. Further purification via silica gel column chromatography (CH_2Cl_2/Methanol = 9 :1) afforded the product (13.5 g, 87.8%) as a light brown solid after oven-drying at 100 °C under vacuum. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.21 (s, br, 1H), 8.47 (s, 1H), 8.08 (d, *J*= 5.5 Hz, 1H), 7.59 (d, *J*= 5.5 Hz, 1H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 144.7, 141.1, 140.7, 139.5, 135.4, 108.8.

***R*-Phenylalaninol (10).** $LiAlH_4$ (4.71 g. 118 mmol) in dry THF (200 mL) under N_2 were cooled to 0 °C. Methanol was added (4.80 mL 118 mmol) followed by portionwise addition of D-phenylalanine (13.0 g, 78.7 mmol). The reaction mixture was stirred for 2 h at rt and then refluxed for 16 h. The reaction is complete when the clear solution turns yellow. Heating was removed and the reaction slurry was cooled with an ice-bath. H_2O (4.7 mL), 15% NaOH (4.7 mL) and H_2O (14.1 mL) were added dropwise and in sequence. Filtration and evaporation of solvent under reduced pressure afforded compound **10** (10.0 g, 84.0%) as a white solid. The NMR spectral data agreed with literature.[67]

(4*R*)-4-Benzyl-2-oxazolidinone (11). Compound **10** (10.0 g, 66.2 mmol), diethyl carbonate (16.1 mL, 133 mmol) and potassium carbonate (0.91 g, 6.6 mmol) were slowly

distilled for 5 h until 15 mL of ethanol-distillate had been collected. The reaction slurry was washed sequentially with water (80 mL), $NaHCO_3$ (80 mL) and brine (80 mL). The combined aqueous phases were extracted with 2x 100 mL CH_2Cl_2. The combined organic phases were dried over $MgSO_4$. Evaporation of solvent under reduced pressure followed by silica gel column chromatography (hexanes/EtOAc = 4:1) afforded compound **11** (3.9 g, 33%) as a white solid that can be recrystallized in EtOAc to white plates. The NMR spectral data agreed with literature.[68]

(4*R*)-1-Oxopropyl-4-benzyl-2-oxazolidinone (12). Compound **11** (2.50 g, 14.1 mmol) was dissolved in 100 mL dry THF under N_2 and cooled to -78 °C. n-Butyllithium (6.0 ml, 2.5 M solution, 15 mmol) was added dropwise by syringe. After 10 minutes propionyl chloride (1.35 ml, 15.5 mmol) was added dropwise by syringe and the reaction slurry was stirred at -78 °C for one hour. The reaction was allowed to slowly warm up to 0 °C and quenched with saturated ammonium chloride solution. THF was evaporated off under reduced pressure and the reaction slurry was extracted 3x with 50 mL CH_2Cl_2. The combined organic phases were washed with 1M NaOH solution (20 mL) and brine (30 mL) and dried over $MgSO_4$. Purification with silica gel column chromatography (hexanes/EtOAc 4:1) yielded compound **12** (3.11 g, 94.5%) as a white oil which solidifies after a short time. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21-7.36 (m, 5H, Ar-H), 4,65-4.71 (m, 1H, N-CH), 4.16-4.24 (m, 2H, $CHCH_2O$), 3.30 (dd, J_1= 13.3 Hz, J_2 = 3.3 Hz, 1H, CH_2-Ph), 3.04 (m, 2 H, CH_2CH_3), 2.75 (dd, 1 H, J_1 = 13.3 Hz, J_2 = 9.6 Hz, CH_2-Ph), 1.20 (t, 3 H, J = 7.3, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 174.1 (C=O), 152.7 (C=O), 135.3 (Ar-H), 129.4 (Ar-H), 129.0 (Ar-H), 127.4 (Ar-H), 66.2, 55.2, 37.9, 29.2, 8.3.

(2*R*, 3*R*, 4*R*)-4-[(1'*S*)-1'-((4''*R*)-4''-Benzyl-2''-oxo-3''-oxazolidinyl)carbonylethyl]-2,3-*O*-*iso*propylidenecyclopentanone (15). Compound **12** (3.00 g, 12.9 mmol) was dissolved in 150 mL dry CH_2Cl_2 under N_2 atmosphere and the solution was cooled to -10 °C. $TiCl_4$ (1.5 mL, 14 mmol) was added dropwise to give orange/yellow slurry. After 2 minutes DIPEA (2.5 mL, 14 mmol) was added dropwise resulting in a dark crimson slurry, which was stirred at 5 °C for 30 minutes and then cooled to -78 °C. At this temperature, a solution of compound **1** (1.78 g, 11.6 mmol) dissolved in 30 mL dry CH_2Cl_2 was added dropwise and the reaction mixture was stirred for 3 hours at -30 °C. Then the slurry was allowed to slowly warm to room temperature and quenched with NH_4Cl (30 mL). After separation of phases the aqueous phase was extracted with 3x 75 mL CH_2Cl_2 and the combined organic phases were washed with brine (30 mL) and dried over $MgSO_4$. Filtration, evaporation of solvent under reduced pressure followed by silica gel chromatography (hexanes/EtOAc = 5:1) yielded isomerically pure compound **15** (1.57 g, 35.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.21 (m, 5H, Ar-H), 4.70-4.69 (m, 1H), 4.64-4.62 (m, 1H), 4.34-4.33 (m, 1H), 4.25-4.18 (m, 1H), 3.88-3.94 (m, 1H), 3.30-3.26 (m, 1H), 2.82-2.71 (m, 3H), 2.33-2.28 (m, 1H), 1.45 (s, 3H), 1.35 (s, 3H), 1.26 (d, *J*= 7.12 Hz, 3H). The NMR spectral data agreed with literature.[77]

(1*S*, 2*S*, 3*R*, *4R*)-4-[(1*S*)-2-Hydroxyisopropyl]-2,3-*O*-*iso*propylidenecyclopentan-1-ol (16). To a solution of **15** (1.75 g, 4.53 mmol) and methanol (0.4 ml, 10 mmol) in dry THF (150 mL) at 0 °C under N_2 was added $LiBH_4$ (2.0 M solution, 4.75 mL, 9.5 mmol) dropwise by syringe. The resulting solution was stirred at 0 °C for one hour and then allowed to slowly warm to room temperature and

quenched with conc. sodium potassium tartrate solution (20 mL). THF was evaporated under reduced pressure and the slurry was diluted with CH_2Cl_2 (100 mL). After separation of phases the aqueous phase was extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic phases were washed with brine (30 mL) and dried over $MgSO_4$. Solvent was removed and column chromatography on a short silica gel column (hexanes/EtOAc 1:2) yielded **16** and **17** (650 mg, 66.5%) as a mixture of epimers in a 1:4 ratio. The NMR spectral data agreed with literature.[77]

(1*S*, 2*S*, 3*R*, *4R*)-4-[(1*S*)-2-*tert*-Butyldimethylsilyloxy*iso*propyl]-2,3-*O*-*iso*propylidenecyclopentan-1-ol (18). To a solution of **16** and epimer **17** (590 mg, 2.73 mmol) in dry CH_2Cl_2 (100 mL) under N_2 was added imidazole (328 mg, 4.82 mmol) then TBSCl (500 mg, 3.31 mmol). The solution was stirred for one hour at room temperature while monitoring the reaction progress via TLC (hexanes/EtOAc = 1:2). After all starting material had vanished the reaction mixture was diluted with CH_2Cl_2 (100 mL) and extracted with brine (30 mL) at which point the solution turned clear. The aqueous phase was extracted 3x with 25 mL CH_2Cl_2 and the combined organic phases were dried over $MgSO_4$. Solvent was removed and column chromatography on silica gel (hexanes/EtOAc = 20:1 gradient to 5:1) yielded isomerically pure **18** (720 mg, 78.4%). The NMR spectral data agreed with literature.[77]

7-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*S*)-2-*tert*-butyldimethylsilyloxy*iso*propyl)cyclopen-1'-yl]-6-chloro-3-deazaadenine (19). A solution of cyclopentanol **18** (500 mg, 1.49 mmol), deaza-base **6** (328 mg, 2.14 mmol) and triphenylphosphine (700 mg, 2.67 mmol) in 100 mL dry THF under N_2 was cooled to 0 °C and di*iso*propyl azodicarboxylate (DIAD, 0.54 mL, 2.7 mmol) was added dropwise

by syringe. The resulting yellow solution was stirred for 2 h at this temperature then refluxed for 5 days. Solvent was removed under reduced pressure and column chromatography on silica gel (hexanes/EtOAc 2:1) yielded **19** (150 mg, 21.6%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (s, 1H), 8.22 (d, *J*=5.6 Hz, 1H), 7.66 (d, *J*=5.6 Hz, 1H), 5.54-5.47 (m, 1H), 4.91-4.87 (t, *J*=7.2 Hz, 1H), 4.65-4.61 (m, 1H), 3.58-3.50 (m, 2H), 2.68-2.62 (m, 1H), 2.23-2.15 (m, 2H), 1.77-1.71 (m, 1H), 1.59 (s, 3H), 1.34 (s, 3H), 1.09 (d, *J*=6.4 Hz, 3H), 0.90 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 151.4, 144.2, 141.1, 133.5, 128.3, 115.0, 114.2, 84.2, 83.0, 66.2, 62.2, 46.2, 39.1, 38.3, 27.4, 25.9, 25.2, 18.2, 15.6, -5.5.

7-[(1'R, 2'S, 3'R, 4'R)-2',3'-*O-Iso*propylidene-4'-((1*S*)-2-hydroxy*iso*propyl)cyclopent-1'-yl]-6-H-3-deazaadenine (20) and 7-[(1'R, 2'S, 3'R, 4'R)-2',3'-*O-Iso*propylidene-4'-((1*S*)-2-hydroxy*iso*propyl)cyclopen-1'-yl]-3-deazaadenine (23). 19 (140 mg, 0.301 mmol) was dissolved in 12 mL anhydrous hydrazine and 2 mL methanol and refluxed for 6 h. The heat was removed and all solvent was removed under reduced pressure. The residue was dissolved in methanol (40 mL) and Raney Nickel (slurry in water) was added. The resulting slurry was refluxed for 3 hours, followed by hot filtration through a celite pad. All solvent was evaporated off and column chromatogaphy (CH_2Cl_2/MeOH = 18:1) on silica gel yielded a mixture of **20** and **21** which were not separable. The mixture of compounds **20** and **21** was dissolved in dry THF (30 mL) and tetrabutylammonium fluoride (TBAF, 1M solution, 0.6 ml, 0.6 mmol) was added at room temperature and the solution was stirred for 2 hours. Monitoring of the reaction progress with TLC (CH_2Cl_2/MeOH = 18:1) showed disappearance of the two starting material and formation of two new and now separable spots. Solvent was

removed under reduced pressure followed by purification via column chromatography on silica gel (CH_2Cl_2/MeOH 18:1) to yield **22** (13 mg, 13% for 2 steps) and **23** (60 mg, 63% for two steps). **23**: ^{1}H NMR (400 MHz, MeOD): δ 9.15 (s, Ar-H, 1H), 8.54 (s, Ar-H, 1H), 8.39 (d, *J*=5.6 Hz, Ar-H, 1H), 7.73 (d, *J*= 5.6 Hz, Ar-H, 1H), 4.86-4.84 (m, 1H), 4.74-4.70 (m, 1H), 4.63-4.60 (m, 1H), 3.65-3.61 (m, 1H), 3.53-3.49 (m, 1H), 2.66 (m, 1H), 2.33 (m, 1H),2.17 (m, 1H), 1.79-1.71 (m, 1H), 1.63 (s, 3H), 1.34 (s, 3H), 1.13 (d, *J*=6.8 Hz, 3H). ^{13}C NMR (100.6 MHz, MeOD): δ 148.5, 144.0, 141.0, 134.7, 131.9, 114.2, 114.1, 84.6, 83.2, 65.1, 62.5, 46.3, 39.1, 34.1, 26.3, 23.9, 14.4. X-ray structure of **23** determined, see Figure 18, page 53. Crystal structure determination was performed by Dr. John Gorden.

7-[(1'R, 2'S, 3'R, 4'R)-2',3'-Dihydroxy-4'-((1*S*)-2-hydroxy*iso*propyl)cyclopen-1'-yl]-6-H-3-deazaadenine (24). 2M hydrochloric acid (5 mL) and methanol (5 mL) were added to **22** (13 mg, 0.040 mmol) and the resulting solution was stirred over night. After TLC (CH_2Cl_2/MeOH = 9:1) indicated completion of the reaction, the solution was neutralized using Amberlite IRA-67 resin. The resin was washed with MeOH and water prior to addition to the reaction mix. The resin was filtered off, the solvent evaporated *in vacuo* and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **24** as a white solid (10 mg, 85%).

7-[(1'R, 2'S, 3'R, 4'R)-2',3'-Dihydroxy-4'-((1*S*)-2-hydroxy*iso*propyl)cyclopen-1'-yl]-3-deazaadenine (25). 2M hydrochloric acid (10 mL) and methanol (10 mL) were added to **23** (60 mg, 0.190 mmol) and the resulting solution was stirred overnight. After TLC (CH_2Cl_2/MeOH = 9:1) indicated completion of the reaction, the solution was neutralized using Amberlite IRA-67 resin. The resin was washed with MeOH and water

prior to addition to the reaction mix. The resin was filtered off, the solvent evaporated in vacuo and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **25** as a white solid (28 mg, 54%).

(2*R*, 3*R*, 4*R*)-4-[(1'*R*)-1'-((4''*S*)-4''-Benzyl-2''-oxo-3''-oxazolidinyl)carbonylethyl]-2,3-*O*-*iso*propylidenecyclopentanone (27). Evans auxiliary **26** (1.1 eq, 1.67 g, 7.15 mmol) was suspended in dry CH_2CH_2 (100 mL) under N_2. Titanium tetrachloride (1.05 eq, 0.77 mL, 6.9 mmol) was added at -30 °C, which resulted in a yellow solution. After 5 minutes, DIPEA (1.2 eq, 1.38 mL, 7.8 mmol) was added dropwise, which resulted in a color change to crimson. After all the DIPEA had been added the reaction slurry was warmed to 5 °C with a sodium chloride saturated ice-bath. The reaction was stirred at this temperature for 30 minutes. Thereafter, the reaction mixture was cooled to -78 °C and cyclopentenone **1** (1 eq, 1.00 g, 6.5 mmol), suspended in 75 mL CH_2Cl_2, was added dropwise. The temperature was allowed to warm to - 30 °C and was stirred at this temperature for 4 hours, then allowed to warm to 0 °C over the course of 2 hours. At this point TLC showed the disappearance of all starting material. The reaction was quenched with 50 mL saturated NH_4Cl, which resulted in a caramel colored solution and precipitation. The phases were separated and the aqueous phase was extracted 3 x with 50 mL CH_2Cl_2 and then the combined organic phases were extracted with brine and dried over $MgSO_4$. Column chromatography on silica gel (ether/ hexanes = 2:1) afforded compound **27** (1.00 g, 39.0%) as white crystals. The retardation factor of the product was R_f = 0.42 for this solvent system. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33-7.18 (m, 5H, Ar-H), 4.81 (d, *J*=5.8 Hz, 1H), 4.64-4.40 (m, 1H), 4.49 (d, *J*=5.8 Hz, 1H), 4.22-4.18 (m, 2H), 3.96 (t, *J*=6.5 Hz, 1H), 3.31-3.25 (dd, *J*= 10.0, 3.3 Hz, 1H), 2.87-2.65

(m, 3H), 2.11-2.15 (dd, *J*= 10.1, 3.3 Hz, 1H), 1.46 (s, 3H), 1.36 (s, 3H), 1.27 (d, *J*=7.0 Hz, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 212.3, 175.6, 153.0, 135.0, 129.4, 129.0, 128.9, 127.5, 112.0, 80.1, 79.0, 66.3, 55.6, 41.4, 40.4, 38.5, 38.0, 26.8, 24.6, 15.3.

(1*S*, 2*S*, 3*R*, *4R*)-4-[(1*R*)-2-Hydroxyisopropyl]-2,3-*O*-*iso*propylidenecyclopentan-1-ol (28). To a solution of **25** (780 mg, 2.02 mmol) in THF (100 mL) at 0 °C was added lithium borohydride (0.092 g, 4.2 mmol) suspended in 25 mL THF, and then methanol (0.2 mL 4.3 mmol), and was stirred at this temperature for 3 hours. The reaction mixture was quenched with a 1M solution of sodium potassium tartrate (25 mL). The cloudy white suspension was diluted with CH_2Cl_2 and the layers were separated. The aqueous layer was extracted 3x with CH_2Cl_2 and the combined organic layers were washed with brine and dried over $MgSO_4$. The filtrate was concentrated in vacuo and purification via silica gel chromatography (EtOAc: hexanes 2:1) afforded **28** with impurities of **29** as a mix of epimers (250 mg, 57.3%) as a clear liquid. The NMR spectral data agreed with literature.[77]

(1*S*, 2*S*, 3*R*, *4R*)-4-[(1*R*)-2-*tert*-Butyldimethylsilyloxy*iso*propyl]-2,3-*O*-*iso*propylidenecyclopentan-1-ol (30). To a solution of **28** and epimer **29** (combined 245 mg, 1.13 mmol) in CH_2Cl_2 was added imidazole (128 mg, 1.88 mmol) and then TBSCl (208 mg, 1.38 mmol) and the solution was stirred for 3 hours. The reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with water and brine until a clear solution was afforded. The separated organic layer was dried over $MgSO_4$. Filtration and concentration was followed by silica gel chromatography (hexanes : EtOAc 15:1) to give 200 mg of diastereomerically pure **30** (52.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.44-4.42 (m, 2H), 3.98 (m, 1H), 3.62-3.58 (m, 1H), 3.39-3.35 (m, 1H), 2.56-2.53 (m, 1H), 2.01-

1.89 (m, 3H), 1.62-1.57 (m, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 0.87-0.86 (m, 12H), 0.16 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 112.3, 84.0, 80.0, 70.5, 67.1, 44.6, 38.0, 35.6, 26.2, 25.9, 24.5, 18.2, 15.3, -5.5. The NMR spectral data agreed with literature.[77]

9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*R*)-2-*tert*-butyldimethylsilyloxy*iso*propyl)cyclopent-1'-yl]-6-chloro-3-deazaadenine (31). To **30** (200 mg, 0.590 mmol), triphenylphosphine (400 mg, 1.48 mmol), 6-chloro-3-deazapurine **6** (186 mg, 1.18 mmol) was added 50 mL THF and the solution was cooled to 0 °C under N_2. Then DIAD (0.3 mL, 2 mmol) was added dropwise via syringe, resulting in a yellow milky solution. After 2.5 h at 0 °C, the solution was heated to boiling, resulting in a clear brown solution, and kept at 80 °C for 48 hours at which point the educt spot had disappeared from the TLC. All solvent was evaporated off and silica gel chromatography (hexanes/ EtOAc = 10:1 gradient to 3:1) afforded **31** (275 mg, 55.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J*=5.7 Hz, Ar-H, 1H), 8.03 (s, 1H), 7.61 (d, *J*=5.7 Hz, Ar-H, 1H), 4.62-4.57 (m, 1H), 4.56-4.53 (m, 1H), 3.67-3.70 (m, 1H), 3.61-3.57 (m, 1H), 2.60-2.55 (m, 1H), 2.25-2.17 (m, 2H), 1.85-1.79 (m, 1H), 1.61 (s, 3H), 1.32 (s, 3H), 1.01 (d, *J*= 6.8 Hz, 3H), 0.91 (s, 9 H), 0.08 (d, *J*=1.9 Hz, 6H).

9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*R*)-2-*tert*-butyldimethylsilyloxy*iso*propyl)cyclopent-1'-yl]-6-H-3-deazaadenine (32). A solution of **31** (250 mg, 0.537 mmol) in anhydrous hydrazine (5.00 mL, 159 mmol) and THF (25 mL) was refluxed overnight under a N_2 atmosphere. After cooling to rt, the solvent was evaporated off *in vacuo*. The residue was dissolved in MeOH (40 mL) and water (20 mL). Four spatula tips of Raney nickel (slurry in water) were added portionwise and the reaction slurry was stirred over-night. Raney nickel was filtered off and all solvent was

evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel (EtOAc/ hexanes = 10:1), which afforded **32** (110 mg, 46%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.13 (s, Ar-H, 1H), 8.47 (s, Ar-H, 1H), 8.03 (d, *J*=5.3 Hz, Ar-H, 1H), 7.70 (d, *J*=5.3 Hz, Ar-H, 1H), 4.66-4.57 (m, 2H), 3.67-3.66 (m, 1H), 3.59-3.57 (m, 1H), 2.55-2.54 (m, 1H), 2.23 (m, 2H), 1.83-1.79 (m, 1H), 1.59 (s, 3H), 1.30 (s, 3H). 0.99 (d, *J*=6.8 Hz, 3H), 0.90 (s, 9H), 0.06 (d, *J*=2.0 Hz, 6H). HRMS (ESI+): m/z Calcd for $C_{23}H_{37}N_3O_3Si$ [$(M + H)^+$]: 432.6539; found 432.6551.

9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*R*)-2-*tert*-butyldimethylsilyloxy*iso*propyl)cyclopent-1'-yl]-3-deazaadenine (33). To a solution of **31** (150 mg, 0.322 mmol) in THF (25 mL) was added anhydrous hydrazine (20.0 mL, 637 mmol) under N_2 atmosphere. The suspension turned cloudy-grey and development of soapy bubbles was observed. After 3 minutes the color changed from grey to yellow. The suspension was refluxed for 2 h. Most of the educt spot was still present on the TLC at this time and it seemed that THF and hydrazine are immiscible. Therefore, all the THF was refluxed off carefully and replaced by 25 mL 1-propanol as solvent. The reaction mixture was refluxed for another 3 h. After cooling to rt, the solvent as well as the hydrazine were evaporated off *in vacuo*. The residue was dissolved in MeOH (20 mL) and water (10 mL). Three spatula tips of Raney nickel (slurry in water) were added portionwise and the reaction slurry was refluxed for one hour. Raney nickel was filtered off on a celite pad and all solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography on silica gel (EtOAc/hexanes = 10:1) to afford **33**. The product was collected as an impure fraction containing a yellow impurity, which was separated off in the next step.

9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*R*)-2-hydroxy*iso*propyl)cyclopent-1'-yl]-3-deazaadenine (**34**). A 1M TBAF solution (0.5 mL, 0.5 mmol) was added to a solution of **33** (145 mg, 0.322 mmol) in THF (50 mL). The reaction mixture was stirred for 3 h at rt. THF was evaporated off *in vacuo* and flash chromatography purification on silica gel (CH_2Cl_2/MeOH = 9:1) afforded **34** as a white solid (70 mg, 66% for two steps from **31**). ^{1}H NMR (400 MHz, MeOD): δ 8.25 (s, Ar-H, 1H), 7.70 (d, *J*=6.2 Hz, Ar-H, 1H), 7.10 (d, *J*= 6.2 Hz, 1H), 4.75 (m, 2H), 4.59 (m, 1H), 3.76-3.72 (m, 1H), 3.46-3.41 (m, 1H), 2.57-2.51 (m, 1H), 2.29-2.28 (m, 1H), 1.94 (d, *J*=1.9 Hz, CH_2-OH, 2H), 1.58 (s, 3H), 1.32 (s, 3H), 1.03 (d, *J*=6.8 Hz, 3H). ^{13}C NMR (100 MHz, MeOD): δ 154.0, 152.0, 140.4, 139.2, 137.9, 113.9, 109.4, 84.6, 83.1, 65.4, 62.0, 46.1, 39.0, 33.0, 26.3, 23.8, 13.9.

9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-2',3'-O-*Iso*propylidene-4'-((1*R*)-2-hydroxy*iso*propyl)cyclopen-1'-yl]-3-deazaadenine (or (5'R)-5'-Methyl-3-deazahomoaristeromycin) (14). To a solution of **34** in MeOH (10 mL) was added 2M HCl solution (10 mL). The reaction mixture was stirred for 2.5 h at rt at which point the reaction showed completion on the TLC. The reaction mixture was then neutralized with Amberlite IRA-67 basic resin. The resin was washed with MeOH and water prior to addition to the reaction mix. The resin was filtered off, the solvent evaporated *in vacuo* and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **14** as a white solid (30 mg, 67%). ^{1}H NMR (400 MHz, MeOD): δ 8.45 (s, Ar-H, 1H), 7.66 (d, *J*=6.2 Hz, Ar-H, 1H), 7.24 (d, *J*=6.3 Hz, Ar-H, 1H), 4.65-7.72 (m, 2H), 4.22-4.19 (m, 1H), 3.67-3.62 (m, 1H), 3.53-3.49 (m, 1H), 3.28-3.26 (m, 1H), 3.14-3.11 (m, 1H), 2.38-2.31 (m, 1H), 2.10-2.01 (m, 1H), 1.00 (d, *J*=6.8 Hz, -CH_3, 3H).

Ethyl-2-((3*aR*,4*R*,6*aR*)-2,2-dimethyl-6-oxotetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)acetate (40). Diisopropylamine (DIPA) (5.00 mL) was freshly distilled and added to 100 mL THF. At -5 °C N-butyllithium (n-BuLi) (2.5 M solution in THF, 15 mL, 37.5 mmol) was added dropwise over 10 minutes. The reaction mixture was stirred for 15 minutes and then cooled to -40 °C. At this temperature ethyl(trimethylsilyl)acetate (5.0 mL, 27 mmol) was added dropwise over 5 minutes. The reaction mixture was stirred at -40 °C for 40 minutes. The reaction mixture was cooled to -78 °C and a solution of hexamethylphosphoramide (HMPA) and THF (1:1, 25 mL) was added. Then, a solution of cyclopentenone **1** (3.85 g, 25.0 mmol) in THF (30 mL) was added dropwise and the resulting solution stirred 2 h. The reaction mixture was then allowed to warm to -40 °C and quenched with saturated NH_4Cl solution (50 mL). The layers were separated and the aqueous layer extracted 3x with 150 mL CH_2Cl_2. The combined organic phases were reduced *in vacuo* until the only solvent left was the high-boiling HMPA. To this crude residue of compound **39** was added a solution of KF (2.00 g, 34.4 mmol) in EtOH / H_2O (2:1, 60 mL). The reaction mixture was stirred at rt for 6 h. Thereafter the layers were separated and the aqueous layer was extracted 3x with 150 mL CH_2Cl_2. The combined organic phases were dried over $MgSO_4$. Filtration and evaporation of solvent followed by column chromatography on silica gel (hexanes/ EtOAc = 4:1) afforded **40** as a clear liquid (2.77 g, 45.9% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.60 (d, *J*=5.6 Hz, 1H), 4.37 (d, *J*=5.5 Hz, 1H), 4.09 (q, 2H), 2.79 (m, 1H), 2.70 (m, 1H), 2.48 (m, 2H), 2.09 (m, 1H), 1.42 (s, 3H), 1.32 (s, 3H), 1.22 (t, 3H). The ^{13}C NMR agreed with literature.[42]

Ethyl 2-((3*aR*,4*R*,6*S*,6*aS*)-6-hydroxy-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)acetate (41). To a solution of **40** (2.00 g, 8.26 mmol) in methanol (25 mL) were added $NaBH_4$ (0.469 g, 12.4 mmol) and $CeCl_3 \cdot 7\ H_2O$ (2.62 g, 7.02 mol) at -10 °C. The milky reaction mixture was stirred for 1.5 h at 0 °C and then quenched with sat. NH_4Cl solution (30 mL). The layers were separated and the aqueous layer extracted 3x with CH_2Cl_2. The combined organic phases were washed with water and brine and dried over $MgSO_4$. Purification via column chromatography on silica gel (hexanes/EtOAc = 1:1) afforded **41** as a clear liquid (1.67 g, 82.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.50 (d, *J*=5.6 Hz, 1H), 4.39 (d, *J*=5.6 Hz, 1H), 4.13 (q, 2H), 3.07 (br, 1H), 2.40 (d, J=7.8 Hz, 1H), 2.29-2.21 (m, 2H), 1.99-1.93 (m, 1H), 1.76-1.70 (m, 1H), 1.51 (s, 3H), 1.34 (s, 3H), 1.25 (t, 3H). The ^{13}C NMR agreed with the literature.[98]

Ethyl 2-((3*aR*,4*R*,6*S*,6*aS*)-6-en-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)acetate (42). To a solution of **41** (1.78 g, 7.30 mmol), PPh_3 (2.87 g, 11.0 mmol) and **6** (1.20 g, 7.80 mmol) in THF (80 mL) was added DIAD (2.5 mL, 13 mmol) dropwise at 0 °C. The reaction was refluxed between 50 °C and 80 °C and reaction progress monitored by TLC. The Mitsunobu reaction was unsuccessful and after a period of 5 days only the elimination product **42** was formed. HRMS (ESI+): m/z Calcd for $C_{12}H_{19}O_4$ $[(M + H)^+]$: 227.2781; found 227.2794.

(3a*R*,6*R*,6a*R*)-2,2-Dimethyl-6-vinyltetrahydrocyclopenta[1,3]dioxol-4-one (43). To a suspension of CuBr · Me_2S (600 mg, 10 mol-%, 2.34 mmol) in THF (50 mL) at -78 °C under N_2 was added vinylmagnesium bromide (1.0 M solution, 35 mL, 35 mmol) dropwise by syringe. The reaction mixture was stirred for 10 minutes and then a solution of cyclopentenone **1** (3.60 g, 23.4 mmol), TMSCl (6.00 mL, 46.8 mmol) and

HMPA (12.4 mL, 70.0 mmol) was added dropwise over 5 minutes. The reaction mixture was stirred at -78 °C for 4 h. The reaction was allowed to warm to 0 °C and quenched with sat. NH_4Cl solution (10 mL). THF was evaporated off *in vacuo* and the residue diluted with EtOAc (75 mL). The layers were separated and the aqueous layer was extracted 2x with 75 mL EtOAc. The combined organic phases were washed with 25 mL brine and dried over $MgSO_4$. Purification via column chromatography on silica gel (hexanes/ EtOAc = 4:1) afforded **43** as a clear liquid (3.00 g, 70.4%). The NMR spectral data was in accordance with the literature.[42,99]

(3a*S*,4*S*,6*R*,6a*R*)-2,2-Dimethyl-6-vinyltetrahydrocyclopenta[1,3]dioxol-4-ol (44). To a suspension of LAH (895 mg, 22.4 mmol) in THF (75 mL) at 0 °C under N_2 was added a solution of **37** (2.33 g, 12.8 mmol) in THF (25 mL) dropwise by syringe. The resulting reaction mixture was allowed to warm to rt and stirred at this temperature for 3 h. The reaction mixture was then carefully quenched sequentially with H_2O (1 mL), NaOH (10% solution, 1 mL) and H_2O (3 mL). A white precipitate formed that was filtered off and washed with CH_2Cl_2. The solvent was then evaporated in vacuo and column chromatogrpahy on silica gel (hexanes/EtOAc 3:1) afforded **44** as a clear liquid (2.07 g, 87.7%). The NMR spectral data was in accordance with the literature.[42]

1*H*-Imidazo[4,5-*c*]pyridine, 4-chloro-1-[(3a*S*,4*R*,6*R*,6a*R*)-6-ethenyltetrahydro-2,2-dimethyl-4*H*-cyclopenta-1,3-dioxol-4-yl] (45). To a solution of **44** (1.216 g, 6.609 mmol) and PPh_3 (2.60 g, 9.92 mmol) in THF (100 mL) under N_2 was added Cl-deazabase **6** (1.01g, 6.61 mmol). This suspension was cooled to 0 °C and DIAD (2.0 mL, 9.02 mmol) was added dropwise by syringe. The cloudy brown solution was then heated at 70-80 °C for 48 h. The reaction mixture turned clear after a few hours of

reflux. The THF was then evaporated off *in vacuo* and purification with silica gel chromatography (hexanes/EtOAc = 1:1) afforded **45** (855 mg, 40.5%) as a colorless oil which can form white crystals at rt over time. A small amount of compound **46** (N-7 regioisomer) (169 mg, 8.0%) in form of a colorless oil was also formed. **45**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (d, *J*=5.7 Hz, 1H), 8.04 (s, 1H), 7.60 (d, *J*=5.7 Hz, 1H), 5.97-5.91 (m, 1H), 5.30-5.18 (m, 2H), 4.71-4.65 (m, 2H), 4.60-4.57 (m, 1H), 2.99-2.90 (m, 1H), 2.70-2.65 (m, 1H), 2.39-2.33 (m, 1H), 1.64 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (62.9 MHz, CDCl3): δ 143.2, 142.0, 141.8, 140.2, 138.3, 137.0, 117.0, 114.8, 106.6, 84.9, 83.9, 62.3, 47.7, 35.7, 27.5, 25.1. X-ray structure of **45** determined, see Figure 20, page 66. Crystal structure determination was performed by Dr. John Gorden.

46: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 (s, 1H), 8.15 (d, *J*=5.6 Hz, 1H), 7.63 (d, *J*=5.6 Hz, 1H), 6.00-5.95 (m, 1H), 5.28-5.17 (m, 2H), 4.75-4.73 (m, 2H), 4.63-4.61 (m, 1H), 2.97-2.92 (m, 1H), 2.74-2.70 (m, 1H), 2.45-2.39 (m, 1H), 1.66 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 142.2, 142.1, 141.1, 139.9, 137.6, 137.0, 116.2, 114.2, 106.5, 84.5, 83.5, 61.9, 47.4, 35.2, 27.1, 24.7. HRMS (ESI+): m/z Calcd for $C_{16}H_{19}N_3O_2Cl$ [(M + H)$^{+}$]: 320.1166; found 320.1158.

2-((3aS,4R,6R,6aR)-4-(4-Chloro-1H-imidazo[4,5-c]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta-[d][1,3]dioxol-6-yl)ethanol (47). To a solution of **45** (600 mg, 1.88 mmol) in THF (50 mL) under N_2 atmosphere at 0 °C was added 9-BBN (0.50 M solution in THF, 7.6 mL, 3.8 mmol) dropwise by syringe. The resulting reaction mixture was allowed to warm to rt and stirred at this temperature for 3 h. Then NaOH (1 N solution, 5.00 mL) and H_2O_2 (30% solution, 5.00 mL) were added sequentially, which resulted in a cloudy solution. After stirring for 30 minutes the reaction mixture was

diluted with CH_2Cl_2 (100 mL) and sat. $NaHCO_3$ solution (20 mL) was added. The phases were separated and the organic phase was dried over $MgSO_4$. Filtration and evaporation of solvent *in vacuo* followed by column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **47** as a clear oil (564 mg, 88.8%). The NMR spectral data was in accordance with the literature.[83]

2-((3*aR*,4*R*,6*R*,6*aS*)-6-(4-Chloro-1H-imidazo[4,5-c]-pyridin-1-yl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)acetaldehyde (48). To a solution of **47** (100 mg, 0.297 mmol) in CH_2Cl_2 over 4 Å molecular sieves was added PDC (114 mg, 0.297 mmol) and the resulting solution was stirred for 48 h while monitoring the reaction progress via TLC. The brown slurry of inorganic chromate salts was filtered and washed with MeOH and then EtOAc. Evaporation of solvent *in vacuo* followed by column chromatography on silica gel (EtOAc/hexanes = 3:1) afforded **48** as a white solid (90 mg, 90.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.1, 143.1, 142.0, 141.7, 140.0, 138.1, 114.8 106.5, 84.5, 83.4, 62.4, 46.3, 38.2, 35.5, 27.4, 25.1.

(*R*)-1-((3*aR*,4*R*,6*R*,6*aS*)-6-(4-Chloro-1H-imidazo[4,5-c]-pyridin-1-yl)-2,2-dimethyltetrahydro-3a*H*-cyclopental[d][1,3]dioxol-4-yl)propan-2-ol (48b). (unsuccessful attempt) (+)-TADDOL (0.070 mmol, 33 mg) and $Ti(OiPr)_4$ (0.1 mL, 0.3) mmol) were mixed in dry toluene (2 mL) at rt under N_2 and stirred at this temperature over-night. This resulted in a yellow slurry, which indicates the formation of TADDOLate **49**.[86] The reaction mixture was then cooled to -25 °C and **48** (90.0 mg, 0.268 mmol) in CH_2Cl_2 (10 mL) was added dropwise by syringe. Dimethylzinc solution was added (2.0 M in toluene, 0.3 mL, 0.6 mmol) by syringe and the reaction was stirred

for a total of 48 h. The reaction progress was monitored by TLC but no formation of product or depletion of starting material was observed. After purification, reactant **48** was isolated quantitatively.

2-((3aS,4R,6R,6aR)-4-(4-Chloro-1H-imidazo[4,5-c]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta-[d][1,3]dioxol-6-yl)-phthalimidoethane (51). Compound **47** (320 mg, 0.950 mmol), PPh_3 (375 mg, 1.43 mmol) and phthalimide (210 mg, 1.43 mmol) were dissolved in THF (30 mL). DIAD (0.37 ml, 1.9 mmol) was added dropwise by syringe and the reaction mixture was stirred at rt for 3 h. The THF was evaporated off affording a yellow oil, which was diluted with 10 mL diethyl ether. Recrystallization from diethyl ether proved unsuccessful and purification was accomplished by column chromatography on silica gel (EtOAc/hexanes = 3:1). Separation from the byproduct triphenylphosphine oxide was extremely difficult. Compound **51** was afforded as a white solid in nearly quantitative yield with some PPh_3O impurity (440 mg, 99.3%). The yield was determined by NMR integration. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, *J*=5.7 Hz, Ar-H, 1H), 8.03 (s, Ar-H, 1H), 7.81-7.66 (m, phthalimide protons, 4H), 7.54 (d, *J*=5.7 Hz, Ar-H, 1H), 4.58-4.57 (m, 2H), 4.39 (m 1H), 3.74 (m, 2H), 3.43-3.41 (m, 1H), 2.76-2.74 (m, 1H), 2.21 (m, 1H), 2.11 (m, 2H), 1.48 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4 (C=O), 142.9, 142.0, 141.5, 140.1, 138.1, 134.0, 133.0, 123.2, 114.5, 106.6, 84.8, 84.6, 65.8, 62.2, 41.7, 36.4, 36.1, 32.2, 27.2. 15.2.

Compound (52). Compound **51** (440 mg, 0.940 mmol) was suspended in ethanol (15 mL) and anhydrous hydrazine (1.5 mL, 50 mmol) under N_2 atmosphere. The reaction mixture was brought to reflux at 70 °C and stirred at this temperature over-night. After cooling to rt the insoluble white solid byproduct (phthalhydrazide) was filtered off and

the solvent was reduced *in vacuo*. The crude product, which was extremely polar, was then washed with EtOAc. EtOAc only dissolves triphenylphosphine oxide impurity (carried over from previous step), which can then be decanted off. After evaporation to dryness, column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded compounds **52**, **53** and **54** in a ratio of 3:7:2 as white solids.

Compound **52**: ^{1}H NMR (600 MHz, D_2O): δ 8.16 (s, Ar-H, 1H), 7.36 (d, *J*=7.2 Hz, Ar-H, 1H), 6.91 (d, *J*=7.4 Hz, Ar-H, 1H), 4.83 (m, 2H), 4.65-4.62 (m, 1H), 3.76-3.72 (m, 1H), 3.14-3.10 (m, 2H), 2.62 (m, 1H), 2.34 (m, 1H), 1.87 (m, 2H), 1.63(s, 3H), 1.34 (s, 3H). ^{13}C NMR (150 MHz, D_2O): δ 144.0, 140.6, 140.5, 139.7, 136.0, 114.9, 107.1, 84.2, 83.8, 67.7, 57.3, 48.7, 40.5, 35.1, 26.2, 24.1.

Compound **53**: ^{1}H NMR (600 MHz, D_2O): δ 8.2 (s, Ar-H, 1H), 7.84 (d, *J*=5.7 Hz, Ar-H, 1H), 7.40 (d, *J*=5.8 Hz, Ar-H, 1H), 4.63 (m, 2H), 4.50 (m, 1H), 2.82 (t, 2H), 2.53-2.49 (m, 1H), 2.23-2.19 (m, 1H), 2.04 (m, 1H), 1.80-1.71 (m, 2H), 1.56 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (150 MHz, D_2O): δ 140.6, 132.8, 130.2, 129.3, 125.6, 115.0, 96.1, 84.4, 83.6, 67.8, 61.2, 40.5, 37.9, 35.4, 26.1, 24.9.

Compound **54**: ^{1}H NMR (600 MHz, D_2O): δ 8.72 (s, Ar-H, 1H), 8.18 (s, Ar-H, 1H), 8.15 (d, *J*=5.8 Hz, Ar-H, 1H), 7.48 (d, *J*=5.8 Hz, Ar-H, 1H), 4.60-4.53 (m, 2H), 4.48-4.45 (m, 1H), 2.68 (t, 2H), 2.43-2.39 (m, 1H), 2.18-2.13 (m, 1H), 2.05 (m, 1H), 1.99 (m, 1H), 1.68-1.60 (m, 2H), 1.55 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, D_2O): δ 140.6, 132.8, 130.2, 129.3, 125.6, 115.0, 96.1, 84.4, 83.6, 67.8, 61.7, 61.2, 48.8, 40.5, 37.9, 26.1, 24.9.

1H-imidazo[4,5-c]pyridin-4-amine (or 3-deazaadenine) (55). To compound **6** (11.0 g, 71.8 mmol) were added anhydrous hydrazine (140 mL, 44.46 mol) and anhydrous 1-propanol (100 mL) and the resulting solution was stirred under reflux

overnight resulting in a clear yellow solution. The reaction progress was monitored with TLC. When all starting material had disappeared on the TLC, the reaction was cooled down to room temperature and 1-propanol and hydrazine were evaporated off *in vacuo*. Water (300 mL) and Raney nickel (slurry in water) were added and the reaction mixture was refluxed for 1 h. The Raney nickel was removed by hot filtration over a celite pad and the solved was reduced *in vacuo*, affording **55** as a yellow solid (9.00 g, 93.6% for two steps). The NMR data agreed with the literature.[59,92]

tert-Butyl 4-(bis(tert-butoxycarbonyl)amino)-1H-imidazo[4,5-c]pyridine-1-carboxylate (or tris-boc-3-deazaadenine) (56). To compound **55** (2.00 g, 14.9 mmol) under N_2 was added generous amounts of THF (500 mL). Then DMAP (190 mg, 1.5 mmol) and di-*tert*-butyl dicarbonate (Boc_2O) (13.0 g, 59.7 mmol) were added and the resulting reaction mixture stirred for 2 days. **55** is very poorly soluble in THF but as it undergoes carbamate formation from mono-boc to di-boc to tri-boc protected product the solubility increases highly and results in a clear solution. The solvent was then evaporated in vacuo and column chromatography on silica gel (EtOAc/hexanes 3:1 gradient to pure EtOAc) afforded **56** as a white foam. Impure fractions were used in the next step and separated off thereafter. The NMR data agreed with the literature.[59,92]

tert-Butyl 1H-imidazo[4,5-c]pyridin-4-ylcarbamate (57). To the solution **56** (2.58 g, 5.94 mmol) in 200 mL THF was added TBAF (2eq, 1.0 M solution, 12 mL, 12 mmol) and the resulting reaction mixture was stirred at rt overnight. Water (75 mL) was added and the solvent was reduced *in vacuo*. The mixture was then diluted with EtOAc (100 mL). The phases were separated and the aqueous phase was extracted with 2x 100 mL EtOAc. The combined organic phases were extracted with brine (50 mL) and dried

over $MgSO_4$. Filtration and evaporation of solvent *in vacuo* followed by column chromatography on silica gel (CH_2Cl_2/MeOH = 18:1) afforded **57** as a white foam (1.77 g, 35.6%). The NMR data agreed with the literature.[59,92]

tert-Butyl 1H-imidazo[4,5-c]pyridin-4-ylcarbamate (57). Reformation from compound 62 after Mitsunobu reaction. Compound **62** (1.68 g, 4.00 mmol) was dissolved in 200 mL THF and TBAF (1.0 M solution, 8.0 mL, 8.0 mmol) were added and the resulting reaction mixture was stirred for 4 hours at rt. Water (75 mL) was added and the solvent was reduced in vacuo. The mixture was then diluted with EtOAc (75 mL). The phases were separated and the aqueous phase was extracted with 2x 75 mL EtOAc. The combined organic phases were extracted with brine (30 mL) and dried over $MgSO_4$. Filtration and evaporation of solvent *in vacuo* followed by column chromatography on silica gel (CH_2Cl_2/MeOH = 18:1) afforded **57** as a white foam (1.23 g, 92.0%). The NMR data agreed with the literature.[59,92]

Compound (61). Compounds **44** (0.975 g, 5.30 mmol), **57** (1.77 g, 5.30 mmol) and PPh_3 (2.08 g, 7.95 mmol) were dissolved in THF (50 mL) under N_2 and the resulting solution cooled to 0 °C. DIAD (1.68 mL, 7.95 mmol) was added dropwise by syringe. The yellow reaction mixture was stirred overnight at rt resulting in a red solution, which was refluxed for 12 h at 70 °C. The solvent was removed *in vacuo* and purification of the crude product via column chromatography on silica gel (hexanes/EtOAc = 2:1 gradient to 1:1) afforded **61** as a white solid (1.69 g, 63.6%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.34 (d, *J*=5.6 Hz, Ar-H, 1H), 8.02 (s, Ar-H, 1H), 7.61 (d, *J*=5.6 Hz, Ar-H, 1H), 5.95-5.92 (dd, CH_2=C**H** (internal),1H), 5.23 (dd, J_1=17.2 Hz, CH-C**H**$_2$ (terminal), 1H), 5.18 (dd, J_1=10.4 Hz, CH-C**H**$_2$ (terminal), 1H), 4.73-4.69 (m, 2H), 4.59-4.57 (m, 1H), 2.93-2.90

(m, 1H), 2.67-2.63 (m, 1H), 2.36-2.32 (m, 1H), 1.64 (s, 3H), 1.43 (s, Boc-hydrogens, 18H), 1.34 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 151.3 (boc group C=O), 144.4, 141.9, 140.9, 140.6, 137.0, 136.9, 116.5, 114.4, 106.9, 84.6, 83.7, 82.8 (Boc-Cq **C(**$CH_3)_3$), 61.9, 47.7, 35.5, 27.8 (boc-CH_3), 27.3, 24.9.

Compound (63). 61 (500 mg, 1.00 mmol) was dissolved in THF (75 mL) under N_2 atmosphere and the resulting solution cooled to 0 °C. At this temperature 9-BBN (0.5 M solution in THF, 2.0 mmol, 4.0 mL) was added dropwise by syringe. The resulting reaction mixture was allowed to warm to rt and stirred at this temperature for 4 h. Then NaOH (1 N solution, 4.00 mL) and H_2O_2 (30% solution, 4.00 mL) were added sequentially, which resulted in a cloudy solution. After stirring for 1 h the reaction mixture was diluted with CH_2Cl_2 (100 mL) and sat. $NHCO_3$ solution (50 mL) was added. The phases were separated and the organic phase was dried over $MgSO_4$. Filtration and evaporation of solvent *in vacuo* followed by column chromatography on silica gel (CH_2Cl_2/MeOH = 18 : 1) afforded **63** as a white solid (420 mg, 81.1%). ^1H NMR (600 MHz, $CDCl_3$): δ 8.31 (d, *J*=5.7 Hz, Ar-H, 1H), 8.08 (s, Ar-H, 1H), 7.65 (d, *J*=5.7 Hz, Ar-H, 1H), 4.70-4.64 (m, 2H), 4.50-4.47 (m, 1H), 3.55 (br, -OH, 1H), 3.78-3.75 (m, 4H), 2.70-2.66 (m, 1H), 2.40-2.34 (m, 1H), 1.19-2.13 (m, 1H), 1.63 (s, 3H), 1.40 (s, Boc-hydrogens, 18H), 1.33 (s, 3H).

Compound (64). To a solution of **63** (220 mg, 0.420 mmol), PPh_3 (168 mg, 0.640 mmol) and phthalimide (94 mg, 0.64 mmol) in THF (50 mL) under N_2 atmosphere was added DIAD (2 eq, 0.17 mL, 0.84 mmol) dropwise by syringe. The resulting reaction mixture was stirred for 3 h at rt. The solvent was evaporated off *in vacuo* and the crude product was purified via column chromatography on silica gel (EtOAc/hexanes = 4:1) to

afford **64** as a white solid (268 mg, 98.5%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.36 (d, *J*=5.6 Hz, Ar-H, 1H), 8.06 (s, Ar-H, 1H), 7.89-7.74 (m, phthalizide-H,4H), 7.64 (d, *J*=5.6 Hz, Ar-H, 1H), 4.71-4.65 (m, 2H), 4.48-4.45 (m, 1H), 3.96-3.88 (m, 1H), 3.85-3.79 (m, 1H), 2.82-2.78 (m, 1H), 2.31-2.26 (m, 2H), 2.23-2.13 (m, 2H), 1.53 (s, 3H), 1.45 (s, boc-hydrogens, 18H), 1.21 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4 (C=O, phthalimide), 151.3 (C=O, Boc), 144.5, 141.9, 140.9, 140.7, 136.9, 134.0, 132.9, 123.2, 114.4, 107.0, 84.7, 84.5, 82.8 (Boc-Cq **C(**$CH_3)_3$), 62.0, 41.8, 36.5, 32.2, 27.9 (boc-CH_3), 27.2, 24.7, 22.0.

2-((3aS,4R,6R,6aR)-4-(4-Amino-1H-imidazo[4,5-c]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta-[*d*][1,3]dioxol-6-yl)ethylamine (65). Compound **64** (96 mg, 0.15 mmol) was suspended in abs. ethanol (5.00 mL) under N_2 atmosphere and hydrazine was added (1.00 mL). The reaction mixture was brought to reflux at 70 °C and stirred at this temperature over-night. After cooling to rt the insoluble white solid byproduct (phthalhydrazide) was filtered off and the solvent was reduced *in vacuo*. The crude product was purified via column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) to afford **65** as a white solid (47 mg, 99.9%). ^{1}H NMR (600 MHz, D_2O): δ 7.99 (s, Ar-H, 1H), 7.65 (d, *J*=6.0 Hz, Ar-H, 1H), 6.88 (d, *J*=6.1 Hz, Ar-H, 1H), 4.55-4.52 (m, 1H), 4.48-4.42 (m, 2H), 2.94 (m, 2H), 2.41-2.37 (m, 1H), 2.16-2.14 (m, 1H), 2.03-2.02 (m, 1H), 1.86-1.77 (m, 2H), 1.59 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 151.8, 141.3, 140.4, 139.4, 126.1, 115.5, 99.9, 84.9, 84.4, 61.7, 41.2, 39.0, 35.8, 32.7, 26.9, 24.8.

[(3*aR*,4*R*,6*R*,6*aS*)-3-(4-Amino-1H-imidazo[4,5-c]pyridin-1-yl)-5-(aminoethyl)-1,2-cyclopentanediol Dihydrochloride (or 6'amino-3-deaza-

homoaristeromycin) (50). To **65** (47 mg, 0.15 mmol) were added HCl (2 M, 1.5 mL) and MeOH (4.0 mL) and the resulting suspension was stirred overnight. After TLC (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) indicated completion of the reaction, the solution was neutralized using Amberlite IRA-67 resin. The resin was washed with MeOH and water prior to addition to the reaction mix. The resin was filtered off, the solvent evaporated *in vacuo* and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **50** as a white solid (36 mg, 89%). ^{1}H NMR (600 MHz, D_2O): δ 8.13 (s, Ar-H, 1H), 7.61 (d, *J*=6.0 Hz, Ar-H, 1H), 6.91 (d, *J*=6.0, Ar-H, 1H), 4.56 (m, 1H), 4.28 (m, 1H), 3.91 (m, 1H), 3.08 (t, *J*=7.6, N-CH_2, 2H), 2.39 (m, 1H), 2.07-1.95 (m, 2H), 1.87-1.80 (m, 1H), 1.60 (m, 1H). ^{13}C NMR (100 MHz, D_2O): δ 150.7, 141.4, 139.1, 138.3, 126.2, 99.1, 75.0, 74.2, 60.8, 54.7, 42.9, 40.2, 38.0 Anal. Calcd for $C_{13}H_{19}N_5O_2 \cdot 2HCl \cdot 0.75CH_3OH \cdot 0.25H_2O$: C, 43.73; H, 6.51; N, 18.54. Found: C, 43.11; H, 6.42; N, 17.78.

1-Bromo-2-((3aS,4R,6R,6aR)-4-(4-Chloro-1H-imidazo[4,5-c]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta-[d][1,3]dioxol-6-yl)ethanol (66). Dry CH_2Cl_2 (50 mL) was cooled to 0 °C and pyridine (0.11 mL, 1.3 mmol) then triflic anhydride (347 mg, 1.23 mmol) were added. After 5 minutes compound **47** (200 mg, 0.593 mmol) was added. The resulting reaction mixture was stirred for 90 minutes at 0 °C. After TLC indicated that the reactant had been consumed, Bu_4NBr was added and the reaction mixture was stirred for 4 hours at 0 °C. $NaHCO_3$ (20 mL) was then added, the phases were separated and the aqueous phase was washed 3x with 20 mL CH_2Cl_2. The combined organic phases were dried over $MgSO_4$. Filtration, evaporation of solvent *in vacuo* followed by column chromatography on silica gel (EtOAc/ hexanes = 4:1) afforded

66 as a white solid (100 mg, 42.2% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J*=5.6, Hz, Ar-H, 1H), 8.04 (s, Ar-H, 1H), 7.60 (d, *J*=5.6 Hz, Ar-H, 1H), 4.71-4.64 (m, 2H), 4.47-4.44 (m, 1H), 3.53 (t, *J*=6.4 Hz, Br-CH_2, 2H), 2.75-2.69 (m, 1H), 2.50-2.43 (m, 1H), 2.28-2.18 (m, 1H), 2.15-2.09 (m, 2H), 1.64 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 142.9, 141.9, 141.6, 140.1, 138.0, 114.8, 106.6, 84.8, 84.2, 62.0, 42.6, 36.5, 35.6, 30.7, 27.4, 25.1.

(65) (route B from 66). To compound **66** (100 mg, 0.250 mmol) were added anhydrous hydrazine (10.0 mL, 318 mmol) and anhydrous 1-propanol (4.0 mL) under N_2 atmosphere and the resulting reaction mixture was refluxed for 12 h. TLC (CH_2Cl_2, MeOH/NH_4OH = 9: 1: 0.1) was used to monitor the reaction progress and showed that the reactant was consumed and a product spot had formed significantly below the reactant spot. At this point all solvents were evaporated off and the residue was dissolved in water (20 mL). Raney nickel (slurring in water, 1 spatula tip) was added and the reaction mixture was refluxed for 1 hour. Hot filtration of the Raney nickel over a celite pad, followed by evaporation of solvent and column chromatography on silica gel afforded **65** as a white solid (65 mg, 82.3%).

2-((3aS,4R,6R,6aR)-4-(4-Amino-1H-imidazo[4,5-c]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta-[*d*][1,3]dioxol-6-yl)ethanol (68). To **47** (330 mg, 0.980 mmol) were added anhydrous hydrazine (40.0 mL, 1.27 mol)) and anhydrous 1-propanol (25.0 mL) under N_2 atmosphere. The reaction mixture was refluxed for 12 h. The reaction progress was monitored via TLC (CH_2Cl_2, MeOH/NH_4OH = 9: 1: 0.1). With the consumption of product a crescent-shaped spot was formed on the TLC with a much lower R_f- value. All

solvent was then evaporated off *in vacuo* and water (25 mL) and Raney nickel (1 spatula tip) were added to the residue. The reaction mixture was refluxed for 1 hour. Raney nickel was removed by hot filtration on a celite pad. Evaporation of solvent *in vacuo* followed by column chromatography on silica gel (CH_2Cl_2, MeOH/NH_4OH = 9: 1: 0.1) afforded **68** as a white solid (280 mg, 89.7% for two steps). The NMR data agreed with the literature.[83]

Compound 69 (unsuccessful attempt). Dry CH_2Cl_2 (50 mL) was cooled to 0 °C and pyridine (0.12 mL, 1.4 mmol) then triflic anhydride (0.22 mL, 1.3 mmol) were added and the resulting solution was stirred for 5 minutes. A solution of **68** (280 mg, 0.880 mmol) in CH_2Cl_2 (30 mL) was added dropwise by syringe and the reaction mixture was stirred for 90 minutes at 0 °C. Then Bu_4NBr (313 mg, 0.970 mmol) was added and the reaction mixture was stirred for 5 hours at 0 °C. A weak $NaHCO_3$ solution was added (20 mL) and the phases were separated. The aqueous phase was extracted 3x with 30 mL CH_2Cl_2 and the combined organic phases were dried over $MgSO_4$. No product could be isolated after purification attempt via column chromatography on silica gel (CH_2Cl_2/ MeOH = 18:1).

(3aα,4α,6α, 6aα)-4-(Hydroxyethyl)-6-[4-[[(dimethylamino)methylene]amino]-lH-imidazo[4,5-c]pyridin-l-yl]-tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxole (70). To a suspension of **68** (200 mg, 0.630 mmol) in dry DMF (10 mL) was added N,N-dimethylformamide dimethylacetal (0.39 mL, 2.8 mmol). The reaction mixture was stirred under N_2 for 12 h after which time TLC showed consumption of **68** and formation of product. The solvent was evaporated off *in vacuo* at 65 °C. Dilution with EtOH helped accelerate the evaporation process. Column chromatography on a short silica gel column

(CH_2Cl_2, MeOH/NH_4OH = 9: 1: 0.1) afforded **70** as a white solid (170 mg, 73.9%). ^1H NMR (600 MHz, D_2O): δ 8.48 (s, N=CH, 1H), 8.16 (s, Ar-H, 1H), 7.78 (d, *J*=6.0 Hz, Ar-H, 1H), 7.20 (d, *J*=6.0 Hz, Ar-H, 1H), 4.66-4.64 (m, 1H), 4.60 (m, 1H), 4.57-4.50 (m, 1H), 3.62 (t, *J*=7.2 Hz, HO-CH_2, 2H), 3.15 (s, N-CH_3, 3H), 3.07 (s, N-CH_3, 3H), 2.47-2.42 (m, 1H), 2.21-2.17 (m, 1H), 2.10-2.04 (m, 1H), 1.82-1.77 (m, 1H), 1.71-1.67 (m, 1H), 1.55 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 158.3 (N=**C**Me_2), 142.5, 141.1, 140.7, 139.0, 136.9, 114.6, 102.2, 99.2, 84.3, 84.0, 71.6, 61.4, 59.8, 48.7, 40.9, 39.6, 34.9, 26.2

Compound 71. Dry CH_2Cl_2 (50 mL) was cooled to 0 °C and pyridine (0.10 mL, 0.73 mmol) then triflic anhydride (0.12 mL, 0.68 mmol) were added and the resulting solution was stirred for 5 minutes. A solution of **70** (170 mg, 0.456 mmol) in CH_2Cl_2 (30 mL) was added dropwise by syringe and the reaction mixture was stirred for 1 hour at 0 °C. Then Bu_4NBr (313 mg, 0.970 mmol) was added and the reaction mixture was stirred for 5 hours at 0 °C. A weak $NaHCO_3$ solution was added (20 mL) and the phases were separated. The aqueous phase was extracted 3x with 30 mL CH_2Cl_2 and the combined organic phases were dried over $MgSO_4$. Filtration, evaporation of solvent followed by column chromatography on silica gel (CH_2Cl_2, MeOH/NH_4OH = 9: 1: 0.1) afforded **71** as a white solid (87 mg, 44%).

6'-Bromo-3-deazahomoaristeromycin (67). To **71** (87 mg, 0.20 mmol) were added HCl (2M, 2.0 mL) and MeOH (4.0 mL) and the resulting suspension was stirred overnight. After TLC (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) indicated completion of the reaction, the solution was neutralized using Amberlite IRA-67 resin. The resin was washed with MeOH and water prior to addition to the reaction mix. The resin was filtered

off, the solvent evaporated *in vacuo* and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **67** as a white solid (36 mg, 89%). ^{1}H NMR (600 MHz, D_2O): δ 8.05 (s, Ar-H, 1H), 7.54 (d, *J*=6.0 Hz, Ar-H, 1H), 6.80 (d, *J*=6.0 Hz, Ar-H, 1H), 4.53-4.47 (m, 1H), 4.24-4.20 (m, 1H), 3.90-3.87 (m, 1H), 3.71 (t, *J*=7.8 Hz, Br-CH_2, 2H), 2.37-2.31 (m, 1H), 2.06-1.95 (m, 1H), 1.92-1.91 (m, 1H), 1.84-1.77 (m, 1H), 1.60-1.51 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 150.8 141.0, 139.0, 138.8, 126.1, 98.9, 75.0, 74.2, 60.7, 55.0, 44.9, 40.2, 38.6. HRMS (ESI+): m/z Calcd for $C_{13}H_{17}N_4O_2Br$ [$(M + H)^+$]: 341.0613; found 341.0627.

Compound 72. To an ice-chilled suspension of **63** (200 mg, 0.390 mmol) in HMPA (3 mL) was added $SOCl_2$ (0.5 mL) and the resulting solution was stirred for 12 h at rt. TLC showed that the product had been completely deprotected by the development of HCl and the spot had moved to the baseline (R_f = 0). A 2N solution of NaOMe in MeOH (20 ml) containing *iso*butyl mercaptan (1.5 mL, 12 mmol) was added to the reaction mixture and stirred at rt overnight. Due to the loss of the protecting groups and the resulting polarity, purification attempts were unsuccessful and the product could not be isolated as the product could not be moved from the baseline of the TLC by even the most polar solvent system that's viable on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1).

(74). (Route B for the synthesis of 72). To ice-chilled CH_2Cl_2 (30 mL) was added dry pyridine (0.1 mL, 1 mmol) then immediately triflic anhydride (0.16 mL, 0.95 mmol). After 5 minutes this solution was added dropwise by syringe to a flask containing **68** (100 mg, 0.315 mmol). The reaction mixture was stirred for 1 hour. Solvent was then evaporated *in vacuo* and the residue was dissolved in 5 mL of a 2N solution of NaOMe in MeOH containing *iso*butyl mercaptan (0.75 mL, 6.0 mmol). The reaction mixture was

stirred overnight at rt. The reaction mixture was then neutralized with glacial acetic acid and the solvent was evaporated *in vacuo* followed by column chromatography of the crude product on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) to afford **74** (65 mg, 53%).

6'-*Iso*butylthio-3-deazahomoaristeromycin (72). To **74** (65 mg, 0.18 mmol) were added HCl (2M, 2.0 mL) and MeOH (4.0 mL) and the resulting suspension was stirred overnight. After TLC (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) indicated completion of the reaction, the solution was neutralized using Amberlite IRA-67 resin. The resin was washed with MeOH and water prior to addition to the reaction mix. The resin was filtered off, the solvent evaporated *in vacuo* and column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 9: 1: 0.1) afforded **72** as a white solid (56 mg, 90%). ^{1}H NMR (600 MHz, D_2O): δ ^{1}H NMR (600 MHz, D_2O): δ 8.20 (s, Ar-H, 1H), 7.61 (d, *J*=6.0 Hz, Ar-H, 1H), 6.95 (d, *J*=6.0 Hz, Ar-H, 1H), 4.62 (m, 1H), 4.29 (m, 1H), 3.85 (m, 1H), 2.39 (m, 1H), 2.16-2.09 (m, 4H), 2.07-1.95 (m, 2H), 1.87-1.80 (m, 1H), 1.60 (m, 1H), 1.33 (m, 1H), 0.95 (d, *J*=6.5 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 151.2, 141.0, 140.4, 139.3, 126.1, 100.2, 75.3, 72.8, 63.4, 42.2, 40.1, 32.8, 32.0, 30.3 26.8, 24.1. 22.0.
HRMS (ESI+): m/z Calcd for $C_{17}H_{26}N_4O_2S$ [$(M + H)^+$]: 351.1855; found 351.1844.

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