CARBOCYCLIC C-NUCLEOSIDES DERIVED FROM FORMYCIN

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CARBOCYCLIC C-NUCLEOSIDES DERIVED FROM FORMYCIN

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Submitted to

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CARBOCYCLIC C-NUCLEOSIDES DERIVED FROM FORMYCIN

Mingzhu He

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Date of Graduation

For my parents

Hong He and Linqing Cao

DISSERTATION ABSTRACT

CARBOCYCLIC C-NUCLEOSIDES DERIVED FROM FORMYCIN

Mingzhu He

Doctor of Philosophy December, 19, 2008
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Analogs of naturally occurring nucleosides have served as structural models for the design of antitumor, antiviral, and antibacterial agents. Some modified nucleosides have become major therapeutic agents for the treatment of viral infectious diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and the herpes viruses. Carbocyclic nucleosides (carbanucleosides) are nucleoside analogs in which the oxygen in the furanoses of traditional nucleosides is replaced by a methylene moiety. One relevant feature of these derivatives is their metabolic stability against phosphorylases as a consequence of the absence of the natural N-glycosidic bond. Much like carbanucleosides, C-nucleosides represent another class of nucleosides resistant to chemical and the enzymatic hydrolytic cleavage of the glycosidic bond. This results from

the structural arrangement in which the ribofuranosyl moiety is liked to a heterocyclic base through a C-C bond rather than the traditional C-N bond. Recently, nucleoside analog discovery has been focused on the hybrid nucleosides, carbocyclic *C*-nucleosides. This has been due to the challenging enantiomeric syntheses posed by carbocyclic C-nucleosides. Consequently, no significant biological activities for this class of compounds have been reported. Thus, it was of interest to undertake a study of carbocyclic C-nucleosides.

The main body of this research work deals with the progress towards carbocyclic formycin analogs as representative carbocyclic C-nucleosides by combining the structural components of formycin and 5'-noraristeromycin, a broadly active antiviral candidate that is devoid of the toxicity of aristeromycin. Synthesis of related *N*-methylated carbocyclic formycin analogs and their structural assignments are also described.

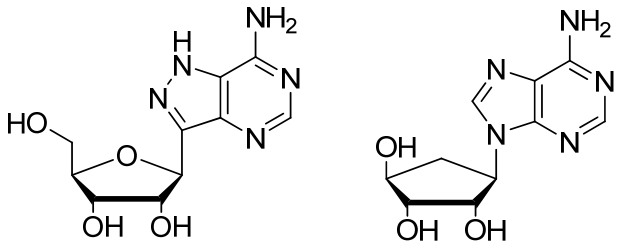

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TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Nucleosides are glycosylamines that involve binding a nucleobase (a nitrogen heterocycle often referred to simply as a base) to a ribofuranose or deoxyribofuranose ring. Examples include adenosine, guanosine, uridine, cytidine, and thymidine (Figure 1). It is known that nucleic acids, DNA and RNA, are the genetic material that cells and viruses use to produce faithful copies of themselves.[1] As the building blocks of DNA and RNA, the naturally occurring molecules of Figure 1 are vital to the functioning and multiplication of cells.[2] They are also important structural moieties in several coenzymes

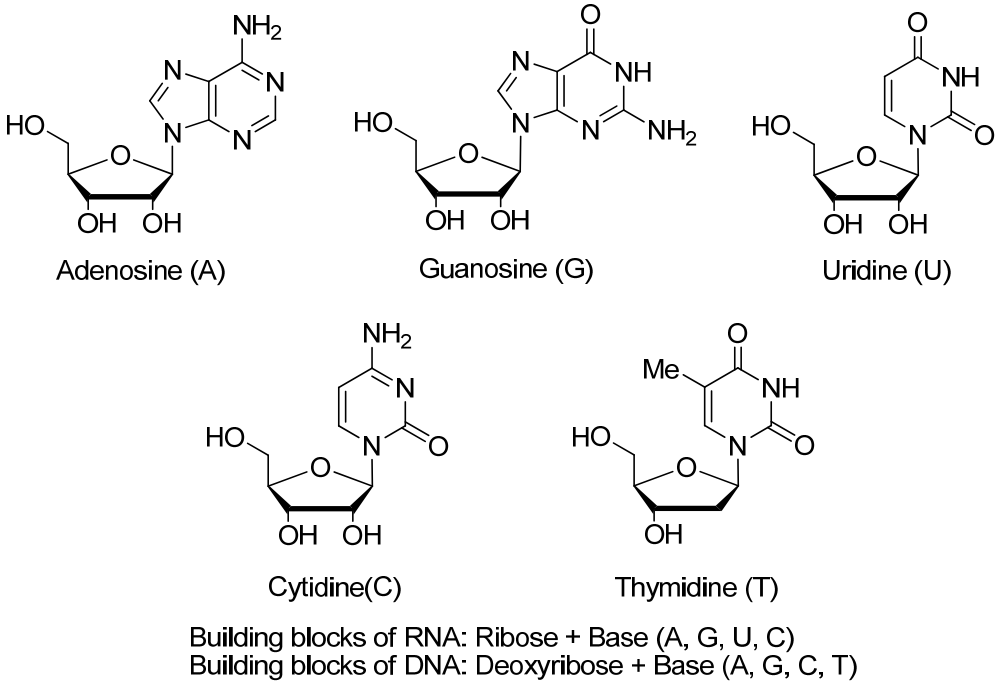


Figure 1. Naturally occurring nucleosides.

such as nicotinamide adenosine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), flavin adenine dinucleotide (FAD) and coenzyme A (Figure 2).

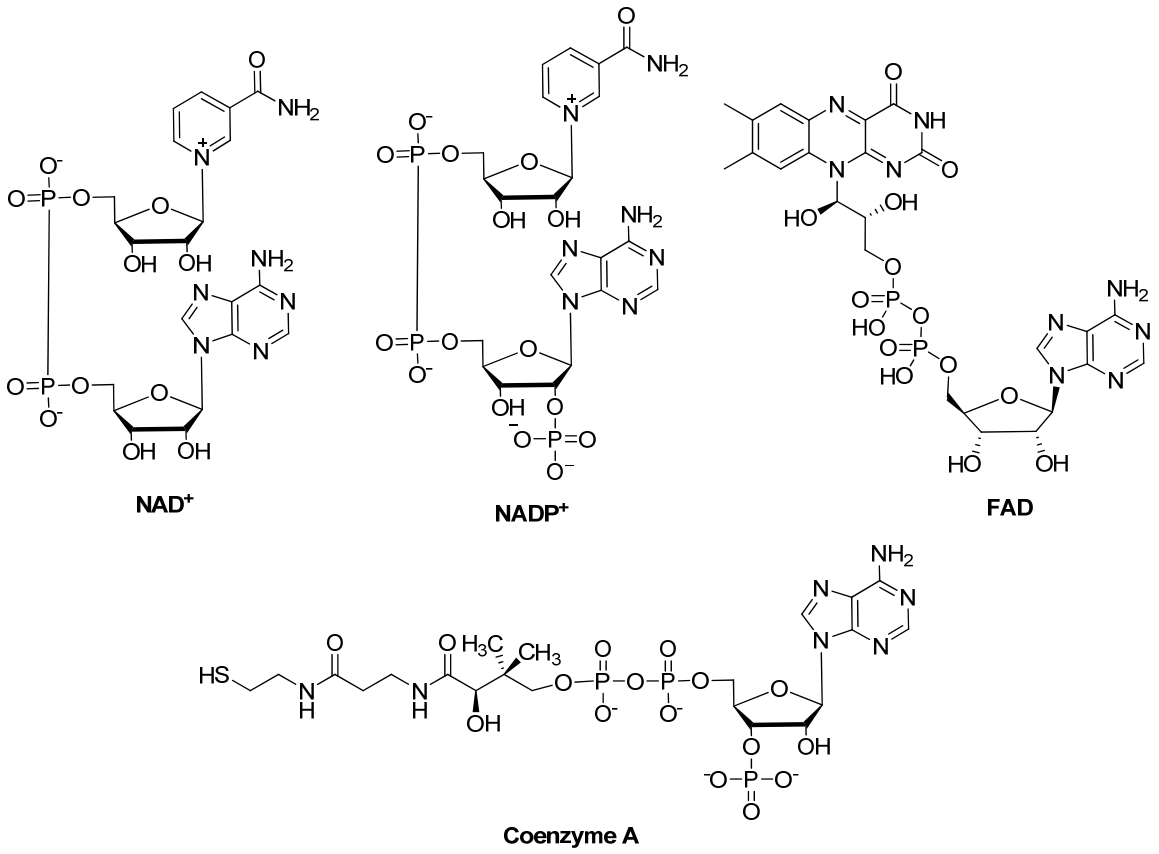


Figure 2. Examples of coenzymes.

Nucleosides also play important roles in fundamental metabolic pathways. For example, adenosine is a major component of adenosine triphosphate (ATP) and is implicated in sleep regulation.[3] Thus, modified nucleosides affect these enzymes and display a range of biological activities potentially.[4-7]

Because of the combined concern over emerging infections diseases and the increased possibility of bioterrorist attack, scientists are in an extensive search for new

drugs against viral infections.[8] In the latter case, particular attention has been paid to the orthopox family of viruses, especially to variola, the causative agent of smallpox or a similar virus, monkeypox virus.[9-13] For emerging threats, antiviral drugs are needed to be developed since vaccines for some widely occurring viruses such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV) are not available[14] or are accompanied with side effects, such as the Hepatitis B virus (HBV) vaccine.[15]

Since the advent of antiviral chemotherapy in 1959 with the discovery of the anti-herpes activity of 5-iodo-2′-deoxyuridine,[16] analogs of the natural nucleosides have served as structure models in the design of antitumor, antiviral, antibacterial agents. Some modified nucleosides have been synthesized and some of them have been clinically approved for treating different viruses.[17] Figure 3 shows the examples, ribavirin (β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) represents a base-modified nucleoside analog and it has a broad spectrum activity against both RNA and DNA viruses. Currently, it is used in the treatment of hepatitis C virus and respiratory syncytial infection.[18,19] The 5′-triphosphate of ribavirin inhibits viral RNA polymerase[20] and the viral specific mRNA capping enzyme guanylyltransferase.[21] Recently, ribavirin was shown to act as a RNA-virus mutagen, forcing RNA viruses into a lethal accumulation of errors, dubbed ‘error catastrophe’.[22,23] And, obviously, this lethal mutagenesis might be enhanced by the inhibitory effect of ribavirin (in its 5′-monophosphate form) on inosine monophosphate (IMP) dehydrogenase and the consequent decrease in cellular guanosine triphosphate (GTP) pools.[22,23]

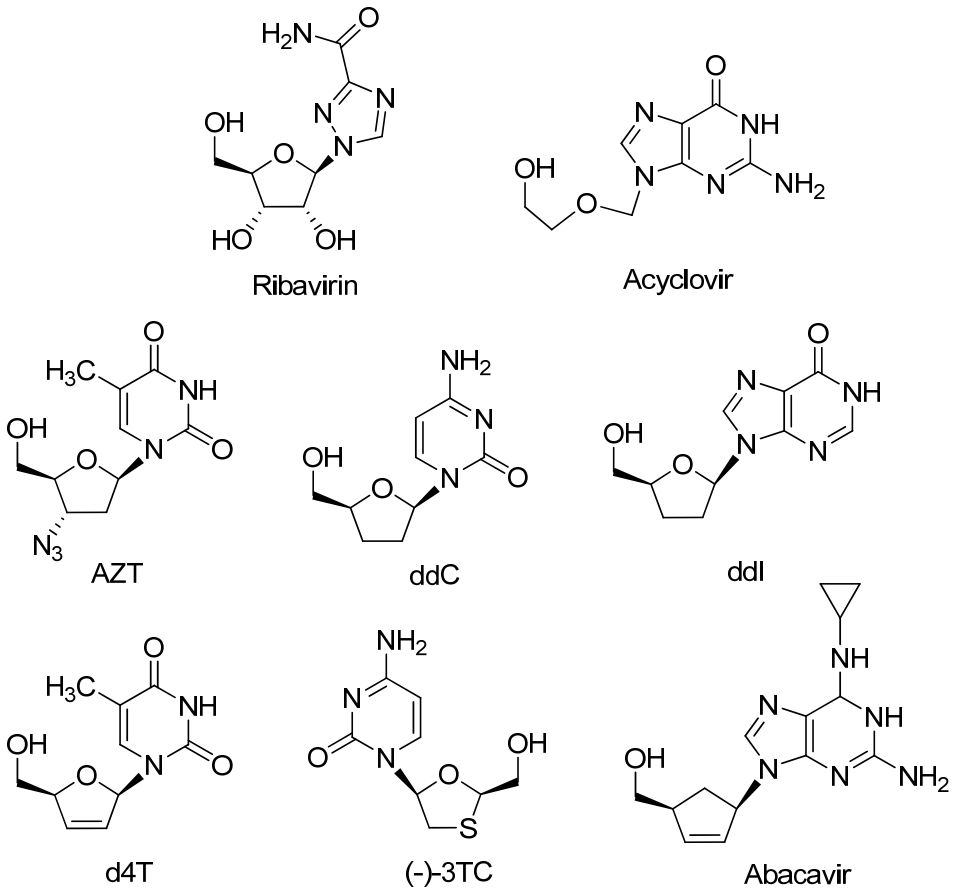


Figure 3. Examples of Modified Nucleosides.

Acyclovir ((9-hydroxyethoxy) methyl guanine), an acyclic nucleoside analog, inhibits the DNA polymerase of herpes simplex virus type 1, HSV-1.[24,25] Before it can interact with viral DNA synthesis, it needs to be phosphorylated intracellularly, in three steps, into the triphosphate form. The first phosphorylation step is ensured by the HSV-encoded thymidine kinase (TK), and is therefore confined to virus-infected cells. Subsequent phosphorylations are achieved by host cellular kinases.[24,26-28]

Other nucleoside variations include the 2′,3′-dideoxynucleoside analogs: 3′-azido-3′-deoxythymidine (AZT),[29,30] 2′,3′-dideoxycytidine (ddC),[31] 2′,3′-dideoxyinosine (ddI),[31] 2′,3′-didehydro-3′-deoxythymidine (d4T),[32,33] (-)-dideoxy-3′-thiacytidine ((-)-3TC),[34,35]

and Abacavir. These six nucleoside analogs have been licensed as anti-HIV drugs, and act by inhibiting the reverse transcriptase of human immunodeficiency virus.[36,37] All of these 2′,3′-dideoxynucleoside analogs act according to a similar 'recipe' as exemplified for AZT (Figure 4). They also must be phosphorylated consecutively inside the host cell by three cellular kinases to form the corresponding 5′-triphosphate derivative; this active metabolite can interact with the reverse transcription (RNA→DNA) reaction resulting in DNA chain termination since they lack the 3′-hydroxyl group necessary for the continued nucleic acid construction via a 3′, 5′-phosphodiester linkage.[38,39]

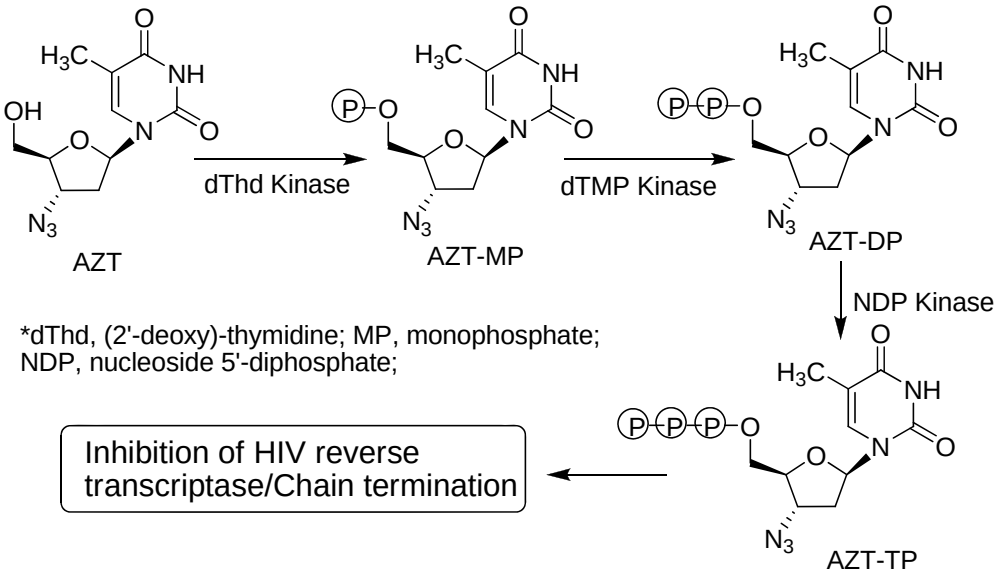


Figure 4. Metabolism of the anti-HIV agent AZT.

Antiviral Activity via Inhibition of *S*-Adenosylhomocysteine Hydrolase

What about an approach to antiviral agents that does not require phosphorylation for antiviral effects? Viruses, like any other replicating species, require the methylation of the 5′-terminal residue of viral mRNA for forming the cap structure necessary for viral protein translation and replication. This 5′-capped structure (Figure 5) consists of a N^7-methylguanosine residue linked at its 5′-hydroxy group to the 5′-end of the mRNA strand by an unusual 5′-5′ triphosphate bridge. Additionally, a methyl group on the 2′-hydroxy group of the penultimate adenine nucleosides is also required. This methylated "cap" is important in mRNA transport from the nucleus and for the following reasons: (1) efficient translation of the mRNA into proteins; (2) protection against ribonucleases and phosphatases; (3) mRNA splicing to ribosomes; and (4) the initiation of transcription of the viral mRNA.[40] Therefore interference of this 5′-capping process will definitely lead to the inhibition of viral replication. The capping process involves three enzymatic reactions (following steps) in which the initial mRNA 5′-triphosphate terminus is first cleaved to a diphosphate RNA (by RNA triphosphatase) followed by guanosine monophosphate (GMP) capping (by RNA guanylytransterase), and, finally, methylation at N7 position of guanine by RNA (guanine-7) methyltransferase.[41]

(1) $pppN(pN)_n \rightarrow ppN(pN)_n + P_i$

(2) $ppN(pN)_n + pppG \rightarrow G(5')pppN(pN)_n + PP_i$

(3) $G(5')pppN(pN)_n + AdoMet \rightarrow \mathbf{m}^7G(5')pppN(pN)_n + AdoHcy$

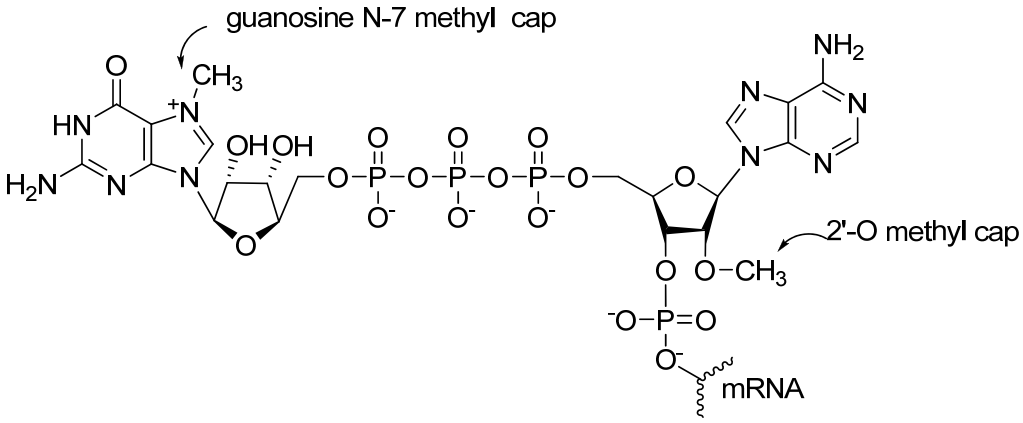


Figure 5. 5′-capped structures.

Both the sugar and base methylations at the 5'-terminus of mRNA are catalyzed by specific methyltransferases, which require *S*-adenosylmethionine (AdoMet) as the methyl donor.[42-44]

As a universal methyl donor for numerous biological methylation processes,[45] AdoMet has been widely known as the second most widely used enzyme cofactor after ATP.[46] The methyl group is transferred when a bionucleophile attacks AdoMet; the nucleophile can be small molecules like tryptamine, medium size molecules such as phospholipids and macromolecules like mRNA.[47]

During the methyl transfer, AdoMet is converted to *S*-adenosyl-L-homocysteine (AdoHcy), which can subsequently be hydrolyzed to form adenosine (Ado) and homocysteine (Hcy) through a reaction catalyzed by AdoHcy hydrolase (Figure 6).[48,49] This is a reversible reaction that normally favors the synthesis reaction (formation of AdoHcy), but the reaction is forced in the hydrolytic direction by further metabolism of adenosine and homocysteine: deamination (to inosine and AMP, respectively) or

phosphorylation of adenosine and remethylation of homocysteine to methionine through a reaction catalyzed by the methylcobalamin-dependent enzyme methionine synthase or combination of homocysteine with serine to form cystathionine. The cycle is completed by the conversion of methionine to AdoMet by AdoMet synthetase, which transfers the adenosyl part of ATP to methionine. This cycle is part of the general metabolism of sulfur-containing amino acid derivatives, itself regulated at the genetic level by AdoMet.[50]

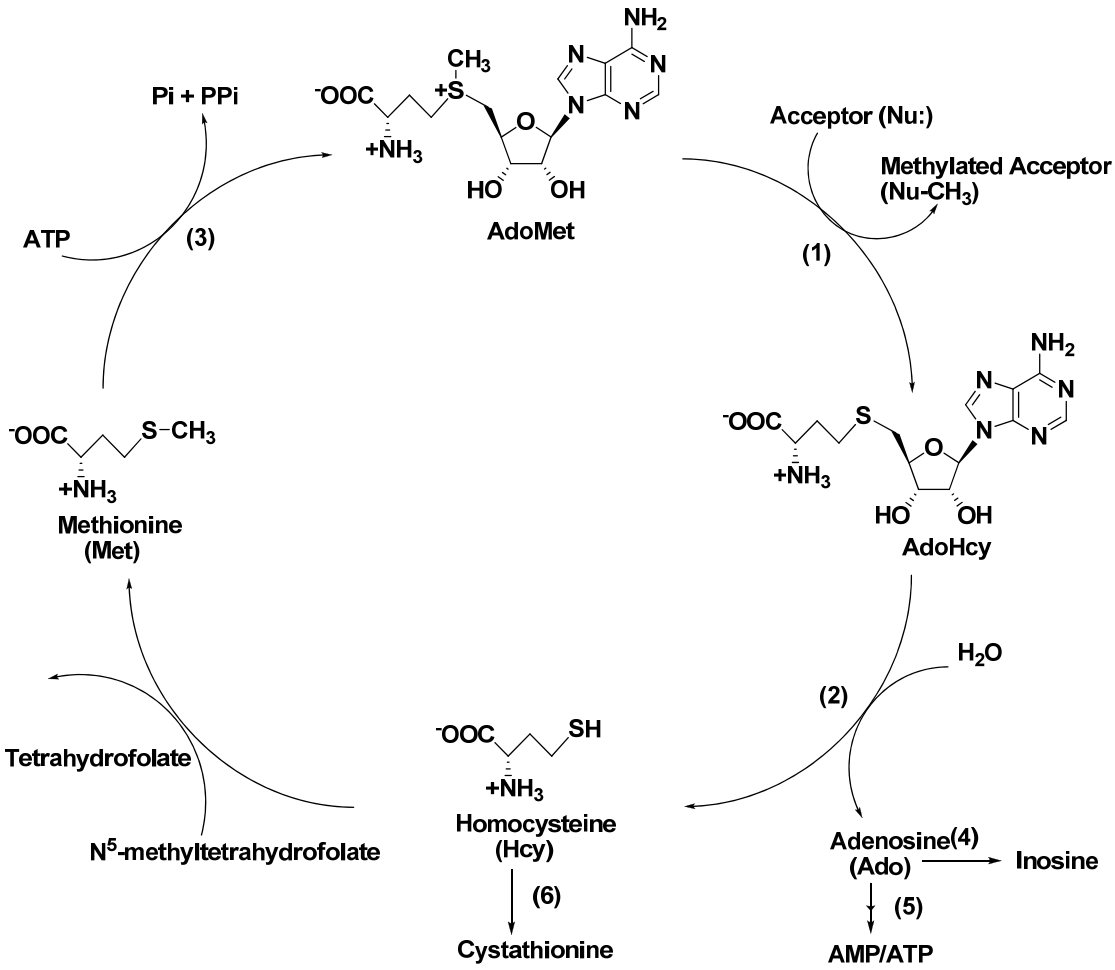


Figure 6. AdoMet metabolic cycle.

Inhibition of AdoHcy hydrolase results in accumulation of AdoHcy, which is both the product and a feedback inhibitor of essential (AdoMet)-dependent methylation reactions (Figure 7). Such methylation reactions are required for final processing of the aforementioned 5′-capped structure of mRNA (as $m^7G(5')pppN(pN)_n$).[42,51,52] The increased concentration of AdoHcy lowers the ratio of AdoMet/AdoHcy and subsequently inhibits AdoMet transferases. This could lead to the inhibition of the transmethylation and 5′-capping, which is essential for viral protein formation and viral replication.[46]

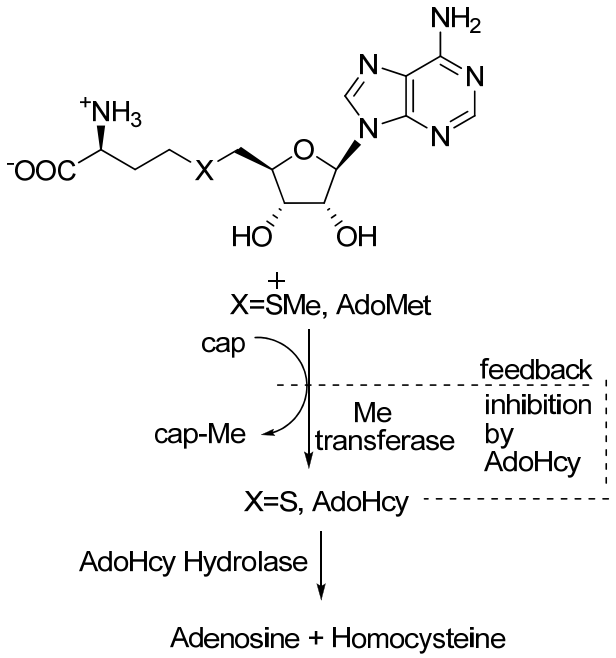


Figure 7. AdoMet/AdoHcy metabolism.

Thus, AdoHcy hydrolase is an attractive pharmacological target as it leads to the inhibition of methyltransferases, and, in turn, it might be expected that the design of AdoHcy hydrolase inhibitors would lead to general suppression of protein synthesis.

Carbocyclic Nucleosides

Nucleoside analogs form the basis of several medicinally important therapies, including antiviral and anticancer treatments. As described in the previous section, for example, acyclovir and AZT are currently in widespread clinical use as anti-herpetic and anti-AIDS medications. However, clinical applications of these nucleosides have been limited by some inherent side effects, such as toxicity and drug resistance, because of their close structural similarity to the natural nucleosides.[53] Another limitation is the instability of the glycosidic bond between the sugar moiety and the heterocyclic base of the nucleoside denied by medicinal agents. This bond undergoes phosphorolysis, which also occurs in normal nucleosides (Figure 8).[54]

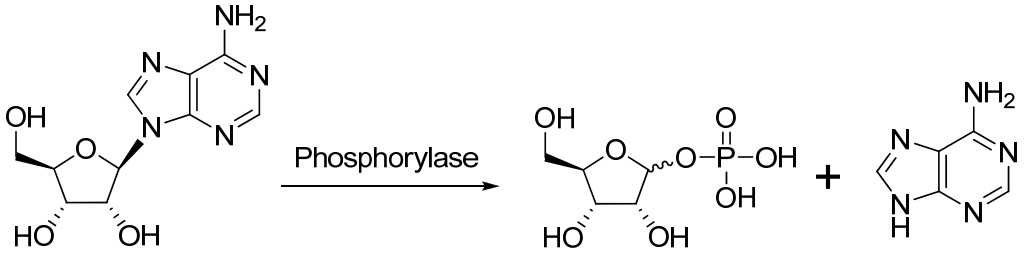


Figure 8 Phosphorolysis of nucleosides

In an effort to overcome this, interest has shifted to the synthesis of carbocyclic nucleosides in which the furanose oxygen of traditional nucleosides has been replaced by a methylene group.[55-59] This change in structure imparted greater stability to the C-N bond against phosphorylases and availed increased lipophilicity, which is an added benefit for oral uptake and cellular penetration.[60-62]

Two of the most important carbocyclic nucleosides are aristeromycin (Ari) and neplanocin A (NpcA), which are naturally occurring. As shown in Figure 9, they are different from each other only by the presence of a double bond between C-4′ and C-6′ of the carbocyclic ring of neplanocin A. Neplanocin A was isolated from the culture broth of *Ampullariella regularis* in 1979[63,64] and later synthesized,[65-70] while aristeromycin was synthesized [65-70] before its isolation from *Streptomyces citricol* in 1969.[71] Both compounds show broad antiviral activities and sparked the explosion of research for additional carbocyclic nucleosides with biological activity. The major mode of action for aristeromycin and neplanocin A is inhibition of AdoHcy hydrolase[47,51] and they represent first generation hydrolase inhibitors. AdoHcy hydrolase is widely recognized as a target for antiviral therapy (*vide infra*) arising from tight inhibitor enzyme binding as well as by the depletion of the essential hydrolase cofactor NAD^+ by consuming it and giving the inactive NADH enzyme form.

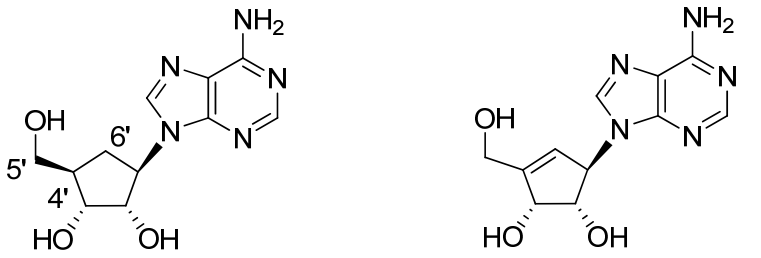
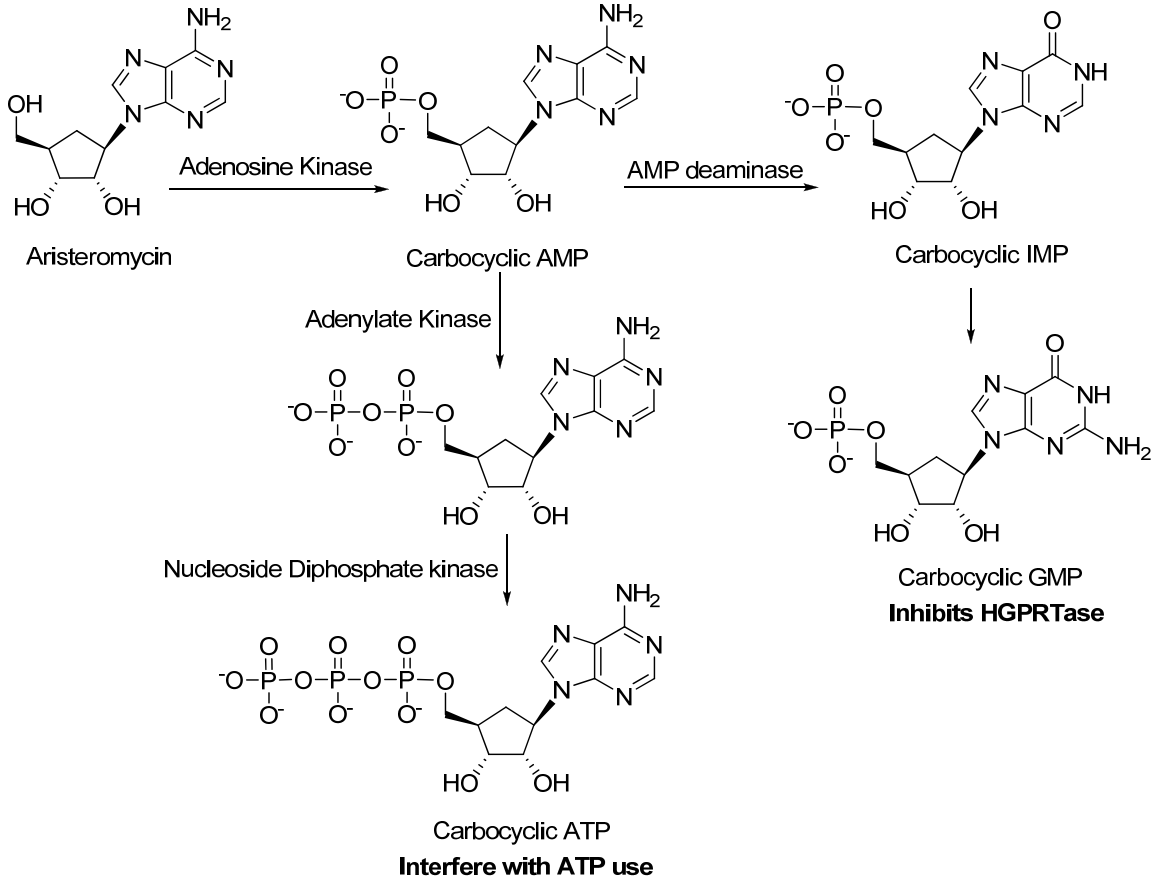


Figure 9. Structures of Aristeromycin and Neplanocin A.

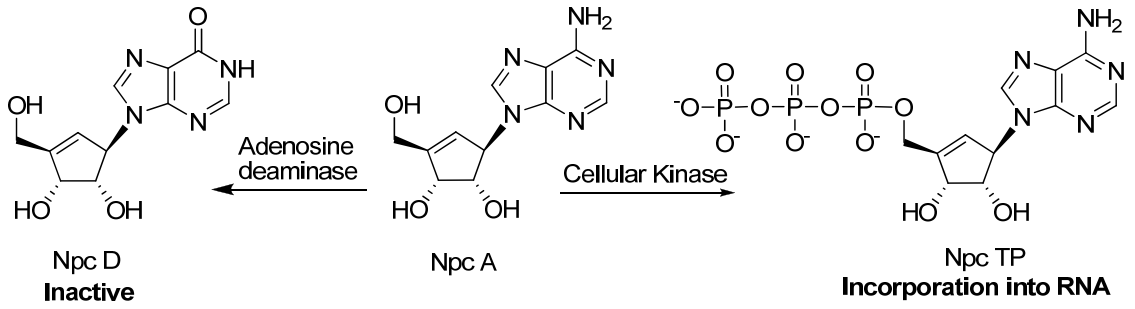
Both aristeromycin and neplanocin A, like adenosine, are phosphorylated by aristeromycinous kinases [72] to their 5′-mono-, 5′-di- and 5′-tri-phosphate forms, which are a possible source of their undesirable toxicities. Adenosine kinase yields the monophosphate form of aristeromycin, resulting carbocyclic adenosine monophosphate

(AMP) serves as a substrate for AMP deaminase that leads to the inosine monophosphate (IMP) analog of aristeromycin.[73] It is then converted to the carbocyclic analog of guanosine monophosphate (GMP), a metabolite that inhibits the crucial cellular enzyme hypoxanthine (guanine)-phosphoribosyltransferase (HGPRTase),[74] which is involved in the purine salvage parthway. On the other hand, carbocyclic AMP yields carbocyclic ATP through series of phosphorylations.[72,75] The structural resemblance to ATP leads it to interfere with the fundamental cellular processes involving ATP and resulting undesirable side effects (Scheme 1).



Scheme 1

Neplanocin A cytoxicity is also thought to form its triphosphate, which is known to be incorporated into RNA[76] and to be converted to neplanocyl methionine, under catalysis by adenosylmethionine synthase, a metabolite that may inhibit cellular RNA methylation.[76,77] Neplanocin A is also a substrate for adenosine deaminase and, consequently, is converted to an inactive form—neplanocin D. (Scheme 2)



Scheme 2

With this discussion in mind, the efforts of several research groups have been channeled into the design of neplanocin A and aristeromycin analogs that would be devoid of phosphorylation while retaining the potent antiviral properties that reside in the inhibition of AdoHcy hydrolase (vide infra). For example, analogs of aristeromycin, 3-deazaaristeromycin[78] and 3-deaza-neplanocin A[79] were synthesized. These compounds (Figure 10) proved to be potent with less cytotoxicity, *vis a vis* the parent nucleoside.[80]

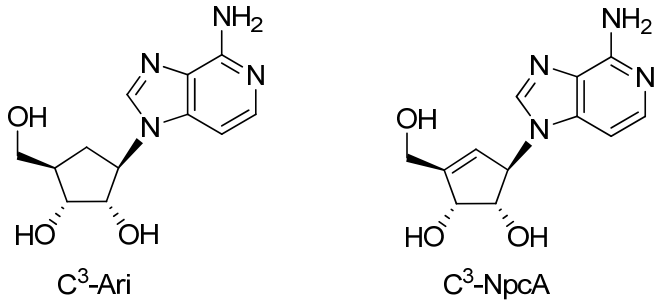


Figure 10. Aristeromycin and neplanocin A analogs with base modified.

The next development in this area came from the Schneller group. They synthesized 5′-noraristeromycin[81] and 5′-deoxyaristeromycin (Figure 11).[82] The latter one showed moderate activity but with no toxicity probably because it was incapable of 5′-phosphorylation, while the former one showed a very potent antiviral activity against vaccinia virus, hepatitis B virus, human cytomegalovirus, measles and influenza, with much less toxicity than aristeromycin and neplanocin A.[83,84] It's believed that 5′-noraristeromycin does not undergo phosphorylation, for one or both of two reasons: (i) the shortened C-4′ chain length does not allow the 4′-hydroxy group to reach the proper position in the binding site of the phosporylating kinase and/or (ii) the 4′-hydroxyl group of 5′-noraristeromycin is secondary and, as a consequence, may be less reactive than the primary 5′-hydroxyl of aristeromycin.

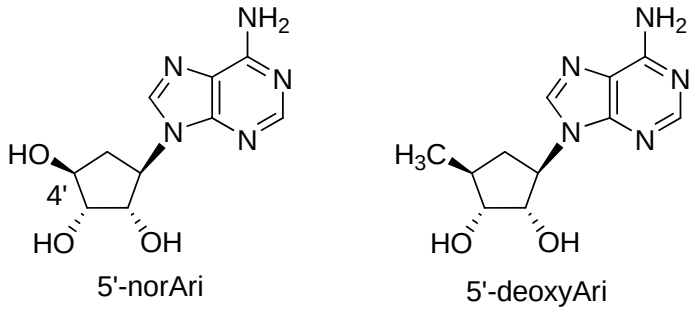


Figure 11. Aristeromycin analogs with side chain modified.

In the aforementioned modified carbocyclic nucleosides, the goal was to retain antiviral activity via inhibition of AdoHcy hydrolase while eliminating the cytoxicity of resulting metabolites. The role of AdoHcy hydrolase in the biological activity of the compounds requires further study.

C-Nucleosides as antiviral agents

C-Nucleosides are a unique class of nucleosides in which the ribofuranosyl moiety is linked to a heterocyclic base through a carbon-to-carbon bond instead of the traditional carbon-to-nitrogen bond. As a result, they are resistant to the chemical and the enzymatic hydrolytic cleavage of the glycosidic bond, much like carbocyclic nucleosides. C-Nucleosides have received considerable attention due not only to the potent metabolic stability but also to the interesting biological activities of the naturally occurring ones.[85]

The first example of a natural C-nucleoside was Pseudouridine (ψ-uridine), which was isolated in 1957[86] as the fifth nucleoside obtained from "soluble RNA," and its structure was established in 1962 as 5-(β-$_D$-ribofuranosyl) uracil (Figure 12).[87-89] It is now known that ψ-uridine is present ubiquitously in active transfer RNA (tRNA),[90] and that certain tRNAs deficient in ψ-uridine are incapable of participating in protein synthesis.[91] This C-nucleoside is formed enzymatically from uridine after assembly of the tRNA chain.[92-95]

Later, a few C-nucleosides were isolated from nature, such as oxazinomycin, pyrazomycin, showdomycin, formycin A and formycin B (see Figure 12). They are antibiotics and exhibit anticancer and/or antiviral activity.[96-99]

Oxazinomycin, which inhibits both gram-positive and gram-negative bacteria and Ehrlich ascites and sarcoma 180 (both solid and ascites) in mice, was discovered independently in two Japanese laboratories.[100,101] Pyrazomycin, isolated from the culture filtrates of *Streptomyces candidus*,[102] has been shown to be an inhibitor of a variety of

viruses and tumors.[103] Showdomycin, elaborated by *Streptomyces showdoensis*,[104] has been found to inhibit gram-positive and gram-negative bacteria and Ehrlich ascites *in vitro* and HeLa *in vitro.*

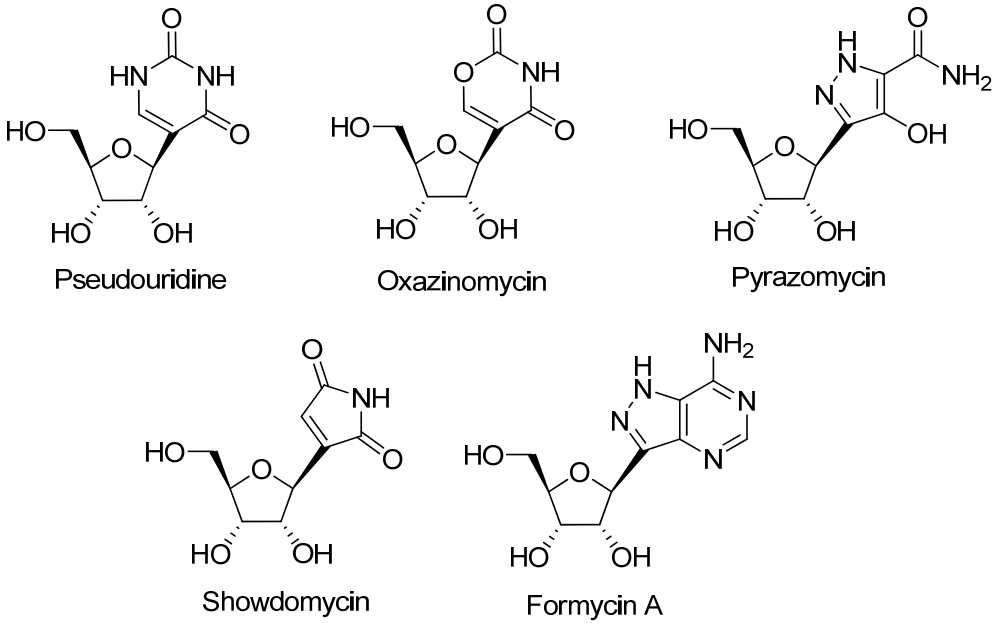


Figure 12. Naturally occurring C-nucleosides.

Formycin A was isolated as an antibiotic from the rice mold *Nocardia interforma* by Hori *et al*.[105] and was identified as a C-nucleoside isomeric with the natural nucleic acid constituent adenosine by Koyama *et al*.[106] Formycin has growth inhibitory effects against Ehrlich carcinoma, mouse leukemia L1210, Yoshida rat sarcoma cells and HeLa cells, and *Xanthomonas oryzae* as well as exhibits some immunosuppressive activity.[100,107]

Interest in formycin A stems from the fact that it can replace adenosine in a variety of biochemical reactions.[108,109] Formycin and its derivatives act as substrates for many adenosine specific enzymes including enzymes of nucleotide metabolism, RNA polymerase, polynucleotide phosphorylase, the pyrophosphorylase of tRNA and

adenosine kinase.[109,110] Formycin A is incorporated into RNA and DNA,[109] and its derivative, formycin 5'-triphosphate (FTP), acts as a source of biological energy and ribopolynucleotides with formycin replacing adenosine, at the binding site of t-RNA to ribosomes have shown no mistranslation of the messenger.[111] FTP can also substitute for ATP in aminoacyl-tRNA synthetase, and it is the first nucleotide analog containing an abnormal base that is capable of functioning in this reaction. Additionally, formycin 5'-triphosphate is a competitive *in vitro* inhibitor of the nucleoside triphosphate reductase of *Lactobacillus leichmanni* in the reduction of ATP, GTP and UTP; however, in the presence of positive effector 2′-deoxyguanosine triphosphate (dGTP), FTP can be reduced at about the same rate as ATP to 2′-deoxyformycin 5'-triphosphate (dFTP) (Figure 13), which is able to mimic dATP as activators of CTP reduction by ribonucleotide reductase.[112]

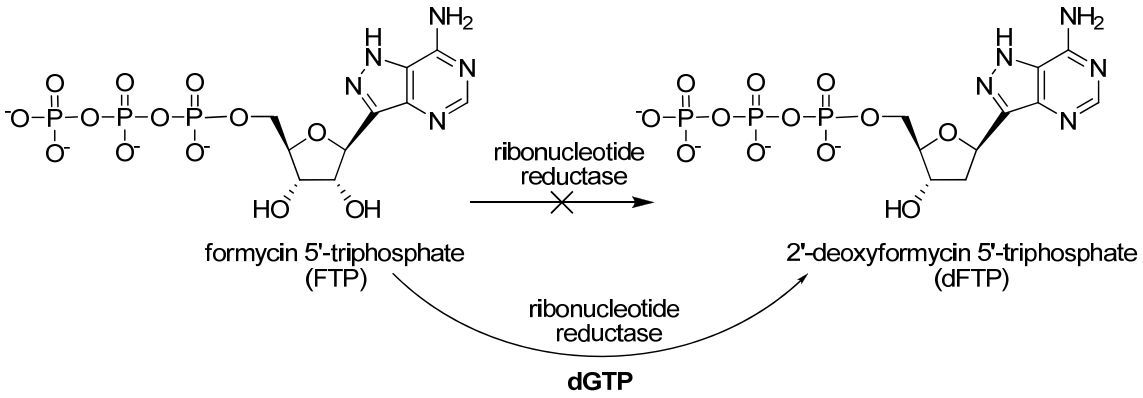


Figure 13. Reduction of FTP by ribonucleotide reductase.

Unfortunately, formycin is also a good substrate for the adenosine catabolic enzyme, adenosine deaminase (ADA)[113] that deaminates formycin, producing the oxygenated form, formycin B, which, in turn, may be converted to the xanthosine analog,

oxoformycin, by hepatic aldehyde oxidase (Figure 14).[114,115] Both formycin B and oxoformycin have shown little or no biological activity and low toxicity in experimental animals.[116] The rapid inactivation of formycin by animal tissues that contain adenosine deaminase has limited investigations of therapeutic activities of this potentially useful antimetabolite.

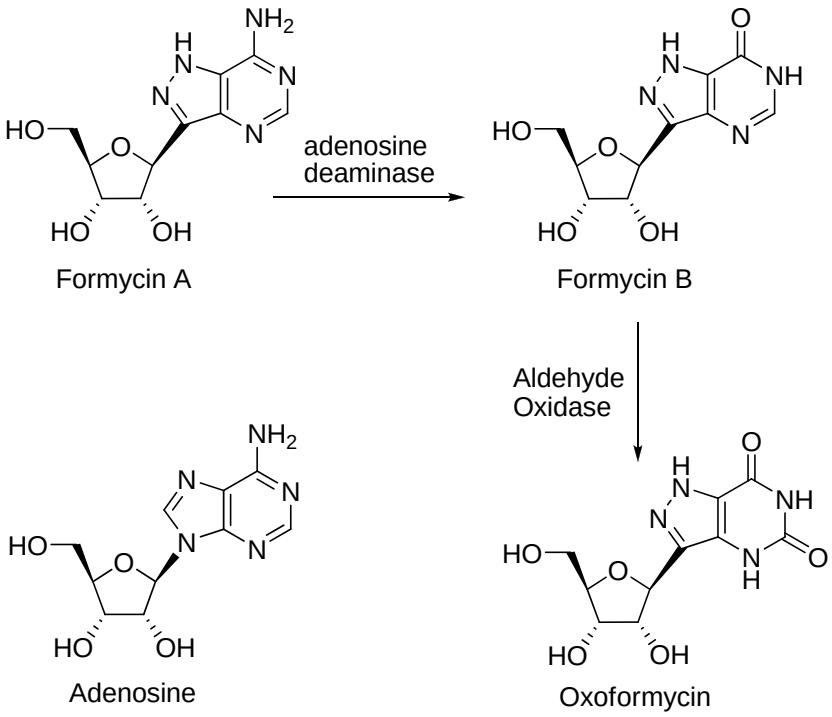


Figure 14. Metabolic conversions of formycin.

In order to increase the effectiveness of formycin as a chemotherapeutic agent, chemically modified formycins were synthesized[117,118] in attempt to decrease or abolish the activity of the formycin molecule with adenosine deaminase, while retaining the activity with adenosine kinase and other key enzymes of adenine nucleotide metabolism. A study[119] suggested that adenosine derivatives which exist in the *anti* conformation would be excellent substrates for adenosine deaminase, whereas those in the *syn*

rotameric conformation would have little or no substrate activity. This report prompted the synthesis of nucleosides designed to restrict rotation around the glycosyl bond of formycin and increase the proportion of nucleoside in the *syn* conformation. This hypothesis predicted that 1-methylformycin, which exists predominantly in the *anti* conformation (since there is no steric hindrance to rotation about the glycosyl bond), would be a good substrate for ADA. On the other hand, marked steric hindrance to rotation occurs with 2-methylformycin. This compound has been found to exist predominantly in the *syn* conformation in the crystalline state (Figure 15).[120]

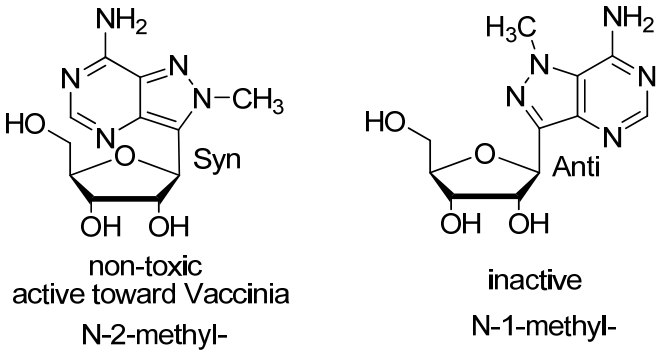


Figure 15. *N*-methyl derivatives of formycin.

The above hypothesis consequently predicted that 2-methylformycin would be a poor substrate for ADA. Crabtree *et al*[121] described the effects which methylation of the individual nitrogen atoms of formycin have on the activity as a substrate for ADA. The data invalidate the hypothesis above: no substrate activity for ADA was detected with 1-methylformcin, whereas, 2-methylformycin displays substrate activity with erythrocyte ADA. However, another report from Makabe *et al*[122]on the enzymatic deamination of certain 1- and 2-alkylformycin showed that both 1-methylformycin and 2-methylformycin were deaminated by Takadiastase ADA and calf intestinal mucosa ADA.

The observed rate of deamination of 1-methylformycin was found to be approximately equal to the rate of deamination of formycin. By contrast, the rate of deamination of 2-methylformycin was very much slower than that of formycin and 1-methylformycin. In fact, no detectable deamination of 2-methylformycin was observed at enzyme concentrations which resulted in complete deamination of formycin and 1-methylformycin. These results suggest that the conformation (either *syn* or *anti*) of an adenosine analog is not the only consideration in determining ADA substrate activity. The lack of substrate activity for certain adenosine analogs maybe also due to specific chemical modifications of the compound which result in a change of the electronic, steric and/or structural parameters. It was also found that 2-methylformycin has relatively strong activity against vaccinia virus. Giziewice *et al* reported the two methylated derivatives had no toxicity on the primary rabbit kidney (PRK) cells used for evaluation of antiviral activity.[123]

Hybrid nucleosides--Carbocyclic C-Nucleosides

On the basis of the interesting chemical and biological properties of C-nucleosides and carbocyclic nucleosides, it was of interest to investigate hybrid nucleosides: carbocyclic C-nucleosides (Figure 16).

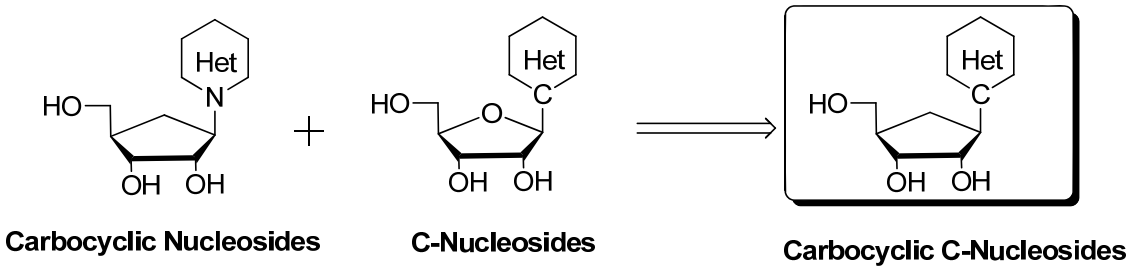
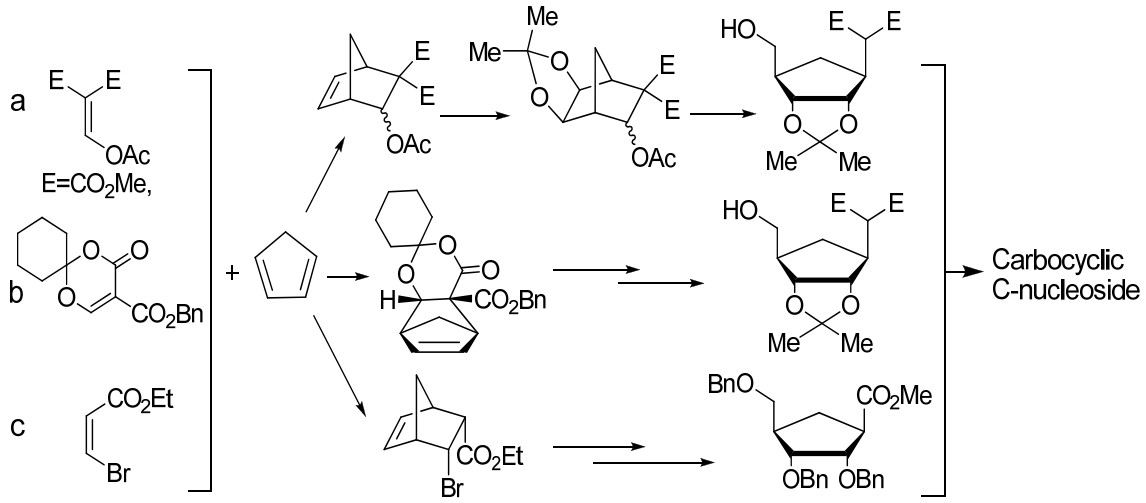


Figure 16. Structure of carbocyclic *C*-nucleoside.

The history of carbocyclic C-nucleosides dates back to the 1960s.[124] However, despite the long history of both carbocyclic and C-nucleosides, only a few carbocyclic C-nucleosides have been prepared, probably due to the synthetic difficulties of these compounds.

To date, the preferred strategy for the synthesis of carbocyclic C-nucleoside analogs proceeds *via* the construction of functionalized carbafuranoses by Diels-Alder reactions (Scheme 3). During exploration of this pathway, a few versatile carbocyclic C-nucleoside precursors also arose.[125-129] In this research, Katagiri, Kaneko, and coworkers developed a method for the synthesis of carbocyclic pyrimidine C-nucleosides[125-127] and carbocyclic oxazinomycins,[130] while Cookson and coworkers[131] described a synthesis of imidazo-[1,5-*a*]pyridine carbocyclic C-nucleosides and their corresponding 2'-deoxy

derivatives. Koizumi and coworkers[132] reported the first enantioselective synthesis of the carbocyclic analog of showdomycin. Stoodley and coworkers[133] reported the synthesis of carbocyclic tiazofurin and its antipode, and Leumann *et al.* synthesized a series of carbocyclic pyrimidine C-nucleosides following the similar routes (Examples shown in Figure 17).[134]



a, From Katagiri group, see ref.125-127; b, From Sato group, see ref.128;
c, From Leahy group, see ref.129

Scheme 3 General strategy for the synthesis of carbocyclic C-nucleosides

Recently, research aimed at the development of the synthesis of carbocyclic 9-deazapurine nucleosides appeared. Chu *et al.* reported the synthesis of carbocyclic 9-deazaaristeromycin,[135] carbocyclic 9-deazainosine and other corresponding analogs.[136] Schneller *et al.* reported the synthesis of 9-deaza-5′-noraristeromycin.[137] Hong and coworkers described the first synthesis of 4'-branched carbocyclic C-nucleoside (Figure 17).[138] Furthermore, many of the carbocyclic C-nucleoside have been synthesized as racemic mixtures (Figure 18), probably due to the synthetic difficulties when seeking

enantiopure substances. Two such analogs of the bioactive C-nucleosides have been described: (±)-carbapyrazofurin,[139] (±)-carbashowdomycin.[140] However, there have been no reports that such compounds are biologically active.

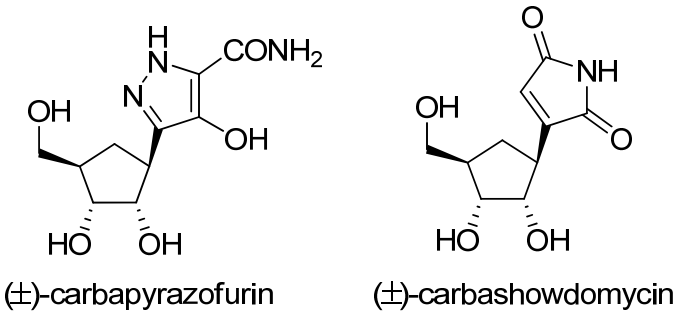


Figure 18. Racemic Carbocyclic C-nucleosides.

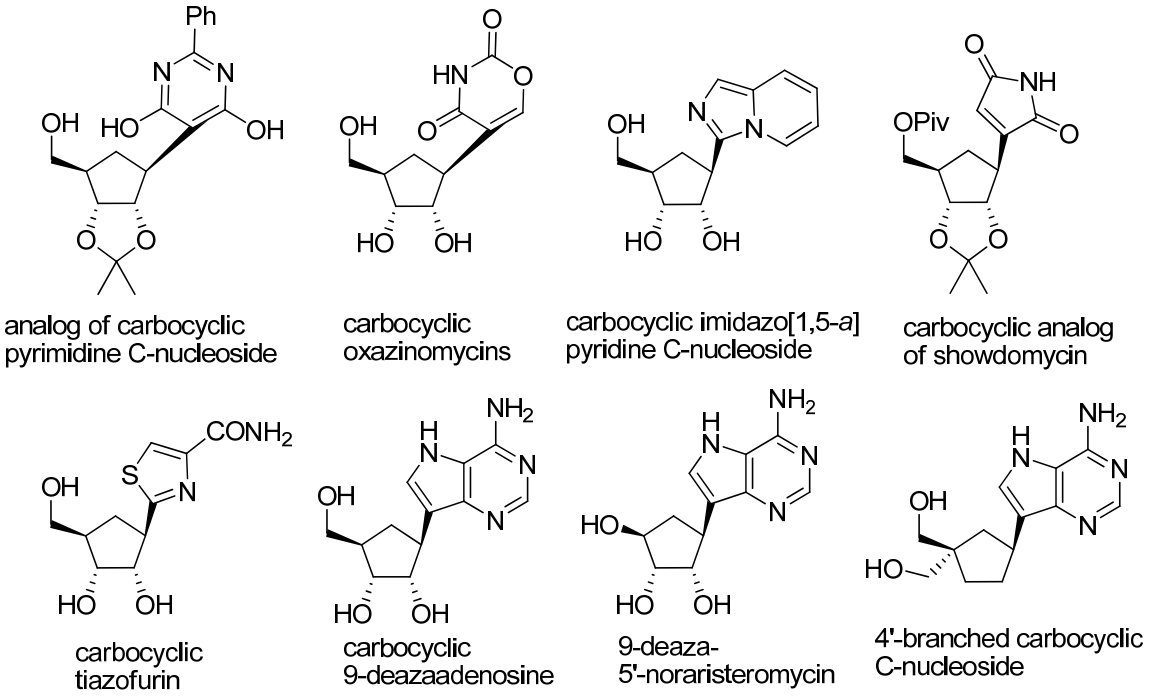


Figure 17. Examples of synthesized carbocyclic C-nucleosides.

Therefore, enantiomeric synthesis of the carbocyclic C-nucleosides offers a synthetic challenge but avails biologically interesting targets. In the latter required, no significant biological activities have been reported. It was of interest to explore further the carbocyclic C-nucleosides because most of the hitherto known carbocyclic C-nucleosides possess only unnatural heterocyclic moieties. In 1997, Leahy and coworkers reported their progress toward the synthesis of novel carbocyclic formycin (Figure 19).[129] They synthesized a common intermediate, which was expected to be used to access carbocyclic nucleoside analogs such as carbocyclic formycin. To date, carbocyclic formycin has not been well explored.

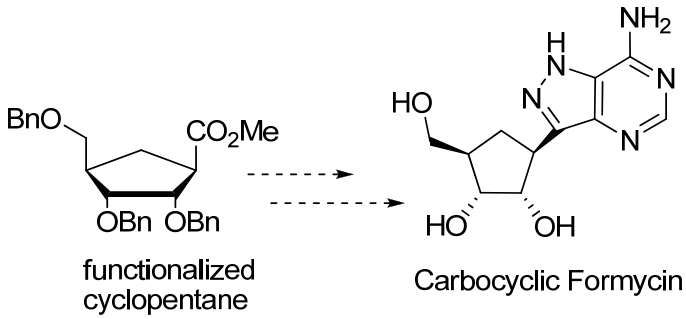
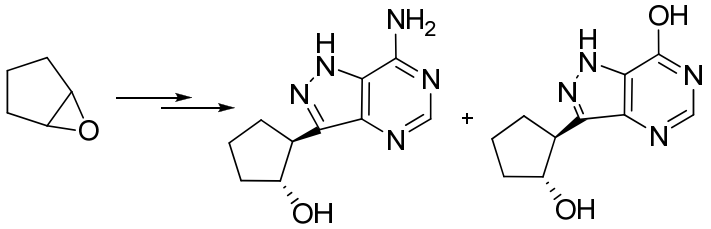


Figure 19. Exploring for the synthesis of Carbocyclic Formycin.

Beginning in 2004, when the Schneller group started to pursue the design and syntheses of novel carbocyclic C-nucleosides, a convenient access to carbocyclic C-nucleosides based on the formycin framework arose.[141-143] Their synthesis of novel carbocyclic formycin analogs were reported as the following (Figure 20).

1) **A model study to carbocyclic formycin A and B analogs**. Zhou, J. *et al* . *Tetrahedron Lett.* **2004**, *45*, 8233-8234.



2) **C-4' Truncated carbocyclic formycin derivatives.** Zhou, J. *et al* *Tetrahedron* **2006**, *62*, 7009-7013

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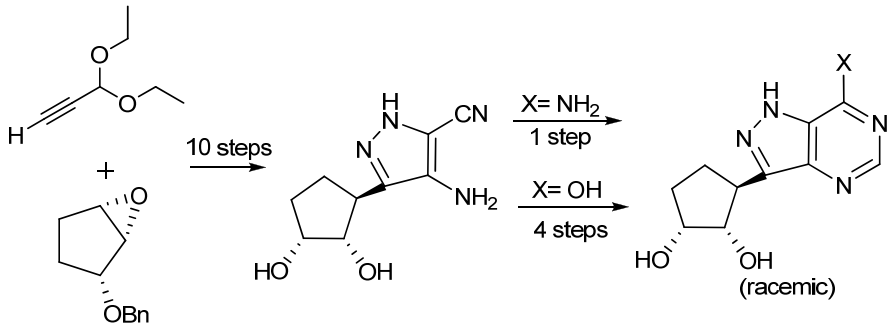


3) **Carbocyclic 4′-*epi*-formycin** Zhou, J. *et al Tetrahedron* **2008**, *64*, 433-438.

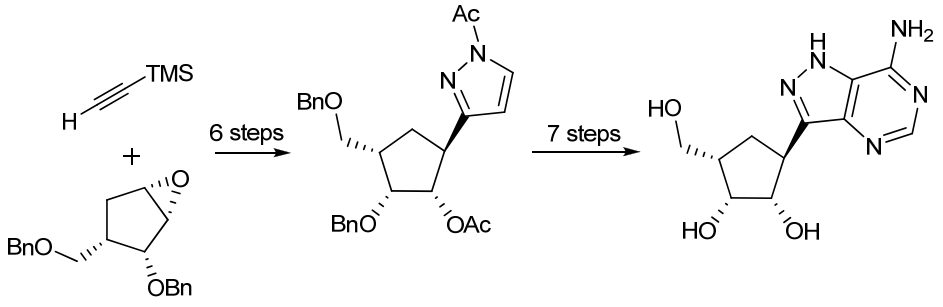


Figure 20. Synthesis of carbocyclic formycin analogs.

When considering formycin derivatives, it should be kept in mind that formycin, like aristeromycin, is limited by its toxicity, which, in some instances, resides in its 5'-

nucleotide derivatives. Based on the previously described non-cytotoxic antiviral properties of 5′-noraristeromycin, this dissertation research focuses on the combination of the structural components of formycin and 5'-noraristeromycin resulting in carbocyclic 5'-norformycin and their *N*-1 and *N*-2 methylated derivatives (Figure 21).

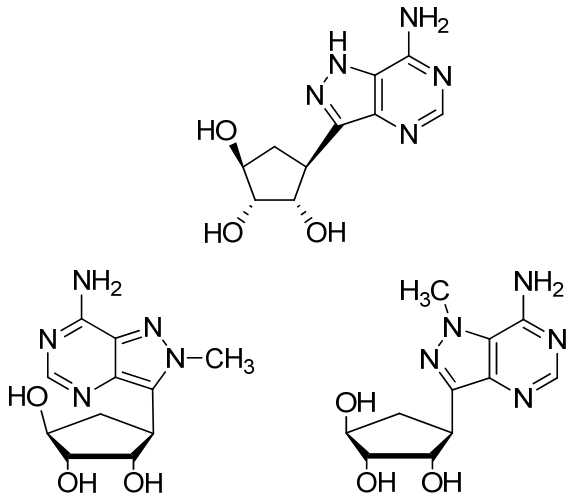


Figure 21. Carbocyclic 5'-norformycin and its methylated versions.

A MODEL STUDY TO *N*-METHYL CARBOCYCLIC FORMYCIN ANALOGS

As was pointed out in the introduction, 1-methyl and 2-methylformycin, which assumed *anti*- and *syn*- conformation respectively, have displayed interesting biological activities,[121] such as 2-methylformycin being active towards vaccinia virus. The methylated version of carbocyclic formycin has never been reported in the literature. Based on the previous work reported in the Schneller group, a model study to *N*-methyl carbocyclic formycin analogs **1** and **2** (Figure 22) was selected as the beginning point of this dissertation.

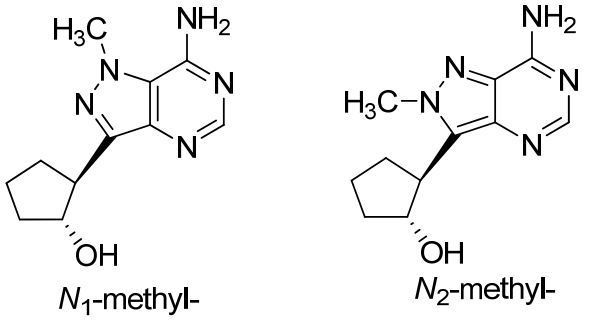
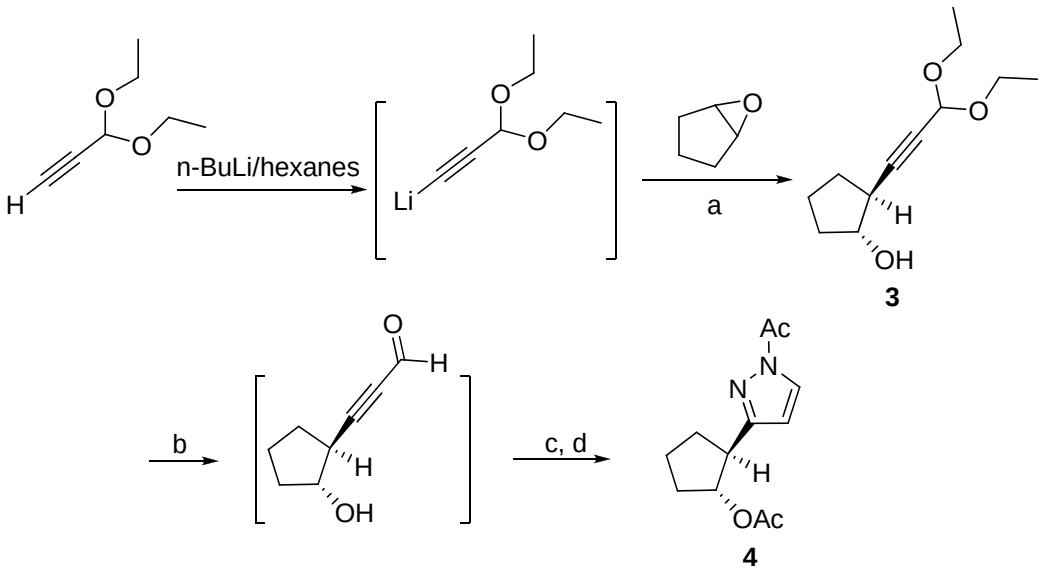


Figure 22. (±)-**1** and (±)-**2**.

The route to **1** and **2** was foreseen as accessible from a readily available epoxide. To move in that direction, the plan was to first introduce a functionally useful group to the "anomeric position" of the requisite cyclopentyl ring through a C-C linkage that

would, concurrently, produce the *vicinal-trans* hydroxyl of epoxide origin. This arrangement was then to be elaborated to a heterocyclic ring of the targets. Most of the biologically active C-nucleosides have been synthesized by this general method, including the synthesis of formycin B[144] and of oxoformycin.[145]

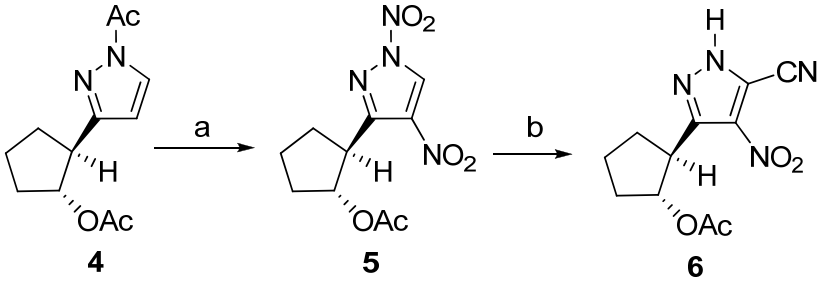
Synthesis of pyrazole derivative. The synthesis started with the addition of lithiated 3,3-diethoxy-1-propyne to cyclopentene oxide at -78 ˚C in the presence of boron trifluoride diethyl etherate,[146,147] affording (±)-**3** (Scheme 4). Hydrolysis of (±)-**3** with a mixture of acetic acid and 10% aqueous hydrochloric acid resulted in acetylenic aldehyde (from step b in Scheme 4). Although an unstable compound, this aldehyde is a versatile intermediate in the synthesis of the target C-nucleosides.[148-150] Treatment of the non-isolated aldehyde with hydrazine monohydrate gave a pyrazole derivative,[151,152] which was acetylated to provide the key intermediate (±)-**4**.



Reagents and conditions: a) $BF_3{\cdot}EtO_2$, 64%; b)10% HCl, AcOH; c) hydrazine monohydrate, AcOH; d). Ac_2O, pyridine, DMAP, 73% for three steps.

Scheme 4 Synthesis of pyrazole ring

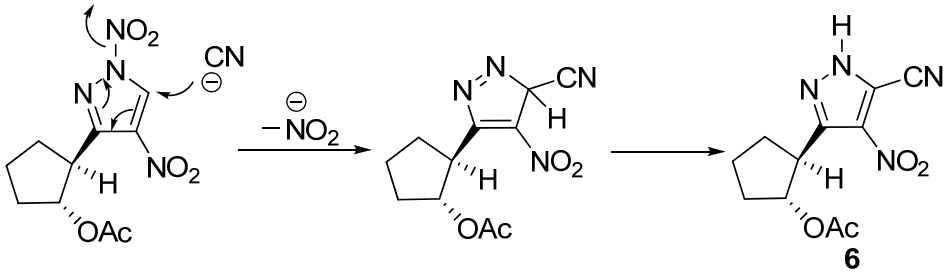
Synthesis of the key nitro nitrile intermediate. Nitration of pyrazole (±)**-4** with ammonium nitrate and trifluoroacetic anhydride in trifluroacetic acid following literature conditions[153] led directly to the 1,4-dinitropyrazole (±)**-5**[154] (Scheme 5). Habraken and Poels[155] have shown that 1,4-dinitropyrazoles react with secondary amines at C-5 in a *cine* substitution with expulsion of the *N*-nitro group as nitrite. Similarly when (±)**-5** was treated with excess potassium cyanide in aqueous ethanol at room temperature, the desired *cine* substitution[156,157] of the *N*-nitro with a cyano occurred, and the key nitro nitrile intermediate (±)**-6** was obtained in a pure crystalline form.



Reagents and conditions: a) ammonium nitrate, TFA, TFAA, 100%
b). KCN, EtOH, 68%.

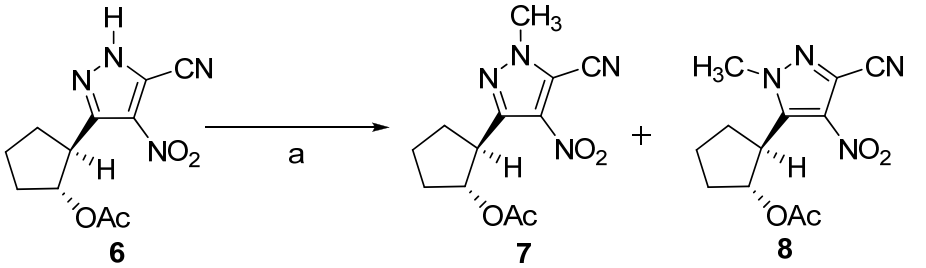
Scheme 5 Synthesis of key nitro nitrile compound

In general, the occurrence of a "cine" substitution (i.e., the entering group comes in *ortho* to the leaving group) is evidence for a 1, 2-addition-elimination mechanism. The actual molecule initially formed is a 3*H*-pyrazole; the ultimate product obtained then arises in a subsequent fast hydrogen rearrangement reaction (Scheme 6).



Scheme 6 mechanism of *cine* substitution

Synthesis of the final *N*-methyl carbocyclic formycin analogs. It was envisioned that the methylation of unsubstituted **(±)-1** may be complex since there are several possible methylation sites. For this reason introduction of the methyl substituent was considered at this stage. Treatment of (±)-**6** with sodium hydride followed by quenching with methyl iodide gave the 1-methyl product **((±)-7)** and 2-methyl product **((±)-8),** which could be separated by column chromatography (Scheme 7).



Reagents and conditions: a) i. NaH, THF; ii. MeI, 60% for **7** and 28% for **8**

Scheme 7 Introducing methyl group to pyrazole ring

To confirm the site of methylation and the stereochemical orientations of the two cyclopentyl ring substituents, an X-ray structural analysis of what was assumed to be the 2-methyl product **(±)-8** (Figure 23) was conducted. This analysis of **(±)-8** not only

confirmed the structure but also showed it possessed a high degree of *syn* character (that is, cyclopentyl ring lies under pyrazole).

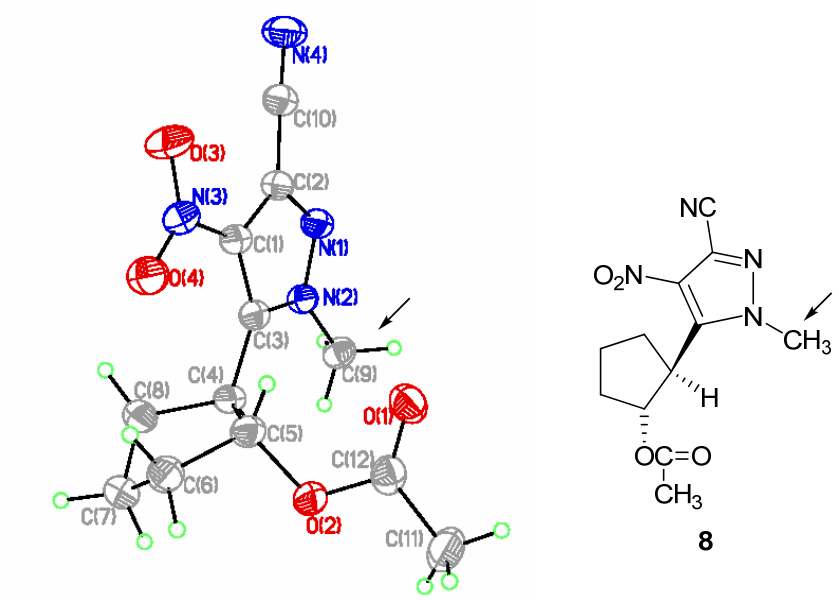
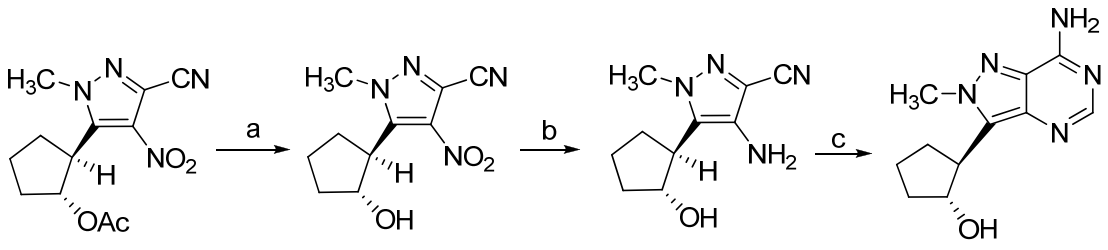


Figure 23. X-ray structure for compound **(±)-8**.

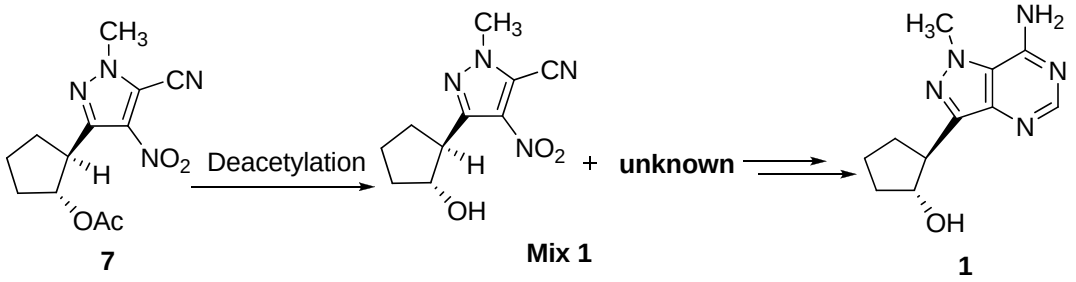
Deacetylation of **(±)-8** to **(±)-9** was conducted in a solution of saturated ammonia in methanol. Hydrogenation of **(±)-9** in the presence of palladium/carbon afforded the reduced product **(±)-10**. Treatment of **(±)-10** with formamidine acetate in refluxing ethanol proceeded with ring annulation to (±)-(1α,2β)-2-(3-[7-amino-2-methyl-1*H*-pyrazolo [4, 3-*d*] pyrimidyl])cyclopentanol, ((±)**-2) (**Scheme 8).



Reagents and conditions: a) NH_3, MeOH, 80%; b) H_2, Pd/C, MeOH,90%; c) $HC(=NH)NH_2 \cdot AcOH$, EtOH, 60%.

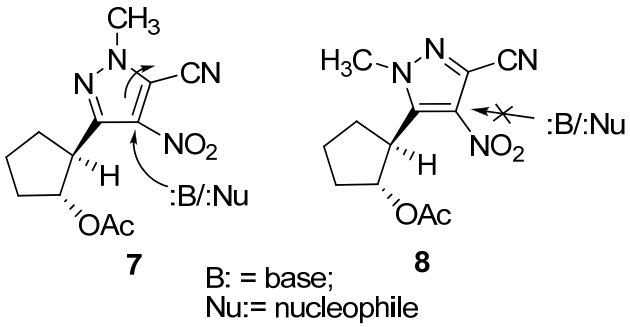
Scheme 8 Synthesis of **(±)-2**

N_1-methyl derivative **(±)-1** was also obtained by treatment of **(±)-7** in the same route as discussed above (Scheme 9). However this was complicated because when **(±)-7** was subjected to the deacetylation, an inseparable mixture containing the desired unprotected secondary alcohol was produced. Subjecting this mixture to steps b and c of Scheme 8 gave (±)-(1α,2β)-2-(3-[7-amino-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidyl]) cyclopentanol (**(±)-1**).



Scheme 9 Synthesis of **(±)-1**

Normally, the deacetylation reaction (Scheme 9) occurs under alkaline conditions, such as catalytic sodium methoxide in methanol, a mixture of potassium carbonate and methanol, or saturated ammonia in methanol, etc. It was assumed *N*-1 methylation decreased the pyrazole ring's stability (possibly nucleophilic causing attack at C-4), probably affording ring opened or functional group changed products. A possible mechanism for this is proposed in Scheme 10.



Scheme 10 Methylated pyrazole ring's stability

Structural assignments for N_1-methyl- and N_2-methylated products

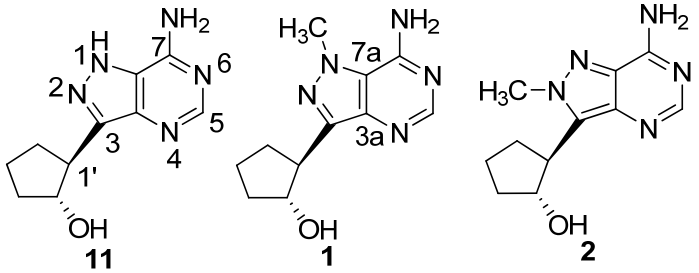
Townsend *et al*. used magnetic circular dichroism (MCD) for assigning the structures to the *N*-methylated C-nucleosides.[158] The MCD spectra of formycin A and 1-methylformycin are very similar but they are quite different from 2-methylformycin. In the model study of this dissertation, similar results were observed, which were based on the comparisons of NMR spectral data (^{1}H and ^{13}C) of the two methylated isomers and the C-nucleoside version lacking the methyl group.[143]

Selected ^{1}H NMR spectral data for the two isomeric methylated products are compiled in Table 1. The methyl signal for the 2-methylated product is upfield from the signal observed for 1-methylated derivative. There was a similar relationship with the H_5 signals. It is of some interest that the signal for the "anomeric ($H_{1'}$) proton" of the 2-methylated isomers wherein rotation about the glycosidic bond (C3-C1′) is restricted is observed approximately 0.1 ppm downfield from the "anomeric" signal observed for the other methyl isomer. In Table 1, the selected ^{13}C NMR data for the two methylated versions were also collected. The signals for C_7 and C_{7a} of 2-methylated product are largely downfield from those of the other two products; however, the signals for C_3 and C_{3a} of the 2-methylated version are upfield by comparison. As with Townsend, the 2-methylated derivative is dissimilar to the 1-methyl and the non methyl group versions. Comparative TLC mobilities of the methylated isomers were presented in Table 2.

Table 1 Selected ^{1}H NMR and ^{13}C NMR Spectral Data [a]

^{1}H NMR	NCH_3	H_5	$H_{1'}$
(±)-11[b]		8.16	3.26-3.18
(±)-1 (1-methyl)	4.15	8.12	3.23-3.14
(±)-2 (2-methyl)	4.10	8.03	**3.32-3.22**

^{13}C NMR[c]	NCH_3	C_3	C_{3a}	C_5	C_7	C_{7a}	$C_{1'}$
(±)-11[b]		147.1	139.6	150.5	151.0	121.8	46.0
1-methyl-**(±)-1**	38.8	145.5	141.0	150.8	151.2	121.8	45.9
2-methyl-**(±)-2**	38.6	**136.8**	**136.1**	151.2	**155.6**	**129.9**	45.0



[a] Me_2SO-d_6 was used as a solvent and chemical shifts are in parts per million from an internal standard.

[b] Data from ref. 143.

[c] ^{13}C assignment for **11**, **1** and **2** is determined by comparison to formycin and methylated formycins.[158]

Table 2 Comparative TLC (Silica gel) Data for the *N*-methylated isomers

	Solvent (CH_2Cl_2: MeOH=10:1 v/v)
1-methyl-**(±)-1**	R=0.22
2-methyl-(±)-**2**	R=0.08

Comparisons of NMR spectral data (^{1}H and ^{13}C) and TLC mobilities of the two isomers allow us to make structural assignments for the subsequent methylated derivatives. For example, after obtaining the two racemic methylated carbocyclic formycin analogs **(±)-12** and **(±)-13** (Figure 24) under the same synthetic route (Scheme 11) discussed above, their structural assignments were confirmed by comparisons of their NMR spectral data and TLC mobilities.

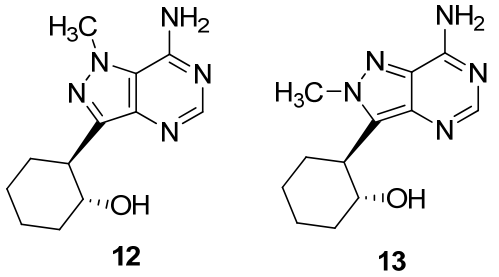
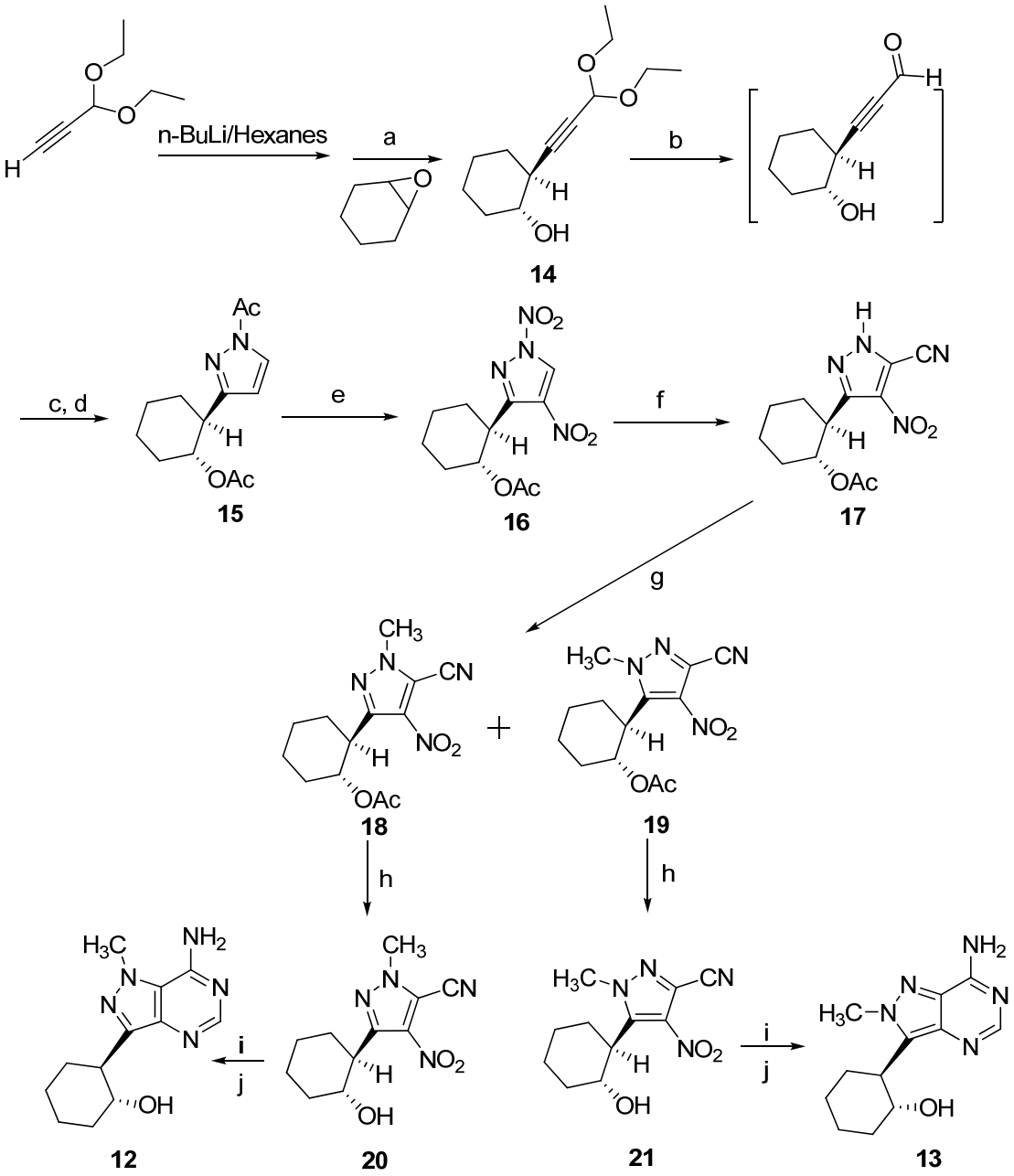


Figure 24. Compounds (±)-**12** and (±)-**13**.

In summary, in the first stage of this dissertation, an efficient means to *N*-methylated carbocyclic formycin analogs has been developed. Application of this pathway employing more functionalized epoxides now allows for access to a comprehensive and diverse library of formycin and formycin-like carbocyclic C-nucleosides as antiviral candidates.

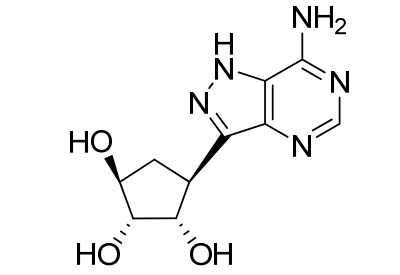


Reagents and conditions: a) BF_3•EtO_2, 64%; b)10% HCl, AcOH; c) hydrazine monohydrate, AcOH; d). Ac_2O, pyridine, DMAP, 70% for three steps; e) ammonium nitrate, TFA, TFAA; f). KCN, EtOH, 65% for two steps; g) i. NaH, THF; ii. MeI, 60.0% for **18** and 25% for **19**; h)NH_3, MeOH, 50% for **20** and 80%for **21** ; i) H_2, Pd/C, MeOH; j) HC(=NH)NH_2AcOH, EtOH, two steps 60% for **12**, 50% for **13**

Scheme 11 Synthesis of Compounds (±)-**12** and (±)-**13**

ENANTIOSELECTIVE SYNTHESIS OF CARBOCYCLIC 5'-NORFORMYCIN

Exploring the key step toward target compound 22. As mentioned in the introduction, the synthesis of carbocyclic C-nucleosides has been challenging.[129,136] With that as back drop, the enantioselective synthesis of carbocyclic 5'-norformycin (**22**) (Figure 25) was undertaken in this dissertation research.

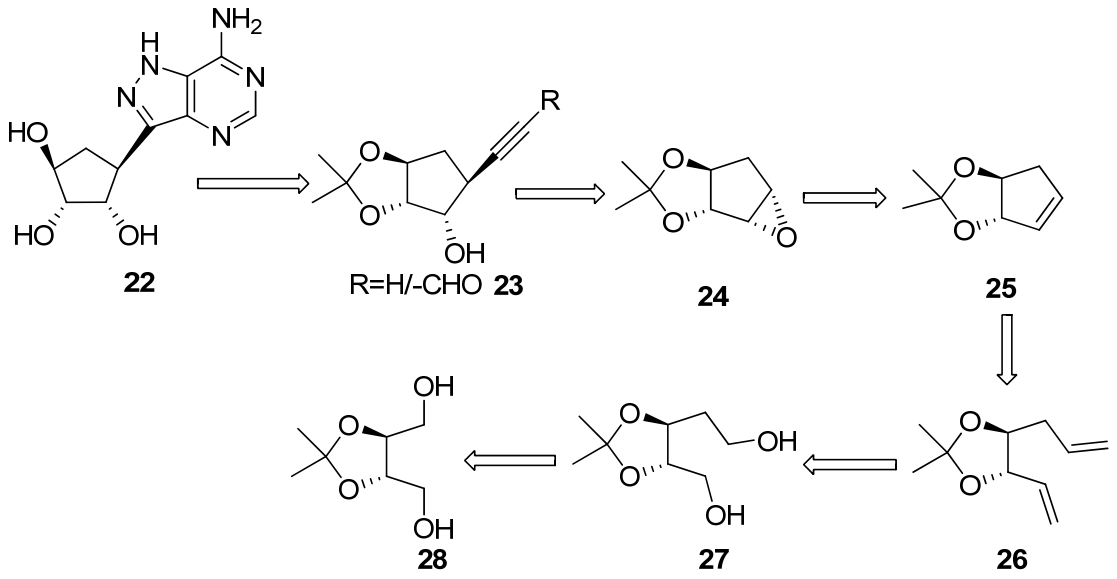


Carbocyclic 5'-norformycin **22**

Figure 25.

Attempts to synthesize the key epoxide. Based on former work developed in the Schneller group, the functionalized epoxide **24** was seen as central to obtaining carbocyclic 5'-norformycin (**22**). A retrosynthetic analysis (Scheme 12) of **24** and previous experience in this project revealed that the base part of target compound **22** could be built from a cyclopentyl alkyne or acetylenic aldehyde (see **23),** which could be

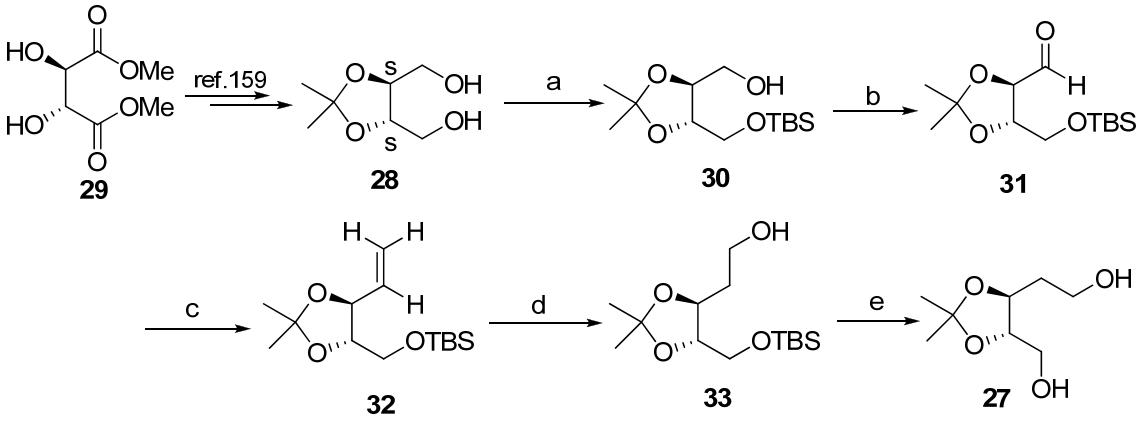
achieved from the epoxide **24**. This key epoxide **24** was foreseen from protected cyclopentene **25** that, in turn, could be produced from diene **26** through a ring closing metathesis (RCM) procedure; and diene **26** could be accessed from chiral diol **28**.



Scheme 12 Retrosynthesis of epoxide **24**

The starting material, (+) 2, 3-O-*iso*propylidene-L-threitol (**28**), is accessible by improving upon a literature route from dimethyl L-tartrate (**29**).[159] While expensive, **28** is also commercially available. The first stage of the synthesis focused on how to selectively introduce the carbon needed for obtaining **27** (Scheme 13). Treatment of diol **28** with excess sodium hydride and one equivalent *tert*-butyldimethylsilyl chloride produced compound **30,** and diol **28** underwent a highly selective mono-silylation in excellent yield.[160] Oxidation of the primary hydroxyl group of **30** with sulfur trioxide-pyridine complex and dimethyl sulfoxide[161] furnished aldehyde **31,** which was converted in good yield to olefin **32** by a Wittig reaction using potassium *tert*-butoxide and

methyltriphenylphosphonium bromide. Submitting olefin **32** to regioselective hydroboration with 9-borabicyclo[3,3,1]nonane (9-BBN) followed by oxidative hydrolysis, smoothly provided **33** in high yield. Removing the silyl group with a 1 M solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) provided the desired diol **27**.



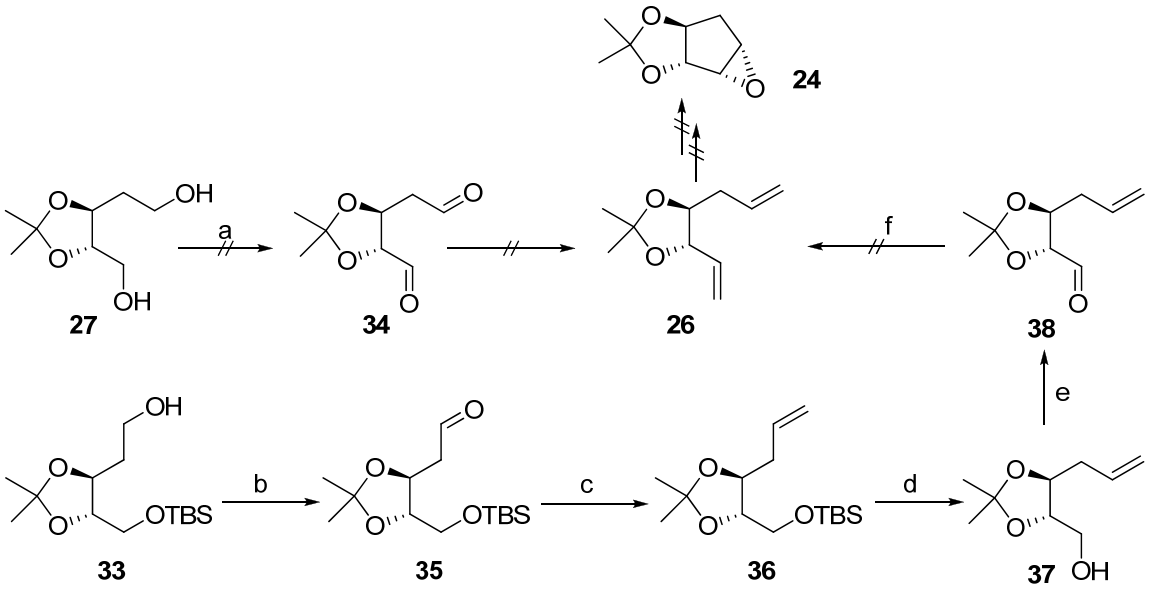
Reaction conditions: a) NaH, 1 eq TBSCl, THF, 85%; b) CH_2Cl_2, DMSO, DIPEA, SO_3-Py, 88%; c) Ph_3PCH_3Br, t-BuOK, Et_2O, 80%; d) 9-BBN,THF, then NaOH, H_2O_2, 94%; e) TBAF,THF,65%

Scheme 13 Synthesis of diol **27**

With diol **27** in hand, the plan was to oxidize the two hydroxyl groups of **27** to the dialdehyde **34** that was to be subjected to Wittig olefination to the diene **26**, which was envisioned as a precursor of the synthesis of epoxide **24** (Scheme 14). Unfortunately, the oxidation of the two primary hydroxyl groups of **27** could not be accomplished. Examples of oxidation conditions attempted are: 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide (EDC),[162] or a Swern-like oxidation (with sulfur trioxide pyridine complex and dimethyl sulfoxide)[161] and a Dess-Martin oxidation.[163]

Considering the likely sensitivity of the dialdehyde, a backup plan considered introducing two carbonyl groups asynchronously. This called for **33** with only one free

hydroxyl group. Oxidation of the primary hydroxyl group of **33** under Swern-like oxidation conditions yielded aldehyde **35,** which was subjected to a Wittig reaction using potassium *tert*-butoxide and methyltriphenylphosphonium bromide to produce olefin **36**. Deprotection of **36** with a 1 M solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran afforded **37.** Oxidation of **37** successfully provided aldehyde **38.** However, the following Wittig olefination was disappointing; the desired diene **26** was not furnished, possibly due to the high instability of both aldehyde and diene (Scheme 14).



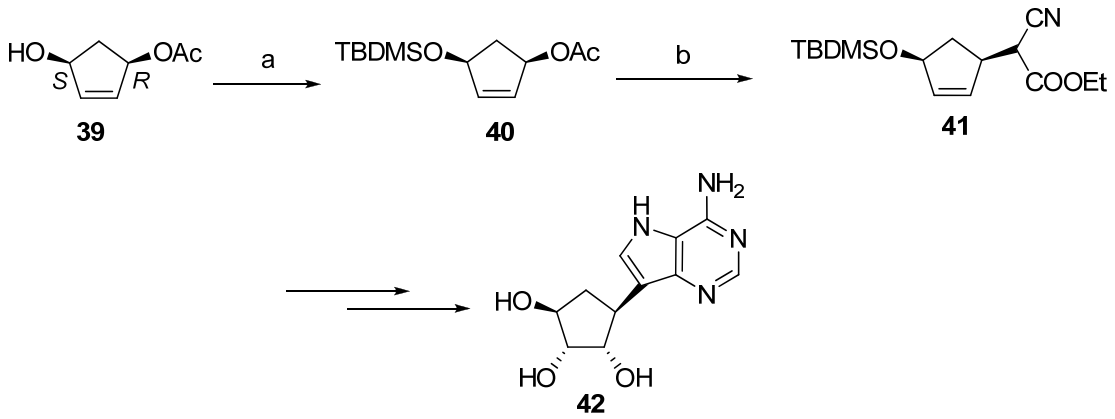
Reaction conditions: a) oxidations b) CH_2Cl_2, DMSO, DIPEA, SO_3-Py; c) Ph_3PCH_3Br, t-BuOK, Et_2O, two step 50%; d)TBAF,THF, 83%; e) CH_2Cl_2, DMSO, DIPEA, SO_3-Py, 60%; f) Ph_3PCH_3Br, t-BuOK, Et_2O

Scheme 14 Attempted synthesis of diene **26**

Although much work has been done on the synthesis of desired epoxide **24**, it should be admitted that the route just outlined was proving impractical. More obstacles were foreseen, such as the difficulty with a requisite ring closing metathesis (RCM) of

diene **26**; enantioselectivity of alkene epoxidation (prior to **24**); and, the stereochemistry of the nucleophilic addition to the epoxide that was to produce the alkyne precursor for heterocyclic ring construction (that is, pyrazolo[4, 3-*d*] pyrimidine). Therefore this route was abandoned in order to explore another pathway to synthesize the target compound not requiring a cyclopentyl epoxide. For this, a procedure to cyclopentyl alkynes was brought forth.[141,143,148-150,157]

Attempts to synthesize the cyclopentyl alkyne from allylic monoacetate 39. In 2003 the Schneller group reported the synthesis of 9-deaza-5′-noraristeromycin (**42**) (Scheme 15).[137] They prepared this carbocylic C-nucleoside from the readily available (+)-(1*R*, 4*S*)-4-hydroxy-2-cyclopentyl monoacetate (**39**).[137] Using Deardorff's method for coupling allylic acetates,[164,165] treatment of the protected monoacetate **40** with ethyl cyanoacetate in the presence of palladium (0) to give the *cis* oriented carbocylic C-nucleoside precursor **41**.

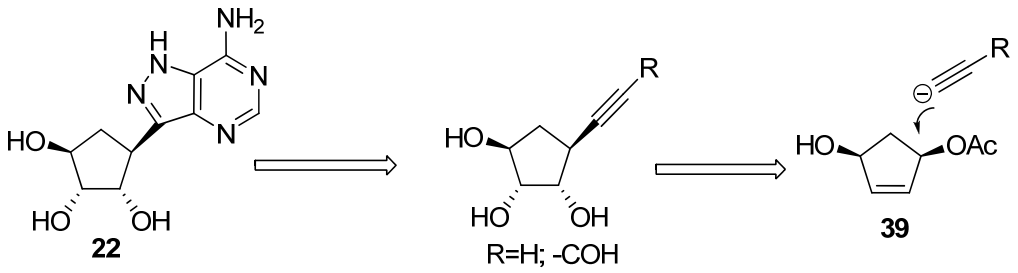


39 **40** **41** **42**

Reaction conditions: a) TBDMSCl, imidazole, DMF, rt, 81%; b) $NCCH_2CO_2Et$, NaH, $(Ph_3P)_4Pd/PPh_3$, THF, rt then to 55 °C, 78%;

Scheme 15 Synthesis of 9-deaza-5′-noraristeromycin

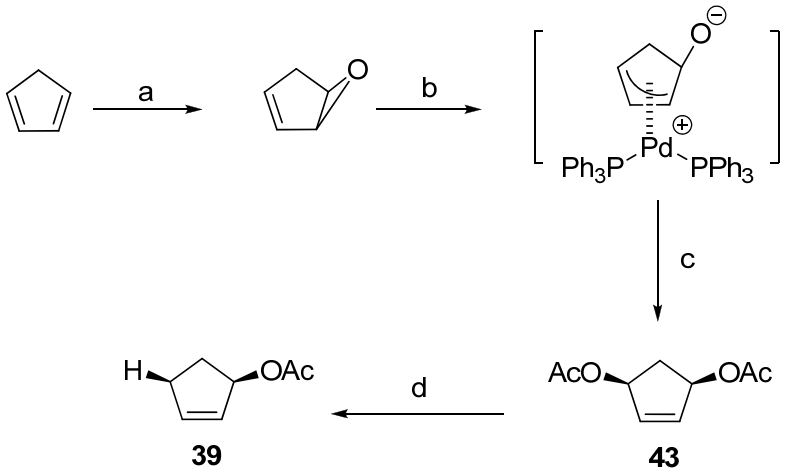
This research work sparked an idea to further study the synthesis of carbocylic 5′-norformycin from (+)-monoacetate **39**. A retrosynthetic analysis was devised (Scheme 16) with the critical alkyne shown as the middle structure.



Scheme 16 Retrosynthesis from monoacetate

(+)-Monoacetate **39** is one of the most important enantiopure precursors for carbocyclic nucleoside synthesis. Access to this material called for enzyme-catalyzed reactions, which themselves are powerful and offer convenient procedures to enantiopure compounds.[166] For the purposes of this project, the enzymatic hydrolysis of prochiral diacetate 4**3,** reported by Laumen and Schneider,[167] provided a means to (+)-monoacetate **39**.

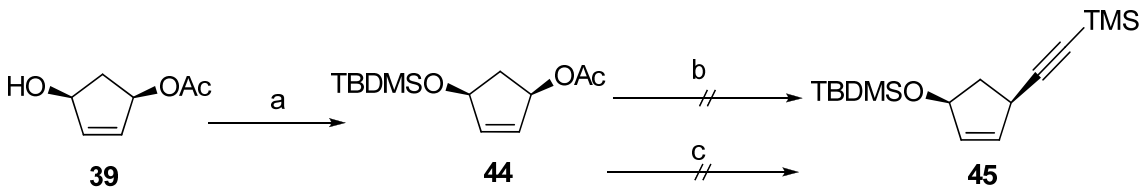
Diacetate **43** was obtained from cyclopentadiene following a literature procedure (Scheme 17).[168] This began with epoxidation of freshly cracked cyclopentadiene, which was followed by *tetrakis*(triphenylphosphine)palladium(0) catalyzed epoxide ring opening in the presence of acetic anhydride. In this latter reaction the presumed palladium intermediate reacted with acetic anhydride to give the *meso*-diacetate **43**. Using the optimized procedure developed in Schneller laboratory,[82] treatment of *meso*-diacetate **43** with *Pseudomonas cepacia* lipase (PCL) afforded the allylic monoacetate **39**. Although PCL normally displays a *pro-R*-hydrolytic preference,[169] it showed a *pro-S*-preference for this substrate.



Reaction conditions: a) Peracetic acid, Na_2CO_3, NaOAc, CH_2Cl_2; b) $Pd(PPh_3)_4$, THF; c)Ac_2O, 85% for three steps; d) *Pseudomonas cepacia* lipase, Buffer, NaOH, 80%

Scheme 17 Synthesis of (+)-monoacetate **39**

Attention then was drawn to palladium (0)-catalyzed allylations that are a common tool in organic synthesis.[170,171] The literature is rife with the use of carbon, oxygen, and nitrogen nucleophiles in this reaction. The use of acetylenic nucleophiles is not so common. To investigate this idea, the secondary hydroxyl group of **39** was protected with *tert*-butyldimethylsilyl chloride. The reaction of **44** using freshly prepared palladium catalyst and trimethylsilylacetylene under the conditions reported[164,165] failed to produce the desired coupling product **45** (Scheme 18).

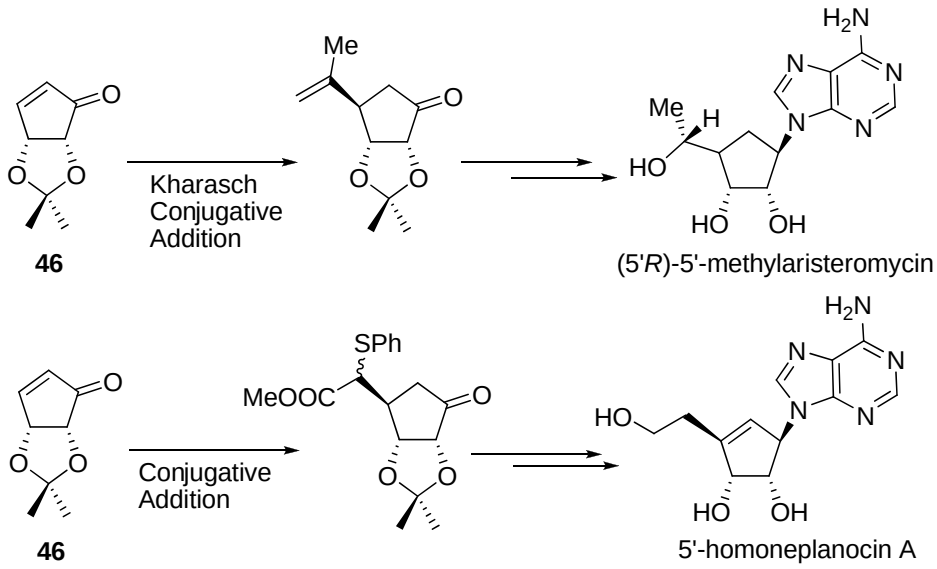


Reaction conditions: a) TBDMSCl, Imidazole, CH_2Cl_2, 90%; b) trimethylsilylacetylene, $Pd(PPh_3)_4$, NaH, THF; c) trimethylsilylacetylene, $Pd(PPh_3)_4$, n-BuLi, THF

Scheme 18 Attempted synthesis of compound **45**

In order to investigate whether the reaction failed due to the alkalinity of sodium hydride, *n*-butyllithium was considered in this reaction instead of sodium hydride. Unfortunately, the coupling also was unsuccessful. A possible reason for this result may lie with the carbon anions lacking sufficient nucleophilicity to attack the π-allyl palladium (II) intermediate to give the desired compound.

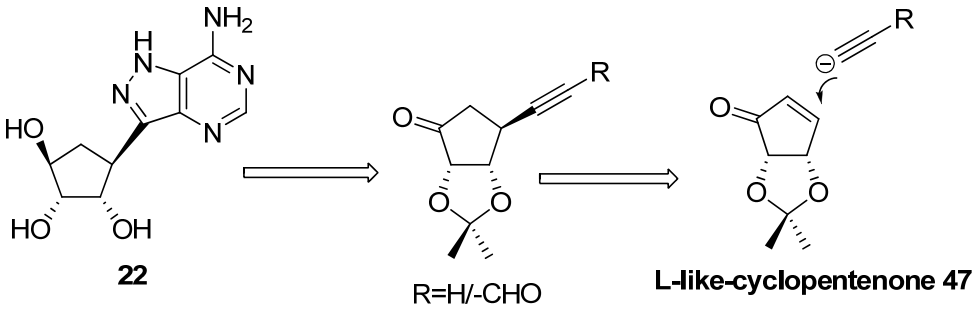
Synthesis of the cyclopentyl alkyne from cyclopentenone. (-)-(4*R*, 5*R*)-4,5-*O*-*Iso*propylidene-2-cyclopentenone (D-like 2-cyclopentenone **(46)**) is another important versatile chiral synthon in carbocyclic nucleosides.[172] The utility of **46** is demonstrated by its application for the preparation of the potential antiviral agents 5′-methylaristeromycin[173] and 5′-homoneplanocin A[174] (Scheme 19) beginning with a 1,4-addition to introduce a modified side chain.



Scheme 19 Examples using conjugative addition to 2-cyclopentenone **46**

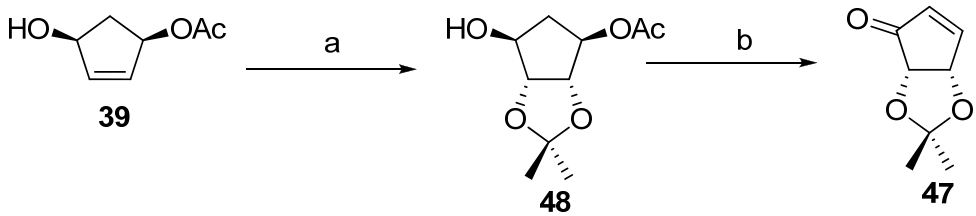
With the promise of the conjugative addition for the goals of this research, a retrosynthetic analysis was designed, and attention focused on the cyclopentyl unit that would lend itself to this purpose (Scheme 20). This suggested that target compound **22**

could be made from the (+)-L-like 2-cyclopentenone **47**. A 1,4-addition reaction could then be performed to give the important intermediate cyclopentyl alkyne. Using a series of procedures developed in the former work, the target synthesis was foreseen.



Scheme 20 Retrosynthesis from L-like-2-cyclopentenone **47**

L-Like 2-cyclopentenone **47**, which is the enantiomer of previously descried D-like cyclopentenone **46,** was easily prepared from the (+)-monoacetate **39** (Scheme 21). Glycolization with 4-methylmorpholine N-oxide (NMO) in the presence of a catalytic amount of osmium tetraoxide followed by protection of the *vicinal*-diol with 2,2-dimethoxypropane afforded **48**. Pyridinium chlorochromate oxidation of **48** proceeded with the fortuitous β-elimination of acetic acid to afford the conjugated L-like 2-cyclopentenone **47.**[175]

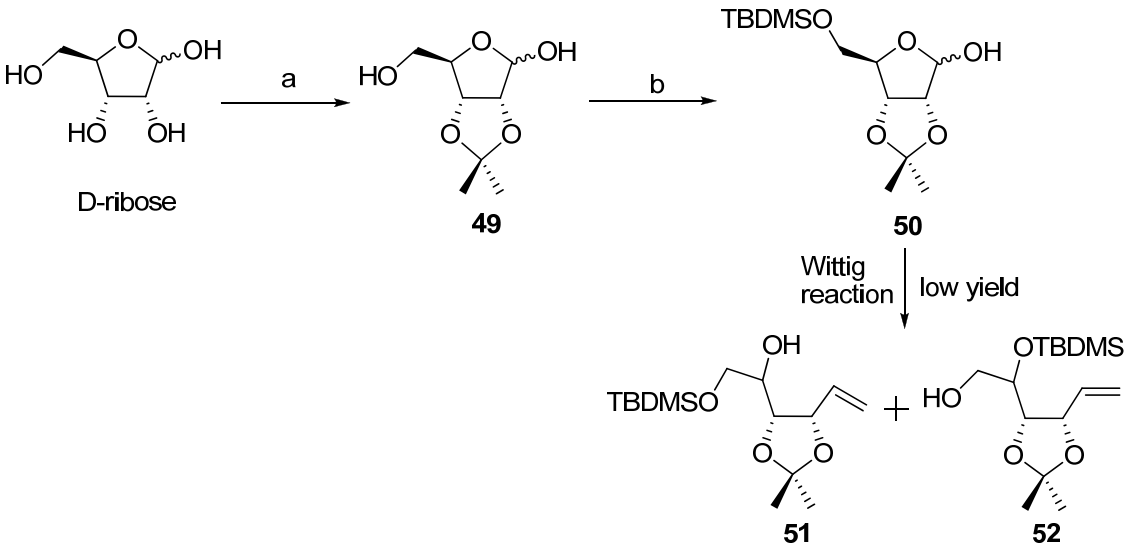


Reagents and conditions: a) i, OsO_4, NMO, Acetone/H_2O, 90%; ii, 2, 2-dimethoxy-propane, *p*-TsOH, Acetone, 75%; b) PCC, Celite, CH_2Cl_2, 78%

Scheme 21 Synthesis of L-like-2-Cyclopentenone **47** from monoacetate **39**

Another route to **47** considered natural sugars with predefined stereochemistry. This route has been found some success from D-ribose in 45% (8 steps) [172] and 38% (6 steps)[176] overall yield, respectively. More recently, Snape *et al.*[177]and Smith *et al.*[178] also developed more scalable routes to both enantiomers of cyclopentenone.

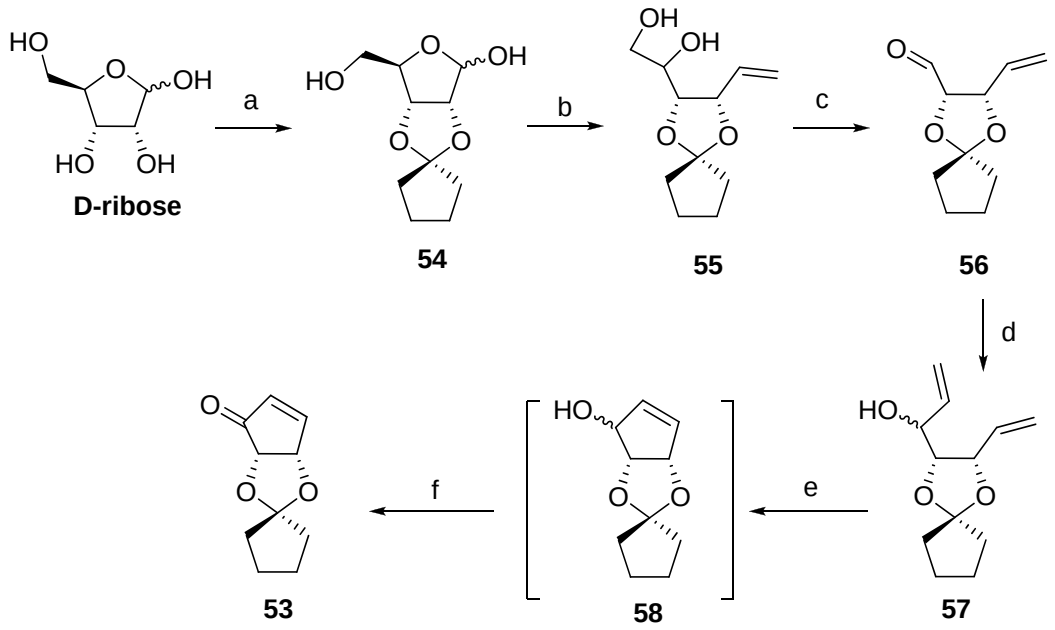
For this research, a new synthetic route to **47** was developed that was guided by the method of Chu and his group (Scheme 22).[172] In that direction, D-ribose was converted to the *iso*propylidene protected derivative **49** with 2,2-dimethoxypropane in the presence of a catalytic amount of sulfuric acid. This was followed by silylation of the primary hydroxyl group using *tert*-butyldimethylsilyl chloride to give lactol **50** in 85% yield. Compound **50** was converted to an olefin **51** by the Wittig reaction. The yield of this step was very low due to the appearance of side product **52** where the silyl group was transferred from a primary position to a secondary position. Chu and his group[172] did not mention this phenomenon.



Reagents and conditions: a) 2, 2-dimethoxy propane, *p*-TsOH, acetone, 85%; b) TBDMSCl, imidazole, CH_2Cl_2, 85%,

Scheme 22 Route from Chu's paper [172]

Finally, a concise and more economic route from D-ribose to the differently protected cyclopentenone (that is **(+)-**(4*S*, 5*S*)-4,5-*O*-cyclopentyl-2-cyclopentenone (**53**)) was accomplished (Scheme 23) in 6 steps (40% yield).



Reagents and conditions: a) cyclopentenone, H^+, 75%; b) CH_3PPh_3Br, t-BuOK, THF, overnight; c) $NaIO_4$, CH_2Cl_2, H_2O, rt, 30 min, 85% for two steps; d) vinylmagnesium bromide, THF, -30 ^oC, 1 h, 85%; e) 1st genertion Grubbs catalyst, CH_2Cl_2, rt, 12 h; f) CH_2Cl_2, DMSO, DIPEA, SO_3-Py, rt, overnight , 78% for two steps.

Scheme 23 Synthesis of cyclopentyl protected L-like-2-cyclocyclopentenone **53**

The cyclopentyl *vicinal* diol protecting group was chosen for **53** since the *iso*propylidene protected derivatives were found to be highly volatile under some conditions. Thus, in presence of a catalytic amount of sulfuric acid, cyclopentyl protected D-ribose **54** was afforded from ribose. A Wittig reaction of **54** with triphenylphosphonium methylidene followed by oxidative cleavage of the *vicinal* diol **55**[179-181] with sodium metaperiodate afforded the vinyl aldehyde **56**[179,182] in 85% yield (two steps). Treatment of **56** with vinylmagnesium bromide gave the diene **57** (85%),

which was subjected to ring-closing methathesis (RCM) reaction using the Grubbs 1st generation catalyst[183,184] (Figure 26) to give **58** in 88% yield. Cyclopentenone **53** was then directly obtained by oxidation of the secondary alcohol under sulfur trioxide-pyridine complex and dimethyl sulfoxide[161] oxidation conditions without isolation of the cyclopentenol **58** in 40% overall yield and six steps from D-ribose. This procedure turned out to be much improved in view of overall yield and number of steps compared to any previously reported procedure.

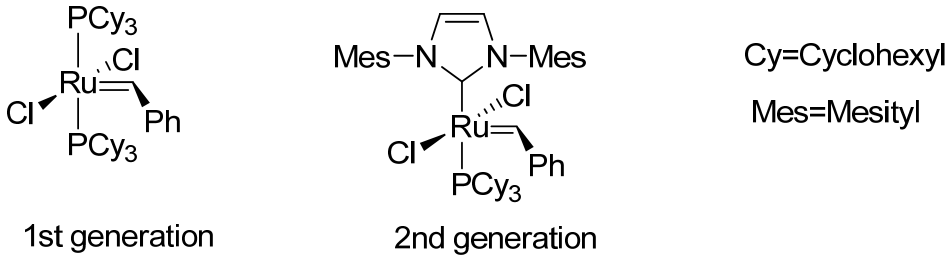
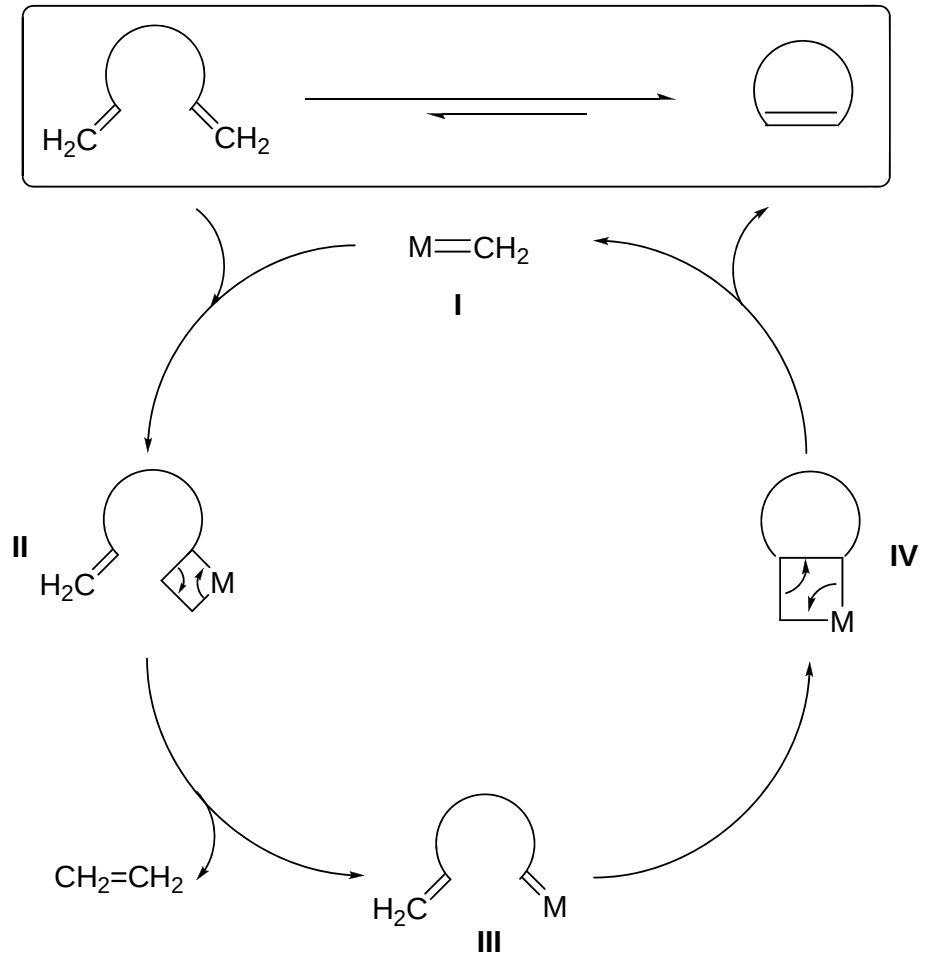


Figure 26. Grubbs Catalyst for RCM.

Since the Grubbs′ catalyst was important to this process, a comment about it is in order. Ruthenium-based Grubbs′ catalysts (Figure **26**) are among the most important catalysts for olefin metathesis (the metal-catalyzed carbon-carbon double bond redistribution[185]). They show great functional tolerance, high catalytic reactivity and moderate air and moisture stability. These advantages make them widely used in many organic syntheses.[185-187] The generally accepted mechanism of their use in ring closure metathesis was proposed by Chauvin[188] and is generalized in Scheme 24. In this process, a metal carbene I is first formed followed by a [2+2]-cycloaddition to afford a metallocyclobutane intermediate II. The subsequent retro [2+2]-cycloaddition releases olefin (ethylene in this case) and gives a new metal carbene III, which leads to a second

metallocyclobutane IV. Subsequent retro ring opening gives a new olefin and the original catalytic metal carbene I to complete the cycle. The key intermediate is the metallacyclobutane II, which can undergo cycloreversion either towards products or back to starting materials. When the olefins of the substrate are terminal, the driving force for RCM is the removal of ethene from the reaction mixture.



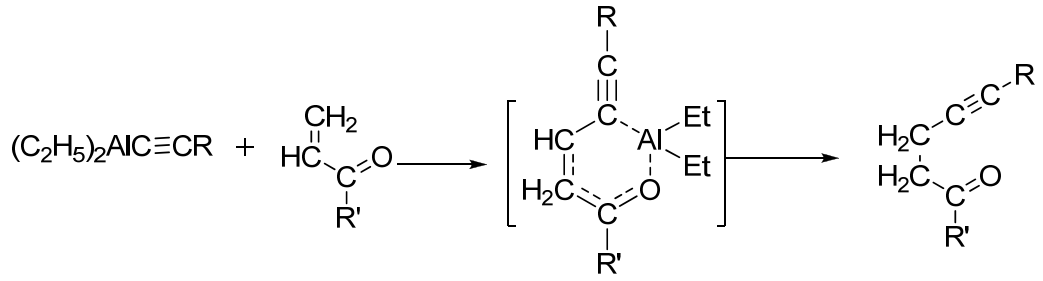
Scheme 24 General mechanism of Ring Closure Metathesis

Key step for the synthesis of target compound 22. With cyclopentenone **53** in hand, exploration of a way to introduce the alkynyl unit through 1,4-conjugative addition for the important intermediate cyclopentyl alkyne was investigated.

Conjugate addition of organometallic compounds to *α, β*-unsaturated ketones is a widely employed reaction in organic synthesis.[189] Organocuprates are commonly employed for the 1,4-addition of alkyl and alkenyl groups to *α, β*-unsaturated ketones;[190] however, cuprates cannot be used in alkynylation reactions owing, presumably, to the strength of the alkynylcopper (I) ligand bond.[191,192].

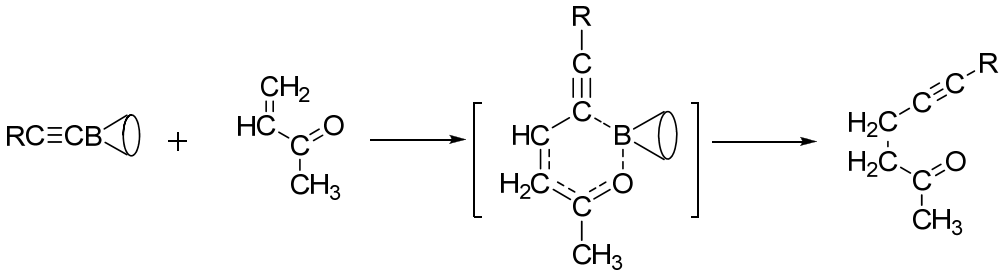
Several alternatives have been reported that achieve conjugative addition to *α, β*-unsaturated enones, but each suffers from limitations for the purposes here. However, a brief overview of contextualization is in order.

Acetylenic alanes[193-195] conjugately add their alkynyl units to *α, β*-unsaturated enones only under certain circumstances. If the *α, β*-unsaturated ketone is able to achieve an *S-cis* conformation, it has been found that 1, 4-addition (Scheme 25) will then proceed in good yield. Cyclic ketones in which the enone system is rigidly constrained to a *transoid* geometry, such as 2-cyclohexenone, react with the alane reagent to give the tertiary carbinol derived from 1, 2- rather than l, 4-addition desired here of the acetylenic unit. A reasonable explanation for this reactivity mode involves the necessity for a six-membered transition state for conjugate addition.[193]



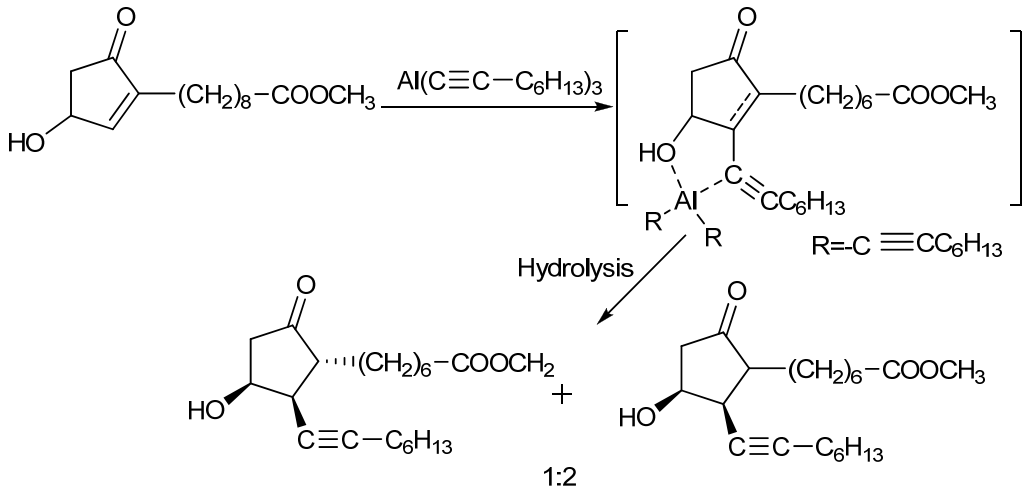
Scheme 25 Conjugate addition of acetylenic alane.

Alkynylboron reagents[196-198] also add only to *α, β* -enones that assume an *S-cis* conformation. As in the case of alkynylalanes, *transoid* ketones gave no indication of the desired 1, 4- reaction. This observation suggested a cyclic transition state analogous to the one proposed for the 1, 4-addition reactions of alkynylalanes (Scheme 26).



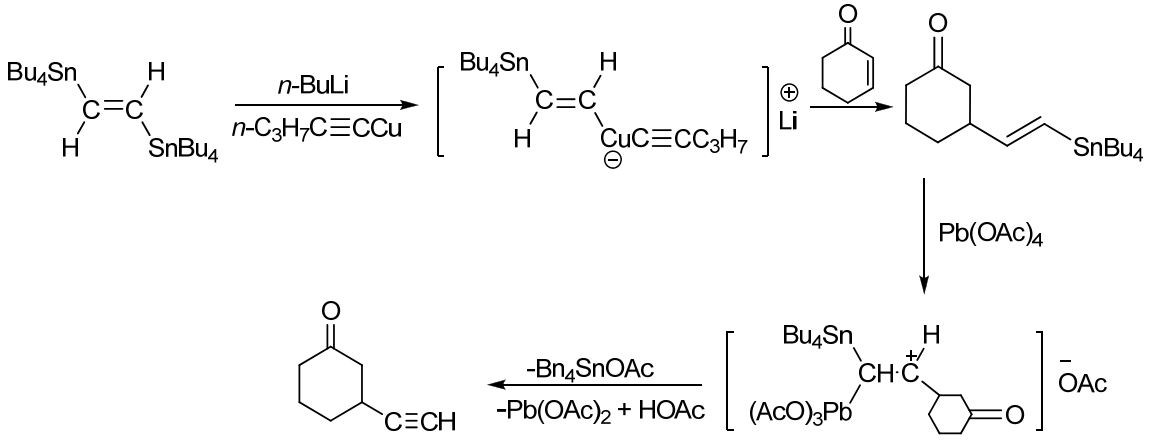
Scheme 26 Conjugate addition of alkynylboron reagents.

In the investigations into the application of 1,4-addition reactions to the synthesis of prostaglandins, Pappo *et al.*[194] were able to perform this reaction on a fixed *S-trans* enone (such as cyclopentenone). Two 1,4-addition products were obtained in approximately a 1:2 ratio (Scheme 27). The fact that the entering octynyl group added *cis* to the hydroxy function indicated participation of that group in the 1,4-addition process by way of a five-membered cyclic intermediate analogous to the structures discussed above. Blockage of the hydroxy function by a tetrahydropyranyl group prevented reaction with the aluminum reagent.



Scheme 27 Conjugate addition of trioctynylaluminum reagent

An indirect method of conjugate addition of an alkynyl group to a fixed *S-trans* enone without neighboring-group participation had been developed by Corey and Wollenburg.[192,199] This procedure involves addition of bis(tri-*n*-butylstanny1)ethylene by cuprate addition and subsequent oxidative elimination of a stannyl group to give, overall, conjugate addition of acetylide to the enone (Scheme 28).



Scheme 28 Conjugate addition of mixed cuprate reagent

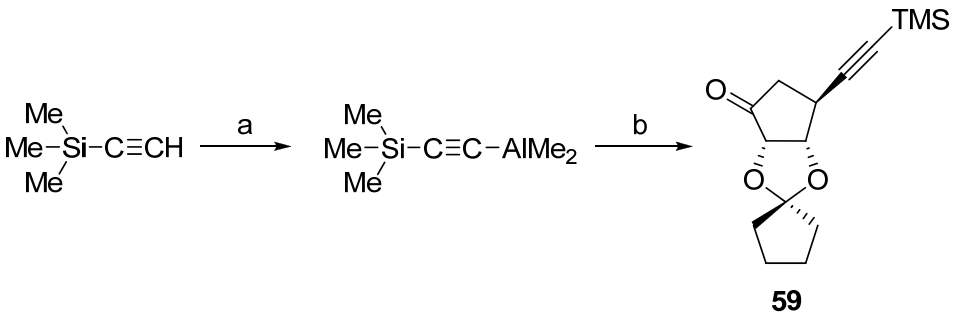
Each of the sequences which achieve conjugate addition of alkynyl units suffers from severe limitations. The enone substrate must be capable of an *S-cis* conformation or, alternatively, must have a conveniently located functional group for direction of an *intramolecular* attack by the organometallic reagent. For those enones which fit neither one of these requirements, it is only possible to conjugately add the ethynyl group, and this must be done by an indirect method.

Schwartz *et al.*[189,200] found that the nickel (II) acetylacetonate ($Ni(acac)_2$)/ di*iso*butylaluminum hydride (DIBAL) system would indeed catalyze the conjugate addition of terminal alkynyl units from dialkylaluminum acetylides to both *S-cis* and *S-trans* enones (Scheme 29). The latter observation was pertinent to this project. Furthermore, the reaction was found to proceed with high stereospecificity.

Reagents and conditions: a) BuLi, dimethylaluminum chloride,ethyl ether;
b) i.$Ni(acac)_2$/ DIBAl; ii. using enones ; iii. KH_2PO_4 / H_2O.

Scheme 29 Nickel-catalyzed 1,4-addition of organoaluminum acetylide to *S-trans* enones

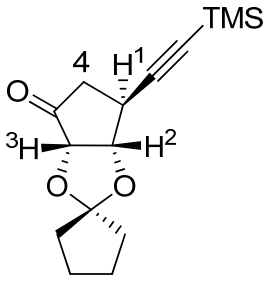
From the above discussion, it was believed that through the above procedure, a nickel-catalyzed conjugate addition of alkynyl groups to **53** could be affected. Fortunately, this addition was successful and the desired cyclopentyl alkyne precursor **59** was obtained (Scheme 30), and the product formed possessed the *anti* stereochemistry.



Reagents and conditions: a) BuLi,dimethylaluminum chloride,ethyl ether;
b) i. $Ni(acac)_2$/ DIBAL; ii. using **53**; iii.KH_2PO_4 / H_2O, 42% for two steps.

Scheme 30 Key step for the synthesis of target compound

The stereochemical assignment for **59** is based on ^1H NMR (Figure 27). From the NMR spectral data, "anomeric" hydrogen (H-1) has no resonance with any one of the hydrogens at positions 2 and 3; in the other hand, the resonance for H-2 with H-3 appears at δ 4.3 as a doublet with coupling constants equal to 5.0 Hz, so does for H-3 with the same coupling constants. All these ^1H NMR data confirmed that the two hydrogens with fixed stereochemistry are *syn* with alkynyl group.



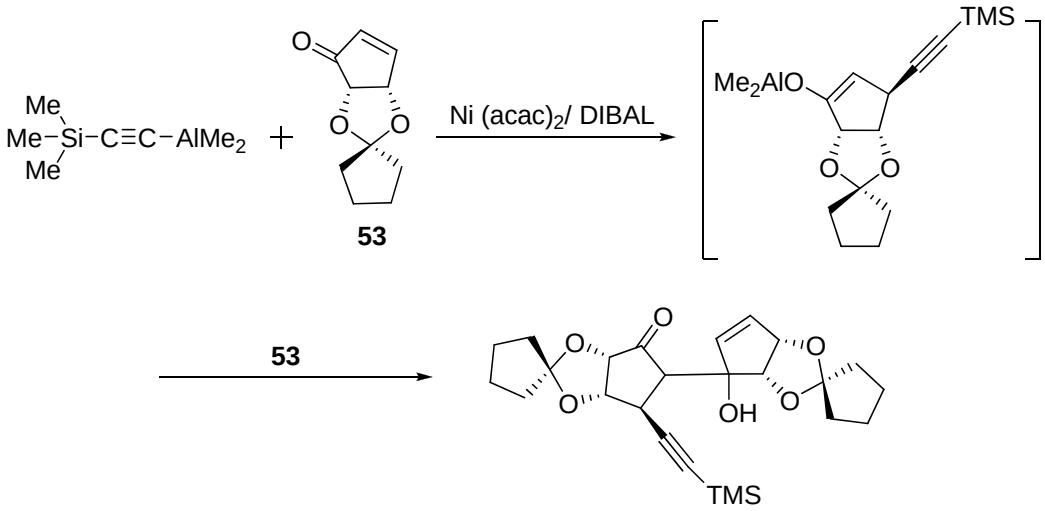
3.3 (dd, J=8.8 Hz, 0.5 Hz, H-1);
4.3 (d, J=5.0 Hz, H-2);
4.7 (d, J=5.0 Hz, H-3);
2.9 (dd, J=8.8 Hz, 18 Hz, H-4); 2.38-2.46 (m, H-4).

Figure 27. ^1H NMR spectral data for compound **59**.

The typical procedure for effecting conjugate addition by this route is illustrated as follows: To nickel(II) acetylacetonate ($Ni(acac)_2$) in ether at -5 ˚C is added 1 M di*iso*butylaluminum hydride in hexane solution. The reaction mixture rapidly turned dark red-brown and it was then cooled to -25 ˚C. To the resultant red-brown solution was added fresh dialkylaluminum acetylide (prepared in the usual manner from the lithium

acetylide and dialkylaluminum chloride) as a solution in ether. 2-Cyclopentenone **53** in ethereal solution was then added dropwise over a period of time. The reaction mixture was allowed to stir at -25 ˚C for several hours. After hydrolysis, the conjugate adduct **59** was afforded.

It was found that optimal yields of the conjugate adduct were obtained when an excess of dialkylaluminum acetylide (2.2 eq) was employed. Using a smaller excess of the aluminum acetylide resulted in a decreased yield of the desired product. It was important to employ an excess of the aluminum acetylide, probably, because the initial product of conjugate addition was an aluminum enolate, which could react with additional unsaturated ketone to give the aldol adduct (Scheme 31). In the presence of excess aluminum acetylide, the added 2-cyclopentenone **53** in the presence of the nickel catalyst preferentially reacted with it. If less aluminum acetylide was employed, oligomers would be generated as by-products.



Scheme 31 Possible unwanted adol side reaction

Finally, the exploration of the key step towards target compound **22** ended with the successful nickel-catalyzed conjugate addition. This represents the first time **53 (**an important enantiopure precursor**)** was called forth for synthesizing carbocyclic C-nucleosides. Also, the 1,4-conjugate addition to a cyclopentenone process is another route towards carbocyclic C-nucleosides avoiding functionalized expoxide openings.

Enantioselective synthesis of carbocyclic 5′-norformycin (22). With cyclopentyl alkyne precursor **59** in hand and calling upon reaction conditions developed earlier in this work, target compound carbocyclic 5′-norformycin (**22**) was believed obtainable.

The synthesis of **22** began with the reduction of the cyclopentyl alkyne precursor **59** using sodium borohydride and cerium chloride (Luche reagent)[201] to afford the α-alcohol **60** (Scheme 32) as the only isomer. The Luche reagent is a prominent reagent for the stereoselective conversion of ketone to secondary alcohols. In this process, Ce(III) acts as a good Lewis acid and is strongly oxophilic, and lead to the chelation intermediate[202] (Figure 28) that promotes the reduction. The existence of the bicyclic cyclopentanone arrangement in **59** guides the borohydride attack from the less hindered β face.

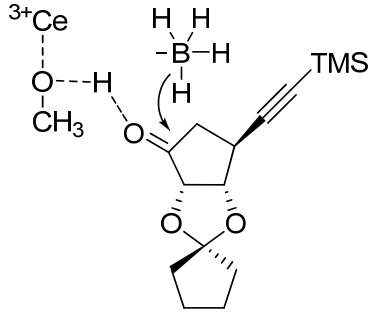
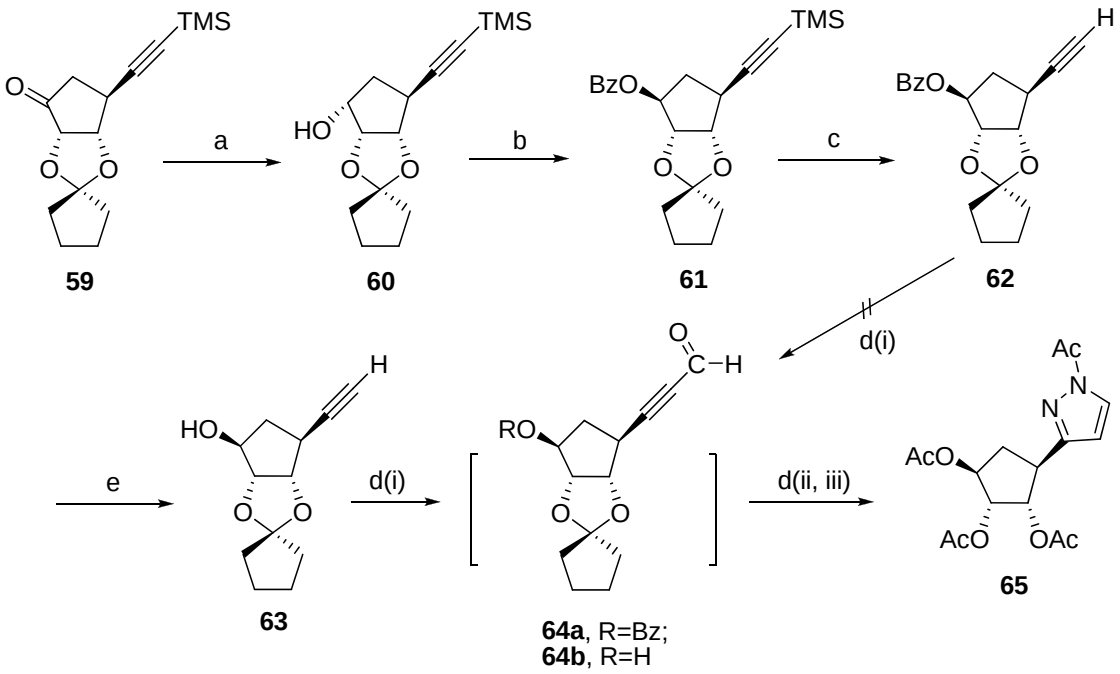


Figure 28. Chelation intermediate to **59**.



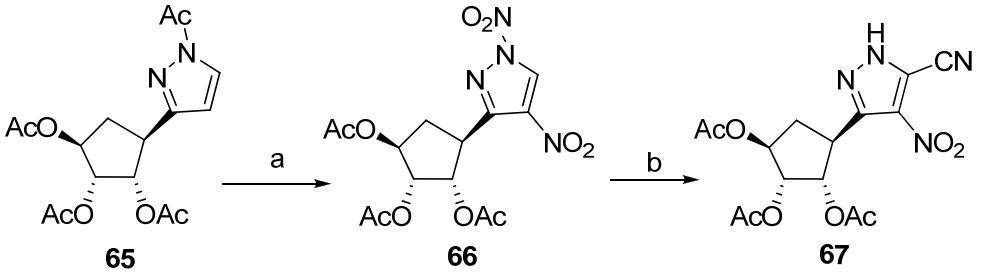
Reagents and conditions: a) $NaBH_4$/ $CeCl_3 \cdot 7H_2O$, MeOH, 75%; b) benzoic acid, DIAD, PPh_3, THF, rt, 70%; c) TBAF, THF, 92%; d) i.BuLi / hexanes, MTBE followed by DMF; ii. AcOH, hydrazine monohydrate; iii. Ac_2O,pyridine, DMAP, 73% for three steps; e) NH_3, MeOH, 98%

Scheme 32 Synthesis of compound **65**

Inversion of the C-4′ center of **60** was accomplished by a Mitsunobu reaction with benzoic acid to give **61**. Removing the trimethylsilyl group of **61** with 1 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave the cyclopentyl alkyne **62**. Directly formylation of **62** (step i of (d)) failed to afford the corresponding propargylic aldehyde **64a** probably due to the instability of benzoic group under the reaction conditions. Thus, treatment of **62** with methanolic ammonia at room temperature produced the unprotected cyclopentyl alkyne **63**. Formylation (step i of (d)) of **63** with subsequent[141-143] reaction of the resultant substituted propargylic aldehyde **64b** with hydrazine monohydrate and followed by acetylation with acetic anhydride catalyzed by

4-dimethylaminopyridine (DMAP) provided the key synthetic intermediate pyrazole **65** (Scheme 32).

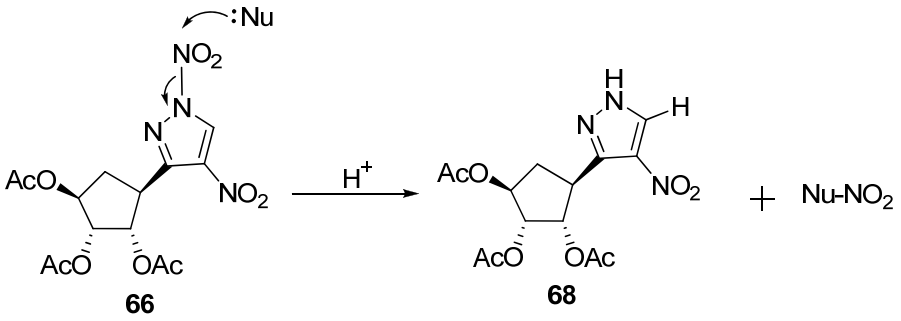
In moving forward towards **22**, the key nitronitrile derivative **67**, was achieved by, first, nitration[153] of **65** with ammonium nitrate and trifluoroacetic anhydride in trifluoroacetic acid to the 1, 4-dinitropyrazole **66 (**Scheme 33**).** This was followed by a *cine*-substitution reaction of **66** with potassium cyanide to afford **67**.[156,157]



Reagents and conditions: a) NH_4NO_3, TFA,TFAA; b) using **66**, KCN, EtOH, EtOAc, 80% for two steps

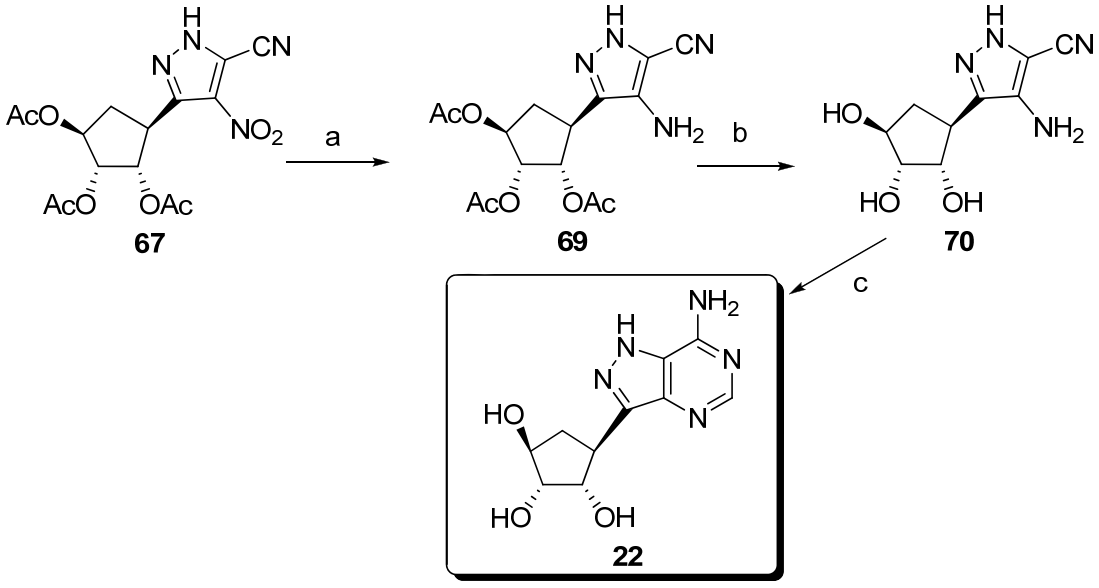
Scheme 33 Synthesis of nitronitrile compound **67**

In exploring the conditions for the *cine*-substitution reaction, often an undesired side product **68** was observed (Scheme 34). This is believed to have arisen *via* a nucleophilic substitution reaction consisting of a displacement of the *N*-nitro group by the nitrile anion and/or ethanol.[155] To limit the amount of **68**, the optimum reaction conditions were explored: for example, (1) reaction time was an important factor in this reaction, requiring quenching within five minutes; (2) a solvent volume ratio of ethanol and ethyl acetate should be nearly 1:1 and, (3) the reaction should be conducted at room temperature.



Scheme 34 Displacement on the *N*-nitro group

Three steps remained to the target compound **22** (Scheme 35). This began with catalytic (Pd) hydrogenation of the nitronitrile derivative **67**. Deacetylation of the resulting aminonitrile derivative **69** with a solution of saturated ammonia in methanol (to **70**) followed by formamidine acetate in refluxing ethanol produced the desired fused pyrimidine target (1*S*,2*R*,3*S*,4*S*)-4-(7-amino-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl) cyclopentane-1,2,3-triol (**22)**.



Reagents and conditions: a) 30 psi H_2, 10% Pd/C, MeOH, 85%; b) NH_3, MeOH, 83%; c) $HC(=NH)NH_2 \bullet AcOH$, EtOH, 54%.

Scheme 35 Synthesis of target compound **22**

Enatioselective Synthesis of *N*-1 and *N*-2 Methylated Carbocyclic 5'-Norformycin Analogs

As mentioned in the introduction section, *N*-1-methylformycin was highly resistant to enzymatic deamination; *N*-2-methylformycin exhibited high activity against vaccinia virus while not affecting cellular DNA and RNA synthesis.[122] Both *N*-methylated formycin analogs were non-cytotoxic. The project for this dissertation research sought to extend these observations to the *N*-methyl carbocyclic 5′-norformycin analogs **71** and **72** (Figure 29).

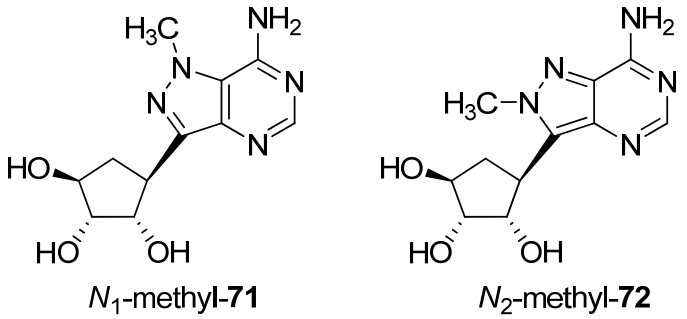
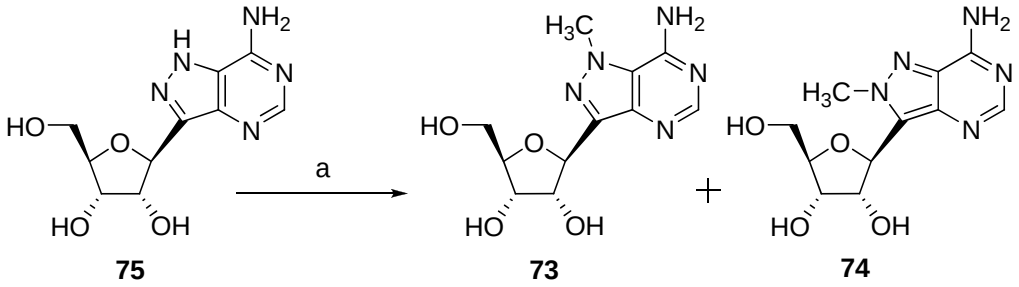


Figure 29. *N*-methyl carbocyclic 5′-norformycin analogs.

The methylated formycin derivatives **73** and **74** (Scheme 36) have been reported by Townsend and his group.[158] In that regard, methylation of formycin (**75**) with methyl iodide in the presence of base afforded 1-methylformycin (**71**) and 2-methylformycin (**72**).

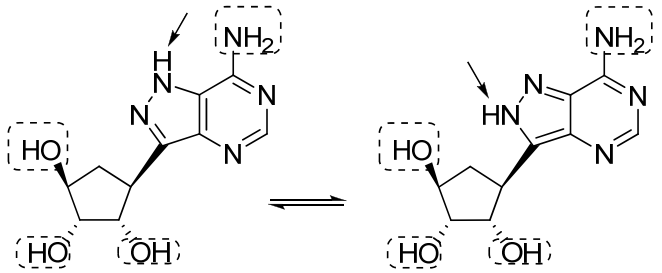


75 **73** **74**

Reaction conditions: a) NaOEt, EtOH, MeI, 3.8% for **73**, 24% for **74**

Scheme 36 Synthesis of methylated formycin derivatives

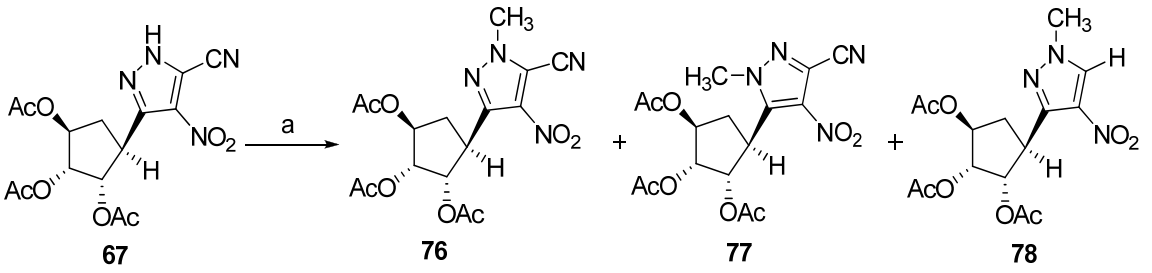
With carbocyclic 5′-norformycin (**22**) in hand, application of the Townsend conditions for achieving **71** and **72** were evaluated. In view of the low yields in this approach (Scheme 36), it was conceived that methylation of carbocyclic 5′-norfomycin (**22**) could result in lower yields of the desired products since there are several possible competing methylation sites (Figure 30).



22 **22**

Figure 30. Possible methylation sites of **22**.

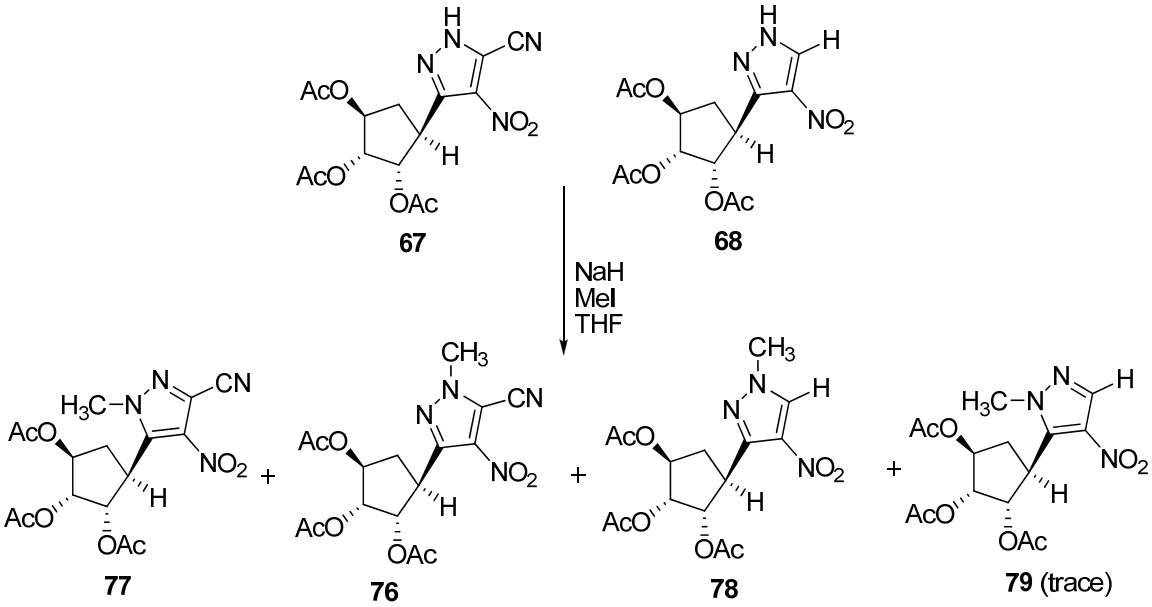
Thus, it was decided to introduce the methyl group at the pyrazole stage using the nitronitrile intermediate **67 (**Scheme 37**).** Treatment of **67** with sodium hydride followed by quenching with methyl iodide gave a major product (*N*-1-methyl **76**), a minor one (*N*-2-methyl **77**) and an undesired side product **78,** which were separated by column chromatography.



Reagents and conditions: a) NaH, CH_3I, THF, 50% for **76**, 5% for **77** and 25% for **78**

Scheme 37 Methylation of **67**

Formation of the side-product **78** could be explained from a possible impurity of **68** with **67**. During the synthesis of nitronitrile compound **67**, a displacement reaction (Scheme 34), in addition to the *cine* substitution, took place with by-product **68** being obtained. By-product **68** and compound **67** have identical R_f values. Only ^{13}C DEPT NMR data can distinguish **68** from **67**. Thus, it is reasonable to believe that the methylation took place with a mixture of compound **67** and **68** (Scheme 38) leading to only three of the maximum four compounds (compound **79** being only in trace amounts).



Scheme 38 Possible reason for side product **79**

Based on the model study discussed in the first part of this dissertation, structural assignments for the above methylated derivatives was confirmed by the comparisons of their TLC mobilities and their NMR spectral data (^{1}H and ^{13}C).

The comparative TLC mobilities of the methylated isomers were the easiest way to distinguish the different isomers and the data are presented in Table 3.

Table 3 Comparative TLC Data for the *N*-methylated isomers

Solvent (hexane/ethyl acetate =3:1 v/v)	
1-methyl **(76)**	R=0.52
2-methyl (**77**)	R=0.34
1-methyl (**78**)	R=0.45

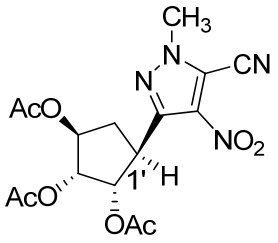
Selected ^{1}H NMR and ^{13}C NMR spectral data for the three isomeric methylated products are compiled in Table 4. The ^{1}H methyl signal for the 1-methyl product **76** is downfield from the signal observed for the 2-methyl derivative **77**; the ^{1}H methyl signal for the by-product **78** was expected to be close with that signal of **76**, but, possibly, because of the absence of the cyano group in **78**, that peak is close to the signal of **77**. The ^{1}H signal for the "anomeric ($H_{1'}$) proton" of the 2-methylated isomer **77** wherein rotation about the glycosidic bond (C3-C1′) is restricted is observed downfield from the "anomeric" signals observed for the 1-methyl isomer **76** and by-product **78**. From some NMR spectral data, by-product **78** can be distinguished from the 1-methyl isomer **76**: from the ^{1}H NMR spectrum, a singlet peak (belonging to H_5 in **78**) is lacking in **76**; from the ^{13}C NMR spectrum, there has no CN signal in **78,** but this signal exists in **76**; from the

^{13}C DEPT NMR spectral data, a positive peak at C_5 in **78** is observed which means C_5 is connected with a hydrogen atom instead of cyano group.

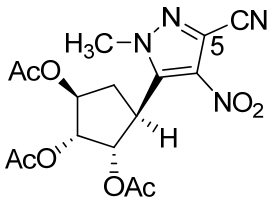
Table 4 Selected ^{1}H NMR and ^{13}C NMR Spectral Data [a]

^{1}H NMR	NCH_3	H_5	H_1'
76 (1-methyl)	4.10	--	4.02-4.08
77 (2-methyl)	4.01	--	4.06-4.14
78 (1-methyl)	3.90	8.11	4.01-4.08

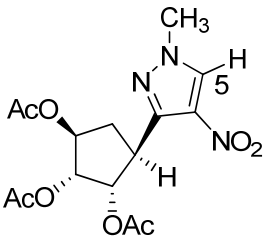
^{13}C NMR [b]	NCH_3	CN	C_5 (DEPT)
76 (1-methyl)	39.90	107.26	115.68 (no peak)
77 (2-methyl)	39.84	107.31	113.92
78 (1-methyl)	40.11	--	**131.15** (positive)



67



68



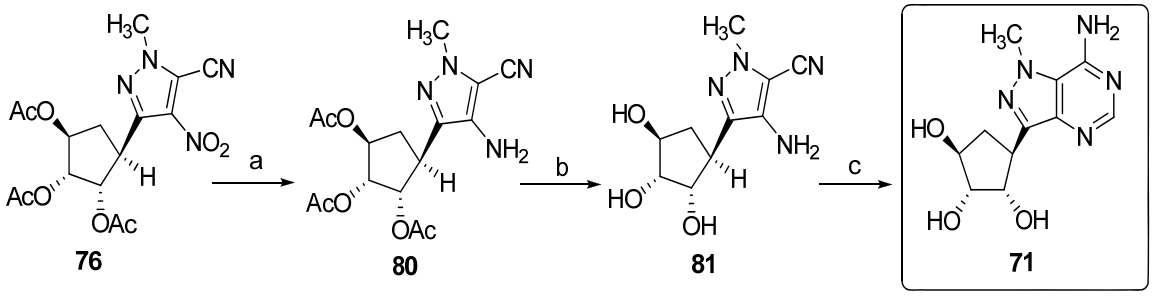
69

[a] $CDCl_3$ was used as a solvent and chemical shifts are in parts per million from an internal standard.

[b] ^{13}C assignment for **76**, **77** and **78** is determined by comparison to methylated formycins[16] and methylated carbocyclicformycin analogs.

With the *N*-1-methylated nitro nitrile compound **76** in hand, steps towards target **71** compound began with hydrogenation of **76** in the presence of palladium/carbon to the

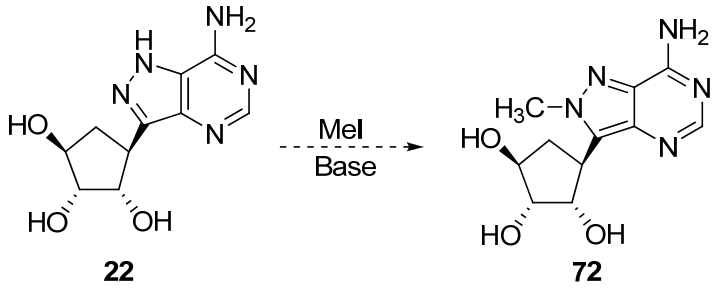
reduced product **80**. Deacetylation of **80** to **81** was conducted in a solution of saturated ammonia in methanol. Treatment of **81** with formamidine acetate in refluxing ethanol produced the desired fused pyrimidine target (1*S*,2*R*,3*S*,4*S*)-4-(7-amino-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)cyclopentane-1,2,3-triol (**71)** (Scheme 39).



Reagents and conditions: a) H_2, Pd/C, MeOH; b) NH_3, MeOH,80% for two steps; c) formamidine acetate, EtOH, 50%.

Scheme 39 Synthesis of *N*-1 methylated 5′-norcarboformycin **71**

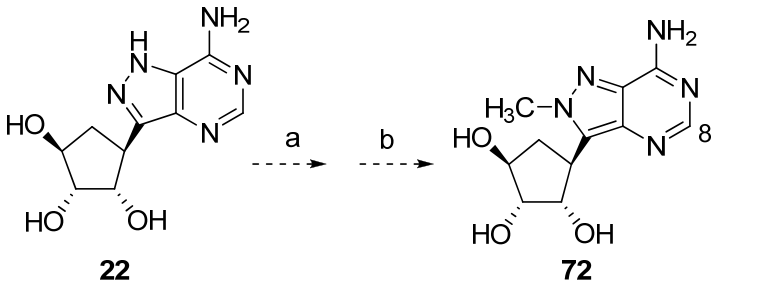
Since the regioselectivity of Scheme 35 gave low yields of the *N*-2-methylated derivative **77**, proceeding to **72** in the same manner, as just described for **71**, was no longer considered to be appropriate. Consequently, it was decided that the synthesis of **72** had to return to the original plan of methylation of **22** to get **72**. (Scheme 40)



Scheme 40 Alternate route to synthesize **72**

Attempted Synthesis of *N*-2 methylated 5′-norcarboformycin 72

It was obvious that the methylation of free 5′-norcarboformycin (**22**) would require treatment of **22** with freshly made sodium ethylate (Scheme 41). Following stirring to effect a clear solution, methyl iodide was then added and the solution was stirred until the starting material was no longer present (TLC). After three additional quantities of methyl iodide were added, the solution was stirred for three days. TLC (R_f) analysis showed the absence of *N*-1 methyl 5′-norcarboformycin (**71**). By contrast, a TLC spot with strong UV absorbency was seen and its R_f value was lower than that of compound **71**. The reaction residue was then analyzed by NMR spectroscopy. Signals (^{1}H methyl signal and H_5 signal) were found. These peaks were different from the *N*-1 methyl derivative. It is speculated that these are due to the presence of *N*-2 methylated 5′-norcarbofomycin (**72**). However, this mixture was complicated and the yield was unsatisfactory. These conditions were not considered further for the methylated derivatives.



22 **72**

Reagents and conditions: a) Na, EtOH; b) MeI, 5% for two steps.

Scheme 41 Synthesis of compound **72**

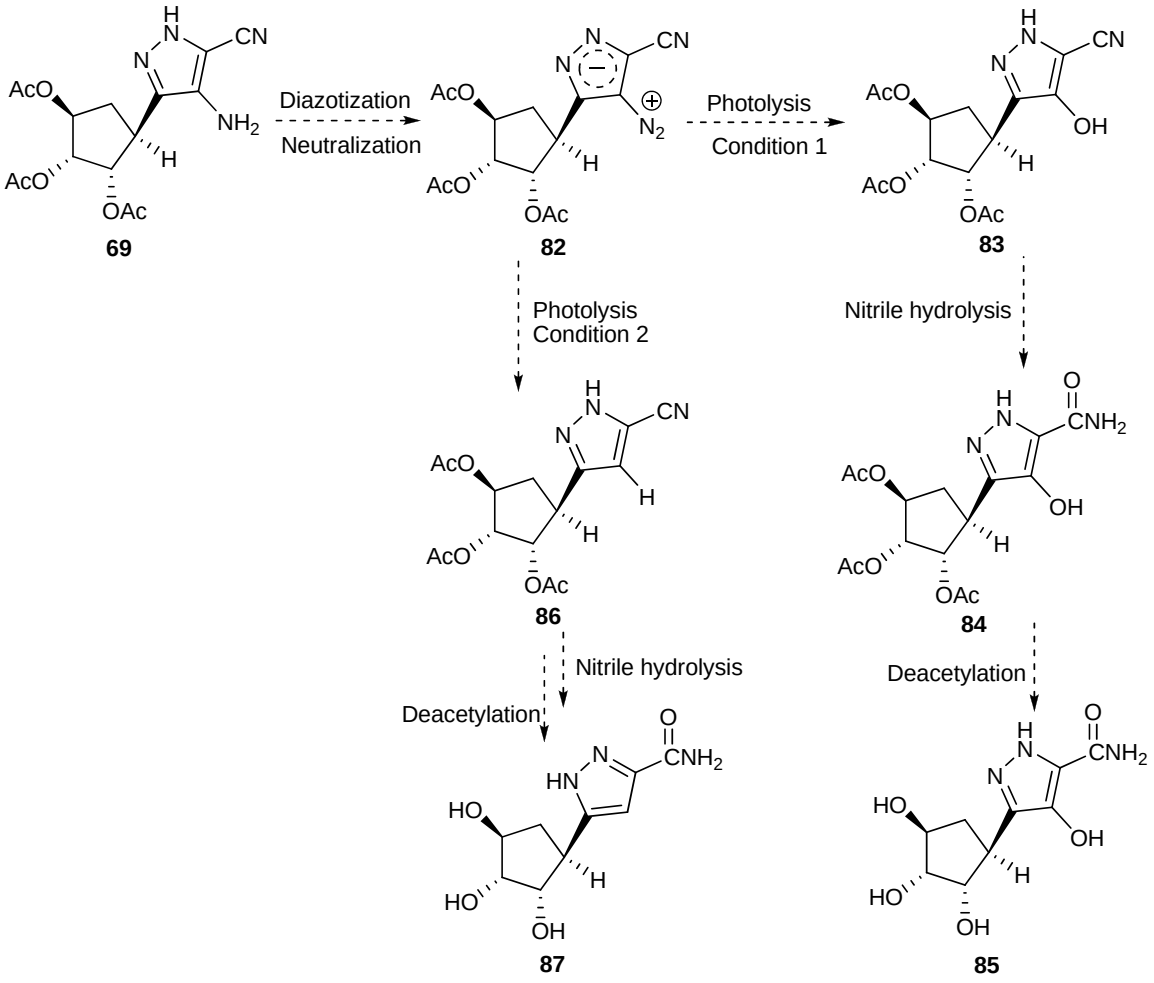
Future Direction: Proposed synthesis of another carbocylic C-nucleoside-Pyrazomycin analogs

As mentioned in the introduction, pyrazomycin was isolated from the culture filtrates of *Streptomyces candidus*[102] and has been shown to be an inhibitor of a variety of viruses and tumors. Pyrazomycin exhibited activity against the vaccinia, herpes simplex, and measles viruses *in vitro*.[102,103] The Friend leukemia virus was also inhibited by pyrazomycin as reported by DeLong *et al*. [203]

In seeking structural analogs of pyrazomycin for improved bioactivity, the 5′-norcarbocyclic system **84** is a worthy target. In that regards, during the synthesis of the 5′-norcarboformycin (**22**), a relevant intermediate 4-aminopyrazole derivative **69**, arose. To achieve **84**, replacement of the C-4 amino group of **69** with a hydroxyl group (or a hydrogen) could lead to the synthesis of 5′-norcarbopyrazomycin **85** and its derivative **87** (Scheme 42).

A classical method[66] exists in aromatic systems to carry out the aforedescribed conversions: that is the 4-aminopyrazole derivative **69** can be diazotized and the diazonium salt solution rendered alkaline to provide a neutral zwitterionic diazopyrazole **82**. On photolysis in aqueous acetone with a medium-pressure mercury lamp and Pyrex filter, nitrogen is evolved and the 4-hydroxypyrazole derivative **83** is obtained. On the

other hand, on photolysis in aqueous dioxane (catalyzed by trifluoroacetic acid) using visible light on **82** will give the deaminated nitrile derivative **86**. Hydrolysis of the nitrile group in **83** and **86** using alkaline hydrogen peroxide, will afford the amides. After deacetylation, the carbopyrazomycin analogs could be accomplished.



Condition 1: a medium-pressure mercury lamp and Pyrex filter; acetone-water (3 : 1 v/v);
Condition 2: visible light; aqueous dioxane containing trifluoroacetic acid.

Scheme 42 Proposed synthesis of **85** and **87**

BIOLOGICAL RESULTS

Target compounds were designed as antiviral agents and compound **1** was evaluated against a wide variety of viruses. The spectrum of viruses used is shown in Table 5.

Table 5 The spectrum of viruses to be assayed

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

The detailed results were showed in Table 6-Table 13. No activity was found. It was also found to have no toxicity effects on the viral host cells.

Table 6 Antiviral Activity of Compounds against HSV-1, HSV-2, HCMV and VZV Based on Cytopathogenic Effect (CPE) Inhibition Assay [a, b]

Compound		**1**
HSV-1[d]	EC50[a]	>300
	EC90[b]	>300
	CC50[c]	>300
HSV-2[d]	EC50	>300
	EC90	>300
	CC50	>300
HCMV[d]	EC50	>300
	EC90	>300
	CC50	>300
VZV[d]	EC50	>300
	EC90	>300
	CC50	>300

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

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

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

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

Table 7. Antiviral Activity of Compounds against RSV A, Parainfluenza and SARS CoV Based on Neutral Red Visual Inhibition Assay

Compound	RSV A[a]		Parainfluenza[b]		SARS CoV[c]	
	EC50	IC50[d]	EC50	IC50	EC50	CC50
1	>100	>100	>100	>100	>100	>100

Units = μM

[a] RSV A was tested on MA-104 cells.

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

[c] SARS CoV was tested on vero cells.

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

Compound	Vaccinia Virus[a]		Cowpox Virus[b]	
	EC50	CC50	EC50	CC50
1	>300	>300	>300	>300

Units = μM

[a] Vaccinia Virus was tested on HFF cells.

[b] Cowpox Virus was tested on HFF cells.

Table 9. Antiviral Activity of Compound 1 in Vero Cell Cultures

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

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

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

Table 10. Antiviral Activity of Compound 1 in HEL Cell Cultures

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

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

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

Table 11. Antiviral Activity of Compound 1 against Cytomegalovirus in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity EC_{50} (μM)[a]		Cytotoxicity (μM)	
	AD-169 strain	Davis strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
1	N. D.[d]	>100	>100	>100
Ganciclovir	7.1	9.0	>400	138
Cidofovir	1.3	3.2	>400	89

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

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

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

[d] Not determined.

[e] No complete inhibition at higher drug concentration.

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

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

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

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

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

Table 13. Antiviral Activity of Compound 1 against HBV

Compound	HBV (Assay: VIR)[a]			
	EC50 (3TC)	EC90 (3TC)	CC50 (3TC)	SI[b] (3TC)
1	>10 (0.047)	>10 (0.14)	>300 (2260)	16900

Units = μM

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

[b]SI = CC50/EC90

CONCLUSION

Interest in carbocyclic formycin analog stems from the fact that the C-nucleoside formycin has shown potentially significant antiviral properties and that formycin can replace adenosine in a variety of biochemical reactions (for example, enzymes of nucleotide metabolism, RNA polymerase, polynucleotide phosphorylase, the pyrophosphorylase of tRNA and adenosine kinase). However, the usefulness of formycin is limited by its toxicity, which, in some instances, resides in its 5'-nucleotide derivatives.

This dissertation reports a combination of the structural components of formycin and the carbocyclic nucleoside 5'-noraristeromycin resulting in carbocyclic 5'-norformycin **22.** The *N*-methylated derivatives are also investigated. Arising in this research was a facile procedure to the enantiopure precursor, L-like 2-cyclopentenone **44** that permitted a convenient means to the desired carbocyclic C-nucleosides through a conjugate addition process. This scheme circumvented the previous, cumbersome approach to carbocyclic C-nucleosides that employed functionalized expoxide ring openings. The synthetic process elaborated herein opens an accessible means to carbocyclic 5'-nor C-nucleosides of potential therapeutic usefulness and a number of other similar analogs.

The formycin literature suggested this investigation be extended to the synthesis of the *N*-1 and *N*-2 methylated carbocyclic 5'-norformycin (**62** and **63**). Thus, synthetic routes to these compounds ((±)-**1,** (±)-**2**, (±)-**12**, (±)-**13**) were developed and a thorough requisite structural analysis (*N*-1 versus *N*-2) carried out.

Furthmore, it is hypothesize that the *N*-2-methylated carbocyclic formycins possess a *syn* character. Application of the result of this research allows for access to a comprehensive and diverse library of formycin and formycin-like carbocyclic C-nucleosides as antiviral candidates.

EXPERIMENTAL SECTION

Materials and methods. Melting points were recorded on a Meltemp II point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 250 Spectrometer (operated at 250 or 62.9 MHz, respectively) or AV 400 Spectrometer (operated at 400 or 100 MHz, respectively). All ^{1}H chemical shifts are reported in δ relative to the internal standard tetramethylsilane (TMS, δ 0.00). ^{13}C chemical shifts are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.23) or relative to DMSO-*d* (center of septet, δ 39.51). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Elemental analyses were performed by the Atlantic Microlabs, Atlanta, Georgia. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm E. Merck silica gel 60-F_{254} coated silica gel plates with visualization by the irradiation with Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Whatman silica gel (average particle size 2-25 μm, 60 Å) and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

(±)-(1α, 2β)-2-(3, 3-Diethoxy-prop-1-ynyl) cyclopentanol (3). To a solution of propiolaldehyde diethyl acetal (97%) (10.7 mL, 75 mmol) in anhydrous tetrahydrofuran (225 mL) at –78 °C under an atmosphere of nitrogen was added *n*-butyllithium (31.5 mL, 79 mmol, 2.5 M solution in hexanes) over 5 min. The reaction was stirred for *ca*. 30 minutes then cyclopentene oxide (98%) (6.5mL, 75 mmol) in anhydrous tetrahydrofuran (75 mL) was added, followed by neat $BF_3 \cdot OEt_2$ (9.5 mL, 75 mmol,) over 15 min. The reaction mixture was stirred at -78 °C for 2 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate solution and partitioned between ethyl acetate and water. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and evaporated *in vacuo* to afford a liquid residue. Purification by flash column chromatography (eluting with 25-33% ethyl acetate/hexanes) furnished the secondary alcohol (10.2 g, 64.1%) as a light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, 6H, *J*=7.1 Hz), 1.56 (m, 1H), 1.72 (m, 3H), 2.08 (m, 2H), 2.32 (br s, 1H), 2.64 (m, 1H), 3.57 (m, 2H), 3.71 (m, 2H), 4.23 (m, 1H), 5.26 (d, 1H, *J*=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 18.6, 31.0, 33.8, 39.6, 58.5, 80.3, 88.5, 91.4, 91.8; Anal. calcd for $C_{12}H_{20}O_3 \cdot 0.1H_2O$: C, 67.29; H, 9.44. Found: C, 67.20; H, 9.18.

(±)-(1α, 2β)-2-(1-acetyl-1H-pyrazol-3-yl)cyclopentanol (4). Acetal **3** (8.5 g, 40 mmol) was dissolved in glacial acetic acid (395 mL) and 10% HCl (95 mL), and the mixture was stirred at room temperature for 1 h. To this, a solution of hydrazine monohydrate (24.7 g, 0.493 mol) in glacial acetic acid (190 mL) was added dropwise over 20 min. The resulting solution was heated at reflux overnight and concentrated in vacuo to afford dark-brown oil. The crude product was redissolved in pyridine (188 mL), acetic anhydride (94.8 mL, 1.02 mol) and DMAP were added, and the resulting solution

was stirred for 16 h at room temperature. Solvent was removed in vacuo, and the crude residue was redissolved in ethyl acetate (394 mL), washed with 10% HCl, water, and brine, dried, concentrated, and chromatographed to afford **4** (6.9 g, 29 mmol, 73%) as a light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 (m, 1H), 1.87 (m, 3H), 2.07 (s, 3H), 2.20 (m, 2H), 2.69 (s, 3H), 3.27 (m, 1H), 5.31 (m, 1H), 6.35 (d, 1H, J=2.8 Hz), 8.19 (d, 1H, J=2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 22.0, 30.8, 33.6, 39.6, 60.6, 79.1, 88.0, 91.3, 91.6, 171.4, 177.4; Anal. calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.11; H, 6.80; N, 11.98.

(±)-(1α, 2β)-2-(1-acetyl-4-nitro-5-cyano-1H-pyrazol-3-yl) cyclopentanol (6). Trifluoroacetic anhydride (29.4 mL) was added dropwise to a stirred solution of **4** (5.00 g, 21.1 mmol) and ammonium nitrate (16.8 g) in trifluoroacetic acid (248 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred overnight. The solvent was evaporated on rotavapor at room temperature, then was diluted with methylene chloride, washed with water, satd. sodium bicarbonate and brine, dried, and concentrated in vacuo to give the dinitro compound **5** (6.00 g, 21.1mmol >100%) as a colorless liquid. The crude product thus isolated was committed to the next step without further purification.

An analytically pure **5** was prepared by chromatography (hexane:ethyl acetate =5: 1). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (m, 4H), 2.02 (s, 3H), 2.25 (m, 1H), 2.34 (m, 1H), 3.86 (m, 1H), 5.42 (m, 1H), 9.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.4, 24.1, 31.7, 32.8, 44.3, 78.0, 125.2, 134.4, 150.7, 171.2; Anal. calcd for $C_{10}H_{12}N_4O_6$: C, 42.26; H, 4.26; N, 19.71. Found: C, 42.33, H, 4.35; N, 19.54.

A solution of the 1,4-dinitro compound (6.00 g, 21.1 mmol) in ethanol (8 mL) and ethyl acetate (8 mL) was added dropwise over 1 min to a stirred solution of potassium cyanide (1.20 g, 18.4 mmol) in ethanol (22 mL) and water (6 mL). Following an additional 5 min at room temperature, the reaction mixture was neutralized with acetic acid (1 mL) and evaporated on the rotavapor, then diluted with ethyl acetate (100 mL), washed with water and brine, dried, concentrated in vacuo to afford **6** (3.81 g, 14.4 mmol, 68.2%) as a dark syrup. Purification by chromatography (hexane:ethyl acetate:methanol=5:2:1) to afford **6** as a white solid, mp 132.5-133.8 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.76 (m, 1H), 1.92 (m, 3H), 2.06 (s, 3H), 2.27 (m, 1H), 3.38 (m, 1H), 3.53 (s, 1H), 3.85 (m, 1H), 5.38 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 20.9, 22.1, 30.6, 31.4, 42.3, 76.2, 110.5, 122.8, 133.4, 145.2, 172.9; Anal. calcd for $C_{11}H_{12}N_4O_4$: C, 50.00; H, 4.58; N, 21.20. Found: C, 50.13; H, 4.79; N, 20.98.

(±)-(1α ,2β)-2-(5-cyano-1-methyl-4-nitro-1H-pyrazol-3-yl)cyclopentyl acetate (7) **and (±)-(1α ,2β)-2-(5-cyano-2-methyl-4-nitro-1H-pyrazol-3-yl)cyclopentyl acetate (8).** To a stirred solution of **6** (1.85 g, 7.01 mmol) in anhydrous THF (150 mL), was added portion wise 60% sodium hydride in mineral oil (0.36 g, 9.0 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min. and then iodomethane (0.64 mL, 1.4 g, 9.5 mmol) was added dropwise. After a period of 24 h the reaction mixture was absorbed by silica gel, and then purified by silica gel column chromatography. Compound **7** (1.16 g, 4.17 mmol, 60.0%) and **8** (0.55 g, 1.98 mmol, 28.4%) was obtained after eluting with a mixture of hexane/ethyl acetate in a ratio of 1:1. Compound **7** was a white syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72 (m, 1H), 1.88 (m, 3H), 2.01 (s, 3H), 2.23 (m, 1H), 2.29 (m, 1H), 3.83 (m, 1H), 4.06 (s, 3H), 5.41 (m, 1H); ^{13}C NMR (100.6

MHz, $CDCl_3$) δ 21.2, 23.7, 31.5, 32.5, 39.7, 43.5, 79.5, 111.2, 115.3, 135.3, 151.0, 170.8; Compound **8** was a white solid, mp 103-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (m, 3H), 2.03 (s, 3H), 2.05 (m, 1H), 2.15 (m, 1H), 2.42 (m, 1H), 3.42 (m, 1H), 3.96 (s, 3H), 5.30 (m, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 21.0, 24.9, 30.8, 32.9, 39.3, 45.0, 80.1, 110.7, 122.0, 133.4, 145.2, 171.2; Anal. calcd for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.07; N, 20.13. Found: C, 52.02; H, 5.11; N, 20.34.

(±)-(1α ,2β)-2-(5-cyano-2-methyl-4-nitro-1*H*-pyrazol-3-yl)cyclopentanol (9). Ammonia gas was introduced to a solution of compound **8** (2.0 g, 7.2 mmol) in MeOH (100 mL). This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was then removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 20:1) to afford **9** (1.4 g, 5.9 mmol, 83%) as a light yellow solid, mp 129-130 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 1.85 (m, 4H), 2.13 (m, 1H), 2.32 (m, 1H), 3.74 (m, 1H), 4.26 (m, 1H), 5.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.2, 28.6, 35.8, 39.7, 45.8, 77.5, 110.8, 121.8, 134.1, 145.9; Anal. calcd for $C_{10}H_{12}N_4O_3$: C, 50.84; H, 5.12; N, 23.72. Found: C, 50.98; H, 5.14; N, 23.72.

(±)-(1α,2β)-2-(5-cyano-2-methyl-4-amino-1*H*-pyrazol-3-yl)cyclopentanol (10). A catalytic amount of Pd/C (1%) was added to a solution of **9** (1.4 g, 5.9 mmol) in methanol (30 mL) at room temperature, the resulting mixture was allowed to shake under 20 psi of hydrogen overnight. After the reaction was complete, concentrated in vacuo to give compound **10** (1.4 g, 6.7 mmol >100%) as a colorless liquid. The crude product thus isolated was used in the next step without further purification.

(±)-(1α,2β)-2-(3-[7-amino--2-methyl-1*H*-pyrazolo[4,3*d*]pyrimidyl]) cyclopentanol (2). A solution of **10** (0.70 g, 3.4 mmol) in ethanol (15 mL) was stirred with formamidine acetate (0.68 g, 6.7 mmol) under reflux for 1 h. The reaction mixture was cooled to room temperature. The solvent was removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 10:1) to afford **2** (0.47 g, 2.0 mmol, 60%) as a light yellow solid, mp = 209 °C (dec); ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.61 (m, 1H), 1.86 (m, 2H), 2.07 (m, 2H), 2.32 (m, 1H), 3.26 (m, 1H), 4.10 (s, 3H), 4.47 (m, 1H), 7.43 (br s, 2H), 8.03 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.3, 28.7, 34.7, 39.1, 45.5, 77.5, 130.3, 136.6, 137.2, 151.6, 156.0; Anal. calcd for $C_{11}H_{15}N_5O$: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.55; H, 6.56; N, 29.86.

(±)-(1α,2β)-2-(3-[7-amino--1-methyl-1*H*-pyrazolo[4,3*d*]pyrimidyl]) cyclopentanol (1). Ammonia gas was introduced to a solution of compound **7** (2.0 g, 7.2 mmol) in MeOH (100 mL). This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 10:1) to afford mixtures including deacetylated product and unknown compounds. Take these mixtures (around 1.5g >100%) followed hydrogenation (A catalytic amount of Pd/C (1%) was added to a solution of the mixtures (1.5 g, 6.3 mmol) in methanol (30 mL) at room temperature, the resulting mixture was allowed to shake under 20 psi of hydrogen overnight. Concentrated in vacuo to give another mixture (1.5 g, >100%) as a yellow liquid. The crude product thus isolated was used in the next step without further purification. A solution of **this mixture** (1.5 g) in ethanol (30 mL) was stirred with formamidine acetate (1.36 g, 13.4 mmol) under reflux for 3 h. The reaction

mixture was cooled to room temperature. The solvent was removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 10:1) to afford **1** (0.24 g, 1.0 mmol, 20% for three steps)as a white solid, mp = 215 °C (dec); ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.62 (m, 1H), 1.75 (m, 2H), 1.92 (m, 2H), 2.07 (m, 1H), 3.18 (m, 1H), 4.15 (s, 3H), 4.37 (m, 1H), 7.26 (br s, 2H), 8.12 (s, 1H); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 22.1, 29.4, 34.1, 38.8, 45.9, 76.8, 121.8, 141.0, 145.5, 150.8, 151.2; Anal. calcd for $C_{11}H_{15}N_5O$: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.46; H, 6.51; N, 29.75.

(±)-(1α, 2β)-2-(3, 3-Diethoxy-prop-1-ynyl) cyclohexanol (14). To a solution of propiolaldehyde diethyl acetal (97%) (10.7 mL, 75 mmol) in anhydrous tetrahydrofuran (225 mL) at –78 °C under an atmosphere of nitrogen was added *n*-butyllithium (31.5 mL, 79 mmol, 2.5 M solution in hexanes) over 5 min. The reaction was stirred for *ca*. 30 minutes then cyclohexane oxide (98%) (7.4 g, 75 mmol) in anhydrous tetrahydrofuran (75 mL) was added, followed by neat $BF_3{\cdot}OEt_2$ (9.5 mL, 75 mmol,) over 15 min. The reaction mixture was stirred at -78 °C for 2 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate solution and partitioned between ethyl acetate and water. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and evaporated *in vacuo* to afford a liquid residue. Purification by flash column chromatography (eluting with 25-33% ethyl acetate/hexanes) furnished the secondary alcohol (10.9 g, 64.2%) as a light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (m, 9H,), 1.35 (m, 1H), 1.62 (m, 1H), 1.70 (m, 1H), 1.97 (m, 2H), 2.27(m, 1H), 2.72 (br s, 1H), 3.48 (m, 1H), 3.57 (m, 2H), 3.71 (m, 2H), 5.26 (d, 1H, *J*=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 24.4, 25.0, 31.9, 33.5, 39.0, 61.0, 73.3, 78.0, 87.8, 91.8; Anal. calcd for $C_{13}H_{22}O_3{\cdot}0.2H_2O$: C, 67.91; H, 9.84. Found: C, 67.71; H, 9.73.

(±)-(1α, 2β)-2-(1-acetyl-1H-pyrazol-3-yl) cyclohexanol (15). Acetal **14** (9.1 g, 40 mmol) was dissolved in glacial acetic acid (395 mL) and 10% HCl (95 mL), and the mixture was stirred at room temperature for 1 h. To this, a solution of hydrazine monohydrate (24.7 g, 0.493 mol) in glacial acetic acid (190 mL) was added dropwise over 20 min. The resulting solution was heated at reflux overnight and concentrated in vacuo to afford dark-brown oil. The crude product was redissolved in pyridine (188 mL), acetic anhydride (94.8 mL, 1.02 mol) and DMAP were added, and the resulting solution was stirred for 16 h at room temperature. Solvent was removed in vacuo, and the crude residue was redissolved in ethyl acetate (394 mL), washed with 10% HCl, water, and brine, dried, concentrated, and chromatographed to afford **15** (7.0 g, 28 mmol, 70%) as a light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (m, 4H), 1.58 (m, 1H), 1.78 (m, 1H), 1.89 (s, 3H), 2.00 (m, 1H), 2.11 (m, 1H), 2.64 (s, 3H), 2.86 (m, 1H), 4.97 (m, 1H), 6.27 (d, 1H, J = 2.8 Hz), 8.11 (d, 1H, J = 2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 22.1, 24.7, 25.4, 32.0, 32.2, 43.1, 75.1, 108.6, 128.9, 159.4, 169.8, 170.6; Anal. calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.34; H, 7.26; N, 11.15.

(±)-(1α, 2β)-2-(1-acetyl-4-nitro-5-cyano-1H-pyrazol-3-yl) cyclohexanol (17). Trifluoroacetic anhydride (29.4 mL) was added dropwise to a stirred solution of **15** (5.28 g, 21.1 mmol) and ammonium nitrate (16.8 g) in trifluoroacetic acid (248 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred overnight. The solvent was evaporated on rotavapor at room temperature, then was diluted with methylene chloride, washed with water, satd. sodium bicarbonate and brine, dried, and concentrated in vacuo to give the dinitro compound **16** (6.30 g, 21.1mmol >100%) as a

colorless liquid. The crude product thus isolated was committed to the next step without further purification.

An analytically pure **16** was prepared by chromatography (hexane:ethyl acetate =4: 1). Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (m, 3H), 1.79 (m, 3H), 1.88 (s, 3H), 2.08 (m, 1H), 2.23 (m, 1H), 3.64 (m, 1H), 5.07 (m, 1H), 9.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.4, 24.5, 25.4, 30.6, 32.1, 41.0, 75.3, 124.7, 135.0, 150.0, 170.4; Anal. calcd for $C_{11}H_{14}N_4O_6$: C, 44.30; H, 4.73; N, 18.79. Found: C, 44.53, H, 4.75; N, 18.79.

A solution of the 1,4-dinitro compound (6.30 g, 21.1 mmol) in ethanol (8 mL) and ethyl acetate (8 mL) was added dropwise over 1 min to a stirred solution of potassium cyanide (1.20 g, 18.4 mmol) in ethanol (22 mL) and water (6 mL). Following an additional 5 min at room temperature, the reaction mixture was neutralized with acetic acid (1 mL) and evaporated on the rotavapor, then diluted with ethyl acetate (100 mL), washed with water and brine, dried, concentrated in vacuo to afford **17** (3.81 g, 13.7 mmol, 65.0%) as a dark syrup. Purification by chromatography (hexane:ethyl acetate:methanol=5:2:1) to afford **17** as a white solid, mp 133.5-134.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (m, 3H), 1.78 (s, 3H), 1.80 (m, 3H), 1.98 (m, 1H), 3.60 (m, 1H), 4.97 (m, 1H), 14.96 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 23.8, 24.5, 29.5, 31.5, 39.7, 73.8, 111.9, 121.5, 133.5, 145.4, 169.5; Anal. calcd for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.07; N, 20.13. Found: C, 51.62; H, 5.21; N, 19.93.

(±)-(1α ,2β)-2-(5-cyano-1-methyl-4-nitro-1H-pyrazol-3-yl)cyclohexyl acetate (18) and (±)-(1α ,2β)-2-(5-cyano-2-methyl-4-nitro-1H-pyrazol-3-yl)cyclohexyl acetate (19). To a stirred solution of **17** (1.9 g, 7.0 mmol) in anhydrous THF (150 mL), was added portion wise 60% sodium hydride in mineral oil (0.36 g, 9.0 mmol) at 0 °C. The

mixture was stirred at room temperature for 30 min. and then iodomethane (0.64 mL, 1.4 g, 9.5 mmol) was added dropwise. After a period of 24 h the reaction mixture was absorbed by silica gel, and then purified by silica gel column chromatography. Compound **18** (1.2 g, 4.3 mmol, 60%) and **19** (0.50 g, 1.7 mmol, 25%) was obtained after eluting with a mixture of hexane/ethyl acetate in a ratio of 2:1. Compound **18** was a yellow solid; mp: 108-110 ^{o}C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.49 (m, 5H), 1. 85 (m, 1H), 1.89 (s, 3H), 2.05 (m, 1H), 2.20 (m, 1H), 3.61 (m, 1H), 4.09 (s, 3H), 5.06 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 20.5, 23.8, 24.5, 29.5, 31.5, 39.7, 43.5, 73.8, 111.9, 121.5, 133.5, 145.4, 169.5; Compound **19** was a yellow solid, mp 155-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (m, 4H), 1.88 (s, 3H), 1.95 (m, 2H), 2.03 (s, 3H), 2.26 (m, 2H), 2.15 (m, 1H), 4.08 (s, 3H), 5.43 (m, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 21.0, 24.9, 30.8, 32.9, 39.3, 45.0, 80.1, 110.7, 122.0, 133.4, 145.2, 171.2.

(±)-(1α ,2β)-2-(5-cyano-1-methyl-4-nitro-1*H*-pyrazol-3-yl) cyclohexanol (20). Ammonia gas was introduced to a solution of compound **18** (1.2 g, 4.3 mmol) in MeOH (100 mL). This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was then removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 10:1) to afford **20** (0.51 g, 2.0 mmol, 50%) as a light yellow solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.48 (m, 2H), 1.85 (m, 4H), 2.13 (m, 1H), 2.32 (m, 1H), 3.74 (m, 1H), 4.09 (s, 3H), 4.26 (m, 1H), 5.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.2, 24.5, 28.6, 35.8, 39.7, 45.8, 77.5, 110.8, 121.8, 134.1, 145.9.

(±)-(1α,2β)-2-(3-[7-amino--1-methyl-1*H*-pyrazolo[4,3*d*]pyrimidyl]) cyclohexanol (12). A catalytic amount of Pd/C (1%) was added to a solution of **20** (0.51

g, 2.0 mmol) in methanol (30 mL) at room temperature, the resulting mixture was allowed to shake under 20 psi of hydrogen overnight. After the reaction was complete, concentrated in vacuo to give reduced compound (0.50 g, 2.0 mmol >100%) as a colorless liquid. The crude product thus isolated was used in the next step without further purification.

A solution of reduced compound (0.50 g, 2.0 mmol) in ethanol (15 mL) was stirred with formamidine acetate (0.29 g, 2.9 mmol) under reflux for 3 h. The reaction mixture was cooled to room temperature. The solvent was removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 6:1) to afford 1**2** (0.30 g, 1.2 mmol, 60%) as a light yellow solid, mp : 234-235 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.46 (m, 3H), 1.77 (m, 4H), 1.96 (m, 1H), 3.95 (td, 1H, J = 5.2, 9.6 Hz), 4.17 (s, 3H), 4.52 (d, 1H, J = 4.8 Hz), 7.20 (br s, 2H), 8.11 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.1, 25.9, 31.6, 36.1, 39.2, 44.8, 71.4, 122.0, 141.7, 146.6, 151.0, 151.4.

(±)-(1α ,2β)-2-(5-cyano-2-methyl-4-nitro-1*H*-pyrazol-3-yl) cyclohexanol (21). Ammonia gas was introduced to a solution of compound **19** (0.5 g, 1.7 mmol) in MeOH (50 mL). This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was then removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 10:1) to afford **21** (0.34 g, 1.4 mmol, 80%) as a light yellow solid; °C ^{1}H NMR (400 MHz, CD_3OD) δ 1.48 (m, 2H), 1.85 (m, 4H), 2.13 (m, 1H), 2.32 (m, 1H), 3.74 (m, 1H), 4. 08 (s, 3H), 4.25 (m, 1H), 5.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2, 24.5, 30.6, 34.5, 39.7, 45.8, 77.3, 113.8, 121.8, 134.1, 145.9.

(±)-(1α,2β)-2-(3-[7-amino--2-methyl-1*H*-pyrazolo[4,3*d*]pyrimidyl]) cyclohexanol (13). A catalytic amount of Pd/C (1%) was added to a solution of **21** (0.34 g, 1.3 mmol) in methanol (15 mL) at room temperature, the resulting mixture was allowed to shake under 20 psi of hydrogen overnight. After the reaction was complete, concentrated in vacuo to give reduced compound (0.34 g, 1.3 mmol >100%) as a colorless liquid. The crude product thus isolated was used in the next step without further purification.

A solution of reduced compound (0.34 g, 1.3 mmol) in ethanol (10 mL) was stirred with formamidine acetate (0.2 g, 2 mmol) under reflux for 3 h. The reaction mixture was cooled to room temperature. The solvent was removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 6:1) to afford **13** (0.17 g, 0.67 mmol, 50%) as a light yellow solid, mp : 172-174; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.49-1.95 (m, 6H), 2.07 (m, 1H), 3.15 (m, 1H), 4.13 (s, 3H), 4.37 (d, 1H, J = 5.2 Hz), 4.82 (m, 1H), 7.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.8, 22.5, 30.4, 34.7, 38.4, 46.1, 77.2, 126.3, 137.5, 142.7, 147.3, 154.2.

((4*S*, 5*S*)-5-((tert-butyldimethylsilyloxy) methyl)-2, 2-dimethyl-1, 3-dioxolan-4-yl) methanol (**30**). To a well stirred 60% sodium hydride in mineral oil suspension (1.56 g, 65.1 mmol) in 30 mL THF at room temperature under N_2 was added the solution of the diol **28** (5.00 g, 31.0 mmol) in 30 mL THF dropwise. The reaction mixture was stirred at room temperature for 45 min, during which time a large amount of opaque white precipitate had formed. TBSCl (4.67 g, 31.0 mmol) in 30 mL THF was added and the stirring was continued for additional 45 min. The reaction mixture was diluted with 100 mL EtOAc and washed with 10% aqueous K_2CO_3, H_2O, brine and dried with $MgSO_4$.

The solvent was removed *in vacuo* and the residue was purified with flash chromatography (silica, 1:3 EtOAc:hexane) to give 7.3 g (85%) of **30** as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.06 (m, 6H), 0.90 (m, 9H), 1.39 (m, 6H), 3.66 (m, 2H), 3.68 (m, 1H), 3.74 (m, 2H), 3.82 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ = -5.5, 18.3, 25.8, 26.9, 27.0, 62.7, 63.7, 78.0, 80.0, 109.1ppm. The spectroscopic data were in accord with those reported in the literature.[168]

(4R, 5S)-5-((tert-butyldimethylsilyloxy) methyl)-2, 2-dimethyl-1, 3-dioxolane-4-carbaldehyde (31). To a -5 ^{o}C solution of **30** (4.01 g, 14.5 mmol) in CH_2Cl_2 (85 mL) and DMSO (12 mL) was added DIPEA (6.20 ml, 35.4 mmol), followed by slow addition of a solution of SO_3• Pyridine (5.9 g, 29 mmol) in DMSO (18 mL). The reaction mixture was stirred at this temperature for 2 h, at which point it was diluted with Et_2O (200 mL) and rinsed with water, 5% $NaHCO_3$, 10% $CuSO_4$ and brine. The organic solution was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue purified by column chromatography (EtOAc/hexanes = 1:3) to afford the aldehyde **31** as a colorless oil (3.5 g, 87 %); ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.08 (m, 6H), 0.89 (m, 9H), 1.39 (d, 3H, J = 0.8 Hz), 1.46 (d, 3H, J = 0.8 Hz); 3.79 (m, 2H), 4.09 (m, 1H), 4.31 (dd, 1H, J = 1.6, 7.2 Hz), 9.75 (d, 1H, J = 2.0Hz) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ -5.5, 18.3, 25.8, 26.3, 26.8, 62.8, 77.5, 81.9, 111.4, 200.7 ppm. The spectroscopic data were in accord with those reported in the literature.[168]

Tert-butyl (((4S, 5S)-2, 2-dimethyl-5-vinyl-1, 3-dioxolan-4-yl) methoxy) di-methylsilane (32). Methyltriphenylphosphonium bromide (4.5 g, 15 mmol) was added in portions to a suspension of *t*-BuOK (1.56 g, 15.9 mmol) in anhydrous ether (50 mL) at 0 ^{o}C. After the resulting suspension was stirred at 0 ^{o}C for 1 h and rt for 1.5 h, it was cooled

to 0 ^{o}C and treated dropwise with a solution of aldehyde **31** (3.40 g, 12.4 mmol) in anhydrous ether (20 mL). The reaction was then stirred overnight at room temperature and filtered to remove a solid. The filtrate was concentrated under reduced pressure and the residue purified by column chromatography (EtOAc/hexanes = 1:7) to give **32** as a colorless oil (2.6 g, 80%). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.02 (m, 6H), 0.85 (m, 9H), 1.35 (d, 6H, *J* = 1.2 Hz), 3.69 (m, 3H), 4.29 (m, 1H), 5.16 (m, 1H), 5.27 (m, 1H), 5.85 (m, 1H) ppm. ^{13}C-NMR(100 MHz, $CDCl_3$/TMS): δ = -5.4, 18.3, 25.9, 26.9, 27.0, 79.3, 81.3, 109.1, 118.0, 135.8 ppm.

2-((4S, 5S)-5-((tert-butyldimethylsilyloxy) methyl)-2, 2-dimethyl-1, 3-dioxolan-4-yl) ethanol (33). To **32** (1.0 g, 3.7 mmol) in THF (20 mL) at 0 ^{o}C under N_2 was added 9-BBN-H (0.5 M in THF, 9.3 mL, 4.6 mmol), and the mixture stirred for 3 hours. Sodium hydroxide (1 M, 5.6 mL) and then H_2O_2 (33% in water, 2.8 mL) were added and the stirring continued for a further 30 mins. The reaction mixture was diluted with EtOAc and then washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo* to give crude product, which was purified by flash column chromatography (EtOAc/hexanes = 1:5) to yield **33** as a colorless oil (1.00 g, 93.5%). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.09 (m, 6H), 0.91(m, 9H), 1.40 (s, 3H), 1.44 (s, 3H), 1.84-1.98 (m, 2H), 3.69-3.73 (m, 1H), 3.79-3.83 (m, 4H), 4.08 (m, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ -5.4, 18.4, 25.9, 26.9, 27.3, 35.6, 60.8, 63.7, 78.6, 80.8, 108.8 ppm.

2-((4S, 5S)-5-(hydroxymethyl)-2, 2-dimethyl-1, 3-dioxolan-4-yl) ethanol (27). To a solution of **33** (1.00 g, 3.45 mmol) in dry THF (40 mL) was added 1.0 M TBAF in THF (6.9 mL, 6.9 mmol) at 0 ^{o}C. The reaction mixture was stirred at rt overnight and the

solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the resulted solution was washed with brine. After drying over anhydrous Na_2SO_4 and filtration, the residue was purified by column chromatography (EtOAc/hexanes = 1:2) to give **27** as a colorless oil (0.39 g, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 1.41 (m, 6H), 1.84-1.87 (m, 2H), 3.03 (brs, 2H), 3.61-3.69 (m, 1H), 3.76-3.82 (m, 4H), 4.06 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 27.1, 27.4, 35.4, 60.3, 62.0, 76.3, 81.4, 109.1.

(((4S,5S)-5-allyl-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)(tert-butyl) dimethylsilane (36). To a -5^{o}C solution of **33** (3.4 g, 11 mmol) in CH_2Cl_2 (70 mL) and DMSO (10 mL) was added DIPEA (4.80 mL, 27.4 mmol), followed by slow addition of a solution of SO_3• pyridine (4.5 g, 22 mmol) in DMSO (15 ml). The reaction mixture was stirred at this temperature for 2 hour, at which point it was diluted with CH_2Cl_2 (200 mL) and rinsed with water, 5% $NaHCO_3$ and brine. The organic solution was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue purified by column chromatography (EtOAc/hexanes = 1:3) to afford the unpure aldehyde **35** as a colorless oil (3.40 g, 100%), The crude product thus isolated was used in the next step without further purification.

Methyltriphenylphosphonium bromide (4.30 g, 14.1 mmol) was added in portions to a suspension of *t*-BuOK (1.47 g, 15.0 mmol) in anhydrous ether (50 mL) at 0 ^{o}C. After the resulting suspension was stirred at 0 ^{o}C for 1 h and rt for 1.5 h, it was cooled to 0 ^{o}C and treated dropwise with a solution of the above aldehyde **35** (3.40 g, 12.4 mmol) in anhydrous ether (20 mL). The reaction was then stirred overnight at room temperature and filtered to remove a solid. The filtrate was concentrated under reduced pressure and the residue purified by column chromatography (EtOAc/hexanes = 1:7) to give **36** as a

colorless oil (1.7 g, 50% for two steps). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.09 (m, 6H), 0.93 (s, 9H), 1.41 (s, 3H), 1.44 (s, 3H), 2.39-2.47 (m, 2H), 3.72-3.79 (m, 3H), 3.80-4.01 (m, 1H), 5.11-5.18 (m, 2H), 5.86-5.93 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ -5.4, 18.4, 25.9, 27.0, 27.4, 37.6, 63.6, 77.8, 80.6, 108.6, 117.4 133.7.

((4S, 5S)-5-allyl-2, 2-dimethyl-1, 3-dioxolan-4-yl) methanol (37). To a solution of **36** (1.2 g, 4.2 mmol) in dry THF (80 mL) was added 1.0 M TBAF in THF (8.4 mL, 8.4 mmol) at 0 ^{o}C. The mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the resulting solution was washed with brine. After drying over anhydrous Na_2SO_4 and filtration, the residue was purified by column chromatography (EtOAc/hexanes = 1:2) to give **37** as a colorless oil (0.60 g, 83%). ^{1}H-NMR (250 MHz, $CDCl_3$/TMS): δ 1.43 (s, 6H), 2.21 (br, 1H), 2.38-2.44 (m, 2H), 3.58-3.65 (m, 1H), 3.77-3.86 (m, 2H), 3.95-4.03 (m, 1H), 5.11-5.20 (m, 2H), 5.77-5.91 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ 27.0, 27.3, 37.3, 61.9, 75.8, 81.1, 108.8, 117.9, 133.5.

(4R, 5S)-5-allyl-2, 2-dimethyl-1, 3-dioxolane-4-carbaldehyde (38). To a -5 ^{o}C solution of **37** (0.17 g, 1.0 mmol) in CH_2Cl_2 (6 mL) and DMSO (0.8 mL) was added DIPEA (0.4 mL), followed by slow addition of a solution of SO_3• pyridine (0.4 g, 2 mmol) in DMSO (1 mL). The reaction mixture was stirred at this temperature for 2 h, at which point it was diluted with CH_2Cl_2 (20 mL) and rinsed with water, 5% $NaHCO_3$ and brine. The organic solution was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue purified by column chromatography (EtOAc/hexanes = 1:3) to afford the aldehyde **38** as a colorless oil (0.10 g, 60%). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 1.51 (m, 6H), 2.39-2.57 (m, 2H), 3.62-3.79 (m, 1H), 4.03-4.11 (m, 1H),

5.13-5.21 (m, 2H), 5.84-5.88 (m, 1H), 9.76 (d, 1H, J = 2Hz). ^{13}C-NMR (100 MHz, $CDCl_3$/TMS): δ 26.2, 27.0, 37.3, 76.2, 84.0, 111.1, 118.7, 132.7, 201.1.

(Z)-Cyclopentene-3, 5-diol diacetate (42). Dicyclopentadiene was cracked by distillation maintaining the distillation head at around 40 °C by controlling the temperature of heating mantle at 160~180 °C. Cyclopentadiene (254 g, 3.80 mol) was obtained which was immediately dissolved in 2200 mL CH_2Cl_2. Sodium carbonate (1000 g, 9.420 mol) was then added portionwise and a suspension was obtained. The suspension was cooled down to -10 °C and then treated with a solution of sodium acetate (20 g, 0.30 mol) in 500mL of peracetic acid (32% in acetic acid) dropwise. The temperature was maintained at -10 °C to -5 °C during the addition. After the addition, the resulting mixture was stirred at room temperture overnight. The mixture was filtered and the filtrate was evaporated to give a pale yellow liquid (250 g) which was the crude monoepoxide (6-Oxabicyclo [3.1.0] hex-2-ene). This crude product was used directly in the next step.

Fresh *tetrakis*(triphenylphosphine)palladium(0)[82] was prepared at first. $PdCl_2$ (5.07 g, 28.6 mmol) and triphenylphosphine (38.01 g, 145.0 mmol) were added to 340 mL anhydrous dimethyl sulfoxide. The mixture was heated to about 160°C under a nitrogen atmosphere until complete solution occurred. The heat was taken away and stirring continued for 5 min. Hydrazine hydrate (4.05 g, 81.0 mmol) was added dropwise in 1 min with rapid stirring. The solution was cooled to room temperture with water bath and yellow crystals appeared. Solid was collected by filtration and washed with 2 × 30 mL ethanol and 2 × 30 mL ether. Product as a light yellow solid (30.5 g) was dried and kept under nitrogen, whose ^{1}H and ^{13}C NMR spectral data agreed with literature.[168]

A solution of crude monoepoxide from last step in 200 mL THF was added dropwise to a dry ice acetone cooled solution of *tetr*akis(triphenylphosphine) palladium(0) (7.0 g) in 600 mL dry THF and acetic anhydride (450 g, 4.41 mol) at 0 °C to 5 °C. After addition, the resulting mixture was stirred at rt overnight. Filtration of the resulting mixture through a pad of silica gel removed the catalyst. Ethyl ether (2 × 100 mL) was used to wash it. Evaporation of the solvent afforded a dark residue. Fractional distillation afforded **42** (150 g, 85.0% for three steps) as a pale yellow oil, whose ^{1}H and ^{13}C NMR spectral data agreed with literature.[82]

(+)-(1*R*, 4*S*)-4-Hydroxy-2-cyclopenten-1-yl acetate ((+)-39). Compound **42** (280 g, 1.52 mol) was added to 0.1 M phosphate buffer (1000 mL). The pH of the resulting suspension was adjusted to 7 by addition of 6 N NaOH dropwise. *Pseudomonas cepacia* lipase (20 g, Amano International Enzyme Corporation) was added to the mixture. The mixture was stirred and the pH of the mixture was kept constant around 7 during the hydrolysis by the continuous addition of 1 N NaOH. After the addition of 1.5 L of NaOH solution, the reaction mixture was filtered through a celite pad. The filtrate was extracted with 3 × 800 mL ethyl acetate. The combined organic phases were dried over anhydrous $MgSO_4$. Evaporation of the solvent under reduced pressure afforded yellow oil. The residual oil was fractionally distilled (bp is around 80 ˚C under reduced pressure to give **(+)-39** (172 g, 80.2%) as a light yellow solid, whose ^{1}H and ^{13}C NMR spectral data agreed with literature.[167]

(1*R*, 4*S*)-4-(tert-butyldimethylsilyl) cyclopent-2-enyl acetate (44). To 1.42 g (10 mmol) of monoacetate **39** dissolved in 20 mL of anhydrous CH_2Cl_2, was added 1.8 g (12 mmol) of tert-butyldimethylchlorosilane and 0.8 g (12 mmol) of imidazole. The

mixture was stirred for 4 h and was subsequently added to 20 mL of 1:l ether/hexanes and 10 mL of water. The organic layer was separated, and the aqueous layer was extracted repeatedly with ether. The combined ether layer was washed with saturated sodium dihydrogen phosphate and dried. Concentration and evaporation of the volatiles gave 2.30 g (90%) of the desired product which was used for the subsequent reaction. The ^{1}H and ^{13}C NMR spectral data agreed with literature.[179]

2,3-*O*-Isopropylidene-D-ribose (49). To a stirred suspension of D-ribose (8.0 g, 53 mmol) in acetone (100 mL) was added dropwise conc. H_2SO_4 (1 mL) at room temperature and the reaction mixture was stirred at room temperature for 2.5 h. The mixture was neutralized with solid $NaHCO_3$, filtered and evaporated under reduced pressure to give colorless syrup. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (1:2) as the eluent to afford **49** as colorless syrup (9.4 g, 93%): ^{1}H NMR (MeOH-d_4), δ = 1.31 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.59 (dd, 1H, *J*=5.6, 12.0 Hz), 3.63 (dd, 1H, *J*=4.8, 12.0 Hz), 4.19 (irregular t, 1H, *J*=4.4, 5.2 Hz), 4.52 (d, 1H, *J*=6.0 Hz), 4.77 (d, 1H, *J*=6.0 Hz), 5.26 (s, 1H). All the spectral data were identical to the literature.[172,179]

(3a*R*,6*R*,6a*R*)-6-((tert-butyldimethylsilyloxy)methyl)-2,2 dimethyltetrahydro -furo [3, 4-d][1, 3] dioxol-4-ol (50). To 16 g (84 mmol) of **40** dissolved in 100 mL of anhydrous CH_2Cl_2 was added 13.9 g (92.4 mmol) of tert-butyldimethylchlorosilane**,** 8.56 g (125 mmol) of imidazole and a catalytic amount of DMAP. The mixture was stirred for 4 h and was subsequently added to 200 mL of 1:l ether/hexane and 100 mL of water. The organic layer was separated, and the aqueous layer was extracted repeatedly with ether. The combined organic layer was washed with saturated sodium dihydrogen phosphate

and dried. Concentration and evaporation of the volatiles gave 21.9 g (85.1%) of the desired product which was used for the subsequent reaction. The ^{1}H and ^{13}C NMR spectral data agreed with literature.[172]

2-(tert-Butyldimethylsilyloxy)-1-((4R,5S)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)ethanol (51) and 2-(tert-butyldimethylsilyloxy)-2-((4S,5S)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)ethanol (52). A suspension of NaH (2.5 g, 0.062 mol, 60% dispersion in mineral oil) in tetrahydrofuran (250 mL) was cooled to 0 °C, and DMSO (7.3 mL, 0.10 mol) was added. After being stirred at room temperature for 0.5 h, the resulting white suspension was cooled to 0 °C and treated with methyltriphenylphosphonium bromide (22.2 g, 62.2 mmol). The reaction mixture was stirred at room temperature for 1 h and then recooled to 0 °C. A solution of lactol **50** (12.8 g, 42.0 mmol) in tetrahydrofuran (80 mL) was added to the resulting reaction mixture at 0 °C. After being heated at reflux for 3 h, the reaction mixture was cooled to room temperature. Diethyl ether (300 mL) was added to the reaction mixture and washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane=1/10), giving compound **51** (3.2 g, 26%) as a colorless oil, and **52 (**3.1 g, 24%) as a colorless oil. For **51** and **52** all the spectral data were identical to the literature.[172,179]

(3a'*R*,6'*R*,6a'*R*)-6'-(hydroxymethyl)tetrahydrospiro[cyclopentane-1,2'-furo [3,4-d][1,3]dioxol]-4'-ol (54). To a stirred suspension of D-ribose (15.0 g, 100 mmol) in cyclopentanone (47.5 g, 565 mmol, 50.0 mL) was added dropwise conc. H_2SO_4 (1 mL) at room temperature and the reaction mixture was stirred at room temperature for 2.5 h. The mixture was neutralized with $NH_3 \bullet H_2O$, evaporated under reduced pressure to give

brown syrup, which was added with CH_2Cl_2 (500 mL) to the reaction mixture and washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (1:2) as the eluent to afford **54** as yellow syrup (16.2 g, 75.0 %): ^{1}H NMR (400 MHz, $CDCl_3$/TMS), δ 1.61-1.80 (m, 6H), 1.94-2.07 (m, 2H), 3.71-3.83 (m, 2H), 4.33 (s, 1H), 4.47 (d, 1H, *J* = 6.0 Hz), 4.80 (d, 1H, *J* = 6.0 Hz), 5.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 23.2, 23.6, 35.7, 35.8, 65.3, 81.7, 86.4, 87.4, 106.5, 121.5.

1-((2*R*,3*S*)-3-vinyl-1,4-dioxaspiro[4.4]nonan-2-yl)ethane-1,2-diol (55). To a stirred suspension of methyl triphenylphosphonium bromide (104 g, 289 mmol) in THF (1.0 L) was added potassium *t*-butoxide (37.9 g, 310 mmol, purity of reagent: 95%) at 0 °C. The reaction mixture was stirred at 0 °C for 20 mins and then at rt for 1 h. After the mixture was allowed to cool to 0 °C, a solution of **54** (21.6 g, 100 mmol) in THF (300 mL) was added and the mixture was stirred at rt overnight. To this mixture was carefully added water (200 mL) and the mixture was extracted with ethyl acetate (4 * 500 mL), dried, filtered and evaporated under reduced pressure to give yellow syrup. This syrup was purified by silica gel column chromatography using hexane and ethyl acetate (1:2–1:2.5) as the eluent to give **55** (23 g) with the contamination of triphenylphosphine oxide, which was used for next reaction without further purification: ^{1}H NMR (400 MHz, $CDCl_3$/TMS), δ 1.65-1.77 (m, 6H), 1.78-1.97 (m, 2H), 2.87 (t, 1H, *J* = 5.6 Hz), 3.01 (d, 1H, *J* = 5.2 Hz), 3.70–3.76 (m, 2H), 3.81-3.87 (m, 1H), 4.05 (dd, *J* = 6.4, 1H, 8.4 Hz), 4.63 (t, 1H, *J* = 6.4 Hz), 5.32 (td, 1H, 1H, *J* = 1.2, 10.4 Hz), 5.46 (td, 1H, *J* = 1.6, 17.2

Hz), 6.03 (ddd, 1H, *J* = 6.8, 10.4, 17.6); ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 23.2, 23.7, 36.7, 37.1, 64.5, 70.0, 78.1, 78.4, 118.9, 132.1, 132.8.

(2*S*,3*S*)-3-vinyl-1,4-dioxaspiro[4.4]nonane-2-carbaldehyde (56). To a solution of crude **55** (23 g) in methylene chloride (320 mL) was added aqueous $NaIO_4$ (160 mL, 22.2 g, 0.650 M solution) at rt and the reaction mixture was stirred at the same temperature for 30 mins. After methylene chloride (300 mL) and water (300 mL) were added, the organic layer was filtered, dried and evaporated under reduced pressure. The residue was purified by short column silica gel chromatography using hexane and ethyl acetate (5:1) as the eluent to give **56** as colorless oil (15.5 g, 85.1 % from **54**). ^{1}H NMR (400 MHz, $CDCl_3$/TMS), δ 1.74-1.81 (m, 6H), 2.04-2.10 (m, 2H), 4.34 (dd, 1H, *J* = 3.2, 7.6 Hz), 4.75 (t, 1H, *J* = 7.6 Hz), 5.34 (td, 1H, *J* = 1.2, 10.4 Hz), 5.48 (td, 1H, *J* = 1.2, 17.2 Hz), 5.77 (ddd, 1H, *J* = 6.8, 10.4, 17.2 Hz), 9.55 (d, 1H, *J* = 3.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 23.1, 24.0, 36.6, 36.8, 79.2, 81.8, 119.8, 121.1, 131.2, 200.7.

1-((2*R*,3*S*)-3-vinyl-1,4-dioxaspiro[4.4]nonan-2-yl)prop-2-en-1-ol (57). To a stirred solution of aldehyde (20.0 g, 110 mmol) in tetrahydrofuran (800 mL) was added vinylmagnesium bromide (1 M solution in tetrahydrofuran, 220 mL, 220 mmol) at -78 °C, and the reaction mixture was stirred at -40 °C for 1 h. After the mixture was allowed to warm to room temperature, the mixture was quenched with saturated ammonium chloride solution and brine, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, filtered, concentrated to dryness. The resulting oil was purified by silica gel column chromatography (hexanes/ethyl acetate=7:1) to give diene **57** (19.6 g, 85.2%) as an inseparable mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) for separated isomers : δ 1.72-1.79 (m, 6H), 1.97-2.02 (m, 2H), 4.00-4.03 (m, 1H), 4.04-4.15 (m, 1H),

4.46-4.58 (m, 1H), 5.22-5.49 (m, 4H), 5.81-6.15 (m, 1H); the other isomer: δ 1.67-1.74 (m, 6H), 1.91-1.94 (m, 2H), 3.91-3.97 (m, 1H), 4.09-4.18 (m, 1H), 4.56-4.61 (m, 1H), 5.19-5.46 (m, 4H), 5.97-6.08 (m, 1H).

(+) - (4*S*, 5*S*)-4, 5-*O*-Cyclopentyl-2-cyclopentenone (53). To a solution of diene **57** (22.0 g, 105 mmol) in anhydrous CH_2Cl_2 (500 mL) was added the first generation Grubbs catalyst (432 mg, 1 mol %) after the solution was flushed with N_2 for 20 min. After stirring at room temperature for 12 h, DMSO (130 mL) DIPEA (17.5 mL, 105 mmol) and SO_3• pyridine (19 g, 93 mmol) were added to the dark brown solution. The reaction mixture was stirred at room temperature overnight and filtered over silical gel pad with CH_2Cl_2. The filtrate was concentrated in vacuum and residue was purified by column chromatography (hexanes/ethyl acetate=5:1) to afford **53** as a white crystal (13.9 g, 74.0% from **57**). $[\alpha]_{20}^{D}$ –69.4 (c = 0.60, $CHCl_3$); mp = 69-70 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62-1.78 (m, 6H), 1.81-1.90 (m, 2H), 4.43 (d, 1H, *J* = 5.6 Hz), 5.25 (ddd, 1H, *J* = 0.8, 2.4, 5.6 Hz), 6.31(d, 1H, *J* = 6.0 Hz), 7.65 (dd, 1H, *J* = 2.4, 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.2, 24.0, 37.3, 37.9, 76.1, 78.1, 124.2, 135.4, 159.8, 203.9; Anal. calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.77; H, 6.66.

Analytic pure **58** was prepared by silica gel column chromatography (hexanes/ethyl acetate=5:1).

α-Alcohol of **58**, colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61-1.71 (m, 6H), 1.72-1.88 (m, 2H), 2.80 (d, 1H, *J* = 9.2 Hz), 4.52 (dd, 1H, *J* =6.4, 9.2 Hz), 4.64 (t, 1H, *J* = 5.6 Hz), 4.98 (dt, 1H, *J* = 0.8, 5.6 Hz), 5.85-5.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.3, 23.4, 37.2, 37.6, 74.1, 77.1, 83.4, 121.7, 131.7, 136.9;

β-Alcohol of **58**, colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.61-1.75 (m, 6H), 1.75-1.88 (m, 2H), 2.25 (d, 1H, *J* = 5.25 Hz), 4.47 (d, 1H, *J* =5.75 Hz), 4.83 (s, 1H), 5.27 (dt, 1H, *J* = 0.75, 5.75 Hz), 5.96 (m, 1H), 6.04 (m, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 23.5, 23.7, 36.8, 37.2, 81.1, 84.3, 85.9, 121.4, 135.4, 135.6.

4-[2-(trirnethylsilyl)ethynyl]-2, 3-*O*-Cyclopentylcyclopentone (59). To 5.46 g (7.70 mL, 55.6 mmol) of trimethylsily1 acetylene in 120 mL of ether at 0 °C was added 22.2 mL (55.6 mmol) of a 1.6 M solution of *n*-BuLi in hexane. The reaction mixture was allowed to stir at -40 °C for 1.5 h and was then added dropwise to an ether solution of 55.6 mL (55.6 mmol) of 1.0 M solution of dimethylaluminum chloride in hexane at room temperature. The reaction mixture was allowed to stir at this temperature for 3.5 h.

To 1.43 g (5.56 mmol) of $Ni(acac)_2$ in 50 mL of ether at -3 °C was added 5.56 mL (5.56 mmol) of a 1.0 M solution of DIBAH in hexane. The reaction mixture was allowed to stir at 0 °C for 10 min and was then cooled to -25 °C, after which the above solution of dimethyl[2-(trimethylsilyl)ethynyl]aluminum was added. Then 5.00 g (27.8 mmol) of cyclopentenone **53** in 100 mL of ether was added dropwise to the reaction mixture over 2.5 h. The reaction mixture was stirred at -30 °C for another 6 h. The reaction mixture was hydrolyzed with saturated aqueous KH_2PO_4 (100 mL) for overnight. The organic layer was extracted with ether, and the ether layer was then washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (hexanes/ethyl acetate=20:1) to afford the conjugate adduct **59** as a yellow crystal (3.2 g, 42%). mp = 64-65 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.15 (s, 9H), 1.68-1.88 (m, 8H), 2.42 (dt, 1H, *J* = 1.75, 18.0 Hz), 2.88 (dd, 1H, *J* = 8.75, 18.0 Hz), 3.32 (dd, 1H, *J* = 0.75, 8.75 Hz), 4.35 (d, 1H, *J* = 5.0 Hz), 4.73 (d, 1H, *J* = 5.0 Hz). ^{13}C NMR (62.9 MHz,

$CDCl_3$/TMS): δ -0.13, 23.0, 23.8, 29.7, 36.1, 36.3, 40.5, 78.0, 81.4, 89.2, 104.5, 122.7, 212.3 ppm. Anal. calcd for $C_{15}H_{22}O_3Si$: C, 64.71; H, 7.96. Found: C, 64.90; H, 7.99.

(1*R*, 2*R*, 3*S*, 4*S*)-4-[2-(trirnethylsilyl)ethynyl]-2, 3-*O*-Cyclopentylcyclopentan-1-ol (60). Sodium borohydride (0.30 g, 7.1 mmol) was added portionwise to a solution of **59** (1.3 g, 4.7 mmol) and cerium(III)chloride heptahydrate (1.5 g, 4.0 mmol) in methanol (50 mL) at 0 °C. After 1.5 hr, the solvent was removed and the residue was neutralized by saturated NH_4Cl solution. Extracted by CH_2Cl_2 (3*50 mL) and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (hexanes/ethyl acetate=10:1) to afford **60** as a white crystal (0.98 g, 75%). mp = 44-45 ^oC; ^1H NMR (250 MHz, $CDCl_3$): δ 0.15 (s, 9H), 1.65-1.81 (m, 6H), 1.87-1.98 (m, 3H), 2.02 (dd, 1H, *J* = 2.3, 6.0 Hz), 2.33 (d, 1H, *J* = 9.5 Hz), 2.87 (d, 1H, *J* = 6.8 Hz), 4.29 (ddd, 1H, *J* = 6.0, 10.0, 15.3 Hz), 4.49 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ -0.01, 22.8, 24.0, 33.3, 35.1, 35.5, 36.5, 71.9, 78.4, 84.0, 87.2, 106.0, 121.1 ppm. Anal. calcd for $C_{15}H_{24}O_3Si$: C, 64.24; H, 8.63 Found: C, 64.12; H, 8.69.

(1*S*, 2*R*, 3*S*, 4*S*)-4-[2-(trimethylsilyl)ethynyl]-2, 3-*O*-Cyclopentylcyclopentan-1-benzoate (61). A solution of triphenylphosphine (1.8 g, 6.8 mmol) in dry THF (50 mL) was cooled to -20 ^oC, and di*iso*propyl azodicarboxylate (1.4 mL, 6.8 mmol) was added over a period of 10 min. This mixture was stirred at -20 ^oC for 20 min to yield a white precipitate of triphenylphosphine-diisopropyl azodicarboxylate complex. To this latter complex as a suspension were added a solution of **60** (1.6 g, 5.7 mmol) in dry THF (10 mL) and benzoic acid (0.84 g, 6.8 mmol). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. After evaporation of the

reaction mixture to dryness, the residue was purified by column chromatography (hexanes/ethyl acetate=10:1) to afford as a white crystal **61** (1.54 g, 69.8%). mp = 92-93 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.08 (s, 9H), 1.68-1.78 (m, 6H), 1.89-1.94 (m, 2H), 2.14 (d, 1H, *J* = 14.0 Hz), 2.51 (ddd, 1H, *J* = 4.8, 8.0, 14.0 Hz), 3.15 (d, 1H, *J* = 8.0 Hz), 4.68 (d, 1H, *J* = 5.8 Hz), 4.75 (d, 1H, *J* = 5.8 Hz), 5.33 (d, 1H, *J* = 4.8 Hz), 7.41-7.55 (m, 2H), 7.55-7.58 (m, 1H), 8.08-8.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ -0.03, 21.6, 22.9, 23.0, 35.4, 35.5, 37.0, 74.4, 79.7, 84.9, 85.6, 107.1, 120.8, 128.3, 129.9, 130.1, 133.0, 165.8. Anal. calcd for $C_{22}H_{28}O_4Si$: C, 68.71; H, 7.34. Found: C, 68.96; H, 7.38.

(1*S*, 2*R*, 3*S*, 4*S*)-4-Ethynyl-2, 3-*O*-Cyclopentylcyclopentan-1-benzoate (62). To a solution of **61** (1.4 g, 3.6 mmol) in dry THF (100 mL) was added 1.0 M TBAF in THF (3.6 mL, 3.6 mmol) at 0 ^{o}C. The mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the resulting solution was washed with brine. After drying (anhydrous Na_2SO_4) and filtration, the residue was purified by column chromatography (EtOAc/hexanes = 1:9) to give **62** as colorless oil (1.0 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68-1.78 (m, 6H), 1.89-1.98 (m, 2H), 2.17 (d, 1H, *J* = 14.0 Hz), 2.21 (d, 1H, *J* = 2.8 Hz), 2.54 (ddd, 1H, *J* = 4.6, 7.8, 14.0 Hz), 3.15 (d, 1H, *J* = 7.8 Hz), 4.68 (d, 1H, *J* = 5.8 Hz), 4.75 (d, 1H, *J* = 5.8 Hz), 5.33 (d, 1H, *J* = 4.6 Hz), 7.44-7.48 (m, 2H), 7.57-7.61 (m, 1H), 8.10-8.13 (m, 2H). ^{13}C NMR (62.9 MHz, $CDCl_3$/TMS): δ 22.9, 24.0, 35.1, 35.3, 35.4, 35.7, 70.6, 79.5, 84.8, 84.9, 85.4, 120.9, 128.3, 129.9, 130.1, 133.1, 165.7. Anal. calcd for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45. Found: C, 73.16; H, 6.50.

(1*S*, 2*R*, 3*S*, 4*S*)-4-ethynyl-2, 3-*O*-Cyclopentylcyclopentan-1-ol (63). Ammonia gas was introduced to a solution of compound **62** (0.32 g, 1.0 mmol) in MeOH (100 mL).

This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was then removed in vacuo and the residue purified by chromatography (EtOAc/hexanes = 1:6) to afford **63** (0.20 g, 98%) as a white solid, mp = 65-66 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62-1.71 (m, 6H), 1.84-1.88 (m, 2H), 1.94 (dt, 1H, *J* = 1.6, 13.6 Hz), 2.22 (d, 1H, *J* = 8.0 Hz), 2.28 (d, 1H, *J* = 2.8 Hz), 2.32 (ddd, 1H, *J* = 4.8, 7.6, 13.6 Hz), 3.00 (d, 1H, *J* = 7.6 Hz), 4.21 (td, 1H, *J* = 0.8, 4.8 Hz), 4.55 (dd, 1H, *J* = 0.4, 6.0 Hz), 4.73 (d, 1H, *J* = 6.0 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$/TMS): δ 22.9, 23.9, 35.2, 35.3, 35.4, 37.2, 71.8, 78.0, 85.0, 85.9, 86.9, 120.5. Anal. calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.30; H, 7.68.

(1S,2R,3S,4S)-4-(1-acetyl-1H-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate (65). To a solution of **63** (1.0 g, 4.8 mmol) in anhydrous *t*-butyl methyl ether (90 mL) with stirring at -78 ^{o}C under N_2 was added *n*-butyllithium (4.8 mL, 12.0 mmol, 2.5 M solution in hexanes), and the reaction mixture stirred at the same temperature for 30 min. An excess of DMF (1.8 mL, 24 mmol) was added in one portion and the cold bath removed. The reaction mixture was allowed to warm to room temperature and aged for 30 min. The TBME solution was poured into a vigorously stirred, biphasic mixture prepared from a 10% aqueous solution of KH_2PO_4 (40 mL) and TBME (40 mL) cooled over ice to ca. +5 ^{o}C. The resulting layers were separated and the organic extract was washed with H_2O. The combined aqueous layers were back extracted with TBME. The combined organic layers were dried (anhydrous Na_2SO_4), filtered, and the filtrate concentrated to give the crude acetylenic aldehyde as an oil. The crude product thus isolated was used in the next step without further purification.

To a solution of crude aldehyde (from the previous process) in glacial AcOH (60 mL) was added a solution of hydrazine monohydrate (2.6 g, 25 mmol) in glacial AcOH (18 mL). The resulting solution was heated at reflux for 24 h and then concentrated in vacuo to afford a dark brown oil. This crude product was dissolved in pyridine (20 mL), and Ac_2O (11.6 mL, 122.4 mmol) and DMAP were added. The resulting solution was stirred for 24 h at room temperature. The solvent was removed in vacuo, and the crude residue dissolved in EtOAc (200 mL), washed with 10% HCl and brine, dried (anhydrous Na_2SO_4), concentrated, and purified by silica gel column chromatography to afford **65** (1.3 g, 73% over three steps) as a light yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.78-1.92 (m, 1H), 1.93 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.55 (s, 3H), 2.62-2.82 (m, 1H), 3.32-3.43 (m, 1H), 5.08-5.15 (m, 1H), 5.24-5.35 (m, 2H), 6.26 (d, 1H, *J* = 2.75 Hz), 8.07 (d, 1H, *J* = 2.75 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$/TMS): δ 20.6, 20.7, 20.9, 21.6, 32.8, 39.4, 75.0, 75.1, 75.2, 108.4, 129.2, 156.5, 169.2, 169.6, 169.8, 170.1 ppm. Anal. calcd for $C_{16}H_{20}N_2O_7$: C, 54.54; H, 5.72; N, 7.95. Found: C, 54.49; H, 5.82; N, 7.82.

(1S,2R,3S,4S)-4-(5-cyano-4-nitro-1H-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate (67). Trifluoroacetic anhydride (2.1 mL, 3.1 g, 15 mmol) was added dropwise to a stirred solution of **65** (0.90 g, 2.6 mmol) and ammonium nitrate (1.9 g, 24 mmol) in TFA (30 mL) at 0 ^{o}C. The resulting solution was allowed to warm to rt and stirred overnight. The solvent was evaporated in vacuo at room temperature and then diluted with CH_2Cl_2, washed with H_2O, saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and the organic phase concentrated in vacuo to give the 1,4-dinitro pyrazole derivative **66** (1.1 g) as a syrup. The crude product thus isolated was used to the next step without further purification.

A purifided **57** was prepared by silica gel column chromatography (EtOAc/hexanes = 1:4). A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.92-2.05 (m, 1H), 2.01 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.86-2.94 (ddd, 1H, *J* = 7.2, 9.6, 14.0 Hz), 4.07-4.12 (m, 1H), 5.19-5.23 (ddd, 1H, *J* = 4.4, 5.6, 7.2 Hz), 5.37-5.39 (t, 1H, *J* = 4.4 Hz), 5.58-5.61 (dd, 1H, *J* = 5.6, 7.2 Hz), 9.08 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 20.5, 20.6, 20.9, 32.6, 38.3, 74.2, 74.6, 74.9, 125.0, 134.1, 147.9, 169.6, 169.9, 170.2.

At room temperature, a solution of the 1,4-dinitro compound in EtOH (9.3 mL) and EtOAc (9.3 mL) was added dropwise over 5 min to a stirred solution of KCN (1.3 g, 20 mmol) in EtOH (23.0 mL) and H_2O (5.5 mL). Following an additional 5 min at room temperature, the reaction mixture was neutralized with AcOH (2.0 mL). After evaporation of the solvent in vacuo, the residue was diluted with EtOAc (110 mL), washed with H_2O and brine, dried (anhydrous Na_2SO_4), and concentrated in vacuo to a residue that was subjected to chromatographic purification (silica gel, CH_2Cl_2/MeOH, 20/1) to afford **67** (0.77 g, 80% over two steps) as a light yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.78-1.84 (ddd, 1H, *J* = 4.4, 9.6, 14.4 Hz), 2.05 (s, 3H), 2.10 (s, 3H), 2.16 (s, 3H), 2.99-3.07 (ddd, 1H, *J* = 7.2, 9.6, 14.4 Hz), 4.18-4.25 (dd, 1H, *J* = 9.6, 18.0 Hz), 5.20-5.24 (ddd, 1H, *J* = 3.6, 5.6, 7.2 Hz), 5.37-5.39 (dd, 1H, *J* = 3.6, 5.6 Hz), 5.68-5.71 (dd, 1H, *J* = 5.6, 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ 20.7, 20.7, 20.9, 33.3, 37.4, 73.1, 74.5, 74.6, 110.5, 122.6, 134.0, 143.9, 170.3, 170.4, 171.8.

(1S,2R,3S,4S)-4-(4-amino-5-cyano-1H-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate (69). A catalytic amount of Pd/C (1%) was added to a solution of **67** (0.70 g, 1.8 mmol) in MeOH (30 mL). The resulting mixture was shaken under 30 psi of H_2 overnight. After the reaction was complete, the solvent was evaporated in vacuo and the

product purified by silica gel chromatography (CH_2Cl_2/EtOAc/MeOH, 8:1:0.5) to afford **69** (0.55 g, 85%) as a syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08-2.11 (m, 9H), 2.72-2.91 (m, 10H), 3.37-3.43 (m, 1H), 4.12 (m, 2H), 5.23 (t, 1H, J = 4.8 Hz), 5.29-5.38 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 20.9, 21.0, 30.6, 36.6, 74.8, 75.0, 75.3, 112.3, 113.9, 134.3, 135.0, 170.2, 170.7, 170.9.

4-Amino-3-((1S,2S,3R,4S)-2,3,4-trihydroxycyclopentyl)-1H-pyrazole-5-carbonitrile (**70**). Anhydrous NH_3 was introduced to a solution of compound **69** (0.50 g, 1.4 mmol) in MeOH (50 mL) at 0 ^{o}C. The reaction mixture was stirred at room temperature. After the starting material was no longer present (TLC), the solvent was removed in vacuo and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH, 6:1) to afford **61** (0.26 g, 80%) as a light yellow solid, mp 174–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (m, 1H), 2.58 (m, 1H), 3.22 (m, 1H), 3.87 (m, 1H), 4.09 (m, 1H), 4.29 (m, 1H), 13.17 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.0, 36.5, 73.6, 74.8, 76.1, 112.2, 114.0, 129.9, 130.2.

(1*S*,2*R*,3*S*,4*S*)-4-(7-Amino-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl) cyclopentane-1,2,3-triol (**22**)**.** A solution of **70** (0.22 g, 1.0 mmol) in EtOH (30 mL) was stirred with formamidine acetate (0.16 g, 1.5 mmol) under reflux for 50 min. The resulting white precipitate was isolated by filtration, washed with EtOH, and dried to afford analytically pure **14** (0.13 g, 54%) as a dark white solid, mp 242–243 ^{o}C (dec), ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72-1.79 (m, 1H), 2.38-2.45 (m, 1H), 3.37(dd, 1H, *J* = 8.0, 17.6 Hz), 3.74 (dd, 1H, *J* = 2.4, 4.4 Hz), 3.91 (dq, 1H, *J* = 2.8, 5.2, 7.6 Hz), 4.33 (dd, 1H, *J* = 5.2, 8.0 Hz), 4.57 (m, 1H), 4.91 (brs, 1H), 5.19 (brs, 1H), 7.28 (brs, 2H), 8.15 (s, 1H), 12.46 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.6, 41.0, 75.6, 76.4, 78.6, 122.4, 139.7, 147.6,

151.1, 151.4. Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot 0.25MeOH$: C, 47.49; H, 5.44; N, 27.01. Found: C, 47.41; H, 5.35; N, 26.92.

(1S,2R,3S,4S)-4-(5-cyano-1-methyl-4-nitro-1*H*-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate (**76**)and **(1S,2R,3S,4S)-4-(5-cyano-2-methyl-4-nitro-1*H*-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate** (**77**). To a stirred solution of **67** (100 mg, 0.270 mmol) in anhydrous THF (10 mL), was added portion wise 60% sodium hydride in mineral oil (14 mg, 0.32 mmol) at 0 ^{o}C. The mixture was stirred at room temperature for 30 min. and then iodomethane (0.02 mL, 0.4 mmol) was added dropwise. After a period of 24 h the reaction mixture was absorbed by silica gel, and then purified by silica gel column chromatography. Compound **76** (52 mg, 0.13 mmol, 50.0%) and **77** (~5 mg, 5%) was obtained after eluting with a mixture of hexane/ethyl acetate in a ratio of 1:1. Compound **76** was a yellow syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 (m, 1H), 2.00 (s, 3H), 2.07 (s, 3H), 2.10 (s, 1H), 2.90 (ddd, 1H, J = 8, 9.6, 14.4 Hz), 4.06 (td, 1H, J = 6.8, 9.2, 14.4 Hz), 4.10 (s, 3H), 5.22 (ddd, 1H, J = 4.8, 6.0, 8.0 Hz), 5.38 (t, 1H, J = 5.2 Hz), 5.60 (dd, 1H, J = 5.2, 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.6, 20.7, 21.0, 33.0, 38.0, 40.0, 74.0, 74.8, 74.9, 107.3, 115.7, 135.4, 148.6, 169.8, 169.9, 170.1; Compound **77** was a yellow syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (m, 1H), 2.02 (s, 3H), 2.06 (s, 3H), 2.12 (s, 1H), 2.87 (m, 1H), 4.01 (s, 3H), 4.06 (m, 1H), 5.22 (m, 1H), 5.46 (t, 1H, J = 5.2 Hz), 5.63 (dd, 1H, J = 5.2, 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.7, 20.9, 21.0, 32.9, 37.8, 39.8, 74.5, 74.8, 74.9, 107.3, 115.7, 135.4, 149.2, 169.4, 170.0, 170.3.

4-amino-1-methyl-3-((1S,2S,3R,4S)-2,3,4-trihydroxycyclopentyl)-1H-pyrazole-5-carbonitrile (81). A catalytic amount of Pd/C (1%) was added to a solution

of **76** (1.4 g, 5.9 mmol) in methanol (30 mL) at room temperature, the resulting mixture was allowed to shake under 25 PSI of hydrogen overnight. After the reaction was complete, concentrated in vacuo to give (1S,2R,3S,4S)-4-(4-amino-5-cyano-1-methyl-1H-pyrazol-3-yl)cyclopentane-1,2,3-triyl triacetate **(80)** (1.4 g, 6.7 mmol >100%) as a colorless liquid, The crude product thus isolated was committed to the next step without further purification.

Anhydrous NH_3 was introduced to a solution of compound **71** (0.50 g, 1.4 mmol) in MeOH (50 mL) at 0 ^oC. The reaction mixture was stirred at room temperature. After the starting material was no longer present (TLC), the solvent was removed in vacuo and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH, 6:1) to afford **81** (0.26 g, 80%) as a light yellow solid, ^1H NMR (400 MHz, CDCl3) δ 1.68 (m, 1H), 2.58 (m, 1H), 3.22 (m, 1H), 3.87 (m, 1H), 4.09 (m, 1H), 4.12 (s, 3H), 4.29 (m, 1H), 13.17 (s, 1H). ^{13}C NMR (100 MHz, CDCl3) δ 33.0, 36.5, 73.6, 74.8, 76.1, 112.2, 114.0, 129.9, 130.2.

(1S,2R,3S,4S)-4-(7-Amino-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-3-yl)cyclopentane-1,2,3-triol (71)

A solution of **81** (0.22 g, 1.0 mmol) in EtOH (30 mL) was stirred with formamidine acetate (0.16 g, 1.5 mmol) under reflux for 50 min. The resulting white precipitate was isolated by filtration, washed with EtOH, and dried to afford analytically pure **62** (0.13 g, 54%) as a gray solid, mp 242–243 ^oC (dec), ^1H NMR (400 MHz, DMSO-d_6) δ 1.72-1.79 (m, 1H), 2.38-2.45 (m, 1H), 3.37(dd, 1H, *J* = 8.0, 17.6 Hz), 3.74 (dd, 1H, *J* = 2.4, 4.4 Hz), 3.91 (dq, 1H, *J* = 2.8, 5.2, 7.6 Hz), 4.00 (s, 3H), 4.33 (dd, 1H, *J* = 5.2, 8.0 Hz), 4.57 (m, 1H), 4.91 (brs, 1H), 5.19 (brs, 1H), 7.28 (brs, 2H), 8.15 (s, 1H),

12.46 (m, 1H). 13C NMR (100 MHz, DMSO) δ 36.4, 40.7, 75.4, 76.3, 78.5, 122.2, 139.8, 147.5, 150.8, 151.1.

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