SYNTHESIS, ANTI-HIV ACTIVITY AND MOLECULAR MODELING STUDIES OF CARBOCYCLIC AND 4'-THIONUCLEOSIDES

by

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(Under the Direction of CHUNG K. CHU)

ABSTRACT

This thesis describes the synthesis, biological evaluation and molecular modeling studies of carbocyclic and 4'-thionucleosides as potential antiviral agents.

Chapter 1 reviews the activity, metabolism, toxicity and the development of resistance of antiviral nucleosides as well as their status in clinical trials.

In Chapter 2, an efficient method for the synthesis of carbocyclic L-2'-deoxynucleosides using D-ribose is presented. The Mitsunobu reaction was used to condense various purine and pyrimidine bases with the carbocyclic sugar moiety.

Chapter 3 describes a comparison study of solid phase synthesis versus solution phase synthesis of carbocyclic L-2'-deoxynucleosides. Solid phase synthesis gave better selectivity of N_1-alkylated products versus O^2-alkylated products in the synthesis of pyrimidine nucleosides, while only N^9-alkylated products were obtained in both solid and solution phase synthesis of purine nucleosides.

Chapter 4 details the synthesis and biological evaluation against HIV-1 in primary human lymphocytes of β-L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides (β-L-2'-F-4'-S-d4Ns). The cytosine, 5-fluorocytosine and adenine

derivatives showed potent anti-HIV activities (EC_{50} 0.12, 0.15 and 1.74 μM, respectively) without significant cytotoxicity. The cytosine derivative (β-L-2'-F-4'-S-d4C), however, showed cross-resistance to 3TC-resistant variant (HIV-1M184V). According to our molecular modeling studies, the steric hindrance between the sugar moiety of the unnatural L-nucleoside and the side chains of Val184 of M184V RT in 3TC-resistant mutant HIV strains destabilizes the RT-nucleoside triphosphate complex, which causes the cross-resistance to 3TC (M184V mutant).

Chapter 5 describes the development of an efficient synthetic method for 3'-thio analogs of oxetanocin A. The thietane ring was synthesized using a carbohydrate template and the condensation between the sugar moiety and various heterocyclic bases was conducted using the Pummerer rearrangement reaction.

Chapter 6 details the synthesis of various L-thietanose nucleosides with different sugar moieties to extend the usage of the synthetic method of the thietanse ring developed in our group.

Chapter 7 desicribes molecular modeling studies showing that the 2',3'-double bond of the sugar moiety of various 2',3'-unsaturated nucleosides interacts with the aromatic moiety of Tyr115 of HIV-1 reverse transcriptase (RT) by hydrophobic π–π interaction. In 3TC-resistant mutant (M184V) RT, 2'-fluoro-2',3'-unsaturated nucleosides with a bulky 4'-substituent experience significant steric hindrance with the side chain of Val184.

INDEX WORDS: carbocyclic nucleosides, 4'-thionucleosides, 3'-thio analogs, thietanose nucleosides, cross-resistance, 3TC-resistant mutant, M184V, HIV and HBV

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I am also very grateful to my family for their endless support and love.

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CHAPTER 1

ADVANCES IN ANTIVIRAL NUCLEOSIDES

INTRODUCTION

Viral infections are significant causes of human mortality and morbidity. Although there are a number of antiviral therapeutic agents available for the treatment of viral infections, the majority of viral infections are not currently curable by chemotherapy and many are still of serious health concerns. Therefore, the discovery and development of safe and effective antiviral therapies have been among the major scientific endeavours. The major focus of these investigations has been the design of effective antiviral agents, which will selectively inhibit a viral (or viral-induced) function without interacting with the host's normal cellular functions.

Viruses are intracellular pathogens sharing many of the nutritional requirements and synthetic pathways of the host cells they infect. Therefore, an ideal antiviral agent would be one that effectively interdicts viral infection as a consequence of interaction with a target that is unique to the virus, and hence of little or no concern to the uninfected host tissues. In principle, such selective antiviral activity can be achieved by targeting virus-specific enzymes such as viral polymerases. In the case of herpes simplex virus, this strategy has lead to the development of several nucleoside analogs, such as idoxouridine, bromovinyldeoxyuridine (BVDU) and acyclovir, of which remarkable antiviral activity is due to a specific interaction of their 5'-triphosphates with the viral DNA polymerase. Retroviruses such as human immunodeficiency virus (HIV) are RNA viruses that replicate through a double-strand DNA intermediate. This novel viral replication cycle requires that retroviruses carry a specific enzyme, reverse transcriptase (RT), since there are no cellular enzymes that can convert single-strand RNA into double-strand DNA. As

a result of the key role of HIV-1 RT in viral replication, it has been a major target for the development of a series of anti-AIDS drugs.

The synthetic nucleoside analogs, which resemble the natural nucleosides, but have an modified deoxy-ribose or ribose moiety, have played a major role in the treatment of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), human cytomegalovirus (HMCV), varicella zoster virus (VZV) and respiratory syncytial virus (RSV). The nucleoside analogs are phosphorylated to the triphosphate form within the infected cell, which compete with natural nucleotides for incorporation into viral DNA or RNA. Incorporation into the growing nucleic acid chain results in irreversible association with the viral polymerase and chain termination. Since idoxuridine (IDU) was approved in the United States for the treatment of herpetic keratitis in 1962, eighteen additional nucleoside analogs have been approved by the FDA. However, these nucleosides have their own limitations and problems in the treatment of diseases such as toxicity and emergence of resistant viral strains, which prompted extensive investigation to discover safer and more effective nucleoside analogs. From these efforts, the past decade has witnessed the emergence of a number of nucleoside analogs with antiviral efficacy. Currently, seven anti-HIV nucleosides analogs and two anti-HBV nucleosides have been approved by the FDA. A number of nucleoside analogs are also under development, which not only have potent antiviral activity against the wild-type as well as the drug-resistant mutant enzyme but also possess reduced cytotoxicity and favorable pharmacokinetic profiles. Thus, this review will be focused on the adverse effects and cross-resistance of the FDA-approved nucleoside reverse transcriptase inhibitors (NRTIs) followed by the detailed discussion of most recent

advances in anti-HIV as well as anti-HBV nucleosides including their antiviral activity, toxicity and cross-resistance. Molecular mechanism of drug-resistance proposed by molecular modeling studies and reports from clinical trials will also be introduced.

ANTI-HIV AGENTS

According to the report of HIV/AIDS epidemic update in 2002 by UNAIDS, globally 42 million people are currently infected by human immunodeficiency virus (HIV).[1] HIV infection is characterized by a prolonged asymptomatic period of years to decades, which is followed by the fatal illness of AIDS. Various complications characterize AIDS, including wasting, neurological impairment, opportunistic infections and malignancies. HIV is one of the lentiviruses which belongs to the family of *retroviridae* defined by the presence of the unique enzyme, reverse transcriptase (RT), which facilitates the production of a DNA copy from an RNA genome (Fig. 1.1).

As a result of the key role of HIV-1 RT in virus replication, it has been a major target for the development of many anti-AIDS agents such as nucleoside reverse transcriptase inhibitors (NRTIs: AZT,[2] 3TC,[3] ddI,[4] ddC,[5] d4T,[6] abacavir[7] and tenofovir disoproxil[8]) (Fig. 1.2) and non-nucleosides RT inhibitors (NNRTIs: nevirapine,[9] delavirdine[10] and efavirenz[11]) (Table 1.1).

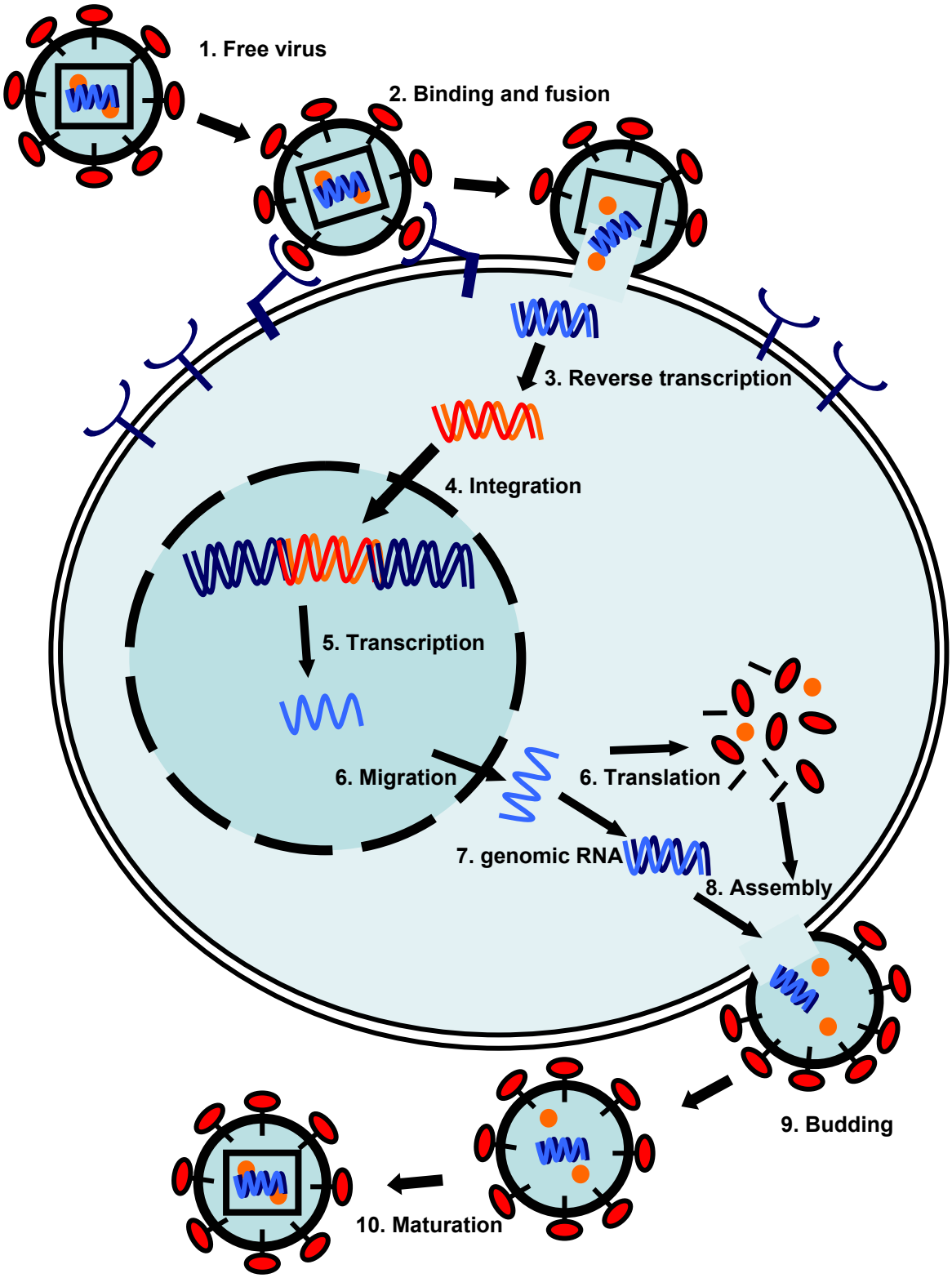


Figure 1.1. Life cycle of HIV-1

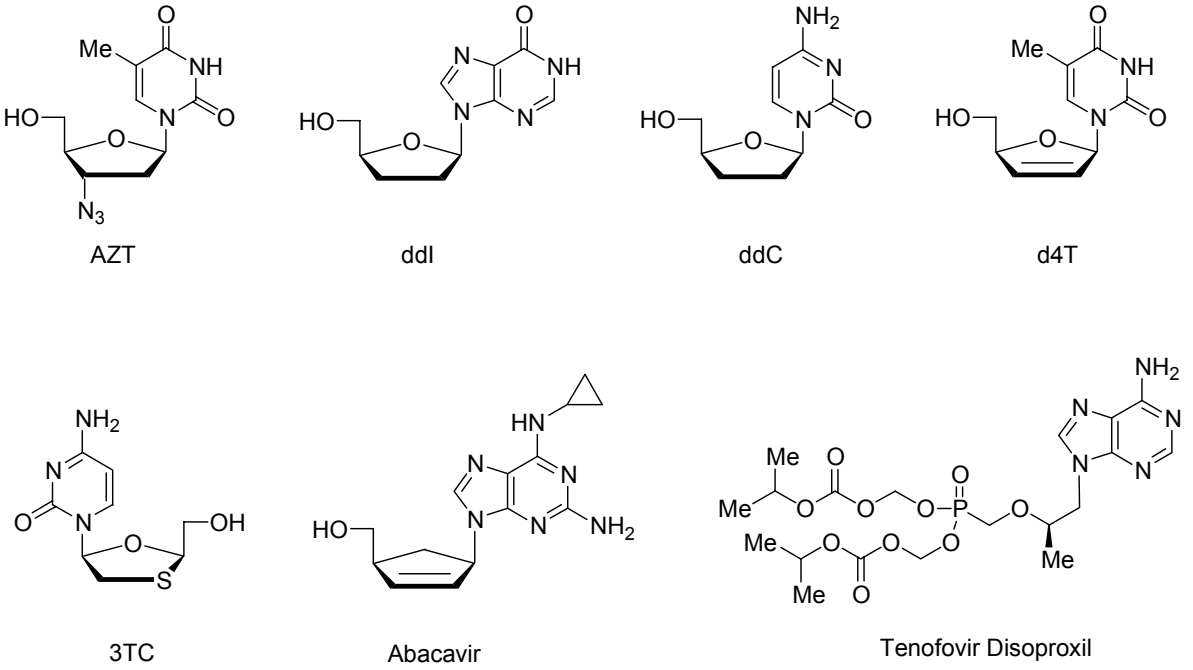


Figure 1.2. FDA-approved anti-HIV NRTIs

However, even in the context of the multi-drug combination therapy now widely in use, the drug-resistant strains of HIV-1 present a major challenge. Cloning and sequencing of nucleoside-resistant isolates have identified mutations in RT that are responsible for the resistance phenotype, as confirmed by site-directed mutagenesis of wild-type HIV clones (Table 1.2).

The simplest mechanism for resistance would be one of discrimination, i.e. some mechanism for RT to exclude the NRTI, while retaining the ability to recognize the analogous natural dNTP substrate. Point mutations such as K65R, T69D, Q151M and M184V/I lead to alterations in affinity of RT for specific NRTIs with little or no change in affinity for the corresponding dNTP substrate. These mutations are located in, or close to, the dNTP substrate binding site, and may therefore affect the initial binding and/or the subsequent positioning of the NRTI for catalysis in such a manner that the mutant RT is able to discriminate between the NRTI and the analogous dNTP substrate (Fig. 1.3).

Table 1.1. FDA-approved anti-HIV drugs in chronological order

Drug	Class[a]	Company	Approved Date
Zidovudine (AZT, Retrovir, ZDV)	NRTI	Glaxo Wellcome	March 19, 1987
Didanosine (ddI, Videx)	NRTI	Bristol Myers-Squibb	October 9, 1991
Zalcitabine (ddC, HIVID)	NRTI	Hoffmann-La Roche	June 19, 1992
Stavudine (d4T, Zerit)	NRTI	Bristol Myers-Squibb	June 17, 1994
Lamivudine (Epivir, 3TC)	NRTI	Glaxo Wellcome	November 17, 1995
Saquinavir mesylate (Invirase, SQV)	PI	Hoffmann-La Roche	December 7, 1995
Ritonavir (Norvir, ABT-538)	PI	Abbott Laboratories	March 1, 1996
Indinavir sulfate (Crixivan, IDV, MK-639)	PI	Merck & Co.	March 14, 1996
Nevirapine (Viramune, BI-RG-587)	NNRTI	Boehringer Ingelheim Pharmaceuticals, Inc.	June 24, 1996
Nelfinavir mesylate (NFV, Viracept)	PI	Agouron Pharmaceuticals	March 14, 1997
Delavirdine mesylate (DLV, Rescriptor)	NNRTI	Pharmacia & Upjohn	April 4, 1997
Combivir (Lamivudine/Zidovudine)	NRTI	Glaxo Wellcome	September 27, 1997
Efavirenz (Sustiva)	NNRTI	DuPont Pharmaceuticals	September 17, 1998
Abacavir (Ziagen)	NRTI	Glaxo Wellcome	December 17, 1998
Agenerase (Amprenavir)	PI	Glaxo Wellcome	April 15, 1999
Kaletra (lopinavir and ritonavir)	PI	Abbott Laboratories	September 15, 2000
Trizivir (Fixed-dose combination of abacavir, zidovudine, and lamivudine	NRTI	Glaxo Wellcome	November 14, 2000
Viread (tenofovir disoproxil fumarate)	NRTI	Gilead Sciences	October 26, 2001
T-20 (Fuzeon, Enfuvirtide)	Viral Fusion Inhibitor	Trimeris / Hoffmann-La Roche	March 15, 2003

[a] NRTI: Nucleoside Reverse Transcriptase Inhibitors, NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors, PI: Protease Inhibitors

Table 1.2. Fold resistances in mutant HIV-1 RT associated with NRTI resistance

Mutation	Resistance Conferred (Fold Resistance)[12]
M41L/T215Y	AZT (60-70)
D67N/K70R/ T215Y/K219Q	AZT (120)
M41L/D67N/ K70R/T215Y	AZT (180)
K65R	ddC (4-10), ddI (4-10), 3TC (17), Abacavir (3), DXG (5.6)
L74V	ddI (5-10), ddC (5-10), Abacavir (4), DXG (3.5)
M184I/V	3TC (>1,000), ddC (2-5), Abacavir (2-5)
V75T	ddC, ddI, d4T (7)
Q151M	Multidrug resistance, Appears in patients with resistance to multiple ddN
K65R/Q151M	DXG (>20)

However, our understanding of the molecular aspects of NRTI resistance has been complicated by the complex patterns of mutations required for resistance to some NRTIs such as AZT.[13,14] Therefore, recent investigations on anti-HIV nucleoside analogs have focused on the discovery of new agents active against the drug-resistant HIV strains. One result of these investigations is tenofovir, recently approved by the FDA.[15]

FDA-approved NRTI drugs

Zidovudine (AZT, Retrovir®) (Fig. 1.2).[2] The screening of nucleosides analogs, which were already being used as polymerase inhibitors to treat malignancies and herpesvirus

infections, led to the first class of antiretroviral drugs. In 1985, zidovudine was discovered as an inhibitor of the reverse transcriptase of HIV and became the first FDA-approved anti-HIV drug. AZT enters cells by passive diffusion,[16,17] and is phosphorylated by thymidine kinase.[18] AZT-monophosphate (AZT-MP) is further phosphorylated to the active species AZT-triphosphate (AZT-TP) by thymidylate kinase and nucleoside diphosphate (NP) kinase (Fig. 1.4).

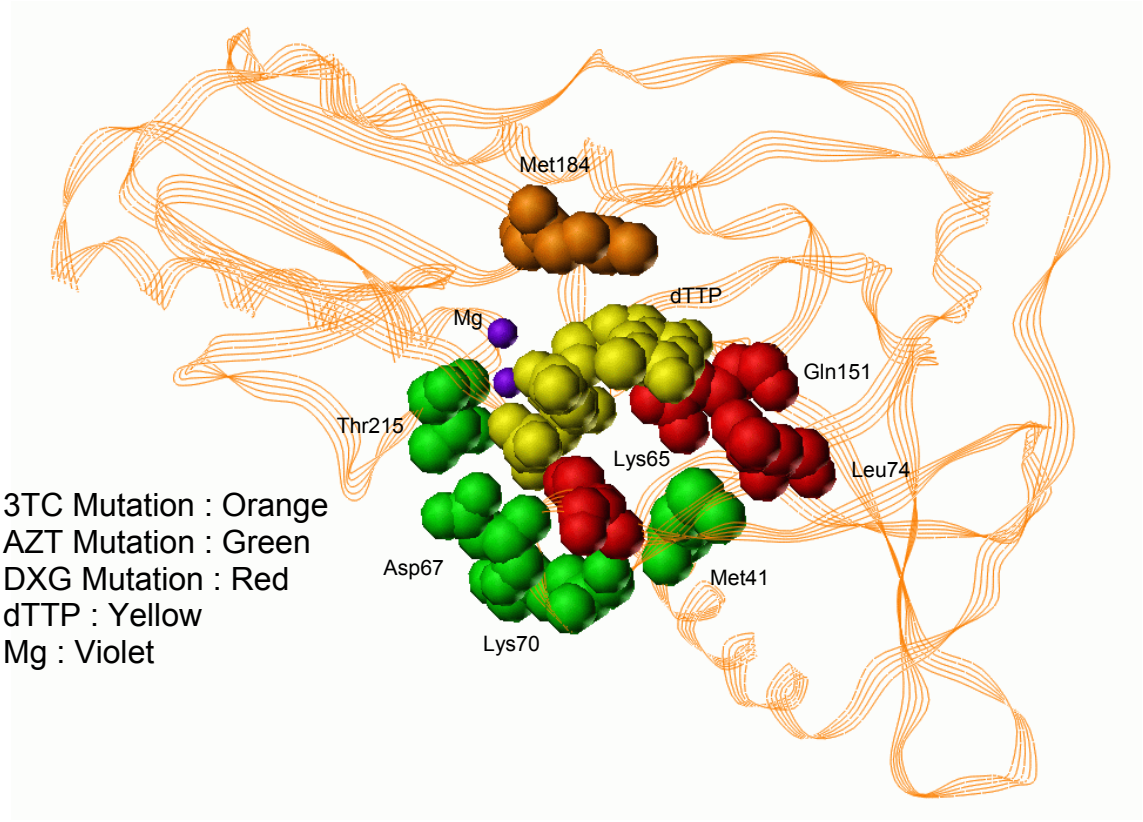


Figure 1.3. Mutations in HIV-1 RT resistant to NRTIs

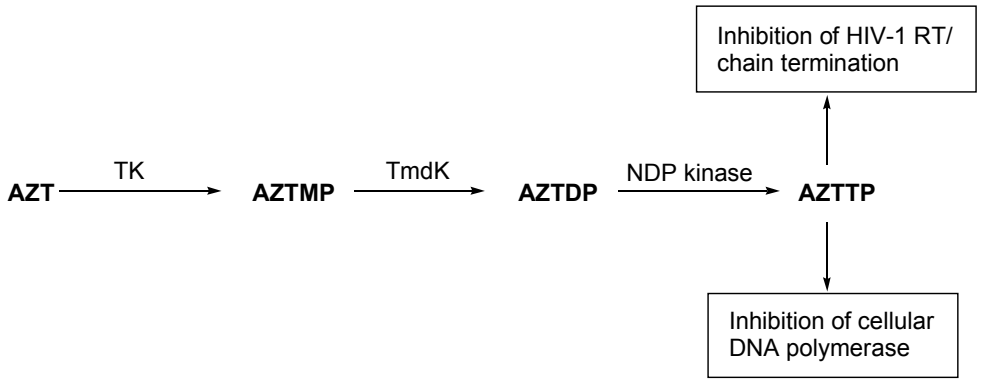


Figure 1.4. Cellular metabolism of AZT

AZT-TP binds to HIV reverse transcriptase (HIV-RT) and acts as a competitive inhibitor and a chain terminating substrate for HIV-RT during either the first or the second strand DNA synthesis.[18] AZT is the most widely used clinical therapeutic against HIV-1, and it is the most potent nucleoside *in vitro* (EC_{50} 0.002 μM in PBM cells) among the NRTI anti-HIV drugs approved so far.[19] An energy-minimized structure of AZT-TP complexed with HIV-1 RT shows that the 3'-azido group of AZT-TP fits nicely in the 3'-OH pocket of RT (Fig. 1.5). Additionally, the 3'-azido group enhances the binding affinity of AZT-TP to HIV-1 RT by hydrogen bonding with the amide backbone of Tyr115 (Fig. 1.5).

The inhibition of cellular DNA polymerase γ by AZT is associated with toxicities such as bone marrow suppression resulting in anemia and leucopenia[20] and hematopoietic toxicity resulting from high intracellular levels of AZT-MP.[21]

A number of AZT–resistant HIV strains have been selected *in vitro* and were also obtained from patients treated with zidovudine. Genetic analysis of the isolates showed 5 amino acid substitutions in the reverse transcriptase, which resulted in decreased susceptibility to zidovudine. Generally, higher levels of resistance were associated with higher numbers of mutations.[14,22] Resistance is most likely to develop in patients with

advanced HIV infection and prolonged zidovudine therapy. Examination of the RT*-T/P-dNTP ternary complex shows that AZT resistance mutations all cluster around the dNTP binding pocket, suggesting a direct influence on AZT binding. However, detailed kinetic analyses were unable to detect significant differences between the AZT-resistant RT and wild-type RT for the incorporation of AZT-TP into the DNA chain.[23,24] Recently, several groups have proposed that AZT resistance can involve the phosphorolytic removal of the chain-terminating AZT from the 3'-terminus of the primer after it has been incorporated into viral DNA.[25-29] This removal, which may be facilitated by the enhanced binding of AZT-resistant RT to an AZT-MP-terminated primer, may be accomplished by two mechanisms that use different substrates to carry out the phosphorolysis reaction.

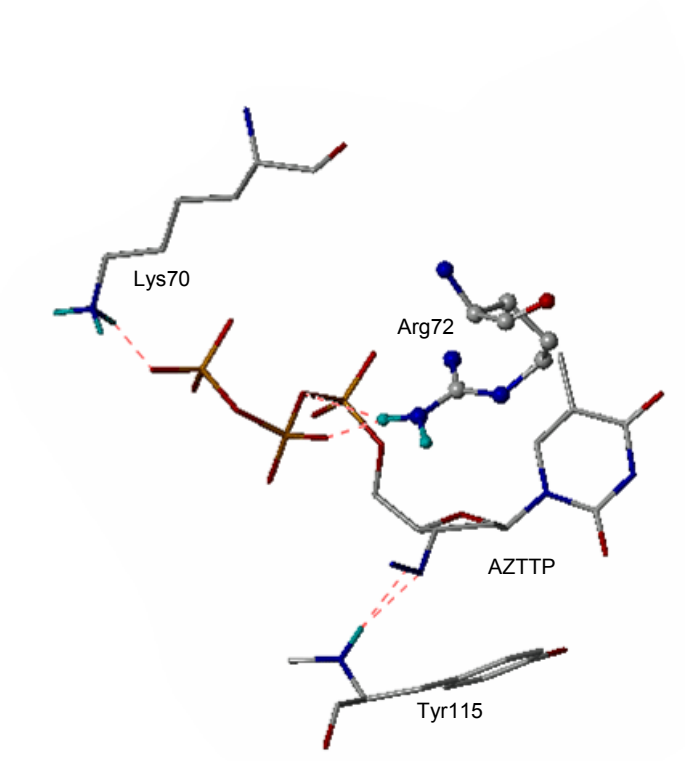


Figure 1.5. AZT at the active site of wild type HIV RT

In the first, the AZT-resistant mutant RT exhibits an increased rate of RT-catalyzed pyrophosphorolysis (the reverse reaction of DNA polymerization). The substrate for pyrophosphorolysis is PPi, which is the normal by-product of DNA synthesis and is also present at an intracellular concentration of about 150 μM.[30] RT-catalyzed pyrophosphorolysis occurs readily at these intracellular levels of pyrophosphate (PPi), and leads to removal of the chain-terminating AZT from the 3'-end of the primer.[25,26] The other mechanism, ATP-dependent mechanism, which uses adenosine triphosphate (ATP) as an acceptor molecule, has also been proposed as a mechanism to remove nucleoside and nucleotide RT chain terminators. Instead of PPi, ATP binds to the active site and removes AZT from the 3'-end of the primer, resulting in the production of dinucleotide polyphosphate.[27-29]

Didanosine (ddI, Videx®) (Fig. 1.2).[4] Didanosine is a potent and selective anti-HIV agent in ATH8 cells.[4] Intracellularly, didanosine is converted by cellular enzymes to its active metabolite, dideoxyadenosine 5'-triphosphate (ddA-TP), which inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate, deoxyadenosine 5'-triphosphate as well as by its incorporation into viral DNA causing termination of viral DNA chain elongation.[4] The pathway of ddI phosphorylation is complex and not well understood than that of AZT phosphorylation.[31] ddI can be aminated by adenosine deaminase to form ddA, or it may be phosphorylated by a cytosolic 5'-nucleotidase to form ddIMP.[32,33] All ddIMP, generated by the exogenous addition of ddI or ddA to cells, is eventually aminated and phosphorylated to ddA-TP by adenylosuccinate synthetase/lyase and purine nucleotide kinase, respectively (Fig. 1.6).[31,32]

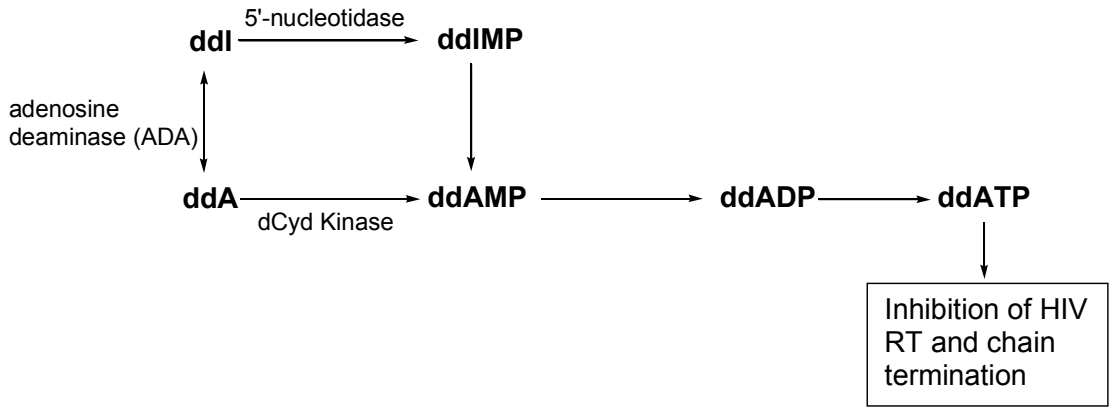


Figure 1.6. Metabolism of ddI

The *in vitro* anti-HIV-1 activity of didanosine was evaluated in a variety of HIV-1 infected lymphoblastic cell lines and monocyte/macrophage cell cultures. The concentration of drug necessary to inhibit viral replication (IC_{50}) ranged from 2.5 to 10 μM in lymphoblastic cell lines and 0.01 to 0.1 μM in monocyte/macrophage cell cultures.[4] Peripheral neuropathy[34] and pancreatitis[35] are the major side effects of didanosine. In addition, human myopathy has been associated with administration of didanosine and other nucleoside analogs.[34] HIV-1 isolates with reduced sensitivity to didanosine have been selected *in vitro* and were also obtained from patients treated with didanosine.[36-38] Genetic analysis of isolates from didanosine-treated patients showed mutations in the reverse transcriptase gene that resulted in the amino acid substitutions K65R,[36] L74V[37] and M184V.[37] The L74V mutation was most frequently observed in clinical isolates. Phenotypic analysis of HIV-1 isolates from 60 patients (some with prior zidovudine treatment) receiving 6 to 24 months of didanosine monotherapy showed that isolates from patients exhibited an average of a 10-fold decrease in susceptibility to didanosine *in vitro* compared to baseline isolates. Clinical isolates that exhibited a

decrease in didanosine susceptibility harbored one or more didanosine-associated mutations.

Zalcitabine (ddC, Hivid®) (Fig. 1.2).[5] Zalcitabine is phosphorylated to ddC-MP, ddC-DP and ddC-TP by deoxycytidine (dCyd) kinase, CMP/dCMP kinase and nucleoside diphosphate (NP) kinase, respectively (Fig. 1.7).[39]

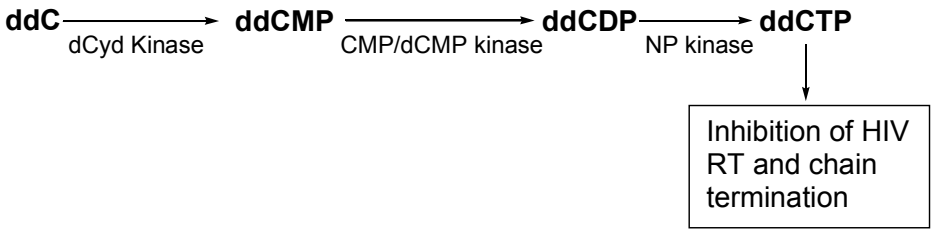


Figure 1.7. Metabolism of ddC

Zalcitabine showed antiviral activity in all acute infections; however, activity was substantially less in chronically infected cells. In *in vitro* drug combination studies with zidovudine or saquinavir, zalcitabine showed additive activity with zidovudine and synergistic activity with saquinavir.[40] The major clinical, dose-limiting toxicity of zalcitabine is peripheral neuropathy, which may occur in up to a third of patients with advanced disease.[41] The incidence in patients with less advanced disease is lower. Zalcitabine-related peripheral neuropathy is a sensorimotor neuropathy characterized initially by numbness and burning dysesthesia involving the distal extremities. These symptoms may be followed by sharp shooting pains or severe continuous burning pain if the drug is not withdrawn. Also, lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs, including zalcitabine, alone or in combination with other antiretrovirals.[42] HIV isolates with

reduced sensitivity to zalcitabine have been isolated within 1 year of therapy from a small number of patients treated with zalcitabine.[36-38,43,44] Genetic analysis of these isolates showed point mutations in the pol gene encoding RT (K65R,[38] T69D,[43] L74V,[37] V75T[44] and M184V[38]).

Stavudine (d4T, Zerit®) (Fig. 1.2).[6] Stavudine, in combination with other antiretroviral agents, is indicated for the treatment of HIV-1 infection in adults and pediatric patients.[45] Stavudine showed anti-HIV activity *in vitro* with EC_{50} values of 0.0088 μM in PBM cells[46] and 0.05 μM MT-2 cells.[47] The phosphorylation of d4T to its monophosphate is the rate–limiting step of the sequential conversion to d4T-TP, which inhibits HIV-RT equipotently with AZT-TP (Fig. 1.8).[39,48] According to the molecular modeling studies, d4T-TP binds to the active site of HIV RT through hydrogen bonding interaction with Arg72 and π-π interaction with Tyr115 (Fig. 1.9).

Like other NRTIs, d4T-TP is also incorporated into the viral DNA, thus terminating DNA synthesis at the incorporation site. The 3',5'-exonuclease ε cannot remove d4T-MP from the 3'-end of DNA once it is incorporated into cellular DNA, whereas, in the case of AZT-MP, the enzyme maintains about 20% of its normal deoxynucleotide excision capability.[49] Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of stavudine because of the inhibition of DNA polymerase γ by d4T-TP.[42] Generalized fatigue, digestive symptoms (nausea, vomiting, abdominal pain, and sudden unexplained weight loss), respiratory symptoms (tachypnea, dyspnea), or neurologic symptoms such as motor weakness might be indicative of lactic acidosis. Also, like ddI and ddC, peripheral neuropathy, manifested by numbness, tingling or pain in the hands or feet, has been reported in patients receiving stavudine.[41]

Stavudine, however, is 10-fold less toxic to human hematopoietic progenitor cells in comparison to AZT.[50]

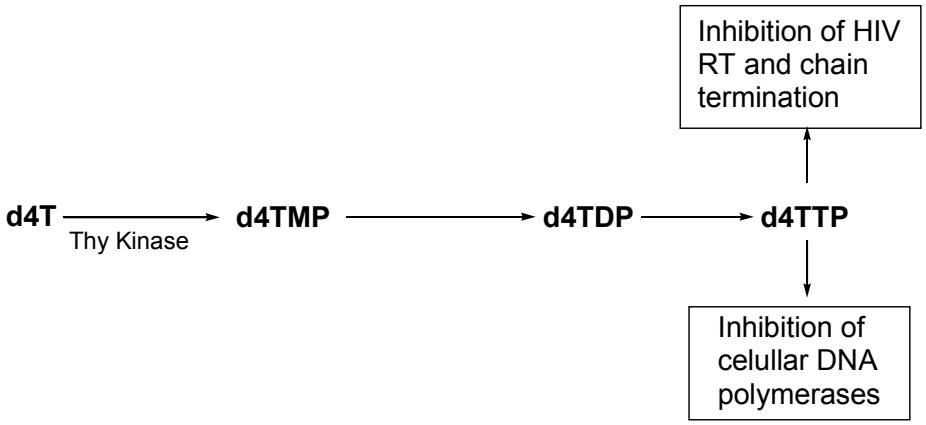


Figure 1.8. Metabolism of d4T

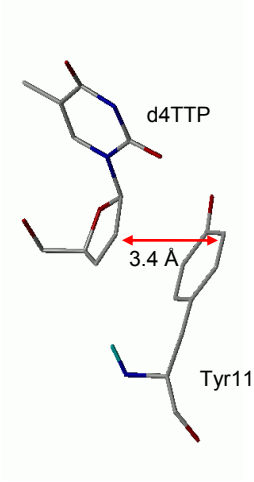


Figure 1.9. Hydrophobic interaction between d4T-TP and Tyr115

HIV-1 isolates with reduced susceptibility to stavudine have been selected *in vitro* and were also obtained from patients treated with stavudine. A recent study confirmed that thymidine analog mutations (TAMs) can arise in patients receiving d4T-based therapy who are naïve with respect to AZT.[51] When recombinant wild-type and mutant HIV-1 RT

with TAMs mutations (D67N, K70R and T215Y) were analyzed for their ability to remove eight nucleoside reverse transcriptase inhibitors in the presence of physiological concentrations of ATP, the order for the rate of removal of the eight inhibitors by the mutant RT enzyme was zidovudine (AZT) > stavudine (d4T) much greater than zalcitabine (ddC) > abacavir > amdoxovir (DAPD) > lamivudine (3TC) > didanosine (ddI) > tenofovir.[52] Thymidine analogs AZT and d4T were the most significantly removed by the mutant enzyme, suggesting that removal of these inhibitors by the ATP-dependent removal mechanism contributes to the AZT and d4T resistance observed in patients with HIV expressing thymidine analog resistance mutations. ATP-dependent removal of tenofovir was 22- to 35-fold less efficient than removal of d4T and AZT, respectively.[52] On the other hand, the 3TC-resistant mutant RT, M184V RT, remains sensitive to stavudine. As the 3TC-resistance by M184V RT is known to be caused by steric hindrance of Val184 with sugar moiety of nucleoside triphosphate, the distance from Val184 to sugar moiety of nucleoside triphosphate is critical. Compared with 3TC-TP/RT complex, the energy-minimized structure of d4T-TP/RT complex shows that the sugar moiety of d4T-TP maintains enough distance from Val184 by virtue of the hydrophobic interaction between 2',3'-double bond of d4T with the nearby aromatic side chain of Tyr115 (Fig. 1.10). This interesting hydrophobic interaction was suggested as an important factor for favorable binding mode of many D-2',3'-unsaturated nucleosides to the wild-type as well as M184V RT.[53]

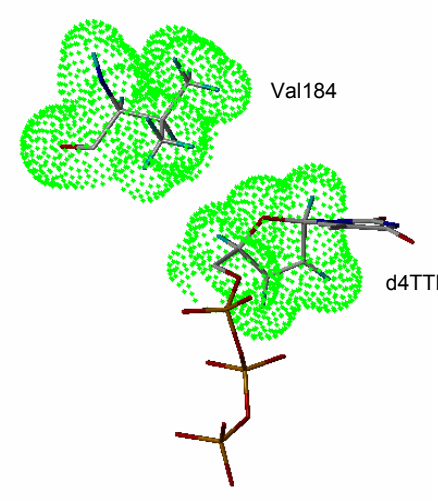


Figure 1.10. d4T-TP does not experience steric hindrance with Val184

Lamivudine (3TC, Epivir®) (Fig. 1.2).[3] Lamivudine has been approved by the FDA for treatment of HIV as well as HBV infections. 3TC differs from other NRTIs in that it is an L-nucleoside and has an oxathiolane ring in place of a ribose. 3TC is phosphorylated to 3TC-TP, the active form, by the sequential actions of dCyd kinase, deoxycytidylate kinase and nucleoside diphosphate (NP) kinase (Fig. 1.11).[54]

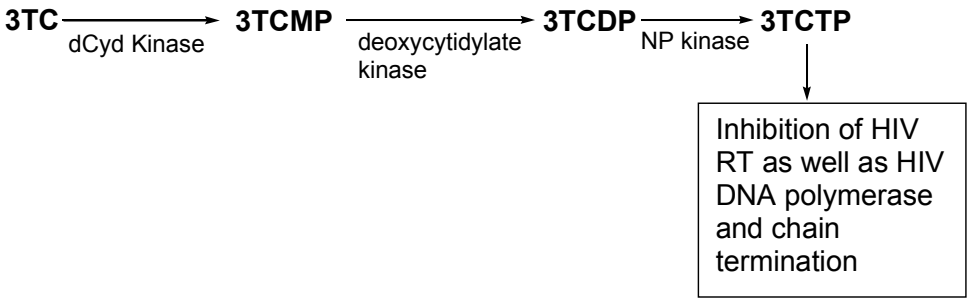


Figure 1.11. Metabolism of 3TC

Like other NRTIs, the triphosphate of 3TC (3TC-TP) competitively inhibits HIV-RT and can be incorporated into viral DNA causing chain termination. In place of the ribose ring,

3TC has a β-L-oxathiolane ring system. Although not as efficient an inhibitor of HIV-1 RT as some of the other NRTIs (K_i = 10.6 ± 1.0 to 12.4 ± 5.1 μM),[55] 3TC has much lower toxicity, apparently because it is not a good substrate for mitochondrial DNA polymerase.[56] Treatment with 3TC rapidly induces resistance in HIV-infected individuals. The resistant isolates showed reduced susceptibility to lamivudine and genotypic analysis showed that the resistance was due to specific substitution mutations in the HIV-1 RT at codon 184 within the YMDD motif.[57] The M184V RT is more than 1,000-fold less efficient in incorporating 3TC-TP into the nascent viral DNA compared with the wild-type enzyme, which is well explained by the recent molecular modeling studies.[58] 3TC-TP was nicely bound to the active site of wild type HIV RT (Fig. 1.12a), but mutation at codon 184 caused steric hindrance between the sugar moiety of 3TC-TP and the side chain of Val184, which made 3TC-TP unable to bind to the active site of mutant RT (Fig. 1.12b).

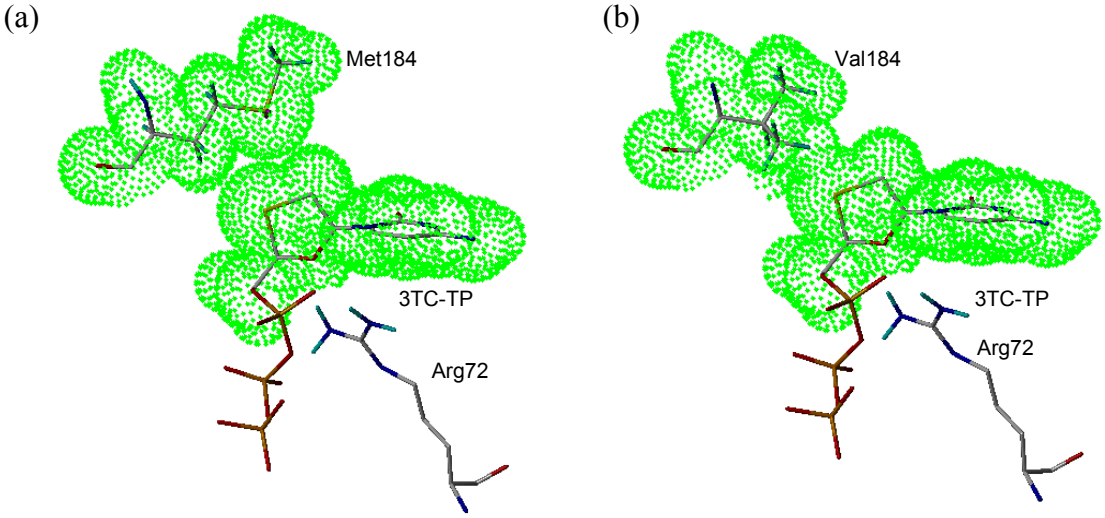


Figure 1.12. Interaction of 3TCTP with (a) M184 in wt HIV RT and (b) V184 in M184V mutant

Lamivudine in combination with zidovudine has synergistic antiretroviral activity in HIV-1 infected cell lines.[59] Thus, although resistance to AZT frequently develops, the addition of 3TC to a therapeutic regimen can cause AZT-resistant strains to revert to AZT-sensitive strains.[60] Combivir® combines 150 mg of 3TC and 300 mg of AZT into a single tablet that is usually taken twice a day. Combivir® tablets have been shown to be bioequivalent to 3TC and AZT taken individually.[61] The incorporation of both drugs into a single tablet simplifies therapy and may improve compliance. Despite the common development of the 184 mutation, many people continue to benefit from combinations that include 3TC. Recent evidence suggests that the 3TC-resistant HIV is less able to mutate again to become resistant to other drugs and it can not mutate to reestablish wild-type replication kinetics.[62]

Abacavir succinate (ABC, 1592U89, Ziagen®) (Fig. 1.2).[7] Abacavir succinate, the 6-cyclopropylamino analog of carbovir (CBV), shows strong inhibition of HIV in PBL cultures with the potency equivalent to AZT, and synergistic anti-HIV activity in combination with AZT.[7a] Also, abacavir shows synergistic activity in combination with amprenavir and nevirapine, and additive activity with didanosine, lamivudine, stavudine and zalcitabine. The intracellular activation of abacavir sequentially included its monophosphorylation by adenosine phosphotransferase, deamination to CBV-MP by cytosolic deaminase, and two further phosphorylation steps to form CBV-TP (Fig. 1.13).[63]

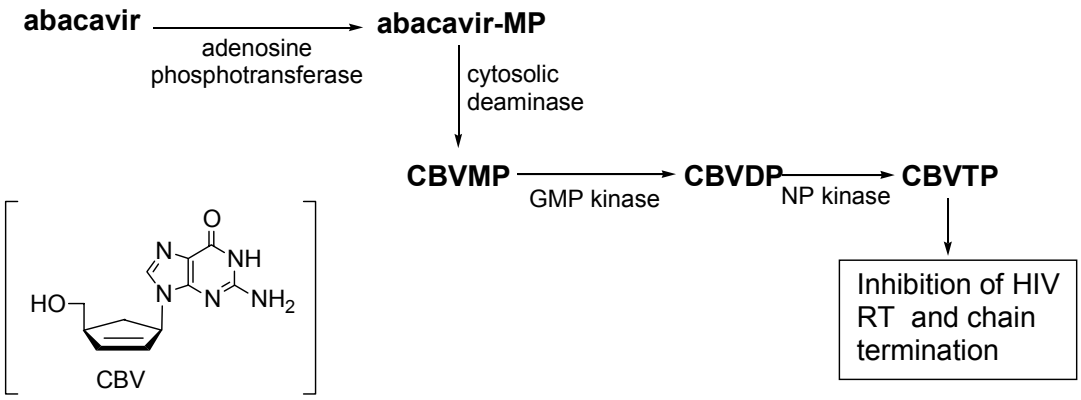


Figure 1.13. Metabolism of Abacavir

Its cytotoxcitiy is low in various human T-cells and bone marrow cells. In addition, toxicities common to other dideoxynucleosides such as peripheral neurapathy and renal, cardiac and hematopoietic toxicity has not been detected during preclinical studies.[64] However, in clinical studies, approximately 5% of adult and pediatric patients receiving abacavir developed a hypersensitivity reaction.[65] Hypersensitivity reactions were characterized by symptoms indicating multi-organ/body system involvement usually appearing within the first 6 weeks of abacavir therapy, although they may appear at any time. Frequently observed signs and symptoms of hypersensitivity include: fever, skin rash, fatigue and gastrointestinal symptoms such as nausea, vomiting, diarrhea or abdominal pain. Abacavir should not be restarted following a hypersensitivity reaction because more severe symptoms will recur within hours and may include life-threatening hypotension and death. Severe or fatal hypersensitivity reactions can occur within hours after reintroduction of abacavir in patients who have no identified history or unrecognized symptoms of hypersensitivity to abacavir therapy.[65] HIV-1 isolates with reduced sensitivity to abacavir have been selected *in vitro* and also were obtained from patients treated with abacavir.[6b] Genetic analysis of isolates from abacavir-treated

patients showed point mutations in the reverse transcriptase gene resulting in amino acid substitutions (K65R, L74V, Y115F and M184V).

Trizivir® combines 300 mg of abacavir (as the sulfate salt), 150 mg of lamivudine and 300 mg of zidovudine into a single tablet that is usually taken twice a day. Trizivir® tablets have been shown to be bioequivalent to abacavir, 3TC and AZT taken individually.[66] Because Trizivir combines a single dose of three drugs into one tablet, it may be easier for some patients to comply with their medication regimen.

Tenofovir disoproxil fumarate (bis(POC)PMPA, Viread®) (Fig. 1.2).[8] Tenofovir disoproxil fumarate (tenofovir DF) has been recently approved by FDA for HIV-positive people who have failed other anti-HIV drugs in the past.[15] Tenofovir DF is a prodrug and orally bioavailable form of tenofovir and requires metabolic processing by esterase to its active metabolite. Tenofovir DF is absorbed and then metabolized to tenofovir, which is, in turn, metabolized to the pharmacologically active metabolite, tenofovir diphosphate. Tenofovir is a phosphonate and its diphosphate is equivalent to the triphosphate of other NRTIs. Tenofovir diphosphate inhibits reverse transcriptase by competing with the natural substrate, deoxyadenosine 5'-triphosphate. Tenofovir diphosphate is also incorporated into HIV viral DNA, causing DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β and γ, and mitochondrial DNA polymerase. Tenofovir disoproxil is active against HIV strains resistant to AZT, 3TC, ddI and ddC with EC_{50} values (0.04-0.001 μM).[67] Molecular modeling studies showed that the tenofovir diphosphate has no steric hindrance with the active site of mutant RT unlike 3TC-TP (Fig. 1.14).[68] The acyclic nature of the TFV

sugar moiety allows flexibility to avoid the steric hindrance with Val184, and thus, TFV binding to M184V RT is energetically indistinguishable from binding to WT RT.

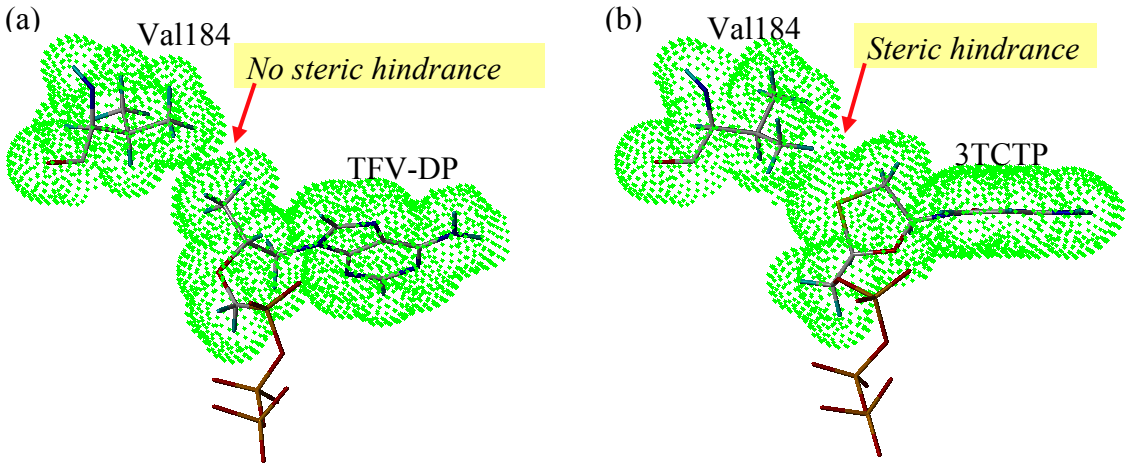


Figure 1.14. (a) Tenofovir diphosphate and (b) 3TC-TP with M184V RT

Also, another molecular modeling study of AZT-resistant RT (D67N, K70R and T215Y) complexed with AZT-TP and TFV-DP clearly indicates that, after incorporation, AZT-MP cannot be easily translocated out of the NRTI binding site because the mutations force the 3'-azido group toward Asp185, which results in steric hindrance during the translocation (Fig. 1.15). TFV-MP, however, is able to adopt multiple conformations to stay away from Asp185 by virtue of its flexible acyclic nature (Fig. 1.16).

In clinical trials, varying degrees of cross-resistance of tenofovir DF to pre-existing zidovudine associated mutations were observed and appeared to depend on the number of specific mutations.[69,70] Patients treated with tenofovir DF whose HIV expressed three or more zidovudine associated mutations that included either the M41L or L210W reverse transcriptase mutation showed reduced responses to tenofovir DF therapy.

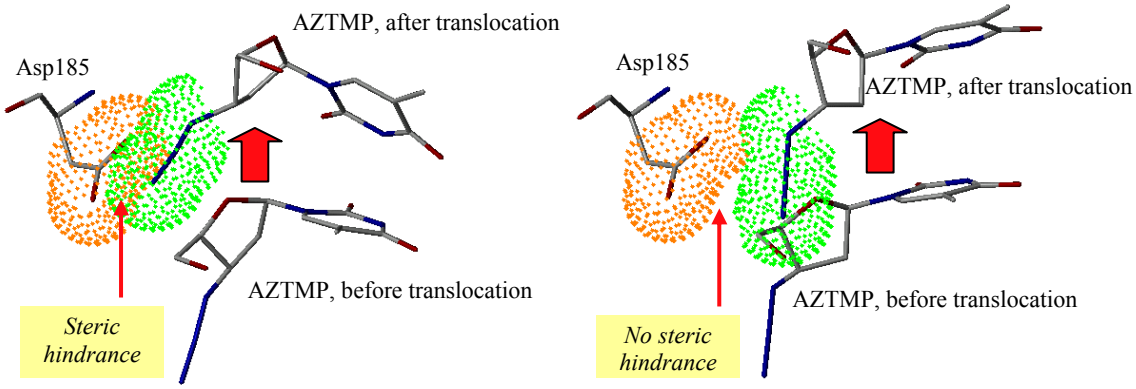


Figure 1.15. AZT-resistance mutation prevents translocation of AZT-MP

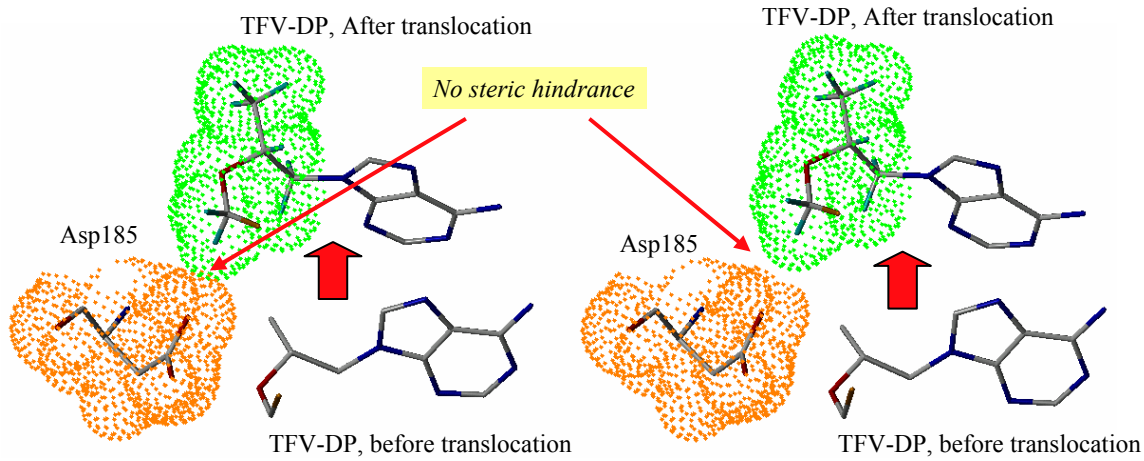


Figure 1.16. TFV-MP does not experience steric hindrance during translocation

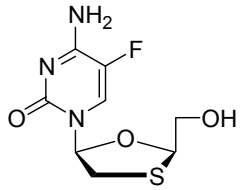
However, the presence of the D67N, K70R, T215Y/F, or K219Q/E/N mutation did not appear to affect responses to tenofovir DF therapy. Virologic response to tenofovir DF was not reduced in patients with HIV that expressed the lamivudine/abacavir associated M184V mutation. In the absence of zidovudine associated mutations, patients with the M184V mutation receiving tenofovir DF showed a -0.84 log10 copies/ml decrease in

their HIV RNA relative to placebo. In the presence of zidovudine associated mutations, the M184V mutation did not affect responses to tenofovir DF treatment. Patients with HIV mutations at K65R or L74V without zidovudine associated mutations appeared to have reduced virologic responses to tenofovir DF.[71] Adverse events reported in patients receiving tenofovir DF included abdominal pain, anorexia, asthenia, diarrhea, dizziness, dyspnea, flatulence, headache, hypophosphatemia, lactic acidosis, nausea, pancreatitis, renal impairment, rash, and vomiting. Renal impairment, which may include hypophosphatemia, has been reported with the use of tenofovir DF. Renal impairment characterized by increased creatinine, renal insufficiency, kidney failure, and Fanconi syndrome has also been observed.[72]

NRTIs under development.

Emtricitabine (FTC, Coviracil®) (Fig. 1.17). Emtricitabine has completed phase III clinical trials against HIV as well as HBV infections. *In vitro* studies have found FTC has significant antiviral effect against HIV and HBV. FTC is four-to-ten times more effective against HIV than 3TC.[73] Schinazi et al. have reported the anti-HIV activity of the racemates as well as the single enantiomers of the 5-fluoro congener of 3TC, FTC.[74] Like 3TC, FTC exhibits 20-fold more potency against HIV-1 in human PBM cells and less toxicity in myeloid cells than its D-enantiomer, and enzymatic studies of FTC show a similar profile to 3TC, in which the D-enationmer of FTC is a substrate for dCyd deaminase, and the L-enantiomer, FTC, is resistant to deamination by the same enzyme.[75]

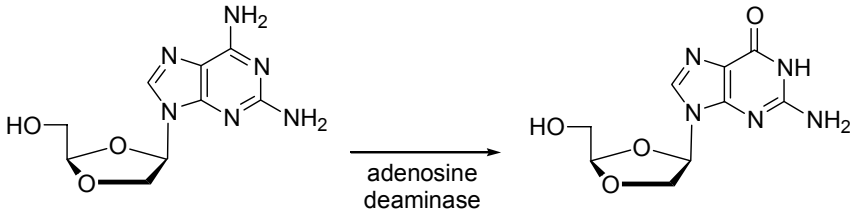
dCyd kinase and nucleoside diphosphate (NP) kinase phosphorylate FTC to its triphosphate,[76] which functions as a chain terminator of viral DNA synthesis, similarly to 3TC. However, due to FTC and 3TC cross resistance,[75b] FTC is unlikely to be a salvage therapy option. The 3TC/FTC-resistant HIV variants were reported to dominate the replicating virus population after two or more cycles of infection in the presence of 3TC or FTC.[75b] These variants are cross-resistant to 3TC and FTC but are susceptible to ddC, AZT, and ddI. FTC is currently being studied in combination with other drugs, including another NRTI and at least one protease inhibitor or non-nucleoside reverse transcriptase inhibitor (NNRTI). A recent study shows that patients with undetectable viral load who switch from twice daily 3TC to a once daily regimen of FTC/ddI/efavirenz are more likely to maintain viral suppression 48 weeks after the switch.[77] Another clinical study was conducted to determine if it was better to combine a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor with either ddI plus d4T or ddI plus FTC. This phase III clinical trial enrolled 571 HIV-positive people who had not taken any anti-HIV drugs before entering the study.[78] All of the patients in this study took efavirenz (Sustiva®), a non-nucleoside reverse transcriptase inhibitor, which was combined with either ddI plus d4T or ddI plus FTC, taken once a day. After six months of treatment, more patients (81%) taking sustiva/ddI/FTC had undetectable viral loads (<50 copies/mL) than those taking sustiva/ddI/d4T (70%). Results from some clinical trials suggest that FTC is as safe and as effective as 3TC and equally as effective as 3TC in patients who have never taken either drug before. Generally, FTC causes a similar range and incidence of side-effects as 3TC, and it appeared to be well tolerated by a majority of patients who took the drug in early clinical trials.



FTC

Figure 1.17. Emtricitabine (FTC, coviracil®)

Amdoxovir (DAPD) (Fig. 1.18). Amdoxovir, which shows potent activity against various mutants of HIV, is currently in phase II clinical trials for the treatment of HIV infections. Pharmacokinetic studies suggest that DAPD is the prodrug of the corresponding guanine derivative, dioxolane-guanine (DXG). DAPD is converted to DXG by adenosine deaminase (Fig. 1.18).[79]



DAPD DXG

Figure 1.18. DAPD (amdoxovir) and DXG

The most important feature of DAPD is its activity against mutants which are resistant to other anti-HIV and anti-HBV agents.[80,81] A Monte Carlo conformational search of dioxolane nucleoside showed that the two different conformations of the dioxolane moiety (3'-endo and 3'-exo) are interchangeable without significant loss of energy (Fig. 1.19).[82]

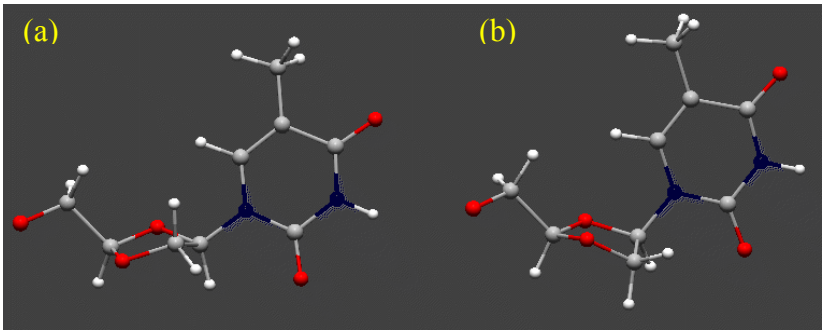


Figure 1.19. Conformational search of D-dioxolane thymine by Monte Carlo method in 5,000 steps found the global minimum as 3'-endo sugar conformation (a), but the 3'-exo conformation (b) was only 0.4 kcal/mol less stable than the global minimum.

Additionally, the 3'-oxygen atom of dioxolane moiety readily accepts hydrogen bonding from the nearby enzyme residues such as Arg72 and Tyr115 (Fig. 1.20). The combined flexible nature of the dioxolane moiety and the functional 3'-oxygen atom generates several energetically indistinguishable conformations of dioxolane nucleosides, which can cope with the conformational change at the active site of RT caused by mutations. In 3TC-resistant (M184V) RT, the sugar conformation of DXG-TP changes to 3'-exo resulting in hydrogen bonding with Tyr115. This interaction tightly holds the dioxolane moiety of DXG-TP away from Val184 to avoid unfavorable steric hindrance (Fig. 1.20c). *In vitro* studies suggest that DAPD is active against viruses with AZT/3TC resistance isolated from patients failing therapy with these drugs, suggesting that it has great potential as a drug for use in salvage therapy. DAPD is also active against virus with multiple insertion mutations at codon 69 and 215 changes. Although three RT mutations have been reported which confer resistance to DXG (namely, L74V, K65R, and Q151M), they cause only a moderate loss of activity (fold increase of EC_{50}: 3.5, 5.6 and 9.6 fold, respectively).

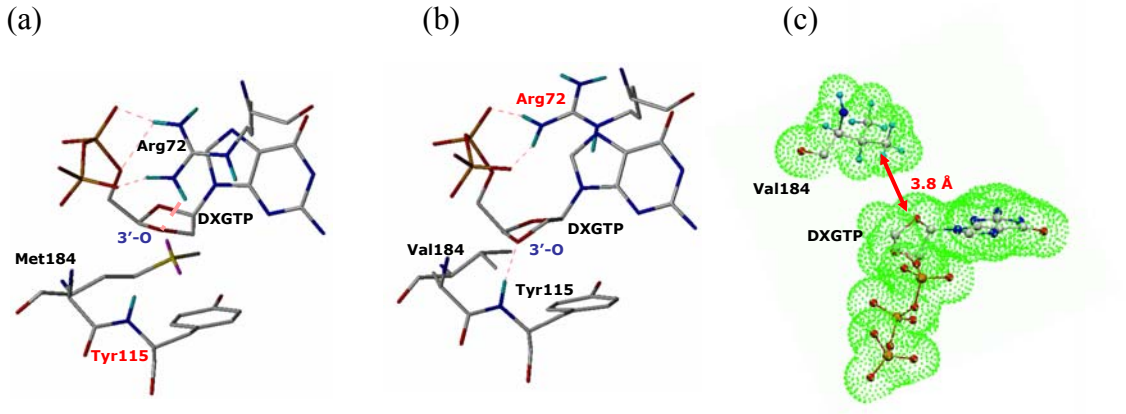


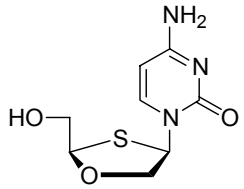
Figure 1.20. DXG-TP at the active site of (a) wild type RT and (b) M184V RT. (c) DXGTP does not experience steric hindrance with Val184.

DAPD is being studied in clinicial trials involving HIV-positive people who are new to anti-HIV treatment and HIV-positive patients who failed other NRTIs in the past. Several phase I/II studies investigating the safety, dosing and efficacy of DAPD have been conducted involving a small number of subjects. The preliminary data suggest that DAPD is a promising new NRTI which may be useful as a salvage therapy. For example, in a study in which DAPD was added to ongoing therapy in a group of highly treatment-experienced patients, viral load fell by nearly two log (or 100-fold) in six individuals taking the 500 mg twice daily dose.[83] Another open-label study in which patients (who had taken many different antiretrovirals) added DAPD to their regimen found that 7 out of 18 patients had gained at least a half log reduction in viral load at week 12.[84] In animal studies, amdoxovir caused kidney and eye problems.[84] In a phase I/II studies, kidney problems were not seen. However, five patients were required to stop the drug because of eye problems (lenticular opacities), but these problems did not appear to have any affect on vision. More studies will be needed to better understand this possible side effect.

NRTIs under pre-clinical study.

SPD754 (Fig. 1.21). SPD754 (formerly known as (-)-dOTC or BCH-10652) is a nucleoside analog which showed promising anti-HIV activity in *in vitro* and animal studies,[85-87] and is now being developed by Shire Pharmaceuticals. Like other nucleoside analogs, SPD754 interferes with the process of HIV replication by imitating the nucleosides or building blocks of DNA, used to produce new HIV genetic material. The drug is inserted into the DNA strand thus inhibiting HIV reproduction.[88] SPD754 is the successor compound to (-)-dOTC, which was suspended in 1999 by Biochem Pharma. The suspension was announced shortly after a phase II/III clinical trial had started to enroll participants. In fact, some toxicities observed in a long-term monkey toxicology (6-9 months) study raised a safety concern regarding use in humans. However, based on preliminary electron microscopy, it was found that this toxicity is not related to mitochondrial toxicity, but may be due to the accumulation of a metabolite that is specific to cynomolgus monkeys. Pre-clinical studies of (-)-dOTC did not find any myelo-toxicity (white blood cells line), its selectivity index is very high, and no mitochondrial toxicity can be suspected. Pre-clinical data indicate that SPD754 is active against HIV strains which have developed resistance to other nucleoside analogs such as AZT and 3TC, with a twofold increase in the mean IC_{50} reported when key mutations at M184V and T215Y were present.[89,90] Resistance is slow to develop in comparison with 3TC, and is associated with changes at codons 65, 75 and 184 (K65R, V75I and M184V), causing cross-resistance to 3TC.[91] Development of these mutations results in a loss of sensitivity of up to 4.3 fold, but the loss of sensitivity required for resistance to develop has not yet

been defined. SPD754 is also able to penetrate the cerebrospinal fluid (CSF), which is considered an HIV sanctuary.[88] The only clinical data regarding SPD754 available are pharmacological data from 27 healthy (HIV negative) male volunteers.[92] These data provide the rationale for once-daily dosing. A small monotherapy study of dOTC was conducted in South Africa to test for efficacy and safety. Thirty people were randomised to dOTC (200 mg, 300 mg or 400 mg twice daily) or placebo. After one week of treatment, viral load fell by over one log (ten-fold) in each treatment group.



(-)-dOTC

Figure 1.21. SPD754 [(-)-dOTC]

D-d4FC (Reverset) and L-d4FC (Elvucitabine) (Fig. 1.22). Reverset (D-d4FC), under development by Pharmasset Pharmaceuticals, was formerly known as DPC-817 whilst under development by Du Pont and then Bristol Myers Squibb. D-d4FC has potent anti-HIV activity *in vitro* with an EC_{50} value of 0.05 μM in PBM cell and anti-HBV activity with an EC_{50} value of 3 nM in 2.2.15 cells without cytotoxicity up to 100 μM in both cell lines.[93,94] Interestingly, its L-isomer (L-d4FC, Elvucitabine) is also active against HIV (EC_{50} 0.034 μM) and HBV replication (EC_{50} 0.01 μM), but has significant cytotoxicity in various cell lines.[94] Another comparison of the antiviral activity of these two enantiomers has been reported, in which L-d4FC was active against HBV in 2.2.15 cells and HIV in MT-2/IIIB cell line with an EC_{50} value of 0.008 and 0.2 μM, respectively,

while D-d4FC showed anti-HBV and anti-HIV activity with an EC_{50} value of >0.3 and 0.2 μM, respectively.[95] These results are different from those published by Faraj *et al*.[93] In any case, as reported, L-d4FC appears to be more toxic than D-d4FC.[95] An important feature of D-d4FC is its activity against 3TC- and AZT-resistant viral strains. Similarly, L-d4FC was tested against clinical isolates bearing all the key NRTI resistance mutations (M41L, M184V, T215Y and Q151M), and showed no loss of sensitivity as measured by the IC_{50}.[96]

The combination of its resistance profile, rapid uptake and conversion to the active triphosphate, and intracellular half-life of 13 to 17 h[96] make D-d4FC a promising anti-HIV candidate. So far, only dose ranging data from a single dose study has been presented.[97] This study compared buffered and enteric coated formulations of the drug, and found that the enteric coated formulation did not produce 5FC, a breakdown product that may cause toxicity. The drug will probably be dosed once daily and phase I/II studies are planned in Europe and North America. L-d4FC is in phase II clinical trials for HIV-infected patients who have developed resistance to lamivudine.

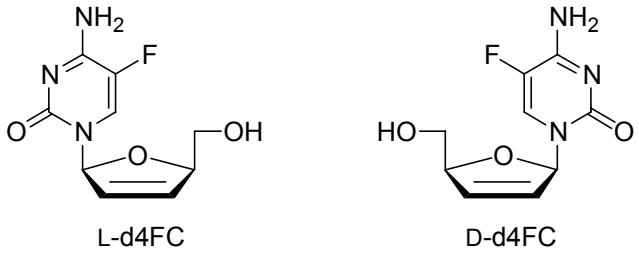


Figure 1.22. L-d4FC and D-d4FC

Racivir {(±)-FTC}. Racivir is a racemic mixture of (+)- and (-)-FTC with a long half life, which is active against both HIV and hepatitis B. It is well tolerated in preclinical safety assessment studies in dogs and rats with excellent oral bioavailability in humans. Its

major advantage over Epivir (3TC) and Coviracil (emtricitabine, FTC) appears to be its relative ease of manufacture. The first study investigated a single dose of racivir at 50, 100, 200 or 400mg, with 6 patients in each treatment group and with 2 placebo patients in each arm. There were no serious adverse events and the most frequent toxicity was headache in 4 patients including one receiving placebo. The pharmacokinetics of the single doses suggested all doses reached the EC_{90} for wild type virus, but the 400mg dose allowed plasma concentrations above the EC_{90} at 24 hours, and also achieved a concentration above the EC_{50} for 3TC-resistant virus. Following this study, a triple combination study of d4T, Sustiva/Stocrin (efavirenz) and racivir at doses of 200, 400 and 600mg took place.[98] Six patients per dosage group were dosed for 14 days. After day 15, treatment was discontinued and viral load continued to be monitored until day 35. All were antiretroviral therapy (ARV)-naïve and had a viral load >5,000 copies/ml and a $CD4^+$ T cell count >50 cells/mm^3. There was no difference in each of the arms in pharmacokinetics at day 1 and 14. The viral load fell by approximately 2 log in all groups and the authors reported that there was no viral rebound by day 28. Rebound above the level of the viral load at day 14 began to be detected by day 35, but viral load still remained more than 1 log below the baseline at this point. These data strongly suggest that racivir will be useful as a once-a-day component of treatment regimens for HIV-1 infected individuals. Racivir is under phase II clinical trial by Pharmasset.

Pyrimidine dioxolane nucleosides (Fig. 1.24). The pyrimidine dioxolanes also show potent antiviral activities against HIV. (±)-Dioxolane-T and other natural pyrimidine base analogs provided promising leads for the inhibition of HIV-1, HBV and herpes virus replication.[99] Recently, the D-isomers of this series have drawn attention because the

AZT/3TC resistant mutant HIV-1 RTs were found to remain sensitive to these derivatives.[100] Originally, (±)-dioxolane cytosine was reported by Belleau et al. as an anti-HIV agent, and subsequently, (±)-dioxolane thymidine was also reported as moderately active against HIV-1.[101,102] Extensive studies of the structure-activity relationships have led to the synthesis of L-dioxolane cytosine (L-OddC) and its 5-fluoro congener (L-F-OddC), which exhibit potent *in vitro* anti-HIV-1 and HBV activities.[103] However, L-OddC is quite toxic and stable to degradation by cytidine deaminase and deoxycytidine deaminase. L-OddC is metabolized in cells by dCyd kinase to its monophosphate, and subsequently to the di- and triphosphate, which inhibits DNA polymerase α, β and γ.[104] L-OddC exhibits potent antitumor activity against various solid tumor cell lines, including prostate, renal, hepatoma and colon.[104] Thus, L-OddC is the first L-nucleoside analog ever shown to have anticancer activity, and is also the first true chain terminator capable of inhibiting tumor growth.[105] L-OddC is currently undergoing phase II clinical trials against leukemia and solid tumors. Another novel compound, reported by Schinazi et al., was D-FDOC, a dioxolane 5-fluorocytosine that also possesses activity against viruses with common nucleoside resistance-conferring mutations.[106] In tissue culture studies, this agent was found to be active against viruses containing a variety of TAMs, as well as M184V. Some degree of resistance was observed in regard to the K65R substitution in both tissue culture and biochemical assays performed with the active triphosphate derivative of this substance. These results suggest that further study of this compound is warranted. The flexible nature of dioxolane moiety allows interconversion between 3'-endo and 3'-exo conformations. Interestingly, energy minimized structures of several dioxolane nucleosides complexed with wild-type as well

as M184V RT indicate that the conformation of dioxolane moiety depends on the attached aglycon (Fig. 1.23). Thus, for example, dioxolane-T-TP favors 3'-exo while D-FDOC-TP is biased to 3'-endo conformation of dioxolane. In either conformation, dioxolane's binding affinity to the active site of RT is significantly influenced by its 3'-oxygen atom which accepts hydrogen bonding from either Arg72 or Tyr115 (Fig. 1.23). As these enzyme residues (Arg72 and Tyr115) are located at the opposite side of Val184, the hydrogen bonding interaction holds the dioxolane nucleoside away from Val184 to avoid unfavorable steric hindrance (Fig. 1.23c). Therefore, molecular modeling study indicates that the D-dioxolane nucleosides are not cross-resistant to M184V RT.

Dideoxynucleoside analogs (Fig. 1.25). The discovery of dideoxynucleosides, such as AZT, ddC and ddI, has triggered an extensive development of this class of compounds. One analog of AZT is AZdU, which was the first nucleoside analog with a uracil base to have anti-HIV activity with EC_{50} of 0.18-0.45 μM.[107] AZdU is sequentially phosphorylated to AZdU-TP, which acts as both an HIV-RT inhibitor and proviral DNA chain terminator.[108] Although its anti-HIV activity is less potent than that of AZT, its toxicity on bone marrow cells is significantly lower than that of AZT.[109] However, AZdU shows cross-resistance with AZT-resistant HIV strains. The clinical trials of AZdU were discontinued due to its extensive metabolism to 5'-glucuronide followed by excretion.[110] AZdMeC, another analog of AZT, shows potent anti-HIV activity in human PBM cells and macrophages and is less toxic than AZT in human bone marrow cells.[111] Metabolic studies indicate that this compound is slowly converted intracellularly to AZT. The major metabolite of AZdMeC is AZT-MP with no formation of AZdMeC-MP. The low toxicity of this compound is related to the lack of formation of AZT-TP in human

bone marrow cells. The clinical trials of AZdMeC was discountinued due to the insufficient conversion to AZT in human by cytidine deaminase.

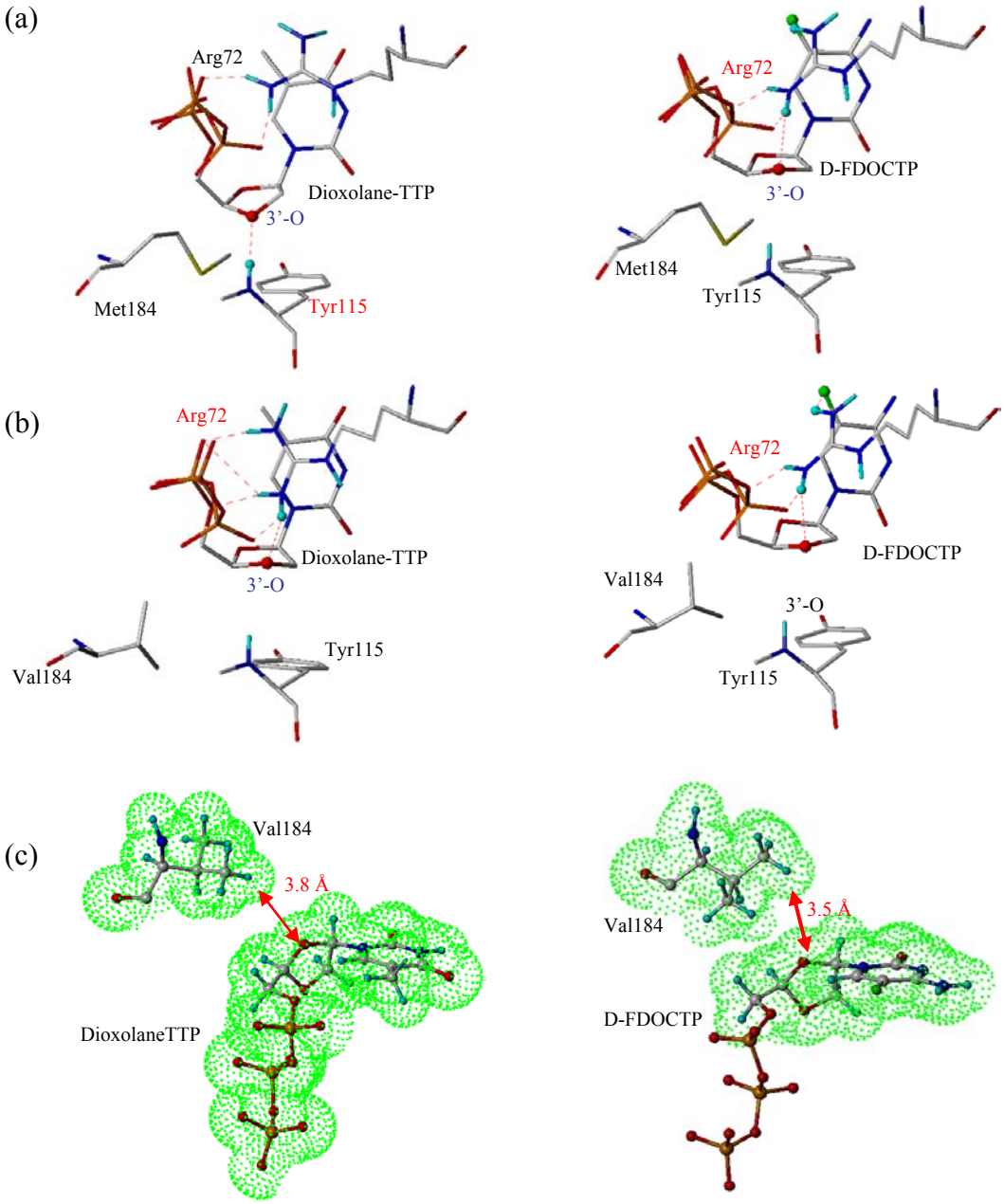


Figure 1.23. Dioxolane nucleosides/ RT complexes. (a) WT RT, (b) M184V RT, (c) No steric hindrance between Val184 and dioxolane moiety.

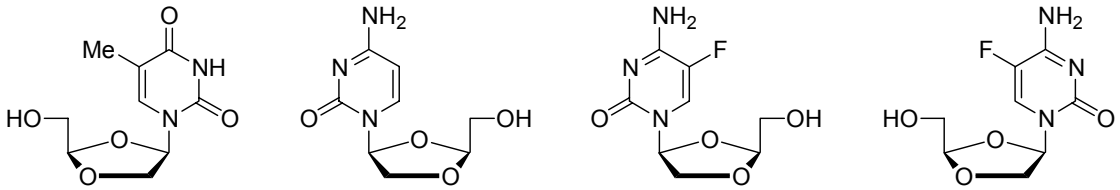


Figure 1.24. Dioxolane nucleosides

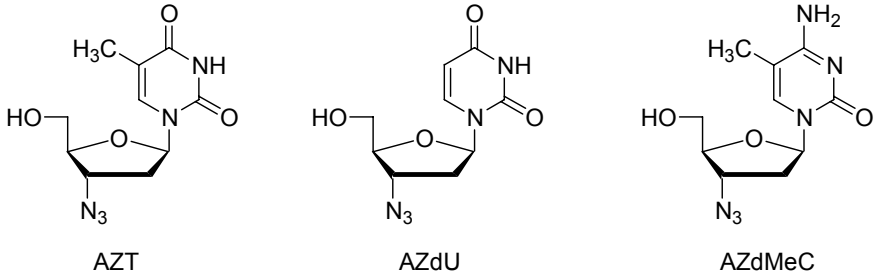


Figure 1.25. 3'-Azido-2',3'-dideoxy nucleosides

4'-Substituted nucleosides (Fig. 1.26). Introduction of a functional group at the 4'-position of 2'-deoxynucleosides elicits potent antiviral activity. Thus, 4'-azido analogs exhibit potent anti-HIV activity with EC_{50} of 0.8-0.003 μM,[112] and, within the series, the guanine analog was found to be most potent. Unfortunately, this class of compounds was highly cytotoxic presumably due to the azido group, which is converted to an amino group. Further evaluations of 4'-azidothymidine (ADRT) in H9, PBL and MT-2 cells infected with HIV have demonstrated a similar inhibitory profile to that of AZT. Interestingly, ADRT retains its activity against AZT-resistant mutants. 4'-*C*-Ethynyl-substituted 2'-deoxynucleosides have shown impressive anti-HIV activity with EC_{50} in the nano- and subnanomolar range, but compounds in this class also display significant toxicities, with IC_{50}'s in the micromolar range.[113] The diaminopurine, guanosine and cytosine derivatives show potent antiviral activities against HIV-1 (EC_{50}: 0.3, 1.4 and 4.8

nM, respectively) along with the high cytotoxicity (IC_{50} of 0.82, 1.5 and 0.92 μM, respectively). The thymidine, 5-bromouridine, 5-iodouridine, 5-fluorocytidine and guanine analogs, although less active, do not show any toxicity in MT-4 cells.

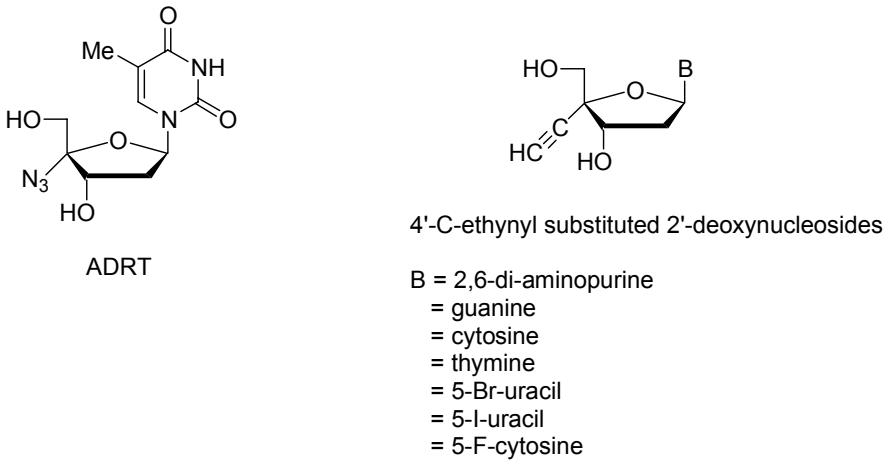


Figure 1.26. 4'-Substituted-2'-deoxy nucleoside analogs

2'- or 3'-Fluoronucleosides (Fig. 1.27). 3'-α-Fluorinated analogs of 2',3'-dideoxynucleosides show anti-HIV activity, whereas 3'-β-fluoro derivatives do not show any significant anti-HIV activity.[114] Particularly, 3'-α-fluoro-2',3'-dideoxyuridine and 3'-α-fluoro-2',3'-dideoxyguanine have anti-HIV activity significantly greater than the parent nucleosides, ddU and ddG.[115] Analogously, 3'-α-fluoro-2',3'-dideoxythymidine (FLT, alovudine) shows potent anti-HIV activity in various cell lines.[116] FLT is phosphorylated intracellularly to FLT-TP, which is one of the most potent inhibitors of HIV-RT *in vitro*.[117] It was first developed by the pharmaceutical company Lederle in 1992, but due to the absence of clear benefit over zidovudine, the company did not pursue further development. However, when information became available on resistance

to AZT, researchers at Medivir tested alovudine activity against HIV variants highly resistant to AZT and found that the compound was active against the drug variants. Medivir purchased the full rights to develop FLT, completed phase I studies and reported phase II results in 2002.[118] In a study of 15 individuals with thymidine analog resistance and detectable viral load, FTC 7.5mg once daily was added to their existing regimen and results were stratified according to baseline thymidine analog mutations and whether or not individuals received concurrent d4T treatment. The greatest viral load reduction was seen in 11 individuals who did not receive d4T (-1.88 log at week 4), and was weaker in patients who received concurrent d4T (-0.54 log). There was no significant difference in response according to the number of mutation by the thymidine analog. FLT's earlier development was also negatively affected by its side-effect profile (anaemia and neutropenia) when used at a dose of 20mg a day.[119,120] However, no serious side effects were seen in the phase II study.[118]

2'-β-Fluorinated analogs of 2',3'-dideoxynucleosides retain anti-HIV activity in comparison to the parent nucleosides. Moreover, both 2'-fluoro-ara-ddA and 2'-fluoro-ara-ddI are stable in acidic conditions under which ddA and ddI decompose instantaneously by acid-catalyzed glycosidic bond cleavage.[121] 2'-Fluoro-ara-ddA and 2'-fluoro-ara-ddI retain the same activity as their parent nucleosides but seem to be slightly more cytotoxic. Phosphorylation of 2'-fluoro-ara-ddA by dCyd kinase forms 2'-fluoro-ara-ddA-MP, which is sequentially phosphorylated to 2'-fluoro-ara-ddA-DP and 2'-fluoro-ara-ddA-TP.[122] 2'-Fluoro-ara-ddC is also effective against several different strains of HIV.[123]

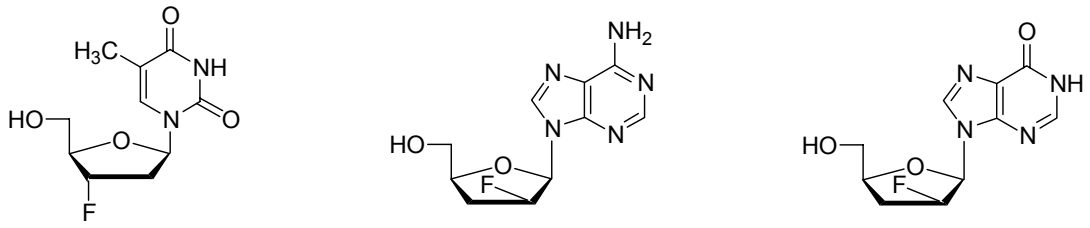
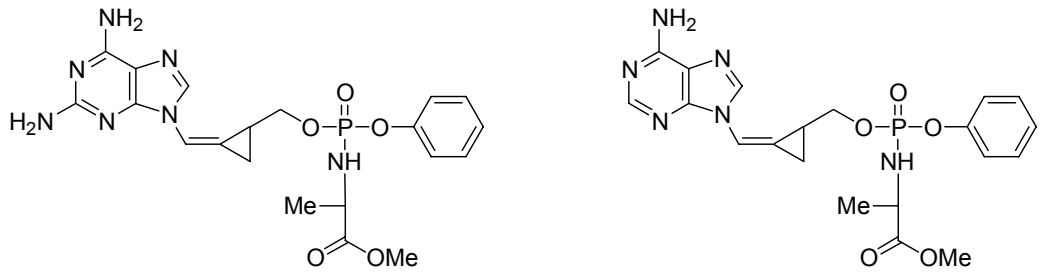


Figure 1.27. 2'- or 3'-Fluoronucleosides

Methylenecyclopropane nucleoside analogs (Fig. 1.28). Nucleoside analogs with a *Z*- or an *E*-methylenecyclopropane moiety were synthesized and examined for activity against HIV-1 *in vitro*.[124,125] The addition of a methyl phenyl phosphoro-L-alaninate moiety to modestly active analogs resulted in potentiation of their anti-HIV-1 activity. Two such compounds, designated QYL-685 (with 2,6-diaminopurine) and QYL-609 (with adenine), were the most potent against HIV-1 *in vitro* with 50% inhibitory concentrations of 0.034 and 0.0026 μM, respectively, in MT-2 cell-based assays.[124] Both compounds were active against zidovudine-resistant, didanosine-resistant, and multidideoxynucleoside-resistant infectious clones *in vitro*.[124] However, when the HIV-1 variants resistant to QYL-685 were induced by exposing HIV-1_{LAI} to increasing concentrations of QYL-685, after 16 passages, the virus (HIV-1_{P16}), which contained M184I mutation, became less sensitive to QYL-685 (104-fold), QYL-609 (>41-fold) and 3TC (>1,100-fold) in comparison to HIV-1_{LAI}.[125] Two infectious clones, HIV-1_{M184I} and HIV-1_{M184V}, were resistant to QYL-685, QYL-609 and 3TC, confirming that the M184I mutation was responsible for the observed resistance. Viral-fitness analyses (competitive HIV-1 replication assays) revealed that in the absence of drugs, M184I and M184V conferred a replication disadvantage on the virus compared to the replication efficiency of the wild-type infectious clone (HIV-1_{wt}). However, in the presence of QYL-685 (4

μM), HIV-1_{M184I} and HIV-1_{M184V} showed greater fitness than HIV-1_{wt}. These data may provide structural and virological relevance with regard to the emergence of M184I and M184V substitutions in HIV-1.[125]



QYL-685 QYL-609

Figure 1.28. Methylenecyclopropane nucleosides (QYL-685 and QYL-609)

2',3'-Unsaturated nucleosides (Fig.1.32). Recently, a number of analogs of 2',3'-didehydro-2',3'-dideoxynucleosdies such as d4T and abacavir have been reported with promising anti-HIV activity. L-d4C has been reported to have antiviral activities against HIV as well as HBV (1.0 μM and 8 nM for HIV and HBV, respectively).[126] The importance of the 2',3'-unsaturated (d4) compounds in anti-HIV chemotherapy has been refocused after a recent study which showed that the instability of the purine analogs can be remarkably improved by the isosteric replacement of 2'-hydrogen by a fluorine atom.[127] Therefore, agents containing the 2',3'-unsaturated sugar moiety with 2'-fluoro substitution has become one of the rational targets in the search for safe, effective and chemically stable antiviral agents. A molecular modeling study of several 2',3'-unsaturated nucleosides including d4T, abacavir, D-2'-fluoro-d4C and D-2'-fluoro-4'-thio-d4C found that the 2',3'-double bonds of these nucleosides are always in the close

proximity to the aromatic side chain of Tyr 115 by hydrophobic π–π interactions (Fig. 1.29). The high binding affinity of the d4 nucleoside triphosphates to the active site of RT, thus obtained, might be one of the reasons for their potent anti-HIV activity.[128]

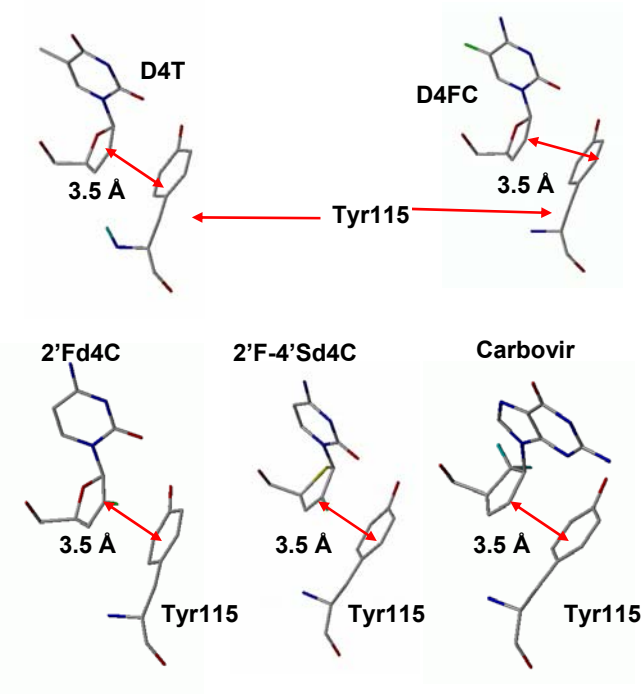


Figure 1.29. Hydrophobic interaction between d4 nucleosides and Tyr115

A comprehensive structure-activity relationship study has been done for the 2'- or 3'-substituted d4N analogs. Among these, D-2'-F-d4C shows moderate anti-HIV with significant toxicity.[127b] Even though this compound and its 5-fluoro analog D-2'-F-d4FC showed only moderate anti-HIV activity, it is noteworthy that the adenosine and inosine derivatives show potent anti-HIV activity and no cross-resistance against the 3TC-resistant mutant (M184V RT).[127b] Among the series of the antipodes, L-2'-F-d4Ns, cytosine and 5-fluorocytosine derivatives exhibit potent anti-HIV and anti-HBV activity,

but they were found to be highly cross-resistant to M184V RT.[127a] Like 3TC, L-2'-F-d4C has steric hindrance with the bulky side chain of Val184 in M184V RT, which results in cross-resistance to 3TC-resistant HIV strains. As the glycosidic bonds of 2'-F-d4Ns are more stable under acidic conditions than d4Ns without 2'-fluorine, it was of interest to investigate the influence of the fluorine atom at the 3' position. D-3'-F-d4C shows only modest anti-HIV activity in H9 cells.[129] However, extensive structure-activity relationship study of the L-3'-F-d4Ns found that the cytosine and 5-fluorocytosine derivatives are potent anti-HIV agents with EC_{50} values of 0.03 μM and 0.007 μM in PBM cells with little or no significant toxicity.[130,131] In spite of the potent anti-HIV activities of this series of compounds, their high cross-resistance to the 3TC-resistant RT limits their further development as new anti-HIV agents.[131] The 4'-thio analogs of d4C have been reported to have marked anti-HIV and anti-HBV activities. Particularly, L-4'-thio-d4C and L-4'-thio-d4FC exhibit significant anti-HIV activity (EC_{50}s 0.8 and 0.4 μM, respectively, in HeLa CD4 cells) as well as anti-HBV activity (EC_{50}s 0.8 and 3.5 μM, respectively) without toxicity.[132] Combination of the substitutions of 2'-F and 4'-S was also achieved, and among the series D- and L-2'-F-4'-thio-d4Cs show potent anti-HIV activity with EC_{50} values of 0.9 μM and 0.12 μM, respectively, with no cytotoxicity up to 100 μM.[133,134] Like their 4'-oxo congeners, the L-isomer was more potent than the D-isomer, but it was highly cross-resistant to M184V RT.[134] However, it is very interesting that, unlike D-2'-F-d4C, D-2'-F-4'-Sd4C also showed significant cross-resistance to the 3TC-resistant RT.[133] It has been generally accepted that, in conjunction with the mechanism of 3TC-resistance which include the steric hindrance of Val184 with the L-oxathiolane sugar moiety, the unnatural L-nucleosides are vulnerable to the cross-

resistance to M184V RT.[135] However, as this was the first D-nucleoside which developed significant cross-resistance to M184V RT, the mechanism of resistance was of particular interest. The molecular modeling study indicates that the steric pressure on the active site of RT conferred by M184V mutation not only blocks the space where the sugar moiety of the L-nucleoside resides, but significantly limits the conformation of D-nucleoside. Thus, the energy-minimized structure of the D-2'-fluoro-4'-thiocytidine/RT complex shows that the steric hindrance of the sugar moiety with Val184 caused by the large van der Waals radius of the 4'-sulfur atom results in the deformation of the active site (Fig. 1.30).[133] Also, abacavir's 4'-CH_2 was found to be slightly overlapping with the van der Waals radius of Val184 resulting in the decreased sensitivity to the 3TC-resistant mutant (Fig. 1.31).[128]

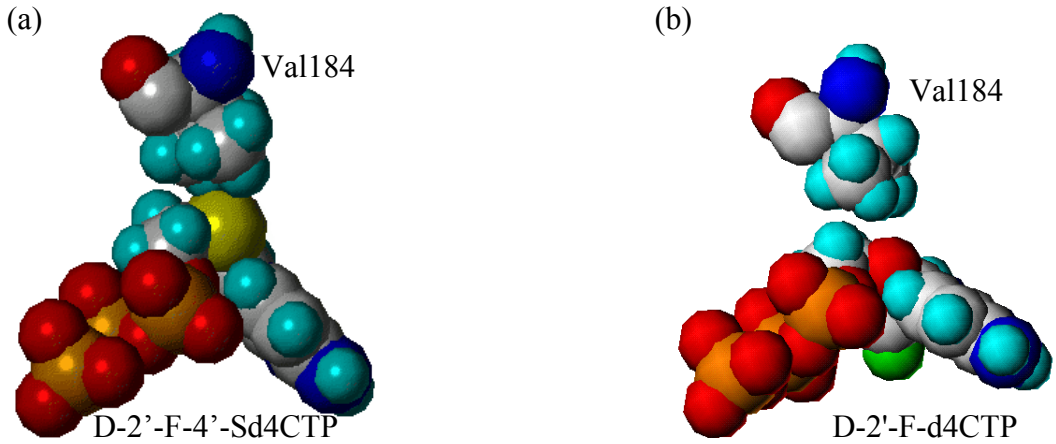


Figure 1.30. Large van der Waals radius of 4'-sulfur atom in D-2'-F-4'-Sd4C clashes with Val184.

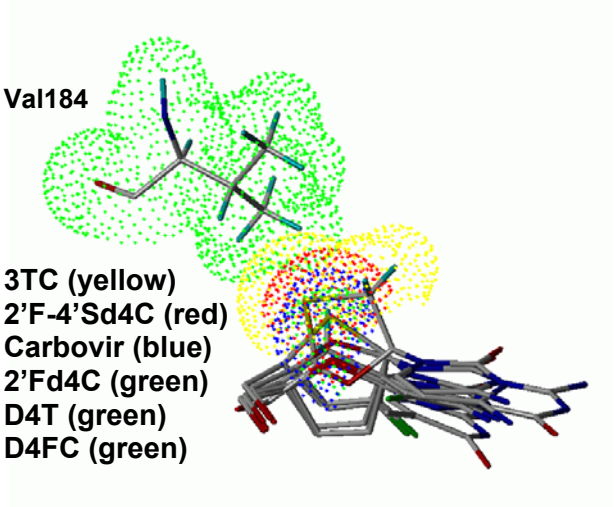


Figure 1.31. Various d4 nucleosides interacting with Val184 in M184V RT

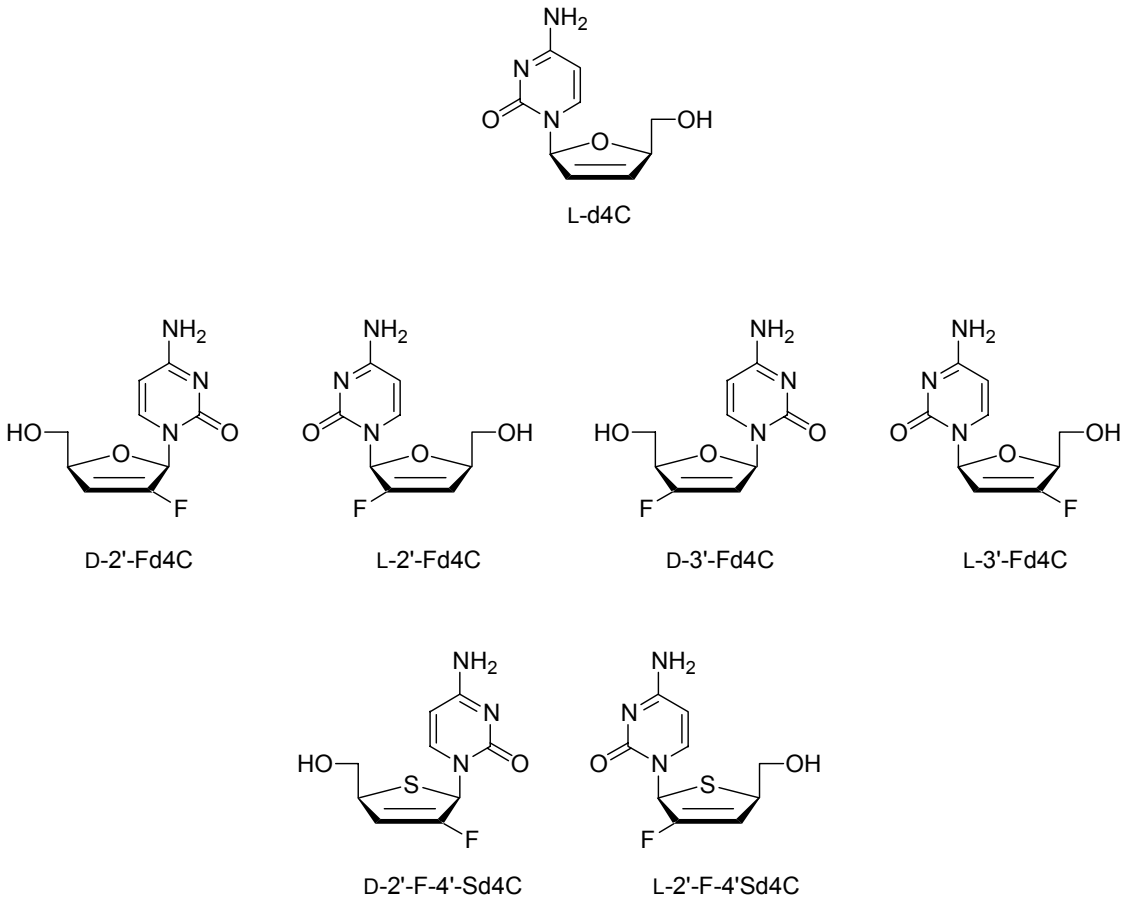


Figure 1.32. 2',3'-Unsaturated (d4) nucleosides

Prodrugs (ddA and d4T prodrugs) (Fig. 1.33). The bottleneck in the metabolic pathway leading from AZT and the other 2',3'-dideoxynucleoside (ddN) analogs to their active 5'-triphosphate form is the first phosphorylation step. Therefore, attempts have been made to design 2',3'-dideoxynucleoside monophosphate (ddNMP) prodrug that, once they have been taken up by the cells, deliver the nucleotide (ddNMP) form. This approach has proven particularly successful for a number of NRTIs such as 2',3'-dideoxyadenosine (ddA) and d4T. The bis(S-acetyl-2-thioethyl)phosphotriester of ddA [bis(SATE)ddAMP] was synthesized and found to be 1,000-fold more potent against HIV than the parent compound ddA.[136] Similarly, aryloxyphosphoramidate derivatives of d4T (i.e. So324, a d4T-MP prodrug containing at the phosphate moiety a phenyl group and the methylester of alanine linked to the phosphate group through a phosphoramidate linkage) have been synthesized.[137-139] After the d4T aryloxyphosphoramidate has been taken up by the cells, d4TMP is released intracellularly and then processed onto its active metabolite d4T-TP.[140] This "thymidine kinase bypass" explains the high anti-HIV activity of d4T aryloxyphosphoramidate derivatives in thymidine kinase deficient cells and resting monocytes/macrophages.[141] Thymidine kinase (in the case of d4T) and adenosine deaminase (in the case of ddA) can also be bypassed by using the cyclic saligenyl approach.[142,143] *Cyclo*saligenyl pronucleotides of d4T and ddA deliver exclusively the nucleotides d4TMP and ddAMP, not only under chemical-simulated hydrolysis conditions but also under intracellular conditions.[144,145] This has been convincingly shown for the *cyclo*saligenyl derivatives of d4TMP in a number of cell lines.[146]

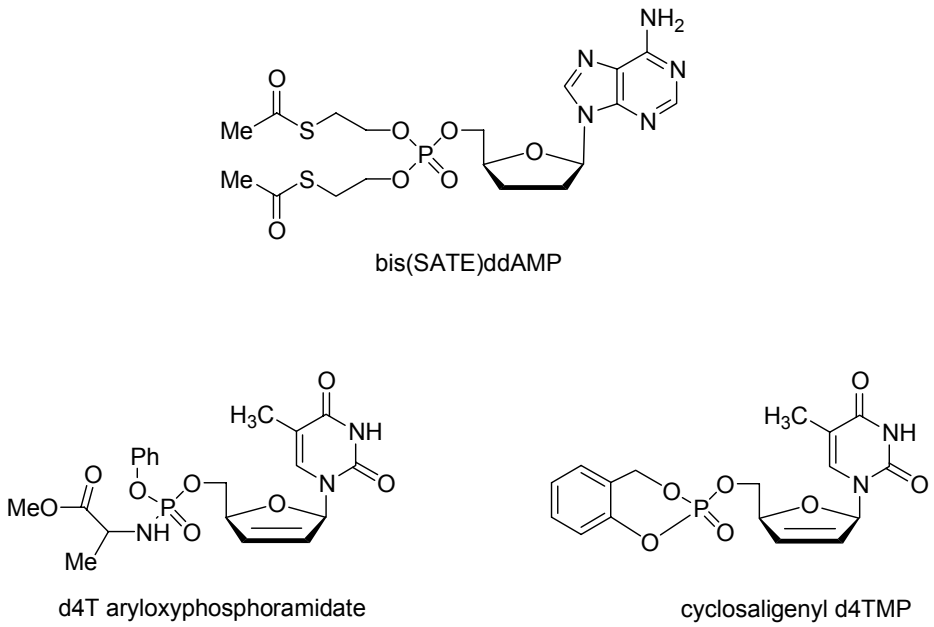


Figure 1.33. Prodrugs of ddA and d4T

ANTI-HBV AGENTS

Hepatitis B virus (HBV) belongs to the family of *hepadnaviridae*, which includes viruses such as woodchuck hepatitis virus, ground squirrel hepatitis virus and duck hepatitis virus. Despite significant progress in the prevention of hepatitis B virus (HBV) transmission by vaccine, there are still more than 350 million chronic HBV carriers worldwide.[147,148] A significant proportion of them develops chronic hepatitis,[149,150] which is associated with high morbidity and mortality because of the frequent development of cirrhosis and/or hepatocellular carcinoma.[151-153] Despite, the availability of a safe and effective vaccine, the world prevalence of HBV-related infection and development of hepatocellular carcinoma have not declined significantly, and treatment with antiviral agents remains the only therapeutic option available to individuals who are chronically

infected with HBV. Until now, alpha interferon (IFN-α, intron A®) and two nucleoside analogs, lamivudine (3TC, epivir-HBV®) and the recently approved adefovir dipivoxil (ADV, bis(POM)PMEA, hepsera®),[154,155] have been approved by the FDA for the treatment of chronic HBV infections. Unfortunately, the rate of response to IFN is low, and drug-associated side effects are significant.[156,157] Nucleoside analogs were first tried as antiviral agents 2 decades ago and are currently used in the treatment of several viral infections, such as HIV, herpes viruses, or HBV infection. The similarity between the HBV and HIV replication cycle (Figure 1.34), as well as the clinical observations of clearance of HBV viremia during therapy of patients with AIDS and co-infection HBV,[158] led to the use of nucleoside analogs in the treatment of chronic HBV infection with satisfactory results. Besides their potent antiviral activity, other advantages of nucleoside analogs include oral administration, excellent tolerance to therapy, absence of serious side effects, and ability to provide treatment to subgroups of chronic hepatitis B patients who are at high risk for side effects after or with contraindications to IFN-α therapy.[159,160] Regarding the safety issue, however, it should be noted that there have also been nucleoside analogs with severe toxicity and even patient deaths.[161] Therefore, intensive preclinical investigation is required before initiation of clinical trials with any of these agents.

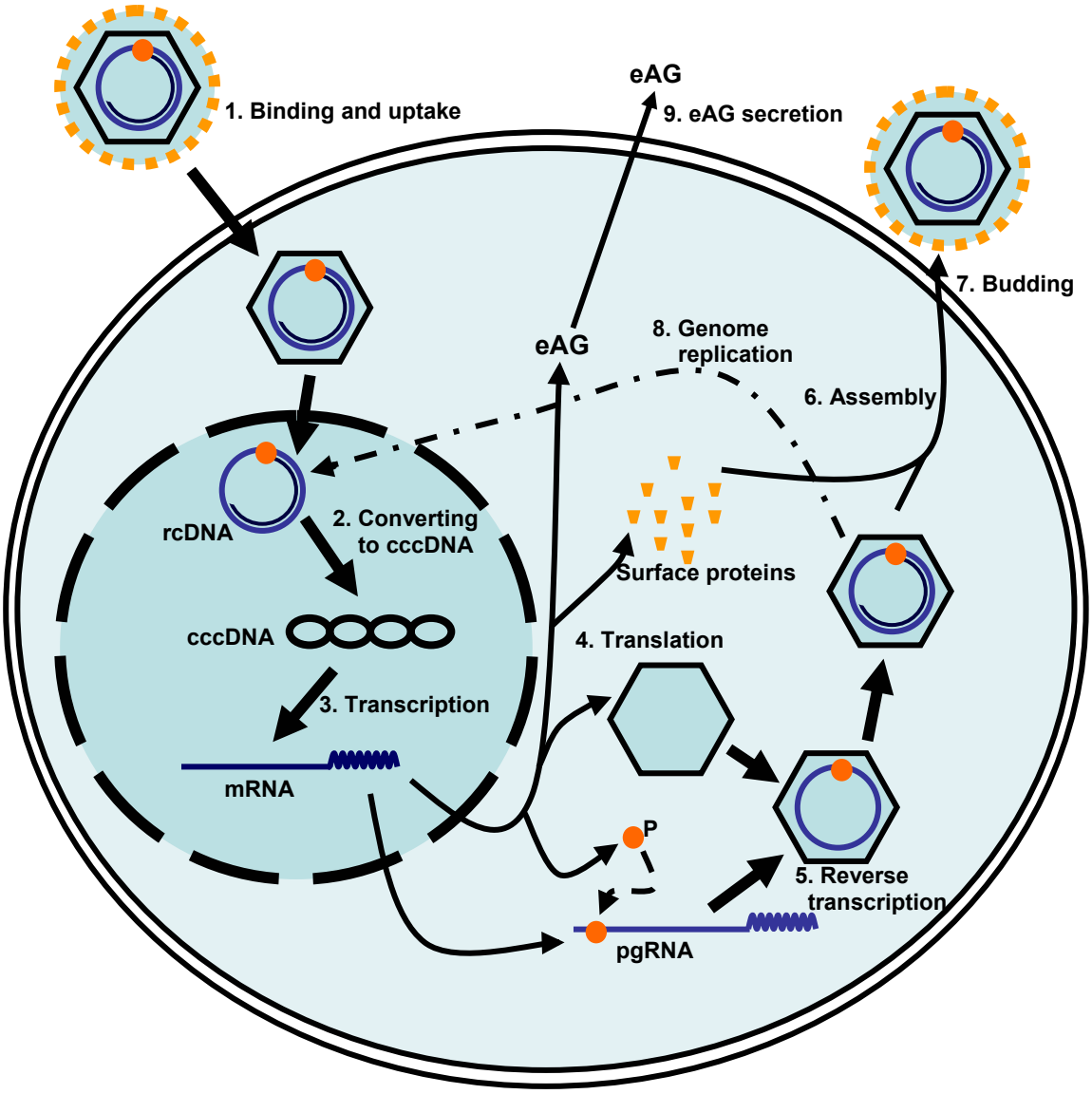


Figure 1.34. Life cycle of HBV

Nucleoside or nucleotide analogs used in the treatment of chronic hepatitis B can be divided into pyrimidine analogs, such as lamivudine and emtricitabine, and into purine analogs, such as ganciclovir, famciclovir, adefovir and entecavir.[162] Fialuridine and lobucavir were withdrawn early because they were found to be associated with fatal adverse events in humans[161] or with development of neoplasms in animals.[162] The main

mechanism of action of nucleoside analogs is the inhibition of viral HBV polymerase, chain termination, and thus the cessation of HBV replication.[162-164] Like anti-HIV NRTIs, most of these drugs are initially converted to nucleoside triphosphates by sequential intracellular phosphorylation by hepatocyte kinases. These nucleoside triphosphates compete with natural nucleoside triphosphates for incorporation into viral DNA by the viral DNA polymerase, resulting in the termination of the elongation of the viral DNA chain.[162,165] Most of nucleoside analogs except L-FMAU (clevudine) used in the treatment of chronic HBV infection do not seem to have any effect on cellular DNA polymerases and have little, if any, effect on cccDNA.[166] According to viral kinetic studies, the HBV plasma half life is about 24 h, and there is a daily turnover of about 50% of the total viral load.[167] The half-life of infected hepatocytes is estimated to be between 10 and 100 days, and their daily turnover ranges between 1% and 7%.[167] In addition, clearance of the cccDNA, which is present within every infected hepatocyte,[168] can be accomplished only indirectly by the hepatocytes death[169] because it is resistant to the actions of antiviral drugs.[167] Such a combination of the relatively high HBV replication rate and the relatively slow death rate of infected hepatocytes suggest that prolonged antiviral therapy is required to eradicate HBV, particularly in patients with long-standing disease and a great percentage of hepatocytes infected by the virus.

Long-term and effective antiviral therapy, as previously stated, is required to eradicate HBV in chronically infected patients. The effectiveness of long-term treatment with nucleoside analogs, however, seems to be progressively decreasing, while viral resistance is progressively increasing because of the emergence of resistant mutant HBV strains. Such mutations change the structure of HBV polymerase resulting in reduction of the

nucleoside analog affinity for the enzyme.[170] Viral resistance is clinically manifested by the virological breakthrough phenomenon defined as reappearance of serum HBV DNA after an initial clearance of viremia despite continuation of therapy. All retroviruses as well as hepadna viruses exhibit an increased rate of emergence of mutations because of both the high viral turnover and the high error rate of viral polymerase. These mutant strains are usually selected under immune pressure, such as the S-escape mutants in patients treated with hepatitis B immune globulin or the precore mutants in patients undergoing anti-HBe seroconversion.[171,172] Thus, resistant HBV mutant strains were expected to emerge in patients with chronic HBV infection treated with nucleoside analogs, similarly to what is observed in patients with HIV infection.[173] The emergence of HBV drug resistance and the severely compromised efficacy of the drugs in most patients after prolonged therapy necessitates the development of alternative therapies that suppress HBV replication *in vivo* more effectively. A more potent antiviral agent should suppress HBV replication as well as slow the emergence of drug-resistant variants. Among the three FDA-approved anti-HBV drugs, two of them are nucleoside analogs and many nucleoside analogs are under clinical studies.

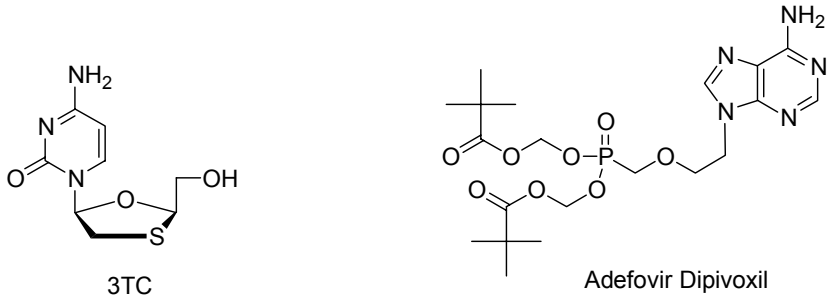


Figure 1.35. FDA-approved anti-HBV nucleosides

Lamivudine (3TC) (Fig. 1.35). Clinicians can now choose to treat the HBV infection with lamivudine, a well-tolerated and orally available drug that has minimal side effects.[174] As with IFN-α, however, a complete antiviral response, as assessed by HBe seroconversion, is seen in only a minority of patients after 1 year of treatment.[175] 3TC is an L-nucleoside and effective in patients who are interferon-α-naïve as well as those who have failed to respond to interferon-α therapy. It has low toxicity and is much better tolerated than interferon, with the advantage of once-daily oral versus parenteral administration. However, of fundamental concern is that while initial treatment of patients with 3TC results in a rapid lowering of HBV DNA levels in the blood, its efficacy is compromised in most patients by the development of drug resistance after prolonged therapy. Lamivudine resistance has been reported in about 15 to 25% of patients after 12 months of treatment, increasing with treatment duration,[175-177] which is usually associated with mutations in the HBV DNA polymerase gene.[178] The development of resistance is caused by the high rate of virus replication and the error rate of the viral polymerase. One of the common mutations associated with lamivudine resistance results in a methionine-to-isoleucine substitution (M204I)[179] in the YMDD nucleotide-binding motif in the catalytic domain of the polymerase. Substitution with valine (M204V) has also been described, almost invariably in conjunction with an upstream leucine-to-methionine (L180M) change in the polymerase B domain.[177,180-184]

Adefovir dipivoxil [bis(POM)PMEA, Hepsera®] (Fig. 1.35). Adefovir, 9-(2-phosphonylmethoxyethyl)adenine (PMEA) is a broad-spectrum antiviral agent, active against retroviruses, hepadnaviruses and herpesviruses.[185-187] However, the possibility of PMEA becoming an orally administered drug is limited by its poor bioavailability,[188] due

to the negative charge of the phosphonate functionality at physiological pH, which limits its gastroenteric absorption. In order to increase the bioavailability and to decrease the toxicity of PMEA, a number of prodrugs have been prepared.[189] *In vitro* studies show that bis-pivaloyloxymethyl derivative (bis-POM-PMEA, adefovir dipivoxil)[190] is able to increase the intracellular concentration of PMEA by 2 logs. It has comparable antiviral activity in HIV-1 infected CEM cells and HCMV-infected MRC-5 cells, and it is even more potent than the parent compound on HSV-1 and HSV-2 in Vero cells.[191] Adefovir dipivoxil was initially developed as a treatment for HIV disease, but proved too toxic to the kidneys and was denied FDA approval in 1999. In clinical trials, oral bioavailability was found to be greater than 40%, but the bis-POM functionality has been found to confer cytostatic effects, probably due to the liberation of formaldehyde and pivalic acid. However, the drug was well tolerated in HBV trials, in which it was administered at much lower doses and for a shorter period than in the earlier HIV studies.[192] Adefovir dipivoxil was approved by the FDA in September 2002 for the treatment of chronic hepatitis B in adults with evidence of active viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease. FDA approval is based upon two double-blind placebo-controlled trials.[193,194] At week 48, 53% of patients receiving adefovir in one study[194]and 63% in the other[193] showed significant improvement in the liver inflammation caused by hepatitis B compared to 25% and 35% of patients receiving the placebo. An improvement in fibrosis (liver scarring) was also observed in people receiving adefovir. It was also showed to be effective against strains of hepatitis B resistant to 3TC.[195] Also, a two-drug combination regimen including adefovir dipivoxil and lamivudine has been shown to be effective in

the therapy of HBV-HIV-coinfected patients.[192] In this combination, the use of adefovir dipivoxil at a suboptimal concentration for HIV activity (10 mg once daily) prevents the occurrence of mutations at codons 65 and 70 of HIV RT, responsible for HIV resistance to adefovir.[192]

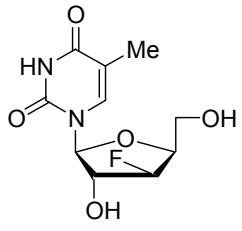
FTC (emtricitabine) (Fig. 1.17). FTC shows potent *in vitro* anti-HIV-1, HIV-2, SIV and FIV activity in various cell cultures. Like 3TC, FTC exhibits 20-fold more potency against HIV-1 in human PBM cells and less toxicity in myeloid progenitor cells than its D-enantiomer. It also shows anti-HBV activity in hepatoma cell lines (HepG2 cells), whereas its D-enantiomer is significantly less potent.[196] However, neither enantiomers showed significant cytotoxicity in human bone marrow progenitor cell assays and any detectable hepatotoxic effects at concentrations above their antiviral activities. Currently, (-)-FTC (coviracil) has completed phase III clinical trials against HIV, and phase III studies as an anti-HBV agent. Racemic FTC (racevir) is in phase II clinical trials as an anti-HIV and anti-HBV agent.

Enzymatic studies of FTC show a similar profile to 3TC, in which the D-enantiomer of FTC is a substrate for dCyd deaminase, and FTC is resistant to deamination by the same enzyme.[197] dCyd kinase and nucleoside diphosphate (NP) kinase phosphorylate FTC to its triphosphate, which functions as chain terminator of viral DNA synthesis, similarly to 3TC. Schinazi *et al.* have also reported that highly 3TC/FTC-resistant HIV-1 variants dominate the replicating virus population after two or more cycles of infection in the presence of 3TC or FTC.[197] These variants are cross-resistant to 3TC and FTC but are susceptible to ddC, AZT, and ddI. DNA sequence analysis of the RT gene amplified from resistant viruses identified a mutation at codon 184 from Met (ATG) to Val (GTG

or GTA) or Ile (ATA). While FTC has a resistance profile similar to 3TC in HIV, a recent randomized, double-blind study evaluating FTC in patients with chronic HBV at 200 mg indicated that it was effective and that there was a low rate of resistance development.[198] FTC produced a sustained virologic and serologic response (29% HBeAg conversion). At 2 years the incidence of mutations was low (19%) among the patients receiving 200mg for the duration of the study, suggesting that potent viral suppression limits the emergence of resistant variants.[198]

L-FMAU (clevudine, 1-(2-fluoro-5-methyl-beta-L-arabino-furanosyl)uracil) (Fig. 1.36). L-FMAU is an exceptionally potent antihepadnaviral agent *in vivo*, and is a candidate for antiviral therapy of chronic HBV infection. Ever since the broad spectrum of biological activity of 2'-F-arabinofuranosyl nucleosides was discovered, a number of structural modifications of these analogs have been carried out.[172] Chu and co-workers have demonstrated that the enantiomer of D-FMAU, L-FMAU (clevudine) has potent anti-HBV as well as anti-EBV activity.[199] L-FMAU was prepared from L-ribose derivatives via either L-xylose or L-arabinose.[199,200] L-FMAU has low cytotoxicity in a variety of cell lines, including MT2, CEM, H1, 2.2.15 and bone marrow progenitor cells.[199] L-FMAU is phosphorylated to L-FMAUMP in 2.2.15 cells by cytosolic TK, dCyd kinase or mt-dPyd kinase and further phosphorylated to L-FMAU-TP acting as a potent inhibitor of HBV DNA polymerase.[201] However, it is not utilized as a substrate by human DNA polymerase α, β, γ or δ. Interestingly, L-FMAU-TP is not a substrate for HBV or EBV DNA polymerases unlike other antiviral nucleosides, which suggests that the anti-HBV and anti-EBV activity of L-FMAU may not be due to its incorporation into HBV and EBV DNA. L-FMAU also has respectable bioavailability in rats.[202,203] Preliminary *in*

vivo toxological studies suggest no apparent toxicity for 30 days at 50 mg/kg/day in mice and for 3 months in woodchucks (10 mg/kg/day).[204] L-FMAU shows potent anti-HBV activity *in vivo* against woodchuck hepatitis virus (WHV) in chronically infected woodchucks and there is no significant virus rebound after cessation of the drug treatment.[205] Thus, four weeks of once-daily oral administration of L-FMAU significantly reduced viremia, antigenemia, intrahepatic WHV replication, and intrahepatic expression of woodchuck hepatitis virus core antigen (WHcAg) in a dose-dependent manner. At the highest dose administered (10 mg/kg/d), significant reductions of intrahepatic WHV RNA and covalently closed circular (ccc)WHV-DNA levels were also observed. The reduction in viremia was remarkably rapid at the higher doses of L-FMAU, with greater than 1,000-fold reductions in WHV-DNA serum levels observed after as little as 2 to 3 days of therapy. Following the withdrawal of therapy, a dose-related delay in viremia rebound was observed. At the highest doses used, viremia remained significantly suppressed in at least one half of the treated animals for 10 to 12 weeks' post-treatment. No evidence of drug-related toxicity was observed in the treated animals. Currently, it is undergoing phase III clinical trials.



L-FMAU

Figure 1.36. L-FMAU (clevudine)

L-2'-deoxynucleosides (Fig. 1.37). Among L-2′-deoxynucleosides, L-dC, L-dT, and L-dA showed selective antiviral activity against HBV with EC_{50} values of 0.24, 0.19, and

0.10~1.9 μM respectively, but not against HIV-1 or other viruses.[206] In the structure-activity relationship (SAR) study of L-dC, substituition of a halogen atom at the 5 position in cytosine ring, without modification of sugar moiety, decreased the potency against HBV but retained the specificity for HBV. In contrast, analogs of L-dC which lacked 3'-OH group on the deoxyribose sugar, such as L-ddC, 3TC, and L-d4C, lost antiviral specificity for HBV and showed activity against HIV.[206] Similarly, replacement of the 3'-OH group with a 3'-fluoro moiety eliminated the antiviral specificity, but the antiviral potency against HBV and HIV was retained. The SARs for the L-dT and L-dA series were similar to those observed for the L-dC series. The specific anti-HBV activity of L-2'-deoxynucleosides was lost on removal or substitution of the 3'-OH group. L-dT and L-dC were phosphorylated by thymidine kinase and 2'-deoxycytidine kinase in human and woodchuck liver extracts. L-dT-TP and L-dC-TP inhibited woodchuck hepatitis virus (WHV) DNA polymerase (IC_{50} 0.24 and 1.8 μM, respectively).[207] In the *in vivo* study of chronically WHV-infected woodchuck model, oral administration of L-dT, L-dC, and L-dA at a dose of 10 mg/kg/day significantly inhibited WHV DNA replication. Serum WHV DNA levels (HBV viremia) decreased in the L-dT-treated animals by as much as 8 logs, which meant WHV DNA levels of pretreated animals were 10^{11} genome equivalents/ml and during the treatment, the levels became 10^3 or detection limit, 300 genome equivalents/ml by quantitative PCR method. After drug withdrawal, viral rebound reached near-pretreatment levels between week 4 and week 8. During treatment of L-dC and L-dA, serum WHV DNA levels decreased by 6 logs and 1.5 logs, respectively, which was also followed by viral rebound within the first week of posttreatment. In laboratory studies and predictive animal models of HBV disease, L-dT

and L-dC each demonstrate potent and highly specific antiviral activity against HBV, and in combination these two agents demonstrate synergistic effect.[208] Currently, L-dT (telvivudine) and the prodrug of L-dC, val-LdC (valtorcitabine), are undergoing phase III and phase I/II studies, respectively, as anti-HBV agents by Idenix Pharmaceuticals.

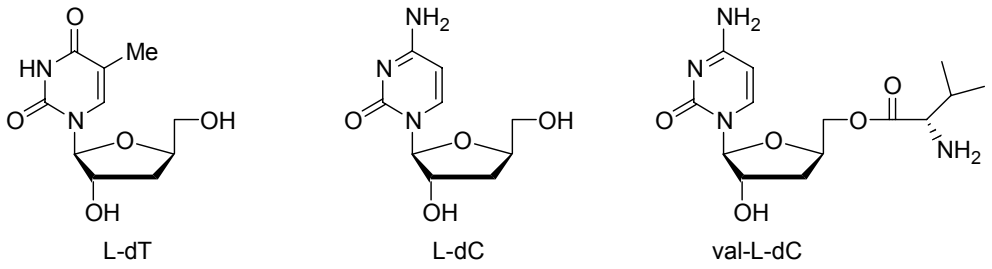


Figure 1.37. L-dT (telvivudine), L-dC and val-LdC (valtorcitabine)

DAPD (Amdoxovir) (Fig. 1.18). (-)-β-D-Dioxolane guanine (DXG) is a nucleoside analog possessing potent activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), and hepatitis B virus (HBV) *in vitro*. Due to the limited aqueous solubility of DXG, (-)-β-D-2,6-diaminopurine dioxolane (DAPD), a more water-soluble prodrug of DXG, is being developed for clinical use. DAPD is susceptible to deamination *in vivo* by the ubiquitously present enzyme adenosine deaminase yielding the active metabolite dioxolane guanosine (DXG).[209] After intravenous administration of DAPD to rats, DXG was rapidly generated from DAPD, with approximately 61% of the dose of DAPD being converted to DXG. After administration of DAPD to monkeys, only concentrations of metabolite DXG could be determined owing to rapid conversion of DAPD to DXG during sample collection. Oral bioavailabitity of DAPD was estimated to be approximately 30%.[209,210] When the inhibitory activity of anti-HBV nucleoside analogs, FTC, L-FMAU, DXG, and DAPD, was compared with lamivudine (3TC) and PMEA, in a cell-free assay for the expression of wild-type duck hepatitis B virus

(DHBV) reverse transcriptase, DAPD-TP was found to be the most active on viral minus strand DNA synthesis, including the priming reaction, followed by 3TC-TP, (-)FTC-TP, and DXG-TP, whereas L-FMAU-TP was a weak inhibitor.[211] In cell culture experiments, however, important differences in IC_{50}s were observed depending on the cell type used, showing that antiviral effect of nucleoside analogs may depend on their intracellular metabolism.[211] IC_{50}s obtained for wild-type DHBV replication in primary duck hepatocytes were much lower than with DHBV transfected LMH cells. IC_{50}s were also significantly lower in 2.2.1.5 and HepG2 cells compared with HBV transfected HuH7 cells. Moreover, L-FMAU inhibited preferentially HBV plus strand DNA synthesis in these cell lines. When the antiviral effect of these inhibitors was also evaluated against 3TC-resistant mutants of the DHBV and HBV polymerases, these mutants were found to be cross-resistant to FTC. In contrast, the double DHBV polymerase mutant was sensitive to DXG-TP and DAPD-TP. Moreover, both purine analogs remained active against DHBV and HBV 3TC-resistant mutants in transfected LMH and HepG2 cells, respectively. Evaluation of the inhibitory effect of various nucleoside analogs on the replication of a lamivudine-resistant HBV variant containing M204V mutation in the polymerase nucleoside-binding domain was performed. For this purpose, the tetracycline-responsive HepAD38 and HepAD79 cells were used, which are stably transfected with either a cDNA copy of the wild-type pregenomic RNA or with cDNA containing the M204V mutation, respectively. Adefovir, tenofovir, lobucavir, L-FMAU, DXG and DAPD proved almost equally effective against both viruses.[212] However, another study to determine the sensitivity of the lamivudine-resistant HBV mutants to L-FMAU and DAPD showed that M204V and M204V plus L180M substitutions, which confer

lamivudine resistance, did not confer cross-resistance to adefovir or DAPD, but conferred cross-resistance to L-FMAU.[213] DAPD is currently under phase II clinical trial for chronic HBV infection.

L-d4FC (Elvucitabine, ACH 126,443) (Fig. 1.22). L-d4T[214], a unnatural isomer of d4T, showed no activity against HIV and HBV, but L-d4 nucleosides with cytosine and 5-fluorocytosine bases, L-d4C and L-d4FC, showed potent activity against HIV (EC_{50} 1 μM for L-d4C and EC_{50} 0.09 μM for L-d4FC) and HBV(EC_{50} 0.01 μM for L-d4C and EC_{50} 0.002 μM for L-d4FC).[215] Like 3TC, L-d4FC is phosphorylated by cytosolic dCyd kinase to its monophosphate which is further phosphorylated to L-d4FC-DP and L-d4FC-TP, which acts as a competitive inhibitor as well as a terminator of viral DNA chain elongation.[216,217] L-d4FC is not susceptible to deoxycytidine deaminase which catabolizes deoxycytidine analogs to deoxyuridine analogs. The relative molecular activity of the 5′-triphosphates of L-d4FC, L-ddFC and ddC on DNA strand elongation by HIV-1 reverse transcriptase (RT) and human DNA polymerases α, β, γ, and ε was evaluated.[218] The K_i values of L-d4FC-TP were 0.20, 1.8, and 4.0 μM for HIV RT, pol γ and pol β, respectively. L-d4FC-TP had no or little inhibitory effect on pol ε or pol α. By comparison, the K_i values of 3TC-TP have been reported as 1, 0.01, and 1.2 μM for HIV RT, pol γ, and pol β, respectively.

In the *in vitro* study, secretion of HBV DNA from 2.2.15 cells was decreased by 50% at a concentration of 1 nM by L-d4FC, which was approximately 15 times more potent than 3TC. The level of formation of phosphorylated metabolites in cells is higher for L-d4FC than for 3TC and L-d4FC metabolites have a much longer retention time in HepG2 cells than 3TC metabolites.[219] These results suggest that the dosage required to suppress HBV

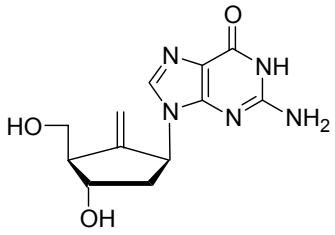
replication in patients will be lower for L-d4FC than for 3TC. Short-term administration of L-d4FC transiently inhibited viral replication *in vivo* in experimentally infected ducklings. Three days after inoculation of virus, drugs were administrated intrapertoneally at 25mg/kg/day for 5 days. The inhibition of the peak of viremia reached 97% for L-d4FC. However, after drug withdrawal, a relapse of viral replication occurred. Long-term administration study could not prevent the relapse of viral replication after drug withdrawal and viral cccDNA was still detected at the end of the treatment, indicating the absence of viral clearance from infected hepatocytes. A 4-week administration of L-d4FC in acutely infected ducklings not only suppressed viral DNA synthesis but also inhibited viral antigen expression in the liver. No hepatocyte or biliary cell expressing the viral pre-S proteins was detected from the liver of L-d4FC-treated animals. In long-term administration study of 3TC, the inhibition of the peak of viremia reached only 80% for 3TC and viral antigens (pre-S envelope proteins) were expressed up to 90%. But a significant relapse of viral replication did not occur after 3TC withdrawal. Interestingly, the greater the antiviral effect during therapy, the higher was the rebound of viral replication after drug withdrawal. This suggested that the suppression of viral replication in acutely infected animals is not associated with a strong specific antiviral response, leading to a delayed peak of viral replication after drug withdrawal. A study of L-d4FC was reported in woodchucks chronically infected with woodchuck hepatitis virus (WHV).[220] L-d4FC was administered at 4 mg/kg to woodchucks for 5 consecutive days as an induction therapy followed by a maintenance therapy twice weekly for 8 more weeks. The results were compared with those of 3TC at a dose of 10 mg/kg by the same schedule. During the 3TC treatment period, both assays

[WHV endogneous polymerase activity (EPA) and DNA] showed a first phase of viremia drop followed by successive phases of a rise and a drop of viremia. In contrast, in L-d4FC treatment group a rapid and significant decrease of the viremia level to the detection limit of the WHV EPA assay or close to the detection limit of the WHV DNA assay was observed and maintained until the end of therapy. However, after drug withdrawal, viremia levels in all groups of treated woodchucks returned to pretherapy values in 4 to 6 weeks for the L-d4FC group and in 10 weeks for the 3TC group. Prolonged L-d4FC treatment for 15 weeks was not able to achieve clearance of intrahepatic viral cccDNA. This was associated with the absence of a decline in the number of infected hepatocytes and the WHV surface antigen titer in serum. Residual viral cccDNA is an active template for viral genome expression, allowing the reinitiation of viral replication when therapy is stopped. Analysis of liver histology showed a decrease in the inflammatory activity of chronic hepatitis and a stability of liver fibrosis in woodchucks treated with L-d4FC, while in control animals, hepatitis activity increased. An electron microscopy study showed the absence of ultrastructural changes of hepatic mitochondria, biliary canaliculi and bile ducts. However, loss of weight was observed in all animals and skin hyperpigmentation related to the accumulation of melanin pigment was reported during L-d4FC treatment and slowly disappeared after drug withdrawal. Although L-d4FC and 3TC administration inhibited viral replication to different extents and acted on liver disease, 4 of 10 in L-d4FC group, 1 of 4 in 3TC group, and 1 of 6 in the control group died of hepatocellular carcionma during the study period. From these continous studies of L-d4FC, it could be concluded that clearance of viral cccDNA and infected cells from the liver is difficult to achieve with monotherapy

with a potent inhibitor of viral replication. L-d4FC is currently undergoing phase II clinical studies by Achillion Pharmaceuticals.

Entecavir (ETV, BMS-200475) (Fig. 1.38). Replacement of the 4'-oxygen atom of the deoxyguanosine with an ethenyl group produces compounds with potent and selective anti-HBV activity in 2.2.15 cells.[221] *In vitro* biochemical studies indicate that entecavir can be efficiently phosphorylated by cellular enzymes to its triphosphate, which is a potent inhibitor of HBV DNA polymerase, inhibiting both priming and elongation steps of HBV DNA replication (EC_{50} 3 nM in 2.2.15 cells and SI ~8000).[221-223] Entecavir (BMS-200475), originally synthesized as an anti-herpesvirus agent, also displayed moderate activity against HSV-1, HSV-2 and VZV. Activity was also seen with HCMV, a herpes virus lacking TK, but no activity was detected against RNA viruses such as HIV or influenza.[221] Human clinical trials have demonstrated the efficacy of ETV for the treatment of chronic HBV infections at doses as low as 0.01 mg daily.[224,225] The *in vivo* efficacy of ETV was previously demonstrated in the woodchuck and duck models of HBV infection.[226,227] Important differences between the therapeutic characteristics of ETV and 3TC were observed in these models, including their roles in the development of antiviral resistance. In the woodchuck model, ETV therapy suppressed the levels of woodchuck hepatitis virus (WHV) DNA in blood by up to 8 log units and reduced hepatic cccDNA levels by up to 4 log units. Even after 14 to 36 months of therapy with ETV, there was no emergence of drug-resistant WHV.[228] These observations are in contrast to those made for 3TC therapy, which not only failed to reduce intrahepatic cccDNA levels in the woodchuck but also led to the emergence of drug-resistant variants with mutations in WHV polymerase.[229] The potential for additional therapeutic benefits

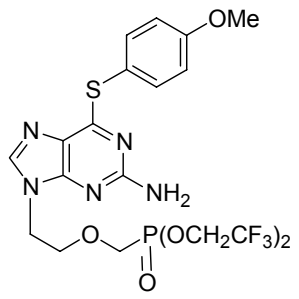
of ETV therapy was indicated by a reduced frequency of hepatocellular carcinoma in the woodchuck model and a prolonged life span of chronically infected animals.[228] While there is limited data published on the *in vitro* efficacy of ETV against 3TC-resistant HBV,[223] recent clinical studies have demonstrated the *in vivo* efficacy of ETV against 3TC-resistant HBV infections.[230] Entecavir is currently undergoing phase III clinical trials as an anti-HBV agent by Bristol-Meyers Squibb.



Entecavir

Figure 1.38. Entecavir (ETV)

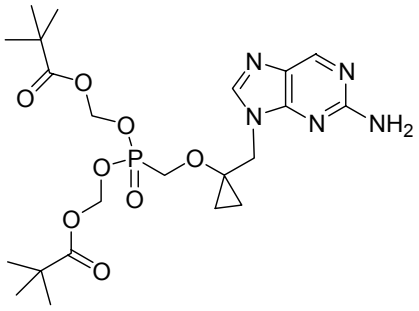
MCC-478 (LY582563) (Fig. 1.39). MCC-478 {2-amino-9-[2-(phosphonomethoxy)ethyl]-6-(4-methoxyphenylthio)purine bis(2,2,2-trifluoroethyl)ester}, a nucleoside phosphonate analog, was synthesized as a derivative of adefovir.[231,232] MCC-478 showed substantially higher (ca. 20-fold, EC_{50} 0.027 μM) anti-HBV activity than that of lamivudine, despite no significant anti-HIV activity.[231] The bis(2,2,2-trifluoroethyl) ester moiety of this series confers good oral bioavailability and the compound has no apparent cytotoxic effects or inhibitory activity against mitochondrial DNA.[231] The replicative intermediates of lamivudine-resistant mutants were also progressively diminished by treatment with increasing doses of MCC-478. Thus, M204I, M204V and L180M/M204V mutants were susceptible to MCC-478 with EC_{50} values of 2.6 μM for M204I, 3.3 μM for M204V and 2.0 μM for L180M/M204V.[232]



MCC-478

Figure 1.39. MCC-478 (LY582563)

LB80380 (Fig. 1.40).[233] LB80380, a nucleoside phosphonate analog, showed potent and selective anti-HIV activity against wild type and lamivudine-resistant HBV with EC_{50} of 0.5-1.0 μM. Woodchucks were treated orally for 28 days at doses of 5 & 15 mg/kg/day of LB80380. WHV DNA viral load was reduced by greater than six logs after four weeks. Mitochondrial toxicity and kidney toxicity were shown to be very low in *in vitro* models. Oral bioavailability of LB80380 is 20% to 60%, which varies according to animal species. LB80380 is in phase II clinical trials for the treatment of chronic human HBV infections.



LB80380

Figure 1.40. LB80380

CHAPTER 2

SYNTHESIS AND ANTIVIRAL ACTIVITY OF CARBOCYCLIC L-2'-DEOXYNUCLEOSIDES

INTRODUCTION

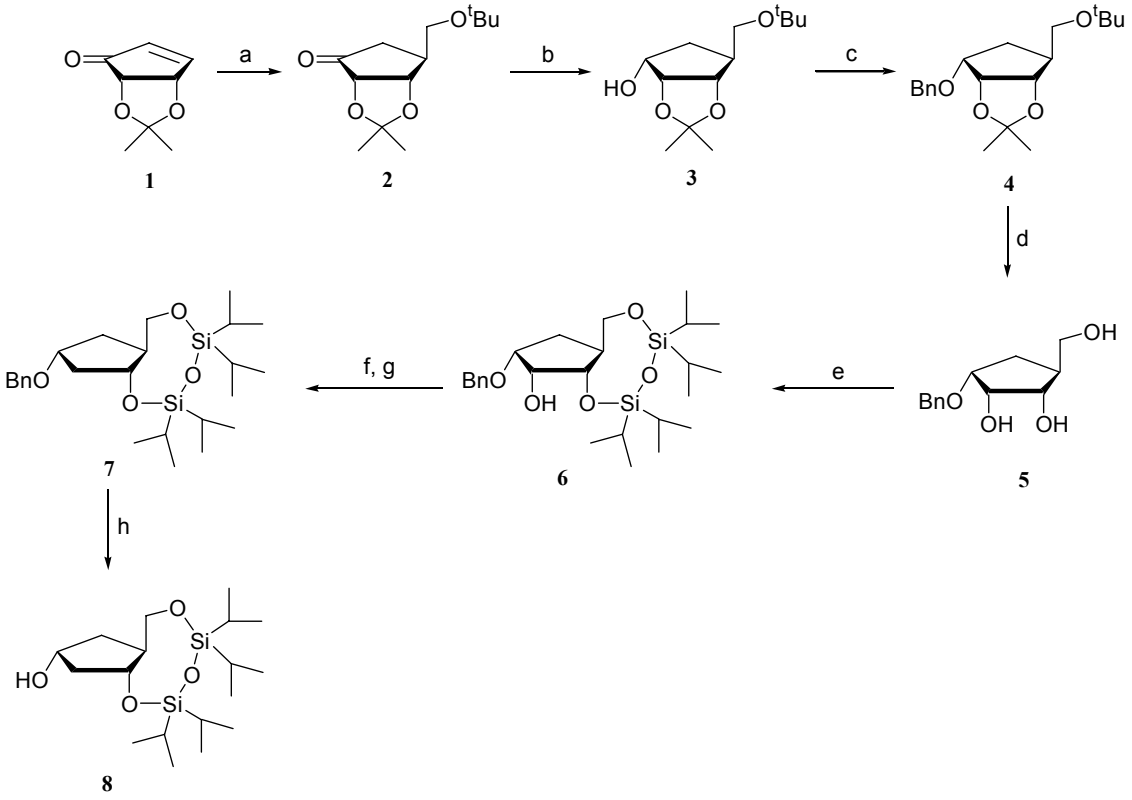
Neplanocin A and aristeromycin are naturally occurring carbocyclic nucleosides which demonstrated antiviral and antitumor activity.[234,235] Carbocyclic nucleosides possess greater metabolic stability to phosphorylase that cleave the glycosidic linkage of normal nucleosides[236,237] and also display a wide range of biological activity.[238] Consequently, extensive efforts have been made to develop carbocyclic nucleosides as therapeutically useful agents against tumors and viruses.[238] Abacavir, one of the successful carbocyclic nucleosides, has been approved for the treatment of HIV infections.[239]Entacavir (BMS-200475) shows potent and selective anti-HBV activity *in vitro* as well as *in vivo*.[240] Carbocyclic analogs of 2'-deoxynucleosides, D-5-bromovinyluridine (BVDU) and D-5-iodouridine (IDU), showed antiviral activity against herpes symplex virus (HSV).[241] Carbocyclic 2'-deoxyguanosine have shown potent antiviral activities against HSV[242]and hepatitis B virus (HBV).[243,244] Since the discovery of lamivudine (3TC),[245,246] a number of L-nucleosides such as emtricitabine (FTC),[247] clevudine (L-FMAU),[248] elvucitabine (L-d4FC)[249] and telvivudine (L-dT)[250] have been discovered as potent antiviral agents. Thus, there has been increasing interest to explore carbocyclic L-nucleosides as potential antiviral agents. However, the difficulty in enantiomeric synthesis of carbocyclic nucleosides was a main obstacle to discover new carbocylcic nucleosides. There have been several efforts to obtain enantiomerically pure carbocyclic nucleosides. Enantiomeric syntheses were obtained only by enzymatic resolution or multistep synthesis. These results encouraged us to synthesize optically active carbocyclic L-2'-

deoxynucleosides and systematically evaluate their activities against several viruses. Herein we report the efficient synthesis of carbocyclic L-2'-deoxynucleosides.

RESULTS AND DISCUSION

Carbocyclic L-2'-deoxynucleosides were synthesized through Mitsunobu reaction using the key intermediate **8** which was obtained from the enone **1** in 8 steps via deoxygenation method reported by Barton et al (Scheme 2.1)[251]. The enone **1** was synthesized by known method[252-254] in 3 steps from D-ribose, and used to synthesize L-aristeromycin and L-carbovir analogs.[255] The enone **1** underwent 1,4-additon using cuprate reagent to give compound **2** in 87% yield, which was reduced to alcohol **3** by using DIBALH reducing reagent in 97% yield. The alcohol **3** was protected with benzyl bromide to give fully-protected tetraol **4** in 88 % yield, which was deprotected with trifluoroacetic acid in water followed by treatment with 1N NaOH to afford compound **5** in 97 % yield. Compound **5** was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIDPSCl)[256] in pyridine to give compound **6**. The alcohol **6** was deoxygenated by treatment with sodium hydride, carbon disulfide and methyl iodide, followed by radical condition of tributyltin hydride and AIBN to give compound **7** in 76 % yield. The benzyl group of compound **7** was removed by using palladium (II) hydroxide and hydrogen balloon to afford the common intermediate **8** in 82 % yield.

Compound **8** was coupled with 6-chloropurine and 2-*N*-acetylamino-6-chloropurine to give compounds **9** and **10**, respectively. Adenine derivative **11** was prepared from **9** by ammonolysis followed by desilylation in 66 % yield (Scheme 2.2). Hypoxanthine



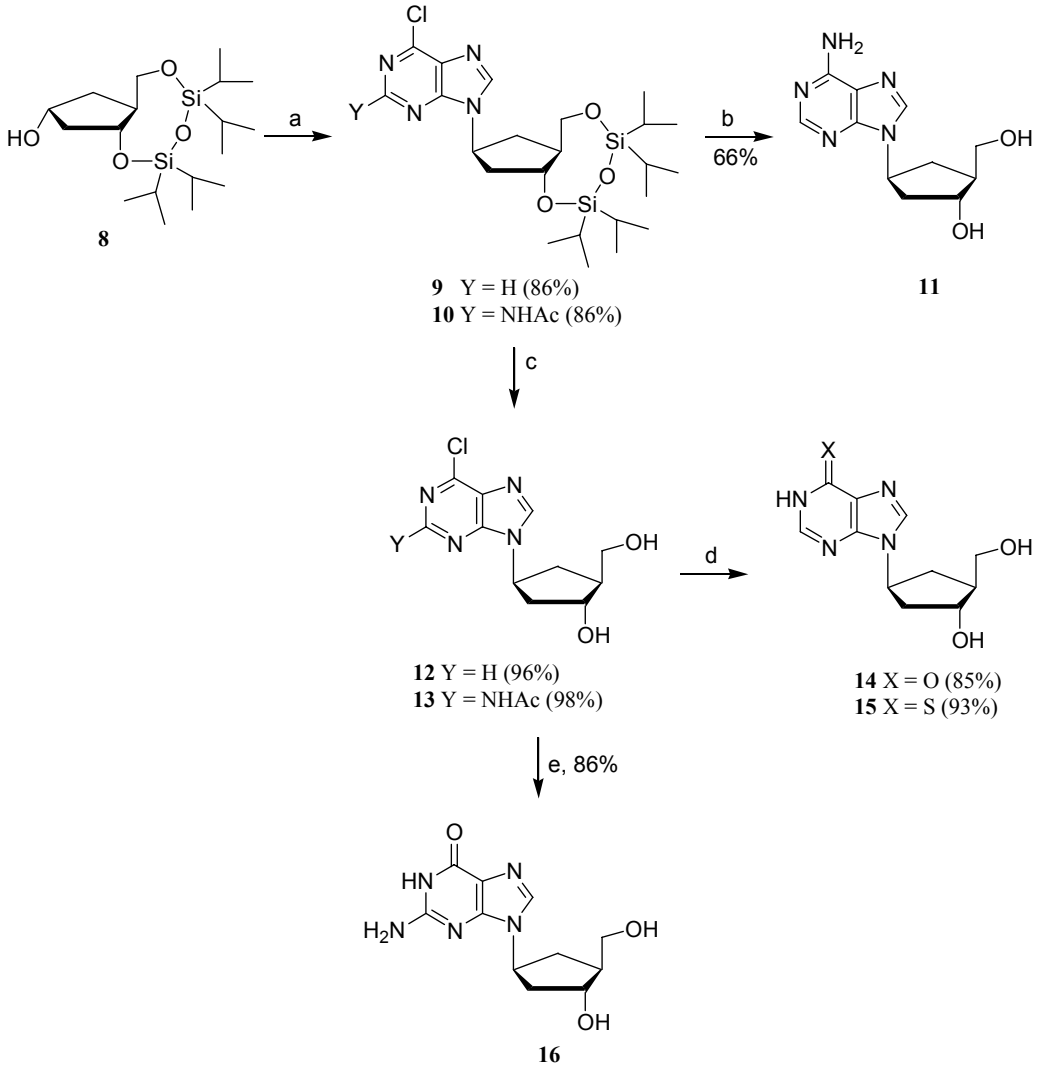
[a]reagents and conditions: a) MeO^{t}Bu, sec-BuLi,; LiBr; CuBr, isopropyl sulfide, b) DIBALH, CH_2Cl_2, -78^{o}C, c) NaH, BnBr, THF, 0 ^{o}C. d) TFA/MeOH, 60 ^{o}C, then 1N NaOH. e) TIPDSCl, pyr, 0 ^{o}C. f) NaH, CS_2, MeI, THF, 0 ^{o}C to rt. g) Bu_3SnH, AIBN, Toluene, reflux. h) $Pd(OH)_2$, MeOH, H_2.

Scheme 2.1. Synthesis of the key intermediate **8**

derivative **14** was obtained from **12** by treatment with 85 % formic acid followed by ammonolysis in 85% yield. Compound **12** was also treated with thiourea in ethanol under reflux conditions to give thiohypoxanthine derivative **15** in 93 % yield. Guanine

derivative **16** was prepared from compound **13** by same procedure as synthesis of hypoxanthine derivative **14** in 86 % yield.

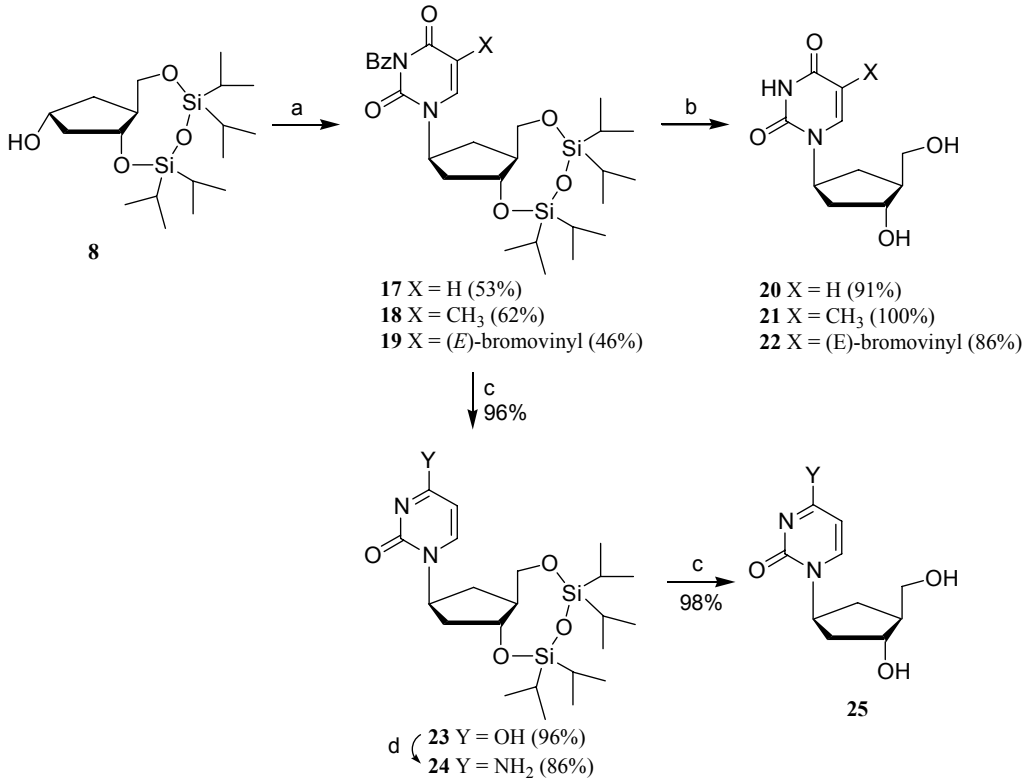
The synthesis of pyrimidine derivatives (**20**, **21**, **22** and **25**) was more complex than purine nucleosides case because of the formation of *O*-2-alkylated by-products. The



Reagents and conditions: a) DEAD, Ph_3P, Purines, DMF/Dioxane, 0°C; b) 1. NH_3, MeOH, 100°C; 2. TBAF, THF; c) TBAF, THF; d) 85% HCO_2H, 60~70°C, then NH_3, MeOH or Thiourea, EtOH, 80°C; e) 85% HCO_2H, 60~70°C, then NH_3, MeOH

Sheme 2.2. Synthesis of purine nucleosides

common intermediate **8** was also condensed with N^3-benzoylpyrimidines under Mitsunobu conditions to give desired pyrimidine nucleosides (**17**, **18** and **19**) along with *O*-2-alkylated by-products which was easily separated firm the desired products (Scheme 2.3).



[a]Reagents and conditions: a) DEAD, Ph_3P, N-Benzoylpyrimidines, DMF/Dioxane, 0°C; b) 1. NH_3, MeOH; 2. TBAF, THF; c) TBAF, THF; d) 1,2,4-triazole, $ClPhOPOCl_2$, pyr, then NH_3, MeOH, 100°C

Scheme 2.3. Synthesis of pyrimidine nucleosides

Protected nucleosides (**17**, **18** and **19**) were debenzoylated and desilylated under the standard conditions to give final carbocyclic L-2'-deoxypyrimidine nucleosides (**20**, **21** and **22**). To synthesize the cytosine derivative **25**, the protected uracil derivative **17** was

treated with methanolic ammonia to give compound **23** in 96 % yield. A well known two-step conversion of uridine to cytidine from compound **23** afforded compound **24** in 86% yield,[257] which was converted to the final cytosine nucleoside **25** in 98% yield by treatment with TBAF.

In summary, we have described the efficient synthesis of optically pure L-carbocyclic 2'-deoxynucleosides by using Mitsunobu reaction of the common intermediate **8** with various purine and pyrimidine bases. Biological evaluation of antiviral activity and cytotoxicity on the synthesized nucleosides (**11**, **14**, **15**, **16**, **20**, **21**, **22** and **25**) was evaluated against HIV-1. 6-Thioinosine analog **15** showed only marginal anti-HIV activity with an EC_{50} of 7.8 μM, while the others showed no anti-HIV activity up to 100 μM (Table 2.1).

Table 2.1. Anti-HIV activity of carbocyclic L-2' deoxynucleosides

Compound	Configuration & Base	Anti-HIV-1 Activity (EC_{50}, μM)	Cytotoxicity (IC_{50}, μM)		
		PBM	PBM	CEM	Vero
11	L-Adenine	>100	67.0	>100	>100
14	L-Hypoxanthine	>100	>100	>100	>100
15	L-6-Thiohypoxanthine	7.8	>100	>100	>100
16	L-Guanine	>100	>100	>100	>100
20	L-Uracil	>100	>100	>100	>100
21	L-Thymine	>100	>100	>100	>100
22	L-Bromovinyluracil	>100	>100	>100	>100
25	L-Cytosine	>100	>100	>100	>100
AZT		0.004	>100	29.0	14.3

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), quin. (quintet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(2*S*,3*S*,4*S*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)-1-cyclopentanone (2).

Potassium *tert*-butoxide (16.43 g, 146.4 mmol) was suspended in anhydrous *tert*-butylmethyl ether (525 mL) under nitrogen. After being cooled to -78 ^{o}C, the well-stirred mixture was treated with *sec*-butyllithium (1.3 M in cyclohexane, 112.7 mL, 146.4 mmol) over 10 min. After the mixture was stirred for 2.5 h at -78 ^{o}C, a solution of LiBr (2 M, 145 mL) in THF was added dropwise over 10 min at -78 ^{o}C, and the resulting solution was stirred at -15 ^{o}C for 30 min. Upon recooling to -78 ^{o}C, a solution of $CuBr{\cdot}Me_2$ (15.05 g, 71.75 mmol) in diisopropyl sulfide (75 mL) was added dropwise over 10 min. The resulting viscous dark solution was stirred for 1 h at -78 ^{o}C and treated with a solution of

the enone **1** (7.4 g, 48 mmol) in THF (67 mL) over 5 min. The reaction mixture was allowed to warm to -30 ^{o}C over 15 min. After 30 min at -30 ^{o}C, 1:1 acetic acid-methanol (168 mL) was added and the mixture poured into 1680 mL of NH_4Cl/NH_4OH (pH = 9). After removal of the aqueous layer, the organic layer was washed with a 1:1 mixture of saturated NH_4Cl and 3% NH_4OH solutions (3,400 mL) and then brine (400 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated, and purified through flash silica gel column chromatography with 2-5% EtOAc in hexanes to give **2** (10.13 g, 87.1%) as a solid: mp 64-65 ^{o}C; $[\alpha]^{26}_{D}$ 185.36^{o} (*c* 1.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.62 (d, *J* = 5.3 Hz, 1H), 4.23 (d, *J* = 5.3 Hz, 1H), 3.50 (dd, *J* = 2.3, 8.5 Hz, 1H), 3.35 (dd, *J* = 2.6, 8.5 Hz, 1H), 2.71 (dd, *J* = 8.9,17.9 Hz, 1H), 2.54 (d, *J* = 8.9 Hz, 1H), 2.05 (d, *J* = 17.9 Hz, 1H), 1.43 (s, 3H), 1.35 (s, 3H), 1.11 (s, 9H); HR-FAB MS obsd: *m*/*z* 243.1596, calcd for $C_{13}H_{23}O_4$ *m*/*z* 243.1596 $(M + H)^{+}$. Anal. Calcd for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.18; H, 9.13.

(1*R*,2*R*,3*S*,4*S*)-4-(*tert*-Butoxymethyl)-2,3-(isopropylidenedioxy)cyclopentan-1-ol (3). Diisobutylaluminum hydride (DIBAL-H, 59.44 mL, 1 M in CH_2Cl_2) was added dropwise over 5 min to a solution of ketone **2** (9.6 g, 39.62 mmol) in anhydrous CH_2Cl_2 (480 mL) at 0-5 ^{o}C. The reaction mixture was stirred for 2.5 h at this temperature and then cautiously quenched with MeOH (480 mL). The colloidal suspension was concentrated, and the white solid was treated with ether (600 mL). The suspension was filtered, washing thoroughly with Et_2O (3,300 mL), and the filtrate was concentrated to give an oil that was purified by flash silica gel column chromatography eluted with 5-10% EtOAc in hexanes to give **3** (7.95 g, 82.1%) as a syrup: $[\alpha]^{25}_{D}$ 11.81^{o} (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.45 (m, 2H), 4.24 (m, 1H), 3.32(dd, *J* = 4.4, 8.8 Hz, 1H), 3.21 (dd, *J* = 4.6,

8.8 Hz, 1H), 2.45 (d, *J* = 8.9 Hz, 1H, D_2O exchangeable), 2.22 (m, 1H), 1.84-1.88 (m, 2H), 1.36 (s, 3H), 1.49 (s, 3H), 1.14 (s, 9H); HR-FAB MS obsd; *m/z* 245.1728, calcd for $C_{13}H_{25}O_4$ *m/z* 245.1753 $(M + H)^+$. Anal. Calcd for $C_{13}H_{24}O_4 \cdot 0.25H_2O$: C, 62.74; H, 9.92. Found: C, 62.73; H, 9.80.

(+)-(*1R, 2R, 3S, 4S*)-1-Benzyloxy-4-(*tert*-butoxymethyl)-2,3-isopropylidenedioxy)cyclopentane (4). To a solution of the alcohol **3** (2.23 g, 9.14 mmol) in 50 ml of THF, NaH as 60 % dispersion (0.439 g, 11.0 mmol) was added at 0 ^oC. After 10 min, BnBr (1.6 ml, 13.7 mmol) was added to the reaction mixture and the ice-bath was removed. After 3 h, the reaction was quenched with 10 ml of water, and the resulting mixture was evaporated. The normal aqueous work-up was done and the crude was purified by silica gel colunm chromatography with 20 % EtOAc in hexanes to give compound **4** (2.70 g, 8.08 mmol and 88 % yield) as a pale yellow oil: $[\alpha]^{25}_D$ 35.51^o (c 1.35, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.41~7.23 (m, 5H), 4.67 (d, *J* = 12.4 Hz, 1H), 4.60 (d, *J* = 12.4 Hz, 1H), 4.52 (t, *J* = 5.2 Hz, 1H), 4.37 (d, *J* = 5.2 Hz, 1H), 4.01~3.95 (m, 1H), 3.22 (dd, *J* = 8.7, 4.8 Hz, 1H), 3.14 (dd, *J* = 8.7, 4.8 Hz, 1H), 2.16~2.04 (m, 2H), 1.72 (dd, *J* = 11.5, 6.5 Hz, 1H), 1.52 (s, 3H), 1.33 (s, 3H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 138.64, 128.29, 127.97, 127.53, 110.66, 82.94, 79.25, 78.34, 72.47, 71.63, 63.39, 42.51, 31.68, 27.32, 26.57, 24.56; FABMS (*m/z*) 335 $(M+H)^+$. Anal ($C_{20}H_{30}O_4$) C, H (for the detail elemental analysis data, see Table 2.1).

(-)-(*1R, 2R, 3S, 4S*)-1-Benzyloxy-4-hydroxymethylcyclopentane-2,3-diol (5). Compound **4** (1.67 g, 4.99 mmol) was treated with 100 ml of TFA:H_2O (2:1) at 50 ^oC for 4 h. The solvent was removed under reduced pressure to dryness and the residue in 100 ml of MeOH was treated with 3 ml of 1 N NaOH at room temperature for 1 h. The

reaction mixture was neutralized with 1 N of HCl using pH paper. The resulting mixture was evaporated, and purified by silica gel column chromatography with 5~10 % MeOH in CHCl3 to give compound **5** (1.16 g, 4.86 mmol and 97 % yield) as white solid: mp 119~120 ^{o}C; $[\alpha]^{25}_{D}$ –11.76^{o} (c 0.40, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.35~7.22 (m, 5H), 4.53 (d, *J* = 11.8 Hz, 1H), 4.45 (t, *J* = 5.2 Hz, 1H, D_2O exchangeable), 4.40 (d, *J* = 11.8 Hz, 1H), 4.30 (d, *J* = 4.4 Hz, 1H, D_2O exchangeable), 4.29 (d, *J* = 7.6 Hz, 1H, D_2O exchangeable), 3.86 (q, *J* = 4.0 Hz, 1H), 3.68 (td, *J* = 8.0, 3.6 Hz, 1H), 3.54 (td, *J* = 7.2, 4.4 Hz, 1H), 2.04~1.96 (m, 1H), 1.78 (td, *J* = 12.8, 8.0 Hz, 1H), 1.63 (ddd, *J* = 13.6, 8.0, 6.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 142.54, 131.83, 131.20, 130.94, 81.90, 76.05, 75.38, 73.81, 66.02, 48.23, 33.90; FABMS (*m/z*) 239 $(M+H)^{+}$. Anal ($C_{13}H_{18}O_4$) C, H.

(+)-(*1R, 2R, 3S, 4S*)-1-Benzyloxy-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane-2-ol (6). To a solution of compound **5** (5.00 g, 21.0 mmol) in 250 ml of pyridine, TIPDSCl (8.0 ml, 25.0 mmol) was dropwisely added at 0 ^{o}C. After 5 h, the reaction was quenched with 10ml of MeOH, the solvent was removed under reduced pressure and the normal aqueous work-up was done. The crude was purified by silica gel column chromatography with 10 % EtOAc in hexanes to give compound **6** (8.00 g, 16.6 mmol and 79 % yield) as oil: $[\alpha]^{23}_{D}$ 4.64^{o} (c 1.61, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.41~7.27 (m, 5H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.09 (t, *J* = 4.2 Hz, 1H), 4.04 (dd, *J* = 7.3, 4.8 Hz, 1H), 3.91 (dd, *J* = 11.8, 3.9 Hz, 1H), 3.88~3.85 (m, 1H), 3.62 (dd, *J* = 11.7, 6.9 Hz, 1H), 2.88 (br s, 1H), 2.49~2.39 (m, 1H), 1.95 (ddd, *J* = 14.0, 9.8, 4.9 Hz, 1H), 1.51 (ddd, *J* = 16.7, 9.2, 7.6 Hz, 1H), 1.08~0.98 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 138.30, 128.36, 127.61, 77.48, 74.06, 72.95, 71.47, 63.91,

45.24, 29.88, 17.57, 17.46, 17.42, 17.30, 17.18, 17.13, 17.04, 13.51, 13.40, 12.92, 12.60; FABMS (*m/z*) 481 $(M+H)^+$. Anal ($C_{25}H_{44}O_5 Si_2$) C, H.

(+)-(*1R, 3R, 4S*)-1-Benzyloxy-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane (7). To a mixture of compound **6** (8.00 g, 16.6 mmol) and NaH as 60 % dispersion (0.66 g, 16.5 mmol) in 400 ml of THF, CS_2 (4.0 ml, 66.5 mmol) and MeI (4.0 ml, 64.3 mmol) were added successively with 30 min of period at 0 ^oC. Then the ice-bath was removed. After 1 h, the reaction was quenched with 10 ml of water and the solvent was removed under reduced pressure. The normal aqueous worke-up was done and the crude was purified by silica gel column chromatography with 3~10 % EtOAc in hexanes to give xantate intermediate (9.24 g, 16.2mmol and 98 % yield) as a yellow syrup: $[\alpha]^{25}_D$ 28.92^o (c 1.56, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.33~7.27 (m, 5H), 6.42 (t, *J* = 4.0 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.40 (d, *J* = 11.4 Hz, 1H), 4.17 (dd, *J* = 9.4, 3.9 Hz, 1H), 4.05 (m, 1H), 3.89 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.75 (dd, *J* = 11.7, 3.6 Hz, 1H), 2.57 (s, 3H), 2.52~2.42 (m, 1H), 1.99 (ddd, *J* = 13.8, 10.3, 6.0 Hz, 1H), 1.86 (dt, *J* = 13.8, 9.0 Hz, 1H), 1.06~0.87 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 216.08, 137.88, 128.38, 127.87, 127.68, 82.69, 75.56, 72.91, 72.05, 62.23, 45.01, 30.66, 18.52,, 17.52, 17.41, 17.36, 17.26, 17.01, 13.50, 13.30, 12.81, 12.75; HR-FABMS (*m/z*) obsd *m/z* 571.2477, calcd for $C_{27}H_{47}O_5S_2Si_2$ *m/z* 571.2404 $(M+H)^+$. Anal. Calcd for $C_{27}H_{46}O_5S_2Si_2$: C, 56.80; H, 8.12. Found: C, 56.54; H, 8.08.

To a refluxing solution of xantate intermediate (9.24 g, 16.2 mmol) in 300 ml of dry toluene, a solution of Bu_3SnH (5.7 ml, 21.2 mmol) and AIBN (0.27 g, 1.6 mmol) in 20 ml of dry toluene was dropwisely added for 30 min. The reaction mixture was refluxed for additional 2 h. The solvent was removed uneder reduced pressure, and the residue was

purified by silica gel column chromatography with 3~10 % EtOAc in hexanes to give compound **7** (5.91 g, 12.7 mmol and 78 % yield) as a syrup: $[\alpha]^{23}_{D}$ 9.13^{o} (c 3.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40~7.27 (m, 5H), 4.78 (d, *J* = 11.8 Hz, 1H), 4.23 (d, *J* = 11.8 Hz, 1H), 4.04~3.96 (m, 2H), 3.93~3.88 (m, 1H), 3.68 (dd, *J* = 11.7, 3.3 Hz, 1H), 2.39 (td, *J* = 12.8, 7.0 Hz, 1H), 2.13~2.04 (m, 1H), 1.86~1.76 (m, 2H), 1.73~1.63 (m, 1H), 1.15~0.85 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 138.62, 128.31, 127.63, 127.42, 75.27, 71.66, 70.35, 60.84, 46.66, 40.77, 32.10, 17.52, 17.42, 17.36, 17.26, 17.18, 17.10, 13.49, 13.35, 12.82, 12.64; FABMS (*m/z*) 465 $(M+H)^{+}$. Anal ($C_{25}H_{44}O_4Si_2$) C, H.

(+)-(*1R, 3R, 4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane-1-ol (8). A mixture of compound **7** (5.91 g, 12.7 mmol) and $Pd(OH)_2$ (0.700 g, 20 % dispersion on carbon) in 100 ml of MeOH was treated with H_2 balloon for overnight. The reaction mixture was filtered through celite pad and concentrated. The residue was purified by silica gel column chromatography with 7.5 % EtOAc in hexanes to give the common intermediate **8** (3.88 g, 1.04 mmol and 82 % yield) as a syrup: $[\alpha]^{23}_{D}$ 20.37^{o} (c 3.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.31~4.22 (m, 1H), 3.98 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.54 (dd, *J* = 11.5, 7.9 Hz, 1H), 2.42~2.30 (m, 1H), 2.20~2.08 (m, 2H), 1.91~1.76 (m, 2H), 1.39 (ddd, *J* = 13.6, 11.2, 5.4 Hz, 1H), 1.15~0.88 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 74.12, 71.66, 63.80, 49.14, 43.18, 36.97, 17.62, 17.50, 17.45, 17.38, 17.32, 17.13, 17.03, 13.58, 13.51, 13.04, 12.46; HR-FABMS (*m/z*) obsd *m/z* 375.2393, calcd for $C_{18}H_{39}O_4Si_2$ *m/z* 373.2387 $(M+H)^{+}$. Anal ($C_{18}H_{38}O_4Si_2$) C, H.

(+)-9-[(*1S,3R,4S)*-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentan-1-yl]-6-chloropurine (9). To a solution of compound **8** (270 mg, 0.721 mmol), 6-chloropurine (167 mg, 1.08 mmol) and Ph_3P (283 mg, 1.08 mmol) in

6 ml of dioxane:DMF (2:1), DEAD (0.17 ml, 1.08 mmol) was dropwisely added at 0 ^{o}C. After 3 h, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with 15~20 % EtOAc in hexanes to give compound **9** (316 mg, 0.618 mmol and 86 % yield) as a syrup: $[\alpha]^{24}_{D}$ 15.87^{o} (c 0.95, MeOH); UV (MeOH) λ_{max} 265.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.13 (s, 1H), 5.11 (quintet, *J* = 8.0 Hz, 1H), 4.75 (q, *J* = 7.6 Hz, 1H), 4.06 (dd, *J* = 11.8, 2.8 Hz, 1H) 3.79 (dd, *J* = 11.7, 3.8 Hz, 1H) 2.44~2.33 (m, 3H), 2.15~2.01 (m, 2H), 1.18~0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 151.62, 151.47, 151.17, 143.93, 132.5, 71.34, 61.09, 52.66, 49.42, 39.69, 33.06, 17.55, 17.44, 17.32, 17.19, 17.15, 17.06, 13.47, 13.40, 12.93, 12.61; HR-FABMS (*m/z*) obsd 511.2276, calcd for $C_{23}H_{40}ClN_4O_3Si_2$ 511.2328 $(M+H)^{+}$. Anal ($C_{23}H_{39}ClN_4O_3Si_2$) C, H.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]adenine (11). A solution of compound **9** (200 mg, 0.391 mmol) in methanolic ammonia was heated to 100 ^{o}C in steel bomb for 6 h. The mixture was concentrated and the residue was purified by silica gel column chromatography with 30 % acetone in hexanes to give aminated product (145 mg, 0.295 mmol and 75 % yield) as a white solid: mp 115~116 ^{o}C; $[\alpha]^{22}_{D}$ 19.17^{o} (c 0.51, MeOH); UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.83 (s, 1H), 5.68 (br s, 2H), 5.06 (quintet, *J* = 8.0 Hz, 1H), 4.68 (q, *J* = 7.6 Hz, 1H), 4.04 (dd, *J* = 11.7, 3.4 Hz, 1H) 3.78 (dd, *J* = 11.7, 4.6 Hz, 1H) 2.40~2.28 (m, 3H), 2.14~2.05 (m, 1H), 1.98 (td, *J* = 12.3, 9.2 Hz, 1H), 1.17~0.92 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 155.37, 152.71, 149.93, 138.94, 120.10, 71.58, 61.48, 51.66, 49.44, 40.04, 33.37, 17.57, 17.46, 17.33, 17.21, 17.15, 17.06, 13.45, 13.39, 12.91, 12.57; HR-FABMS (*m/z*) obsd

492.2796, calcd for $C_{23}H_{42}N_5O_3Si_2$ 492.2826 $(M+H)^+$. Anal. Calcd for $C_{23}H_{41}N_5O_3Si_2$: C, 56.17; H, 8.40; N, 14.24. Found: C, 56.08; H, 8.34; N, 14.15.

A solution of the aminated product (135 mg, 0.275 mmol) in 10 ml of THF was treated with 0.55 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 20 % MeOH in CH_2Cl_3 to give adenine derivative **11** (60 mg, 0.241 mmol and 88 % yield) as a white solid: mp 134~138 ^oC; $[\alpha]^{22}_D$ -7.58^o (c 0.38, MeOH); UV (H_2O) λ_{max} 259.5 nm (ε 20 000, pH 2), 261.0 nm (ε, 21 000, pH 7), 261.0 nm (ε, 21 000, pH 11); ^1H NMR (MeOH-*d*$_4$) δ 8.37 (s, 1H), 8.32 (s, 1H), 5.28 (tt, *J* = 9.4, 8.0 Hz, 1H), 4.44 (dt, *J* = 6.7, 4.0 Hz, 1H), 3.91 (dd, *J* = 10.9, 5.7 Hz, 1H) 3.78 (dd, *J* = 10.9, 6.2 Hz, 1H) 2.68 (dt, *J* = 12.8, 7.4 Hz, 1H), 2.53 (ddd, *J* = 13.4, 9.4, 6.8 Hz, 1H), 2.40~2.28 (m, 2H), 2.05 (dt, *J* = 12.9, 9.6 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 157.66, 153.83, 151.07, 141.56, 120.85, 74.20, 64.77, 55.43, 51.08, 42.00, 35.47; HR-FABMS (*m/z*) obsd 250.1295, calcd for $C_{11}H_{16}N_5O_2$ 250.1304 $(M+H)^+$. Anal ($C_{11}H_{15}N_5O_2 \cdot 0.15CH_2Cl_2$) C, H.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]-6-chloropurine (12). A solution of compound **9** (316 mg, 0.618 mmol) in 10 ml of THF was treated with 1.24 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 50 % MeOH in CH_2Cl_2 to give compound **12** (160 mg, 0.595 mmol and 96 % yield) as a white solid: mp 140~142 ^oC; $[\alpha]^{22}_D$ -16.57^o (c 0.60, MeOH); UV (H_2O) λ_{max} 265.5 nm; ^1H NMR (MeOH-*d*$_4$) δ 8.51 (s, 1H), 8.45 (s, 1H), 5.05 (tt, *J* = 9.5, 8.0 Hz, 1H), 4.12 (dt, *J* = 6.6, 4.2 Hz, 1H), 3.53 (dd, *J* = 10.9, 5.8 Hz, 1H) 3.46 (dd, *J* = 10.9, 6.3 Hz, 1H) 2.36 (dt, *J* = 12.8, 7.8 Hz, 1H), 2.28 (ddd, *J* = 13.4, 9.4, 6.7 Hz, 1H), 2.07 (ddd, *J* = 12.0, 7.7, 3.4 Hz,

1H), 2.03~1.93 (m, 1H), 1.77 (dt, *J* = 12.9, 9.6 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 153.58, 153.03, 151.56, 147.57, 133.15, 74.12, 64.70, 56.36, 51.07, 41.66, 35.14; HR-FABMS (*m/z*) obsd 269.0807, calcd for $C_{11}H_{14}ClN_4O_2$ 269.0805 $(M+H)^+$. Anal ($C_{11}H_{13}ClN_4O_2$) C, H.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]-*N*-Acetyl-2-amino-6-chloropurine (13). To a solution of compound **8** (157 mg, 0.419 mmol), 2-acetylamino-6-chloropurine (110 mg, 0.626 mmol) and Ph_3P (165 mg, 0.629 mmol) in 6 ml of dioxane:DMF (2:1), DEAD (0.10 ml, 0.633 mmol) was dropwisely added at 0 ^oC. After 3 h, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with 20 % EtOAc in hexanes to give compound **10** (205 mg, 0.361 mmol and 86 % yield) with some reduced DEAD impurity. A solution of compound **10** (95 mg, 0.167 mmol) in 5 ml of THF was treated with 0.33 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 20 % MeOH in CH_2Cl_3 to give compound **13** (53 mg, 0.163 mmol and 98 % yield) as a white solid: mp 201~203 ^oC; $[\alpha]^{27}_D$ -27.09^o (c 0.29, MeOH); UV (MeOH) λ_{max} 259.5 nm 288.0 nm; ^{1}H NMR (MeOH-*d*$_4$) δ 8.47 (s, 1H), 5.22 (quintet, *J* = 8.7 Hz, 1H), 4.37 (dt, *J* = 6.6, 4.2 Hz, 1H), 3.75 (dd, *J* = 10.9, 5.6 Hz, 1H) 3.71 (dd, *J* = 10.9, 6.0 Hz, 1H) 2.54 (dt, *J* = 12.7, 7.7 Hz, 1H), 2.46 (ddd, *J* = 13.4, 9.2, 6.8 Hz, 1H), 2.33 (s, 3H), 2.26 (ddd, *J* = 12.3, 8.0, 3.6 Hz, 1H), 2.22~2.13 (m, 1H), 2.02 (dt, *J* = 12.8, 9.7 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 171.71, 154.20, 153.64, 151.96, 146.55, 129.71, 74.13, 64.63, 56.11, 51.02, 41.85, 34.98, 25.34; HR-FABMS (*m/z*) obsd 326.1025, calcd for $C_{13}H_{17}ClN_5O_3$ 326.1020 $(M+H)^+$.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]hypoxanthine (14). Compound **12** (37 mg, 0.14 mmol) was treated with 10 ml of 85 % HCO_2H at 60~70 ^oC for 3 h. The reaction mixture was concentrated to dryness and the residue was treated with 20 ml of methanolic ammonia at room temperature for 1 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 20 % MeOH in CH_2Cl_2 to give hypoxanthine derivative **14** (29 mg, 0.12 mmol and 85 % yield) as a white solid: mp 200~202 ^oC; $[\alpha]^{24}_D$ -10.67^o (c 0.74, MeOH); UV (H_2O) λ_{max} 250.0 nm (ε 8 600, pH 2), 250.0 nm (ε 9 500, pH 7), 254.0 nm (ε 9 500, pH 11); ^1H NMR (MeOH-*d*$_4$) δ 8.20 (s, 1H), 8.06 (s, 1H), 5.18 (tt, *J* = 9.6, 7.9 Hz, 1H), 4.32 (dt, *J* = 6.3, 4.2 Hz, 1H), 3.75 (dd, *J* = 10.9, 5.8 Hz, 1H) 3.68 (dd, *J* = 10.9, 6.3 Hz, 1H) 2.59~2.51 (m, 1H), 2.43 (ddd, *J* = 13.4, 9.4, 6.7 Hz, 1H), 2.30~2.15 (m, 2H), 1.92 (dt, *J* = 12.8, 9.7 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 159.48, 150.61, 146.55, 141.07, 126.02, 74.15, 64.73, 55.77, 51.10, 42.12, 35.63; HR-FABMS (*m/z*) obsd 251.1160, calcd for $C_{11}H_{15}N_4O_3$ 251.1144 $(M+H)^+$, Anal ($C_{11}H_{14}N_4O_3$) C, H, N.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]-6-thiohypoxanthine (15). To a solution of compound **12** (44 mg, 0.16 mmol) in 3 ml of ethanol, thiourea (24 mg, 0.33 mmol) was added and the reaction mixture was refluxed for 1 h. The resulting mixture was concentrated to half of volume and CH_2Cl_2 (3 ml) was added. The solid was filtered and washed with 3 ml of ethanol to give thiohypoxanthine derivative **15** (40 mg, 0.15 mmol and 93 % yield) as a white solid: mp 300 ^oC, dec.; $[\alpha]^{24}_D$; UV (H_2O) λ_{max}; ^1H NMR (DMSO-*d*$_6$) δ 16.65 (br s, 1H), 8.33 (s, 1H), 8.12 (s, 1H), 4.94 (quintet, *J* = 8.7 Hz, 1H), 4.77 (d, *J* = 3.6 Hz, 1H), 4.60 (br t, 1H), 4.04~3.98 (m, 1H), 3.44 (dd, *J* = 10.3, 5.4 Hz, 1H) 3.35 (dd, *J* = 10.7, 6.2 Hz, 1H) 2.29 (dt, *J* = 12.5, 7.9 Hz, 1H), 2.20~2.13 (m,

1H), 2.03~1.88 (m, 2H), 1.64 (dt, *J* = 12.3, 9.5 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 176.14, 144.95, 144.35, 141.91, 135.69, 71.78, 62.99, 53.67, 49.68, 40.85, 34.30; HR-FABMS (*m/z*) obsd 267.0910, calcd for $C_{11}H_{15}N_4O_2S$ 267.0916 $(M+H)^+$. Anal ($C_{11}H_{14}N_4O_2S$) C, H, N.

(-)-9-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]guanine (16). Compound **13** (47 mg, 0.144 mmol) was treated with 20 ml of 85 % HCO_2H at 100 °C for overnight. The reaction mixture was concentrated to dryness and the residue was treated with 20 ml of methanolic ammonia at room temperature for 3 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 15~25 % MeOH in CH_2Cl_2 to give guanine derivative **16** (33 mg, 0.124 mmol and 86 % yield) as a white solid: mp 250 °C, dec.; $[\alpha]^{24}_D$ -17.55° (c 0.26, MeOH); UV (H_2O) λ_{max} 253.5 nm (ε 10 000, pH 2), 253.0 nm (ε 10 000, pH 7), 268.0 nm (ε 9 600, pH 11); ^{1}H NMR (MeOH-d_4) δ 7.91 (s, 1H), 5.09~5.00 (m, 1H), 4.35 (dt, *J* = 6.5, 4.1 Hz, 1H), 3.78 (dd, *J* = 10.9, 5.7 Hz, 1H) 3.75 (dd, *J* = 10.9, 6.3 Hz, 1H) 2.53 (dt, *J* = 12.6, 7.9 Hz, 1H), 2.41 (ddd, *J* = 13.4, 9.5, 6.8 Hz, 1H), 2.27~2.16 (m, 2H), 1.92 (dt, *J* = 12.8, 9.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 159.91, 155.35, 153.50, 138.44, 118.34, 74.18, 64.77, 54.90, 51.07, 41.97, 35.36; HR-FABMS (*m/z*) obsd 266.1253, calcd for $C_{11}H_{16}N_5O_3$ 266.1253 $(M+H)^+$. Anal ($C_{11}H_{15}N_5O_3 \cdot 0.6CH_2Cl_2$) C, H, N.

(+)-1-[(*1S,3R,4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentan-1-yl]-*N*^{3}-benzoyluracil (17). To a solution of intermediate **8** (139 mg, 0.371 mmol), *N*^{3}-benzoyluracil (120 mg, 0.555 mmol) and Ph_3P (146 mg, 0.557 mmol) in 6 ml of dioxane:DMF (2:1), DEAD (0.09 ml, 0.571 mmol) was dropwisely added at 0 °C. After 3 h, the solvent was removed under reduced pressure and the residue

was purified by silica gel column chromatography with 10~20 % EtOAc in hexanes to give compound **17** (112 mg, 0.195 mmol and 53 % yield) as a syrup:$[\alpha]^{24}_{D}$ 22.20^{o} (c 1.69, $CHCl_3$); UV (MeOH) λ_{max} 253.0 nm; ^{1}H NMR ($CDCl_3$) δ 7.93 (d, *J* = 8.1 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.51 (d, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 7.6 Hz, 1H), 5.85 (d, *J* = 8.1 Hz, 1H), 5.14~5.02 (m, 1H), 4.40 (q, *J* = 8.1 Hz, 1H), 4.00 (dd, *J* = 11.8, 3.4Hz, 1H), 3.72 (dd, *J* = 11.8, 4.0 Hz, 1H), 2.30~2.18 (m, 2H), 2.10~1.93 (m, 2H), 1.70~1.55 (m, 1H), 1.15~0.95 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 168.82, 161.89, 149.75, 140.60, 135.06, 131.41, 130.42, 129.13, 102.75, 71.07, 60.76, 53.05, 48.62, 38.46, 32.11, 17.46, 17.38, 17.36, 17.30, 17.17, 17.06, 16.97, 13.47, 13.30, 12.76, 12.51; HR-FABMS (*m/z*) obsd 573.2719, calcd for $C_{29}H_{45}N_2O_6Si_2$ 573.2816 $(M+H)^{+}$. Anal ($C_{29}H_{44}N_2O_6Si_2$) C, H, N.

(+)-1-[(*1S,3R,4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentan-1-yl]-*N*^{3}-benzoylthymine (18). To a solution of intermediate **8** (50 mg, 0.13 mmol), *N*^{3}-benzoylthymine (46 mg, 0.20 mmol) and Ph_3P (52 mg, 0.20 mmol) in 3 ml of dioxane:DMF (2:1), DEAD (0.05 ml, 0.20 mmol) was dropwisely added at 0 ^{o}C. After 3 h, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with 10~20 % EtOAc in hexanes to give compound **18** (48 mg, 0.082 mmol and 62 % yield) as a syrup: $[\alpha]^{26}_{D}$ 20.19^{o} (c 2.2, $CHCl_3$); UV (MeOH) λ_{max} 252.5 nm; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, *J* = 7.4 Hz, 2H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.08 (s, 1H), 5.17~5.06 (m, 1H), 4.41 (q, *J* = 8.3 Hz, 1H), 4.00 (dd, *J* = 11.8, 3.2Hz, 1H), 3.73 (dd, *J* = 11.8, 3.5 Hz, 1H), 2.28~2.16 (m, 2H), 2.05~1.90 (m, 2H), 1.95 (s, 3H), 1.63 (td, *J* = 12.6, 9.8 Hz, 1H), 1.15~0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 170.16, 163.30, 150.76, 137.28, 135.78, 132.44, 131.24, 129.91, 112.27, 71.51, 60.79, 52.45, 48.93, 38.69, 32.30, 29.98, 17.66, 17.56, 17.53,

17.48, 17.46, 17.33, 17.21, 17.12, 13.62, 13.43, 12.76, 12.67; HR-FABMS (*m/z*) obsd 587.2980, calcd for $C_{30}H_{47}N_2O_6Si_2$ 587.2986 $(M+H)^+$. Anal ($C_{30}H_{46}N_2O_6Si_2$) C, H, N.

(+)-1-[(*1S,3R,4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentan-1-yl]-N^3-benzoyl-(*E*)-5-bromovinyluracil (19). To a solution of intermediate **8** (150 mg, 0.400 mmol), (*E*)-N^3-benzoyl-5-bromovinyluracil (160 mg, 0.498 mmol) and Ph_3P (157 mg, 0.599 mmol) in 6 ml of dioxane:DMF (2:1), DEAD (0.10 ml, 0.64 mmol) was dropwisely added at 0 ^oC. After 3 h, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography with 10~20 % EtOAc in hexanes to give compound **19** (125 mg, 0.184 mmol and 46 % yield) as a syrup: $[\alpha]^{22}_D$ 20.39^o (c 1.07, CH_2Cl_2); UV (MeOH) λ_{max} 253.5 nm, 300.3 nm; ^1H NMR ($CDCl_3$) δ 7.92 (dd, *J* = 8.5, 1.3 Hz, 2H), 7.67 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.51 (t, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 13.6 Hz, 1H), 7.20 (s, 1H), 6.62 (d, *J* = 13.6 Hz, 1H), 5.16~5.07 (m, 1H), 4.42 (q, *J* = 8.3 Hz, 1H), 4.00 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.73 (dd, *J* = 11.8, 3.7 Hz, 1H), 2.32~2.18 (m, 2H), 2.07~1.92 (m, 2H), 1.63 (td, *J* = 12.6, 9.7 Hz, 1H), 1.17~0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 168.81, 160.70, 149.01, 138.66, 135.63, 131.70, 130.83, 129.65, 128.18, 112.37, 118.18, 71.43, 60.88, 53.57, 49.09, 39.02, 32.69, 17.95, 17.86, 17.84, 17.77, 17.62, 17.50, 17.41, 13.96, 13.74, 13.22, 12.99; FABMS (*m/z*) 677 $(M+H)^+$, 679 $(M+3)^+$.

(-)-1-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]uracil (20). Compound **17** (112 mg, 0.195 mmol) was treated with 10 ml of methanolic ammonia at room temperature for 2 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 40 % EtOAc in hexanes to give debenzoylated intermedate **23** (88 mg, 0.188 mmol and 96 % yield) as a syrup: $[\alpha]^{23}_D$ 25.05^o (c 2.23,

CH_2Cl_2); UV (MeOH) λ_{max} 267.0 nm; ^{1}H NMR (MeOH-d_4) δ 8.93 (br s, 1H), 7.18 (d, J = 8.1 Hz, 1H), 5.74 (dd, J = 8.0, 2.3 Hz, 1H), 5.11~5.02 (m, 1H), 4.39 (q, J = 8.3 Hz, 1H), 4.00 (dd, J = 11.8, 3.4Hz, 1H), 3.73 (dd, J = 11.8, 3.8 Hz, 1H), 2.26~2.14 (m, 2H), 2.04~1.90 (m, 2H), 1.59 (td, J = 12.6, 9.8 Hz, 1H), 1.15~0.95 (m, 28H); ^{13}C NMR (MeOH-d_4) δ 162.91, 150.88, 140.95, 102.80, 71.08, 60.65, 52.76. 48.60, 38.38, 32.02, 17.47, 17.37, 17.30, 17.18, 17.09, 17.00, 13.48, 13.30, 12.78, 12.55; FABMS (*m/z*) 469 $(M+H)^+$. Anal ($C_{22}H_{40}N_2O_5Si_2$·0.1hexane) C, H, N.

A solution of the debenzoylated intermedate **23** (79 mg, 0.168 mmol) in 10 ml of THF was treated with 0.34 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 20 % MeOH in CH_2Cl_2 to give uracil derivative **20** (37 mg, 0.160 mmol and 95 % yield) as a white solid: mp 150~152 ^oC; $[\alpha]^{25}_D$ -4.39^o (c 1.34, MeOH); UV (H_2O) λ_{max} 267.5 nm (ε, 4 800, pH 2), 268.0 nm (ε, 4 700, pH 7), 266.0 nm (ε, 4 300, pH 11); ^{1}H NMR (MeOH-d_4) δ 7.64 (d, J = 8.0 Hz, 1H), 5.64 (d, J = 8.0 Hz, 1H), 5.01 (quintet, J = 9.0 Hz, 1H), 4.41 (dt, J = 6.8, 4.4 Hz, 1H), 3.63 (dd, J = 10.8, 5.5 Hz, 1H), 3.56 (dd, J = 10.8, 6.2 Hz, 1H), 2.22 (dt, J = 12.6, 7.5 Hz, 1H), 2.07~1.92 (m, 3H), 1.52 (dt, J = 12.7, 10.0 Hz, 1H); ^{13}C NMR (MeOH-d_4) δ 166.71, 153.21, 144.59, 102.97, 73.99, 64.06, 56.59, 50.66, 40.43, 33.91; HR-FABMS (*m/z*) obsd 227.1059, calcd for $C_{10}H_{15}N_2O_4$ 227.1032 $(M+H)^+$. Anal ($C_{10}H_{14}N_2O_4$) C, H, N.

(-)-1-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]thymine (21).

Compound **18** (25 mg, 0.043 mmol) was treated with 5 ml of methanolic ammonia at room temperature for 2 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 20 % EtOAc in hexanes to give debenzoylated

intermediate (21 mg, 0.043 mmol and quant. yield) as a syrup: $[\alpha]^{26}_{D}$ 31.01° (c 1.89, MeOH); UV (MeOH) λ_{max} 271.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.92 (br s, 1H), 6.91 (s, 1H), 5.08~4.98 (m, 1H), 4.32 (q, *J* = 8.5 Hz, 1H), 3.93 (dd, *J* = 11.7, 3.3 Hz, 1H), 3.66 (dd, *J* = 11.7, 3.3 Hz, 1H), 2.18~2.06 (m, 2H), 1.94~1.81 (m, 2H), 1.84 (s, 1H), 1.53 (td, *J* = 12.7, 9.7 Hz, 1H), 1.17~0.86 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 163.57, 150.84, 136.74, 111.51, 71.00, 60.26, 51.78, 48.54, 38.33, 31.98, 28.67, 17.50. 17.42, 17.39, 17.34, 17.31, 17.19, 17.09, 17.00, 13.49, 12.64, 12.58, 12.56; HR-FABMS (*m/z*) obsd 483.2632, calcd for $C_{23}H_{43}N_2O_5Si_2$ 483.2711 $(M+H)^+$. Anal. Calcd for $C_{23}H_{42}N_2O_5Si_2$: C, 57.22; H, 8.77; N, 5.80. Found: C, 57.32; H, 8.79; N, 5.64.

A solution of the debenzoylated intermediate (107 mg, 0.222 mmol) in 10 ml of THF was treated with 0.45 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 10~20 % MeOH in CH_2Cl_2 to give thymine derivative **21** (55 mg, 0.22 mmol and quant. yield) as a white solid: mp 170~171 °C; $[\alpha]^{25}_{D}$ -6.54° (c 1.00, MeOH); UV (H_2O) λ_{max} 273.0 nm (ε, 14 000, pH 2), 273.5 nm (ε, 14 000, pH 7), 271.0 nm (ε, 11 000, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 7.61 (d, *J* = 1.1 Hz, 1H), 5.16 (quintet, *J* = 9.0 Hz, 1H), 4.26 (dt, *J* = 6.8, 4.5 Hz, 1H), 3.79 (dd, *J* = 10.9, 5.5 Hz, 1H), 3.72 (dd, *J* = 10.9, 6.1 Hz, 1H), 2.34 (dt, *J* = 12.6, 7.7 Hz, 1H), 2.23~1.97 (m, 3H), 1.98 (d, *J* = 1.1 Hz, 3H), 1.68 (dt, *J* = 12.6, 10.1 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 166.83, 153.36, 140.23, 111.95, 74.01, 64.65, 56.21, 50.68, 48.40, 33.86, 12.76; HR-FABMS (*m/z*) obsd 241.1299, calcd for $C_{11}H_{17}N_2O_4$ 241.1260 $(M+H)^+$. Anal ($C_{11}H_{16}N_2O_4$) C, H, N.

(-)-1-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]-(*E*)-5-bromovinyluracil (22). Compound **17** (120 mg, 0.177 mmol) was treated with 10 ml of

methanolic ammonia at room temperature for 2 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 20 % EtOAc in hexanes to give debenzoylated intermediate (98 mg, 0.171 mmol and 97 % yield) as a syrup: $[\alpha]^{22}_{D}$ 34.54° (c 0.56, CH_2Cl_2); UV (MeOH) λ_{max} 252.0 nm, 297.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.55 (br s, 1H), 7.42 (d, *J* = 13.6 Hz, 2H), 7.10 (s, 1H), 6.60 (d, *J* = 13.6 Hz, 1H), 5.13~5.03 (m, 1H), 4.41 (q, *J* = 8.4 Hz, 1H), 4.00 (dd, *J* = 11.8, 3.3 Hz, 1H), 3.73 (dd, *J* = 11.8, 3.5 Hz, 1H), 2.27~2.13 (m, 2H), 2.03~1.90 (m, 2H), 1.61 (td, *J* = 12.7, 9.8 Hz, 1H), 1.16~0.94 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 160.75, 149.35, 138.69, 128.03, 111.97, 110.23, 70.97, 60.36, 52.97, 48.64, 38.49, 32.14, 17.53, 17.46, 17.43, 17.36, 17.33, 17.21, 17.11, 17.02, 13.54, 13.33, 12.80, 12.58; FABMS (*m/z*) 573 $(M+H)^+$, 575 $(M+3)^+$. Anal. (Calcd for $C_{24}H_{41}BrN_2O_5Si_2 \cdot 0.2EtOAc$: C, 50.38; H, 7.26; N, 4.74. Found: C, 50.68; H, 7.28; N, 4.75

A solution of the debenzoylated intermediate (81 mg, 0.14 mmol) in 10 ml of THF was treated with 0.29 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 10 % MeOH in CH_2Cl_2 to give carbocyclic BVDU **22** (42 mg, 0.126 mmol and 89 % yield) as a white solid: mp 190 °C, dec.; $[\alpha]^{25}_{D}$ -6.67° (c 1.31, MeOH); UV (H_2O) λ_{max} 296.5 nm (ε, 15 000, pH 2), 252.5 nm (ε, 18 000, pH 2), 297.0 nm (ε, 15 000, pH 7), 252.5 nm (ε, 18 000, pH 7), 289.0 nm (ε, 15 000, pH 11), 258.0 nm (ε, 18 000, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 7.77 (s, 1H), 7.34 (d, *J* = 13.6 Hz, 1H), 6.82 (d, *J* = 13.6 Hz, 1H), 5.06 (quintet, *J* = 9.0 Hz, 1H), 4.17 (dt, *J* = 6.8, 4.5 Hz, 1H), 3.68 (dd, *J* = 10.8, 5.4 Hz, 1H), 3.62 (dd, *J* = 10.8, 6.1 Hz, 1H), 2.27 (dt, *J* = 12.5, 7.5 Hz, 1H), 2.15~1.93 (m, 3H), 1.59 (dt, *J* = 12.7, 10.0 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 164.07, 152.21, 142.57, 130.78,

112.57, 109.05, 73.97, 64.61, 56.91, 50.67, 40.54, 33.92; HR-FABMS (*m/z*) obsd 331.0291, calcd for $C_{12}H_{16}BrN_2O_4$ 331.0293 $(M+H)^+$. Anal ($C_{12}H_{15}BrN_2O_4$) C, H, N.

(+)-1-[(*1S,3R,4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentan-1-yl]cytosine (24). To a solution of compound **23** (88 mg, 0.188 mmol) and 1,2,4-triazole (78 mg, 1.13 mmol) in 4 ml of pyridine, chlorophenyl dichlorophosphate (0.09 ml, 0.553 mmol) was added at 0 ^oC. After 24 h, the solvent was removed under reduced pressure and the residue was treated with 10 ml of methanolic ammonia at 100 ^oC in steel bomb for 4 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 50 % EtOAc in hexanes to give compound **24** (76 mg, 0.162 mmol and 86 % yield) as a syrup: $[\alpha]^{24}_D$ 28.31^o (c 0.73, CH_2Cl_2); UV (MeOH) λ_{max} 275.5 nm; ^1H NMR ($CDCl_3$) δ 7.20 (d, *J* = 7.2 Hz, 1H), 5.69 (d, *J* = 7.3 Hz, 1H), 5.13~5.07 (m, 1H), 4.30 (q, *J* = 8.1 Hz, 1H), 3.92 (dd, *J* = 11.7, 3.4 Hz, 1H), 3.65 (dd, *J* = 11.7, 4.1 Hz, 1H), 2.18~2.07 (m, 2H), 1.95~1.86 (m, 2H), 1.46 (td, *J* = 12.5, 10.0 Hz, 1H), 1.05~0.82 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 165.05, 156.60, 141.75, 95.16, 71.40, 61.10, 53.05, 48.75, 38.89, 32.52, 17.47, 17.40, 17.37, 17.30, 17.18, 17.08, 16.99, 13.44, 13.29, 12.76, 12.52; FABMS (*m/z*) 468 $(M+H)^+$. Anal ($C_{22}H_{41}N_3O_4Si_2 \cdot 0.15$hexane) C, H, N.

(-)-1-[(*1S,3R,4S*)-3-Hydroxy-4-hydroxymethylcyclopentan-1-yl]cytosine (25).

A solution of compound **24** (72 mg, 0.154 mmol) in 10 ml of THF was treated with 0.31 ml of 1 N TBAF solution in THF at room temperature for 30 min. The reaction mixture was concentrated and purified by silica gel column chromatography with 20 % MeOH in CH_2Cl_2 to give cytosine derivative **25** (34 mg, 0.151 mmol and 98 % yield) as a white solid: mp 193~196 ^oC; $[\alpha]^{25}_D$ -2.82^o (c 0.94, MeOH); UV (H_2O) λ_{max} 284.0 nm (ε, 12

000, pH 2), 275.0 nm (ε, 8 000, pH 7), 275.0 nm (ε, 8 000, pH 11); ^{1}H NMR (MeOH-d_4) δ 7.53 (d, J = 7.4 Hz, 1H), 5.75 (d, J = 7.4 Hz, 1H), 4.96 (quintet, J = 8.9 Hz, 1H), 4.03 (dt, J = 6.7, 4.7 Hz, 1H), 3.54 (dd, J = 10.9, 5.5 Hz, 1H), 3.48 (dd, J = 10.9, 6.2 Hz, 1H), 2.14 (dt, J = 12.6, 7.6 Hz, 1H), 1.97~1.87 (m, 3H), 1.41 (dt, J = 12.5, 10.1 Hz, 1H); ^{13}C NMR (MeOH-d_4) δ 167.60, 159.46, 144.60, 96.50, 74.10, 64.72, 57.31, 50.81, 40.85, 34.38; HR-FABMS (*m/z*) obsd 226.1186, calcd for $C_{10}H_{16}N_3O_3$ 226.1192 $(M+H)^+$. Anal ($C_{10}H_{15}N_3O_3 \cdot 0.05CH_2Cl_2$) C, H, N.

Table 2.2. Elemental Analysis Data

Cmpd	Formula	Calc for C	Calc for H	Calc for N	Found for C	Found for H	Found for N
4	$C_{20}H_{30}O_4$	71.82	9.04	-[a]	71.99	9.07	-[a]
5	$C_{13}H_{18}O_4$	65.53	7.61	-[a]	65.60	7.64	-[a]
6	$C_{25}H_{44}O_5Si_2$	62.45	9.22	-[a]	62.30	9.23	-[a]
7	$C_{25}H_{44}O_4Si_2$	64.60	9.54	-[a]	64.38	9.40	-[a]
8	$C_{18}H_{38}O_4Si_2$	57.70	10.22	-[a]	57.59	10.10	-[a]
9	$C_{23}H_{39}ClN_4O_3Si_2$	54.04	7.69	10.96	54.09	7.65	10.94
11	$C_{11}H_{15}N_5O_2 \cdot 0.15CH_2Cl_2$	51.11	5.89	26.73	51.25	6.18	26.97
12	$C_{11}H_{13}ClN_4O_2$	49.17	4.88	20.85	49.37	4.77	20.92
14	$C_{11}H_{14}N_4O_3$	52.79	5.64	22.39	52.70	5.34	22.13
15	$C_{11}H_{14}N_4O_2S$	49.61	5.31	21.04	49.59	5.33	20.92
16	$C_{11}H_{15}N_5O_3 \cdot 0.6CH_2Cl_2$	44.06	5.16	22.15	44.36	5.06	22.28
17	$C_{29}H_{44}N_2O_6Si_2$	60.80	7.74	4.89	60.99	7.64	4.79
18	$C_{30}H_{46}N_2O_6Si_2$	61.40	7.90	4.77	61.20	7.99	4.59
20	$C_{10}H_{14}N_2O_4$	53.09	6.24	12.38	52.83	6.31	12.20
21	$C_{11}H_{16}N_2O_4$	54.99	6.71	11.66	54.76	6.64	11.50
22	$C_{12}H_{15}BrN_2O_4$	43.52	4.57	8.46	43.68	4.55	8.36
23	$C_{22}H_{40}N_2O_5 \cdot 0.1C_6H_{14}$	56.86	8.74	5.87	56.01	8.65	5.67
24	$C_{22}H_{41}N_3O_2 \cdot 0.15C_6H_{14}$	57.22	9.04	8.74	57.04	8.82	8.34
25	$C_{10}H_{15}N_3O_3 \cdot 0.05CH_2Cl_2$	52.60	6.63	18.31	52.79	6.82	18.02

CHAPTER 3

SOLID PHASE SYNTHESIS OF CARBOCYCLIC L-2'-DEOXYNUCLEOSIDE[1]

[1] Choo, H., Y. Chong and C. K. Chu. 2001. *Organic Letters*. 3:1471-1473.

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INTRODUCTION

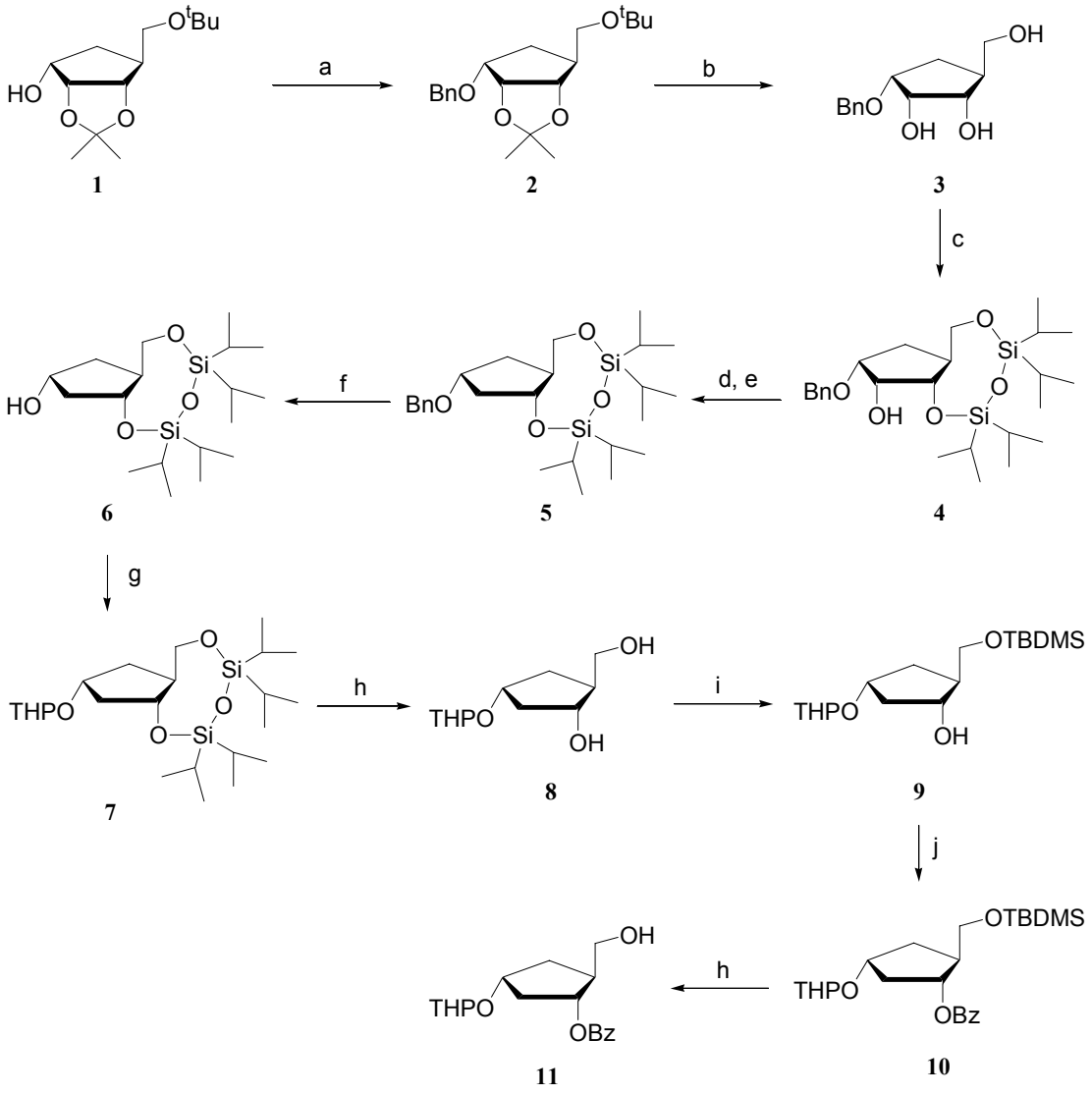
Nucleosides have been playing a major role combating tumor and virus, and a variety of modifications of natural nucleosides have been made to discover novel antitumor and/or antiviral agents.[258] Among nucleosides, carbocyclic analogs have been one of the interesting classes of compounds due to the nonglycosidic nature of the bond between carbocyclic moiety and heterocyclic base, which results in metabolic stability to phosphorylases. Several methods[259] of condensation between carbocyclic moiety and heterocyclic bases have been reported: 1) a classical S_N2 type reaction with tosylate or mesylate, 2) an epoxide ring opening method, 3) a palladium-catalyzed coupling reaction, and 4) Mitsunobu reaction.[260] The Mitsunobu reaction has been widely utilized for the synthesis of both purine and pyrimidine derivatives. However, solution phase synthesis needs to be improved. For example, the by-products, reduced DEAD and triphenylphosphine oxide, are very difficult to separate from the products after reaction, and a regioisomeric mixture of N_1- and O^2-alkylated pyrimidines is usually produced. However, there has not been much effort to solve this regioselectivity problem. Recently, Crimmins *et al* reported regioselective synthesis of purine nucleosides by using the palladium-catalyzed coupling method in solid phase synthesis.[261] Herein we report a comparison of solid phase synthesis with solution phase synthesis of carbocyclic nucleosides by Mitsunobu reaction, in which we focused on the regioselectivity of the condensation. Furthermore, this method can be proposed as a general strategy for a library of carbocylic nucleosides.

Previously, we have reported the syntheses of several carbocyclic L-nucleosides such as L-aristeromycin and L-carbovir[262] from alcohol **1**, which was synthesized by a known method[263] in 5 steps from D-ribose. By using the alcohol **1**, 2'-deoxy carbocyclic moiety **11** was synthesized as shown in Scheme 3.1. Appropriately protected carbocyclic moiety **11** was proposed as the key intermediate because the protecting groups could be kept stable under the reaction conditions on solid phase and removed at the time when the product is generated from the resin after the Mitsunobu reaction.

RESULTS AND DISSCUSION

The alcohol **1** was protected by benzyl bromide to give the fully-protected intermediate **2** in 88% yield, which was deprotected by trifluoroacetic acid in water followed by treatment with 1N NaOH to give compound **3** in 97% yield (Scheme 3.1). Compound **3** was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane(TIPDSCl)[264] in pyridine to give compound **4** which was converted to a xanthate by successive treatment with sodium hydride, carbon disulfide and methyl iodide. It was deoxygenated by tributyltin hydride and AIBN to give compound **5** in 76% yield in two steps. The benzyl group of compound **5** was removed by using palladium(II) hydroxide and hydrogen to give compound **6**. Compound **6** was protected by treatment with DHP and PPTS followed by deprotection of TIPDS group to give compound **8** in 71% yield in two steps. To differentiate 5'-OH from 3'-OH, TBDMS group was introduced to compound **8** to give compound **9** in 99%

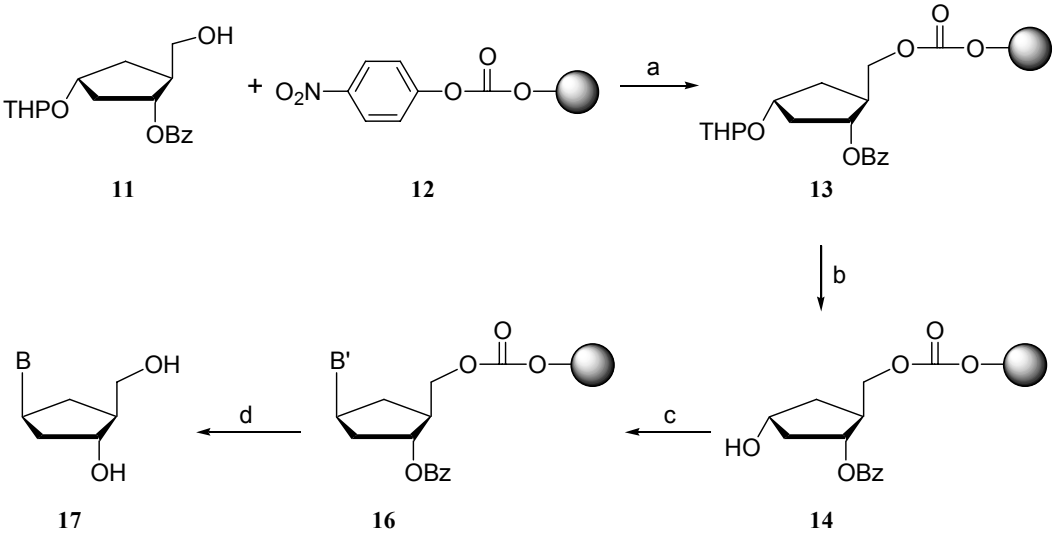
yield. The key intermediate **11** was obtained by benzoylation followed by deprotection of TBDMS group in 98% yield in two steps.



[a]Reagents and conditions: a) NaH, BnBr, THF, 0 ^{o}C; b) TFA/H_2O, 60 ^{o}C, then 1N NaOH; c) TIPDSCl, pyr, 0 ^{o}C; d) NaH, CS_2, MeI, THF, 0 ^{o}C to rt; e) Bu_3SnH, AIBN, Toluene, reflux; f) $Pd(OH)_2$, MeOH, H_2; g) DHP, PPTS ,CH_2Cl_2, rt; h) TBAF, THF, rt; i) TBDMSCl, Imidazole, THF, 0 ^{o}C to rt; j) BzCl, pyr, 0 ^{o}C

Scheme 3.1. Synthesis of carbocyclic moiety **11**

The intermediate **11**, which was used in 3 equiv, was loaded to *p*-nitrophenyl carbonate resin **12**[265] by treatment with 10 equiv of DIPEA and 1.0 equiv of 4-DMAP in CH_2Cl_2 under reflux for 24 h (Scheme 3.2). The unreacted **11** was recycled after the reaction. Resin **13** was deprotected by PPTS in a 1:1 mixture of 1-butanol and 1,2-dichloroethane at 60°C for 24 h.[266] The temperature was very critical in this step. If the temperature was below 55°C, the reaction did not go to completion even after 24 hrs. Completion of the reaction was confirmed by the cleavage of a small portion of resin **14**. Resin **14** was condensed with 10 equiv of heterocyclic bases **15a-e** (Fig. 3.1) under Mitsunobu conditions. Carbocyclic nucleosides **17** were obtained from resin **16** by treatment with 10 equiv of K_2CO_3 in THF/MeOH (2:1) at room temperature for 24 hrs. The crude product was purified by silica gel chromatography to give the pure carbocyclic nucleosides **17** (Table 3.1).



[a]Reagents and conditions : a) DMAP, DIPEA, CH_2Cl_2, 40°C; b) PPTS, 1-Butanol/1,2-Dichloroethane, 60°C; c) B'H (**15a-e**), DEAD, Ph_3P, DMF/dioxane or DMF, rt; d) K_2CO_3, THF/MeOH, rt

Scheme 3.2. Solid phase syntheis of carbocyclic L-2'-deoxynucleosides **17** [a]

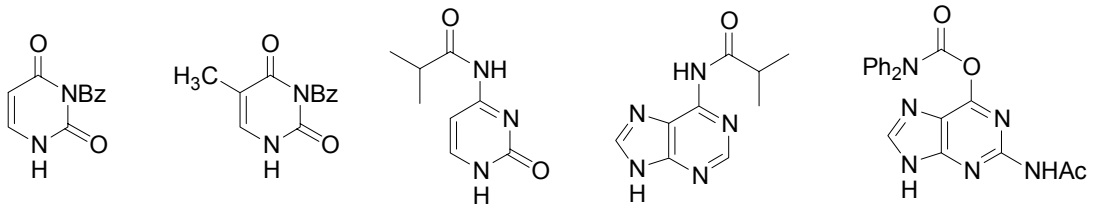


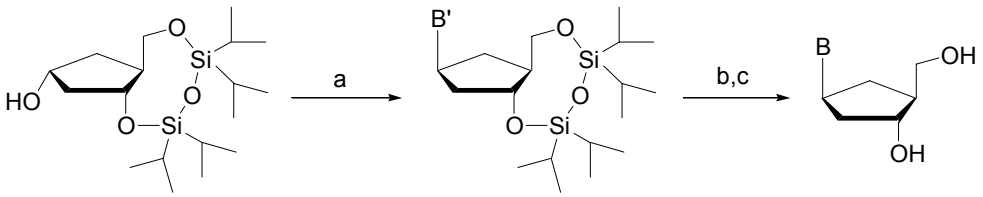
Figure 3.1. Structures of heterocyclic bases (B'H)

Table 3.1. Comparison between solid and solution phase syntheses

B'H (**15**)	**17** B =	Solid Phase		Solution Phase	
		HPLC ratio[a]	%yield[b]	Isolation ratio[a]	%yield[c]
a	uracil	97/3	51%[d]	64/36	79%[d]
b	thymine	85/15	100%[d]	70/30	88%[d]
c	cytosine	20/80	97%[e]	17/83	39%[e]
d	adenine	100/0	55%[e]	100/0	43%[e]
e	guanine	100/0	62%[e]	100/0	47%[e]

[a]ratio between N_1- and O^2-alkylated pyrimidines or N_9- and N_7- alkylated purines, [b]yield in 4 steps from **12** to **17**, [c]yield in 3 steps from **6** to **17**, [d]Solvent: DMF/1,4-dioxane(1:2), [e]Solvent: DMF only

The results of solid phase synthesis of carbocyclic nucleosides **17** were compared with those of solution phase synthesis (Scheme 3.3), where Mitsunobu reaction was used as a condensation method. Even though the solvent system of DMF/1,4-dioxane (1:2) was good in view of the yield,[267] it could not be generally used because the solubility of some protected bases (**15c-e**) was poor in this solvent. Therefore, in some cases (**15c-e**), the solvent was changed to DMF.



6 **18** **17**

[a]Reagents and conditions: a) DEAD, Ph_3P, B'H **15**, solvent, b) NH_3, MeOH, c) TBAF, THF

Scheme 3.3. Solution phase synthesis of carbocyclic nucleosides **17**

Regioselectivity of N_1- and O^2-alkylated pyrimidines was increased in the case of uracil and thymine derivatives in the solid phase synthesis (Table 3.1). The regioselectivity can be explained by relative stability of two resonance forms of the conjugate anion of pyrimidines. As noted by Crimmins *et al*,[265] the different environment of the solid phase, where aromatic rings of polystyrene resin solvate the reaction sites, is probably responsible for the improved regioselectivity. However, in the case of cytosine derivative, the fully aromatized resonance form of the conjugate anion is more stable than the other one, and the preference for the aromatized resonance form results in the formation of O^2-alkylated cytosine as a major product. This preference does not seem to be effected by the unique environment of solid phase, and can explain the similar ratios of N_1- and O^2-alkylated cytosine in both solid and solution phase syntheses. In the case of purine derivatives, there was no regioisomeric problem in both syntheses.

This study on solid phase synthesis encouraged us to optimize and standardize the conditions in the solid phase synthesis in order to apply this method to automation. Therefore, DMF was chosen as a general solvent because of the favorable solubility of all the heterocyclic bases (**15a-e**). The results are shown in Table 3.2. Changing the solvent

from a 1:2 mixture of DMF and 1,4-dioxane to DMF did not affect the regioisomeric ratios significantly, which also supported the explanation of the increased regioselectivity in solid phase synthesis.

Table 3.2. Optimized solid phase synthesis

B'H (**15**)	**17** B =	HPLC ratio[a]	%yield[b]
a	uracil	98/2	74%
b	thymine	90/10	80%
c	cytosine	30/70	82%
d	adenine	100/0	69%
e	guanine	100/0	58%

[a]ratio between N_1- and O^2-alkylated pyrimidines or N_9- and N_7- alkylated purines, [b]yield in 4 steps from **12** to **17**

In summary, we have demonstrated that pyrimidine and purine derivatives of carbocyclic nucleosides were successfully synthesized on solid phase through Mitsunobu reaction. This method could be applied for the synthesis of other nucleoside libraries.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported as s

(singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(+)-(*1R,2R,3S,4S*)-1-Benzyloxy-4-(*tert*-butoxymethyl)-2,3-isopropylidenedioxy)cyclopentane (2). To a solution of the alcohol **1** (2.23 g, 9.14 mmol) in 50 ml of THF was added NaH as 60 % dispersion (0.439 g, 11.0 mmol) at 0 ^{o}C. After 10 min, BnBr (1.6 ml, 13.7 mmol) was added to the reaction mixture and the ice-bath was removed. After 3 hr, the reaction was quenched with 10 ml of water, and the resulting mixture was evaporated. The normal aqueous work-up was done and the crude was purified by silica gel colunm chromatography with 20 % EtOAc in hexanes to give compound **2** (2.70 g, 8.08 mmol and 88 % yield) as a pale yellow oil: $[\alpha]^{25}_{D}$ 35.51^{o} (c 1.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.41~7.23 (m, 5H), 4.67 (d, J = 12.4 Hz, 1H), 4.60 (d, J = 12.4 Hz, 1H), 4.52 (t, J = 5.2 Hz, 1H), 4.37 (d, J = 5.2 Hz, 1H), 4.01~3.95 (m, 1H), 3.22 (dd, J = 8.7, 4.8 Hz, 1H), 3.14 (dd, J = 8.7, 4.8 Hz, 1H), 2.16~2.04 (m, 2H), 1.72 (dd, J = 11.5, 6.5 Hz, 1H), 1.52 (s, 3H), 1.33 (s, 3H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 138.64, 128.29, 127.97, 127.53, 110.66, 82.94, 79.25, 78.34, 72.47, 71.63, 63.39, 42.51, 31.68, 27.32, 26.57, 24.56; FABMS (*m/z*) 335 $(M+H)^{+}$. Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 71.99; H, 9.07.

(-)-(*1R,2R,3S,4S*)-1-Benzyloxy-4-hydroxymethylcyclopentane-2,3-diol (3). Compound **2** (1.67 g, 4.99 mmol) was treated with 100 ml of TFA and H_2O (2:1) at 50 ^oC for 4 hr. The solvent was removed under reduced pressure to dryness and the residue in 100 ml of MeOH was treated with 3 ml of 1 N NaOH at room temperature for 1 h. The reaction mixture was neutralized with 1 N of HCl using pH paper. The resulting mixture was evaporated, and purified by silica gel column chromatography with 5~10 % MeOH in CHCl3 to give compound **3** (1.16 g, 4.86 mmol and 97 % yield) as white solid: mp 119~120 ^oC; $[\alpha]^{25}_D$ –11.76^o (c 0.40, MeOH); ^1H NMR (DMSO-d_6) δ 7.35~7.22 (m, 5H), 4.53 (d, J = 11.8 Hz, 1H), 4.45 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 4.40 (d, J = 11.8 Hz, 1H), 4.30 (d, J = 4.4 Hz, 1H, D_2O exchangeable), 4.29 (d, J = 7.6 Hz, 1H, D_2O exchangeable), 3.86 (q, J = 4.0 Hz, 1H), 3.68 (td, J = 8.0, 3.6 Hz, 1H), 3.54 (td, J = 7.2, 4.4 Hz, 1H), 2.04~1.96 (m, 1H), 1.78 (td, J = 12.8, 8.0 Hz, 1H), 1.63 (ddd, J = 13.6, 8.0, 6.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 142.54, 131.83, 131.20, 130.94, 81.90, 76.05, 75.38, 73.81, 66.02, 48.23, 33.90; FABMS (*m/z*) 239 $(M+H)^+$. Anal. Calcd for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.60; H, 7.64.

(+)-(*1R,2R,3S,4S*)-1-Benzyloxy-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane-2-ol (4). To a solution of compound **3** (5.00 g, 21.0 mmol) in 250 ml of pyridine was added TIPDSCl (8.0 ml, 25.0 mmol) dropwisely at 0 ^oC. After 5 hr, the reaction was quenched with 10ml of MeOH, the solvent was removed under reduced pressure and the normal aqueous work-up was done. The crude was purified by silica gel column chromatography with 10 % EtOAc in hexanes to give compound **4** (8.00 g, 16.6 mmol and 79 % yield) as oil: $[\alpha]^{23}_D$ 4.64^o (c 1.61, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.41~7.27 (m, 5H), 4.64 (d, J = 12.0 Hz, 1H), 4.57 (d, J = 12.0 Hz, 1H), 4.09

(t, *J* = 4.2 Hz, 1H), 4.04 (dd, *J* = 7.3, 4.8 Hz, 1H), 3.91 (dd, *J* = 11.8, 3.9 Hz, 1H), 3.88~3.85 (m, 1H), 3.62 (dd, *J* = 11.7, 6.9 Hz, 1H), 2.88 (br s, 1H), 2.49~2.39 (m, 1H), 1.95 (ddd, *J* = 14.0, 9.8, 4.9 Hz, 1H), 1.51 (ddd, *J* = 16.7, 9.2, 7.6 Hz, 1H), 1.08~0.98 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 138.30, 128.36, 127.61, 77.48, 74.06, 72.95, 71.47, 63.91, 45.24, 29.88, 17.57, 17.46, 17.42, 17.30, 17.18, 17.13, 17.04, 13.51, 13.40, 12.92, 12.60; FABMS (*m/z*) 481 $(M+H)^+$. Anal. Calcd for $C_{25}H_{44}O_5Si_2$: C, 62.45; H, 9.22. Found: C, 62.30; H, 9.23.

(+)-(*1R,3R,4S*)-1-Benzyloxy-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane (5). To a mixture of compound **4** (8.00 g, 16.6 mmol) and NaH as 60 % dispersion (0.66 g, 16.5 mmol) in 400 ml of THF was added CS_2 (4.0 ml, 66.5 mmol) and MeI (4.0 ml, 64.3 mmol) successively with 30 min of period at 0 ^oC. Then the ice-bath was removed. After 1 hr, the reaction was quenched with 10 ml of water and the solvent was removed under reduced pressure. The normal aqueous worke-up was done and the crude was purified by silica gel column chromatography with 3~10 % EtOAc in hexanes to give xantate intermediate (9.24 g, 16.2mmol and 98 % yield) as a yellow syrup: $[\alpha]^{25}_D$ 28.92^o (c 1.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.33~7.27 (m, 5H), 6.42 (t, *J* = 4.0 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.40 (d, *J* = 11.4 Hz, 1H), 4.17 (dd, *J* = 9.4, 3.9 Hz, 1H), 4.05 (m, 1H), 3.89 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.75 (dd, *J* = 11.7, 3.6 Hz, 1H), 2.57 (s, 3H), 2.52~2.42 (m, 1H), 1.99 (ddd, *J* = 13.8, 10.3, 6.0 Hz, 1H), 1.86 (dt, *J* = 13.8, 9.0 Hz, 1H), 1.06~0.87 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 216.08, 137.88, 128.38, 127.87, 127.68, 82.69, 75.56, 72.91, 72.05, 62.23, 45.01, 30.66, 18.52,, 17.52, 17.41, 17.36, 17.26, 17.01, 13.50, 13.30, 12.81, 12.75; HR-FABMS (*m/z*) obsd *m/z* 571.2477, calcd for

$C_{27}H_{47}O_5S_2Si_2$ *m/z* 571.2404 $(M+H)^+$. Anal. Calcd for $C_{27}H_{46}O_5S_2Si_2$: C, 56.80; H, 8.12. Found: C, 56.54; H, 8.08.

To a refluxing solution of xantate intermediate (9.24 g, 16.2 mmol) in 300 ml of dry toluene was dropwisely added a solution of Bu_3SnH (5.7 ml, 21.2 mmol) and AIBN (0.27 g, 1.6 mmol) in 20 ml of dry toluene for 30 min. The reaction mixture was refluxed for additional 2 hr. The solvent was removed uneder reduced pressure, and the residue was purified by silica gel column chromatography with 3~10 % EtOAc in hexanes to give compound **5** (5.91 g, 12.7 mmol and 78 % yield) as a syrup: $[\alpha]^{23}_D$ 9.13° (c 3.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40~7.27 (m, 5H), 4.78 (d, *J* = 11.8 Hz, 1H), 4.23 (d, *J* = 11.8 Hz, 1H), 4.04~3.96 (m, 2H), 3.93~3.88 (m, 1H), 3.68 (dd, *J* = 11.7, 3.3 Hz, 1H), 2.39 (td, *J* = 12.8, 7.0 Hz, 1H), 2.13~2.04 (m, 1H), 1.86~1.76 (m, 2H), 1.73~1.63 (m, 1H), 1.15~0.85 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 138.62, 128.31, 127.63, 127.42, 75.27, 71.66, 70.35, 60.84, 46.66, 40.77, 32.10, 17.52, 17.42, 17.36, 17.26, 17.18, 17.10, 13.49, 13.35, 12.82, 12.64; FABMS (*m/z*) 465 $(M+H)^+$. Anal. Calcd for $C_{25}H_{44}O_4$ Si_2: C, 64.60; H, 9.54. Found: C, 64.38; H, 9.40.

(+)-(*1R,3R,4S*)-3,4-(1,1,3,3-tetraisopropyldisiloxyidene-1-oxy-3-oxymethyl)cyclopentane-1-ol (6). A mixture of compound **5** (5.91 g, 12.7 mmol) and $Pd(OH)_2$ (0.700 g, 20 % dispersion on carbon) in 100 ml of MeOH was treated with H_2 balloon for overnight. The reaction mixture was filtered through celite pad and concentrated. The residue was purified by silica gel column chromatography with 7.5 % EtOAc in hexanes to give the common intermediate **6** (3.88 g, 1.04 mmol and 82 % yield) as a syrup: $[\alpha]^{23}_D$ 20.37° (c 3.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.31~4.22 (m, 1H), 3.98 (dd, *J* = 11.6, 4.0 Hz, 1H), 3.54 (dd, *J* = 11.5, 7.9 Hz, 1H), 2.42~2.30 (m, 1H),

2.20~2.08 (m, 2H), 1.91~1.76 (m, 2H), 1.39 (ddd, *J* = 13.6, 11.2, 5.4 Hz, 1H), 1.15~0.88 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 74.12, 71.66, 63.80, 49.14, 43.18, 36.97, 17.62, 17.50, 17.45, 17.38, 17.32, 17.13, 17.03, 13.58, 13.51, 13.04, 12.46; HR-FABMS (*m/z*) obsd *m/z* 375.2393, calcd for $C_{18}H_{39}O_4Si_2$ *m/z* 373.2387 $(M+H)^+$. Anal. Calcd for $C_{18}H_{38}O_4Si_2$: C,57.70; H, 10.22. Found: C,57.59; H, 10.10.

(*1R,3R,4S*)-3,4-(1,1,3,3-Tetraisopropyldisiloxyidene-1,-oxy-3-oxymethyl)-1-tetrahydropyranyloxycyclopentane (7). To a solution of compoud **6** (0.534g, 1.43 mmol) and PPTS (0.036g, 0.14 mmol) in 20 mL of CH_2Cl_2 was added DHP (0.16 mL, 1.7 mmol) dropwise. After 4 hrs, the reaction was almost completed and TEA was added to reaction mixture. The resulting mixture was evaporated under reduced pressure and purified with 5 % EtOAc and 1 % TEA in hexanes by silica gel column chromatography to give compound 7 (0.560g, 1.22 mmol, 85% yield): ^{1}H NMR ($CDCl_3$) δ 4.57~4.52 (m, 2H), 4.17~4.03 (m, 2H), 3.98~3.86 (m, 4H), 3.84~3.78 (m, 2H), 3.60 (dt, J = 11.7, 4.3 Hz, 2H), 3.44~3.37 (m, 2H), 2.37~2.27 (m, 2H), 2.06~1.93 (m, 2H), 1.80~1.40 (m, 18H), 1.07~0.85 (m, 56H); ^{13}C NMR ($CDCl_3$) δ 97.68, 97.36, 77.10, 72.79, 72.48, 72.01, 62.79, 61.60, 47.28, 47.01, 42.00, 40.90, 33.38, 32.23, 31.54, 31.48, 25.93, 25.90, 20.34, 19.99, 17.95, 17.85, 17.76, 17.66, 17.59, 17.52, 13.88, 13.77, 13.22, 13.04, 13.01; FABMS (*m/z*) 459 $(M+H)^+$. Anal. Calcd for $C_{23}H_{46}O_5Si_2$: C, 60.21; H, 10.11. Found: C, 60.48; H, 10.15.

(*1R,3R,4S*)-4-Hydroxymethyl-1-tetrahydropyranyloxy-cyclopentane-1-ol (8).

A solution of compound **7** (0.560g, 1.22 mmol) in 20 mL of THF was treated with 2.5 mL of 1N TBAF in THF for 10 min. The resultion mixture was evaporated and purified with 10% MeOH and 1% TEA in CH_2Cl_2 by silica gel column chromatography to give

the compound **8** (0.223g, 1.03 mmol, 84% yield): ^{1}H NMR ($CDCl_3$) δ 4.63~4.58 (m, 2H), 4.31~4.24 (m, 2H), 4.05~3.98 (m, 2H), 3.91~3.82 (m, 2H), 3.69 (td, J = 10.6, 5.5 Hz, 2H), 3.54~3.45 (m, 4H), 3.09 (br s, 1H), 2.96 (br s, 1H), 2.42 (br s, 1H), 2.37~2.24 (m, 4H), 2.14~1.87 (m, 6H), 1.84~1.65 (m, 4H), 1.58~1.42 (m, 8H), 1.36 (ddd, *J* = 14.3, 9.2, 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 97.59, 97.22, 76.38, 76.27, 76.23, 76.04, 65.47, 65.35, 62.84, 48.85, 48.79, 41.68, 40.56, 34.59, 33.40, 31.10, 31.03, 25.36, 25.32, 19.76, 19.74; HR-FABMS (*m/z*) obsd *m/z* 217.1438, calcd for $C_{11}H_{21}O_4$ *m/z* 217.1439 $(M+H)^+$. Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.23; H, 9.54.

(*1R,3R,4S*)-4-*tert*-Butyldimethylsilyloxymethyl-1-tetrahydropyranyloxy-cyclopentane-3-ol (9). A solution of compound **8** (0.203g, 0.939 mmol) in THF (10 mL) was treated with imidazole (0.077g, 1.02 mmol) and TBDMSCl (0.134g, 0.89 mmol) at 0^{o}C for overnight. The resulting mixture was evaporated and purified with 10% EtOAc and 1% TEA in hexanes by silica gel column chromotography to give compound 9 (0.306g, 0.926 mmol, 99% yield): ^{1}H NMR ($CDCl_3$) δ 4.59 (br t, *J* = 3.4, 2H), 4.28~4.22 (m, 2H), 3.97~3.90 (m, 2H), 3.86~3.79 (m, 2H), 3.69 (ddd, J = 13.7, 9.8, 4.9 Hz, 2H), 3.48~3.38 (m, 4H), 2.84 (br s, 1H), 2.75 (br s, 1H), 2.30~2.16 (m, 2H), 2.06~1.42 (m, 19H), 1.38 (ddd, *J* = 14.4, 9.1, 5.6 Hz, 1H), 0.83 (s, 18H), 0.000 (s, 3H), -0.003 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 97.86, 97.38, 77.29, 76.97, 66.33, 66.03, 63.24, 63.05, 49.00, 48.86, 42.10, 40.81, 34.69, 33.44, 31.53, 31.49, 26.28, 25.83, 25.80, 20.21, 20.09, 18.61, -5.06, -5.12; FABMS (*m/z*) 331 $(M+H)^+$. Anal. Calcd for $C_{17}H_{34}O_4Si$: C, 61.77; H, 10.37. Found: C, 61.81; H, 10.48.

(*1R,3R,4S*)-3-Benzoyl-4-*tert*-butyldimethylsilyloxymethyl-1-tetrahydropyranyloxy-cyclopentane (10). A solution of compound **9** (0.169g, 0.511 mmol) in pyridine (5 mL)

was treated with BzCl (0.07 mL, 0.56 mmol) at 0oC for1 hr. The resulting mixture was evaporated and purified with 5-10% EtOAc and 1% TEA in hexanes by silica gel column chromatography to give compound **10** (0.217g, 0.499 mmol, 98% yield): ^{1}H NMR ($CDCl_3$) δ 8.01 (t, *J* = 6.9 Hz, 4H), 7.51 (t, J = 7.1 Hz, 2H), 7.39 (t, J = 7.6 Hz, 1H), 5.18~5.11 (m, 2H), 4.63 (dd, J = 4.4, 2.6 Hz, 1H), 4.58 (dd, J = 4.6, 2.9 Hz, 1H) 4.34~4.26 (m, 2H), 3.90~3.78 (m, 2H), 3.72 (dd, J = 9.8, 4.9 Hz, 1H), 3.68 (dd, J = 10.0, 5.2 Hz, 1H), 3.61 (dd, J = 9.6, 4.6 Hz, 1H), 3.60 (dd, J = 10.0, 4.9 Hz, 1H), 3.48~3.41 (m, 2H), 2.58~2.42 (m, 3H), 2.30 (ddd, *J* = 14.1, 7.3, 5.8 Hz, 1H), 2.08~1.41 (m, 18H), 0.833 (s, 9H), 0.831 (s, 9H),0.00 (s, 3H), -0.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.87, 133.15, 133.11, 131.11, 130.00, 129.96, 128.65, 128.63, 97.86, 97.79, 76.27, 76.08, 64.19, 64.15, 63.26, 62.85, 46.70, 46.30, 40.54, 39.04, 35.40, 33.96, 31.55, 26.28, 25.90, 20.32, 20.06, 18.65, -5.08; FABMS (*m/z*) 435 $(M+H)^+$. Anal. Calcd for $C_{24}H_{38}O_5Si$: C, 66.32; H, 8.81. Found: C, 66.36; H, 8.88.

(*1R,3R,4S*)-3-Benzoyl-4-hydroxymethyl-1-tetrahydropyranyloxy-cyclopentane (11). A solution of compound 11 (0.200g, 0.460 mmol) in THF (10 mL) was treated with 0.8 mL of 1N TBAF in THF at room temperature for 10 min. The resulting mixture was evaporated and purified with 20% EtOAc and 1% TEA in hexanes by silica gel column chromatography to give compound **11** (0.147g, 0.459 mmol, 100% yield): ^{1}H NMR ($CDCl_3$) δ 8.07~8.00 (m, 4H), 7.56 (t, J = 7.1 Hz, 2H), 7.43 (t, J = 7.6 Hz, 4H), 5.22~5.15 (m, 2H), 4.70~4.65 (m, 2H), 4.37~4.28 (m, 2H), 3.95~3.85 (m, 2H), 3.72~3.57 (m, 4H), 3.55~3.53 (m, 2H), 2.93 (br s, 2H), 2.60~2.42 (m, 3H), 2.31 (ddd, J = 13.9, 7.5, 5.7 Hz, 1H), 2.17~1.98 (m, 4H), 1.88~1.47 (m, 14H); ^{13}C NMR ($CDCl_3$) δ 167.27, 133.08, 133.06, 130.16, 129.68, 129.61, 128.32, 128.30, 97.47, 97.22, 77.43, 75.17, 74.95, 63.94,

63.70, 62.69, 62.52, 47.30, 46.75, 39.57, 38.01, 35.34, 33.78, 31.11, 31.06, 25.45, 19.71, 19.65; HR-FABMS (*m/z*) obsd *m/z* 321.1700, calcd for $C_{18}H_{25}O_5$ *m/z* 321.1720 $(M+H)^+$. Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.47; H, 7.55. Found: C, 67.41; H, 7.64.

General procedure for scheme 3-2. DIPEA (10 eq) and 4-DMAP (1 eq) were added to a mixture of resin **12** and alcohol **11** (3 eq) in CH_2Cl_2 at rt. The mixture was refluxed under N_2 for 24hrs. After cooling, the resulting resin **13** was filtered, washed and dried. Resin **13** and PPTS (10 eq) were added to a 1:1 mixture of 1,2-dichloroethane and 1-butanol at rt. The resulting mixture was stirred overnight at 60°C. After cooling, the resulting resin **14** was filtered, washed and dried. PPh_3 (10 eq) and **15** (10 eq) were added to a mixture of resin **14** in appropriate solvent. To this mixture was added DEAD (10 eq) dropwise. After stirring for 24hrs at rt, the resulting resin **16** was filtered, washed and dried. A mixture of resin **16** in THF/MeOH (2:1) was treated with solid K_2CO_3 (10 eq) at rt for 24hrs. The reaction mixture was filtered through a short silica gel pad. The filtrate was concentrated and purified by column chromatography on silica gel.

General procedure for scheme 3-3. A solution of compound **6** (1.0 eq) in DMF/1,4-dioxane (1:2) or DMF was treated with appropriate heterocyclic base **15** (1.5 eq), Ph_3P (1.5 eq) and DEAD (1.5 eq), successively. After 4~6 hrs, the reaction was completed. The solvent was evaporated under reduced pressure and separated between O^2-alkylated and N_1-alkylated products in the case of pyrimidines, but the reduced DEAD was difficult to remove. Succesive deprotections of bezoyl, acetyl, or diphenyl cabamate group and TIPDS group with NH_3/MeOH and TBAF gave the desired product.

***N*$_1$-Alkylated uracil (17a).** mp 150~152 °C; $[\alpha]^{25}_D$ -4.39° (c 1.34, MeOH); UV (H_2O) λ_{max} 267.5 nm (ε, 4 800, pH 2), 268.0 nm (ε, 4 700, pH 7), 266.0 nm (ε, 4 300, pH 11);

^{1}H NMR (MeOH-d_4) δ 7.64 (d, J = 8.0 Hz, 1H), 5.64 (d, J = 8.0 Hz, 1H), 5.01 (quintet, J = 9.0 Hz, 1H), 4.41 (dt, J = 6.8, 4.4 Hz, 1H), 3.63 (dd, J = 10.8, 5.5 Hz, 1H), 3.56 (dd, J = 10.8, 6.2 Hz, 1H), 2.22 (dt, J = 12.6, 7.5 Hz, 1H), 2.07~1.92 (m, 3H), 1.52 (dt, J = 12.7, 10.0 Hz, 1H); ^{13}C NMR (MeOH-d_4) δ 166.71, 153.21, 144.59, 102.97, 73.99, 64.06, 56.59, 50.66, 40.43, 33.91; HR-FABMS (*m/z*) obsd 227.1059, calcd for $C_{10}H_{15}N_2O_4$ 227.1032 $(M+H)^+$. Anal. Calcd for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.83; H, 6.31; N, 12.20.

***O*^{2}-Alkylated uracil (17a).** $[\alpha]^{25}_D$ –17.17^o (c 0.72, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.71 (d, J = 6.7 Hz, 1H), 6.00 (d, J = 6.8 Hz, 1H), 5.51~5.43 (m, 1H), 4.12 (q, J = 6.6 Hz, 1H), 3.66 (dd, J = 10.9, 5.7 Hz, 1H), 3.54 (dd, J = 10.8, 6.8 Hz, 1H), 2.50~2.41 (m, 1H), 2.17~1.96 (m, 3H), 1.64 (ddd, J = 13.5, 7.6, 4.3 Hz, 1H); HR-FABMS (*m/z*) obsd 227.1028, calcd for $C_{10}H_{15}N_2O_4$ 227.1032 $(M+H)^+$

***N*$_1$-Alkylated thymine (17b).** mp 170~171 ^{o}C; $[\alpha]^{25}_D$ -6.54^o (c 1.00, MeOH); UV (H_2O) λ_{max} 273.0 nm (ε, 14 000, pH 2), 273.5 nm (ε, 14 000, pH 7), 271.0 nm (ε, 11 000, pH 11); ^{1}H NMR (MeOH-d_4) δ 7.61 (d, J = 1.1 Hz, 1H), 5.16 (quintet, J = 9.0 Hz, 1H), 4.26 (dt, J = 6.8, 4.5 Hz, 1H), 3.79 (dd, J = 10.9, 5.5 Hz, 1H), 3.72 (dd, J = 10.9, 6.1 Hz, 1H), 2.34 (dt, J = 12.6, 7.7 Hz, 1H), 2.23~1.97 (m, 3H), 1.98 (d, J = 1.1 Hz, 3H), 1.68 (dt, J = 12.6, 10.1 Hz, 1H); ^{13}C NMR (MeOH-d_4) δ 166.83, 153.36, 140.23, 111.95, 74.01, 64.65, 56.21, 50.68, 48.40, 33.86, 12.76; HR-FABMS (*m/z*) obsd 241.1299, calcd for $C_{11}H_{17}N_2O_4$ 241.1260 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.76; H, 6.64; N, 11.50.

***O*^{2}-Alkylated thymine (17b).** $[\alpha]^{25}_D$ –19.89^o (c 1.44, MeOH); UV (H_2O) λ_{max} 270.5 nm; ^{1}H NMR (MeOH-d_4) δ 7.56 (s, 1H), 5.45~5.38 (m, 1H), 4.11 (q, J = 6.6 Hz, 1H), 3.66

(dd, *J* = 10.9, 5.7 Hz, 1H), 3.53 (dd, *J* = 10.8, 6.8 Hz, 1H), 2.48~2.38 (m, 1H), 2.16~1.95 (m, 3H), 1.91 (d, *J* = 0.8 Hz, 3H), 1.62 (ddd, *J* = 13.6, 7.6, 4.3 Hz, 1H); HR-FABMS (*m/z*) obsd 241.1179, calcd for $C_{11}H_{17}N_2O_4$ 241.1188 $(M+H)^+$.

***N*$_1$-Alkylated cytosine (17c).** mp 193~196 ^oC; $[\alpha]^{25}_D$ –2.82^o (c 0.94, MeOH); UV (H_2O) λ_{max} 284.0 nm (ε, 12 000, pH 2), 275.0 nm (ε, 8 000, pH 7), 275.0 nm (ε, 8 000, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 7.53 (d, *J* = 7.4 Hz, 1H), 5.75 (d, *J* = 7.4 Hz, 1H), 4.96 (quintet, *J* = 8.9 Hz, 1H), 4.03 (dt, *J* = 6.7, 4.7 Hz, 1H), 3.54 (dd, *J* = 10.9, 5.5 Hz, 1H), 3.48 (dd, *J* = 10.9, 6.2 Hz, 1H), 2.14 (dt, *J* = 12.6, 7.6 Hz, 1H), 1.97~1.87 (m, 3H), 1.41 (dt, *J* = 12.5, 10.1 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 167.60, 159.46, 144.60, 96.50, 74.10, 64.72, 57.31, 50.81, 40.85, 34.38; HR-FABMS (*m/z*) obsd 226.1186, calcd for $C_{10}H_{16}N_3O_3$ 226.1192 $(M+H)^+$. Anal. Calcd for $C_{10}H_{15}N_3O_3 \cdot 0.05CH_2Cl_2$: C, 52.60; H, 6.63; N, 18.31. Found: C, 52.79; H, 6.82; N, 18.02.

***O*^{2}-Alkylated cytosine (17c).** $[\alpha]^{25}_D$ –14.55^o (c 0.66, MeOH); UV (H_2O) λ_{max} 267.0 nm; ^{1}H NMR (MeOH-*d*$_4$) δ 7.80 (d, *J* = 5.4 Hz, 1H), 6.10 (d, *J* = 5.9 Hz, 1H), 4.39~4.33(m, 1H), 4.12 (q, *J* = 6.5Hz, 1H), 3.68 (dd, *J* = 10.8, 6.0Hz, 1H), 3.52 (dd, *J* = 10.7, 7.2 Hz, 1H), 2.47~2.39 (m, 1H), 2.13~1.95 (m, 3H), 1.60~1.50 (m, 1H); HR-FABMS (*m/z*) obsd 226.1200, calcd for $C_{10}H_{16}N_3O_3$ 226.1192 $(M+H)^+$.

***N*$_9$-Alkylated adenosine (17d).** mp 134~138 ^oC; $[\alpha]^{22}_D$ -7.58^o (c 0.38, MeOH); UV (H_2O) λ_{max} 259.5 nm (ε 20 000, pH 2), 261.0 nm (ε, 21 000, pH 7), 261.0 nm (ε, 21 000, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 8.37 (s, 1H), 8.32 (s, 1H), 5.28 (tt, *J* = 9.4, 8.0 Hz, 1H), 4.44 (dt, *J* = 6.7, 4.0 Hz, 1H), 3.91 (dd, *J* = 10.9, 5.7 Hz, 1H) 3.78 (dd, *J* = 10.9, 6.2 Hz, 1H) 2.68 (dt, *J* = 12.8, 7.4 Hz, 1H), 2.5+ (ddd, *J* = 13.4, 9.4, 6.8 Hz, 1H), 2.40~2.28 (m, 2H), 2.05 (dt, *J* = 12.9, 9.6 Hz, 1H); ^{13}C NMR (MeOH-*d*$_4$) δ 157.66, 153.83, 151.07,

141.56, 120.85, 74.20, 64.77, 55.43, 51.08, 42.00, 35.47; HR-FABMS (*m/z*) obsd 250.1295, calcd for $C_{11}H_{16}N_5O_2$ 250.1304 $(M+H)^+$. Anal. Calcd for $C_{11}H_{15}N_5O_2 \cdot 0.15CH_2Cl_2$: C, 51.11; H, 5.89; N, 26.73. Found: C, 51.25; H, 6.18; N, 26.97.

***N*$_9$-Alkylated guanosine (17e).** mp 250 ^oC, dec.; $[\alpha]^{24}_D$ -17.55^o (c 0.26, MeOH); UV (H_2O) λ_{max} 253.5 nm (ε 10 000, pH 2), 253.0 nm (ε 10 000, pH 7), 268.0 nm (ε 9 600, pH 11); ^1H NMR (MeOH-*d*$_4$) δ 7.91 (s, 1H), 5.09~5.00 (m, 1H), 4.35 (dt, *J* = 6.5, 4.1 Hz, 1H), 3.78 (dd, *J* = 10.9, 5.7 Hz, 1H) 3.75 (dd, *J* = 10.9, 6.3 Hz, 1H) 2.53 (dt, *J* = 12.6, 7.9 Hz, 1H), 2.41 (ddd, *J* = 13.4, 9.5, 6.8 Hz, 1H), 2.27~2.16 (m, 2H), 1.92 (dt, *J* = 12.8, 9.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 159.91, 155.35, 153.50, 138.44, 118.34, 74.18, 64.77, 54.90, 51.07, 41.97, 35.36; HR-FABMS (*m/z*) obsd 266.1253, calcd for $C_{11}H_{16}N_5O_3$ 266.1253 $(M+H)^+$. Anal. Calcd for $C_{11}H_{15}N_5O_3 \cdot 0.6CH_2Cl_2$: C, 44.06; H, 5.16; N, 22.15. Found: C, 44.36; H, 5.06; N, 22.28.

CHAPTER 4

SYNTHESIS, ANTI-HIV ACTIVITY AND MOLECULAR MECHANISM OF DRUG RESISTANCE OF L-2',3'-DIDEHYDRO-2',3'-DIDEOXY-2'-FLUORO-4'-THIONUCLEOSIDES[1]

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[1] Choo, H., Y. Chong, Y. Choi, J. Mathew, R. F. Schinazi and C. K. Chu. 2003. *Journal of Medicinal Chemistry*. 46:389-398.

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ABSTRACT

β-L-2',3'-Didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides (β-L-2'-F-4'-S-d4Ns) have been synthesized and evaluated against HIV-1 in primary human lymphocytes. The key intermediate **8**, which was prepared from 2,3-*O*-isopropylidene-L-glyceraldehyde **1** in 13 steps, was condensed with various pyrimidine and purine bases followed by elimination and deprotection to give the target compounds, β-L-2'-F-4'-S-d4Ns (**17**~**20** and **27**~**30**). The antiviral activity of the newly synthesized compounds was evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells, among which the cytosine **17**, 5-fluorocytosine **18** and adenine **27** derivatives showed potent anti-HIV activities (EC_{50} 0.12, 0.15 and 1.74 μM, respectively) without significant cytotoxicity up to 100 μM in human PBM, CEM and Vero cells. The cytosine derivative **17** (β-L-2'-F-4'-S-d4C), however, showed cross-resistance to 3TC-resistant variant (HIV-1_{M184V}). Molecular modeling studies suggest that the similar pattern of antiviral activity with that of β-L-2'-F-d4N stemmed from their conformational and structural similarities. The isosteric substitution of sulfur for 4'-oxygen was well tolerated in the catalytic site of HIV-1 reverse transcriptase in the wild-type virus. However, the steric hindrance between the sugar moiety of the unnatural L-nucleoside and the side chains of Val184 of M184V RT in 3TC-resistant mutant HIV strains destabilizes the RT-nucleoside triphosphate complex, which causes the cross-resistance to 3TC (M184V mutant).

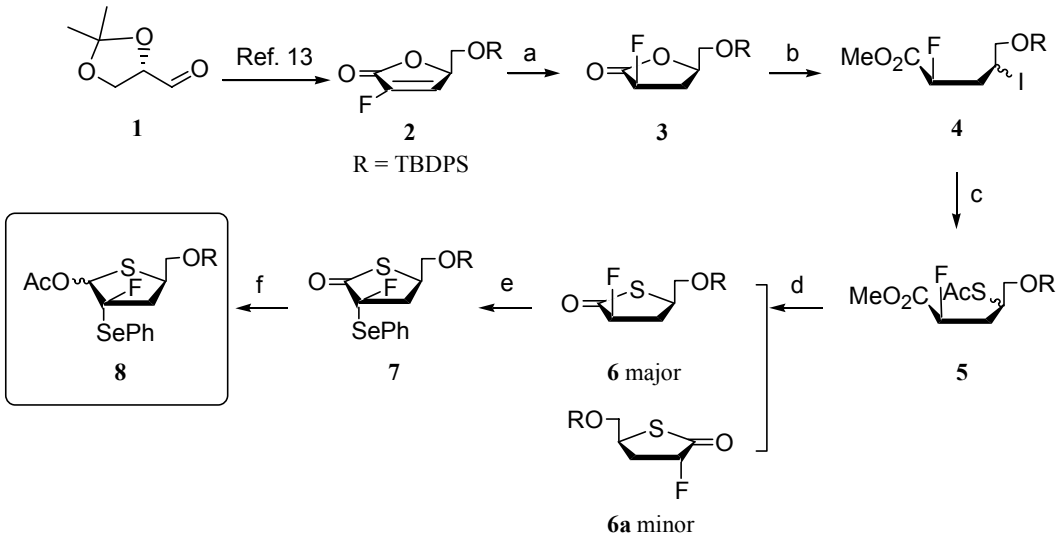
INTRODUCTION

A number of L-nucleosides such as 3TC,[268,269] FTC,[270] L-FMAU,[271] L-d4FC[272] and L-dT[273] have been discovered as potent antiviral agents. Particularly, 2',3'-unsaturated nucleosides such as L-d4C,[272] L-d4FC[272] and L-d4A[274] have drawn our attention due to their high antiviral potency. A combination of the unnatural L-configuration and 2',3'-unsaturated sugar moiety with 2'-fluoro substitution was worth to explore, as the 2'-fluoro moiety can stabilize the glycosidic bond. We have recently discovered 2',3'-unsaturated L-2'-fluoronucleosides (β-L-2'-F-d4Ns) as potent antiviral agents against HIV-1 and hepatitis B virus (HBV).[275] Thus, to expand our efforts to discover more potent and less toxic antiviral agents, it was of interest to study the effect of isosteric replacement of 4'-oxygen by a sulfur atom. While there have been numerous efforts to modify the sugar as well as the heterocyclic bases of natural nucleosides for the purpose of developing new antiviral agents, 2',3'-unsaturated 4'-thionucleosides have not been well investigated due to the synthetic difficulties.[276] In a recent communication,[277] we reported a stereoselective synthesis of L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thiocytidine (β-L-2'-F-4'-S-d4C), which showed potent anti-HIV activity (EC_{50} 0.12 μM) in human peripheral blood monomuclear (PBM) cells without cytotoxicity up to 100 μM. In this article, various pyrimidine and purine nucleosides were synthesized for the structure-activity relationship study. The cytosine derivative **17** (β-L-2'-F-4'-S-d4C) was also evaluated against 3TC-resistance variant (HIV-1_{M184V}). In order to understand the molecular basis of the antiviral activity as well as the cross-resistance by the mutant RT strain (HIV-1_{M184V}), molecular modeling studies were conducted using the published

crystal structure of HIV-1 reverse transcriptase.[278,279] Herein, we report the full accounts of the synthesis and biological evaluation of the titled nucleosides along with their molecular modeling studies.

RESULTS AND DISCUSSION

Chemistry. Several synthetic strategies were explored for the synthesis of our target nucleosides: (1) introduction of fluorine at the 2'-carbon by the modified Horner-Emmons reaction using a fluorinated phosphonate reagent which has been successfully used by our group for syntheses of several fluorinated nucleosides,[280,281] (2) introduction of sulfur at the 4'-carbon by double inversion,[282] and (3) introduction of a phenylselenyl group at the 2'-carbon resulting in β-selectivity during condensation with heterocyclic bases as well as generation of a double bond to give d4 nucleosides.[283] (*R*)-2-Fluorobutenolide **2** was prepared from 2,3-*O*-isopropylidene-L-glyceraldehyde **1** in three steps by the known method (Scheme 4.1).[280] Streoselective hydrogenation of (*R*)-2-fluorobutenolide **2** by using 5% Pd/C smoothly underwent to give β-2-fluorolactone **3** in 95% yield. Hydrolysis of β-2-fluorolactone **3** using NaOH in aqueous EtOH followed by methylation of the resulting carboxylic acid gave a hydroxymethylester, which was treated with iodine, triphenylphosphine and imidazole in toluene at 60^{o}C for 4 h to give a 6:1 epimeric mixture of the iodoesters **4** in 82% overall yield. The inseparable epimeric mixture of iodoesters **4** was converted to an epimeric mixture of thiolacetates **5** in 91% yield by nucleophilic substitution with potassium thiolacetate in DMF.



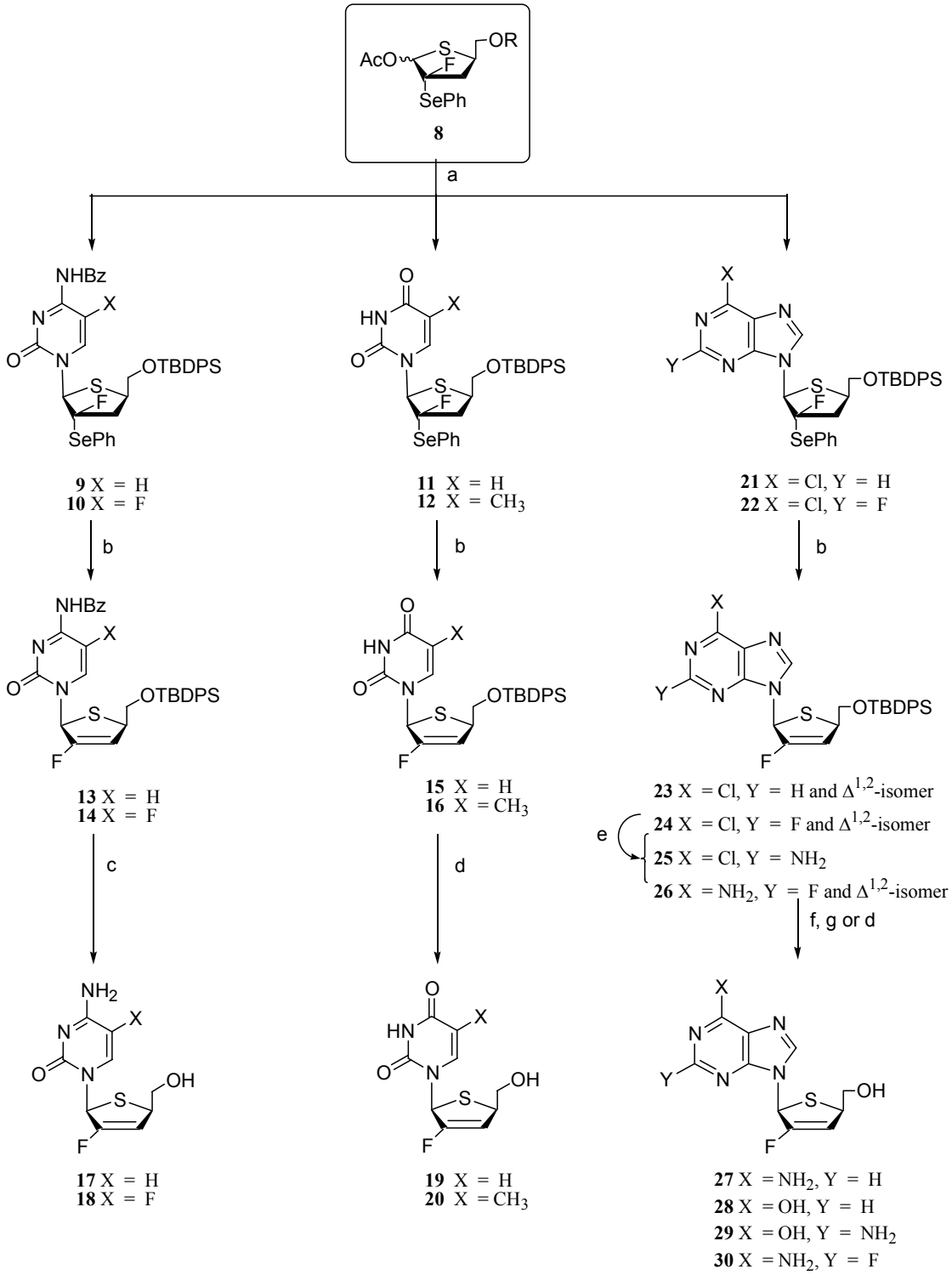
[a]Keys: (a) H_2, Pd(0)/C, EtOAc, (b) i) NaOH, EtOH, ii) dimethyl sulfate, DMSO, iii) I_2, Ph_3P, imidazole, toluene, (c) KSAc, DMF, (d) i) DIBAL-H, toluene, -78°C, ii) DMSO, Ac_2O, (e) LiHMDS, TMSCl, PhSeBr, -78°C, (f) i) DIBAL-H, toluene, -78°C, ii) Ac_2O, TEA, CH_2Cl_2

Scheme 4.1. Synthesis of the key intermediate **8**

Reductive cyclization of the thiolacetates **5** using DIBAL-H in toluene at -78°C followed by Moffatt-type oxidation provided the corresponding thiolactone **6** in 54% yield as well as the separable minor epimer **6a**. The thiolatone **6** was treated with LiHMDS and TMSCl followed by PhSeBr to give the phenylselenylthiolatone **7** stereoselectively (Scheme 4.1). DIBAL reduction of the 2-fluoro-2-phenylselenothiolactone **7** followed by acetylation using Ac_2O and triethylamine in CH_2Cl_2 gave the key intermediate **8** in 83% yield. The acetate **8** was then condensed with various pyrimidine heterocyclic bases under Vorbrüggen conditions to give the corresponding pyrimidine analogs **9**~**12** in 39~59% yield (Scheme 4.2 and Table 4.1). During the condensation, the β-anomer was obtained exclusively by virtue of the bulky α–phenylselenyl group.[281] Oxidation of the phenylselenyl group by mCPBA at -78°C followed by treatment with pyridine gave the

corresponding *syn*-eliminated 2',3'-unsaturated compounds **13**~**16** in moderate to good yields (68~80%). The desired cytidine and 5-fluorocytidine analogs **17** and **18** were obtained by successive treatment with TBAF in THF and methanolic ammonia. On the other hand, the TBDPS protecting groups at 5' positions of the sugar moieties of the compounds **15** and **16** were removed by the treatment with TBAF in THF to give the desired 2'-fluoro-4'-thio-2',3'-unsaturated uracil and thymine nucleosides **19** and **20**, respectively. To synthesize the purine analogs **27**~**30**, the key intermediate **8** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give the corresponding nucleosides **21** and **22** in 73% and 67% yield, respectively (Scheme 4.2 and Table 4.1). The 6-chloropurine derivative **21** was treated with mCPBA followed by pyridine to give a *syn*-eliminated inseparable mixture of 2',3'-unsaturated nucleoside **23** and its $\Delta^{1,2}$-isomer in 86% yield (3:1 determined by ^{1}H NMR). Under the same reaction conditions, the 2-fluoro-6-chloropurine derivative **22** also gave an inseparable mixture of the *syn*-eliminated product **24** and its $\Delta^{1,2}$-isomer in 71% yield.

A mixture of compound **23**, its $\Delta^{1,2}$-isomer and methanolic ammonia was heated at 100^{o}C in a steel bomb to give the corresponding adenosine analog, which was deprotected by TBAF in THF to give the 2'-fluoro-4'-thio-2',3'-unsaturated adenosine **27** in 57% yield. The $\Delta^{1,2}$-isomer, however, was unstable under the reaction conditions and decomposed. The 2',3'-unsaturated 6-chloropurine derivative **23** was treated with sodium methoxide and 2-mercaptoethanol in refluxing methanol to give a hypoxanthine derivative, which was converted to the final 2'-fluoro-4'-thio-2',3'-unsaturated inosine derivative **28** in 81% yield.



[a]Keys: (a) HMDS, CH_3CN, pyrimidines or purines; TMSOTf, rt, (b) mCPBA,-78^oC; pyridine, rt, (c) i) TBAF, THF, ii) NH_3, MeOH, rt, (d) TBAF, THF, rt, (e) NH_3 bubbling, DME, rt, (f) i) NH_3, MeOH, 80^oC, ii) TBAF, THF, rt, (g) i) $HSCH_2CH_2OH$, NaOMe, 60^oC, ii) TBAF, THF, rt

Scheme 4.2. Synthesis of the target nucleosides

Table 4.1. ^{1}H NMR Data

Compd	H-1'	H-3'	H-4'	H-5'
9[a]	6.91 (d, *J* = 15.6 Hz)	2.62-2.47 (m)	3.68-3.58 (m)	3.87-3.72 (m)
	Other protons	8.82 (br s, 1H), 8.12 (d, *J* = 6.5 Hz, 1H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.69-7.27 (m, 19H), 1.02 (s, 9H)		
10[a]	6.73 (d, *J* = 16.3 Hz)	2.63-2.46 (m)	3.67-3.58 (m)	3.92-3.76 (m)
	Other protons	12.70 (br s, 1H), 8.33 (d, *J* = 7.5 Hz, 2H), 7.87 (dd, *J* = 5.9, 1.7 Hz, 1H), 7.72-7.28 (m, 18H), 1.07 (s, 9H)		
11[a]	6.63 (d, *J* = 14.7 Hz)	2.55-2.41 (m)	3.62-3.58 (m)	3.87-3.72 (m)
	Other protons	9.26 (br s, 1H), 7.72 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.68-7.30 (m, 15H), 5.56 (dd, *J*= 8.2, 1.9 Hz, 1H), 1.03 (s, 9H)		
12[a]	6.72 (d, *J* = 18.1 Hz)	2.62~2.44 (m)	3.67~3.57 (m)	3.87~3.73 (m)
	Other protons	9.13 (br s, 1H), 7.74~7.25 (m, 16H), 1.83 (s, 3H), 1.07 (s, 9H)		
13[a]	7.03 (s)	5.82 (s)	4.23~4.15 (m)	3.83 (dd, *J* = 10.4, 6.1 Hz) 3.78 (dd, *J* = 10.4, 6.3 Hz)
	Other protons	8.95 (br s, 1H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.69~7.38 (m, 15H), 1.10 (s, 9H)		
14[a]	6.95 (s)	5.80 (s)	4.23~4.15 (m)	3.84 (dd, *J* = 10.5, 6.2 Hz) 3.79 (dd, *J* = 10.2, 6.6 Hz)
	Other protons	12.80 (br s, 1H), 8.30 (d, *J* = 7.3 Hz, 2H), 7.72~7.38 (m, 14H), 1.10 (s, 9H)		
15[a]	6.93 (s)	5.75 (s)	4.22~4.14 (m)	3.85 (dd, *J* = 10.6, 5.9 Hz) 3.81 (dd, *J* = 10.5, 5.6 Hz)
	Other protons	9.34 (br s, 1H), 7.70~7.35 (m, 11H), 5.44 (dd, *J* = 8.1, 1.2 Hz, 1H), 1.09 (s, 9H)		
16[a]	6.96 (s)	5.81 (s)	4.22~4.13 (m)	3.85 (dd, *J* = 10.5, 6.5 Hz) 3.77 (dd, *J* = 10.2, 7.0 Hz)
	Other protons	9.45 (br s, 1H), 7.72~7.35 (m, 10H), 7.02 (s, 1H), 1.74 (s, 3H), 1.08 (s, 9H)		
17[b]	6.93 (s)	5.89-5.85 (m)	4.17-4.12 (m)	3.78 (dd, *J* = 11.7, 4.6 Hz) 3.69 (ddd, *J* = 11.6, 4.3, 1.2 Hz)
	Other protons	8.05 (dd, *J* = 7.5, 1.0 Hz, 1H), 5.97 (d, *J* = 7.5 Hz, 1H)		
18[b]	6.92 (s)	5.84 (s)	4.17 (s)	3.84 (dd, *J* = 11.8, 3.7 Hz) 3.68 (br d, *J* = 10.6 Hz)
	Other protons	8.39 (d, J = 6.6 Hz, 1H)		
19[b]	6.87 (s)	5.89 (s)	4.19-4.13 (m)	3.80 (dd, *J* = 11.7, 4.5 Hz) 3.71 (ddd, *J* = 11.7, 4.1, 1.4 Hz)
	Other protons	8.11 (dd, *J* = 8.1, 0.8 Hz, 1H), 5.77 (d, *J* = 8.1 Hz, 1H)		
20[b]	6.87 (s)	5.86 (s)	4.17 (s)	3.84 (dd, *J* = 11.9, 4.0 Hz) 3.77 (ddd, *J* = 11.9, 3.7, 1.7 Hz)
	Other protons	8.03 (s, 1H), 1.88 (d, *J* = 1.2 Hz, 3H)		
21[a]	6.55 (d, *J* = 14.7 Hz)	2.67~2.46 (m)	3.72~3.62 (m)	3.88 (ps t, *J* = 8.5 Hz) 3.80 (ps t, *J* = 8.7 Hz)
	Other protons	8.60 (s, 1H), 8.23 (d, J = 1.7 Hz, 1H), 7.63~7.01 (m, 15H), 0.96 (s, 9H)		
22[a]	6.37 (d, *J* = 13.9 Hz)	2.68~2.57 (m) 2.52 (td, *J* = 12.9, 7.7 Hz)	3.72~3.64 (m)	3.89 (dd, *J* = 9.7, 6.9 Hz) 3.80 (td, *J* = 7.8, 1.3 Hz)
	Other protons	8.20 (s, 1H), 7.62~7.06 (m, 15H), 0.98 (s, 9H)		
23[a]	6.87 (s)	5.87 (s)	4.33~4.24 (m)	3.96 (dd, *J* = 10.2, 6.8 Hz) 3.86 (dd, *J* = 10.0, 7.5 Hz)
	Other protons	8.69 (s, 1H), 8.12 (s, 1H), 7.67 (t, *J* = 14.4 Hz, 4H), 7.50~7.36 (m, 6H), 1.08 (s, 9H)		

[a]$CDCl_3$, [b]CD_3OD, [c]Not Applicable

Table 4.1. ^{1}H NMR Data (Continued)

Cpd	H-1'	H-3'	H-4'	H-5'
$\Delta^{1,2}$-isomer of **23**[a]	na[c]	3.25 (ddd, *J* = 16.8, 9.3, 3.3 Hz, 1H), 3.03 (ddd, *J* = 16.6, 5.7, 1.2 Hz, 1H)	4.03~3.83 (m, 3H)	
	Other protons	8.81 (s, 1H), 8.18 (s, 1H), 7.70~7.35 (m, 10H), 1.08 (s, 9H)		
24[a]	6.68 (s)	5.79 (s)	4.25~4.18 (m)	3.86 (td, J = 10.2, 6.5 Hz) 3.82~3.77 (m)
	Other protons	8.01 (s, 1H), 7.63~7.28 (m, 10H), 1.01 (s, 9H)		
$\Delta^{1,2}$-isomer of **24**[a]	na[c]	3.24 (ddd, *J* = 16.3, 9.3, 3.1 Hz, 1H), 3.01 (ddd, *J* = 16.8, 5.1, 0.6 Hz, 1H)	4.04~3.80 (m, 3H)	
	Other protons	8.12 (s, 1H), 7.75~7.31 (m, 10H), 1.08 (s, 9H)		
25[a]	6.65 (s)	5.83 (s)	4.26~4.22 (m)	3.90 (dd, *J* = 10.1, 7.2 Hz) 3.81 (dd, *J* = 9.8, 7.3 Hz)
	Other protons	7.74 (s, 1H), 7.70~7.37 (m, 10H), 5.18 (s, 2H), 1.07 (s, 9H)		
26[a]	6.72 (s)	5.83 (s)	4.29~4.19 (m)	3.94 (dd, *J* = 10.2, 6.6 Hz) 3.87 (dd, *J* = 10.1, 7.1 Hz)
	Other protons	7.80 (s, 1H), 7.74~7.31 (m, 10H), 6.95 (br s, 2H), 1.08 (s, 9H)		
$\Delta^{1,2}$-isomer of **26**[a]	na[c]	3.20 (ddd, *J* = 16.5, 9.2, 3.1 Hz, 1H), 3.00 (dd, *J* = 16.7, 4.6 Hz, 1H)	4.02~3.79 (m, 3H)	
	Other protons	7.84 (s, 1H), 7.74~7.31 (m, 10H), 6.95 (br s, 2H), 1.08 (s, 9H)		
27[b]	6.89 (s)	5.93 (s)	4.28 (s)	3.90 (dd, *J* = 11.5, 4.2 Hz) 3.81 (dd, *J* = 11.4, 3.2 Hz)
	Other protons	8.52 (s, 1H), 8.20 (s, 1H)		
28[b]	6.90 (s)	5.93 (s)	4.26 (s)	3.88 (dd, *J* = 11.6, 4.7 Hz) 3.80 (dd, *J* = 10.7, 3.2 Hz)
	Other protons	8.51 (s, 1H), 8.07 (s, 1H)		
29[b]	6.61 (s)	5.83 (s)	4.22-4.14 (m)	3.84-3.74 (m)
	Other protons	7.98 (s, 1H)		
30[b]	6.77 (s)	5.92 (s)	4.28-4.21 (m)	3.89 (dd, *J* = 11.6, 4.7 Hz) 3.85-3.79 (m)
	Other protons	8.44 (s, 1H)		

[a]$CDCl_3$, [b]CD_3OD, [c]Not Applicable

On the other hand, a solution of **24** and its $\Delta^{1,2}$-isomer in ethylene glycol dimethyl ether (DME) was bubbled with dry ammonia at room temperature for 16 h to give the 2-amino-6-chloropurine **25** and 2-fluoro-6-aminopurine derivative **26** in 49% and 28% yield, respectively. The $\Delta^{1,2}$-isomer of **24**, however, decomposed under the reaction as well as purification conditions presumably because of its instability. The 2-amino-6-chloropurine derivative **25** was hydrolyzed by using sodium methoxide and 2-mercaptoethanol in

refluxing methanol to give the final 2',3'-unsaturated 2'-fluoro-4'-thio-guanosine **29** in 61% yield. The 2',3'-unsaturated 2'-fluoro-4'-thio-2-fluoroadenosine **30** was obtained by deprotection of the TBDPS group of the 2-fluoro-6-aminopurine derivative **26** by TBAF in THF in 70% yield.

Anti-HIV Activity. The synthesized pyrimidine (**17**~**20**) and purine (**27~30**) nucleosides were evaluated for their anti-HIV activity and cytotoxicity *in vitro*, and results are summarized in Table 4.2. The anti-HIV activity was evaluated in PBM cells infected with HIV-1, and AZT was included as a positive control.[284] The cytotoxicity of the synthesized nucleosides was also assessed in human PBM, CEM and Vero cells. Among

Table 4.2. Activity of L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides

Compound	Configuration & Base	Anti-HIV-1 Activity (EC_{50}, μM)	Cytotoxicity (IC_{50}, μM)		
		PBM	PBM	CEM	Vero
17	L-Cytosine	0.12	>100	>100	>100
18	L-5-F-Cytosine	0.15	>100	>100	>100
19	L-Uracil	>100	>100	>100	>100
20	L-Thymine	>100	>100	>100	>100
27	L-Adenine	1.7	>100	>100	>100
28	L-Hypoxanthine	15.5	>100	>100	>100
29	L-Guanine	43.5	>100	41.5	66.4
30	L-2-F-Adenine	11.5	13.0	10.4	66.1
AZT		0.004	>100	29.0	14.3

these nucleosides, two pyrimidine nucleosides, cytidine **17** (EC_{50} 0.12 μM) and 5-fluorocytidine **18** (EC_{50} 0.15 μM) showed the most potent anti-HIV-1 activity, and the purine nucleosides, adenosine **27** (EC_{50} 1.7 μM), inosine **28** (EC_{50} 15.5 μM), guanosine **29** (EC_{50} 43.5 μM) and 2-fluoroadenosine **30** (EC_{50} 11.5 μM), also showed moderate antiviral activity whereas significant cytotoxicity was observed in 2-fluoroadenosine **30** (IC_{50} 13.0, 10.4 and 66.1 μM for PBM, CEM and Vero cells, respectively). However, all other synthesized nucleosides showed no significant cytotoxicity. Despite the replacement of an oxygen atom (β-L-2'-F-d4N) by a sulfur atom (β-L-2'-F-4'-S-d4N), the anti-HIV activity was generally maintained.

Antiviral activity against Lamivudine (3TC)-resistant (HIV-1_{M184V}) mutant strain. Although 3TC is the most commonly used nucleoside in combination therapy for HIV-1 infection,[285] 3TC monotherapy results in the selection of 3TC-resistant viral variant, resulting in prompt rebound in plasma viral load.[286] High-level resistance to 3TC is conferred by a single mutation at codon 184 (M184V) in the catalytic domain of HIV-1 RT, and the M184V substitution increases 50% inhibitory concentration of 3TC at least 1,000-fold.[287,288] This viral resistance to 3TC prompted the discovery of nucleosides with antiviral activity against HIV-1 isolates containing common 3TC resistance mutation. It is noteworthy that the recently US FDA approved anti-HIV drug, tenofovir, showed unique activity against AZT- as well as 3TC-resistant mutant HIV strains.[289] In view of this resistance issue, antiviral activity of the cytosine analog (17) was evaluated against the lamivudine-resistant mutant strain (HIV-1_{M184V}) along with other 2',3'-unsaturated nucleosides such as β-L-2'-F-d4C and β-D-2'-F-d4C[290] (Table 4.3). Among these nucleosides, only β-D-2'-F-d4C[290] showed antiviral activity against the M184V mutant,

Table 4.3. Correlation of relative binding energy difference (ΔE_{rel}) with fold increase

Compound	WT (xxBRU)		M184V		FI[b]	ΔE_{rel}[c] (kcal/mol)
	Activity (EC_{90}, μM)	E_{rel}[a] (kcal/mol)	Activity (EC_{90}, μM)	E_{rel}[a] (kcal/mol)		
β-L-2'-F-4'-S-d4C **17**	1.4	86.0	>100	-21.5	>100	107.5
β-L-2'-F-d4C	2.1	36.8	>100	-70.0	>100	106.8
β-D-2'-F-d4C	8.0	44.3	1.8	-6.5	0.2	50.8

[a] relative binding energy (E_{rel}) = binding energy of nucleoside triphosphate – binding energy of dCTP, [b] EC_{90} HIV-1_{M184V}/EC_{90} HIV-1_{xxBRU}, [c] relative binding energy difference (ΔE_{rel}) = E_{rel}(WT) – E_{rel}(M184V).

whereas its L-congener (β-L-2'-F-d4C)[275] as well as 4'-sulfur containing nucleoside (β-L-2'-F-4'-S-d4C **17**) were significantly cross-resistant. From this study, it is apparent that the unnatural L-sugar configuration of 2'-fluoro-2',3'-unsaturated nucleosides cannot be accommodated by the mutation at the codon 184 of HIV-1 RT. Therefore, it was of interest to investigate the molecular basis of the drug-resistance by M184V mutant to various 2'-F-2',3'-unsaturated nucleosides (*vide infra*).

Molecular Modeling Studies. In order to understand the molecular basis of the antiviral activity and resistance profiles of the two types of nucleosides (2'-F-4'-S-d4N vs 2'-F-d4N) as well as the role of the isosterically substituted sulfur atom, we conducted molecular modeling studies of the representative cytidine analogs (2'-F-d4C and 2'-F-4'-S-d4C). Our molecular modeling studies mainly focused on the interactions of the nucleoside triphosphates with the viral polymerase, HIV-1 RT. The crystal structure of HIV-1 RT, complexed with thymidine triphosphate and DNA duplex reported by Huang et al, enabled us to perform molecular modeling studies of the triphosphates of several

nucleoside reverse transcriptase inhibitors (NRTIs). Previously, we qualitatively demonstrated that the calculated binding affinity of NRTIs correlates with anti-HIV activity.[291] In our current studies, quantum mechanical *ab initio* calculations at the level of RHF/3-21G* using Spartan 5.1.1 (Wavefunctions, Inc.) were performed on the cytidine analogs (β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C), and the geometry-optimized structures were compared (Fig. 4.1.a). The two geometry-optimized structures were nicely superimposed each other except positions of the 4'-oxygen and 4'-sulfur. Because the sulfur atom has a longer van der Waals radius than the oxygen atom, the 4'-sulfur was positioned slightly outside of the 4'-oxygen when two cytidine analogs were superimposed. However, the distance between the heterocyclic base and 5'-hydroxy group, which has been considered as the key factor[292,293] in binding of the nucleosides to the nucleoside kinases, is similar in the two classes as shown in Fig. 4.1.a. From this result, we may assume that both β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C may be potentially the substrates of nucleoside kinases, such as 2'-deoxycytidine kinase. After the rate-limiting initial phosphorylation of the synthesized nucleosides, their triphosphates could be readily synthesized by cellular nucleotide kinases.[294] Recently it was also reported that L-nucleoside diphosphates are phosphorylated by 3-phosphoglycerate kinase.[295] The triphosphates of geometry-optimized β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C, β-L-2'-F-4'-S-d4CTP and β-L-2'-F-d4CTP, were manually docked into the truncated catalytic site of HIV-1 RT (Lys1~Pro243 in p66 subunit), and the resulting enzyme- inhibitor complexes were energy-minimized using Kollman-All-Atom force field.[296] Both minimized structures showed that the triphosphates of β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C bound tightly to the active site of HIV-1 RT (Fig. 4.1.c and 4.1.d).

In addition to the structural similarity, which was shown in Fig. 4.1.a, their binding modes to the HIV-1 RT were also similar in several aspects. Arg72 at the active site of HIV-1 RT plays a key role in binding the triphosphates of β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C: The guanidinium moiety of Arg72 approaches the active site in order to establish multiple hydrogen bonds with the triphosphates of β-L-2'-F-4'-S-d4CTP and β-L-2'-F-d4CTP, resulting in stabilization of the nucleoside triphosphates. The backbone amides, which are part of 3'-OH pocket residues (Asp113 and Ala114), also stabilize the triphosphates through the hydrogen bonding interaction. Even though the 4'-sulfur atom in β-L-2'-F-4'-S-d4C **17** is closer to Arg72 compared to the 4'-oxygen in β-L-2'-F-d4C, there was no destabilizing steric hindrance between the 4'-sulfur atom and the guanidinium moiety of Arg72, which allowed stable orientation of β-L-2'-F-4'-S-d4C **17** at the active site (Fig. 4.1.b). The conformational analysis and binding affinity studies indicate that β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C have similar structures, and the substitution of a sulfur atom is well tolerated at the polymerase level, which could explain their similar antiviral potency in the wild-type RT. However, the M184V mutation in HIV-1 RT causes a serious problem in positioning the L-configured nucleoside triphosphates at the active site because the branched methyl groups of Val184 tend to occupy the space where the sugar moiety of L-2'-F-2',3'-unsaturated nucleosides projects. The resulting steric hindrance destabilized the L-2'-F-2',3'-unsaturated nucleoside triphosphate-RT complex (Fig. 4.1.e), which can be confirmed by the highly decreased relative binding energies of L-nucleosides (Table 4.3).

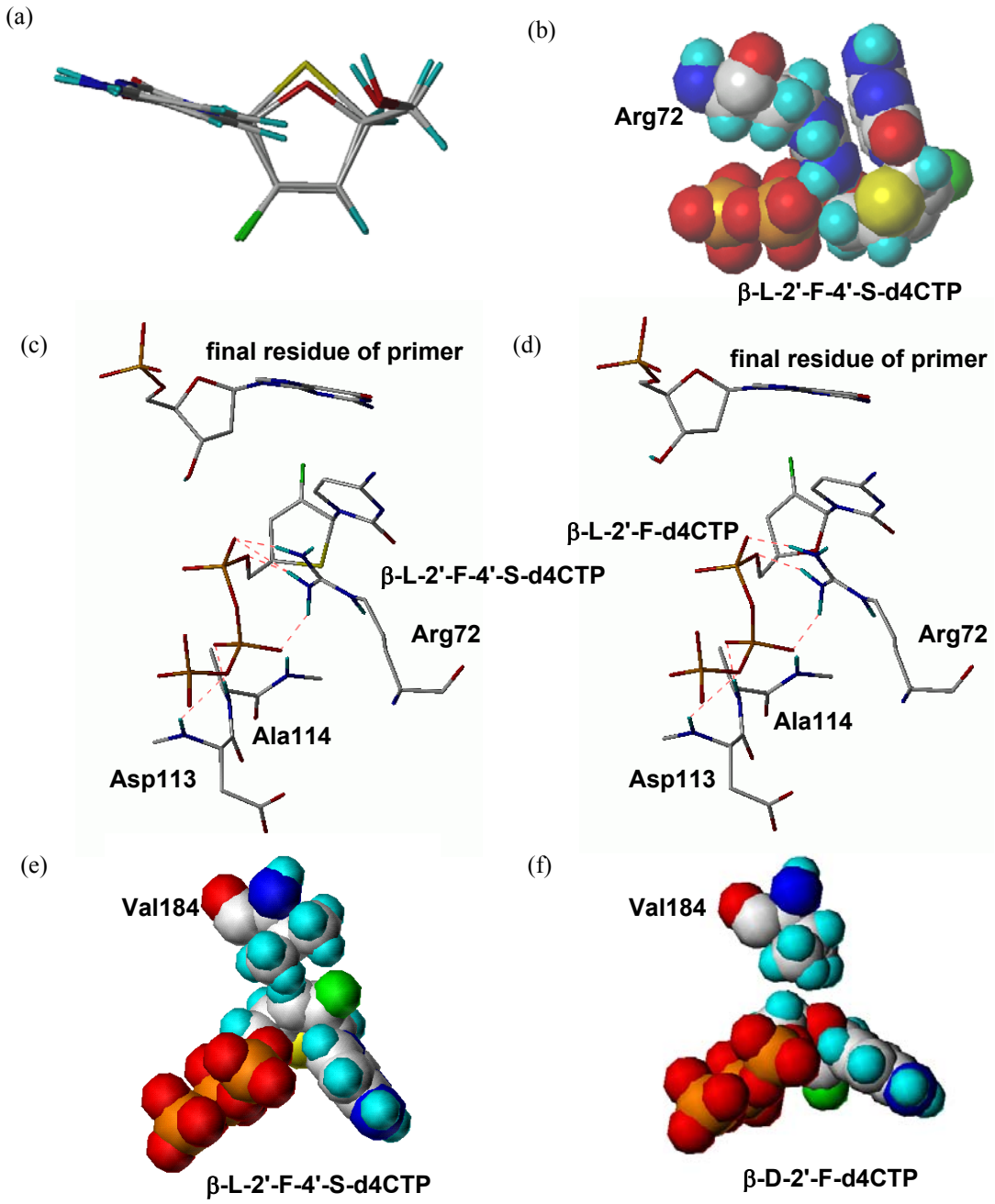


Figure 4.1. Molecular modeling studies: (a) Superimposed structures of β-L-2'-F-d4C and β-L-2'-F-4'-S-d4C. (b) The CPK structure showing interaction of β-L-2'-F-4'-S-d4CTP with Arg72. (c) The minimized structure of β-L-2'-F-4'-S-d4CTP after docking to the active site of HIV-1 reverse transcriptase. (d) The minimized structure of β-L-2'-F-d4CTP after docking to the active site of HIV-1 reverse transcriptase. (e) β-L-2'-F-4'-S-complex after mutation from M184 to V184 showing a steric hindrance between the sugar moiety of β-L-2'-F-4'-S-d4CTP and the side chain of V184. (f) β-D-2'-F-d4CTP/RT complex after mutation showing no steric hindrance.

On the other hand, the structure of β-D-2'-F-d4CTP-RT complex does not show any significant steric hinderance between Val184 and the sugar moiety of nucleoside triphosphate because the natural D-configured sugar moiety was located at the opposite side (Fig. 4.1.f). The potent anti-HIV-1_{M184V} activity of β-D-2'-F-d4C supports this concept.

In summary, we have developed an efficient synthetic methodology for β-L-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides (β-L-2'-F-4'-S-d4Ns), and discovered that the pyrimidine analogs, β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-4'-S-d4-5FC **18** showed potent antiviral activity against HIV-1. The cytidine analog, β-L-2'-F-4'-S-d4C **17**, however, did not show any significant antiviral activity against the 3TC-resistant mutant RT. Our molecular modeling studies revealed that the same pattern of the anti-HIV-1 activity of β-L-2'-F-4'-S-d4N and β-L-2'-F-d4N can be explained by their conformational and structural similarities. The unnatural L-configuration of sugar moiety was found to provide steric hindrance with the side chain of Val184 in 3TC-resistant RT, which destabilized the RT-nucleoside analog complex.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). UV

spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(*2R*,*4R*)-(-)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-butyrolactone (3). A solution of compound **2** (25 g, 67.5 mmol) in 500 mL of EtOAc was treated with 2.5 g of palladium on carbon (5% w/w) under H_2 atmosphere for 3 h. After filtration through a celite pad, the filtrate was concentrated and purified by silica gel column chromatography with 7% EtOAc in hexanes to give compound **3** (23.8 g, 64.1 mmol, 95% yield) as a white solid: mp 88~89°C; $[\alpha]^{24}_D$ -15.3° (c 0.835, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.69~7.38 (m, 10H), 5.27 (dt, *J* = 51.4, 8.9 Hz, 1H), 4.54~4.47 (m, 1H), 3.92 (dd, *J* = 11.7, 2.4 Hz, 1H), 3.74 (dd, *J* = 11.7, 3.7 Hz, 1H), 2.67 (ddt, *J* = 13.2, 8.6, 6.6 Hz, 1H), 2.54 (ddt, *J* = 24.4,13.2, 9.0 Hz, 1H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.19 (d, *J* = 21.3 Hz), 135.62, 135.51, 132.73, 132.40, 129.97, 129.95, 127.97, 127.84, 89.82 (d, *J* = 193.0 Hz), 76.31 (d, *J* = 6.1 Hz), 63.94, 30.26 (d, *J* = 20.0 Hz), 26.64, 19.21; FABMS *m/z* 373 $(M+H)^+$; Anal. ($C_{21}H_{25}FO_3Si$) C, H.(for the detail data, see Table 4.4)

(*2R*,*4R/S*)-5-*tert*-Butyldiphenylsilyloxy-2-fluoro-4-iodopentanoic acid methyl ester (4). A mixture of compound **3** (12.2 g, 32.6 mmol) in 5% aqueous EtOH was treated with solid NaOH (1.44 g, 36.0 mmol) at rt for 2 h. The resulting mixture was concentrated and co-evaporated two times with 250 mL of toluene to dryness. The crude carboxylate

sodium salt was dissolved in 15 mL of DMSO and treated with dimethyl sulfate (3.71 mL, 39.2 mmol) at 0°C. After addition, the ice-bath was removed. The reaction mixture was stirred for 1 h and then, poured to ice-cooled water (500 mL) and extracted with ethyl ether (3X200 mL). The combined organic layer was washed with water (3X200 mL), dried over $MgSO_4$, and concentrated to dryness. The crude methyl ester was treated with I_2 (14.9 g, 58.7 mmol), imidazole (6.67 g, 98.0 mmol), and Ph_3P (17.1 g, 65.3 mmol) in toluene (300 mL) at 60°C for 4 h. Aqueous $NaHCO_3$ (200 mL) was added to the resulting mixture and iodine was added portionwise until the iodine color persisted to remove remaining Ph_3P. Aqueous $Na_2S_2O_3$ was added dropwise until the iodine color disappeared to remove the remaining iodine. The resulting mixture was poured to a separatory funnel, diluted with 300 mL of toluene, washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography with 7% EtOAc in hexanes to give compounds **4** (13.7 g, 26.6 mmol, 82% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) for the major δ 7.75~7.35 (m, 10H), 5.10 (ddd, *J* = 48.4, 7.5, 5.2 Hz, 1H), 4.33~4.18 (m, 1H), 3.95~3.78 (m 2H), 3.82 (s, 3H) 2.80~2.40 (m, 2H), 1.10 (s, 9H), for the minor δ 7.75~7.35 (m, 10H), 5.18 (ddd, *J* = 49.2, 10.8, 2.3 Hz, 1H), 4.33~4.18 (m, 1H), 3.95~3.78 (m 2H), 3.83 (s, 3H), 2.45~2.10 (m, 2H), 1.09 (s, 9H); HRMS (FAB) obsd, *m/z* 515.0972, calcd for $C_{22}H_{29}FIO_3Si$, *m/z* 515.0915 $(M+H)^+$; Anal. ($C_{22}H_{28}FIO_3Si \cdot 0.05C_6H_{14}$) C, H.

(*2R,4S/R*)-4-Acetylsulfanyl-5-*tert*-butyldiphenylsilyloxy-2-fluoropentanoic acid methyl ester (5). A solution of compounds **4** (13.7 g, 26.6 mmol) in 20 mL of DMF was treated with solid KSAc (6.08 g, 53.2 mmol) at rt for 8 h. The resulting mixture was diluted with EtOAc (500 mL), washed with water (2X200 mL), dried over $MgSO_4$,

filtered, concentrated and purified by column chromatography with 10% EtOAc in hexanes to give products **5** (11.14 g, 24.1 mmol, 91% yield) as a red-brown oil: ^{1}H NMR ($CDCl_3$) for the major δ 7.67~7.35 (m, 10H), 5.12~4.92 (m, 1H), 3.93~3.68 (m, 3H), 3.81 (s, 3H), 2.44~2.10 (m, 2H), 2.32 (s, 3H), 1.057 (s, 9H), for the minor δ 7.67~7.35 (m, 10H), 5.12~4.92 (m, 1H), 3.93~3.68 (m, 3H), 3.81 (s, 3H), 2.44~2.10 (m, 2H), 2.30 (s, 3H), 1.062 (s, 9H); HRMS (FAB) obsd, *m/z* 463.1789, calcd for $C_{24}H_{32}FO_4SSi$, *m/z* 463.1775 $(M+H)^+$; Anal. ($C_{24}H_{31}FO_4SSi$) C, H, S.

(*2R*,*4R*)-(-)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-thiobutyrolactone (6). A solution of compounds **5** (11.1 g, 24.1 mmol) in toluene (200 mL) was treated with 53 mL of 1M DIBAL-H in hexane at -78^{o}C for 1 h. The reaction was quenched with 12 mL of MeOH and warmed up to room temperature for 1 h and aq. $NaHCO_3$ (23 mL) and EtOAc (200 mL) were added to the mixture. The resulting mixture was filtered and the filtrate was concentrated to driness. The crude thiolactol was treated with Ac_2O (34 mL) and DMSO (35 mL) at rt for 24 h. The reaction mixture was poured to a separatory funnel containing ice-cooled water (300 mL) and extracted with ethyl ether (3X300 mL). The combined organic layer was washed with water (3X300 mL), dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography with 5% Et_2O in hexanes to give the products **6** (5.08 g, 13.1 mmol, 54% yield) and **6a** (0.85 g, 2.2 mmol, 9% yield) as a yellow oil: For **6**: $[\alpha]^{25}_D$ -29.4^o (c 1.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68~7.37 (m, 10H), 5.07 (ddd, *J* = 50.5, 10.2, 6.9 MHz, 1H), 3.96~3.78 (m, 3H), 2.72~2.62 (m, 1H), 2.18~ 2.07 (m, 1H), 1.07 (s,9H);^{13}C NMR ($CDCl_3$) δ 200.70 (d, *J* = 17.4 MHz), 135.50, 132.66, 132.58, 129.99, 127.85, 93.26 (d, *J* = 196.9 MHz), 66.61, 43.97 (d, *J* = 7.1 MHz), 32.58 (d, *J* = 19.6 MHz), 26.68, 19.21; Anal. ($C_{21}H_{25}FO_2SSi$) C,

H, S; For **6a**: $[\alpha]^{25}_{D}$ 50.7° (c 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.66~7.40 (m, 10H), 5.18 (dt, *J* = 44.4, 7.0 Hz, 1H), 4.02 (quint, *J* = 5.0 Hz) 3.86 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.84 (dd, *J* = 10.8, 5.0 Hz, 1H), 2.88~2.78 (m, 2H), 1.07 (s, 9H); Anal. ($C_{21}H_{25}FO_2SSi$) C, H, S.

(*2S*,*4R*)-(-)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl-γ-thiobutyrolactone (7). To a solution of compound **6** (5.08 g, 13.1 mmol) in THF (60 mL), 15.7 mL of 1M LiHMDS in THF was added slowly at -78°C, and the reaction mixture was stirred at the same temperature for 1 h. TMSCl (2.16 mL, 17 mmol) was added dropwise to the reaction mixture and the mixture was allowed to warm to rt. The resulting mixture was stirred at rt for 30 min and cooled to -78°C. A solution of PhSeBr (4.69 g, 19.6 mmol) in THF (20 mL) was rapidly added and the mixture was stirred at -78°C for 1 h. The mixture was diluted with ethyl ether (300 mL), washed with water (4X100 mL), dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography with 3% Et_2O in hexanes to give desired product **7** (5.28 g, 9.69 mmol, 74% yield) as a pale yellow syrup: $[\alpha]^{24}_{D}$ -56.4° (c 0.542, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.70~7.35 (m, 15H), 3.97~3.77 (m, 3H), 2.49 (dd, *J* = 13.3, 4.5 Hz, 1H), 2.22 (td, *J* = 14.4, 10.5 Hz, 1H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 196.34 (d, *J* = 22.7 Hz), 137.03, 135.53, 132.63, 132.50, 130.03, 129.39, 127.87, 124.79, 105.13 (d, *J* = 260.6 Hz), 65.67, 44.59 (d, *J* = 2.9 Hz), 39.13 (d, *J* = 21.4 Hz), 26.70, 19.21; HRMS (FAB) obsd, *m/z* 545.0873, calcd for $C_{27}H_{30}FO_2SSeSi$, *m/z* 545.0885 $(M+H)^+$; Anal. ($C_{27}H_{29}FO_2SSeSi{\cdot}0.15CHCl_3$) C, H, S.

(*1S/R*,*2S*,*4R*)-1-*O*-Acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranoside (8). A solution of compound **7** (4.98 g, 9.14

mmol) in toluene (100 mL) was treated with 18.3 mL of 1M DIBAL-H in hexane at -78^{o}C for 1 h. The reaction was quenched with 4 mL of MeOH and warmed up to room temperature for 1 h and aq. $NaHCO_3$ (8 mL) and EtOAc (100 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to dryness. A solution of the crude thiolactol in CH_2Cl_2 (100 mL) was treated with Ac_2O (2.6 mL, 28 mmol), TEA (3.8 mL, 28 mmol) and catalytic amount of 4-DMAP at rt for 3 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 3% Et_2O in hexanes to give the acetate **8** (4.48 g, 7.61 mmol, 83% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.70~7.32 (m, 15H), 6.03, 5.99 (d & s, J = 7.6 Hz, 1H), 3.76~3.60 (m, 3H), 2.60~2.53 (m, 1H), 2.41~2.31 (m, 1H) 2.13, 2.01 (2s, 3H), 1.04, 0.99 (2s, 9H); Anal. ($C_{29}H_{28}FO_3SSeSi$) C, H, S.

General procedure for condensation reaction of the acetate **8** with pyrimidines. The preparation of cytosine derivative **9** is representative.

(-)-*N*^{4}-Benzoyl-1-[(*1S*,*2S*,*4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]cytosine (9). A mixture of *N*^{4}-benzoylcytosine (0.548 g, 2.55 mmol) in HMDS (15 mL) and CH_3CN (15 mL) was refluxed for 5 h. After removing solvent using vacuum pump, a solution of the acetate **8** (0.500 g, 0.849 mmol) in 15 mL of CH_3CN was added to the reaction flask containing the silylated *N*^{4}-benzoylcytosine, and then TMSOTf (0.31 mL, 1.7 mmol) was added dropwise at room temperature. After 16 h, the reaction was quenched with 1 mL of sat. $NaHCO_3$ and the resulting mixture was concentrated to one fifth of volume. The crude mixture was diluted with 100 mL of CH_2Cl_2, washed with aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography with

30% EtOAc in hexanes to give cytosine derivative **9** (0.300 g, 0.404 mmol, 48% yield) as a foam: $[\alpha]^{24}_{D}$ -168.38° (c 0.486, CH_2Cl_2); UV(MeOH) λ_{max} 308 nm; ^{13}C NMR ($CDCl_3$) δ 161.94, 155.42, 147.17, 136.96, 135.60, 135.54, 133.21, 132.83, 129.96, 129.92, 129.41, 129.20, 129.03, 127.78,127.60, 125.73, 106.39 (d, *J* = 248.8 Hz), 96.87, 66.74 (d, *J* = 17.6 Hz), 66.29, 47.21, 42.39 (d, *J* = 22.2 Hz), 26.73, 19.15; Anal. ($C_{38}H_{38}FN_3O_3SSeSi \cdot 0.2Et_2O$) C, H, N, S.

(-)-*N*^{4}-Benzoyl-1-[(*1S*,*2S*,*4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (10). See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **10** was obtained on 0.849-mmol scale in 59% yield: $[\alpha]^{24}_{D}$ -209.1° (c 0.693, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 332.5 nm; ^{13}C NMR ($CDCl_3$) δ 151.88 (d *J* = 19.2 Hz), 147.15, 139.01 (d, *J* = 237.9 Hz), 136.78, 135.90, 135.49, 135.47, 133.00, 132.71, 130.01, 129.96, 129.91, 129.51, 129.18, 128.24, 127.76, 126.85 (d, *J* = 34.4 Hz), 124.74, 105.60 (d, *J* = 247.5 Hz), 67.01 (d, *J* = 18.6), 66.43, 47.19, 41.64 (d, *J* = 21.0 Hz), 26.68, 19.09; FABMS *m/z* 762 $(M+H)^+$; Anal. ($C_{38}H_{37}F_2N_3O_3SSeSi$) C, H, N, S.

(-)-1-[(*1S*,*2S*,*4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]uracil (11). See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **11** was obtained on 0.849-mmol scale in 48% yield: $[\alpha]^{23}_{D}$ -129.2° (c 0.578, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 262.5 nm; ^{13}C NMR (CDCl3) δ 162.77, 150.77, 141.80 (d, J = 5.0 hz), 136.94, 135.58, 135.51, 132.80, 132.77, 129.93, 129.61, 129.26, 127.79, 125.12, 106.24 (d, J = 248.7 Hz), 102.31, 66.06, 65.99 (d, J = 14.6 Hz), 46.86, 41.58 (d, J = 21.8 Hz), 26.75, 19.18; Anal. ($C_{31}H_{33}FN_2O_3SSeSi$) C, H, N, S.

(-)-1-[(*1S,2S,4R*)-5-O-(tert-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]thymine (12). See the general procedure for condensation reaction of the acetate 8 with pyrimidines. The title compound 12 was obtained on 0.849-mmol scale in 38% yield: $[\alpha]^{26}_{D}$ -130.6° (c 0.464, CH_2Cl_2); UV(CH_2Cl_2) λmax (CH_2Cl_2) 266.0 nm; ^{13}C NMR ($CDCl_3$) δ 163.25, 150.93, 137.22 (d, *J* = 6.1 Hz), 136.88, 135.48, 132.94, 132.90, 129.87, 129.46, 129.15, 127.74, 125.16, 110.67, 106.15 (d, *J* = 247.03 Hz), 66.76, 65.85 (d, *J* = 18.3 Hz), 46.79, 42.04 (d, *J* = 21.0 Hz), 26.70, 19.17, 12.59; FABMS *m/z* 655 $(M+H)^+$; Anal. ($C_{32}H_{35}FN_2O_3SSeSi$) C, H, N, S.

General procedure for *syn*-elimination reaction using mCPBA to give 2',3'-unsaturated nucleosides. The preparation of cytosine derivative **13** is representative.

(+)-*N*^{4}-Benzoyl-1-[(*1S,4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]cytosine (13). To a solution of compound **9** (0.169 g, 0.228 mmol) in 5 mL of CH_2Cl_2, a solution of mCPBA (57~86%, 69 mg) in 5 mL of CH_2Cl_2 was added at -78°C and the mixture was stirred at -78°C for 30 min. Pyridine (0.07 mL, 0.9 mmol) was then added and the resulting mixture was stirred at rt for 2 h. The reaction mixture was diluted with 50 mL of CH_2Cl_2, washed with aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 30% EtOAc in hexanes to give eliminated product **13** (0.107 g, 0.183 mmol, 80% yield) as a foam: $[\alpha]^{24}_{D}$ 128.81° (c 0.515, CH_2Cl_2); UV(MeOH) λ_{max} 308 nm; ^{13}C NMR ($CDCl_3$) δ 171.09, 166.78, 162.14, 155.06, 155.04 (d, *J* = 280.8 Hz), 144.49, 135.58, 135.50, 133.19, 132.96, 132.76, 132.43, 130.11, 128.97, 127.89, 127.58, 111.29 (d, *J* = 16.6 Hz), 98.40, 67.75, 61.91 (d, *J* = 23.6 Hz), 46.83 (d, *J* = 7.2 Hz), 26.82, 19.24; Anal. ($C_{32}H_{32}FN_3O_3SSi{\cdot}0.1C_6H_{14}$) C, H, N, S.

(+)-N^4-Benzoyl-1-[(*1S,4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (14). See the general procedure for *syn*-elimination reaction. The title compound **14** was obtained on 0.493-mmol scale in 73% yield: $[\alpha]^{26}_D$ 152.58° (c 0.485, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 331.0 nm; ^{13}C NMR ($CDCl_3$) δ 152.88 (d, *J* = 281.3 Hz), 152.08 (d, *J* = 19.1 Hz), 147.13, 140.42 (d, *J* = 241.0 Hz), 135.86, 135.59, 135.50, 133.17, 132.59, 132.39, 130.18, 130.15, 130.11, 128.33, 127.93, 124.05 (d, *J* = 36.2 Hz), 111.24 (d, *J* = 16.5 Hz), 67.77, 61.85 (d, *J* = 23.54 Hz), 47.05 (d, *J* = 7.1 Hz), 26.79, 19.23; FABMS *m/z* 604 $(M+H)^+$; Anal. ($C_{32}H_{31}FN_3O_3SSi$) C, H, N, S.

(+)-1-[(*1S,4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]uracil (15). See the general procedure for *syn*-elimination reaction. The title compound **15** was obtained on 0.411-mmol scale in 68% yield: $[\alpha]^{26}_D$ 77.7° (c 0.492, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 262.5 nm; ^{13}C NMR ($CDCl_3$) δ 162.78, 154.79 (d, *J* = 280.9 Hz), 150.59, 139.55, 135.59, 135.49, 132.79, 132.37, 130.13, 130.10, 127.92, 127.90, 110.70 (d, *J* = 16.9 Hz), 104.01, 67.33, 60.74 (d, *J* = 23.7 Hz), 46.88 (d, *J* = 7.2 Hz), 26.87, 19.31; HRMS (FAB) obsd, *m/z* 483.1572, calcd for $C_{25}H_{28}FN_2O_3SSi$, *m/z* 483.1574 $(M+H)^+$; Anal. ($C_{25}H_{27}FN_2O_3SSi$) C, H, N, S.

(+)-1-[(*1S,4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]thymine (16). See the general procedure for *syn*-elimination reaction. The title compound **16** was obtained on 0.326-mmol scale in 76% yield: $[\alpha]^{26}_D$ 50.7° (c 0.530, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 266.0 nm; ^{13}C NMR ($CDCl_3$) δ 163.47, 154.97 (d, *J* = 281.2 Hz), 150.78, 135.52, 135.43, 134.52, 132.79, 132.51, 130.03, 127.84, 112.65, 110.45 (d, *J* = 16.8 Hz), 68.23, 60.49 (d, *J* = 23.7 Hz), 46.74 (d, *J* = 7.1 Hz),

26.74, 19.23, 12.54; HRMS (FAB) obsd, *m/z* 497.1725, calcd for $C_{26}H_{30}FN_2O_3SSi$, *m/z* 497.1730 $(M+H)^+$; Anal. ($C_{26}H_{29}FN_2O_3SSi$) C, H, N, S.

General procedure for successive deprotections of protected unsaturated nucleosides. The preparation of cytidine analog **17** is representative.

(+)-1-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]cytosine (17). A solution of the unsaturated cytidine **13** (0.180 g, 0.307 mmol) in 15 mL of THF was treated with 0.37 mL of 1M TBAF in THF for 2 h. The mixture was concentrated, and filtered through a short pad of silica gel. The filtrate was concentrated and, without further purification, the crude product was treated with methanolic ammonia at rt for 30 h. After removal of solvent, the residue was purified by silica gel column chromatography with 5% MeOH in CH_2Cl_2 to give cytidine analog **17** (0.066 g, 0.27 mmol, 88% yield) as a white solid: mp 89-91°C (dec.); $[\alpha]^{24}_D$ 205.3° (c 0.140, MeOH); UV(H_2O) λ_{max} 279.5 nm (ε 19,900, pH 2). 272.0 nm (ε 15,900, pH 7), 272.5 nm (ε 16,200, pH 11); ^{13}C NMR (MeOH-d_4) δ 167.45, 158.35, 156.58 (d, J = 276 Hz), 142.97, 112.57 (dd, J = 17, 3 Hz), 97.44, 65.72, 62.73 (d, J = 24 Hz), 48.95 (d, J = 10 Hz); Anal. ($C_9H_{10}FN_3O_2S$) C, H, N, S.

(+)-1-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (18). See the general procedure for successive deprotections. The title compound **18** was obtained on 0.346-mmol scale in 88% yield: mp 189~190°C; $[\alpha]^{24}_D$ 169.2° (c 0.159, MeOH); UV(H_2O) λ_{max} 286.0 nm (ε 8,600, pH 2). 282.0 nm (ε 7,500, pH 7), 282.0 nm (ε 6,900, pH 11); ^{13}C NMR (DMSO-d_6) δ 160.38 (d, J = 15 Hz), 157.51 (d, J = 274 Hz), 156.04, 140.00 (d, J = 241 Hz), 129.8 (d, J = 32 Hz), 115.45 (d, J = 17 Hz), 66.85, 64.56 (d, J = 24 Hz), 51.05 (d, J = 8 Hz); Anal. ($C_9H_9F_2N_3O_2S$) C, H, N, S.

General procedure for desilylation reaction of *tert*-butyldiphenyl ether by using TBAF. The preparation of uridine analog **19** from compound **15** is representative.

(+)-1-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]uracil (19). A solution of compound **15** (0.135 g, 0.280 mmol) in THF (10 mL) was treated with 0.33 mL of 1M TBAF in THF at room temperature for 2 h. The mixture was concentrated and purified by silica gel column chromatography with 3% MeOH in $CHCl_3$ to give the desired product **19** (0.063g, 0.258 mmol, 92% yield) as a white solid: mp 184~185°C; $[\alpha]^{24}_D$ 132.9° (c 0.103, MeOH); UV(H_2O) λ_{max} 263.0 nm (ε 9,100, pH 2). 263.0 nm (ε 9,700, pH 7), 263.5 nm (ε 7,800, pH 11); ^{13}C NMR (DMSO-d_6) δ 166.33, 157.05 (d, J = 274 Hz), 154.20, 143.96, 115.58 (d, J = 16 Hz), 106.74, 67.50, 63.68 (d, J = 24 Hz), 51.20 (d, J = 8 Hz); Anal. ($C_9H_9FN_2O_3S$) C, H, N, S.

(+)-1-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]thymine (20). See the general procedure for desilylation reaction and trituation with Et_2O. The title compound **20** was obtained on 0.248-mmol scale in 91% yield: mp 174~176°C; $[\alpha]^{24}_D$ 50.4° (c 0.180, MeOH); UV(H_2O) λ_{max} 268.5 nm (ε 5,700, pH 2). 268.5 nm (ε 5,500, pH 7), 269.0 nm (ε 4,500, pH 11); ^{13}C NMR (DMSO-d_6) δ 166.99, 157.13 (d, J = 274 Hz), 154.20, 139.29, 115.44 (d, J = 17 Hz), 114.36, 67.25, 63.42 (d, J = 24 Hz), 51.15 (d, J = 8 Hz), 15.74; Anal. ($C_{10}H_{11}FN_2O_3S{\cdot}0.35Et_2O$) C, H, N, S.

General procedure for condensation reaction of the acetate **8** with purines. The preparation of 6-chloropurine derivative **21** is representative.

(-)-9-[(*1S,2S,4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-6-chloropurine (21). A mixture of 6-chloropurine (0.789 g,

5.10 mmol) and ammonium sulfate (0.112 g, 0.85 mmol) in 30 mL of HMDS was refluxed for 5 h. HMDS was evaporated to give yellow solid. To this flask containing the silylated 6-chloropurine, a solution of the acetate **8** (1.0 g, 1.70 mmol) in 1,2-dichloroethane (30 mL) was added. The resulting slurry was cooled to -25°C and TMSOTf (0.62 mL, 3.40 mmol) was added dropwise at -25°C. The reaction mixture was stirred for 3 h at -25~-10°C, for 8 h at rt, and for 5 h at 40°C. The resulting mixture was diluted with 300 mL of CH_2Cl_2, washed with aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography with 11% EtOAc in hexanes to give compound **21** (0.847 g, 1.24 mmol, 73% yield) as a pale yellow foam: $[\alpha]^{24}_D$ -90.0° (c 1.287, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.5 nm; ^{13}C NMR ($CDCl_3$) δ 151.76, 151.73, 150.77, 144.92 (d, *J* = 5.7 Hz), 136.24, 135.53, 135.46, 132.77, 132.68, 131.13, 129.95, 129.89, 129.35, 128.91, 127.75, 124.26, 105.04 (d, *J* = 248.1 Hz), 66.56, 65.60 (d, *J* = 20.4 Hz), 47.30, 41.59 (d, *J* = 20.9 Hz), 26.69, 19.10; Anal. ($C_{32}H_{32}ClFN_4OSSeSi$) C, H, N, S.

(-)-9-[(*1S*,*2S*,*4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-6-chloro-2-fluoropurine (22). See the general procedure for condensation reaction of the acetate **8** with purines. The title compound **22** was obtained on 1.70-mmol scale in 67% yield: $[\alpha]^{24}_D$ -86.5° (c 1.44, $CHCl_3$); UV($CHCl_3$) λ_{max} 266.0 nm; ^{13}C NMR ($CDCl_3$) δ 157.03 (d, *J* = 220.8 Hz), 153.55 (d, *J* = 16.7 Hz), 152.44 (d, *J* = 17.3 Hz), 145.53, 136.28, 135.61, 135.54, 132.82, 132.72, 130.05, 129.99, 129.80 (d, *J* = 4.6 Hz), 129.51, 129.03, 127.84, 124.20, 105.00 (d, *J* = 248.3 Hz), 66.56, 65.92 (d, *J* = 21.1 Hz), 47.38, 41.51 (d, *J* = 20.9 Hz), 26.76, 19.19; Anal. ($C_{32}H_{31}ClF_2N_4OSSeSi$) C, H, N, S.

9-[(*1S,4R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloropurine (23) and 9-[(*4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloropurine ($\Delta^{1,2}$-isomer) . See the general procedure for *syn*-elimination reaction. A mixture of compounds **23** and its $\Delta^{1,2}$-isomer (3:1) were obtained on 1.10-mmol scale in 86% yield as a pale yellow foam: UV(CH_2Cl_2) λ_{max} 264.5 nm; Anal. ($C_{26}H_{26}ClFN_4OSSi$) C, H, N, S.

9-[(*1S,4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloro-2-fluoropurine (24) and 9-[(*4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloro-2-fluoropurine ($\Delta^{1,2}$-isomer) . See the general procedure for *syn*-elimination reaction. A mixture of compound **24** and its $\Delta^{1,2}$-isomer were obtained on 1.12-mmol scale in 71% yield as a pale yellow foam: UV(CH_2Cl_2) λ_{max} 266.0 nm; Anal. ($C_{26}H_{25}ClF_2N_4OSSi$) C, H, N, S.

(+)-2-Amino-9-[(*1S,4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloropurine (25), 6-amino-9-[(*1S,4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoropurine (26) and 6-amino-9-[(*4R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoropurine ($\Delta^{1,2}$-isomer). Dry ammonia gas was bubbled into a stirred solution of a mixture of compound **24** and its $\Delta^{1,2}$-isomer (0.430 g, 0.79 mmol) in 1,2-dimethoxyethane (25 mL) at rt for 5h. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC chromatography with 20% EtOAc in hexanes to give pure compound **25** (0.209 g,

0.387 mmol, 49% yield) and a mixture of compound **26** and its $\Delta^{1,2}$-isomer (0.116 g, 0.221 mmol, 28% yield): for compound **25**; $[\alpha]^{24}_{D}$ 74.0° (c 0.236, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 300.0 nm; ^{13}C NMR ($CDCl_3$) δ 159.11, 154.78 (d, *J* = 281.2 Hz), 153.48, 151.49, 139.92, 135.57, 135.48, 132.76, 132.48, 130.12, 130.09, 127.91, 127.88, 125.30, 109.95 (d, *J* = 16.5 Hz), 68.01, 58.33 (d, *J* = 24.0 Hz), 47.46 (d, *J* = 7.1 Hz), 26.76, 19.19; Anal. ($C_{26}H_{27}ClFN_5OSSi\cdot 0.3EtOAc$) C, H, N, S: for a mixture of compounds **26** and $\Delta^{1,2}$-isomer: UV($CHCl_3$) λ_{max} 261.0 nm; ^{1}H NMR see Table 4.4.

(-)-9-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]adenine (27). A mixture of compound **23** and its $\Delta^{1,2}$-isomer (0.241 g, 0.46 mmol) was treated with methanolic ammonia at 80°C for 12 h using steel bomb. Solvent was evaporated and the residue was dried under vacuum for 3 h. A solution of the dried crude product in 30 mL of THF was treated with 0.5 mL of 1M TBAF in THF at rt for 3 h. The resulting mixture was concentrated and purified by preparative TLC chromatography with 5~10% MeOH in CH_2Cl_2 to give the desired compound **27** (0.070 g, 0.26 mmol 57% yield) as a white solid: mp 192~194°C; $[\alpha]^{26}_{D}$ -38.1° (c 0.165, MeOH); UV(H_2O) λ_{max} 258.0 nm (ε 9,500, pH 2). 259.0 nm (ε 8,900, pH 7), 259.5 nm (ε 8,600, pH 11); ^{13}C NMR (MeOH-d_4) δ 157.46, 156.31 (d, *J* = 278.2 Hz), 154.01, 150.51, 141.21, 120.10, 111.64 (d, *J* = 17.0 Hz), 65.65, 60.38 (d, *J* = 24.6 Hz), 49.81 (d, *J* = 9.1 Hz); HRMS (FAB) obsd, *m/z* 268.0669, calcd for $C_{10}H_{11}FN_5OS$, *m/z* 268.0668 $(M+H)^+$; Anal. ($C_{10}H_{10}FN_5OS$) C, H, N, S.

(-)-9-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]hypoxanthine (28). A mixture of compound **23** and its $\Delta^{1,2}$-isomer (0.216 g, 0.41 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.12

mL, 1.65 mmol) and NaOMe (0.091 g, 1.69 mmol) at 60°C for 24 h. The resulting mixture was quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel with 4% MeOH in CH_2Cl_2. The filtrate was concentrated to dryness. The crude product was treated with 0.84 mL of 1M TBAF in THF at rt for 3 h. The reaction mixture was concentrated, and purified by preparative TLC chromatography with 7% MeOH in CH_2Cl_2 to give the desired compound **28** (0.089 g, 0.33 mmol, 81% yield) as a white solid: mp 143~145°C (dec.); $[\alpha]^{23}_D$ -23.7° (c 0.324, 7:1, CH_2Cl_2:MeOH); UV(H_2O) λ_{max} 246.5 nm (ε 12,000, pH 2). 248.0 nm (ε 10,600, pH 7), 253.5 nm (ε 10,900, pH 11); ^{13}C NMR (MeOH-*d*$_4$) δ 158.86, 156.22 (d, *J* = 278.3 Hz), 150.03, 147.13, 140.65, 125.28, 111.76 (d, *J* = 17.1 Hz), 65.48, 60.51 (d, *J* = 24.5 Hz), 49.82 (d, *J* = 7.9 Hz); HRMS (FAB) obsd, *m/z* 269.0517, calcd for $C_{10}H_9FN_4O_2S$, *m/z* 269.0509 $(M+H)^+$; Anal. ($C_{10}H_9FN_4O_2S$) C, H, N, S.

(+)-9-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]guanosine (29). See the procedure for the preparation of compound **28**. The title compound **29** was obtained on 0.16-mmol scale in 61% yield: mp 210°C (dec.); $[\alpha]^{25}_D$ 75.2° (c 0.15, DMSO); UV(H_2O) λ_{max} 256.0 nm (ε 11,100, pH 2). 255.0 nm (ε 12,500, pH 7), 266.0 nm (ε 10,700, pH 11); ^{13}C NMR (DMSO-*d*$_6$) δ 160.12, 157.49 (d, *J* = 278.1 Hz), 157.44, 154.47, 138.61, 119.83, 114.34 (d, *J* = 17.0 Hz), 67.90, 61.00 (d, *J* = 24.5 Hz), 51.73 (d, *J* = 7.6 Hz), 44.06 (d, *J* = 9.1 Hz); Anal. ($C_{10}H_{10}FN_5O_2S{\cdot}0.2H_2O$) C, H, N, S.

(-)-9-[(*1S,4R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoroadenosine (30). See the general procedure of desilylation and trituation with Et_2O. The title compound **30** was obtained on 0.20-mmol scale in 70% yield: mp >300°C;

$[\alpha]^{26}_{D}$ -17.8° (c 0.200, MeOH); UV(H_2O) λ_{max} 260.5 nm (ε 12,700, pH 2). 260.5 nm (ε 12,700, pH 7), 261.5 nm (ε 12,200, pH 11); Anal. ($C_{10}H_9F_2N_5OS$) C, H, N, S.

Antiviral Assay. Human peripheral blood mononuclear (PBM) cells (obtained from Atlanta Red Cross) were isolated by Ficoll-Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2-3 days prior to use. HIV-1_{LA}I obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infetious clones HIV-1_{xxBru} and HIV-$1_{M184Vpitt}$ were obtained from Dr. John Mellors (University of Pittsburgh). Infections were done in bulk for one hour, either with 100 $TCID_{50}$ /1 x 10^7 cells for a flask (T25) assay or with 200 $TCID_{50}$ / 6 x 10^5 cells/well for a 24 well plate assay. Cells were added to a plate or flask containing a ten-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/ml penicillin, 80 μg/ml streptomycin, 0.0008% DEAE-Dextran, 0.045% sodium bicarbonate, and 26 IU/ml recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in a humidified 5% CO_2-air at 37°C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

Table 4.4. Elemental analysis data

Cmpd	Formula	Calc for C	Calc for H	Calc for N	Calc for S	Found for C	Found for H	Found for N	Found for S
3	$C_{21}H_{25}FO_3Si$	67.71	6.76	-[a]	-[a]	67.71	6.78	-[a]	-[a]
4	$C_{22}H_{28}FIO_3Si \cdot 0.05C_6H_{14}$	51.63	5.58	-[a]	-[a]	51.84	5.53	-[a]	-[a]
5	$C_{24}H_{31}FO_4SSi$	62.31	6.75	-[a]	6.93	62.36	6.69	-[a]	7.04
6	$C_{21}H_{25}FO_2SSi$	64.91	6.48	-[a]	8.25	65.15	6.58	-[a]	8.12
6a	$C_{21}H_{25}FO_2SSi$	64.91	6.48	-[a]	8.25	64.94	6.42	-[a]	8.16
7	$C_{27}H_{29}FO_2SSeSi \cdot 0.15CHCl_3$	58.07	5.23	-[a]	5.70	57.88	5.36	-[a]	5.76
8	$C_{29}H_{28}FO_3SSeSi$	59.27	5.66	-[a]	5.46	59.24	5.68	-[a]	5.42
9	$C_{38}H_{38}FN_3O_3SSeSi \cdot 0.2Et_2O$	61.51	5.32	5.55	4.23	61.88	5.55	5.56	4.21
10	$C_{38}H_{37}F_2N_3O_3SSeSi$	59.99	4.90	5.52	4.21	59.97	5.05	5.44	4.23
11	$C_{31}H_{33}FN_2O_3SSeSi$	58.20	5.20	4.38	5.01	58.18	5.33	4.33	4.95
12	$C_{32}H_{35}FN_2O_3SSeSi$	58.79	5.40	4.29	4.90	58.85	5.46	4.20	4.98
13	$C_{32}H_{32}FN_3O_3SSi \cdot 0.1C_6H_{14}$	65.88	5.66	7.07	5.39	66.01	5.88	6.82	5.20
14	$C_{32}H_{31}F_2N_3O_3SSi$	63.66	5.18	6.96	5.31	63.69	5.30	6.78	5.26
15	$C_{25}H_{27}FN_2O_3SSi$	62.21	5.64	5.80	6.64	62.41	5.68	5.71	6.52
16	$C_{26}H_{29}FN_2O_3SSi$	62.87	5.89	5.64	6.46	62.54	5.82	5.36	6.13
17	$C_9H_{10}FN_3O_2S$	44.44	4.14	17.27	13.18	44.26	3.92	16.96	13.03
18	$C_9H_9F_2N_3O_2S$	41.38	3.47	16.08	12.27	41.45	3.60	15.95	12.14
19	$C_9H_9FN_2O_3S$	44.26	3.71	11.47	13.13	44.22	3.81	11.15	12.84
20	$C_{10}H_{11}FN_2O_3S \cdot 0.35Et_2O$	48.18	5.14	9.86	11.28	48.49	4.88	10.07	11.59
21	$C_{32}H_{32}ClFN_4OSSeSi$	56.34	4.73	8.21	4.70	56.00	4.81	8.04	4.73
22	$C_{32}H_{31}ClF_2N_4OSSeSi$	54.89	4.46	8.00	4.58	54.81	4.64	7.81	4.63
23	$C_{26}H_{26}ClFN_4OSSi$	59.47	4.99	10.67	6.11	59.56	5.30	10.33	5.93
24	$C_{26}H_{25}ClF_2N_4OSSi$	57.50	4.64	10.32	5.90	57.42	5.02	9.93	5.65
25	$C_{26}H_{27}ClFN_5OSSi \cdot 0.3EtOAc$	57.66	5.24	12.36	5.66	57.87	5.35	12.41	5.62
27	$C_{10}H_{10}FN_5OS$	44.94	3.77	26.20	12.00	44.83	3.88	25.83	11.85
28	$C_{10}H_9FN_4O_2S$	44.77	3.38	20.88	11.95	44.86	3.65	20.75	11.89
29	$C_{10}H_{10}FN_5O_2S \cdot 0.2H_2O$	41.87	3.65	24.41	11.18	42.17	3.35	24.37	11.16
30	$C_{10}H_9F_2N_5OS$	42.10	3.18	24.55	11.24	41.99	3.16	24.36	11.28

[a] Not Applicable

Supernatants were centrifuged at 12,000 rpm for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 µl virus solubilization buffer (VSB) containing 0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.05 M Tris, pH 7.8. Ten µl of each sample were added to 75 µl RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M $MgCl_2$, 0.006 M dithiothreitol, 0.006 mg/ml poly $(rA)_n$ oligo $(dT)_{12-18}$, 96 µg/ml dATP, and 1 µM of 0.08 mCi/ml ^{3}H-thymidine triphosphate (Moravek Biochemicals, Brea, CA)) and incubated at 37°C for 2 h. The reaction was stopped by the addition of 100 µl 10% trichloroacetic acid containing 0.05% sodium pyrophosphate. The acid insoluble product was harvested onto filter paper using a Packard Harvester (Meriden, CT), and the RT activity was read on a Packard Direct Beta Counter (Meriden, CT). The RT results were expressed in counts per minute (CPM) per milliliter. The antiviral 50% effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) were determined from the concentration-response curve using the median effect method.[297]

Cytotoxicity assays. The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD.) and Vero (African green monkey kidney) cells. PBM cells were obtained from whole blood of healthy seronegative donors (HIV-1 and hepatitis B virus) by single-step Ficoll-Hypaque discontinous gradient centrifugation. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5 x 10^3, 2.5 x 10^3 and 5 x 10^4 cells/well respectively. All of the cells were plated in 96-well cell culture plates containing ten-fold serial dilutions of the test drug. The cultures were incubated for 3, 4, and 5 days for Vero,

CEM, and PBM cells, respectively in a humidified 5% CO_2-air at 37°C. At the end of incubation, MTT tetrazolium dye solution (Cell titer 96®, Promega, Madison, WI) was added to each well and incubated overnight. The reaction was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570 nm using an ELISA plate reader (Bio-tek instruments, Inc., Winooski, VT, Model # EL 312e). The 50% inhibition concentration (IC_{50}) was determined from the concentration-response curve using the median effect method.[297]

Molecular modeling studies. (a) Conformational analysis: The initial conformations of β-L-2'-F-4'-S-d4C **17** and β-L-2'-F-d4C were constructed by builder module in Spartan 5.1.1 (Wavefunctions, Inc. Irvine, CA), and all calculations were performed on a Silicon Graphics O2 workstation. The initial conformations were cleaned up and geometry-optimized through quantum mechanical *ab initio* calculation using RHF/3-21G* basis in Spartan 5.1.1. (b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme-ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd). A model of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template-primer) duplex. The geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of $n+1^{th}$ nucleotide in template overhang was modified to the base complementary

to the incoming NRTIs. Thus, the adenine moiety in the original X-ray structure (1rtd) was modified to guanine. The inhibitor triphosphates were manually docked into the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure. Gästeiger-Hückel charge was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to enzyme site from the biopolymer module in Sybyl 6.7. Fluorine parameters were obtained from the literatures[298,299] and MM2 parameters and put to the parameter files. In order to eliminate local strains resulting from merging inhibitors, residues inside 6 Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom force field until iteration number reached 5,000.

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CHAPTER 5

SYNTHESIS OF 3'-THIO NUCLEOSIDES

INTODUCTION

Oxetanocin A, a natural product from *Bacillus megaterium*, is a unique nucleoside which bears not only an oxetanose instead of a furanose but also a hydroxymethyl group instead of a hydroxy group in the sugar moiety (Fig. 5.1).[300] It has been found to be active against HIV and herpes viruses and effective as an antitumor agent.[301] Due to its unique structure and biological activity, numerous modifications have been conducted on the sugar moiety as well as heterocyclic base of oxetanocin A in order to discover effective and nontoxic antiviral nucleosides.[302] However, thietanose nucleosides in which the oxygen in the oxetane ring of oxetanocin A is replaced by a sulfur atom have not been extensively investigated presumably due to the synthetic difficulties in formation of a thietane ring as well as the condensation between a thietane ring moiety and a heterocyclic base. Until now, only two syntheses of thietanose nucleosides, **1** and **2**, have been reported (Fig. 5.1).[303]

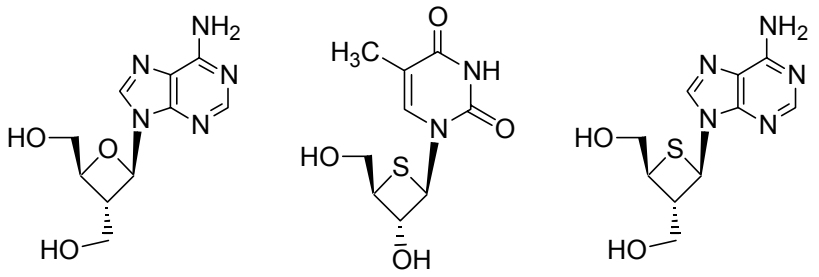


Figure 5.1. Oxetanocin A and thietanose nucleosides

The thietane rings of these nucleosides were obtained in poor to moderate yield, which was not efficient enough to conduct the structure-activity relationship study of thietanose nucleosides.[303] Recently, a very efficient method to synthesize a four-membered thietane ring using a carbohydrate template was reported by Voss et al,[304] which can be adapted to syntheses of various thietanose nuclaosides. By using this method, we report an efficient synthesis of a 3'-thio analog **2** of oxetanocin A as well as 3'-thio analogs with different heterocyclic bases as potential antiviral and/or antitumor agents.

RESULTS AND DISCUSSION

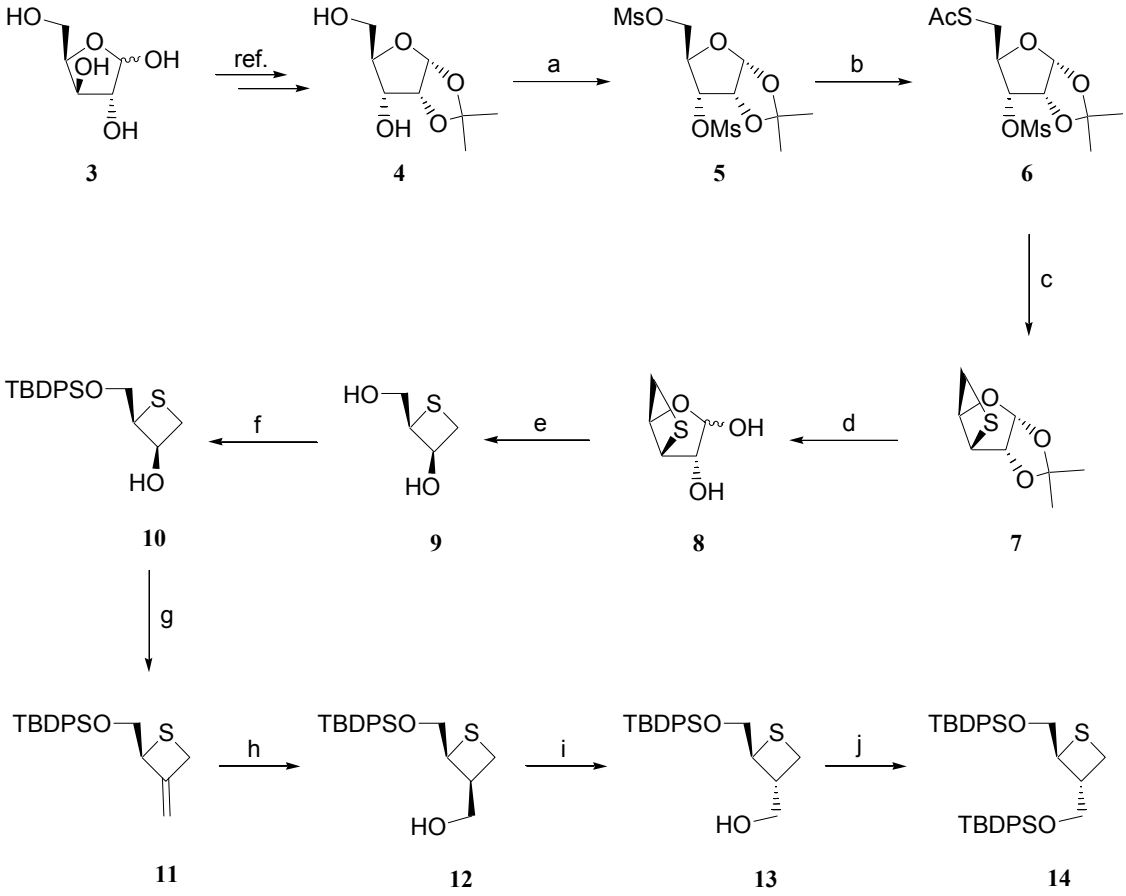
Chemistry. An efficient synthesis of a thietanose ring, via 1-thioacetyl-3-mesylate reported by Voss et al,[304] was adapted to our synthesis with some modifications. The 1-thioacetyl-3-mesylate was synthesized in 2 easy steps by dimesylation followed by selective monothioacetylation instead of selective thioacetylation by Mitsunobu reaction followed by mesylation (Scheme 5.1). D-Xylose **3** was converted to diol **4** in 5 steps by a well-known method.[305] Diol **4** was mesylated with MsCl, TEA and DMAP to give the 1,3-Dimesylate **5** in 90% yield. 1,3-Dimesylate **5** was selectively converted to mono-thioacetate **6** by treatment with KSAc in DMF at room temperature in 81 % yield. A solution of the mono-thioacetate **6** and $NaHCO_3$ in previously deoxygenated aqueous EtOH was refluxed under N_2 atmosphere for 6 h to give compound **7** in 98% yield. Compound **7** was deprotected by treatment with 2% TFA followed by neutralization with Amberlite(OH^-) resin to give compound **8** in 95% yield. Compound **8** was oxidatively

cleaved to diol **9**, which was selectively protected with TBDPSCl, TEA and DMAP to give **10**.

To introduce a hydroxymethyl group at the 3-position of the thietane ring of compound **10**, Hegedus' method was used, which was reported in the synthesis of (-)-cyclobut-A.[306] Compound **10** was oxidized to ketone intermediate which was methylenated with Petasis reagent, Cp_2TiMe_2,[307] to give **11**. Hydroboration followed by oxidation afforded compound **12** with β-hydroxymethyl group. Among borane reagents such as BH_3·THF, BH_3·SMe_2, and 9-BBN, only BH_3·SMe_2 gave a good yield possibly because the thietane ring has a sulfur atom vulnerable to oxidation. The epimerization of the hydroxymethyl group was carried out through successive reactions without purification: swern oxidation, epimerization of the intermediate aldehyde and reduction. Compound **13** was obtained in 47% overall yield from compound **12**. Compound **13** was protected with TBDPSCl to give **14** in 94% yield.

Condensation of the sugar moiety **14** with heterocyclic bases was conducted by Pummerer rearrangement after formation of a sulfoxide with some modification of the previous method (Scheme 5.2 and 5.3).[308] Silylated heterocyclic bases, such as thymine, uracil, 6-chlolopurine and 6-chloro-2-fluoropurine, were obtained under reflux conditions using HMDS and acetonitrile instead of using TMSOTf and TEA. These silylated heterocyclic bases was condensed with a sulfoxide of compound **14** in the presence of TMSOTf, TEA and catalytic amound of ZnI_2 to give the corresponding nucleosides (**15**, **16** and **21**) in 40-46% yield.

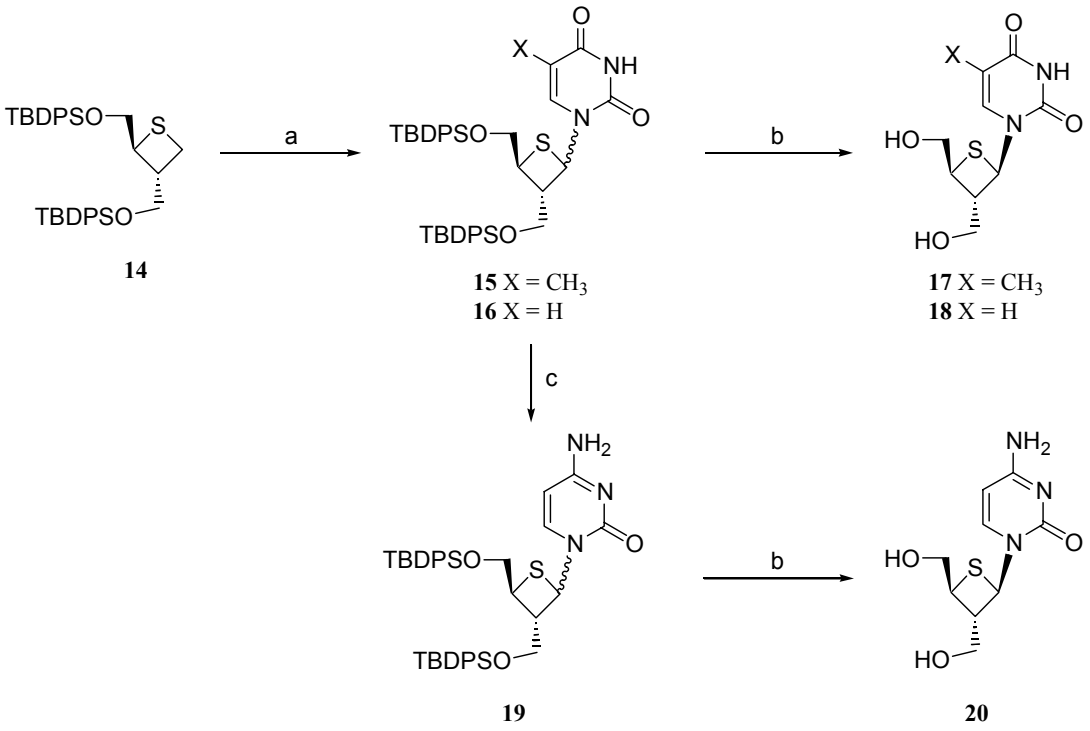
Condensation of compound **14** with thymine and uracil gave a α/β mixture of protected nucleosides **15** and **16** in 46% and 40% yield, respectively. The nucleoside analogs **15** and **16** were treated with TBAF in THF at room temperature to afford thymine and uracil



[a]keys a) MsCl, pyr. DMAP, CH_2Cl_2, b) KSAc, DMF, c) $NaHCO_3$, $EtOH/H_2O$, reflux, d) 4% TFA; OH^- resin e) $NaIO_4$; $NaBH_4$, MeOH, f) TBDPSCl, TEA, DMAP, CH_2Cl_2, g) i. Ac_2O, DMSO, ii. Petasis reagent, THF/Et_2O (1:1), reflux, h) BH_3SMe_2, THF; H_2O_2, 1N NaOH, i) i. Swern Oxidation, ii. NaOMe, MeOH; NaBH4, j) TBDPSCl, immidazole, CH_2Cl_2

Scheme 5.1. Synthesis of the key intermediate **12**

derivatives **17** and **18** which were separated from α isomers. The uracil derivative **15** was aminated by treatment with 2,4,6-triisopropylbenzenesulfonyl chloride, TEA and DMAP in acetonitrile followed by aqueous ammonia solution to give cytosine derivative **19** which was deprotected with TBAF to give cytosine nucleoside **20** (Scheme 5.2).

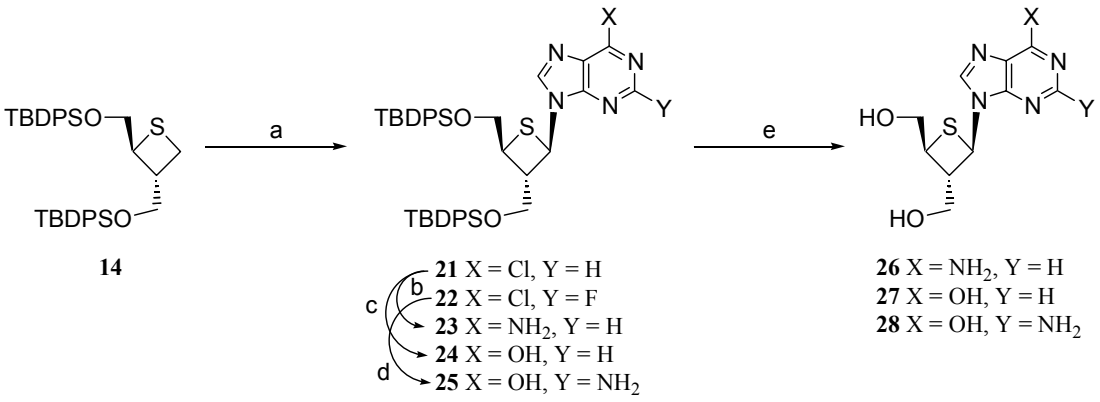


[a]keys a) i. mCPBA, CH_2Cl_2, ii. thymine or uracil, HMDS, CH_3CN; TMSOTf, TEA, ZnI_2, toluene, b) TBAF, THF, c) 2,4,6-triisopropylbenzenesulfonyl chloride, TEA, DMAP, CH_3CN; NH_4OH

Scheme 5.2. Synthesis of 3'-thio pyrimidine analogs

Compound **14** was condensed with 6-chloropurine or 6-chloro-2-fluoropurine to give compounds **21** and **22**. (Scheme 5.3). Compounds **21** and **22** as well as their α-isomers were obtained in 40-45% yield with a β/α ratio of 5:4. Compound **21** was converted to

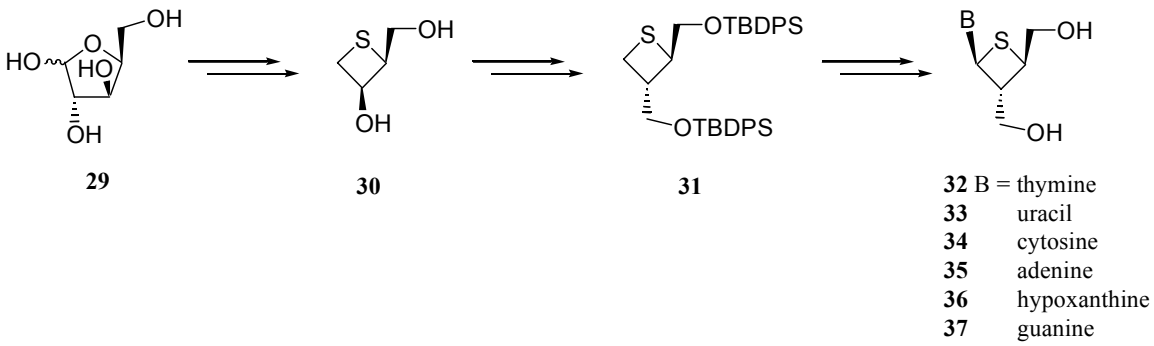
adenine derivative **23** in 72% yield by using NaN_3 in DMF followed by hydrogenolysis with 10 % Pd(0) on carbon in methanol. Compound **21** was also converted to inosine analog **24** in 85% yield by treatment with NaOMe and 2-mercaptoethanol in methanol. Compound **22** was converted to guanosine analog **25** in 74 % yield by treatment with NaOMe and 2-mercaptoethanol in methanol followed by amination in ethanolic ammonia at 60°C. Compounds **23**, **24** and **25** were deprotected using TBAF to give final nucleosides **26**, **27** and **28** in 89-94 % yield.



[a]keys a) i. mCPBA, CH_2Cl_2, ii. 6-chloropurine or 6-cholor-2-fluoropurine, HMDS, CH_3CN; TMSOTf, TEA, ZnI_2, toluene, b) i. NaN_3, DMF, ii. H_2, Pd(0), MeOH, c) NaOMe, 2-mecaptoethanol, MeOH, d) i. NaOMe, 2-mecaptoethanol, MeOH, ii. NH_3, EtOH, 60°C, e) TBAF, THF

Scheme 5.3. Synthesis of 3'-thio purine analogs

L-3'-Thio analogs of oxetancin A were were also synthesized under similar reaction conditions starting from the opposite enantiomer, L-xylose **29** (Scheme 5.4). L-Xylose **29** was converted to the key intermediate **31**, an enantiomer of compound **14**, which was obtained through the thietane diol **30**, an enantiomer of compound **7**. After coupling of **31** with the appropriately silylated pyrimidine and purine bases, L-3'-thio analogs (**32-37**)of oxetanocin A were obtained using the same procedures for D-isomers.



Scheme 5.4. Synthesis of L-3'-thio analogs of oxetanocin A

Anti-HIV activity. The antiviral activity of the synthesized D- and L-nucleosides (**17**, **18**, **20**, **26**-**28** and **32**-**37**) was evaluated against HIV-1 in human PBM cells *in vitro* with using AZT as a positive control and the results are summarized in Table 5.1.

Table 5.1. Anti-HIV activity of D- and L-3'-thio analogs of oxetanocin A

Compd	Conf.	Base	HIV (PBM)		Toxicity (IC_{50}, μM)		
			EC_{50} (μM)	EC_{90} (μM)	PBM	CEM	vero
17	D	Thymine	>100	>100	>100	>100	>100
18	D	Uracil	6.9	23.1	8.5	4.9	>100
20	D	Cytosine	1.3	5.3	≤1.0	4.3	54.8
26	D	Adenine	>100	>100	>100	>100	>100
27	D	Hypoxanthine	>100	>100	>100	>100	>100
28	D	Guanine	>100	>100	>100	>100	>100
32	L	Thymine	>100	>100	>100	>100	>100
33	L	Uracil	>100	>100	>100	>100	>100
34	L	Cytosine	14.1	47.6	13.1	45.6	>100
35	L	Adenine	>100	>100	>100	>100	>100
36	L	Hypoxanthine	>100	>100	>100	>100	>100
37	L	Guanine	>100	>100	>100	>100	>100
AZT			0.004	0.01	>100	14.3	29.0

Among the synthesized nucleosides, D-uridine (**18**), D-cytidine (**20**) and L-cytidine (**34**) analogs show anti-HIV activity with EC_{50}s of 6.9, 1.3 and 14.1 μM, respectively, but these three nucleoside analogs are cytotoxic in PBM cells and CEM cells. However, the other nucleosides show neither anti-HIV activity nor cytotoxicity up to 100 μM. It is notable that compounds **17**, **18** and **32** are pyrimidine nucleosides with four-membered ring sugar moiety to show antiviral activity. Only a few pyrimidine nucleosides with four-membered rings show antiviral activity such as the thymidine analog of oxetanocin A and its carbocyclic 5-(2-halovinyl)uridine analogs.

Molecular modeling studies. The molecular modeling studies of synthesized 3'-thioadenosine **26** as well as the parent nucleoside, oxetanocin A, were conducted to compare the conformations of these two nucleosides to the active conformation of the thymidine triphosphate in the crystal structure of HIV-1 reverse transcriptase (PDB code 1RTD).[309] The most stable conformation of 3'-thionucleoside **26** was obtained by a Monte Carlo conformational search using the molecular graphics and simulation program MacroModel, version 7.0 (Schrödinger, Inc.). The crystal structure of oxetanocin A was obtained from the Cambridge Structural Database. The three conformational structures of 3'-thionucleoside **26**, oxetanocin A and TTP were superimposed using Sybyl 6.7 in Fig. 5.2. The C3'-C4'bond (in blue) in 3'-thionucleoside **26** is displaced by 0.6 Å from the C4'-C5'bond (in orange) in TTP and 3'-sulfur atom in 3'-thionucleoside **26** extrudes from the 3'-oxygen in oxetanocin A and the 4'-oxygen in TTP. Another noticeable feature is that 2'-hydroxymethyl groups of thietanose adenosine **26** and oxetanocin A are located in almost the same position as the 3'-hydroxy of TTP. It is well known that there is a 3'-hydroxy pocket for natural substrates (dNTP) in the active site of HIV RT,

strengthening the binding of natural substrates to the active site through hydrogen bonding. This 3'-hydroxy pocket also plays an important role in binding of AZTTP to HIV RT, which was demonstrated in our previous molecular modeling studies. Thus, the 2'-hydroxymethyl groups of thietanose adenosine **24** might be nicely located in the 3'-OH pocket, which could favorably contribute to their binding affinity to RT.

However, D-adenosine analog **26** does not show any anti-HIV activity. Instead, D-cytidine analog **20** is active against HIV, which has the same sugar conformation as D-adenosine analog **26** based on the Monte Carlo conformational search. To understand the difference of anti-HIV activity between the two nucleoside analogs, molecular modeling studies using the crystal structure of HIV-1 RT (PDB code 1RTD) were conducted replacing TTP with the corresponding triphosphates of the nucleoside analogs **20** and **26**. Each triphosphate was docked to the active site of HIV-1 RT and the resulting complex was minimized using Kollman-All Atom force field. In the minimized structures, both nucleoside triphosphates are well bound to the active site of HIV-1 RT without any steric hindrance with amino acid residues of the active site of RT. As expected, each hydroxy moiety of 2'-hydoxymethyl group ($-CH_2OH$) of the two thietanose nucleoside triphosphates is hydrogen-bonded to Tyr 115 of 3'-hydroxy pocket in the active site of HIV RT as well as their own β-phospahte (Figure 5.3). From the molecular modeling results, it could be suggested that there would be no difference in the ability of both the nucleosides triphospahtes to bind to the active site of HIV RT. Thus, the lack of anti-HIV activity of the adenosine analog **26** might result from other cellular factors such as discrimination by kinases which are responsible for the metabolism to the triphosphates of the nucleoside analogs.

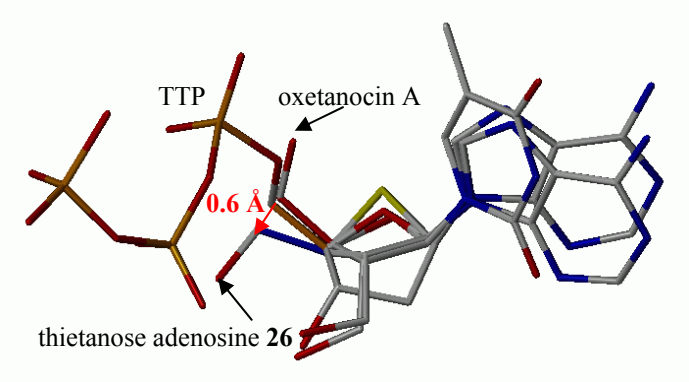


Figure 5.2. Superimposed structures of 3'-thionucleoside **26**, oxetanocin A and TTP where the C3'-C4' bond in 3'-thionucleoside **26** is in blue, the C3'-C4' bond in oxetanocin A is in red, and the C4'-C5' bond in TTP is in orange.

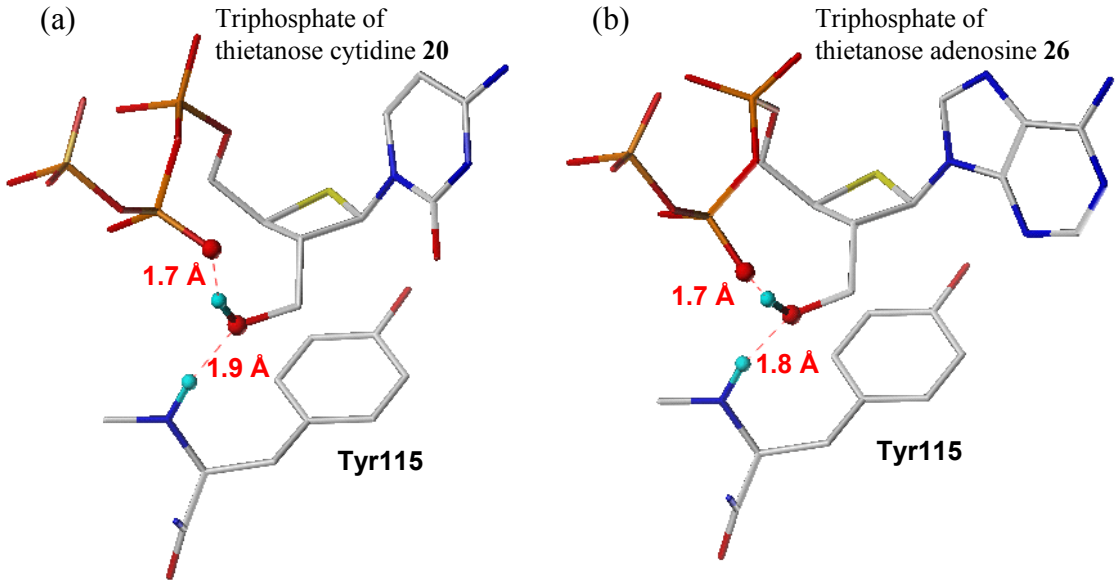


Figure 5.3. The minimized structures of HIV reverse transcriptase complexed with (a) the triphospahte of cytidine analog **20** and (b) the triphosphate of adenosine analog **26** show that both triphosphates are bound to the active site of HIV RT, interacting with Tyr115 and their own β-phosphate through hydrogen bonding.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), quin. (quintet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

1,2-*O*-Isopropylidene-3,5-di-*O*-mesyl-α-D-ribofuranoside (5). A solution of compound **4** (33.2g, 175 mmol) and pyridine (56.6 mL, 700 mmol) in CH_2Cl_2 (500 mL) was treated with MsCl (33.8 mL, 437 mmol) and DMAP (4.3g, 35 mmol) at 0°C for 18 h. The reaction mixture was diluted with CH_2Cl_2 (500 mL), washed with 1N HCl, aq $NaHCO_3$, and brine, and dried over $MgSO_4$. The filtrate was concentrated and crystallized with a 1:10 mixture of CH_2Cl_2 and hexanes to give the product **5** (29.3g, 0.0816 mol, 90%) as a white solid: mp 115~116°C; $[\alpha]^{22}_{D}$ 77.8° (c 0.43, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.84 (d, *J* = 3.4 Hz, 1H), 4.82~4.74 (m, 2H), 4.55 (dd, *J* = 11.8, 1.9 Hz, 1H), 4.41~4.30 (m, 2H), 3.16 (s, 3H), 3.08 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 114.11, 103.98,

77.24, 74.96, 74.86, 66.14, 38.66, 37.63, 26.61, 26.53; Anal. ($C_{10}H_{18}O_9S_2$) C, H. (for the detailed ^{1}H NMR and elemental analysis data, see Tables 5.2 and 5.3)

5-*S*-Acetyl-1,2-*O*-isopropylidene-3-*O*-mesyl-α-D-ribofuranoside (6). A solution of compound **5** (81.5g, 235 mol) in DMF (400 mL) was treated with KSAc (29.5g, 258 mmol) at room temperature. After 16 h, the reaction mixture was diluted with EtOAc, washed with water (2 X 1 L), and dried over $MgSO_4$. The filtrate was concentrated and purified with 20% EtOAc in hexanes by silica gel column chromatography to give product **6** (62.1g, 190 mmol, 81%) as a brownish solid: mp 112~113°C; $[\alpha]^{23}_D$ 68.5 ° (c 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.77 (d, *J* = 3.7 Hz, 1H), 4.75 (t, *J* = 4.2 Hz, 1H), 4.48 (dd, *J* = 8.8, 4.6 Hz, 1H), 4.31~4.25 (m, 1H), 3.35 (dd, *J* = 14.5, 4.1 Hz, 1H), 3.23 (*J* = 14.5, 5.1 Hz, 1H), 3.16 (s, 3H), 2.36 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 194.60, 113.72, 103.82, 77.88, 77.40, 75.45, 38.77, 30.43, 29.29, 26.55, 26.51; Anal. ($C_{11}H_{18}O_7S_2$) C, H, S.

3,5-Thioanhydro-1,2-*O*-isopropylidene-α-D-xylofuranoside (7). A mixture of EtOH (100 mL) and water (5 mL) was refluxed under Ar for 2 h and cooled. Compound **6** (2.12g, 6.50 mmol) and solid $NaHCO_3$ (1.09g, 13.0 mmol) was added to the solvent and the resulting mixture was refluxed for 8 h. The reaction mixture was cooled, diluted with Et_2O, filtered, and purified with 5% Et_2O in hexanes by silica gel column chromatography to give the product **7** (1.20g, 6.37 mmol, 98%) as oil: $[\alpha]^{22}_D$ 78.7° (c 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.41 (d, *J* = 3.4 Hz, 1H), 5.26 (t, *J* = 5.3 Hz, 1H), 4.71 (d, *J* = 3.4 Hz, 1H), 4.08 (d, *J* = 5.3 Hz, 1H), 3.49 (dd, *J* = 10.6, 5.4 Hz, 1H), 2.84 (d, *J* = 10.6 Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 113.82, 108.11, 86.68, 82.78, 46.19, 31.97, 27.86, 27.27; Anal. ($C_8H_{12}O_3S$) C, H, S.

3,5-Thioanhydro-α-D-xylofuranose (8). Compound **7** (1.10g, 5.31 mmol) was with 4% TFA solution in water at room temperature for 8 h, the reaction mixture was neutralized with Amberite (OH) resin which was prewashed with water (2X100 mL). After filtration, the filtrate was concentrated and purified with 50% EtOAc in hexanes by silica gel column chromatography to give the product **8** (0.750g, 5.06 mmol, 95%): ^{1}H NMR ($CDCl_3$) for major δ 6.11 (d, *J* = 2.3 Hz, 1H), 5.16~5.12 (m, 1H), 4.17 (d, *J* = 2.5 Hz, 1H), 3.95 (d, *J* = 5.9 Hz, 1H), 3.50 (dd, *J* = 10.9, 6.3 Hz, 1H), 3.00 (d, *J* = 10.9 Hz, 1H), for minor δ 5.56 (s, 1H), 5.29~5.24 (m, 1H), 4.37 (s, 1H), 3.95 (d, *J* = 5.9 Hz, 1H), 3.64 (dd, *J* = 10.9, 6.1 Hz, 1H), 3.12 (d, *J* = 10.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) for major δ 98.95, 78.42, 75.92, 47.79, 32.58, for minor δ 107.76, 82.96, 80.27, 47.73, 33.72; Anal. ($C_5H_8O_3S$) C, H, S.

(*2R*,*3S*)-2-Hydroxymethyl-thietan-3-ol (9). A solution of compound **8** (1.92g, 12.9 mmol) in MeOH (50 mL) was treated with an aqueous solution of $NaIO_4$ (3.04g, 14.2 mmol) in water (25 mL) dropwise at 0^{o}C for 10 min with vigorous stirring. After 20 min, well-grounded $NaBH_4$ was added portionwise to the reaction mixture at 0^{o}C. After 30 min, the resulting mixture was filtered and the filtrate was neutralized with 1N HCl and concentrated. The residue was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1) and the white solid was filtered off. The filtrate was concentrated and purified with 3~5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the products **9** (1.21g, 10.1 mmol, 78%): $[\alpha]^{24}_{D}$ 166.2^{o} (c 0.63, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.00 (quin. *J* = 8.1 Hz, 1H), 4.09~3.98 (m, 2H), 3.85 (d, *J* = 8.9 Hz, 1H), 3.76~3.71 (m, 1H), 3.37~3.24 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 68.89, 62.70, 51.31, 37.56; Anal. ($C_4H_8O_2S{\cdot}0.35H_2O$) C, H, S.

(*2R,3S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-ol (10). A solution of compound **9** (5.00g, 41.6 mmol), TEA (11.6 mL, 83.2 mmol), and DMAP (0.51g, 4.2 mmol) in CH_2Cl_2 was treated with TBDPSCl (9.8 mL, 37.4 mmol) at 0°C for 2 h. The resulting mixture was concentrated and purified with 5% EtOAc in hexanes by silica gel column chromatography to give the product **10** (12.7g, 35.3 mmol, 85%) as oil: $[\alpha]^{20}_D$ 74.1° (c 0.91, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.84~7.38 (m, 10H), 5.06 (quin, *J* = 8.1 Hz, 1H), 4.19~4.10 (m, 2H), 3.88 (d, *J* = 9.7 Hz, 1H), 3.73~3.67 (m, 1H), 3.54 (t, *J* = 8.3 Hz, 1H), 3.33 (t, *J* = 8.3 Hz, 1H), 1.12 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.89, 135.54, 132.58, 132.09, 130.07, 130.04, 127.95, 127.87, 69.62, 65.12, 50.48, 38.11, 26.84, 19.19.; Anal. ($C_{20}H_{26}O_2SSi$) C, H, S.

***tert*-Butyl-[(*2S*)-3-methylene-thietan-2-ylmethoxy]-diphenylsilane (11).** Compound **10** (12.7g, 35.3 mmol) was treated with DMSO (45 mL) and Ac_2O (30 mL) at room temperature for overnight. The reaction mixture was quenched with ice, diluted with Et_2O (500 mL), washed with water. The organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified with 3% EtOAc in hexanes by silica gel column chromatography to give the intermediate ketone (11.5g, 32.3 mmol, 92%) as oil.

A mixture of titanocene dichloride (38.7g, 115 mmol) in Et_2O (1 L)was treated with 1.6 M MeLi solution (214 mL) in Et_2O at room temperature for 2 h. The reaction mixture was quenched with ice and extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. This reagent (Petasis reagent) was dissolved in THF (80 mL). A solution of the intermediate ketone (10.0g, 28.2 mmol) in Et_2O (50 mL) was treated with the freshly prepared Petasis reagent solution. The resulting mixture was refluxed at 60°C bath temperature. After 48 h, the reaction mixture

was cooled to room temperature, diluted with hexanes, and filtered through the silica gel pad. The filtrate was concentrated and purified with 2% EtOAc in hexanes by silica gel column chromatography to give the product **11** (4.12g, 11.6 mmol, 41%) as oil: $[\alpha]^{22}_{D}$ 73.6^{o} (c 0.80, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.72~7.65 (m, 4H), 7.47~7.37 (m, 6H), 4.86 (d, *J* = 2.1 Hz, 1H), 4.71 (d, *J* = 2.1 Hz, 1H), 4.44~4.37 (m, 1H), 4.00 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.87 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.84~3.74 (m, 2H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 146.13, 135.67, 135.64, 133.48, 133.39, 129.71, 129.70, 127.69, 109.08, 68.12, 52.85, 33.28, 26.79, 19.27; Anal. ($C_{21}H_{26}OSSi$) C, H, S.

[(*2S,3R*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (12). A solution of compound **11** (4.12g, 11.5 mmol) in THF (200 mL) was treated with 2M BH_3SMe_2 solution (11.5 mL) in THF at 0^{o}C for 30 min. The reaction mixture was warmed up to room temperature and stirred for 2 h. The reaction mixture was quenched with 3% H_2O_2 (26 mL) and 1N NaOH (23 mL). Afher 2 h, the reaction mixture was treated with sat. $Na_2S_2O_3$, and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The filtrate was concentrated and purified with 10% EtOAc in hexanes by silica gel column chromatography to give the product **12** (3.35g, 8.99 mmol, 78% yield) as well as compound **13** (0.51g, 1.37 mmol, 12% yield): $[\alpha]^{22}_{D}$ 43.4^{o} (c 1.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.78~7.32 (m, 10H), 4.28 (t, *J* = 10.1 Hz, 1H), 4.01 (t, *J* = 9.9 Hz, 1H), 3.80~3.42 (m, 5H), 3.02~2.83 (m, 2H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.50, 135.46, 132.24, 130.09, 127.92, 65.00, 62.74, 43.39, 42.21, 26.73, 24.23, 19.06; Anal. ($C_{21}H_{28}O_2SSi$) C, H, S.

[(*2R,3S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (13). Oxalyl chloride (1.56 mL, 17.9 mmol) was added dropwise to a solution of DMSO (1.90

mL, 26.8 mmol) in CH_2Cl_2 (30 mL) at -78°C. After 10 min, a solution of compound **12** (3.33g, 8.94 mmol) in CH_2Cl_2 (20 mL) was added to the reaction mixture at -78°C. After 20 min, TEA (5.0 mL, 36 mmol) was added at -78°C. After 10 min, the reaction mixture was warmed up to room temperature for 1 h. The resulting mixture was diluted with CH_2Cl_2, and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. The aldehyde was dissolved in MeOH (30 mL), and treated with NaOMe (1.45g, 26.8 mmol) for 16 h. The resulting mixture was treated with $NaBH_4$ (1.01g, 26.7 mmol) for 2 h. The reaction mixture was neutralized with 1N HCl, concentrated to a tenth volume of the solution, extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, concentrated, filtered and purified by silica get column chromatography with 10% EtOAC in hexanes to give the product **13** (1.58g, 4.24 mmol, 47% yield): $[\alpha]^{24}_D$ 40.7° (c 0.81, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.70~7.36 (m, 10H), 3.82~3.58 (m, 5H), 3.22~3.10 (m, 1H), 3.01~2.93 (m, 2H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.59, 135.57, 132.84, 132.77, 130.08, 129.92, 127.93, 127.81, 68.55, 65.75, 46.75, 44.57, 26.77, 23.58, 19.14; Anal. ($C_{21}H_{28}O_2SSi$) C, H, S.

(*2R,3S*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietane (14). A solution of compound **13** (2.00g, 5.37 mmol) and imidazole (0.77g, 11 mmol) in CH_2Cl_2 was treated with TBDPSCl (1.54 mL, 5.92 mmol) at room temperature for 3 h. The reaction mixture was conceontrated and purified with 2% EtOAc in hexanes to give the product **14** (3.10g, 5.07 mmol, 94%) as oil: $[\alpha]^{22}_D$ 45.0° (c 0.38, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.70~7.33 (m, 20H), 3.90 (dd, *J* = 9.7, 6.1 Hz, 1H), 3.77 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.73~3.65 (m, 3H), 3.16~2.95 (m, 3H), 1.05 (s, 9H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.61, 135.56,

133.51, 129.70, 129.66, 127.72, 127.68, 68.64, 66.25, 44.64, 44.10, 26.85, 26.80, 23.97, 19.31, 19.27; Anal. ($C_{37}H_{46}O_2SSi_2$) C, H, S.

1-[(*2R*,*3S*,*4R/S*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-thymine (15). A solution of compound **14** (0.250g, 0.409 mmol) in CH_2Cl_2 was treated with a solution of 77% mCPBA (0.092g, 0.411 mmol) in CH_2Cl_2 dropwise at -78^{o}C. The mixture was stirred at -78^{o}C for 30 min. The reaction was quenched with aq. $NaSSO_3$, diluted with CH_2Cl_2, washed with aq $Na_2S_2O_3$, aq $NaHCO_3$, 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 thymine (0.155g, 1.23 mmol) in acetonitrile (10 mL) and HMDS (10 mL) was refluxed for 3 h until the solution became clear. After evaporation, the residue was treated successively with a solution of the crude sulfoxide in toluene (10 mL), TEA (0.11 mL, 0.79 mmol), TMSOTf (0.16 mL, 0.88 mmol), and ZnI_2 (0.039g, 0.12 mmol) at 0^{o}C. After 24 h, the reaction was quenched with water at 0^{o}C and the reaction mixture was diluted with CH_2Cl_2. The organic layer was dried over $NaSO_4$, filtered, concentrated and purified with 30% EtOAc in hexane by preparative TLC to give the product **15** (0.138g, 0.188 mmol, 46%) as an α/β mixture (1:3): UV(CH_2Cl_2) λ_{max} 270.0 nm; ^{1}H NMR ($CDCl_3$) δ 9.03, 8.99 (2br s, 1H), 7.98, 7.80 (2s, 1H), 7.72~7.30 (m, 20H), 6.30 (d, *J* = 6.8 Hz, 0.75H), 6.20 (d, *J* = 7.7 Hz, 0.25H), 3.88 (dd, *J* = 9.8, 5.9 Hz, 0.25H), 3.81~3.45 (m, 5H), 3.26~3.19 (m, 0.75H), 1.87, 1.83 (2s, 3H), 1.07, 1.044, 1.036, 0.98 (4s, 18H); Anal. ($C_{42}H_{50}N_2O_4SSi_2$) C, H, N, S.

1-[(*2R*,*3S*,*4R/S*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-uracil (16). See the procedure for condensation reaction of the compound **14** with thymine. The title compound **16** was obtained on 0.301-mmol scale in 40% yield: UV(CH_2Cl_2) λ_{max}

265.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.58, 8.53 (2br s, 1H), 8.19 (d, *J* = 7.8 Hz, 0.3H), 8.16 (d, *J* = 7.8 Hz, 0.7H), 7.70~7.33 (m, 20H), 6.24 (d, *J* = 6.8 Hz, 0.7H), 6.20 (d, *J* = 7.8 Hz, 0.3H), 5.69 (d, *J* = 8.8 Hz, 0.3H), 5.60 (d, *J* = 7.8 Hz, 0.7H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H); Anal. ($C_{41}H_{48}N_2O_4SSi_2$) C, H, N, S.

1-[(*2R*,3*S*,*4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-thymine (17). A solution of compound **15** (0.130g, 0.177 mmol) in THF (10 mL) was treated with 0.35 mL of 1M TBAF solution in THF at 0^{o}C. After 3 h, the reaction mixture was concentrated and purified by prep TLC with 7% MeOH in CH_2Cl_2 to give compound **17** (0.029g, 0,112 mmol, 63% yield) and α-isomer (0.011g, 0.043 mmol, 24% yield): mp >200^{o}C (dec.); $[\alpha]^{26}_D$ -493^o (c 0.050, MeOH:DMF (1:1)); UV(H_2O) λ_{max} 264.0 nm (ε 9,400, pH 2). 262.0 nm (ε 9,900, pH 7), 265.5 nm (ε 7,100, pH 11); ^{1}H NMR (MeOH) δ 8.30 (s, 1H), 6.08 (d, *J* = 6.8 Hz, 1H), 3.76 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.72 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.58 (d, *J* = 3.9 Hz, 2H), 3.54~3.49 (m, 1H), 3.32~3.26 (m, 1H), 1.95 (s, 3H); Anal. ($C_{10}H_{14}N_2O_4S$) C, H, N, S.

1-[(*2R*,3*S*,*4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-uracil (18). See the procedure for deprotection reaction of the compound **17** with 1M TBAF solution. The title compound **18** was obtained on 0.083-mmol scale in 59% yield: mp >200^{o}C (dec.); $[\alpha]^{22}_D$ -35.4^o (c 0.17, MeOH); UV(H_2O) λ_{max} 265.5 nm (ε 9,200, pH 2), 265.5 nm (ε 8,500, pH 7), 265.5 nm (ε 6,900, pH 11); ^{1}H NMR (MeOH) δ 8.46 (d, J = 7.8 Hz, 1H), 6.07 (d, J = 6.8 Hz, 1H), 5.81 (d, J = 7.8 Hz, 1H), 3.76 (dd, J = Hz, 1H), 3.71 (dd, J = Hz, 1H), 3.60 (d, J = Hz, 2H), 3.54~3.49 (m, 1H), 3.28~3.21 (m, 1H); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

1-[(*2R*,*3S*,*4R/S*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-cytosine (19). A solution of compound **16** (0.120g, 0.166 mmol) in acetonitrile was treated with TEA (0.07 mL, 0.50 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (0.151g, 0.499 mmol), and DMAP (0.061 g, 0.50 mmol) at room temperature. After 16 h, ammonia water was added to the resulting mixture. After 3h, the mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography with 5% MeOH in CH_2Cl_2 to give a α/β mixture of compound **19** (0.090g, 0.125 mmol, 75% yield): UV(CH_2Cl_2) λ_{max} 285.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.31 (d, *J* = 7.8 Hz, 0.3H), 8.25 (d, *J* = 7.8 Hz, 0.7H), 7.69~7.28 (m, 20H), 6.26 (d, *J* = 6.8 Hz, 0.7H), 6.24 (d, *J* = 7.8 Hz, 0.3H), 5.62 (d, *J* = 6.8 Hz, 0.3H), 5.58 (d, *J* = 6.8 Hz, 0.7H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H); Anal. ($C_{41}H_{49}N_3O_3SSi_2$) C, H, N, S.

1-[(*2R*,3*S*,*4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-cytosine (20). See the procedure for deprotection reaction of the compound **17** with 1M TBAF solution. The title compound **20** was obtained on 0.118-mmol scale in 59% yield:mp 125~126°C; $[\alpha]^{23}_D$ -59.2° (c 0.14, MeOH); UV(H_2O) λ_{max} 282.5 nm (ε 12,500, pH 2), 274.5 nm (ε 9,700, pH 7), 275.5 nm (ε 9,600, pH 11); ^{1}H NMR (MeOH) δ 8.43 (d, *J* = 6.8 Hz, 1H), 6.04 (s, *J* = 6.8 Hz, 1H), 6.00 (d, *J* = 7.8 Hz, 1H), 3.75 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.66~3.58 (m, 1H), 3.51~3.47 (m, 1H), 3.14 (quin, *J* = 6.8 Hz, 1H); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

9-[(*2R*,3*S*,*4R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloropurine (21). See the procedure for condensation reaction of the compound **14** with thymine. The title compound **21** as well as α-isomer were obtained on 0.622-mmol scale

in 45% yield as a 5:4 mixture of compound **21** and α-isomer: $[\alpha]^{23}_{D}$ 16.8^{o} (c 0.65, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.87 (s, 1H), 8.86 (s, 1H), 7.66~7.27 (m, 20H), 6.56 (d, *J* = 7.1 Hz, 1H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.05, 152.54, 148.07, 142.71, 135.55, 135.48, 135.44, 132.77, 132.70, 132.54, 130.07, 130.04, 130.2, 127.91, 127.89, 127.86, 127.84, 66.50, 62.92, 55.33, 50.79, 37.60, 26.86, 26.77, 19.23, 19.17; Anal. ($C_{42}H_{47}ClN_4O_2SSi_2$) C, H, N, S.

9-[(*2R*,3*S*,*4R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloro2-fluoropurine (22). See the procedure for condensation reaction of the compound **14** with thymine. The title compound **22** as well as α-isomer were obtained on 0.797-mmol scale in 40% yield as a 5:4 mixture of compound **22** and α-isomer: $[\alpha]^{24}_{D}$ 9.3^{o} (c 0.21, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 254.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1H), 7.64~7.24 (m, 20H), 6.47 (d, *J* = 6.8 Hz, 1H), 3.83~3.65 (m, 5H), 3.36~3.28 (m, 1H), 1.06 (s, 9H), 1.03 (s, 9H); Anal. ($C_{42}H_{46}ClFN_4O_2SSi_2$) C, H, N, S.

9-[(*2R*,3*S*,*4R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-adenine (23). A solution of compound **21** (0.202g, 0.265 mmol) in DMF (2 mL) was treated with NaN_3 (0.172g, 2.65 mol) at room temperature for 16h. The resulting mixure was directly filtered through silica gel pad using eluent as a 1:1 mixture of hexanes and EtOAc. The filtrate was concentrated to dryness. A solution of the crude azide in MeOH (15 mL) was treated with 0.030g of Pd(0)/C and H_2 balloon for 48 h. The resulting mixture was filtered through celite pad and the filtrate was concentrated and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **23** (0.143g, 0.192 mmol, 72% yield): $[\alpha]^{23}_{D}$ 41.5^{o} (c 0.17, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 272.0 nm; ^{1}H NMR ($CDCl_3$)

δ 8.44 (s, 1H), 7.85 (s, 1H), 7.66~7.30 (m, 20H), 6.06 (br s, 2H), 6.03 (d, *J* = 8.6 Hz, 1H), ;3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.64, 153.31, 150.83, 145.57, 135.51, 135.40, 135.30, 132.76, 132.57, 132.30,132.19, 130.20, 130.13, 130.04, 130.00, 127.97, 127.88, 127.82, 65.87, 61.96, 56.65, 51.77, 38.09, 26.87, 26.78, 19.16; Anal. ($C_{42}H_{49}N_5O_2SSi_2$) C, H, N, S.

9-[(*2R*,3*S*,*4R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-hypoxanthine (24). A solution of compound **21** (0.134g, 0.176 mmol), 2-mercaptoethanol (0.049 mL, 0.70 mmol) and NaOMe (0.038g, 0.70 mmol) in MeOH was refluxed for 6 h. The mixture was cooled to room temperature, neutralized with AcOH, concentrated, and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **24** (0.112g, 0.150 mmol, 85% yield): $[\alpha]^{24}_D$ 27.9^o (c 0.21, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 262.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H), 7.72 (s, 1H), 7.68~7.12 (m, 20H), 6.56 (d, *J* = 5.9 Hz, 1H), 3.89 (dd *J* = 10.7, 5.9 Hz, 1H), 3.85~3.68 (m, 4H), 3.39~3.32 (m, 1H), 1.05 (s, 9H), 1.01 (s, 9H); Anal. ($C_{42}H_{48}N_4O_3SSi_2$) C, H, N, S.

9-[(*2R*,3*S*,*4R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-guanine (25). A solution of compound **22** (0.138g, 0.177 mmol), 2-mercaptoethanol (0.049 mL, 0.70 mmol) and NaOMe (0.019g, 0.35 mmol) in MeOH was refluxed for 4 h. The mixture was cooled to room temperature, neutralized with AcOH, concentrated, and filtered through silica gel pad. The filtrate was concentrated to dryness. The residue was treated with ethanolic ammonia at 60^{o}C for 6 h. The reaction mixture was concentrated and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **25** (0.115g, 0.151 mmol, 74% yield): $[\alpha]^{24}_D$ 17.8^o (c 0.12, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H), 7.75~7.24 (m, 20H), 6.36 (d, *J* = 6.8 Hz,

1H), 3.95~3.63 (m, 5H), 3.37~3.30 (m, 1H), 1.04 (s, 9H), 0.99 (s, 9H); Anal. ($C_{42}H_{49}N_5O_3SSi_2$) C, H, N, S.

9-[(*2R,3S,4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-adenine (26). See the procedure for deprotection reaction of the compound **15** with 1M TBAF solution. The title compound **26** was obtained on 0.188-mmol scale in 94% yield: mp >200°C; $[\alpha]^{25}_D$ 95.2° (c 0.057, MeOH); UV(H_2O) λ_{max} 272.5 nm (ε 15,000, pH 2). 270.5 nm (ε 10,000, pH 7), 271.5 nm (ε 10,000, pH 11); ^{1}H NMR (MeOH) δ 8.84 (s, 1H), 8.24 (s, 1H), 6.17 (d, *J* = 6.6 Hz, 1H), 3.80 (d, *J* = 4.6 Hz, 2H), 3.73 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H); Anal. ($C_{10}H_{13}N_5O_2S$) C, H, N, S.

9-[(*2R,3S,4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-hypoxanthine (27). See the procedure for deprotection reaction of the compound **15** with 1M TBAF solution. The title compound **27** was obtained on 0.150-mmol scale in 89% yield: mp 213~214°C; $[\alpha]^{26}_D$ 22.0° (c 0.13, MeOH); UV(H_2O) λ_{max} 256.5 nm (ε 7,600, pH 2). 258.0 nm (ε 7,200, pH 7), 263.0 nm (ε 7,600, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 8.91 (s, 1H), 8.02 (s, 1H), 6.36 (d, *J* = 5.9 Hz, 1H), 3.83 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.78 (dd, *J* = 11.7, 4.9 Hz, 1H),3.77~3.70 (m, 2H), 3.60 (q, *J* = 4.9 Hz, 1H), 3.48 (quin, *J* = 5.9 Hz, 1H); Anal. ($C_{10}H_{12}N_4O_3S$) C, H, N, S.

9-[(*2R,3S,4R*)-2,3-Dihydroxymethyl-thietan-4-yl]-guanine (28). See the procedure for deprotection reaction of the compound **15** with 1M TBAF solution. The title compound **28** was obtained on 0.132-mmol scale in 91% yield: mp >200°C (dec.); $[\alpha]^{23}_D$ -73.7° (c 0.11, DMSO); UV(H_2O) λ_{max} 251.5 nm (ε 7,300, pH 2). 266.0 nm (ε 6,900, pH 7), 266.0 nm (ε 7,100, pH 11); ^{1}H NMR (DMSO-*d*$_6$) δ 13.48 (br s, 1H), 8.79 (s, 1H), 6.23 (d, *J* =

6.8 Hz, 1H), 5.07 (t, J = 4.9 Hz, 1H), 4.99 (br s, 1H), 3.79~3.46 (m, 5H), 3.30~2.26 (m, 1H); Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N, S.

1-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-thymine (32). mp >200°C (dec.); $[\alpha]^{26}_D$ 82.8° (c 0.13, DMSO); Anal. ($C_{10}H_{14}N_2O_4S$) C, H, N, S.

1-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-uracil (33). mp >200°C (dec.); $[\alpha]^{22}_D$ 31.2° (c 0.23, MeOH); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

1-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-cytosine (34). mp 126~127°C; $[\alpha]^{23}_D$ 64.7° (c 0.11, MeOH); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

9-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-adenine (35). mp >200°C (dec.); $[\alpha]^{25}_D$ -93.3° (c 0.11, MeOH); Anal. ($C_{10}H_{13}N_5O_2S$) C, H, N, S.

9-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-hypoxanthine (36). mp 213~214°C; $[\alpha]^{26}_D$ -23.9° (c 0.13, MeOH); Anal. ($C_{10}H_{12}N_4O_3S$) C, H, N, S.

9-[(*2S,3R,4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-guanine (37). mp >200°C (dec.); $[\alpha]^{23}_D$ 70.7° (c 0.12, DMSO); Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N, S.

Table 5.2. ^{1}H NMR Data

Compd	H-1'	H-5/6 (for pyrimidines)	H-2/8 (for purines)
15[a]	6.30 (d, *J* = 6.8 Hz, 0.75H) 6.20 (d, *J* = 7.7 Hz, 0.25H)	7.98, 7.80 (2s, 1H)	-[b]
	Other protons	9.03, 8.99 (2br s, 1H), 7.98, 7.80 (2s, 1H), 7.72~7.30 (m, 20H), 3.88 (dd, *J* = 9.8, 5.9 Hz, 0.25H), 3.81~3.45 (m, 5H), 3.26~3.19 (m, 0.75H), 1.87, 1.83 (2s, 3H), 1.07, 1.044, 1.036, 0.98 (4s, 18H)	
16[a]	6.24 (d, *J* = 6.8 Hz, 0.7H) 6.20 (d, *J* = 7.8 Hz, 0.3H)	8.19 (d, *J* = 7.8 Hz, 0.3H) 8.16 (d, *J* = 7.8 Hz, 0.7H) 5.60 (d, *J* = 7.8 Hz, 0.7H) 5.69 (d, *J* = 8.8 Hz, 0.3H)	-[b]
	Other protons	8.58, 8.53 (2br s, 1H), 7.70~7.33 (m, 20H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H),3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H)	
17[c]	6.08 (d, *J* = 6.8 Hz, 1H)	8.30 (s, 1H)	-[b]
	Other protons	3.76 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.72 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.58 (d, *J* = 3.9 Hz, 2H), 3.54~3.49 (m, 1H), 3.32~3.26 (m, 1H), 1.95 (s, 3H)	
18[c]	6.07 (d, J = 6.8 Hz, 1H)	8.46 (d, J = 7.8 Hz, 1H) 5.81 (d, J = 7.8 Hz, 1H)	-[b]
	Other protons	3.76 (dd, J = Hz, 1H), 3.71 (dd, J = Hz, 1H), 3.60 (d, J = Hz, 2H), 3.54~3.49 (m, 1H), 3.28~3.21 (m, 1H)	
19[a]	6.26 (d, *J* = 6.8 Hz, 0.7H) 6.24 (d, *J* = 7.8 Hz, 0.3H)	8.31 (d, *J* = 7.8 Hz, 0.3H) 8.25 (d, *J* = 7.8 Hz, 0.7H) 5.62 (d, *J* = 6.8 Hz, 0.3H) 5.58 (d, *J* = 6.8 Hz, 0.7H)	-[b]
	Other protons	7.69~7.28 (m, 20H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H)	
20[c]	6.04 (s, *J* = 6.8 Hz, 1H)	8.43 (d, *J* = 6.8 Hz, 1H) 6.00 (d, *J* = 7.8 Hz, 1H)	-[b]
	Other protons	3.75 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.66~3.58 (m, 1H), 3.51~3.47 (m, 1H), 3.14 (quin, *J* = 6.8 Hz, 1H)	
21[a]	6.56 (d, *J* = 7.1 Hz, 1H)	-[b]	8.87 (s, 1H), 8.86 (s, 1H)
	Other protons	7.66~7.27 (m, 20H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H)	
22	6.47 (d, *J* = 6.8 Hz, 1H)	-[b]	8.85 (s, 1H)
	Other protons	7.64~7.24 (m, 20H), 3.83~3.65 (m, 5H), 3.36~3.28 (m, 1H), 1.06 (s, 9H), 1.03 (s, 9H)	
23[a]	6.03 (d, *J* = 8.6 Hz, 1H)	-[b]	8.44 (s, 1H), 7.85 (s, 1H)
	Other protons	7.66~7.30 (m, 20H), 6.06 (br s, 2H),;3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H)	
24[a]	6.56 (d, *J* = 5.9 Hz, 1H)	-[b]	8.45 (s, 1H), 7.72 (s, 1H)
	Other protons	7.68~7.12 (m, 20H), 3.89 (dd *J* = 10.7, 5.9 Hz, 1H), 3.85~3.68 (m, 4H), 3.39~3.32 (m, 1H), 1.05 (s, 9H), 1.01 (s, 9H)	
25	6.36 (d, *J* = 6.8 Hz, 1H)	-[b]	8.36 (s, 1H)
	Other protons	7.75~7.24 (m, 20H), 3.95~3.63 (m, 5H), 3.37~3.30 (m, 1H), 1.04 (s, 9H), 0.99 (s, 9H)	
26[c]	6.17 (d, *J* = 6.6 Hz, 1H)	-[b]	8.84 (s, 1H), 8.24 (s, 1H)
	Other protons	3.80 (d, *J* = 4.6 Hz, 2H), 3.73 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H)	
27[c]	6.36 (d, *J* = 5.9 Hz, 1H)	-[b]	8.91 (s, 1H), 8.02 (s, 1H)
	Other protons	3.83 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.78 (dd, *J* = 11.7, 4.9 Hz, 1H),3.77~3.70 (m, 2H), 3.60 (q, *J* = 4.9 Hz, 1H), 3.48 (quin, *J* = 5.9 Hz, 1H)	
28	6.23 (d, *J* = 6.8 Hz, 1H)	-[b]	8.79 (s, 1H)
	Other protons	13.48 (br s, 1H), 5.07 (t, J = 4.9 Hz, 1H), 4.99 (br s, 1H), 3.79~3.46 (m, 5H), 3.30~2.26 (m, 1H)	

[a]$CDCl_3$, [b]Not Applicable, [c]CD_3OD

Table 5.3. Elemental analysis data

Cmpd	Formula	Calc for C	Calc for H	Calc for N	Calc for S	Found for C	Found for H	Found for N	Found for S
5	$C_{10}H_{18}O_9S_2$	34.68	5.24	-[a]	-[a]	34.51	5.22	-[a]	-[a]
6	$C_{11}H_{18}O_7S_2$	40.48	5.56	-[a]	19.65	40.67	5.37	-[a]	19.59
7	$C_8H_{12}O_3S$	51.04	6.43	-[a]	17.03	50.83	6.36	-[a]	17.00
8	$C_5H_8O_3S$	40.53	5.44	-[a]	21.64	40.64	5.43	-[a]	21.77
9	$C_4H_8O_2S{\cdot}0.35H_2O$	37.99	6.93	-[a]	25.35	38.02	6.98	-[a]	25.00
10	$C_{20}H_{26}O_2SSi$	66.99	7.31	-[a]	8.94	66.87	7.41	-[a]	8.98
11	$C_{21}H_{26}OSSi$	71.13	7.39	-[a]	9.04	71.24	7.37	-[a]	8.85
12	$C_{21}H_{28}O_2SSi$	67.69	7.57	-[a]	8.61	67.89	7.66	-[a]	8.50
13	$C_{21}H_{28}O_2SSi$	67.69	7.57	-[a]	8.61	67.51	7.61	-[a]	8.81
14	$C_{37}H_{46}O_2SSi_2$	76.73	7.79	-[a]	5.39	77.09	7.59	-[a]	5.24
15	$C_{42}H_{50}N_2O_4SSi_2$	68.62	6.86	3.81	4.36	68.38	6.51	3.58	4.33
16	$C_{41}H_{48}N_2O_4SSi_2$	68.29	6.71	3.8	4.45	68.38	6.31	3.58	4.33
17	$C_{10}H_{14}N_2O_4S$	46.50	5.46	10.85	12.41	46.67	5.08	10.64	12.54
18	$C_9H_{12}N_2O_4S$	44.25	4.95	11.47	13.13	44.05	5.17	11.27	12.89
19	$C_{41}H_{49}N_3O_3SSi_2$	68.39	6.86	5.84	4.45	68.64	7.26	5.84	4.66
20	$C_9H_{12}N_2O_4S$	44.43	5.39	17.27	13.18	44.25	5.26	16.97	12.99
21	$C_{42}H_{47}ClN_4O_2SSi_2$	66.07	6.20	7.34	4.20	66.29	6.34	7.06	4.03
22	$C_{42}H_{46}ClFN_4O_2SSi_2$	64.55	5.95	7.17	4.10	64.64	6.14	6.64	3.92
23	$C_{42}H_{49}N_5O_2SSi_2$	67.79	6.64	9.41	4.31	67.67	6.25	9.37	4.08
24	$C_{42}H_{48}N_4O_3SSi_2$	67.70	6.49	7.52	4.30	67.64	6.26	7.43	4.16
25	$C_{42}H_{49}N_5O_3SSi_2$	66.37	6.50	9.21	4.22	66.49	6.73	8.95	4.28
26	$C_{10}H_{13}N_5O_2S$	44.93	4.90	26.20	12.00	45.25	5.17	25.97	11.79
27	$C_{10}H_{12}N_4O_3S$	44.77	4.51	20.88	11.95	45.04	4.37	20.53	11.75
28	$C_{10}H_{13}N_5O_3S{\cdot}0.1C_6H_{15}N$	43.39	4.98	24.34	10.93	43.43	5.29	24.22	10.86
32	$C_{10}H_{14}N_2O_4S{\cdot}0.2MeOH$	46.28	5.64	10.46	11.97	45.91	5.57	10.77	12.03
33	$C_9H_{12}N_2O_4S$	44.25	4.95	11.47	13.13	44.35	5.12	11.07	12.80
34	$C_9H_{13}N_3O_3S$	44.43	5.39	17.27	13.18	44.21	5.07	16.97	12.93
35	$C_{10}H_{13}N_5O_2S$	44.93	4.90	26.20	12.00	45.25	5.17	25.97	11.79
36	$C_{10}H_{12}N_4O_3S$	44.77	4.51	20.88	11.95	44.55	4.22	20.80	11.57
37	$C_{10}H_{13}N_5O_3S$	42.39	4.63	24.72	11.32	42.43	4.73	24.95	11.28

[a] Not Applicable

CHAPTER 6

SYNTHESIS OF L-3'-THIETANOSE NUCLEOSIDES

INTRODUCTION

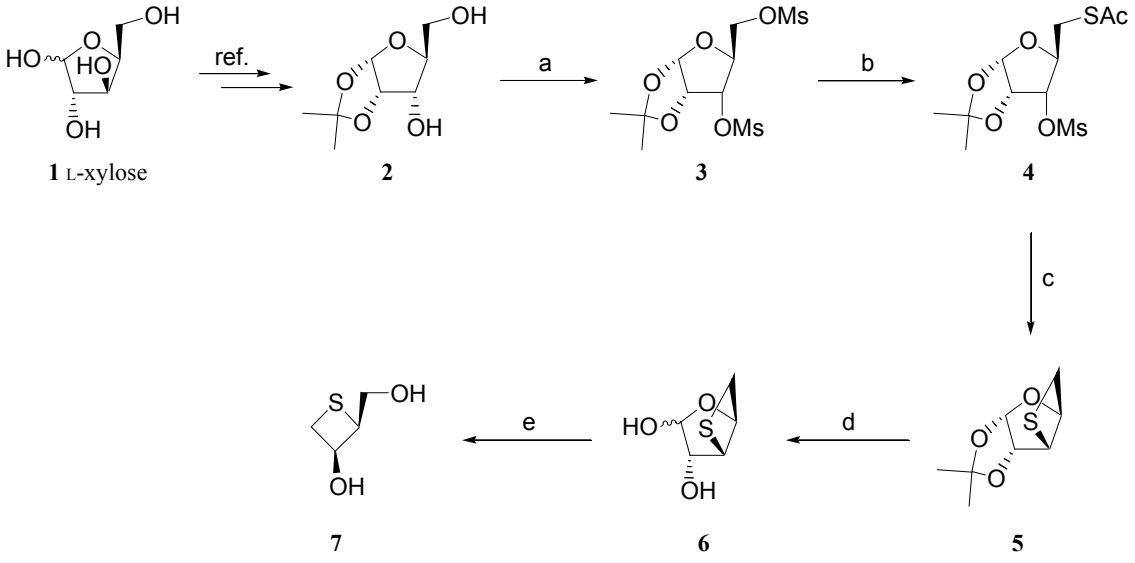
Various nucleosides have been reported as potent inhibitors against HIV, HSV, HBV, VZV, RSV, and HCMV. In the search for effective and nontoxic antiviral nucleosides, several modifications have been accomplished on the carbohydrate moiety of the naturally occurring nucleosides. Mostly L-nucleosides show no cytotoxicity, while they have potent antiviral activity in some cases. Here are excellent examples:3TC,[310,311] FTC,[312] L-FMAU[313] and L-dT.[314] 3TC has been approved for the treatment of HIV and HBV infections by the FDA. FTC, L-FMAU and L-dT are now under clinical trials for the treatment of HIV and/or HBV infections. Discovery of new antiviral L-nucleosides is very interesting, especially in terms of drug safety issue.

An unusual nucleoside, oxetanocin A, which has a four-membered oxetanose ring instead of a five-membered furanose ring, showed antiviral and antitumor activity.[315] Due to the unique structure and biological activity of this compound, extensive structure-activity studies have been conducted to discover safer and more effective antiviral drug candidates.[316] Thietane analogs of oxetanocin A in which the ring oxygen in the sugar moiety is replaced by a sulfur atom are very interesting because thionucleosides are usually more stable to phosphorylase which cleaves the glycosidic bond in nucleosides and makes potent nucleosides inactive.[317] However, the thietane analogs have not been extensively investigated presumably due to the difficulty in synthesis of a four-membered thietane ring as well as the lack of efficient condensation method of thietane derivatives with heterocyclic bases. Until now, only two syntheses of thietanose nucleosides have been reported.[318,319]

On the basis of these considerations, efficient synthesis and biological evaluation of L-thietanose nucleosides are valuable to conduct the extensive structure-activity relationship studies in search for new potent antiviral nucleosides. In this paper, we report an efficient and novel synthesis of L-thietanose nucleosides with different substitutions at 2'-position.

RESULTS AND DISCUSSION

An efficient synthesis of a thietanose ring by substitution reaction of 1-thioacetyl-3-mesylate reported by Voss et al.[320] was adapted to our synthesis with some modifications. For this purpose, L-xylose **1** was converted to diol **2** in 5 steps by a well-known method (Scheme 6.1).[321] Diol **2** was mesylated with MsCl, TEA and DMAP to give the 1,3-bismesylate **3** in 90% yield. Since the direct bis-substitution reaction of **3** with sodium sulfide did not provide good result, the two-step substitution method[320] was used: 1,3-bismesytae **3** was selectively converted to mono-thioacetate **4** by treatment with KSAc in DMF at room temperature in 80 % yield. A solution of the mono-thioacetate **4** and $NaHCO_3$ in previously deoxygenated EtOH and water was refluxed under N_2 atmosphere for 6 h to give compound **5** in 98% yield. Compound **5** was deprotected by treatment with 2% TFA followed by neutralization with Amberlite (OH^-) resin to give triol **6** in 95% yield.



1 L-xylose **2** **3** **4** **5** **6** **7**

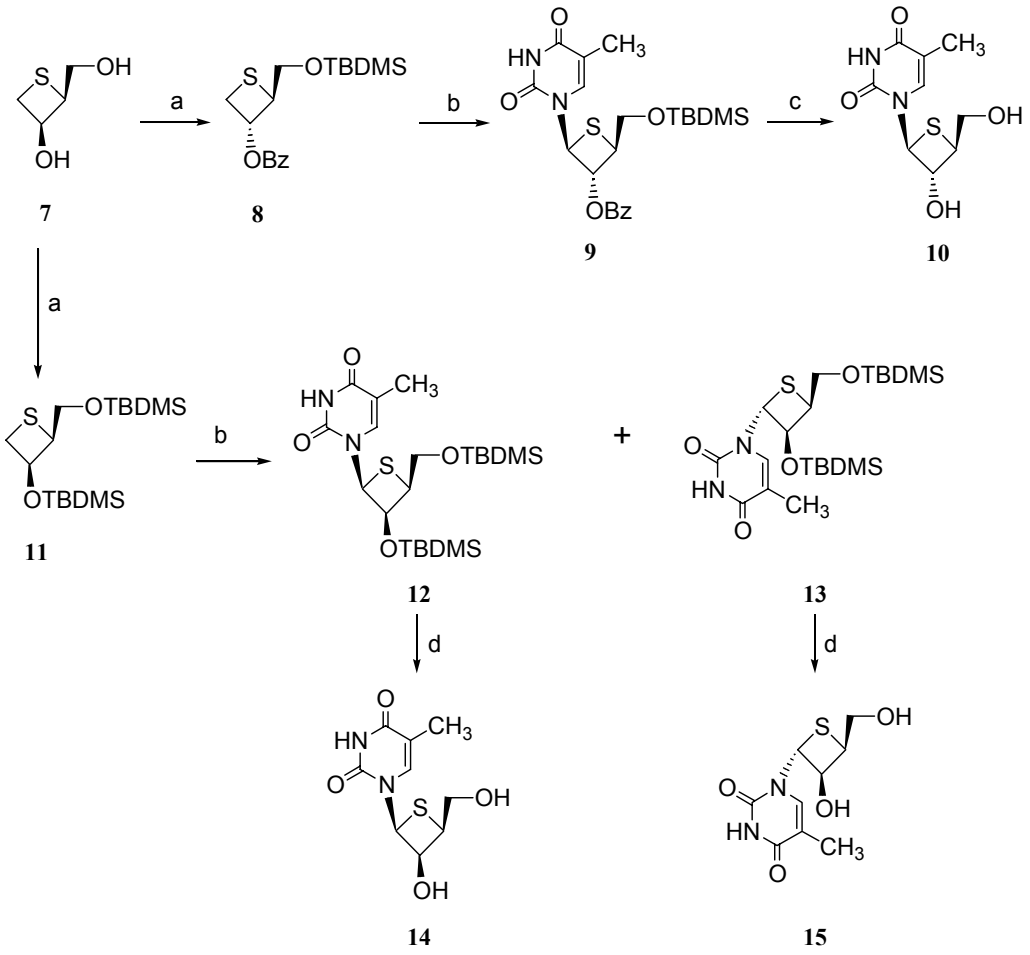
[a]keys a) MsCl, pyr. DMAP, CH_2Cl_2, b) KSAc, DMF, c) $NaHCO_3$, $EtOH/H_2O$, reflux, d) 4% TFA; OH^- resin e) $NaIO_4$; $NaBH_4$, MeOH

Scheme 6.1. Synthesis of the key intermediate **7**

Compound **6** was oxidatively cleaved to the key intermediate **7** from which various 2'-substituted thietanose nucleosides can be synthesized. For optimum yield of this 1,2-diol cleavage, several reaction conditions such as an amount of $NaIO_4$ (1.2 eq), well-stirring, reaction time (1 h), and temperature (0^oC) turned out to be critical.

From the key intermediate **7**, several thietanose nucleosdes were synthesized including L-antipodes of two thietanose nucleosides reported before (Schemes 6.2 and 6.3). The diol **7** underwent the primary alcohol protection with TBDMSCl and Mitsunobu reaction with benzoic acid to give compound **8** in 46% overall yield. Condensation reaction of compound **8** with thymine was carried out according to the O'Neil and Hamilton's protocol[328] with some modification. In this condensation, thymine was treated with HMDS in refluxing acetonitrile and the volatiles were evaporated to dryness to give the

silylated thymine. The sulfoxide of compound **8**, which was prepared by oxidation of **8** with mCPBA, was condensed with the silylated thymine in the presence of TMSOTf, TEA, and a catalytic amount of ZnI_2 in toluene to give exclusively β-isomer **9** in 40% yield. The thymine derivative **9** was successively deprotected with TBAF and methanolic ammonia to give L-thietanose thymine **10** in 85% yield (Scheme 6.2).

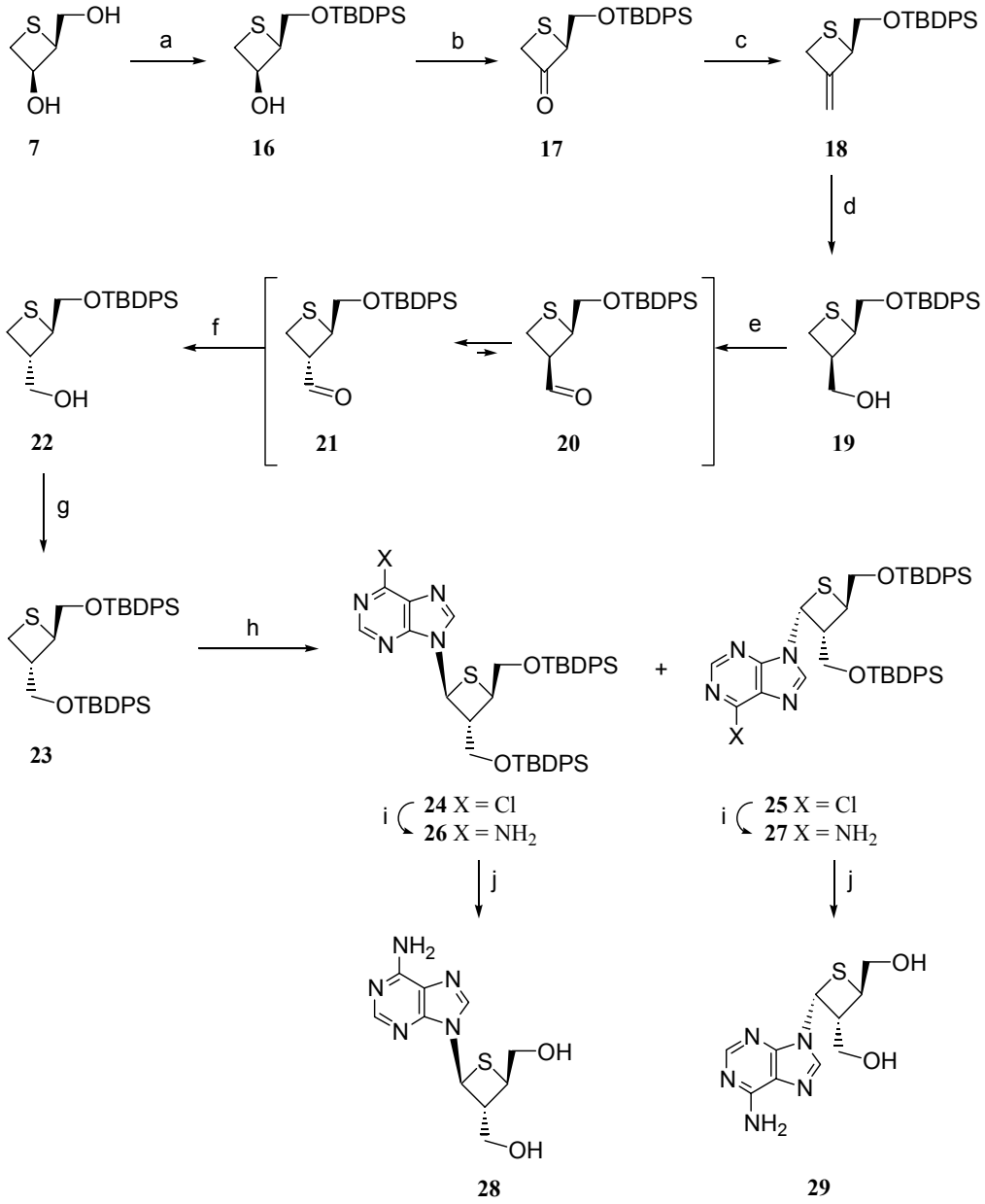


[a]keys a) TBDMSCl, imidazole, CH_2Cl_2; BzOH, DEAD, Ph_3P, THF, b) mCPBA, CH_2Cl_2; Thymine, TMSOTf, TEA, cat.ZnI_2, toluene, c) TBAF, THF; NH_3, MeOH, d) TBAF, THF

Scheme 6.2. Synthesis of L-thietanose nucleosides

Compound **7** was also converted to bis-TBDMS-protected compound **11** in 93% yield, which was oxidized to the corresponding sulfuxide with treatment with mCPBA. The sufoxide was condensed with silylated thymine as described above to give β-anomer **12** and α-anomer **13** in 41% yield with an anomeric ratio (β:α) of 1:3. β-Anomer **12** and α-anomer **13** were deprotectd by TBAF to the final nucleosides **14** and **15** in 89% and 94% yields, respectively (Scheme 6.2).

To expand the scope of our new strategy, this method was applied to the synthesis of a 2'-hydroxymethylthietanose nucleoside. Compound **4** was monoprotected with TBDPSCl, TEA and DMAP to give compound **16** in 87% yield, which was oxidized under Moffatt-type conditions to give ketone **18** in 98% yield. The methylenation of the ketone **18** was accomplished with Petasis' reagent (Cp_2TiMe_2) to give compound **18** in 50 % yield. Unfortunately, compound **19** underwent hydroboration by treatment with $BH_3 \cdot SMe_2$ followed by oxidation with 3% H_2O_2 and 1N NaOH to give undesired *cis*-dialkyl product **19** as a major in 64% yield, with only minor amount of the desired *trans*-dialkyl compound **22** in 11% yield. The sterically hindered nature of the β-side of compound **18** does not allow the approach of BH_3 from the β-side, favoring the formation of the undesired configuration of the 2-hydroxymethyl group, which can be supported by the same result of the synthesis of (-)-cyclobut-A.[329] In order to obtain the correct configuration at the 2-position, the major product **19** was oxidized and equilibrated under basic conditions. Therefore, Swern oxidation of compound **19** followed by epimerization with NaOMe and reduction with $NaBH_4$ gave the desired product **22** in 47% overall three-step yield. The primary hydroxyl group in compound **22** was protected in 98% yield by treating with TBDPSCl and imidazole in methylene chloride to give compound **23**.



[a]*keys* a) TBDPSCl, TEA, DMAP, CH_2Cl_2, b) Ac_2O, DMSO, c) Petasis reagent, d) BH_3SMe_2, THF, e) Ac_2O, DMSO; NaOMe, MeOH, f) $NaBH_4$, MeOH, g) TBDPSCl, imidazole, CH_2Cl_2, h) (i) mCPBA, CH_2Cl_2, (ii) 6-chloropurine, TMSOTf, TEA, cat.ZnI_2, toluene, i) (i) NaN_3, DMF, (ii) H_2, Pd(0), MeOH, j) TBAF, THF

Scheme 6.3. Synthesis of L-3'-thio analog of oxetanocin A

The compound **23** was oxidized to a sufoxide which was condensed with silylated 6-chloropurine to give β-anomer **24** and α-anomer **25** with an anomeric ratio (β:α) of 5:4 in 45% yield. Compounds **24** and **25** was converted to adenine derivatives **26** and **27** using azide method. Compoud **24** and **25** was treated respectively with NaN_3 in DMF at room temperature followed by hydrogenolysis using H_2 and Pd(0) in methanol to give compounds **26** and **27** in 76% and 84% yield, respectively. After deprotection of compounds **26** and **27**, the final nucleosides **28** and **29** were obtained in 93% and 90% yield, respectively.

The synthesized nucleosides **10**, **14**, **15**, **28** and **29** were biologically evaluated against HIV-1 in human PBM cells *in vitro*. However, none of the nucleoside analogs are active against HIV-1.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), quin. (quintet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column

chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(-)-[*2R,3R,4S,5S*]-Methanesulfonic acid 5-methanesulfonyloxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yl ester (3). A solution of compound **2** (17.2g, 90.4 mmol) and pyridine (29.3 mL, 362 mmol) in CH_2Cl_2 (200 mL) was treated with MsCl (17.5 mL, 226 mmol) and DMAP (2.2g, 18 mmol) at 0^oC for 18 h. The reaction mixture was diluted with CH_2Cl_2 (300 mL), washed with 1N HCl, aq $NaHCO_3$ and brine, and dried over $MgSO_4$. The filtrate was concentrated and purified with 30~50% EtOAc in hexanes by silica gel column chromatography to give the product **3** (29.3g, 0.0816 mol, 90%) as a white solid: mp 115~116^oC; $[\alpha]^{22}_D$ -80.2^o (c 0.57, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.84 (d, J = 3.4 Hz, 1H), 4.82~4.74 (m, 2H), 4.55 (dd, J = 11.8, 1.9 Hz, 1H), 4.41~4.30 (m, 2H), 3.16 (s, 3H), 3.08 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 114.11, 103.98, 77.24, 74.96, 74.86, 66.14, 38.66, 37.63, 26.61, 26.53; Anal. ($C_{10}H_{18}O_9S_2$) C, H (for the detail elemental analysis data and ^1H NMR, see Tables 6.1 and 6.2).

(-)-[*2R,3R,4S,5S*]-Thioacetic acid S-(6-methanesulfonyloxy-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl) ester (4). A solution of compound **3** (6.08g, 17.5 mol) in DMF (30 mL) was treated with KSAc (2.20g, 19.3 mmol) at room temperature. After 3 h, the reaction mixture was diluted with EtOAc, washed with water (2 X 100mL), and dried over $MgSO_4$. The filtrate was concentrated and purified with 20% EtOAc in hexanes by silica gel column chromatography to give product **4** (4.57g, 14.0

mmol, 80%) as a brownish solid: mp 112~113°C; $[\alpha]^{23}_{D}$ -70.0° (c 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.77 (d, *J* = 3.7 Hz, 1H), 4.75 (t, *J* = 4.2 Hz, 1H), 4.48 (dd, *J* = 8.8, 4.6 Hz, 1H), 4.31~4.25 (m, 1H), 3.35 (dd, *J* = 14.5, 4.1 Hz, 1H), 3.23 (*J* = 14.5, 5.1 Hz, 1H), 3.16 (s, 3H), 2.36 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 194.60, 113.72, 103.82, 77.88, 77.40, 75.45, 38.77, 30.43, 29.29, 26.55, 26.51; Anal. ($C_{11}H_{18}O_7S_2$) C, H, S.

(-)-4,4-Dimethyl-tetrahydro-3,5,6-trioxa-2-thia-cyclobuta[a]pentalene (5). A mixture of EtOH (100 mL)and water (5 mL) was refluxed under Ar for 2 h and cooled. Compound **4** (2.12g, 6.50 mmol) and solid $NaHCO_3$ (1.09g, 13.0 mmol) was added to the solvent and the resulting mixture was refluxed for 8 h. The reaction mixture was cooled, diluted with Et_2O, filtered, and purified with 5% Et_2O in hexanes by silica gel column chromatography to give the product **5** (1.20g, 6.37 mmol, 98%) as oil: $[\alpha]^{23}_{D}$ -89.4° (c 1.76, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 6.41 (d, *J* = 3.4 Hz, 1H), 5.26 (t, *J* = 5.3 Hz, 1H), 4.71 (d, *J* = 3.4 Hz, 1H), 4.08 (d, *J* = 5.3 Hz, 1H), 3.49 (dd, *J* = 10.6, 5.4 Hz, 1H), 2.84 (d, *J* = 10.6 Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 113.82, 108.11, 86.68, 82.78, 46.19, 31.97, 27.86, 27.27; Anal. ($C_8H_{12}O_3S$) C, H, S.

(-)-2-Oxa-6-thia-bicyclo[3.2.0]heptane-3,4-diol (6). Compound **5** (1.10g, 5.31 mmol) was with 4% TFA solution in water at room temperature for 8 h, the reaction mixture was neutralized with amberite ($^-$OH) resin which was prewashed with water (2X100 mL). After filtration, the filtrate was concentrated and purified with 50% EtOAc in hexanes by silica gel column chromatography to give the product **6** (0.750g, 5.06 mmol, 95%) as oil: ^{1}H NMR ($CDCl_3$) for major δ 6.11 (d, *J* = 2.3 Hz, 1H), 5.16~5.12 (m, 1H), 4.17 (d, *J* = 2.5 Hz, 1H), 3.95 (d, *J* = 5.9 Hz, 1H), 3.50 (dd, *J* = 10.9, 6.3 Hz, 1H), 3.00 (d, *J* = 10.9

Hz, 1H), for minor δ 5.56 (s, 1H), 5.29~5.24 (m, 1H), 4.37 (s, 1H), 3.95 (d, *J* = 5.9 Hz, 1H), 3.64 (dd, *J* = 10.9, 6.1 Hz, 1H), 3.12 (d, *J* = 10.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) for major δ 98.95, 78.42, 75.92, 47.79, 32.58, for minor δ 107.76, 82.96, 80.27, 47.73, 33.72; Anal. ($C_5H_8O_3S$) C, H, S.

(-)-[*2R,3S*]-2-Hydroxymethyl-thietan-3-ol (7). A solution of compound **6** (1.92g, 12.9 mmol) in MeOH (50 mL) was treated with an aqueous solution of $NaIO_4$ (3.04g, 14.2 mmol) in water (25 mL) dropwise at 0^{o}C for 10 min with keeping stirring well. After 20 min, well-grounded $NaBH_4$ was added portionwise to the reaction mixture at 0^{o}C. After 30 min, the resulting mixture was filtered and the filtrate was neutralized with 1N HCl and concentrated. The residue was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1) and the white solid was filtered off. The filtrate was concentrated and purified with 3~5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the products **7** (1.21g, 10.1 mmol, 78%) and **8** (0.194g, 1.29 mmol, 10%): for compound **7**: $[\alpha]^{24}_D$ -173.5^o (c 0.84, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.00 (quin. *J* = 8.1 Hz, 1H), 4.09~3.98 (m, 2H), 3.85 (d, *J* = 8.9 Hz, 1H), 3.76~3.71 (m, 1H), 3.37~3.24 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 68.89, 62.70, 51.31, 37.56; Anal. ($C_4H_8O_2S{\cdot}0.35H_2O$) C, H, S.

(-)-[*2R,3S*]-Benzoic acid 2-(*tert*-butyl-dimethyl-silanyloxymethyl)-thietan-3-yl ester (8). A solution of compound **7** (0.333g, 2.77 mmol) and imidazole (0.377g, 4.95 mmol) in CH_2Cl_2 was treated with TBDMSCl (0.397g, 2.63 mmol) at 0^{o}C for 16 h. The reaction was quenched with 1 mL of MeOH. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water, and dried over $NaSO_4$. The filtrate was concentrated and purified with 10% EtOAc in hexanes by silica gel column chromatography to give the TBDMS-protected intermediate (0.380g, 1.43 mmol, 52%). A solution of the TBDMS-

protected intermediate (0.356g, 1.34 mmol), benzoic acid (0.196g, 1.60 mmol) and Ph_3P (0.421g, 1.61 mmol) in THF (5 mL) was treated with DIAD (0.32 mL, 1.63 mmol) at 0°C for 30 min. The reaction mixture was concentrated and purified with 25% EtOAc in hexanes by silica gel column chromatography to give the product **9** (0.403g, 1.19 mmol, 89%) as oil: $[\alpha]^{24}_D$ -72.7° (c 0.83, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.03 (d, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 7.5 HZ, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 6.04 (q, *J* = 8.1 Hz, 1H), 4.19 (dd, *J* = 10.8, 7.1 Hz, 1H), 3.96 (dd, *J* = 10.9, 5.7 Hz, 1H), 3.84 (q, *J* = 6.7 Hz, 1H), 3.67 (t, *J* = 8.7 Hz, 1H), 3.36 (t, *J* = 8.7 Hz, 1H), 0.81 (s, 9H), 0.003 (s, 3H), -0.021 (s, 3H);^{13}C NMR ($CDCl_3$) δ 165.08, 133.19, 129.80, 129.62, 128.52, 68.19, 63.17, 50.10, 33.18, 25.76, 18.24, -5.38, -5.49; Anal. ($C_{17}H_{26}O_3SSi$) C, H, S.

(-)-[*2R,3S,4S*]-Benzoic acid 2-(*tert*-butyl-dimethyl-silanyloxymethyl)-4-(5-methyl-2,4-dioxo-3,4-dihydro-*2H*-pyrimidin-1-yl)-thietan-3-yl ester (9). A solution of compound **9** (0.321g, 0.948 mmol) in CH_2Cl_2 was treated with a solution of 77% mCPBA (0.250g, 1.12 mmol) in CH_2Cl_2 dropwise at -78°C. The mixture was stirred at -78°C for 30 min. The reaction was quenched with aq. $NaSSO_3$, diluted with CH_2Cl_2, washed with aq $NaSSO_3$, aq $NaCO_3$, and brine, and dried over $MgSO_4$. The filtrate was concentrated to dryness. The crude sulfoxide (0.320g, 0.903 mmol, 95%) was used for the next reaction without further purification. A mixture of thymine in acetonitrile and HMDS was refluxed for 3 h until the solution became clear. After evaporation, the residue was treated successively with a solution of the crude sulfoxide (0.220g, 0.621 mmol) in toluene (5 mL), TEA(0.17 mL, 1.21 mmol), TMSOTf (0.25 mL, 0.90 mmol), and ZnI_2 (0.059g, 0.19 mmol) at 0°C. After 24 h, the reaction was quenched with aq $NaHCO_3$ and the reaction mixture was filtered and the filtrate was extracted with CH_2Cl_2.

The organic layer was dried over $NaSO_4$, filtered, concentrated and purified with 30% EtOAc in hexane by preparative TLC to give the product **9** (0.115g, 0.249 mmol, 40%): $[\alpha]^{25}_D$ -161.0^o (c 0.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.01(d, *J* = 8.1 Hz, 2H), 7.74 (s, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 6.77 (d, *J* = 7.6 Hz, 1H), 5.90 (t, *J* = 8.1 Hz, 1H), 4.07 (dd, *J* = 11.1, 5.6 Hz, 1H), 3.98 (dd, *J* = 11.1, 4.5 Hz, 1H), 3.89~3.83 (m, 1H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.10, 163.20, 150.28, 135.29, 133.71, 130.03, 128.57, 128.45, 112.32, 73.36, 62.66, 57.96, 44.07, 25.73, 18.23, 12.75, -5.45, 5.52; Anal. ($C_{22}H_{30}N_2O_5SSi$) C, H, N, S.

(-)-[*2R,3S,4S*]-1-(3'-Hydroxy-4'-hydroxymethyl-thietan-2'-yl)-thymine (10). A solution of compound **9** (0.124g, 0.268 mmol) in THF (10 mL) was treated with 1 N solution of TBAF (0.32 mL, 0.32 mmol) in THF at room temperature for 2 h. The reaction mixture was concentrated and filtered through silica gel pad. The filtrate was concentrated to dryness. The resulting crude intermediate was treated with methanolic ammonia (20 mL) at room temperature for 16 h. After concentration, the residue was purified with 7% MeOH in $CHCl_3$ by silica gel column chromatography to give the product **10** (0.056g, 0.229 mmol, 85%) as a white solid: mp 188~189^{o}C; $[\alpha]^{23}_D$ -242.3^o (c 0.16, MeOH); UV(H_2O) λ_{max} 271.0 nm (ε 10,200, pH 2). 270.5 nm (ε 10,200, pH 7), 272.0 nm (ε 9,100, pH 11); ^{1}H NMR (DMSO-*d6*) δ 11.41 (s, 1H, D_2O exchangeable), 7.99 (s, 1H), 6.12 (d, J = 7.5 Hz, 1H, D_2O exchangeable), 6.11 (d, J = 6.8 Hz, 1H), 4.97 (q, J = 7.6 Hz, 1H), 4.79 (t, J = 5.5 Hz, 1H), 3.95~3.87 (m, 1H), 3.77~3.68 (m, 1H), 3.64~3.62 (m, 1H), 1.83 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.0, 154.11, 140.16, 114.11, 76.01, 65.06, 64.45, 50.65, 15.76; Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

(-)-[*2R,3R*]-3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(*tert*-butyl-dimethyl-silanyloxymethyl)-thietane (11). A solution of compound **7** (0.263g, 2.19 mmol) and imidazole (0.447g, 6.57 mmol) in CH_2Cl_2 (10 mL) was treated with TBDMSCl (0.825g, 5.47 mmol) at 0°C for 16 h. The reaction mixture was concentrated and purified with 1~2% EtOAc in hexanes by silica gel column chromatography to give the product **11** (0.707g, 2.03 mmol, 93%): $[\alpha]^{24}_D$ -90.9° (c 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.95 (quin. *J* = 7.9 Hz, 1H), 4.14 (dd, *J* = 10.9, 5.1 Hz, 1H), 4.00 (dd, *J* = 10.9, 8.1 Hz, 1H), 3.60 (q, *J* = 6.8 Hz, 1H), 3.37 (t, *J* = 8.4 Hz, 1H), 3.15 (t, *J* = 7.9 Hz, 1H), 0.90 (s, 9H), 0.87 (s, 9H), 0.085 (s, 6H), 0.040 (s, 3H), 0.036 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 67.62, 63.39, 54.29, 37.23, 26.00, 25.68, -4.91, -5.02, -5.12, -5.21; Anal. ($C_{16}H_{36}O_2SSi_2$) C, H, S.

(-)-[*2R,3R,4S*]-1-[3-(*tert*-Butyl-dimethyl-silanyloxy)-4-(*tert*-butyl-dimethyl-silanyhloxymethyl)-thietan-2-yl-thymine (12) and (-)-[*2R,3R,4R*]-1-[3-(*tert*-butyl-dimethyl-silanyloxy)-4-(*tert*-butyl-dimethyl-silanyhloxymethyl)-thietan-2-yl]-thymine (13). See the procedure for condensation reaction of the compound **10** with thymine. The title compounds **12** and **13** was obtained on 0.644-mmol scale in 41% yield: For compound **12**: $[\alpha]^{21}_D$ -7.7° (c 0.52, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 270.5 nm; ^{1}H NMR (DMSO-*d6*) δ 8.86 (s, 1H), 7.94 (s, 1H), 6.37 (d, *J* = 6.4 Hz, 1H), 5.13 (t, *J* = 6.4 Hz, 1H), 4.04 (dd, *J* = 9.9, 6.1 Hz, 1H), 3.88~3.74 (m, 2H), 1.97 (s, 3H), 0.89 (s, 9H), 0.82 (s, 9H), 0.088 (s, 3H), 0.076 (s, 3H), 0.004 (s, 3H), -0.134 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.43, 150.85, 139.83, 109.25, 74.58, 62.55, 54.20, 46.14, 25.88, 25.52, 18.39, 17.92, 12.55, -5.24, -5.27, -5.60; Anal. ($C_{21}H_{40}N_2O_4SSi_2$) C, H, N, S. for compound **13**: $[\alpha]^{23}_D$ -96.8° (c 0.28, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 271.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1H), 7.66 (s, 1H), 6.35 (d, *J* = 7.4 Hz, 1H), 4.71 (t, *J* = 7.6 Hz, 1H), 4.08 (dd, *J* = 10.9, 4.3 Hz, 1H),

3.99 (dd, *J* = 10.9, 6.9 Hz, 1H), 3.66~3.56 (m, 1H), 1.99 (s, 3H), 0.93 (s, 9H), 0.83 (s, 9H), 0.11 (s, 6H), -0.024 (s, 3H), -0.043 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.32, 150.22, 135.56, 111.88, 74.57, 63.02, 61.94, 47.81, 25.88, 25.44, 18.37, 17.89, 12.62, -5.08, -5.23, -5.27, -5.34; Anal. ($C_{21}H_{40}N_2O_4SSi_2$) C, H, N, S.

(-)-[*2R,3R,4S*]-1-[3-(Hydroxy-4-hydroxymethyl)-thietan-2-yl]-thymine (14). A solution of compound **12** (0.026g, 0.0550 mmol) in THF (5 mL) was treated with 1N TBAF solution (0.11 mL) in THF at 0^{o}C for 30 min. The solvent was removed and the residue was purified with 3~5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the product **14** (0.012g, 0.0491 mmol, 89%) as a white solid: mp 205^{o}C (dec.); $[\alpha]^{21}_D$ -32.1^o (c 0.13, MeOH); UV(H_2O) λ_{max} 272.5 nm (ε 10,400, pH 2). 272.0 nm (ε 10,400, pH 7), 271.5 nm (ε 8,700, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 8.21 q, *J* = 1.3 Hz, 1H), 6.23 (d, *J* = 6.5 Hz, 1H), 5.15 (t, *J* = 6.6 Hz, 1H), 3.98 (dd, *J* = 11.1, 6.2 Hz, 1H), 3.90 (q, *J* = 6.6 Hz, 1H), 3.78 (dd, *J* = 11.1, 6.4 Hz, 1H), 1.93 (d, *J* = 1.1 Hz, 3H); ^{13}C NMR (MeOH-*d*$_4$) δ 166.45, 152.99, 141.50, 109.60, 74.89, 62.53, 56.67, 47.01, 12.51; Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

(-)-[*2R,3R,4R*]-1-[3-(Hydroxy-4-hydroxymethyl)-thietan-2-yl]-thymine (15). A solution of compound **13** (0.157g, 0.332 mmol) in THF (5 mL) was treated with 1N TBAF solution (0.70 mL, 0.70 mmol) in THF at 0^{o}C for 30 min. The solvent was removed and the residue was purified with 3~5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the product **15** (0.076g, 0.311 mmol, 94%) as a white solid: mp 200~201^{o}C ; $[\alpha]^{21}_D$ -244.0^o (c 0.12, MeOH); UV(H_2O) λ_{max} 271.0 nm (ε 10,200, pH 2). 271.0 nm (ε 10,100, pH 7), 272.0 nm (ε 8,700, pH 11); ^{1}H NMR (MeOH-*d*$_4$) δ 7.94 (q, *J* = 1.3 Hz, 1H), 6.29 (d, *J* = 7.4 Hz, 1H), 4.06 (dd, *J* = 8.4, 7.4 Hz, 1H), 4.06 (dd, *J* = 12.0,

5.1 Hz, 1H), 3.93 (dd, *J* = 12.0, 6.6 Hz, 1H), 3.74~3.67 (m, 1H), 1.93 (d, *J* = 1.3 Hz, 3H); ^{13}C NMR (MeOH-d_4) δ 165.98, 152.46, 138.05, 112.55, 75.14, 63.56, 62.66, 47.51, 12.43; Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

(-)-[*2R,3R*]-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-ol (16). A solution of compound **7** (2.387g, 19.9 mmol), TEA (11.1mL, 79.6 mmol), and DMAP (0.48g, 3.9 mmol) in CH_2Cl_2 was treated with TBDPSCl (4.9 mL, 18.8 mmol) at 0^{o}C for 2 h. The resulting mixture was concentrated and purified with 5% EtOAc in hexanes by silica gel column chromatography to give the product **16** (6.13g, 17.1 mmol, 86%) as oil: $[\alpha]^{22}_D$ -78.6^o (c 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.84~7.38 (m, 10H), 5.06 (quin, *J* = 8.1 Hz, 1H), 4.19~4.10 (m, 2H), 3.88 (d, *J* = 9.7 Hz, 1H), 3.73~3.67 (m, 1H), 3.54 (t, *J* = 8.3 Hz, 1H), 3.33 (t, *J* = 8.3 Hz, 1H), 1.12 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.89, 135.54, 132.58, 132.09, 130.07, 130.04, 127.95, 127.87, 69.62, 65.12, 50.48, 38.11, 26.84, 19.19.; Anal. ($C_{20}H_{26}O_2SSi$) C, H, S.

(-)-[*2R*]-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-one (17). Compound **16** (0.967g, 2.70 mmol) was treated with DMSO (5mL) and Ac_2O (3.5mL) at room temperature for overnight. The reaction mixture was quenched with ice, diluted with Et_2O (x mL), washed with water. The organic layer was dried over $NaSO_4$, concentrated and purified with 3% EtOAc in hexanes by silica gel column chromatography to give the product **17** (0.895g, 2.51 mmol, 93%) as oil: $[\alpha]^{25}_D$ -66.1^o (c 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.74~7.35 (m, 10H), 4.82 (q, J = 4.3 Hz, 1H), 4.28 (dd, J = 16.3, 3.6 Hz, 1H), 4.17 (d, J = 15.8 Hz, 1H), 4.15 (dd, J = 11.2, 4.6 Hz, 1H), 3.90 (dd, J = 11.2, 3.6 Hz, 1H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 196.97, 135.69, 135.58, 132.80, 132.75, 129.84, 127.77, 127.56, 73.44, 63.08, 52.80, 26.67, 19.29.

(-)-[*2R*]-*tert*-Butyl-(3-methylene-thietan-2-ylmethoxy)-diphenyl-silane (18). A mixture of titanocene dichloride (0.810g, 3.25 mmol) in Et_2O (20mL)was treated with 1.4 N MeLi solution (5.1mL, 7.1 mmol) in Et_2O at room temperature for 2 h. The reaction mixture was quenched with ice and extracted with Et_2O. The organic layer was dried over $NaSO_4$ and concentrated to dryness. This reagent was dissolved in Et_2O (1 mL) and to this solution, a solution of compound **17** (0.290g, 0.813 mmol) in THF (1 mL) was added. The resulting mixture was refluxed at 60^oC, the bath temperature. After 48 h, the reaction mixture was cooled to room temperature, diluted with hexanes, and filtered through the silica gel pad. The filtrate was concentrated and purified with 2% EtOAc in hexanes by silica gel column chromatography to give the product **18** (0.190g, 0.53 mmol, 66%) as oil: $[\alpha]^{23}_D$ -71.8^o (c 1.60, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.72~7.65 (m, 4H), 7.47~7.37 (m, 6H), 4.86 (d, *J* = 2.1 Hz, 1H), 4.71 (d, *J* = 2.1 Hz, 1H), 4.44~4.37 (m, 1H), 4.00 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.87 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.84~3.74 (m, 2H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 146.13, 135.67, 135.64, 133.48, 133.39, 129.71, 129.70, 127.69, 109.08, 68.12, 52.85, 33.28, 26.79, 19.27; Anal. ($C_{21}H_{26}OSSi$) C, H, S.

(-)-[*2R,3R*]-[2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (19). A solution of compound **18** (0.505g, 1.42 mmol) in THF (10 mL) was treated with 2N BH_3SMe_2 solution (1.42 mL, 2.84 mmol) in THF at 0^oC for 30 min. The reaction mixture was warmed up to room temperature and stirred for 2 h. The reaction mixture was quenched with 3% H_2O_2 and 1N NaOH. Afher 2 h, the reaction was treated with sat. $NaSSO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The filtrate was concentrated and purified with 10% EtOAc in hexanes by silica gel column chromatography to give the product **19** (0.337g, 0.904 mmol, 64%) as

oil: $[\alpha]^{22}_D$ -32.1° (c 0.82, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.78~7.32 (m, 10H), 4.28 (t, *J* = 10.1 Hz, 1H), 4.01 (t, *J* = 9.9 Hz, 1H), 3.80~3.42 (m, 5H), 3.02~2.83 (m, 2H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.50, 135.46, 132.24, 130.09, 127.92, 65.00, 62.74, 43.39, 42.21, 26.73, 24.23, 19.06; Anal. ($C_{21}H_{28}O_2SSi$) C, H, S.

(-)-[*2R,3S*]-[2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (22). Compound **19** (0.337g, 0.904 mmol) was treated with DMSO (2 mL) and Ac_2O (1.6 mL) at room temperature for 18 h. The reaction was quenched with ice and extracted with Et_2O and the organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude aldehyde was treated with 0.25 N NaOMe solution in MeOH (7 mL, 1.8 mmol) for 24 h. The reaction mixture was treated with $NaBH_4$ (0.250g, 13.8 mmol) for 30 min. The mixture was neutralized with 1 N HCl and concentrated. The resulting residue was dissolved with CH_2Cl_2/MeOH mixture and solid was filtered off. The filtrate was concentrated and purified with 10% EtOAc in hexanes by silica gel column chromatography to give the product **22** as oil: $[\alpha]^{22}_D$ -39.5° (c 0.91, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.70~7.36 (m, 10H), 3.82~3.58 (m, 5H), 3.22~3.10 (m, 1H), 3.01~2.93 (m, 2H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.59, 135.57, 132.84, 132.77, 130.08, 129.92, 127.93, 127.81, 68.55, 65.75, 46.75, 44.57, 26.77, 23.58, 19.14; Anal. ($C_{21}H_{28}O_2SSi$) C, H, S.

(-)-[*2R,3S*]-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietane (23). A solution of compound **22** (0.175g, 0.470 mmol) and imidazole (0.064g, 0.94 mmol) in CH_2Cl_2 was treated with TBDPSCl (0.15 mL, 0.58 mmol) at room temperature for 3 h. The reaction mixture was conceontrated and purified with 2% EtOAc in hexanes to give the product **23** (0,280g, 0.458 mmol, 97%) as oil: $[\alpha]^{24}_D$ -44.7° (c 0.43, CH_2Cl_2); ^1H NMR ($CDCl_3$)

δ 7.70~7.33 (m, 20H), 3.90 (dd, *J* = 9.7, 6.1 Hz, 1H), 3.77 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.73~3.65 (m, 3H), 3.16~2.95 (m, 3H), 1.05 (s, 9H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.61, 135.56, 133.51, 129.70, 129.66, 127.72, 127.68, 68.64, 66.25, 44.64, 44.1, 26.85, 26.80, 23.97, 19.31, 19.27; Anal. ($C_{37}H_{46}O_2SSi_2$) C, H, S.

9-[(*2S*,3*R*,*4S*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloropurine (24) and 9-[(*2R*,3*R*,*4S*)-2,3-bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloropurine (25). See the procedure for condensation reaction of the compound **9** with thymine. The title compounds **24** as well as α-isomer **25** were obtained on 0.370-mmol scale in 40% yield as a 5:4 mixture of compound **24** and α-isomer **25**: for compound **24** :$[\alpha]^{22}_D$ -16.8^o (c 0.58, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.5 nm; ^{1}H NMR ($CDCl_3$) δ 8.87 (s, 1H), 8.86 (s, 1H), 7.66~7.27 (m, 20H), 6.56 (d, *J* = 7.1 Hz, 1H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.05, 152.54, 148.07, 142.71, 135.55, 135.48, 135.44, 132.77, 132.70, 132.54, 130.07, 130.04, 130.2, 127.91, 127.89, 127.86, 127.84, 66.50, 62.92, 55.33, 50.79, 37.60, 26.86, 26.77, 19.23, 19.17; Anal. ($C_{42}H_{47}ClN_4O_2SSi_2$) C, H, N, S: for compound **25**:$[\alpha]^{22}_D$ 21.4^o (c 0.68, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.5 nm; ^{1}H NMR ($CDCl_3$) δ 9.03 (s, 1H), 8.78 (s, 1H), 7.65~7.15 (m, 20H), 6.61 (d, J = 7.3 Hz, 1H), 3.83 (dd, J = 10.7, 4.6 Hz, 1H), 3.77 (dd, J = 10.7, 5.1 Hz, 1H), 3.59~3.47 (m, 2H), 3.45~3.41 (m, 1H), 3.35 (dd, J = 10.7, 6.1 Hz, 1H), 1.02 (s, 9H), 0.67 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.58, 152.46, 148.78, 142.73, 135.62, 135.55, 135.26, 135.16, 132.87, 132.80, 132.13, 132.01, 130.05, 129.96, 129.88, 127.83, 66.61, 62.00, 53.04, 49.70, 40.38, 26.79, 26.40, 19.28, 18.64; Anal. ($C_{42}H_{47}ClN_4O_2SSi_2$) C, H, N, S.

9-[(*2S*,3*R*,*4A*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-adenine (26). A solution of compound **24** (0.090g, 0.118 mmol) in DMF (1 mL) was treated with NaN_3 (0.077g, 0.118 mol) at room temperature for 16h. The resulting mixure was directly filtered through silica gel pad using eluent as a 1:1 mixture of hexanes and EtOAc. The filtrate was concentrated to dryness. A solution of the crude azide in MeOH (15 mL) was treated with 0.020g of Pd(0)/C and H_2 balloon for 48 h. The resulting mixture was filtered through celite pad and the filtrate was concentrated and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **26** (0.065g, 0.087 mmol, 74% yield): $[\alpha]^{23}_D$ -40.7^o (c 0.15, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 272.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.44 (s, 1H), 7.85 (s, 1H), 7.66~7.30 (m, 20H), 6.06 (br s, 2H), 6.03 (d, *J* = 8.6 Hz, 1H), ;3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.64, 153.31, 150.83, 145.57, 135.51, 135.40, 135.30, 132.76, 132.57, 132.30,132.19, 130.20, 130.13, 130.04, 130.00, 127.97, 127.88, 127.82, 65.87, 61.96, 56.65, 51.77, 38.09, 26.87, 26.78, 19.16; Anal. ($C_{42}H_{49}N_5O_2SSi_2$) C, H, N, S.

9-[(*2S*,3*R*,*4A*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-adenine (27). See the procedure for preparation of the compound **26**. The title compound **27** was obtained on 0.161-mmol scale in 80% yield: $[\alpha]^{26}_D$ 15.3^o (c 0.34, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 272.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1H), 8.50 (s, 1H), 7.68~7.22 (m, 20H), 6.21 (d, J = 6.9 Hz, 1H), 5.18 (br s, 2H), 3.88 (dd, J = 10.7, 4.9 Hz, 1H), 3.82 (dd, J = 10.7, 5.9 Hz, 1H), 3.66~3.59 (m, 2H), 3.52~3.44 (m, 2H), 1.06 (s, 9H), 0.80 (s, 9H); Anal. ($C_{42}H_{49}N_5O_2SSi_2$) C, H, N, S.

9-[(*2S*,3*R*,*4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-adenine (28). See the procedure for deprotection reaction of the compound **15** with 1M TBAF solution. The title compound **28** was obtained on 0.094-mmol scale in 84% yield: mp >200°C; $[\alpha]^{25}_{D}$ -93.3° (c 0.11, MeOH); UV(H_2O) λ_{max} 272.5 nm (ε 15,000, pH 2). 270.5 nm (ε 10,000, pH 7), 271.5 nm (ε 10,000, pH 11); ^{1}H NMR (MeOH) δ 8.84 (s, 1H), 8.24 (s, 1H), 6.17 (d, *J* = 6.6 Hz, 1H), 3.80 (d, *J* = 4.6 Hz, 2H), 3.73 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H); Anal. ($C_{10}H_{13}N_5O_2S$) C, H, N, S.

9-[(*2R*,3*R*,*4S*)-2,3-Dihydroxymethyl-thietan-4-yl]-adenine (29). See the procedure for deprotection reaction of the compound **15** with 1M TBAF solution. The title compound **29** was obtained on 0.117-mmol scale in 80% yield: mp >200°C; $[\alpha]^{25}_{D}$ 168.4° (c 0.12, MeOH); UV(H_2O) λ_{max} 274.0 nm (ε 12,000, pH 2). 271.5 nm (ε 8,900, pH 7), 271.5 nm (ε 8,600, pH 11); ^{1}H NMR (MeOH) δ 9.04 (s, 1H), 8.23 (s, 1H), 6.36(d, J = 7.8 Hz, 1H), 3.93 (dd, J = 10.7, 5.6 Hz, 1H), 3.82 (dd, J = 11.7, 6.8 Hz, 1H), 3.74 (dd, J = 11.7, 6.8 Hz, 1H), 3.64 (quin, J = 7.3 Hz, 1H), 3.35~3.30 (m, 2H); Anal. ($C_{10}H_{13}N_5O_2S{\cdot}0.1CH_3OH$) C, H, N, S.

Table 6.1. ^{1}H NMR Data

Compd	H-1'	H-5/6 (for pyrimidines)	H-2/8 (for purines)
9[a]	6.77 (d, *J* = 7.6 Hz, 1H)	7.74 (s, 1H)	-[b]
	Other protons	8.98 (s, 1H), 8.01(d, *J* = 8.1 Hz, 2H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 5.90 (t, *J* = 8.1 Hz, 1H), 4.07 (dd, *J* = 11.1, 5.6 Hz, 1H), 3.98 (dd, *J* = 11.1, 4.5 Hz, 1H), 3.89~3.83 (m, 1H), 0.87 (s, 9H), 0.03 (s, 6H);	
10[c]	6.11 (d, J = 6.8 Hz, 1H)	7.99 (s, 1H)	-[b]
	Other protons	11.41 (s, 1H, D_2O exchangeable), 6.12 (d, J = 7.5 Hz, 1H, D_2O exchangeable), 4.97 (q, J = 7.6 Hz, 1H), 4.79 (t, J = 5.5 Hz, 1H), 3.95~3.87 (m, 1H), 3.77~3.68 (m, 1H), 3.64~3.62 (m, 1H), 1.83 (s, 3H);	
12[a]	6.37 (d, *J* = 6.4 Hz, 1H)	7.94 (s, 1H)	-[b]
	Other protons	8.86 (s, 1H), 5.13 (t, *J* = 6.4 Hz, 1H), 4.04 (dd, *J* = 9.9, 6.1 Hz, 1H), 3.88~3.74 (m, 2H), 1.97 (s, 3H), 0.89 (s, 9H), 0.82 (s, 9H), 0.088 (s, 3H), 0.076 (s, 3H), 0.004 (s, 3H), -0.134 (s, 3H)	
13[a]	6.35 (d, *J* = 7.4 Hz, 1H)	7.66 (s, 1H)	-[b]
	Other protons	8.26 (s, 1H), 4.71 (t, *J* = 7.6 Hz, 1H), 4.08 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.99 (dd, *J* = 10.9, 6.9 Hz, 1H), 3.66~3.56 (m, 1H), 1.99 (s, 3H), 0.93 (s, 9H), 0.83 (s, 9H), 0.11 (s, 6H), -0.024 (s, 3H), -0.043 (s, 3H)	
14[d]	6.23 (d, *J* = 6.5 Hz, 1H)	8.21 (q, *J* = 1.3 Hz, 1H)	-[b]
	Other protons	5.15 (t, *J* = 6.6 Hz, 1H), 3.98 (dd, *J* = 11.1, 6.2 Hz, 1H), 3.90 (q, *J* = 6.6 Hz, 1H), 3.78 (dd, *J* = 11.1, 6.4 Hz, 1H), 1.93 (d, *J* = 1.1 Hz, 3H)	
15[d]	6.29 (d, *J* = 7.4 Hz, 1H)	7.94 (q, *J* = 1.3 Hz, 1H)	-[b]
	Other protons	4.06 (dd, *J* = 8.4, 7.4 Hz, 1H), 4.06 (dd, *J* = 12.0, 5.1 Hz, 1H), 3.93 (dd, *J* = 12.0, 6.6 Hz, 1H), 3.74~3.67 (m, 1H), 1.93 (d, *J* = 1.3 Hz, 3H)	
24[a]	6.56 (d, *J* = 7.1 Hz, 1H)	-[b]	8.87 (s, 1H), 8.86 (s, 1H)
	Other protons	7.66~7.27 (m, 20H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H)	
25[a]	6.61 (d, J = 7.3 Hz, 1H)	-[b]	9.03 (s, 1H), 8.78 (s, 1H)
	Other protons	7.65~7.15 (m, 20H), 3.83 (dd, J = 10.7, 4.6 Hz, 1H), 3.77 (dd, J = 10.7, 5.1 Hz, 1H), 3.59~3.47 (m, 2H), 3.45~3.41 (m, 1H), 3.35 (dd, J = 10.7, 6.1 Hz, 1H), 1.02 (s, 9H), 0.67 (s, 9H)	
26[a]	6.03 (d, *J* = 8.6 Hz, 1H)	-[b]	8.44 (s, 1H), 7.85 (s, 1H),
	Other protons	7.66~7.30 (m, 20H), 6.06 (br s, 2H), 3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H)	
27[a]	6.21 (d, J = 6.9 Hz, 1H)	-[b]	8.62 (s, 1H), 8.50 (s, 1H)
	Other protons	7.68~7.22 (m, 20H), 5.18 (br s, 2H), 3.88 (dd, J = 10.7, 4.9 Hz, 1H), 3.82 (dd, J = 10.7, 5.9 Hz, 1H), 3.66~3.59 (m, 2H), 3.52~3.44 (m, 2H), 1.06 (s, 9H), 0.80 (s, 9H)	
28[d]	6.17 (d, *J* = 6.6 Hz, 1H)	-[b]	8.84 (s, 1H), 8.24 (s, 1H)
	Other protons	3.80 (d, *J* = 4.6 Hz, 2H), 3.73 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H)	
29[d]	6.36 (d, J = 7.8 Hz, 1H)	-[b]	9.04 (s, 1H), 8.23 (s, 1H)
	Other protons	3.93 (dd, J = 10.7, 5.6 Hz, 1H), 3.82 (dd, J = 11.7, 6.8 Hz, 1H), 3.74 (dd, J = 11.7, 6.8 Hz, 1H), 3.64 (quin, J = 7.3 Hz, 1H), 3.35~3.30 (m, 2H)	

[a]$CDCl_3$, [b]Not Applicable, [c]DMSO-d_6, [d]CD_3OD

Table 6.2. Elemental analysis data

Cmpd	Formula	Calc for C	Calc for H	Calc for N	Calc for S	Found for C	Found for H	Found for N	Found for S
3	$C_{10}H_{18}O_9S_2$	34.68	5.24	-[a]	-[a]	34.51	5.22	-[a]	-[a]
4	$C_{11}H_{18}O_7S_2$	40.48	5.56	-[a]	19.65	40.67	5.37	-[a]	19.59
5	$C_8H_{12}O_3S$	51.04	6.43	-[a]	17.03	50.83	6.36	-[a]	17.00
6	$C_5H_8O_3S$	40.53	5.44	-[a]	21.64	40.64	5.43	-[a]	21.77
7	$C_4H_8O_2S{\cdot}0.35H_2O$	37.99	6.93	-[a]	25.35	38.02	6.98	-[a]	25.00
8	$C_{17}H_{26}O_3SSi$	60.31	7.74	-[a]	9.47	60.37	7.74	-[a]	9.50
9	$C_{22}H_{30}N_2O_5SSi$	57.12	6.54	6.06	6.93	57.30	6.67	5.85	6.81
10	$C_9H_{12}N_2O_4S$	44.25	4.95	11.07	13.13	44.54	5.12	11.20	12.74
11	$C_{16}H_{36}O_2SSi_2$	55.11	10.41	-[a]	9.20	55.58	10.54	-[a]	9.13
12	$C_{21}H_{40}N_2O_4SSi_2$	53.35	8.53	5.93	6.78	53.63	8.60	5.73	6.92
13	$C_{21}H_{40}N_2O_4SSi_2$	53.35	8.53	5.93	6.78	53.10	8.46	5.91	6.93
14	$C_9H_{12}N_2O_4S$	44.25	4.95	11.07	13.13	44.00	5.08	11.16	12.77
15	$C_9H_{12}N_2O_4S$	44.25	4.95	11.07	13.13	44.27	5.10	11.20	12.89
16	$C_{20}H_{26}O_2SSi$	66.99	7.31	-[a]	8.94	66.87	7.41	-[a]	8.98
18	$C_{21}H_{26}OSSi$	71.13	7.39	-[a]	9.04	70.86	7.37	-[a]	8.86
19	$C_{21}H_{28}O_2SSi$	67.69	7.57	-[a]	8.61	67.68	7.72	-[a]	8.33
22	$C_{21}H_{28}O_2SSi$	67.69	7.57	-[a]	8.61	67.46	7.61	-[a]	8.48
23	$C_{37}H_{46}O_2SSi_2$	72.73	7.59	-[a]	5.25	72.73	7.66	-[a]	5.11
24	$C_{42}H_{47}N_4O_2ClSSi_2{\cdot}1.1EtOAc$	64.77	6.54	6.51	3.73	64.43	6.31	6.67	3.95
25	$C_{42}H_{47}N_4O_2ClSSi_2$	66.07	6.20	7.34	4.20	66.09	6.49	6.90	3.90
26	$C_{42}H_{49}N_5O_2SSi_2$	67.79	6.64	9.41	4.31	67.87	6.83	9.05	4.08
27	$C_{42}H_{49}N_5O_2SSi_2$	67.79	6.64	9.41	4.31	67.83	6.81	9.15	4.05
28	$C_{10}H_{13}N_5O_2S$	44.93	4.90	26.20	12.00	44.75	4.99	25.91	11.74
29	$C_{10}H_{13}N_5O_2S{\cdot}0.5CH_3OH$	44.51	5.34	24.72	11.32	44.18	5.03	24.47	11.23

CHAPTER 7

THE ROLE OF 2',3'-UNSATURATION ON THE ANTIVIRAL ACTIVITY OF ANTI-HIV NUCLEOSIDES AGAINST 3TC-RESISTANT MUTANT (M184V)[1]

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INTRODUCTION

Concerns about the development of the high level viral resistance to 3TC have prompted studies of the molecular mechanism of resistance, which is conferred by a single mutation at codon 184 in the catalytic domain of HIV-1 reverse transcriptase (RT).[324,325] The sterically demanding nature of Val184 imposes severe restriction to the conformation of nucleoside reverse transcriptase inhibitor (NRTI) triphosphates, in which only a selected number of NRTIs maintain the antiviral activity against M184V RT. It is noteworthy that no unnatural L-nucleosides have been reported to inhibit M184V RT, and, until now, only three classes of NRTIs have been reported to be active against M184V RT, which include dioxolane/oxathiolane (DAPD[326]/dOTC[327]), acyclic (Tenofovir disoproxil)[328] and 2',3'-unsaturated nucleosides [D4T,[329] D4FC,[330] D-2',3'-unsaturated-2'-fluorocytidine (2'Fd4C)[331]]. Among these, the 2',3'-unsaturated nucleosides including 2'-fluorine substitution[331,332] have been studied to provide the structure-activity relationships against M184V RT, where D-2',3'-unsaturated cytidine analogs show potent activity against M184V RT[331] but the isosteric replacement of 4'-oxygen with a sulfur atom (D-2',3'-unsaturated 2'-fluoro-4'-thiocytidine, 2'F-4'Sd4C)[332] develops significant cross-resistance. On the other hand, abacavir, which has a 4'-CH_2 group instead of an oxygen atom, exhibits only a 2-5 fold increase to M184V RT,[333] suggesting that the 4'-substituent in the 2',3'-substituted nucleosides is critical in determining the cross-resistance to the M184V RT. Thus, it was of interest to determine how the 2',3'-unsaturated moiety and its substitution contribute to the binding mode of the unsaturated nucleosides at the active site of M184V RT.

METHODS

Six NRTI (3TC, D4T, D4FC, 2'Fd4C, 2'F-4'Sd4C and Carbovir) triphosphates were docked into the active site of HIV-1 RT, and the resulting enzyme-ligand complex was studied by the energy minimization method (Fig. 7.1).[334] Carbovir triphosphate was included in this study because, according to the literature, abacavir monophosphate is metabolized to carbovir monophosphate which is converted to the active moiety, carbovir triphosphate.[333]

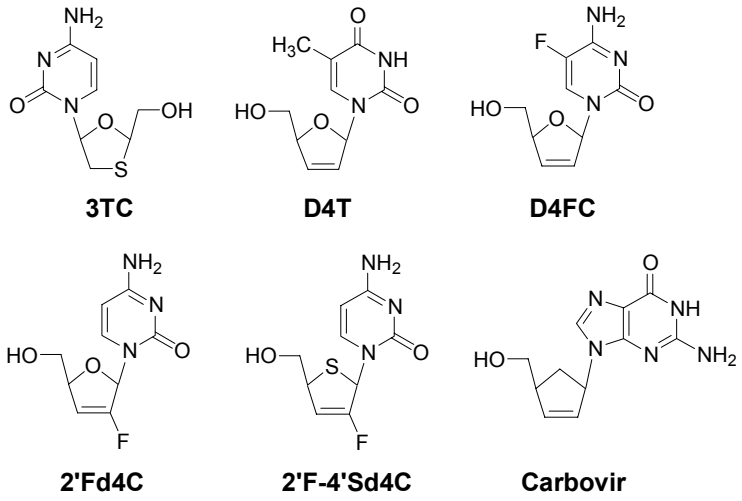

Figure 7.1. 3TC and five 2',3'-unsaturated NRTIs under study

(a) Conformational analysis: The initial conformations of the NRTIs were constructed by the builder module in Spartan 5.1.1 (Wavefunctions, Inc. Irvine, CA). The initial conformations were cleaned up and geometrically optimized through quantum mechanical *ab initio* calculations using RHF/3-21G* basis in Spartan 5.1.1.

(b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling of the

enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme-ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[341] A model of the NRTI binding site was constructed, which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template-primer) duplex.[334] The geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of 5th nucleotide in the template of the 7:4 (template-primer) duplex was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd)[335] was modified to guanine or cytosine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[335] Gästeiger-Hückel charge was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to enzyme site from the biopolymer module in Sybyl. Fluorine parameters were obtained from the literature[336,337] and MM2 parameters, and put to the parameter files. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and the mutated residue (Val 184) were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field until the iteration number reached 5,000.

The energy-minimized structures were analyzed to provide the characteristic binding mode of the 2',3'-unsaturated D-nucleosides to HIV-1 RT.

RESULTS AND DISCUSSION

Although 3TC is one of the most commonly used nucleoside analogs in first-line combination therapy for the treatment of HIV-1 infections,[338] 3TC monotherapy results in the high-level resistance conferred by a single mutation at codon 184, which increases the EC_{50} value of 3TC at least 1,000-fold.[324] As reported previously, the crystal structure of M184V RT without bound DNA template-primer duplex and deoxyribonucleoside triphosphate shows apparently no significant difference from that of the wild type (WT) RT.[339] In addition, the active site of HIV-1 RT is tight enough not to allow any significant conformational change.[340] Therefore, as the Met184 in the wild type RT directly faces the nucleoside triphosphate binding site, the structural difference between the Met184 and Val184 should exert its effect on the binding event of the nucleoside triphosphate at the active site of M184V RT in such a manner that the M184V RT is able to discriminate between the NRTI and analogs, which can cause steric hindrance with the Val184.

Significant efforts have been directed toward the 2',3'-unsaturated nucleosides because of their potent antiviral activity and, particularly, their antiviral potency toward M184V RT (Table 7.1). Among those, D4FC and 2'Fd4C show potent antiviral activity against M184V RT.[330,331] On the other hand, abacavir has moderate cross-resistance to M184V RT (2-5 fold increase),[333] but 2'F-4'Sd4C shows significant cross-resistance.[332]

Table 7.1. *In vitro* antiviral activity of the NRTIs against WT and M184V RT

	WT	M184V	FI
3TC[331]	0.042	>50	>1,200
D4T[335]	0.45	0.5	1.1
D4FC[336]	0.18	0.14	0.8
2'Fd4C[337]	8.0 [a]	1.8 [a]	0.23
2'F-4'Sd4C[338]	5.0[a]	125[a]	25
ABC[339]	5.3	15.5	2.9

[a] EC_{90} in μM

In order to investigate the binding mode of each NRTI to RT, the six NRTI structures, obtained from the quantum mechanical *ab initio* calculations using RHF/3-21G* basis in Spartan 5.1.1, were superimposed and compared. 3TC has the largest van der Waals radius of the sugar moiety which is directly projected to Val184 in the HIV RT. The van der Waals radii of 4'-CH_2 in carbovir and 4'-sulfur in 2'F-4'Sd4C are similar, and the 4'-oxygen atom in D4T, D4C and 2'Fd4C has the smallest van der Waals radius (Fig. 7.2). As the 4'-substituent of the sugar moiety of NRTI triphosphate is in close contact to Val184, it might be possible to correlate the cross-resistance of NRTIs to M184V RT with the relative sizes of the 4'-substituents. D4T, D4FC and 2'Fd4C with an oxygen atom at the 4'-position are still active or more active against M184V RT than against WT RT, while abacavir (ABC) with 4'-CH_2 substituent has little cross-resistance to M184V RT. However, different resistance profiles of 2'F-4'Sd4C and ABC, which have the 4'-substituents similar in size, deserved more investigation.

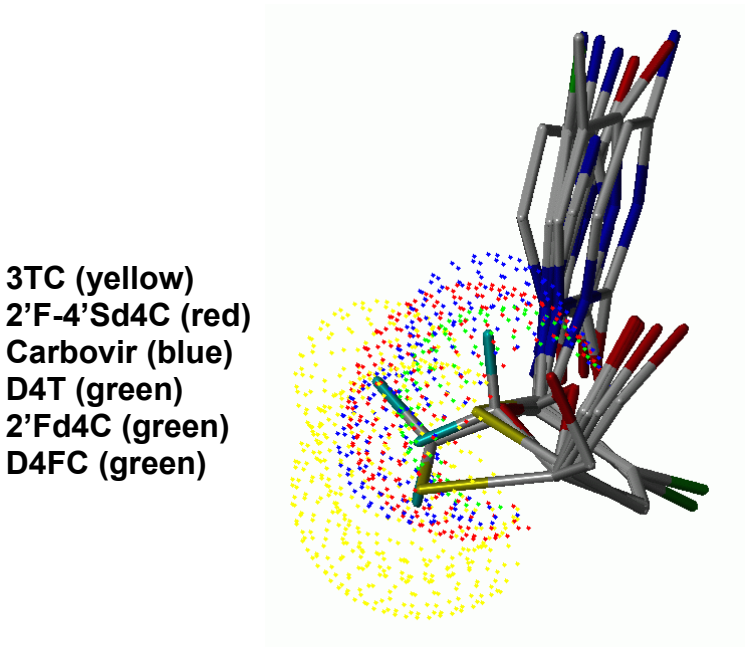

Figure 7.2. Structures of 3TC /D4T/2'F4'Sd4C/2'Fd4C/carbovir/D4FC

Comparison of the minimized structures of ternary complexes (NRTI-DNA-WT RT) shows several interesting features: (a) The 2'-fluoro 2',3'-unsaturated nucleosides (2'Fd4C and 2'F-4'Sd4C) show their sugar moieties slightly rotated to locate their 4'-substituents closer to Met184 (Fig. 7.3). This conformational difference is believed to be the result of the possible steric hindrance between the 2'-fluorine atom and the adjacent amino acid (Gln151). (b) Every 2',3'-unsaturated nucleoside studied shows their 2',3'-double bond at close proximity (~ 3.5 Å) with right orientation to the aromatic side chain of Tyr115, which can be understood as a hydrophobic π-π interaction (Fig. 7.4).[332] This π-π interaction strengthens the binding between the HIV RT and the triphosphates. This interaction may partially explain the significant anti-HIV activity of the d4Ns against the M184V mutant by the π-π interaction.

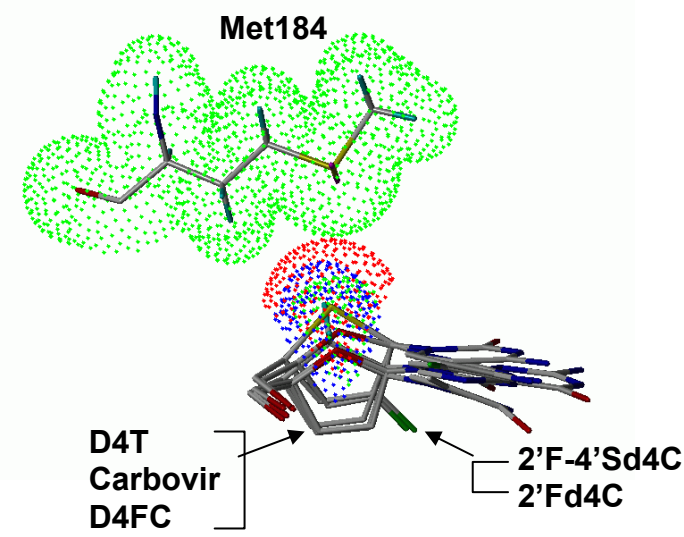

Figure 7.3. The rotation of the sugar moiety of 2'Fd4C and 2'F-4'Sd4C pushes the 4'-substituent close to Met184. The 4'-oxygen in 2'Fd4C (green) is far away from Met184, but the 4'-sulfur atom in 2'F-4'Sd4C (red) is in close contact to Met184.

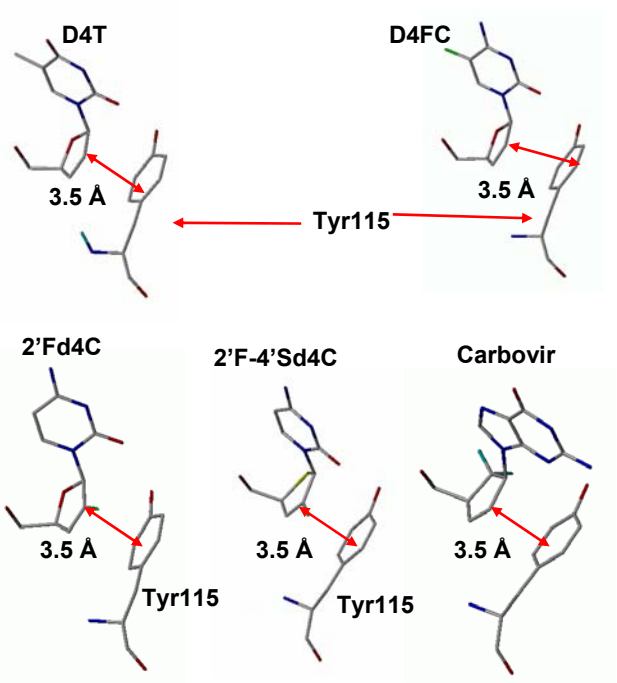

Figure 7.4. 2',3'-double bonds of the 2',3'-unsaturated nucleosides (D4T, D4FC, 2'Fd4C, 2'F-4'Sd4C and Carbovir) are separated from the aromatic side chain of Tyr115 by 3.5Å in parallel by the hydrophobic π-π interaction.

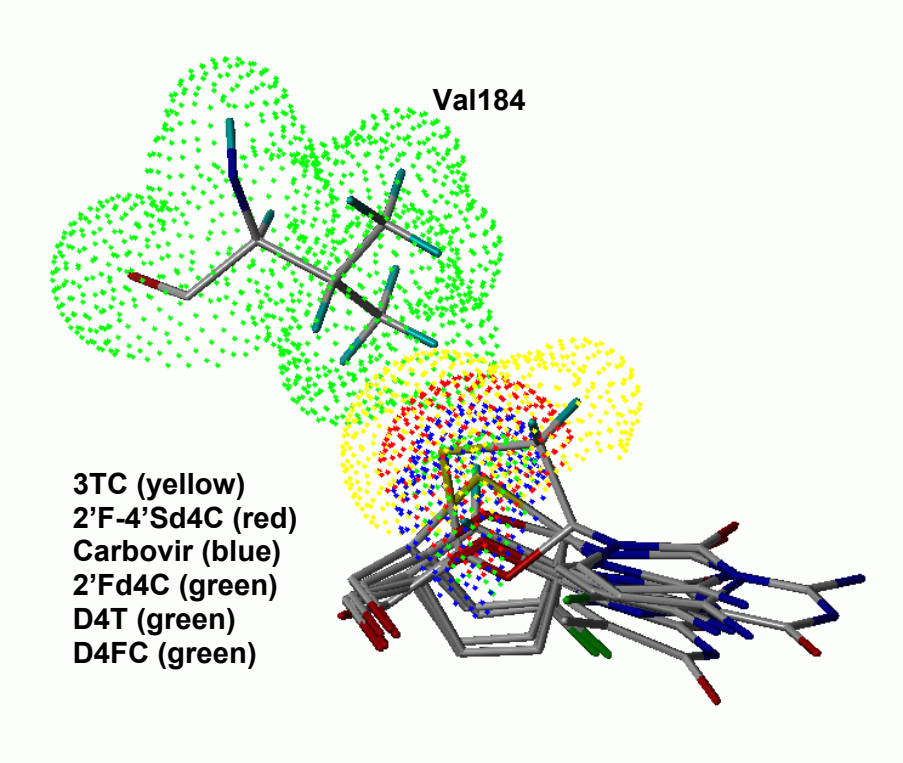

Figure 7.5. Even though the carbovir has a bulky 4'-CH_2 group (blue), it does not experience serious steric hindrance with Val184. However, 2'F-4'Sd4C with bulky 4'-sulfur (red) experience steric hindrance because of the rotation by 2'-fluoro substituent. L-Configured 3TC (yellow) also has big steric hindrance with Val184.

In the structures of ternary complexes (NRTI-DNA-M184V RT), 3TC is located too close to the Val184 resulting in significant steric hindrance (Fig. 7.5), suggesting that 3TC has difficulty in binding to the active site of M184V RT. As we mentioned before, the sugar moieties of 2'Fd4C and 2'F-4'Sd4C rotated to Met184 in WT RT. This conformational change was manageable when the 4'-substituent was an oxygen atom (2'Fd4C), but the 4'-sulfur atom (2'F-4'Sd4C), which has larger van der Waals radius than oxygen atom, cannot avoid the unfavorable steric hindrance with the side chain of Val184, resulting in high cross-resistance to M184V RT (Fig. 7.5). However, the 4'-CH_2

group in carbovir is not close enough to Val184 because there is no 2'-fluoro substituent (Fig. 7.4). Therefore, the steric hindrance between the sugar moiety of carbovir and Val184, if any, should not be significant.

In summary, the π-π interaction can contribute, in part, to the observed strong binding of the 2',3'-unsaturated nucleosides at the active site of RT, resulting in potent anti-HIV activity including against M184V mutant. Every 2',3'-unsaturated nucleoside analog studied showed the same pattern of interaction with the adjacent aromatic side chain of Tyr115 in the hydrophobic π-π interaction. However, the 2'-fluorine substitution rotates the sugar moiety to push the 4'-substituent close to Met184. Therefore, 2'-fluoro 2',3'-unsaturated nucleoside with a bulky substituent at the 4'-position experienced steric hindrance with Val84, and thereby reduced antiviral potency, which was confirmed by the significant cross-resistance of 2'F-4'Sd4C to M184V RT.

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