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Yongfeng Li

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Date

Part 1: Synthesis and Anti-HIV Activity of Novel Cyclobutyl Nucleoside and Nucleotide Analogs

Part 2: Synthesis of Fluorescent Nucleoside Analogs

Part 3: Synthesis of Abacavir

By

Yongfeng Li
Doctor of Philosophy

Chemistry

Dennis C. Liotta
(Advisor)

Frank McDonald
(Committee Member)

Albert Padwa
(Committee Member)

Accepted

Lisa A. Tedesco, Ph.D.
Dean of the Graduate School

Date

Part 1: Synthesis and Anti-HIV Activity of Novel Cyclobutyl Nucleoside and Nucleotide Analogs

Part 2: Synthesis of Fluorescent Nucleoside Analogs

Part 3: Synthesis of Abacavir

By

Yongfeng Li

B.S. (1994) & M.S. (1997)
Lanzhou University, P.R. China

Advisor: Dennis C. Liotta, Ph.D.

An abstract of a dissertation
submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
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Abstract

Part 1: Synthesis and Anti-HIV Activity of Novel Cyclobutyl Nucleoside and Nucleotide Analogs, Part 2: Synthesis of Fluorescent Nucleoside Analogs, Part 3: Synthesis of Abacavir

By Yongfeng Li

Part 1 of this dissertation describes the synthesis and anti-HIV activity of several novel four-membered ring nucleoside and nucleotide analogs as anti-HIV agents. The synthesis of these analogs features a [2+2] cycloaddition or [3+1] cycloaddition to construct the carbocyclic four-membered ring or thietane ring. An efficient S_N2 strategy was used to couple the carbocyclic four-membered ring with a variety of nucleoside bases, including pyrimidine and purine bases. In addition, the Pummerer-type rearrangement was used to synthesize the thietanose nucleosides. The EC_{50} values of those compounds in human PBM cell lines ranged from 14.6 to 100 μM and some of them were shown toxicity in PBM, CEM and Vero cells. Interestingly, most of the cyclobutyl nucleoside analogs did not show any anti-HIV activity; however, some of the cyclobutyl phosphonate nucleoside analogs did show some mild anti-HIV activity and toxicity. For the thietanose nucleosides, some of them showed moderate activity and cytotoxicity. The other nucleosides showed neither anti-HIV activity nor cytotoxicity up to 100 μM.

Part 2 of this dissertation reported the synthesis of fluorescent nucleoside analogs with modified sugar moieties (*e.g.*, sugars other than ribose and 2'-deoxyribose). Novel variants of fluorescent thymidine analog 6-methyl-3-(β-D-2'-

deoxyribofuranosyl) furano-[2,3-*d*]pyrimidin-2-one were synthesized, such as, AZT, D4T and DDC, by a Sonogashira reaction and a copper catalyzed cycloaddition. These compounds can be used as promising tools to research metabolites inside living cells and study biochemical reactions in situ, such as the 5'-phosphorylation of the nucleosides by cellular deoxynucleoside kinases.

Part 3 of this dissertation discusses the improvement on the preparation of abacavir ((1S,4R)-4-(2-amino-6-(cyclopropylamino)-9H-purin-9-yl)cyclopent-2-enyl)methanol by utilizing commercially available and inexpensive starting materials and that proceeds with high regioselectivity and stereochemical control. After formation of a novel π-allylpalladium complex, the bicyclic precursor can be opened with complete regio- and stereo-specificity to yield the desired, biologically active β-anomeric nucleoside.

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Abbreviations

abs	Absolute
AcOH	Acetic acid
Ac_2O	Acetic anhydride
Ac	Acetyl
Bn	Benzyl
Bz	Benzoyl
9-BBN	9-Borabicylo[3.3.1]nonane
bp	Boiling point
n-BuLi	*n*-Butyllithium
t-BuLi	*t*-Butyllithium
cat	Catalytic
CAN	Ceric ammonium nitrate
CBN	Cyclobutyl nucleoside
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
1,2-DCE	1,2-Dichloroethane
DCC	1,3-Dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DAST	(Diethylamino)sulfur trifluoride
DEAD	Diethyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminum hydride

DMAP	N,N-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	N,N-Dimethylformamide
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
EC50	Effective concentration of a drug that is required for 50% inhibition of viral replication *in vitro*
EC90	Effective concentration of a drug that is required for 90% inhibition of viral replication *in vitro*
EI-MS	Electron Ionisation Mass Spectroscopy
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
HIV-RT	HIV-reverse transcriptase
HMPT	Hexamethylphosphorous triamide
HMDS	1,1,1,3,3,3-Hexamethyldisilazane
HPLC	High Pressure Liquid Chromatography
HR	High Resolution
IC50	Concentration of an inhibitor that is required for 50% inhibition of an enzyme *in vitro*
IR	Infrared Spectroscopy
LDA	Lithium diisopropylamide
L-Selectride	Lithium tri-sec-butylborohydride
LS-Selectride	Lithium trisiamylborohydride y
*m*CPBA	*meta*-Chloroperoxy benzoic acid
Mins	Minutes

mp	Melting point
MS	Mass Spectroscopy
MsCl	Methanesulfonyl chloride
NaHMDS	Sodium hexamethyldisilazide
NFSI	N-Fluorobenzenesulfonimide
NIS	N-Iodosuccinimide
NMO	N-methylmorpholine N-oxide
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
PMB	*p*-Methoxybenzyl
Py	Pyridine
RT	Room temperature
Sat.	Saturated
Selectfluor®	1-(Chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)
TBAF	Tetrabutylammonium fluoride
Tf_2O	Trifluoromethanesulfonic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPSCl	*t*-Butyldiphenylsilyl chloride
TsCl	*p*-Toluenesulfonyl chloride

Part 1: Synthesis and Anti-HIV Activity of Novel Cyclobutyl Nucleoside and Nucleotide Analogs

1.1 Statement of purpose

Acquired immune deficiency syndrome (AIDS) has become one of the major causes of death in the world. It is estimated that over 40 million people are infected with the human immunodeficiency virus (HIV), which is the causative agent of AIDS.[1] In 1985, 3'-azido-3'-deoxythymidine (AZT) was approved as the first synthetic nucleoside to inhibit the replication of HIV. Since then, a number of other synthetic nucleoside analogs have been proven to be effective against HIV. After phosphorylation to the triphosphate form by cellular kinases, the nucleotides are incorporated into a growing strand of viral DNA and cause chain termination due to the absence of the 3'-hydroxyl group.

Despite the enormous success of nucleoside based therapy of HIV infection, there is still no cure for AIDS. One reason is that the prolonged clinical use of nucleoside analogs gives rise to resistant viruses that contain mutations in the reverse transcriptase (RT).[2] For example, the M184V/I mutation in HIV-1 RT is associated with high level resistance to lamivudine (3TC) and emtricitabine (FTC). Structural studies suggest that the mechanism of resistance of HIV-1 RT carrying the M184V/I mutation involves steric hindrance, which would prevent further incorporation of nucleoside analogs such as 3TC and FTC in the nucleotide forms.[3]

We postulated that the more rigid and smaller cyclobutyl ring would enable the nucleoside analog to fit into the more sterically hindered active site of

RT containing the M184V/I mutants. Nucleoside analogs of this type received a great deal of attention several years ago with the discovery of naturally occurring Oxetanocin A, which shows activity against HIV, and Lobucavir (Cyclobut-G) with activity against Hepatitis B virus (HBV). However, virtually all of the reported derivatives possess the 2',3'-*bis*-hydroxyl motif that enables them to be phosphorylated and incorporated into growing strands of DNA. We are interested in preparing a series of 2'-substituted cyclobutyl nucleosides that only have the 3'-hydroxymethyl for phosphorylation, but not the 2'-hydroxymethyl motif. We reasoned that these nucleoside analogs could fit into the more sterically hindered active site of M184I/V mutant forms of RT. In addition, a series of *O*-phosphonates and their prodrug forms will be synthesized to circumvent with phosphorylation.

1.2 Introduction

In 1981, AIDS (the acquired immunodeficiency syndrome) was first described in the United States with the unexplained appearance of Kaposi's sarcoma, Pneumocystis carinii pneumonia and other opportunistic infections in previously healthy homosexual males. The affected individuals displayed a specific immune deficiency resulting from the depletion of CD4+ lymphocytes. In 1983, the etiological cause of AIDS was determined to be HIV (the human immunodeficiency virus).[4, 5] In 1985, for the first time, it was reported that the synthetic nucleoside AZT (3'-azido-3'-deoxythymine) inhibits the replication of human immunodeficiency virus.[6] Since then, a number of drugs including synthetic nucleoside analogs have been approved by FDA to be effective against HIV, which can be divided into six classes: (1) NRTI (nucleoside reverse transcriptase inhibitor), FDA approved drugs are: AZT, DDI, DDC, D4T, 3TC, FTC, Abacovir (Figure 1); (2) NtRTI (nucleotide reverse transcriptase inhibitor), FDA approved drugs are: Tenofovir disoproxil fumarate (Figure 2); (3) NNRTI (non-nucleoside reverse transcriptase inhibitor), FDA approved drugs are: Nevirapine, Delavirdine, Efavirenz, Etravirine (Figure 3); (4) PI (protease inhibitor), FDA approved drugs are: Saquinavir, Indinavir, Ritonavir, Nelfinavir, Amprenavir, Lopinavir (Figure 4). (5) Fusion and entry inhibitors, FDA approved drugs are: Fuzeon and Selzentry; (6) Integrase inhibitors, FDA approved drug is: Isentress (Figure 5). The accepted standard of medical treatment for HIV infection involves the use of a combination of three of these drugs.[7] The use of combination therapy or drug "cocktails" has profoundly reduced the morbidity and

mortality associated with AIDS. However, the approved anti-HIV drugs and the combination(s) of these drugs have significant limitations including toxicities associated with long term administration, pharmacokinetic interactions with other agents, poor compliance due to complex dosing regimens and the development of viral resistance.[8] These limitations have necessitated the continued search for new anti-HIV agents with improved clinical profiles.

Figure 1: Structures of nucleoside analog reverse transcriptase inhibitors (NRTIs)

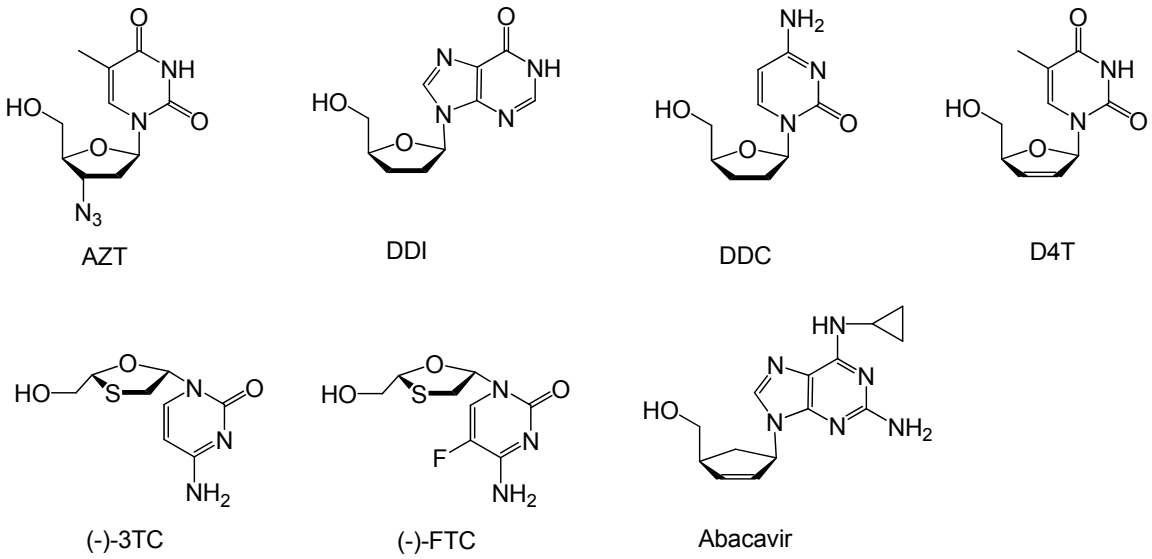
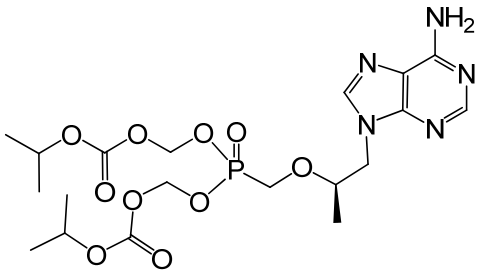
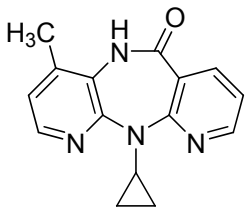


Figure 2: Structures of nucleotide analog reverse transcriptase inhibitors (NtRTI)

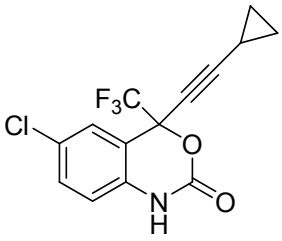


Tenofovir disoproxil fumarate

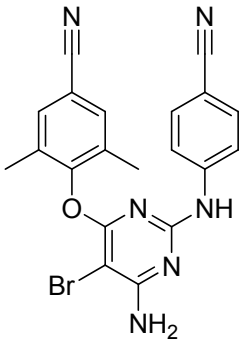
Figure 3: Structures of non-nucleoside reverse transcriptase inhibitors (NNRTIs)



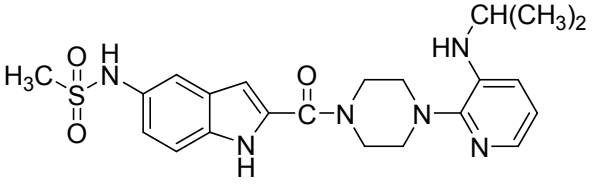
Nevirapine



Efavirenz



Etravirine



Delavirdine

Figure 4: Structures of protease inhibitors (PIs)

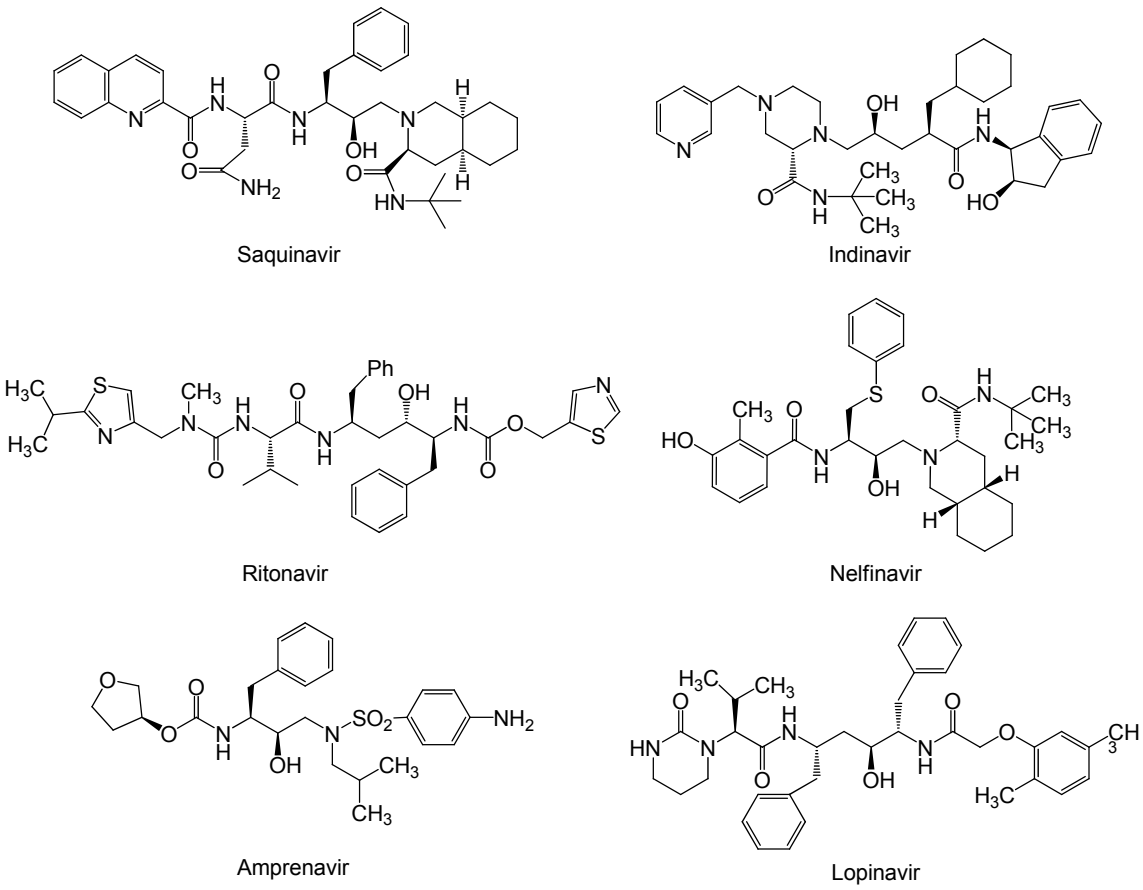
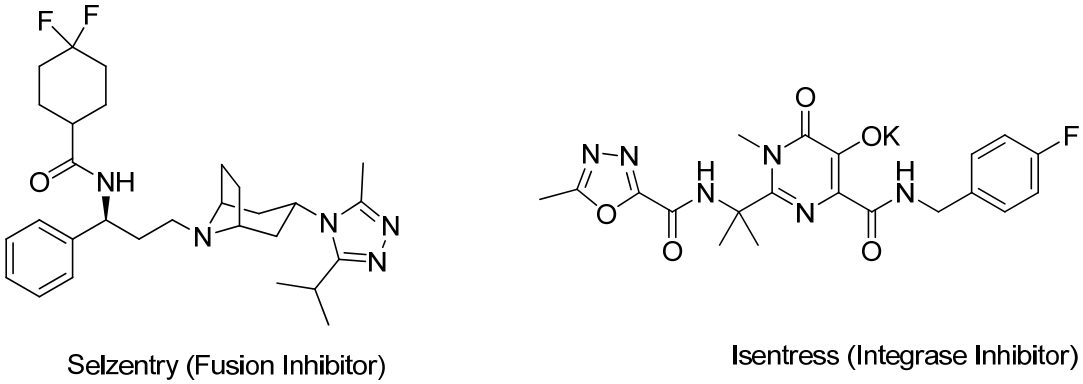
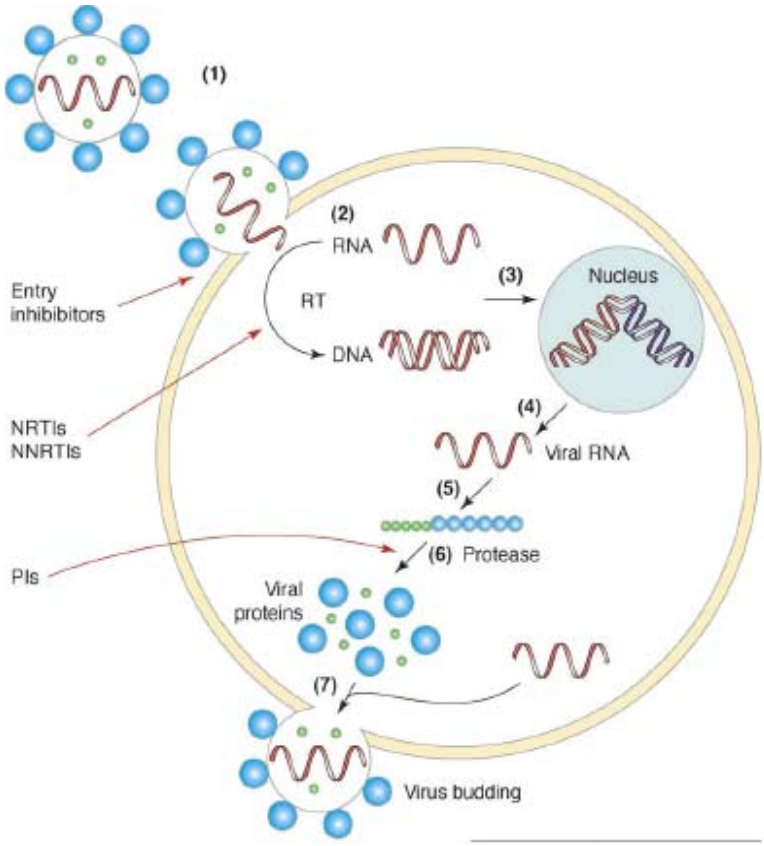


Figure 5: Structures of fusion and integrase inhibitors



1.2.1 HIV replication cycle

Figure 6: Targets of Retroviral Therapy at Different Steps of the HIV Lifecycle



In order to design effective anti-HIV drugs, complete knowledge of the mechanism of viral proliferation is required. In the life circle of the virus, there are several stages.

1. Binding and Fusion: HIV begins its life cycle when it binds to a CD4 receptor and one of two co-receptors on the surface of a $CD4^+$ T- lymphocyte. The virus then fuses with the host cell. After fusion, the virus releases RNA, its genetic material, into the host cell.

2. Reverse Transcription: An HIV enzyme called reverse transcriptase converts the single- stranded HIV RNA into double-stranded HIV DNA.

3. Integration: The newly formed HIV DNA enters the host cell's nucleus, where an HIV enzyme called integrase "hides" the HIV DNA within the host cell's own DNA. The integrated HIV DNA is called provirus. The provirus may remain inactive for several years, producing few or no new copies of HIV.

4. Transcription: When the host cell receives a signal to become active, the provirus uses a host enzyme called RNA polymerase to create copies of the HIV genomic material, as well as shorter strands of RNA called messenger RNA (mRNA).

5. Translation: The mRNA is used as a blueprint to make long chains of HIV proteins.

6. Assembly: An HIV enzyme called protease cuts the long chains of HIV proteins into smaller individual proteins. As the smaller HIV proteins come together with copies of HIV's RNA genetic material, a new virus particle is assembled.

7. Budding: The newly assembled virus pushes out ("buds") from the host cell. During budding, the new virus steals part of the cell's outer envelope. This envelope, which acts as a covering, is studded with protein/sugar combinations called HIV glycoproteins. These HIV glycoproteins are necessary for the virus to bind CD4 and co- receptors. The new copies of HIV can now move on to infect other cells.

1.2.2 The mechanism of NRTIs

Nucleoside reverse transcriptase inhibitors are the first class of compounds to be used in anti-HIV-1 therapy and are a cornerstone in highly active antiretroviral therapy (HAART).[9] All approved nucleoside reverse transcriptase inhibitors are 2', 3'-dideoxy derivatives of the natural nucleoside substrates of DNA polymerases and are all thought to inhibit reverse transcriptase activity in a similar fashion.[10-14] After absorption into the cell, they are phosphorylated at the 5'-hydroxyl group by cellular kinases to form the monophosphate, diphosphate and ultimately triphosphate esters. Following this, the inhibition can occur through two discrete mechanisms.[6, 15-17] The first of these is direct competitive inhibition of the DNA assembling enzyme (cellular DNA polymerase or viral reverse transcriptase), in which the 2', 3'-dideoxy derivatives compete with the natural substrates (2'-deoxynucleotides) for the enzyme's active site. The second mechanism of action is chain termination through incorporation of the 2', 3'-dideoxy derivatives into the growing DNA strand. Chain termination is caused by the lack of a 3'-hydroxyl group that is necessary to form a 3'-5' phosphodiester bond with the next nucleoside substrate in the elongating DNA strand.[18] During this process, pyrophosphate is expelled as a leaving group at the same time. If the above process is reversed by the attack of the pyrophosphate, NRTI will be expelled from the elongating DNA chain with the re-appearance of the 3'-hydroxyl group, making the drug inactive. Although the latter mechanism of chain termination is considered to be the more likely mechanism of inhibition, it has not been rigorously established whether

competitive inhibition of the DNA assembling enzyme contributes to the anti-HIV activity of these compounds.

1.2.3 Challenges of NRTIs

The efficacy of a nucleoside analog is dependent upon several factors, including its oral bioavailability, cellular uptake, the intracellular anabolism to its triphosphate derivatives, the ability to compete with natural nucleotides as a substrate for RT, and the degree of drug resistance developed by the virus.[19, 20]

Currently, appearance of drug resistant viruses is an inevitable consequence of prolonged exposure of HIV-1 to antiretroviral therapy. This is believed to be caused by both a high turnover of HIV-1 in patients and by low fidelity of viral RT.[21, 22] To achieve efficient inhibition of HIV-1 replication in patients, and to delay appearance of drug resistant virus, drug combinations, which include NRTIs, NNRTIs and/or PIs, have been used effectively in treating HIV-1 infection.[23, 24] However, recent studies show that HIV-1 can become resistant to multiple drugs in patients undergoing combination therapy, although resistance takes longer to develop than in a single drug regimens.[25, 26] Drug resistant viruses have been well documented in patients undergoing either monotherapy or combination therapy with two drugs that include AZT, DDI, DDC and 3TC.[2, 27-30] A single amino acid substitution within the RT enzyme is sufficient to cause resistance *in vitro*, as in the case of the M184V mutation with 3TC, although a combination of mutations is required to confer high-level resistance to AZT.[28, 31-34] Of particular concern is the fact that mutant viruses might be transmitted during *de novo* infections;[20,35] therefore, it is of utmost importance to

identify new agents that are active against these drug resistant strains of HIV-1 and are well tolerated by individuals living with HIV-1.

There are two main biochemical mechanisms that lead to NRTI resistance, namely sterical inhibition and phosphorylysis.[36] *Sterical inhibition* is caused by mutations enabling the reverse transcriptase to recognize structural differences between NRTIs and deoxyribonucleotide triphosphate (dNTPs). Incorporation of NRTIs is then prevented in favor of dNTPs (e.g. in the presence of the mutations M184V, Q151M, L74V, or K65R). *Phosphorylysis* via ATP (adenosine triphosphate) or pyrophosphate leads to the removal of the NRTIs already incorporated in the growing DNA chain. This is the mechanism for RTs with the following mutations: M41L, D67N, K70R, L210W, T215Y and K219Q. Phosphorylysis leads to cross-resistance between NRTIs, the degree of which may differ between substances (AZT, d4T > ABC > ddC, ddI > 3TC).

For several NRTIs, such as 3TC, and for NNRTIs, a high degree of resistance can develop following only a single mutation. For this reason, such drugs should only be used in highly effective regimens. However, the 3TC specific mutation, M184V, also reduces viral replication capacity (often referred to as reduced viral fitness) by 40 - 60%. After 52 weeks with 3TC monotherapy, the viral load remained 0.5 log below the initial levels despite early development of the M184V mutation. Compared to treatment interruptions, continuous monotherapy with 3TC delays virological and immunological deterioration.

The M184V/I mutation, which is associated with 3TC resistance, is also the key resistance mutation for FTC. Consequently, FTC is unlikely to have anti-

HIV effect in people who have already developed resistance to 3TC. However, the M184V mutation can enhance the antiviral efficacy of other drugs such as AZT, because it slows the ability of the virus to develop resistance to those other drugs. The mutation does not affect susceptibility to other NRTIs or NNRTIs. No other mutations have been associated with high-level FTC resistance, including other NRTI and NNRTI mutations. However, the ddC-associated mutation K65R may slightly reduce the efficacy of FTC, which stays in the blood for much longer than 3TC, and makes the development of resistance to FTC less likely than 3TC.

1.3 Background

1.3.1 Synthesis of carbocyclic four- membered rings

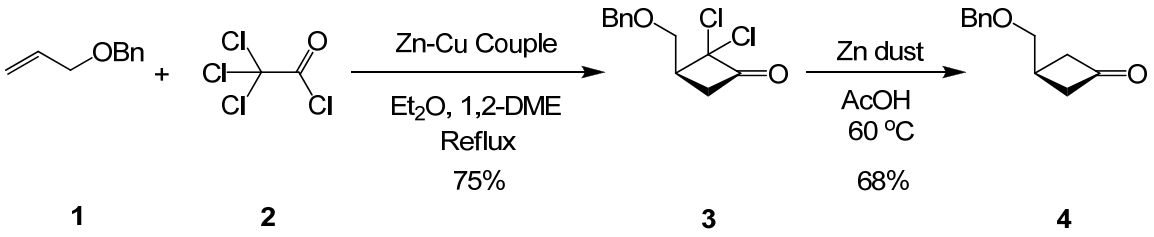
Studies have shown that cyclobutane itself has a puckered structure with a dihedral angle of *ca* 30±6^{o} and a barrier to inversion of *ca* 1.4 kcal/mol.[37, 38] A carbocyclic four-membered ring is very strained, which makes it hard to synthesize. In general, a carbocyclic four-membered ring can be constructed either directly from cycloaddition reactions ([2+2] or [3+1]) or directly from ring expansion reaction of the three-membered ring or indirectly from five-membered ring contraction reactions. The methodologies used in the synthesis of carbocyclic four-membered ring nucleosides are summarized below.

1.3.1.1 [2+2] cycloaddition

Method 1: Ketene cycloaddition with alkene

Under thermal conditions, dichloroketene generated *in situ* from trichloroacetyl chloride **2** using copper activated zinc dust reacts with electron-rich alkenes to form *gem*-dichloro-substituted cyclobutanone **3** in good to excellent yields.[39] Dichloroketene is considered to be more reactive than unsubstituted ketene with alkenes; in addition, dichloroketene is less prone to dimerization than unsubstituted ketenes. After removal of chlorine by zinc dust, cyclobutanone **4** can be obtained.

Scheme 1: [2+2] Cycloaddition between allyl benzyl ether 1 and dichloroketene

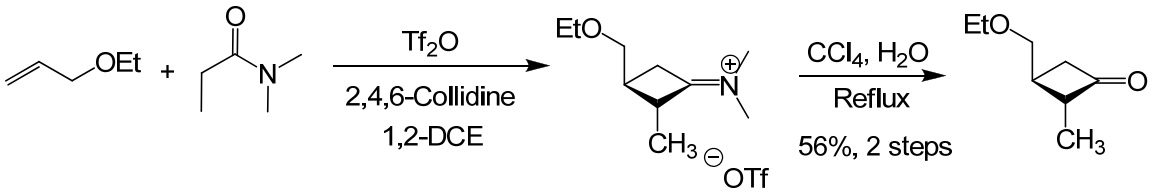


1 **2** **3** **4**

Method 2: Keteniminium salt cycloaddition with alkene

Under thermal conditions, keteniminium triflates can react with alkenes to give the intermediate cyclobutaniminium salt **7**, which is hydrolyzed to provide cyclobutanone **8**.[40] Since keteniminium salts are more electrophilic than ketenes, less nucleophilic alkenes can be used. The method works well for alkenes or acetylenes bearing alkyl, alkenyl or aryl groups, however, they do not react with enol ethers or enamines.

Scheme 2: Cycloaddition between allyl ethyl ether **5** and keteniminium salt

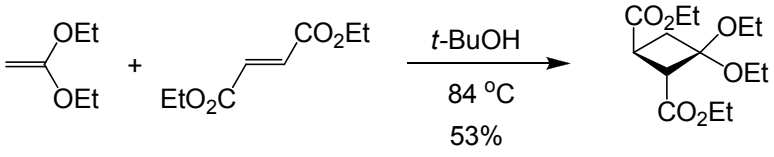


5 **6** **7** **8**

Method 3: Ketene acetal cycloaddition with alkene

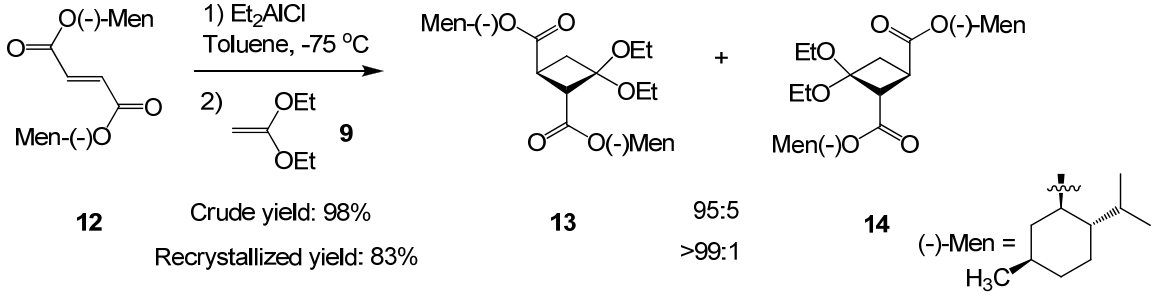
Under thermal conditions, diethyl fumarate **10** reacts with ketene diethylacetal **9** in *t*-BuOH for 72 hrs to provide the racemic cyclobutane diester **11** in moderate yield after distillation.[41] Using a Lewis acid, usually a organoaluminum catalyst, (-)-dimenthyl fumarate **12** reacts with ketene diethylacetal **9** at low temperature to give almost enantiomerically pure cyclobutane diester **13**.[42]

Scheme 3: [2+2] Cycloaddition between diethyl fumarate **10** and ketene diethylacetal **9**



9 **10** **11**

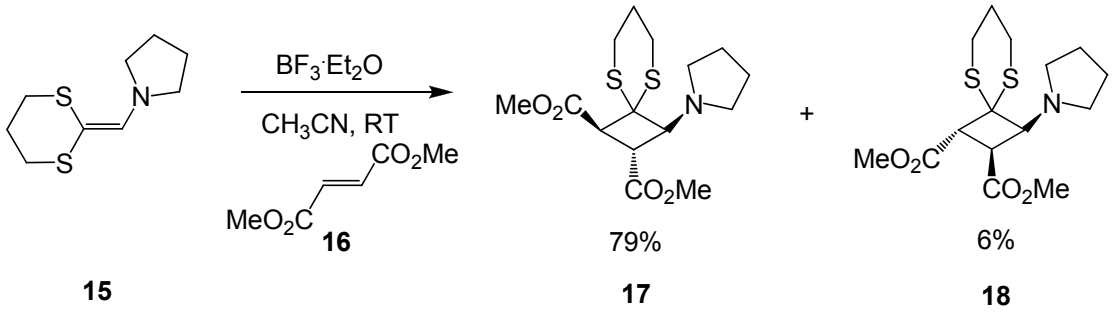
Scheme 4: Asymmetric [2+2] cycloaddition between dimenthyl fumarate **12** and ketene diethylacetal **9**



Method 4: Enamine cycloaddition with alkene

Catalyzed by Lewis acid, β,β-disubstituted enamine **15** reacts with electrophilic alkenes, such as dimethyl maleate **16**, to provide the highly substituted stable cyclobutanes **17** and **18**.[43]

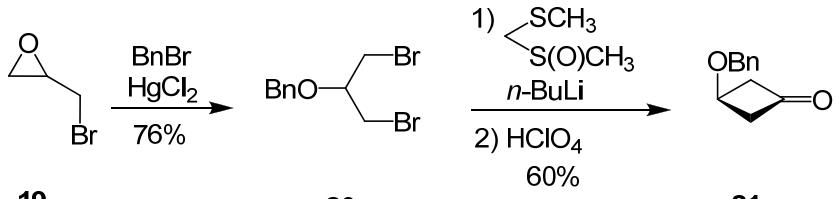
Scheme 5: [2+2] Cycloaddition between enamine **15** and dimethyl maleate **16**



1.3.1.2 [3+1] cycloaddition

In the following transformation, base-promoted condensation of the dibromide **20** with methyl methylthiomethyl sulfoxide and subsequent hydrolysis as described by Tsuchihashi furnished the cyclobutanone product **21**.[44]

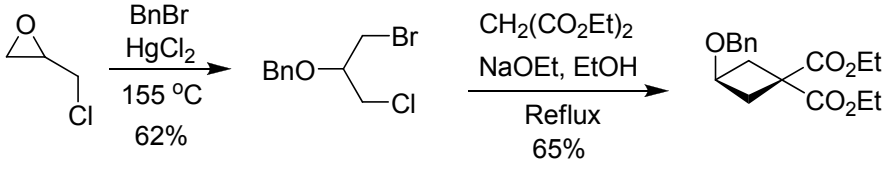
Scheme 6: Synthesis of cyclobutanone **21** by [3+1] cycloaddition



19 **20** **21**

Another similar example was reported by Helal: alkylation of diethylmalonate with 2-benzyloxy-1-bromo-3-chloropropane **23** gave the substituted cyclobutane **24**.[45]

Scheme 7: Synthesis of 3-benzyloxy-cyclobutane-1, 1-dicarboxylic acid diethyl ester **24** by [3+1] cycloaddition



22 **23** **24**

1.3.2 Synthesis of nucleoside triphosphates

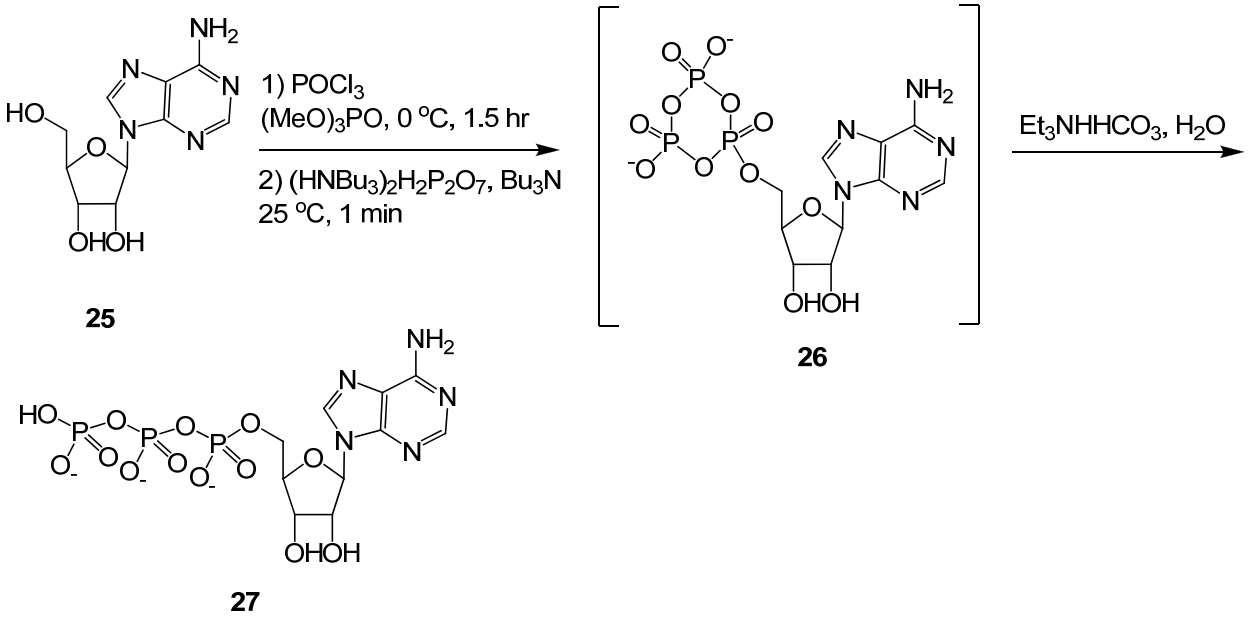
Nucleoside triphosphates are difficult to make, isolate, characterize, and store due to several factors.[46] Firstly, the preparation involves charged ionic reagents, as well as lipophilic substrates. Therefore, it is difficult to find appropriate reaction media and a purification procedure to isolate the charged water-soluble product from a mixture of hydrophilic and hydrophobic impurities. Secondly, the right choice of solvent system is very important to stabilize the product. Hydrolysis of the triphosphate is accelerated under basic and acidic conditions. Methods for the synthesis of nucleoside triphosphates evolved rapidly in the 1950s, but up to now, a general method for preparing nucleoside triphosphates for all nucleoside derivatives is still not available. Landmarks in the development of contemporary synthesis of nucleoside triphosphates are summarized below:

1.3.2.1 "One-pot, three-step" triphosphorylation

Early work on the phosphorylation of nucleosides using phosphorus oxytrichloride was hampered by lack of regioselectivity. An innovation was made by Yoshikawa and co-workers who discovered that the rate of the phosphorylation of nucleosides was accelerated by using trimethyl- or triethylphosphate as the solvent.[47] Based on Yoshikawa's monophosphorylation procedure, Ludwig generated the nucleoside dichlorophosphates by reacting the nucleoside with phosphorus oxytrichloride using trimethyl- or triethylphosphate as the solvent, which reacted directly with *bis*(tri-*n*-butylammonium) pyrophosphate

in dry DMF to give the triphosphate product **27** after quenching with triethylammonium bicarbonate. Ludwig noted that decreased yields were obtained if an amine base was not added.[47]

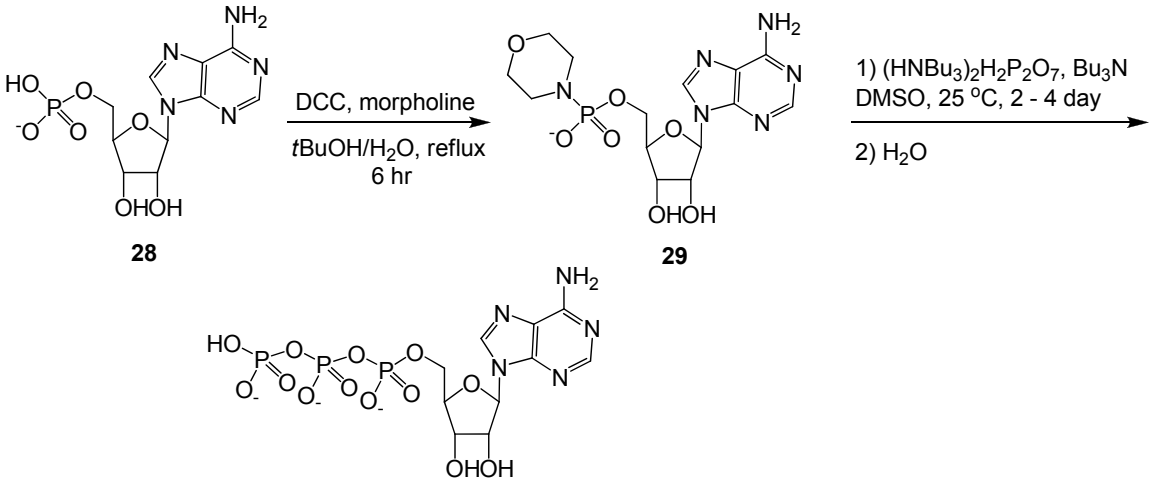
Scheme 8: Yoshikawa and Ludwig's one-pot three-step synthesis of triphosphate **27**



1.3.2.2 Monophosphate activation

It is difficult to ascertain the efficiency of the monophosphorylation step in the "one-pot, three-step" triphosphorylation reaction. This is not a problem, however, if the nucleoside monophosphates are isolated *en route* to nucleoside triphosphates. In the following example, monophosphate **28** is activated by morpholine as phosphoramidates **29**, which reacts with nucleophilic pyrophosphate to provide the triphosphate **27**.[48]

Scheme 9: Triphosphate synthesis through phosphoramidates **29**



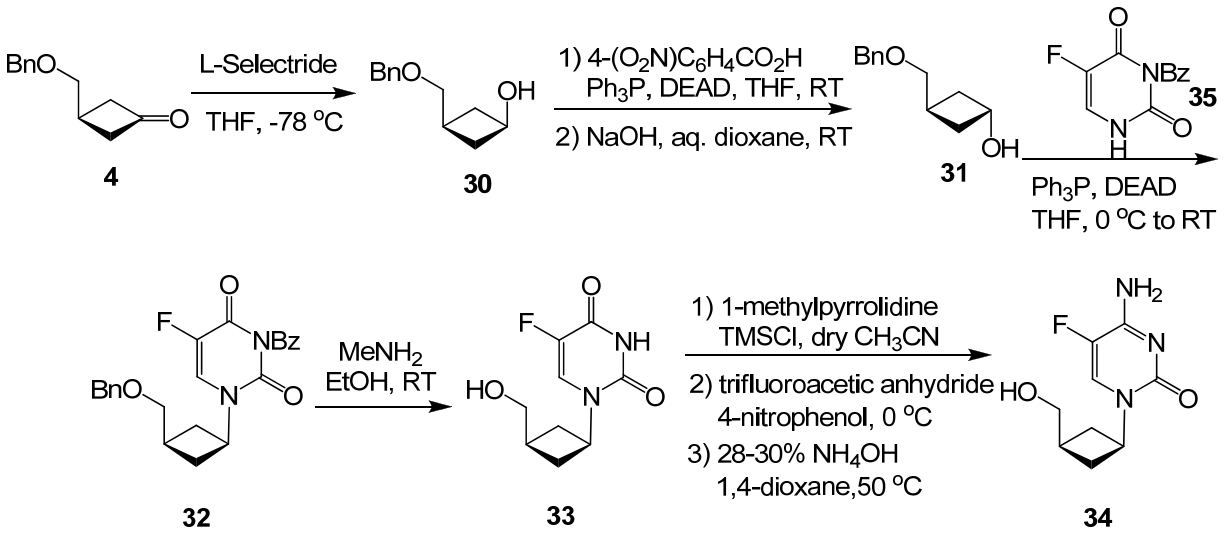
1.3.3 Synthesis and anti-HIV activity of 3'-hydroxymethyl cyclobutyl nucleosides

1.3.3.1 Synthesis

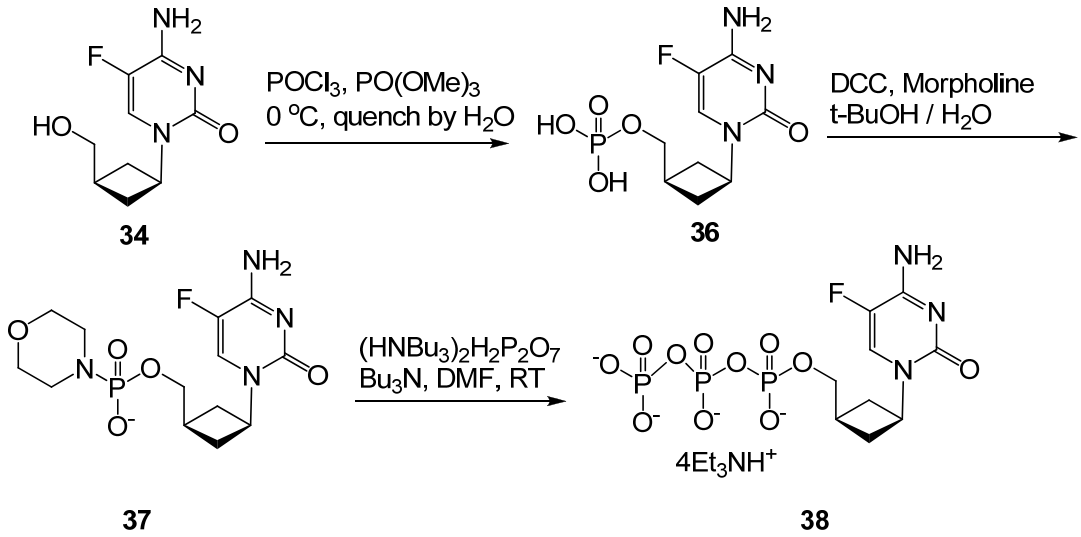
In 1995, the Reese group reported the synthesis of 9-[*cis*-3-(hydroxymethyl)cyclobutyl]-adenine and -guanine analogs. The synthesis featured a cycloaddition reaction between dichloroketene and allyl benzyl ether **1** and a Mitsunobu coupling reaction between the cyclobutanol **30** and the nucleoside base with inversion of the stereocenter.[49] Later in 1998, they reported the synthesis of 1-[*cis*-3-(hydroxymethyl)cyclobutyl]-uracil, –cytosine and –thymine, using similar key reactions.[50] In the Liotta group, 5-fluoro-1-[*cis*-3-(hydroxymethyl)-cyclobutyl]-cytosine **34** and its triphosphate **38** were synthesized in 2004 and the nucleoside itself followed the Reese synthesis, while the

triphosphate followed the procedure using activated morpholine as a key intermediate.

Scheme 10: Synthesis of 5-fluoro-1-[*cis*-3-(hydroxymethyl)cyclobutyl]cytosine **34**

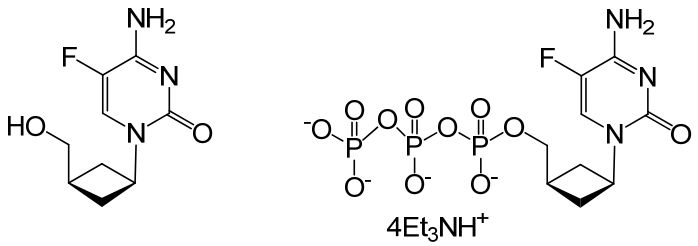


Scheme 11: Synthesis of 5-fluoro-1-[*cis*-3-(hydroxymethyl)cyclobutyl]cytosine triphosphate **38**



1.3.3.2 Anti-HIV activity

Figure 7: Cyclobutyl nucleoside analogs reported from the Liotta group



DLS183
34

DLS183-TP
38

In the Liotta group, the above compounds were evaluated for their anti-HIV activity and cytotoxicity in PBM, CEM and Vero cells, according to the known procedures.[51-53] It turned out that compound **DLS183** (**34**) were not active up to 100 μM, although it didn't exhibit any cytotoxicity up to 100 μM. However, its triphosphate form, **DLS183-TP** (**38**), showed good anti-HIV activity comparable with 3TC against wild type HIV RT, recombinant wild type HIV RT, as well as the M184I and M184V mutants, according to the RT assay described by Eriksson, Chu and Schinazi.[54] The results are shown in Tables 1 - 4 and Figures 7 - 9.

Table 1: Inhibition of HIV-RT from viral-lysate (WT)

	cpm/ml	**SD**	**%Inhibition**
WT cont	276994.506	27124.182	
3TC-TP 10 μM	124341.312	5110.884	55
3TC-TP 1 μM	215883.012	32964.78	22
3TC-TP 0.1 μM	268420.2	31938.252	3.1
DLS183-TP 10μM	134616.36	3065.154	51
DLS183-TP 1μM	228126.312	34687.278	18
DLS183-TP 0.1μM	280781.826	3857.472	0.01
No enzyme	71173.2		

Table 2: Inhibition of HIV-RT from viral-lysate (M/I)

	cpm/ml	SD	%Inhibition
M/I cont	341761.452	9427.008	
3TC-TP 10 µM	298628.406	769.008	13
3TC-TP 1 µM	329605.62	25588.608	0.03
3TC-TP 0.1 µM	329435.346	16435.77	0.03
DLS183-TP 10µM	160719.12	5023.86	53
DLS183-TP 1µM	272130.486	19976.004	20
DLS183-TP 0.1µM	407041.44	67797.246	0.01

Table 3: Inhibition of HIV-RT from viral-lysate (M/V)

	cpm/ml	SD	%Inhibition
M184V control	312707.646	22531.224	
3TC-TP 10 µM	336946.272	51714.012	0.01
3TC-TP 1 µM	288267.666	7702.512	7
3TC-TP 0.1 µM	270239.712	21601.71	13
DLS183-TP 10µM	152688.27	9583.962	51
DLS183-TP 1µM	245062.692	16673.31	22
DLS183-TP 0.1µM	297603.432		5

Table 4: Comparison of Inhibition of HIV RT in Cell-Free Assays

HIV RT*	**Inhibition of RT Activity (IC_{50}, µM)****			
	3TC-TP		DLS183-TP	
Recombinant HIV RT (WT)	2.99	0.7	4.74	0.34
HIV RT (WT)	6.53	1.46	6.85	1.79
HIV RT (M/I)	>10		6.06	0.75
HIV RT (M/V)	>10		6.91	1.50

* All the HIV RT used, except the recombinant RT, was obtained from viral lysates from PBMC infected with respective HIV.

** Values represented are from triplicates from one experiment.

Figure 8: Inhibition of HIV-RT (WT)

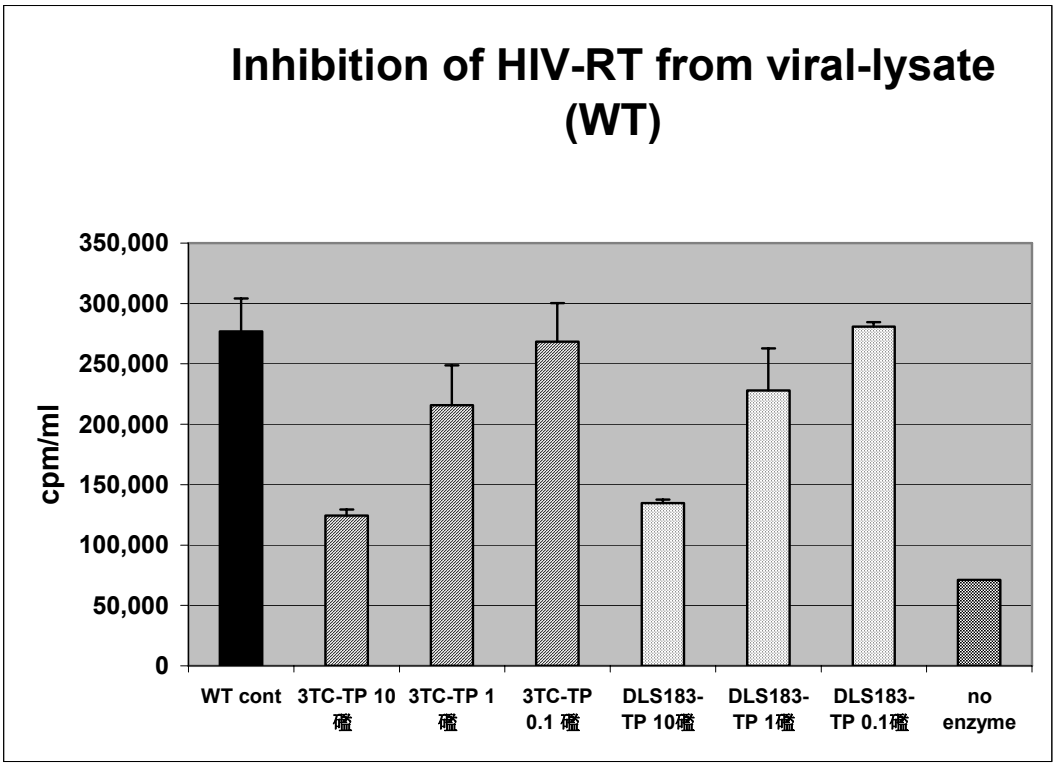


Figure 9: Inhibition of HIV-RT (M/I)

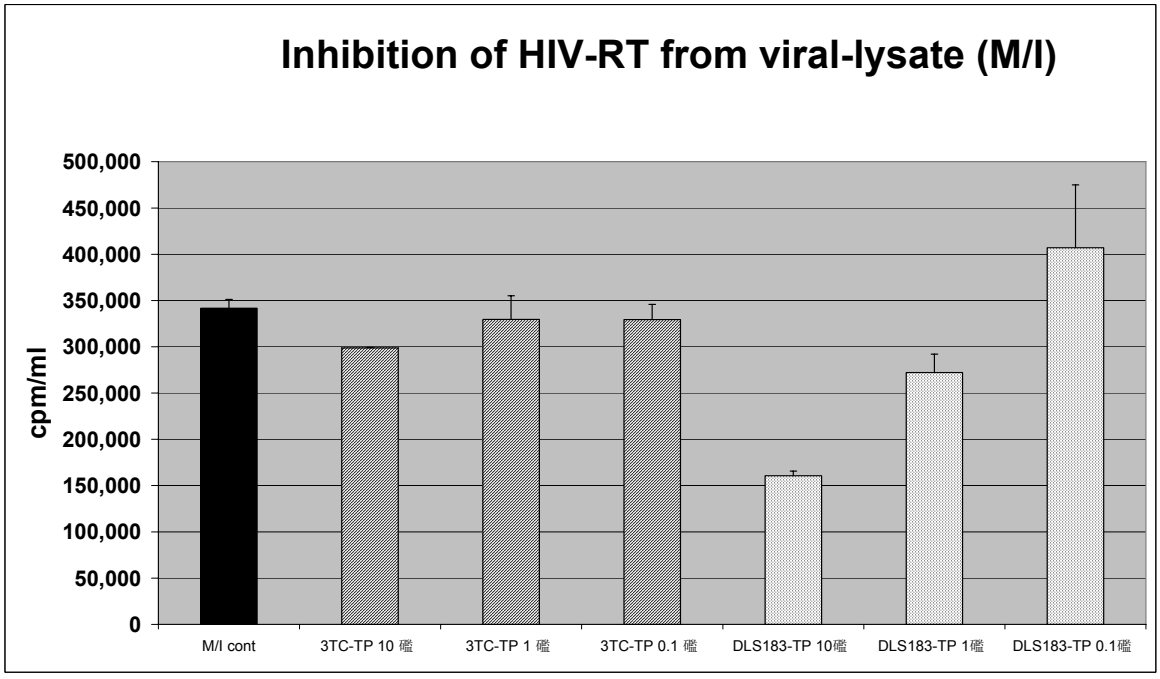
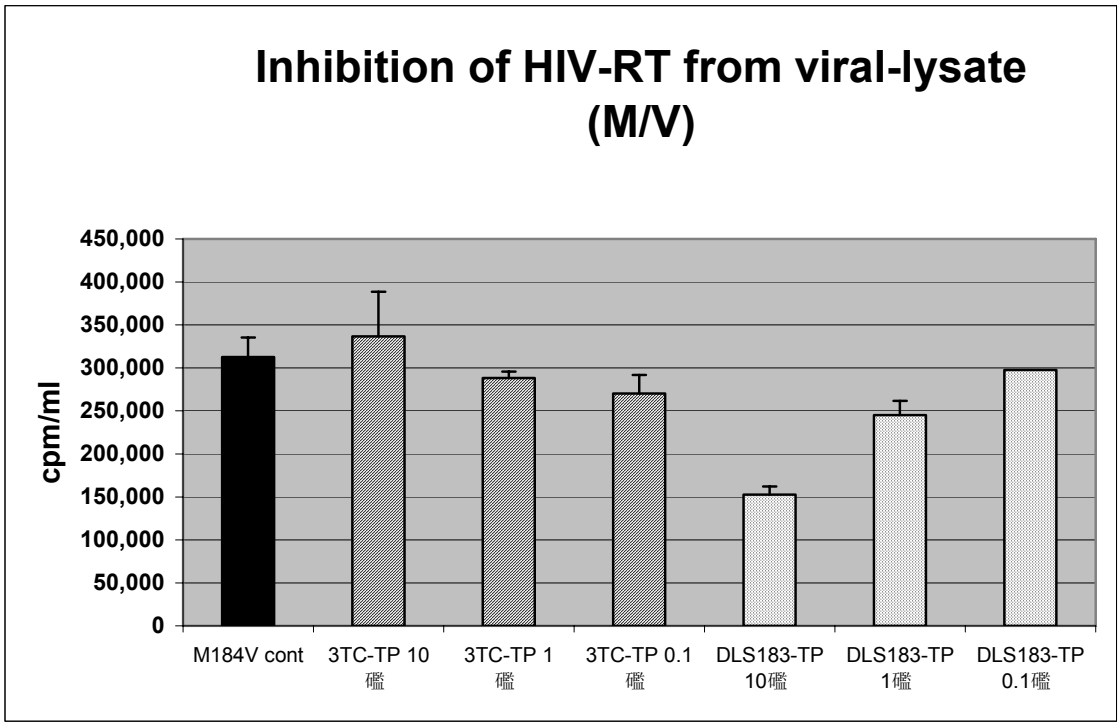


Figure 10: Inhibition of HIV-RT (M/V)

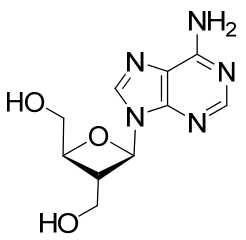


1.4 Design and synthesis of cyclobutyl nucleosides

The fact that the cyclobutyl nucleoside itself is not active against HIV while its triphosphate is quite active against HIV and mutated forms of HIV suggests that the nucleoside cannot be phosphorylated by the cellular kinases and this may be caused by the differences of the structure and the conformation of the cyclobutyl nucleosides. It is known from the literature that the naturally occurring Oxetanocin A **39** showed very good anti-HIV activity, although it is not stable towards nucleoside phosphorylases. It is also known that Lobucavir (Cyclobut-G) **40** showed anti-HBV activity although it has been withdrawn from the clinical trials because of its toxicity. Both of these compounds are four-membered ring

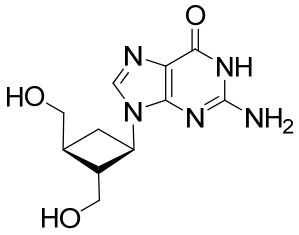
nucleoside analogs and both of them not only have the 3'-hydroxymethyl group but also have the 2'-hydroxymethyl group which enables them to be phosphorylated and incorporated into growing strands of DNA. However, we are interested in preparing analogs that process the former phosphorylation capability, but not the latter one. Based on this hypothesis, several carbocyclic cyclobutyl nucleoside analogs were synthesized and evaluated in order to find more active compounds against HIV. The structures of the analogs are shown below (Figure 11). The 5-fluoro-1-[cis-3-(hydroxymethyl)-cyclobuyl]-cytosine was synthesized by our group using a reported procedure.[55] To compare the electronic properties of the 2'-substituted analogs, the 2'-fluorocyclobutyl and 2'-methylcyclobutyl nucleosides were synthesized and evaluated by our group.

Figure 11: Cyclobutyl nucleoside analogs



Oxetanocin A

39



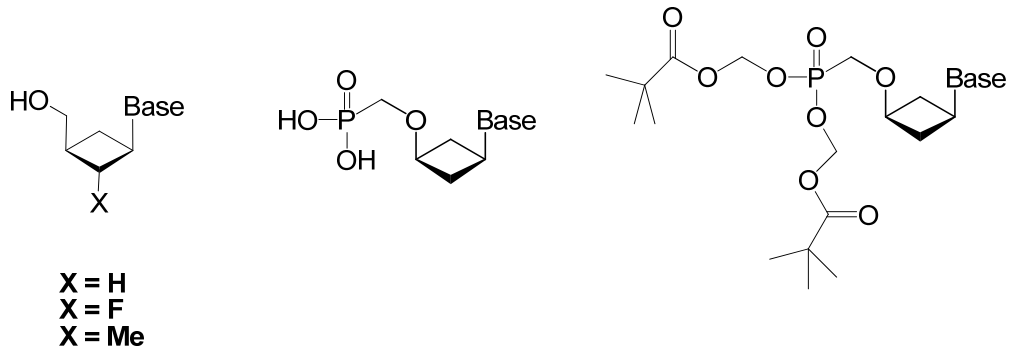
Lobucavir

40

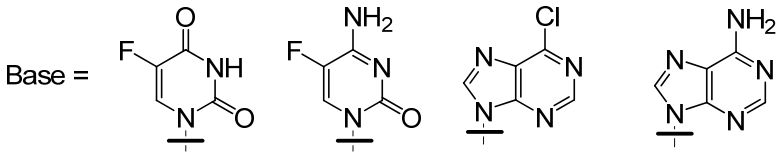
To circumvent the problems with phosphorylation, a series of 5'-phosphonate analogs were synthesized and evaluated to mimic naturally occurring monophosphates. Actually, a nucleoside phosphonate is a nucleoside monophosphate analog; however, a phosphonate has the advantage over its

phosphate counterpart of being metabolically stable, as its phosphorus-carbon bond is not susceptible to phosphatase hydrolysis. More importantly, the presence of a 5'-phosphonate allows the first phosphorylation step required for nucleoside activation to be skipped, therefore bypassing this inefficient and rate-limiting step in the conversion to the 5'-triphosphate. Like a nucleoside monophosphate, a nucleoside phosphonate can be further phosphorylated by cellular nucleotide kinases. The concept of nucleoside phosphonate has been applied to design chain terminators for anti-HIV chemotherapy and proven to be valid. 9-(2-phosphonylmethoxypropyl)adenine (PMPA) and 9-(2-phosphonyl methoxyethyl)adenine (PMEA) are two effective and potent nucleoside phosphonate chain terminators for HIV reverse transcriptase (RT).[56-58] Mechanism of action studies show that incorporation of PMPA diphosphate (PMPApp) by HIV-RT is almost as efficient as that of dATP or ddATP.[59] Despite this success, the concept of nucleoside phosphonates has not been fully explored for cyclobutyl nucleosides. In this study, we designed and synthesized a series of 5'- phosphonates and their prodrug forms.

Figure 12: Structures of cyclobutyl nucleoside analogs



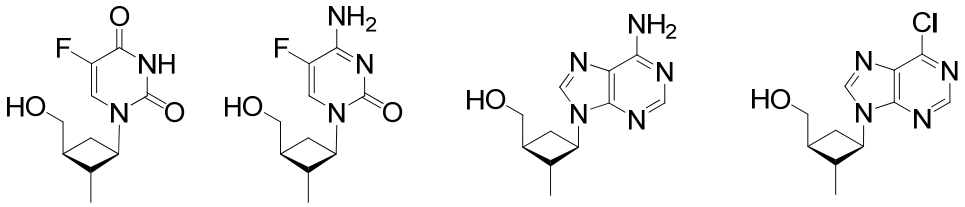
X = H
X = F
X = Me



1.4.1 Design and synthesis of 2'-methyl substituted cyclobutyl nucleosides

1.4.1.1 Synthesis of 2'-α-methyl-3'-hydroxymethyl cyclobutyl nucleosides

Figure 13: Structures of 2'-α-methyl-3'-hydroxymethyl cyclobutyl nucleosides

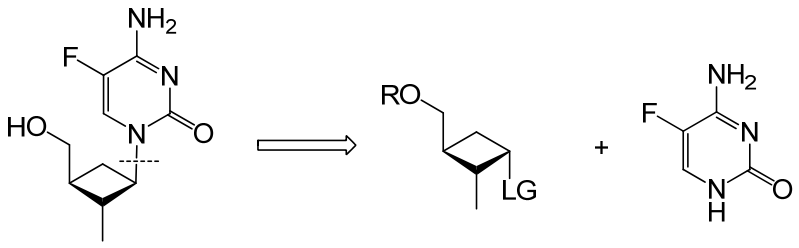


41 **42** **43** **44**

It is known from previous work in our group that the 5-fluoro-1- [*cis*-3 -(hydroxymethyl)-cyclobutyl]-cytosine **34** displayed no anti-HIV activity (Figure 7).[55] The X-ray crystallography data showed that the two substituents on the cyclobutyl ring are both equatorial. This may cause the nucleoside to occupy space than those nucleosides with two axial substituents. In order to populate the diaxial conformation, a 2'-α-fluorine was installed on the ring; however, the 2'-fluoro analogs didn't show any activity. We postulate that the ring size is the main problem that causes the inactivity. Therefore, a methyl group in the 2-position will be introduced. In order to synthesize these compounds, a disconnection approach (Scheme 12) can be envisioned. In this example, 5-fluorocytosine represents the nucleoside base.

1.4.1.2 Synthesis of 2'-methyl-cyclobutyl nucleoside analogs

Scheme 12: Disconnection approaches to the synthesis of pyrimidine analogs



42 **45** **46**

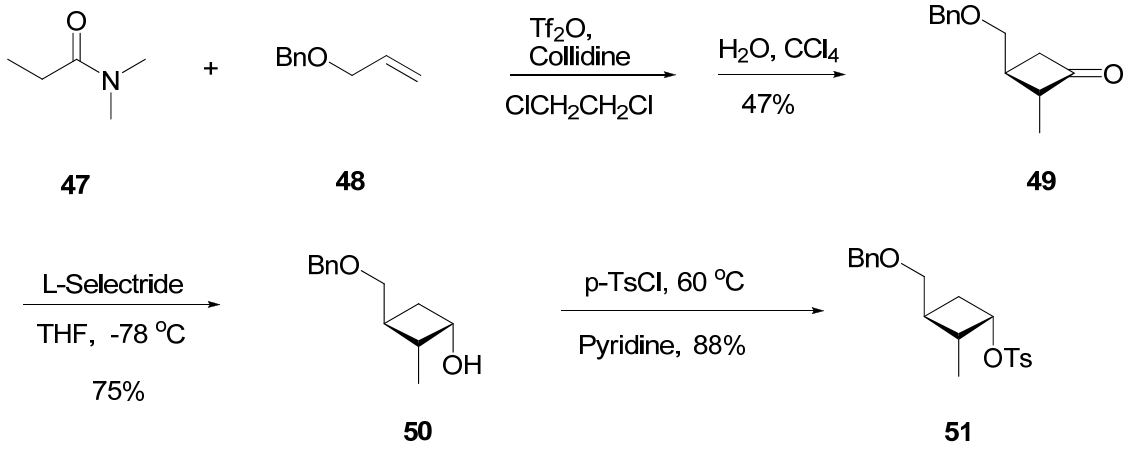
In this approach, the methylated cyclobutyl ring activated by a good leaving group reacts with 5-fluorocytosine **46** in an S_N2 fashion. The advantage of this approach is that the stereochemistry can be set at this step; however, the regiochemistry between the N1 attack and the O2 attack will be an issue. In addition, an elimination reaction of the cyclobutyl ring can also occur. Therefore, we decided to use 5-fluorouracil as the 5-fluorocytosine precursor to synthesize the target product.

Synthesis of the 2-methyl cyclobutanol

As shown in Scheme 13, a keteniminium salt was used as the key intermediate to form the cyclobutanone **49**. The keteniminium triflate generated in situ from N,N-dimethylpropionamide **47** reacted with allyl benzyl ether **48** to give the intermediate cyclobutaniminium salt which is hydrolyzed to provide cyclobutanone **49** as the major diastereomer with the methyl group trans to the

benzyl group (dr = 9:1). Reduction of the cyclobutanone **49** gave primarily one diastereomer **50** with the methyl group and the hydroxyl group cis to each other as confirmed by ^{1}H NOE analysis. The tosylate **51** was then formed by reaction with p-TsCl and pyridine.

Scheme 13: [2+2] cycloaddition to form the 2'-methyl cyclobutanol **48**

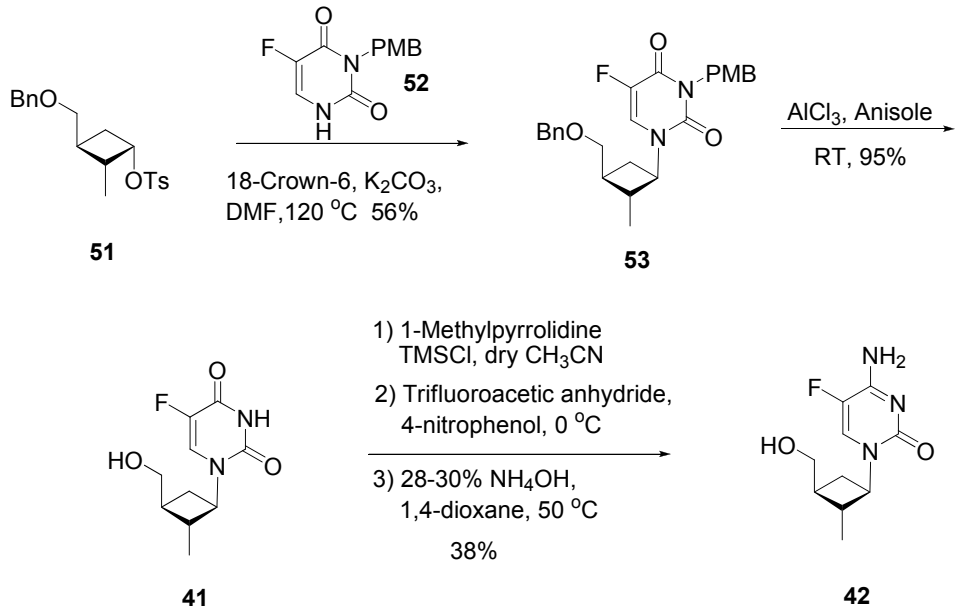


Synthesis of 2'-methyl-cyclobutyl pyrimidine analogs

The reaction of the activated cyclobutyl derivative **51** with the PMB-protected 5-fluorouracil **52**[60] under basic conditions by an S_N2 substitution mechanism was carried out to give the desired protected nucleoside **53**. When tosylate **51** was reacted with commercially available 5-fluorocytosine, the major product obtained was the O2-coupled product with trace amounts of N1-coupled product. The wrong regioselectivity is probably caused by the stronger nucleophilicity of O2 compared with N1 of 5-fluorocytosine. The next deprotection step was then carried out by using Lewis acid $AlCl_3$[61-63]. The para-methoxybenzyl

and the benzyl groups were removed cleanly with good yields to give uridine **41**. In order to make the 5-fluorocytosine derivative, the above 5-fluorouracil compound **41** was transformed into the desired compound **42** in three steps as reported by Reese.[39, 50]

Scheme 14: Synthesis of 2'-methyl-cyclobutyl pyrimidine analogs

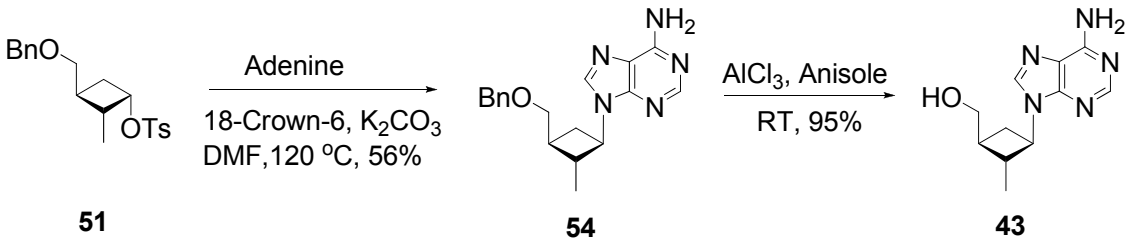


Synthesis of 2'-methyl-cyclobutyl purine analogs

Under basic conditions, tosylate **51** reacted with adenine to give mainly the *N*-9-coupled product **54** with minor *N*-7-coupled byproduct, which could be separated cleanly by silica gel flash chromatography (Scheme 15). The structures were assigned based on ^{1}H NMR and ^{13}C NMR where the chemical shifts of H8 and C8 on the adenine ring are more downfield for the N7-coupled product than that of the N9-coupled product. Removal of the benzyl group by

$AlCl_3$ gave the desired product **43,** and this deprotection condition gave better yield than hydrogenolysis condition which didn't lead to completion of the reaction.

Scheme 15: Synthesis of 2'-methylcyclobutyl adenosine **43**



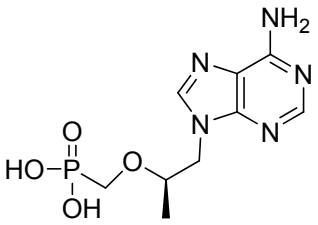
51 **54** **43**

1.4.2 Design and synthesis of cyclobutyl pyrimidine phosphonate analogs

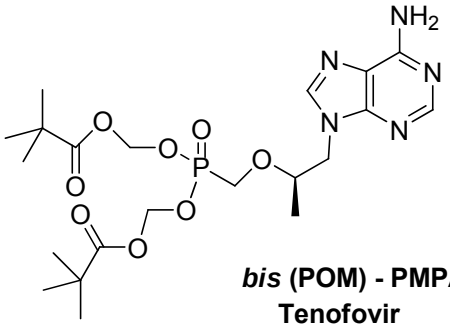
In the literature, several phosphate isosteres have been adapted to prepare nucleoside phosphonates. As shown in Figure 14, the acyclic nucleoside phosphonates, in which the furanose ring of a nucleotide is replaced with an acyclic side chain and the $POCH_2$ unit of the monophosphate is replaced with a bioisostere, PCH_2O, have attracted considerable attention for their broad-spectrum antiviral activity against several DNA and RNA viruses.[64] Bis(POM)-PMPA (Tenofovir disoproxil fumarate) was approved by FDA on 2001 as a nucleoside reverse transcriptase inhibitor to treat HIV and also is currently in late-stage clinical trials for the treatment of hepatitis B. All of the phosphonates mimic the overall shape and geometry of a nucleoside monophosphate. We applied the same strategy to a cyclobutyl nucleotide scaffold and designed a series of

phosphonate analogs. Since the ionic character of a phosphonic acid would present an obstacle for cellular permeability, pivaloate ester prodrug forms were synthesized. To elucidate the mechanism of the incorporation into DNA, a diphosphophosphonate was also made.

Figure 14: Acyclic phosphonates



PMPA

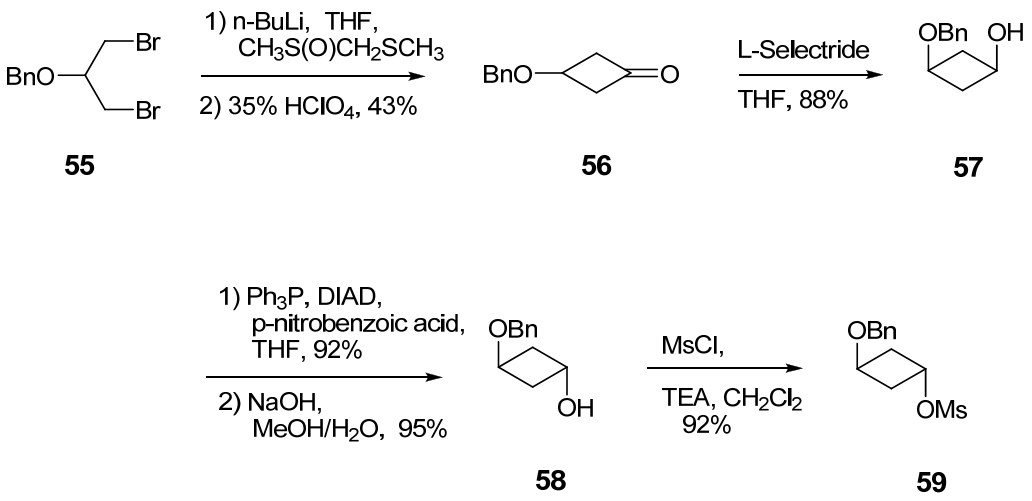


***bis* (POM) - PMPA**
Tenofovir

Synthesis of the cyclobutanol

The preparation of the 3-benzyloxycyclobutanone **56** was based on the procedure reported by Ogura et al.[65] The methyl methylthiomethyl sulfoxide was deprotonated by *N* -butyllithium, and then reacted with dibromo compound **55** to afford *syn*- and *anti*-dithioketal S-oxide intermediate, which was hydrolyzed by perchloric acid to give compound **56** (Scheme 16). Reduction of the cyclobutanone **56** with bulky base L-Selectride in THF solution gave *cis*-compound **57**, which was converted to *trans*-3-benzyloxycyclobutanol **58** via Mitsunobu reaction followed by hydrolysis of the resulting ester. The alcohol was activated by reacting with MsCl to form the good leaving group mesylate **59**.

Scheme 16: Synthesis of the trans-3-benzyloxycyclobutanol **58**

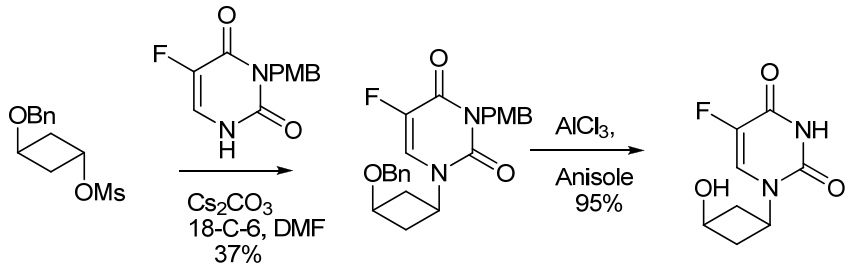


55 **56** **57** **58** **59**

Synthesis of pyrimidine nucleosides

Under basic condition, the mesylate was reacted with PMB protected 5-fluorouracil to give the protected nucleoside **60**, which was deprotected by Lewis acid $AlCl_3$ in anisole to give the compound **61** (Scheme 17).

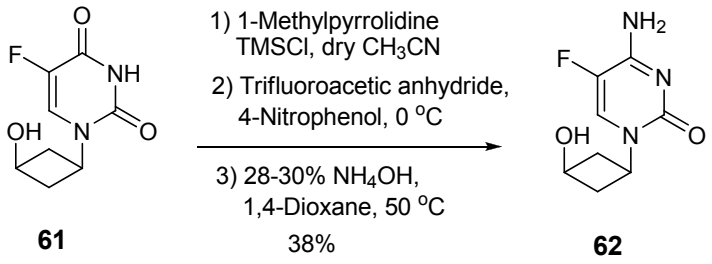
Scheme 17: Synthesis of the nucleoside **61**



59 **60** **61**

In order to make the 5-fluorocytosine derivative, the above 5-fluorouracil compound **61** was used as the starting material and transformed into the desired compound **62** after three steps as reported by Reese.

Scheme 18: Synthesis of the nucleoside **62**

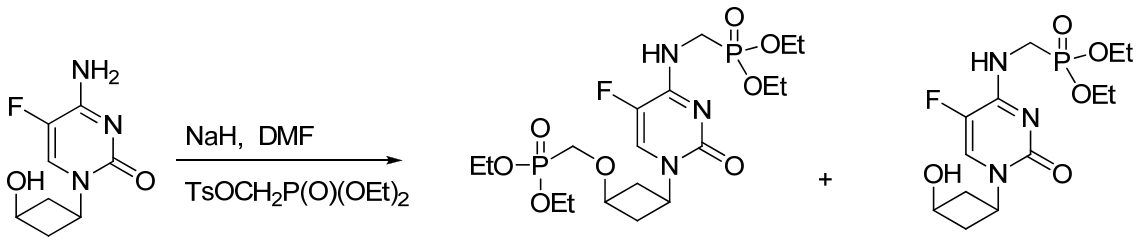


61 **62**

Synthesis of the phosphonate ester

For the phosphonate synthesis, initial attempts to alkylate the oxygen at C3 of cytidine **62** with diethyl p-tolylsulfonyloxymethyl phosphonate in the presence of sodium hydride resulted in a mixture of N-coupling product **64** and both N-coupling and O-coupling product **63** (Scheme 19). The phosphorylation of the cytidine, which has hydroxyl and amino groups in the same molecule, takes place competitively at both the amino and hydroxyl functions.[66]

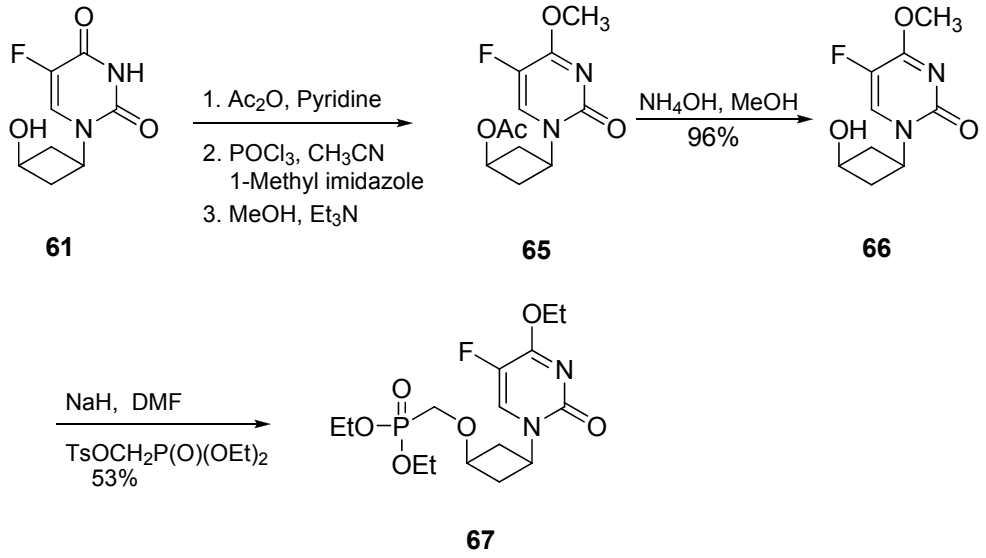
Scheme 19: Phosphorylation from cytidine



62 **63** 32% **64** 26%

In order for the alkylation reaction to work, proper protecting groups were needed to be introduced to uridine. Starting from uridine **61**, the 3'-hydroxyl group was first protected by an acetyl group, and then a methyl group was introduced to the 4-O-position of the uridine in two steps by base-aided condensation with phosphorus oxychloride in acetonitrile. The subsequent reaction of the resulting phosphate with methanol gave the 4-*O*-methoxy compound **65**, which was hydrolyzed to give **66**. Condensation of this compound with p-tolylsulfonyloxymethyl phosphonate in the presence of sodium hydride led to selective O-phosphorylation product **67**. However, according to NMR, the methyl group protecting the base had been largely replaced by an ethyl group. This could be the result of a nucleophilic exchange with ethanolate, formed under the basic NaH conditions.[67] The methyl and ethyl base-protected derivatives were difficult to separate and the mixture was used as such for the next reaction.

Scheme 20: Phosphorylation from uridine



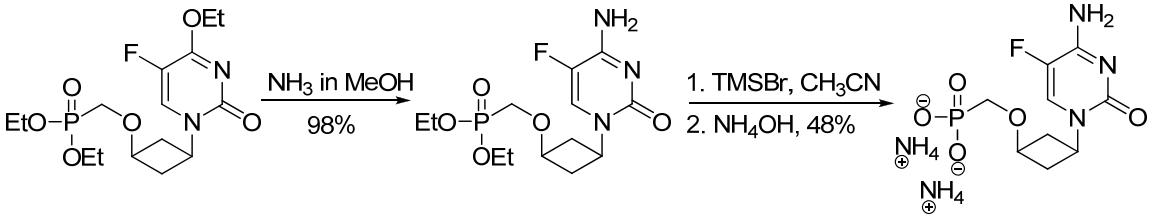
61 **65** **66**

67

Synthesis of the phosphonic acid and prodrug

Amination of the ester **67** in 7N ammonia in methanol at 60 ˚C overnight gave the 5-fluoro cytidine **68**, which was hydrolyzed by treatment with bromotrimethylsilane[68] to afford the phosphonic acid, and followed by treatment with ammonium hydroxide to give the salt **69**.

Scheme 21: Synthesis of the phosphonic acid

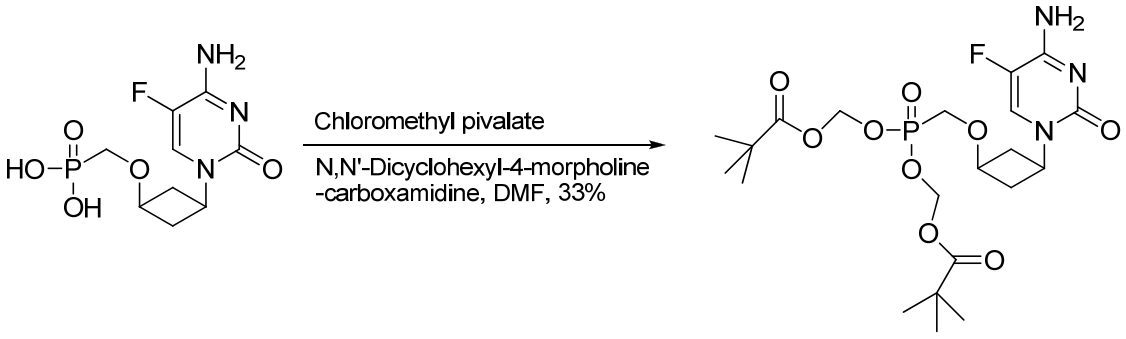


67 **68** **69**

Since the salt **69** is charged and poorly penetrates into cells,[69] we have investigated the potential of neutral bis[(acyloxy)methyl] organophosphonate as membrane-permeable prodrugs of the parent ionic phosphonate. The mechanism of the prodrug should be as follows: after entering cells by passive diffusion, one of the (acyloxy)-methyl groups should be cleaved by nonspecific carboxylate esterases to generate the hydroxymethyl analog. This intermediate should be inherently chemically labile and spontaneously dissociate by elimination of one molecule of formaldehyde to yield the corresponding mono[(acyloxy)methyl] phosphodiester. Repetition of this sequence with the second (acyloxy)-methyl group should generate the parent dianionic phosphate.[70]

To synthesize the prodrug **70,** the reported method was followed.[71, 72] The phosphonic acid was activated by a bulky base *N,N'*-dicyclohexyl-4-morpholine-carboxamidine, and then reacted with chloromethyl pivalate in DMF to give the coupling product **70** in moderate yield.

Scheme 22: Synthesis of prodrug **70**



69 **70**

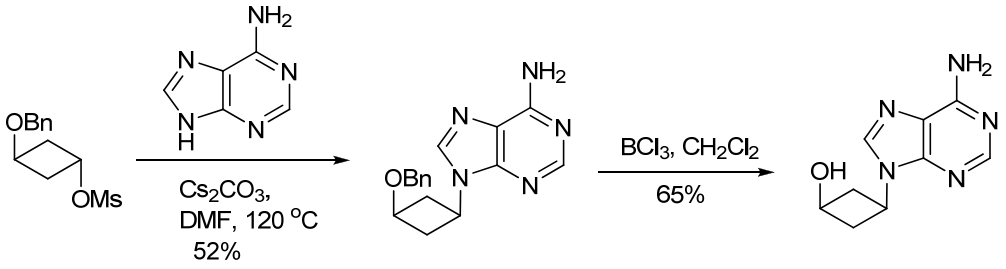
1.4.3 Design and synthesis of cyclobutyl adenine phosphonate analogs

The purine analogs synthesized in this series were evaluated for their anti-HIV activity and cytotoxicity in PBM, CEM, and Vero cells. Both the adenosine and guanidine analogs showed moderate anti-HIV activity. More importantly, however, is the fact that each synthesized analog proved to be non-cytotoxic up to 100 μM. Because these analogs are relatively harmless to the cell, this series is worth further effort to improve the anti-HIV activity.

Synthesis of the purine nucleoside

Under basic condition, the mesylate **59** was reacted with adenine to give the protected nucleoside **71**, which was deprotected with Lewis acid BCl_3 in dichloromethane to give the compound **72**.

Scheme 23: Synthesis of adenosine 72

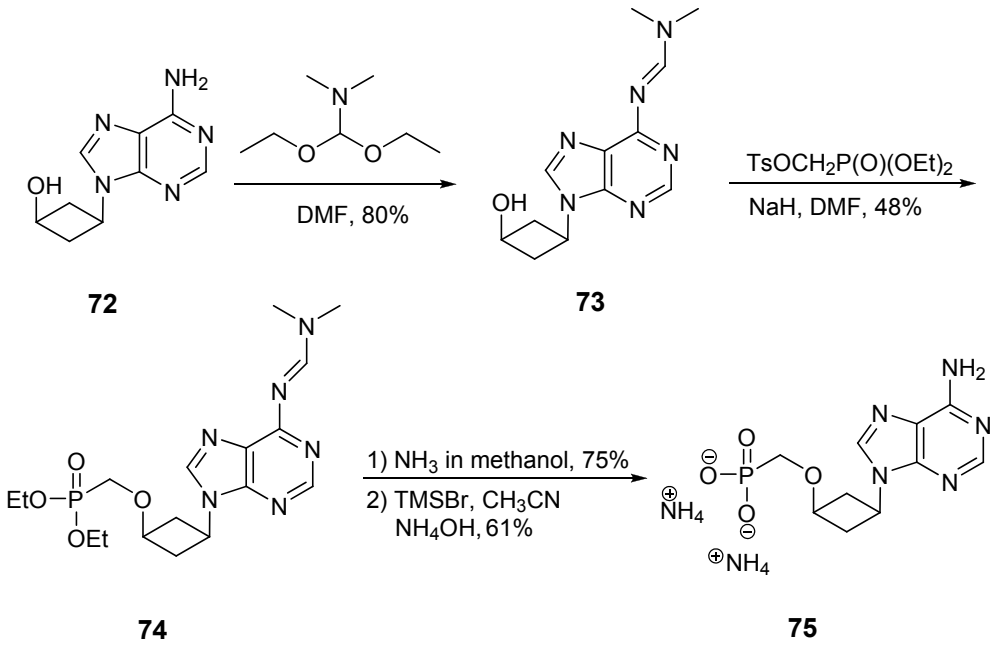


59 **71** **72**

Synthesis of the phosphonic acid and prodrug

Initial attempts to alkylate the oxygen at C4 of **72** with diethyl *p*-tolylsulfonyloxymethyl phosphonate in the presence of NaH resulted in a mixture of several inseparable products. Therefore, we decided to first convert **72** into its *N*,*N*-dimethylformamidine derivative **73**. Further treatment of the protected nucleoside **73** with phosphonate ester led to clean O-alkylation. Deprotection of the amidine group with methanolic ammonia gave phosphonate ester **74**. Hydrolysis of **74** by treatment with bromotrimethylsilane afforded the phosphonic acid, which was neutralized with ammonium hydroxide to give the salt **75** in good yield.

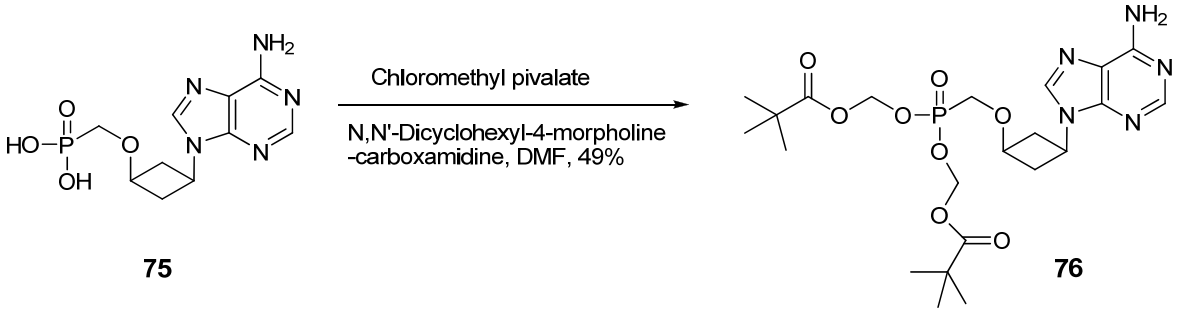
Scheme 24: Synthesis of the phosphonic acid



72 **73** **74** **75**

To synthesize the prodrug **76**, the reported method was followed. [71, 72] The phosphonic acid was activated by a bulky base *N,N'*-dicyclohexyl-4-morpholine-carboxamidine, and then reacted with chloromethyl pivalate in DMF to give the coupling product **76** with good yield.

Scheme 25: Synthesis of the prodrug

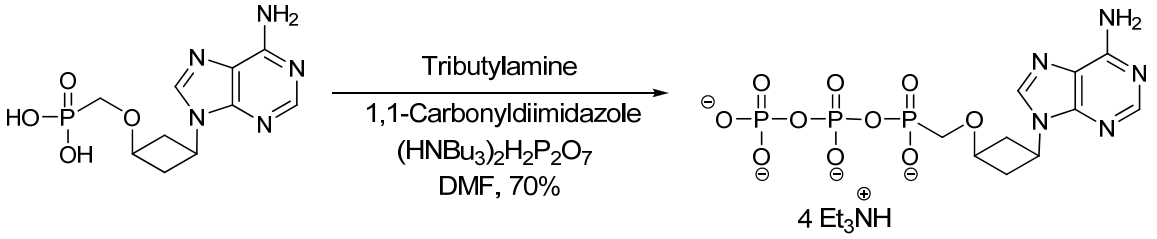


75 **76**

Synthesis of the diphosphophosphonate

The compound **75** was converted to its diphosphate **77** to be able to study its interaction with reverse transcriptase. Therefore, the phosphonic acid was treated with bis(tri-*n*-butylammonium) pyrophosphate in the presence of 1,1-carbonyldiimidazole (CDI) and tributylamine to give the diphosphophosphonate **77**.[73] The diphosphophosphonate obtained from the reaction contained a 2',3'-cyclic carbonate moiety because of the coupling between 2'- and 3'- hydroxyl groups of **75** with CDI. The cyclic carbonate was hydrolyzed by treatment with triethylammonium bicarbonate (TEAB) buffer to give the diphosphophosphonate, which was isolated by ion-exchange chromatography and monitored by reverse-phase HPLC.

Scheme 26: Synthesis of diphosphophosphonate **77**



75 **77**

1.4.4 Design and synthesis of cyclobutyl guanosine phosphonate analogs

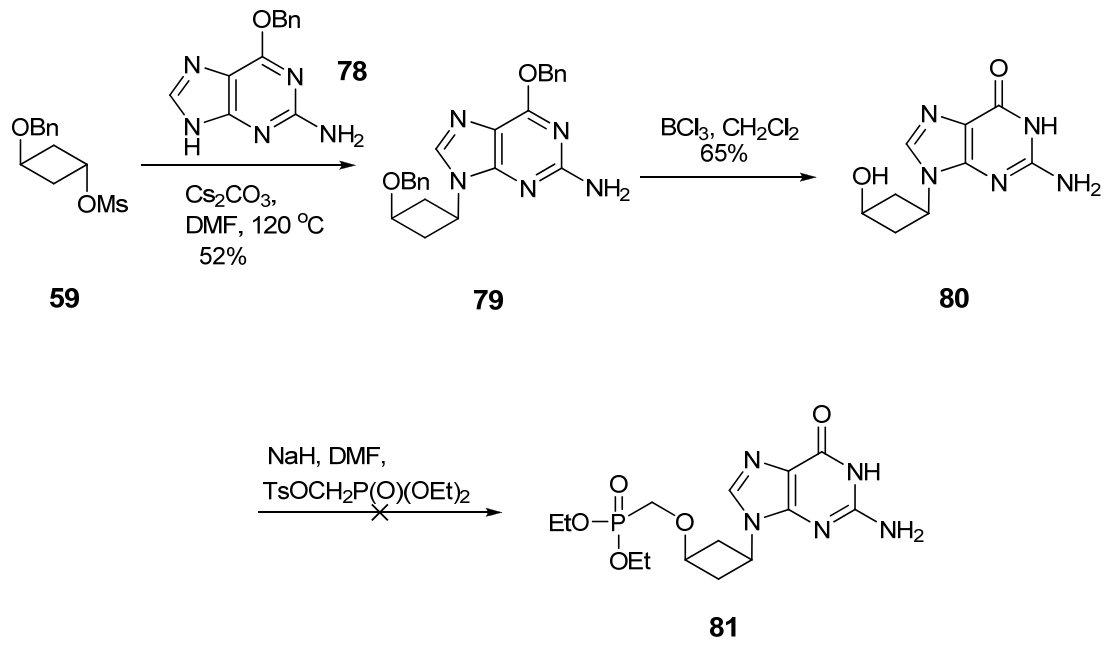
The cyclobutyl purine analogs had been previously synthesized by the Liotta group and tested for their anti-HIV activity and cytotoxicity in PBM, CEM, and Vero cells. The guanosine analogs showed better activity than adenosine

analogs. Therefore, we proposed that the base size is still very important for the anti-HIV activity because modified nucleosides may be recognized by the kinases to be further phosphorylized. The series of guanosine is worth further effort to improve the activity.

Synthesis of the guanidine phosphonate

Under basic condition, the mesylate **59** was reacted with O^6-benzylguanine[74] to give the protected nucleoside **79**, which was deprotected with Lewis acid BCl_3 in dichloromethane to give the compound **80**. However, the reaction of the nucleoside and diethyl *p*-tolylsulfonyloxymethyl in the presence of NaH resulted in a mixture of several inseparable products.

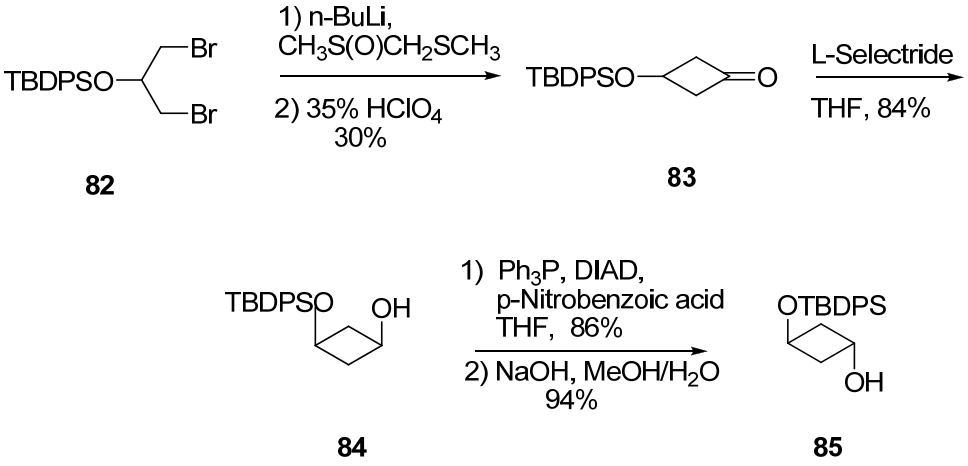
Scheme 27: Synthesis of the guanosine analogs



Synthesis of the diisopropyl (*trans*-3-hydroxycyclobutoxy)methyl phosphonate

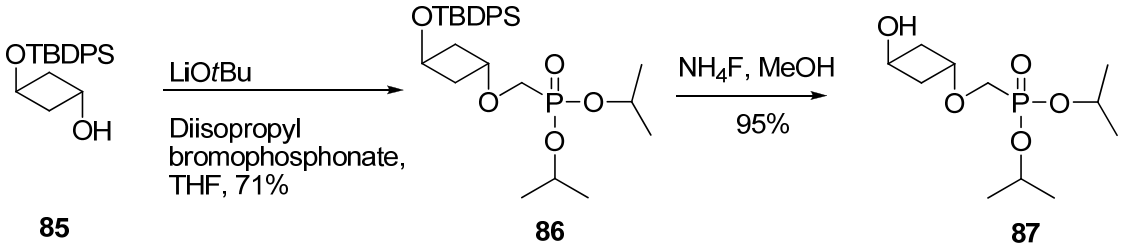
Finally, we decided to change the starting material and use a phosphonate ester as key intermediate. The preparation of the 3-(*tert*-butyldiphenylsilyloxy)cyclobutanone **83** was based on the procedure reported by Ogura et al.[65] The methyl methylsulfinyl methylsulfide was deprotonated by *N* -butyllithium, and then reacted with dibromo compound **82** to afford *syn*- and *anti*-dithioketal S-oxide intermediate, which was hydrolyzed by perchloric acid to give compound **83**. Reduction of the cyclobutanone **83** with bulky base L-Selectride in THF solution gave *cis*-compound **84**, which was converted to *trans*-3-(*tert*-butyldiphenylsilyloxy)cyclobutanol **85** via Mitsunobu reaction followed by hydrolysis of the resulting ester.

Scheme 28: Synthesis of the cyclobutanol **85**



Deprotonated by lithium *tert*-butoxide, compound **85** was reacted with diisopropyl bromophosphonate in THF to give the diisopropyl (*trans*-3-(tert-butyldiphenylsilyloxy)cyclobutoxy)methylphosphonate **86**, which was deprotected by ammonium fluoride in methanol to afford the alcohol **87**.

Scheme 29: Synthesis of the phosphonate **87**

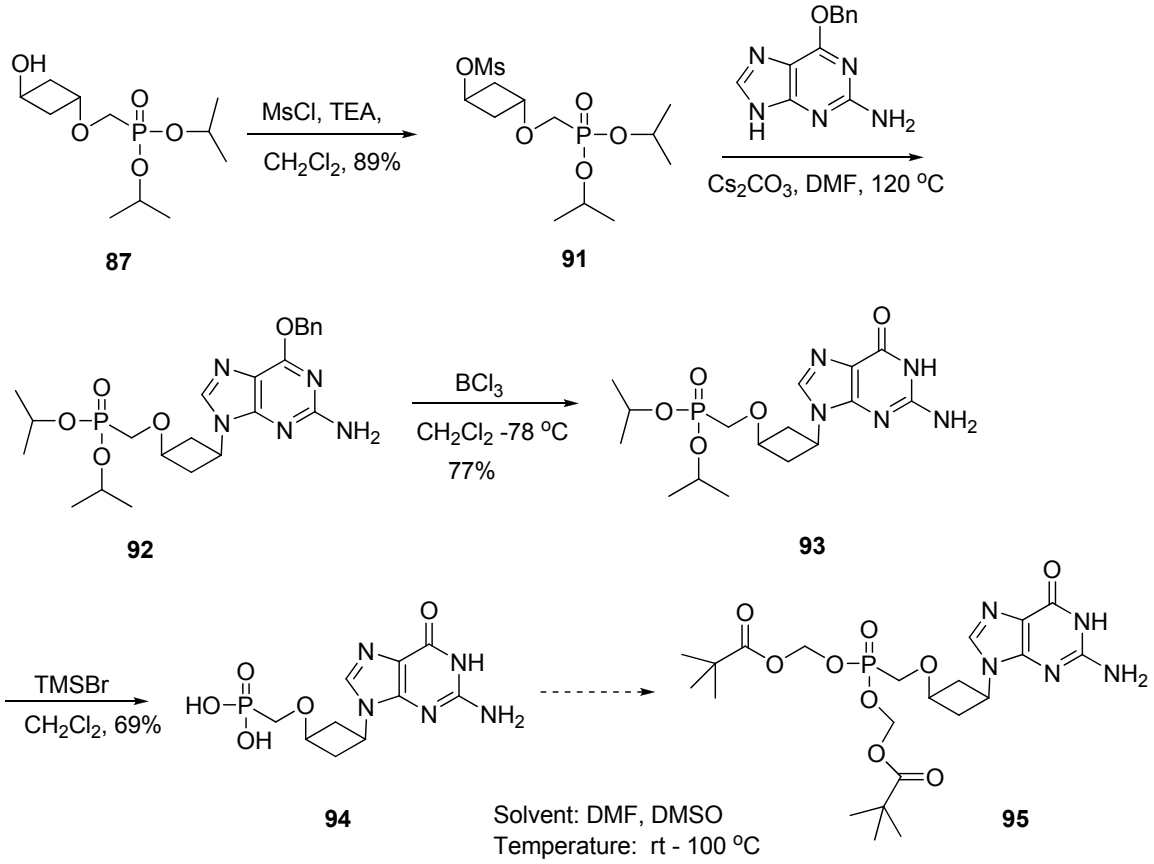


85 **86** **87**

Synthesis of the guanosine phosphonate

The alcohol **87** was activated by forming the mesylate **91** with MsCl. Under basic conditions, the mesylate **91** reacted with O^6-benzylguanine[74] to give the protected nucleoside **92**, which was deprotected by Lewis acid BCl_3 in dichloromethane to give the nucleoside **93**. Hydrolysis of **93** by treatment with bromotrimethylsilane afforded the phosphonic acid, which was neutralized by ammonium hydroxide to give the phosphonic salt **94** in good yield. The conversion into prodrug form using the previously described procedure failed because of the poor solubility of the phosphonic acid **94**, which couldn't be dissolved in DMF or DMSO, even under refluxing condition.

Scheme 30: Synthesis of the phosphonic acid **94** and prodrug **95**



87 91 92 93 94 95

1.4.5 Design and synthesis of thietanose nucleoside

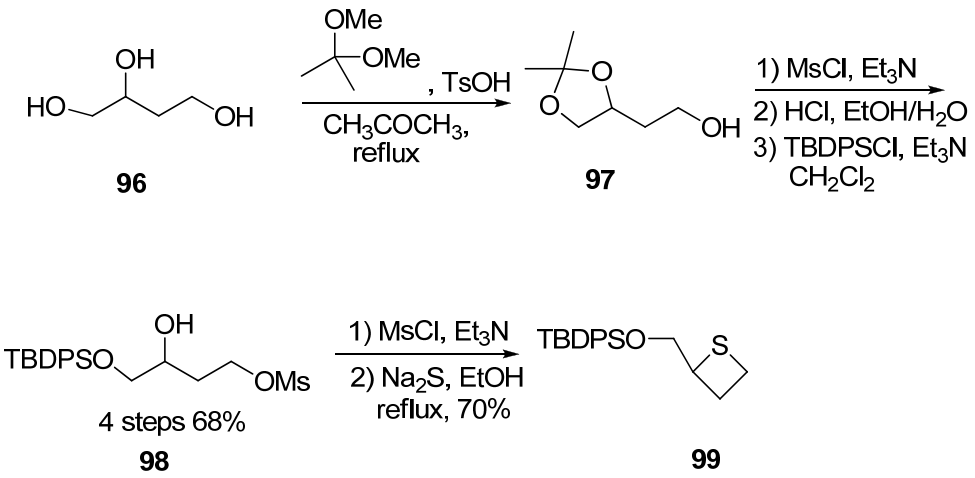
Thietanose nucleosides which have a four-membered thietane ring, are of interest because the replacement of oxygen in the sugar ring with a sulfur usually increases the nucleoside's stability toward acidic hydrolysis as well as against phosphorylases which cleave the glycosyl bond, thus resulting in the inactivation of nucleosides. However, only a few synthetic methodologies for the synthesis of thietanose nucleosides have been reported in the literature, presumably due to synthetic difficulties.[75, 76] Formation of the thietane ring as well as the condensation reaction with heterocyclic bases occurred in poor to moderate

yields, which was not sufficient to conduct SAR studies of thietanose nucleosides. For the synthesis of various thietanose nucleosides, an efficient synthetic method for a common intermediate is required, via which various analogs can be prepared for the study of the SARs of the thietanose nuleosides.

Synthesis of the thietanose derivative

The 1,2-diol of 1,2,3-butanetriol **96** was protected to form the acetonide **97**, which was then converted to the monomesylate. Deprotection by acid and selective silylation of the primary alcohol gave the secondary alcohol **98**. Reaction with MsCl to form the dimesylate, and refluxing with Na_2S in ethanol afforded the thietane ring compound **99**. [77, 78]

Scheme 31: Synthesis of the thietane ring **99**

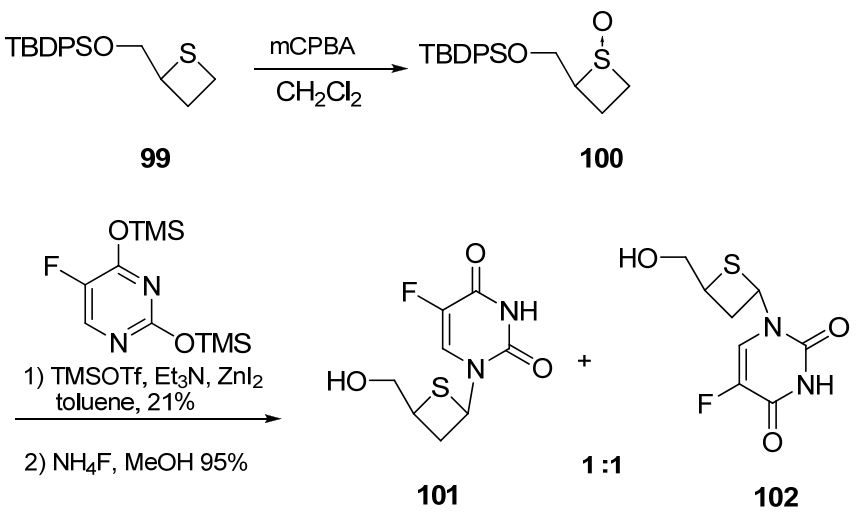


Synthesis of the uridine derivatives

Condensation of the thietanose derivative **99** with silylated uracil base was conducted by the Pummerer-type rearrangement after formation of a sulfoxide with mCPBA.[79, 80] Silylated base was obtained under refluxing conditions using

HMDS and acetonitrile, which was subsequently condensed with the corresponding sulfoxide **100** in the presence of TMSOTf, triethylamine, and a catalytic amount of ZnI_2 to provide the protected nucleoside derivative as α/β mixture with ratio of 1:1 in 21% yield. The TBDPS group was then deprotected by ammonium fluoride to give pure **101** and **102**, which were separated by silica-gel chromatography. The thietanose nucleoside **101** is the *cis* isomer as confirmed by ^{1}H NOE analysis, and the **102** is the *trans* isomer.

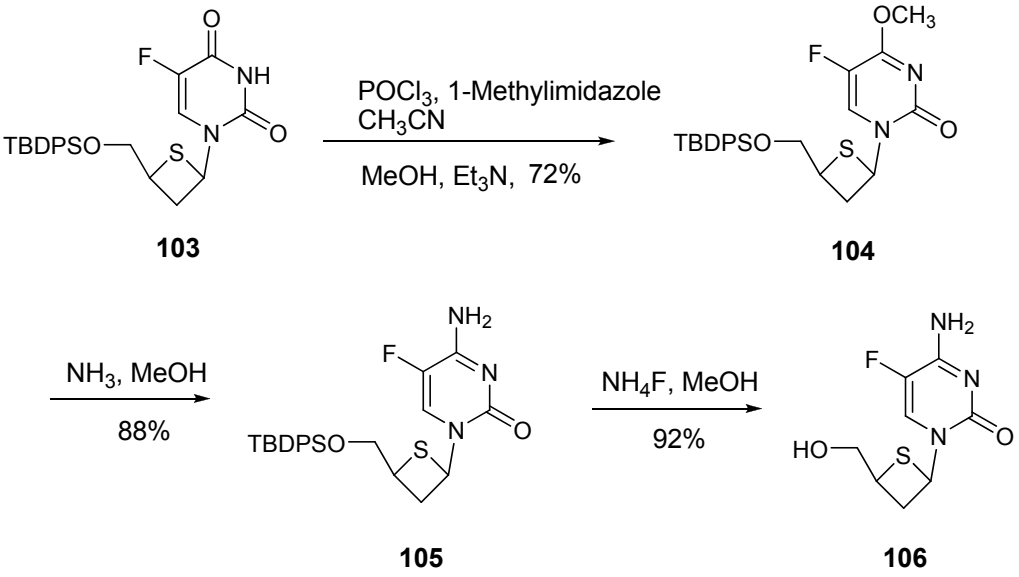
Scheme 32: Pummerer-type rearrangement to form thietanose nucleosides



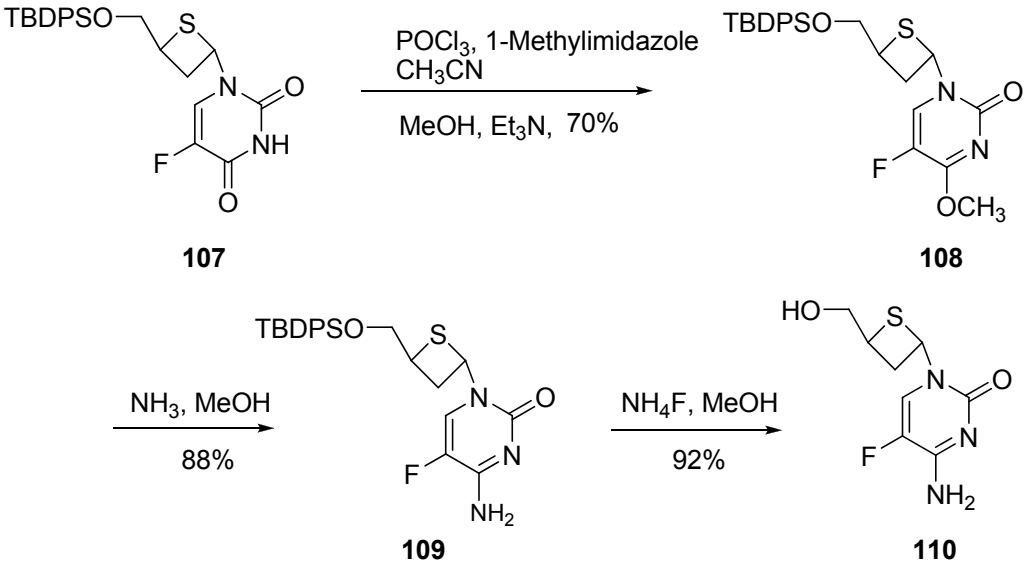
Synthesis of the cytidine derivatives

The uracil derivatives of protected **103** or **107** were aminated by treatment with phosphorous oxychloride in the presence of base catalyst, methanol and triethylamine in acetonitrile, followed by methanolic ammonia solution, to give the cytosine **105** or **109**. The nucleoside analogs were treated with ammonium fluoride to afford cytidine thietanose nucleosides *cis* **106** and *trans* **110**.

Scheme 33: Synthesis of the *cis*-cytidine thietanose nucleosides



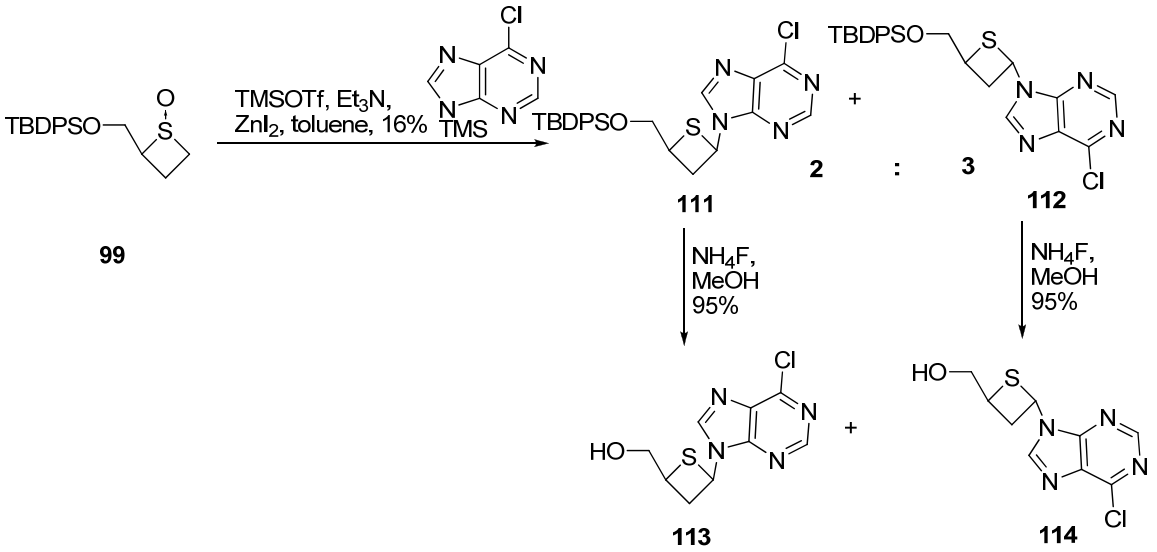
Scheme 34: Synthesis of the *trans*-cytidine thietanose nucleosides



Synthesis of the purine derivatives

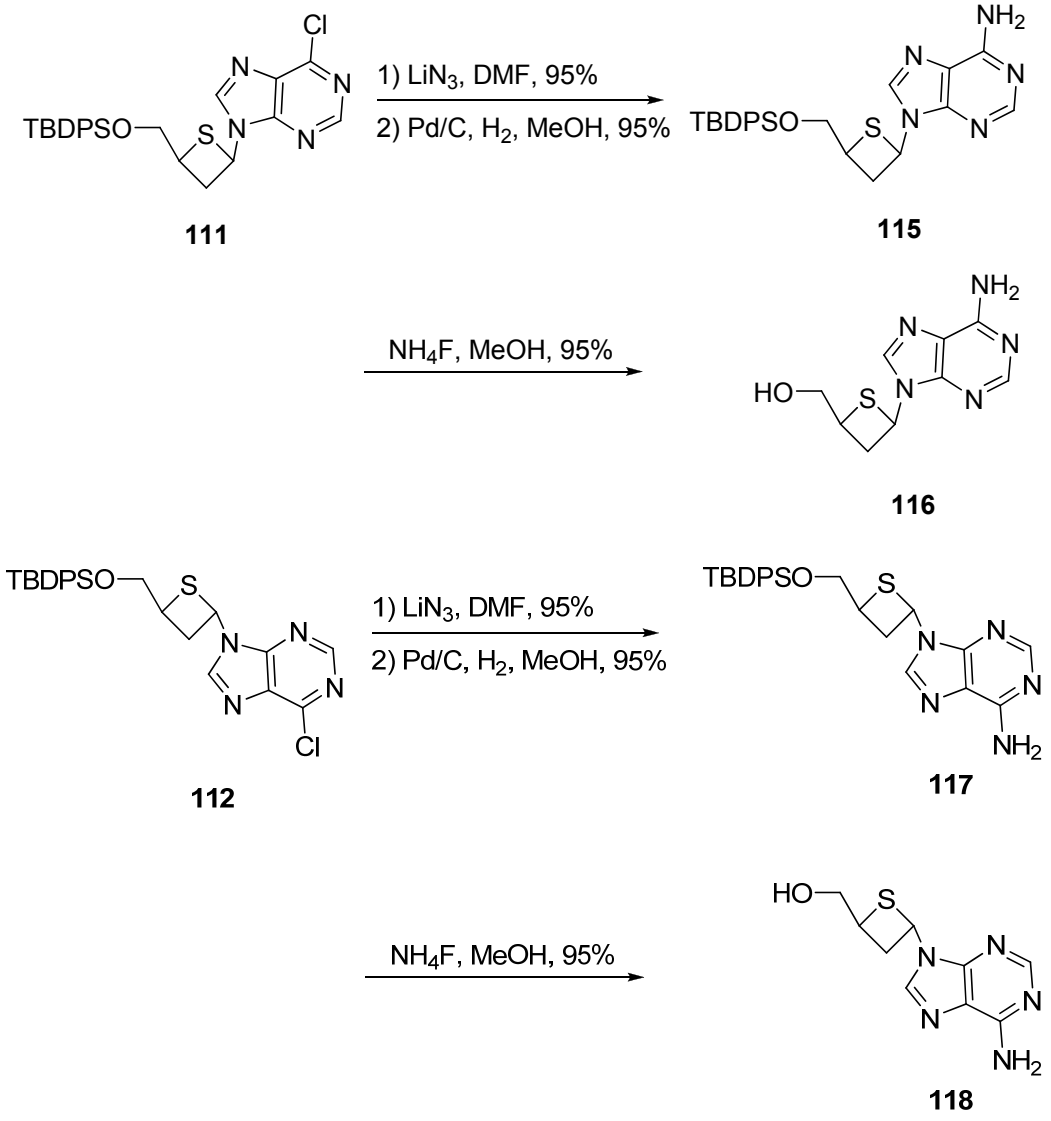
Condensation of the thietanose derivative **99** with silylated 6-chloropurine base was conducted by the Pummerer-type rearrangement after formation of a sulfoxide with mCPBA.[79, 80] The silylated base was obtained under refluxing conditions using HMDS and acetonitrile, which was then condensed with the corresponding sulfoxide in the presence of TMSOTf, triethylamine, and a catalytic amount of ZnI_2 to provide the protected nucleoside derivative as *cis/trans* mixture with a ratio of 2:3 in 16 % yield. The TBDPS group was then deprotected by ammonium fluoride to give pure **113** and **114**, which were separated by silica-gel chromatography.

Scheme 35: Synthesis of the 6-chloro-purine nucleosides **113** and **114**



Protected compounds **111** and **112** were converted to the adenine derivative **115** and **117** in 90% yield by using NaN_3 in DMF, followed by hydrogenation with 10% Pd(0) on carbon in methanol. Ammonium fluoride was used to deprotect those compounds in methanol to form the nucleosides **116** and **118**.

Scheme 36: Synthesis of the adenosine **116** and **118**



1.5 Anti-HIV activity

The antiviral activity of the synthesized analogs were evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells in vitro using AZT as a positive control, and the results are summarized in Table 5.

Table 5: Anti-HIV activity and toxicity of cyclobutyl nucleosides

Compound	Activity (PBM)		Toxicity (IC_{50} μM)		
	EC_{50} (μM)	EC_{90} (μM)	PBM	CEM	Vero
41	> 100	> 100	> 100	> 100	> 100
42	> 100	> 100	> 100	> 100	> 100
43	> 100	> 100	> 100	> 100	> 100
61	> 100	> 100	> 100	> 100	> 100
62	> 100	> 100	> 100	> 100	> 100
66	> 100	> 100	> 100	> 100	> 100
68	> 100	> 100	> 100	> 100	> 100
69	> 100	> 100	> 100	> 100	> 100
70	**49.1**	> 100	**31.6**	> 100	**97.8**
72	> 100	> 100	> 100	> 100	> 100
73	**55.1**	> 100	> 100	> 100	> 100
74	> 100	> 100	> 100	> 100	> 100
75	**34.4**	> 100	> 100	> 100	> 100
76	> 100	> 100	> 100	> 100	> 100
101	**80.3**	> 100	> 100	> 100	> 100
102	**14.6**	**49.5**	> 100	> 100	**40.1**
106	> 100	> 100	> 100	> 100	> 100
110	> 100	> 100	> 100	> 100	> 100
113	> 100	> 100	> 100	> 100	> 100
114	> 100	> 100	> 100	> 100	> 100
116	> 100	> 100	> 100	> 100	> 100
118	> 100	> 100	> 100	> 100	> 100

The above data showed that all the 2'-methylcyclobutyl nucleoside analogs were inactive against HIV (EC_{50} > 100 μM) although none of them are

toxic (IC_{50} > 100 μM). However, some of the cyclobutyl phosphonate (adenine) nucleoside analogs showed some moderate anti-HIV activity (EC_{50} = 34.1 μM) and one cyclobutyl nucleoside (5-fluorocytosine) prodrug showed some moderate anti-HIV activity (EC_{50} = 49.1 μM) and some cytotoxicoty (IC_{50} = 31.6 μM).

The anti - HIV activity of the synthesized thietanose nucleosides (**101** - **118**) was evaluated. Among them, analogs show moderate anti-HIV activity, with EC_{50} = 80.3, 14.6 μM, respectively. However, these nucleoside analogs are also cytotoxic in PBM and CEM cells.

1.6 Incorporation of CBN-TP (77) into DNA using reverse transcriptase

The antiviral activity of phosphonate nucleosides is mostly explained by their intracellular metabolism to the diphosphate forms followed by incorporation into the viral genome and chain termination.[81] We used the HIV reverse transcriptase as an assay to compare the incorporation ability of CBN-TP versus the natural A-TP. This should give us an idea about the selectivity of the anti-HIV compound since the HIV reverse transcriptase plays a key role in the replication of the viral genome. The incorporation studies were done with a DNA template and a DNA primer, as reverse transcriptase is able to synthesize doublestranded DNA (although reverse transcriptase is also able to synthesize a DNA strand using RNA as template).

The HIV reverse transcriptase accepted CBN-TP as easily as the natural building block (~6 μM), and its maximum rate of incorporation around is 10-fold slower than dATP (~1.3 per sec.). These studies have proven that CBN-TP can be incorporated into DNA, functioning as a chain terminator.

1.7 Discussion

The concept of nucleoside phosphonate chain terminators has been successfully utilized in designing novel and potent drugs against retroviruses. Notably, tenofovir disoproxil fumarate, a prodrug of PMPA adefovir dipivoxil, a prodrug of PMEA; and cidofovir have been approved by the FDA for the treatment of HIV/AIDS, HBV, and CMV infections, respectively. In this study, we extended the idea to the cyclobutyl nucleosides. Therefore, a series of cyclobutyl phosphonate analogues were designed and synthesized. They exhibited varied antiviral activity in an HIV assay with an EC_{50} ranging from 35 to 100 μM. To eliminate the potential cell penetration issues associated with the anionic phosphonate moiety, we further modified compounds **69** and **75** by masking their anionic charges with two pivalate groups and prepared two bis-pivalate prodrugs **70** and **76**. For compound **75** and its prodrug **76**, the anti-HIV activity is not improved. Compared to **69**, the anti-HIV activity of **70** is moderately improved, but its cytotoxicity is drastically increased. The result is not totally unexpected. It implicates that the nucleoside phosphonate **69** may possess a broad activity against host polymerases. The cytotoxicity is exposed once it gets efficiently delivered into cells. To understand the mechanism of action of the cyclobutyl

nucleoside phosphonates, a diphosphophosphonate derivative **77** was synthesized so that it could be tested in an HIV-RT nucleotide incorporation assay. The result showed that it could be incorporated into the HIV-RT, implying that it can potentially serve as chain terminators.

PMPA is an acyclic nucleoside analog that structurally differs from canonical nucleotides. Its diphosphate derivative is comparable to ATP as a substrate for HIV-RT. It is likely that the acyclic sugar moiety in PMPA is flexible enough for the cellular kinases to recognize and phosphorylate it, in contrast to the relatively rigid cyclobutyl ring in our compounds. It appears that the initial cellular nucleoside kinases cannot recognize the modified nucleoside analogs due to their rigidity and these structural and conformational differences from the natural nucleoside substrates, which results in a low, if any, level of the triphosphate. Our future work will be focused on the synthesis of different prodrugs of various cyclobutyl nucleoside analogs to circumvent the problems of phosphorylation.

In summary, several novel four-membered ring nucleoside analogs were successfully synthesized. These cyclobutyl nucleoside monophosphonate (CBNMP) mimics demonstrated some promising but relatively weak anti-HIV activity. Further mechanistic studies indicate that the diphosphophosphonate can potentially function as a chain terminator, thereby proving the principle that the concept is applicable to the cyclobutyl nucleoside. But challenges remain as the initial phosphorylation reaction is not good enough to compete with natural

nucleosides and further medicinal chemistry efforts are needed to come up with novel and potent nucleoside phosphonate chain terminators for HIV.

For the thietanose nucleosides, some of them showed moderate activity and cytotoxicity. The other nucleosides showed neither anti-HIV activity nor cytotoxicity up to 100 mM. It is well known that nucleosides/nucleotides analogs can inhibit human DNA polymerases, which may cause side effects such as cytotoxicity, mitochondrial toxicity, etc.[82] Consequently, if compounds **101** and **102** can be phosphorylated to triphosphates, the low selectivity of these nucleotides between the HIV reverse transcriptase (RT) and cellular polymerase may explain the observed antiviral activity as well as the cytotoxicity.

Therefore, in order to find a better lead compound and explore the nucleoside phosphorylation and its structure relationship, more four-membered nucleoside analogs will be made. We believe that the design and synthesis of analogs of four-membered nucleoside that are efficiently phosphorylated in cells could have great potential in inhibiting HIV mutants that result in branched side chains positioned near the RT active site.

Part 2: Synthesis of Fluorescent Nucleoside Analogs

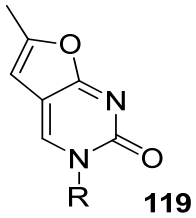
2.1 Statement of purpose

The ability to detect nucleoside and nucleoside analogs with high sensitivity in complex mixtures such as a cell's cytoplasm would greatly benefit studies of cellular uptake and metabolism.[83-87] While the nucleobases of natural nucleosides do possess intrinsic fluorescence properties at physiological conditions, direct measurements are impractical due to the compounds' low quantum yields and overlapping absorption maxima with aromatic amino acids in proteins and small-molecule metabolites such as flavines and NADH. Due to such cellular autofluorescence, fluorescent substrates or reporters with absorption maxima of >300 nm are highly sought after to minimize background and improve signal-to-noise ratios.[88-90]

Among the first modified nucleosides with improved fluorescent properties were the *etheno*-derivatives of adenosine **121**[91] and cytidine. A tricyclic guanine derivative **123** has also been described. While these fluorescent analogs are accepted by a number of nucleotide-utilizing proteins, numerous additional fluorophores have since been synthesized to address specific biological questions. More importantly, the preparation of new fluorescent nucleosides (fNAs) is being driven by the need for fluorescent probes with improved spectral properties but that still closely mimic the natural nucleobases in size and hydrogen-bonding patterns. Among these second and third-generation compounds, the furano and pyrrolo-pyrimidines **119** and **120**[92] and the pterines

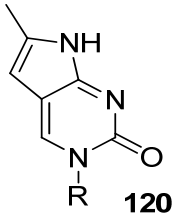
122 and **124**[93-96] are of particular interest for monitoring in vitro and in vivo phosphorylation of NA prodrugs. These fNAs show close structural similarities with natural nucleobases, and their red-shifted excitation and emission wavelengths further reduce the interference of cellular autofluorescence in experiments.

Figure 15: Structures of fluorescent nucleoside analogs



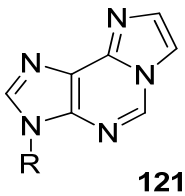
119

λ_{ex} = 331 nm
λ_{em} = 413 nm



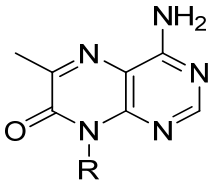
120

λ_{ex} = 335 nm
λ_{em} = 415 nm



121

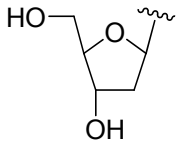
λ_{ex} = 305 nm
λ_{em} = 415 nm

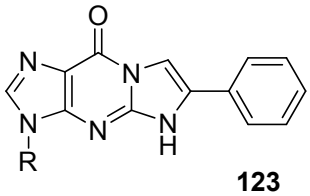


122

λ_{ex} = 320 nm
λ_{em} = 430 nm

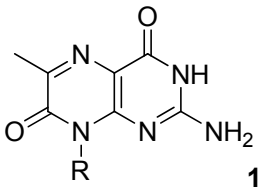
R =





123

λ_{ex} = 306 nm
λ_{em} = 482 nm



124

λ_{ex} = 320 nm
λ_{em} = 430 nm

2.2 Introduction and background

Viral diseases, as well as the recent threat of weaponized viruses, represent a continuous global health problem. Worldwide, more that 40 million people have become infected with HIV since its emergence twenty years ago, and the annual death toll has risen to 2.5 million. Over the years, several generations of antiviral drugs have been introduced, targeting viral proteins involved in host cell entry and genomic integration, as well as DNA replication and proteolytic processing of viral precursor proteins.

The most dominant group of antiviral drugs is nucleoside analogs (NAs). From a functional perspective, NAs are actually prodrugs whose bioavailability depends on intracellular phosphorylation to the triphosphate. Administered in their uncharged nucleoside form, these compounds utilize the host's nucleoside salvage pathway for membrane passage and subsequent sequential phosphorylation by deoxynucleoside kinases (dNK) (*e.g.*, thymidine kinase), deoxynucleotide monophosphate kinases (dNMPK) (*e.g.*, thymidylate kinase), and deoxynucleotide diphosphate kinase (dNDP kinase). Resembling the natural building blocks of DNA and RNA, the triphosphate anabolites then turn into competitive substrates for a virus' low-fidelity polymerase or reverse transcriptase. The incorporation of NA-triphosphates results in the immediate termination of the replication process, preventing further viral proliferation. The mammalian cell's replication machinery on the other hand has a higher fidelity, protecting the host from the lethal effects of these suicide substrates.

While theoretically sound, this process is flawed in practice by the NA's dependence on the phosphorylative activation by the cellular nucleoside and nucleotide kinases. While nucleoside transport proteins show relatively broad specificity for nucleosides and NAs, the high substrate specificity of the human nucleoside & nucleotide kinases reduces the effective turnover of many prodrugs. In particular, the two initial phosphorylation reactions by human dNKs and dNMPKs limit triphosphate formation, which results in the accumulation of NAs and NA monophosphates inside the cell. Consequently, many prodrug candidates that show promising activity in primer extension experiments *in vitro* fail to express measurable effects *in vivo*.

The stage at which NAs build up varies between individual analogs. For instance, 2',3'-didehydro-2',3'-dideoxy thymidine (d4T) is a poor substrate for dNKs and accumulates as the nucleoside, while 3'-azido-thymidine (AZT) is turned over to the monophosphate (AZTMP) but can not effectively be phosphorylated by the cell's dNMPKs. Making matters worse, the accumulation of precursor not only lessens the effectiveness of NAs but can actually trigger an adverse cellular response. Drug-induced expression of cellular multidrug resistant protein that *actively exports* the nucleoside prodrug out of the cell has been observed. As for AZTMP, the buildup of this intermediate has been shown to interfere with the host metabolism, suppressing kinase activity and possibly causing cytotoxic effects through AZT metabolism to the 3'-amino derivative.

Dramatic effects on cellular metabolism have been observed upon long-term drug treatment. In response to extended exposure to NAs, phenotypic

(temporary) and genotypic (permanent) changes were detected in the host cells. A link between declining gene expression levels for dNKs and DNA methylation has been proposed, slowing NA activation and further raising the concentration levels of the intermediates. In addition, studies of high NA levels in mammalian cell cultures and animals suggest a link between NAs and genotoxic effects in the host. In summary, the inefficient phosphorylation of NAs by cellular kinases is a significant contributor to the serious side effects associated with NA-based antiviral treatment.

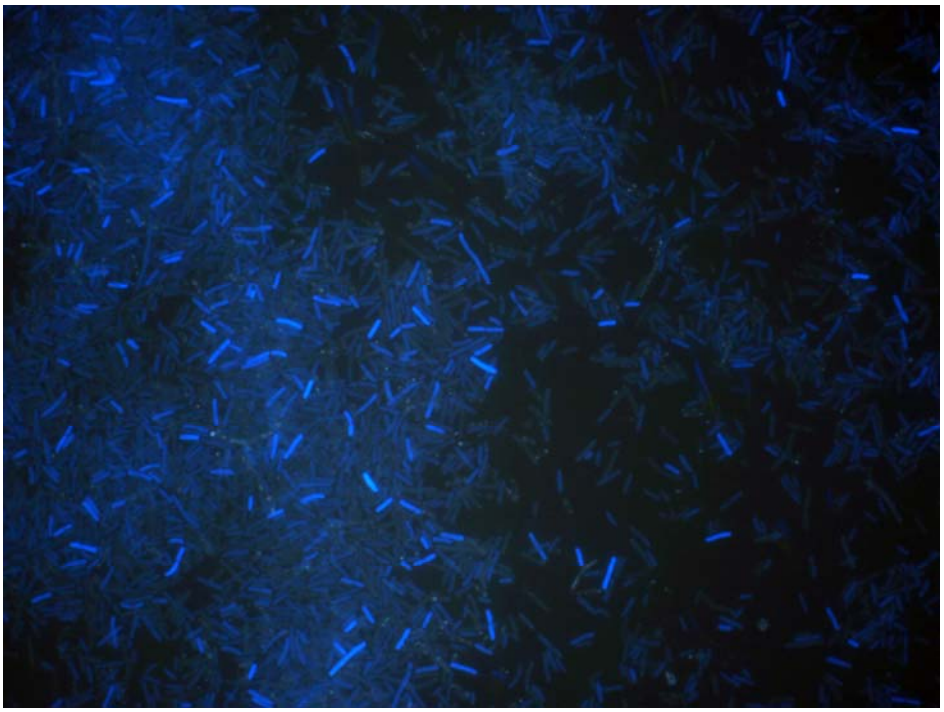
In vivo experiments with fluorescent nucleoside analogs

Our collaborator, Dr. Stefen Lutz, found a very good application of 2'-deoxynucleosides (**119**) as a promising tool to research the metabolite inside a living cell and study biochemical reactions in situ, such as the 5'-phosphorylation of the nucleosides by cellular deoxynucleoside kinases.

Uncharged compound **119** can effectively enter and exit the cellular environment via broad-specificity nucleoside transporter proteins, while the negative charge of one or more phosphate groups in 2'-deoxynucleotides prevent the latter from leaving the cell. Fluorescent NAs that are phosphorylated by deoxynucleoside and deoxynucleotide kinases will become "trapped" inside the cellular compartment, resulting in the increased autofluorescence of these cells. Fluorescence microscopy can be used to qualitatively evaluate the intracellular accumulation and distribution/localization of such a fluorescent probe in single cells. More quantitatively, *flow cytometry* can be employed to assess a cell

population and, in combination with fluorescence-activated cell sorting (FACS), isolate subgroups of cells with interesting properties.

Figure 16: Digital images of fluorescence microscopy of *E. coli* KY895 with pDIM-t*Dm*dNK compound **119**



E. coli KY895 (pDIM-t*Dm*dNK) w/ 1 mM IPTG induction ex:350 nm (100x magnification)

2.3 Novel fluorescent nucleoside analogs with ribose modification

Very few versions of fluorescent nucleoside analogs with modified sugar moieties (*e.g.*, sugars other than ribose and 2'-deoxyribose) have been reported.[97] Searching for acyclovir and ganciclovir derivatives with increased antiviral activity, a range of tricyclic guanine NA derivatives with substitutions in the appended ring, such as the highly fluorescent 6-phenyl analog **123** have

been developed.[98] In vivo studies of these fNAs in HSV-infected mammalian cells, conducted in parallel with the synthetic efforts, suggested the efficient phosphorylation of several of the analogs by deoxynucleoside kinases. More recent in vitro experiments, however, show that turnover of fNA **123** by the HSV-kinase is 10 to 100-fold lower in comparison to ganciclovir.

Focusing exclusively on the pharmacological properties of these modified nucleosides, their potential use in connection with studies of the cellular metabolism and, in particular, for the identification of dNKs is completely unexplored. Arabinosyl derivatives of the furano-pyrimidine have also been prepared. The evaluation of this fluorescent araT against varicella zoster virus (VZV) however revealed significantly reduced biological activity of the furano product compared to araT. Similar substrate limitations for type-II dNK have also been observed, as discussed in greater detail in the examples below. Without being bound by theory, it is believed that these observations are linked to the furano-pyrimidine rather than the ribose modifications.

2.4 Pyrimidine analogs

The fluorescent thymidine analog 6-methyl-3-(β-D-2'-deoxyribofuranosyl) furano-[2,3-*d*]pyrimidin-2-one **119** and the cytidine analog 6-methyl-3-(β -D-2'-deoxyribofuranosyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-one **120** have been used to study the structural and functional properties of nucleic acids and more recently nucleoside membrane transport proteins. When incorporated in synthetic oligonucleotides, **120** forms hydrogen-bonding interactions equivalent to its

natural counterpart and causes no significant structural disturbance.[99-101] Similar "native-like" behavior is also exhibited by **119** and **120** in enzymatic assays, and both compounds show low cytotoxicity in vivo.[102]

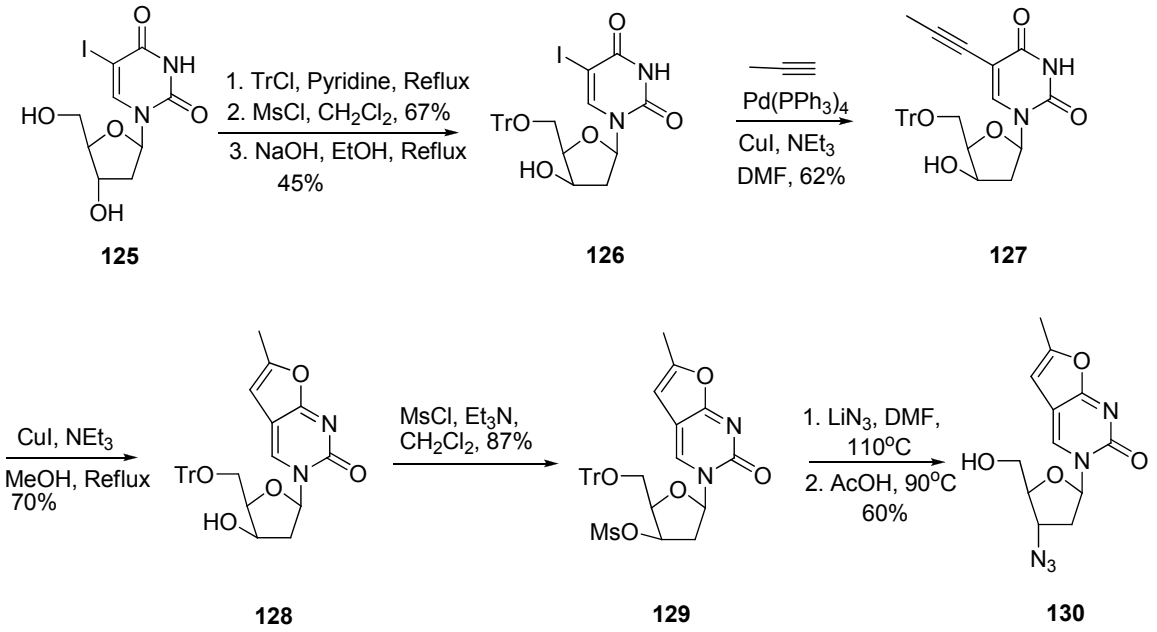
Novel variants of compounds **119** and **120** can be prepared according to reported methods,[103-105] namely fNA 3-(4-azido-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro [2,3-d]pyrimidin-2(3H)-one (**130**) (a variant of compound **119** that is a combination of AZT sugar derivative and fluorescent nucleobase), and fNA 3-(5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methyl-3H-pyrrolo [2,3-d]pyrimidin-2(7H)-one. A combination of different sugar derivative and fluorescent nucleobases in Figure 14 can be prepared as illustrated in Schemes 37-39.

Synthesis of 3-(4-azido-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro [2,3-d]pyrimidin-2(3H)-one

The synthetic methods for the modified AZT analogs containing reactive functionalities tethered to the C-5 position involve the formation of the key intermediate. Selective 5'– protection of 5-iodo-2'-deoxyuridine (IDU, **125**) with trityl chloride followed by treatment with mesyl chloride gave the protected IDU. Following the published procedure the configuration of the 3'- group was readily inverted to give the xylo compound **126** using refluxing ethanolic sodium hydroxide.[106] Treatment of **126** with propyne in the presence of catalyst tetrakis(triphenylphosphine)palladium and copper(I) iodide in anhydrous DMF gave the Sonogashira product **127**.[107, 108] Copper catalyzed cycloaddition was

carried out to afford the fluorescent compound **128**,[97, 109] which was treated with MsCl to give the mesylate **129**. The azido substituted fluorescent nucleoside **130** was obtained as single product after a S_N2 reaction by NaN_3 and acidic deprotection by acetic acid.[110]

Scheme 37: Synthesis of 3-(4-azido-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one **130**

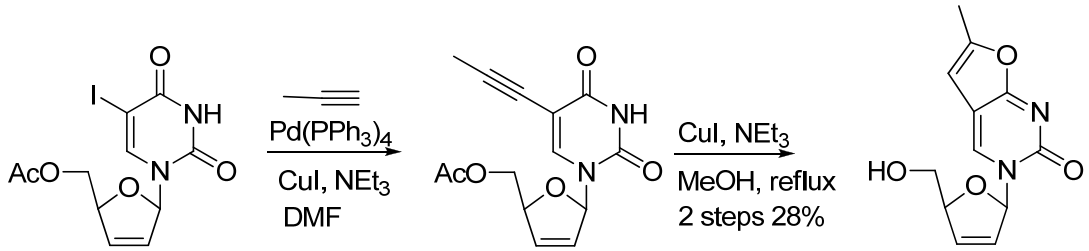


Synthesis of 3-(5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one

The D4T variant of compounds **119** and **120** can be prepared by following Scheme 38. Starting from known compound **131**[111] and following our established

procedure, synthon **131** was readily converted into the **133** using a two-step Pd- and Cu-catalysed process. Because of the poor stability of D4T sugar ring, the intermediate should be treated very carefully.

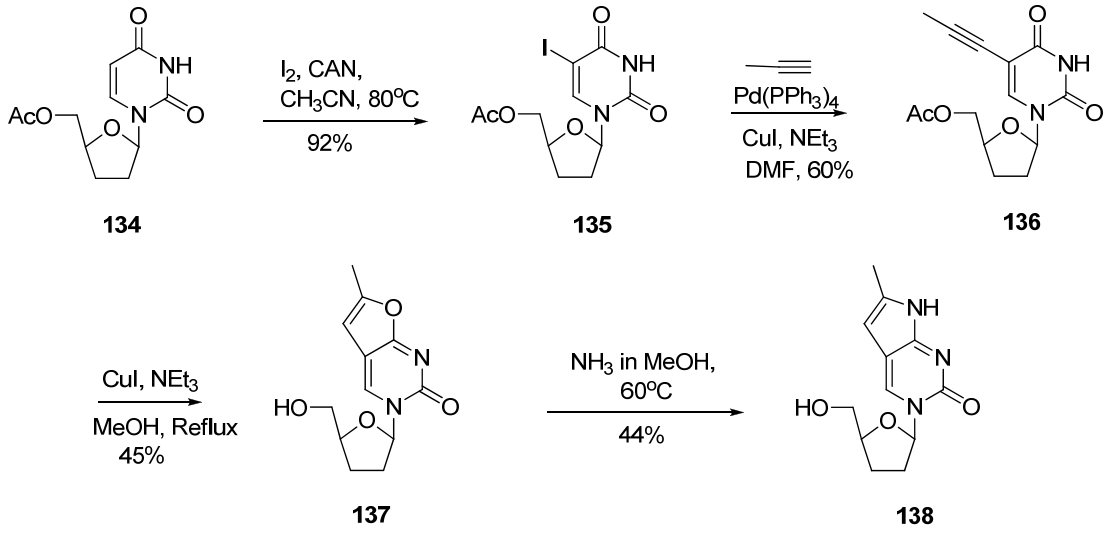
Scheme 38: Synthesis of 3-(5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one **133**



Synthesis of 3-(5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one

The DDC variant of compounds **137** and **138** can be prepared by starting with known compound **134**. Halogenation of **134** to its iodo counterpart **135** was achieved using elemental iodine and CAN at 60 ^{o}C.[112] Using our established procedure, the C-5 modified target compound **136** were synthesized via a palladium catalyzed coupling of propyne with the iodo uridine **135**. Cyclization of the alkalized uridine under basic conditions in the presence of catalyst CuI gave the product **137**, which was treated with ammonic methanol to afford the cytidine variation **138**.[102]

Scheme 39: Synthesis of 3-(5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one **138**



2.5 Conclusion

The application of fNAs as molecular probes provides a powerful new tool for studying the uptake and metabolism of antiviral prodrugs. The examples above demonstrate the successful synthesis of novel fluorescent pyrimidine prodrugs that showed substrate properties similar to the natural nucleosides in assays with type-I dNKs. Separately, fNAs were tested in bacterial cultures, taking advantage of intracellular fluorophore accumulation as a result of phosphorylation. The same feature can be utilized to assay large combinatorial libraries of dNKs, identifying and isolating enzymes with activity for NA prodrugs by FACS. The latter high-throughput screen represents a significant improvement over previous selections and screening techniques, allowing for the first time the

direct positive selection for fNA phosphorylation activity. These examples conclusively demonstrate the successful identification of mutant kinases with novel activity.

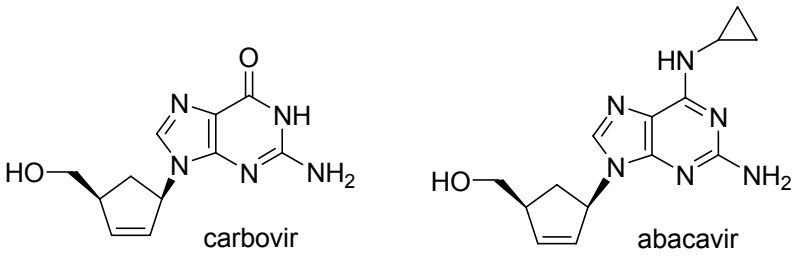
Part 3: Synthesis of Abacavir

3.1 Statement of purpose

A process for the preparation of abacavir ((1S,4R)-4-(2-amino-6-(cyclopropylamino)-9H-purin-9-yl)cyclopent-2-enyl)methanol and carbovir (2-amino-9-((1R,4S)-4-(hydroxymethyl)cyclopent-2-enyl)-9H-purin-6-ol) is provided that utilizes commercially available and inexpensive starting materials and that proceeds with high regioselectivity and stereochemical control (Figure 11). This process represents a significant advance in preparation of biologically active nucleosides, because after formation of a novel π-allylpalladium complex, the bicyclic precursor can be opened with complete regio- and stereo-specificity to yield the desired, biologically active β-anomeric nucleoside. It is believed that this is the first report of the synthesis of nucleosides in which the regiochemistry and stereochemistry of the glycosidic linkage are controlled via a bicycloamide opening. The high degree of regiocontrol and stereocontrol throughout the synthesis may decrease the cost of the product relative to other known manufacturing methods of abacavir. Furthermore, it should be possible to prepare easily on large scale from inexpensive materials.

The process starts with two inexpensive, commercially available compounds, chlorosulfonyl isocyanate and cyclopentadiene. The total process includes [2+2] cycloaddition, kinetic resolution, tosylation and π-allylpalladium formation. This process can be used to prepare a wide range of unsaturated carbocyclic nucleosides, through selection of the heterocyclic bases.

Figure 17: The structures of Abacavir and Carbavir



3.2 Introduction and background

Acquired immune deficiency syndrome (AIDS) has rapidly become one of the major causes of death in the world. It is estimated that over 40 million people have developed human immunodeficiency virus (HIV) infections, which is the causative agent of AIDS.[113] In 1985, 3'-azido-3'-deoxythymidine (AZT) was approved as the first synthetic nucleoside to inhibit the replication of HIV. Since then, a number of other synthetic nucleoside analogs have been proven to be effective against HIV. After cellular phosphorylation to the triphosphate form by cellular kinases, the nucleotides are incorporated into a growing strand of viral DNA and cause chain termination due to the absence of the 3'-hydroxyl group.

Carbocyclic nucleosides are structurally analogs of nucleosides in which the furanose oxygen is replaced by a methylene group. Because of the structural resemblance to native nucleosides, carbonucleosides can behave as inhibitors of the enzymes. On the other hand, the bonds between the sugars and nucleoside bases are not susceptible to be hydrolyzed by phosphorylases or phosphotransferases, which endows them with a wide spectrum of biological activity.[114]

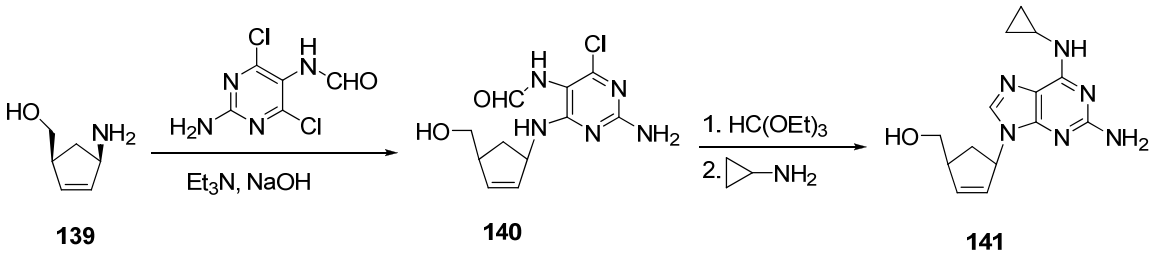
Abacavir (Ziagen)[115, 116], a carbocyclic nucleoside, was approved by the Food and Drug Administration (FDA) in 1998 as a nucleoside reverse transcriptase inhibitor to treat HIV-1 infection. Treatment with abacavir, alone or in combination with other anti- HIV agents decreases the viral load greater than 99% as well as significantly improves the CD4 cell count in patients with HIV infection, and effectiveness was maintained at least 48 weeks. Therefore,

continuous improvement in the enantioselective syntheses of abacavir is required due to its therapeutic significance.

Several methods have been disclosed for preparing this kind of carbocyclic nucleoside, which includes the following methods:[117-121]

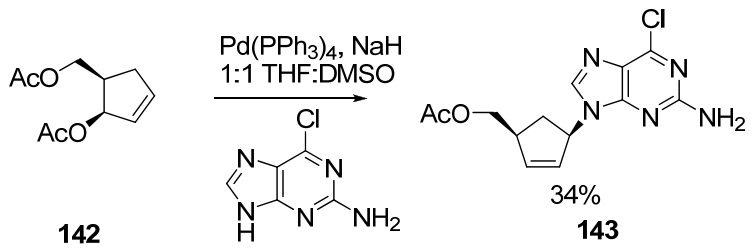
1. Using a cycloalkene substituted with an amino group as a starting material, the desired nucleoside base is constructed on the nitrogen atom of the amino group.[122]

Scheme 40: Vince's method to Abacavir



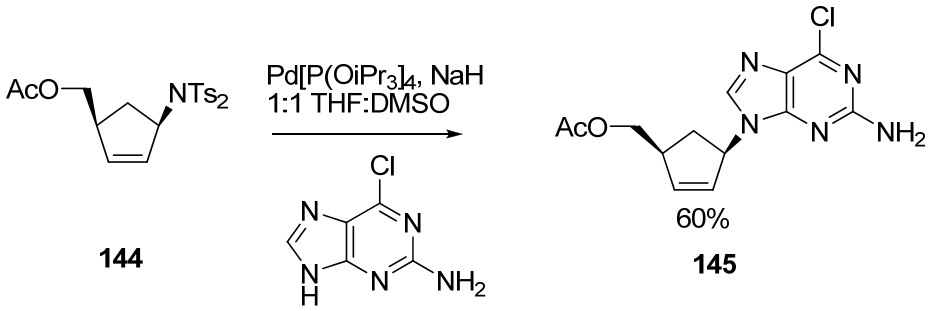
2. A purine structure is directly introduced into a 1-alkoxy-2-cyclopentene derivative **142** in the presence of a palladium catalyst. [117-119]

Scheme 41: Crimmins' method to Abacavir



3. A purine structure is directly introduced into a 2-cyclopentene-1-yl-*N,N*-ditosylimide derivative **144** in the presence of a palladium catalyst. [120, 121]

Scheme 42: Jung's method to Abacavir



However, each of the above methods includes an obstacle which would cause expensive production on an industrial scale. In the above method 1, the construction of a nucleoside structure on the *N*-atom needs many steps, which in turn increases the production cost. Methods 2 and 3 are advantageous in that not as many steps are needed to synthesize the target compounds; however, their strategies require enantiomerically pure cyclopentene derivatives, which are synthesized in many steps.

The problems described above that are encountered in the preparation of pharmaceutically active nucleosides increase the public cost of health care. In fact, the high cost of the antiviral, and in particular anti-HIV nucleosides prevents many of those in need from being able to obtain the drug at all.

Therefore, it is an objective of the present method to provide a synthesis of abacavir and carbovir from inexpensive, readily available starting materials and provide a synthetic methodology of abacavir and carbovir that is efficient and

generous and does not result in the production of a significant amount of undesired isomers.

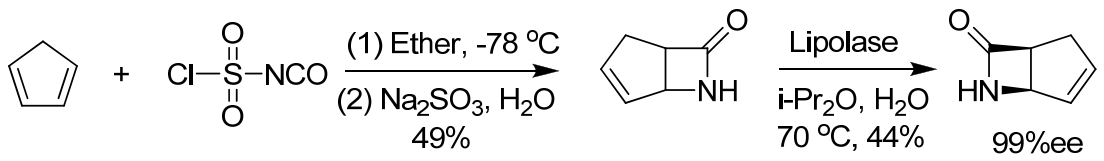
3.3 Synthesis of abacavir

The present method utilized a cyclopentenecarboxamide derivative, which is useful as an intermediate for abacavir and carbovir nucleoside synthesis. In addition, the method presents a highly regioselective and stereoselective way for preparing a cyclopentenecarboxamide *via* π-allylpalladium complex formation. The steps involved in the process are outlined below.

Synthesis of (1S,5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one

The starting materials for the preparation of 6-aza-bicyclo[3.2.0]hept-3-en-7-one **148** are cyclopentadiene **146** and chlorosulfonyl isocyanate **147**, as shown in Scheme 43. The β-lactam is obtained in 49% yield by a [2+2] cycloaddition and hydrolysis.[123] The optically active (1S,5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one **149** is prepared by an easy and efficient lipase (lipase B from *Candida Antarctica*)-catalyzed enantioselective ring opening of racemic β-lactam with 47% yield and 99% ee.[124]

Scheme 43: Synthesis of (1S,5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one **149**

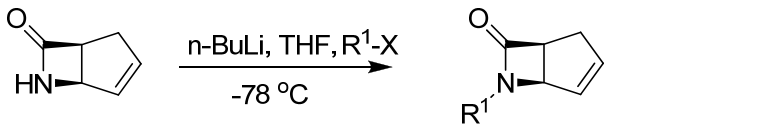


146 **147** **148** **149**

Synthesis of corresponding bicycloamide derivative

A method for preparing a bicycloamide derivative is explained below. The bicycloamide derivative **150** can be obtained by reacting, in the presence of an organolithium compound at -78 ˚C, (1S,5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one **149** with a compound R^1-X, wherein R^1 is an electron withdrawing group having a sulfur or a carbon atom directly bond to a nitrogen atom of the amide group, and X is a a halogen atom. The above reaction is shown in Scheme 44.

Scheme 44: Synthesis of bicycloamide derivative



R^1: electron-withdrawing group

149 **150**

Both the bicycloamide derivative **149** and (1S,5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one **150** are somewhat unstable compounds. Thus, when the reaction to produce the bicycloamide derivative using **149** as a starting material is carried out in the presence of sodium hydride at room temperature as in conventional methods, it is difficult to obtain an objective bicycloamide derivative in satisfactory yields.

To the contrary, when Scheme 44 is carried out at a low temperature in the presence of an organolithium base, the bicycloamide derivative can be produced in good yields. Thus, the reaction was carried out at a low temperature of -78 ˚C to 0 ˚C. The organolithium bases included, for instance, alkyl lithium compounds such as methyllithium, n-butyllithium, and t-butyllithium, and lithium amide bases such as lithium bis(trimethylsilyl)amide, lithium diisopropylamide，and the yields were almost the same.

Examples for compounds representing **150** are as follows. In the case where R^1 is an R-SO_2- group, examples include p-toluenesulfonyl chloride, o-nitrobenzenesulfonyl chloride, or (R)-(-)-10-camphorsulfonyl chloride. R^1 also can be R-CO- group, such as (S)-1-phenylethyl carbonochloridate and (S)-2-phenylpropanoyl chloride.

Table 6: Bicycloamide derivative with different R^1 group

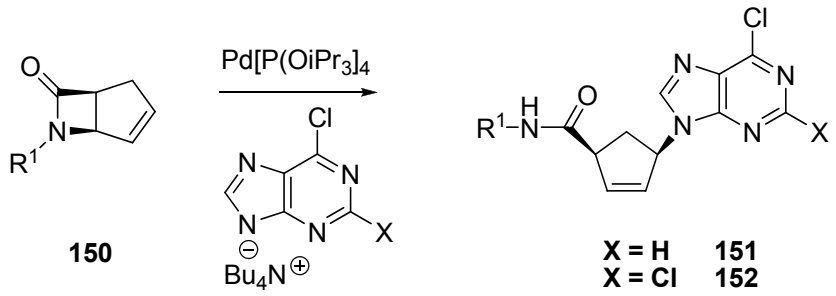
R^1	Product	Yield (%)
p-toluenesulfonyl	**150a**	64
o-nitrobenzenesulfonyl	**150b**	26
(R)-(-)-10-camphorsulfonyl	**150c**	78
(S)-2-phenylpropanoyl	**150d**	82

Synthesis of the cyclopentencarboxamide derivative

A method for preparing a cyclopentencarboxamide derivative **151** is explained below. In this reaction, the cyclopentencarboxamide derivative **151** can

be obtained by the reaction of bicycloamide derivative **150** with tetrabutyl salt of nucleoside bases in the presence of a palladium catalyst in THF at room temperature, as shown in Scheme 45.

Scheme 45: Synthesis of cyclopentencarboxamide derivative



150

X = H 151
X = Cl 152

First, we carried out the coupling reaction of **150** with 6-chloropurine in the presence of Pd^0. Compound **150a** reacted with 6-chloropurine tetrabutylammonium salt in the presence of $Pd[P(OiPr)_3]_4$ in THF at rt for 1 hr to give **151a** in 60% yield. The fact that **151a** was obtained as the major product in this reaction shows that the Pd^0 catalyst approaches from the exo-side of **150a** to form the π-allylpalladium complex, which reacted stereoselectively with nucleoside base to form **151a**. Compound **150c** didn't react with the same base under the same reaction conditions. Next, to obtain Abacavir, we investigated conversion of **150** to **152** by changing R^1 groups. The yields are shown in table 7. The tosyl group as a electron-withdrawing group, was proven to be the best one compared to others.

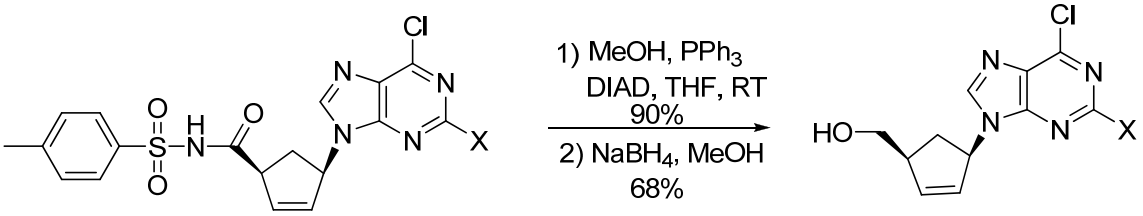
Table 7: Coupling reactions with different R^1 group

R^1	Product	Yield (%)
p-toluenesulfonyl	**151a / 152a**	54
o-nitrobenzenesulfonyl	**152b**	22
(R)-(-)-10-camphorsulfonyl	**152c**	0
(S)-2-phenylpropanoyl	**152d**	0

Synthesis of Abacavir

In the next step, the sulfonyl amide groups of **151a** or **152a** would be converted to the hydroxymethyl group. The direct conversion by using reductive reagents such as $NaBH_4$ in the presence of a metal salt was unsuccessful. Therefore, compound **151a** was transformed to the N-methylated derivative by the Mitsunobu reaction, followed by reduction with a mild reducing agent, such as sodium borohydride, to produce the known hydroxymethyl product in 68% yield.

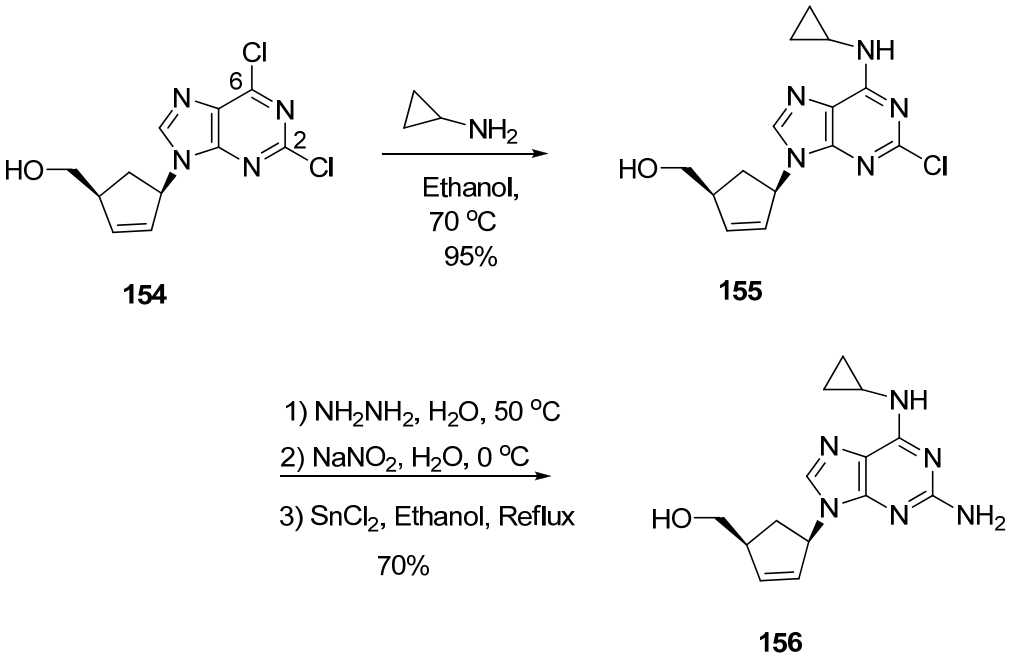
Scheme 46: Synthesis of Abacavir precursor



X = H, 151a
X = Cl, 152a

X = H, 153
X = Cl, 154

Scheme 47: Synthesis of Abacavir



The transformation of **152a** to Abacavir was carried out as follows; **152a** was converted to **154** using the same method as for **153**. As shown in the literature,[125] the reactivity of the chlorine atoms in 2,6-dichloropurine are different because of the difference in electron density. The 2-position has two adjacent nitrogen atoms, which make it more electron rich and less reactive with nucleophilic reagents. On the contrary, the 6-position just has one adjacent nitrogen atom and one carbon atom, which allows the substitution reaction to happen more easily.[126] Since the 6-chlorine atom is much more reactive than the 2-chlorine atom in the purine base, it is logical to assume that the monosubstitution of 2,6-dichloropurine nucleoside by a nucleophilic reagent should yield a 6-substituted product which, after isolation, should be capable of undergoing further substitution of the 2-position with a variety of nucleophilic

reagents. Thus, a method for the preparation of Abacavir is available. When compound **154** was heated to 70 ˚C with cyclopropylamine in ethanol, the 6-chloro substituent was replaced with cyclopropylamine to afford compound **155**.[127, 128]

We initially attempted to react compound **155** with methanolic ammonia at different temperatures in a sealed flask[129] but unfortunately the desired compound was not formed. Because an azido group can be easily reduced to an amino group, we decided to use an azido group to displace the 2-chloro group. Initial attempts to displace this 2-chloro group by reacting compound **155** with LiN_3 in refluxing EtOH failed. We then used the more nucleophilic reagent NH_2NH_2 to displace the 2-chloro group to form the 2-hydrazino derivative, which on treatment with sodium nitrite in an acid medium gave the 2-azidoadenosine.[130] A normal method to reduce the 2-azido group is hydrogenolysis,[131] but the double bond in the sugar ring will be sensitive to some of those conditions. Therefore a mild and convenient reducing reagent, stannous chloride,[132] was then used to reduce the azide to provide Abacavir **156.** The total yield for the three step sequence was 70%.

3.4 Conclusion

An enantioselective synthesis of Abacavir has been developed from easily available chiral β-lactam. The key step features the rapid assembly of the carbocyclic core of Abacavir via a simple [2+2] cycloaddition by cyclopentadiene and chlorosulfonyl isocyanate. Enantiopure β-lactam is prepared through the

lipolase-catalysed ring opening and an electron withdrawing group attached to the nitrogen of the β-lactam is necessary to assist C-N bond cleavage. A π-allylpalladium complex was a crucial strategic element which participated in glycosylation by ring strain-assisted C-N bond fission to assist the formation of the precursor of Abacavir. Such a synthesis will supply a more convergent and practical method for the synthesis of Abacavir.

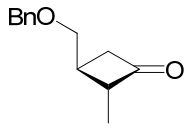
4. Experimental Section

General Notes

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Specific reactions were performed in oven-dried glassware under an atmosphere of argon gas. All solvents used were anhydrous or kept dry over activated 4 Å molecular sieves. Reaction progress was monitored through thin layer chromatography (TLC) on pre-coated glass purchased from EM Science. Unless otherwise stated, organic extracts were dried over commercially available anhydrous magnesium sulfate and the solvents removed with a rotary evaporator. Brine refers to saturated sodium chloride solution. Infrared spectra were recorded on Nicolet Avatar 370 DTGS FT-IR spectrometer as thin films on sodium chloride plates. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Mercury 300, Varian 400 or Varian 600 spectrometer. ^{19}F NMR and ^{31}P NMR spectra were recorded on Varian 400 spectrometer. Unless otherwise stated, all NMR spectra were recorded in deuterated chloroform ($CDCl_3$), methylene alcohol (CD_3OD), acetonitrile (CD_3CN), methyl sulfoxide ($(CD_3)_2SO$), deuterium oxide (D_2O) and referenced to the residual peak; chemical shifts (δ) are reported in parts per million, and the coupling constants (J) are reported in Hertz. Mass spectra were obtained on either a VG 70-S Nier Johnson or JEOL Mass Spectrometer. HPLC analyses were conducted on a Varian ProStar system.

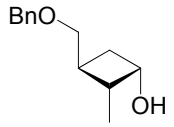
4.1 Experimental section of part 1

3-(benzyloxymethyl)-2-methylcyclobutanone (47)



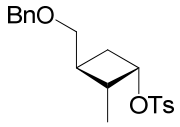
To a solution of *N,N*-Dimethylpropiamide (2.1 mL, 1.89 mmol) in 1,2-dichloroethane (20 mL) at -25 ˚C was added freshly distilled Tf_2O (3.9 mL, 2.27 mmol) at such a rate that the temperature didn't rise above -20 ˚C. The colorless homogeneous reaction mixture was stirred at -20 ˚C for 10 mins. After addition of a mixture of 2,4,6-trimethylpyridine (3.0 mL, 2.27 mmol) and allylbenzyl ether (5.8 mL, 4.54 mmol) in 1,2-dichloroethane (25 mL) over a period of 10 mins, then let the mixture slowly warm up to r.t., and then refluxed for 17 hrs. The solvent was removed giving a black oil, which was dried by vacuum. After addition of 10 mL of CCl_4 and 10 mL of water to the black oil and refluxing for 6 hrs, a mixture of 20 mL of saturated $NaHCO_3$ and 20 mL of CH_2Cl_2 were added to quench the reaction. After separation and evaporation, the crude was purified by flash column chromatography (ethyl acetate: hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **47** (1.25 g, 34% yield) as a colorless oil. IR (cm^{-1}) 3536 (br), 2800-3100 (m), 1777 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, 3H, J = 7.2 Hz), 2.22 (m, 1H), 2.88-3.00 (m, 2H), 3.10 (m 1H), 3.63 (d, 2H, J = 6.4 Hz), 4.56 (s, 2H), 7.34 (m, 5H); ^{13}C NMR (100 MHz $CDCl_3$) δ 13.4, 32.8, 37.0, 38.4, 67.3, 73.2, 73.8, 76.9, 77.3, 77.6, 127.8, 128.6, 138.8; HRMS (FAB) *m/z* 205.1222, calcd for $C_{13}H_{17}O_2$ 205.1223 (M^+H).

3-(benzyloxymethyl)-2-methylcyclobutanol (48)



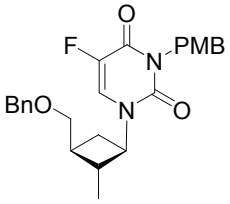
A solution of lithium tri-sec-butylborohydride (1.0 M in THF, 7.3 mL, 7.34 mmol) in THF was added under argon and dropwise over a period of 10 mins to a stirred solution of butanone (1.25 g, 6.12 mmol) in dry THF (20 mL) at -78 ˚C. The product was then allowed to warm up to RT and saturated $NaHCO_3$ (8 mL) was added over a period of 2 mins. The result solution was then cooled and 30% aqueous hydrogenperoxide (3 mL) was added dropwise at such a rate that the temperature was maintained at 25-30 ˚C. Water and ethyl acetate were then added. The organic layer was separated, washed with water, dried and evaporated under rotary-vap to give the crude alcohol, which was purified by flash column chromatography (ethyl acetate:hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **48** (1.2 g, 95% yield) as colorless oil. IR (cm^{-1}) 3393 (br), 2800-3100 (m), 1456 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (d, 3H, J = 7.2 Hz), 2.00-2.15 (m, 2H), 2.29 (m, 1H), 3.45 (m 1H), 3.32 (m, 1H), 4.51 (s, 2H), 7.24-7.34 (m, 5H); ^{13}C NMR (100M Hz, $CDCl_3$) δ 13.4, 32.8, 37.0, 38.4, 67.3, 73.2, 73.8, 76.9, 77.3, 77.6, 127.8, 128.6, 138.8; HRMS (FAB) *m/z* 207.1379, calcd for $C_{13}H_{19}O_2$ 207.1380 (M^+H).

3-(benzyloxymethyl)-2-methylcyclobutyl 4-methylbenzenesulfonate (49)



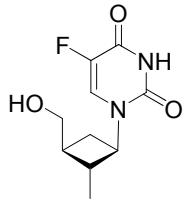
Compound **48** (1.00 g, 4.90 mmol) and triethylamine (0.65 mL, 8.83 mmol) were dissolved in dry THF (20 mL) and *p*-methylbenzenesulfonyl chloride (1.70 g, 8.83 mmol) in dry THF (5 mL) was added to the solution. The mixture was stirred for 1 hr at r.t. and then filtered through a celite pad. The filtrate was evaporated under reduced pressure to afford light yellow oil which was purified by flash column chromatography (ethyl acetate: hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **49** (1.4 g, 89% yield). IR (cm^{-1}) 2800-2950 (m), 1357 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (d, 3H, J = 6.8 Hz), 2.03-2.14 (m, 2H), 2.42 (s, 1H), 2.46 (m 1H), 3.38 (d, 2H, J = 5.6 Hz), 4.47 (s, 2H), 4.93 (dd, 1H, J =6.0, 6.8 Hz), 7.25-7.35 (m, 8H), 7.75 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 21.9, 30.5, 37.2, 38.5, 72.4, 73.3, 76.3, 127.7, 127.8, 128.0, 128.6, 130.0, 134.3, 138.6, 144.8; HRMS (FAB) *m/z* 361.1470, calcd for $C_{20}H_{25}O_4{}^{32}S_1$ 361.1468 (M^+H).

3-((benzyloxymethyl)-2-methylcyclobutyl)-5-fluoro-3-(4-methoxybenzyl) pyrimidine-2,4(1H,3H)-dione (50)



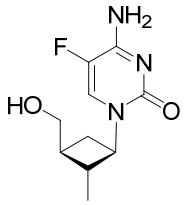
To the PMB protected 5-fluorouracil (1.68 g, 6.72 mmol) was added 10 mL of dry DMF followed by compound **49** (2.00 g, 5.60 mmol), 18-crown-6 (1.77 g, 6.72 mmol) and K_2CO_3 (0.93 g, 6.72 mmol). After stirring at 120 ˚C overnight, the mixture was cooled and extracted by EtOAc. After evaporation, the residue was purified by flash column chromatography (ethyl acetate: hexane = 1:1). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **50** (0.68 g, 56% yield) as white solid. Melting point: 169 - 171 ˚C; IR (cm^{-1}) 2958 (br), 1711 (s), 1674 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, 3H, J = 6.8 Hz), 1.88-1.92 (m, 2H), 2.39 (m, 1H), 3.48 (m 1H), 3.76 (s, 1H), 4.47 (s, 3H), 4.51 (m, 1H), 4.54 (s, 2H), 5.05 (dd, 2H, J = 13.6, 13.2 Hz), 6.82 (m, 2H), 7.25-7.45 (m, 5H), 7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 28.6, 36.0, 40.1, 44.7, 54.6, 55.5, 71.1, 73.5, 113.9, 123.4, 123.7, 127.7, 128.0, 128.7, 131.3, 138.4, 139.2, 141.5, 150.2, 159.5; HRMS (FAB) *m/z* 439.2018, calcd for $C_{25}H_{28}O_4N_2F_1$ 439.2028 (M^+H).

5-fluoro-1-(3-(hydroxymethyl)-2-methylcyclobutyl)pyrimidine-2,4(1H,3H)-dione (51)



To a 10 mL flask with $AlCl_3$ (2.40 g, 18 mmol), dry anisole 5 mL was added, and the solution became to red. To another 100 mL flask with compound **50** (0.75 g, 1.8 mmol) was added 15 mL of anisole, and the first one was added dropwise to the second one. After reacting 1 hr, anhydrous methanol (20 mL) was added at 0 ˚C till the solvent changed to white. After the solvent was evaporated under pressure, the residue was purified by flash column chromatography (CH_2Cl_2: MeOH = 1:1). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **51** (0.68 g, 95% yield) as white solid. Melting point: 153 - 155 ˚C; IR (cm^{-1}) 3370 (br), 2953 (br), 2357 (s), 2336 (s), 1699 (s); ^1H NMR (400MHz, CD_3OD) δ 1.11 (d, 3H, J = 6.4 Hz), 1.79-1.83 (m, 2H), 2.95 (m, 2H), 2.18 (s 1H), 2.08 (m, 1H), 2.74 (m, 1H), 3.54 (s, 2H), 4.35 (m, 1H), 7.68 (d, 2H, J = 6.8 Hz), 9.27 (br, 1H); ^{13}C NMR (100 MHz, CD3OD) δ 17.5, 28.2, 37.9, 40.0, 54.3, 63.6, 126.3, 126.6, 139.4, 141.7, 150.3, 159.0; HRMS (FAB) *m/z* 229.0982, calcd for $C_{10}H_{14}O_3N_2F$ 229.0983 (M^+H). Anal. Calcd for $C_{10}H_{13}O_3N_2F$ + 0.3 CH_3OH: C, 52.02; H, 6.02; N, 11.78. Found: C, 51.96; H, 5.96; N, 11.53.

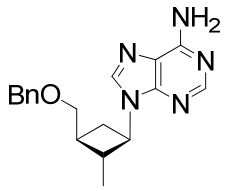
4-amino-5-fluoro-1-((1R,2R,3S)-3-(hydroxymethyl)-2-methylcyclobutyl) pyrimidin-2(1H)-one (52)



Compound **51** (138 mg, 0.46 mmol), 1-methylpyrrolidine (0.5 mL, 4.60 mmol), chlorotrimethylsilane (0.19 mL, 1.38 mmol) and dry acetonitrile (20 mL) were stirred at r.t.. After 1 hr, the reactants were cooled to 0 ˚C and trifluoroacetic anhydride (0.35 mL, 2.30 mmol), was added dropwise over a period of 5 mins. After a further period of 30 mins at 0 ˚C, 4-nitrophenol (0.21 g, 1.38 mmol) was added and the cooled reactants were stirred for 3 hrs. The products were then poured into saturated aqueous $NaHCO_3$ and the resulting mixture was extracted with dichloromethane. The combined organic extracts were dried and evaporated under reduced pressure. The residue was dissolved in dioxane and concentrated aqueous NH_3 was added. The reactants were heated in a sealed flask at 50 ˚C for overnight. The resulting solution was concentrated and fractionated by column chromatography on silica gel (CH_2Cl_2: MeOH = 1:1) to afford compound **52** (55 mg, 38% yield). Melting point: 138 - 140 ˚C; IR (cm^{-1}) 3329 (br), 3174 (br), 2925 (br), 1679 (s), 1601 (s), 1515 (s); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01 (d, 3H, J = 6.4 Hz), 1.65-1.69 (m, 2H), 3.28-3.43 (m 2H), 4.30 (m, 1H), 4.40 (m, 1H), 7.34 (br, 1H), 7.57 (br, 1H), 7.92 (d, 2H, J = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.9, 24.3, 33.6, 35.9, 49.3, 51.1, 59.1, 122.4, 122.6, 132.0, 133.6, 152.0, 153.4, 153.5; HRMS (FAB) *m/z* 228.1138, calcd for $C_{10}H_{15}O_2N_3F_1$ 228.1143

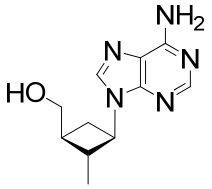
(M^+H). Anal. Calcd for $C_{10}H_{14}O_2N_3F$: C, 52.86; H, 6.21; N, 18.49; Found: C, 52.96; H, 6.12; N, 18.53.

3-(benzyloxymethyl)-2-methylcyclobutyl)-9H-purin-6-amine (53)



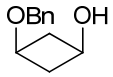
To the adenine (0.45 g, 3.33 mmol) was added under argon 10 mL of dry DMF followed by compound **49** (1.0 g, 2.79 mmol), 18-crown-6 (0.89 g, 3.33 mmol) and K_2CO_3 (0.47 g, 3.33 mmol). After stirring at 120 ˚C overnight, the mixture was cooled and extracted by EtOAc. After evaporation, the residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **50** (0.44 g, 50% yield) as white solid. Melting point: 135 - 137 ˚C; IR (cm^{-1}) 3329 (br), 3162 (br), 2925 (br), 1638 (s), 1593 (s); ^1H NMR (400 MHz, DMSO-d_6) δ 1.10 (d, 3H, J = 6.4 Hz), 2.00 (m, 1H), 2.13 (m 1H), 2.55-2.67 (m, 2H), 3.50 (d, 2H J = 6.4 Hz), 4.40-4.50 (m, 3H), 6.60 (br, 2H), 7.35 (m, 5H), 7.90 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ 18.5, 30.4, 36.7, 42.5, 52.5, 70.8, 72.3, 73.3, 120.1, 127.7, 127.8, 127.9, 128.6, 138.6, 138.9, 150.3, 153.0, 156.2; HRMS (FAB) *m/z* 324.1819, calcd for $C_{18}H_{22}O_1N_5$ 324.1819 (M^+H).

3-(6-amino-9H-purin-9-yl)-2-methylcyclobutyl) methanol (54)



To a 25 mL flask with compound **53** (0.12 g, 0.37 mmol) inside, dry CH_2Cl_2 (4 mL) was added under argon to give a colorless solution. This was cooled to –78 ^oC, BCl_3 (1.0 M in CH_2Cl_2, 2 mL, 2 mmol) was added drop by drop. After 8 hrs, the reaction was quenched by adding 7 N NH_3 in MeOH (4.7 mL, 33 mmol) slowly. The products were then concentrated under reduced pressure and the residue was purified by silica gel flash chromatography (CH_2Cl_2: MeOH = 10:1) to give 0.05 g product in 48% yield. Melting point: 178 – 180 ˚C; IR (cm^{-1}) 3329 (br), 3162 (br), 2913 (s), 1650 (s), 1585 (s); ^1H NMR (400 MHz, CD_3OD) δ 1.21 (d, 3H, J = 6.8 Hz), 2.15 (m, 1H), 2.33 (dd, 1H J = 10.0, 10.8 Hz), 2.56 (m, 1H), 2.76 (M, 1H), 4.30 (m, 1H), 4.49 (m, 1H), 8.18 (s, 1H), 8.26 (s, 1H); ^{13}C NMR (100MHz, CD_3OD) δ 17.0, 29.7, 35.4, 42.5, 52.7, 139.9, 149.6, 152.4, 156.1, 161.7; HRMS (FAB) *m/z* 234.1356, calcd for $C_{11}H_{16}O_1N_5$ 234.1349 (M^+H). Anal. Calcd for $C_{11}H_{15}O_1N_5$: C, 56.64; H, 6.48; N, 30.02; Found: C, 56.54; H, 6.42; N, 30.21.

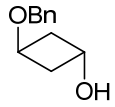
cis-3-(benzyloxy)cyclobutanol (57)



A 2.4 eq portion of *N*-butyllithium (1.6 M in hexane, 243 mL) was added dropwise to a solution containing 2.4 eq of methyl methylsulfinyl methylsulfide (41 mL, 0.39 mmol) in 400 mL of tetrahydrofuran at -10 ˚C. The reaction mixture was stirred at -10 ˚C for 2 hrs and then cooled to -78 ˚C. The yellow reaction mixture was maintained at -78 ˚C as a 1 eq portion of the ((1,3-dibromopropan-2-yloxy)methyl)benzene (50 g, 0.16 mmol) in 85 mL of tetrahydrofuran was added dropwise. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was added to brine and extracted twice with ethyl acetate. The combined organic layers were subject to the usual work up to provide 60 mL of dark red-brown liquid. This mixture of syn- and anti-dithioketal S-oxide intermediates was purified in three portions via silica gel column chromatography. Less polar impurities were eluted first with 3:7 ethyl acetate: hexane followed by elution of product with pure ethyl acetate. A total of 23.8 g of intermediate was obtained in this manner. The syn- and anti-dithioketal S-oxide intermediates (23.8 g, 0.09 mol) were dissolved in 600 mL of diethyl ether and treated with 34 mL of 35% perchloric acid. After overnight stirring, the reaction mix was neutralized with sodium bicarbonate followed by purification via silica gel column chromatography (15:85 ethyl acetate: hexane) provided the ketone (11.8 g, 41% yield) as an orange-yellow liquid: ^{1}H NMR (400MHz, $CDCl_3$) δ 3.29 (m, 4H), 4.35–4.42 (m, 1H), 4.53 (s, 2H), 7.30–7.40 (m, 5H). Under argon a solution of lithium tri-sec-butylboranuide (1.0 M in THF, 23.5 mL, 0.023 mmol) in THF was

added dropwise over a period of 10 mins to a stirred solution of butanone (4.0 g, 0.02 mmol) in dry THF (20 mL) at -78 ˚C. The product was then allowed to warm up to r.t. and saturated $NaHCO_3$ (8 mL) was added over a period of 2 mins. The result solution was then cooled and 30% aqueous hydrogen peroxide (3 mL) was added dropwise at such a rate so as to maintain the temperature at 25 - 30 ˚C, water and ethyl acetate were then added. The organic layer was separated, washed with water, dried and evaporated under rotary-vap to give the crude alcohol, which was purified by flash column chromatography (ethyl acetate: hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **57** (3.8 g, 95% yield) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.90 - 1.98 (m, 2H), 2.68 - 2.75 (m, 2H), 2.63 (dt, 1H, J = 6.8 Hz), 3.90 (m, 1H), 4.42 (s, 2H), 7.20 - 7.34 (s, 5H).

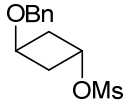
***trans*-3-(benzyloxy)cyclobutanol (58)**



To a 100 ml flask with *cis*-3-(benzyloxy)cyclobutanol **57** (1.65 g, 9.47 mmol), 4-nitrobenzoic acid (3.16 g, 18.90 mmol), Ph_3P (5.21 g, 19.90 mmol) and dry THF 25 mL were added under argon. Then the reaction mixture was cooled to 0 ˚C and DIAD (3.9 mL, 20 mmol) was added drop by drop to give a yellow solution. This was allowed to warm up to room temperature gradually and was left stirring for 77 hrs, after which time the solvent was removed and applied directly to the silica gel flash chromatography (hexane: ethyl acetate = 20:1) to give the desired product with a little impurity and this was redissolved in 1,4-dioxane (6.6 mL).

This was treated with aqueous NaOH (0.4 mol/L, 4.3 mL, 1.70 mmol) at room temperature. After 30 min, 0.07 mL of AcOH was added and the products were concentrated to small volume under reduced pressure. The residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$ and solvent evaporation gave the crude product that was purified by silica gel flash chromatography (Hexane: EtOAc = 3:1) to give the product (1.04 g, 52%). IR (cm^{-1}) 3000 (br), 1336 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 2.18 (m, 2H), 2.37 (m, 2H), 4.29 (m, 1H), 4.21 (s, 2H), 4.56 (m, 1H), 7.26 – 7.35 (m, 5H); ^{13}C NMR (100 MHz $CDCl_3$) δ 39.7, 65.2, 70.5, 70.7, 127.9, 128.0, 128.6, 138.3; HRMS (FAB) *m/z* 179.1064, calcd for $C_{11}H_{15}O_2$ 179.1067 (M^+H).

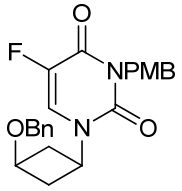
trans-3-(benzyloxy)cyclobutyl methanesulfonate (59)



To a 50 mL flask with *trans*-3-(benzyloxymethyl)cyclobutanol **58** (66 mg, 0.37 mmol) inside, dry CH_2Cl_2 (20 mL) was added to give a clear solution, and then Et_3N (0.26 mL, 1.9 mmol) was added to the above solution. After 10 mins, reaction mixture was cool to 0 ˚C, MsCl (0.04 mL, 0.5 mmol) was added drop by drop and it was left stirring with the temperature gradually going up to room temperature. After 3 hrs, the reaction was quenched by adding H_2O. Then the organic phase was separated, washed with brine once and dried over $MgSO_4$. Solvent evaporation gave the crude product that was purified by flash column chromatography (ethyl acetate: hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **59** (3.8 g, 95%)

as colorless oil. IR (cm^{-1}) 1344 (s), 1171 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 2.54 (t, 4H, J = 5.6, 6.0 Hz), 2.99 (s, 3H), 4.31 (dt, 1H, J = 5.6, 5.0 Hz), 4.42 (s, 2H), 5.22 (m, 1H), 7.26 – 7.37 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.0, 69.9, 70.9, 73.8, 128.0, 128.1, 128.7, 137.7; HRMS (FAB) *m/z* 257.0841, calcd for $C_{12}H_{17}O_4S_1$ 257.0842 (M^+H).

1-cis-3-(benzyloxy)cyclobutyl)-5-fluoro-3-(4-methoxybenzyl)pyrimidine-2,4(1H,3H)-dione (60)



To the PMB protected 5-fluorouracil (1.68 g, 6.72 mmol) was added 10 mL of dry DMF under Argon followed by compound **59** (1.86 g, 5.60 mmol), 18-crown-6 (1.77 g, 6.72 mmol) and K_2CO_3 (0.93 g, 6.72 mmol). After stirring at 120 ˚C overnight, the mixture was cooled and extracted by EtOAc three times. After washing by water and the organic solvent were evaporated, and then the residue was purified by flash column chromatography (ethyl acetate: hexane = 1:1). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **60** (1.00 g, 44 % yield) as white solid. Melting point: 136 – 137 ˚C; IR (cm^{-1}) 1654 (s), 1371 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 2.05-2.10 (m, 2H), 2.77-2.83 (m, 2H), 3.76 (s, 3H), 3.87 (m, 1H), 4.44 (s, 2H), 4.57 (m, 1H), 5.04 (s, 2H), 6.81 (d, 2H, J = 8.4 Hz), 7.30 – 7.45 (m, 8H); ^{13}C NMR (100 MHz $CDCl_3$) δ 37.8, 44.3, 44.8, 55.5, 66.3, 71.1, 114.0, 122.8, 123.2, 128.2, 128.3, 128.6, 128.8,

131.2, 137.6, 139.3, 141.7, 150.0, 159.5; HRMS (FAB) *m/z* 411.1714, calcd for $C_{23}H_{24}O_4N_2F$ 411.1715 (M^+H). Anal. Calcd for $C_{23}H_{23}O_4N_2F$ + 0.4 H_2O: C, 66.14; H, 5.74; N, 6.71; Found: C, 66.19; H, 5.66; N, 6.64.

The stereochemistry was established by X-ray crystallography analysis.

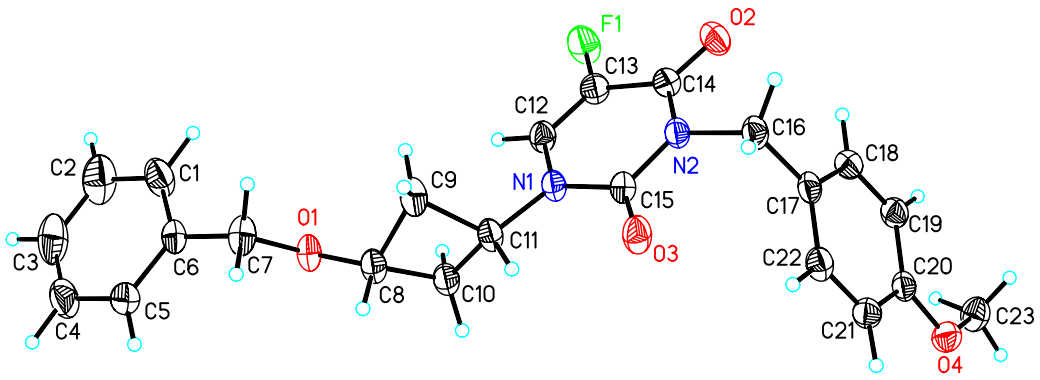


Table 8: Crystal data and structure refinement for **60**

Identification code	ylpmbs	
Empirical formula	C23 H23 F N2 O4	
Formula weight	410.43	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.015(2) Å	= 66.574(3)°.
	b = 10.682(2) Å	= 69.149(4)°.
	c = 12.568(3) Å	= 66.476(3)°.
Volume	989.9(4) $Å^3$	
Z	2	
Density (calculated)	1.377 Mg/m^3	
Absorption coefficient	0.101 mm^{-1}	
F(000)	432	
Crystal size	0.64 x 0.52 x 0.32 mm^3	
Theta range for data collection	1.82 to 28.45°.	
Index ranges	-12<=h<=12, -14<=k<=14, -16<=l<=16	
Reflections collected	14825	
Independent reflections	4951 [R(int) = 0.0258]	
Completeness to theta = 28.45°	99.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9684 and 0.9382	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4951 / 0 / 363	
Goodness-of-fit on F^2	1.041	
Final R indices [I>2sigma(I)]	R1 = 0.0450, wR2 = 0.1202	
R indices (all data)	R1 = 0.0524, wR2 = 0.1266	
Largest diff. peak and hole	0.324 and -0.222 $e.Å^{-3}$	

Table 9: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for **60**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

	x	y	z	U(eq)
F(1)	6792(1)	4472(1)	665(1)	47(1)
N(1)	9234(1)	3977(1)	2589(1)	25(1)
N(2)	10969(1)	3020(1)	1014(1)	26(1)
O(1)	5866(1)	6455(1)	5204(1)	30(1)
O(2)	9897(1)	3132(1)	-434(1)	40(1)
O(3)	12009(1)	2876(1)	2481(1)	34(1)
O(4)	13894(1)	-3741(1)	2349(1)	33(1)
C(1)	2888(2)	9279(2)	5259(1)	44(1)
C(2)	1349(2)	9995(2)	5835(2)	57(1)
C(3)	1084(2)	9968(2)	6994(2)	54(1)
C(4)	2366(2)	9251(2)	7562(1)	51(1)
C(5)	3913(2)	8540(1)	6985(1)	38(1)
C(6)	4180(1)	8546(1)	5826(1)	28(1)
C(7)	5870(2)	7795(1)	5199(1)	35(1)
C(8)	7488(1)	5608(1)	4818(1)	30(1)
C(9)	8378(2)	6034(1)	3486(1)	33(1)
C(10)	7602(2)	4190(1)	4716(1)	32(1)
C(11)	9032(1)	4426(1)	3608(1)	28(1)
C(12)	7899(1)	4373(1)	2120(1)	29(1)
C(13)	8094(2)	4103(1)	1125(1)	31(1)
C(14)	9680(2)	3388(1)	486(1)	29(1)
C(15)	10819(1)	3263(1)	2066(1)	25(1)
C(16)	12655(2)	2252(1)	436(1)	29(1)
C(17)	12988(1)	656(1)	914(1)	27(1)
C(18)	12488(2)	-53(1)	452(1)	31(1)
C(19)	12754(2)	-1519(1)	902(1)	30(1)
C(20)	13556(1)	-2300(1)	1834(1)	26(1)

C(21)	14072(2)	-1605(1)	2302(1)	30(1)
C(22)	13791(1)	-145(1)	1843(1)	30(1)
C(23)	13225(2)	-4471(2)	1988(1)	36(1)

Table 10: Bond lengths [Å] and angles [°] for **60**

F(1)-C(13)	1.3415(14)
N(1)-C(12)	1.3694(15)
N(1)-C(15)	1.3838(14)
N(1)-C(11)	1.4680(14)
N(2)-C(14)	1.3864(16)
N(2)-C(15)	1.3975(14)
N(2)-C(16)	1.4844(14)
O(1)-C(8)	1.4071(14)
O(1)-C(7)	1.4303(15)
O(2)-C(14)	1.2237(15)
O(3)-C(15)	1.2113(14)
O(4)-C(20)	1.3620(15)
O(4)-C(23)	1.4253(16)
C(1)-C(6)	1.3781(19)
C(1)-C(2)	1.383(2)
C(2)-C(3)	1.380(3)
C(3)-C(4)	1.370(3)
C(4)-C(5)	1.386(2)
C(5)-C(6)	1.3866(18)
C(6)-C(7)	1.5028(16)
C(8)-C(10)	1.5311(17)
C(8)-C(9)	1.5438(18)
C(9)-C(11)	1.5394(17)
C(10)-C(11)	1.5386(17)
C(12)-C(13)	1.3301(17)
C(13)-C(14)	1.4393(16)
C(16)-C(17)	1.5042(17)
C(17)-C(18)	1.3868(17)
C(17)-C(22)	1.3902(17)

C(18)-C(19)	1.3872(18)
C(19)-C(20)	1.3908(17)
C(20)-C(21)	1.3893(17)
C(21)-C(22)	1.3795(18)

C(12)-N(1)-C(15)	121.75(10)
C(12)-N(1)-C(11)	119.87(9)
C(15)-N(1)-C(11)	118.14(9)
C(14)-N(2)-C(15)	125.93(9)
C(14)-N(2)-C(16)	117.28(9)
C(15)-N(2)-C(16)	116.75(9)
C(8)-O(1)-C(7)	111.24(9)
C(20)-O(4)-C(23)	117.23(10)
C(6)-C(1)-C(2)	120.61(14)
C(3)-C(2)-C(1)	120.22(16)
C(4)-C(3)-C(2)	119.61(13)
C(3)-C(4)-C(5)	120.33(14)
C(4)-C(5)-C(6)	120.34(14)
C(1)-C(6)-C(5)	118.89(12)
C(1)-C(6)-C(7)	121.11(12)
C(5)-C(6)-C(7)	119.98(12)
O(1)-C(7)-C(6)	109.55(10)
O(1)-C(8)-C(10)	115.13(10)
O(1)-C(8)-C(9)	119.02(11)
C(10)-C(8)-C(9)	89.28(9)
C(11)-C(9)-C(8)	86.53(9)
C(8)-C(10)-C(11)	87.01(9)
N(1)-C(11)-C(10)	119.95(10)
N(1)-C(11)-C(9)	118.69(10)
C(10)-C(11)-C(9)	89.17(9)
C(13)-C(12)-N(1)	120.62(10)
C(12)-C(13)-F(1)	121.13(11)
C(12)-C(13)-C(14)	123.04(11)
F(1)-C(13)-C(14)	115.82(10)
O(2)-C(14)-N(2)	122.57(11)
O(2)-C(14)-C(13)	124.44(12)

N(2)-C(14)-C(13)	112.99(10)
O(3)-C(15)-N(1)	122.79(10)
O(3)-C(15)-N(2)	121.59(10)
N(1)-C(15)-N(2)	115.62(10)
N(2)-C(16)-C(17)	111.81(9)
C(18)-C(17)-C(22)	118.26(11)
C(18)-C(17)-C(16)	121.06(11)
C(22)-C(17)-C(16)	120.68(11)
C(17)-C(18)-C(19)	121.66(11)
C(18)-C(19)-C(20)	119.13(11)
O(4)-C(20)-C(21)	115.47(11)
O(4)-C(20)-C(19)	124.68(11)
C(21)-C(20)-C(19)	119.85(11)
C(22)-C(21)-C(20)	120.10(11)
C(21)-C(22)-C(17)	121.00(11)

Symmetry transformations used to generate equivalent atoms:

Table 11: Anisotropic displacement parameters ($Å^2 \times 10^3$) for **60**. The anisotropic displacement factor exponent takes the form: $-2^2[h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
F(1)	33(1)	66(1)	49(1)	-32(1)	-20(1)	1(1)
N(1)	21(1)	29(1)	28(1)	-16(1)	-4(1)	-4(1)
N(2)	24(1)	25(1)	26(1)	-12(1)	-2(1)	-5(1)
O(1)	25(1)	31(1)	39(1)	-21(1)	-1(1)	-7(1)
O(2)	43(1)	46(1)	31(1)	-20(1)	-10(1)	-6(1)
O(3)	23(1)	41(1)	43(1)	-24(1)	-9(1)	-2(1)
O(4)	39(1)	27(1)	35(1)	-12(1)	-14(1)	-6(1)
C(1)	41(1)	54(1)	48(1)	-32(1)	-15(1)	-4(1)
C(2)	33(1)	64(1)	91(1)	-47(1)	-22(1)	1(1)
C(3)	36(1)	52(1)	72(1)	-39(1)	14(1)	-14(1)
C(4)	72(1)	42(1)	31(1)	-19(1)	9(1)	-20(1)
C(5)	50(1)	30(1)	34(1)	-11(1)	-11(1)	-9(1)
C(6)	28(1)	26(1)	33(1)	-15(1)	-2(1)	-9(1)
C(7)	29(1)	32(1)	45(1)	-20(1)	1(1)	-10(1)
C(8)	25(1)	34(1)	35(1)	-20(1)	-6(1)	-4(1)
C(9)	29(1)	34(1)	39(1)	-21(1)	2(1)	-12(1)
C(10)	36(1)	29(1)	29(1)	-14(1)	-2(1)	-7(1)
C(11)	24(1)	32(1)	31(1)	-19(1)	-6(1)	-4(1)
C(12)	22(1)	31(1)	33(1)	-15(1)	-6(1)	-3(1)
C(13)	27(1)	34(1)	33(1)	-13(1)	-12(1)	-4(1)
C(14)	32(1)	28(1)	26(1)	-10(1)	-6(1)	-7(1)
C(15)	23(1)	24(1)	29(1)	-12(1)	-4(1)	-6(1)
C(16)	25(1)	29(1)	29(1)	-13(1)	2(1)	-7(1)
C(17)	22(1)	29(1)	26(1)	-14(1)	1(1)	-5(1)
C(18)	32(1)	33(1)	26(1)	-12(1)	-9(1)	-4(1)
C(19)	31(1)	32(1)	31(1)	-15(1)	-10(1)	-6(1)
C(20)	23(1)	28(1)	26(1)	-13(1)	-3(1)	-4(1)
C(21)	28(1)	34(1)	31(1)	-14(1)	-10(1)	-4(1)
C(22)	27(1)	34(1)	33(1)	-18(1)	-6(1)	-7(1)
C(23)	34(1)	31(1)	45(1)	-16(1)	-10(1)	-8(1)

Table 12: Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **60**

	x	y	z	U(eq)
H(1)	3100(20)	9327(19)	4401(17)	56(5)
H(2)	460(30)	10520(20)	5415(19)	68(6)
H(3)	10(30)	10470(20)	7385(19)	71(6)
H(4)	2220(30)	9210(20)	8370(20)	71(6)
H(5)	4820(20)	8010(20)	7411(17)	58(5)
H(7A)	6150(20)	8390(20)	4376(17)	55(5)
H(7B)	6751(19)	7627(16)	5587(14)	36(4)
H(8)	8202(18)	5476(15)	5322(13)	29(3)
H(9A)	9190(20)	6533(18)	3243(15)	45(4)
H(9B)	7560(20)	6524(17)	2991(14)	41(4)
H(10A)	7850(20)	3325(19)	5372(16)	46(4)
H(10B)	6597(19)	4318(15)	4505(13)	34(4)
H(11)	10107(19)	3987(16)	3809(13)	33(4)
H(12)	6838(18)	4824(15)	2529(13)	31(4)
H(16A)	12706(19)	2585(16)	-410(14)	36(4)
H(16B)	13437(18)	2528(15)	575(12)	29(3)
H(18)	11950(19)	496(17)	-187(14)	38(4)
H(19)	12403(19)	-1971(17)	581(14)	37(4)
H(21)	14643(19)	-2169(17)	2916(14)	38(4)
H(22)	14177(19)	307(17)	2178(14)	38(4)
H(23A)	12030(20)	-4051(17)	2119(14)	42(4)
H(23B)	13701(19)	-4429(16)	1191(15)	37(4)
H(23C)	13540(20)	-5460(20)	2450(16)	48(4)

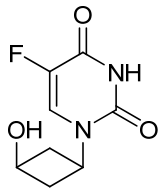
Table 13: Torsion angles [°] for **60**

C(6)-C(1)-C(2)-C(3)	-0.8(3)
C(1)-C(2)-C(3)-C(4)	1.4(3)
C(2)-C(3)-C(4)-C(5)	-0.9(2)
C(3)-C(4)-C(5)-C(6)	-0.1(2)
C(2)-C(1)-C(6)-C(5)	-0.2(2)
C(2)-C(1)-C(6)-C(7)	-178.44(14)
C(4)-C(5)-C(6)-C(1)	0.6(2)
C(4)-C(5)-C(6)-C(7)	178.90(12)
C(8)-O(1)-C(7)-C(6)	-169.48(10)
C(1)-C(6)-C(7)-O(1)	-79.49(16)
C(5)-C(6)-C(7)-O(1)	102.25(14)
C(7)-O(1)-C(8)-C(10)	-176.23(11)
C(7)-O(1)-C(8)-C(9)	-72.09(14)
O(1)-C(8)-C(9)-C(11)	-139.80(11)
C(10)-C(8)-C(9)-C(11)	-21.19(9)
O(1)-C(8)-C(10)-C(11)	143.19(11)
C(9)-C(8)-C(10)-C(11)	21.19(10)
C(12)-N(1)-C(11)-C(10)	49.96(16)
C(15)-N(1)-C(11)-C(10)	-135.61(12)
C(12)-N(1)-C(11)-C(9)	-57.27(15)
C(15)-N(1)-C(11)-C(9)	117.16(12)
C(8)-C(10)-C(11)-N(1)	-144.33(11)
C(8)-C(10)-C(11)-C(9)	-21.26(10)
C(8)-C(9)-C(11)-N(1)	145.23(10)
C(8)-C(9)-C(11)-C(10)	21.08(9)
C(15)-N(1)-C(12)-C(13)	-1.22(18)
C(11)-N(1)-C(12)-C(13)	173.01(11)
N(1)-C(12)-C(13)-F(1)	179.20(11)
N(1)-C(12)-C(13)-C(14)	0.3(2)
C(15)-N(2)-C(14)-O(2)	-179.38(11)
C(16)-N(2)-C(14)-O(2)	-1.71(17)
C(15)-N(2)-C(14)-C(13)	1.26(17)
C(16)-N(2)-C(14)-C(13)	178.92(10)

C(12)-C(13)-C(14)-O(2)	-179.65(13)
F(1)-C(13)-C(14)-O(2)	1.41(19)
C(12)-C(13)-C(14)-N(2)	-0.30(18)
F(1)-C(13)-C(14)-N(2)	-179.24(10)
C(12)-N(1)-C(15)-O(3)	-178.33(11)
C(11)-N(1)-C(15)-O(3)	7.35(17)
C(12)-N(1)-C(15)-N(2)	2.00(16)
C(11)-N(1)-C(15)-N(2)	-172.32(10)
C(14)-N(2)-C(15)-O(3)	178.22(11)
C(16)-N(2)-C(15)-O(3)	0.54(16)
C(14)-N(2)-C(15)-N(1)	-2.11(16)
C(16)-N(2)-C(15)-N(1)	-179.78(9)
C(14)-N(2)-C(16)-C(17)	-90.02(13)
C(15)-N(2)-C(16)-C(17)	87.87(13)
N(2)-C(16)-C(17)-C(18)	85.17(13)
N(2)-C(16)-C(17)-C(22)	-94.11(13)
C(22)-C(17)-C(18)-C(19)	0.75(17)
C(16)-C(17)-C(18)-C(19)	-178.55(10)
C(17)-C(18)-C(19)-C(20)	-0.58(18)
C(23)-O(4)-C(20)-C(21)	173.18(10)
C(23)-O(4)-C(20)-C(19)	-6.82(16)
C(18)-C(19)-C(20)-O(4)	-179.76(10)
C(18)-C(19)-C(20)-C(21)	0.24(17)
O(4)-C(20)-C(21)-C(22)	179.91(10)
C(19)-C(20)-C(21)-C(22)	-0.09(17)
C(20)-C(21)-C(22)-C(17)	0.27(18)
C(18)-C(17)-C(22)-C(21)	-0.59(17)
C(16)-C(17)-C(22)-C(21)	178.71(10)

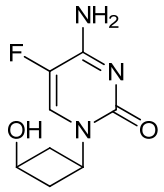
Symmetry transformations used to generate equivalent atoms:

5-fluoro-1-(*cis*-3-hydroxycyclobutyl)pyrimidine-2,4(1H,3H)-dione (61)



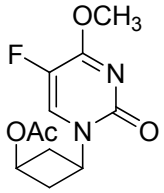
To a 10 mL flask with $AlCl_3$ (1.74 g, 13.0 mmol), dry anisole (5 mL) was added, and the solution became to red. To another 100 mL flask with compound **60** (1.07 g, 2.61 mmol) was added (15 mL) anisole, and the first one was added dropwise to the second one. After stirring 1 hr, anhydrous methanol (20 mL) was added at 0 ˚C till the solvent changed to white. After the solvent was evaporated under pressure, the residue was purified by flash column chromatography (CH_2Cl_2: MeOH = 8:1). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **61** (0.50 g, 95% yield) as white solid. Melting point: 179 – 180 ˚C; IR (cm^{-1}) 3320 (br), 3010 (br), 1624 (s), 1371 (s); ^1H NMR (600 MHz, CD_3OD) δ 2.08-2.13 (m, 2H), 2.74-2.78 (m, 2H), 3.29 (s, 3H), 4.02 (dt, 1H, J = 6.6, 7.2 Hz), 4.33 (dt, 1H, J = 8.4 Hz), 4.59 (br, 1H), 7.94 (d, 2H, J = 7.2 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 39.2, 43.5, 59.6, 115.4, 126.6, 139.8, 141.3, 150.1, 158.4; HRMS (FAB) *m/z* 201.0676, calcd for $C_8H_{10}O_3N_2F$ 201.0675 (M^+H). Anal. Calcd for $C_8H_{10}O_3N_2F$: C, 48.00; H, 4.53; N, 14.00; Found: C, 48.12; H, 4.56; N, 14.09.

4-amino-5-fluoro-1-(*cis*-3-hydroxycyclobutyl)pyrimidin-2(1H)-one (62)



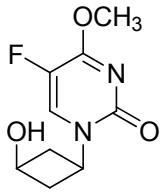
Compound **61** (92 mg, 0.46 mmol), 1-methylpyrrolidine (0.5 mL, 4.60 mol), chlorotrimethylsilane (0.19 mL, 1.38 mmol) and dry acetonitrile (20 mL) were stirred at r.t.. After 1 hr, the reactants were cooled to 0 ˚C and trifluoroacetic anhydride (0.35 mL, 2.30 mmol), was added dropwise over a period of 5 mins. After a further period of 30 mins at 0 ˚C, 4-nitrophenol (0.21 g, 1.38 mmol) was added and the cooled reactants were stirred for 3 hrs more. The products were then poured into saturated aqueous $NaHCO_3$ and the resulting mixture was extracted with dichloromethane. The combined organic extracts were dried and evaporated under reduced pressure. The residue was dissolved in dioxane and concentrated aqueous NH_3 was added. The reactants were heated in a sealed flask at 50 ˚C overnight. The resulting solution was concentrated and fractionated by column chromatography on silica gel (CH_2Cl_2: MeOH = 1:1) to afford compound **62** (35 mg, 38% yield). Melting point: 156 – 157 ˚C; IR (cm^{-1}) 3340 (br), 3210 (br), 1674, 1502, 1205, 1129; ^1H NMR (400MHz, CD_3OD) δ 1.97-2.07 (m, 2H), 2.73-2.84 (m, 2H), 4.03 (m, 1H), 4.31 (m, 1H), 7.87 (d, 2H, J = 6.4 Hz); ^{13}C NMR (100MHz CD_3OD) δ 39.5, 44.2, 59.6, 126.7, 127.0, 136.0, 138.4, 156.1; HRMS (FAB) *m/z* 200.0829, calcd for $C_8H_{11}O_2N_3F_1$ 200.0830 (M^+H).

cis-3-(5-fluoro-4-methoxy-2-oxopyrimidin-1(2H)-yl)cyclobutyl acetate (65)



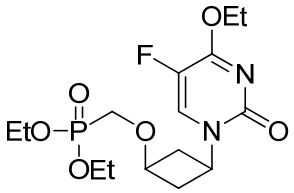
Acetic anhydride (1.6 mL, 16.25 mmol) was added to the ice-cooled solution of compound **62** (325 mg, 1.63 mmol) in pyridine (10 mL), and the mixture was stirred for overnight at r.t.. The solvent was then removed by pressure, and ethanol was added twice to co-evaporate the residue. The crude compound obtained (340 mg) was used to next step without further purification. The acetyl nucleoside (340 mg, 1.4 mmol) was dissolved in anhydrous acetonitrile (30 mL) and phosphorous oxychloride (0.39 mL, 4.2 mmol), 1-methylimidazole (1.12 mL, 14.0 mmol) was added dropwise at 0 ˚C, and then the reaction mixture was stirred at r.t. for 2 hrs. After the mixture was cooled in an ice bath, 1.8 mL of triethyl amine and 7 mL of methanol were added. The mixture was stirred overnight at r.t. and then evaporated. The crude was purified by column chromatography on silica gel (EtOAc: Hexane = 4:1) to afford compound **65** (290 mg, 83% yield). Melting point: 135 – 136 ˚C; IR (cm^{-1}) 1730, 1677, 1636, 1496, 1344, 1332, 1245; ^{1}H NMR (400MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.16 (m, 2H), 3.02 (m, 2H), 4.04 (s, 3H), 4.66 (m, 1H), 4.83 (m, 1H), 7.51 (d, 2H, J = 5.6 Hz); ^{13}C NMR (100MHz $CDCl_3$) δ 21.1, 37.5, 45.2, 55.4, 62.0, 127.6, 127.9, 135.7, 138.2, 154.2, 162.5, 170.4; HRMS (FAB) *m/z* 257.0929, calcd for $C_{11}H_{14}O_4N_2F_1$ 257.0932 (M^+H). Anal. Calcd for $C_{11}H_{13}O_4N_2F_{1:}$ C, 51.56; H, 5.11; F, 7.41; N, 10.93; Found: C, 51.60; H, 5.16; N, 10.87.

5-fluoro-1-(*cis*-3-hydroxycyclobutyl)-4-methoxypyrimidin-2(1H)-one (66)



The compound **65** (290 mg, 13.49 mmol) was dissolved in 10 mL of methanol and 4 mL 33% ammonia hydroxide was added. After stirring for 2 hrs at r.t., the solvent was then evaporated, and resultant was purified by column chromatography on silica gel (EtOAc) to afford compound **66** (280 mg, 95% yield). M. p. 124 - 126˚C; IR (cm^{-1}) 3381, 1666, 1631, 1538, 1491, 1409, 1333; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.09-2.16 (m, 1H), 2.90-2.97 (m, 2H), 4.03 (s, 3H), 4.12 (m, 1H), 4.51 (m, 1H), 7.63 (d, 2H, J = 5.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.2, 44.8, 55.3, 60.6, 128.7, 129.0, 135.8, 138.2, 154.6, 162.3, 162.4; HRMS (FAB) *m/z* 215.0824, calcd for $C_9H_{12}O_3N_2F_1$ 215.0827 (M^+H); Anal. Calcd for $C_9H_{11}O_3N_2F$ + 0.8 H_20: C, 47.29; H, 5.56; N, 12.25. Found: C, 47.44; H, 5.50; N, 12.32.

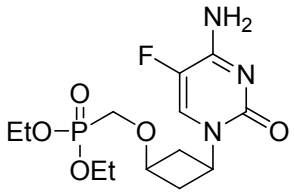
Diethyl- *cis*-3-(4-ethoxy-5-fluoro-2-oxopyrimidin-1(2H)-yl)cyclobutoxy) methylphosphonate (67)



A mixture of compound **66** (280 mg, 1.31 mmol) in 40 mL of anhydrous DMF was cooled to 0 ˚C and treated with sodium hydride (60%, 130 mg, 3.28mmol). After

10 mins, diethyl phosphonomethyl tosylate (1.02 g, 3.28 mmol) was added. The reaction mixture was stirred at r.t. for 3 days and quenched by adding methanol and acetic acid to neutralize the mixture. After the solvent was evaporated, the residue was purified by column chromatography on silica gel (EtOAc: MeOH = 9:1) to afford compound **67** (260 mg, 58% yield). IR (cm^{-1}) 1666, 1642, 1491, 1333，1030; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29-1.39 (m, 9H), 2.01-2.09 (m, 2H), 2.87-2.91 (m, 2H), 3.66 (d, 2H, J = 9.2), 3.91 (m, 1H), 4.13 (m, 4H), 4.47 (dd, 2H, J = 6.8), 4.61 (m, 1H), 7.59 (d, 1H, J = 5.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 16.7, 16.7, 37.4, 44.2, 61.9, 62.8, 63.6, 64.5, 69.2, 69.3, 127.8, 128.1, 135.8, 138.2, 154.4, 162.0, 162.1; HRMS (FAB) *m/z* 379.1427, calcd for $C_{15}H_{25}O_6N_2F_1P_1$ 379.1429 (M^+H).

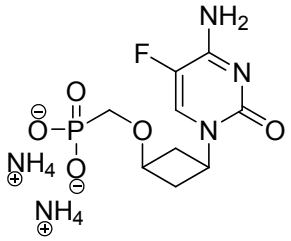
Diethyl ((*cis*)-3-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)cyclobutoxy) methylphosphonate (68)



A mixture of compound **67** (260 mg, 0.75 mmol) and 60 ml of 7 N ammonia in methanol was put into a sealed flask, and then stirred at 65 ˚C for overnight. After the reaction mixture was cooled to r.t., the solvent was removed under reduced pressure. The crude was purified by column chromatography on silica gel (EtOAc: MeOH = 9:1) to afford compound **67** (260 mg, 96% yield). Melting point: 79 – 80 ˚C; IR (cm^{-1}) 3317, 3183，1677, 1607, 1502, 1234, 1018; ^{1}H NMR (400 MHz,

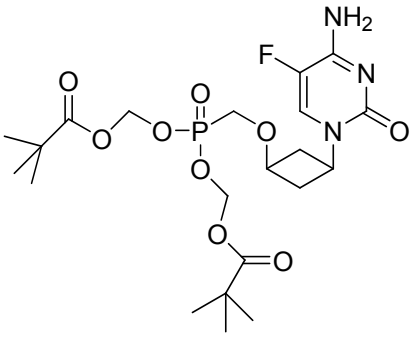
CD_3OD) δ 1.33 (t, 3H, J = 6.8 Hz), 2.14 (m, 2H), 3.79 (d, 2H, J = 9.2 Hz), 3.94 (m, 1H), 4.16 (m, 4H), 4.41 (m, 1H), 7.91 (d, 2H, J = 6.4 Hz); ^{13}C NMR (100 MHz CD_3OD) δ 15.6, 15.6, 36.6, 44.4, 60.7, 62.4, 62.9, 63.0, 69.1, 69.2, 126.7, 127.0, 136.0, 138.4, 156.1, 158.0, 158.1; HRMS (FAB) *m/z* 350.1274, calcd for $C_{13}H_{22}O_5N_3F_1P_1$ 350.1276 (M^+H).

ammonium ((*cis*)-3-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl) cyclobutoxy) methyl phosphonate (69)



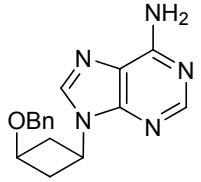
Compound **68** (35 mg, 0.11 mmol) was dissolved in 10 mL of anhydrous acetonitrile, and bromotrimethylsilane (0.14 mL, 1.1 mmol) was added dropwise. After the mixture was stirred at r.t. overnight, ammonia hydroxide was added to quench the reaction. After evaporating the solvent, the residue was diluted with water and methanol, and separated by preparative HPLC with C-18 reverse phase column (99% water in methanol- 50% water in methanol for 60 mins, retention time was 14 minute) to give product **69** (23 mg, 70%). Melting point: > 300 ˚C (dec); IR (cm^{-1}) 3066, 2879, 1690, 1607, 1450, 1059; ^1H NMR (400MHz, D_2O) δ 2.09-2.16 (m, 1H), 2.90-2.97 (m, 2H), 4.03 (s, 3H), 4.12 (m, 1H), 4.51 (m, 1H), 7.63 (d, 2H, J = 5.6 Hz); ^{13}C NMR (100MHz, D_2O) δ 40.2, 44.8, 55.3, 60.6, 128.7, 129.0, 135.8, 138.2, 154.6, 162.3, 162.4; HRMS (FAB) *m/z* 294.0648, calcd for $C_9H_{14}O_5N_3F_1P_1$ 294.0650 (M^+H).

(((*cis*-3-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)cyclobutoxy)methyl) phosphoryl)bis(oxy)bis(methylene) bis(2,2-dimethylpropanoate) (70)



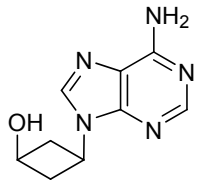
The salt **69** was neutralized with HOAc, and purified by preparative HPLC with C-18 reverse phase column (99% water in methanol- 50% water in methanol for 60 mins, retention time was 10 minute) to obtain the pure phosphonic acid to perform the prodrug synthesis. To a solution of 15 mg (0.05 mmol) of the phosphonic acid in 4 mL of anhydrous DMF were added *N,N*–dicyclohexyl-4-morpholine carboxamidine (29.3 mg, 0.10 mmol) and chloromethyl pivalate (0.037 mL, 0.25 mmol). The heterogeneous mixture became homogeneous after 15 mins and was stirred at r.t. for 36 hrs. After evaporating solvent, the residue was purified by silica gel chromatography eluting with 10% methanol in dichloromethane to give the product **70** (10 mg, 38% yield). Melting point: 130 – 131 ˚C; IR (cm^{-1}) 3340, 3206, 1753, 1677, 1146, 957; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (s, 18H), 2.01-2.09 (m, 2H), 2.79-2.85 (m, 2H), 3.75 (d, 2H, J = 8.8 Hz), 3.90 (dt, 1H, J = 6.8, 7.2 Hz), 4.57 (m, 1H), 5.69 (m, 6H), 7.49 (d, 2H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0, 37.4, 38.9, 43.4, 62.1, 63.8, 69.3, 69.5, 81.9, 81.9, 125.8, 126.1, 135.6, 138.1, 154.9, 157.7, 157.8, 177.1, HRMS (FAB) *m/z* 522.2008, calcd for $C_{21}H_{34}O_9N_3F_1P_1$ 522.2011 (M^+H).

9-(*cis*-3-(benzyloxy)cyclobutyl)-9H-purin-6-amine (71)



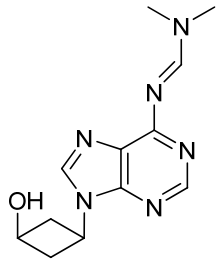
To adenine (0.54 g, 4.00 mmol) was added under argon 10 mL of dry DMF followed by compound **59** (0.85 g, 3.32 mmol), 18-crown-6 (1.05 g, 4.00 mmol) and K_2CO_3 (0.55 g, 4.00 mmol). After stirring at 120 ˚C overnight, the mixture was cooled and extracted by EtOAc three times. After washing by water and the organic solvent was evaporated, and then the residue was purified by flash column chromatography (CH_2Cl_2: MeOH = 9:1). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **60** (0.45 g, 46% yield) as white solid. Melting point: 161 – 162 ˚C; IR (cm^{-1}) 3325 (br), 2472 (br), 2070, 1617, 1119, 972; ^1H NMR (400 MHz, CD_3OD) δ 2.44-2.53 (m, 2H), 2.78-2.87 (m, 2H), 3.93 (m, 1H), 4.39 (s, 2H), 4.53 (m, 1H), 7.08 – 7.25 (m, 5H), 8.11 (s, 1H), 8.21 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 39.4, 43.1, 44.4, 68.0, 71.8, 95.2, 77.8, 95.2, 103.2, 129.0, 129.3, 129.6, 142.2; HRMS (FAB) *m/z* 296.1506, calcd for $C_{16}H_{18}N_5O$ 296.1508 (M^+H).

cis-3-(6-amino-9H-purin-9-yl)cyclobutanol (72)



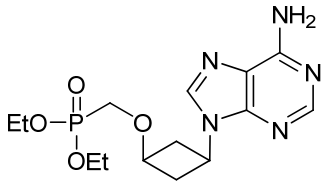
In a 25 mL flask with 9-(*cis*-3-(benzyloxy)cyclobutyl)-9H-purin-6-amine **71** (1.00 g, 3.40 mmol) inside, anhydrous CH_2Cl_2 10 mL was added to give a white emulsion under argon. This flask was then cooled to –78 ^oC and after 10 mins, BCl_3 (1.0 M in CH_2Cl_2, 17 mL, 17 mmol) was added dropwise. The reaction mixture was allowed to stir at -78 $^\circ C$ for 6 hrs, and then quenched by adding 7N NH_3 in MeOH (5 mL) dropwise. The mixture was concentrated under reduced pressure and was purified by silica gel flash chromatography (CH_2Cl_2: MeOH = 5:1) to give the desired product **72** (500 mg, 75%) as white solid. Melting point: 228 – 230 $^\circ C$; IR (cm^{-1}) 3325 (br), 2467, 2362, 2070, 1618, 1120, 957; ^1H NMR (400 MHz, CD_3OD) δ 2.49-2.54 (m, 2H), 2.94-2.99 (m, 2H), 4.17 (m, 1H), 4.56 (m, 1H), 8.17 (s, 1H), 8.23 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 29.3, 41.1, 59.5, 70.0, 119.2, 140.1, 149.6, 152.1, 155.8; HRMS (FAB) *m/z* 206.1034, calcd for $C_9H_{12}O_1N_5$ 206.1036 (M^+H). Anal. Calcd for $C_9H_{11}O_1N_5$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.60; H, 5.50; N, 34.10.

N'-(9-(cis-3-hydroxycyclobutyl)-9H-purin-6-yl)-N,N-dimethylformimidamide (73)



To the *cis*-3-(6-amino-9H-purin-9-yl) cyclobutanol **72** (0.30 g, 1.46 mmol) was added under argon 10 mL of anhydrous DMF followed by 1,1-diethoxy-N,N-dimethylmethanamine (0.66 g, 4.38 mmol). After stirring at r.t. overnight, the mixture was concentrated, and the residue was purified by flash column chromatography (CH_2Cl_2: MeOH = 95:5). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **73** (430 mg, 94% yield) as white solid. Melting point: 137 – 138 ˚C; IR (cm^{-1}) 3329 (br), 3189 (br), 2920, 1648, 1601, 1100; ^1H NMR (400MHz, $CDCl_3$) δ 2.51-2.60 (m, 2H), 2.93-3.10 (m, 2H), 3.10 (s, 3H), 3.13 (s, 1H), 4.20 (dt, 1H, J = 6.8, 7.2 Hz), 4.52 (dt, 1H, J = 8.0, 8.4 Hz), 7.89 (s, 1H), 8.42 (s, 1H), 8.85 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 35.3, 41.1, 41.5, 42.2, 61.2, 70.6, 126.6, 140.8, 151.5, 152.3, 158.5, 159.9; HRMS (FAB) *m/z* 257.0841, calcd for $C_{12}H_{17}O_4S_1$ 257.0842 (M^+H).

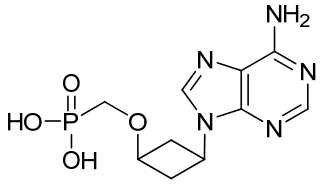
diethyl(cis-3-(6-(dimethylamino)methyleneamino)-9H-purin-9-yl) cyclobutoxy)methylphosphonate (74)



A mixture of compound **73** (100 mg, 0.35 mmol) in anhydrous DMF (30 mL) was cooled to 0 ˚C and treated with sodium hydride (60%, 41.7 mg, 1.05 mmol). After 10 mins, diethyl phosphonomethyl tosylate (270 mg, 1.05 mmol) was added. The reaction mixture was stirred at r.t. for 3 days and quenched by adding methanol and acetic acid to neutralize the mixture. After the solvent was evaporated, the residue was purified by column chromatography on silica gel (EtOAc/MeOH 9:1) to afford the intermediate (260 mg, 58% yield) as white solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.34 (m, 6H), 2.58 (m, 2H), 2.05 (m, 2H), 3.75 (d, 2H, J = 9.0), 3.07 (m, 1H), 4.17 (m, 4H), 4.67 (m, 1H), 8.0 (s, 1H), 8.51 (s, 1H), 8.96 (s, 1H); A mixture of intermediate (45 mg, 0.13 mmol) and 60 mL 7 N ammonia in methanol was put into a sealed flask, and then stirred at 65 ˚C overnight. After the reaction mixture was cooled to r.t., the solvent was removed by reduced pressure to afford the compound **74** as a white solid (41 mg, 91% yield). Melting point: 130-131 ˚C; IR (cm^{-1}) 3334 (br), 3171 (br), 2978, 1643, 1596, 1240, 1018; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (m, 6H), 2.41-2.44 (m, 2H), 2.83-2.87 (m, 2H), 3.61 (d, 2H, J = 9.2 Hz), 3.88 (dt, 1H, J = 6.8, 7.2 Hz), 4.00 (m, 4H), 4.83 (m, 1H), 6.86 (s, 2H), 7.83 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.56, 16.61, 38.2, 40.6, 61.7,

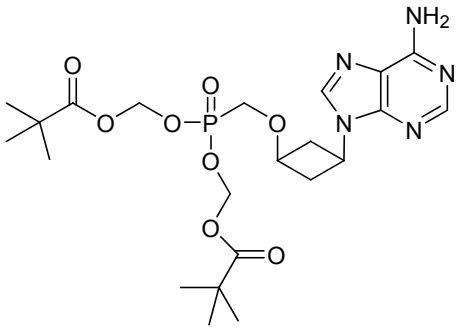
62.7, 62.8, 63.3, 69.3, 69.4, 119.8, 138.6, 149.9, 152.9, 156.3, 164.1; HRMS (FAB) *m/z* 356.1480, calcd for $C_{14}H_{23}O_4N_5P$ 356.1482 (M^+H).

ammonium (*cis* -3-(6-amino-9H-purin-9-yl)cyclobutoxy)methylphosphonate (75)



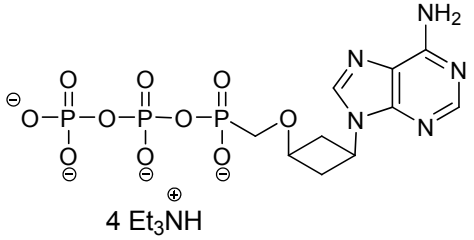
Compound **74** was dissolved in 10 mL anhydrous acetonitrile, and bromo trimethylsilane (0.17 mL, 1.3 mmol) was added dropwise. After the mixture was stirred at r.t. overnight, ammonia hydroxide was added to quench the reaction. After evaporating the solvent, the residue was diluted by water and methanol, and separated by preparative HPLC with C-18 reverse phase column (99% water in methanol- 50% water in methanol 60 mins, retention time was 18 mins) to give product **75** (30 mg, 70%). Melting point: 130 – 131 ˚C; IR (cm^{-1}) 3340, 3206, 1753, 1677, 1146, 957; ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (s, 18H), 2.01-2.09 (m, 2H), 2.79-2.85 (m, 2H), 3.75 (d, 2H, J = 8.8 Hz), 3.90 (dt, 1H, J = 6.8, 7.2 Hz), 4.57 (m, 1H), 5.69 (m, 6H), 7.49 (d, 2H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0, 37.4, 38.9, 43.4, 62.1, 63.8, 69.3, 69.5, 81.9, 81.9, 125.8, 126.1, 135.6, 138.1, 154.9, 157.7, 157.8, 177.1, HRMS (FAB) *m/z* 300.0855, calcd for $C_{10}H_{15}O_4N_5P_1$ 300.0856 (M^+H). Anal. Calcd for $C_{10}H_{15}O_4N_5P_1$ + 0.3 H_2O: C, 39.43; H, 4.83; N, 22.99. Found: C, 39.24; H, 4.85; N, 23.12.

(((*cis*-3-(6-amino-9H-purin-9-yl)cyclobutoxy)methyl)phosphoryl)bis(oxy) bis(methylene) bis(2,2-dimethylpropanoate) (76)



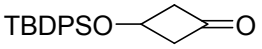
To a solution of **75** (61 mg, 0.22 mmol) of the phosphonic acid in a 4 ml of anhydrous DMF were added *N,N*–dicyclohexyl-4-morpholine carboxamidine (0.20 mg, 0.66 mmol) and chloromethyl pivalate (0.16 mL, 1.10 mmol). The heterogeneous mixture became homogeneous after 15 mins and was stirred at r.t. for 36 hr. After evaporating solvent, the residue was purified by silica gel chromatography eluting with 15% methanol in dichloromethane to give the product **76** (43 mg, 42% yield). Melting point: 148 - 149 ˚C; IR (cm^{-1}) 3329, 3165, 1747, 1642, 1590, 1251, 965; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 12H), 2.60 (m, 2H), 3.02 (m, 2H), 3.83 (d, 2H, J = 8.8 Hz), 4.08 (m, 1H), 4.65 (m, 1H), 5.72 (dt, 1H, J = 5.2, 7.2 Hz), 5.90 (br, 2H), 8.00 (s, 1H), 8.32 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.1, 38.5, 39.0, 40.6, 62.2, 63.8, 69.5, 69.6, 81.9, 82.0, 120.1, 139.0, 150.3, 152.7, 155.4, 177.1; HRMS (FAB) *m/z* 528.2210, calcd for $C_{22}H_{35}O_8N_5P_1$ 528.2218 (M^+H). Anal. Calcd for $C_{22}H_{34}O_8N_5P_1$: C, 50.09; H, 6.50; N, 13.28. Found: C, 50.01; H, 6.57; N, 13.33.

(*cis*-3-(6-amino-9H-purin-9-yl)cyclobutoxy)methylphosphonic diphosphoric anhydride triethylammonium salt (77)



Compound **74** (30.0 mg, 0.10 mmol) and tributylamine in water (10 mL) were mixed and the solvent was coevaporated under reduced pressure twice. The residue was repeatedly evaporated with anhydrous ethanol and toluene. To the resulting powder were added DMF (4 mL) and 1,1'-carbonyldiimidazole (107 mg, 0.66 mmol). After stirring at r.t. for 3.5 hr, the reaction mixture was quenched by addition of methanol (40 mL). Bis(tri-n-butyl ammonium)-pyrophosphate (264.2 mg, 0.58 mmol) was added and the stirring was continued for 16 hr. The reaction was quenched by adding 5 mL 0.1 M TEAB and this was directly applied to the DEAE-sephadex dianion exchange column (11 mm X 220 mm, eluent from 0.1 M TEAB to 0.7 M TEAB). After analyzing the fractions by HPLC with C-18 reverse phase column (250 mm X 4.6 mm), all the products were collected and lyophilized to give the triethylammonium salt of 4'-O-triphosphate of (*cis* -3-(6-amino-9H-purin-9-yl)cyclobutoxy)methylphosphonate **77** (345 mg, 40% yield) as a white solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.06 (m, 44H), 2.32 (m, 2H), 2.95 (m, 30H), 3.61 (d, 2H, J = 8.8 Hz), 4.00 (dt, 1H, J = 6.8, 7.2 Hz), 4.34 (dt, 1H, J = 7.6, 9.2 Hz), 7.90 (s, 1H), 8.10 (s, 1H); ^{31}P NMR (D_2O, 162 MHz): δ 8.4 (αP, d, 1P, J = 48.9 Hz), -10.4 (γP, dd, 1P, J = 22.0, 199.1 Hz), -2 (βP, dt, 1P, J = 21.4, 20.1, 5.5 Hz); HRMS (FAB) *m/z* 458.0033, calcd for $C_{10}H_{15}O_{10}N_5P_3$ 458.0026 (M^-H).

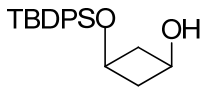
3-(tert-butyldiphenylsilyloxy)cyclobutanone (83)



A 2.4 eq portion of n-butyllithium (1.6 M in hexane, 121 mL) was added dropwise to a solution containing 2.4 eq of methyl (methylthio)methylsulfoxide (20.5 mL, 0.19 mol) in 400 mL of tetrahydrofuran at -10 ˚C. The reaction mixture was stirred at -10 ˚C for 2 hrs and then cooled to -78 ˚C. The yellow reaction mixture was maintained at -78 ˚C as a 1 eq portion of the dibromo compound (36.50 g, 0.08 mol) in 85 mL of tetrahydrofuran was added dropwise. The reaction mixure was allowed to warm to room temperature overnight. The reaction mixure was added to brine and extracted twice with ethyl acetate. The combined organic layers were subjected to the usual workup to provide 30 mL of dark red-brown liquid. This mixture of syn- and anti-dithioketal S-oxide intermediates was purified in three portions via silica gel column chromatography. Less polar impurities were eluted first with 3:7 ethyl acetate: hexane followed by elution of product with pure ethyl acetate. A total of 14.0 g of intermediate was obtained in this manner. The syn- and anti-dithioketal S-oxide intermediates were dissolved in 300 mL of diethyl ether and treated with 18 mL of 35% perchloric acid. After overnight stirring, the reaction mixure was neutralized with sodium bicarbonate followed by purification via silica gel column chromatography (ethyl acetate: hexane = 15:85), providing the ketone (11.6 g, 45% yield) as an orange-yellow liquid. IR (cm^{-1}) 3329 (br), 3189 (br), 2920, 1648, 1601, 1100; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 9H), 3.13 (m, 4H), 4.58 (m, 1H), 7.39 (m, 6H), 7.64 (m, 4H); ^{13}C NMR (100

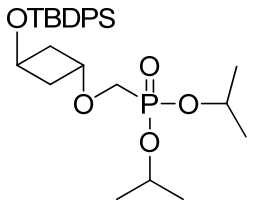
MHz, $CDCl_3$) δ 19.2, 26.9, 57.3, 59.3, 138.1, 130.2, 133.5, 135.7, 206.1; HRMS (FAB) *m/z* 325.1575, calcd for $C_{20}H_{25}O_2Si$ 325.1582 (M^+H).

cis-3-(tert-butyldiphenylsilyloxy)cyclobutanol (84)



A solution of lithium tri-sec-butylborohydride (1.0 M in THF, 23.5 mL, 0.023 mmol) in THF was added under argon and dropwise over a period of 10 mins to a stirred solution of cyclobutanone **83** (4.0 g, 0.02 mmol) in dry THF (20 mL) at -78 ˚C. The product was then allowed to warm up to r.t. and saturated $NaHCO_3$ (8 mL) was added over a period of 2 mins. The result solution was then cooled and 30% aqueous hydrogen peroxide (3 mL) was added dropwise at such a rate so as to maintain the temperature at 25 - 30 ˚C, water and ethyl acetate were then added. The organic layer was separated, washed with water, dried and evaporated under rotary-vap to give the crude alcohol, which was purified by flash column chromatography (ethyl acetate: hexane = 1:4). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **84** (3.8 g, 95% yield) as colorless oil. IR (cm^{-1}) 3311 (br), 2926, 2844, 1111, 1053, 675; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 9H), 2.01 (m, 2H), 2.58 (m, 2H), 3.66-3.83 (m, 2H), 7.38 (m, 6H), 7.64 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.2, 26.9, 44.7, 59.6, 59.8, 127.8, 129.8, 134.2, 135.7; HRMS (FAB) *m/z* 342.1976, calcd for $C_{20}H_{30}O_2Si$ 342.1980 (M^+H).

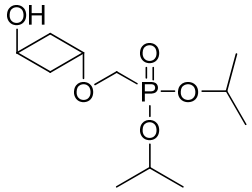
diisopropyl (*trans*-3-(tert-butyldiphenylsilyloxy)cyclobutoxy)methyl phosphonate (86)



To a 100 mL flask with *cis*-3-(tert-butyldiphenylsilyloxy)cyclobutanol **84** (3.07 g, 9.47 mmol), 4-nitrobenzoic acid (3.16 g, 18.9 mmol) and Ph_3P (5.21 g, 19.9 mmol) inside, dry THF (25 mL) was added under argon. Then the reaction mixture was cooled to 0 ˚C and DIAD (3.9 mL, 20 mmol) was added drop by drop to give a yellow solution. This mixture was allowed to warm up to room temperature gradually and left stirring for 10 hrs, after which time the solvent was removed and applied directly to the silica gel flash chromatography (Hexane: EtOAc = 20:1) to give the desired product with a little impurity and then was redissolved in 1,4-dioxane (6.6 mL). This mixture was treated with aqueous NaOH (0.4 mol/L, 4.3 mL, 1.7 mmol) at room temperature. After 30 mins, 0.07 mL of AcOH was added and the products were concentrated to small volume under reduced pressure. The residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$ and solvent evaporation gave the crude product (3.00 g) that was used in the next reaction without further purification. The crude compound was dissolved in 20 mL DMF, lithium tert-butoxide (0.83 g, 10 mmol) was added, and the resulting mixture was stirred for 10 mins. To the mixture was added diisopropyl bromomethyl phosphonate (2.40 g, 9.26 mmol) and then the temperature was raised to 50 ˚C and the mixture was stirred overnight. DMF was

removed under reduced pressure, and the residue was purified by flash column chromatography (ethyl acetate: hexane = 1:2). The appropriate fractions were combined, and the solvent was removed in vacuo to give product **86** (4.0 g, 86% yield) as colorless oil. IR (cm^{-1}) 2978, 2932, 2850, 1240, 1111, 983, 691; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 9H), 1.24 (m, 12H), 2.21 (dd, 4H, J = 4.8, 6.0 Hz), 3.51 (d, 2H, J = 9.2 Hz), 4.18 (dt, 1H, J = 5.2, 10.0 Hz) 4.48 (dt, 1H, J = 6.4, 12.8 Hz), 4.68 (m, 1H), 7.36 (m, 6H), 7.59 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 19.2, 21.3, 24.1, 24.2, 24.3, 27.0, 39.7, 60.6, 62.4, 64.1, 66.3, 71.7, 71.2, 73.1, 73.3, 127.8, 129.8, 134.2, 135.6; HRMS (FAB) *m/z* 505.2535, calcd for $C_{27}H_{42}O_5PSi$ 505.2534 (M^+H).

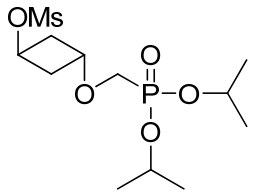
diisopropyl (*trans*-3-hydroxycyclobutoxy)methylphosphonate (87)



The diisopropyl (*trans*-3-(tert-butyldiphenylsilyloxy)cyclobutoxy)methyl phosphornate **86** (4.00 g, 7.98 mmol) was dissolved in methanol (50 mL), and ammonium fluoride (1.18 g, 31.9 mmol) was added to the solution. The reaction mixture was heated to reflux for overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give product (1.80 g, 85%). IR (cm^{-1}) 3311 (br), 3399 (br), 2978, 2938, 1246, 1106, 989; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26

(m, 12H), 2.11 (m, 2H), 2.26 (m, 2H), 3.53 (d, 2H, J = 9.6 Hz), 4.17 (m, 1H), 4.43 (br, 1H), 4.68 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.1, 24.1, 24.2, 39.2, 62.6, 63.8, 64.4, 71.3, 71.4, 73.4, 73.5; HRMS (FAB) *m/z* 267.1358, calcd for $C_{11}H_{24}O_5P_1$ 267.1356 (M^+H).

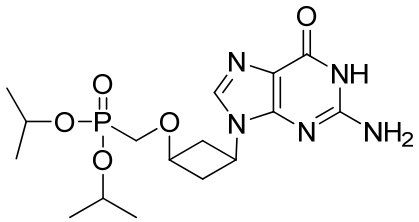
trans-3-((diisopropoxyphosphoryl)methoxy)cyclobutyl methanesulfonate (91)



To a 50 mL flask with diisopropyl(*trans*-3-hydroxycyclobutoxy)methyl phosphonate **87** (1.20 g, 4.51 mmol) inside, dry CH_2Cl_2 was added to give a clear solution under argon. Then Et_3N (1.00 mL, 6.77 mmol) was then added to the above solution. After 10 mins, the reaction mixture was cooled to 0 ˚C, MsCl (0.53 mL, 6.77 mmol) was added dropwise and the mixture was left stirring with the temperature going up to room temperature gradually. After 3 hrs, the reaction was quenched by adding H_2O, and then the organic phase was separated, washed with brine once and dried over $MgSO_4$. Solvent evaporation gave the crude product that was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give product (2.0 g, 93% yield) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (m, 12H), 2.48 (t, 4H, J = 5.4, 6.0 Hz), 2.93 (s, 3H), 3.54 (d, 2H, J = 9.6 Hz), 4.26 (dt, 1H, J = 4.8, 5.4 Hz), 4.69 (m, 1H), 5.13 (dt, 1H, J = 6.6, 6.0 Hz); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 24.1, 24.2, 24.3, 37.6, 38.3, 63.0, 64.1, 71.3, 71.4, 72.7, 72.8, 73.2; HRMS (FAB) *m/z* 345.1095, calcd for $C_{12}H_{26}O_7SP_1$ 345.1094 (M^+H).

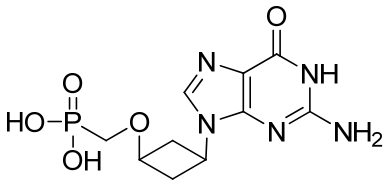
diisopropyl (*cis*-3-(2-amino-6-oxo-1H-purin-9(6H)-yl)cyclobutoxy)methyl phosphonate (93)



To the O^6-benzylguanine (368 mg, 1.53 mmol) was added 10 mL of dry DMF under argon followed by *trans*-3-((diisopropoxyphosphoryl)methoxy)cyclobutyl methanesulfonate **91** (350 mg, 1.02 mmol), Cs_2CO_3 (488 mg, 1.53 mmol). After stirring at 120 ˚C overnight, the mixture was cooled and solvent was evaporated. The residue was purified by flash column chromatography (MeOH: CH_2Cl_2 = 15:85). The appropriate fractions were combined, and the solvent was removed in vacuo to give product (220 mg, 44% yield) as white solid. The compound was then dissolved in 20 mL of 95% methanol, and was hydrogenated by hydrogen gas of 50 psi gas pressures in the presence of 5 % Pd/C (20 mg) in a hydrogenator. After 3 hrs, the mixture was filtered and concentrated to dryness to give the product as white solid (198 mg, 98 % yield). Melting point: 201-202 ˚C, ^{1}H NMR (400 MHz, CD_3OD) δ 1.33 (d, 12H, J = 6.4 Hz), 2.54 (m, 2H), 2.92 (m, 2H), 3.76 (d, 2H, J = 8.8 Hz), 4.04 (dt, 1H, J = 4.8, 6.4 Hz), 4.46 (m, 3H), 4.62 (m, 2H), 7.85 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 23.1, 37.4, 40.7, 61.5, 63.2,

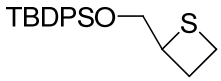
69.3, 69.5, 72.0, 72.1, 116.7, 136.8, 151.9, 153.9, 158.4; HRMS (FAB) *m/z* 400.1690, calcd for $C_{16}H_{27}O_5N_5P$ 400.1697 (M^+H).

(*cis*-3-(2-amino-6-oxo-1H-purin-9(6H)-yl)cyclobutoxy)methylphosphonic acid (94)



The diisopropyl (*cis*-3-(2-amino-6-oxo-1H-purin-9(6H)-yl)cyclobutoxy)methyl phosphonate (80 mg, 0.20 mmol) was dissolved in (10 mL) anhydrous acetonitrile, and bromo trimethylsilane (0.14 mL, 1.1 mmol) was added dropwise. After the mixture was stirred at r.t. overnight, water was added to quench the reaction. After evaporating the solvent, the residue was diluted by water and methanol, and separated by preparative HPLC with C-18 reverse phase column (99% water in methanol- 50% water in methanol for 60 mins, retention time was 15 mins) to give product **94** (50 mg, 70%); Melting point: > 200 ˚C (dec); IR (cm^{-1}) 3055 (br), 1689, 1409, 1117, 1047; ^1H NMR (400 MHz, D_2O) δ 2.34 (m, 2H), 2.85 (m, 2H), 3.55 (d, 2H, J = 9.6 Hz), 3.95 (dt, 1H, J = 4.8, 6.4 Hz), 4.42 (m, 1H), 8.82 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 36.9, 42.9, 62.3, 63.9, 69.0, 69.1, 107.8, 136.3, 150.0, 155.1, 155.3; HRMS (FAB) *m/z* 316.0805, calcd for $C_{10}H_{15}O_5N_5P$ 316.0811 (M^+H).

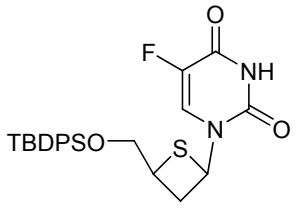
tert-butyldiphenyl(thietan-2-ylmethoxy)silane (99)



The 1,2,4-butanetriol (2.2 g, 20 mmol) and catalyst TsOH (10 mg) was dissolved in acetone (100 mL), and the reaction mixture was refluxed for 2 hrs. After evaporation, the residue was dissolved in 100 mL dichloromethanol and the solution was cooled to 0 ˚C. MsCl (1.94 mL, 24 mmol) and triethylamine (3.46 mL, 24 mmol) were added to the solution, and the mixture was stirred for 3 hrs. After extraction, and evaporation of the solvent, the crude product was dissolved in 0.5 N HCl solution (30 mL) and methanol (100 mL). After stirring for 30 mins, the solvent was evaporated under reduced pressure. The residue was repeatly evaporated with anhydrous ethanol and toluene. To the resulting oil was added dichloromethane (50 mL) and triethylamine (3.46 mL, 24 mmol), and then cooled to 0 ˚C. TBDPSCl (5.12 mL, 20 mmol) was added dropwise to the solution, and then the mixture was stirred at r.t. overnight. After extraction, and concentration, the residue was purified by silica gel chromatography eluting with 1:1 ethyl acetate and hexane to give the intermediate **98** (5.74 g, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 9H), 1.76-2.87 (m, 2H), 2.40 (br, 1H), 2.95 (s, 3H), 3.50 (m, 1H), 3.63 (m, 1H), 3.88 (m, 1H), 4.37-4.45 (m, 2H), 7.40 (m, 6H), 7.65 (m, 4H); To the compound **98** (4.8 g, 11 mmol) was added 50 mL of dichloromethane and triethylamine (2.38 mL, 16.5 mmol), and the mixture was cooled to 0 ˚C, MsCl (1.28 mL, 16.5 mmol) was added dropwise. After stirring at r.t. overnight, water was added to quench the reaction. After extraction, evaporation, the crude dimesylate would be used in the next reaction without further purification. To the

solution of the 5.00 g crude dimesylate in 100 mL of ethanol, Na_2S (1.17 g, 15.00 mmol) was added, and then the mixture was refluxed for 20 hrs. After cooling the mixture, the solvent was evaporated, extracted by ether to give the crude product, which was purified by silica gel chromatography eluting with 1:9 ethyl acetate and hexane to give the thietane compound **99** as colorles oil. IR (cm^{-1}) 2920, 2851, 1426, 1094, 703; ^1H NMR (400 MHz, $CDCl_3$) 1.05 (s, 9H), 2.63 (m, 1H), 2.92 (m, 1H), 3.03 (m, 1H), 3.13 (m, 1H), 3.71- 3.90 (m, 3H), 7.39 (m, 6H), 7.67 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 22.4, 27.0, 29.8, 42.0, 69.3, 127.9, 129.9, 133.8, 135.8; HRMS (FAB) *m/z* 343.1547, calcd for $C_{20}H_{27}O_1S_1Si_1$ 343.1546 (M^+H).

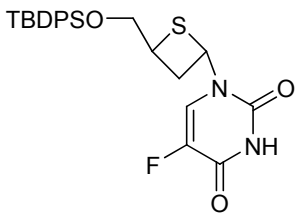
1-*cis* - (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidine -2,4(1H,3H)-dione (103)



A solution of compound **99** (0.15 g, 0.41 mmol) in CH_2Cl_2 was treated with a solution of 77% mCPBA (0.092 g, 0.41 mmol) in CH_2Cl_2 dropwise at -78 °C. The mixture was stirred at -78 °C for 30 mins. The reaction was quenched with aqueous $Na_2S_2O_3$, diluted with CH_2Cl_2, washed with aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. The filtrate was concentrated to dryness. The crude sulfoxide **100** was used for the next reaction without further purification. A mixture of uracil (0.16 g, 1.23 mmol) in acetonitrile (10 mL) and

HMDS (10 mL) was refluxed for 3 hrs until the solution became clear. After evaporation, the residue was treated successively with a solution of the crude sulfoxide in toluene (10 mL), triethylamine (0.11 mL, 0.79 mmol), TMSOTf (0.16 mL, 0.88 mmol), and ZnI_2 (0.039 g, 0.12 mmol) at 0 °C. After 24 hrs, the reaction was quenched with water at 0 °C, and the reaction mixture was diluted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified with 3:7 EtOAc and hexane by silica gel chromatography to give the product **103** (0.023 g, 0.045 mmol, 11% yield) and **107** (0.023 g, 0.045 mmol, 11%) as a *cis*/*trans* ratio 1:1. Melting point: 176 - 178 ˚C; IR (cm^{-1}) 2920, 2851, 1676, 1603, 1073, 703; ^1H NMR (400 MHz, $CDCl_3$) 1.08 (s, 9H), 2.94 (m, 1H), 3.15 (m, 1H), 3.59 (m, 1H), 3.92 (m, 2H), 6.29 (m, 1H), 7.44 (m, 6H), 7.67 (m, 4H), 8.22 (dd, 1H, J = 3.6, 6.0 Hz), 9.88 (d, 1H, J = 4.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 27.0, 36.6, 38.3, 52.1, 68.2, 124.6, 125.0, 128.1, 130.2, 133.3, 135.9, 139.8, 142.2, 149.4, 156.9, 157.2; HRMS (FAB) *m/z* 471.1569, calcd for $C_{24}H_{28}O_3N_2FSSi$ 471.1569 (M^+H).

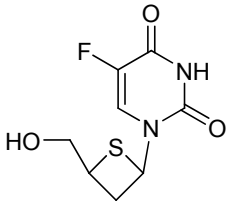
1-*trans* - (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidine -2,4(1H,3H)-dione (107)



The *trans* isomer **107**. Melting point: 154 - 156 ˚C; IR (cm^{-1}) 2910, 2851, 1675, 1600, 1073, 703; ^1H NMR (400 MHz, $CDCl_3$) 1.05 (s, 9H), 2.81 (m, 1H), 3.21 (m,

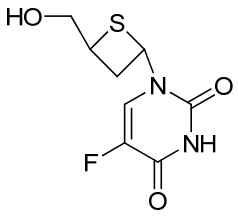
1H), 3.64-3.78 (m, 2H), 6.26 (m, 1H), 7.44 (m, 6H), 7.67 (m, 4H), 8.22 (dd, 1H, J = 2.8, 6.0 Hz), 9.70 (d, 1H, J = 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 27.1, 35.3, 38.0, 48.9, 67.0, 124.8, 125.1, 128.1, 128.1, 130.2, 130.3, 133.0, 135.8, 139.6, 141.9, 149.4, 156.8, 157.1, 171.5; HRMS (FAB) *m/z* 471.1569, calcd for $C_{24}H_{28}O_3N_2F_1S_1Si_1$ 471.1569 (M^+H).

5-fluoro-1-(*cis*-4-(hydroxymethyl)thietan-2-yl)pyrimidine-2,4(1H,3H)-dione (101)



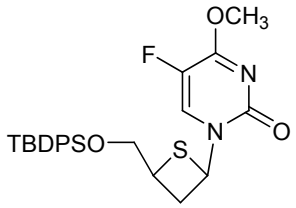
The 1-*cis* - (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidine - 2,4(1H,3H)-dione **103** (100 mg, 0.21 mmol) was dissolved in methanol (20 mL), and ammonium fluoride (38 mg, 1.05 mmol) was added to the solution. The reaction mixture was heated to reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give the desired product (45 mg, 90% yield). Melting point: 163 - 164 ˚C; IR (cm^{-1}) 3340 (br), 1695, 1660, 1251, 1012; ^{1}H NMR (400 MHz, CD_3OD) 2.83 (m, 1H), 2.94 (m, 1H), 3.44 (m, 2H), 3.61 (m, 2H), 6.04 (m, 1H), 8.14 (dd, 1H, J = 1.2, 6.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 35.5, 37.3, 64.8, 125.6, 126.1, 139.6, 141.9, 149.4, 158.1, 158.9; HRMS (FAB) *m/z* 231.0245, calcd for $C_8H_8O_3N_2FS$ 231.0234 (M^-H).

5-fluoro-1-(*trans*-4-(hydroxymethyl)thietan-2-yl)pyrimidine-2,4(1H,3H)-dione (102)



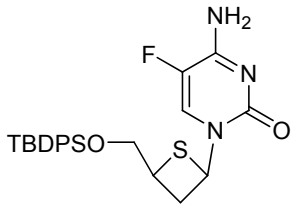
The 1-*trans* - (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidine -2,4(1H,3H)-dione **107** (100 mg, 0.21 mmol) was dissolved in methanol (20 mL), and ammonium fluoride (38 mg, 1.05 mmol) was added to the solution. The reaction mixture was heated to reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give the desired product (42 mg, 88%). Melting point: 173 - 174 ˚C; IR (cm^{-1}) 3434 (br), 3078 (br), 1695, 1660, 1257; ^{1}H NMR (400 MHz, CD_3OD) 2.96 (m, 1H), 3.26 (m, 2H), 3.66 (m, 3H), 6.17 (m, 1H), 8.71 (d, 1H, J = 6.4 Hz); ^{13}C NMR (100MHz, D_2O) δ 35.5, 36.8, 64.8, 125.6, 126.0, 139.4, 141.7, 149.7, 158.1, 158.3; HRMS (FAB) *m/z* 231.0245, calcd for $C_8H_8O_3N_2F_1S_1$ 231.0234 (M^-H).

1-(*cis*-4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoro-4-methoxy pyrimidin-2(1H)-one (104)



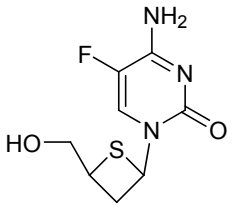
The 5-fluoro-1-(*cis*-4-(hydroxymethyl)thietan-2-yl)pyrimidine-2,4(1H,3H)-dione **103** (220 mg, 0.45 mmol) was dissolved in anhydrous acetonitrile (20 mL) and phosphorous oxychloride (0.12 mL, 1.36 mmol), 1-methylimidazole (0.36 mL, 4.50 mmol) were added dropwise at 0 ˚C, and then the reaction mixture was stirred at r.t. for 2 hrs. After the mixture was cooled in an ice bath, 0.6 mL of triethylamine and 2.2 mL of methanol were added. The mixture was stirred overnight at r.t. and then evaporated. The crude residue was purified by column chromatography on silica gel (EtOAc: Hexane = 1:1) to afford the methyl protected nucleoside **104** (163 mg, 72% yield). Melting point: 135 - 136 ˚C; IR (cm^{-1}) 2943, 2850, 1689, 1491, 1106; ^{1}H NMR (400 MHz, $CDCl_3$) 1.06 (s, 9H), 2.84 (m, 1H), 3.21 (m, 2H), 3.60 (m, 1H), 3.91 (m, 2H), 4.04 (s, 3H), 6.24 (m,1H), 7.47 (m, 6H), 7.66 (m, 4H), 8.39 (d, 1H, J = 5.6 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.5, 27.0, 36.9, 38.7, 53.5, 55.5, 68.4, 128.0, 128.2, 128.5, 130.1, 133.3, 135.8, 138.3, 153.9, 162.7, 162.8; HRMS (FAB) *m/z* 485.1722, calcd for $C_{25}H_{30}O_3FSSi$ 485.1725 (M^+H).

4-amino-1-*cis*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidin-2(1H)-one (105)



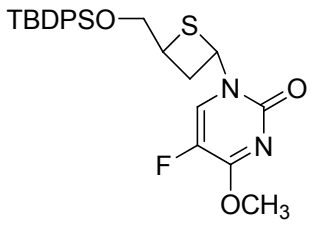
A mixture of compound **104** (140 mg, 0.28 mmol) and 60 mL of 7 N ammonia in methanol was put into a sealed flask, and then stirred at 65 ˚C overnight. After the reaction mixture was cooled to r.t., the solvent was removed by reduced pressure. The crude residue was purified by column chromatography on silica gel (EtOAc: MeOH = 9:1) to afford compound **105** (122 mg, 96% yield). Melting point: 147 - 149 ˚C; IR (cm^{-1}) 3311 (br), 2932, 2850, 1683, 1514, 1111, 703; ^{1}H NMR (400 MHz, $CDCl_3$) 1.06 (s, 9H), 2.84 (m, 1H), 3.15 (m, 2H), 3.60 (m, 1H), 3.90 (m, 2H), 6.19 (br, 1H), 6.26 (m, 1H), 7.40 (m, 6H), 7.67 (m, 4H), 8.19 (d, 1H, J = 6.0 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.5, 27.0, 36.5, 38.7, 50.7, 52.9, 68.5, 125.6, 126.0, 127.9, 128.0, 129.8, 130.1, 133.4, 135.7, 135.8, 135.9, 138.4, 154.5, 158.2, 158.3; HRMS (FAB) *m/z* 470.1726, calcd for $C_{20}H_{25}O_4{}^{32}S_1$ 470.1728 (M^+H).

4-amino-5-fluoro-1-*(cis*-4-(hydroxymethyl)thietan-2-yl)pyrimidin-2(1H)-one (106)



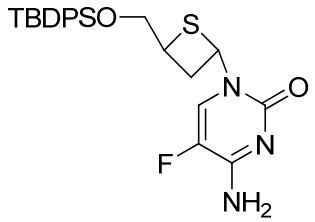
The 4-amino-1-*cis*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoro pyrimidin-2(1H)-one **105** (110 mg, 0.23 mmol) was dissolved in 20 mL of methanol, and ammonium fluoride (39 mg, 1.13 mmol) was added to the solution. The reaction mixture was heated at reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography with 95% ethyl acetate and 5% methanol. The appropriate fractions were combined, and the solvent was removed in vacuo to give product (46 mg, 90% yield). Melting point: 208 - 210 ˚C; IR (cm^{-1}) 3340 (br), 3165 (br), 2920, 1683, 1600, 1502; ^{1}H NMR (400 MHz, DMSO-d_6) 2.89 (m, 1H), 3.10 (m, 1H), 3.50-3.68 (m, 2H), 5.05 (m, 1H), 6.04 (m, 1H), 6.99 (br, 1H), 7.59 (br, 1H), 7.82 (br, 1H), 8.47 (d, 1H, J = 7.2 Hz); ^{13}C NMR (100 MHz, D_2O) δ 35.5, 36.8, 48.9, 64.8, 126.6, 126.9, 139.4, 141.7, 155.1, 157.8; HRMS (FAB) *m/z* 232.0550, calcd for $C_8H_{11}FN_3O_2{}^{32}S_1$ 232.0551 (M^+H). Anal. Calcd for $C_8H_{10}FN_3O_2{}^{32}S_1$: C, 41.55; H, 4.36; N, 18.17. Found: C, 41.37; H, 4.48; N, 17.99.

1-(*trans*-4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoro-4-methoxy pyrimidin-2(1H)-one (108)



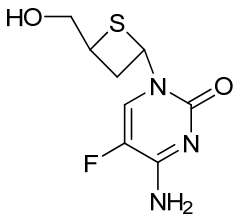
The 5-fluoro-1-(*trans*-4-(hydroxymethyl)thietan-2-yl)pyrimidine-2,4(1H,3H)-dione **107** (240 mg, 0.49 mmol) was dissolved in anhydrous acetonitrile (20 mL) and phosphorous oxychloride (0.15 mL, 1.48 mmol) and 1-methylimidazole (0.40 mL, 4.90 mmol) were added dropwise at 0 ˚C, and then the reaction mixture was stirred at r.t. for 2 hrs. After the mixture was cooled in an ice bath, 0.6 mL of triethyl amine and 2.2 mL of methanol were added. The mixture was stirred overnight at r.t. and then the solvent was evaporated. The crude was purified by column chromatography on silica gel (EtOAc: Hexane = 1:1) to afford the methyl protected nucleoside **108** (175 mg, 70% yield). Melting point: 148 - 150 ˚C; IR (cm^{-1}) 2943, 2850, 1689, 1491, 1106; ^{1}H NMR (400 MHz, $CDCl_3$) 1.08 (s, 9H), 2.70 (m, 1H), 3.32 (m, 1H), 3.71 (m, 3H), 4.04 (s, 3H), 6.27 (m,1H), 7.42 (m, 6H), 7.66 (m, 4H), 8.35 (d, 1H, J = 5.6 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.5, 27.1, 36.7, 38.5, 50.4, 55.5, 67.3, 128.1, 128.5, 128.8, 130.2, 130.3, 133.1, 135.7, 135.8, 135.8, 138.2, 153.8, 162.6, 162.8; HRMS (FAB) *m/z* 485.1722, calcd for $C_{25}H_{30}O_3F_1S_1Si_1$ 485.1725 (M^+H).

4-amino-1-*trans*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoropyrimidin-2(1H)-one (109)



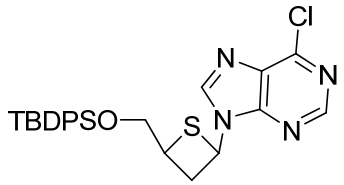
A mixture of compound **108** (150 mg, 0.30 mmol) and 60 ml of 7 N ammonia in methanol was put into a sealed flask, and then stirred at 65 ˚C overnight. After the reaction mixture was cooled to r.t., the solvent was removed by pressure. The crude was purified by column chromatography on silica gel (EtOAc: MeOH 9:1) to afford compound **109** (138 mg, 96% yield). Melting point: 177 - 178 ˚C; IR (cm^{-1}) 3311 (br), 2932, 2850, 1683, 1514, 1111, 703; ^{1}H NMR (400 MHz, $CDCl_3$) 1.09 (s, 9H), 2.70 (m, 1H), 3.26 (m, 1H), 3.72 (m, 3H), 5.95 (br, 1H), 6.27 (m, 1H), 7.38 (m, 6H), 7.66 (m, 4H), 8.17 (d, 1H, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 27.1, 35.7, 39.2, 50.4, 68.3, 126.5, 126.7, 128.5, 130.6, 133.3, 136.1, 136.3, 136.5, 138.2, 154.8, 158.6, 158.7; HRMS (FAB) *m/z* 470.1726, calcd for $C_{24}H_{29}FN_3O_2SSi$ 470.1728 (M^+H).

4-amino-5-fluoro-1-*(cis*-4-(hydroxymethyl)thietan-2-yl)pyrimidin-2(1H)-one (110)



The 4-amino-1-*trans*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoro pyrimidin-2(1H)-one **109** (100 mg, 0.21 mmol) was dissolved in 20 mL of methanol, and ammonium fluoride (38 mg, 1.03 mmol) was added to the solution. The reaction mixture was heated to reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give the desired product (42 mg, 90% yield). Melting point: 126 - 127 ˚C; IR (cm^{-1}) 3340 (br), 3165 (br), 2920, 1683, 1600, 1502; ^{1}H NMR (400 MHz, DMSO-d_6) 2.52 (m, 1H), 3.16 (m, 1H), 3.50-3.62 (m, 3H), 5.05 (m, 1H), 5.87 (m, 1H), 8.30 (d, 1H, J = 6.4 Hz); ^{13}C NMR (100 MHz, D_2O) δ 35.3, 37.0, 50.6, 65.5, 127.1, 127.4, 136.2, 138.7, 155.9, 158.0; HRMS (FAB) *m/z* 232.0550, calcd for $C_8H_{11}FN_3O_2S$ 232.0551 (M^+H). Anal. Calcd for $C_8H_{10}FN_3O_2S$ + 0.4 H_2O: C, 40.30; H, 4.57; N, 17.62. Found: C, 40.27; H, 4.48; N, 17.49.

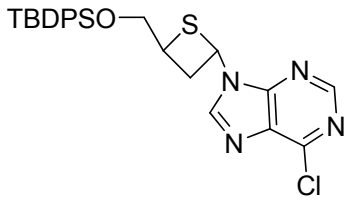
9-*cis*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-6-chloro-9H-purine (111)



A solution of *tert*-butyldiphenyl(thietan-2-ylmethoxy)silane **99** (0.15 g, 0.41 mmol) in CH_2Cl_2 (30 mL) was treated dropwise with a solution of 77% mCPBA (0.092 g, 0.41 mmol) in CH_2Cl_2 (4 mL) at -78 °C. The mixture was stirred at -78 °C for 30 mins. The reaction was quenched with aqueous $Na_2S_2O_3$, diluted with CH_2Cl_2, washed with aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. The filtrate was concentrated to dryness. The crude sulfoxide was used for the next reaction without further purification. A mixture of 6-chloropurine (0.19 g, 1.23 mmol) in acetonitrile (10 mL) and HMDS (10 mL) was refluxed for 3 hrs until the solution became clear. After evaporation, the residue was treated successively with a solution of the crude sulfoxide in toluene (10 mL), triethylamine (0.11 mL, 0.79 mmol), TMSOTf (0.16 mL, 0.88 mmol), and ZnI_2 (0.039 g, 0.12 mmol) at 0 °C. After 24 hrs, the reaction was quenched with water at 0 °C, and the reaction mixture was diluted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified with 3:7 EtOAc and hexane by silica gel chromatography to give the first product (0.062 g, 0.12 mmol, 10% yield) as a *cis* isomer **111**. Melting point: 185 – 186 ˚C; IR (cm^{-1}) 2932, 2850, 1596, 1537, 1467, 1374, 1106, 703; ^1H NMR (400 MHz, $CDCl_3$) 1.05 (m, 9H), 2.81 (dt, 1H, J = 6.4, 7.2 Hz), 3.55 (m, 1H), 3.83 (m, 3H), 6.40 (dd, 1H, J = 6.0,

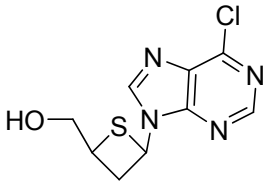
2.0 Hz), 7.39 (m, 6H), 7.63 (m, 4H), 8.85 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.5, 27.1, 36.2, 40.0, 50.0, 68.2, 122.3, 128.1, 130.2, 130.3, 133.0, 133.1, 135.7, 135.8, 142.9, 148.0, 152.9, 162.8; HRMS (FAB) *m/z* 495.1434, for $C_{25}H_{28}ClN_4O^{32}SSi$ 495.1436 (M^+H).

9-*trans*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-6-chloro-9H-purine (112)



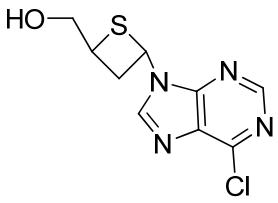
The *trans* isomer **112** (94 mg, 15% yield). Melting point: 165 – 166 ˚C; IR (cm^{-1}) 2932, 2850, 1596, 1537, 1467, 1374, 1106, 703; ^{1}H NMR (400 MHz, $CDCl_3$) 1.09 (m, 9H), 3.11 (m, 1H), 3.41 (m, 1H), 3.82 (m, 1H), 3.92 (m, 2H), 6.45 (dd, 1H, J = 5.4, 2.4 Hz), 7.41 (m, 6H), 7.69 (m, 4H), 8.85 (s, 1H), 9.10 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.9, 27.7, 36.9, 40.6, 50.8, 68.8, 123.3, 129.1, 131.2, 130.9, 133.9, 133.9, 136.7, 136.8, 141.9, 147.3, 153.9, 161.8; HRMS (FAB) *m/z* 495.1334, calcd for $C_{25}H_{28}ClN_4OSSi$ 495.1436 (M^+H).

(*cis*-4-(6-chloro-9H-purin-9-yl)thietan-2-yl)methanol (113)



The less polar *cis* isomer **111** (60 mg, 0.12 mmol) was dissolved in 20 mL of methanol, and ammonium fluoride (55 mg, 1.10 mmol) was added to the solution. The reaction mixture was heated at reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give product (28 mg, 94% yield). Melting point: 169 – 170 ˚C; IR (cm^{-1}) 3332 (br), 2920, 1636, 1600, 1374; ^{1}H NMR (400 MHz, CD_3OD) 3.23 (m, 1H), 3.65 (m, 1H), 3.90 (m, 3H), 4.05 (br, 1H), 6.40 (m, 1H), 8.80 (s, 1H), 9.40 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 37.3, 38.7, 49.7, 64.8, 122.3, 143.1, 149.2, 152.8, 162.3; HRMS (FAB) *m/z* 257.0270, calcd for $C_9H_{10}ClN_4O^{32}S$ 257.0261 (M^+H).

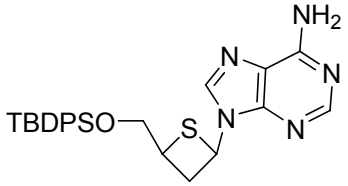
(*trans*-4-(6-chloro-9H-purin-9-yl)thietan-2-yl)methanol (114)



The *trans* isomer 9-*trans*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-6-chloro-9H-purine **112** (60 mg, 0.12 mmol) was dissolved in 20 mL of methanol, and ammonium fluoride (55 mg, 1.10 mmol) was added to the solution. The

reaction mixture was heated to reflux for overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give the desired product (25 mg, 90% yield). Melting point: 150 – 151 ˚C; IR (cm^{-1}) 3332 (br), 2920, 1636, 1600, 1374; ^{1}H NMR (400 MHz, $CDCl_3$) 2.52 (m, 1H), 2.91 (br, 1H), 3.21 (m, 1H), 3.57 (m, 1H), 3.98 (m, 3H), 6.49 (dd, 1H, J = 4.8, 7.8), 8.90 (s, 1H), 9.14 (s,1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 38.4, 39.9, 52.2, 65.8, 122.5, 143.2, 147.7, 153.0, 162.8; HRMS (FAB) *m/z* 257.0254, calcd for $C_9H_{10}ClN_4OS$ 257.0261 (M^+H).

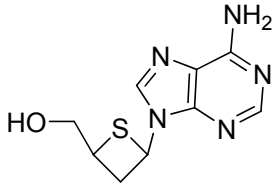
9- *cis*- (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-9H-purin-6-amine (115)



A solution of (*cis*-4-(6-chloro-9H-purin-9-yl)thietan-2-yl)methanol **111** (50 mg, 0.098 mmol) in DMF (2 mL) was treated with NaN_3 (24.6 mg, 0.98 mmol) at r.t. for 16 hrs. The resulting mixture was directly filtered through a silica gel pad using as eluent a 1:1 mixture of hexane and ethyl acetate. The filtrate was concentrated to dryness. A solution of the crude azide in methanol (15 mL) was treated with 5 mg of Pd (0)/C and H_2 balloon for 24 hr. The resulting mixture was filtered through a Celite pad, and the filtrate was concentrated and purified by chromatography with 2% methanol in dichloromethane to give the product (37

mg, 70% yield). Melting point: 156 – 158 ˚C; IR (cm^{-1}) 2932, 2856, 1631, 1590, 1106; ^{1}H NMR (400 MHz, $CDCl_3$) 1.14 (s, 9H), 3.20 (m, 1H), 3.63 (m, 2H), 4.02 (m, 2H), 6.22 (m, 1H), 6.55 (br, 2H), 7.42 (m, 6H), 7.70 (m, 4H), 8.00 (s, 1H), 8.51 (s,1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 19.6, 27.1, 36.8, 41.4, 53.4, 67.2, 111.7, 128.2, 130.3, 133.1, 135.9, 146.0, 151.8, 153.8, 162,4; HRMS (FAB) *m/z* 476.1933, calcd for $C_{25}H_{30}N_5O^{32}SSi$ 476.1935 (M^+H).

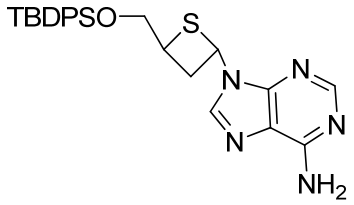
(*cis*-4-(6-amino-9H-purin-9-yl)thietan-2-yl)methanol (116)



The 9- *cis*- (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-9H-purin-6-amine (35 mg, 0.07 mmol) was dissolved in 10 mL of methanol, and ammonium fluoride (26 mg, 0.70 mmol) was added to the solution. The reaction mixture was heated at reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography with 10% methanol in dichloromethane. The appropriate fractions were combined, and the solvent was removed in vacuo to give product (16 mg, 93% yield). Melting point: 164 – 165 ˚C; IR (cm^{-1}) 3311 (br), 3189 (br), 1637, 1602, 1392; ^{1}H NMR (400 MHz, CD_3OD) 3.17 (m, 1H), 3.44 (m, 1H), 3.67-3.85 (m, 3H), 6.33 (t, 1H, J = 7.8 Hz), 8.22 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz CD_3OD) δ 36.7, 39.8, 50.0, 64.6, 110.9, 145.6, 152.1, 152.8, 159.9 HRMS (FAB) *m/z* 238.0755, calcd for $C_9H_{12}N_5O^{32}S_1$

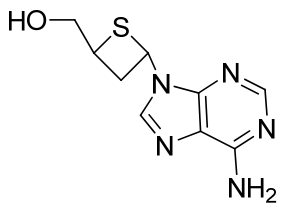
238.0757 (M^+H). Anal. Calcd for $C_9H_{11}N_5O^{32}S_1$ + 0.5 H_2O: C, 43.89; H, 4.91; N, 28.44. Found: C, 43.96; H, 4.84; N, 28.19.

9- *trans*- (4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-9H-purin-6-amine (117)



A solution of (*trans*-4-(6-chloro-9H-purin-9-yl)thietan-2-yl)methanol **112** (75 mg, 0.15 mmol) in DMF (2 mL) was treated with NaN_3 (97.5 mg, 1.50 mmol) at r.t. for 16 hrs. The resulting mixture was directly filtered through a silica gel pad using as eluent a 1:1 mixture of hexane and ethyl acetate. The filtrate was concentrated to dryness. A solution of the crude azide in methanol (15 mL) was treated with 10 mg of Pd (0)/C and a H_2 balloon for 24 hrs. The resulting mixture was filtered through a Celite pad, and the filtrate was concentrated and purified by chromatography with 2% methanol in dichloromethane to give the product (48 mg, 71% yield). Melting point: 190 – 192 ˚C; IR (cm^{-1}) 3332 (br), 2920, 1636, 1600, 1374; ^1H NMR (400 MHz, $CDCl_3$) 1.14 (s, 9H), 3.22 (m, 1H), 3.65 (m, 2H), 4.05 (m, 2H), 6.23 (dd, 1H, J = 8.4, 7.2 Hz), 6.45 (br, 2H), 7.45 (m, 6H), 7.73 (m, 4H), 8.00 (s, 1H), 8.55 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 20.6, 30.2, 35.0, 46.9, 60.6, 101.9, 121.6, 123.8, 126.6, 129.3, 139.5, 145.2, 147.3, 156.0; HRMS (FAB) *m/z* 476.1934, calcd for $C_{25}H_{30}N_5O^{32}SSi$ 476.1935 (M^+H).

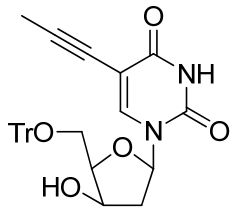
(*trans*-4-(6-amino-9H-purin-9-yl)thietan-2-yl)methanol (118)



The 4-amino-1-*trans*-(4-((tert-butyldiphenylsilyloxy)methyl)thietan-2-yl)-5-fluoro pyrimidin-2(1H)-one **117** (48 mg, 0.10 mmol) was dissolved in 20 mL of methanol, and ammonium fluoride (38 mg, 1.00 mmol) was added to the solution. The reaction mixture was heated at reflux overnight. After cooling to r.t., the mixture was evaporated to dryness. The residue was purified by flash column chromatography (ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give the desired product (24 mg, 92% yield). Melting point: 170 - 171 ˚C; IR (cm^{-1}) 3311 (br), 3189 (br), 1637, 1602, 1392; ^{1}H NMR (400 MHz, CD_3OD) 3.30 (m, 2H), 3.76 (m, 1H), 3.91 (m, 2H), 6.39 (dd, 1H, J = 6.8, 8.0 Hz), 8.26 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD)) δ 36.9, 39.3, 52.5, 66.0, 111.1, 145.4, 152.4, 152.8, 160.1; HRMS (FAB) *m/z* 238.0756, calcd for $C_9H_{12}N_5O^{32}S$ 238.0757 (M^+H).

4.2 Experimental section of part 2

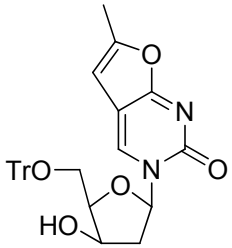
1-(4-hydroxy-5-(trityloxymethyl)-tetrahydrofuran-2-yl)-5-(prop-1-ynyl) pyrimidine-2,4(1H,3H)-dione (127)



A stirred solution of compound **126** (506 mg, 0.85 mmol) in anhydrous DMF (10 mL), in a three neck flask was deoxygenated with argon for 1.5 hr. Catalyst tetrakis(triphenylphosphine)palladium (100 mg, 0.085 mmol) and copper(I) iodide(30 mg, 0.17 mmol) were added. Argon was removed by vacuum, and anhydrous triethylamine (0.25 mL, 1.70 mmol) was injected and the flask was filled up with propyne (1.5 ml, 25.5 mmol). The reaction mixture was protected from light and stirred for 22 hrs. Then triphenylphosphine on polystyrene (500 mg) was added to deactivate the catalyst. The mixture was stirred under argon for 3 hrs. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The crude product was purified by flash column chromatography (initial eluent: ethyl acetate: hexane = 1:1, followed by: ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give compound **127** (260 mg, 62% yield). Melting point: 208 – 209 ˚C; IR (cm^{-1}) 3440 (br), 3195 (br), 3056 (br), 1691, 1446, 1286, 1061, 706; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (s, 3H), 2.26 (d, 1H, J = 14.4 Hz), 2.48-2.57 (m, 1H), 3.39 (s, 1H), 3.45-3.71 (m, 2H), 4.10 (m, 1H), 4.36 (s, 1H), 6.13 (d, 1H, J = 6.6 Hz), 7.25-7.35 (m, 9H), 7.48 (d, 6H, J = 7.5), 7.99 (s, 1H), 9.69 (s, 1H); ^{13}C NMR

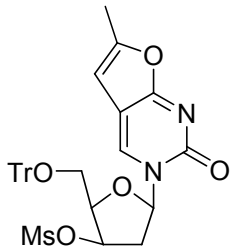
(100 MHz, $CDCl_3$) δ 4.9, 41.5, 62.6, 70.7, 70.8, 84.3, 86.5, 87.6, 90.5, 100.0, 127.5, 128.3, 128.8, 143.6, 149.9, 163.0, 179.0; HRMS (FAB) *m/z*, 509.2070, calcd for $C_{31}H_{29}N_2O_5$ 509.2068 (M^+H).

3-(4-hydroxy-5-(trityloxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one (128)



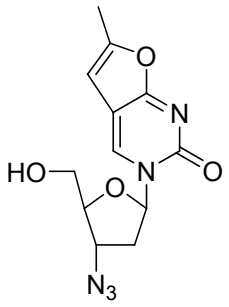
To a stirred solution of compound **127** (260 mg, 0.52 mmol) in methanol (70 mL) and triethylamine (30 mL) was added copper (I) iodide (19 mg, 0.10 mmol). The mixture was refluxed for 4 hrs. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (initial eluent: ethyl acetate, followed by ethyl acetate: methanol = 9:1). The combined fractions were combined and the solvent was removed in vacuo to give the pure product (180 mg, 70% yield). Melting point: 180 – 181 ˚C; IR (cm^{-1}) 3342 (br), 3060 (br), 1674, 1572, 1446, 1074, 706; ^1H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 2.45-2.57 (m, 2H), 3.52 (dd, 1H, J = 3.2, 10.4 Hz), 3.79 (dd, 1H, J = 6.8, 10.8 Hz), 4.279 (m, 1H), 4.39 (m, 1H), 5.91 (d, 1H, J = 1.2 Hz), 6.12 (d, 1H, J = 5.6 Hz), 7.23-7.32 (m, 9H), 7.52 (d, 6H, J = 7.2 Hz), 8.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 42.1, 63.2, 70.9, 85.8, 87.4, 89.4, 100.2, 107.0, 127.4, 128.2, 128.8, 129.0, 137.1, 143.9, 155.0, 155.0, 171.6; HRMS (FAB) *m/z* 509.2068 calcd for $C_{31}H_{29}O_5N_2$ 509.2068 (M^+H).

5-(6-methyl-2-oxofuro[2,3-d]pyrimidin-3(2H)-yl)-2-(trityloxymethyl)-tetrahydrofuran-3-yl methanesulfonate (129)



To an ice-cooled solution of compound **128** (100 mg, 0.20 mmol) and triethylamine (0.057 mL, 0.40 mmol) in dichloromethane (15 mL) was added methanesulfonyl chloride (0.030 mL, 0.40 mmol) dropwise. The reaction mixture was kept at r.t. for 1 hr. The solvent was removed under vacuo, and the crude product was purified by flash chromatography (initial eluent: ethyl acetate, followed by ethyl acetate: methanol = 9:1). The combined fractions were combined and the solvent was removed under vacuo to give the crude product as foamy solid (100 mg, 87% yield). Melting point: 179 – 180 ˚C; IR (cm^{-1}) 3620 (br), 3520 (br), 1670, 1642, 1576, 1172; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31 (s, 1H), 2.58 (d, 1H, J = 16 Hz), 2.91 (m, 1H), 3.45 (dd, 1H, J = 12.8, 6.4 Hz), 3.69 (m, 1H, J = 10.4, 6.4 Hz), 4.41 (m, 1H), 5.21 (t, 1H, J = 3.6, 4.0 Hz), 5.97 (d, 1H, J = 1.2 Hz), 6.23 (m, 1H), 7.29 (m, 9H), 7.48 (m, 6H), 8.07 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 38.6, 40.8, 61.9, 79.6, 83.2, 87.7, 87.8, 99.9, 107.8, 127.7, 128.3, 128.9, 134.5, 143.5, 154.9, 156.1, 172.2; HRMS (FAB) *m/z* 587.1847, calcd for $C_{32}H_{31}O_7N_2S$ 587.1849 (M^+H).

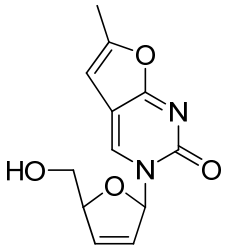
3-(4-azido-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one (130)



Compound **129** (80 mg, 0.14 mmol) and LiN_3 (12 mg, 0.20 mmol) were suspended in DMF (5 mL) and the mixture was heated to 110 ˚C for 2.5 hrs. The organic homogeneous mixture was poured into water and ethyl acetate was added. The aqueous layer was extracted with ethyl acetate and the organic extract was washed with water, then with brine. After drying (Na_2SO_4), the solvent was evaporated in vacuo to give the product as a foamy solid (58 mg, 80% yield). A solution of the foamy solid (40 mg, 0.075 mmol) in aqueous acetic acid (80%, 20 mL) was heated to 90 ˚C for 15 mins. After cooling the solvent was removed under reduced pressure, and the residue was purified by flash chromatography with ethyl acetate (1.5% methanol) as eluent. Evaporation of the appropriate fractions afforded the title compound (13 mg, 60% yield), which was crystallized from ethyl acetate. Melting point: decomposed at 250 ˚C; UV (H_2O) λ_{max} 331 nm; IR (cm^{-1}) 3469 (br), 2839, 1667, 1638, 1573, 1482; ^1H NMR (400 MHz, $CDCl_3$) δ 2.27 (d, 3H, J = 7.2 Hz), 2.30-2.36 (m, 1H), 2.57-2.64 (m, 1H), 3.29 (brs, 1H), 3.71-3.75 (m, 1H), 3.90-3.95 (m, 2H), 4.18 (dd, 1H, J = 6.8, 13.2 Hz), 6.10-6.14 (m, 1H), 8.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 39.2 58.6, 60.5, 85.7, 88.0, 100.1, 108.4, 136.2, 155.2, 156.2, 171.9; HRMS (FAB)

m/z 292.1036, calcd for $C_{12}H_{14}O_4N_5$ 292.1039 (M^+H). Anal. Calcd for $C_{12}H_{13}O_4N_5$: C, 49.48; H, 4.50; N, 24.04. Found: C, 49.59; H, 4.63; N, 23.98.

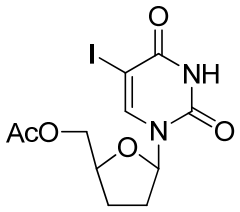
3-(5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one (133)



A stirred solution of compound **131** (321 mg, 0.85 mmol) in anhydrous DMF (10 mL), in a three neck flask was deoxygenated with argon for 1.5 hrs. Catalyst tetrakis(triphenylphosphine)palladium (100 mg, 0.085 mmol) and copper(I) iodide (30 mg, 0.17 mmol) were added. Argon was removed by vacuum, and anhydrous triethylamine (0.25 mL, 1.70 mmol) was injected and the flask was filled up with propyne (1.5 mL, 25.5 mmol). The reaction mixture was protected from light and stirred for 22 hrs. Then triphenylphosphine on polystyrene (500 mg) was added to deactivate the catalyst. The mixture was stirred in argon for 3 hrs. The resin was filtered and washed with methanol, and the combined filtrate was evaporated to dryness. The crude product was purified by flash column chromatography (initial eluent: ethyl acetate: hexane = 1:1, followed by: ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give compound **132** (148 mg, 60% yield). To a stirred solution of compound **132** (100 mg, 0.34 mmol) in methanol (70 mL) and triethylamine (30

mL) was added copper (I) iodide (12 mg, 0.07 mmol). The mixture was refluxed for 4 hrs. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (initial eluent: ethyl acetate, followed by ethyl acetate: methanol = 9:1. The combined fractions were combined and the solvent was removed in vacuo to give the pure product (64 mg, 70% yield). Melting point: 123 - 125 ˚C; UV (H_2O) λ_{max} 331 nm; IR (cm^{-1}) 3440 (br), 3195, 1670, 1642; ^1H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H), 3.61 (m, 2H), 4.88 (s, 1H), 5.05 (t, 1H, J = 5.6 Hz), 6.01 (d, 1H, J = 6.0 Hz), 6.38 (m, 2H), 6.95 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (100 MHz, -d^6) δ 14.27, 62.78, 88.88, 92.61, 100.95, 107.50, 127.22, 135.24, 138.19, 154.98, 155.60, 172.18; HRMS (FAB) *m/z* 271.0684, calcd for $C_{12}H_{12}O_4N_2Na$ 271.0689 (M^+Na).

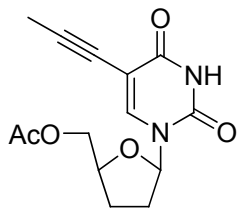
(5-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-2-yl)methyl acetate (135)



A mixture of compound **134** (1.27 g, 0.50 mmol), iodine (76 mg, 0.31 mmol), CAN (137 mg, 0.25 mmol), and MeCN (8 mL) was stirred at 80 ˚C for 1h. Reaction progress was monitored by TLC. Solvent was evaporated, and the residue was partitioned between a cold solution of EtOAc (20 mL), brine (10 mL), and 5% $NaHSO_3$ / H_2O (5 mL X 2), dried ($MgSO_4$) and evaporated. The crude 5-iodo products were purified by flash chromatography with ethyl acetate as eluant

to give 1.75 g product in 92% yield. Melting point: 154 - 156 ˚C; IR (cm^{-1}) 3230 (br), 3080 (br), 1736, 1701, 1683; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78-1.88 (m, 1H), 1.98-2.04 (m, 1H), 2.09-2.19 (m, 1H), 2.41-2.51 (m, 1H), 4.31-4.36 (m, 3H), 6.02 (dd, 1H, J = 3.2, 6.4 Hz), 8.04 (s, 1H), 9.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 25.1, 33.4, 64.3, 79.8, 87.2, 96.5, 139.6, 149.9, 159.4, 170.9; HRMS (FAB) *m/z* 380.9945, calcd for $C_{11}H_{14}O_5N_2I$ 380.9942 (M^+H). Anal. Calcd for $C_{11}H_{13}O_5N_2I$ + 0.2 CH_3OH: C, 34.80; H, 3.60; N, 7.25; Found: C, 34.97; H, 3.50; N, 7.09.

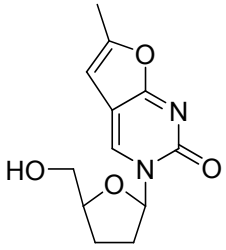
(5-(2,4-dioxo-5-(prop-1-ynyl)-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-2-yl)methyl acetate (136)



A stirred solution of compound **135** (323 mg, 0.85 mmol) in anhydrous DMF (10 mL), in a three neck flask was deoxygenated with argon for 1.5 hrs. Catalyst tetrakis(triphenylphosphine)palladium (100 mg, 0.085 mmol) and copper (I) iodide (30 mg, 0.17 mmol) were added. Argon was removed by vacuum, and anhydrous triethylamine was injected and the flask was filled up with propyne (1.5 mL, 25.5 mmol). The reaction mixture was protected from light and stirred for 22 hrs. Then triphenylphosphine on polystyrene (500 mg) was added to deactivate the catalyst. The mixture was stirred under argon for 3 hr. The resin was filtered and washed with methanol, and the combined filtrate was

evaporated to dryness. The crude product was purified by flash column chromatography (initial eluent: ethyl acetate: hexane = 1:1, followed by: ethyl acetate). The appropriate fractions were combined, and the solvent was removed in vacuo to give compound **136** (149 mg, 60%) as white solid. Melting point: 173 - 175 ˚C; IR (cm^{-1}) 3178 (br), 3088 (br), 1687, 1278; ^1H NMR (400 MHz, $CDCl_3$) δ 1.77-1.90 (m, 1H), 2.00 (s, 3H), 2.00-2.16 (m, 2H), 2.19 (s, 3H), 2.40-2.50 (m, 1H), 4.29-4.40 (m, 3H), 6.03 (dd, 1H, J = 2.8, 6.4 Hz), 7.92 (s, 1H), 8.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.8, 20.9, 25.1, 33.4, 64.3, 71.0, 79.7, 86.92, 90.65, 100.24, 141.90, 149.42, 162.15, 170.88; HRMS (FAB) *m/z* 293.1132, calcd for HRMS (FAB) *m/z* 293.1132, calcd for $C_{14}H_{17}O_5N_2$ 293.1137 (M^+H). Anal. Calcd for $C_{14}H_{16}O_5N_2$ + 0.2 H_2O: C, 56.83; H, 5.59; N, 9.47. Found: C, 56.78; H, 5.58; N, 9.45.

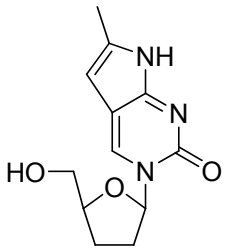
3-(5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methylfuro[2,3-d]pyrimidin-2(3H)-one (137)



To a stirred solution of compound **136** (0.80 g, 2.74 mmol) in methanol (70 mL) and triethylamine (30 mL) was added copper (I) iodide (0.10 g, 0.55 mmol). The mixture was refluxed for 4 hrs. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (initial eluent: ethyl acetate,

followed by ethyl acetate/methanol (9:1). The combined fractions were combined and the solvent was removed in vacuo to give the crude product, which was recrystallized from methanol to give pure product as white solid (0.31g, 45% yield). Melting point: 130 - 131 ˚C; UV (H_2O) λ_{max} 331 nm; IR (cm^{-1}) 3450 (br), 1666, 1629,1572, 1180; ^1H NMR (400MHz, $CDCl_3$) δ 1.87-1.92 (m, 2H), 2.20 (m, 1H), 2.34 (s, 1H), 2.56-2.63 (m, 1H), 3.83 (dd, 1H, J = 4.2, 12 Hz), 4.11 (d, 1H, J = 12 Hz), 4.274 (br, 1H), 6.10 (d, 1H, J = 1.2 Hz), 6.18 (d, 1H, J = 6.0 Hz), 8.60 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 14.2, 23.9, 34.1, 62.4, 83.7, 89.2, 100.4, 107.7, 137.1, 155.2, 155.4, 171.8; HRMS (FAB) *m/z* 251.1024, calcd for $C_{12}H_{15}O_4N_2$ 251.1026 (M^+H). Anal. Calcd for $C_{12}H_{14}O_4N_2$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.67; H, 5.58; N, 11.04.

3-(5-(hydroxymethyl)-tetrahydrofuran-2-yl)-6-methyl-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one (138)

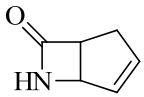


To a 40 mL of 7 N ammonium in methanol was added compound **137** (80 mg, 0.32 mmol) in a pressure bottle. After the mixture was heated to 60 ˚C for 12 hrs, the solvent was removed in vacuo, and the crude product was purified by flash chromatography (initial eluent: ethyl acetate, followed by ethyl acetate/methanol (9:1). The combined fractions were combined and the solvent was removed in

vacuo to give the crude product, which was recrystallized from methanol to give pure product (35 mg, 44% yield). Melting point: 54 – 55 ˚C; UV (H_2O) λ_{max} 335 nm; IR (cm^{-1}) 3350 (br), 1670, 1560, 1090; ^1H NMR (400MHz, $CDCl_3$ + a drop of CD_3OD) δ 1.91-1.93 (m, 2H), 2.18 (m, 1H), 2.41(s, 3H), 2.54 (m, 1H) 3.82 (dd, 1H, J = 4.0, 12 Hz), 4.08 (dd, 1H, J = 2.8, 12.4 Hz), 4.26 (m, 1H), 4.80 (d, 1H, J = 1.6 Hz), 6.22 (dd, 1H, J = 2.4, 6.8 Hz), 8.44 (s, 1H); ^{13}C NMR (100MHz, $CDCl_3$) δ 13.5, 24.2, 33.9, 62.5, 83.2, 88.8, 97.9, 110.6, 134.9, 138.6, 155.5, 158.6; HRMS (FAB) *m/z* 250.1183, calcd for $C_{12}H_{16}O_3N_3$ 250.1186 (M^+H). Anal. Calcd for $C_{12}H_{15}O_3N_3$: C, 53.92; H, 6.41; N, 15.72. Found: C, 53.95; H, 6.20; N, 15.93.

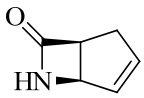
4.3 Experimental section of part 3

(±)-6-aza-bicyclo[3.2.0]hept-3-en-7-one (148)



A solution of freshly distilled cyclopentadiene (33.0 mL, 489 mmol) in anhyd Et_2O (70 mL) was added dropwise to a solution of chlorosulfonyl isocynate (21.3 mL) in anhyd Et_2O (200 mL) with vigorous stirring at -78 ˚C. After stirring at this temperature the mixture was treated at 0 ˚C with the solution of aq Na_2SO_3 (25%; 300 mL) and then with aq KOH (10%) to give pH = 8. After stirring the mixture for 30 mins at 0 ˚C, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3x100 mL). The combined organic layers were dried and evaporated in vacuo, and the residue purified by flash chromatography (hexane: EtOAc, 2:1) to give **148** (12.90 g, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43-2.51 (m, 1H), 2.70-2.78 (m, 1H), 3.81-3.89 (m, 1H), 4.50-4.55 (m, 1H), 5.91-5.99 (m, 1H), 6.00-6.04 (m, 1H), 6.35 (br, 1H)

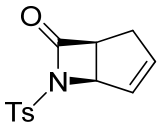
(1S, 5R)-6-aza-bicyclo[3.2.0]hept-3-en-7-one (149)



Crystalline racemic **148** (4.00 g, 36.61 mmol) was dissolved in diisopropyl ether (80 mL). Lipolase (lipase B from *Candida Antarctica*) (4.00 g, 50 mg/mL) and water (0.32 mL, 17.80 mmol) were added and the mixture was shaken in an incubator shaker at 70 ˚C for 5 hrs. The reaction was stopped by filtering off the enzyme. The solvent was evaporated off and the residue (1S,5R)-6-aza-

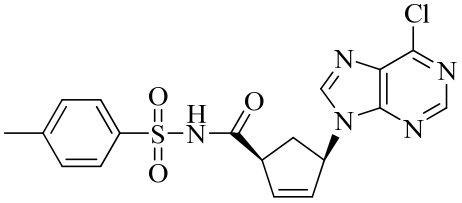
bicyclo[3.2.0]hept-3-en-7-one **149** crystallized out. (1.80 g, 45% yield); recrystallized from diisopropyl ether; $[\alpha]^{25}_{D}$ = -34.7 (C = 0.45, $CHCl_3$), lit.[124] -34.8 (C = 0.45, $CHCl_3$); ee > 99% (Mosher ester determination); Melting point: 76 – 77 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 2.44 (s, 3H), 2.46-2.53 (m, 1H), 2.65-2.70 (m, 1H), 3.80-3.84 (m, 1H), 4.99-5.01 (m, 1H), 6.02 (s, 1H), 6.48 (s, 1H).

(1S,5R)-6-tosyl-6-aza-bicyclo[3.2.0]hept-3-en-7-one (150a)



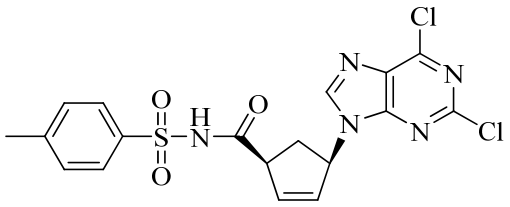
A solution of **149** (2.00 g, 18.32 mmol) in anhyd THF (33 mL) was added dropwise to a stirred mixture of 1.6 M n-BuLi in hexane (19.5 mL, 31.21 mmol) and anhydrous THF (33 mL) at -78 ˚C under Argon. The mixture was stirred at -78 ˚C for 1 hr and *p*- toluenesulfonyl chloride (4.65 g, 24.40 mmol) was added. The reaction temperature was raised gradually to room temperature. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (hexane: EtOAc, 4:1) to give white solid **150a** (2.90 g, 60% yield). $[\alpha]^{25}_{D}$ = -129.9 (C = 2.50, $CHCl_3$); Melting point: 93 - 95 ˚C; IR (cm^{-1}) 3068, 2921, 1781, 1348, 1119; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 2.44 (s, 3H), 2.46-2.53 (m, 1H), 2.65-2.70 (m, 1H), 3.80-3.84 (m, 1H), 4.99-5.01 (m, 1H), 6.02 (s, 2H), 7.33 (d, 2H, J = 8.0 Hz), 7.84 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.3, 145.2, 138.9, 136.5, 130.1, 128.4, 127.5, 66.2, 52.0, 31.2, 21.9; HRMS (FAB) *m/z* 264.0688, calcd for $C_{13}H_{14}NO_3S$ 264.0689.

(1S,4R)-4-(6-chloro-9H-purin-9-yl)-N-tosylcyclopent-2-enecarboxamide (151a)



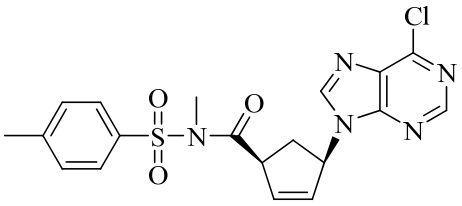
To a stirred solution of tetrabutylammonium salt of 2,6-dichloropurine (0.60 g, 1.5 mmol) in anhydrous THF (20 mL) which was prepared from 2,6-dichloropurine and tetrabutylammonium hydroxide was dissolved in 10 mL of DMF, palladium acetate (34 mg, 0.15 mmol) and triisopropyl phosphate (0.21 mL, 0.91 mmol) were added and stirred under argon at r.t. for 1hr. A solution of **150a** (0.40 g, 1.5 mmol) in anhydrous THF (5 mL) was added dropwise to the resultant mixture, which was then stirred for 2 hrs. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (CH_2Cl_2-Methanol, 95:5) to give yellowish solid **152a** (0.45 g, 57% yield). Melting point: 200 - 202 ˚C; IR (cm^{-1}) 3068, 2966, 1715, 1683, 1589, 1560, 1339; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.98-2.05 (m, 1H), 2.34 (s, 3H), 2.67-2.75 (m, 1H), 3.67 (m, 1H), 5.70 (m, 1H), 6.07 (m, 1H), 6.15 (m, 1H), 7.35 (d, 2H, J = 8.0 Hz), 7.75 (d, 2H, J=8.0 Hz), 8.28 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.8, 29.9, 33.5, 51.5, 60.2, 128.2, 130.2, 132.0, 135.4, 136.8, 145.0, 146.1, 149.7, 152.1, 171.8; HRMS (FAB) *m/z* 417.0659, calcd for $C_{18}H_{17}ClN_5O_3S$ 417.0660.

(1S,4R)-4-(2,6-dichloro-9H-purin-9-yl)-N-tosylcyclopent-2-enecarboxamide (152a)



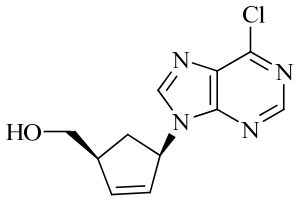
To a stirred solution of tetrabutylammonium salt of 2,6-dichloropurine (0.67 g, 1.5 mmol) in anhydrous THF (20 mL) which was prepared from 2,6-dichloropurine and tetrabutylammonium hydroxide was dissolved in 10 mL of DMF, palladium acetate (34 mg, 0.15 mmol) and triisopropyl phosphate (0.21 mL, 0.91 mmol) were added and stirred under argon at r.t. for 1hr. A solution of **150a** (0.40 g, 1.5 mmol) in anhydrous THF (5 mL) was added dropwise to the resultant mixture, which was then stirred for 2 hrs. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (CH_2Cl_2-Methanol, 95:5) to give yellowish solid **152a** (0.33 g, 49% yield). $[\alpha]^{25}_D$ = -81.6 (C = 0.50, CH_3OH); Melting point: 214 - 215 ˚C; IR (cm^{-1}) 3256, 2966, 1686, 1683, 1609, 1503; ^1H NMR (400 MHz, $CDCl_3$ and a drop of CD_3OD): δ 2.09-2.15 (m, 1H), 2.38 (s, 3H), 2.72-2.80 (m, 1H), 3.58-3.60 (m, 1H), 5.73-5.76 (m, 1H), 5.88-5.91 (m, 1H), 6.11-6.13 (m, 1H), 7.27 (d, 2H, J = 8.0 Hz), 7.85 (d, 2H, J=8.0 Hz), 8.24 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ and a drop of CD_3OD): δ 21.9, 33.8, 51.4, 59.5, 128.4, 129.8, 130.8, 131.4, 135.7, 135.7, 145.4, 145.5, 151.8, 152.8, 170.9; HRMS (FAB) *m/z* 452.0342, calcd for $C_{18}H_{16}Cl_2N_5O_3S$ 452.0340. Anal. Calcd for $C_{18}H_{15}Cl_2N_5O_3S$: C, 47.80; H, 3.34; N, 15.48. Found: C, 48.08; H, 3.30; N, 15.46.

(1S,4R)-4-(6-chloro-9H-purin-9-yl)-N-methyl-N-tosylcyclopent-2-enecarboxamide (153a)



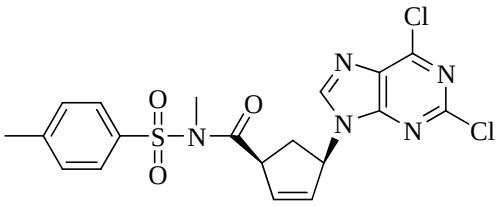
To a solution of **151a** (0.60 g, 1.44 mmol) in THF-CH_2Cl_2(1:1) was added methanol (0.2 mL), PPh_3 (1.33 g, 5.76 mmol) , and diisopropyl azodicarboxylate (0.98 mL, 5.74 mmol) under argon atmosphere with stirring at room temperature, after being stirred for 30 mins, the solvent was evaporated in vacuo to give a residue, which was submitted to column chromatography. Elution with hexane-EtOAc (1:1) gave 0.59 g of product **153** (95% yield). Melting point: 201 - 202 ˚C; IR (cm^{-1}) 1732, 1695, 1585, 1560, 1360, 1164; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.10-2.13 (m, 1H), 2.40 (s, 3H), 2.81-2.87 (m, 1H), 3.20 (s, 3H), 4.50 (m, 1H), 5.83 (m, 1H), 5.96 (m, 1H), 6.11 (m, 1H), 7.31 (d, 2H, J = 8.0 Hz), 7.69 (d, 2H, J = 8.0 Hz), 8.25 (s, 1H), 8.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 33.6, 35.8, 51.1, 59.4, 127.5, 130.4, 131.0, 131.9, 135.9, 136.6, 144.7, 145.7, 150.9, 151.6, 151.9, 173.9; HRMS (FAB) *m/z* 432.0838, calcd for $C_{19}H_{19}ClN_5O_3S$ 432.0832.

((1S,4R)-4-(2,6-dichloro-9H-purin-9-yl)cyclopent-2-enyl)methanol (153)



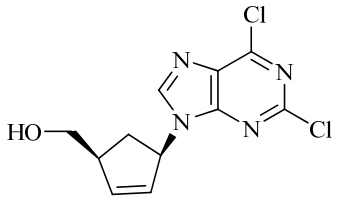
To a solution of **153a** (0.57 g, 1.35 mmol) in methanol was added $NaBH_4$ (51.3 mg, 1.35 mmol) portionwise with stirring at -20 ºC. During this period, the internal temperature was kept below 0 ºC. The mixture was then stirred at room temperature for 5 hr. After the reaction was neutralized with AcOH, the solvent was evaporated off in vacuo. To the residue was added water, and the mixture was extracted with EtOAc. The extract was dried over $MgSO_4$ and condensed in vacuo to give a residue, which was purified on silica gel column chromatography. Elution with CH_2Cl_2-MeOH (95:5) afforded 0.33 g of product **153** (95% yield). Melting point: 154 - 156 ˚C; IR (cm^{-1}) 3370, 1589, 1686, 1556, 1335, 1204; ^1H NMR (400 MHz, $CDCl_3$): δ 1.85-1.95 (m, 1H), 2.83-2.91 (m, 1H), 3.10-3.11 (m, 1H), 3.72 (m, 1H), 3.88 (m, 1H), 5.75-5.80 (m, 1H), 5.84-5.87 (m, 1H), 6.21-6.24 (m, 1H), 8.41 (s, 1H), 8.78 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.1, 47.8, 60.9, 64.5, 129.4, 131.9, 140.1, 145.1, 151.0, 151.5, 151.8; HRMS (FAB) *m/z* 251.0650, calcd for $C_{11}H_{12}ClN_4O_1$ 251.0645.

(1S,4R)-4-(2,6-dichloro-9H-purin-9-yl)-N-methyl-N-tosylcyclopent-2-enecarboxamide (154a)



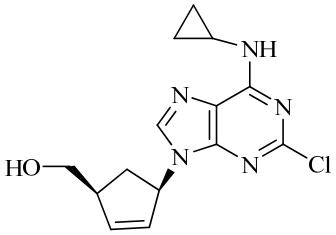
To a solution of **152a** (0.65 g, 1.44 mmol) in THF-CH_2Cl_2(1:1) was added methanol (0.2 mL), PPh_3 (1.33 g, 5.76 mmol) , and diisopropyl azodicarboxylate (0.98 mL, 5.74 mmol) under argon atmosphere with stirring at room temperature, after being stirred for 30 mins, the solvent was evaporated in vacuo to give a residue, which was submitted to column chromatography. Elution with hexane-EtOAc (1:2) gave 0.63 g of product **154a**. $[\alpha]^{25}_D$ = -39.8 (C = 0.38, CH_3OH); Melting point: 210 - 212 ˚C; IR (cm^{-1}) 3256, 2966, 1686, 1683, 1609, 1503; ^1H NMR (400 MHz, $CDCl_3$): δ 2.12-2.16 (m, 1H), 2.45 (s, 3H), 2.82-2.90 (m, 1H), 3.25 (s, 3H), 4.54-4.56 (m, 1H), 5.80-5.83 (m, 1H), 5.96-5.99 (m, 1H), 6.16-6.18 (m, 1H) 7.36 (d, 2H, J = 8.0 Hz), 7.73 (d, 2H, J = 8.0 Hz), 8.31 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 33.7, 35.8, 51.2, 59.6, 127.5, 130.5, 130.8, 131.1, 135.9, 137.1, 145.5, 145.8, 151.8, 152.9, 153.0, 173.9; HRMS (FAB) *m/z* 466.0501, calcd for $C_{19}H_{18}Cl_2N_5O_3S$ 466.0500. Anal. Calcd for $C_{19}H_{17}Cl_2N_5O_3S$: C, 48.93; H, 3.67; N, 15.02. Found: C, 48.98; H, 3.56; N, 15.06.

((1S,4R)-4-(2,6-dichloro-9H-purin-9-yl)cyclopent-2-enyl)methanol (154)[133]



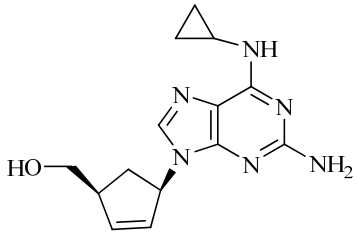
To a solution of **154** (0.63 g, 1.35 mmol) in methanol was added $NaBH_4$ (51.3 mg, 1.35 mmol) portionwise with stirring at -20 ºC. During this period, the internal temperature was kept below 0 ºC. The mixture was then stirred at room temperature for 5 hr. After the reaction was neutralized with AcOH, the solvent was evaporated off in vacuo. To the residue was added water, and the mixture was extracted with EtOAc. The extract was dried over $MgSO_4$ and condensed in vacuo to give a residue, which was purified on silica gel column chromatography. Elution with CH_2Cl_2-MeOH (95:5) afforded 0.29 g of product **155** (65% yield). $[\alpha]^{25}_D$ = -44.6 (C = 0.45, $CHCl_3$); Melting point: 157 - 159 ˚C; IR (cm^{-1}) 3256, 2966, 1686, 1683, 1609, 1503; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85-1.91 (m, 1H), 2.53 (s, 1H), 2.84-2.92 (m, 1H), 3.10-3.11 (m, 1H), 3.72 (m, 1H), 3.88 (m, 1H), 5.75-5.80 (m, 1H), 5.84-5.87 (m, 1H), 6.21-6.24 (m, 1H), 8.42 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.1, 47.7, 60.8, 64.4, 129.2, 131.1, 140.3, 145.7, 151.6, 152.8, 152.9; HRMS (FAB) *m/z* 285.0304, calcd for $C_{11}H_{11}Cl_2N_4O_1$ 285.0302.

((1S,4R)-4-(2-chloro-6-(cyclopropylamino)-9H-purin-9-yl)cyclopent-2-enyl)methanol (155)



To a solution of **154** (200 mg, 0.70 mmol) in ethanol (20 mL) was added cyclopropylamine (0.14 mL, 2.1 mmol), the mixture was then heated to 70 ˚C for 5 hrs. After evaporating the solvent, the crude product **155** was purified on silica gel column chromatography. Elution with CH_2Cl_2-MeOH (95:5) afforded 0.18 g of product **155** (90% yield). $[\alpha]^{25}_D$ = -94.2 (C = 0.50, CH_3OH); Melting point: 197 - 199 ˚C; IR (cm^{-1}) 3322, 3256, 1686, 1683, 1503; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.62 (m, 2H), 0.90 (m, 2H), 1.88 (m, 1H), 2.84 (m, 1H), 3.07 (m, 3H), 3.70 (m, 1H), 3.83 (m, 1H), 5.61-5.66 (m, 1H), 5.81-5.83 (m, 1H), 6.14-6.16 (m, 1H), 6.21 (brs, 1H), 7.81 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.7, 34.1, 47.7, 60.6, 65.0, 119.3, 128.4, 129.3, 130.2, 138.9, 139.4, 156.6; HRMS (FAB) *m/z* 306.1116, calcd for $C_{14}H_{17}Cl_1N_5O_1$ 306.1116.

((1S,4R)-4-(2-amino-6-(cyclopropylamino)-9H-purin-9-yl)cyclopent-2-enyl)methanol (156)



The compound **155** was dissolved in hydrazine monohydrate (10 mL) and MeOH (5 mL). After heating at 50 ˚C for overnight, the solution was concentrated to dry and coevaporated with 2-propanol (2 x 30 mL) until a white gum was obtained. The residue was dissolved in a 10% aqueous acetic acid solution (10 mL) and cooled in an ice bath. Sodium nitrite (75 mg, 1.10 mmol) was added, and the mixture was stirred for 1 hr. After evaporating the solvent, the crude product was dissolved in ethanol and Tin (II) chloride dihydrate (315 mg, 1.41 mmol) was added. After heating to reflux for 2 hrs, the mixture was cooled and evaporated. The residue was purified on silica gel column chromatography. Elution with CH_2Cl_2-MeOH (95:5) afforded 133 mg of product **156** (70% yield). $[\alpha]^{25}_D$ = -37.9 (C = 0.29, CH_3OH), lit.[119] -37.2 (C = 0.45, CH_3OH); Melting point: 210 - 212 ˚C; IR (cm^{-1}) 3321, 3207, 1589, 1474; ^1H NMR (400 MHz, DMSO-d_6): δ 0.54-0.64 (m, 4H), 1.51-1.58 (m, 1H), 2.52-2.60 (m, 1H), 2.83 (m, 1H), 3.00 (br, 1H), 3.40-3.42 (m, 2H), 4.73 (m, 1H), 5.37 (m, 1H), 5.83 (m, 2H), 6.07 (m, 1H), 7.57 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 7.1, 22.0, 35.0, 48.4, 58.8, 64.8, 114.2, 130.7, 135.5, 138.7, 156.6, 160.7; HRMS (FAB) *m/z* 287.1611, calcd for $C_{14}H_{19}N_6O_1$ 287.1610.

5. References and Notes

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