

TOWARDS THE DISCOVERY OF ANTIVIRAL NUCLEOSIDES; SYNTHESIS,
ANTIVIRAL ACTIVITY AND MOLECULAR MECHANISM OF DRUG
RESISTANCE

by

YOUHOON CHONG

(Under the Direction of CHUNG K. CHU)

ABSTRACT

Nucleoside analogues continue to be important drug regimens as part of antiviral therapy. However, because of drug toxicity, and the lack of a durable response due to resistance, there is clearly a need for new compounds to cope with these drawbacks. For this purpose, we developed novel nucleoside analogues and several protocols for molecular modeling studies which can provide a plausible mechanism of antiviral drug resistance conferred by mutation in HIV-1 reverse transcriptase (RT) as well as HBV polymerase. Thus, our recent efforts in the area of molecular modeling studies of HIV-1 RT and drug resistance by mutant RT were reviewed in Chapter 1. In Chapter 2, a divergent synthesis of D- and L- carbocyclic 4'-fluoro-2',3'-dideoxynucleosides is described. Development of an efficient process for preparing a synthetically useful intermediate, L-2-deoxy ribose is described in Chapter 3, followed by a discussion of the enantiomeric synthesis of D-2'-fluoro-4'-thio-2',3'-unsaturated nucleosides and their potent antiviral activities in Chapter 4. Chapter 5 deals with a structure-activity relationship study of L-3'-fluoro-2',3'-unsaturated nucleosides, and another structure-activity relationship study of D-3'-

fluoro-4'-thio-2',3'-unsaturated nucleosides is described in Chapter 6. Several molecular modeling studies of HIV-1 RT and HBV polymerase complexed with various nucleoside analogues are discussed in the next five chapters: AZT, 3TC and D-dioxolane guanine (DXG) against AZT/3TC resistant RT (Chapter 7), L-FMAU against HBV polymerase (Chapter 8), dioxolane nucleosides against 3TC-resistant RT (Chapter 9), 3'-substituted L-nucleosides against 3TC-resistant HBV polymerase (Chapter 10) and fluorinated cytidine analogues against HIV-1 RT (Chapter 11). Finally, various attempts for the synthesis of hereto unknown 1'-fluororibonucleoside are detailed in Chapter 12.

INDEX WORDS: Carbocyclic nucleosides, 2-deoxy ribose, 4'-thionucleosides, 3'-fluoro-2',3'-unsaturated nucleosides, 3'-fluoro-4'-thionucleosides, molecular modeling, human immunodeficiency virus (HIV), drug resistance, hepatitis B virus (HBV), L-FMAU and homology modeling.

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CHAPTER 1

INTRODUCTION, A LITERATURE REVIEW

Introduction

Viruses, contrary to bacteria and parasites, meet their metabolic needs by using both their host's and their own enzymes. The fact that the viral enzymes are often quite similar to their cellular equivalents is one of the reasons that makes the development of effective, nontoxic drugs against viral infections a rather challenging task. One way to overcome this problem has been to identify, within viral enzymes, those which have properties not shared by the host counterparts. These enzymes could then be selective targets for agents which impair viral replication without harming the host.

Retroviruses such as the 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 to double-strand DNA. RT is a multifunctional enzyme that has two types of DNA polymerase activity, RNA-dependent DNA polymerase (RDDP, unlike cellular enzymes) to synthesize a DNA strand copy of the viral genomic RNA template and DNA-dependent DNA polymerase (DDDP, like cellular enzymes) to complete the synthesis of double-strand viral DNA. Additionally, RT possesses an intrinsic RNaseH activity to degrade the genomic RNA component of the DNA/RNA duplex intermediate formed during RT RDDP synthesis. 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 drugs. Examples include the nucleoside/nucleotide inhibitors (NRTIs): zidovudine,¹ lamivudine,² didanosine,³ zalcitabine,⁴ stavudine,⁵ abacavir,⁶ tenofovir disoproxil,⁷ as well as the non-nucleoside inhibitors (NNRTIs),

nevirapine,⁸ delavirdine⁹ and efavirenz.¹⁰ However, even in the context of the multi-drug combination therapy now standard in care, the mutability and resultant adaptability of HIV-1 RT present a major challenge to the design of effective antiviral strategies because many initially potent drugs lose efficacy over time.

In the case of the NNRTIs, most of the resistance mutations associated with the p66 subunit are located proximal to the drug-binding site, resulting in direct steric perturbations. In contrast, until the recent publication of the crystal structure of a covalently trapped catalytic complex of HIV-1 RT,¹¹ some aspects of the structural basis for the mechanisms of resistance for NRTI drugs have largely remained a puzzle because many of the mutation sites that give rise to resistance are distal (between 10-20 Å) to the putative nucleoside triphosphate binding site on the enzyme. In addition, this work¹¹ reveals that there is a conformational change of the so-called fingers and thumb domains of the p66 subunit of HIV-1 RT that, together with a loop movement, results in a more 'closed' conformation of the central cleft of p66, instead of the 'open' conformation seen in previous HIV-1 RT crystal structures. The presence of this unanticipated conformation places certain NRTI drug resistance mutations much closer to the nucleoside triphosphate binding site of the enzyme than previously predicted, thereby providing a simple steric explanation for their mechanism of resistance. However, the structure of the wild type enzyme complexed with the natural substrate (dNTP) fails to delineate the mechanism of resistance conferred to many structurally different NRTIs by mutation in the amino acid residues of the enzyme. In addition, in certain cases such as AZT-resistant mutants, simple combination of the mutations relatively far away from the dNTP binding site does not provide any structural information of resistance. Therefore, even though there is a

critical need for a comprehensive understanding of the molecular mechanism of anti-HIV drug resistance by mutant RTs, lack of crystallographic and structural information of the mutant RTs complexed with DNA duplex as well as NRTI triphosphate has prevented detailed investigations. As an aid to understanding how mutations affect RT structure and function, several molecular modeling studies have been undertaken on RT modified at specific residues.¹² Presumably, mutation may either deform the active site conformation through structural realignment or destabilize inhibitor binding. Therefore, under the given limitations of the computational method, assessment of the structural adjustment resulting from these local changes and the accompanying energetic consequences may provide insight into the design of more effective NRTIs. This review discusses our recent efforts to correlate the structure with enzyme function in the HIV-1 RT, which may lead to the understanding of the NRTI drug resistance conferred by mutations.

Inhibition of HIV-1 RT by nucleoside reverse transcriptase inhibitors (NRTIs).

HIV-1 RT differs from cellular DNA polymerases in two respects. First, HIV RT readily utilizes many chemically altered analogues of the natural deoxynucleoside triphosphate (dNTP). Second, HIV RT lacks a formal 'proofreading' activity.¹³ These characteristics are important from a drug design view point for HIV. NRTIs are analogues of the normal dNTP substrates of DNA polymerases. The 2',3'-dideoxynucleosides such as ddC and ddI lack a 3'-OH on the sugar, whereas other analogues such as 3'-azido-3'-deoxythymidine (AZT) have the 3'-OH replaced by other functional groups that do not

allow the primer extension. Efficiency of the inhibition of HIV-1 RT is dependent on four major factors: (i) the efficiency of cellular uptake of the analogue, (ii) the efficiency of the initial phosphorylation by cellular kinases, (iii) the level of competitive inhibition with the natural substrate, and (iv) the efficiency of triphosphate binding to RT and subsequent incorporation into the growing viral DNA.¹⁴

Cellular uptake: Most nucleoside analogues, such as ddC and 3TC, enter cultured cells by active transport.¹⁵ In contrast, a more efficient mechanism, i.e., passive or nonfacilitated diffusion has been observed for entry of AZT and ddI.¹⁶ Phosphorylation of nucleoside analogues precedes antiviral activity in HIV-infected cells.

Phosphorylation: NRTIs require intracellular metabolic transformation for antiviral activity, namely conversion to the triphosphate, a process catalyzed by cellular kinases. The first phosphorylation step, often considered as rate limiting, is dependent on cytosolic thymidine kinase 1 (TK1), cytosolic deoxycytidine kinase (dCK), mitochondrial thymidine kinase 2 (TK2) or mitochondrial deoxyguanosine kinase (dGK), depending on the nucleoside considered.¹⁷ Conversion of AZT to AZT 5'-monophosphate (AZT-MP) by thymidine kinase occurs with about the same efficiency as the synthesis of TMP by the same enzyme, but the process and kinetics of phosphorylation are not identical for other thymidine analogues.¹⁸ The pathways of ddA and ddI phosphorylation are more complex and less well understood than those of either AZT or ddC phosphorylation;^{18,19} ddI can be aminated by adenosine deaminase to form ddA, or it may be phosphorylated by a cytosolic 5'-nucleotidase to form ddIMP.^{20,21} All ddIMP, generated by the exogenous addition of ddI or ddA to cells, is eventually aminated and phosphorylated to ddATP by adenosine deaminase and purine nucleoside kinases, respectively.^{19,20} In

contrast, many β -L-cytidine analogues such as 3TC,²² FTC²² and L-dC²³ are substrates of dCK regardless of the nature of the pentose ring. In addition to the activation of cytosine nucleoside derivatives, human dCK also catalyzes the phosphorylation of D- and L-enantiomers of β -dA, β -araA, and β -dG with enantioselectivities favoring the unnatural L-enantiomer for adenosine derivatives and the natural D-enantiomer for 2'-deoxyguanosine.²⁴ In the case of the carbocyclic analogues of deoxyguanosine, the enzyme (dGK) catalyzes the phosphorylation of both enantiomers with a preference for the L-antipode.²⁵

Competitive inhibition and incorporation into the growing viral DNA: After conversion to the active triphosphate, NRTIs must compete with the natural dNTPs, both for recognition by RT as a substrate (binding) and for subsequent incorporation into the nascent viral DNA chain (catalysis). NRTIs thus inhibit RT-catalyzed proviral DNA synthesis by two mechanisms.²⁶ First, they are competitive inhibitors for binding and/or catalytic incorporation with respect to the analogous dNTP substrate. Second, they terminate further viral DNA synthesis, due to lack of a 3'-OH group. Even though the chain termination is the principal mechanism of NRTI antiviral action, both mechanisms are thought to be required for the inhibition of HIV replication in host cells.^{14,27} HIV-1 RT DNA synthesis follows an ordered 'bi-bi' mechanism involving several RT mechanistic species (Fig. 1.1).^{28,29,30,31} Free RT first binds to the template/primer (T/P) to form a tight RT-T/P_n binary complex, which is followed by the binding of dNTP, to form the RT-T/P_n-dNTP ternary complex (Fig. 1.1). Binding of dNTP appears to be a two-stage process.³² The initial interaction may be nonselective with each of the four dNTPs binding with relatively similar affinities. The second stage is more selective, and involves

the correct positioning of only specific dNTP complementary to the template base. The selectivity involved in positioning and incorporating the correct nucleotide is primarily controlled by the free energy of base pairing with the complementary template nucleotide base. Since NRTIs and the analogous dNTPs have identical base structures, in most cases RT has little opportunity to readily affect the discrimination between natural substrates and inhibitors at this stage. Formation of the initial RT-T/P_n-dNTP induces a rate-limiting change in protein conformation to form a very tight ternary complex, RT^{*}-T/P_n-dNTP (Fig. 1.1).

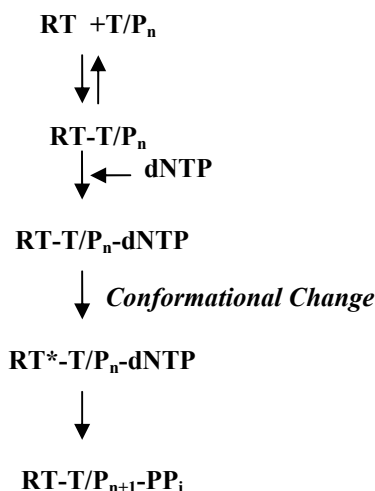


Figure 1.1. Reaction mechanism for RT-catalyzed DNA synthesis.

The formation of this ternary complex allows the critical transition state to be reached, enabling a nucleophilic attack by the 3'-OH primer terminus on the α-phosphate of the bound dNTP. This results in phosphodiester-bond formation and extension of the viral DNA strand by one nucleotide, along with formation of the RT-T/P_{n+1}-PP_i ternary

complex (Fig. 1.1). The PP_i product then dissociates, leaving the RT-T/ P_{n+1} binary complex, which is kinetically indistinguishable from the RT-T/ P_n binary complex. RT can then either dissociate from the newly elongated RT/ P_{n+1} (distributive mode of polymerization) or translocate along the bound template, bind the next complementary dNTP and continue DNA synthesis (processive mode of polymerization). Competitive inhibition by ddNTP results from stronger binding of RT to ddNTP than to dNTP, and, therefore, the strength of a competitive inhibitor of RT is directly related to the ratio of its K_i (inhibition constant or enzyme-inhibition dissociation constant) for ddNTP to its K_m (Michaelis constant, a measure of dissociation) for dNTP. In RT reactions employing homopolymeric template-primer (T/P), the K_i for AZT-TP was nearly 10-fold less than the K_m for TTP, making this analogue a potent competitive inhibitor of RT.^{33,34,35} With the possible exception of the acyclic analogue phosphonylmethoxyethyladenine,³⁶ nearly all ddNTP having anti-RT activities were shown to be competitive inhibitors of HIV-1 RT: ddCTP, 3TC-TP and ddATP ($K_i/K_m < 10$) were relatively poor competitive inhibitors of HIV-1 RT compared with AZT-TP, carbovir-TP and ddGTP ($K_i/K_m > 19$). Nevertheless, competitive inhibition by NRTIs was not sufficient to inactivate HIV-1 RT.^{14,37}

Chain termination of HIV-1 reverse transcription has been observed with nearly all ddNTP studied in cell-free RNA-dependent DNA polymerase (RDDP) and DNA-dependent DNA polymerase (DDDP) reactions.^{35,38,39,40,41} Incorporation of ddNMP by RT and subsequent chain termination are preceded by a NRTI-TP binding event. The key kinetic steps that define the incorporation of dNTP (and NRTIs) are the nucleotide binding event, the rate-limiting conformational change, the efficiency of incorporation and RT-T/ P_{n+1} dissociation constant.⁴²

HIV-1 RT structure and function.

Crystal structures for three of the RT mechanistic forms have been solved, which include substrate-free enzyme (RT),^{43,44} the RT-T/P binary complex^{45,46} and an RT*-T/P-dNTP ternary complex.¹¹ These structural data have provided considerable insight into the conformational changes associated with DNA polymerization, the molecular structure of the RT active site, and the relative position of certain critical residues during catalysis, all of which are important for understanding the molecular basis of HIV-1 resistance to NRTIs. The HIV-1 RT gene encodes a 66-kDa protein; however, the presumed biologically relevant form of HIV-1 RT is a heterodimer comprised of subunits of 66 and 51kDa (termed p66 and p51).⁴⁷ The p51 subunit is produced during viral assembly and maturation *via* HIV-1 protease-mediated cleavage of the C-terminal domain of a p66 subunit. The first crystal structure of HIV-1 RT revealed that the p66 subunit was arranged with right-handed fingers (residues 1-85)-palm (86-117, 156-237)-thumb (238-318) domain structure containing a cleft that is similar to that first observed for the Klenow fragment of PolI⁴⁸ (Fig. 1.2). The DNA polymerase catalytic aspartate residues (D110, D185 and D186) are in the palm subdomain. In addition, the p66 has two additional major subdomains, the 'connection' (residues 319-426) and the C-terminal ribonuclease H (RNase H) domains. The latter subdomain is missing in the p51 subunit. Whereas the overall folding of the subdomains is similar in both p66 and p51 subunits, the spatial arrangement of the subdomains differs markedly.^{49,50} The p66 subunit adopts an 'open', catalytically competent conformation that can 'grasp' a nucleic acid template, whereas the p51 subunit is in a 'closed' conformation. The p51 subunit is considered to

play a largely structural role, although it may also be important in interacting with the transfer RNA (tRNA) primer used for the initiation of HIV-1 DNA synthesis.⁴⁹

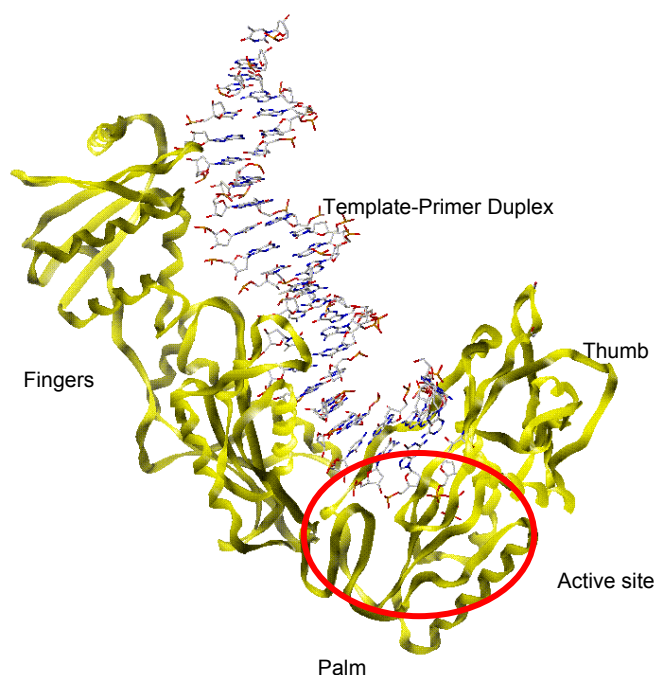


Figure 1.2. Three dimensional structure of HIV-1 RT•T/P•TTP ternary complex

However, only the palm domains of these two proteins have similar three-dimensional folds. The RT structure revealed that nevirapine, a NNRTI, can bind in the palm domain close but not overlapping to the putative dNTP binding site. This and further RT-inhibitor complex structures nicely explained the NNRTI resistance mutations,^{51,52} which were clustered around the drug binding pocket. However, the NRTI mutations that had been reported at that time appeared to be positioned on both the fingers and palm domains, at some distance from the presumed dNTP site (Fig. 1.3). An RT structure with a bound double-stranded DNA template-primer together with a Fab fragment confirmed the previous ‘open’ RT p66 conformation.⁴⁵ However, comparison of the structures of free

RT with the RT-T/P binary complex shows that interaction of RT with the nucleic acid induces a conformational rotation of the p66 thumb subdomain. As the complex reaches the transition state (RT^{*}-T/P-dNTP ternary complex) by binding dNTP, further conformational changes in RT are induced.¹¹ In particular, parts of the fingers subdomain rotate inwards toward the palm subdomain and the polymerase active site, effectively ‘clamping’ the dNTP into the active site region. Therefore, in order for the enzyme-ligand complex to be catalytically active, there must be some conformational changes in RT leading to a tight binding of the substrate. Two fingers residues make important contacts with the dNTP substrate. The ϵ -amino group of Lys65 and the guanidinium group of Arg72 interact with the γ - and α -phosphates of the bound dNTP substrate, respectively (Fig. 1.4a).

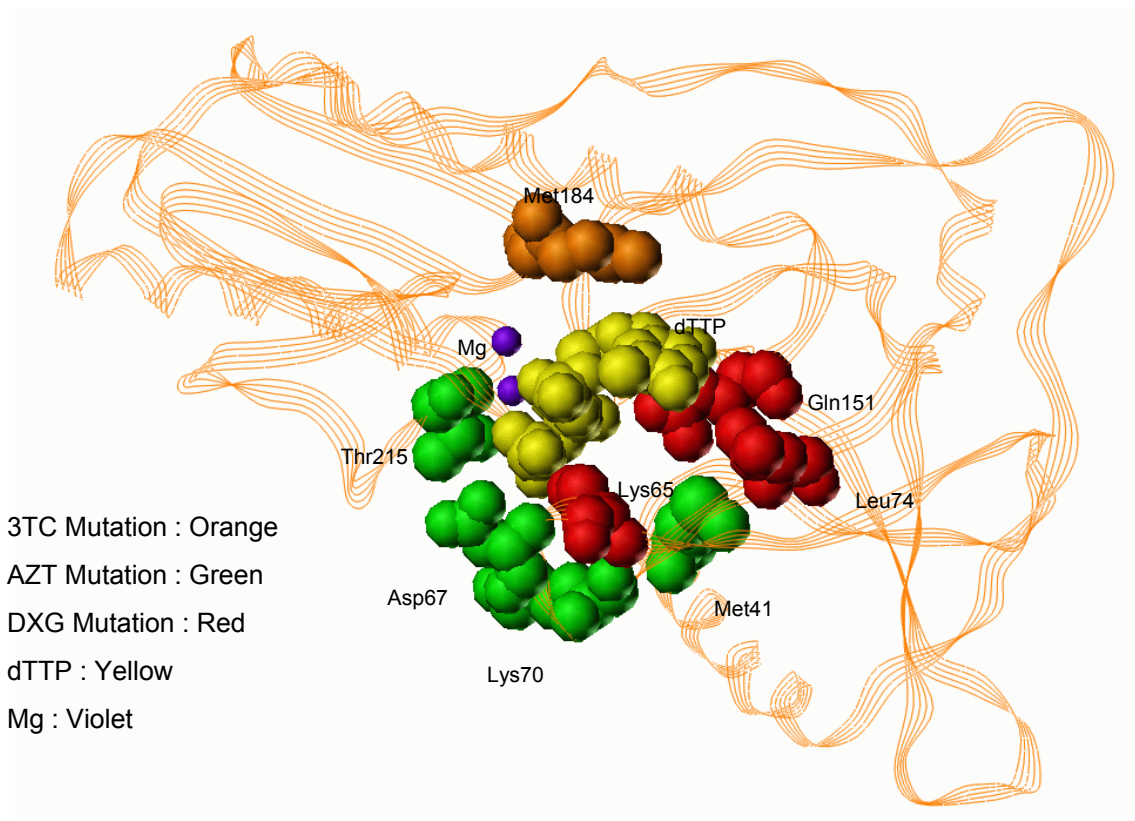


Figure 1.3. Mutations in HIV-1 RT resistant to NRTIs.

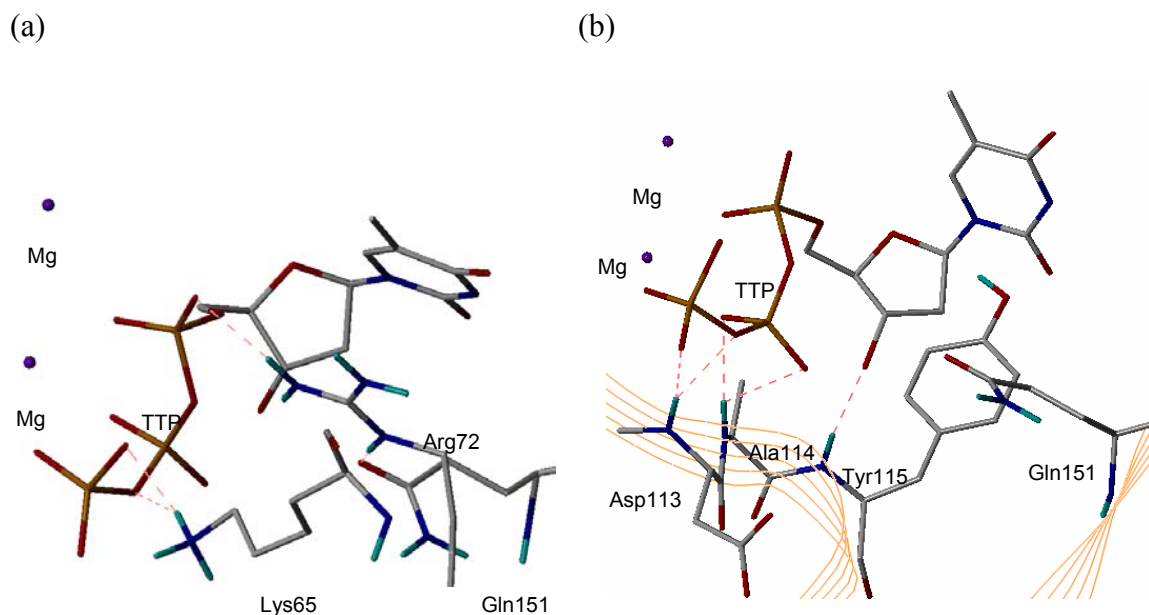


Figure 1.4. Active site of HIV-1 RT.

The dNTP triphosphate moiety also interacts with the main-chain amide-NH of residues Asp113 and Ala114 (Fig. 1.4b), and with the magnesium (Mg^{2+}) divalent metal ion cofactor bound to the polymerase active site aspartates (Asp110, Asp185 and Asp186) (Fig. 1.5). The divalent metal ions facilitate the nucleophilic attack by the 3'-OH of the primer terminus on the α -phosphorous of the incoming dNTP, and stabilize the transition state.⁵³ Only two of the active site aspartates (Asp110 and Asp185) are coordinated to Mg^{2+} in the HIV-1 RT ternary complex (Fig. 1.5).¹¹ The third aspartate (Asp186) may be involved in positioning the primer terminus by interacting with the 3'-OH of the primer terminal nucleotide, which projects into a small pocket formed by the side chains of Asp113, Ala114, Tyr115, Phe116 and Gln151 (Fig. 1.4b). Interestingly, the 3'-OH of the nucleotide forms hydrogen bonds with the backbone -NH of Tyr115 and triphosphate moiety of the dNTPs (Fig. 1.4b), which also plays an important role accommodating the dNTP in HIV mutant RT.

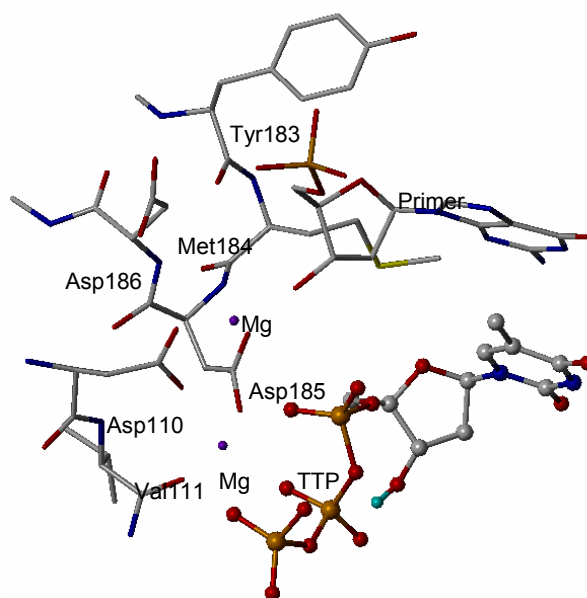


Figure 1.5. YMDD motif and Mg-coordination geometry of HIV-1 RT.

Mechanism of HIV-1 resistance to NRTIs.

The low fidelity of HIV-1 RT and the high rate of replication of HIV result in mutations at a frequency of about 10^{-4} per cycle.³⁷ Mutant viruses, many of which are replication defective,⁵⁴ are continuously produced by $CD4^+$ cells that contain stably integrated proviral DNA. This heterogeneity allows for early selection of replication-competent viruses by antiviral drugs, permitting the emergence of drug-resistant forms.^{55,56,57,58,59,60} Nucleoside-resistant isolates have been identified in patients receiving antiretroviral nucleoside drugs.^{61,62,63} In addition, resistant variants can be generated through tissue culture passage of drug-sensitive strains in the presence of increasing drug concentrations.^{62,64,65,66,67} 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 1.1). 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,¹¹ 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. 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.^{68,69} In the absence of structural data for NRTI-resistant RTs, three mechanisms have been proposed to account for the molecular basis of the NRTI resistance phenotype. These include selective alterations in NRTI binding and/or incorporation, template/primer (T/P) repositioning and NTP-mediated as well as phosphorolytic removal of an incorporated chain-terminating NRTI residue from the 3'-end of the nascent viral DNA.⁷⁰ The different resistance phenotypes seem to correlate with different sets of mutations in RT, and the effect of an individual set of mutations on resistance to a specific NRTI can only be presumed by kinetic studies and building up the enzyme active site by molecular modeling studies. However, the understanding of how mutations affect RT structure and function, and the ensuing loss of potent antiviral activities of certain NRTIs have not been demonstrated in conjunction with their binding modes, which would provide

invaluable insight into the design of more effective NRTIs active against mutant RTs.

Under the assumption that mutation may either deform the active site conformation through structural realignment or destabilize inhibitor binding, we tried to assess the structural adjustment resulting from mutations and the accompanying energetic consequences.

Table 1.1. Fold resistances in mutant HIV-1 RT associated with NRTI resistance.

Mutation	Resistance Conferred (Fold Resistance) ⁷¹
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)

Molecular modeling studies of mutant RTs.

Understanding the mechanisms involved in HIV resistance to 3TC and AZT (Fig. 1.6), particularly in the context of combination treatment, is of enormous practical importance,

since both compounds are important components of many currently employed drug regimens. For the past several years, we have tackled this challenging question by comparing the wild-type and computer-generated mutant HIV-1 RT complexed with DNA duplex as well as various NRTI-TP in the energy-minimized states.^{72,73} The comparison of the binding modes of AZTTP, 3TCTP and various NRTI-TPs which are known to be active against mutant RTs provided interesting insights to the combat against the NRTI-resistant RTs.

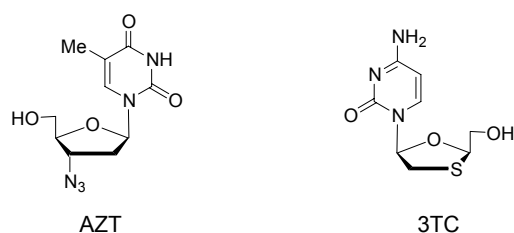


Figure 1.6. Structures of AZT and 3TC.

Computational methods.

All molecular modeling of the enzyme-substrate complexes was carried out using SYBYL 6.6 (Tripos Inc. St. Louis, MO), and the Monte Carlo conformational search⁷⁴ was performed by Macromodel 7.0 (Schrödinger, Inc).

Enzyme sites. 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).¹¹ Given the large size of the HIV-1 RT, calculations of the entire protein-ligand complex were not practical. Therefore, a model

of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit. Hydrogen atoms were added, and the clipped residues (Lys1 and Pro243) were capped with acetyl groups. The template-primer duplex had to be truncated as well. Interestingly, 5:2 (template:primer, ratio of bases) duplex was not stable enough presumably because of the insufficient base stacking interaction to maintain the duplex structure. Thus, a 7:4 (template:primer) duplex, which was shown to be stable enough to give a reliable result was used for the modeling studies.⁷⁵ The missing 3'-OH group in G22 [the 3'-end (n^{th}) nucleotide in the primer strand] in the original X-ray coordinates (1rtd)¹¹ was added, so that it could properly coordinate the adjacent Mg atom. Three clipped residues in template and primer strands [G3, C19(template)/ G9(primer)] were capped with methyl groups.⁷²

NRTIs. The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates (AZT⁷⁶ and ddC⁷⁷) or conformational analysis⁷⁵ of the modified crystal structures (3TC from FTC⁷⁸/ L-d4FC and L-3'Fd4C from d4C⁷⁹). The X-ray coordinates of D-dioxolane cytosine⁸⁰ were used as a template for construction of DXG structure, whose heterocyclic base moiety, cytosine, was modified to guanine before docking into the enzyme. Also, the heterocyclic moiety of $n+1^{\text{th}}$ nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. The enzyme site and the inhibitor were merged to form a catalytic complex and then the NRTI triphosphate was situated in such an orientation that it could be paired with its complementary base in the template strand by adjusting the torsional angles to those found in the X-ray structure.¹¹

Method. Gasteiger-Hückel charge^{81,82} was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom

charges^{83,84} were loaded to enzyme site from the biopolymer option in SYBYL 6.6 (Tripos Inc. St. Louis, MO). Fluorine parameters were obtained from the literature^{85,86} and MM2 parameters. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed⁷² until energy change from one iteration to the next was less than 0.05 kcal/mol (hot region: 6 Å , interesting region : 12 Å). The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field⁸⁷ until iteration number reached 5,000.

Calculation of Relative Binding Energy (E_{rel}). The binding affinities were estimated by means of the binding energy differences (relative binding energy, E_{rel}) between the NRTI triphosphate-RT complex and the corresponding natural substrate, 2'-deoxynucleoside triphosphates (dNTPs)-RT complex. The binding energy (E_b) of each nucleoside triphosphate was calculated by the Eq 1.

$$E_b = E(\text{ligand}) + E(\text{enzyme}) - E(\text{enzyme-inhibitor complex}) \dots\dots (\text{Eq 1})$$

Where, E_b = binding energy of the NRTI triphosphate or dNTP to RT

$E(\text{enzyme})$ = energy of RT-DNA duplex complex

$E(\text{ligand})$ = energy of NRTI triphosphate or dNTP

$E(\text{enzyme-ligand complex})$ = energy of RT-DNA duplex-NRTI triphosphate ternary complex or RT-DNA duplex-dNTP ternary complex

The relative binding energy (E_{rel}) of the NRTI triphosphate was calculated by the difference of the binding energy between NRTI triphosphate and the corresponding dNTP (Eq 2).

$$E_{rel} = E_b(\text{NRTI triphosphate}) - E_b(\text{dNTP}) \dots\dots (\text{Eq 2})$$

Therefore, a favorable binding affinity would be represented by a large positive E_{rel} and a poor binding NRTI triphosphate would have a small or negative E_{rel} .

Monte Carlo conformational search. The Monte Carlo conformational search of D-dioxolane thymine was performed in 5,000-step, in the presence of GB/SA water model using MM3 force field (Macromodel).⁷⁴

M184V RT (3TC resistance).

The M184V mutation in RT that confers high-level resistance to 3TC is located in the YMDD motif that constitutes a portion of the polymerase-active site of RT and other polymerases.⁸⁸ Mutated RT that contains the M184V mutation can discriminate between 3TC-TP and dCTP, in such a way that the incorporation rates of 3TC-MP are 20- to 100-fold less than with the wild-type enzyme.^{89,90,91,92} Using the M184I RT/DNA and wild-type RT/DNA crystal structures, Sarafianos *et al.*⁹³ have modeled the interactions of dCTP and 3TCTP at the active site of the M184I enzyme, assuming similar binding of the triphosphate moieties and similar base-pairing interactions of the incoming inhibitors with a modeled template overhang. The predicted position of the nucleotide with respect to the active site YIDD motif in this model is strongly supported by the structure of the polymerase active site in the ternary HIV-1 RT/DNA/dTTP complex.¹¹ The 3TCTP and dCTP nucleoside ring configurations are enantiomeric, so that alignment of the triphosphates and bases would cause the nucleoside ring of the β -L-inhibitor to project 1.5-2.0 Å further toward residue 184 than would the ribose ring of dCTP. Therefore, the most significant difference between the interactions of wild-type HIV-1 RT and the 3TC-resistant mutant HIV-1 RT (M184V RT) with ribose and β -L-oxathiolane nucleoside

inhibitors would be a very close contact between the C γ 2 methyl of Val and the protruding oxathiolane ring. This unfavorably close contact (2.5 Å in M184I vs. 3.9 Å in wild-type) would result in severe steric hindrance, preventing proper positioning of 3TCTP for catalysis, consistent with the data of Feng and Anderson. A recent pre-steady-state kinetic analysis of the D- and L- isomeric 3TC compounds as well as the corresponding 2',3'-dideoxy and natural nucleoside triphosphate provided a mechanistic basis for understanding the inhibition of RT by 3TC.⁸⁹ Thus, the maximum rate of incorporation (k_{pol}) followed the decreasing order of dCTP > ddCTP > (+)-BCH-189 (D-counterpart of 3TC) > 3TC while the K_d values determined for the DNA/RNA primer-template followed the decreasing order of 3TC $\cong$ (+)-BCH-189 $\cong$ ddCTP > dCTP. The corresponding efficiency of incorporation followed the decreasing order of dCTP > ddCTP > (+)-BCH-189 > 3TC, which suggests that perturbations on the ribose ring of cytidine analogues (C $\rightarrow$ S) decrease the rate and efficiency of incorporation but enhance the binding affinity. Our computer-generated three dimensional structure of M184V RT shows almost no difference from the wild-type (WT) RT even at the active site because by simply rotating its side chain to the open NRTI binding site the mutated residue (Val184) can acquire stable conformation. As a result, the deoxy ribose sugar moiety of TTP is in close proximity to Val184 (3.3 Å) but not enough to be sterically hindered (Fig. 1.7a). However, even though a natural substrate such as TTP can manage to fit into the active site without unfavorable steric hindrance, it is obvious that the approach of the sterically demanding Val184 into the NRTI binding site significantly limits the conformation of the NRTI sugar moiety, which can be confirmed by the binding mode of 3TC triphosphate (Fig. 1.7a). For the sake of proper base pairing with the complementary

base in the template strand, the unnatural L-configuration of 3TC locates its oxathiolane sugar moiety at the upper open space of the NRTI binding site in the WT RT, where Met184 contributes to the binding of 3TCTP by an extended van der Waals contact to the oxathiolane sugar moiety of 3TCTP. However, in M184V RT, 3TCTP cannot be accommodated into the binding site, which is already occupied by the side chain of Val184 (Fig. 1.7b). In this context, it is noteworthy that, to date, no L-configured nucleoside has been reported to have antiviral activity against the M184V RT.

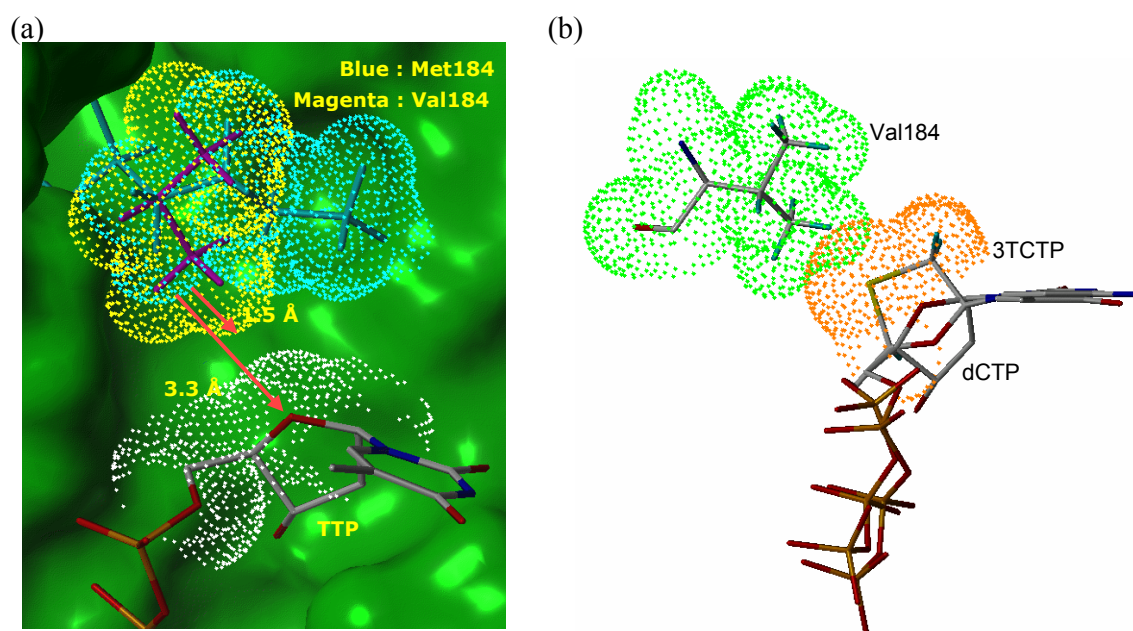


Figure 1.7. Active sites of M184V RT and wild-type RT.

Concerns about the development of viral resistance to 3TC^{94,95} prompted the discovery of structurally related nucleosides with activity against HIV isolates containing common 3TC resistance mutations. Until now, three classes of NRTIs have been reported to be active against M184V RT, which include dioxolane/oxathiolane (DAPD⁹⁶/dOTC⁹⁷),

acyclic (Tenofovir disoproxil)⁹⁸ and 2',3'-unsaturated nucleosides [d4T,⁹⁹ d4FC,¹⁰⁰ D-2',3'-unsaturated-2'-fluorocytidine (2'Fd4C)¹⁰¹].

*2',3'-Unsaturated (d4) nucleosides.*⁷³ Among these, the 2',3'-unsaturated nucleosides including 2'-fluorine substitution^{101,102} have been studied to provide the structure-activity relationships against M184V RT, where D-2',3'-unsaturated cytidine analogs show potent activity against M184V RT¹⁰¹ but the isosteric replacement of 4'-oxygen with a sulfur atom (D-2',3'-unsaturated 2'-fluoro-4'-thiocytidine, 2'F-4'Sd4C)¹⁰² develops significant cross-resistance (Table 1.2). On the other hand, abacavir, which has a 4'-CH₂ group instead of an oxygen atom, exhibits only a 2- to 5-fold increase to M184V RT (Table 1.2),⁹⁹ suggesting that the 4'-substituent in the 2',3'-substituted nucleosides is critical in determining cross-resistance to the M184V RT.

Table 1.2. In vitro antiviral activity (EC₅₀, µM) of the NRTIs against WT and M184V RT.

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

^a EC₉₀ in µM

Thus, it was of interest to find out 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. In order to investigate the binding mode of each NRTI to RT, various 2',3'-unsaturated NRTI structures (Fig. 1.8), obtained from the quantum mechanical *ab initio* calculations using RHF/3-21G* basis in Spartan 5.1.1, were superimposed and compared.

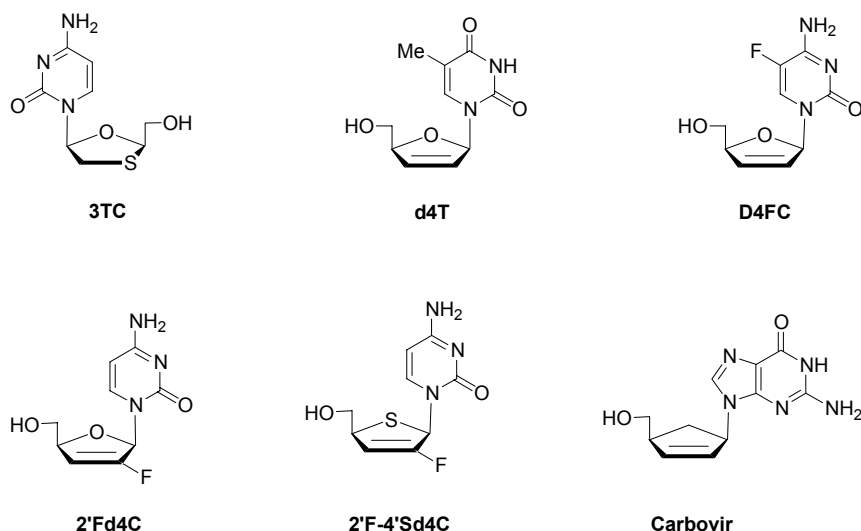


Figure 1.8. Structures of 2',3'-unsaturated NRTIs.

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'-CH₂ in carbovir and 4'-sulfur in 2'F-4'Sd4C are similar, and the 4'-oxygen atoms in D4T, D4C and 2'Fd4C has the smallest van der Waals radius (Fig. 1.9). 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 ABC with 4'-CH₂ 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.

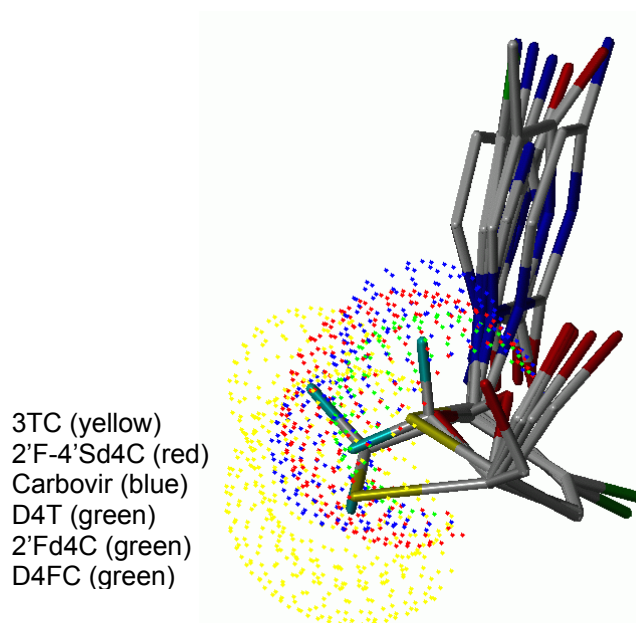


Figure 1.9. Superimposed structures of 3TC /D4T/2'F4'Sd4C/2'Fd4C/Carbovir/D4FC.

Comparison of the minimized structures of ternary complexes (NRTI-DNA-WT RT) shows several interesting features: (a) 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. 1.10). This π - π interaction strengthens the binding between the HIV RT and the triphosphates, and may partially explain the significant anti-HIV activity of the d4Ns against the M184V mutant by the π - π interaction.

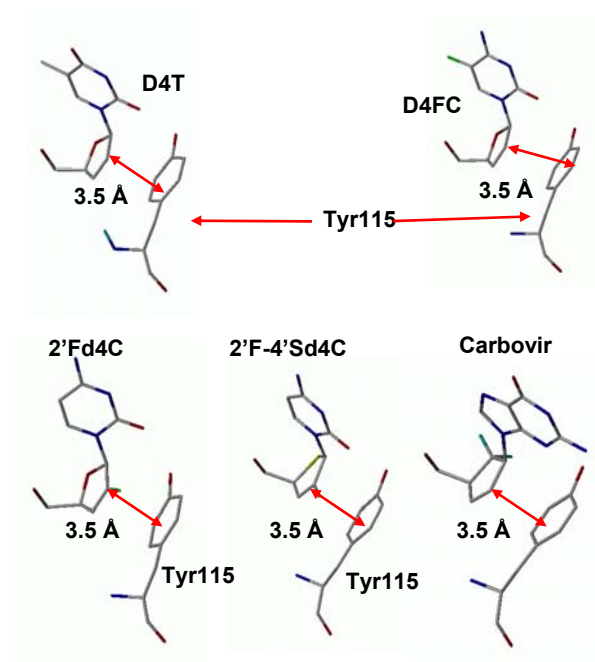


Figure 1.10. Hydrophobic π - π interaction.

(b) 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. 1.11). 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). 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 a larger van der Waals radius than the oxygen atom, cannot avoid the unfavorable steric hindrance with the side chain of Val184, resulting in high cross-resistance to M184V RT (Fig. 1.11). However, the 4'-CH₂ group in carbovir is not close enough to Val184 because there is no 2'-fluoro substituent (Fig. 1.12). Therefore, the steric hindrance between the sugar moiety of carbovir and Val184, if any, should not be significant.

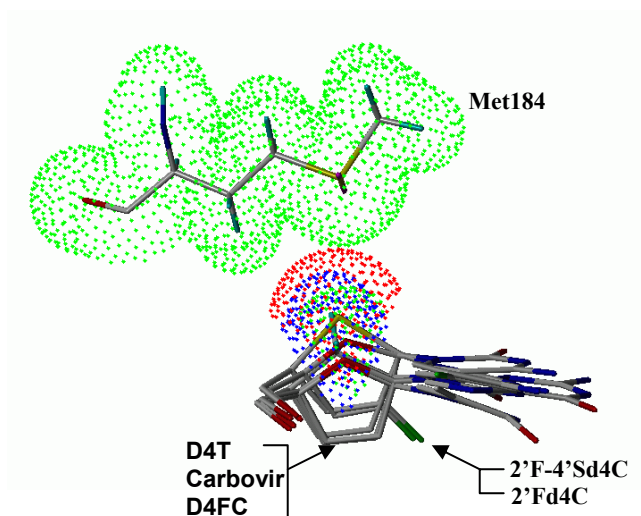


Figure 1.11. 4'-oxygen in 2'Fd4C (green) vs 4'-sulfur atom in 2'F-4'Sd4C (red).

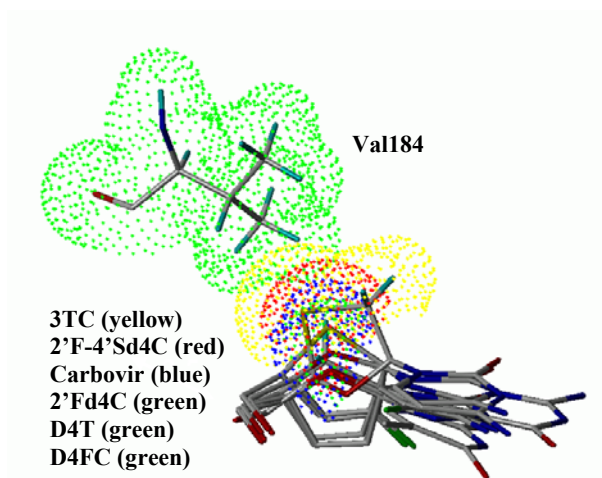


Figure 1.12. 4'-CH₂ in carbovir is not close to Val184 because there is no 2'-fluorine.

Potent anti-HIV activity of the fluorinated 2',3'-unsaturated (d4) nucleosides prompted extensive structure-activity relationship studies of D- and L- 2'-fluoro-d4 nucleosides,^{101,103} 2'-fluoro-4'-thio nucleosides,^{102,104} 3'-fluoro-d4 nucleosides^{105,106} and 3'-fluoro-4'-thio nucleosides^{107,108} (Fig. 1.13).

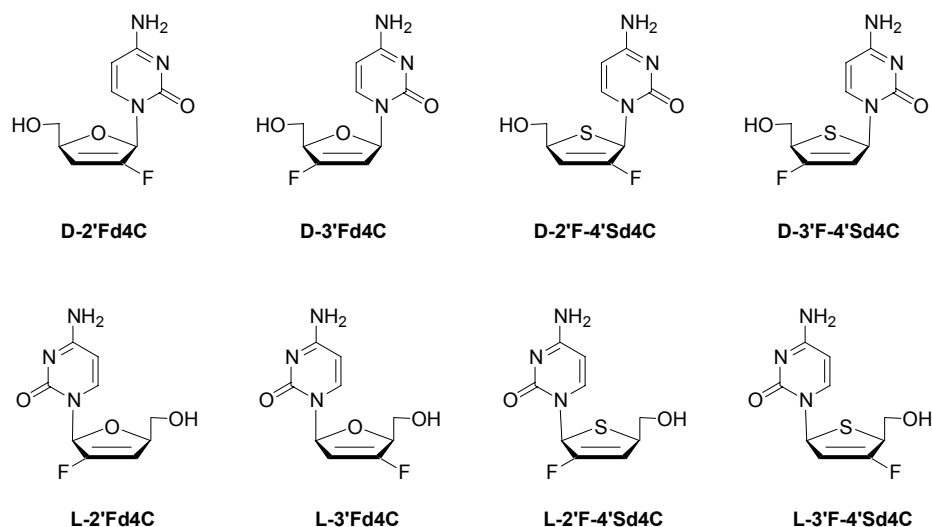


Figure 1.13. 2'- or 3'-fluorinated 2',3'-unsaturated nucleosides.

Unfortunately, in spite of the remarkable anti-HIV activity against the wild-type RT, no L-nucleoside was found to be active against M184V RT, and the molecular modeling studies showed that the 2'- or 3'-fluorinated d4 L-nucleoside also cannot avoid the steric hindrance with Val184.^{101,104,105,106} The large binding pocket of the wild type HIV-1 RT was found to provide enough space for L-nucleoside to bind at the active site (Fig. 1-14b), and it is noteworthy that the potent anti-HIV activity of L-3'-Fd4C correlates with its high relative binding energy by virtue of the participation of the 3'-fluorine atom into the hydrogen bonding with the amide backbone of Asp185 (Fig. 1-14a).¹⁰⁵ This favorable binding mode, however, cannot be maintained when the triphosphate of the L-3'-fluoro 2',3'-unsaturated nucleoside binds to the active site of M184V RT because the bulky side chain of Val184 occupies the space needed for binding and pushes the 3'-fluorine atom of L-3'-Fd4CTP away from the amide backbone of Asp185, out of hydrogen bonding distance, resulting in poor binding energy and thereby cross-resistance (Fig. 1-14c).¹⁰⁵

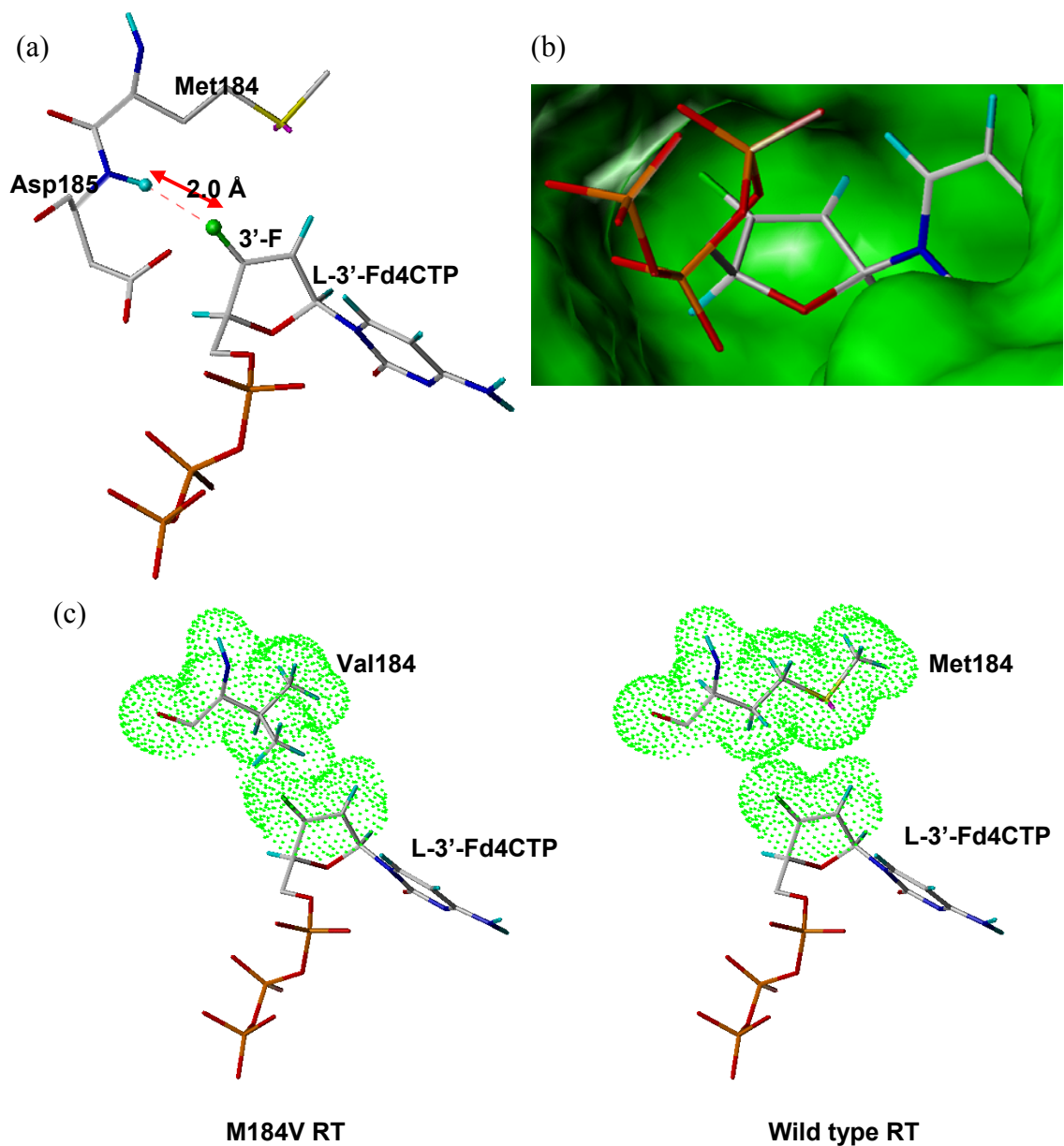


Figure 1.14. (a) Binding mode of L-3'-Fd4CTP at the active site of HIV-1 RT. 3'-F is hydrogen bonded to the amide backbone of Asp185. (b) Binding site of HIV-1 RT. Deep and spacious enough to accommodate the unnatural L-nucleoside triphosphates. (c) Steric hindrance of L-3'-Fd4CTP with the bulky side chain of Val184 in M184V RT (left). In wild type RT, L-3'-Fd4CTP is in favorable distance from Met184 which may enable a stabilizing hydrophobic interaction (right).

The large van der Waals radius of the sulfur atom at the 4'-position of the sugar moiety was also found to result in the steric hindrance with Val184. Thus, M184V RT was found to be insensitive to fluorinated 4'-thio d4 nucleoside.¹⁰² Interestingly, even though the binding mode of D-3'-Fd4CTP to the wild-type RT is indistinguishable from that of D-2'-Fd4CTP due to their conformational similarity, the low anti-HIV activity of D-3'-Fd4C is exceptional in this series.¹⁰⁶ Presumably, D-3'-Fd4C is not easily metabolized to its monophosphate by cellular kinases such as deoxycytidine kinase (dCK) because of the unfavorable interaction of the 3'-fluorine with the enzyme active site. Among the 2'- or 3'-fluorinated 2',3'-unsaturated nucleosides, therefore, only D-2'-Fd4C maintains its high activity against M184V RT.¹⁰¹

*D-dioxolane nucleosides.*¹⁰⁹ Since the discovery of D-dioxolane guanosine and its prodrug, D-dioxolane 2,6-diaminopurine (DAPD), which are active against AZT- as well as 3TC-resistant mutants,^{110,111} nucleosides which have dioxolane sugar moiety have been focused in the pursuit of new drug candidates for anti-HIV therapy. Among the series of the nucleosides, thymidine, 5-fluorocytidine and guanidine analogues (Fig. 1.15) showed potent anti-HIV activity against 3TC-resistant mutant RT (Table 1.3).

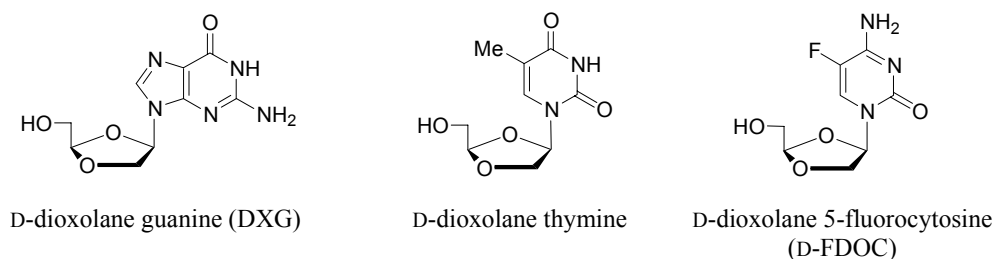


Figure 1.15. Structures of D-dioxolane nucleosides.

In this context, it is of great interest to understand the role of the dioxolane moiety as well as the heterocyclic bases for the anti-HIV activity of these nucleosides against 3TC-resistant mutant RT.

Table 1.3. Antiviral activities of D-dioxolane nucleosides against M184V RT.

	3TC		D-Dioxolane T		DXG		D-FDOC	
	EC ₅₀ ¹¹⁰	FI	EC ₅₀ ¹¹²	FI	EC ₅₀ ¹¹⁰	FI	EC ₅₀ ¹¹²	FI
WT	0.041	--	0.200	--	0.21	--	0.045	--
M184V	>50	>1,200	0.088	0.44	0.44	2.1	0.030	0.67

The steric pressure on the active site of RT conferred by the M184V mutation not only blocks the space where the sugar moiety of the L-nucleoside resides, but significantly limits the conformation of D-nucleosides. As was seen in our previous study, the D-2'-fluoro-4'-thio-2',3'-unsaturated nucleoside is a good example in this category because the cytidine nucleoside, potent against WT RT, shows high cross-resistance to M184V RT.¹⁰² As its 4'-oxa analogue, the D-2'-fluoro-2',3'-unsaturated nucleoside, is known to maintain its antiviral activity against M184V RT, the characteristic mechanism of resistance of D-2'-fluoro-4'-thio-2',3'-unsaturated nucleoside is quite interesting. The energy-minimized structure of the D-2'-fluoro-4'-thio cytidine/RT complex shows that, in spite of the same conformation of D-2'-fluoro-4'-thio-2',3'-unsaturated cytidine as that of its 4'-oxa derivative, the steric clash with Val184 caused by the longer C-S bond as well as the larger van der Waals radius of the 4'-sulfur atom result in a deformation of the active site.¹⁰²

Molecular modeling studies indicate that, unlike other nucleosides cross-resistant to M184V RT, the D-dioxolane nucleosides are able to escape the stress of Val184 by adjusting their conformation with no energy cost. Also, the 3'-oxygen atom of D-dioxolane sugar moiety is critical in locating the nucleoside triphosphate away from Val184 by interacting with the active site residues of RT. In every minimized structure of D-dioxolane nucleoside triphosphate/RT complexes, the 3'-oxygen atom of D-dioxolane sugar moiety was always found to be in specific interaction with the nearby enzyme residues regardless of its conformation (Fig. 1.16), and the conformational analysis showed that the interconversion of the dioxolane's conformation from one to another can happen without significant loss of energy (Fig. 1.17). The specific interaction between dioxolane sugar moiety and the enzyme residues effectively holds the nucleoside triphosphate out of the clashing distance with Val184 (Fig. 1.16c).

In this context, it is interesting that, in the crystal structure of TTP bound to the WT RT, the 3'-OH of TTP is in specific interaction with its own triphosphate moiety as well as the nearby enzyme residue Tyr115 (Fig. 1.18a).¹¹

As Tyr115 is located on the other side of the active site of RT from Val184, it is possible that the 2'-deoxy sugar moiety of TTP can stay away from Val184 by virtue of the interaction between 3'-OH and Tyr115. Also AZT, which has the same conformation as TTP, does not experience any cross-resistance to M184V RT¹¹³ because the 3'-azido group of AZT is in the right position to interact with Tyr115 (Fig. 1.18b).⁷² On the other hand, moderate cross-resistance of ddI and ddC,⁶⁶ which do not have any functionality inside their sugar ring to interact with the enzyme residues in a specific manner, to M184V RT supports our claim.

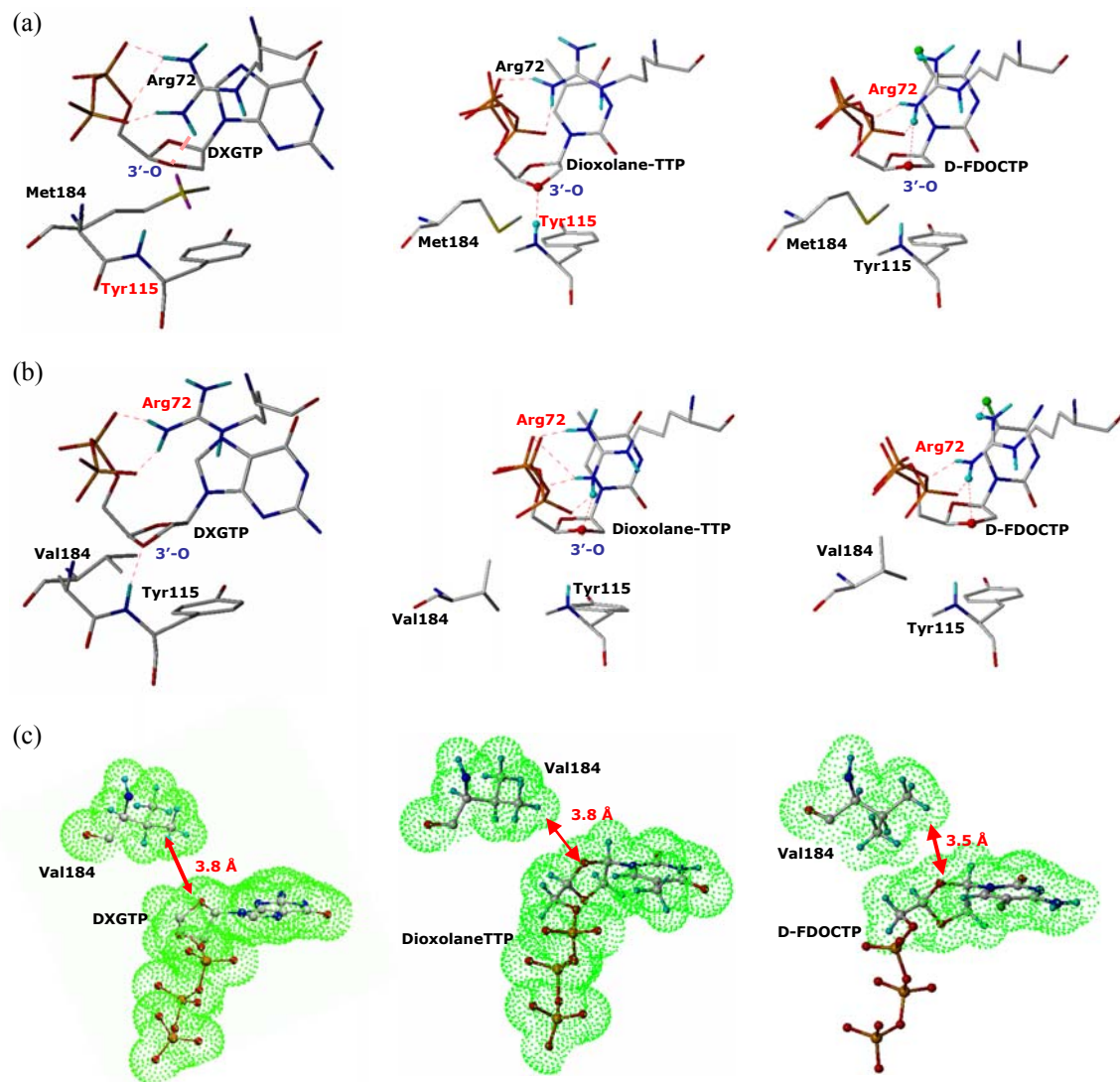


Figure 1.16. D-dioxolane nucleoside triphosphates-RT complexes with (a) WT RT, (b) M184V RT. (c) The dioxolane sugar moieties stay away from Val184.

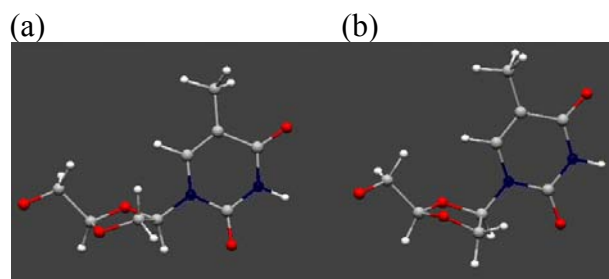


Figure 1.17. Two different conformations of D-dioxolane thymine: (a) 3'-exo (b) 3'-endo

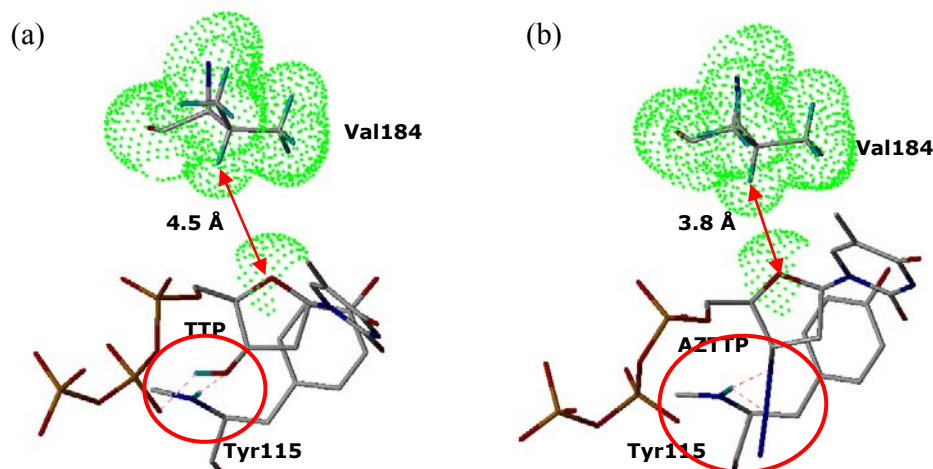


Figure 1.18. AZT (b), which has the same conformation as TTP (a), does not experience any cross-resistance to M184V RT. The 3'-azido group of AZT is in the right position to interact with Tyr115 to hold the nucleoside triphosphate away from Val184.

M41L/D67N/K70R/T215Y (AZT-resistance).⁷²

Tissue culture and clinical data have revealed that multiple mutations in the “fingers” and “palm” subdomains of RT are required to confer resistance to AZT.⁶⁹ Thus, AZT resistance correlates with multiple mutations in RT, including M41L, D67N, K70R, L210W, T215Y/F and K219Q.^{56,114,115,116} The extent of AZT resistance is related to the combination of mutations present. In general, two or more of these mutations are normally needed in RT to show high-level AZT resistance.^{68,69} 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 AZTTP into the DNA chain.^{91,117,118}

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.^{119,120,121,122,123} 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. In the first, the AZT-resistant mutant RT exhibits an increased rate of RT-catalyzed pyrophosphorolysis (the reverse reaction of DNA polymerization).^{119,120,122,123} The substrate for pyrophosphorolysis is PPi, which is the normal product of DNA synthesis and is also present at an intracellular concentration of about 150 μ M.¹²⁴ 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.^{119,120} However, the weakness of the pyrophosphorolysis mechanism is that the reaction regenerates AZTTP, which may then be reincorporated into the viral DNA. In addition, there is a report which failed to observe differences in pyrophosphorolytic activity between wild-type and AZT-resistant RT.¹²³ Meyer et al. have shown that HIV-1 RT also possesses an interesting capacity to carry out ribonucleotide-dependent phosphorolysis (dinucleotide polyphosphate synthesis).¹²³ This reaction, by the same chemistry involved in pyrophosphorolysis, excises the terminal nucleotide from the 3'-end of the primer with the concomitant formation of a dinucleoside tetraphosphate. The advantage of the ribonucleotide-dependent phosphorolysis mechanism is that the phosphorolytic removal of AZT results in the formation of dinucleoside tetraphosphate which, unlike the AZTTP product of pyrophosphorolysis, cannot be reincorporated into the viral DNA. However, in contrast to

PPi, HIV-1 RT has only a very low affinity for ribonucleotides such as ATP.¹²² Furthermore, it should be envisioned that ATP binds in an orientation ‘backward’ from that of the normal dNTP substrate in order for the γ -phosphate of ATP to be in proximity to the phosphodiester bond between the last two nucleotides of the primer strand, to enable the ribonucleotide-dependent phosphorolysis reaction to proceed.⁷⁰ On the other hand, it is not clear how the AZT-resistant mutant RT acquires the enhanced affinity to AZT-MP-terminated primer. Thus, even though the phosphorolytic removal of nucleoside chain terminators by pyrophosphate acceptor molecules (PPi or ATP) has been proposed as a mechanism of AZT resistance, the several limitations of the mechanism mentioned above questions another possible route for the resistance conferred by mutations.

In this regard, the crystal structure of the AZT-resistant substrate-free RT containing the D67N/K70R/T215F/K219Q mutations⁵¹ draws our attention because it suggests that the T215F and K219Q mutations might induce long-range conformational changes that impact the active site aspartate residues, which is likely to provide a subtle method of introducing discrimination when the drug molecule mimics a substrate. As confirmed by many studies, TTP and AZT-TP have virtually identical conformations when bound to RT.¹²⁵ Therefore, unlike 3TC, the scope for mutating contact residues is limited strictly because significant changes are likely to be incompatible with the maintenance of an active enzyme.⁵¹ The mutations sites 41, 67, 70 and 215 are surrounding the dNTP binding site, but do not contact the dNTP directly (Fig. 1.19). Among the four mutation sites, only 41 is in close distance from the bottom half of the so-called 3'-OH pocket composed of Asp113, Ala114, Tyr115 (Fig. 1.19 and Fig. 1.20).

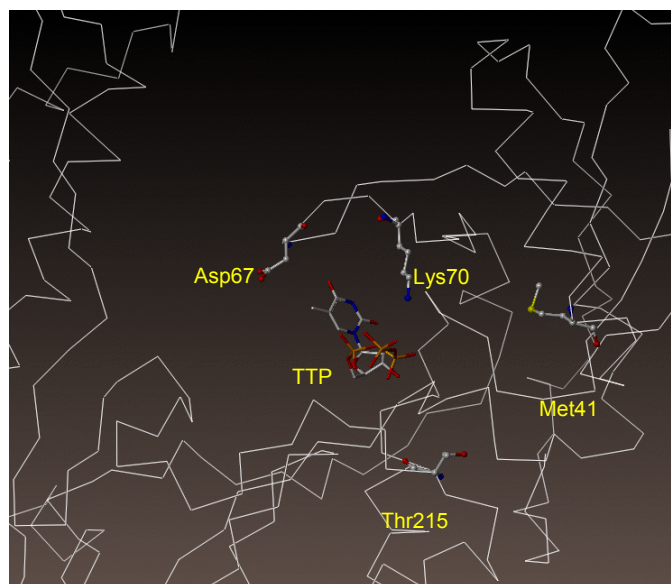


Figure 1.19. Mutation sites of AZT-resistant RT surrounds dNTP-binding site.

Since Lys70 is in direct contact to dNTP, the mutation to arginine, which has a short side chain, may fail to interact with the γ -phosphate of dNTP. The remaining two residues, 67 and 215, are relatively far away from the dNTP site, and the effect of the mutation in these two amino acid residues is interesting. Our energy-minimization study of quadruple mutant RT (M41L/ D67N/ K70R/ T215Y) complexed with TTP shows that the mutation does not change the conformations of dNTP and the 3'-OH pocket (Asp113, Ala114 and Tyr115) of RT (Fig. 1.20a). The mutations M41L and T215Y do not seem to accompany significant conformational change either (Fig. 1.20a). However, 67 and 70 show significant conformational differences between WT and mutant (Fig. 1.20a).

Taken together, unlike the 3'-OH pocket, the coordination geometry of the two Mg^{2+} ions by Asp185, Asp110 and Val111 show significant movement (Fig. 1.20b). Therefore, of conformational interest is how these changes affects the binding event of AZTTP at the

dNTP binding site of RT. The calculated relative binding energies (E_{rel}) of AZTTP and DXGTP complexed with WT as well as AZT-resistant RT shows that AZT is a potent competitive inhibitor against WT RT, but its affinity to the mutant RT is significantly decreased (Table 1.4).

Interestingly, DXGTP, which is known to maintain its antiviral activity against the AZT-resistant RT, shows only a moderate decrease in the relative binding energy to the mutant RT (Table 1.4). Therefore, it seems that the significant conformational change in the Mg^{2+} coordination geometry conferred by the combination of mutations selectively affect the binding of AZTTP.

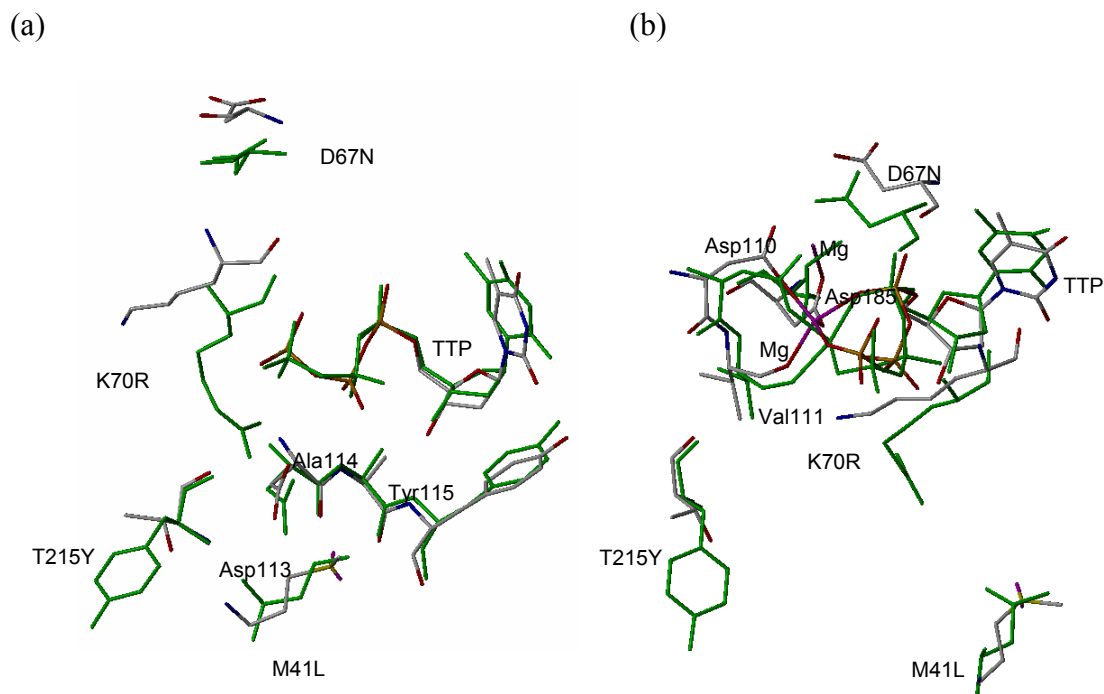


Figure 1.20. Energy-minimized structure of AZT-resistant RT complexed with TTP (Atom type: Wild type, green: mutant).

Table 1.4. AZT- resistant RT: correlation of binding energy (E_{rel}) with activity (EC_{50})

	AZT			DXG		
	EC_{50}^{110} (μM)	Fold Increase (EC_{50})	E_{rel} ($kcal/mol$)	EC_{50}^{110} (μM)	Fold Increase (EC_{50})	E_{rel} ($kcal/mol$)
WT	0.0041	--	146.4	0.21	--	12.0
M41L/D67N/ K70R/T215Y	0.082	20	73.4	0.24	1.1	8.0

The mutations at 41 and 215 create small conformational changes around their positions, and several residues such as Glu40, Glu44, Lys46, Gly112 and Ser117 experience conformational changes, which propagate ultimately to the three catalytic residues Asp110, Asp185 and Asp186 through Asp110, Val111, Gly112 and Asp113 (Fig. 1.20b). However, this conformational change affects the binding mode of nucleoside triphosphates at the active site in quite a different way. A close comparison with TTP binding to the AZT-resistant mutant enzyme revealed an interesting change in the conformation of the enzyme-ligand complex mediated by the interaction between the triphosphate moiety of AZTTP and the Arg70 residue of RT (Fig. 1.21a). The lack of 3'-OH in AZTTP as well as the mutation K70R results in a shift of the entire triphosphate moiety outward from the binding site (0.8 Å). As a result, the Arg70, which interacts with the γ -phosphate, shifts significantly (~ 3 Å) out of the binding pocket in order to maintain its hydrogen bonding to the γ -phosphate (Fig. 1.21a). This movement of Arg70 exerts significant effects to the three dimensional structure of the enzyme-ligand (AZTTP) complex (Fig. 1.21a): the hydrogen bond between Arg70 and Asp113 is not effective any more and the movement of Arg70 unfolds the palm domain resulting in a loose and ineffective binding mode. Therefore, a slight change caused by the binding of the AZTTP

moiety propagates to Arg70, resulting in a significant overall change in enzyme-ligand complex without deforming the “3’-OH pocket”, which emulates essentially for the natural substrate (TTP) binding to the mutant RT.

D-Dioxolane nucleosides. The favorable binding of DXGTP to the quadruple AZT mutant-RT can be explained by two factors. As was in the quadruple mutant-AZTTP complex, the conformational change originated from 41 and 215 propagates to the three catalytic residues Asp110, Asp185 and Asp186 (Fig. 1.21b). In addition, as Asp67 mutates to Asn67, three residues Asn67, Lys219 and Asp110 form a salt bridge, which shifts Asp110 1.2 Å out of the binding site (Fig. 1.21b). Interestingly, this shift can be found in the binding mode of no other nucleoside triphosphates (AZTTP, TTP and dGTP) to the same mutant enzyme.

Therefore, the unique binding mode of DXGTP deserves more attention. Due to the lack of a 3’-OH in DXGTP sugar moiety, DXGTP fails to stabilize its β -phosphate by hydrogen bonding. As a result, an adjacent residue, Lys65, moves toward the hydrogen bonding distance to the β -phosphate. However, it should be noted that in AZTTP binding mode, the movement resides in Arg70 not in Lys65 (Fig. 1.21a), which requires further explanation. Compared to the AZTTP binding, the interaction between Arg72 and the triphosphate moiety may be the critical factor in determining the residue which should move. In DXGTP binding, Arg72 rotates toward the triphosphate to form hydrogen bonds with both α - and β -phosphates. As a result, the triphosphate moiety has a more contracted conformation compared with that of AZTTP binding, and the γ -phosphate does not move out significantly. The conformational change in Lys65 propagates to Asn67 through Arg70, Thr69 and Ser68. As the conformation of Asn67 changes, the adjacent residue

Lys219 pulls down to form a stable salt bridge among Asn67, Lys219 and Asp110 (Fig. 1.21b).

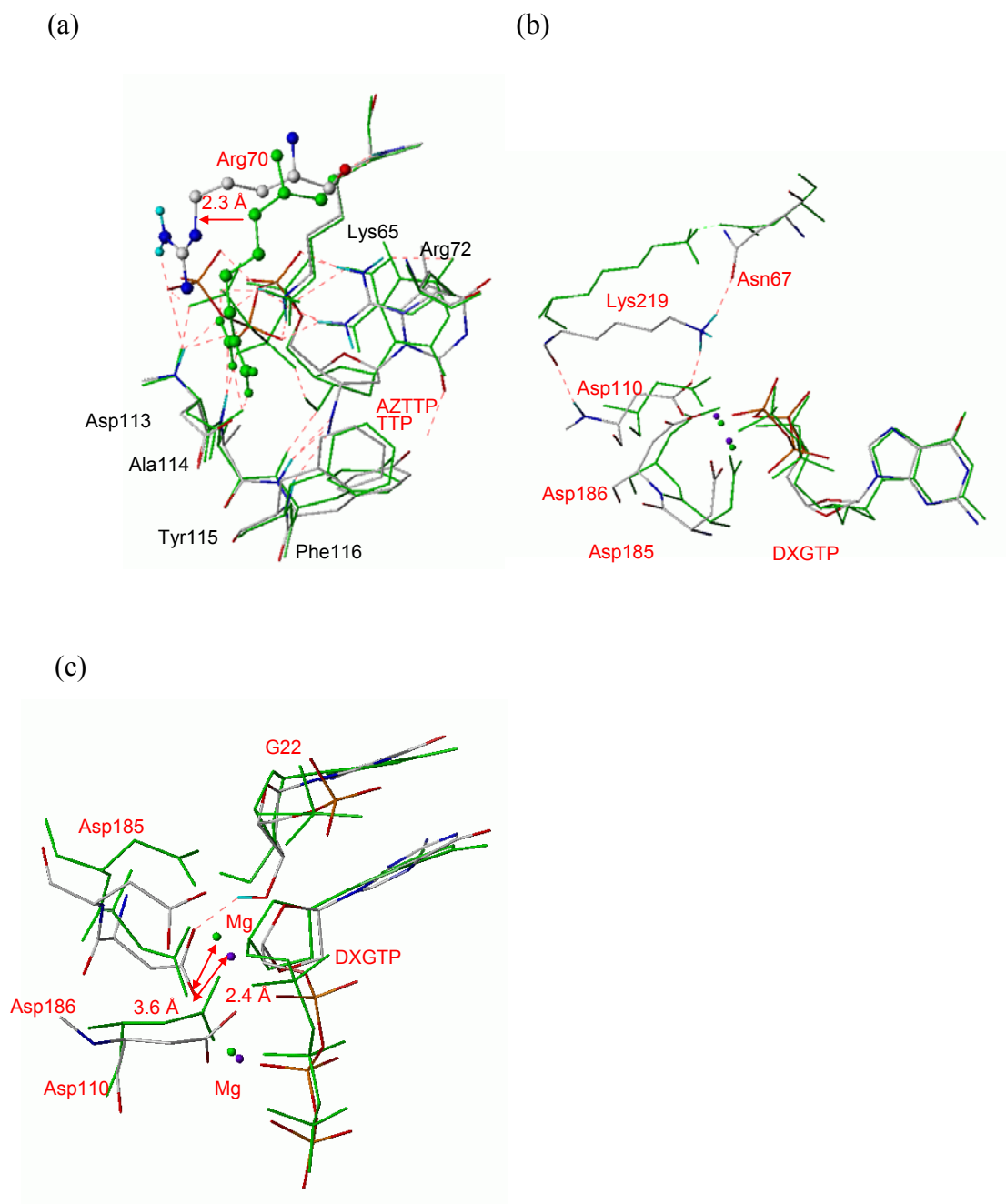


Figure 1.21. Comparison of AZT-resistant mutant complexed with AZTTP and DXGTP. (Green: Mutant RT/dNTP complex, Atom type: Mutant RT/ NRTI-TP complex)

The combined effect of propagation of conformational change and salt bridge formation results in conformational changes in three catalytic aspartate residues (110, 185, and 186), which improves the chelation of the two Mg atoms (Fig. 1.21c). The two Mg²⁺, one catalytic and the other nucleotide-binding are believed to facilitate an S_N2 nucleophilic attack by the 3'-OH of the primer terminus on the α -phosphorus of the incoming dNTP and to stabilize the negative charge in the transition state. Therefore, changes in conformation as well as binding mode of the three conserved Asp residues could contribute to an alignment of Mg atoms at the transition state and an increase in the polymerization rate or processivity. Another change followed by a salt bridge formation is the distance between fingers and palm domains. Fingers and palm domains move 1.1 Å and 0.4 Å toward each other to result in a decreased distance and therefore a tight binding state.

K65R mutant RT.⁷²

Several ddN resistance mutations arise in the flexible β 3- β 4 loop situated in the fingers domain of the p66 subunit of RT.¹²⁶ Five residues, that form part of this loop (Ile63, Lys64, Lys65, Tyr71 and Arg72), are conserved in all retroviral RTs⁶⁸ and among those, two fingers residues make important contacts with the dNTP substrate. The ϵ -amino group of Lys65 and the guanidinium group of Arg72 make salt bridges with the γ - and α -phosphates of the bound dNTP substrate, respectively. These interactions help positioning the dNTP for base-pairing with the complementary template residue as well

as for catalysis of phosphodiester bond formation with the 3'-OH of the primer terminal nucleotide. While the available structural data have provided considerable information on the structure of RT as well as the relevant amino acid residues critical for catalysis, it has not provided the molecular mechanism how mutations on RT influence the dNTP binding, thereby the anti-HIV potency. Even though this mutant is very rare, DXG experiences modest cross-resistance, which is well correlated with the decrease in its relative binding energy (E_{rel}) to the mutant enzyme, K65R RT (Table 1.5).

Table 1.5. DXG-resistant RTs : correlation of binding energy (E_{rel}) with activity (EC_{50})

	DXG		
	EC_{50} (μM) ¹²⁷	Fold Increase (EC_{50})	E_{rel} (<i>kcal/mol</i>)
WT	2.19	--	12.0
K65R	14.0	6.4	-37.1
L74V	6.3	2.9	0.2
K65R/ Q151M	>50.0	22.8	-44.4

This decrease in binding energy can be explained by the outward rotation of Arg72 from the binding site in DXGTP binding mode (Fig. 1.22b). In WT RT/DXGTP complex (Fig. 1.22b), unlike the WT RT/dGTP complex (Fig. 1.22a) where the inward movement of Arg72 is not allowed because of the 3'- β -hydrogen atom of the deoxyribose sugar ring, Arg72 is significantly involved in bridging the DXGTP and Gln151 by the formation of multiple hydrogen bondings. As a result, DXGTP has a very tight binding mode in WT RT. In K65R RT, however, as the longer and bulkier side chain of Arg65 occupies the binding site, the nearby Arg72 is pushed away from the binding site. This conformational

change, initiated by the movement of Arg65 toward the binding site, results in the loss of hydrogen bonds between Arg72 and DXGTP dioxolane moiety and Gln151 and thereby a loose complex (Fig. 1.22d).

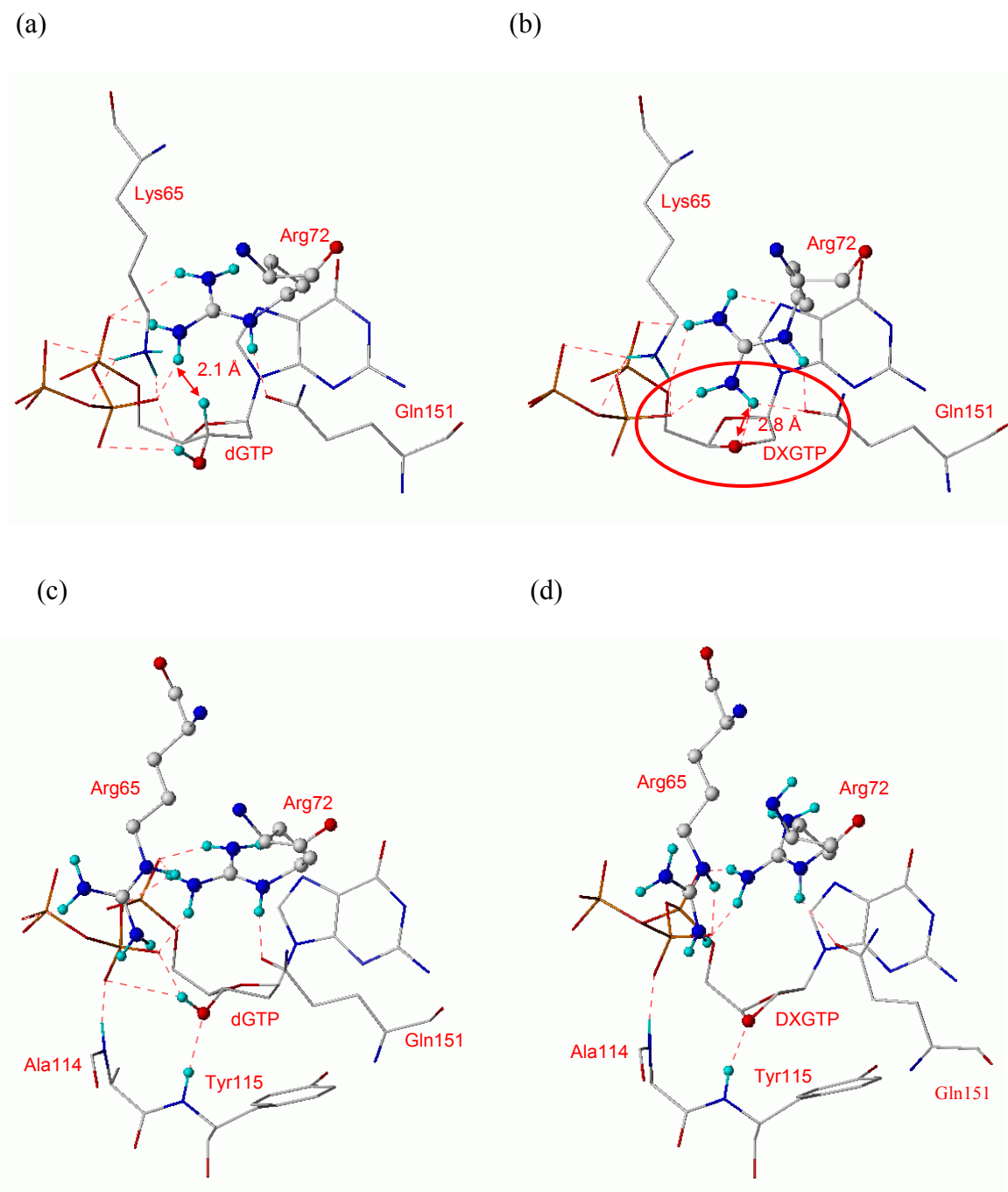


Figure 1.22. (a) WT RT/dGTP complex, (b) WT RT/DXGTP complex, (c) K65R RT/dGTP complex, (d) K65R/DXGTP complex.)

L74V mutant RT.⁷²

In the structure of RT-DNA-dNTP,¹¹ Leu74 is not in a position to directly affect dNTP binding. However, it does interact with the template nucleotide that is base-paired to the incoming dNTP and is also proximal to Gln151, which is a key residue in the dNTP-binding pocket. The L74V mutation has been thought to reduce sensitivity to ddI and ddC by altering template position and/or by affecting the orientation of Arg72 and Gln151 side chains.^{35,128} Leu74 upholds the template nucleotide by hydrophobic interactions so that the template nucleotide could be base-paired with the incoming dNTP. Therefore, the mutation of this residue from leucine to valine results in the misalignment of the template residue for base pairing and a significant conformational change in adjacent residues such as Gln151 and Arg72 (Fig. 1.23b).

In DXGTP binding, the combined effect of the misalignment of base pairing and the conformational change in Arg72 result in the downward shift of the dioxolane ring and the concurrent deformation of the active site (Fig. 1.24a), which is reflected on the decreased relative binding energy of DXGTP to L74V RT (Table 1.5). As a result, the triphosphate moiety of DXGTP adopts a new conformation, which hampers the formation of optimal geometry for S_N2-type polymerization reaction (Fig. 1.24b): the distance between the nucleophile (3'-OH at the end of primer strand) and the reaction center (phosphorous atom at the α -phosphate moiety) is lengthened (3.66 Å vs 3.42 Å in WT) and the angle among the nucleophile, reaction center and the leaving group (β - and γ -phosphates) (160.4°) deviates from that of optimal value (177.0° in WT) (Table 1.6).

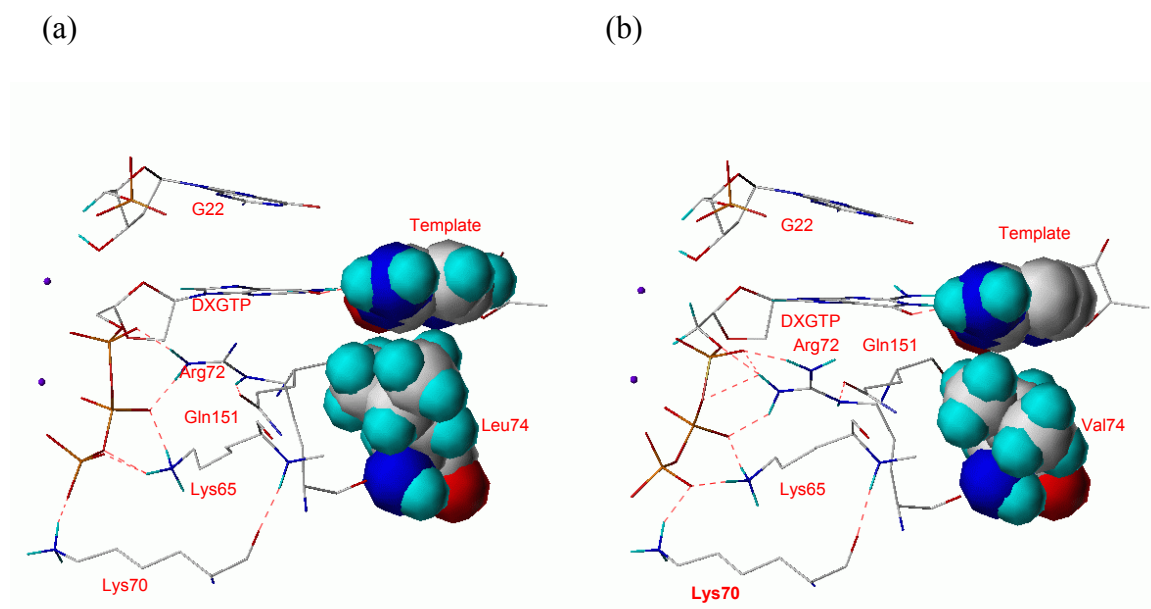


Figure 1.23. (a) WT RT/DXGTP, (b) L74V RT/ DXGTP

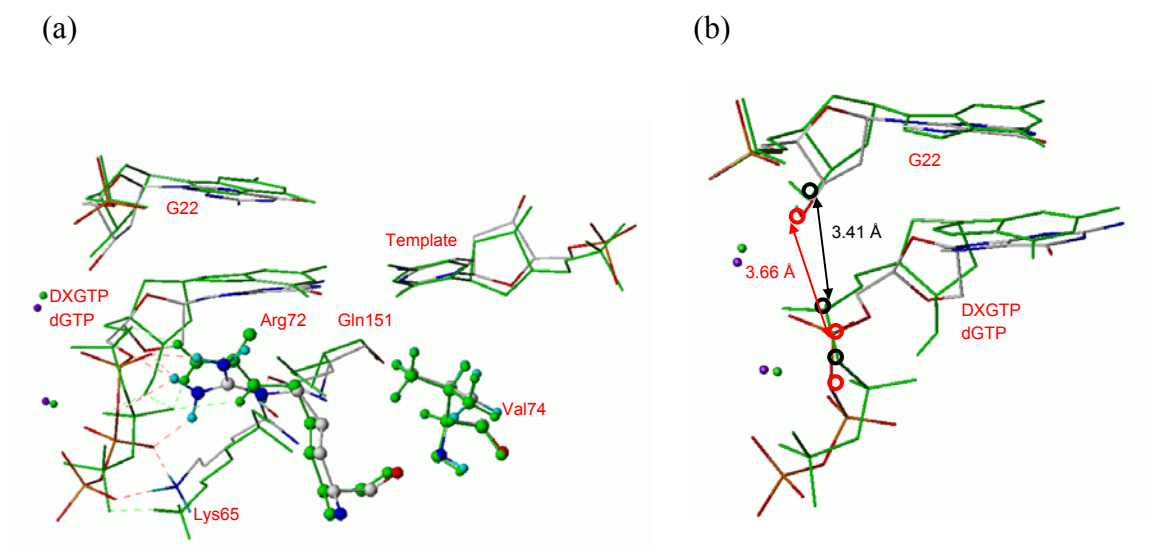


Figure 1.24. Deformation of the active site and S_N2 type polymerization geometry.
(Green: dGTP, Atom type: DXGTP)

Table 1.6. Comparison of geometry for S_N2 type nucleophilic attack.

	Wild-type		K65R		L74V		K65R/Q151M	
	DXG	dGTP	DXG	dGTP	DXG	dGTP	DXG	dGTP
Distance ^a (Å)	3.42	3.23	3.36	3.32	3.66	3.41	4.40	3.37
Angle ^b (degree)	177.0	174.9	177.3	176.0	160.4	173.6	158.8	176.4

^a Distance between the nucleophile (3'-oxygen of the terminal residue in primer strand) and the reaction center (α phosphorous atom in NRTI triphosphates)

^b Angle among the nucleophile, reaction center and the leaving group (β and γ phosphates in NRTI triphosphate)

K65R/Q151M RT.⁷²

In the crystal structure of the ternary complex, Gln151 interacts with residues that either directly interact with dNTP (e. g. Arg72, which hydrogen bonds with dNTP) or affect the position of important structural elements (e. g. the main chain carbonyl oxygen of residue 73 is hydrogen-bonded to Gln151, bridging the palm and fingers subdomains).¹¹ The Q151M mutation might therefore engender multidrug resistance to AZT, d4T and ddC/ddI by inducing both direct and indirect changes at the dNTP-binding pocket (Table 1.1).^{129,130} A site-directed mutagenesis study found that a combination mutation of K65R and Q151M was fully resistant to DXGTP and this mutant exhibits a significant decrease in antiviral activity of DXGTP (Table 1.5). The decreased relative binding energy (E_{rel} ,

Table 1.5) and the minimized RT-DXGTP structure shows two important features which this mutation invokes at the dNTP binding site. The bulky Met151 pushes neighboring Arg65 away from the binding site (Fig. 1.25).

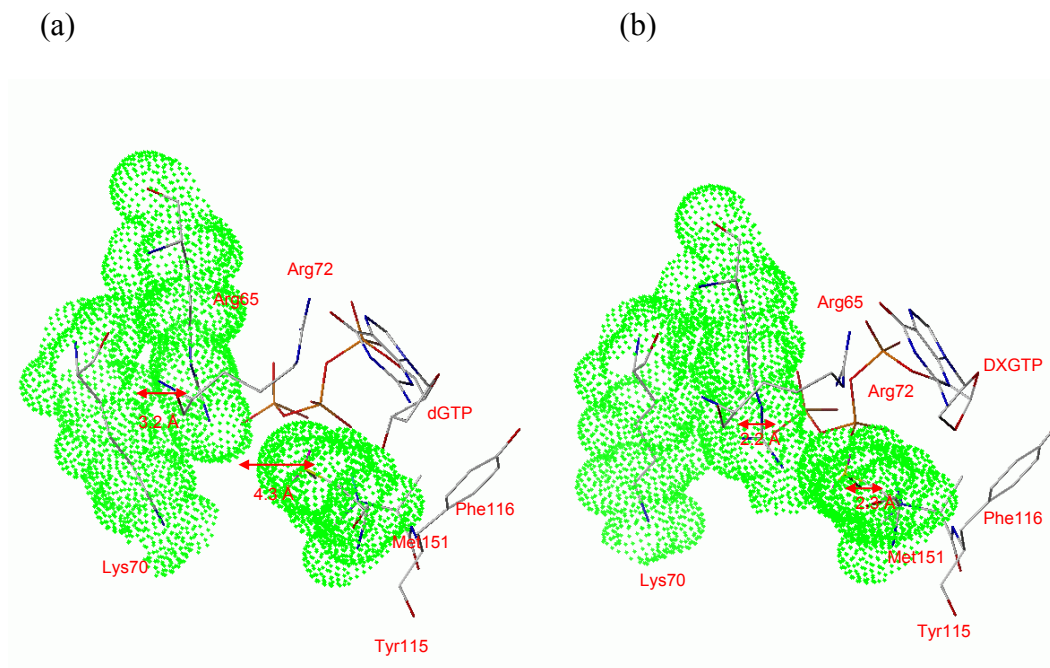


Figure 1.25. K65R/L74V mutant complex with (a) dGTP and (b) DXGTP.

In dGTP binding (Fig. 1.25a), the pushed Arg65 also rotates itself in order to prevent another steric hindrance with the adjacent residue Lys70. However, in DXGTP binding (Fig. 1.25b), Arg65 is heavily involved in the stabilization of β -phosphate and can neither move out nor rotate to prevent the unfavorable steric hindrance with Met151 and Lys70, which results in a high-energy binding state (Fig. 1.25b). In addition to that, as with the L74V mutation, the geometry for S_N2 type polymerization reaction is significantly deformed as a result of conformational change in both Arg65 and the triphosphate moiety in DXGTP: the nucleophile and the reaction center are separated by 4.40 Å, and the angle

around the reaction center (158.8°) deviates significantly from that of the WT (Table 1.6) (Fig. 1.26). Therefore, the incorporation of DXGTP into the growing DNA chain in K65R/Q151M mutant enzyme can be expected to be more difficult than in any other mutant enzymes studied.

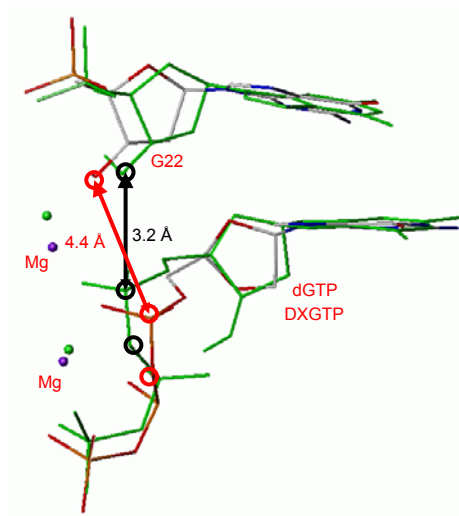


Figure 1.26. K65R/L74V RT complex. S_N2 type polymerization geometry.

CHAPTER 2

DIVERGENT SYNTHESIS OF D- AND L- CARBOCYCLIC 4'-FLUORO-2',3'- DIDEOXYNUCLEOSIDES AS POTENTIAL ANTIVIRAL AGENTS¹

¹Chong, Y., G. Gumina and C. K. Chu. 2000. *Tetrahedron: Asymmetry*. 11:4835-4875.
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Introduction

Nucleoside analogues display a wide range of biological activities and have attracted particular attention as antitumor and antiviral agents.¹³¹ In spite of the initial success obtained with several nucleosides, the instability of the glycosidic bond of the classical 4'-oxonucleosides in the physiological conditions¹³² as well as undesirable side effects and toxicities of certain nucleosides have prompted the search for other antiviral or antitumor agents with improved biological and chemical properties. Consequently, extensive modifications have been made to both the heterocyclic base as well as the sugar moiety,¹³³ and the latter has been extensively substituted by acyclic¹³⁴ and carbocyclic moieties.¹³⁵ Among the several approaches to modify the structure of nucleosides, carbocyclic nucleosides attracted great interests because the replacement of the furanose ring oxygen offered greater metabolic stability to the endogenous phosphorylases which cleave the natural glycosidic linkage.¹³⁶ Another interesting feature of carbocyclic nucleosides is that a number of carbocyclic adenosine analogues are assumed to exert their antiviral action through the inhibition of *S*-adenosylhomocysteine hydrolase, and this mechanism might be exploited in a combination therapy in association with nucleosides acting with different mechanisms.¹³⁷ The recent approval of abacavir (Figure 2.1) by FDA for the treatment of HIV shows the therapeutic significance of carbocyclic nucleosides.¹³⁸

However, since the conformation of the five-membered ring is believed to play a critical role in modulation of biological activity, the structural change following the removal of the oxygen is significant and generally results in the decreased biological activity.

Introduction of a small and highly electronegative fluoride atom to a nucleoside structure has been employed to confer biologically active conformations to the carbocyclic nucleosides.¹³⁹

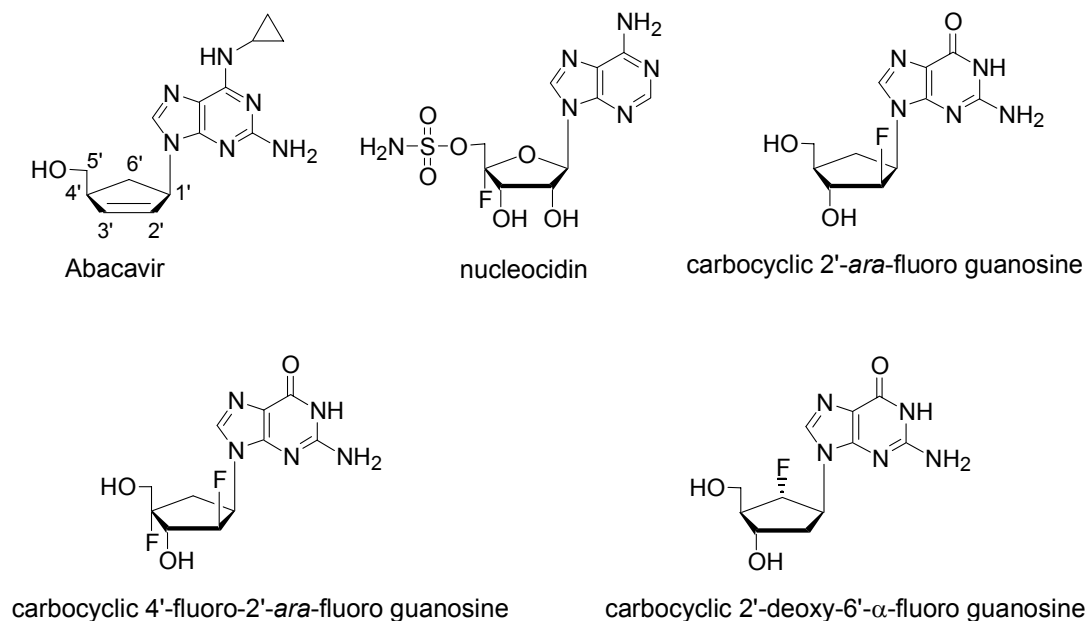


Figure 2.1. Examples of biologically active nucleosides

The isosteric relationship between oxygen and a fluorine¹⁴⁰ prompted various syntheses of fluoro-substituted carbocyclic nucleosides. Several fluoride-containing carbocyclic nucleosides at 2', 3', and 6' positions have been reported.¹⁴¹ Thus, 2'-*ara*-fluoro and 6'- α -fluoro substituent have been shown to confer potent antiherpes activity to some purine analogues¹⁴² (Figure 2.1). In contrast, substitution at the 4'-position of carbocyclic nucleosides has been largely ignored despite the presence of a 4'-fluoro substituent in the naturally occurring furanose antibiotic nucleocidin (Figure 2.1). To our knowledge, carbocyclic 4'-fluoro-2'-deoxyguanosine from aristeromycin¹⁴³ and carbocyclic 4'- α -

fluoro-2'-*ara*-fluoro guanosine¹⁴⁴ (Figure 2.1), which showed potent anti HSV-1 and anti HSV-2 activity, are the only examples of 4'-fluoro-substituted carbocyclic nucleosides. The importance of carbocyclic nucleosides as antiviral agents, as well as the potential advantage of fluorine substitution, prompted us to combine these two features into novel carbocyclic 4'-fluoro-2',3'-dideoxy nucleosides.^{145,146}

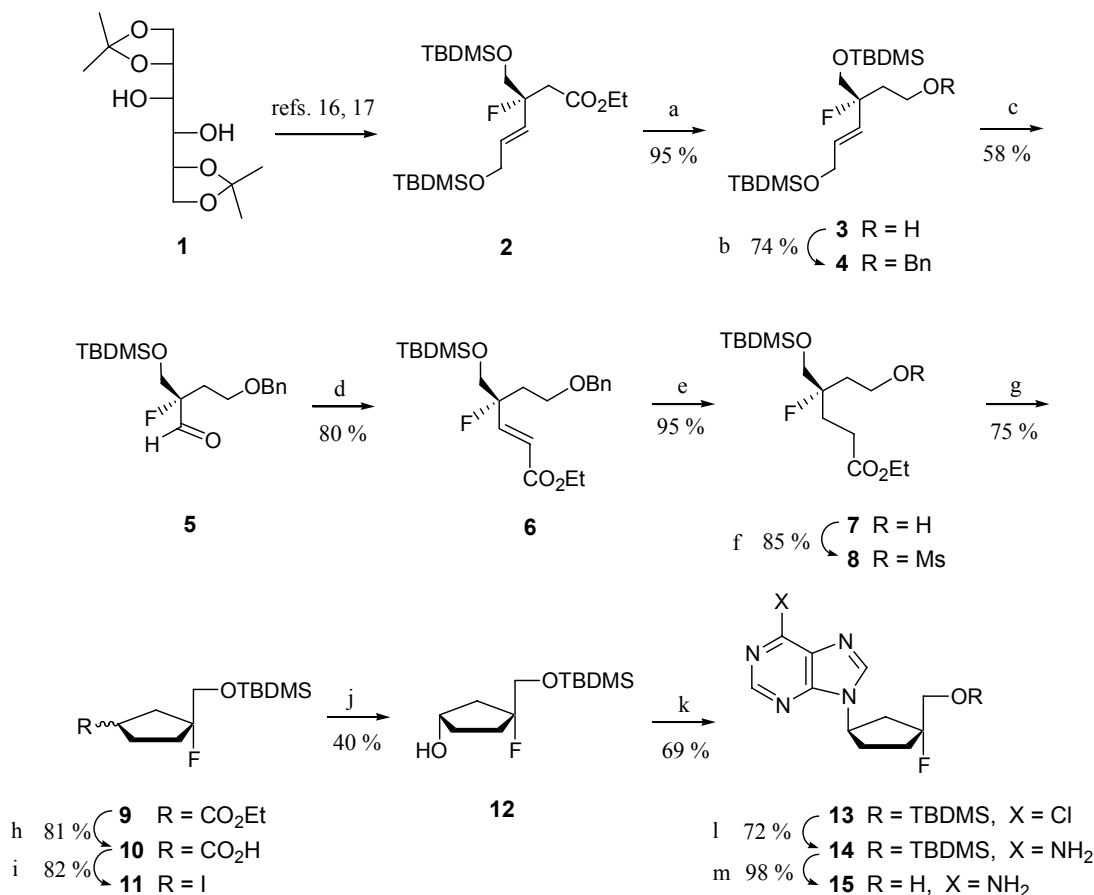
Previously, we have reported preliminary synthetic results of 4'-substituted carbocyclic nucleosides as a communication.^{16b} Herein we report an efficient and general approach to the asymmetric synthesis of D- and L-carbocyclic 4'-fluoro-2',3'-dideoxy nucleosides by using the common intermediate **2** (Scheme 2.1)¹⁴⁷ obtained by [3,3]-sigmatropic rearrangement of a fluoro allylic alcohol. Starting from this common intermediate, we developed a divergent synthetic pathway leading to both D- and L-nucleosides. The synthesis of L-isomer was accomplished by way of classical five-membered ring cyclization method, whereas a more convenient ring closing metathesis route was employed for the synthesis of the D-isomer. In both cases, the Mitsunobu condensation between an α -cyclopentanol and heterocyclic bases was generally used.

Results and Discussion

Even though the critical intermediate **2** was easily obtained, the cyclopentanol derivative **12** could not be prepared straightforwardly because the tertiary fluoride group was very susceptible for β -elimination under basic conditions. Several classical methods such as Dieckmann condensation¹⁴⁸ of diesters and intramolecular Horner-Emmons reaction of β -

ketophosphonate¹⁴⁹ were tried, but failed because the highly acidic α proton of the ester **2** was easily abstracted to result in the formation of a diene.¹⁵⁰ As reported previously,^{16b} the susceptibility of the protected ester **2** toward elimination made the synthesis of L-isomer more difficult than expected (Scheme 2.1). In order to synthesize L-nucleosides, ester **2** was reduced to alcohol **3** in 95 % yield by treatment with a solution of lithium aluminium hydride in THF at low temperature ($-40\text{ }^{\circ}\text{C}$) and anhydrous work-up. At temperatures higher than $-20\text{ }^{\circ}\text{C}$, the eliminated by-product was obtained as an inseparable mixture with **3**. Alcohol **3** was benzylated to a fully protected triol **4**, which was treated with ozone in methanol at $-78\text{ }^{\circ}\text{C}$, followed by decomposition of the ozonide by dimethyl sulfide to give aldehyde **5**. The Horner-Emmons-type reaction was performed by the treatment of **5** with the sodium salt of triethyl phosphonoacetate in THF. *E*-Alkene **6**, obtained in 80 % yield, was quantitatively converted to the intermediate **8** by catalytic hydrogenolysis followed by mesylation of the resulting alcohol **7**. Treatment of **8** with sodium hydride in THF under refluxing conditions generated the enolate that cyclized through an intramolecular nucleophilic substitution reaction to produce epimeric esters **9** in 75 % yield. The esters **9** were hydrolyzed to acids **10** by treatment with a solution of sodium hydroxide in 1:1 water/ethanol, followed by careful acidification. Oxidative iododecarboxylation of **10** could be achieved by the method reported by Barton *et al*¹⁵¹ to afford epimeric iodides **11**, which were smoothly hydrolyzed by heating at $100\text{ }^{\circ}\text{C}$ in a solution of sodium bicarbonate in 15 % water/HMPA. Surprisingly, only the α -alcohol could be isolated from the hydrolysis of the mixture **11** (epimeric ratio $\approx 1:1$). Under the reaction conditions, the α -iodide was probably not hydrolyzed and longer reaction times or higher temperatures caused its

decomposition. A solution of the alcohol **12** was added to the mixture containing 6-chloropurine and the preformed complex triphenylphosphine/DEAD in THF, and the reaction was performed at room temperature in the dark for 6 h.

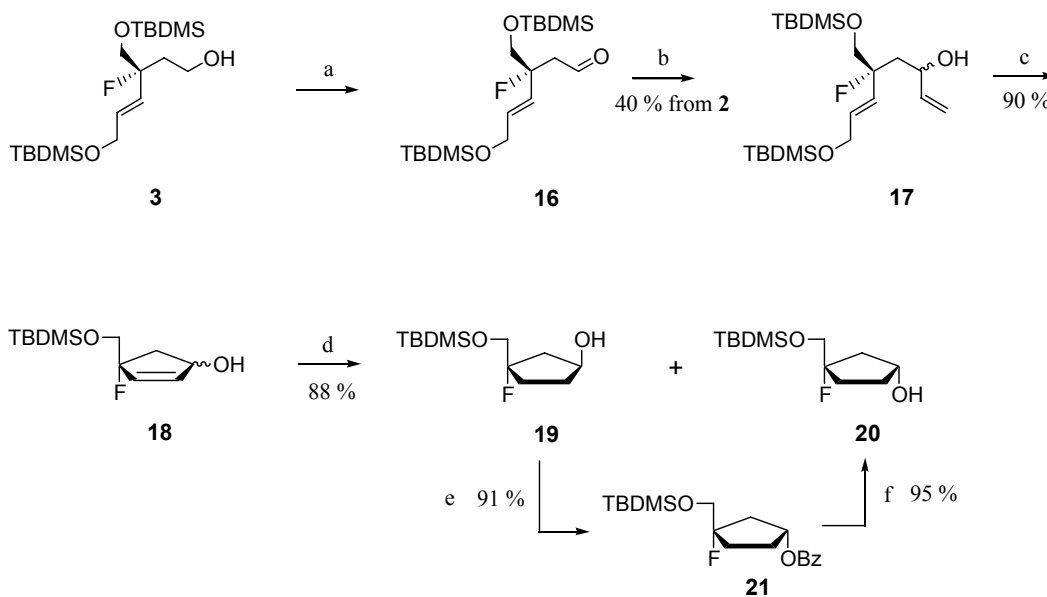


Reagents and conditions : (a) LAH, THF, -40 ~ -35 °C; (b) NaH, BnBr, THF; (c) O₃, MeOH, -78 °C, then DMS; (d) (EtO)₂P(O)CH₂CO₂Et, NaHMDS, THF, -78 °C; (e) H₂, Pd/C, cyclohexane; (f) MsCl, pyridine, CH₂Cl₂, 0 °C; (g) NaH, THF, reflux; (h) NaOH/H₂O, EtOH, rt; (i) Pb(OAc)₄, CCl₄, *hν*, reflux, 15min.; I₂, CCl₄, *hν*, reflux, 2h; (j) NaHCO₃, 15 % (v/v) water/HMPA, 100 °C; (k) 6-chloropurine, DEAD, PPh₃, THF; (l) NH₃, MeOH, 100°C, steel bomb; (m) TBAF, THF, rt

Scheme 2.1. Synthesis of (1'R, 4'R)- 9-[4-fluoro-4-(hydroxymethyl)cyclopentan-1-yl]adenine (**15**)

Removal of the solvent and flash chromatography gave **13** in 80 % yield. The 6-chloropurine derivative **13** was converted to a protected adenosine analogue **14** by treatment with a saturated solution of ammonia in methanol in a steel bomb at 100 °C for 2.5 h. Desilylation of the primary alcohol by treatment with tetrabutylammonium fluoride (TBAF) in THF resulted in the final L- carbocyclic 4'-fluoro-2',3'-dideoxyadenosine **15** in 98 % yield.

Due to the difficulties and low yield for the L-isomer as described above, we decided to develop a more efficient method of synthesis for D-isomers, in which the ring closing metathesis reaction¹⁵² of the 1,6-diene allowed a more facile synthesis of D-enantiomer (Scheme 2.2).



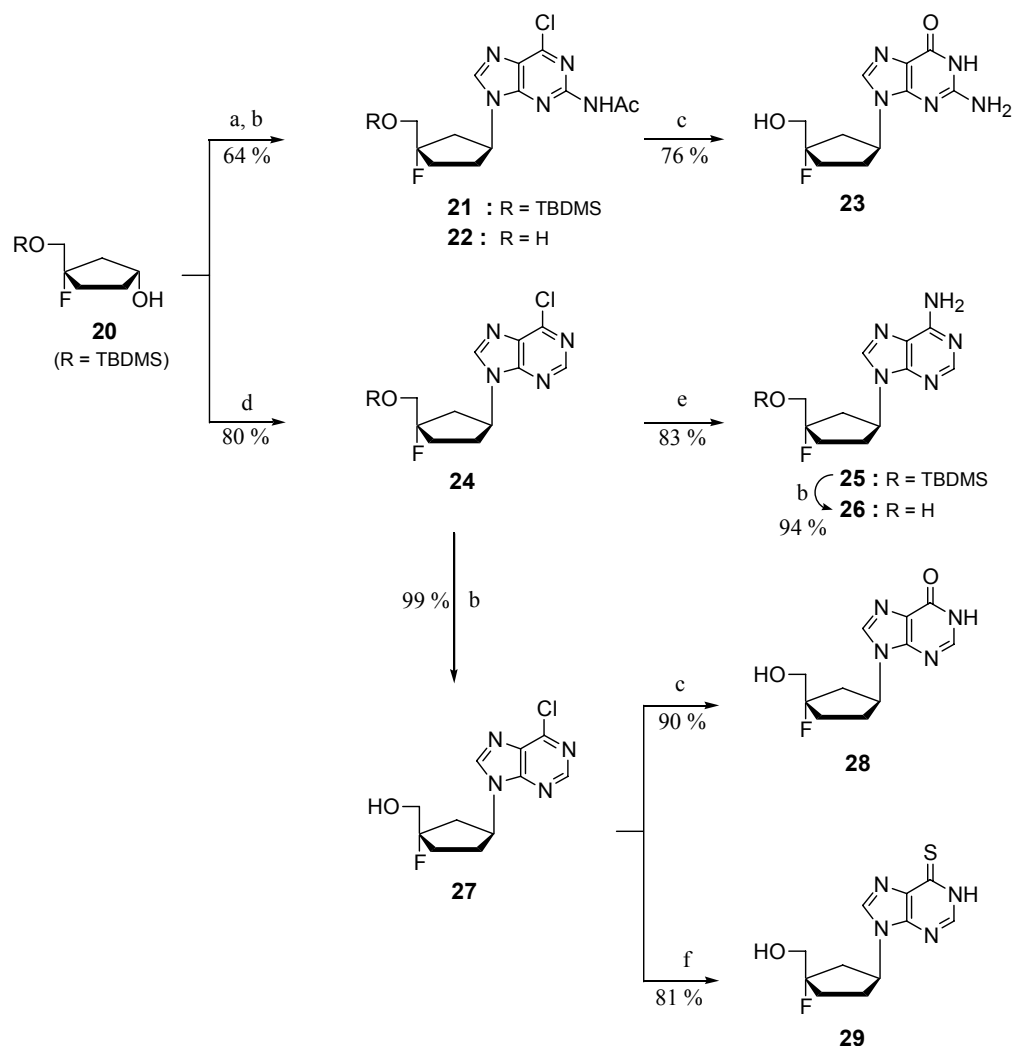
Reagents and conditions : (a) PCC, 4Å^o MS, CH₂Cl₂; (b) vinylmagnesium bromide, THF, -78°C; (c) Grubbs catalyst, CH₂Cl₂; (d) H₂, Pd/C, cyclohexane; (e) DEAD, PPh₃, PhCOOH, THF; (f) LAH, THF, -40 ~ -35°C

Scheme 2.2. Synthesis of (1R, 4S)- 4-tert-butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-ol (**20**)

The common intermediate, alcohol **3**, was oxidized by PCC, and the resulting aldehyde **16** was subjected to carbonyl addition by vinylmagnesium bromide to result in the 1:1 diastereomeric mixture of 1,6-dienes **17**. Due to the instability of intermediates **3** and **16**, the best yield was achieved when they were reacted immediately after filtration without any further purification. Under these conditions, compound **17** was obtained in 40 % yield from **3**. A ring closing metathesis, catalyzed by Grubbs catalyst¹⁵³ [benzylidene-bis(tricyclohexylphosphine)-dichlororuthenium], afforded cyclopentenols **18** as a diastereomeric mixture, whose α/β ratio (1:1) was consistent with the diastereomeric ratio of **17**. Even though this reaction proceeded very smoothly and in high (90 %) yield, the instability of the cyclopentenols **18** made it difficult to fully characterize their structures. Therefore, the structure could be confirmed after saturation of the double bond. Reduction of the double bond in **18** was accomplished by catalytic hydrogenation on 10 % Pd/C in cyclohexane to result in two easily separable isomers, **19** and **20** in 88 % yield. The β -Alcohol **19**, however, could be converted into the α -epimer **20** by Mitsunobu reaction followed by LAH reduction in 86 % overall yield (Scheme 2.2). The absolute configuration of α -alcohol **20** was confirmed by comparing the spectroscopic data (¹H and ¹³C spectra) as well as optical rotation values with those of its enantiomer (**12**, Scheme 2.1).^{16b} The α -cyclopentanol **20** was condensed with various protected purine and pyrimidine bases under Mitsunobu conditions. The success of Mitsunobu reactions in the syntheses of nucleoside analogues is known to depend on the conditions employed.¹⁵⁴ Therefore, appropriate choice of solvent system, temperature, and procedures are critical for the regioselectivity as well as chemical yield. In purine synthesis (Scheme 2.3), THF and a 2:1 mixture of dioxane and DMF were used as

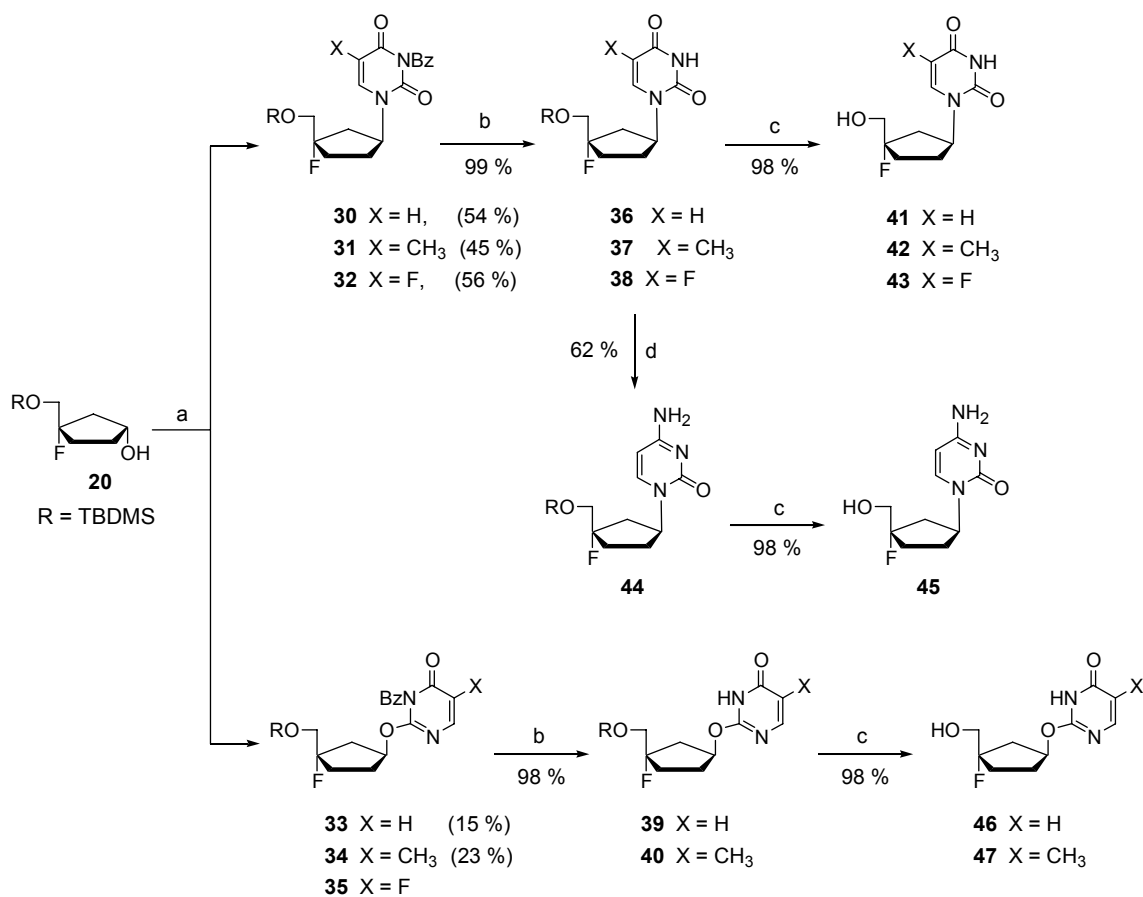
solvents for the coupling of the alcohol **20** with 6-chloropurine and *N*²-acetylamino-6-chloropurine, respectively. The use of dioxane-DMF mixture allowed better solubility of the heterocyclic bases and better yields. Slow addition of DEAD to a mixture of alcohol **20**, triphenylphosphine, and corresponding purine base in anhydrous solvent gave a yellow solution which was stirred for 6 ~ 12 hours at room temperature to give protected purine analogues (**21** and **24**) in 65 ~ 80 % yield. Because of the interference of the reduced form of DEAD, 1,2-dicarbethoxyhydrazine, the structure of **21** could not be characterized until it was desilylated to give **22**. The guanosine analogue **23** was prepared from **22** after treating it with formic acid^{24a} followed by methanolic ammonia. The 6-chloropurine derivative **24** was converted into three different nucleosides by using conventional nucleoside chemistry. Thus, the adenosine analogue **26** was prepared by amination of the 6-chloropurine analogue **24** followed by desilylation, while treatment of the desilylated 6-chloropurine analogue **27** with formic acid^{24a} and thiourea gave the inosine **28** and the 6-mercaptopurine **29** analogues, respectively. On the other hand, the syntheses of pyrimidine nucleosides were more complex than the purine case due to the formation of *O*²-alkylated by-products (Scheme 2.4, **33**, **34** and **35**). The formation of *O*²-alkylated compounds was inevitable in the reaction conditions tested, but the ratio of *N*- to *O*-alkylation was dramatically improved by changing the solvent from THF to 2:1 mixture of dioxane-DMF ; the ratio of **30** : **33** changed from 1.3 : 1 in THF to 3.6 : 1 in dioxane-DMF. The structures of *O*²-alkylated compounds (**33** and **34**) were confirmed by ¹H and ¹³C NMR after removing the *N*₃-benzoyl groups because debenzoylated compounds (**39** and **40**) allowed direct comparison with ¹H and ¹³C spectra reported for other *O*²-alkylated pyrimidine nucleosides;^{24c,d} the H-2' chemical shifts of **39** and **40** (δ

5.51, 5.46, respectively) were appreciably downfield from those of the N_1 Mitsunobu products **36** and **37** (δ 5.02, 5.00, respectively). Furthermore, the C-1' chemical shifts of **39** and **40** (δ 80.14, 79.84, respectively) were also quite downfield from those of the N_1 Mitsunobu products **36** and **37** (δ 59.87, 57.76, respectively). Unfortunately, the O^2 -alkylated product of 5-fluorouridine analogue **35** could not be isolated because it easily decomposed during column chromatography on silica gel.



Reagents and conditions : (a) DEAD, PPh_3 , N^2 -acetylamino-6-chloropurine, THF, ; (b) TBAF, THF; (c) HCO_2H , 90°C , then NH_3 , MeOH, rt; (d) DEAD, PPh_3 , 6-chloropurine, THF, ; (e) NH_3 , MeOH, 100°C , steel bomb; (f) thiourea, EtOH, reflux.

Scheme 2.3. Synthesis of purine nucleosides



Reagents and conditions : (a) DEAD, PPh_3 , N_3 -benzoylpyrimidine, DMF-dioxane (2:1), 0°C ; (b) NH_3 , MeOH, 0°C ; (c) TBAF, THF, r.t.; (d) 1,2,4-triazole, 4-chlorophenyldichlorophosphate, pyridine, 0°C , then NH_3 , MeOH, 100°C , steel bomb.

Scheme 2.4. Synthesis of pyrimidine nucleosides

The products of the Mitsunobu reaction (**30**, **31** and **32**) were converted into nucleoside analogues **41**, **42** and **43** *via* successive treatment with methanolic ammonia and tetrabutylammonium fluoride. The cytidine analogue **45** was synthesized by amination of uridine analogue **36** because it is known that the *O*-alkylation is the predominant pathway^{24c} in the Mitsunobu reaction of protected cytosine with cyclopentanol. The well-known two step conversion of uridine to cytidine¹⁵⁵ was adopted to give the protected

cytidine analogue **44** in 62 % yield. The final cytidine analogue **45** was obtained by desilylation.

Anti-HIV activity and cytotoxicity evaluations on the synthesized nucleosides (**15**, **23**, **26**, **28**, **29**, **41**, **42**, **43** and **45**) were performed in human peripheral blood mononuclear cells (PBMC). The adenine analogue **26** and guanine analogue **23** exhibited weak activity against HIV with EC_{50} values of 27.6 and 32.3 μ M, respectively, while no other synthesized nucleosides showed significant anti-HIV activity. The toxicities of these nucleosides were also assessed, and these compounds did not exhibit any significant toxicities at concentration up to 100 μ M in CEM, PBM and Vero cells.

In summary, we have developed a synthetic method stereoselectively introducing tertiary fluoride group and a *de novo* synthesis of key intermediates, α - and β -cyclopentanol (**12** and **20**). Condensation of these intermediates with protected purine and pyrimidine bases, followed by ammonolysis and deprotection, readily provided optically pure D- and L-carbocyclic 4'-fluoro-2',3'-dideoxynucleosides. The convenience of our approach lies in the possibility of obtaining a number of different analogues by the reaction of a unique key intermediate with several heterocyclic bases.

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 ^1H NMR and 100 MHz for ^{13}C NMR with tetramethylsilane as the internal

standard. 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 silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(-)-(3*R*)-3-*tert*-Butyldimethylsilyloxymethyl-6-*tert*-butyldimethylsilyloxy-3-

fluorohex-4-enoic acid ethylester (2). This compound was prepared from 1,2:5,6-di-*O*-isopropylidene-D-mannitol in 8 steps. The reaction conditions are described in detail in supplementary material: $[\alpha]^{24}_{\text{D}} -1.26^\circ$ (c 1.63, CHCl_3); ^1H NMR (CDCl_3) δ 5.83 (m, 2H), 4.14 (br m, 2H) 4.06 (q, $J = 7.1$ Hz, 2H), 3.71 (m, 2H), 2.82 (dd, $J = 18.4, 15.2$ Hz, 1H), 2.71 (dd, $J = 15.2, 15.0$ Hz, 1H), 1.18 (t, $J = 7.1$ Hz, 3H), 0.84 (s, 9H), 0.83 (s, 9H), 0.00 (s, 6H), -0.01 (s, 6H); ^{13}C NMR (CDCl_3) δ 169.88 (d, $J = 10.9$ Hz), 131.24 (d, $J = 9.6$ Hz), 127.38 (d, $J = 19.2$ Hz), 95.64 (d, $J = 179.3$ Hz), 67.30 (d, $J = 26.5$ Hz), 63.22, 60.84, 40.48 (d, $J = 26.0$ Hz), 26.29, 26.18, 18.76, 18.65, 14.57, -5.05, -5.17; Anal. Calcd for $\text{C}_{21}\text{H}_{43}\text{FO}_4\text{Si}_2$: C, 58.02; H, 9.97. Found: C, 58.30; H, 9.98.

(+)-(3*R*)-3-*tert*-Butyldimethylsilyloxymethyl-6-*tert*-butyldimethylsilyloxy-3-

fluorohex-4-en-1-ol (3). To a stirred solution of 300 mg (0.69 mmol) of **2** in 50 ml of dry diethylether 1.04 ml (1.04 mmol) of 1.0 M solution of lithium aluminium hydride was added at -50°C . The reaction mixture was stirred at $-40 \sim -35^\circ\text{C}$ for 1.5 h, and then quenched with 5 ml of EtOAc. After 30 minutes the reaction mixture was diluted with 50

ml of hexane and allowed to slowly warm to rt. It was filtered through a short TLC grade silica gel pad washing with a 4:1 mixture of hexane and ethyl acetate. Evaporation of the solvents under the reduced pressure gave 270 mg (0.69 mmol) of **3** as a light yellow oil which was used for the next step without further purification: $[\alpha]_D^{25} +7.66^\circ$ (c 0.92, CHCl₃); ¹H NMR (CDCl₃) δ 5.89 (dt, *J* = 15.6, 4.0 Hz, 1H), 5.77 (ddt, *J* = 21.6, 15.6, 1.6 Hz, 1H), 4.21 (dt, *J* = 4.0 Hz, 1.6 Hz, 2H), 3.81 (m, 1H), 3.73 (m, 1H), 3.69 (dd, *J* = 13.2, 11.1 Hz, 1H), 3.63 (dd, *J* = 22.7, 11.1 Hz, 1H), 2.35 (br s, 1H), 2.12 (dddd, *J* = 17.3, 14.8, 7.3, 4.9 Hz, 1H), 1.98 (dddd, *J* = 25.3, 14.8, 6.7, 4.8 Hz, 1H), 0.91 (s, 9H), 0.90 (s, 9H), 0.08 (s, 6H), 0.07 (s, 6H); ¹³C NMR (CDCl₃) δ 130.4 (d, *J* = 10.1 Hz), 127.95 (d, *J* = 19.8 Hz), 97.21 (d, *J* = 175.3 Hz), 68.00 (d, *J* = 28.4 Hz), 62.72, 58.18 (d, *J* = 5.2 Hz), 38.63 (d, *J* = 21.4 Hz), 25.89, 25.76, 18.36, 18.27, -5.44, -5.55; FABMS (*m/z*) 393 (M+H)⁺. Anal. Calcd for C₁₉H₄₃FO₄Si₂: C, 58.11; H, 10.52. Found: C, 58.07; H, 10.51.

(+)-(4*R*)-6-Benzyloxy-1-*tert*-butyldimethylsilyloxy-4-*tert*-

butyldimethylsilyloxymethyl-4-fluorohex-2-ene (4). To a stirred, ice cooled suspension of sodium hydride (0.60 g of 60% dispersion in mineral oil, 15.0 mmol) in anhydrous THF (20 ml), a solution of **3** (2.37 g, 6.0 mmol) in anhydrous THF (20 ml) was added. The mixture was stirred at rt for 1 h, then tetrabutylammonium iodide (TBAI, 0.22 g, 0.6 mmol) was added and, upon recooling at 0 °C, benzyl bromide (1.1 ml, 9.2 mmol) was added. The reaction mixture was stirred at rt overnight, then cooled to 0 °C, quenched with water (20 ml), and extracted with ethyl acetate (3 x 50 ml) to give a crude product which was purified by flash chromatography (49:1 hexanes/ethyl acetate) to yield 2.17 g (4.49 mmol, 74%) of **4** as a colorless oil: $[\alpha]_D^{26} 1.07^\circ$ (c 1.42 CHCl₃); ¹H NMR (CDCl₃) δ 7.32 (m, 5H), 5.86 (dt, *J* = 28.8, 4.0 Hz, 1H), 5.79 (m, 1H), 4.58 (dd, *J* = 16.2, 12.2 Hz,

1H), 4.48 (s, 1H), 4.20 (m, 2H), 3.77-3.50 (m, 2H), 2.27-1.88 (m, 2H), 0.91 (s, 9H), 0.90 (s, 9H), 0.07 (s, 6H), 0.06 (s, 6H); ^{13}C NMR (CDCl_3) δ 138.06, 130.27 (d, $J = 10.0$ Hz), 128.34, 127.98 (d, $J = 20.3$ Hz), 127.65, 127.61, 96.58 (d, $J = 176.4$ Hz), 73.01, 68.00 (d, $J = 28.0$ Hz), 65.78 (d, $J = 5.3$ Hz), 62.91, 38.73 (d, $J = 22.0$ Hz), 25.91, 25.82, 18.37, 18.27, -5.29, -5.42, -5.54.

(-)-(4S)-4-O-Benzoyloxy-2-tert-butyldimethylsilyloxymethyl-2-fluorohexanal (5). A solution of **4** (5.80 g, 12.0 mmol) in methanol (150 ml) was treated with O_3 at -78°C , until a slight blue color persisted (30~45 min). The solution was then degassed with argon, allowed to warm to rt and methyl sulfide (2.7 ml, 36.8 mmol) was added. The mixture was stirred at 0°C for 2 h, then methanol was removed under reduced pressure and the residue was treated with water (10 ml), and methylene chloride (3x50 ml) was used to take up the product. The combined organic layers were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude product was purified by flash chromatography (19:1 hexanes/ethyl acetate) to yield 2.36 g (6.93 mmol, 58 %) of **5** as a colorless oil: $[\alpha]_D^{28} -15.09^\circ$ (c 2.38 CHCl_3); ^1H NMR (CDCl_3) δ 9.70 (dd, $J = 6.2, 0.6$ Hz, 1H), 7.36~7.25 (m, 5H), 4.45 (s, 2H), 3.92 (dd, $J = 25.8, 11.6$ Hz, 1H), 3.80 (dd, $J = 19.8, 11.6$ Hz, 1H), 3.65 (td, $J = 10.0, 3.8$ Hz, 1H), 3.58~3.52 (m, 1H), 2.27~2.11 (m, 1H), 2.05~1.96 (m, 1H), 0.87 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (CDCl_3) δ 200.87 (d, $J = 40.1$ Hz), 137.78, 128.37, 127.62, 127.53, 99.81 (d, $J = 185.3$ Hz), 72.91, 66.15 (d, $J = 22.2$ Hz), 63.95 (d, $J = 5.7$ Hz), 33.03 (d, $J = 21.4$ Hz), 25.67, 18.22, -5.61; FABMS (m/z) 341 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{18}\text{H}_{29}\text{FO}_3\text{Si}\cdot 0.24\text{CH}_2\text{Cl}_2$: C, 60.70; H, 8.23. Found: C, 60.38; H, 8.48.

(+)-(4R)-Ethyl (E)-6-O-benzyloxy-4-tert-butyldimethylsilyloxymethyl-4-fluoro-2-enoate (6). To a solution of triethyl phosphonoacetate (1.33 ml, 6.7 mmol) in anhydrous THF (20 ml), a 1.0 M solution of NaHMDS in THF (6.7 ml, 6.7 mmol) was added at -78 °C. After the mixture was stirred at -78 °C for 1 h, a solution of **5** (2.30 g, 6.75 mmol) in anhydrous THF was added. Stirring was continued at -78 °C for 1 more hour, after which the reaction was quenched with saturated ammonium chloride solution (2 ml), allowed to reach rt, and extracted with ethyl ether (3x10 ml). The combined organic layer was washed with brine, dried over MgSO₄, filtered and concentrated. The crude product was purified by flash chromatography (9:1 hexanes/ethyl acetate) to yield 2.22 g (5.41 mmol, 80 %) of **6** as a colorless oil: $[\alpha]_D^{27}$ 2.90° (c 3.56 CHCl₃); ¹H NMR (CDCl₃) δ 7.38~7.25 (m, 5H), 6.94 (dd, *J* = 22.4, 15.8 Hz, 1H), 6.08 (d, *J* = 15.8 Hz, 1H), 4.48 (d, *J* = 13.9 Hz, 1H), 4.45 (d, *J* = 13.9 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 1H), 3.70~3.65 (m, 2H), 3.64~3.51 (m, 2H), 2.26~2.01 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ¹³C NMR (CDCl₃) δ 165.95, 146.01 (d, *J* = 20.0 Hz), 138.07, 128.33, 127.62, 127.57, 120.88 (d, *J* = 10.2 Hz), 96.54 (d, *J* = 181.0 Hz), 73.06, 67.31 (d, *J* = 27.6 Hz), 65.17 (d, *J* = 5.1 Hz), 60.49, 34.89 (d, *J* = 22.1 Hz), 25.73, 18.22, -5.50, -5.58; FABMS (*m/z*) 411 (M+H)⁺. Anal. Calcd for C₂₂H₃₅FO₄Si: C, 64.35; H, 8.59. Found: C, 64.45; H, 8.70.

(+)-(4S)-Ethyl 4-tert-butyldimethylsilyloxymethyl-4-fluoro-6-hydroxyhexanoate (7). To a solution of **6** (2.56 g, 6.2 mmol) in cyclohexane (100 ml) Pd/C (10 %, 1.33 g, 1.2 mmol) was added. The mixture was thoroughly deoxygenated, then saturated with hydrogen and stirred for 24 h. The charcoal was, then, removed by filtration through a short Celite pad, which was thoroughly washed with hexanes. Evaporation of the solvent

gave a crude product which was purified by flash chromatography to yield 1.91 g (5.92 mmol, 95 %) of **7** as a colorless oil: $[\alpha]_D^{29}$ 2.19° (c 2.85 CHCl₃); ¹H NMR (CDCl₃) δ 4.14 (q, *J* = 7.1, 2H), 3.84~3.73 (m, 2H), 3.71~3.61 (m, 2H), 2.49 (bs, 1H), 2.45~2.40 (m, 2H), 1.26 (t, *J* = 7.1, 3H), 0.90 (s, 9H), 0.09 (s, 6H); ¹³C NMR (CDCl₃) δ 173.18, 97.19 (d, *J* = 172.7 Hz), 66.32 (d, *J* = 30.7 Hz), 60.57, 57.96 (d, *J* = 7.4 Hz), 38.05 (d, *J* = 21.5 Hz), 30.40 (d, *J* = 21.8 Hz), 28.13 (d, *J* = 5.8 Hz), 25.71, 18.17, 14.16 -5.55, -5.60; FABMS (*m/z*) 323 (M+H)⁺. Anal. Calcd for C₁₅H₃₁FO₄Si: C, 55.87; H, 9.69. Found: C, 56.11; H, 9.73.

(-)-(4S)-Ethyl 4-*tert*-butyldimethylsilyloxymethyl-4-fluoro-6-hydroxy-6-methanesulfonyloxyhexanoate (8). To a solution of **7** (200 mg, 0.62 mmol) and pyridine (0.18 ml, 2.23 mmol) in anhydrous methylene chloride (5 ml), mesyl chloride (0.15 ml, 1.94 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 24 h, then quenched with methanol (0.3 ml), concentrated under reduced pressure, and purified by flash chromatography (9:1 hexanes/ethyl acetate) to yield 210 mg (0.52 mmol, 85 %) of **8** as a colorless oil: $[\alpha]_D^{27}$ -0.31° (c 2.04 CHCl₃); ¹H NMR (CDCl₃) δ 4.39 (t, *J* = 7.1 Hz, 2H), 4.14 (q, *J* = 7.1, 2H), 3.64 (dd, *J* = 12.2, 10.9 Hz, 1H), 3.60 (dd, *J* = 14.3, 10.9 Hz, 1H), 3.02 (s, 3H), 2.45~2.39 (m, 2H), 2.18 (td, *J* = 7.1, 3.9 Hz, 1H), 2.13 (td, *J* = 7.1, 2.3 Hz, 1H), 2.08~1.98 (m, 2H), 1.26 (t, *J* = 7.1, 3H), 0.90 (s, 9H), 0.08 (s, 6H); ¹³C NMR (CDCl₃) δ 172.89, 95.98 (d, *J* = 175.0 Hz), 65.75 (d, *J* = 30.3 Hz), 65.26 (d, *J* = 7.8 Hz), 60.63, 37.48, 34.22 (d, *J* = 22.5 Hz), 30.23 (d, *J* = 22.2 Hz), 28.10 (d, *J* = 5.9 Hz), 25.72, 18.13, 14.15, -5.57, -5.61; FABMS (*m/z*) 401 (M+H)⁺.

Ethyl (1*R*, 3*R*)-3-*tert*-butyldimethylsilyloxymethyl-3-fluorocyclopentancarboxylate (9) and Ethyl (1*S*, 3*R*)-3-*tert*-butyldimethylsilyloxymethyl-3-fluorocyclopent-1-yl

carboxylate.(9). To a suspension of sodium hydride (175 mg of 60 % dispersion in mineral oil, 4.37 mmol) in anhydrous THF (20 ml), a solution of **8** (350 mg, 0.87 mmol) in anhydrous THF (20 ml) was added. The reaction was refluxed overnight, then cooled to 0 °C and quenched with saturated ammonium chloride solution (20 ml). After 5 more ml of water were added to dissolve precipitated salt, the mixture was extracted with ethyl acetate (3 x 40 ml). The combined organic layer was washed with brine (10 ml), dried over MgSO₄ and concentrated under reduced pressure to give a crude product which was purified by flash chromatography (49:1 hexanes/ethyl acetate) to yield α - and β -**9** as colorless oil (combined yield: 200 mg, 0.66 mmol, 75 %): **For major isomer**, $[\alpha]_D^{27}$ -1.18° (c 1.81 CHCl₃); ¹H NMR (CDCl₃) δ 4.13 (q, J = 7.1, 2H), 3.74 (dd, J = 14.5, 11.0 Hz, 1H), 3.70 (dd, J = 15.2, 11.0 Hz, 1H), 3.11~3.02 (m, 1H), 2.21~1.82 (m, 6H), 1.26 (t, J = 7.1, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ¹³C NMR (CDCl₃) δ 175.65, 106.24 (d, J = 176.1 Hz), 66.24 (d, J = 29.5 Hz), 60.50, 42.28, 38.14 (d, J = 23.6 Hz), 33.82 (d, J = 23.2 Hz), 28.08, 25.81, 18.28, 14.20 -5.45; FABMS (m/z) 305 (M+H)⁺. Anal. Calcd for C₁₅H₂₉FO₃Si: C, 59.17; H, 9.60. Found: C, 59.46; H, 9.59. **For minor isomer**, $[\alpha]_D^{27}$ 5.73° (c 1.33 CHCl₃); ¹H NMR (CDCl₃) δ 4.15 (q, J = 7.1, 2H), 3.67 (d, J = 15.0 Hz, 2H), 2.83~2.74 (m, 1H), 2.20 (dd, J = 26.3, 8.6 Hz, 1H), 2.14~2.06 (m, 1H), 2.03~1.91 (m, 2H), 1.85~1.69 (m, 2H), 1.26 (t, J = 7.1, 3H), 0.89 (s, 9H), 0.07 (s, 6H); ¹³C NMR (CDCl₃) δ 174.88, 105.18 (d, J = 178.0 Hz), 66.09 (d, J = 32.5 Hz), 60.54, 43.26, 37.63 (d, J = 24.4 Hz), 34.61 (d, J = 24.0 Hz), 28.13, 25.81, 18.25, 14.21 -5.47; FABMS (m/z) 305 (M+H)⁺. Anal. Calcd for C₁₅H₂₉FO₃Si: C, 59.17; H, 9.60. Found: C, 59.35; H, 9.53.

(4R)-3-tert-Butyldimethylsilyloxymethyl-3-fluorocyclopentancarboxylic acid (10). To a solution of **9** (0.90 g, 2.95 mmol) in ethanol (50 ml), a solution of sodium hydroxide

(0.41 g in 50 ml, 10.25 mmol) was added. The mixture was stirred at rt for 5 h, then concentrated under reduced pressure to about 50 ml, cooled to 0 °C, quenched with glacial acetic acid (1.2 ml, 20.96 mmol), and rapidly extracted with ethyl acetate (3x200 ml). The combined extracts were washed with water (20 ml) and brine (20 ml), then dried over MgSO₄ and concentrated *in vacuo*. The crude product thus obtained was rapidly purified by flash chromatography (1:1 hexanes/ethyl acetate) to yield 0.66 g (2.39 mmol, 81 %) of the epimeric mixture **10** as a colorless oil: ¹H NMR (CDCl₃) δ 10.52 (bs, 1H), 3.78~3.63 (m, 4H), 3.16~3.08 (m, 1H), 2.91~2.82 (m, 1H) 2.27~1.83 (m, 12H), 0.90 (s, 18H), 0.07 (s, 12H); ¹³C NMR (CDCl₃) δ (major isomer) 181.59, 106.12 (d, *J* = 176.3 Hz), 66.06 (d, *J* = 30.0 Hz), 42.08, 38.04 (d, *J* = 23.7 Hz), 33.81 (d, *J* = 23.1 Hz), 27.96, 25.79, 18.25, -5.47; δ (minor isomer) 180.93, 105.19 (d, *J* = 177.0 Hz), 65.91 (d, *J* = 32.7 Hz), 42.87, 37.43 (d, *J* = 24.4 Hz), 34.16 (d, *J* = 24.0 Hz), 28.00, 25.79, 18.26, -5.47; HR-FABMS (*m/z*) obsd 277.1603, calcd for C₁₃H₂₆FO₃Si 277.1635 (M+H)⁺. Anal. Calcd for C₁₃H₂₅FO₃Si·0.11C₆H₁₄: C, 56.82; H, 9.21. Found: C, 57.17; H, 9.22.

(4*R*)-1-*tert*-Butyldimethylsilyloxymethyl-1-fluoro-3-iodopentane (11). A mixture of **10** (0.66 g, 2.39 mmol) and lead tetraacetate (3.18 g, 7.17 mmol) in carbon tetrachloride (20 ml) was stirred under reflux while illuminated with a 250 W tungsten lamp for 15 min. Then, while maintaining the same conditions of reflux and illumination, a solution of iodine (1.83 g, 7.20 mmol) in carbon tetrachloride (60 ml) was added in small portions until no more discoloration was observed. The reaction was continued for one more hour, then allowed to cool to rt. The lead salts were filtered off and the filtrate was decolorized by shaking with a 2% solution of sodium thiosulfate. The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo* to a crude product which was purified by

flash chromatography (hexanes) to yield 0.70 g (1.95 mmol, 82 %) of the epimeric mixture **11** as a colorless oil: ^1H NMR (CDCl_3) δ 4.44~4.36 (m, 1H), 4.10~4.02 (m, 1H), 3.75 (dd, $J = 14.3, 10.9$ Hz, 1H), 3.71 (dd, $J = 15.2, 10.9$ Hz, 1H), 3.63 (d, $J = 14.0$ Hz, 2H), 2.67~1.72 (m, 12H), 0.91 (s, 9H), 0.89 (s, 9H), 0.08 (s, 6H) 0.06 (s, 6H); ^{13}C NMR (CDCl_3) δ 105.44 (d, $J = 178.0$ Hz), 104.24 (d, $J = 179.9$ Hz), 66.53 (d, $J = 31.8$ Hz), 66.19 (d, $J = 35.0$ Hz), 48.56 (d, $J = 23.9$ Hz), 47.79 (d, $J = 24.4$ Hz), 38.81, 38.42, 35.11 (d, $J = 24.4$ Hz), 34.35 (d, $J = 24.0$ Hz), 25.82, 25.78, 21.74, 19.39, 18.29, 18.21, -5.43, -5.49; FABMS (m/z) 359 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{12}\text{H}_{24}\text{FIO}_2\text{Si} \cdot 0.02\text{C}_6\text{H}_{14}$: C, 40.43; H, 6.80. Found: C, 40.76; H, 6.87.

(+)-(1S, 3R)-3-tert-Butyldimethylsilyloxymethyl-3-fluorocyclopentan-1-ol (12). A mixture of **11** (690 mg, 1.93 mmol) and sodium bicarbonate (0.33 g, 3.93 mmol) in 15 % (v/v) water/HMPA was heated at 100 °C overnight. The mixture was then cooled to 0 °C, diluted with water (20 ml), and extracted with ether (3 x 40 ml). The combined organic phases were washed with brine (10 ml), dried over MgSO_4 , and concentrated under reduced pressure to a crude product which was purified by flash chromatography (6:1 hexanes/ethyl acetate) to yield 190 mg (0.76 mmol, 40 %) of the only diastereomer **12** as a colorless oil: $[\alpha]_{\text{D}}^{27}$ 2.21° (c 3.13, CHCl_3); ^1H NMR (CDCl_3) δ 4.33 (m, 1H), 3.69 (dd, 1H, $J = 15.6, 10.7$ Hz), 3.65 (dd, 1H, $J = 16.4, 10.7$ Hz), 2.14-1.74 (m, 6H), 0.89 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (CDCl_3): δ 106.17 (d, $J = 175.0$ Hz), 73.28, 66.19 (d, $J = 32.0$ Hz), 43.68 (d, $J = 21.5$ Hz), 34.51, 32.66 (d, $J = 24.1$ Hz), 25.78, 18.23, -5.48. HR-FABMS (m/z) obsd 249.1699, calcd for $\text{C}_{12}\text{H}_{26}\text{FO}_2\text{Si}$ 249.1686 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{12}\text{H}_{25}\text{FO}_2\text{Si}$: C, 58.02; H, 10.14. Found: C, 57.80; H, 9.96.

(-)-9-[(1*R*,4*R*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentyl]-6-

chloropurine (13). A suspension of triphenylphosphine (590 mg, 2.25 mmol) and 6-chloropurine (348 mg, 2.25 mmol) in anhydrous THF (10 ml) was treated with DEAD (355 μ l, 2.25 mmol) at rt in the dark for 1 h. A solution of **12** (140 mg, 0.56 mmol) in anhydrous THF was, then, added and the mixture was stirred in the dark at rt for 6 h. Evaporation of solvent gave a crude product which was purified by flash chromatography (4:1 hexanes/ethyl acetate) to yield 149 mg (0.39 mmol, 69 %) of **13** as a white solid : mp 74-76 °C; $[\alpha]_D^{28}$ -12.84° (c 1.17 CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 265.5 nm ; ¹H NMR (CDCl₃) δ 8.73 (s, 1H), 8.18 (s, 1H), 5.28~5.20 (m, 1H), 3.83 (dd, *J* = 17.4, 10.6 Hz, 1H), 3.80 (dd, *J* = 19.2, 10.6 Hz, 1H), 2.67 ~ 2.52 (m, 3H), 2.47~2.32 (m, 1H), 2.26~2.17 (m, 1H), 2.13~1.99 (m, 1H), 0.92 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ¹³C NMR (CDCl₃) δ 151.64, 151.62, 151.16, 143.91, 132.25, 104.32 (d, *J* = 176.4 Hz), 65.58 (d, *J* = 32.7 Hz), 55.68, 40.41 (d, *J* = 23.7 Hz), 32.88 (d, *J* = 23.4 Hz), 30.86, 25.79, 18.27, -5.45; FABMS (*m/z*) 385 (M+H)⁺. Anal. Calcd for C₁₇H₂₆ClFN₄OSi: C, 53.04; H, 6.81; N, 14.55; Cl, 9.21. Found: C, 53.28; H, 6.79; N, 14.35; Cl, 9.35.

(-)-9-[(1*R*,4*R*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentyl]adenine (14).

140 mg (0.36 mmol) of **13** was dissolved in 15 ml of a stock solution of ammonia in methanol, and then stirred at 100 °C in a steel bomb for 2.5 h. Evaporation of volatiles under reduced pressure afforded a crude off-white solid, which was purified by flash chromatography (49:1 chloroform/methanol) to yield 96 mg (0.26 mmol, 72 %) of **14** as a white solid : mp 152-154 °C; $[\alpha]_D^{26}$ -17.87° (c 2.17 CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 261.5 nm ; ¹H NMR (CDCl₃) δ 8.34 (s, 1H), 7.86 (s, 1H), 5.70 (br s, 2H), 5.21~5.12 (m, 1H), 3.83 (dd, *J* = 17.6, 10.6 Hz, 1H), 3.69 (dd, *J* = 19.2, 10.6 Hz, 1H), 2.66 ~ 1.96 (m, 6H), 0.92

(s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (CDCl_3) δ 155.45, 152.64, 150.02, 139.02, 120.19, 104.46 (d, $J = 176.6$ Hz), 65.79 (d, $J = 32.4$ Hz), 54.85, 40.57 (d, $J = 23.7$ Hz), 32.92 (d, $J = 23.4$ Hz), 31.06, 25.80, 18.28, -5.45; FABMS (m/z) 366 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{17}\text{H}_{28}\text{FN}_5\text{OSi}$: C, 55.86; H, 7.72; N, 19.16. Found: C, 55.68; H, 7.65; N, 19.07.

(-)-9-[(1*R*,4*R*)-4-fluoro-4-hydroxymethylcyclopentyl]adenine (15). 78.0 mg (0.21 mmol) of **14** was stirred in a 1.0 M solution of TBAF in THF (1ml) at rt for 30 min. Evaporation of solvent under reduced pressure gave an oil that was purified by flash chromatography (methanol/chloroform 1:19) to yield 52.6 mg (0.197 mmol, 92 %) of **15** as a white solid : mp 167-168 °C; $[\alpha]_D^{29}$ -12.73° (c 0.37, MeOH); UV (MeOH) λ_{max} 260.0 (ϵ 20890, pH 2), 261.5 (ϵ 19800, pH 7), 261.5 (ϵ 17980, pH 11); ^1H NMR (CD_3OD): δ 8.12 (s, 1H), 8.08 (s, 1H), 5.08 (m, 1H), 3.69 (dd, 1H, $J = 16.2, 12.1$ Hz), 3.65 (dd, 1H, $J = 17.4, 12.1$ Hz), 2.43-2.34 (m, 3H), 2.33-2.15 (m, 1H), 2.13-2.04 (m, 1H), 2.00-1.86 (m, 1H). ^{13}C NMR (CD_3OD): δ 157.3, 153.5, 150.7, 141.2, 120.5, 105.9 (d, $J = 176.6$ Hz), 66.7 (d, $J = 27.4$ Hz), 56.1, 41.6 (d, $J = 24.1$ Hz), 33.7 (d, $J = 23.8$ Hz), 31.5. HR-FABMS, (m/z) obsd 252.1268; Calcd for $\text{C}_{11}\text{H}_{15}\text{FON}_5$, m/z 252.1261 (MH^+). Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{FON}_5$: C, 52.58; H, 5.62; N, 27.87. Found: C, 52.74; H, 5.73; N, 27.72.

(3*R*)-3-*tert*-Butyldimethylsilyloxymethyl-6-*tert*-butyldimethylsilyloxy-3-fluorohex-4-en-1-al (16). The primary alcohol (270 mg, 0.69 mmol) **3** obtained above was dissolved in 50 ml of dry methylene chloride. To this colorless solution was added 223 mg (1.03 mmol) of pyridinium chlorochromate and 223 mg of 4A $^\circ$ molecular sieves. The resulting dark brown mixture was vigorously stirred at rt for 2h and then filtered through a short

TLC grade silica gel pad washing with methylene chloride. Evaporation under the reduced pressure gave 270 mg (0.69 mmol) of the crude aldehyde **16** as a pale yellow oil which was used for the next step without further purification: ^1H NMR (C_6D_6) δ 9.63 (q, $J = 2.3$ Hz, 1H), 5.85 (m, 2H), 3.94 (br, s, 2H), 3.60 (dd, $J = 14.4, 11.2$ Hz, 1H), 3.49 (dd, $J = 25.8, 11.2$ Hz, 1H), 2.60 (ddd, $J = 16.8, 16.2, 2.3$ Hz, 1H), 2.50 (ddd, $J = 21.9, 16.2, 2.3$ Hz, 1H), 0.94 (s, 9H), 0.89 (s, 9H), -0.02 (s, 3H), -0.04 (s, 3H); ^{13}C NMR (C_6D_6) δ 196.56 (d, $J = 7.3$ Hz), 130.41 (d, $J = 9.7$ Hz), 125.69 (d, $J = 19.7$ Hz), 94.52 (d, $J = 177.2$ Hz), 66.65 (d, $J = 26.1$ Hz), 61.25, 47.59 (d, $J = 21.5$ Hz), 24.67, 24.54, 17.11, 17.04, -6.82, -6.94.

(5*R*,3*R*)/(5*R*,3*S*)-5-*tert*-Butyldimethylsilyloxymethyl-8-*tert*-butyldimethylsilyloxy-5-fluoro-3-hydroxyocta-1,6-diene (17**).** To a stirred solution of 270 mg (0.69 mmol) of the aldehyde **16** in dry THF, 0.95 ml (0.95 mmol) of 1.0 M solution of vinylmagnesium bromide solution in THF was slowly added at -78°C . After 1.5 h, 5 ml of water was added, and the reaction mixture was slowly warmed to rt. The aqueous phase was extracted three times (3 x 10 ml) with diethyl ether, and the combined organic layer was dried over MgSO_4 , filtered, and evaporated to give a yellow crude product which was purified by column chromatography on silica gel (15:1 hexane/diethyl ether) to give 115 mg (0.275 mmol, 40 % yield from compound **3**) of the desired 1,6-diene **17**: ^1H NMR (CDCl_3) δ 5.83 ~ 5.66 (m, 6H), 5.10 (dt, $J = 17.2, 1.5$ Hz, 2H), 4.95 (dt, $J = 10.4, 3.8, 1.5$ Hz, 2H), 4.22 ~ 4.17 (m, 2H), 4.15 ~ 4.13 (br m, 4H), 3.65 (dd, $J = 21.0, 11.1$ Hz, 1H), 3.62 (dd, $J = 15.3, 10.8$ Hz, 1H), 3.59 (dd, $J = 16.8, 11.1$ Hz, 1H), 3.54 (dd, $J = 19.5, 10.8$ Hz, 1H), 1.94~1.78 (m, 4H), 0.84 (s, 9H), 0.83 (s, 9H), 0.82 (s, 9H), 0.81 (s, 9H), 0.0041 (s, 6H), -0.0053 (s, 6H), -0.0106 (s, 6H), -0.0151 (s, 6H); ^{13}C NMR (CDCl_3)

δ 143.41, 143.37, 131.47 (d, $J = 10.2$ Hz), 131.37 (d, $J = 10.2$ Hz), 130.58 (d, $J = 19.9$ Hz), 130.19 (d, $J = 20.0$ Hz), 114.67, 114.40, 99.12 (d, $J = 177.1$ Hz), 98.02 (d, $J = 176.9$ Hz), 70.18, 70.12, 69.84 (d, $J = 28.3$ Hz), 69.36 (d, $J = 27.4$ Hz), 64.49, 64.42, 44.37, 44.16, 26.86, 26.79, 19.64, 19.60, -4.79, -4.85; HR-FABMS (m/z) obsd 419.2793, calcd for $C_{21}H_{44}FO_3Si_2$ 419.2813 ($M+H$)⁺. Anal. Calcd for $C_{21}H_{43}FO_3Si_2$: C, 60.24; H, 10.35. Found: C, 60.38; H, 10.37.

(1*S*,4*S*)-4-*tert*-Buthyldimethylsilyloxymethyl-4-fluorocyclopent-3-en-1-ol (18). The Grubbs catalyst (20 mg, 0.024 mmol) was weighed and dissolved in 35 ml of dry methylene chloride in a well-dried two-necked flask equipped with N₂ inlet. To this solution a solution of 100 mg (0.24 mmol) of the 1,6-diene **17** in 15 ml of dry methylene chloride was slowly added over 10 minutes under N₂ atmosphere. The wine red solution was stirred for 30 more minutes, and the resulting dark brown solution was evaporated and purified by column chromatography on silica gel (6:1:0.01 hexane/diethyl ether/triethyl amine) to give 53 mg (0.215 mmol, 90 % yield) of the cyclopentenol **18** as a 1 : 1 mixture of diastereomers, which was immediately used for the next step. Due to the instability of this compound, the structure of this compound was characterized only by NMR spectra: ¹H NMR (CD₃OD) δ 6.04 (dt, $J = 5.7, 2.2$ Hz, 1H), 5.99 (dt, $J = 5.7, 1.9$ Hz, 1H), 5.86 (dt, $J = 5.7, 1.5$ Hz, 1H), 5.82 (dt, $J = 5.5, 1.6$ Hz, 1H), 4.83 (br s, 1H), 4.53 (br m, 1H), 3.74 (dd, $J = 18.0, 11.4$ Hz, 1H), 3.71 (dd, $J = 19.8, 11.4$ Hz, 1H), 3.63 (dd, $J = 14.4, 10.9$ Hz, 1H), 3.55 (dd, $J = 17.8, 10.9$ Hz, 1H), 2.52 (dt, $J = 14.0, 7.4$ Hz, 1H), 2.26 (ddd, $J = 21.7, 14.7, 6.8$ Hz, 1H), 1.75 (ddd, $J = 20.6, 14.7, 3.7$ Hz, 1H), 1.69 (ddd, $J = 25.7, 14.0, 4.6$ Hz, 1H), 0.83 (s, 9H), 0.81 (s, 9H), 0.00 (s, 6H), 0.00 (s, 6H), -0.019 (s, 3H), -0.027 (s, 3H); ¹³C NMR (CDCl₃) δ 142.81 (d, $J = 8.8$ Hz), 141.39 (d, $J =$

9.2 Hz), 134.29 (d, $J = 23.4$ Hz), 133.98 (d, $J = 18.3$ Hz), 108.25 (d, $J = 174.4$ Hz), 106.17 (d, $J = 180.0$ Hz), 76.22, 75.32, 69.14 (d, $J = 30.8$ Hz), 68.57 (d, $J = 34.6$ Hz), 44.35 (d, $J = 18.7$ Hz), 43.72 (d, $J = 25.2$ Hz), 26.73, 26.67, 19.64, 19.54, -4.87, -4.90, -4.93, -4.99.

For the 1:1 mixture of diastereomers

(-)-(1*S*,3*S*)-3-*tert*-Butyldimethylsilyloxymethyl-3-fluorocyclopentan-1-ol (19) and (+)-(1*R*,3*S*)-3-*tert*-Butyldimethylsilyloxymethyl-3-fluorocyclopentan-1-ol (20)

A solution of **18** (1.35 g, 5.48 mmol) in 50 ml cyclohexane was treated with Pd/C (135 mg, 10 % w/w). The mixture was thoroughly deoxygenated, and then saturated with hydrogen. After stirring for 2 hrs at rt the reaction mixture was filtered through a celite pad and evaporated to give a 1:1 mixture of diastereomeric cyclopentanol which was separated by column chromatography on silica gel (10:1 hexanes/ethyl acetate). 621 mg (2.50 mmol, 46 % yield) of β -alcohol **19** and 570 mg (2.30 mmol, 42 % yield) of α -alcohol **20** were obtained as colorless oil: For **19**, $[\alpha]_D^{25} -0.95^\circ$ (c 0.97, CHCl₃); ¹H NMR (CDCl₃) δ 4.44(br d, 1H, H-1), 3.69 (d, $J = 12.7$ Hz, 2H, H-6), 2.55 ~ 1.66 (m, 6H, H-2, H-4, H-5), 0.91 (s, 9H, Si-C(CH₃)₃), 0.09 (s, 6H, Si-CH₃); ¹³C NMR (CDCl₃) δ 104.9 (d, $J = 80.5$ Hz), 73.18, 67.20 (d, $J = 34.8$ Hz), 45.53 (d, $J = 22.5$ Hz), 34.75, 32.61 (d, $J = 23.9$ Hz), 26.15, 18.72, -5.09; FABMS (m/z) obsd 249; Anal. Calcd for C₁₂H₂₅FO₂Si: C, 58.02; H, 10.14. Found: C, 58.20; H, 9.98. For **20**, $[\alpha]_D^{24} -2.31^\circ$ (c 0.90, CHCl₃); ¹H NMR (CDCl₃) δ 4.33 (br s, 1H, H-1), 3.68 (dd, $J = 15.4, 10.2$ Hz, 1H, H-6), 3.66 (dd, $J = 16.2, 10.2$ Hz, 1H, H-6), 2.14 ~ 1.79 (m, 6H, H-2, H-4, H-5), 0.89 (s, 9H, Si-C(CH₃)₃), 0.06 (s, 6H, Si-CH₃); ¹³C NMR (CDCl₃) δ 106.12 (d, $J = 175.1$ Hz), 73.23, 66.18 (d, $J = 32.3$ Hz), 43.66 (d, $J = 21.6$ Hz), 34.48, 32.65 (d, $J = 23.6$ Hz), 25.77, 18.22, -5.50;

FABMS (m/z) obsd 249; Anal. Calcd for $C_{12}H_{25}FO_2Si$: C, 58.02; H, 10.14. Found: C, 58.28; H, 10.12.

(-)-(1*R*, 3*S*)-1-Benzoyloxy-3-*tert*-butyldimethylsilyloxymethyl-3-fluorocyclopentane (21). To a stirred solution of 37 mg (0.149 mmol) of β isomer **19**, triphenyl phosphine (195 mg, 0.743 mmol), and benzoic acid (91 mg, 0.745 mmol) in 10 ml of dry THF 0.117 ml (0.743 mmol) of DEAD in 5 ml of THF was slowly added. The resulting yellow solution was stirred at rt for 6 hrs. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (40:1 hexanes/diethyl ether) to give 48 mg (0.136 mmol, 91 % yield) of the α benzoyloxycyclopentanol **21** as a colorless oil: $[\alpha]^{25}_D$ -15.70° (c 1.49 $CHCl_3$); ^1H NMR (CD_3OD) δ 7.98 ~ 7.96 (m, 2H), 7.49 ~ 7.45 (m, 1H), 7.37 ~ 7.33 (m, 2H), 5.33 (m, 1H), 3.62 (dd, J = 14.4, 1.60 Hz, 2H), 2.32 ~ 1.79 (m, 6H), 0.82 (s, 9H), 0.00 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 166.37, 132.87, 130.48, 129.64, 128.31, 104.41 (d, J = 179.0 Hz), 75.74, 66.28 (d, J = 32.9 Hz), 40.94 (d, J = 23.5 Hz), 33.03 (d, J = 23.7 Hz), 31.44, 25.82, 18.27, -5.45; FABMS (m/z) obsd 353, for $C_{19}H_{30}FO_3Si$. Anal. Calcd for $C_{19}H_{29}FO_3Si$: C, 64.74; H, 8.29. Found: C, 64.85; H, 8.23.

9-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-*N*²-acetylamino-6-chloropurine (21) and (+)-9-[(1*S*, 4*S*)-4-fluoro-4-hydroxymethylcyclopentan-1-yl]-*N*²-acetylamino-6-chloropurine (22). To a suspension of triphenylphosphine (108 mg, 0.41 mmol), *N*²-acetylamino-6-chloropurine (106 mg, 0.50 mmol), and **20** (85 mg, 0.342 mmol) in 6 ml of anhydrous dioxane and 3 ml of DMF, 0.08 ml (0.51 mmol) of DEAD was added over 30 minutes at 0 °C. The yellow mixture was stirred for 6 hrs at room temperature. Solvent was removed under

reduced pressure and the residue was purified by column chromatography on silica gel (hexane : ethyl acetate = 2 : 1) to give 97 mg of a inseparable mixture of the desired compound **21** and reduced DEAD. This mixture was directly subjected to the next step. The mixture obtained above was dissolved in anhydrous THF (10 ml) and treated with 0.22 ml (0.22 mmol) of 1.0 M TBAF in THF. The solution was stirred for 30 minutes at room temperature and evaporated to give a colorless residue which was purified by column chromatography on silica gel (30:1 CH₂Cl₂/MeOH) to give 70 mg (0.214 mmol, 63 % yield from **20**) of **22** as a white solid: mp 208 ~ 210 °C; $[\alpha]_D^{25}$ -127.8° (c 0.54 MeOH); UV (MeOH) $\lambda_{\max}$ 288.0 nm; ¹H NMR (CD₃OD) δ 8.71 (s, 1H), 5.50 (quintet, *J* = 8.3 Hz, 1H), 4.03 (dd, *J* = 18.4, 12.2 Hz, 1H), 4.00 (dd, *J* = 19.8, 12.2 Hz, 1H), 2.81 ~ 2.19 (m, 6H), 2.53 (s, 3H); ¹³C NMR (CD₃OD) δ 154.11, 153.63, 151.82, 146.79, 129.68, 106.54 (d, *J* = 176.1 Hz), 67.01 (d, *J* = 27.4 Hz), 57.23, 41.62 (d, *J* = 23.8 Hz), 34.25 (d, *J* = 23.4 Hz), 31.69, 25.09; HR-FABMS (*m/z*) obsd 328.0995, calcd for C₁₃H₁₆ClFN₅O₂ 328.0992 (M+H)⁺. Anal. Calcd for C₁₃H₁₅ClFN₅O₂: C, 47.64; H, 4.61; N, 21.37. Found: C, 47.76; H, 4.68; N, 21.19.

(+)-9-[(1*S*,4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]guanine (23**).** Compound **22** (64 mg, 0.196 mmol) was dissolved in 15 ml of 85 % formic acid and the resulting colorless solution was stirred at 80 °C for 6 h. After evaporation of volatiles, the remaining oil was treated with methanolic ammonia (15 ml), and the solution was stirred overnight. Solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (6:1 CH₂Cl₂/MeOH) to give 40 mg (0.15 mmol, 76 % yield) of **23** as a white solid: mp 220 °C, dec.; $[\alpha]_D^{24}$ 6.38° (c 0.54 MeOH); UV (H₂O) $\lambda_{\max}$ 253.5 nm (ϵ 8 080, pH 2), 252.5 nm (ϵ 8 263, pH 7), 266.5 nm (ϵ 7 123, pH 11); ¹H NMR

(CD₃OD) δ 8.09 (s, 1H, H-8), 5.26 (quintet, J = 8.4 Hz, 1H, H-1'), 4.02 ~ 3.97 (m, 2H, H-5'), 2.71 ~ 2.20 (m, 6H, H-2', H-3', H-6'); ¹³C NMR (CD₃OD) δ 198.77, 159.87, 155.36, 138.59, 118.43, 106.34 (d, J = 175.9 Hz), 67.06 (d, J = 27.5 Hz), 56.03, 41.95 (d, J = 23.7 Hz), 34.13 (d, J = 23.8 Hz), 31.77; HR-FABMS (m/z) obsd 268.1204, calcd for C₁₁H₁₃FN₅O₂ 268.1209 (M+H)⁺. Anal. Calcd for C₁₁H₁₄FN₅O₂·0.9CHCl₃: C, 38.15; H, 4.01; N, 18.69. Found: C, 38.18; H, 4.56; N, 18.78.

(+)-9-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-6-chloropurine (24**).** To a suspension of triphenylphosphine (602 mg, 2.30 mmol) and 6-chloropurine (372 mg, 2.41 mmol) in 5 ml of anhydrous THF, a solution of 0.36 ml (2.29 mmol) of DEAD in THF was added over 30 minutes at rt. After 1 h, 285 mg (1.15 mmol) of **20** in 10 ml of THF was added to the yellow mixture, and then the resulting mixture was stirred for 6 hrs at room temperature. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (10:1 hexanes/diethyl ether) to give 355 mg (0.922 mmol, 80 % yield) of the desired compound **24** as a white solid: mp 80 ~ 82 °C; [α]_D²⁶ 13.40° (c 3.80 CHCl₃); UV (MeOH) λ_{max} 266.0 nm; ¹H NMR (CDCl₃) δ 8.70 (s, 1H), 8.17 (s, 1H), 5.22 (quintet, J = 7.9 Hz, 1H), 3.82 (dd, J = 12.3, 10.5 Hz, 1H), 3.78 (dd, J = 14.2, 10.5 Hz, 1H), 2.65 ~ 1.97 (m, 6H), 0.90 (s, 9H), 0.08 (s, 6H); ¹³C NMR (CDCl₃) δ 151.65, 151.62, 151.14, 143.95, 132.26, 104.34 (d, J = 176.7 Hz), 65.56 (d, J = 32.6 Hz), 55.70, 40.42 (d, J = 23.7 Hz), 32.89 (d, J = 23.3 Hz), 30.86, 25.80, 18.28, -5.44; HR-FABMS (m/z) obsd 385.1642, calcd for C₁₇H₂₇ClFN₄OSi 385.1626 (M+H)⁺. Anal. Calcd for C₁₇H₂₆ClFN₄OSi: C, 53.04; H, 6.81; N, 14.55. Found: C, 53.56; H, 6.90; N, 14.44.

(+)-9-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]adenine

(25). Compound **24** (140mg, 0.36 mmol) was treated with methanolic ammonia and heated to 110 °C in a steel bomb for 6 h. Solvent was removed under reduced pressure and the remaining residue was purified by column chromatography on silica gel (50:1 CH₂Cl₂/MeOH) to give 110 mg (0.30 mmole, 83 % yield) of the aminated compound **25** as a white solid: mp 150 ~ 152 °C; $[\alpha]_D^{26}$ 18.05° (c 0.62 CHCl₃); UV (MeOH) $\lambda_{\max}$ 266.0 nm; ¹H NMR (CDCl₃) δ 8.24 (s, 1H), 7.77 (s, 1H), 5.65 (br s, 2H), 5.07 (quintet, *J* = 7.9 Hz, 1H), 3.73 (dd, *J* = 13.1, 10.6 Hz, 1H), 3.69 (dd, *J* = 14.7, 10.6 Hz, 1H), 2.52 ~ 1.87 (m, 6H), 0.82 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ 154.36, 151.62, 149.03, 138.06, 119.20, 103.42 (d, *J* = 176.3 Hz), 64.79 (d, *J* = 32.4 Hz), 53.85, 39.57 (d, *J* = 23.7 Hz), 31.92 (d, *J* = 23.4 Hz), 30.06, 24.80, 17.28, -6.46; HR-FABMS (*m/z*) obsd 366.2114, calcd for C₁₇H₂₉FN₅OSi 366.2125 (M+H)⁺. Anal. Calcd for C₁₇H₂₈FN₅OSi: C, 55.86; H, 7.72; N, 19.16. Found: C, 55.79; H, 7.69; N, 19.16.

General procedure for the desilylation reaction of *tert*-butyldimethylsilyl ether by using TBAF. Preparation of adenine analogue 26 from 25 is representative.

(+)-9-[(1*S*,4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]adenine (26). To a stirred solution of 100 mg (0.274 mmol) of **25** in 5 ml anhydrous THF, 0.27 ml (0.27 mmol) of 1.0 M solution of TBAF in THF was added. After 1 h, solvent was removed under reduced pressure and the remaining residue was purified by column chromatography on silica gel (10:1 CH₂Cl₂/MeOH) to give 65 mg (0.259 mmol, 95 % yield) of **26** as a white solid: mp 170 ~ 172 °C; $[\alpha]_D^{27}$ 10.51° (c 0.67 MeOH); UV (H₂O) $\lambda_{\max}$ 260.0 nm (ϵ 20 858, pH 2), 261.0 nm (ϵ 14 624, pH 7), 261.0 nm (ϵ 12 639, pH 11); ¹H NMR (CD₃OD) δ 8.39 (s, 1H, H-8), 8.35 (s, 1H, H-2), 5.34 (ddd, *J* = 17.6, 10.0, 8.0 Hz, 1H, H-1'), 3.93

(dd, $J = 17.3, 12.4$ Hz, 1H, H-5'), 3.90 (dd, $J = 19.6, 17.3$ Hz, 1H, H-5') 2.70 ~ 2.18 (m, 6H, H-2', H-3', H-6'); ^{13}C NMR (CDCl_3) δ 158.78, 154.96, 152.17, 142.76, 122.03, 107.36 (d, $J = 176.1$ Hz), 68.16 (d, $J = 27.5$ Hz), 57.61, 43.10 (d, $J = 23.7$ Hz), 35.23 (d, $J = 23.9$ Hz), 32.96; HR-FABMS (m/z) obsd 252.1244, calcd for $\text{C}_{11}\text{H}_{15}\text{FN}_5\text{O}$ 252.1260 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{FN}_5\text{O}\cdot 0.2\text{MeOH}$: C, 52.21; H, 5.79; N, 27.18. Found: C, 52.06; H, 5.61; N, 27.03.

(+)-9-[(1*S*,4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]-6-chloropurine (27). See the general procedure for deprotection of TBDMS group using TBAF. mp 142 ~ 144 °C; $[\alpha]_D^{24}$ 5.50° (c 0.80 MeOH); UV (MeOH) λ_{max} 266.0 nm; ^1H NMR (CD_3OD) δ 8.99 (s, 1H), 8.91 (s, 1H), 5.57 (ddd, $J = 17.6, 9.7, 7.9$ Hz, 1H), 4.04 (dd, $J = 17.4, 12.1$ Hz, 1H), 4.01 (dd, $J = 18.7, 12.1$ Hz, 1H), 2.86 ~ 2.27 (m, 6H); ^{13}C NMR (CD_3OD) δ 194.17, 193.68, 192.26, 188.30, 173.87, 146.94 (d, $J = 176.5$ Hz), 107.58 (d, $J = 27.4$ Hz), 98.06, 82.39 (d, $J = 23.9$ Hz), 74.78 (d, $J = 23.7$ Hz), 72.22; HR-FABMS (m/z) obsd 271.0759, calcd for $\text{C}_{11}\text{H}_{13}\text{ClFN}_4\text{O}$ 271.0761 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{11}\text{H}_{12}\text{ClFN}_4\text{O}\cdot 0.6\text{CH}_3\text{COCH}_3$: C, 50.32; H, 5.15; N, 18.34. Found: C, 50.23; H, 4.92; N, 18.31.

(+)-9-[(1*S*,4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]hypoxanthine (28).

Compound **27** (48 mg, 0.177 mmol) was dissolved in 10 ml formic acid (85 %) and the resulting colorless solution was stirred for 2.5 hrs at 70°C. After thoroughly evaporating the formic acid under reduced pressure, the remaining residue was treated with methanolic ammonia. The resulting solution was stirred overnight at room temperature. After solvent evaporation, the remaining residue was purified by column chromatography on silica gel (20:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to give 40 mg (0.159 mmole, 90 % yield) of **28** as a

white solid: mp 220°C, dec.; $[\alpha]^{23}_{\text{D}}$ 22.01° (c 0.60 MeOH); UV (H₂O) λ_{max} 250.0 nm (ϵ 16 612, pH 2), 251.0 nm (ϵ 16 913, pH 7), 252.0 nm (ϵ 17 930, pH 11); ¹H NMR (CD₃OD) δ 8.33 (s, 1H, H-8), 8.19 (s, 1H, H-2), 5.35 (ddd, J = 17.7, 9.9, 7.9 Hz, 1H, H-1'), 3.92 (dd, J = 17.3, 12.2 Hz, 1H, H-5'), 3.89 (dd, J = 18.5, 12.2 Hz, 1H, H-5'), 2.74 ~ 2.16 (m, 6H, H-2', H-3', H-6'); ¹³C NMR (CD₃OD) δ 159.43, 150.55, 146.64, 141.14, 126.16, 106.30 (d, J = 176.1 Hz), 67.00 (d, J = 27.5 Hz), 56.81, 42.22 (d, J = 23.8 Hz), 34.12 (d, J = 23.6 Hz), 32.03; HR-FABMS (m/z) obsd 253.1102, calcd for C₁₁H₁₄FN₄O₂ 253.1100 (M+H)⁺. Anal. Calcd for C₁₁H₁₃FN₄O₂·0.2MeOH: C, 52.01; H, 5.38; N, 21.66. Found: C, 51.80; H, 5.18; N, 21.40.

(+)-9-[(1*S*,4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]-6-mercaptopurine (29).

A solution containing 50 mg (0.185 mmol) of **27** and 21 mg (0.276 mmol) of thiourea in 4 ml ethyl alcohol was refluxed for 1.5 h. After solvent evaporation under reduced pressure, the remaining off-white solid was purified by fractional recrystallization in ethyl alcohol to give 40 mg (0.149 mmol, 81 % yield) of **29** as a white solid: mp 268 ~ 270 °C; $[\alpha]^{24}_{\text{D}}$ 16.50° (c 0.17 MeOH); UV (H₂O) λ_{max} 322.5 nm (ϵ 24 531, pH 2), 320.5 nm (ϵ 24 374, pH 7), 310.5 nm (ϵ 22 724, pH 11); ¹H NMR (DMSO) δ 14.00 (br s, 1H, N-H), 8.65 (s, 1H, H-8), 8.44 (s, 1H, H-2), 5.43 (t, J = 5.8 Hz, 1H, 5'-OH), 5.31 (dt, J = 17.7, 8.0 Hz, 1H, H-1'), 3.87 ~ 3.80 (m, 2H, H-5'), 2.66 ~ 2.07 (m, 6H, H-2', H-3', H-6'); ¹³C NMR (DMSO) δ 176.20, 145.04, 144.33, 142.00, 135.79, 105.58 (d, J = 175.7 Hz), 64.93 (d, J = 27.2 Hz), 54.44, 40.84 (d, J = 23.1 Hz), 32.70 (d, J = 23.5 Hz), 30.42; HR-FABMS (m/z) obsd 269.0885, calcd for C₁₁H₁₄FN₄OS 269.0892 (M+H)⁺. Anal. Calcd for C₁₁H₁₃FN₄O₂·0.3MeOH: C, 48.83; H, 5.15; N, 20.16. Found: C, 48.74; H, 5.02; N, 20.38.

(+)-1-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-*N*₃-benzoyluracil (30**).** To a solution of triphenylphosphine (647 mg, 2.47 mmol), *N*₃-benzoyluracil (533 mg, 2.47 mmol), and **20** (245 mg, 0.986 mmol) in 6 ml of anhydrous dioxane and 3 ml DMF, 0.4 ml (2.54 mmol) of DEAD was added over 30 min at 0 °C. The resulting homogeneous yellow solution was stirred for 3 hrs at room temperature. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (1:1 hexanes/ethyl acetate) to give 240 mg (0.537 mmol, 54 % yield) of the desired *N*₁-alkylated product **30** as a colorless oil, and 64 mg (0.143 mmol, 15 % yield) of a inseparable mixture of the *O*²-alkylated product **33** and reduced DEAD as a pale yellow oil, respectively: For **30**, $[\alpha]_D^{25}$ 1.65° (c 1.30 CHCl₃); UV (MeOH) $\lambda_{\max}$ 253.0 nm; ¹H NMR (CDCl₃) δ 7.85 (d, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 4.98 (quintet, *J* = 8.2 Hz, 1H), 3.67 (dd, *J* = 11.8, 10.5 Hz, 1H), 3.63 (dd, *J* = 13.8, 10.5 Hz, 1H), 2.35 ~ 2.24 (m, 2H), 2.16 ~ 2.00 (m, 2H), 1.92 ~ 1.76 (m, 2H), 0.82 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ 168.81, 161.97, 149.69, 141.33, 131.40, 130.46, 129.16, 104.13 (d, *J* = 176.7 Hz), 102.48, 65.67 (d, *J* = 33.0 Hz), 57.25, 39.16 (d, *J* = 23.7 Hz), 32.94 (d, *J* = 23.2 Hz), 29.81, 25.80, 18.26; HR-FABMS (*m/z*) obsd 447.2104, calcd for C₂₃H₃₂FN₂O₄Si 447.2115 (M+H)⁺. Anal. Calcd for C₂₃H₃₁FN₂O₄Si: C, 61.86; H, 7.00; N, 6.27. Found: C, 61.78; H, 7.00; N, 6.05.

(-)-1-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-*N*₃-benzoylthymine (31**).** To a solution of triphenylphosphine (186 mg, 0.709 mmol), *N*₃-benzoylthymine (171 mg, 0.743 mmol), and **20** (88 mg, 0.354 mmol) in 2 ml of anhydrous dioxane and 1 ml DMF, 0.11 ml (0.699 mmol) of DEAD was added over 30

min at 0 °C. The resulting homogeneous yellow solution was stirred for 3 hrs at room temperature. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (1:1 hexanes/ethyl acetate) to give 73 mg (0.16 mmol, 45 % yield) of the desired *N*₁-alkylated product **31** as a colorless oil, and 37 mg (0.081 mmol, 23 % yield) of a inseparable mixture of the *O*²-alkylated product **34** and reduced DEAD as a pale yellow oil, respectively: $[\alpha]_D^{24}$ -8.11° (c 2.20 CHCl₃); UV (MeOH) $\lambda_{\max}$ 254.0 nm; ¹H NMR (CDCl₃) δ 7.84 (d, *J* = 8.1 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 2H), 7.01 (br d, *J* = 1.2 Hz, 1H), 4.98 (quintet, *J* = 8.6 Hz, 1H), 3.66 (dd, *J* = 12.5, 10.6 Hz, 1H), 3.62 (dd, *J* = 14.6, 10.6 Hz, 1H), 2.30 ~ 1.97 (m, 4H), 1.92 ~ 1.75 (m, 2H), 1.87 (br d, *J* = 1.2 Hz, 3H), 0.83 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ 169.53, 163.10, 150.15, 137.58, 135.41, 132.01, 130.86, 129.55, 111.58, 104.56 (d, *J* = 176.9 Hz), 66.21 (d, *J* = 32.3 Hz), 57.21, 39.47 (d, *J* = 23.4 Hz), 33.43 (d, *J* = 23.0 Hz), 29.90, 26.23, 18.66, 13.06, -5.02;. Anal. Calcd for C₂₄H₃₃FN₂O₄Si: C, 62.58; H, 7.22; N, 6.08. Found: C, 62.75; H, 7.22; N, 5.89.

(-)-1-[(1*S*, 4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-*N*₃-benzoyl-5-fluorouracil (32**).** To a solution of triphenylphosphine (154 mg, 0.587 mmol), *N*₃-benzoyl-5-fluorouracil (167 mg, 0.713 mmol), and **20** (71 mg, 0.286 mmol) in 2 ml of anhydrous dioxane and 1 ml DMF, 0.113 ml (0.718 mmol) of DEAD was added over 30 min at 0 °C. The resulting homogeneous yellow solution was stirred for 3 hrs at room temperature. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (4:1 hexanes/ethyl acetate) to give 75 mg (0.161 mmol, 56 % yield) of the desired *N*₁-alkylated product **32** as a colorless oil: $[\alpha]_D^{24}$ -0.64° (c 4.80 CHCl₃); UV (MeOH) $\lambda_{\max}$ 265.0 nm; ¹H NMR (CD₃OD) δ 7.92 (d, *J*

= 7.4 Hz, 2H), 7.68 (t, J = 7.8 Hz, 1H), 7.52 (t, J = 7.8 Hz, 2H), 7.46 (d, J = 6.0 Hz, 1H), 5.10 (quintet, J = 8.2 Hz, 1H), 3.76 (dd, J = 11.3, 10.5 Hz, 1H), 3.72 (dd, J = 13.3, 10.5 Hz, 1H), 2.41 ~ 1.85 (m, 6H), 0.92 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (CD_3OD) δ 167.75, 156.26 (d, J = 27.2 Hz), 148.69, 140.53 (d, J = 240.0 Hz), 135.87, 131.41, 130.97, 129.69, 104.25 (d, J = 177.1 Hz), 66.15 (d, J = 33.6 Hz), 57.50, 39.55 (d, J = 23.9 Hz), 33.36 (d, J = 23.3 Hz), 30.26, 26.23, 18.72, -5.05; HR-FABMS (m/z) obsd 465.2029, calcd for $\text{C}_{23}\text{H}_{31}\text{F}_2\text{N}_2\text{O}_4\text{Si}$ 465.2021 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{23}\text{H}_{30}\text{F}_2\text{N}_2\text{O}_4\text{Si}\cdot 0.2\text{C}_6\text{H}_{14}$: C, 60.33; H, 6.86; N, 5.81. Found: C, 60.66; H, 6.89; N, 5.77.

General Procedure for N_3 -debenzoylation. The synthesis of uridine analogue **36 from compound **30** is representative.**

(+)-1-[(1*S*,4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]uracil

(36). The N_3 -benzoylated uridine analogue **30** (240 mg, 0.537 mmol) was treated with 10 ml of methanolic ammonia for 3 hrs at 0 °C. After solvent evaporation, the remaining residue was purified by column chromatography on silica gel (1:1 hexanes/ethyl acetate) to give 181 mg (0.528 mmol, 98 % yield) of **36** as a white solid: $[\alpha]_D^{28}$ 2.54° (c 1.10 CHCl_3); UV (MeOH) λ_{max} 266.0 nm; ^1H NMR (CDCl_3) δ 9.55 (br s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 5.65 (d, J = 8.0 Hz, 1H), 5.02 (quintet, J = 8.3 Hz, 1H), 3.67 (dd, J = 10.5, 8.8 Hz, 1H), 3.63 (dd, J = 13.8, 10.5 Hz, 1H), 2.31 ~ 1.69 (m, 6H), 0.82 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (CDCl_3) δ 163.88, 151.31, 141.84, 104.49 (d, J = 176.5 Hz), 103.02, 66.15 (d, J = 33.31 Hz), 56.87, 39.59 (d, J = 23.5 Hz), 33.36 (d, J = 23.3 Hz), 30.24, 26.22, 18.68, -5.04; HR-FABMS (m/z) obsd 343.1840, calcd for $\text{C}_{16}\text{H}_{28}\text{FN}_2\text{O}_3\text{Si}$ 343.1853 ($\text{M}+\text{H}$) $^+$. Anal. Calcd for $\text{C}_{16}\text{H}_{27}\text{FN}_2\text{O}_3\text{Si}\cdot 0.11\text{C}_6\text{H}_{14}$: C, 56.85; H, 8.17; N, 7.96. Found: C, 56.98; H, 8.03; N, 7.76.

(-)-1-[(1*S*, 4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]thymine (37). See the general procedure for deprotection of benzoyl group using methanolic ammonia. $[\alpha]_D^{24} -4.75^\circ$ (c 0.50, CHCl₃); UV (MeOH) $\lambda_{\max}$ 271.0 nm; ¹H NMR (CDCl₃) δ 8.29 (s, 1H), 6.90 (s, 1H), 5.00 (tt, $J = 8.3, 8.2$ Hz, 1H), 3.66 (dd, $J = 11.9, 10.6$ Hz, 1H), 3.62 (dd, $J = 14.0, 10.6$ Hz, 1H), 2.28~1.68 (m, 6H), 1.83 (s, 3H), 0.82 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ 163.78, 150.99, 137.52, 111.53, 104.41 (d, $J = 176.5$ Hz), 66.17 (d, $J = 32.9$ Hz), 56.41, 39.50 (d, $J = 23.4$ Hz), 33.43 (d, $J = 23.3$ Hz), 30.04 (d, $J = 13.8$ Hz), 26.22, 18.65, 12.99, -5.03; HR-FABMS (m/z) obsd 357.2008, calcd for C₁₇H₃₀FN₂O₃Si 357.2009 (M+H)⁺. Anal. Calcd for C₁₇H₂₉FN₂O₃Si·0.2C₆H₁₄: C, 58.49; H, 8.58; N, 7.50. Found: C, 58.23; H, 8.41; N, 7.15.

(+)-1-[(1*S*, 4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]-5-fluorouracil (38). See the general procedure for deprotection of benzoyl group using methanolic ammonia. $[\alpha]_D^{24} 1.91^\circ$ (c 4.00 CHCl₃); UV (MeOH) $\lambda_{\max}$ 273.5 nm; ¹H NMR (CDCl₃) δ 9.76 (br s, 1H), 7.24 (d, $J = 5.9$ Hz, 1H), 5.06 (quintet, $J = 8.3$ Hz, 1H), 3.66 (dd, $J = 10.5, 10.5$ Hz, 1H), 3.62 (dd, $J = 12.6, 10.5$ Hz, 1H), 2.32 ~ 2.22 (m, 2H), 2.15 ~ 1.79 (m, 3H), 1.73 ~ 1.64 (m, 1H), 0.81 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CD₃OD) δ 156.80 (d, $J = 26.4$ Hz), 149.57, 140.63 (d, $J = 238.4$ Hz), 125.38 (d, $J = 32.6$ Hz), 103.72 (d, $J = 177.0$ Hz), 65.71 (d, $J = 33.9$ Hz), 56.31, 39.13 (d, $J = 23.7$ Hz), 32.95 (d, $J = 23.4$ Hz), 29.90, 25.78, 18.27, -5.51; Anal. Calcd for C₁₆H₂₆F₂N₂O₃Si·0.1C₆H₁₄: C, 54.02; H, 7.48; N, 7.59. Found: C, 54.09; H, 7.22; N, 7.63.

(+)-*O*²-[(1*S*, 4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]uracil (39). See the general procedure for deprotection of benzoyl group using methanolic ammonia. $[\alpha]_D^{23} 27.40^\circ$ (c 4.46 CHCl₃); UV (MeOH) $\lambda_{\max}$ 267.5 nm; ¹H NMR (CDCl₃) δ

7.69 (d, $J = 6.6$ Hz, 1H), 6.03 (d, $J = 6.6$ Hz, 1H), 5.51 ~ 5.48 (m, 1H), 3.67 (d, $J = 17.9$ Hz, 2H), 2.40 ~ 1.83 (m, 6H), 0.82 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (CDCl_3) δ 166.12, 157.38, 155.69, 109.15, 105.38 (d, $J = 177.0$ Hz), 80.14, 66.97 (d, $J = 29.3$ Hz), 41.80 (d, $J = 24.0$ Hz), 32.77 (d, $J = 23.5$ Hz), 31.12, 26.22, 18.70, -5.03; FABMS (m/z) obsd 343; Anal. Calcd for $\text{C}_{16}\text{H}_{27}\text{FN}_2\text{O}_3\text{Si}$: C, 56.11; H, 7.95; N, 8.18. Found: C, 56.26; H, 7.99; N, 7.95.

(+)- O^2 -[(1*S*, 4*S*)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]thymine (40). See the general procedure for deprotection of benzoyl group using methanolic ammonia. $[\alpha]_{\text{D}}^{23}$ 23.40° (c 1.07 CHCl_3); UV (MeOH) λ_{max} 271.0 nm; ^1H NMR (CDCl_3) δ 7.53 (br s, 1H), 5.46 (br s, 1H), 3.67 (d, $J = 17.1$ Hz, 2H), 2.43 ~ 1.81 (m, 6H), 1.93 (s, 3H), 0.82 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (CDCl_3) δ 175.15, 155.32, 151.68, 118.52, 105.41 (d, $J = 176.8$ Hz), 79.84, 66.92 (d, $J = 29.7$ Hz), 41.90 (d, $J = 23.8$ Hz), 32.88 (d, $J = 23.7$ Hz), 31.30, 26.21, 18.68, 12.82, -5.00; FABMS (m/z) obsd 357; Anal. Calcd for $\text{C}_{17}\text{H}_{29}\text{FN}_2\text{O}_3\text{Si} \cdot 0.2\text{C}_6\text{H}_{14}$: C, 58.49; H, 8.58; N, 7.50. Found: C, 58.75; H, 8.45; N, 7.16.

(-)-1-[(1*S*, 4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]uracil (41). See the general procedure for deprotection of TBDMS group using TBAF. mp 75 °C; $[\alpha]_{\text{D}}^{25}$ -2.92° (c 1.31, MeOH); UV (H_2O) λ_{max} 271.5 nm (ϵ 1 602, pH2), 271.0 nm (ϵ 1 308, pH7), 270.0 nm (ϵ 1 238, pH11); ^1H NMR (CDCl_3) δ 7.74 (d, $J = 8.0$ Hz, 1H, H-6), 5.75 (d, $J = 8.0$ Hz, 1H, H-5), 5.10 (ddd, $J = 16.0, 10.6, 8.2$ Hz, 1H, H-1'), 3.78 (dd, $J = 15.1, 12.1$ Hz, 1H, H-5'), 3.73 (dd, $J = 19.4, 12.1$ Hz, 1H, H-5'), 2.39~1.96 (m, 6H, H-2', H-3', H-6'); ^{13}C NMR (CDCl_3) δ 166.71, 153.15, 144.82, 106.14 (d, $J = 175.9$ Hz), 103.00, 67.03 (d, $J = 27.2$ Hz), 58.21, 40.38 (d, $J = 23.4$ Hz), 33.90 (d, $J = 23.7$ Hz), 30.04; HR-FABMS

(*m/z*) obsd 229.0989, calcd for C₁₀H₁₄FN₂O₃ 229.0988 (M+H)⁺. Anal. Calcd for C₁₀H₁₄FN₂O₃·0.2H₂O: C, 51.81; H, 5.83; N, 12.08. Found: C, 51.74; H, 5.93; N, 11.70.

(+)-1-[(1*S*, 4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]thymine (42). See the general procedure for deprotection of TBDMS group using TBAF. mp 156 ~ 158 °C; [α]_D²² 4.60° (c, 1.32 MeOH); UV (H₂O) $\lambda_{\max}$ 267.0 nm (ϵ 21 659, pH 2), 267.0 nm (ϵ 21 233, pH 7), 265.0 nm (ϵ 16 569, pH 11); ¹H NMR (CD₃OD) δ 7.68 (d, *J* = 1.1 Hz, 1H, H-6), 5.22 (ddd, *J* = 18.9, 10.8, 8.1 Hz, 1H, H-1'), 3.88 (dd, *J* = 18.4, 12.1 Hz, 1H, H-5'), 3.86 (dd, *J* = 19.4, 12.1 Hz, 1H, H-5'), 2.46 ~ 2.05 (m, 6H, H-2', H-3', H-6'), 2.05 (d, *J* = 1.1 Hz, 3H, CH₃); ¹³C NMR (CD₃OD) δ 166.83, 153.31, 140.45, 112.00, 106.16 (d, *J* = 175.9 Hz), 67.07 (d, *J* = 27.4 Hz), 57.76, 40.32 (d, *J* = 23.4 Hz), 33.89 (d, *J* = 23.4 Hz), 29.98, 12.74; HR-FABMS (*m/z*) obsd 243.1154, calcd for C₁₁H₁₆FN₂O₃ 243.1144 (M+H)⁺. Anal. Calcd for C₁₁H₁₅FN₂O₃: C, 54.54; H, 6.24; N, 11.56. Found: C, 54.24; H, 6.20; N, 11.34.

(-)-1-[(1*S*, 4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]-5-fluorouracil (43). See the general procedure for deprotection of TBDMS group using TBAF. mp 142 ~ 144 °C; [α]_D²⁵ -4.20° (c 1.21 MeOH); UV (H₂O) $\lambda_{\max}$ 273.0 nm (ϵ 8 758, pH 2), $\lambda_{\max}$ 273.5 nm (ϵ 8 217, pH 7), $\lambda_{\max}$ 271.5 nm (ϵ 6 839, pH 11); ¹H NMR (CD₃OD) δ 7.97, (d, *J* = 6.9 Hz, 1H, H-6), 5.09 (dt, *J* = 18.4, 8.0 Hz, 1H, H-1'), 3.75 (dd, *J* = 21.0, 12.1 Hz, 1H, H-5'), 3.72 (dd, *J* = 19.6, 12.1 Hz, 1H, H-5'), 2.36 ~ 1.87 (m, 6H, H-2', H-3', H-6'); ¹³C NMR (CD₃OD) δ 159.83 (d, *J* = 25.7 Hz), 151.84, 142.26 (d, *J* = 232.4 Hz), 128.68 (d, *J* = 33.7 Hz), 106.04 (d, *J* = 176.0 Hz), 67.05 (d, *J* = 27.3 Hz), 58.19, 40.20 (d, *J* = 23.5 Hz), 33.79 (d, *J* = 23.5 Hz), 29.91; HR-FABMS (*m/z*) obsd 247.0888, calcd for C₁₀H₁₃F₂N₂O₃

247.0889 (M+H)⁺. Anal. Calcd for C₁₀H₁₂F₂N₂O₃·0.1CH₃CH₂OCH₂OCH₃: C, 49.25; H, 5.17; N, 11.05. Found: C, 49.39; H, 5.23; N, 10.81.

(+)-O²-[(1S, 4S)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]uracil (46). See the general procedure for deprotection of TBDMS group using TBAF. [α]_D²² 20.86° (c 0.91 MeOH); UV (MeOH) $\lambda_{\max}$ 267.5 nm; ¹H NMR (CD₃OD) δ 7.72 (d, *J* = 6.7 Hz, 1H, H-6), 6.01 (d, *J* = 6.7 Hz, 1H, H-5), 5.56 (m, 1H, H-1'), 3.66 (d, *J* = 19.7 Hz, 2H, H-5'), 2.46 ~ 1.91 (m, 6H, H-2', H-3', H-6'); HR-FABMS (*m/z*) obsd 229.0988, calcd for C₁₀H₁₄FN₂O₃ 229.0983 (M+H)⁺; Anal. Calcd for C₁₀H₁₃FN₂O₃ : C, 52.63; H, 5.74; N, 12.27. Found: C, 52.91; H, 5.96; N, 12.00.

(+)-O²-[(1S, 4S)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]thymine (47). See the general procedure for deprotection of TBDMS group using TBAF. [α]_D²³ 31.72° (c 0.19 MeOH); UV (MeOH) $\lambda_{\max}$ 270.0 nm; ¹H NMR (CD₃OD) δ 7.57 (d, *J* = 1.0 Hz, 1H, H-6), 5.51 (m, 1H, H-1'), 3.66 (d, *J* = 19.8 Hz, 2H, H-5'), 2.44 ~ 1.92 (m, 6H, H-2', H-3', H-6'), 1.92 (d, *J* = 1.0 Hz, 3H, CH₃); HR-FABMS (*m/z*) obsd 243.1145, calcd for C₁₁H₁₆FN₂O₃ 243.1158 (M+H)⁺.

(+)-1-[(1S, 4S)-4-*tert*-Butyldimethylsilyloxymethyl-4-fluorocyclopentan-1-yl]cytosine (44). To a solution of **36** (73 mg, 0.213 mmol) and 1,2,4-triazole (97 mg, 1.40 mmol) in anhydrous pyridine (10 ml), 4-chlorophenyldichlorophosphate (0.111 ml, 0.682 mmol) was slowly added at 0 °C. The resulting solution was stirred for 24 hrs at rt, and then evaporated to dryness *in vacuo*. The residue was dissolved in CHCl₃, and the solution was washed with water (5 ml x 2) and saturated aq. NaHCO₃ solution. After drying over MgSO₄, the remaining residue was carefully dried and used for the next step without further purification. The crude product obtained above was treated with methanolic

ammonia and heated to 100 °C for 6 hrs in a steel bomb. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel (2:1 hexanes/acetone) to give 45 mg (0.132 mmol, 62 % yield) of the aminated product **44** as a pale yellow syrup: $[\alpha]_D^{25}$ 6.23° (c 1.59 CHCl₃); UV (MeOH) $\lambda_{\max}$ 276.0 nm; ¹H NMR (CDCl₃) δ 7.25 (d, *J* = 7.3 Hz, 1H), 5.74 (d, *J* = 7.3 Hz, 1H), 5.04 (quintet, *J* = 8.3 Hz, 1H), 3.68 (dd, *J* = 13.0, 10.5 Hz, 1H), 3.64 (dd, *J* = 14.7, 10.5 Hz, 1H), 2.30 ~ 1.70 (m, 6H), 0.82 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ 165.11, 156.42, 143.23, 104.76 (d, *J* = 176.7 Hz), 94.90, 66.44 (d, *J* = 32.5 Hz), 57.88, 39.79 (d, *J* = 23.3 Hz), 33.42 (d, *J* = 23.2 Hz), 30.35, 26.25, 18.70, -5.02; HR-FABMS (*m/z*) obsd 342.2018, calcd for C₁₆H₂₉FN₃O₂Si 342.2013 (M+H)⁺. Anal. Calcd for C₁₆H₂₈FN₃O₂Si·0.4CH₃COCH₃: C, 56.64; H, 8.40; N, 11.52. Found: C, 56.62; H, 8.33; N, 11.23.

(-)-1-[(1*S*, 4*S*)-4-Fluoro-4-hydroxymethylcyclopentan-1-yl]cytosine (45). See the general procedure for deprotection of TBDMS group using TBAF. $[\alpha]_D^{25}$ -2.10° (c 1.37 MeOH); UV (H₂O) $\lambda_{\max}$ 283.0 nm (ϵ 7 718, pH 2), $\lambda_{\max}$ 274.0 nm (ϵ 5 321, pH 7), $\lambda_{\max}$ 274.0 nm (ϵ 5 223, pH 11); ¹H NMR (CD₃OD) δ 7.77 (d, *J* = 7.4 Hz, 1H, H-6), 5.99 (d, *J* = 7.4 Hz, 1H, H-5), 5.17 (ddd, *J* = 18.5, 10.5, 8.0 Hz, 1H, H-1'), 3.80 (dd, *J* = 18.5, 12.1 Hz, 1H, H-5'), 3.77 (dd, *J* = 19.5, 12.1 Hz, 1H, H-5'), 2.41 ~ 1.92 (m, 6H, H-2', H-3', H-6'); ¹³C NMR (CD₃OD) δ 167.60, 159.24, 144.94, 106.26 (d, *J* = 175.9 Hz), 96.55, 67.14 (d, *J* = 27.4 Hz), 59.01, 40.72 (d, *J* = 23.4 Hz), 34.04 (d, *J* = 23.8 Hz), 30.46; HR-FABMS (*m/z*) obsd 228.1155, calcd for C₁₀H₁₅FN₃O₂ 228.1148 (M+H)⁺. Anal. Calcd for C₁₀H₁₄FN₃O₂·0.39CH₂Cl₂: C, 47.93; H, 5.72; N, 16.14. Found: C, 48.09; H, 6.04; N, 15.96.

CHAPTER 3

EFFICIENT SYNTHESIS OF 2-DEOXY-L-*ERYTHRO*-PENTOSE (2-DEOXY-L-RIBOSE) FROM L-ARABINOSE¹

¹Chong, Y. and C. K. Chu. 2002. *Carbohydrate Research*. 337:397-402.
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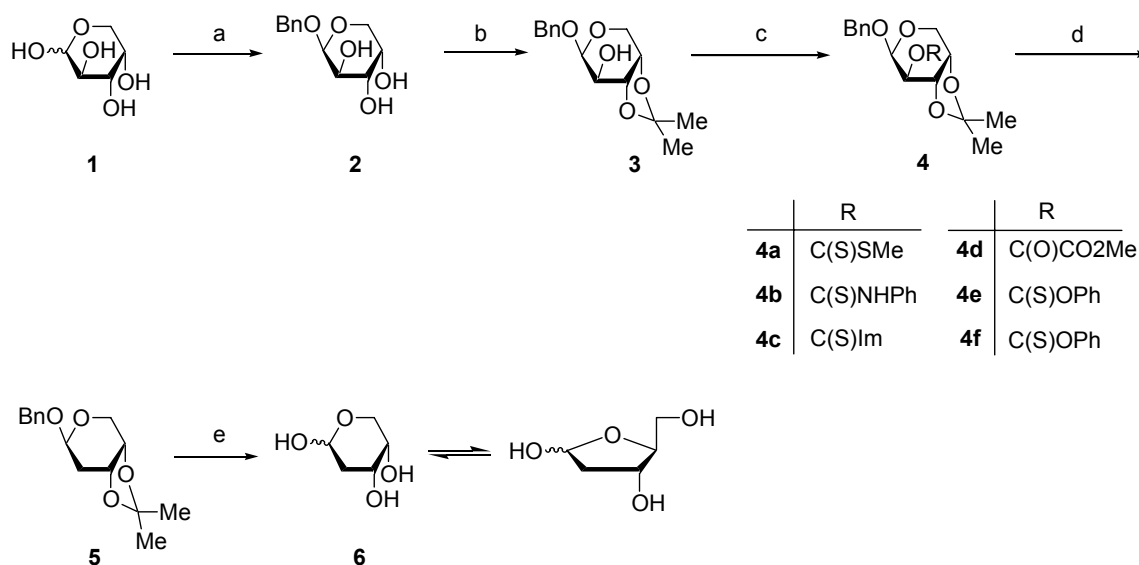
Introduction

The use of L-carbohydrates and related nucleosides in medicinal application has greatly increased due to their potent biological activity and lower toxicity compared to their D-counterpart.^{156,157,158,159,160,161} Among these, L-FMAU (clevudine),² L-thymidine (L-T),³ L-3'-thiacytidine (3TC),⁴ L-5-fluoro-3'-thiacytidine (FTC),^{4a,5} L-2',3'-dideoxycytidine (L-ddC)⁶ and L-5-fluoro-2',3'-dideoxycytidine (L-FddC)^{6b,6c} showed potent antiviral activity with greatly reduced toxicity. For these reasons, modified nucleosides in the unnatural L-configuration have become an important synthetic target, which requires ready access to L-carbohydrates, especially L-ribose and its derivatives. However, the high cost of L-ribose and 2-deoxy-L-*erythro*-pentose (2-deoxy-L-ribose) has hampered their use as starting material for the synthesis of L-nucleosides in large quantities. We herein report a short, five-step synthesis of 2-deoxy-L-ribose (**6**) from an inexpensive and readily available starting material L-arabinose (**1**).

Results and discussion

Even though several syntheses^{162,163,164,165} of 2-deoxy-L-*erythro*-pentose (2-deoxy-L-ribose) using L-arabinose⁷, L-ribose^{7c} or L-ascorbic acid⁸ as the starting materials are known, none of these methods have proved to be an economical and efficient procedure for the preparation of 2-deoxy-L-ribose in large quantities. Our previous experience in the large-scale synthesis of L-FMAU from L-arabinose via L-ribose¹⁶⁶ showed a possibility of

finding a very practical route to 2-deoxy-L-ribose since deoxygenation of readily obtainable intermediate benzyl 3,4-*O*-isopropylidene- β -L-arabinoside **3** (Scheme 3.1) would directly give rise to our target compound **6**. Because of the sterically hindered nature of secondary hydroxyl group in carbohydrates, Barton-type radical deoxygenation conditions were tested first (Table 3.1).



Scheme 3.1. Synthesis of 2-deoxy-L-erythro-pentose (2-deoxy-L-ribose, **6**) from L-arabinose (**1**)

The secondary alcohol **3** was reacted with several different types of reagents to give radical precursors **4a** - **4f**. The radical precursors **4a** - **4f** were treated with tri-*n*-butyltin hydride and AIBN under two different conditions. As was described in the literature,^{7b,e} normal radical deoxygenation conditions (Method A, Table 3.1) gave rise to the desired deoxygenated product **5** in a relatively low yield due to the hydrolysis of the starting material and formation of side products. Changing the reaction conditions circumvented this problem (Method B, Table 3.1). To a refluxing solution of radical precursor **4** in dry

toluene, a solution of tri-*n*-butyltin hydride and AIBN in dry toluene was slowly added over 1 h. After stirring 1 h more under reflux, volatiles were evaporated under reduced pressure, and the remaining residue was filtered through a short silica gel pad to give a crude product **5** which was used for the next step without further purification. Method B proved to be better than method A under every reaction tested (Table 3.1). Even though the best yield was obtained through *S*-methyl xanthate^{7b,e} (**4a**, Entry 1, Table 3.1), the high flammability of carbon disulfide restricted the use of this method for a large-scale synthesis. Other reagents such as phenyl isothiocyanate (**4b**, Entry 2, Table 3.1),¹⁶⁷ 1,1'-thiocarbonyldiimidazole (**4c**, Entry 3, Table 3.1),¹⁶⁸ methyl oxalyl chloride (**4d**, Entry 4, Table 3.1),¹² and phenoxythiocarbonyl chloride (**4e**, Entry 5, Table 3.1)¹⁶⁹ did not give promising results because either they were not reactive enough or the reagents used were too expensive. The most efficient deoxygenation was achieved by preparing the phenoxythiocarbonyl ester¹⁴ *in situ* (**4f**, Table). Phenol and pyridine in anhydrous methylene chloride was treated with thiophosgene to give a phenoxythiocarbonyl chloride, which was directly reacted with the alcohol **3** to afford phenoxythiocarbonyl ester **4f**. To a refluxing solution of phenoxythiocarbonyl ester **4f** in dry toluene, a solution of tri-*n*-butyltin hydride and AIBN in toluene was slowly added over 1.5 hour. The resulting dark brown solution was stirred under reflux for additional 1 hour to give the deoxygenated compound **5**, which was treated with 4% trifluoroacetic acid in water at 40°C for 10 hours. The reaction mixture was washed several times with ethyl acetate to remove benzyl alcohol, and then the aqueous phase was filtered through Amberlite® IRA-400 (OH) ion-exchange resin. The water was thoroughly evaporated under reduced pressure and then coevaporated with ethanol and toluene several times to give the desired

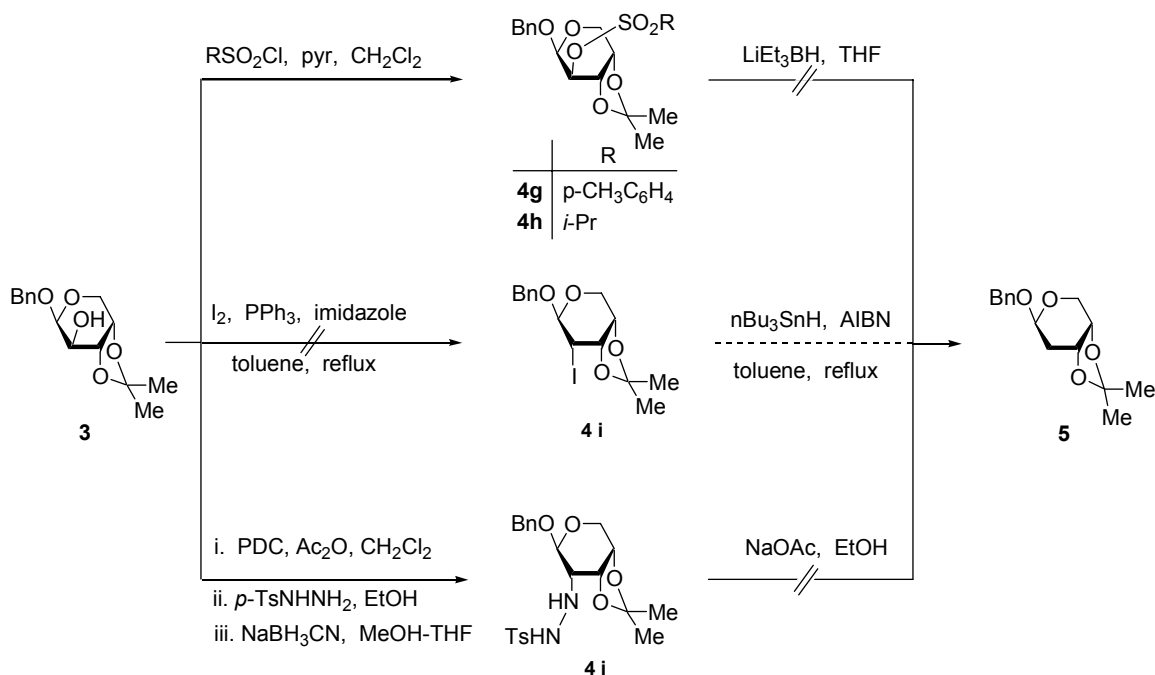
2-deoxy-L-ribose **6** in 70% yield based on the starting alcohol **3**. These conditions were proved to be effective up to the 100g (0.357 mol) scale, and the chemical yield became even better (70%) as the scale of the reaction was increased (**4f**, Entry 6, Table 3.1).

Table 3.1. Free radical deoxygenation of compound **3**

Entry	Radical Precursor	% yield	
		Method A ^a	Method B ^b
1	4a	54	85 84 ^c
2	4b	45	55
3	4c	0 ^d	36
4	4d	—	0 ^e
5	4e	55 ^f	59 ^f
6	4f	—	56 ^g 70 ^{g, h}

- To a stirred solution of compound **4** in dry toluene were added nBu₃SnH and AIBN. The resulting solution was stirred for 2 h under reflux.
- To a stirred solution of compound **4** in dry toluene was *slowly added a solution of nBu₃SnH and AIBN in dry toluene over 1.5 h*. After addition, the reaction mixture was stirred for additional 1 h under reflux.
- 50 g scale.
- Intermediate **4c** was converted to a side product whose structure remains to be determined.
- Intermediate **4d** was hydrolyzed to the starting material **3**.
- Intermediate **4e** was prepared by the reaction of **3** with the commercially available phenyl chlorothionoformate
- Intermediate **4f** was prepared by the reaction of thiophosgene with phenol followed by **3**
- 100 g scale, The yield was obtained after hydrolysis to give 2-deoxy-L-ribose **6** because the separation of compound **5** was not practical

Several reductive deoxygenation conditions were also tested (Scheme 3.2). Thus, the secondary alcohol **3** was converted into alkyl sulfonates (**4g** and **4h**) and treated with Super-Hydride[®] in refluxing THF. However, the sterically hindered nature of sulfonates (**4g** and **4h**) did not allow the reductive cleavage of C-O bond. Instead, the sulfonates were converted into the starting alcohol **3** by hydrolysis. Attempted iodination followed by free radical deoxygenation strategy did not work at all because the initial iodination reaction under the Mitsunobu conditions was completely blocked (Scheme 3.2).



Scheme 3.2. Attempted reductive deoxygenation of compound **3**

Reductive deoxygenation of the corresponding ketone under the Wolff-Kishner conditions was tested (Scheme 3.2). Intermediate **3** was oxidized to the corresponding ketone, which was treated with *p*-toluenesulfonylhydrazide to give a *cis*- and *trans*- mixture of tosylhydrazone **4j**. However, sodium cyanoborohydride mediated reduction of the

hydrazone **4j** did not go to completion, leaving one of the two double bond isomers unreacted. The corresponding hydrazide obtained in a low yield (33%) was treated with sodium acetate trihydrate in refluxing ethanol, but it did not afford the desired deoxygenated compound **5**. Rather, the starting material decomposed under the reaction conditions.

In conclusion, after survey of available methods for radical deoxygenation, we have modified and improved a commonly used procedure to provide 2-deoxy-L-ribose (**6**) in higher yield from L-arabinose, which can be readily adapted to the large-scale synthesis.

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 ^1H NMR and 100MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). 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 silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(+)-1-*O*-Benzyl-3,4-*O*-isopropylidene- β -L-arabinoside (**3**) Benzyl alcohol (1000 mL) was saturated with hydrogen chloride for 40 min at 0 °C and L-arabinose (200 g, 1.33 mol) was added and the mixture was stirred at room temperature for 10 h, during which periods 1-*O*-benzyl- β -L-arabinoside (**2**) precipitated. To the mixture, EtOAc (1.5 L) was slowly added while stirring for additional precipitation. Filtration of the resulting solid, washing with EtOAc and drying in air gave the compound **2** as a white solid, which was used for the next step without further purification.

A mixture of 1-*O*-benzyl- β -L-arabinoside **2**, 2,2-dimethoxypropane (410 mL, 3.33 mol), and *p*-TsOH·H₂O (5.1 g, 0.027 mol) in acetone (2.5 L) was stirred at room temperature for 2 h. The reaction mixture was then neutralized with Et₃N and evaporated under reduced pressure to give (+)-1-*O*-benzyl-3,4-*O*-isopropylidene- β -L-arabinoside (**3**) as a yellow syrup, which was filtered through a short silica gel (2-20 micron) pad (20 cm height, 10 cm in diameter) washing with a 3:1 hexane-EtOAc. 343 g (1.22 mol, 92 % yield based on L-arabinose **1**) of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene- β -L-arabinoside (**3**) was obtained as a white solid: mp 52 °C; $[\alpha]_D^{23}$ 180.03 ° (c 20.3, CHCl₃); ¹H NMR (CDCl₃) δ 7.39-7.30 (m, 5H), 4.94 (d, *J* = 3.6 Hz, 1H), 4.79 (d, *J* = 11.7 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.25-4.21 (m, 1H), 4.21 (q, *J* = 6.1 Hz, 1 H), 4.01 (dd, *J* = 13.2, 2.4 Hz, 1 H), 3.94 (dd, *J* = 13.1, 1.1 Hz, 1 H), 3.80 (d, *J* = 3.2 Hz, 1H), 2.28 (br s, 1 H), 1.53 (s, 3 H), 1.36 (s, 3 H); ¹³C NMR (CDCl₃) δ 137.0, 128.6, 128.1, 128.0, 109.2, 96.9, 75.9, 72.9, 70.0, 69.7, 59.8, 27.9, 25.8; HRFABMS (*m/z*) obsd 281.1415; calcd for C₁₅H₂₁O₅ 281.1389 (M + H)⁺; Anal. Calcd for C₁₅H₂₀O₅: C, 64.27; H, 7.19 Found: C, 64.04; H, 7.15

Preparation of precursors (4a – 4f) for free radical deoxygenation. 1-O-Benzyl-3,4-O-isopropylidene-2-O-[(methylthio)thiocarbonyl]-β-L-arabinoside (4a) To a stirred solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene-β-L-arabinoside (**3**) (42.7 g, 0.152 mol) in anhydrous THF (500 mL), NaH (6.1 g, 0.152 mol) was slowly added at 0 °C. After the initial violent reaction subsided, the ice-bath was removed. After stirring for 30 min at rt, carbon disulfide (18.3 mL, 0.304 mol) was added at once. The resulting yellow suspension was stirred for 30 min, methyl iodide (19.0 mL, 0.304 mol) was slowly added at rt, and the resulting yellow solution was stirred for 30 more min at rt. Water (50 mL) was added, and the volume of the reaction mixture was reduced to half by evaporation. The aqueous phase was extracted three times with EtOAc, and the combined organic phase was washed with brine, dried over MgSO₄, filtered, and evaporated to give 57.7 g of the crude 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-[(methylthio)thiocarbonyl]-β-L-arabinoside (**4a**) as yellow syrup, which was used for the next step without further purification.

1-O-Benzyl-3,4-O-isopropylidene-2-O-[N-phenylthioxocarbamoyl]-β-L-arabinoside (4b) To a stirred solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene-β-L-arabinoside **3** (1.0 g, 3.57 mmol) and phenyl isothiocyanate (0.47 mL, 3.93 mmol) in anhydrous THF (20 mL), NaH (157 mg, 3.93 mmol) was slowly added, and the reaction was monitored by TLC. After 2 h, the solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and water. The organic layer was separated and dried over MgSO₄. Evaporation of the solvent gave 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-[*N*-phenylthioxocarbamoyl]-β-L-arabinoside (**4b**) as yellow syrup, which was used for the next step without further purification.

1-O-Benzyl-3,4-O-isopropylidene-2-O-[1-imidazolyl(thiocarbonyl)]-β-L-arabinoside

(4c) A solution of 1,1'-thiocarbonyldiimidazole (668 mg, 3.75 mmol) and (+)-1-*O*-benzyl-3,4-*O*-isopropylidene-β-*L*-arabinoside **3** (500 mg, 1.78 mmol) in dry THF (30 mL) was stirred under reflux overnight. After evaporation of the solvent, the remaining yellow residue was filtered through a short silica gel pad washing with a 3:1 hexane-EtOAc. The filtrate was concentrated to give 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-[1-imidazolyl(thiocarbonyl)]-β-*L*-arabinoside (**4c**), which was used for the next step without further purification.

1-O-Benzyl-3,4-O-isopropylidene-2-O-[methyloxalyl]-β-L-arabinoside (4d) To a stirred solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene-β-*L*-arabinoside **3** (0.5 g, 1.78 mmol) and pyridine (0.6 mL, 7.14 mmol) in anhydrous CH₂Cl₂ (10 mL), methyl chlorooxalate (0.2 mL, 2.14 mmol) was added at rt. The colorless solution was stirred for 2 h at rt, and then diluted with 200 mL of CH₂Cl₂. The organic layer was washed with water, 2 N HCl, and brine, dried over MgSO₄, filtered, and evaporated to give 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-[methyloxalyl]-β-*L*-arabinoside (**4d**), which was used for the next step without further purification.

1-O-Benzyl-3,4-O-isopropylidene-2-O-[phenoxythiocarbonyl]-β-L-arabinoside (4e) To a stirred solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene-β-*L*-arabinoside **3** (0.5 g, 1.78 mmol) and pyridine (0.6 mL, 7.14 mmol) in anhyd CH₂Cl₂ (15 mL), phenyl chlorothionoformate (0.3 mL, 2.14 mmol) was added at rt. The resulting yellow solution was stirred for 2 h at rt until the color changed to green. After evaporation of the solvent, the residue was filtered through a short silica gel pad using a 10:1 hexane-EtOAc as the eluent. The filtrate was concentrated to give 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-

[phenoxythiocarbonyl]- β -L-arabinoside (**4e**), which was used for the next step without further purification.

*1-O-Benzyl-3,4-O-isopropylidene-2-O-[phenoxythiocarbonyl]- β -L-arabinoside (**4f**)*

To a stirred solution of thiophosgene (40.8 mL, 0.535 mol) in dry CH₂Cl₂ (500 mL), a solution of phenol (53.75 g, 0.571 mol) and pyridine (57.7 mL, 0.714 mol) in 250 mL of CH₂Cl₂ was slowly added (over 30 min) at 0 °C. The resulting dark wine-red solution was stirred for 30 min at rt. To this solution, a solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene- β -L-arabinoside **3** (100 g, 0.357 mol) and pyridine (57.7 mL, 0.714 mol) in dry CH₂Cl₂ (500 mL) was added over 30 min. The resulting dark-green solution was stirred for 1 h at rt. The reaction mixture was diluted with 1 L of CH₂Cl₂ and washed with water (100 mL x 5), satd NaHCO₃ solution, and brine. The organic layer was dried over MgSO₄, filtered, and concentrated to give crude dark brown 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-phenoxythiocarbonyl- β -L-arabinoside **4f**, which was used for the next step without further purification.

*(+)-1-O-Benzyl-2-deoxy- β -L-erythro-pentopyranoside (**5**). Method A. Conversion of **4a** to **5** is representative.* A solution of crude 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-[(methylthio)thiocarbonyl]- β -L-arabinoside (**4a**) (2.0 g, 5.44 mmol), nBu₃SnH (2.19 mL, 8.15 mmol), and AIBN (90 mg, 0.54 mmol) in toluene was heated to reflux, and then a solution of AIBN (90 mg, 0.54 mmol) in 5 mL of toluene was slowly added over 30 min. The resulting solution was refluxed for 1 h more. After solvent evaporation, the resulting residue was purified by column chromatography on silica gel (20:1 Hex:EtOAc) to give (+)-1-*O*-benzyl-2-deoxy- β -L-erythro-pentopyranoside **5** (768 mg, 2.93 mmol, 54% yield) as a colorless oil.

(+)-1-*O*-Benzyl-2-deoxy- β -L-erythro-pentopyranoside (**5**). *Method B. Conversion of 4f to 5 is representative.* To a refluxing solution of the crude 1-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-phenoxythiocarbonyl- β -L-arabinoside **4f** obtained above in anhydrous toluene (1.5 L), a solution of nBu₃SnH (114.0 mL, 0.535 mol) and AIBN (11.8 g, 0.071 mol) in 500 mL of anhyd toluene was added over 1 h. After the addition, the brown solution was stirred for additional 30 min under reflux. The reaction mixture was concentrated under reduced pressure and filtered through a short silica gel (2-20 micron) pad (20 cm height, 10 cm in diameter) washing with a 6:1 hexane-EtOAc. The filtrate was washed with 5% NaOH solution, water, and brine, dried over MgSO₄, filtered, and evaporated to give 88.9 g of a mixture of (+)-1-*O*-benzyl-2-deoxy- β -L-erythro-pentopyranoside (**5**) and phenol as a colorless syrup. An analytical sample was obtained by column chromatography on silica gel (12:1 hexane-EtOAc): $[\alpha]_D^{23}$ 122.23° (*c* 8.3, CHCl₃); ¹H NMR (CDCl₃) δ 7.47-7.35 (m, 5 H), 5.08 (t, *J* = 5.3 Hz, 1 H), 4.88 (d, *J* = 11.9 Hz, 1 H), 4.60 (d, *J* = 11.9 Hz, 1 H), 4.56 (q, *J* = 5.7 Hz, 1 H), 4.25 (dt, *J* = 6.5, 2.6 Hz, 1 H), 4.00 (dd, *J* = 12.9, 2.8 Hz, 1 H), 3.87 (dd, *J* = 12.9, 2.3 Hz, 1 H), 2.27 (dt, *J* = 14.7, 4.6 Hz, 1 H), 1.96 (ddd, *J* = 14.7, 6.0, 4.7 Hz, 1 H), 1.61 (s, 3 H), 1.44 (s, 3 H); ¹³C NMR (CDCl₃) δ 137.8, 128.3, 127.7, 127.5, 108.4, 95.6, 71.9, 69.8, 69.1, 61.1, 31.4, 27.2, 25.3; HRFABMS (*m/z*) obsd 265.1420, calcd for C₁₅H₂₁O₄ 265.1440 (M + H)⁺. Anal. Calcd for C₁₅H₂₀O₄: C, 68.16; H, 7.63. Found: C, 67.93; H, 7.72

2-Deoxy-L-erythro-pentose (2-Deoxy-L-ribose, **6**) A mixture of crude (+)-1-*O*-benzyl-2-deoxy- β -L-erythro-pentopyranoside **5** (73 g) in 4% aq trifluoroacetic acid was stirred for 10 h at 40 °C. The reaction mixture was poured into a separatory funnel and washed with EtOAc (3 x 500 mL). The aqueous layer was filtered through Amberlite® IRA 400 (OH⁻

form) ion-exchange resin and evaporated to dryness. The remaining syrup was coevaporated with ethanol and toluene two times and dried *in vacuo* to give 26 g (0.194 mol, 70% yield based on (+)-1-*O*-benzyl-3,4-*O*-isopropylidene- β -L-arabinoside **3**) of 2-deoxy-L-ribose (**6**) as a pale yellow syrup. Anal. Calcd for C₅H₁₀O₄·0.1H₂O : C, 44.18; H, 7.56 Found C, 43.87; H, 7.26.

N-(6-Benzyloxy-2,2-dimethyl-dihydro-[1,3]dioxolo[4,5-*c*]pyran-7-yl)-*N'*-(4-toluenesulfonyl)-hydrazine (**4j**) To a stirred solution of (+)-1-*O*-benzyl-3,4-*O*-isopropylidene- β -L-arabinoside **3** (3.0 g, 10.71 mmol) in dry CH₂Cl₂, pyridinium dichromate (PDC, 2.14 g, 5.68 mmol) and Ac₂O (2.93 mL, 31.05 mmol) were added at 0 °C. The resulting dark-brown mixture was stirred for 5 h under reflux, and then 0.5 equiv (2.14 g, 5.68 mmol) of PDC was added. The reaction mixture was stirred for 3 h under reflux. Solvent was evaporated to one third, and then EtOAc (100 mL) was added to the mixture. The mixture was filtered through a Celite pad, and the filtrate was purified by filtration through a silica gel pad washing with EtOAc. Evaporation under reduced pressure gave the corresponding ketone as a pale-yellow syrup, which was used for the next step without further purification.

A solution of the crude ketone obtained above (3.0 g, 10.8 mmol) and *p*-toluenesulfonhydrazide in abs EtOH was stirred for 2 h under reflux. The reaction mixture was cooled down and concentrated to give a yellow syrup which was purified by column chromatography on silica gel (4:1 hexane-EtOAc) to give a 2:1 *cis*- and *trans*-mixture of *N*-(6-benzyloxy-2,2-dimethyl-dihydro-[1,3]dioxolo[4,5-*c*]pyran-7-ylidene)-*N'*-(4-toluenesulfonyl)-hydrazine (3.52 g, 7.89 mmol, 73% yield based on compound **3**) as a pale-yellow foam. The major isomer : ¹H NMR (CDCl₃) δ 9.91 (s, 1H), 7.74 (d, *J* = 8.2

Hz, 2H), 7.39 ~ 7.14 (m, 7H), 5.07 (s, 1H), 4.82 (d, $J = 5.5$ Hz, 1H), 4.59 (d, $J = 11.5$ Hz, 1H), 4.44 (d, $J = 11.5$ Hz, 1H), 4.16 (d, $J = 5.3$ Hz, 1H), 4.04 (m, 1H), 3.90 (d, $J = 13.4$ Hz, 1H), 2.32 (s, 3H), 1.26 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (CDCl_3) δ 145.17, 143.91, 136.45, 135.32, 129.33, 128.45, 128.21, 127.75, 127.48, 110.32, 97.93, 73.55, 71.11, 69.12, 57.28, 26.14, 25.69, 21.29; The minor isomer : ^1H NMR (CDCl_3) δ 9.56 (s, 1H), 7.58 (d, $J = 8.2$ Hz, 2H), 7.39 ~ 7.14 (m, 7H), 5.35 (s, 1H), 4.72 (d, $J = 11.4$ Hz, 1H), 4.61 ~ 4.52 (m, 2H), 4.44 (d, $J = 11.5$ Hz, 1H), 3.63 ~ 3.55 (m, 2H), 2.30 (s, 3H), 1.41 (s, 3H), 1.23 (s, 3H)

To a stirred solution of *N*-(6-benzyloxy-2,2-dimethyl-dihydro-[1,3]dioxolo[4,5-*c*]pyran-7-ylidene)-*N'*-(4-toluenesulfonyl)-hydrazine (770 mg, 1.72 mmol) in a 1:1 mixture of THF (10 mL) and MeOH (10 mL), a trace of methyl orange and sodium cyanoborohydride (108 mg, 1.72 mmol) were added, and then methanolic HCl (satd) was added dropwise keeping the color of the solution reddish yellow transition-point. The mixture was stirred at rt for 1h. A second portion of sodium cyanoborohydride (108 mg, 1.72 mmol) was added, followed by dropwise addition of methanolic HCl to maintain the pH at 3.8. The reaction mixture was stirred for another 1 h, and then neutralized with satd NaHCO_3 . The mixture was evaporated to dryness, dissolved in water, and extracted three times with CH_2Cl_2 . The combined organic layers were washed with brine, dried over MgSO_4 , filtered, and evaporated to give a yellow syrup, which was purified by column chromatography on silica gel (3:1 hexane-EtOAc) to give *N*-(6-benzyloxy-2,2-dimethyl-dihydro-[1,3]dioxolo[4,5-*c*]pyran-7-yl)-*N'*-(4-toluenesulfonyl)-hydrazine **4j** (256 mg, 0.57 mmol, 33% yield) and unreacted starting material (the major double bond isomer, 356 mg, 0.80 mmol, 46 % yield) : For compound **4j** $[\alpha]_D^{25}$ 95.5 ° (c 3.6, CHCl_3); ^1H

NMR (CDCl₃) δ 7.99 (d, J = 8.3 Hz, 2H), 7.61~7.50 (m, 5H), 7.45 (d, J = 8.3 Hz, 2H), 6.79 (s, 1H), 5.00 (d, J = 11.8 Hz, 1H), 4.89 (d, J = 6.9 Hz, 1H), 4.72 (d, J = 11.8 Hz, 1H), 4.63 (dd, J = 7.2 Hz, 3.3 Hz, 1H), 4.37 (dt, J = 7.2 Hz, 2.7 Hz, 1H), 4.17 (br, 1H), 3.89 (dd, J = 12.8 Hz, 2.9 Hz, 1H), 3.79 (dd, J = 12.8 Hz, 2.6 Hz, 1H), 3.03 (br, 1H), 2.62 (s, 3H), 1.63 (s, 3H), 1.50 (s, 3H); ¹³C NMR (CDCl₃) δ 143.95, 137.08, 134.76, 129.49, 128.52, 128.23, 127.91, 109.70, 98.73, 72.80, 71.91, 69.33, 62.32, 58.27, 26.35, 24.94, 21.52; HRFABMS (m/z) obsd 449.1764, calcd for C₂₂H₂₉N₂O₆S 449.1746 (M + H)⁺; Anal. Calcd for C₂₂H₂₈N₂O₆S: C, 58.91; H, 6.29; N, 6.25. Found: C, 59.17; H, 6.39; N, 6.22; for the recovered starting material ; $[\alpha]_D^{26}$ 199.7 ° (c 4.5, CHCl₃); ¹H NMR (CDCl₃) δ 9.87 (s, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.29~7.19 (m, 7H), 5.06 (s, 1H), 4.83 (d, J = 5.6 Hz, 1H), 4.58 (d, J = 11.5 Hz, 1H), 4.46 (d, J = 11.5 Hz, 1H), 4.15 (d, J = 5.5 Hz, 1H), 4.05 (m, 1H), 3.90 (d, J = 13.4 Hz, 1H), 2.33 (s, 3H), 1.26 (s, 3H), 0.99 (s, 3H); ¹³C NMR (CDCl₃) δ 145.09, 144.03, 136.58, 135.49, 129.46, 128.38, 127.93, 127.89, 127.66, 110.51, 98.14, 73.72, 71.28, 69.35, 57.44, 26.30, 25.87, 21.47

CHAPTER 4

STEREOSELECTIVE SYNTHESIS AND ANTIVIRAL ACTIVITY OF D-2',3'- DIDEHYDRO-2',3'-DIDEOXY-2'-FLUORO-4'-THIONUCLEOSIDES¹

¹Chong, Y., H. Choo, Y. Choi, J. Matthew, R. F. Schinazi and C. K. Chu. 2002. *J. Med. Chem.* 45: 4888-4898.

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Introduction

Nucleoside analogue reverse transcriptase inhibitors continue to be important drug regimens as part of highly active antiretroviral therapy (HAART). However, because of the drug toxicity^{170,171} and the lack of a durable response due to resistance,¹⁷² there is clearly a need for new compounds to cope with these drawbacks. Particularly, compounds with activity against HIV isolates that are resistant to currently available therapies as well as agents with beneficial pharmacokinetic profiles that allow infrequent dosing are under active investigation.

The past decade has witnessed the emergence of a class of 2',3'-unsaturated nucleosides such as d4T,^{173,174} L-d4C,^{175,176} L-d4FC^{175,176} and abacavir^{177,178} as interesting therapeutic candidates for anti-HIV therapy because of their potent antiviral activity. The importance of this class of compounds has been confirmed after a recent study which showed that the instability of the purine analogues can be remarkably improved by the isosteric replacement of 2'-hydrogen by fluorine atom.¹⁷⁹ Therefore agents containing the 2',3'-unsaturated sugar moiety with 2'-fluoro substitution has become one of the rational targets in search for safe, effective and chemically stable antiviral agents. Thus, it was of interest to synthesize the nucleosides with isosteric replacement of 4'-oxygen by sulfur atom. The first example of 4'-thionucleosides was reported in 1964 by Reist et al., who synthesized the 4'-thio counterpart of naturally occurring adenosine.¹⁸⁰ Since then, several classes of 4'-thionucleosides have been reported,¹⁸¹ including 9-(4-thio-D-xylofuranosyl)adenine,¹⁸² 9-(4-thio-D-arabinofuranosyl)adenine¹⁸² and 4'-thio-araC.¹⁸³ However, the difficulty of synthesizing optically pure 4'-thionucleosides has impaired

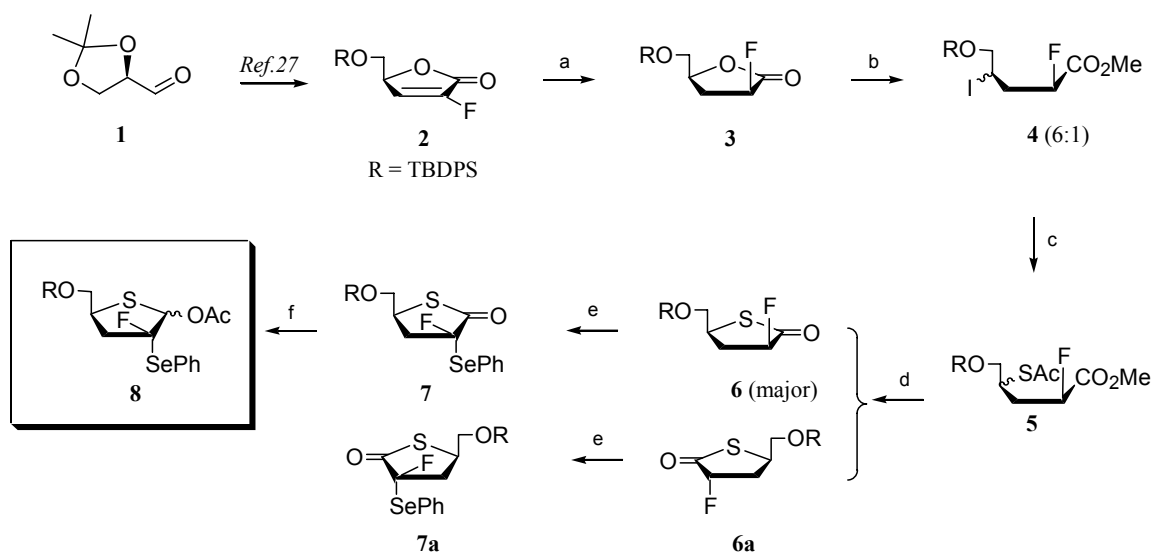
additional syntheses of these analogues, and only a few examples have been known until recently.¹⁸⁴ Moreover, biologically interesting 2'-deoxy-4'-thionucleosides had not been synthesized until Walker¹⁸⁵ and Secrist¹⁸⁶ independently reported the syntheses of pyrimidine 2'-deoxy-4'-thionucleosides in 1991, which were followed by an alternative synthesis of 2'-deoxy-4'-thionucleosides using the Sharpless asymmetric epoxidation,¹⁸⁷ synthesis of 4'-thio-2',3'-dideoxynucleosides,¹⁸⁸ and the syntheses of 4'-thioarabinonucleosides¹⁸⁹ as well as 2'-modified 2'-deoxy-4'-thiocytidines.¹⁹⁰ The synthesized 4'-thio-2'-deoxy, 4'-thio-2',3'-dideoxy and 4'-thioarabino nucleosides have shown to have potent anti-herpes, anti-HIV and anti-cytomegalovirus activities, respectively, and some analogues, especially 4'-thiothymidine and 2'-deoxy-4'-thiocytidine, have exhibited potent cytotoxicity.¹⁸⁵⁻¹⁸⁸ Interestingly, the 4'-thionucleosides are usually resistant to hydrolytic cleavage of glycosyl linkage catalyzed by nucleoside phosphorylase,¹⁹¹ which is one of the advantages of 4'-thionucleosides compared with several metabolically unstable "4'-oxy" antiviral agents which are substrates for nucleoside phosphorylase.¹⁹² Even though 4'-thionucleosides have recently received considerable attention as potential antiviral agents, their 2',3'-unsaturated analogues have not been well investigated probably because of the synthetic difficulties. A report by Young et al., however, reemphasized the importance of this class of nucleosides, in which L-4'-thio-d4C analogues showed marked anti-HBV as well and anti-HIV activity.¹⁹³ In a recent communication, we reported the stereoselective synthesis of the β -L-2'-F-4'-Sd4C which showed potent anti-HIV activity (EC_{50} 0.12 μ M) in human peripheral blood mononuclear (PBM) cells.¹⁹⁴ By using the same synthetic strategy, a series of D-counterparts were synthesized for the structure-activity relationship

studies. Various pyrimidine and purine nucleosides were synthesized from the key intermediate, (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranoside **8**, which was prepared from the 2,3-*O*-isopropylidene-D-glyceraldehyde **1** in 13 steps. The antiviral activities of the synthesized compounds were evaluated against HIV-1 in human PBM cells. The antiviral activity of cytidine **17** and 5-fluorocytidine **18** analogues were also examined against the clinically important lamivudine-resistant variants (HIV-1_{M184V}). In order to understand the reduced antiviral activity of the synthesized β -D-2'-F-4'-Sd4N compared with β -D-2'-Fd4N¹⁷⁹ and the cross-resistance of the lamivudine-resistant variants (HIV-1_{M184V}) to β -D-2'-F-4'-Sd4C (**17**) and β -D-2'-F-4'-Sd4FC (**18**), molecular modeling studies including a quantum mechanical geometry optimization of β -D-2'-F-4'-Sd4C (**17**) as well as energy-minimization of HIV-1 RT/ β -D-2'-F-4'-Sd4C (**17**) complex were performed. Herein, we report the full accounts of the synthesis, biological evaluation and molecular modeling studies of the titled nucleosides.

Results and Discussion

Chemistry. Introduction of a sulfur atom at the 4'-position of a sugar ring has been hampered by the laborious synthetic procedures. The synthetic methods known to date employ either nucleophilic attack of dimesylate by disulfide anion,^{189,190} ring closure of dithioacetal¹⁸⁹ or reductive cyclization of thioacetic acid ester.¹⁸⁸ 2-Fluoro-2-buten-4-olide **2**, which was used as the key intermediate for the synthesis of 2'-fluoro-2',3'-

unsaturated nucleosides,¹⁷⁹ can also be used for this purpose because the butenolide ring can be converted into the appropriate intermediates (dimesylate, dithioacetal and thioacetic acid ester) for any of those three reactions after some manipulations. The attempted syntheses of these intermediates from the 2-fluoro-2-buten-4-olide **2**, however, gave very complicated mixtures presumably because of the instability of fluorovinyl moiety. The transformation was possible only after saturation of the butenolide **2** by catalytic hydrogenation. Therefore, 2-fluoro- γ -butyrolactone **3** was used as a platform for the generation of the key intermediate (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenenyl-4-thio- β -D-ribofuranoside **8** (Scheme 4.1).

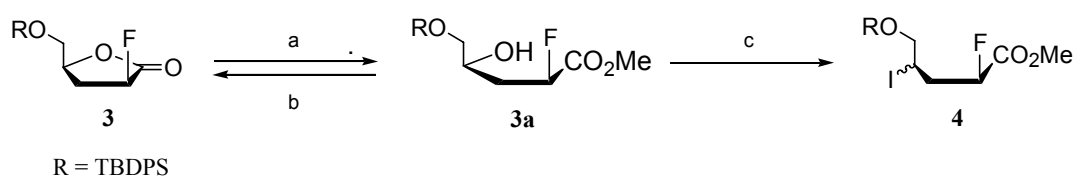


^aKeys: (a) H₂, Pd(0)/C, EtOAc, (b) i) NaOH, EtOH, ii) dimethyl sulfate, DMSO, iii) I₂, Ph₃P, imidazole, toluene, (c) KSAc, DMF, (d) i) DIBAL-H, toluene, -78°C, ii) DMSO, Ac₂O, (e) LiHMDS, TMSCl, PhSeBr, -78°C, (f) i) DIBAL-H, toluene, -78°C, ii) Ac₂O, TEA, CH₂Cl₂

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

(*R*)-2-Fluorobutenolide **2** was prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde **1** in three steps by a known method (Scheme 4.1).¹⁹⁵ (*S*)-2-Fluorobutenolide **2** was

hydrogenated to β -2-fluorolactone **3** by treatment with 5% Pd/C under H₂ in a quantitative yield. The ¹H NMR of the crude compound **3** showed no contamination by the epimer at C2. The lactone **3** was converted to iodoester **4** in three consecutive steps. Hydrolysis using NaOH in aqueous EtOH followed by methylation of the corresponding carboxylic acid gave hydroxy methylester **3a** (Scheme 4.2), which was treated with iodine, triphenylphosphine, and imidazole in toluene at 60°C for 4 h to give a 6:1 epimeric mixture of the iodoester **4** in 83% overall yield (Scheme 4.1). In order to confirm which carbon center has epimerized, the hydroxyl methylester **3a** was subjected to reclosure of the ring to give the starting 2-fluorobutenolide **3** under basic conditions (Scheme 4.2). The spectroscopic data (¹H and ¹³C NMR) of this compound matched that of the starting compound, which suggested that there was no significant epimerization during saponification followed by methylation. Iodination under several different reaction conditions, however, confirmed that high temperature and longer reaction time resulted in partial epimerization at C4. Treatment of the hydroxyl methylester **3a** under Mitsunobu conditions at 60°C for 4 h was found to be the optimum conditions to minimize the epimerization.

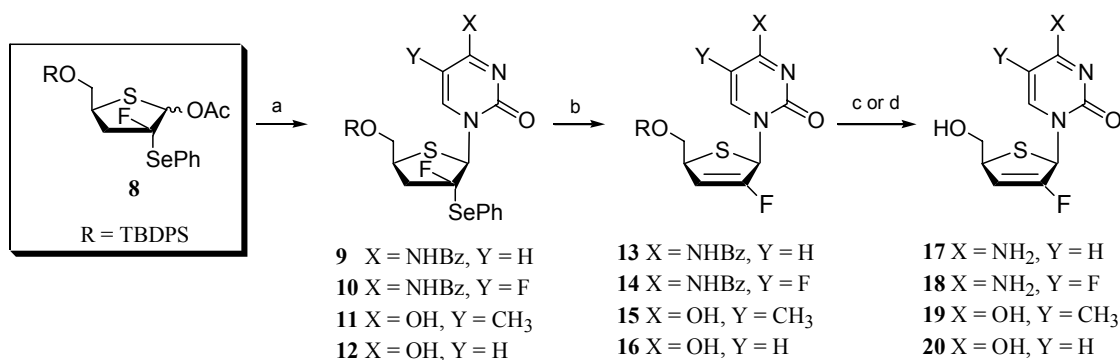


^aKeys: (a) i) NaOH, EtOH, ii) dimethyl sulfate, DMSO, (b) pyridine, rt, c) I₂, Ph₃P, imidazole, toluene

Scheme 4.2. Epimerization at C4 during iodination

The epimeric mixture of iodoester **4** was subjected to nucleophilic attack by potassium thioacetate in DMF to give an epimeric mixture of thioacetates **5**. DIBAL-mediated

reduction of the thioacetates **5** followed by Moffat-type oxidation provided the corresponding thiolactone **6** and **6a**, in 54% yield. At this stage, the two epimers could be separated by column chromatography on silica gel. During the ensuing phenylselenylation, the C2 position was sp^2 -hybridized by trapping the enolate as silylenolether. The phenylselenenyl group approached the least hindered α face of the silylenolether to give the corresponding enantiomers **7** and **7a** (Scheme 4.1). The optical rotation values of these two compounds were matched with opposite signs {for **7**; $[\alpha]_D^{24}$ 54.0° (c 0.606, CHCl₃) and for **7a**; $[\alpha]_D^{24}$ -56.4° (c 0.542, CHCl₃)}, which finally confirmed the epimerization at C4 during iodination under Mitsunobu conditions. The epimerized product **7a**, therefore, can be recycled as the key intermediate for the synthesis of L-antipodes. DIBAL reduction of the 2-fluoro-2-phenylselenothiolactone **7** followed by acetylation using Ac₂O and triethylamine in CH₂Cl₂ gave the key intermediate (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranoside **8** in 90% yield (Scheme 4.1). Condensation of **8** with various pyrimidine heterocyclic bases under Vorbrüggen conditions gave the corresponding pyrimidine analogues **9** ~ **12** in 40~60% yield (Scheme 4.3). The condensation gave the β -anomer exclusively by virtue of the bulky α -phenylselenenyl group.¹⁹⁶ Oxidation of the phenylselenide group by mCPBA at low temperature followed by treatment with pyridine gave the corresponding *syn*-eliminated 2',3'-unsaturated compounds **13** ~ **16** in high yields (70~80%) (Scheme 4.3). The protecting groups on the heterocyclic base and the 5' position of the sugar moiety were sequentially deblocked by the treatment with TBAF in THF and methanolic ammonia to give the desired 2'-fluoro-4'-thio-2',3'-unsaturated pyrimidine nucleosides **17** ~ **20** (Scheme 4.3).

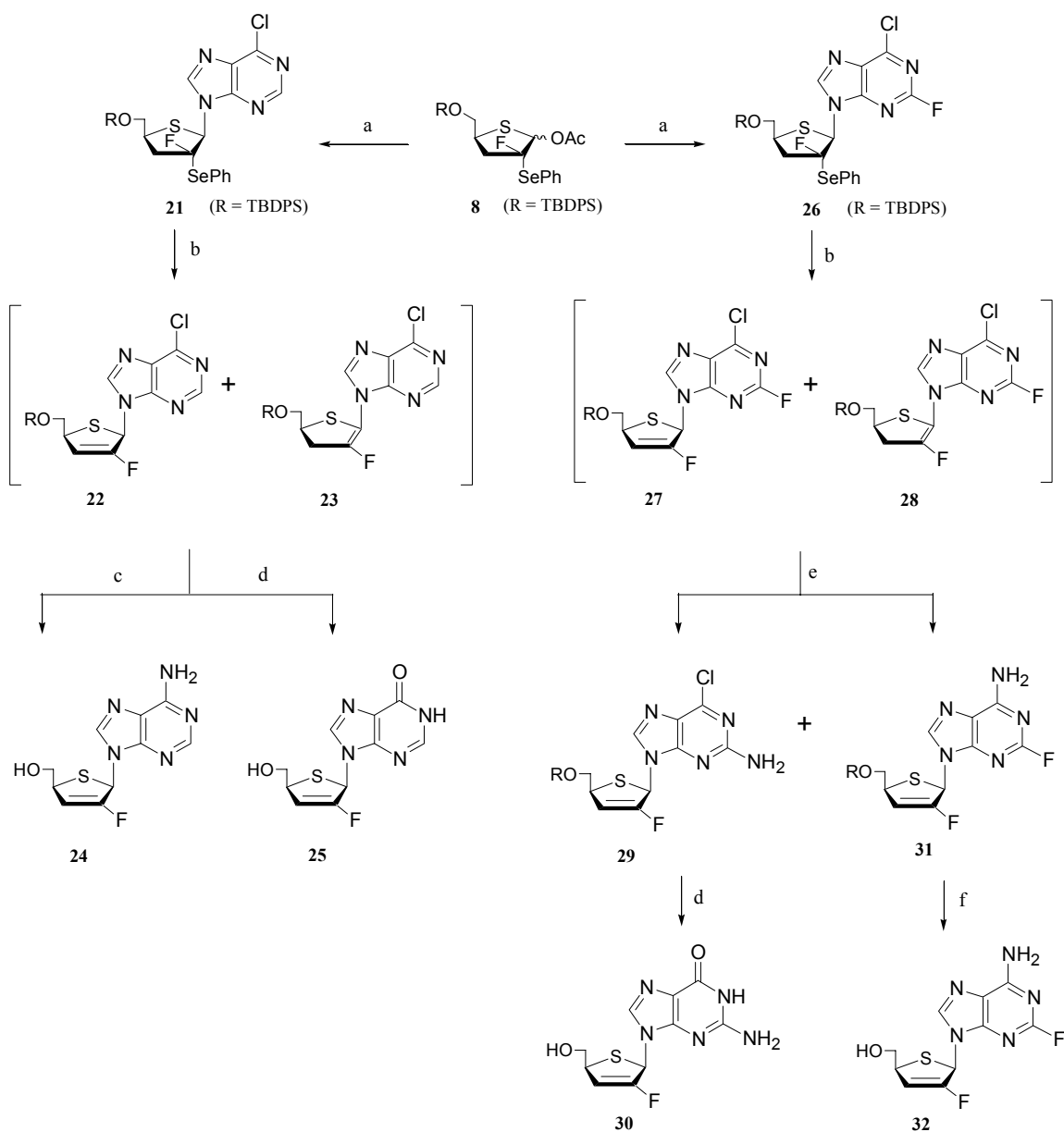


^aKeys: (a) HMDS, CH_3CN , pyrimidines; TMSOTf, (b) mCPBA, -78°C ; pyridine, rt, (c) i) TBAF, THF, ii) NH_3 , MeOH, rt, (d) TBAF, THF

Scheme 4.3. Synthesis of the pyrimidine nucleosides **17~20a**

The synthesis of purine analogues (**24**, **25**, **30** and **32**) needed more careful treatments (Scheme 4.4). The key intermediate **8** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give the corresponding nucleosides **21** and **26** in 65% and 66% yield, respectively. To achieve a clean and high-yield conversion, the temperature of the reaction mixture had to be carefully controlled in such a manner that, after addition of TMSOTf at 0°C , the reaction mixture was stirred for 6 h at rt and then for 2 h at 60°C . The 6-chloropurine derivative **21** was *syn*-eliminated by successive treatment with mCPBA and pyridine to give an inseparable mixture of 2',3'-unsaturated nucleoside **22** and its $\Delta^{1,2}$ -isomer **23** in 81% yield (3:1 determined by ^1H NMR). Amination of the mixture of **22** and **23** by treatment with methanolic ammonia at 100°C in a steel bomb gave the adenosine analogue, which was deprotected by TBAF in THF to give 2'-fluoro-4'-thio-2',3'-unsaturated adenosine **24** in 61% yield. The $\Delta^{1,2}$ -isomer **23**, however, did not survive under reaction conditions and silica gel column chromatography due to its instability. On the other hand, the 2',3'-unsaturated 6-chloropurine derivative **22** was hydrolyzed in the presence of sodium methoxide and 2-mercaptoethanol in refluxing

methanol to give the hypoxanthine derivative, which was converted to the desired 2'-fluoro-4'-thio-2',3'-unsaturated inosine **25** in 46% yield (yield is not optimized).



^aKeys: (a) HMDS, (NH₄)₂SO₄, purines; TMSOTf, (b) mCPBA, -78°C; pyridine, rt, (c) i) NH₃, MeOH, 80°C, ii) TBAF, THF, (d) i) HSCH₂CH₂OH, NaOH, 60°C, ii) TBAF, THF, (e) NH₃ bubbling, DME, rt, (f) TBAF, THF

Scheme 4.4. Synthesis of the purine nucleosides **24~25**, **30** and **32a**

The 2-fluoro-6-chloropurine derivative **26** was also treated with mCPBA followed by pyridine to give an inseparable mixture of the *syn*-eliminated product **27** and its $\Delta^{1,2}$ -isomer **28** in 71% yield. Dry ammonia gas was bubbled into a solution of **27** and **28** in ethylene glycol dimethyl ether (DME) at room temperature for 16 h to give the 2-amino-6-chloropurine derivative **29** and 2-fluoro-6-aminopurine derivative **31** in 45% and 25% yield, respectively, which were readily separated by silica gel column chromatography. Unfortunately, the $\Delta^{1,2}$ -isomer **28** was not stable enough to survive the reaction and purification conditions. The 2-amino-6-chloropurine derivative **29**, thus obtained, was hydrolyzed by using sodium methoxide and 2-mercaptoethanol in refluxing methanol to give the final 2'-fluoro-4'-thio-2',3'-unsaturated guanosine **30** in 61% yield. The 2-fluoro-6-aminopurine derivative **31**, on the other hand, was deprotected by TBAF in THF to afford the 2'-fluoro-4'-thio-2',3'-unsaturated 2-fluoroadenosine **32** in 70% yield.

Structure-Activity Relationships. The synthesized pyrimidine (**17~20**) and purine (**24**, **25**, **30** and **32**) nucleosides were evaluated against HIV-1 in human PBM cells *in vitro*, and AZT was included as a positive control. The results are summarized in Table 4.1. Among the tested nucleosides, two pyrimidine nucleosides, cytidine **17** (EC₅₀ 1.3 μ M) and 5-fluorocytidine **18** (EC₅₀ 11.6 μ M), and all purine nucleosides synthesized, adenosine **24** (EC₅₀ 8.1 μ M), inosine **25** (EC₅₀ 43.6 μ M), guanosine **30** (EC₅₀ 80.5 μ M) and 2-fluoroadenosine **32** (EC₅₀ 1.2 μ M), showed moderate to potent antiviral activities. It is noteworthy that 2-fluoroadenosine analogue **32** showed antiviral potency as well as high cytotoxicity (IC₅₀ 1.5, 1.1 and 7.6 μ M for PBM, CEM and Vero, respectively) whereas no other compound showed cytotoxicity up to 100 μ M. Compared with the β -D-2'-Fd4N nucleosides,¹⁷⁹ there was a general trend of antiviral activities throughout the

series, where cytidine, 5-fluorocytidine and adenosine analogues were most potent and almost all purine nucleosides showed moderate to potent activities. However, the β -D-2'-Fd4N nucleosides¹⁷⁹ were consistently more potent than their 4'-thio congeners, which suggests that the two types of nucleosides may have similar structural features and may be recognized in the same fashion at the kinase level, but the way they interact with the viral polymerase (HIV-1 reverse transcriptase) must be somewhat different (*vide infra* for molecular modeling studies).

Table 4.1. Anti-HIV activity and cytotoxicity of D-2',3'-didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides

Compound	Activity (EC ₅₀ , μ M)	Cytotoxicity (IC ₅₀ , μ M)		
	HIV-1	PBM	CEM	Vero
17	1.3	>100	>100	>100
18	11.6	>100	>100	>100
19	92.3	>100	>100	53.0
20	>100	>100	>100	>100
24	8.1	>100	>100	>100
25	43.6	>100	>100	>100
30	80.5	>100	>100	>100
32	1.2	1.5	1.1	7.6
AZT	0.004	>100	14.3	28.0

Antiviral activity against lamivudine-resistant (HIV-1_{M184V}) mutant strain.

Lamivudine (3TC, (-)- β -2',3'-dideoxy-3'-thiacytidine) is an important component of the highly active antiretroviral therapy (HAART). However, single mutation at residue 184 (M184V) of the reverse transcriptase (RT) in HIV causes high-level resistance to 3TC and contribute to the failure of anti-AIDS combination therapy.^{197,198} Therefore, there is an urgent need to discover new drug candidates active against the M184V mutant HIV-1 RT. The cytosine (**17**) and 5-fluorocytosine analogues (**18**), along with two positive controls, AZT and 3TC, were evaluated against the lamivudine-resistant mutant strain (HIV-1_{M184V}) in human PBM cells *in vitro* (Table 4.2).

Table 4.2. Activity of selected nucleosides against lamivudine-resistant virus (HIV-1_{M184V}) in human PBM cells.

Compound	xxBRU (EC ₉₀ , μ M)	M184V (EC ₉₀ , μ M)	FI ^a
17	5.0	$\approx$ 125	25
β -D-2'-F-d4FC ^b	9.9	34.7	3.5
AZT	0.01	0.003	0.3
3TC	0.08	535	6,688

^a FI is the fold increase (EC₉₀ HIV-1_{M184V}/ EC₉₀ HIV-1_{xxBRU})

^b Ref. 11(b) : Lee et al. *J. Med. Chem.* **2002**, 45, 1313~1320

Compared with the 4'-oxygen congeners such as β -D-2'-Fd4FC,¹⁷⁹ the β -D-2'-F-4'-Sd4C (**17**) showed significantly reduced anti-HIV activity against HIV-1_{M184V} (Table 4.2),

which suggests the isosteric substitution of 4'-oxygen with 4'-sulfur made the β -D-2'-F-4'-Sd4N cross-resistant to the M184V mutation in HIV-1 RT (*vide infra* for molecular modeling studies).

Molecular Modeling. Since the conformational difference between the nucleoside analogues is one of the critical factors in determining their antiviral activity, it was of interest to compare the conformations of β -D-2'-Fd4N and the corresponding 4'-thio congeners. For this purpose, a quantum mechanical calculation (RHF/3-21G*) was performed on cytidine analogues (β -D-2'-Fd4C¹⁷⁹ and β -D-2'-F-4'-Sd4C **17**), and the geometry-optimized structures were compared (Figure 4.1-a). The two structures were superimposable except for the 4'-positions; the strain inside the 4'-thio sugar ring caused by the longer C-S bond length resulted in moving the 4'-sulfur atom by 0.5Å down to the plane formed by C1', C2', C3' and C4'. Another reason for this out-of-plane moving the 4'-sulfur atom is that, unlike 4'-oxygen, the 4'-sulfur atom cannot form a hydrogen bond with 5'-OH due to the lack of the gauche effect. However, the critically important configurations of the N1 and C5' atoms of a nucleoside are in good accordance, which may imply the similar tolerance to the two types of nucleosides by the kinases. On the other hand, when the triphosphates of β -D-2'-Fd4C¹⁷⁹ and β -D-2'-F-4'-Sd4C **17** bind to the active site of HIV-1 reverse transcriptase,¹⁹⁹ the isosteric replacement of 4'-oxygen with 4'-sulfur atom exerts effects on the interaction between the nucleoside triphosphates and the active site residues, particularly Tyr115 and Met184 (Figure 4.1-b and 1-c). The energy-minimized structures²⁰⁰ of β -D-2'-Fd4C and β -D-2'-F-4'-Sd4C **17** bound to HIV-1 RT shows that nucleoside inhibitors are located in a well-defined binding pocket formed by Arg72, Met184 and 3'-OH pocket residues (Asp113, Tyr115, Phe116 and

Gln151) (Figure 4.1-b). The role of Arg72 is notable because, as it moves into the binding pocket, it stabilizes the bound nucleotide through hydrogen bonding with the triphosphate moiety as well as nonspecific hydrophobic interaction with the heterocyclic moiety of the nucleoside triphosphate (Figure 4.1-b).

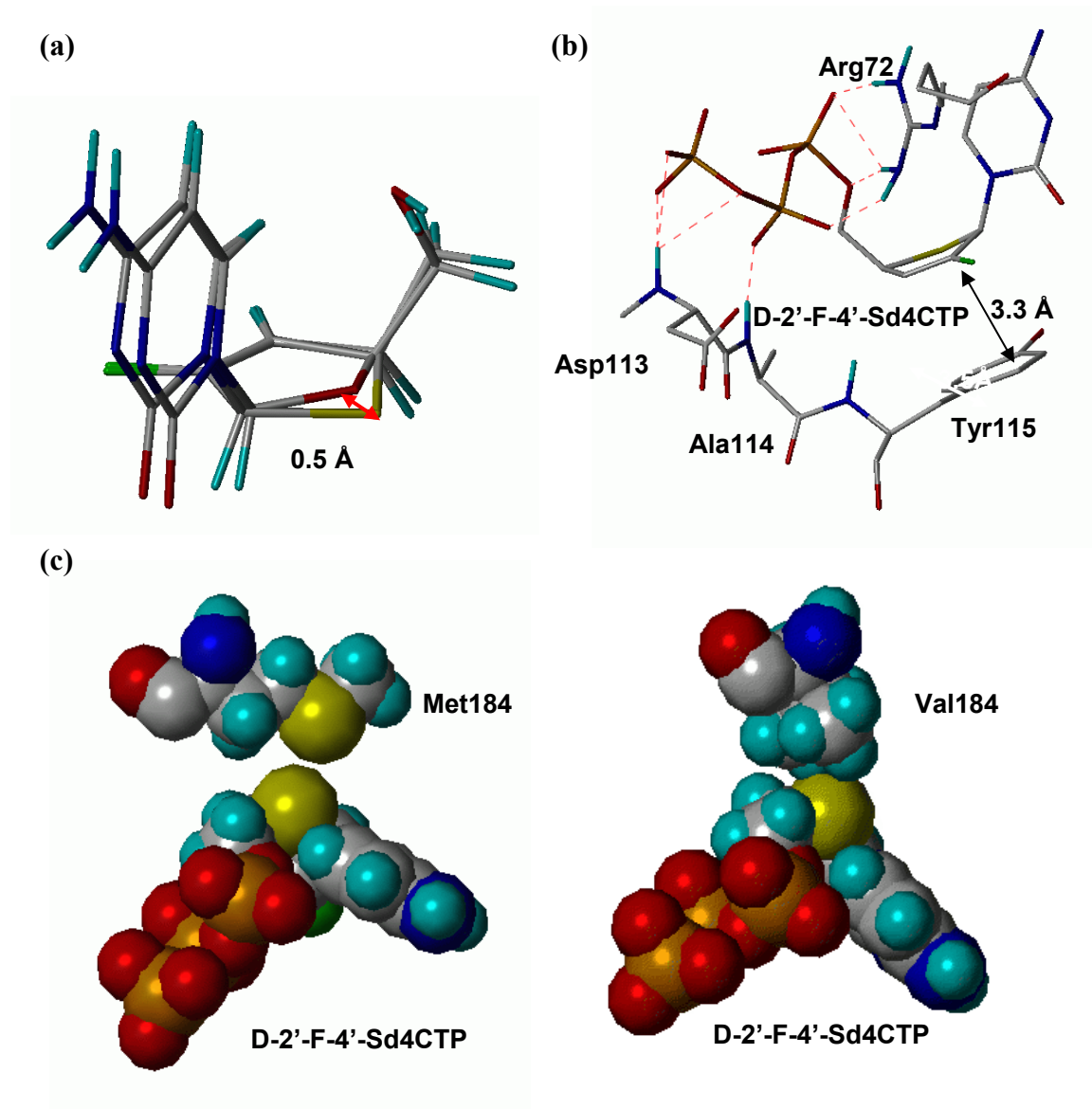


Figure 4.1. (a) Superimposed, geometry-optimized structures of D-2'-F-4'-Sd4C (**17**) and D-2'-Fd4C. (b) Binding mode of D-2'-F-4'-Sd4C (**17**) to the active site of HIV-1 RT. (c) Energy minimized structure of D-2'-F-4'-Sd4C (**17**), complexed with the wild-type HIV-1 RT (left) and M184V mutant RT (right).

An additional characteristic binding mode of these d4-nucleotides is the possible π - π interaction²⁰¹ between C2'-C3' fluorovinyl moiety of the nucleotide and the aromatic ring of nearby Tyr115, which could be one reason for the high antiviral activity of 2',3'-unsaturated nucleosides¹⁷³⁻¹⁷⁸ (Figure 4.1-b). Fluorine substitution at C2' position would make the C2'-C3' double bond electron poor and, thus, increase the π - π interaction with the electron-rich aromatic ring of Tyr115. In spite of almost the same binding mode of β -D-2'-Fd4C and β -D-2'-F-4'-Sd4C **17**, the decreased antiviral activity of β -D-2'-F-4'-Sd4C can be explained by the decrease in π - π interaction. The replacement of electronegative oxygen by sulfur depolarizes charges inside the sugar ring in β -D-2'-F-4'-Sd4C **17**, which may result in reduced π - π interaction between the fluorovinyl moiety and aromatic ring of Tyr115. Because of its out-of-plane location and large van der Waals radius, the 4'-sulfur atom in the β -D-2'-F-4'-Sd4N sugar moiety is in close contact to Met184 (Figure 4.1-c). As a result, the mutation of Met184 to Val184, which has a bulky side chain, results in a significant steric hindrance between the 4'-sulfur atom in the β -D-2'-F-4'-Sd4N sugar moiety and the side chain of Val184, which might be one of the reasons for the high cross-resistance of HIV-1_{M184V} to β -D-2'-F-4'-Sd4C (**17**) and β -D-2'-F-4'-Sd4FC (**18**) (Table 4.2, Figure 4.1-c).

Metabolic Stability. In an attempt to understand metabolic stability of the adenosine analogue **24**, adenosine deaminase (ADA) binding efficiencies and deamination kinetics studies were performed.²⁰² As shown in Table 4.3, the adenine derivative **24** was found to be tightly bound ($K_M = 18.3 \mu\text{M}$) to the mammalian adenosine deaminase (from calf intestinal mucosa, EC.3.5.4.4), but the turnover number ($k_{cat} = 0.94 \text{ s}^{-1}$) was very low, which indicates that even though the adenine derivative **24** can strongly bind to ADA, it

can barely be catalyzed by ADA to the corresponding inosine derivative. It is noteworthy that, compared with other substrates of ADA, β -D-2'-F-4'-Sd4A **24** has very low K_M and k_{cat} values, and the catalytic efficiency (k_{cat}/K_M) is as low as that of β -FddA.²⁰³ Metabolic stability of adenosine analogue **24** has important implications because adenosine deaminase is one of the major deactivating enzymes of the purine catabolism pathway.²⁰⁴ In summary, we have developed an efficient synthesis of optically pure β -D-2'-F-4'-Sd4 nucleosides. The isosteric replacement of 4'-oxygen in β -D-2'-Fd4 nucleosides with 4'-sulfur caused substantial changes in the binding mode of β -D-2'-F-4'-Sd4 to the active site of HIV-1 RT, resulting in reduced *in vitro* anti-HIV-1 activity. The lamivudine-resistant mutant strain (HIV-1_{M184V}) showed significant cross-resistance to the β -D-2'-F-4'-Sd4 nucleosides, which could be explained by the larger van der Waals radius as well as the close proximity to Met184 of the 4'-sulfur atom.

Table 4.3. Kinetic Constants for Adenosine Analogues as Substrates of ADA.^a

Compound	K_M (μ M)	k_{cat} (s^{-1})	k_{cat}/K_M (μ M ⁻¹ s ⁻¹)	$t_{1/2}$ (min)
D-2'-F-4'-Sd4A (24)	18.3	0.94	0.05	33
D-2'-F-d4A ²⁸	23.0	NA ^b	NA ^b	42
Adenosine	24.5	76.4	3.1	0.5

^a Kinetic data were obtained spectrophotometrically at 25°C by following the decrease in absorbance at 265 nm.

^b Not Available.

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 ^1H NMR and 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. 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. High resolution 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*,4*S*)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro- γ -butyrolactone (3). To a solution of lactone **2** (14.8 g, 39.9 mmol) in 200 mL of EtOAc, 2.0 g of Pd/C (5% w/w) under H_2 atmosphere was added and the mixture was stirred for 3 h. After filtration of the reaction mixture through a celite pad, the filtrate was concentrated, purified by silica gel column chromatography with 5% EtOAc in hexanes to give compound **3** (13.9 g, 37.5 mmol, 94 % yield) as a white solid: mp 87~89°C; $[\alpha]_D^{24}$ 15.5° (c 0.85, CHCl_3); ^1H NMR (CDCl_3) δ 7.70~7.35 (m, 10H), 5.26 (dt, J = 51.2, 8.8 Hz, 1H), 4.54~4.47 (m, 1H), 3.93 (dd, J = 11.4, 2.3 Hz, 1H), 3.71 (dd, J = 11.4, 3.6 Hz, 1H), 2.65 (ddt, J = 13.1, 8.4, 6.6 Hz, 1H), 2.54 (ddt, J = 24.2, 13.2, 9.0 Hz, 1H), 1.04 (s, 9H); ^{13}C NMR (CDCl_3) δ 171.30 (d, J

= 21.3 Hz), 135.59, 135.30, 132.52, 132.20, 129.76, 129.35, 127.82, 127.63, 89.30 (d, J = 191.0 Hz), 76.24 (d, J = 6.1 Hz), 63.73, 30.14 (d, J = 20.0 Hz), 26.58, 19.12; Anal. ($C_{21}H_{25}FO_3Si$) C, H.

(2*S*,4*S*/*R*)-5-*tert*-Butyldiphenylsilyloxy-2-fluoro-4-iodopentanoic acid methyl ester

(4). A mixture of compound **3** (7.49 g, 20.1 mmol) in 5% aqueous EtOH was treated with solid NaOH (0.885 g, 22.1 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 (2.29 mL, 24.2 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 (7.65 g, 30.1 mmol), imidazole (4.11 g, 60.4 mmol), and Ph_3P (10.55 g, 40.2 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** (7.52 g, 14.6 mmol, 73% yield) as pale yellow oil: ^1H NMR ($CDCl_3$) for major δ 7.71~7.33 (m, 10H), 5.08 (ddd, J = 47.9, 7.2, 5.0 Hz, 1H), 4.31~4.17 (m, 1H), 3.96~3.76 (m, 2H), 3.79 (s, 3H) 2.75~2.08 (m, 2H), 1.10 (s, 9H), for minor δ 7.71~7.33 (m, 10H), 5.16 (ddd, J = 48.2, 10.3, 2.1 Hz, 1H), 4.31~4.17 (m, 1H),

3.96~3.76 (m 2H), 3.81 (s, 3H), 2.75~2.08 (m, 2H), 1.08 (s, 9H); HRMS (FAB) obsd, m/z 515.0945, calcd for $C_{22}H_{29}FIO_3Si$, m/z 515.0915 ($M+H$)⁺; Anal. ($C_{22}H_{28}FIO_3Si \cdot 0.2C_6H_{14}$) C, H.

(2*S*,4*R*/*S*)-4-Acetylsulfanyl-5-*tert*-butyldiphenylsilyloxy-2-fluoropentanoic acid methyl ester (5). A solution of compounds **4** (11.72 g, 22.8 mmol) in 12 mL of DMF was treated with solid KSAc (5.2 g, 45.7 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** (9.2 g, 19.9 mmol, 87% yield) as a red-brown oil: ¹H NMR ($CDCl_3$) for major δ 7.61~7.32 (m, 10H), 5.22~4.88 (m, 1H), 3.89~3.65 (m, 3H), 3.78 (s, 3H), 2.41~2.07 (m, 2H), 2.29 (s, 3H), 1.05 (s, 9H), for major δ 7.61~7.32 (m, 10H), 5.22~4.88 (m, 1H), 3.89~3.65 (m, 3H), 3.78 (s, 3H), 2.41~2.07 (m, 2H), 2.27 (s, 3H), 1.06 (s, 9H); HRMS (FAB) obsd, m/z 463.1770, calcd for $C_{24}H_{32}FO_4SSi$, m/z 463.1775 ($M+H$)⁺; Anal. ($C_{24}H_{31}FO_4SSi$) C, H, S.

(2*S*,4*S*)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro- γ -thiobutyrolactone (6) and (2*S*,4*R*)-(-)-4-*tert*-butyldiphenylsilyloxymethyl-2-fluoro- γ -thiobutyrolactone (6a). A solution of compounds **5** (9.2 g, 19.9 mmol) in toluene (200 mL) was treated with 43.7 mL of 1M DIBAL-H in hexane at -78°C for 1 h. The reaction was quenched with 9.6 mL of MeOH and warmed up to room temperature for 1 h and aq. $NaHCO_3$ (19 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 (19 mL) and DMSO (20 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 (3 x 300 mL).

The combined organic layer was washed with water (3 x 300 mL), dried over MgSO₄, filtered, concentrated and purified by silica gel column chromatography with 5% Et₂O in hexanes to give the product **6** (4.2 g, 10.8 mmol, 54% yield) and **6a** (0.7 g, 1.8 mmol, 9% yield) as yellow oil: For **6**: $[\alpha]_D^{25}$ 28.2° (c 1.08, CHCl₃); ¹H NMR (CDCl₃) δ 7.68~7.37 (m, 10H), 5.07 (ddd, *J* = 50.5, 10.2, 6.9 Hz, 1H), 3.96~3.78 (m, 3H), 2.70~2.62 (m, 1H), 2.18~2.05 (m, 1H), 1.07 (s, 9H); ¹³C NMR (CDCl₃) δ 200.69 (d, *J* = 18.0 Hz), 135.53, 132.68, 132.60, 130.01, 127.86, 93.27 (d, *J* = 197.0 Hz), 66.64, 43.98 (d, *J* = 7.0 Hz), 32.61 (d, *J* = 19.4 Hz), 26.69, 19.23; Anal. (C₂₁H₂₅FO₂SSi) C, H, S, For **6a**: $[\alpha]_D^{27}$ -46.7° (c 0.5, CHCl₃); ¹H NMR (CDCl₃) 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. (C₂₁H₂₅FO₂SSi) C, H, S.

(2*R*,4*S*)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl-γ-

thiobutyrolactone (7). To a solution of compound **6** (4.74 g, 12.2 mmol) in THF (60 mL), 14.7 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 (2.01 mL, 15.9 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 (4.37 g, 18.3 mmol) in THF (20 mL) was rapidly added and the mixture was stirred at -78°C for 1h. The mixture was diluted with ethyl ether (300 mL), washed with water (4X100 mL), dried over MgSO₄, filtered, concentrated and purified by silica gel column chromatography with 3% Et₂O in hexanes to give desired product **7** (4.77 g, 8.76 mmol, 72% yield) as a pale yellow syrup: $[\alpha]_D^{24}$ 54.0° (c 0.606, CHCl₃); ¹H NMR (CDCl₃) δ 7.68~7.35 (m, 15H), 3.96~3.89 (m, 1H), 3.87 (dd, *J* = 10.2, 5.1 Hz, 1H), 3.80 (dd, *J* =

10.1, 7.1 Hz, 1H), 2.49 (dd, $J = 13.4, 4.0$ Hz, 1H), 2.22 (td, $J = 13.5, 10.5$ Hz, 1H), 1.06 (s, 9H); ^{13}C NMR (CDCl_3) δ 196.32 (d, $J = 23.1$ Hz), 137.01, 135.52, 132.63, 132.50, 130.02, 129.99, 129.38, 127.87, 124.78, 105.12 (d, $J = 260.6$ Hz), 65.66, 44.58 (d, $J = 2.9$ Hz), 39.13 (d, $J = 21.4$ Hz), 26.69, 19.20; Anal. ($\text{C}_{27}\text{H}_{29}\text{FO}_2\text{SSeSi}$) C, H, S.

(2*S*,4*R*)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl- γ -

thiobutyrolactone (7a). See the previous procedure for reaction of the compound **7** with phenylselenyl bromide. The title compound **7a** was obtained on 9.69-mmol scale in 74 % yield as a pale yellow syrup: $[\alpha]_D^{24} -56.4^\circ$ (c 0.542, CHCl_3); ^1H 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 $\text{C}_{27}\text{H}_{30}\text{FO}_2\text{SSeSi}$, m/z 545.0885 ($\text{M}+\text{H}^+$); Anal. ($\text{C}_{27}\text{H}_{29}\text{FO}_2\text{SSeSi} \cdot 0.15\text{CHCl}_3$) C, H, S.

(1*R*/5*S*,2*R*,4*S*)-1-*O*-Acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-

phenylselenyl-4-thio- β -D-ribofuranoside (8). A solution of compounds **7** (4.77 g, 8.76 mmol) in toluene (100 mL) was treated with 17.5 mL of 1M DIBAL-H in hexane at -78°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 (2.48 mL, 26.3 mmol), TEA (3.66 mL, 26.3 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% Et₂O in hexanes to give the acetate **8** (4.4 g, 7.48 mmol, 85% yield) as a pale yellow oil: ¹H NMR (CDCl₃) 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. (C₂₉H₂₈FO₃SSeSi) 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-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranosyl]cytosine (9**).** A mixture of *N*⁴-benzoylcytosine (0.505 g, 2.35 mmol) in HMDS (15 mL) and CH₃CN (15 mL) was heated under reflux for 5 h. After removing solvent using vacuum pump, a solution of the acetate **8** (0.460 g, 0.786 mmol) in 15 mL of CH₃CN was added to the reaction flask containing the silylated *N*⁴-benzoylcytosine, then TMSOTf (0.28 mL, 1.4 mmol) was added dropwise at rt. After 16 h, the reaction was quenched with 1 mL of sat. NaHCO₃ and the resulting mixture was concentrated. The crude mixture was diluted with 100 mL of CH₂Cl₂, washed with aq. NaHCO₃, dried over MgSO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography with 30% EtOAc in hexanes to give cytosine derivative **9** (0.269 g, 0.362 mmol, 46 % yield) as a foam: [α]²⁴_D 179.9° (c 0.50, CH₂Cl₂); UV(MeOH) λ_{max} 308 nm; ¹³C NMR (CDCl₃) 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. (C₃₈H₃₈FN₃O₃SSeSi) C, H, N, S.

(+)-*N*⁴-Benzoyl-1-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranosyl]-5-fluorocytosine (10**).** See the general

procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **10** was obtained on 0.850-mmol scale in 52 % yield: $[\alpha]^{24}_{\text{D}}$ 202.6° (c 0.410, CH₂Cl₂); UV(CH₂Cl₂) λ_{max} 332.5 nm; ¹³C NMR (CDCl₃) 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; HRMS (FAB) obsd, m/z 762, calcd for C₃₈H₃₈F₂N₃O₃SSeSi, m/z 762.15 (M+H)⁺; Anal. (C₃₈H₃₇F₂N₃O₃SSeSi) C, H, N, S.

(+)-1-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranosyl]thymine (11**).** See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **11** was obtained on 0.850-mmol scale in 45 % yield: $[\alpha]^{25}_{\text{D}}$ 137.8° (c 0.42, CH₂Cl₂); UV(CH₂Cl₂) λ_{max} (CH₂Cl₂) 266.0 nm; ¹³C NMR (CDCl₃) 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; HRMS (FAB) obsd, m/z 655, calcd for C₃₂H₃₆FN₂O₃SSeSi, m/z 655.1287 (M+H)⁺; Anal. (C₃₂H₃₅FN₂O₃SSeSi·0.4H₂O) C, H, N, S.

(+)-1-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranosyl]uracil (12**).** See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **12** was obtained on 0.766-mmol scale in 53 % yield: $[\alpha]^{23}_{\text{D}}$ 136.5° (c 0.36, CH₂Cl₂); UV(CH₂Cl₂) λ_{max} 262.5 nm; ¹³C 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.

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-[(1*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]cytosine (13**).** To a solution of compound **9** (0.229 g, 0.310 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 $-78^\circ C$ and the mixture was stirred at $-78^\circ C$ 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 40% EtOAc in hexanes to give eliminated product **13** (0.107 g, 0.225 mmol, 72 % yield) as a foam: $[\alpha]_D^{25} -140.2^\circ$ (c 0.366, 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. ($C_{32}H_{32}FN_3O_3SSi$) C, H, N, S.

(-)- N^4 -Benzoyl-1-[(1*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-5-fluorocytosine (14**).** See the general procedure for *syn*-elimination reaction. The title compound **14** was obtained on 0.465-mmol scale in 69 % yield: $[\alpha]_D^{25} -150.5^\circ$ (c 0.345, 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; FAB-MS obsd, m/z 604, calcd for $C_{32}H_{32}FN_3O_3SSi$, m/z 604.1823 ($M+H$)⁺; Anal. ($C_{32}H_{31}F_2N_3O_3SSi$) C, H, N, S.

(-)-1-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]thymine (15). See the general procedure for *syn*-elimination reaction. The title compound **15** was obtained on 0.375-mmol scale in 69 % yield: $[\alpha]^{26}_D -43.2^\circ$ (c 0.34, 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; FAB-MS obsd, m/z 497, calcd for $C_{26}H_{30}FN_2O_3SSi$, m/z 497.1652 ($M+H$)⁺; Anal. ($C_{26}H_{29}FN_2O_3SSi \cdot 0.6H_2O$) C, H, N, S.

(-)-1-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]uracil (16). See the general procedure for *syn*-elimination reaction. The title compound **16** was obtained on 0.391-mmol scale in 58 % yield: $[\alpha]^{25}_D -72.1^\circ$ (c 0.35, 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; FAB-MS obsd, m/z 483, calcd for $C_{25}H_{28}FN_2O_3SSi$, m/z 483.1496 ($M+H$)⁺; Anal. ($C_{25}H_{27}FN_2O_3SSi$) C, H, N, S.

General procedure for two successive deprotections of protected unsaturated nucleosides. This is representative of the preparation of cytidine analogs **17** and **18**.

(-)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]cytosine (17). A solution of the protected cytidine **13** (0.135 g, 0.225 mmol) in 15 mL of THF was

treated with 0.31 mL of 1M TBAF in THF for 2 h. The mixture was concentrated, and filtered through a short pad of silica gel. After the filtrate was concentrated, 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 CH₂Cl₂ to give cytidine analog **17** (0.048 g, 0.186 mmol, 84% yield) as a white solid: mp 89-91°C (dec.); $[\alpha]^{24}_D$ -208.3° (c 0.44, MeOH); UV(H₂O) $\lambda_{\max}$ 279.5 nm (ϵ 19,900, pH 2). 272.0 nm (ϵ 15,900, pH 7), 272.5 nm (ϵ 16,200, pH 11); Anal. (C₉H₁₀FN₃O₂S 0.2H₂O) C, H, N, S.

(-)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-5-fluorocytosine (18**).** See the general procedure for two successive deprotections. The title compound **18** was obtained on 0.296-mmol scale in 90 % yield: mp 187-189°C; $[\alpha]^{25}_D$ -171.3° (c 0.42, MeOH); UV(H₂O) $\lambda_{\max}$ 286.0 nm (ϵ 8,600, pH 2). 282.0 nm (ϵ 7,500, pH 7), 282.0 nm (ϵ 6,900, pH 11); Anal. (C₉H₉F₂N₃O₂S) C, H, N, S.

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

(-)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]thymine (19**).** A solution of compound **15** (0.119 g, 0.235 mmol) in THF (10 mL) was treated with 0.25 mL of 1M TBAF in THF at rt for 2 h. The mixture was concentrated and purified by silica gel column chromatography with 3% MeOH in CH₂Cl₂ to give the desired product **19** (0.055g, 0.206 mmol, 88% yield) as a white solid: mp 174~176°C; $[\alpha]^{25}_D$ -46.5° (c 0.29, MeOH); UV(H₂O) $\lambda_{\max}$ 268.5 nm (ϵ 5,700, pH 2). 268.5 nm (ϵ 5,500, pH 7), 269.0 nm (ϵ 4,500, pH 11); Anal. (C₁₀H₁₁FN₂O₃S 0.1Et₂O) C, H, N, S.

(-)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]uracil

(20). See the general procedure for desilylation reaction. The title compound **20** was obtained on 0.193-mmol scale in 89 % yield: mp 184~185°C; $[\alpha]^{25}_{\text{D}}$ -136.4° (c 0.47, MeOH); UV(H₂O) 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); Anal. (C₉H₉FN₂O₃S·0.3MeOH) 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-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenenyl-4-thio- β -D-ribofuranosyl]-6-chloropurine (21). A mixture of 6-chloropurine (0.947 g, 6.12 mmol) and ammonium sulfate (0.135 g, 1.02 mmol) in 60 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** (1.2 g, 2.04 mmol) in 1,2-dichloroethane (30 mL) was added. The resulting slurry was cooled to -25°C and TMSOTf (0.74 mL, 4.08 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 CH₂Cl₂, washed with aq. NaHCO₃, dried over MgSO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography with 11% EtOAc in hexanes to give compound **21** (1.01 g, 1.48 mmol, 73% yield) as a pale yellow foam: $[\alpha]^{22}_{\text{D}}$ 75.4° (c 1.08, CHCl₃); UV(CH₂Cl₂) λ_{max} 264.5 nm; ¹³C NMR (CDCl₃) δ 151.84, 150.87, 144.98 (d, *J* = 5.7 Hz), 136.32, 135.60, 135.53, 132.85, 132.76, 131.21, 130.02, 129.96, 129.44, 128.99, 128.79, 127.82, 124.34, 105.10 (d, *J* = 248.1 Hz), 66.64, 65.68 (d, *J* = 20.3 Hz), 47.33, 41.68 (d, *J* = 20.7 Hz), 26.74, 19.17; Anal. (C₃₂H₃₂ClFN₄OSSeSi) C, H, N, S.

(+)-9-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranosyl]-6-chloro-2-fluoropurine (26). See the general procedure for condensation reaction of the acetate **8** with purines. The title compound **26** was obtained on 1.70-mmol scale in 67% yield: $[\alpha]_D^{23}$ 81.0° (c 1.37, CHCl₃); UV(CHCl₃) $\lambda_{\max}$ 270.0 nm; ¹³C NMR (CDCl₃) δ 157.00 (d, *J* = 220.8 Hz), 153.53 (d, *J* = 17.1 Hz), 152.4 (d, *J* = 17.3 Hz), 145.53, 136.26, 135.59, 135.52, 132.80, 132.70, 130.03, 129.97, 129.79 (d, *J* = 4.9 Hz), 129.49, 129.00, 127.83, 124.19, 104.99 (d, *J* = 248.5 Hz), 66.53, 65.90 (d, *J* = 20.7 Hz), 47.36, 41.48 (d, *J* = 20.7 Hz), 26.75, 19.17; Anal. (C₃₂H₃₁ClF₂N₄OSSeSi·0.2Hexane) C, H, N, S.

9-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-6-chloropurine (22) and 9-[(4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-6-chloropurine (23). See the general procedure for *syn*-elimination reaction. A mixture of title compounds **22** and **23** (3:1) were obtained on 1.48-mmol scale in 86% yield as a pale yellow foam: UV(CH₂Cl₂) $\lambda_{\max}$ 264.5 nm; Anal. (C₂₆H₂₆ClFN₄OSSi) C, H, N, S.

9-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-6-chloro-2-fluoropurine (27) and 9-[(4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-6-chloro-2-fluoropurine (28). See the general procedure for *syn*-elimination reaction. A mixture of title compounds **27** and **28** were obtained on 1.14-mmol scale in 71% yield as a pale yellow foam: UV(CH₂Cl₂) $\lambda_{\max}$ 266.0 nm; Anal. (C₂₆H₂₅ClF₂N₄OSSi) C, H, N, S.

(-)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]adenine (24). A mixture of compound **22** and compound **23** (0.342 g, 0.65 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 0.3 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 CH₂Cl₂ to give the desired compound **24** (0.099 g, 0.37 mmol 57% yield) as a white solid: mp 190~191°C; $[\alpha]_D^{25}$ -33.7° (c 0.164, 7:1 CH₂Cl₂:MeOH); UV(H₂O) $\lambda_{\max}$ 257.5 nm (ϵ 9,600, pH 2). 259.0 nm (ϵ 9,500, pH 7), 259.0 nm (ϵ 9,800, pH 11); ¹³C NMR (MeOH-*d*₄) δ 157.43, 156.31 (d, *J* = 277.9 Hz), 153.98, 150.51, 141.22, 120.10, 111.65 (d, *J* = 17.1 Hz), 65.63, 60.38 (d, *J* = 24.7 Hz), 49.73 (d, *J* = 7.7 Hz); Anal. (C₁₀H₁₀FN₅OS) C, H, N, S.

(+)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]hypoxanthine (25). A mixture of compound **22** and compound **23** (0.323 g, 0.62 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.17 mL, 2.46 mmol) and NaOMe (0.136 g, 2.52 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 CH₂Cl₂. The filtrate was concentrated to dryness. The crude product was treated with 0.68 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 CH₂Cl₂ to give the desired compound **25** (0.135 g, 0.50 mmol, 81% yield) as a white solid: mp 143~145°C (dec.); $[\alpha]_D^{27}$ 21.5° (c 0.132, 7:1, CH₂Cl₂:MeOH); UV(H₂O) $\lambda_{\max}$ 247.5 nm (ϵ 10,200, pH 2). 248.0 nm (ϵ 10,000, pH 7), 253.5 nm (ϵ 10,700, pH 11); ¹³C NMR (MeOH-*d*₄) δ 158.85, 156.22 (d, *J* = 248.4 Hz), 150.04,

147.13, 140.66, 125.29, 111.77 (d, $J = 17.0$ Hz), 65.50, 60.51 (d, $J = 24.8$ Hz), 49.83 (d, $J = 7.3$ Hz); Anal. ($C_{10}H_9FN_4O_2S$) C, H, N, S.

(-)-2-Amino-9-[(1*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-6-chloropurine (29) and 6-amino-9-[(1*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-

ribofuranosyl]-2-fluoropurine (31). Dry ammonia gas was bubbled into a stirred solution of a mixture of compounds **27** and **28** (0.430 g, 0.79 mmol) in 1,2-dimethoxyethane (25 mL) at rt for 5 h. 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 **29** (0.190 g, 0.352 mmol, 45% yield) and a mixture of compound **31** and its $\Delta^{1,2}$ -isomer (0.116 g, 0.22 mmol, 28% yield): for compound **29**; $[\alpha]_D^{24} -70.4^\circ$ (c 0.40, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 303.5 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. ($C_{26}H_{27}ClFN_5OSSi$) C, H, N, S: a mixture of compound **31** and its $\Delta^{1,2}$ -isomer; UV($CHCl_3$) λ_{max} 261.0 nm.

(-)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]guanosine (30). See the procedure for the preparation of compound **25**.

The title compound **30** was obtained on 0.29-mmol scale in 61% yield: mp 210°C (dec.); $[\alpha]_D^{25} -73.7^\circ$ (c 0.152, DMSO); UV(H_2O) λ_{max} 254.0 nm (ϵ 11,600, pH 2). 255.0 nm (ϵ 12,400, pH 7), 265.0 nm (ϵ 12,500, 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, 60.99 (d, $J = 24.5$ Hz), 51.73 (d, $J = 7.6$ Hz), 44.06 (d, $J = 9.1$ Hz); Anal. ($C_{10}H_{10}FN_5O_2S$) C, H, N, S.

(+)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio- β -D-ribofuranosyl]-2-

fluoroadenosine (32). See the general procedure of desilylation. The title compound **32** was obtained on 0.22-mmol scale in 70% yield: mp >300°C; $[\alpha]_D^{26}$ 17.1° (c 0.15, MeOH); UV(H₂O) λ_{max} 261.0 nm (ϵ 12,700, pH 2). 261.0 nm (ϵ 12,900, pH 7), 260.5 nm (ϵ 13,100, pH 11); ¹³C NMR (MeOH-*d*₄) δ 160.82 (d, *J* = 269.8 Hz), 159.67, 159.02, 155.19, 152.65, 141.63, 118.69, 111.99 (d, *J* = 17.1 Hz), 65.96 (d, *J* = 1.8 Hz), 60.77 (d, *J* = 24.3 Hz), 50.04; Anal. (C₁₀H₉F₂N₅OS) C, H, N, S.

Molecular modeling study. (a) Conformational analysis: The initial conformations of D- β -2'-F-d4C and D- β -2'-F-4'-S-d4C **17** 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* 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).¹⁹⁹ 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+1th nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray

structure (1rtd)¹⁹⁹ was modified to guanine. 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.¹⁹⁹ Gasteiger-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 literatures^{205,206} 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 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.

Adenosine deaminase kinetics study. Assays were performed at 25°C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range of 15-100 µM and with 0.15 unit of adenosine deaminase (EC.3.5.4.4. from calf intestinal mucosa). The assays were monitored with a UV spectrometer at 265 nm. Initially, the qualitative assays were performed with D-β-2'-F-4'-S-d4A **24** (200 µM) in the presence of 0.24 unit of adenosine deaminase for 120 min to determine whether it is a substrate of this enzyme. The concentration (c_t) of each substrate at a certain time (t) was calculated from the absorbance (A_t) at that time (t), where it was assumed that the total change of absorbance ($A_0 - A_\infty$) was directly related to the disappearance of the substrate.³⁵ Initial hydrolysis rates for each substrate concentration were measured manually through graphical curve fitting of the concentration vs time data for reaction of each substrate. From Lineweaver-Burke plot of these initial rates, $V_{\max}$ (maximum velocity) and K_M (Michaelis constant)

were obtained for each substrate and k_{cat} was also calculated with 0.15 unit of the enzyme (M.W. 33,000) in 2 mL of buffer solution. The $t_{1/2}$ of D- β -2'-F-4'-S-d4A **24** and adenosine were also measured at 20 μ M with 0.15 unit of the enzyme.

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 infectious 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 TCID₅₀ / 1×10^7 cells for a flask (T25) assay or with 200 TCID₅₀ / 6×10^5 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 μ g/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% CO₂-air at 37°C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12,000 rpm for 2 hr to pellet the virus. The pellet was solubilized with vortexing in 100 μ l 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 μ l of each sample were added to 75 μ l RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M MgCl₂, 0.006 M dithiothreitol, 0.006 mg/ml poly (rA)_n oligo (dT)₁₂₋₁₈, 96 μ g/ml dATP, and 1 μ M of 0.08 mCi/ml ³H-thymidine triphosphate (Moravek Biochemicals, Brea, CA) and incubated at 37°C for 2 hr. The reaction was stopped by the addition of 100 μ l 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 (EC₅₀) and 90% effective concentration (EC₉₀) were determined from the concentration-response curve using the median effect method.²⁰⁷

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 discontinuous gradient centrifugation. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5×10^3 , 2.5×10^3 and 5×10^4 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% CO₂-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 hr to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570nm 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.²⁰⁷

Supplemental Material

Elemental Analysis Data

Cmpd No	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 ₃ Si	67.71	6.76	-- ^a	-- ^a	67.70	6.79	-- ^a	-- ^a
4	C ₂₂ H ₂₈ FIO ₃ Si·0.2C ₆ H ₁₄	52.41	5.84	-- ^a	-- ^a	52.30	5.55	-- ^a	-- ^a
5	C ₂₄ H ₃₁ FO ₄ SSi	62.31	6.75	-- ^a	6.93	62.03	6.63	-- ^a	6.98
6	C ₂₁ H ₂₅ FO ₂ SSi	64.01	6.48	-- ^a	8.25	64.88	6.51	-- ^a	8.15
6a	C ₂₁ H ₂₅ FO ₂ SSi	64.01	6.48	-- ^a	8.25	65.15	6.58	-- ^a	8.12
7	C ₂₇ H ₂₉ FO ₂ SSeSi	59.65	5.38	-- ^a	5.90	60.10	5.46	-- ^a	5.85
7a	C ₂₇ H ₂₉ FO ₂ SSeSi·0.15CHCl ₃	58.07	5.23	-- ^a	5.70	57.88	5.36	-- ^a	5.76
8	C ₂₉ H ₂₈ FO ₃ SSeSi	59.27	5.66	-- ^a	5.46	59.61	5.72	-- ^a	5.57
9	C ₃₈ H ₃₈ FN ₃ O ₃ SSeSi	61.44	5.16	5.66	4.32	61.52	5.42	5.47	4.21
10	C ₃₈ H ₃₇ F ₂ N ₃ O ₃ SSeSi	59.99	4.90	5.52	4.21	59.96	5.07	5.42	4.23
11	C ₃₂ H ₃₅ FN ₂ O ₃ SSeSi·0.4H ₂ O	58.15	5.47	4.23	4.85	58.03	5.49	4.21	4.95

12	C ₃₁ H ₃₃ FN ₂ O ₃ SSeSi	58.20	5.20	4.38	5.01	58.17	5.35	4.32	4.93
13	C ₃₂ H ₃₂ FN ₃ O ₃ SSi	65.88	5.66	7.07	5.39	66.04	5.85	6.81	5.17
14	C ₃₂ H ₃₁ F ₂ N ₃ O ₃ SSi	63.66	5.18	6.96	5.31	63.71	5.32	6.76	5.27
15	C ₂₆ H ₂₉ FN ₂ O ₃ SSi·0.6H ₂ O	61.54	6.00	5.50	6.30	61.31	5.87	5.34	6.11
16	C ₂₅ H ₂₇ FN ₂ O ₃ SSi	62.21	5.64	5.80	6.64	62.38	5.65	5.69	6.48
17	C ₉ H ₁₀ FN ₃ O ₂ S·0.2H ₂ O	43.79	4.25	17.02	12.99	43.87	4.31	16.92	12.86
18	C ₉ H ₉ F ₂ N ₃ O ₂ S	41.38	3.47	16.08	12.27	41.29	3.52	15.99	12.18
19	C ₁₀ H ₁₁ FN ₂ O ₃ S·0.1Et ₂ O	47.02	4.55	10.54	12.07	47.02	4.56	10.48	11.89
20	C ₉ H ₉ FN ₂ O ₃ S·0.3CH ₃ OH	44.00	4.05	11.04	12.63	44.01	3.89	11.22	12.76
21	C ₃₂ H ₃₂ ClFN ₄ OSSeSi	56.34	4.73	8.21	4.70	56.69	5.12	7.95	4.51
22	C ₂₆ H ₂₆ ClFN ₄ OSSi	59.47	4.99	10.67	6.11	59.58	5.03	10.41	6.09
24	C ₁₀ H ₁₀ FN ₅ OS	44.94	3.77	26.20	12.00	45.23	4.07	25.98	11.79
25	C ₁₀ H ₉ FN ₄ O ₂ S	44.77	3.38	20.88	11.95	44.53	3.67	21.15	11.91
26	C ₃₂ H ₃₁ ClF ₂ N ₄ OSSeSi·0.2C ₆ H ₁₄	55.58	4.75	7.81	4.47	55.55	4.68	7.74	4.36

27	C ₂₆ H ₂₅ ClF ₂ N ₄ OSSi	57.50	4.64	10.32	5.90	57.18	4.50	10.13	5.68
29	C ₂₆ H ₂₇ ClFN ₅ OSSi	57.82	5.04	12.97	5.94	57.73	4.99	12.98	5.82
30	C ₁₀ H ₁₀ FN ₅ O ₂ S	42.40	3.56	24.72	11.32	42.27	3.74	24.56	11.09
32	C ₁₀ H ₉ F ₂ N ₅ OS	42.10	3.18	24.55	11.24	42.28	3.37	24.39	11.19

^a Not applicable.

Supplemental Material (¹H NMR Data^a)

Cpd	H-1'	H-3'	H-4'	H-5'	Other protons
9	6.91 (d, <i>J</i> = 15.6 Hz)	2.62-2.47 (m)	3.68-3.58 (m)	3.87-3.72 (m)	8.82 (br s, 1H), 8.12 (d, <i>J</i> = 6.5 Hz, 1H), 7.92 (d, <i>J</i> = 7.3 Hz, 2H), 7.69-7.27 (m, 19H), 1.02 (s, 9H)
10	6.73 (d, <i>J</i> = 16.3 Hz)	2.63-2.46 (m)	3.67-3.58 (m)	3.92-3.76 (m)	12.70 (br s, 1H), 8.33 (d, <i>J</i> = 7.5 Hz, 2H), 7.87 (dd, <i>J</i> = 5.9, 1.7 Hz, 1H), 7.72-7.28 (m, 18H), 1.07 (s, 9H)
11	6.63 (d, <i>J</i> = 14.7 Hz)	2.55-2.41 (m)	3.62-3.58 (m)	3.87-3.72 (m)	9.26 (br s, 1H), 7.72 (dd, <i>J</i> = 8.2, 2.0 Hz, 1H), 7.68-7.30 (m, 15H), 5.56 (dd, <i>J</i> = 8.2, 1.9 Hz, 1H), 1.03 (s, 9H)
12	6.72 (d, <i>J</i> = 18.1 Hz)	2.62~2.44 (m)	3.67~3.57 (m)	3.87~3.73 (m)	9.13 (br s, 1H), 7.74~7.25 (m, 16H), 1.83 (s, 3H), 1.07 (s, 9H)

13	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)	8.95 (br s, 1H), 7.91 (d, $J = 7.5$ Hz, 2H), 7.69~7.38 (m, 15H), 1.10 (s, 9H)
14	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)	12.80 (br s, 1H), 8.30 (d, $J = 7.3$ Hz, 2H), 7.72~7.38 (m, 14H), 1.10 (s, 9H)
15	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)	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	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)	9.45 (br s, 1H), 7.72~7.35 (m, 10H), 7.02 (s, 1H), 1.74 (s, 3H), 1.08 (s, 9H)
17	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)	8.05 (dd, $J = 7.5$, 1.0 Hz, 1H), 5.97 (d, $J = 7.5$ Hz, 1H)
18	6.92 (s)	5.84 (s)	4.17 (s)	3.84 (dd, $J = 11.8$, 3.7 Hz) 3.68 (br d, $J = 10.6$	8.39 (d, $J = 6.6$ Hz, 1H)

				Hz)	
19	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)	8.11 (dd, $J = 8.1$, 0.8 Hz, 1H), 5.77 (d, $J = 8.1$ Hz, 1H)
20	6.87 (t, $J = 1.0$ Hz)	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)	8.03 (s, 1H), 1.88 (d, $J = 1.2$ Hz, 3H)
21	6.63 (d, $J = 14.7$ Hz)	2.75~2.63 (m)	3.80~3.73 (m)	3.96 (t, $J = 8.2$ Hz) 3.88 (td, $J = 7.6$, 1.8 Hz)	8.69 (s, 1H), 8.31 (d, $J = 2.9$ Hz, 1H), 7.70~7.13 (m, 15H), 1.05 (s, 9H)
22	6.87 (s)	5.87 (s)	4.33~4.25 (m)	3.96 (dd, $J = 10.2$, 6.8 Hz) 3.86 (t, $J = 8.8$ Hz)	8.69 (s, 1H), 8.12 (s, 1H), 7.69~7.35 (m, 10H), 1.08 (s, 9H)
23	--	3.25 (ddd, $J = 16.6$, 9.2, 3.1 Hz) 3.03 (dd, $J = 16.6$, 5.2 Hz)	4.05~3.78 (m, 3H)		8.82 (s, 1H), 8.19 (s, 1H), 7.69~7.35 (m, 10H), 1.08 (s, 9H)
24	6.88 (s)	5.93 (s)	4.31~4.24 (m)	3.90 (dd, $J = 11.6$, 4.4 Hz) 3.81 (ddd, $J = 11.5$, 4.1, 1.2 Hz)	8.52 (s, 1H), 8.20 (s, 1H)

25	6.90 (s)	5.93 (s)	4.28~4.23 (m)	3.88 (dd, $J = 11.7, 4.7$ Hz) 3.80 (dd, $J = 10.5, 4.0$ Hz)	8.51 (s, 1H), 8.07 (s, 1H)
26	6.45 (d, $J = 14.0$ Hz)	2.76~2.63 (m) 2.60 (td, $J = 14.8, 7.9$ Hz)	3.80~3.72 (m)	3.97 (dd, $J = 9.7, 7.3$ Hz) 3.89 (ps td, $J = 7.6, 1.2$ Hz)	8.27 (d, $J = 2.9$ Hz, 1H), 7.70~7.14 (m, 15H), 1.06 (s, 9H)
27	6.68 (s)	5.86 (s)	4.32~4.23 (m)	3.94 (dd, $J = 10.2, 6.0$ Hz) 3.87~3.83 (m)	8.08 (s, 1H), 7.69~7.36 (m, 10H), 1.08 (s, 9H)
28	--	3.24 (ddd, $J = 16.3, 9.3, 3.1$ Hz) 3.01 (ddd, $J = 16.8, 5.1, 0.6$ Hz)	4.04~3.80 (m, 3H)		8.12 (s, 1H), 7.75~7.31 (m, 10H), 1.08 (s, 9H)
29	6.65 (s)	5.82 (s)	4.25~4.19 (m)	3.90 (dd, $J = 10.1, 7.0$ Hz) 3.81 (dd, $J = 9.8, 7.4$ Hz)	7.74 (s, 1H), 7.68~7.36 (m, 10H), 5.37 (s, 1H), 1.07 (s, 9H)
30	6.73 (s)	5.93 (s)	4.27~4.23 (m)	3.86 (dd, $J = 11.3, 4.6$ Hz) 3.81 (ddd, $J = 11.5, 4.6, 1.1$ Hz)	8.14 (s, 1H)

31	6.71 (s)	5.82 (s)	4.27~4.19 (m)	3.93~3.82 (m)	7.77 (s, 1H), 7.73~7.38 (m, 10H), 6.40 (br s, 2H), 1.08 (s, 9H)
32	6.77 (s)	5.92 (s)	4.27-4.22 (m)	3.89 (dd, $J = 11.7, 4.9$ Hz) 3.82 (ddd, $J = 12.0, 4.6, 1.2$ Hz)	8.44 (s, 1H)

^a CD₃OD;ppm;s, singlet; d, doublet; dd, double doublet; t, triplet, br s, broad singlet; m, multiplet; td, triple doublet; ddd, double double doublet.

CHAPTER 5

L-2',3'-DIDEHYDRO-2',3'-DIDEOXY-3'-FLUORONUCLEOSIDES: SYNTHESIS, ANTI-HIV ACTIVITY, CHEMICAL AND ENZYMATIC STABILITY AND THE MECHANISM OF RESISTANCE¹

¹ Chong, Y., G. Gumina, J. S. Mathew, R. F. Schinazi and C. K. Chu. 2003. *J. Med. Chem.* 46: 3245-3256.

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Introduction

A number of fluorine-substituted nucleoside analogues have demonstrated their potent antiviral activity as well as favorable chemical and pharmacological properties by virtue of the small but highly electronegative nature of the fluorine atom, which is also capable of participating in hydrogen bonding. Thus, fluorination at the 5-position of the pyrimidine base²⁰⁸ as well as at the 2'- and/or 3'- position of the sugar moiety of the nucleoside analogue have been extensively studied in the pursuit of safe, effective and chemically stable antiviral agents.^{209,210} Among those, the most interesting antiviral activities were found when a fluorine was substituted in 2',3'-dideoxy-2',3'-didehydro nucleosides. Both the 5-fluorocytidine analogue^{1c,1e} and the 2'-fluoro analogue^{2b-2d} showed potent anti-HIV activity without significant cytotoxicity. It is interesting to note that the antiviral activities of nucleosides containing the 2',3'-unsaturated sugar moiety with a 2'-fluoro substitution have increased stabilization of the glycosidic bond. As this stabilization of the glycosidic bond results from the destabilization of the oxonium ion resulting from the cleavage of the glycosidic bond by the highly electronegative fluorine atom (Scheme 5.4), it was of interest to investigate the influence of the fluorine atom at the 3' position. In view of the fact that the L-isomers tend to have potent antiviral activity with reduced toxicity in comparison to their D-counterparts, in a recent communication, we reported the stereoselective synthesis of the β -L-3'-F-d4C which showed potent anti-HIV activity (EC_{50} 0.03 μ M) in PBM cells.²¹¹ By using a similar synthetic strategy, a series of L-nucleosides were synthesized from the key intermediate, (4*S*)-1-*O*-acetyl-5-*O*-benzoyl-2,3-dideoxy-2,2-difluoro- β -D-ribofuranoside **7**, and their antiviral activities were

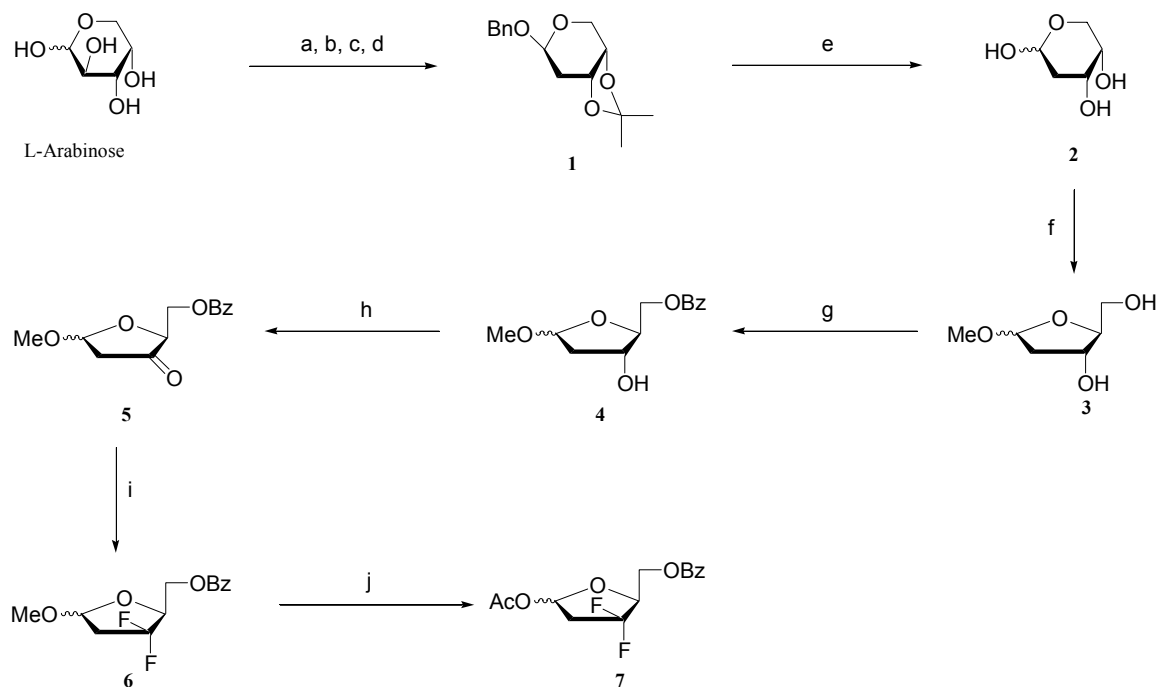
evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells. The most potent cytidine **24** and 5-fluorocytidine **26** analogues were also evaluated against a lamivudine-resistant molecular infectious clone (HIV-1_{M184V}).

Results and Discussion

Chemistry. Synthesis of the nucleoside analogues with a fluorine atom at the 3'-position of a 2',3'-unsaturated sugar ring has been hampered by the lack of an efficient method for introducing the fluoro-vinyl group at the 3'-position. The only synthetic method known to date employs the oxidation followed by difluorination of the 3'-OH group at the nucleoside level.²¹² Elimination of HF by sodium methoxide afforded the desired 3'-fluoro-2',3'-unsaturated thymidine analogue. However, for the extensive structure-activity relationship study, a more general synthetic method such as the introduction of the 3,3-difluoro functionality before condensation with the heterocyclic base needed to be developed. In addition, the preparation of the key intermediate **7** required improvement from our previous report due to the high cost of L-xylose as well as lengthy synthetic steps. Therefore, 2-deoxyribose, which can be prepared from inexpensive L-arabinose in large quantity and high yield,²¹³ was methylated followed by benzylation to give the 5-*O*-benzoyl methyl furanoside **4**.

Pyridinium dichromate-mediated oxidation of compound **4** followed by difluorination with DAST provided the intermediate **6**, which was transglycosylated to the key intermediate (4*S*)-1-*O*-acetyl-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-D-ribofuranoside **7**

in high yield (95 %, Scheme 5.1) by treatment with acetic anhydride and acetic acid in the presence of sulfuric acid.

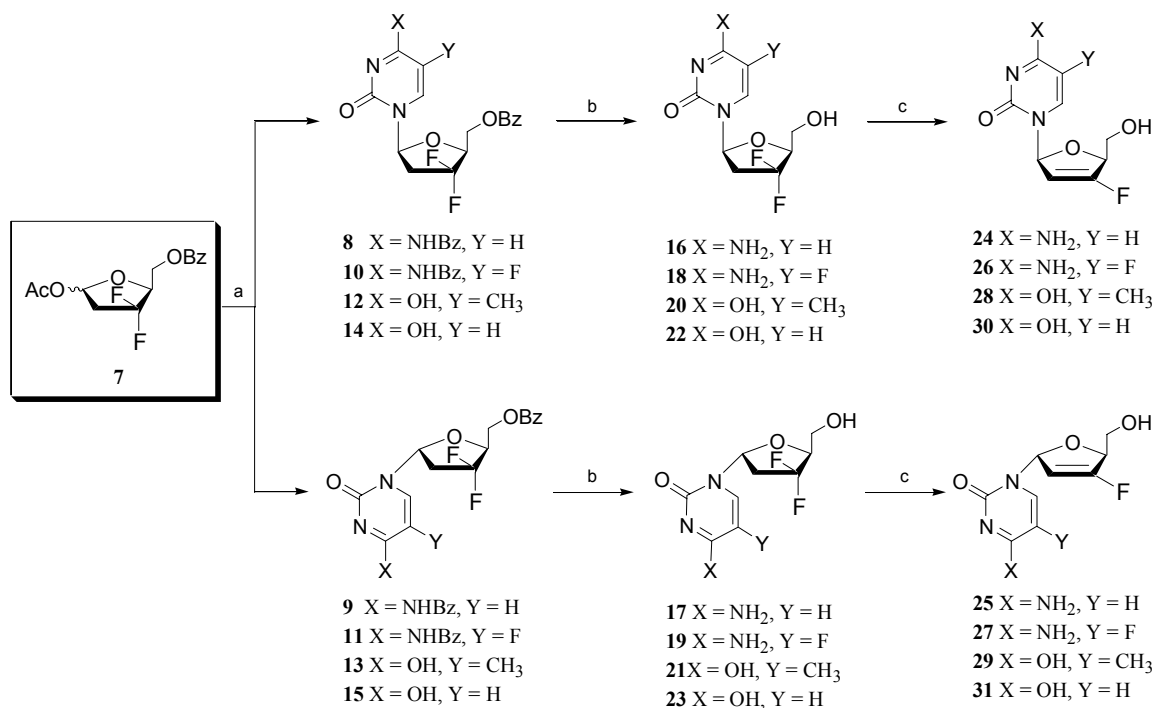


^aKeys: (a) BnOH, HCl (g); (b) 2,2-dimethoxypropane, p-TsOH, acetone; (c) CSCl₂, PhOH, pyr, CH₂Cl₂; (d) nBu₃SnH, AIBN, toluene, reflux; (e) 4% TFA, 40°C; (f) HCl, MeOH; (g) BzCl, pyr; (h) PDC, Ac₂O, CH₂Cl₂; (i) DAST, CH₂Cl₂, reflux; (j) Ac₂O, H₂SO₄, AcOH

Scheme 5.1. Synthesis of the key intermediate **7a**

Condensation of **7** with various pyrimidine heterocyclic bases under Vorbrüggen conditions gave α/β mixtures of the corresponding pyrimidine analogues **8-15** in 40~60% yield (Scheme 5.2). The protecting groups on the heterocyclic base and the 5' position of the sugar moiety were deblocked by methanolic ammonia to give the corresponding 2',3'-dideoxy-3',3'-difluoro pyrimidine nucleosides **16-23** (Scheme 5.2), which underwent smooth elimination to the final 3'-fluoro 2',3'-unsaturated pyrimidine nucleosides **24-29** by treatment with NaOMe in DMF. However, the instability of the

uridine analogues (**30** and **31**) under the reaction conditions resulted in decomposition, which was circumvented by using a 2:1 mixture of DMF and dioxane to give the desired compounds (**30** and **31**) in moderate yields (54% and 41%, respectively).



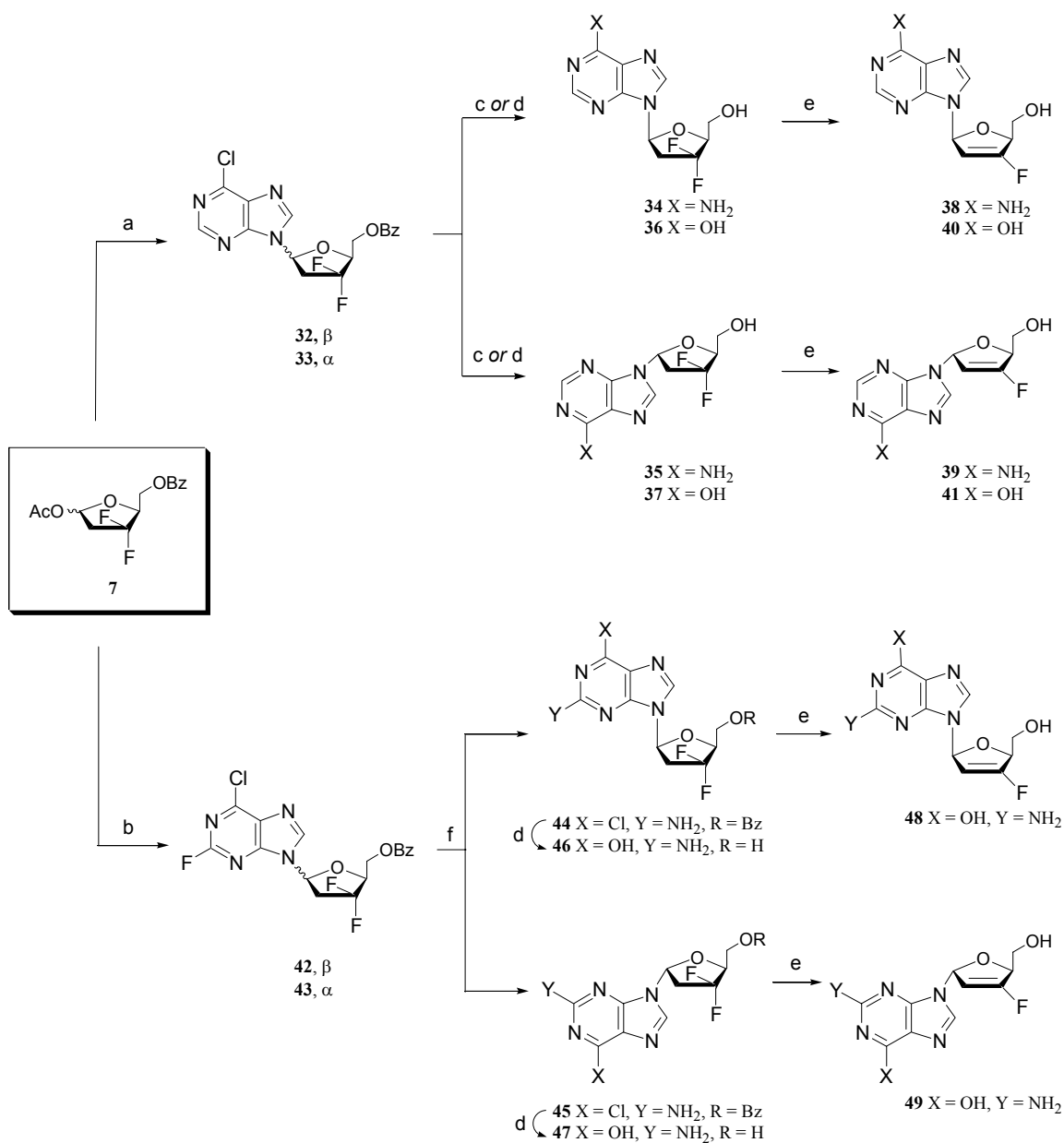
^aKeys: (a) HMDS, CH₃CN, pyrimidines; TMSOTf, (b) NH₃, MeOH, rt, (c) NaOMe, DMF (or 2:1 DMF:dioxane), rt

Scheme 5.2. Synthesis of the Pyrimidine Nucleosides.

For the preparation of the purine nucleosides, the key intermediate **7** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give α/β mixtures of the corresponding nucleosides **32/33** and **42/43** in 47% and 78% yield, respectively (Scheme 5.3). To achieve a clean and high-yield conversion, the temperature of the reaction mixture had to be carefully controlled in such a manner that, after addition of TMSOTf at 0°C, the reaction mixture was stirred for 6 h at room temperature and then for another 2 h at 60°C. Amination of the mixture of **32** and **33** by treatment with ammonium hydroxide at 60°C

in a steel bomb gave the adenosine analogue (**34** and **35**), whereas hydrolysis in the presence of sodium methoxide and 2-mercaptoethanol in refluxing methanol gave the inosine analogues (**36** and **37**). Dry ammonia gas was bubbled into a solution of **42** and **43** in ethylene glycol dimethyl ether (DME) at room temperature for 16 h to give the 2-amino-6-chloropurine derivatives (**44** and **45**) in 29% and 28% yield, respectively, which were readily separated by silica gel column chromatography. The 2-amino-6-chloropurine derivatives (**44** and **45**), thus obtained, were hydrolyzed by using sodium methoxide and 2-mercaptoethanol in refluxing methanol to give 2',3'-dideoxy-3',3'-difluoro guanosine derivatives (**46** and **47**) in 51% and 80% yield, respectively. The 2',3'-dideoxy-3',3'-difluoro purine nucleosides (**34-37** and **46-47**) were treated with NaOMe in DMF to give the final 3'-fluoro-2',3'-unsaturated purines (**38-41** and **48-49**). However, these nucleosides slowly decomposed during purification (column chromatography on silica gel followed by fractional crystallization in EtOH) resulting in low yields (33 ~ 46 %).

Structure-Activity Relationships. Using AZT as a positive control, the EC₅₀ values of the synthesized nucleosides were evaluated against HIV-1 in human PBM cells *in vitro*, and the results are summarized in Table 5.1 and Table 5.2. In the 3',3'-difluoro series (Table 5.1), only the cytidine **16** (EC₅₀ 11.5 μM) and 5-fluorocytidine **18** (EC₅₀ 8.8 μM) analogues showed moderate antiviral activity. However, the 2',3'-unsaturated nucleosides (Table 5.2) showed more potent anti-HIV-1 activity in comparison to the corresponding 3',3'-difluoro analogues, among which cytidine **24** (EC₅₀ 0.089 μM), 5-fluorocytidine **26** (EC₅₀ 0.018 μM) and guanosine **48** (EC₅₀ 38.5 μM) showed moderate to potent antiviral activities.



^aKeys: (a) HMDS, (NH₄)₂SO₄, 6-Chloropurine; TMSOTf, CH₃CN, (b) HMDS, (NH₄)₂SO₄, 2-Fluoro-6-chloropurine; TMSOTf, CH₃CN, (c) NH₄OH, 60°C, (d) HSCH₂CH₂OH, NaOMe, 60°C, (e) NaOMe, DMF, rt, (f) NH₃ bubbling, DME, rt.

Scheme 5.3. Synthesis of the Purine Nucleosides.

Even though the cytidine analogue **24** showed minimal cytotoxicity in PBM cells (IC₅₀ 86.9 μ M), the selectivity index (SI, 976) is very high. No other synthesized nucleoside showed significant cytotoxicity up to 100 μ M except the 2',3'-unsaturated uridine **30**

(IC₅₀ 30.1 μ M for CEM) and guanosine **48** (IC₅₀ 17.4, 58.4 and 36.5 μ M in PBM, CEM and Vero cells, respectively) analogues.

Table 5.1. Anti-HIV-1 activity of L-2'-deoxy-3',3'-difluoronucleosides

Compd	Base (Config.)	Activity (EC ₅₀ , μ M)	Cytotoxicity (IC ₅₀ , μ M)		
			PBM	CEM	Vero
16	Cytosine (β)	11.5	>100	>100	>100
17	Cytosine (α)	>100	>100	>100	>100
18	5-F-Cytosine (β)	8.8	>100	>100	>100
19	5-F-Cytosine (α)	>100	>100	>100	>100
20	Thymine (β)	>100	>100	>100	>100
21	Thymine (α)	>100	>100	>100	>100
22	Uracil (β)	>100	>100	>100	>100
23	Uracil (α)	>100	>100	>100	>100
34	Adenine (β)	>100	>100	>100	>100
35	Adenine (α)	>100	>100	>100	>100
36	Hypoxanthine (β)	>100	>100	>100	>100
37	Hypoxanthine (α)	>100	>100	>100	>100
46	Guanine (β)	>100	>100	>100	>100
47	Guanine (α)	>100	>100	>100	>100

Table 5.2. Anti-HIV-1 activity of L-2',3'-dideoxy-2',3'-didehydro-3'-fluoronucleosides

Compd	Base (Config.)	Activity (EC ₅₀ , μ M)	Cytotoxicity (IC ₅₀ , μ M)		
			PBM	CEM	Vero
24	Cytosine (β)	0.089	86.9	>100	>100
25	Cytosine (α)	>100	>100	>100	>100
26	5-F-Cytosine (β)	0.018	>100	>100	>100
27	5-F-Cytosine (α)	30.0	>100	>100	>100
28	Thymine (β)	>100	>100	>100	>100
29	Thymine (α)	>100	>100	>100	>100
30	Uracil (β)	>100	>100	30.1	>100
31	Uracil (α)	>100	>100	>100	>100
38	Adenine (β)	>100	>100	>100	>100
39	Adenine (α)	>100	>100	>100	>100
40	Hypoxanthine (β)	>100	>100	>100	>100
48	Guanine (β)	38.5	17.4	58.4	36.5
49	Guanine (α)	>100	>100	>100	>100

Antiviral activity against lamivudine-resistant (HIV-1_{M184V}) mutant strain. Single point mutation at residue 184 (M184V) of the reverse transcriptase (RT) in HIV causes high-level resistance to lamivudine (3TC) and contributes to the failure of anti-AIDS therapy.^{214,215} Therefore, in order to discover novel drug candidates active against the M184V mutant HIV-1 RT, the cytosine (**24**) and 5-fluorocytosine analogues (**26**), along with two positive controls, AZT and 3TC, were evaluated against the lamivudine-resistant mutant strain (HIV-1_{M184V}) in human PBM cells *in vitro* (Table 5.3).

Unfortunately, the results of the study suggests that the 3'-fluoro-2',3'-unsaturated nucleosides are significantly cross-resistant to the M184V mutant. Interestingly, the 5-fluorocytosine analogue (**26**) was 11.6-fold more cross-resistant than the cytosine analogue (**24**), suggesting that in addition to the sugar conformation, the base moiety also plays a role.

Chemical Stability. It is conceivable that the 2'-fluoro-2',3'-unsaturated compound, whose fluorine atom is closer to the glycosidic bond than the 3'-fluoro analogue, is more effective in stabilizing the nucleoside toward acidic environment. In order to prove this hypothesis, D-2'-Fd4C,^{2c} L-3'-Fd4C **24** and L-d4C^{1e} were treated in a pH 2 buffer solution, and their stabilities were measured by TLC every 10 min (initial 3 h) to 6 h (Table 5.4). As expected, L-d4C started decomposition to the corresponding sugar moiety and aglycon (cytosine), marking its half-life at 1 h. Compared with D-2'-Fd4C, which showed indefinite stability under acidic buffer solution, L-3'-Fd4C **24** slowly decomposed, but its half-life (48 h) was much longer than that for L-d4C.

Table 5.3. Activity of selected nucleosides against lamivudine-resistant virus (HIV-1_{M184V}) in human PBM cells.

Compound	xxBRU (EC ₅₀ , μM)	M184V		FI ^a
		(EC ₅₀ , μM)	(EC ₉₀ , μM)	
24	0.023	2.4	15.7	104
26	0.009	10.9	67.4	1,211
AZT	0.01	0.003	0.025	0.3
3TC	0.02	535	>535	26,750

^a FI is the fold increase (EC₅₀ HIV-1_{M184V}/ EC₅₀ HIV-1_{xxBRU})

^b Not available

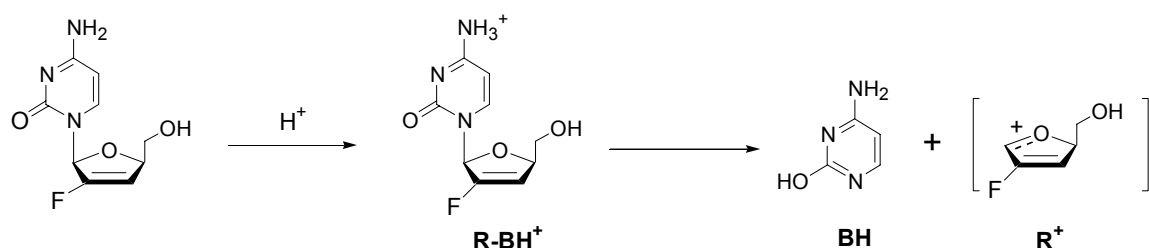
Table 5.4. Comparison between the calculated energy required for the dissociation of the glycosidic bond of the protonated cytidine nucleosides with their stabilities at pH 2 buffer solution.

Compound	t _{1/2} (h) ^a	ΔE _{calc} ^b (kcal/mol)
D-2'-Fd4C ^{2c}	> 72	57.55
L-3'-Fd4C (24)	48	50.71
L-d4C	1	40.56

^a Half-life at pH 2 buffer solution.

^b Calculated energy required for dissociation of the glycosidic bond, ΔE_{calc} = E(R⁺) + E(BH) – E(R-BH⁺) (Figure 5.1).

In many cases, the stability of the nucleoside analogue, particularly the stability of the glycosidic bond, is an important factor governing the biological activity as well as the therapeutic usefulness of the nucleoside drug candidate. Therefore, for the purpose of designing novel nucleoside antiviral agents, an indirect method of measuring the stability of the glycosidic bond was of interest. For this purpose, we tried to evaluate the energy required for the dissociation of the glycosidic bond as a result of a protonation under acidic conditions (Scheme 5.4).



Scheme 5.4. Dissociation of the glycosidic bond of 2',3'-unsaturated nucleosides

The energy of each chemical species was determined by quantum mechanical (RHF, 6-31G*) method, and the energy required for the bond dissociation reaction (ΔE) was calculated to find a potential correlation with the chemical stability of the nucleoside analogues. As shown in Table 5.4, the stability of the resulting oxonium ion (Scheme 5.4) correlates with the energy required for the bond dissociation. The fluorine substitution at the 2' or 3' position destabilizes the corresponding oxonium ion species and the destabilization was greater in the 2'-F substitution because of the shorter distance between the oxonium ion center and the fluorine atom.

Molecular Modeling. The triphosphate of the quantum mechanically geometry-optimized L-3'-Fd4C was docked into the active site of HIV-1 RT, and the resulting

complex was minimized²¹⁶ to give a characteristic binding mode of this nucleoside triphosphate, in which the 3'-fluorine atom is strongly interacting with the amide backbone of Asp185 by hydrogen bonding (Figure 5.1a). The large binding pocket of the HIV-1 RT, which accommodates the unnatural L-configured nucleosides such as 3TC,²¹⁷ L-d4FC^{1e} and L-2'-Fd4C,^{2b} was found to provide enough space for L-3'-Fd4CTP to bind at the active site without any steric hindrance (Figure 5.1b). This favorable binding mode, however, cannot be maintained when the triphosphate of the synthesized 3'-fluoro 2',3'-unsaturated nucleoside binds to the active site of M184V RT because the bulky side chain of Val184 occupies the space needed for binding and pushes the 3'-fluorine atom of L-3'-Fd4CTP away from the amide backbone of Asp185, out of hydrogen bonding distance, resulting in poor binding energy and thereby cross-resistance (Figure 5.1c).

Metabolic Stability. The metabolic stability toward deaminases such as cytidine deaminase and adenosine deaminase is another measure for the successful therapeutic candidates because, from a therapeutic point of view, along with nucleoside phosphorylases, these are the two major deactivating enzymes of the nucleoside catabolism pathway.²¹⁸ Due to the unavailability of the commercial cytidine deaminase, only the adenosine deaminase (ADA) binding efficiencies and deamination kinetics studies of the adenosine analogue **38** were conducted.²¹⁹ As expected, the unnatural L-configured adenine derivative **38** was found not to be deaminated by the enantioselective adenosine deaminase ensuring the metabolic stability of the unnatural L-nucleosides against the enzyme. As other L-cytidine analogues,²²⁰ it is unlikely that the L-cytidine derivatives with 3'-F substitution are substrates for cytidine deaminase.

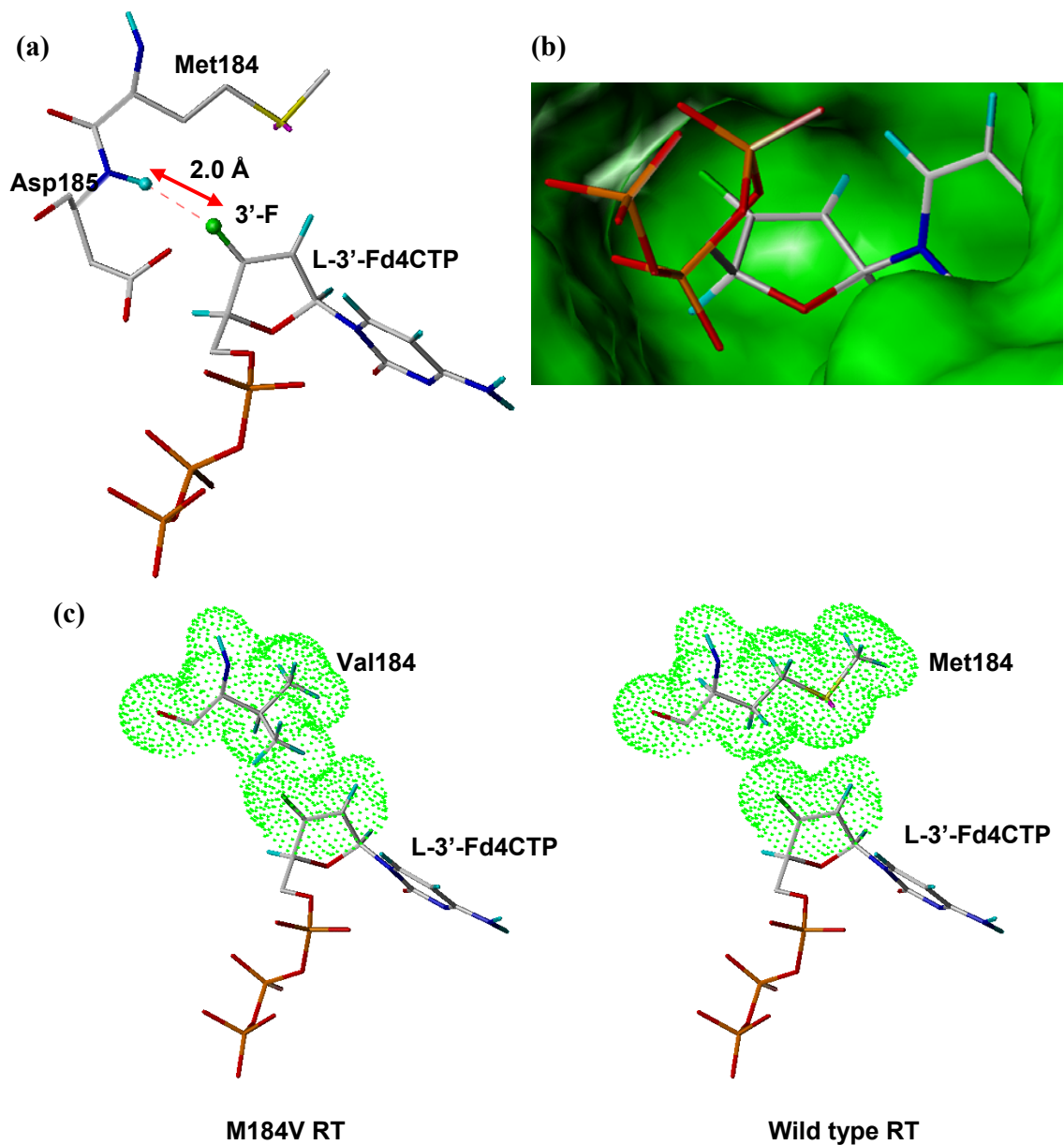


Figure 5.1. (a) Binding mode of L-3'-Fd4CTP at the active site of HIV-1 RT. 3'-F is hydrogen bonded to the amide backbone of Asp185. (b) Binding site of HIV-1 RT. Deep and spacious enough to accommodate the unnatural L-nucleoside triphosphates. (c) Steric hindrance of L-3'-Fd4CTP with the bulky side chain of Val184 in M184V RT (left). In wild type RT, L-3'-Fd4CTP is in favorable distance from Met184 which may enable a stabilizing hydrophobic interaction (right).

In summary, we have developed an efficient synthesis of optically pure β -L-3'-Fd4 nucleosides in an attempt to evaluate the effect of substitution of the fluorine atom at the 3'-position of the 2',3'-unsaturated nucleosides, and it was found that 3'-F substitution also provides stability to the glycosidic bond. The stability of the glycosidic bond achieved by the 3'-fluorine substitution was found to be strong enough to provide potent antiviral activity to the cytidine **24** and 5-fluorocytidine **26** analogues even though it was not as strong as that of 2'-fluorine. Additionally, the molecular modeling study showed that the 3'-fluorine atom participates in binding of the L-3'-Fd4CTP at the active site of the HIV-1 RT by forming a hydrogen bond with the amide backbone of Asp185. However, the 5-fluorocytidine analogue **26** showed significant cross-resistance to the lamivudine-resistant HIV-1 RT, which could be explained by the disrupted binding mode by the steric hindrance with the bulky side chain of Val184.

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 ^1H NMR and 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. 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. 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-Methyl 2-deoxy-L-ribofuranoside (3). Methanolic HCl (1%, 50.4 ml made by adding 1.7 ml AcCl to 100 ml MeOH) was added to a solution of **2**²¹³ (25.2 g, 0.19 mol) in MeOH (250 ml). The reaction mixture was stirred at rt for 25 min and neutralized with Amberlite® IRA 400 (OH⁻) resin. After filtration followed by evaporation of the volatiles, the residual syrup was used for the next step without further purifications.

(+)-5-*O*-Benzoyl-2-deoxy-1-methyl-β-L-ribofuranose (4). **Methyl 2-deoxy-L-ribofuranoside 3** (1.1 g, 7.46 mmol) was dissolved in dry pyridine (10 ml), cooled to 0 °C and treated with BzCl. The mixture was stirred at rt for 10 h, and then diluted with cold water. The aqueous phase was extracted with CH₂Cl₂, and the combined organic layers were washed twice with aqueous NaHCO₃ solution, 2 N HCl and water. The organic layer was dried over MgSO₄, filtered and evaporated to give a crude, which was purified by column chromatography on silica gel (hexane:EtOAc = 2:1) to give 1.35 g (5.37 mmol, 72% yield) of **4** as a pale yellow oil (1:1 epimeric mixture): ¹H NMR (CDCl₃, 400 MHz) δ 8.08-8.07 (m, 2H), 8.03-8.01 (m, 2H), 7.60-7.56 (m, 2H), 7.47-7.43 (m, 4H), 5.15 (d, *J* = 4.5 Hz, 1H), 5.11 (dd, *J* = 5.3, 1.5 Hz, 1H), 4.57 (td, *J* = 6.8, 4.8 Hz, 1H), 4.43-4.36 (m, 4H), 4.26 (d, *J* = 6.1 Hz, 1H), 4.20 (q, *J* = 5.2 Hz, 1H), 3.41 (s, 3H), 3.32 (s, 3H), 2.32 (ddd, *J* = 13.3, 6.8, 1.5 Hz, 1H), 2.22-2.10 (m, 3H); HRMS (FAB) obsd, *m/z* 253.1083, calcd for C₁₃H₁₇O₅, *m/z* 253.1076 (M+H⁺). Anal. Calcd for C₁₃H₁₆O₅: C, 61.90; H, 6.39. Found: C, 61.57, H, 6.38.

(+)-Methyl 5-*O*-benzoyl-2-deoxy- β -L-glycero-pentofuranosid-3-ulose (5). Chromic anhydride (2.73 g, 27.30 mmol) was added to a stirred mixture of anhydrous pyridine (4.5 ml, 55.64 mmol) and anhydrous dichloromethane (50 ml) at rt, and stirring was continued at rt for 15 min. A solution of **4** (1.72 g, 6.82 mmol) in anhydrous dichloromethane (20 ml) was then added, followed immediately by acetic anhydride (2.6 ml, 27.51 mmol). After 15 min, the dark brown solution was treated with ethyl acetate (400 ml) and the resulting mixture was filtered through a TLC-grade silica gel pad, which was washed with ethyl acetate (one portion of 500 ml). The filtrate was concentrated under reduced pressure, co-evaporated with toluene (3x100 ml), and solvents were removed *in vacuo* to give 1.50 g (88%) of **5** as an orange oil: ^1H NMR (CDCl_3 , 400 MHz) δ 8.12-8.10 (m, 4H), 7.63-7.58 (m, 2H), 7.49-7.45 (m, 4H), 5.41 (t, J = 5.0 Hz, 2H), 4.80 (dd, J = 12.1, 2.7 Hz, 1H), 4.69 (dd, J = 11.7, 3.2 Hz, 1H), 4.51-4.47 (m, 2H), 4.44-4.42 (m, 1H), 4.31 (t, J = 3.0 Hz, 1H), 3.50 (s, 3H), 3.43 (s, 3H), 2.85 (dd, J = 18.3, 5.7 Hz, 1H), 2.71 (ddd, J = 18.2, 5.4, 0.6 Hz, 1H), 2.56 (d, J = 12.6 Hz, 1H), 2.52 (d, J = 12.4 Hz, 1H); HRMS (FAB) obsd, m/z 251.0920, calcd for $\text{C}_{13}\text{H}_{15}\text{O}_5$, m/z 251.0919 (MH^+). Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{O}_5 \cdot 0.07\text{C}_6\text{H}_5\text{CH}_3$: C, 63.12; H, 5.72. Found: C, 63.51, H, 5.37.

(+)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-1-methyl- β -L-ribofuranose (6). (Diethylamino)sulfur trifluoride (3.0 ml, 22.71 mmol) was added to a solution of **5** (1.40 g, 5.59 mmol) in anhydrous dichloromethane (25 ml) and the reaction mixture was refluxed for 36 h. The resulting solution was then carefully poured into an ice-cooled saturated solution of sodium bicarbonate (50 ml) and extracted with diethyl ether (4 x 100 ml). The combined organic extracts were washed with brine (50 ml), dried over MgSO_4 , filtered and concentrated to a crude oil that is purified by silica gel flash column

chromatography (1:9 ethyl acetate/hexanes) to give 1.01 g (66%) of **6** as a yellow oil: ^1H NMR (CDCl_3) δ 7.90-7.18(m, 4H), 5.10(m, 1H), 4.54-4.29(m, 3H), 3.31, 3.29(s, 3H), 3.28 (s, 3H), 2.67-2.21(m, 2H); HRMS (FAB) obsd, m/z 273.0950, calcd for $\text{C}_{13}\text{H}_{15}\text{F}_2\text{O}_4$, m/z 273.0938 ($\text{M}+\text{H}^+$). Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{F}_2\text{O}_4$: C, 57.35; H, 5.18. Found: C, 57.64, H, 5.30.

1-Acetyl-5-O-benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranose (7). Concentrated sulfuric acid (80 μl , 1.50 mmol) was added to an ice-cold solution of **6** (200 mg, 0.73 mmol) and acetic anhydride (300 μl , 3.20 mmol) in glacial acetic acid, and the reaction was stirred at 0 $^\circ\text{C}$ for 5 min, then at rt for 10 more min. The resulting mixture was poured into an ice-cold saturated solution of sodium bicarbonate (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic extracts were washed with water (30 ml), brine (30 ml), dried over MgSO_4 , filtered, concentrated and co-evaporated with toluene (3 x 10 ml) to give 210 mg (95%) of 5:3 epimeric mixture **7** as a crude yellow oil which was used in the following reaction without further purification: ^1H NMR (CDCl_3) δ 8.06-8.02 (m, 4H), 7.57 (m, 2H), 7.47-7.43 (m, 4H), 6.45 [d, 1H, H_1 (*major isomer*), $J = 5.3$ Hz], 6.41 [d, 1H, H_1 (*minor isomer*), $J = 5.9$ Hz], 4.65-4.49 (m, 6H, H_4 , H_5), 2.11 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (CDCl_3) (*major isomer*) δ 169.72, 165.91, 133.28, 129.68, 128.43, 127.50 (dd, $J = 254.9$, 251.6 Hz), 95.34 (dd, $J = 7.7$, 4.5 Hz), 79.10 (dd, $J = 33.0$, 24.7 Hz), 61.01 (dd, $J = 7.7$, 2.4 Hz), 41.91 (t, $J = 25.1$ Hz) 21.03; (*minor isomer*) δ 169.62, 165.91, 133.27, 129.73, 128.41, 127.35 (dd, $J = 255.1$, 251.3 Hz), 95.83 (dd, $J = 7.6$, 3.8 Hz), 80.31 (dd, $J = 32.0$, 25.0 Hz), 62.12 (dd, $J = 7.8$, 3.5 Hz), 41.27 (t, $J = 24.8$ Hz) 21.00; MS (FAB) m/z 301 ($\text{M}+\text{H}^+$).

General procedure for condensation reaction of the acetate **7** with pyrimidines. The preparation of cytosine derivatives **8** and **9** is representative.

(-)-*N*⁴-Benzoyl-1-[(1*S*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-*L*-ribofuranosyl] cytosine (8**) and (+)-*N*⁴-benzoyl-1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-*L*-ribofuranosyl] cytosine (**9**).** A mixture of *N*⁴-benzoylcytosine (225 mg, 1.05 mmol) and ammonium sulfate (7 mg, 0.053 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was heated under reflux for 4 h, then the solvent was removed *in vacuo* at 30-35 °C. To the residual oil, a solution of **7** (210 mg, 0.70 mmol) in anhydrous acetonitrile (10 ml) was added followed, upon cooling to 0 °C, by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.19 ml, 1.05 mmol). The resulting mixture was stirred at rt overnight, then diluted to 50 ml with dichloromethane and poured into an ice-cold saturated solution of NaHCO₃ (25 ml). The organic phase was separated, washed with brine (5 ml), dried over MgSO₄, filtered and concentrated to a crude that was purified by flash column chromatography (3:97 MeOH/CHCl₃) to give 80 mg of **8** (25%) as the first eluted product and 100 mg of **9** (31%) as the second eluted product. Both isomers are colorless oils, which become white solids upon trituration with diethyl ether and a drop of methanol. For the compound **8**; white solid, mp 166-167 °C (dec.); [α]²⁴_D -89.75° (*c* 0.31, CHCl₃); UV (MeOH) λ_{max} 259.5, 301.0; ¹³C NMR (CDCl₃, 100 MHz) δ 166.48, 165.91, 162.58, 154.55, 143.35, 133.59, 133.31, 129.63, 129.07, 128.60, 127.53, 125.71 (t, *J*_{C-F} = 253.6 Hz), 96.90, 84.39 (dd, *J*_{C-F} = 6.6, 3.6 Hz), 79.80 (dd, *J*_{C-F} = 31.5, 25.1 Hz), 60.77 (d, *J*_{C-F} = 4.8 Hz), 41.69 (t, *J*_{C-F} = 23.7 Hz); HRMS (FAB) obsd, *m/z* 456.1377, calcd for C₂₃H₂₀F₂N₃O₅, *m/z* 456.1371 (MH⁺). Anal. (C₂₃H₁₉F₂N₃O₅) C, H, N. For the compound **9**: white solid, mp 176-178 °C (dec.); [α]²⁴_D 42.61° (*c* 1.19, CHCl₃); UV (MeOH) λ_{max}

259.0; ^{13}C NMR (CDCl_3 , 100 MHz) δ 166.54, 165.80, 162.77, 154.62, 143.48, 133.51, 133.19, 129.58, 128.94, 128.60, 127.58, 126.51 (dd, $J_{\text{C-F}} = 255.7, 249.3$ Hz), 96.71, 86.83 (dd, $J_{\text{C-F}} = 8.1, 2.0$ Hz), 80.78 (dd, $J_{\text{C-F}} = 31.7, 25.2$ Hz), 61.47 (t, $J_{\text{C-F}} = 5.3$ Hz), 41.14 (t, $J_{\text{C-F}} = 24.0$ Hz); HRMS (FAB) obsd, m/z 456.1374, calcd for $\text{C}_{23}\text{H}_{20}\text{F}_2\text{N}_3\text{O}_5$, m/z 456.1371 (MH^+). Anal. ($\text{C}_{23}\text{H}_{19}\text{F}_2\text{N}_3\text{O}_5$) C, H, N.

(-)- N^4 -Benzoyl-1-[(1*S*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-5-fluorocytosine (10) and (+)- N^4 -Benzoyl-1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-5-fluorocytosine (11). See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **10** and **11** were obtained on 1.67-mmol scale in 59% yield: For the compound **10**; $[\alpha]_{\text{D}}^{28} -67.91^\circ$ (c 0.87, CH_2Cl_2); UV(CHCl_3) λ_{max} 326.5 nm; ^{13}C NMR (CDCl_3) δ 165.85, 152.25 (d, $J = 18.4$ Hz), 146.90, 140.06 (d, $J = 240.6$ Hz), 135.44, 133.60, 133.19, 129.90, 129.55, 129.01, 128.61, 128.34, 125.51 (t, $J = 253.9$ Hz), 124.09, 123.75, 82.69, 79.50 (dd, $J = 31.4, 24.9$ Hz), 60.58 (d, $J = 4.1$ Hz), 40.80 (t, $J = 23.8$ Hz); Anal. ($\text{C}_{23}\text{H}_{18}\text{F}_3\text{N}_3\text{O}_5$) C, H, N.; For the compound **11**; $[\alpha]_{\text{D}}^{27} 41.49^\circ$ (c 0.15, CHCl_3); UV(CHCl_3) λ_{max} 327.0 nm; ^{13}C NMR (CDCl_3) δ 165.80, 152.49 (d, $J = 16.5$ Hz), 146.91, 139.98 (d, $J = 240.2$ Hz), 135.53, 133.63, 133.23, 129.94, 129.62, 129.05, 128.68, 128.40, 126.35 (dd, $J = 257.7, 249.5$ Hz), 124.53, 124.23, 85.60, 80.79 (dd, $J = 31.1, 25.1$ Hz), 61.42 (d, $J = 5.6$ Hz), 40.80 (t, $J = 24.3$ Hz); Anal. ($\text{C}_{23}\text{H}_{18}\text{F}_3\text{N}_3\text{O}_5 \cdot 0.2\text{C}_6\text{H}_{14}$) C, H, N.

N^4 -Benzoyl-1-[(1*S*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl] thymine (12) and N^4 -Benzoyl-1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl] thymine (13). See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **12** and **13** were obtained on 0.62-mmol

scale in 53% yield: UV (MeOH) $\lambda_{\max}$ 263.5; ^{13}C NMR (CDCl_3 , 100 MHz) (*major isomer*) δ 165.87, 163.78, 150.25, 135.05, 133.47, 126.67 (dd, $J_{\text{C-F}} = 255.7, 251.1$ Hz), 111.71, 84.84 (t, $J_{\text{C-F}} = 5.9$ Hz), 80.15 (dd, $J_{\text{C-F}} = 31.3, 24.5$ Hz), 61.51 (dd, $J_{\text{C-F}} = 6.7, 4.1$ Hz), 40.34 (t, $J_{\text{C-F}} = 24.3$ Hz), 12.59; (*minor isomer*) δ 165.84, 163.45, 150.25, 133.82, 133.59, 125.91 (dd, $J_{\text{C-F}} = 255.1, 252.3$ Hz), 112.39, 81.18 (t, $J_{\text{C-F}} = 5.9$ Hz), 79.11 (dd, $J_{\text{C-F}} = 31.6, 24.6$ Hz), 60.88 (dd, $J_{\text{C-F}} = 6.1, 3.1$ Hz), 40.47 (t, $J_{\text{C-F}} = 23.9$ Hz), 12.32; HRMS (FAB) obsd, m/z 367.1122, calcd for $\text{C}_{17}\text{H}_{17}\text{F}_2\text{N}_2\text{O}_5$, m/z 367.1106 (MH^+). Anal. ($\text{C}_{17}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_5 \cdot 0.03 \text{CHCl}_3$) C, H, N.

1-[(1*S*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]uracil (14) and 1-[(1*R*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]uracil (15). See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **14** and **15** were obtained on 1.67-mmol scale in 35 % yield: UV(CHCl_3) $\lambda_{\max}$ 256.5 nm; Anal. ($\text{C}_{16}\text{H}_{14}\text{F}_2\text{N}_2\text{O}_5$) C, H, N.

General procedure for the deprotection reaction of the pyrimidine analogues **8** ~ **15** with methanolic ammonia. The preparation of cytosine derivative **16** is representative.

(-)-1-[(1*S*, 4*S*)-2,3-Dideoxy-3,3-difluoro- β -L-ribofuranosyl]cytosine (16). A mixture of **8** (70 mg, 0.15 mmol) in saturated ammonia/methanol solution (10 ml) was stirred at rt for 4 h, and then concentrated to dryness under reduced pressure. The residue was purified by preparative TLC (1:9 methanol/chloroform) to give 38 mg of **16** (100%) as a white solid.: mp 194-196 °C (dec.); $[\alpha]_{\text{D}}^{24} -51.89^\circ$ (c 1.15, MeOH); UV (MeOH) $\lambda_{\max}$ 276.5 (ϵ 18160) (pH 2), 268.0 (ϵ 13280) (pH 7), 268.5 (ϵ 13580) (pH 11); ^{13}C NMR (DMSO-d_6 , 100 MHz) δ 168.19, 158.46, 142.62, 128.71 (dd, $J_{\text{C-F}} = 255.1, 247.4$ Hz), 97.04, 84.73 (dd, $J_{\text{C-F}} = 6.7, 4.9$ Hz), 83.74 (dd, $J_{\text{C-F}} = 29.5, 25.0$ Hz), 60.73 (t, $J_{\text{C-F}} = 4.8$

Hz), 42.14 (t, $J_{\text{C-F}} = 23.4$ Hz), 12.29; HRMS (FAB) obsd, m/z 248.0850, calcd for $\text{C}_9\text{H}_{12}\text{F}_2\text{N}_3\text{O}_3$, m/z 248.0847 (MH^+). Anal. ($\text{C}_9\text{H}_{11}\text{F}_2\text{N}_3\text{O}_3 \cdot 0.1 \text{ H}_2\text{O}$) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]cytosine (17). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compound **17** was obtained on 0.09-mmol scale in 100% yield: White solid, mp 198-200 (dec.); $[\alpha]_{\text{D}}^{25}$ 28.23° (c 0.27, MeOH); UV (MeOH) λ_{max} 277.5 (ϵ 15010) (pH 2), 269.0 (ϵ 10130) (pH 7), 269.0 (ϵ 10150) (pH 11); ^{13}C NMR (CD_3OD , 100 MHz) δ 168.37, 158.54, 142.57, 129.28 (dd, $J_{\text{C-F}} = 254.3, 247.2$ Hz), 96.48, 87.75 (dd, $J_{\text{C-F}} = 8.7, 3.6$ Hz), 84.93 (dd, $J_{\text{C-F}} = 29.3, 25.3$ Hz), 61.24 (t, $J_{\text{C-F}} = 5.4$ Hz), 42.63 (t, $J_{\text{C-F}} = 23.9$ Hz), 12.29; HRMS (FAB) obsd, m/z 248.0829, calcd for $\text{C}_9\text{H}_{12}\text{F}_2\text{N}_3\text{O}_3$, m/z 248.0847 (MH^+). Anal. ($\text{C}_9\text{H}_{11}\text{F}_2\text{N}_3\text{O}_3$) C, H, N.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-5-fluorocytosine (18) and (+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-5-fluorocytosine (19).

See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compounds **18** and **19** were obtained on 1.29-mmol scale in 47% and 43% yield, respectively: For the compound **18**; $[\alpha]_{\text{D}}^{25}$ -54.71° (c 0.99, CH_3OH); UV(H_2O) λ_{max} 283.5 nm (ϵ 10,050, pH 2). 278.0 nm (ϵ 8,430, pH 7), 278.0 nm (ϵ 8,350, pH 11); ^{13}C NMR (CD_3OD) δ 150.30 (d, $J = 14.1$ Hz), 146.82, 129.14 (d, $J = 241.0$ Hz), 118.67 (dd, $J = 255.9, 247.1$ Hz), 116.73 (d, $J = 33.3$ Hz), 74.93 (dd, $J = 7.8, 3.5$ Hz), 73.82 (dd, $J = 29.6, 25.1$ Hz), 50.69 (t, $J = 4.9$ Hz), 32.02 (t, $J = 23.4$ Hz); HRMS (FAB) obsd, m/z 266.08, calcd for $\text{C}_9\text{H}_{11}\text{F}_3\text{N}_3\text{O}_3$, m/z 266.07 ($\text{M}+\text{H}$) $^+$; Anal. ($\text{C}_9\text{H}_{10}\text{F}_3\text{N}_3\text{O}_3$) C, H, N. For the compound **19**; mp 159-160 °C; $[\alpha]_{\text{D}}^{25}$ 19.68° (c 0.23, CH_3OH); UV(H_2O) λ_{max} 284.0 nm (ϵ 7,494, pH 2). 278.5 nm (ϵ 5,816, pH 7), 278.5 nm

(ϵ 6,030, pH11); ^{13}C NMR (CD_3OD) δ 159.86 (d, $J = 14.0$ Hz), 156.28, 138.51 (d, $J = 242.3$ Hz), 128.65 (dd, $J = 255.1, 247.2$ Hz), 126.20 (d, $J = 34.1$ Hz), 87.27 (d, $J = 5.5$ Hz), 84.44 (dd, $J = 29.0, 25.5$ Hz), 60.69 (t, $J = 5.3$ Hz), 42.02 (t, $J = 24.1$ Hz); Anal. ($\text{C}_9\text{H}_{10}\text{F}_3\text{N}_3\text{O}_3$) C, H, N.

(+/-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]thymine (20) and (-)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]thymine (21). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compounds **20** and **21** were obtained on 0.38 mmol scale in 55% and 45% yield, respectively: For the compound **20**; White solid: mp 140-148 °C (dec.); $[\alpha]_{\text{D}}^{22} -31.27^\circ$ (c 0.21, CHCl_3); UV (MeOH) λ_{max} 264.0 (ϵ 11700) (pH 2), 263.5 (ϵ 12170) (pH 7), 264.0 (ϵ 8660) (pH 11); ^{13}C NMR (DMSO-d_6 , 100 MHz) δ 163.59, 150.36, 135.40, 127.26 (dd, $J_{\text{C-F}} = 255.8, 246.7$ Hz), 110.20, 80.97 (dd, $J_{\text{C-F}} = 28.5, 24.4$ Hz), 80.32 (dd, $J_{\text{C-F}} = 7.1, 4.1$ Hz), 58.50, 38.54 (t, $J_{\text{C-F}} = 23.6$ Hz), 12.29; HRMS (FAB) obsd, m/z 263.0849, calcd for $\text{C}_{10}\text{H}_{13}\text{F}_2\text{N}_2\text{O}_4$, m/z 263.0843 (MH^+). Anal. ($\text{C}_{10}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_4 \cdot 0.14 \text{Et}_2\text{O}$) C, H, N. For the compound **21**; White solid: mp 145-148 °C (dec.); $[\alpha]_{\text{D}}^{24} -26.50^\circ$ (c 0.15, CHCl_3); UV (MeOH) λ_{max} 265.5 (ϵ 12820) (pH 2), 263.5 (ϵ 12590) (pH 7), 264.0 (ϵ 10030) (pH 11); ^{13}C NMR (DMSO-d_6 , 100 MHz) δ 163.77, 150.38, 136.32, 127.77 (dd, $J_{\text{C-F}} = 252.3, 249.2$ Hz), 109.82, 83.47 (t, $J_{\text{C-F}} = 6.0$ Hz), 81.75 (dd, $J_{\text{C-F}} = 28.5, 24.3$ Hz), 58.76, 38.57 (t, $J_{\text{C-F}} = 23.6$ Hz), 12.17; HRMS (FAB) obsd, m/z 263.0835, calcd for $\text{C}_{10}\text{H}_{13}\text{F}_2\text{N}_2\text{O}_4$, m/z 263.0843 (MH^+). Anal. ($\text{C}_{10}\text{H}_{12}\text{F}_2\text{N}_2\text{O}_4 \cdot 0.5 \text{MeOH}$) C, H, N.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]uracil (22) and (-)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]uracil (23). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The

title compounds **22** and **23** were obtained on 0.57-mmol scale in 42% and 56% yield, respectively: For the compound **22**; mp 138~140 °C; $[\alpha]_D^{26}$ -25.86° (c 0.21, MeOH); UV(H₂O) $\lambda_{\max}$ 259.0 nm (ϵ 8,725, pH 2). 259.0 nm (ϵ 9,145, pH 7), 259.0 nm (ϵ 6,638, pH 11); ¹³C NMR (CD₃OD) δ 165.92, 152.01, 141.79, 128.09 (dd, J = 255.5, 247.4 Hz), 103.35, 83.20 (dd, J = 29.6, 24.7 Hz), 83.08 (dd, J = 8.0, 4.3 Hz), 60.21 (t, J = 4.7 Hz), 40.87 (t, J = 23.6 Hz); Anal. (C₉H₁₀F₂N₂O₄) C, H, N. For the compound **23**; mp 194~196 °C; $[\alpha]_D^{25}$ -5.3° (c 0.32, MeOH); UV(H₂O) $\lambda_{\max}$ 259.0 nm (ϵ 8,833, pH 2). 259.0 nm (ϵ 9,150, pH 7), 259.0 nm (ϵ 6,629, pH 11); ¹³C NMR (CD₃OD) δ 166.58, 152.42, 142.50, 129.14 (dd, J = 254.4, 247.5 Hz), 103.23, 86.95 (dd, J = 8.1, 4.0 Hz), 84.78 (dd, J = 29.2, 25.2 Hz), 61.09 (t, J = 5.4 Hz), 41.82 (t, J = 24.2 Hz); Anal. (C₉H₁₀F₂N₂O₄) C, H, N.

General procedure for the elimination reaction of the pyrimidine analogues **16** ~ **23** with sodium methoxide. The preparation of cytosine derivative **24** is representative.

(+)-1-[(1*S*, 4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]cytosine (24**).**

A mixture of **16** (30 mg, 0.12 mmol) and sodium methoxide (50 mg, 0.37 mmol) in anhydrous DMF (5 ml) was stirred at rt overnight, then quenched with 1 ml of a saturated aqueous solution of ammonium chloride. Evaporation of volatiles *in vacuo* gave a crude, which was purified by a short silica gel flash column chromatography (CHCl₃ to 1:15 MeOH/CHCl₃) to yield 15 mg (54%) of **24** as a white solid.: mp 182-183 °C (dec.); $[\alpha]_D^{22}$ 6.67° (c 0.54, MeOH); UV (MeOH) $\lambda_{\max}$ 276.0 (ϵ 11990) (pH 2), 267.5 (ϵ 8010) (pH 7), 264.5 (ϵ 8060) (pH 11); ¹³C NMR (CD₃OD, 100 MHz) δ 168.31, 163.40 (d, J_{C-F} = 284.8 Hz), 159.05, 144.05, 102.34 (d, J_{C-F} = 9.7 Hz), 95.68, 88.42 (d, J_{C-F} = 14.9 Hz), 81.96 (d, J_{C-F} = 24.7 Hz), 61.82 (d, J_{C-F} = 2.2 Hz); HRMS (FAB) obsd, m/z 228.0778, calcd for C₉H₁₁FN₃O₃, m/z 228.0784 (MH⁺). Anal. (C₉H₁₀FN₃O₃) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- α -L-ribofuranosyl]cytosine (25).

See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **25** was obtained on 0.06-mmol scale in 51% yield: White solid: mp 135 °C (dec.); $[\alpha]_D^{24}$ 169.67° (*c* 1.17, MeOH); UV (MeOH) $\lambda_{\max}$ 276.5 (ϵ 14140) (pH 2), 268.0 (ϵ 9510) (pH 7), 268.5 (ϵ 9660) (pH 11); ^{13}C NMR (CD_3OD , 100 MHz) δ 167.67, 163.00 (d, $J_{\text{C-F}}$ = 285.1 Hz), 158.31, 142.36, 101.93 (d, $J_{\text{C-F}}$ = 10.0 Hz), 96.82, 89.55 (d, $J_{\text{C-F}}$ = 15.1 Hz), 81.80 (d, $J_{\text{C-F}}$ = 24.9 Hz), 61.88 (d, $J_{\text{C-F}}$ = 2.2 Hz); HRMS (FAB) obsd, *m/z* 228.0773, calcd for $\text{C}_9\text{H}_{11}\text{FN}_3\text{O}_3$, *m/z* 228.0784 (MH⁺). Anal. ($\text{C}_9\text{H}_{10}\text{FN}_3\text{O}_3 \cdot 0.82\text{MeOH}$) C, H, N.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-5-fluoro-

cytosine (26). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **26** was obtained on 0.12-mmol scale in 95% yield: mp 124 °C; $[\alpha]_D^{25}$ -25.47° (*c* 0.10, CH_3OH); UV(H_2O) $\lambda_{\max}$ 284.0 nm (ϵ 10,057, pH 2). 277.5 nm (ϵ 8,191, pH 7), 277.5 nm (ϵ 8,519, pH 11); ^{13}C NMR (CD_3OD) δ 162.99 (d, J = 285.1 Hz), 159.91 (d, J = 14.2 Hz), 156.91, 138.37 (d, J = 242.0 Hz), 127.44 (d, J = 33.0 Hz), 101.83 (d, J = 10.0 Hz), 88.32 (d, J = 15.0 Hz), 81.51 (d, J = 24.8 Hz), 61.08; Anal. ($\text{C}_9\text{H}_9\text{F}_2\text{N}_3\text{O}_3$) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- α -L-ribofuranosyl]-5-fluoro-

cytosine (27). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **27** was obtained on 0.15-mmol scale in 94% yield: mp 146 °C (dec.); $[\alpha]_D^{26}$ 143.04° (*c* 0.13, CH_3OH); UV(H_2O) $\lambda_{\max}$ 284.0 nm (ϵ 7,837, pH 2). 277.5 nm (ϵ 6,280, pH 7), 278.0 nm (ϵ 6,033, pH 11); ^{13}C NMR (CD_3OD) δ 163.23 (d, J = 281.8 Hz), 159.89 (d, J = 14.0 Hz), 156.77, 138.77 (d, J =

243.7 Hz), 126.25 (d, $J = 31.8$ Hz), 101.75 (d, $J = 10.1$ Hz), 90.06 (d, $J = 15.2$ Hz), 81.86 (d, $J = 25.2$ Hz), 61.86; Anal. ($C_9H_9F_2N_3O_3$) C, H, N.

(+)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]thymine (28).

See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **28** was obtained on 0.11-mmol scale in 54% yield: White solid: mp 146-148 °C (dec.); $[\alpha]_D^{22}$ 32.19° (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} 264.0 (ϵ 10080) (pH 2), 264.5 (ϵ 10130) (pH 7), 264.5 (ϵ 7710) (pH 11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.01, 161.54 (d, $J_{C-F} = 287.8$ Hz), 150.50, 136.85, 110.83, 99.64 (d, $J_{C-F} = 10.2$ Hz), 85.65 (d, $J_{C-F} = 14.8$ Hz), 79.98 (d, $J_{C-F} = 24.5$ Hz), 60.56 (d, $J_{C-F} = 1.8$ Hz), 12.28; HRMS (FAB) obsd, m/z 243.0789, calcd for $C_{10}H_{12}FN_2O_4$, m/z 243.0781 (MH^+). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

(+/-)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]thymine (29).

See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **29** was obtained on 0.10-mmol scale in 51% yield White solid: mp 146-148 °C (dec.); $[\alpha]_D^{22}$ -18.68° (c 0.22, CH_3OH); UV (MeOH) λ_{max} 264.0 (ϵ 10075) (pH 2), 264.5 (ϵ 10130) (pH 7), 264.5 (ϵ 7708) (pH 11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.61, 161.53 (d, $J_{C-F} = 289.0$ Hz), 150.83, 134.66, 111.96, 99.99 (d, $J_{C-F} = 10.1$ Hz), 86.97 (d, $J_{C-F} = 14.9$ Hz), 80.25 (d, $J_{C-F} = 24.6$ Hz), 61.42, 12.56; HRMS (FAB) obsd, m/z 243.0773, calcd for $C_{10}H_{12}FN_2O_4$, m/z 243.0781 (MH^+). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

(+)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-dideoxy-3-fluoro- β -L-ribofuranosyl]uracil (30).

A solution of **22** (56 mg, 0.23 mmol) in a 1:2 mixture of anhydrous dioxane (1ml) and DMF (2ml) was treated with sodium methoxide (49 mg, 0.90 mmol) at rt, and the resulting

mixture was stirred at 50~55 °C for 6h. After diluting with MeOH, the mixture was filtered through a short silica gel pad washing with 10:1 CH₂Cl₂:MeOH. The filtrate was evaporated to dryness under reduced pressure, and the residue was purified by a short silica gel flash column chromatography (20:1 CH₂Cl₂/MeOH) to yield 20 mg (0.09 mmol, 54% yield) of **30** as a white solid: mp 92~94 °C; $[\alpha]_D^{22}$ 11.74° (c 0.61, MeOH); UV(H₂O) $\lambda_{\max}$ 259.0 nm (ϵ 26,681, pH 2). 258.5 nm (ϵ 15,591, pH 7), 259.0 nm (ϵ 11,073, pH 11); ¹³C NMR (CD₃OD) δ 166.27, 163.31 (d, J = 285.4 Hz), 152.56, 143.16, 102.59, 101.19 (d, J = 10.8 Hz), 87.13 (d, J = 15.2 Hz), 81.53 (d, J = 24.5 Hz), 61.25 (d, J = 2.6 Hz); Anal. (C₉H₉FN₂O₄) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- α -L-ribofuranosyl]uracil (31).

See the procedure for the preparation of the uridine analogue **30** by elimination reaction of **22** with sodium methoxide. The title compound **31** was obtained on 0.36-mmol scale in 41% yield: mp 88~90 °C; $[\alpha]_D^{23}$ 184.6° (c 0.30, MeOH); UV(H₂O) $\lambda_{\max}$ 259.0 nm (ϵ 28,099, pH 2). 258.5 nm (ϵ 15,700, pH 7), 259.5 nm (ϵ 12,669, pH 11); ¹³C NMR (CD₃OD) δ 166.11, 163.41 (d, J = 285.7 Hz), 152.31, 142.09, 103.34, 101.30 (d, J = 10.8 Hz), 88.77 (d, J = 15.4 Hz), 81.91 (d, J = 25.2 Hz), 61.77; Anal. (C₉H₉FN₂O₄·0.2C₂H₅OC₂H₅) C, H, N.

9-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-6-chloropurine (32) and 9-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-6-chloropurine (33).

A mixture of 6-chloropurine (1.03 g, 6.66 mmol), ammonium sulfate (44 mg, 0.33 mmol) and HMDS (20ml) was refluxed for 6h. After cooling to rt, volatiles were evaporated under reduced pressure. To the residue, a solution of **7** in anhydrous CH₃CN was added, and the mixture was cooled to 0 °C, whereupon TMSOTf was added

dropwise. The mixture was stirred for 8 h at 0 °C, 10 h at rt and then 3 h at 60 °C. The reaction mixture was poured into an aqueous NaHCO₃ solution and extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered and evaporated to give a yellow residue which was purified by column chromatography on silica gel (1:2 Hexane:EtOAc) to give 610 mg (1.55 mmol, 47% yield) of a inseparable mixture of **32** and **33** as a pale yellow oil: UV(CH₂Cl₂) λ_{max} 266.6 nm; HRMS (FAB) obsd, m/z 395.0723, calcd for C₁₇H₁₈ClF₂N₄O₃, m/z 395.0719 (M+H⁺); Anal. (C₁₇H₁₃ClF₂N₄O₃·0.3H₂O) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-adenine (34) and (-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-adenine (35). A mixture of compound **32** and compound **33** (0.1 g, 0.25 mmol) was treated with aqueous ammonia in a steel bomb at 60 °C for 8 h. Solvent was evaporated and the residue was purified by column chromatography (20:1 CH₂Cl₂:MeOH) to give the desired compounds **34** (95 mg, 0.35 mmol 42% yield) and **35** (100 mg, 0.37 mmol, 44% yield) as white solids. For the compound **34**; mp 198~200 °C; $[\alpha]_{\text{D}}^{25}$ 18.21° (c 0.45, CH₃OH); UV(H₂O) λ_{max} 256.0 nm (ϵ 12,797, pH 2). 258.0 nm (ϵ 13,143, pH 7), 258.5 nm (ϵ 12,876, pH11); ¹³C NMR (CD₃OD) δ 160.70, 157.58, 153.86, 150.21, 141.17, 128.22 (t, J = 257.6 Hz), 120.60, 83.81 (dd, J = 29.2, 24.6 Hz), 83.35 (d, J = 7.7 Hz), 60.96 (t, J = 5.7 Hz), 40.90 (t, J = 24.1 Hz); Anal. (C₁₀H₁₁F₂N₅O₂) C, H, N.

For the compound **35**; mp 152~154 °C; $[\alpha]_{\text{D}}^{24}$ -36.67° (c 0.47, CH₃OH); UV(H₂O) λ_{max} 256.0 nm (ϵ 14,121, pH 2). 258.0 nm (ϵ 14,226, pH 7), 258.5 nm (ϵ 14,598, pH11); ¹³C NMR (CD₃OD) δ 157.40, 154.03, 150.38, 140.79, 128.88 (t, J = 252.1 Hz), 120.61, 84.13

(t, $J = 6.0$ Hz), 83.71 (dd, $J = 29.3, 24.7$ Hz), 60.36 (dd, $J = 6.8, 3.1$ Hz), 41.15 (t, $J = 25.0$ Hz); Anal. ($C_{10}H_{11}F_2N_5O_2$) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-hypoxanthine (36) and (-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-hypoxanthine (37). A mixture of compounds **32** and **33** (0.222 g, 0.56 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.16 mL, 2.25 mmol) and NaOMe (0.125 g, 2.31 mmol) at 60 °C for 6 h. The resulting mixture was quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel. The filtrate was concentrated and purified by column chromatography (10:1 CH_2Cl_2 :MeOH) to give the desired compounds **36** (0.06 g, 0.22 mmol, 39% yield) and **37** (0.056 g, 0.21 mmol, 38% yield) as white solids: For the compound **36**; mp 166~168 °C; $[\alpha]^{24}_D$ 5.82° (c 0.21, CH_3OH); UV(H_2O) λ_{max} 252.5 nm (ϵ 18,306, pH 2). 249.0 nm (ϵ 19,825, pH 7), 257.5 nm (ϵ 20,988, pH11); ^{13}C NMR (CD_3OD) δ 158.85, 149.75 (dd, $J = 276.7, 264.3$ Hz), 147.10, 140.36, 125.74, 111.57, 83.80 (t, $J = 24.6$ Hz), 83.00 (d, $J = 6.0$ Hz), 60.68, 41.25 (t, $J = 24.5$ Hz); Anal. ($C_{10}H_{10}F_2N_4O_3$) C, H, N. For the compound **37**; mp 103~105 °C; $[\alpha]^{25}_D$ -39.39° (c 0.323, CH_3OH); UV(H_2O) λ_{max} 250.0 nm (ϵ 19,258, pH 2). 250.0 nm (ϵ 19,480, pH 7), 258.0 nm (ϵ 22,384, pH11); ^{13}C NMR (CD_3OD) δ 159.28, 150.84 (t, $J = 227.0$ Hz), 147.33, 140.76, 126.48, 109.46, 84.90 (d, $J = 5.8$ Hz), 84.15 (dd, $J = 29.0, 24.6$ Hz), 60.71 (t, $J = 4.0$ Hz), 41.83 (t, $J = 24.9$ Hz); Anal. ($C_{10}H_{10}F_2N_4O_3$) C, H, N.

(-)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-adenine (38).

A solution of **34** (30 mg, 0.11 mmol) in a 2:1 mixture of anhydrous dioxane (1.5 ml) and DMF (0.7 ml) was treated with NaOMe (18 mg, 0.33 mmol) at 0 °C, and the resulting mixture was stirred for 15h at rt. The crude was filtered through a short silica gel pad and

the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (10:1 CH₂Cl₂:MeOH) to give the desired product **38** (20 mg, 0.08 mmol, 73% yield) as a white solid: mp 148 °C (dec.); [α]_D²⁵ -11.78° (c 0.18, CH₃OH); UV(H₂O) λ_{max} 258.0 nm (ϵ 11,310, pH 2), 258.5 nm (ϵ 12,814, pH 7), 258.5 nm (ϵ 13,283, pH11); ¹³C NMR (CD₃OD) δ 163.23 (d, J = 285.1 Hz), 157.42, 153.84, 150.30, 141.56, 120.21, 101.33 (d, J = 11.3 Hz), 86.39 (d, J = 15.5 Hz), 82.16 (d, J = 24.7 Hz), 61.64; Anal. (C₁₀H₁₀FN₅O₂) C, H, N.

(+)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- α -L-ribofuranosyl]-adenine (39).

See the procedure for the preparation of the adenosine analogue **38** by elimination reaction of **34** with sodium methoxide. The title compound **39** was obtained on 0.16-mmol scale in 62% yield: mp 146 °C (dec); [α]_D²⁴ 86.06° (c 0.66, CH₃OH); UV(H₂O) λ_{max} 258.5 nm (ϵ 10,076, pH 2), 258.5 nm (ϵ 11,042, pH 7), 258.5 nm (ϵ 10,812, pH11); ¹³C NMR (CD₃OD) δ 163.70 (d, J = 285.5 Hz), 157.36, 154.08, 150.41, 140.64, 120.41, 101.10 (d, J = 11.4 Hz), 87.19 (d, J = 15.7 Hz), 81.88 (d, J = 25.2 Hz), 61.66; Anal. (C₁₀H₁₀FN₅O₂) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-

hypoxanthine (40). A solution of **36** (52 mg, 0.19 mmol) in a 2:1 mixture of anhydrous dioxane (1 ml) and DMF (0.5 ml) was treated with NaOMe (31 mg, 0.57 mmol) at 0 °C, and the resulting mixture was stirred for 12 h at rt. A mixture of NaOMe (31 mg, 0.57 mmol) in 0.5 ml of DMF was added to the mixture, and the mixture was stirred for 12 h at rt. The crude was filtered through a short silica gel pad and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (10:1 CH₂Cl₂:MeOH) to give the desired product **40** (15 mg, 0.06 mmol, 32% yield) as a white

solid and the starting material **36** (17 mg, 0.06 mmol) recovered: mp 200 °C (dec.); $[\alpha]^{24}_D$ 6.74° (c 0.17, CH₃OH); UV(H₂O) $\lambda_{\max}$ 247.5 nm (ϵ 6,346, pH 2). 248.5 nm (ϵ 6,505, pH 7), 256.5 nm (ϵ 11,296, pH11); ¹³C NMR (CD₃OD) δ 160.65 (d, J = 271.7 Hz), 150.03, 147.00, 140.93, 125.25, 118.01, 101.19 (d, J = 11.3 Hz), 86.26 (d, J = 15.1 Hz), 82.20 (d, J = 24.6 Hz), 61.45; Anal. (C₁₀H₉FN₄O₃) C, H, N.

9-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-6-chloro-2-fluoropurine (42) and 9-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-6-chloro-2-fluoropurine (43). A mixture of 6-chloro-2-fluoropurine (1.26 g, 7.33 mmol), ammonium sulfate (220 mg, 1.67 mmol) and HMDS (40 ml) was heated under reflux for 4 h until a clear suspension was obtained. After cooling to rt, volatiles were evaporated under reduced pressure. To the off-white solid, a solution of **7** (1 g, 3.33 mmol) in anhydrous CH₃CN was added, and the mixture was cooled to 0 °C, whereupon TMSOTf (0.96 ml, 5.33 mmol), was added dropwise. The mixture was stirred for 1 h at 0 °C, 24 h at rt and then 3 h at 60 °C. To a separatory funnel containing 100 ml of aqueous NaHCO₃ solution, the reaction mixture was slowly added. After diluting with CH₂Cl₂ and phase separation, the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, evaporated and purified by column chromatography on silica gel (4:1 hexane:EtOAc) to give 1.07 g (2.6 mmol, 78% yield) of an inseparable mixture of **42** and **43** as a pale yellow foam : UV(CH₂Cl₂) $\lambda_{\max}$ 269.5 nm; Anal. (C₁₇H₁₂ClF₃N₄O₃·0.04C₆H₁₄) C, H, N.

(+)-1-[(1*R*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-2-amino-6-chloropurine (44) and (+)-1-[(1*S*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-2-amino-6-chloropurine (45). Anhydrous ammonia was bubbled into a

solution of a mixture of **42** and **43** (152 mg, 0.37 mmol) in dry dimethoxyethane (20 ml) at rt for 6h. After evaporation of volatiles, the residue was filtered through a short silica gel pad to remove inorganic salts, and the filtrate was concentrated and purified by column chromatography on silica gel (3:1 hexane:EtOAc) to give the desired products **44** (43 mg, 29% yield) and **45** (42 mg, 28% yield) as white foams: For the compound **44**; $[\alpha]_D^{23}$ 10.18° (c 0.17, CH₂Cl₂); UV(CH₂Cl₂) $\lambda_{\max}$ 300.0 nm; ¹³C NMR (CDCl₃) δ 165.89, 159.11, 153.09, 151.73, 140.33, 133.35, 129.61, 129.15, 128.42, 126.83 (t, J = 253.8 Hz), 125.59, 82.40 (t, J = 5.3 Hz), 79.47 (dd, J = 31.1, 24.1 Hz), 61.05 (d, J = 7.3 Hz), 39.51 (t, J = 25.2 Hz); Anal. (C₁₇H₁₄ClF₂N₅O₃) C, H, N. For the compound **45**; $[\alpha]_D^{22}$ 6.27° (c 2.011, CH₂Cl₂); UV(CH₂Cl₂) $\lambda_{\max}$ 300.0 nm; ¹³C NMR (CDCl₃) δ 165.92, 159.16, 153.07, 151.64, 139.74, 133.38, 129.53, 129.10, 128.43, 126.19 (dd, J = 256.4, 251.2 Hz), 125.33, 81.24 (t, J = 5.8 Hz), 79.41 (dd, J = 31.3, 24.3 Hz), 61.37 (t, J = 4.3 Hz), 39.60 (t, J = 24.4 Hz); Anal. (C₁₇H₁₄ClF₂N₅O₃) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl] guanine (46**).** A solution of **44** (166 mg, 0.41 mmol) in anhydrous MeOH (20 ml) was treated with 2-mercaptoethanol (0.11 ml, 1.62 mmol) and NaOMe (90 mg, 1.66 mmol), and the resulting mixture was stirred for 18 h at 60 °C. After cooling to rt, 0.2 ml of AcOH was added. The volatiles were removed under reduced pressure, and the remaining inorganic salts were removed by filtering the residue through a short silica gel pad washing with 8:1 CH₂Cl₂:MeOH. The filtrate was concentrated to give a white solid, which was dissolved in a small amount of DMF and purified by column chromatography on silica gel (8:1 CH₂Cl₂:MeOH) to give 60 mg (0.21 mmol, 51% yield) of **46** as a white solid: mp >300 °C; $[\alpha]_D^{25}$ 25.49° (c 0.189, DMF); UV(H₂O) $\lambda_{\max}$ 253.5 nm (ϵ 12,628, pH 2). 251.5

nm (ϵ 11,901, pH 7), 258.0 nm (ϵ 10,140, pH11); ^{13}C NMR (DMSO- d_6) δ 160.30, 157.48, 154.57, 138.49, 130.93 (dd, $J = 255.4, 246.4$ Hz), 120.09, 84.83 (t, $J = 28.2$ Hz), 82.80 (t, $J = 4.1$ Hz), 62.36, 42.17 (t, $J = 23.7$ Hz); Anal. ($\text{C}_{10}\text{H}_{11}\text{F}_2\text{N}_5\text{O}_3$) C, H, N.

(+)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl] guanine(47). See the procedure for the preparation of the guanosine analogue **46** from **44** by hydrolysis reaction using 2-mercaptoethanol and sodium methoxide. The title compound **47** was obtained on 0.27-mmol scale in 80% yield: mp >250 $^\circ\text{C}$; $[\alpha]^{24}_{\text{D}}$ 24.40 $^\circ$ (c 0.157, DMF); UV(H_2O) λ_{max} 254.0 nm (ϵ 14,309, pH 2), 254.0 nm (ϵ 14,464, pH 7), 258.0 nm (ϵ 12,693, pH 11); ^{13}C NMR (CD_3OD) δ 165.97, 161.12 (dd, $J = 272.3, 246.3$ Hz), 152.70, 137.79, 128.81, 117.96, 83.56 (dd, $J = 29.5, 24.9$ Hz), 82.55 (d, $J = 8.9$ Hz), 60.84 (t, $J = 5.5$ Hz), 40.83 (t, $J = 24.1$ Hz); Anal. ($\text{C}_{10}\text{H}_{11}\text{F}_2\text{N}_5\text{O}_3 \cdot 0.9\text{H}_2\text{O}$) C, H, N.

(+)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl] guanine (48).

A solution of **46** (110 mg, 0.38 mmol) in anhydrous DMF (1.5 ml) was treated with NaOMe (83 mg, 1.53 mmol), and the resulting mixture was stirred for 24 h at rt. Two more equivalents (41 mg) of sodium methoxide was added, and the mixture was stirred for 12 h at rt. The mixture was filtered through a short silica gel pad washing with 7:1 CH_2Cl_2 :MeOH. The filtrate was evaporated to dryness and the residue was dissolved in a small amount of DMF. The undissolved solid was filtered off and the filtrate was evaporated to dryness again. The residue was dissolved in a small amount of DMF and purified by column chromatography on silica gel (CH_2Cl_2 :MeOH = 8:1) to give a pale yellow gel, which was trituated with CH_2Cl_2 and Et_2O to give the desired product **48** (35 mg, 0.13 mmol, 46% yield) as an off-white solid: mp >250 $^\circ\text{C}$; $[\alpha]^{24}_{\text{D}}$ 44.25 $^\circ$ (c 0.677, CH_3OH); UV(H_2O) λ_{max} 248.5 nm (ϵ 18,111, pH 2), 251.5 nm (ϵ 17,481, pH 7), 260.0

nm (ϵ 19,537, pH11); ^{13}C NMR (CD_3OD) δ 163.45 (d, $J = 285.0$ Hz), 159.35, 149.12, 137.41, 117.96 (d, $J = 10.7$ Hz), 109.70, 101.27 (d, $J = 11.4$ Hz), 86.87 (d, $J = 15.7$ Hz), 81.73 (d, $J = 25.1$ Hz), 61.67; Anal. ($\text{C}_{10}\text{H}_{10}\text{FN}_5\text{O}_3 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

(+)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- α -L-ribofuranosyl] guanine (49).

See the procedure for the preparation of the guanosine analogue **48** from **46** by elimination reaction using sodium methoxide in DMF. The title compound **49** was obtained on 0.10-mmol scale in 33% yield : mp 200 °C (dec); $[\alpha]_{\text{D}}^{23}$ 21.75° (c 0.15, DMF); UV(H_2O) λ_{max} 253.0 nm (ϵ 20,778, pH 2), 249.0 nm (ϵ 11,259, pH 7), 266.5 nm (ϵ 9,759, pH11); ^{13}C NMR (CD_3OD) δ 164.97, 162.14, 160.71 (d, $J = 215.84$ Hz), 156.00, 138.86, 118.03, 101.48 (d, $J = 11.0$ Hz), 86.17 (d, $J = 15.3$ Hz), 82.42 (d, $J = 24.6$ Hz), 62.15; Anal. ($\text{C}_{10}\text{H}_{10}\text{FN}_5\text{O}_3$) C, H, N.

Stability studies. Energy required for the cleavage of the glycosidic bond: The initial conformations of L-d4C, L- β -2'-Fd4C and L- β -3'-Fd4C **24** 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* calculations using RHF/6-31G* basis in Spartan 5.1.1. Proton (H^+) was added to the O^2 atom of the cytidine analogues to build R-BH $^+$ (Scheme 5.4), which was again geometry-optimized by using the same basis function (RHF/6-31G*). The aglycon (BH) and oxonium ion of the sugar moiety (R^+) were also independently optimized to give the corresponding minimized energies. The dissociation enthalpy of the glycosidic bond (Table 5.4) was calculated by using the minimized energies. [$\Delta E_{\text{calc}} = E(\text{R}^+) + E(\text{BH}) - E(\text{R-BH}^+)$]

Molecular modeling study. 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).²²¹ 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 $n+1^{\text{th}}$ nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd)²²¹ was modified to guanine. 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.²²¹ Gasteiger-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 the enzyme site from the biopolymer module in Sybyl. Fluorine parameters were obtained from the literature^{222,223} 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 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.

Adenosine deaminase study. Assays were performed at 25°C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range of 15-100 μ M and with 0.15 unit of adenosine deaminase (EC.3.5.4.4. from calf intestinal mucosa). The assays were monitored with a UV spectrometer at 265 nm. Initially, the qualitative assays were performed with D- β -2'-Fd4A **38** (200 μ M) in the presence of 0.24 unit of adenosine deaminase for 120 min to determine whether it is a substrate of this enzyme. The concentration (c_t) of each substrate at a certain time (t) was calculated from the absorbance (A_t) at that time (t), where it was assumed that the total change of absorbance ($A_0 - A_\infty$) was directly related to the disappearance of the substrate.²²⁴

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_{LAI} obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infectious 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 TCID₅₀ / 1×10^7 cells for a flask (T25) assay or with 200 TCID₅₀ / 6×10^5 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 μ g/ml streptomycin, 0.0008% DEAE-Dextran, 0.045% sodium bicarbonate, and 26 IU/ml recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT and/or 3TC were 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% CO₂-air at 37°C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

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. 10 µl of each sample were added to 75 µl RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M MgCl₂, 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 ³H-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 (EC₅₀) and 90% effective concentration (EC₉₀) were determined from the concentration-response curve using the median effect method.²²⁵

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

discontinuous gradient centrifugation. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5×10^3 , 2.5×10^3 and 5×10^4 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% CO₂-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 (IC₅₀) was determined from the concentration-response curve using the median effect method.²²⁵

Supplemental Material

Elemental Analysis Data

Cmpd No	Formula	Calc for C	Calc for H	Calc for N	Found for C	Found for H	Found for N
8	C ₂₃ H ₁₉ F ₂ N ₃ O ₅	60.66	4.21	9.23	60.34	4.18	8.91
9	C ₂₃ H ₁₉ F ₂ N ₃ O ₅	60.66	4.21	9.23	60.44	4.09	8.99
10	C ₂₃ H ₁₈ F ₃ N ₃ O ₅	58.35	3.83	8.88	58.62	3.86	8.76
11	C ₂₃ H ₁₈ F ₃ N ₃ O ₅ ·0.2C ₆ H ₁₄	59.24	4.27	8.56	59.26	4.37	8.26
12 & 13	C ₁₇ H ₁₆ F ₂ N ₂ O ₅ ·0.03CHCl ₃	55.30	4.37	7.57	54.92	4.40	7.37
14 & 15	C ₁₆ H ₁₄ F ₂ N ₂ O ₅	54.55	4.01	7.95	54.72	4.12	7.77
16	C ₉ H ₁₁ F ₂ N ₃ O ₃ ·0.1H ₂ O	43.41	4.53	16.88	43.63	4.50	16.48
17	C ₉ H ₁₁ F ₂ N ₃ O ₃	43.41	4.53	16.88	43.33	4.50	16.53
18	C ₉ H ₁₀ F ₃ N ₃ O ₃	40.76	3.80	15.85	40.98	3.89	15.61
19	C ₉ H ₁₀ F ₃ N ₃ O ₃	40.76	3.80	15.85	41.03	3.88	15.64
20	C ₁₀ H ₁₂ F ₂ N ₂ O ₄ ·0.14Et ₂ O	46.53	4.95	10.28	46.28	4.90	9.88
21	C ₁₀ H ₁₂ F ₂ N ₂ O ₄ ·0.5CH ₃ OH	45.33	5.07	10.07	45.49	4.96	9.76
22	C ₉ H ₁₀ F ₂ N ₂ O ₄	43.55	4.06	11.29	43.75	4.17	10.99
23	C ₉ H ₁₀ F ₂ N ₂ O ₄	43.55	4.06	11.29	43.61	4.19	11.11
24	C ₉ H ₁₀ FN ₃ O ₃	47.58	4.44	18.50	47.47	4.44	18.17
25	C ₉ H ₁₀ FN ₃ O ₃ ·0.82CH ₃ OH	46.53	5.28	16.58	46.81	4.99	16.18
26	C ₉ H ₉ F ₂ N ₃ O ₃	44.09	3.70	17.14	44.17	3.74	17.13
27	C ₉ H ₉ F ₂ N ₃ O ₃	44.09	3.70	17.14	43.89	3.68	16.91
28	C ₁₀ H ₁₁ FN ₂ O ₄	49.59	4.58	11.57	49.33	4.30	11.78
29	C ₁₀ H ₁₁ FN ₂ O ₄	49.59	4.58	11.57	49.21	4.20	11.80
30	C ₉ H ₉ FN ₂ O ₄	47.37	3.98	12.28	47.29	3.70	11.99
31	C ₉ H ₉ FN ₂ O ₄ ·0.2Et ₂ O	48.92	4.83	11.19	48.96	4.74	11.48
32 & 33	C ₁₇ H ₁₃ ClF ₂ N ₄ O ₃ ·0.3H ₂ O	51.03	3.43	14.00	50.69	3.24	13.95
34	C ₁₀ H ₁₁ F ₂ N ₅ O ₂	44.28	4.09	25.82	44.35	4.12	25.80
35	C ₁₀ H ₁₁ F ₂ N ₅ O ₂	44.28	4.09	25.82	44.24	4.11	25.67
36	C ₁₀ H ₁₀ F ₂ N ₄ O ₃	44.12	3.70	20.58	44.07	3.60	20.61
37	C ₁₀ H ₁₀ F ₂ N ₄ O ₃	44.12	3.70	20.58	44.46	3.98	20.41
38	C ₁₀ H ₁₀ FN ₅ O ₂	47.81	4.01	27.88	48.03	3.76	27.53
39	C ₁₀ H ₁₀ FN ₅ O ₂	47.81	4.01	27.88	47.58	4.26	27.62

40	$\text{C}_{10}\text{H}_9\text{FN}_4\text{O}_3$	47.62	3.60	22.22	47.52	3.45	21.90
42 & 43	$\text{C}_{17}\text{H}_{12}\text{ClF}_3\text{N}_4\text{O}_3 \cdot 0.04\text{C}_6\text{H}_{14}$	49.75	3.04	13.46	49.85	3.06	13.07
44	$\text{C}_{17}\text{H}_{14}\text{ClF}_2\text{N}_5\text{O}_3$	49.83	3.44	17.09	49.62	3.24	16.83
45	$\text{C}_{17}\text{H}_{14}\text{ClF}_2\text{N}_5\text{O}_3$	49.83	3.44	17.09	50.03	3.58	16.85
46	$\text{C}_{10}\text{H}_{11}\text{F}_2\text{N}_5\text{O}_3$	41.82	3.86	24.38	41.50	4.06	24.03
47	$\text{C}_{10}\text{H}_{11}\text{F}_2\text{N}_5\text{O}_3 \cdot 0.9\text{H}_2\text{O}$	39.58	4.25	23.08	39.46	4.21	22.70
48	$\text{C}_{10}\text{H}_{10}\text{FN}_5\text{O}_3 \cdot 0.5\text{H}_2\text{O}$	43.48	4.01	25.35	43.50	4.25	25.05
49	$\text{C}_{10}\text{H}_{10}\text{FN}_5\text{O}_3$	44.95	3.77	26.21	44.84	3.77	26.11

Supplemental Material

¹H NMR Data^a

Cpd	H-1'	H-2'	H-4'	H-5'	Other protons
8	6.30 (t, $J = 6.1$ Hz)	2.57 (m, $J = 15.6, 10.5, 5.3$ Hz), 3.26 (dtd, $J = 15.6, 12.8, 7.0$ Hz)	4.57 (ddd, $J = 18.0, 9.7, 4.8$ Hz),	4.75 (dd, $J = 12.5, 4.3$ Hz), 4.71 (dd, $J = 12.5, 5.5$ Hz)	8.81 (bs, 1H), 8.10-7.88 (m, 5H), 7.65-7.44 (m, 7 H)
9	6.29 (dd, $J = 7.3, 3.2$ Hz)	3.21 (dddd, $J = 15.4, 12.8, 7.3, 5.4$ Hz), 2.82 (dtd, $J = 15.4, 7.5, 3.2$ Hz)	4.82 (m)	4.69 (dd, $J = 12.4, 3.8$ Hz), 4.57 (dd, $J = 12.4, 4.6$ Hz)	8.89 (bs, 1H), 8.06-7.89 (m, 5H), 7.65-7.47 (m, 7 H)
10	6.32 (t, $J = 6.2$ Hz)	3.16 ~ 3.03 (m), 2.59 ~ 2.47 (m)	4.73 (dd, $J = 12.5, 4.0$ Hz)	4.67 (dd, $J = 12.5, 5.2$ Hz), 4.52 ~ 4.44 (m)	8.24 (d, $J = 7.6$ Hz, 2H), 8.03 (d, $J = 8.0$ Hz, 2H), 7.72 (d, $J = 5.6$ Hz, 1H), 7.60 ~ 7.54 (m, 2H), 7.48 ~ 7.43 (m, 4H)
11	6.30 (dd, $J = 7.0, 3.7$ Hz)	3.20 ~ 3.06 (m), 2.82 ~ 2.71 (m)	4.82 ~ 4.76 (m)	4.64 (dd, $J = 12.5, 3.7$ Hz), 4.56 (dd, $J = 12.0, 4.5$ Hz)	8.26 (d, $J = 7.4$ Hz, 2H), 8.04 (d, $J = 7.2$ Hz, 2H), 7.63 ~ 7.45 (m, 8H)
12 & 13	6.42 (t, $J = 7.0$ Hz),	3.10-2.94 (m), 2.83-2.76 (m),	4.81-4.73 (m)	4.62-4.53 (m)	9.60 [bs, 1H (<i>major isomer</i>)], 9.55 (bs, 1H (<i>minor isomer</i>)), 8.04 (m,

	6.33 (dd, $J = 7.5, 5.0$ Hz)	2.57- 2.43 (m)			4H), 7.59 (m, 2 H), 7.49 (m, 4H), 7.22 [d, 1H, H ₆ (<i>minor isomer</i>), $J = 1.2$ Hz], 7.18 [d, 1H, H ₆ (<i>major isomer</i>), $J = 1.2$ Hz]
14 & 15	6.42 (t, $J = 7.0$ Hz), 6.33 (dd, $J = 7.5, 5.0$ Hz)	3.10-2.94 (m), 2.83-2.76 (m), 2.57- 2.43 (m)	4.81-4.73 (m)	4.62-4.53 (m)	9.60 [bs, 1H (<i>major isomer</i>)], 9.55 (bs, 1H (<i>minor isomer</i>)), 8.04 (m, 4H), 7.59 (m, 2 H), 7.49 (m, 4H), 7.22 [d, 1H, H ₆ (<i>minor isomer</i>), $J = 1.2$ Hz], 7.18 [d, 1H, H ₆ (<i>major isomer</i>), $J = 1.2$ Hz]
16	6.27 (t, $J = 6.8$ Hz)	2.90 (m), 2.51 (m)	4.17 (m)	3.83 (m)	7.97 (d, 1H, H ₆ , $J = 7.3$ Hz), 5.93 (d, 1H, H ₅ , $J = 7.3$ Hz)
17	6.21 (dd, $J = 7.4, 3.6$ Hz)	3.01 (m), 2.63 (m)	4.49 (m)	3.79 (dd, $J = 12.5, 3.6$ Hz), 3.74 (ddd, $J = 12.5, 4.0, 1.5$ Hz)	7.69 (d, 1H, H ₆ , $J = 7.5$ Hz), 5.90 (d, 1H, H ₅ , $J = 7.5$ Hz)
18	6.24 (m)	2.90 (heptet, $J = 7.0$ Hz), 2.53 (ddt, $J = 28.0, 13.2, 7.6$ Hz)	4.19 (ddt, $J = 14.9, 7.1, 3.5$ Hz)	3.89 ~ 3.81 (m)	8.18 (d, $J = 6.8$ Hz, 1H)
19	6.19 (dd, $J = 6.9, 3.1$ Hz)	3.02 (ddt, $J = 27.9, 13.2, 7.4$ Hz), 2.70 ~ 2.59 (m)	4.56 ~ 4.50 (m)	3.82 ~ 3.73 (m)	7.83 (d, $J = 6.6$ Hz, 1H)
20	6.21 (t, $J =$	2.83 (m),	4.11 (m)	3.67 (m, 2H)	11.43 (bs, 1H), 7.64 (s, 1H, H ₆),

	7.2 Hz)	2.65(m)			5.25 (t, 1H, 5'-OH, $J = 4.4$ Hz), 1.78 (s, 3H)
21	6.21 (t, $J = 6.4$ Hz)	2.97-2.73 (m)	4.50 (m)	3.59 (m)	11.41 (bs, 1H), 7.52 (s, 1H, H ₆), 5.13 (t, 1H, 5'-OH, $J = 4.9$ Hz), 1.78 (s, 3H)
22	6.29 (t, $J = 7.2$ Hz)	2.87 (tdd, $J = 14.5, 8.1, 6.7$ Hz), 2.62 (tdd, $J = 19.6, 13.8, 7.8$ Hz)	4.16 (ddt, $J = 15.1, 8.0, 3.8$ Hz)	3.87 ~ 3.79 (m, 2H)	7.98 (d, $J = 8.2$ Hz, 1H), 5.74 (d, $J = 8.2$ Hz, 1H)
23	6.27 (dd, $J = 7.6, 4.1$ Hz)	3.07 ~ 2.93 (m), 2.79 ~ 2.68 (m)	4.50 (ddd, $J = 16.4, 7.4, 3.8$ Hz)	3.81 ~ 3.73 (m)	7.67 (d, $J = 8.1$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H)
24	7.01 (m)	5.46 (m)	4.76 (m)	3.80 (m), 3.79 (m)	8.14 (d, 1H, H ₆ , $J = 7.5$ Hz), 5.90 (d, 1H, H ₅ , $J = 7.5$ Hz)
25	6.99 (m)	5.47 (m)	4.96 (m)	3.81 (dd, $J = 12.7, 2.5$ Hz), 3.67 (m, $J = 12.7, 2.9$ Hz)	7.63 (d, 1H, H ₆ , $J = 7.4$ Hz), 5.93 (d, 1H, H ₅ , $J = 7.4$ Hz)
26	6.94 (br s)	5.45 (br s)	4.75 ~ 4.71 (m)	3.79 (d, $J = 1.9$ Hz)	8.33 (d, $J = 6.8$ Hz, 1H)
27	6.97 ~ 6.95 (m)	5.47 (br s)	5.00 ~ 4.95 (m)	3.81 (dd, $J = 12.7, 2.5$ Hz), 3.67 (dd, $J = 12.7,$	7.77 (d, $J = 6.3$ Hz, 1H)

				2.8 Hz)	
28	7.05 (s)	5.26 (s)	4.73 (s)	3.94 (m)	8.94 (bs, 1H), 7.70 (s, 1H, H ₆), 3.30 (bs, 1H, 5'-OH), 1.86 (s, 3H)
29	7.07 (s)	5.33 (s)	4.91 (s)	3.91 (m)	8.01 (bs, 1H), 7.34 (s, 1H, H ₆), 3.48 (bs, 1H, 5'-OH), 1.94 (s, 3H)
30	6.92 (br s)	5.44 (s)	4.72 (br s)	3.77 (br s)	8.10 (d, $J = 8.1$ Hz, 1H), 5.66 (d, $J = 8.1$ Hz, 1H)
31	6.98 ~ 6.96 (m)	5.46 (t, $J = 1.6$ Hz)	4.97 ~ 4.94 (m)	3.81 (dd, $J = 12.7$, 2.5 Hz), 3.68 (dd, $J = 12.7$, 2.7 Hz)	7.64 (d, $J = 8.1$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H)
32 & 33	6.52 (t, $J = 6.8$ Hz, 1H), 6.56 (dd, $J = 7.7$, 4.9 Hz, 1H)	3.61 ~ 3.48 (m, 1H), 3.24 ~ 3.16 (m, 1H), 3.35 ~ 3.30 (m, 1H), 3.24 ~ 3.16 (m, 1H)	4.99 ~ 4.92 (m, 1H), 4.82 ~ 4.78 (m, 1H)	4.70 (dd, $J = 12.4$, 4.4 Hz, 1H), 4.59 (dd, $J = 12.3$, 5.6 Hz, 1H), 4.65 ~ 4.57 (m, 2H)	8.80 (s, 1H), 8.28 (s, 1H), 8.04 (dd, $J = 7.1$, 1.5 Hz, 2H), 7.61 ~ 7.56 (m, 1H), 7.48 ~ 7.41 (m, 2H), 8.69 (s, 1H), 8.32 (s, 1H), 7.98 (dd, $J = 7.1$, 1.4 Hz, 2H), 7.61 ~ 7.56 (m, 1H), 7.48 ~ 7.41 (m, 2H),
34	6.43 (dd, $J = 8.5$, 6.4 Hz)	3.34 ~ 3.19 (m)	4.29 ~ 4.24 (m)	3.90 ~ 3.83 (m)	8.34 (s, 1H), 8.20 (s, 1H), 3.04 ~ 2.95 (m, 1H)
35	6.54 (dd, $J = 7.7$, 4.5 Hz)	3.39 ~ 3.27 (m), 3.20 ~ 3.06 (m)	4.67 ~ 4.59 (m)	3.84 (dd, $J = 12.2$, 3.8 Hz), 3.77 (dd, $J = 12.3$, 4.5 Hz)	8.24 (s, 1H), 8.20 (s, 1H)

36	6.45 (dd, $J = 8.0, 6.6$ Hz)	3.30 ~ 3.12 (m), 3.10 ~ 3.00 (m)	4.30 ~ 4.20 (m)	3.85 (br s)	8.36 (s, 1H), 8.08 (s, 1H)
37	6.42 (dd, $J = 7.7, 4.6$ Hz)	3.20 ~ 2.95 (m)	4.55 ~ 4.45 (m)	3.70 (dd, $J = 12.4, 3.9$ Hz), 3.63 (ddd, $J = 12.4, 4.9, 1.3$ Hz)	8.05 (s, 1H), 7.94 (s, 1H)
38	7.01 ~ 6.99 (m)	5.67 (t, $J = 1.6$ Hz)	4.88 ~ 4.83 (m)	3.79 (d, $J = 2.2$ Hz)	8.40 (s, 1H), 8.19 (s, 1H)
39	6.82 ~ 6.79 (m)	5.48 (br s)	4.86 ~ 4.83 (m)	3.64 (dd, $J = 12.8, 2.4$ Hz), 3.52 (dd, $J = 12.8, 2.8$ Hz)	8.05 (s, 1H), 8.00 (s, 1H), 3.42 (d, $J = 2.6$ Hz, 1H)
40	7.02 (br s)	5.68 (br s)	4.61 (s)	3.78 (br s)	8.40(s, 1H), 8.06 (s, 1H)
42 & 43	6.52 (dd, $J = 7.6, 4.6$ Hz, 1H), 6.46 (t, $J = 6.6$ Hz, 1H)	3.38 (tdd, $J = 14.8, 13.4, 4.6$ Hz, 1H), 3.21 (ddt, $J = 22.4, 12.4, 7.6$ Hz, 1H), 3.49 ~ 3.33 (m, 1H), 3.28 ~ 3.14 (m, 1H)	4.94 ~ 4.86 (m, 1H), 4.64 ~ 4.55 (m, 1H)	4.68 (dd, $J = 12.3, 4.2$ Hz, 1H), 4.59 (ddd, $J = 12.3, 5.6, 0.9$ Hz, 1H), 4.78 (dd, $J = 11.7, 4.0$ Hz, 1H), 4.68 (dd, $J = 12.3, 4.1$ Hz, 1H)	8.27 (s, 1H), 8.04 (d, $J = 7.1$ Hz, 2H), 7.60 (t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 2H), 8.26 (s, 1H), 8.04 (d, $J = 6.8$ Hz, 2H), 7.67 (t, $J = 7.6$ Hz, 1H), 7.54 (t, $J = 7.6$ Hz, 2H)
44	6.35 (dd, $J = 6.9, 5.2$ Hz)	3.46 ~ 3.36 (m), 3.14 ~ 3.02 (m)	4.90 ~ 4.83 (m)	4.64 (dd, $J = 12.2, 4.1$ Hz), 4.53 (dd, $J = 12.2,$	8.00 (d, $J = 8.2$ Hz, 2H), 7.90 (s, 1H), 7.55 (td, $J = 8.2, 1.2$ Hz, 1H), 7.41 (t, $J = 7.2$ Hz, 2H), 5.53 (br s,

				6.1 Hz)	2H)
45	6.29 (t, $J = 7.0$ Hz)	3.24 (ddd, $J = 30.4, 14.5, 7.1$ Hz), 3.03 (ddt, $J = 20.9, 10.0, 7.1$ Hz)	4.57 ~ 4.48 (m)	4.75 (dd, $J = 12.1, 4.5$ Hz), 4.62 (dd, $J = 12.1, 5.9$ Hz)	7.97 (d, $J = 7.7$ Hz, 2H), 7.91 (s, 1H), 7.54 (t, $J = 7.5$ Hz, 1H), 7.40 (t, $J = 7.7$ Hz, 2H), 5.49 (br s, 2H)
46	6.13 (t, $J = 7.0$ Hz)	3.23 ~ 3.11 (m), 3.08 ~ 2.97 (m)	5.22 (t, $J = 4.1$ Hz)	4.17 ~ 4.11 (m), 3.70 ~ 3.60 (m)	10.80 (br s, 1H), 7.94 (s, 1H), 6.58 (br s, 2H)
47	6.26 (dd, $J = 8.4, 6.4$ Hz)	3.22 ~ 3.11 (m), 2.99 ~ 2.88 (m)	4.24 ~ 4.17 (m,)	3.84 (br s)	7.99 (s, 1H)
48	6.76 ~ 6/74 (m)	5.52 (br s)	4.98 ~ 4.96 (m)	3.74 (dd, $J = 12.7, 2.6$ Hz), 3.63 (dd, $J = 12.7, 2.8$ Hz)	7.76 (s, 1H)
49	6.83 (br s)	5.61 (s)	4.80 (br s)	3.77 (d, $J = 2.3$ Hz), 3.30 (t, $J = 1.5$ Hz)	8.05 (s, 1H)

^a CD₃OD;ppm;s, singlet; d, doublet; dd, double doublet; t, triplet, br s, broad singlet; m, multiplet; td, triple doublet; ddd, double double doublet.

CHAPTER 6

SYNTHESES, ANTI-HIV ACTIVITY AND MOLECULAR MODELING STUDY OF D-2',3'-DIDEHYDRO-2',3'-DIDEOXY-3'-FLUORO-4'-THIONUCLEOSIDES

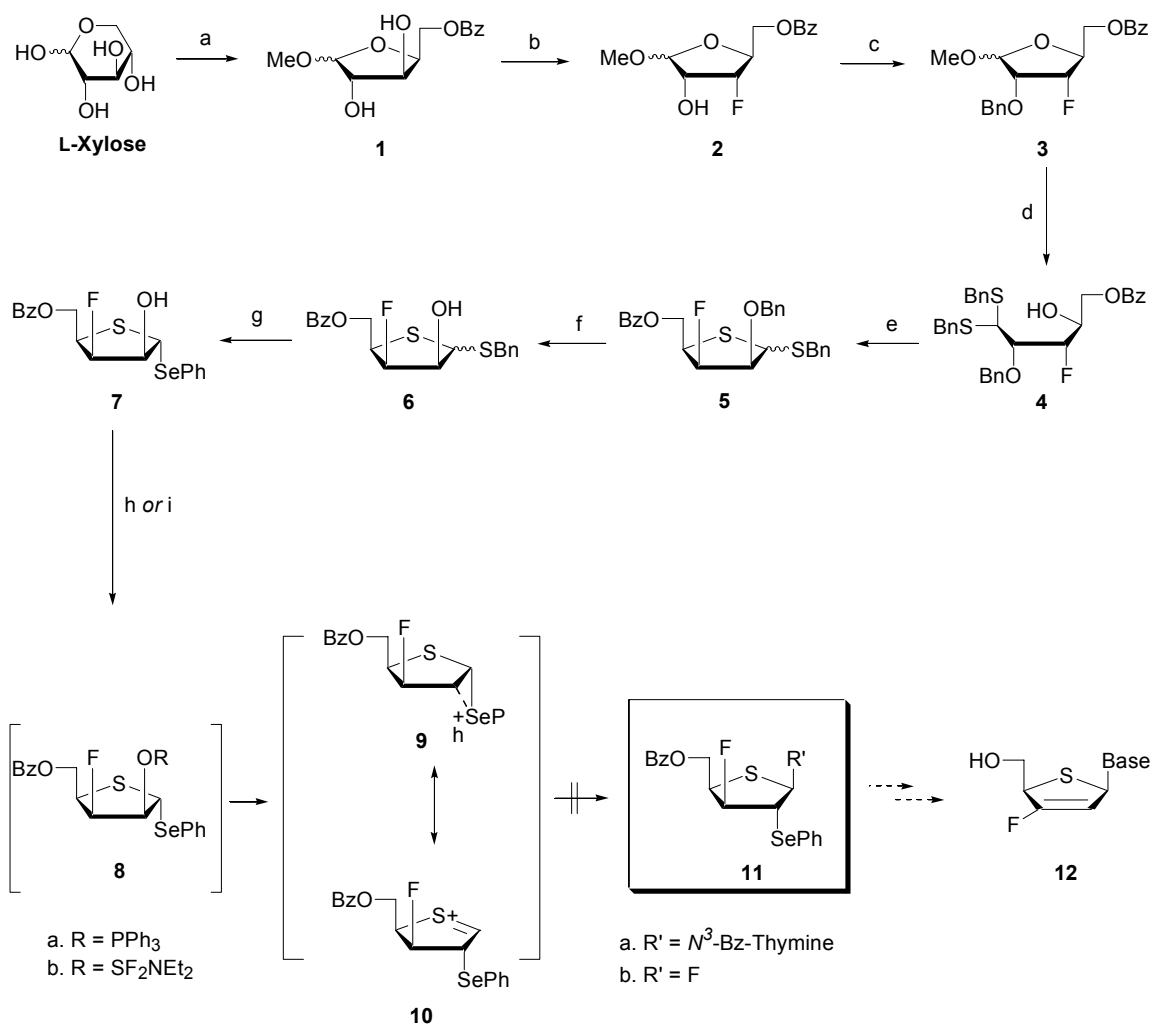
Introduction

Antiviral agents containing 2',3'-unsaturated sugar moiety with 3'-fluoro substitution has shown potent antiviral activity against HIV-1 without significant cytotoxicity.²²⁶ Thus, it was of interest to synthesize the nucleosides with isosteric replacement of 4'-oxygen by sulfur atom. The 4'-thionucleosides are usually resistant to hydrolytic cleavage of glycosyl linkage catalyzed by nucleoside phosphorylase,²²⁷ which is known to deactivate several "4'-oxy" antiviral agents.²²⁸ Even though the 4'-thionucleosides have recently received considerable attention as potential antiviral agents,^{229,230,231,232,233} their 2',3'-unsaturated analogues have not been well investigated, presumably because of the synthetic difficulties. Particularly, introduction of the fluorine atom into the 2' or 3' position of the 2',3'-unsaturated sugar moiety has always been challenging, and, to our knowledge, the recent syntheses of D- and L- 2'-fluoro-4'-thio-2',3'-unsaturated nucleosides²³⁴ and L-3'-fluoro-2',3'-unsaturated nucleosides are the only examples.²²⁶ These recent syntheses used mild conditions for this purpose such as oxidative elimination of 2'-phenylselenide and elimination of HF from 3',3'-difluoro nucleoside, respectively,. In this study, by adapting the synthetic protocols, which was used in the synthesis of L-3'-fluoro-2',3'-unsaturated nucleosides, various D-3'-fluoro-4'-thio-2',3'-unsaturated nucleosides were prepared and their anti-HIV activities were evaluated against human peripheral blood mononuclear (PBM) cells. In order to understand the inactivity of the D-2',3'-unsaturated 3'-fluoro-4'-thionucleosides, the binding mode of D-2',3'-unsaturated 3'-fluoro-4'-thiocytidine triphosphate to the wild type RT was investigated by molecular modeling studies.

Herein, we report the full accounts of the synthesis, biological evaluation and molecular modeling studies of the titled nucleosides.

Results and Discussion

Chemistry. Enantioselective synthesis of a 3'-fluoro-2',3'-unsaturated nucleoside is extremely limited by the lack of the synthetic methodologies to introduce the fluorine atom at the 3'-position of the 2',3'-double bond. To date, our recent synthesis of L-3'-fluoro-2',3'-unsaturated nucleosids,²²⁶ which introduced the 2',3'-double bond at the late stage of the synthesis is the only example, which can be adapted to the preparation of the 2',3'-unsaturated 3'-fluoro nucleoside whose 4'-oxygen is isosterically replaced with 4'-sulfur.²²⁶ On the other hand, in order to avoid the use of strongly basic conditions at the late stage of the synthesis, which occasionally degrade relatively unstable nucleoside analogues, the mild *syn*-elimination conditions of phenylselenyl group²³⁴ was worth while to be tested on the 4'-thio nucleoside with *trans*-2'-phenylselenyl-3'-fluoro combination (**11**, Scheme 6.1). For this purpose, recently reported 1,2-migration of selenoglycoside **7**,^{235,236} which can be directly prepared from 2'-deoxy-2'-fluoro ribofuranoside **2**, was believed to be an excellent precursor for the key intermediate **11** (Scheme 6.1). Also, 2'-deoxy-2'-fluoro ribofuranoside **2** could be used for the synthesis of the L-counterpart of the target nucleoside, L-2',3'-dideoxy-2',3'-didehydro-3'-fluoro-4'-thionucleoside as well through the bicyclic intermediate **22** (Scheme 6.2).²³⁷

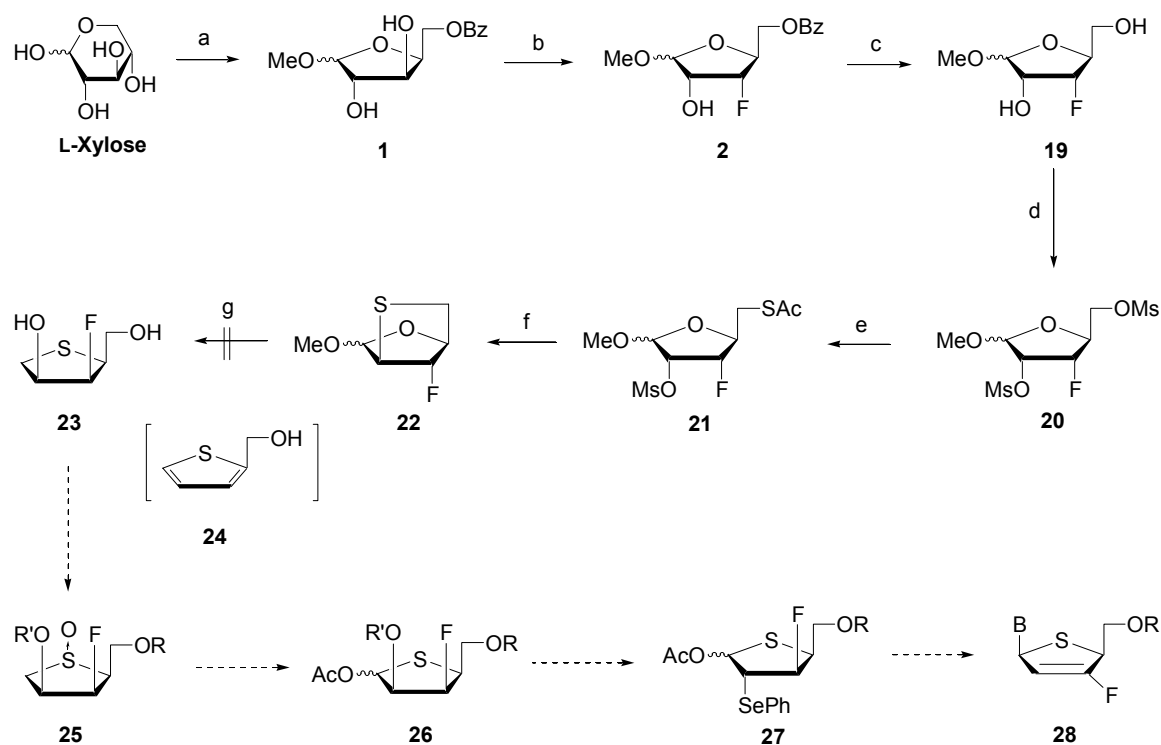


Reagents and Conditions: (a) i. MeOH, HCl; ii. BzCl, TEA, CH₂Cl₂; (b) DAST, CH₂Cl₂; (c) NaH, BnBr, TBAI, THF; (d) BnSH, BF₃·OEt₂; (e) I₂, Imidazole, PPh₃, tol, reflux; (f) BCl₃, CH₂Cl₂; (g) i. Hg(OAc)₂, AcOH; ii. Ac₂O; iii. PhSeH, BF₃·OEt₂; iv. NH₃, MeOH; (h) N³-Bz-Thymine, PPh₃, DIAD, DMF; (i) DAST, CH₂Cl₂, rt, 3h.

Scheme 6.1. Attempted synthesis of D-3'F-4'S-d4 nucleosides

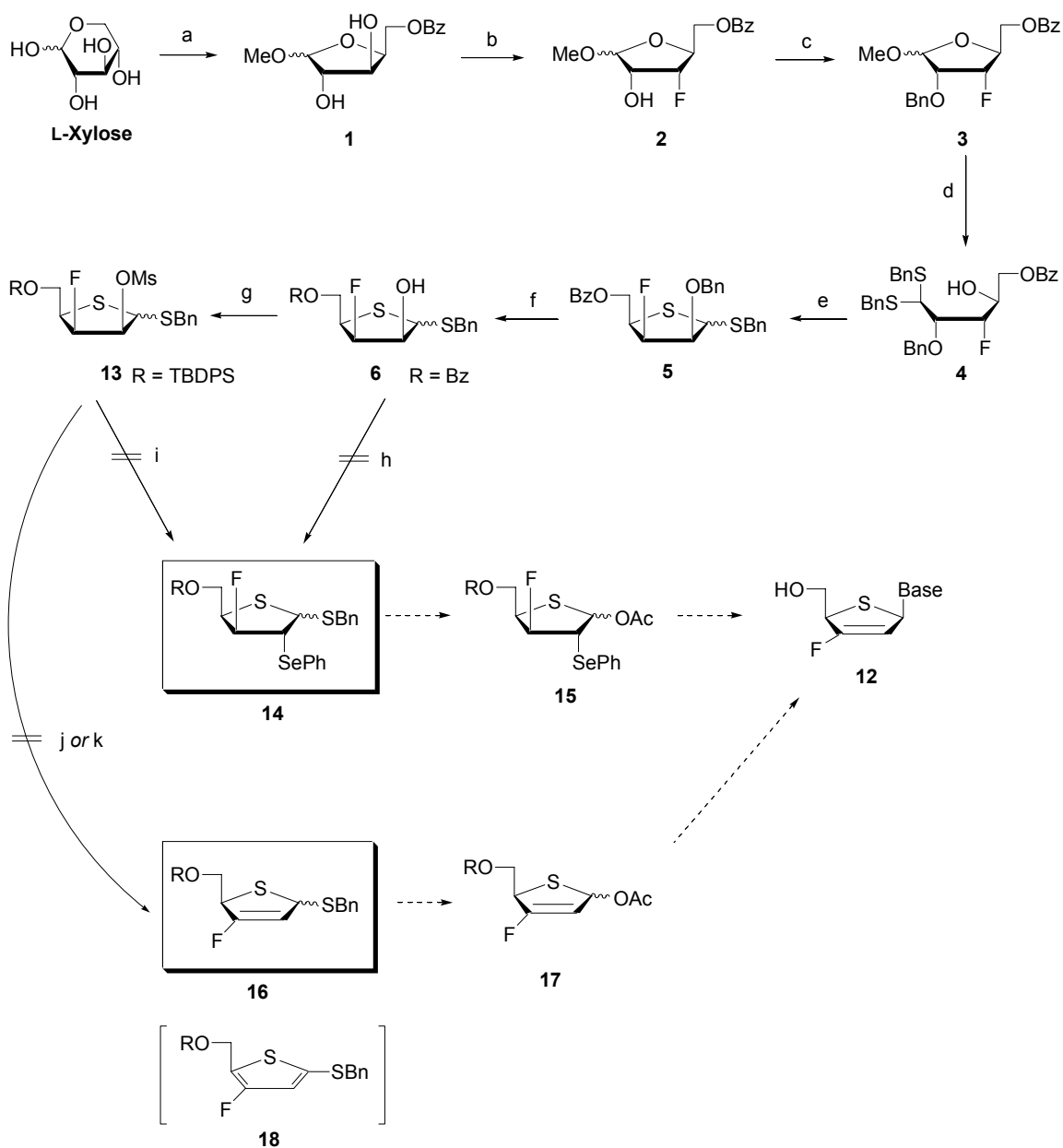
Despite the widespread interest in the chemistry of deoxyfluoro nucleosides, generally applicable chemical methods have not been available until Mikhailopulo *et al.*²³⁸ reported the direct conversion of methyl 5-*O*-benzoyl-β-D-xylofuranoside to compound **2** by the treatment with DAST (Scheme 6.1). Therefore, L-xylose was treated with methanol in the

presence of HCl to give the methyl xylofuranoside, which was monoprotected by BzCl to give compound **1**. Treatment of **1** with 6 equivalents of DAST in CH₂Cl₂ gave a clean conversion to the 3-deoxy-3-fluoro ribofuranoside **2** in 60 % yield. The hydroxyl group at 2-position was protected by a benzyl group to give **3**, which was treated with BnSH in the presence of a Lewis acid. This reaction was so sensitive to reaction conditions that only carefully controlled use of BF₃-THF complex in dry CH₂Cl₂ provided a reasonable yield (60 %).



Reagents and Conditions: (a) i. MeOH, HCl; ii. BzCl, TEA, CH₂Cl₂; (b) DAST, CH₂Cl₂; (c) NH₃, MeOH; (d) MsCl, pyr; (e) KSAc, DMF; (f) NaHCO₃; (g) 2% TFA; NaBH₄

Scheme 6.2. Attempted synthesis of D-3'F-4'S-d4 nucleosides



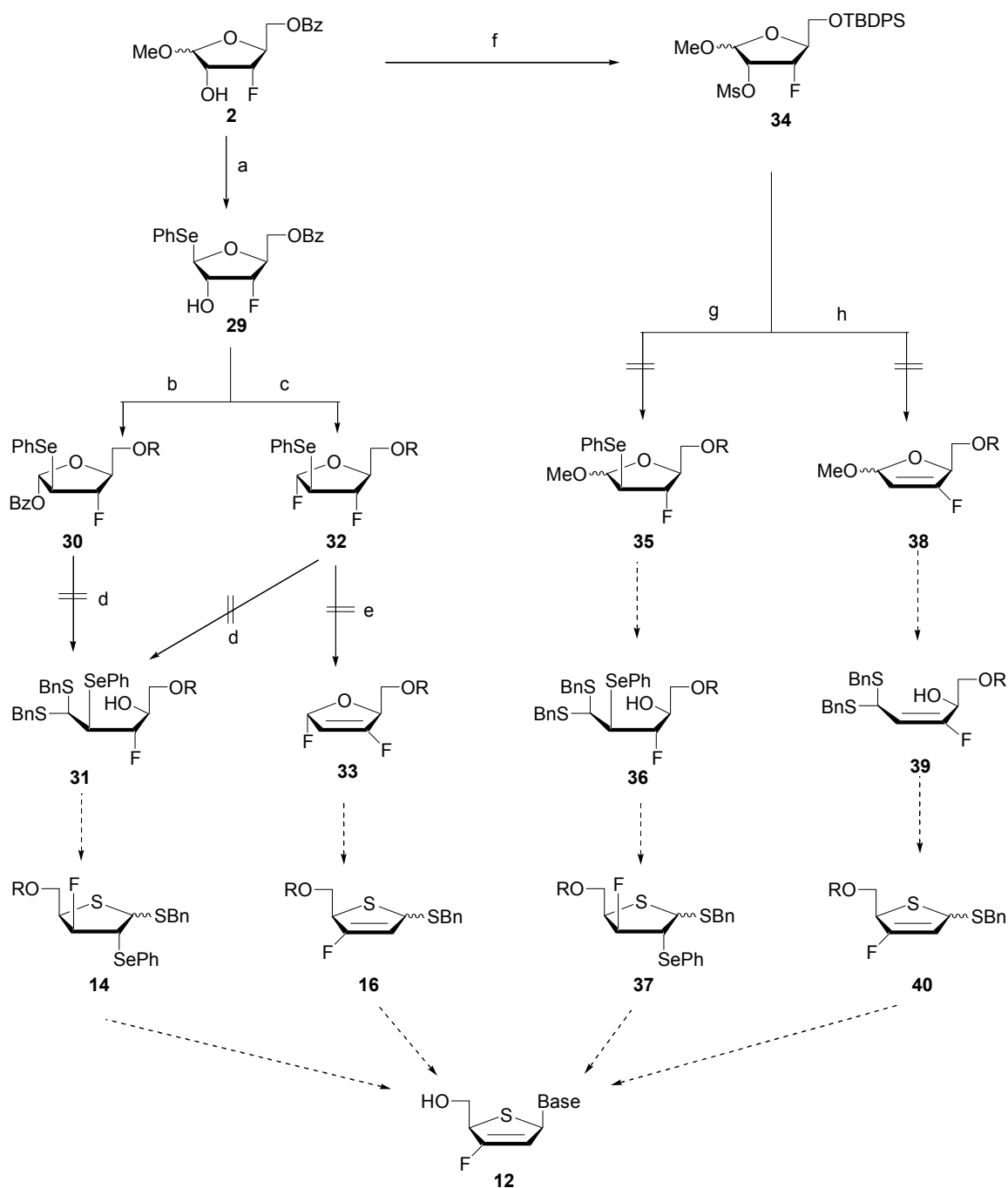
Scheme 6.3. Attempted synthesis of D-3'F-4'S-d4 nucleosides

The dithioacetal, thus obtained, underwent smooth cyclization to **5** by the treatment with iodine, triphenylphosphine and imidazole.²³⁹ After deprotection of the 2-*O*-benzyl group

by BCl_3 , compound **6** was re-protected as its acetate. Treatment of the acetate with $\text{Hg}(\text{OAc})_2$ in AcOH followed by Ac_2O provided the diacetate, which was phenylselenylated and deprotected to give **7**. The intermediate **7** was subjected to two different 1,2-migration conditions: reaction with *N*³-benzoyl thymine under Mitsunobu conditions²³⁵ and treatment with DAST.²³⁶ Both conditions were known to work in normal “4-oxa” nucleoside systems, but neither of them gave the desired products suggesting that the 4'-thio group prevents the 1,2-migration of phenylselenyl group. Therefore, direct introduction of the phenylselenyl group at the 2-position without 1,2-migration was attempted (Scheme 6.3).

However, neither the treatment of the free alcohol **6** with *o*- NO_2 -PhSeCN in the presence of nBu_3P nor the reaction of the corresponding mesylate **13** with phenylselenyl anion gave the desired *trans*-substituted 2-fluoro-3-phenylseleno sugar moiety. Compound **13** was also subjected to *trans*-elimination with strong bases to the 3-fluoro-2,3-unsaturated intermediate **16**, but the more labile proton at 1 position was deprotonated earlier than the proton at 3-position resulting in the aromatization of the sugar ring to form a stable thiophene derivative **18**. Since these failures were believed to be associated with the sulfur atom at the 4-position, the possibility of introducing the phenylselenyl group before conversion to the “4-thio” sugar was investigated (Scheme 6.4). Thus, 3-deoxy-3-fluoro methyl ribofuranoside **2** was protected with acetyl group, reacted with phenylselenol in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ and then the acetyl group was deprotected by methanolic ammonia to give the corresponding selenoglycoside **29** in good yield. Interestingly, the selenoglycoside **29** underwent smooth 1,2-migration under the Mitsunobu conditions as well as DAST conditions to give **30** and **32**, respectively,

confirming the idea that the sulfur atom at 4-position of the compound **7** prevented the 1,2-migration of the phenylselenenyl group.



Scheme 6.4. Attempted synthesis of D-3'F-4'S-d4 nucleosides

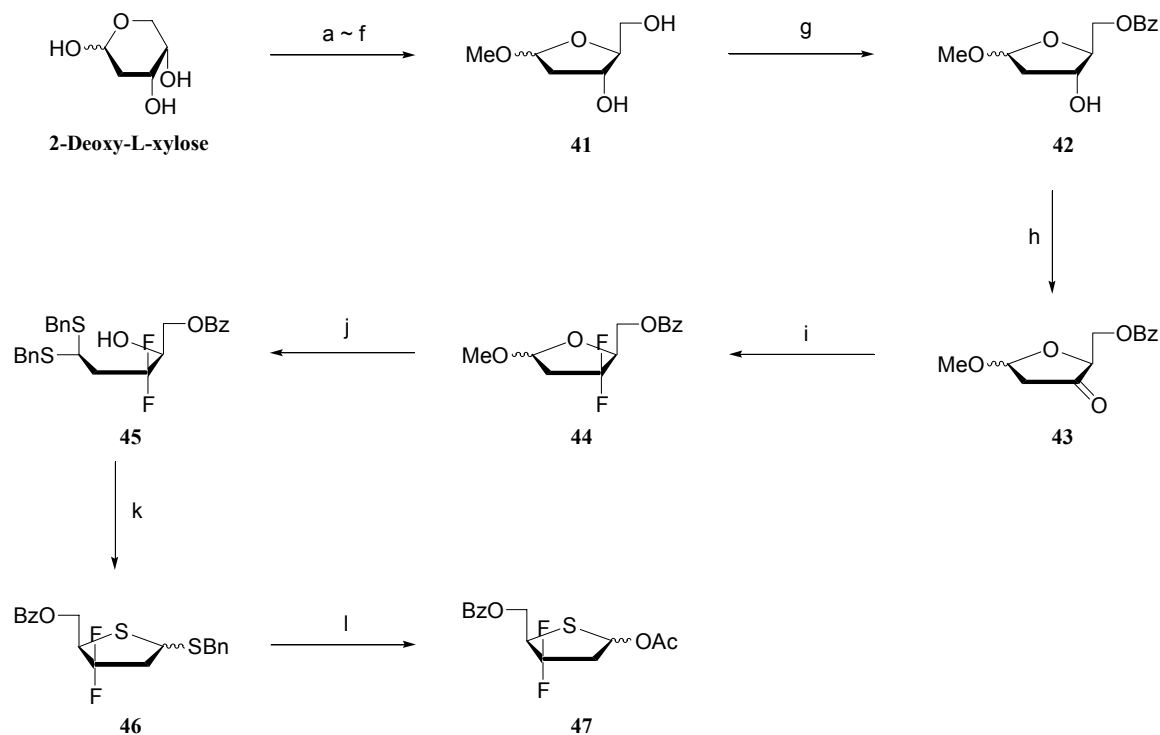
However, neither the compound **30** nor **32** gave the dithioacetal **31** by reaction with BnSH in the presence of BF₃·OEt₂ or HCl. The oxidative *syn*-elimination of the compound **32** did not give the eliminated product **33** either, suggesting that the proton at 2-position is so strongly bound to the C2 atom that the selenoxide, formed by reaction with mCPBA, cannot initiate the elimination process. This finding that the *trans*-2-phenylseleno-3-fluoro moiety is resistant to *syn*-elimination was disappointing because, according to our scheme, the final nucleoside cannot be prepared without the *syn*-elimination. Other approaches to directly attack the mesylate **34** with phenylselenenyl anion or to eliminate the mesylate by using strong bases failed as well.

On the other hand, the 3-deoxy-3-fluoro ribofuranoside **2** was deprotected and mesylated to give the bismesylate **20**, which was treated with KSAc followed by NaHCO₃ to give the bicyclic intermediate **22** (Scheme 6.2).²³⁷ Hydrolysis followed by NaBH₄ reduction, however, did not give the desired diol **23** which could be used as a substrate for Pummerer rearrangement. After hydrolysis, the resulting bicyclic lactol was so unstable that it underwent aromatization to give the thiophene derivative **24**.

In summary, hereto unknown 1,2-migration of selenoglycoside containing sulfur atom at 4-position as well as *syn*-elimination of *trans*-2-phenylseleno-3-fluoro moiety were tested to find an efficient synthetic route for 3'-fluoro-4'-thio-2',3'-unsaturated nucleoside. However, our study indicates that the sulfur atom at 4-position of selenoglycoside interferes 1,2-migration, and *trans*-2-phenylseleno-3-fluoro moiety is highly resistant to *syn*-elimination.

The synthesis of the target compound was accomplished by the elimination reaction of the hydrogen fluoride from 4'-thio derivative of the 3',3'-difluoro-2',3'-dideoxy-2',3'-

didehydro nucleosides²²⁶ under various basic conditions (Scheme 6.5 ~ Scheme 6.7). For this purpose, the key intermediate **47** was prepared from methyl 2-deoxyribose **41**, which can be prepared from 2-deoxy-L-arabinose in large quantity and high yield, in 6 steps (Scheme 6.5).²⁴⁰

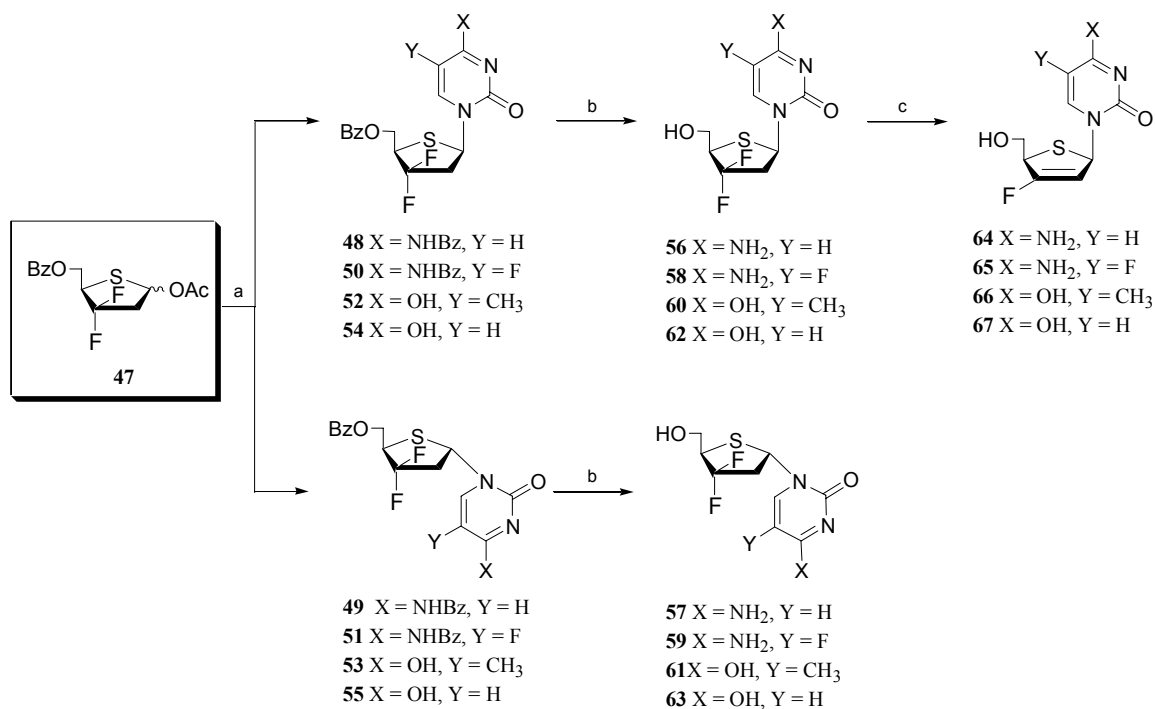


Reagents and conditions (a) BnOH, HCl (g); (b) 2,2-dimethoxypropane, *p*-TsOH, acetone; (c) CScI₂, PhOH, pyr, CH₂Cl₂; (d) nBu₃SnH, AIBN, toluene, reflux; (e) 4%TFA, 40°C; (f) HCl, MeOH; (g) BzCl, pyr; (h) PDC, Ac₂O, CH₂Cl₂; (i) DAST, CH₂Cl₂, reflux; (j) BnSH, BF₃·OEt₂, CH₂Cl₂, 0°C to rt; (k) I₂, PPh₃, Imidazole, toluene-CH₃CN, reflux; (l) Hg(OAc)₂, AcOH

Scheme 6.5. Synthesis of the key intermediate **47**

Thus, methyl 2-deoxyribose **41** was protected with benzoyl chloride and pyridine to give the 5-*O*-benzoyl methyl furanoside **42**. Pyridinium dichromate-mediated oxidation of compound **42** followed by difluorination with DAST provided the 3,3-difluoro methyl furanoside **44**, which was treated with benzyl mercaptan in the presence of a Lewis acid

to give 4-hydroxy dithioacetal **45**. The 4'-sulfur atom was then introduced to the furanoside moiety by cyclizing the 4-hydroxy dithioacetal **45** under the Mitsunobu-type ring closure conditions. Transglycosylation of the compound **46** by the treatment with mercury(II) acetate in acetic acid provided the key intermediate **47**, which was condensed with various pyrimidine and purine heterocyclic bases under Vorbrüggen conditions (Scheme 6.6 and Scheme 6.7, respectively).



^aKeys: (a) HMDS, CH₃CN, pyrimidines; TMSOTf, (b) NH₃, MeOH, rt, (c) ^tBuOK, DMF (or 2:1 DMF:dioxane), rt

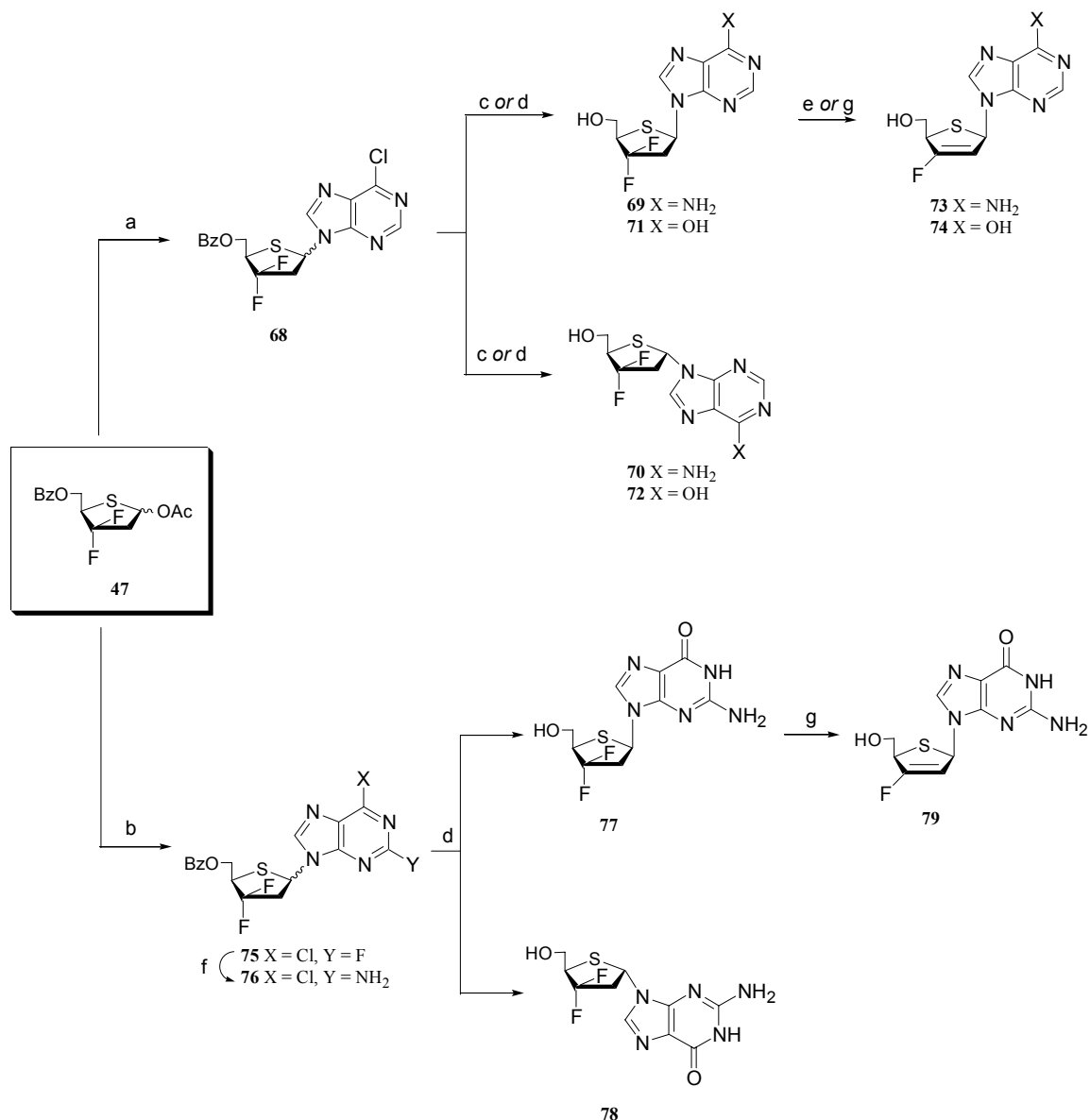
Scheme 6.6. Synthesis of the Pyrimidine Nucleosides.

The key intermediate **47** was condensed with the silylated pyrimidine bases in the presence of TMSOTf in dry CH₃CN to give α/β mixtures of the protected pyrimidine nucleosides **48-55** in 40 ~ 70% yield (Scheme 6.6). After deblocking the protecting groups on the pyrimidine heterocyclic base and the 5' position of the sugar moiety by

methanolic ammonia. The resulting 2',3'-dideoxy-3',3'-difluoro-4'-thio pyrimidine nucleosides (**56**, **58**, **60** and **62**) were treated with NaOMe in various solvents as had been previously reported for the synthesis of 3'-fluoro 2',3'-unsaturated nucleosides.²²⁶ However, unlike 3'-fluoro 2',3'-unsaturated nucleosides, the 4'-thio analogues (**56**, **58**, **60** and **62**) were found to be highly resistant to the elimination of HF under the same conditions leaving the starting material unchanged. The final 2',3'-unsaturated 3'-fluoro-4'-thio pyrimidine nucleosides **64-67** were obtained by the treatment with a stronger base, ^tBuOK, in DMF or in a 2:1 mixture of DMF and dioxane at room temperature, but careful control of the reaction was critical because longer reaction time usually resulted in decomposition of the starting material and poor yield. Therefore, after stirring the reaction mixture for 3 to 5 hours at room temperature, the reaction was stopped and, after purification, the recovered starting material was recycled.

For the preparation of the purine nucleosides, the key intermediate **47** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give α/β mixtures of the corresponding nucleosides **68** and **75** in 47% and 78% yield, respectively (Scheme 6.7). In each case, even after stirring for 12h at room temperature, the kinetically preferred adducts, N^3 - and N^7 -adducts, were predominant. Therefore, the conversion to the thermodynamic product, N^9 -adduct, was accomplished by stirring the reaction mixture for additional 12h at 60°C. The α/β mixture of 6-chloropurine derivatives (**68**) was treated with saturated ammonia in dry MeOH at 100°C in a steel bomb to give the adenosine analogue (**69** and **70**), whereas hydrolysis in the presence of sodium methoxide and 2-mercaptoethanol in refluxing methanol gave the inosine analogues (**71** and **72**). Unlike the pyrimidine case, however, elimination of hydrogen fluoride from the 2',3'-dideoxy-

3',3'-difluoro-4'-thio purine nucleosides (**69** and **71**) by treatment with ^tBuOK in DMF or a 2:1 mixture of DMF and dioxane did not give the final 3'-fluoro-4'-thio-2',3'-unsaturated purines. Instead, the starting material was completely decomposed under the reaction conditions.



^aKeys: (a) HMDS/CH₃CN, 6-Chloropurine, reflux; TMSOTf, CH₃CN, (b) HMDS/CH₃CN, 2-Fluoro-6-chloropurine, reflux; TMSOTf, CH₃CN, 0°C to 60°C, (c) NH₃, MeOH, 100°C, (d) HSCH₂CH₂OH, NaOMe, MeOH, 60°C, (e)

^tBuOK, 1,4-dioxane, 0°C, (f) NH₃ bubbling, DME, rt, (g) NaOMe, 1,4-dioxane-DMF (1:1), 0°C; ^tBuOK.

Scheme 6.7. Synthesis of the Purine Nucleosides.

In order to find milder reaction condition, several protocols were tried. Unfortunately, nucleosides with different purine bases showed different reaction profiles under the same reaction conditions. Therefore, after screening various elimination conditions, potassium *tert*-butoxide in 1,4-dioxane at 0 °C turned out to be good enough for the elimination reaction of the adenosine analogue **69**, which was not applicable to the same reaction of hypoxanthine (**71**) and guanine derivatives (**77**) due to the limited solubility of 1,4-dioxane. A combination of NaOMe and *t*BuOK at 0°C, however, proved to give the corresponding 2',3'-unsaturated nucleosides (**73** and **74**) without any remaining starting material or decomposition. Presumably, deprotonation of the acidic protons by pretreatment of the 2',3'-dideoxy-3',3'-difluoro-4'-thio purine nucleosides (**71** and **77**) with 2.2 equivalents of NaOMe followed by the elimination of the hydrogen fluoride by stronger base (*t*BuOK) at low temperature proceeds in a more controlled manner. On the other hand, dry ammonia gas was bubbled into a solution of **75** in ethylene glycol dimethyl ether (DME) at room temperature for 16 h to give the inseparable α/β mixture of 2-amino-6-chloropurine derivatives (**76**) in 29% yield. The 2-amino-6-chloropurine derivatives (**76**), thus obtained, were hydrolyzed by using sodium methoxide and 2-mercaptoethanol in refluxing methanol to give 2',3'-dideoxy-3',3'-difluoro-4'-thio guanosine analogues (**77** and **78**), which were readily separable by column chromatography on silica gel, in 51% and 80% yield, respectively. Under the same elimination conditions using a combination of NaOMe and *t*BuOK at 0°C, the β -2',3'-dideoxy-3',3'-difluoro-4'-thio guanosine analogues (**77**) was cleanly converted to the final 3'-fluoro-4'-thio-2',3'-unsaturated guanosine (**79**) in 33% yield.

Structure-Activity Relationships. The antiviral activities of the synthesized nucleosides

were evaluated against HIV-1 in human PBM cells *in vitro*. Interestingly, in contrast to the rarely negative effect of fluorine substitution at the 2',3'-unsaturated double bond of the sugar moiety,²⁴¹ the synthesized D-2',3'-unsaturated 3'-fluoro-4'-thionucleosides did not show significant antiviral activity against HIV-1. Only D-guanosine analogue (**79**) showed marginal anti-HIV activity (EC_{50} 23.4 μ M) (*vide infra* for molecular modeling studies).

Molecular Modeling. In order to investigate the characteristic binding mode of D-3'-F-4'-Sd4 nucleoside, molecular modeling studies were performed by using the wild type (PDB entry 1rtd)²⁴² HIV-1 RT complexed with D-3'-F-4'-Sd4C triphosphate (TP). The geometrically optimized conformation of D-3'-F-4'-Sd4C, which was obtained by a quantum mechanical calculation (RHF/6-31G**), was located into the active site of HIV-1 RT, and the resulting complex was minimized²⁴³ until there was no significant change in atomic coordinates. The quantum mechanically geometry-optimized structures of D-3'-F-4'-Sd4C and D-2'-F-4'-Sd4C show almost the same conformation, which implies that the discrimination of D-3'-F-4'-Sd4C at the kinase level is not a plausible answer for inactivity of this compound to HIV-1 RT. The 3'-fluorine atom in the 2',3'-unsaturated D-nucleoside triphosphate, however, is located too close to the β -phosphate of its own triphosphate moiety when it is bound to the active site of HIV-1 RT (Fig. 6.1). The highly negatively charged two moieties strongly repulse each other to force a conformational change in the sugar moiety, but the possible steric hindrance between the bulky 4'-sulfur atom of the sugar and Met184 does not allow the sugar moiety to escape from the unfavorable electrostatic interaction. Rather, the minimized structure of D-3'-F-4'-Sd4CTP complexed with HIV-1 RT shows a disrupted base-pairing with the

complementary base in the template strand (Fig. 6.1). Therefore, because of the repulsive electrostatic interaction, the binding mode of the 3'-fluorinated D-2',3'-unsaturated nucleoside triphosphate is distorted to lose its binding affinity to HIV-1 RT. In this regard, it is also interesting to note that, D-3'-Fd4C showed only moderate anti-HIV activity whereas its L-congener was one of the most potent nucleosides in the fluorinated 2',3'-unsaturated nucleoside series.

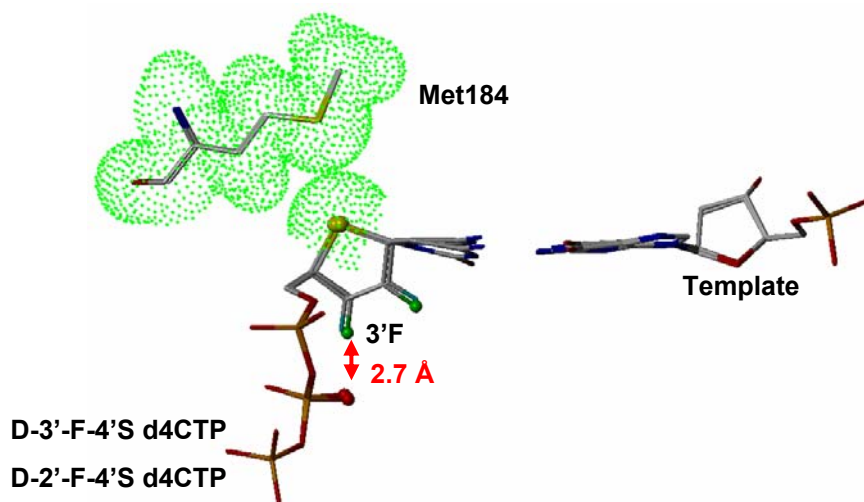


Figure 6.1. Negatively charged 3'-fluorine and β -phosphate moieties (2.7 Å apart) strongly repulse each other to force a conformational change in the sugar moiety, but the possible steric hindrance between the bulky 4'-sulfur atom of the sugar and Met184 does not allow the sugar moiety to escape from the unfavorable electrostatic interaction. Rather, the minimized structure of D-3'-F-4'-Sd4CTP complexed with HIV-1 RT shows a disrupted base-pairing with the complementary base in the template strand.

In summary, various D-3'-F-4'Sd4 nucleosides were prepared, and their anti-HIV activities were evaluated. Unfortunately, the synthesized D-isomers did not show any significant anti-HIV activity presumably because of the decreased binding affinity of the

corresponding triphosphates conferred by the unfavorable electrostatic interaction between the 3'-fluorine atom and its own triphosphate moiety.

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 ^1H NMR and 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. 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. High resolution 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*R*,3*S*,4*R*)-Benzoic acid 3-fluoro-4-hydroxy-5-methoxy-tetrahydro-furan-2-ylmethyl ester (2). For detail reaction conditions, see the reference 8. (Mikhailopulo, I. A.; Sivets, G. G. A novel route for the synthesis of deoxy fluoro sugars and nucleosides. *Helv. Chim. Acta.* **1999**, 82, 2052-2065). For β -isomer: ^1H NMR (CDCl_3) δ 8.06 (d, J = 7.2 Hz, 2H), 7.57 (t, J = 7.4 Hz, 1H), 7.44 (t, J = 7.9 Hz, 2H), 5.19 (dt, J = 54.0, 4.8 Hz, 1H), 4.91 (s, 1H), 4.60 – 4.51 (m, 2H), 4.43 (dd, J = 12.2, 5.2 Hz, 1H), 4.26 (br s, 1H),

3.29 (s, 3H); ^{13}C NMR (CDCl_3) δ 166.21, 133.23, 129.67, 128.41, 107.90, 92.24 (d, J = 187.0 Hz), 78.35 (d, J = 25.6 Hz), 74.34 (d, J = 15.3 Hz), 64.10 (d, J = 4.4 Hz) 55.52; Anal. Calcd for $\text{C}_{13}\text{H}_{15}\text{FO}_5$: C, 57.78; H, 5.59. Found: C, 57.89; H, 5.62. For α -isomer: ^1H NMR (CDCl_3) δ 7.98 (d, J = 7.3 Hz, 2H), 7.58 (t, J = 7.3 Hz, 1H), 7.45 (t, J = 7.8 Hz, 2H), 4.97-4.96 (m, 1H), 4.90 (ddd, J = 55.0, 5.5, 0.4 Hz, 1H), 4.58 (dt, J = 25.9, 2.9 Hz, 1H), 4.50 (dd, J = 12.0, 3.8 Hz, 1H), 4.43 (dd, J = 12.0, 3.5 Hz, 1H), 4.20 (tt, J = 16.9, 5.2 Hz, 1H), 3.49 (s, 3H), 2.84 (dd, J = 11.7, 1.0 Hz, 1H); ^{13}C NMR (CDCl_3) δ 165.96, 133.38, 129.54, 128.53, 102.25, 90.41 (d, J = 185.4 Hz), 80.46 (d, J = 25.3 Hz), 72.30 (d, J = 16.6 Hz), 63.73 (d, J = 10.0 Hz), 55.74.

(2*R*,3*S*,4*R*)-Benzoic acid 4-benzyloxy-3-fluoro-5-methoxy-tetrahydro-furan-2-ylmethyl ester (3). Sodium hydride (537 mg, 13.43 mmol) was added to a solution of **2** (2.42 g, 8.95 mmol) in dry THF and the mixture was stirred for 20 min at 0°C. BnBr (2.66 ml, 22.39 mmol) was slowly added and the mixture was treated with TBAI (662 mg, 1.79 mmol). After stirring for 24 h at rt, water was added. After phase separation, the aqueous phase was extracted three times with EtOAc and the combined organic phase was dried over MgSO_4 , filtered and evaporated to give a yellow oil which was purified by column chromatography on silica gel (7:1 hexane:EtOAc) to give 1.54 g (4.28 mmol, 48 % yield) of β -isomer and 0.76 g (2.1 mmol, 24 % yield) of α -isomer, respectively.: For β -isomer: $[\alpha]_{\text{D}}^{23}$ 44.4° (c 1.128, CH_3OH); ^1H NMR (CDCl_3) δ 8.24 (d, J = 7.9 Hz, 2H), 7.77 (t, J = 7.9 Hz, 1H), 7.63 (t, J = 8.1 Hz, 2H), 7.58-7.49 (m, 5H), 5.37 (dt, J = 53.2, 4.5 Hz, 1H), 5.16 (br s, 1H), 4.97 (d, J = 11.9 Hz, 1H), 4.87 (d, J = 11.8 Hz, 1H), 4.84 – 4.81 (m, 1H), 4.78 – 4.75 (m, 1H), 4.61 (dd, J = 11.8, 4.5 Hz, 1H), 4.24 – 4.21 (m, 1H), 3.53 (s, 3H); ^{13}C NMR (CDCl_3) δ 166.13, 133.26, 129.90, 129.71, 129.66, 128.46,

128.40, 127.96, 127.81, 106.72, 91.45 (d, $J = 192.0$ Hz), 80.40 (d, $J = 14.0$ Hz), 78.82 (d, $J = 25.4$ Hz), 72.91, 63.95, 55.60; Anal. Calcd for $C_{20}H_{21}FO_5$: C, 66.66; H, 5.87. Found: C, 66.77; H, 6.07. For α -isomer: $[\alpha]^{23}_D -57.7^\circ$ (c 0.704, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.78 (d, $J = 7.4$ Hz, 2H), 7.50 (t, $J = 7.4$ Hz, 1H), 7.34 ~ 7.24 (m, 7H), 4.86 (d, $J = 4.4$ Hz, 1H), 4.83 (ddd, $J = 56.3, 5.4, 1.1$ Hz, 1H), 4.71 (d, $J = 12.4$ Hz, 1H), 4.61 (d, $J = 12.4$ Hz, 1H), 4.57 ~ 4.56 (m, 0.5H), 4.51 ~ 4.49 (m, 0.5H), 4.38 (dd, $J = 12.1, 3.8$ Hz, 1H), 4.33 (dd, $J = 12.1, 3.6$ Hz, 1H), 3.74 (dt, $J = 24.5, 5.1$ Hz, 1H), 3.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.75, 133.10, 129.30, 129.15, 128.39, 128.32, 128.21, 128.12, 128.08, 101.84, 88.71 (d, $J = 190.7$ Hz), 80.32 (d, $J = 25.3$ Hz), 77.09 (d, $J = 18.5$ Hz), 72.47, 63.52 (d, $J = 9.4$ Hz), 55.45; Anal. Calcd for $C_{20}H_{21}FO_5$: C, 66.66; H, 5.87. Found: C, 66.81; H, 6.06.

(-)-(2*R*,3*S*,4*R*)-Benzoic acid 4-benzyloxy-5,5-bis-benzylsulfanyl-3-fluoro-2-hydroxypentyl ester (4). A solution of **3** (1.54 g, 4.27 mmol) and BnSH (2.0 ml, 17.10 mmol) in dry CH_2Cl_2 was treated with $BF_3 \cdot THF$ (0.11 ml, 0.85 mmol) and then the mixture was stirred for 4h at $0^\circ C$. The reaction mixture was quenched with $NaHCO_3$ and diluted with CH_2Cl_2 . The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 . The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated to give a colorless oil which was purified by column chromatography on silica gel (10:1 hexane:EtOAc) to give 1.8 g (3.12 mmol, 73 % yield) of **4** as a colorless oil: $[\alpha]^{24}_D -6.08^\circ$ (c 1.57, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.96 (d, $J = 8.2$ Hz, 2H), 7.51 (t, $J = 6.9$ Hz, 1H), 7.39 (t, $J = 15.2$ Hz, 2H), 7.27 ~ 7.05 (m, 15H), 4.88 (d, $J = 11.0$ Hz, 1H), 4.62 (dt, $J = 46.0, 5.9$ Hz, 1H), 4.60 (d, $J = 11.0$ Hz, 1H), 4.31 ~ 4.22 (m, 2H), 4.07 ~ 3.97 (m, 1H), 3.96 ~ 3.90 (m, 1H), 3.79 (d, $J = 3.6$ Hz, 1H), 3.69 (s, 4H),

2.58 (d, $J = 4.8$ Hz, 1H); ^{13}C NMR (CDCl_3) δ 166.61, 137.78, 137.69, 137.00, 133.15, 129.69, 129.07, 128.63, 128.49, 128.37, 128.23, 128.11, 127.27, 127.08, 91.14 (d, $J = 177.8$ Hz), 81.21 (d, $J = 25.6$ Hz), 74.92, 69.66 (d, $J = 22.6$ Hz), 65.30 (d, $J = 6.5$ Hz), 51.28, 36.64, 35.75; Anal. Calcd for $\text{C}_{33}\text{H}_{33}\text{FO}_4\text{S}_2$: C, 68.72; H, 5.77; S, 11.12. Found: C, 69.02; H, 6.03; S, 10.73.

(2*R*,3*R*,4*S*)-Benzoic acid 4-benzyloxy-5-benzylsulfanyl-3-fluoro-tetrahydrothiophen-2-ylmethyl ester (5). To a solution of **4** (2.47 g, 4.28 mmol) in a 2:1 mixture of dry toluene and CH_3CN were added PPh_3 (3.37 g, 12.85 mmol), I_2 (2.72 g, 10.71 mmol) and imidazole (1.17 g, 17.13 mmol). The reaction mixture was vigorously stirred at 90°C for 24 h, after which time the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20:1 hexane:EtOAc) to give 1.2 g (2.56 mmol, 60 % yield) of **5** as a mixture of α and β isomers. For the major: ^1H NMR (CDCl_3) δ 7.94 (d, $J = 7.1$ Hz, 2H), 7.51 (t, $J = 7.4$ Hz, 1H), 7.38 (t, $J = 7.9$ Hz, 2H), 7.31 ~ 7.19 (m, 10 H), 5.03 (dt, $J = 52.2, 3.4$ Hz, 1H), 4.58 (s, 2H), 4.55 (dd, $J = 11.1, 3.5$ Hz, 1H), 4.48 (d, $J = 7.8$ Hz, 1H), 4.38 (ddd, $J = 11.0, 7.2, 1.9$ Hz, 1H), 3.86 ~ 3.76 (m, 4H); ^{13}C NMR (CDCl_3) δ 180.02, 133.24, 129.69, 129.01, 128.68, 128.58, 128.43, 128.17, 127.95, 127.34, 119.85, 90.78 (d, $J = 190.5$ Hz), 85.88 (d, $J = 17.2$ Hz), 73.34, 63.37 (d, $J = 9.4$ Hz), 51.28 (d, $J = 4.0$ Hz), 51.28 (d, $J = 4.0$ Hz), 44.65 (d, $J = 18.7$ Hz), 36.21; Anal. Calcd for $\text{C}_{26}\text{H}_{25}\text{FO}_3\text{S}_2$: C, 66.64; H, 5.38; S, 13.69. Found: C, 66.89; H, 5.39; S, 13.39. For the minor: ^1H NMR (CDCl_3) δ 7.96 (d, $J = 7.2$ Hz, 2H), 7.50 (t, $J = 7.3$ Hz, 1H), 7.37 (t, $J = 7.5$ Hz, 2H), 7.30 ~ 7.19 (m, 10H), 5.13 (ddd, $J = 52.4, 4.4, 3.0$ Hz, 1H), 4.67 (dd, $J = 1.1, 8.0$ Hz, 1H), 4.58 (d, $J = 11.9$ Hz, 1H), 4.49 (ddd, $J = 10.9, 7.1, 2.1$ Hz, 1H), 4.36 ~ 4.31 (m, 2H), 4.00 (ddd, $J = 26.8, 6.3, 2.8$ Hz,

1H), 3.86 (d, $J = 13.6$ Hz, 1H), 3.76 (d, $J = 9.6$ Hz, 1H), 3.82 ~ 3.70 (m, 1H); ^{13}C NMR (CDCl_3) δ 179.41, 133.19, 129.71, 129.30, 128.68, 128.60, 128.55, 128.41, 128.17, 128.10, 127.21, 92.73 (d, $J = 194.6$ Hz), 81.68 (d, $J = 16.7$ Hz), 72.21, 63.79 (d, $J = 11.1$ Hz), 51.68, 44.53 (d, $J = 18.6$ Hz), 36.49.

(2*R*,3*R*,4*S*)-Benzoic acid 5-benzylsulfanyl-3-fluoro-4-hydroxy-tetrahydro-thiophen-

2-ylmethyl ester (6). A solution of **5** (988 mg, 2.11 mmol) in dry CH_2Cl_2 was treated with 1.0M solution of BCl_3 (8.43 ml, 8.43 mmol) at -78°C and then the mixture was stirred for 4 h at the same temperature. To the reaction mixture, pyridine (1 ml) and MeOH (3 ml) was added, and it was stirred overnight at rt. After evaporation of volatiles under the reduced pressure, the residue was dissolved in CH_2Cl_2 and the organic layer was washed with 2N HCl, aqueous NaHCO_3 and brine, dried over MgSO_4 , filtered and evaporated to give a crude, which was purified by column chromatography on silica gel (6:1 hexane:EtOAc) to give 795 mg (2.1 mmol, 100 % yield) of **6** as a 3.6:1 mixture of α and β isomers. For the major: ^1H NMR (CDCl_3) δ 8.02 (d, $J = 7.3$ Hz, 3H), 7.57 (t, $J = 7.3$ Hz, 1H), 7.44 (t, $J = 7.6$ Hz, 2H), 7.39 ~ 7.26 (m, 6H), 6.04 (d, $J = 5.2$ Hz, 1H), 5.21 (dt, $J = 51.3, 3.6$ Hz, 1H), 4.77 ~ 4.65 (m, 4H), 4.25 (ddd, $J = 20.3, 5.2, 3.0$ Hz, 1H), 4.05 ~ 3.94 (m, 1H), 2.09 (s, 3H); Anal. Calcd for $\text{C}_{21}\text{H}_{21}\text{FO}_5\text{S}$: C, 62.36; H, 5.23; S, 7.93. Found: C, 62.38; H, 5.52; S, 7.64. For the minor: ^1H NMR (CDCl_3) δ 8.02 (d, $J = 7.3$ Hz, 3H), 7.57 (t, $J = 7.3$ Hz, 1H), 7.44 (t, $J = 7.6$ Hz, 2H), 7.39 ~ 7.26 (m, 6H), 6.21 (d, $J = 4.8$ Hz, 1H), 5.23 (dt, $J = 52.7, 3.5$ Hz, 1H), 4.52 ~ 4.47 (m, 4H), 4.28 ~ 4.21 (m, 1H), 4.05 ~ 3.94 (m, 1H), 2.16 (s, 3H).

(2*R*,3*R*,4*S*)-Benzoic acid 3-fluoro-4-hydroxy-5-phenylselanyl-tetrahydro-thiophen-2-ylmethyl ester (7). To a solution of **6** (600 mg, 1.59 mmol) in AcOH (10 ml) was added Hg(OAc)₂ (1.26 g, 3.96 mmol), and the mixture was stirred for 24 h at rt. The reaction mixture was diluted with CH₂Cl₂ and washed successively with water, aqueous NaHCO₃ and 5 % KCN solution. The organic layer was dried over MgSO₄, filtered and concentrated to give the crude, which was purified by column chromatography on silica gel (hexane:EtOAc = 2:1) to give 230 mg (0.73 mmol, 46 % yield) of the corresponding acetate as a mixture of α/β isomers. The obtained acetates were dissolved in dry CH₂Cl₂ and treated with phenylselenol and BF₃OEt₂. After stirring for 1 h at rt, TEA was added and the reaction mixture was concentrated to dryness. The residual yellow oil was purified by column chromatography on silica gel (7:1 hexane:EtOAc) to give 160 mg (0.39 mmol, 53 % yield) of the α -isomer and 50 mg (0.12 mmol, 16 % yield) of β -isomer, respectively. For β -isomer: $[\alpha]^{22}_{\text{D}}$ 400.5° (c 0.154, CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.94 (d, J = 7.3 Hz, 2H), 7.62 (d, J = 6.9 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 7.37 (t, J = 7.5 Hz, 2H), 7.33 ~ 7.23 (m, 3H), 5.08 (dt, J = 52.9, 3.2 Hz, 1H), 4.56 (d, J = 10.2 Hz, 1H), 4.53 (dd, J = 11.1, 2.9 Hz, 1H), 4.36 (ddd, J = 11.1, 6.9, 2.0 Hz, 1H), 3.99 ~ 3.88 (m, 1H), 3.85 ~ 3.72 (m, 1H), 2.39 (d, J = 7.5 Hz, 1H); ¹³C NMR (CDCl₃) δ 166.36, 139.19, 133.29, 129.66, 129.53, 129.36, 129.25, 128.98, 128.44, 93.36 (d, J = 186.8 Hz), 80.42 (d, J = 18.3 Hz), 63.40 (d, J = 11.1 Hz), 48.27, 45.10 (d, J = 18.8 Hz). For α -isomer: $[\alpha]^{23}_{\text{D}}$ -320.3° (c 0.12, CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.97 (d, J = 7.7 Hz, 2H), 7.55 ~ 7.50 (m, 3H), 7.39 (t, J = 7.8 Hz, 2H), 7.24 ~ 7.22 (m, 3H), 5.08 (dt, J = 52.3, 3.0 Hz, 1H), 4.73 (d, J = 6.4 Hz, 1H), 4.63 (dd, J = 11.1, 8.1 Hz, 1H), 4.46 (ddd, J = 10.9, 6.9, 2.0 Hz, 1H), 4.32 (dddd, J = 28.2, 10.5, 6.4, 2.4 Hz, 1H), 3.83 (dtd, J = 26.4, 7.5, 3.8

Hz, 1H), 3.46 (d, $J = 11.6$ Hz, 1H); ^{13}C NMR (CDCl_3) δ 165.95, 134.00, 133.30, 131.16, 129.69, 129.51, 129.35, 128.45, 128.17, 93.90 (d, $J = 187.2$ Hz), 75.83 (d, $J = 17.3$ Hz), 63.12 (d, $J = 10.3$ Hz), 55.28, 45.34 (d, $J = 19.1$ Hz), 29.67.

(2*R*,3*S*,4*R*)-Methanesulfonic acid 3-fluoro-4-methanesulfonyloxy-5-methoxy-tetrahydro-furan-2-ylmethyl ester (20). Compound **2** (3 g, 11.1 mmol) was treated with a saturated ammonia in MeOH (30 ml) and the resulting solution was stirred for 5 h at rt. After evaporation of the volatiles, the residue was purified by column chromatography on silica gel (1:1 hexane:EtOAc) to give 1.5 g (9.03 mmol, 81 % yield) of the corresponding diol, which was used for the next step without further purifications. A mixture of the diol (1.5 g, 9.03 mmol) obtained above, pyridine (2.6 ml, 31.6 mmol) and DMAP (220 mg, 1.81 mmol) in dry CH_2Cl_2 was treated with MsCl (2.1 ml, 27.1 mmol) and the mixture was stirred for 24 h at rt. Aqueous NaHCO_3 solution was added and, after phase separation, the aqueous phase was extracted three times with CH_2Cl_2 . The combined organic layer was dried over MgSO_4 , filtered and evaporated to give a colorless oil, which was purified by column chromatography on silica gel (1:1 hexane:EtOAc) to give 1.61 g (4.99 mmol, 55 % yield) of β -isomer and 436 mg (1.35 mmol, 15 % yield) of α -isomer of the compound **20** as colorless oils. For β -isomer: $[\alpha]_D^{22} 29.4^\circ$ (c 3.03, CH_2Cl_2); ^1H NMR (CDCl_3) δ 5.25 (dt, $J = 52.1, 5.0$ Hz, 1H), 5.10 (br s, 1H), 5.00 (t, $J = 3.6$ Hz, 1H), 4.53 ~ 4.43 (m, 1H), 4.40 (t, $J = 5.0$ Hz, 2H), 3.44 (s, 3H), 3.15 (s, 3H), 3.12 (s, 3H); ^{13}C NMR (CDCl_3) δ 105.72, 89.14 (d, $J = 196.4$ Hz), 79.03 (d, $J = 13.6$ Hz), 77.92 (d, $J = 25.6$ Hz), 67.71 (d, $J = 3.9$ Hz), 56.01, 38.59, 37.78; Anal. Calcd for $\text{C}_8\text{H}_{15}\text{FO}_8\text{S}_2$: C, 29.81; H, 4.69. Found: C, 29.70; H, 4.72. For α -isomer: $[\alpha]_D^{23} -78.4^\circ$ (c 1.64, CH_2Cl_2); ^1H NMR (CDCl_3) δ 5.13 (d, $J = 4.5$ Hz, 1H), 5.02 (ddd, $J = 55.3, 5.8, 1.9$

Hz, 1H), 4.74 (dt, $J = 21.0, 5.4$ Hz, 1H), 4.49 (dq, $J = 25.0, 2.1$ Hz, 1H), 4.36 (d, $J = 2.9$ Hz, 2H), 3.45 (s, 3H), 3.11 (s, 3H), 3.01 (s, 3H); ^{13}C NMR (CDCl_3) δ 101.28, 87.71 (d, $J = 194.3$ Hz), 79.90 (d, $J = 26.0$ Hz), 73.99 (d, $J = 14.9$ Hz), 67.37 (d, $J = 8.9$ Hz), 55.99, 38.73, 37.74; Anal. Calcd for $\text{C}_8\text{H}_{15}\text{FO}_8\text{S}_2$: C, 29.81; H, 4.69. Found: C, 30.14; H, 4.72.

(2*R*,3*S*,4*R*)-Thiolacetic acid *S*-(3-fluoro-4-methanesulfonyloxy-5-methoxy-

tetrahydro-furan-2-ylmethyl) ester (21). A mixture of β -**20** (1.59 g, 4.92 mmol) and KSAc (562 mg) in dry DMF (4 ml) was stirred vigorously for 24 h at rt and then diluted with water. The reaction mixture was extracted three times with EtOAc and the combined organic layer was washed with brine, dried over MgSO_4 , filtered and evaporated to give a dark brown residue, which was purified by column chromatography on silica gel (2:1 hexane:EtOAc) to give 780 mg (2.58 mmol, 52 % yield) of the compound β -**21** as a wine-red oil along with the unreacted starting material β -**20** (554 mg, 1.72 mmol). For β -isomer: $[\alpha]_D^{25}$ 58.1° (c 1.525, CH_2Cl_2); ^1H NMR (CDCl_3) δ 5.03 (br s, 1H), 5.02 (dt, $J = 52.8, 4.8$ Hz, 1H), 4.9 (t, $J = 4.6$ Hz, 1H), 4.37 ~ 4.28 (m, 1H), 3.41 (s, 3H), 3.21 (dd, $J = 14.1, 6.7$ Hz, 1H), 3.15 (dd, $J = 14.1, 6.4$ Hz, 1H), 3.10 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (CDCl_3) δ 105.55, 91.44 (d, $J = 196.1$ Hz), 79.85 (d, $J = 14.2$ Hz), 79.47 (d, $J = 24.7$ Hz), 55.88, 38.58, 31.58, 30.53; Anal. Calcd for $\text{C}_9\text{H}_{15}\text{FO}_6\text{S}_2$: C, 35.75; H, 5.00; S, 21.21. Found: C, 35.85; H, 4.82; S, 21.29. For α -isomer, 392 mg (1.22 mmol) of the α -**20** gave 270 mg (0.89 mmol, 73 % yield) of α -**21** as a wine-red oil.: $[\alpha]_D^{25}$ -54.4° (c 0.74, CH_2Cl_2); ^1H NMR (CDCl_3) δ 5.12 (d, $J = 4.0$ Hz, 1H), 4.76 (dd, $J = 54.1, 5.8$ Hz, 1H), 4.67 (dt, $J = 5.4$ Hz, 1H), 4.66 (dt, $J = 5.4$ Hz, 1H), 3.48 (s, 3H), 3.30 (dd, $J = 14.4, 4.6$ Hz, 1H), 3.16 (s, 3H), 3.10 (dd, $J = 14.7, 6.2$ Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (CDCl_3)

δ 101.16, 89.26 (d, J = 194.0 Hz), 80.76 (d, J = 25.6 Hz), 74.17 (d, J = 15.0 Hz), 55.79, 38.85, 30.38, 30.30; Anal. Calcd for $C_9H_{15}FO_6S_2$: C, 35.75; H, 5.00; S, 21.21. Found: C, 36.02; H, 4.92; S, 21.00.

(-)-(2*S*,3*S*,4*R*,5*S*)-7-Fluoro-3-methoxy-2-oxa-5-thia-bicyclo[2.2.1]heptane (22). A mixture of 2-methoxyethanol (12 ml) and water (0.7 ml) was refluxed under N_2 for 90 min and, during cooling, N_2 was bubbled through. Compound β -**21** (240 mg, 0.79 mmol) and $NaHCO_3$ (133 mg, 1.59 mmol) were added under N_2 and the solution was refluxed for 48 h. After evaporation of the volatiles under the reduced pressure, the residue was diluted with EtOAc and then filtered through a short Celite/ $MgSO_4$ pad. After concentration, the filtrate was purified by column chromatography on silica gel (4:1 hexane:EtOAc) to give 90 mg (0.55 mmol, 70 % yield) of **22** as a yellow oil. Because of the low boiling point of the compound **22**, it was used for the next step without drying.: $[\alpha]_D^{21}$ -46.6° (c 0.938, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.53 (s, 1H), 5.30 (d, J = 55.8 Hz, 1H), 4.47 (s, 1H), 3.59 (s, 3H), 3.37 (q, J = 2.3 Hz, 1H), 2.92 (q, J = 11.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 107.56, 97.29 (d, J = 206.6 Hz), 77.41 (d, J = 18.3 Hz), 57.43, 47.56 (d, J = 15.6 Hz), 31.18 (d, J = 7.1 Hz).

(+)-(2*S*,3*R*,4*S*,5*R*)-Benzoic acid 3-fluoro-4-hydroxy-5-phenylselanyl-tetrahydrofuran-2-ylmethyl ester (29). Acetic anhydride (0.2 ml, 2.0 mmol) was added to a solution of β -**2** (270 mg, 1.0 mmol) and pyridine (0.2 ml, 2.5 mmol) in dry CH_2Cl_2 , and the solution was stirred for 3 h. After evaporation to dryness, the residue was coevaporated with a mixture of 1:1 toluene:EtOH five times and dried in high vacuum for 5 h to give 278 mg (0.89 mmol) of the corresponding acetate, which was used for the

next step without further purification. Phenylselenol (0.14 ml, 1.34 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$ (0.23 ml, 1.78 mmol) were added to a solution of the acetate (278 mg, 0.89 mmol) in dry CH_2Cl_2 , and the solution was stirred for 3 h at rt. Triethylamine (2 ml) was added and the reaction mixture was evaporated to give a yellow residue, which was purified by column chromatography on silica gel (hexane:EtOAc 8:1) to give 325 mg (0.74 mmol, 74 % yield) of the selenoglycoside. The obtained selenoglycoside was carefully treated with methanolic ammonia for 50 min at rt and then concentrated to give a crude, which was purified by column chromatography on silica gel (6:1 hexane:EtOAc) to give 225 mg (0.57 mmol, 77 % yield) of the compound **29** as a pale yellow syrup: $[\alpha]^{24}_{\text{D}} 125.40^\circ$ (c 0.936, CHCl_3); ^1H NMR (CDCl_3) δ 7.96 (d, $J = 7.2$ Hz, 2H), 7.65 ~ 7.57 (m, 3H), 7.44 (t, $J = 7.5$ Hz, 2H), 7.26 ~ 7.22 (m, 3H), 5.40 (dd, $J = 6.8, 1.7$ Hz, 1H), 5.02 (ddd, $J = 54.5, 4.4, 1.9$ Hz, 1H), 4.54 (dm J 24.9 Hz, 1H), 4.38 (d, $J = 4.3$ Hz, 2H), 4.23 ~ 4.10 (m, 1H), 2.36 (dd, $J = 7.7, 3.9$ Hz, 1H); ^{13}C NMR (CDCl_3) δ 166.41, 135.98, 133.78, 130.12, 129.71, 129.56, 128.95, 128.91, 127.01, 92.38 (d, $J = 184.6$ Hz), 84.72, 81.10 (d, $J = 23.8$ Hz), 75.48 (d, $J = 16.7$ Hz), 63.83 (d, $J = 9.2$ Hz).

(2*S*,3*S*,4*S*,5*S*)-Benzoic acid 3-fluoro-5-benzoxo-4-phenylselanyl-tetrahydro-furan-2-ylmethyl ester (30). Diisopropylazodicarboxylate (0.15 ml, 0.73 mmol) was added to a solution of **29** (145 mg, 0.37 mmol), BzOH (134 mg, 1.1 mmol) and PPh_3 (192 mg, 0.73 mmol) in DMF (5 ml), and the resulting solution was stirred for 24 h at rt. The volatiles were evaporated under the reduced pressure, and the residue was purified by column chromatography on silica gel (hexane:EtOAc = 15:1) to give 90 mg (0.21 mmol, 57 % yield) of the 1,2-rearranged product **30** along with 55 mg (0.14 mmol) of the unreacted starting material **29**: ^1H NMR (CDCl_3) δ 7.95 (d, $J = 7.4$ Hz, 2H), 7.88 (d, $J = 7.4$ Hz,

2H), 7.54 (m, 3H), 7.43 (t, $J = 7.5$ Hz, 1H), 7.36 (t, $J = 7.7$ Hz, 2H), 7.26 ~ 7.19 (m, 5H), 6.66 (d, $J = 5.2$ Hz, 1H), 5.43 (ddd, $J = 55.3, 6.6, 4.0$ Hz, 1H), 5.03 ~ 4.87 (m, 1H), 4.53 ~ 4.44 (m, 3H), 4.02 (dt, $J = 22.3, 6.4$ Hz, 1H); ^{13}C NMR (CDCl_3) δ 166.42, 165.22, 134.86, 133.64, 130.37, 130.11, 129.89, 129.71, 129.54, 129.03, 128.81, 128.75, 128.30, 98.79 (d, $J = 190.5$ Hz), 98.30 (d, $J = 7.2$ Hz), 81.80 (d, $J = 25.8$ Hz), 64.10 (d, $J = 4.6$ Hz), 49.35 (d, $J = 21.2$ Hz).

(2*S*,3*R*,4*S*,5*S*)-Benzoic acid 3,5-difluoro-4-phenylselanyl-tetrahydro-furan-2-ylmethyl ester (32). DAST (0.04 ml, 0.28 mmol) was added to a solution of **29** (75 mg, 0.19 mmol) in CH_2Cl_2 at 0°C , and the resulting mixture was stirred for 0.5 h at 0°C . The reaction mixture was quenched with aqueous NaHCO_3 , and the aqueous phase was extracted with CH_2Cl_2 three times. The combined organic layer was dried over MgSO_4 , filtered and concentrated to give a pale yellow oil, which was purified by column chromatography on silica gel (hexane:EtOAc=8:1) to give 63 mg (0.16 mmol, 84 % yield) of the 1,2-rearranged difluoro compound **32** as a colorless oil: ^1H NMR (CDCl_3) δ 8.02 (d, $J = 7.4$ Hz, 2H), 7.53 ~ 7.48 (m, 2H), 7.38 (t, $J = 7.7$ Hz, 2H), 7.31 ~ 7.23 (m, 4H), 5.87 (d, $J = 62.8$ Hz, 1H), 5.06 (dd, $J = 54.8, 2.4$ Hz, 1H), 4.77 (dm, $J = 26.5$ Hz, 1H), 4.47 (d, $J = 5.6$ Hz, 2H), 3.94 (dd, $J = 21.3, 11.3$ Hz, 1H); ^{13}C NMR (CDCl_3) δ 166.02, 134.30, 133.37, 129.86, 129.79, 129.31, 128.88, 128.42, 126.68, 114.91 (d, $J = 232.3$ Hz), 96.07 (d, $J = 191.0$ Hz), 84.49 (d, $J = 27.9$ Hz), 63.36 (d, $J = 7.0$ Hz), 48.73 (dd, $J = 25.8, 19.4$ Hz).

5-*O*-Benzoyl-2-deoxy-1-methyl- β -L-ribofuranose (42). Methyl 2-deoxy-L-ribofuranoside **41**²¹³ (1.1 g, 7.46 mmol) was dissolved in dry pyridine (10 ml), cooled to 0°C and treated with BzCl . The mixture was stirred at rt for 10 h, and then diluted with

cold water. The aqueous phase was extracted with CH₂Cl₂, and the combined organic layers were washed twice with aqueous NaHCO₃ solution, 2 N HCl and water. The organic layer was dried over MgSO₄, filtered and evaporated to give a crude, which was purified by column chromatography on silica gel (hexane:EtOAc = 2:1) to give 1.35 g (5.37 mmol, 72% yield) of **42** as a pale yellow oil (1:1 epimeric mixture): ¹H NMR (CDCl₃, 400 MHz) δ 8.08-8.07 (m, 2H), 8.03-8.01 (m, 2H), 7.60-7.56 (m, 2H), 7.47-7.43 (m, 4H), 5.15 (d, *J* = 4.5 Hz, 1H), 5.11 (dd, *J* = 5.3, 1.5 Hz, 1H), 4.57 (td, *J* = 6.8, 4.8 Hz, 1H), 4.43-4.36 (m, 4H), 4.26 (d, *J* = 6.1 Hz, 1H), 4.20 (q, *J* = 5.2 Hz, 1H), 3.41 (s, 3H), 3.32 (s, 3H), 2.32 (ddd, *J* = 13.3, 6.8, 1.5 Hz, 1H), 2.22-2.10 (m, 3H); HRMS (FAB) obsd, *m/z* 253.1083, calcd for C₁₃H₁₇O₅, *m/z* 253.1076 (M+H⁺). Anal. Calcd for C₁₃H₁₆O₅: C, 61.90; H, 6.39. Found: C, 61.57, H, 6.38.

Methyl 5-*O*-benzoyl-2-deoxy-β-L-glycero-pentofuranosid-3-ulose (43). Chromic anhydride (2.73 g, 27.30 mmol) was added to a stirred mixture of anhydrous pyridine (4.5 ml, 55.64 mmol) and anhydrous dichloromethane (50 ml) at rt, and stirring was continued at rt for 15 min. A solution of **42** (1.72 g, 6.82 mmol) in anhydrous dichloromethane (20 ml) was then added, followed immediately by acetic anhydride (2.6 ml, 27.51 mmol). After 15 min, the dark brown solution was treated with ethyl acetate (400 ml) and the resulting mixture was filtered through a TLC-grade silica gel pad, which was washed with ethyl acetate (one portion of 500 ml). The filtrate was concentrated under reduced pressure, co-evaporated with toluene (3x100 ml), and solvents were removed *in vacuo* to give 1.50 g (88%) of **43** as an orange oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.12-8.10 (m, 4H), 7.63-7.58 (m, 2H), 7.49-7.45 (m, 4H), 5.41 (t, *J* = 5.0 Hz, 2H), 4.80 (dd, *J* = 12.1, 2.7 Hz, 1H), 4.69 (dd, *J* = 11.7, 3.2 Hz, 1H), 4.51-4.47 (m, 2H), 4.44-4.42 (m, 1H), 4.31

(t, $J = 3.0$ Hz, 1H), 3.50 (s, 3H), 3.43 (s, 3H), 2.85 (dd, $J = 18.3, 5.7$ Hz, 1H), 2.71 (ddd, $J = 18.2, 5.4, 0.6$ Hz, 1H), 2.56 (d, $J = 12.6$ Hz, 1H), 2.52 (d, $J = 12.4$ Hz, 1H); HRMS (FAB) obsd, m/z 251.0920, calcd for $C_{13}H_{15}O_5$, m/z 251.0919 (MH^+). Anal. Calcd for $C_{13}H_{14}O_5 \cdot 0.07C_6H_5CH_3$: C, 63.12; H, 5.72. Found: C, 63.51, H, 5.37.

5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-1-methyl- β -L-ribofuranose (44).

(Diethylamino)sulfur trifluoride (3.0 ml, 22.71 mmol) was added to a solution of **43** (1.40 g, 5.59 mmol) in anhydrous dichloromethane (25 ml) and the reaction mixture was refluxed for 36 h. The resulting solution was then carefully poured into an ice-cooled saturated solution of sodium bicarbonate (50 ml) and extracted with diethyl ether (4 x 100 ml). The combined organic extracts were washed with brine (50 ml), dried over $MgSO_4$, filtered and concentrated to a crude oil that is purified by silica gel flash column chromatography (1:9 ethyl acetate/hexanes) to give 1.01 g (66%) of **44** as a yellow oil: ^1H NMR ($CDCl_3$) δ 7.90-7.18(m, 4H), 5.10(m, 1H), 4.54-4.29(m, 3H), 3.31, 3.29(s, 3H), 3.28 (s, 3H), 2.67-2.21(m, 2H); HRMS (FAB) obsd, m/z 273.0950, calcd for $C_{13}H_{15}F_2O_4$, m/z 273.0938 ($M+H^+$). Anal. Calcd for $C_{13}H_{14}F_2O_4$: C, 57.35; H, 5.18. Found: C, 57.64, H, 5.30.

(-)-Benzoic acid 5,5-bis-benzylsulfanyl-3,3-difluoro-2-hydroxy-pentyl ester (45). A solution of **44** (5.77g, 21.2 mmol) and benzyl mercaptane (10.0 ml, 84.8 mmol) in dry CH_2Cl_2 was treated with $BF_3 \cdot OEt_2$ at $0^\circ C$. The mixture was stirred for 30 min at $0^\circ C$ and for 6h at rt. The reaction mixture was then diluted with CH_2Cl_2 and poured into a separatory funnel containing saturated aqueous $NaHCO_3$ solution, and then extracted three times with CH_2Cl_2 . The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give a dark yellow residue which was purified by column

chromatography on silica gel (15:1 Hexane : EtOAc) to give 24.1 g (49.4 mmol, 65% yield) of **45** as a yellow oil: $[\alpha]_D^{23}$ -22.12° (*c* 1.12, CHCl₃); ¹H NMR (CDCl₃) δ 8.05 (t, 2H, *J* = 6.26 Hz), 7.60 (t, 1H, *J* = 6.26 Hz), 7.48 (t, 2H, *J* = 7.04 Hz), 7.31 ~ 7.14 (m, 10H), 4.47 (dd, 1H, *J* = 11.7, 2.3 Hz), 4.39 (dd, 1H, *J* = 11.7, 7.0 Hz), 3.85 ~ 3.69 (m, 8H), 2.84 (d, 1H, *J* = 6.26 Hz), 2.65 ~ 2.45 (m, 2H); ¹³C NMR (CDCl₃) δ 167.29, 137.99, 137.86, 133.80, 130.55, 130.20, 129.93, 129.52, 129.39, 129.06, 129.03, 128.87, 127.67, 127.62, 122.23 (t, *J* = 249.3 Hz), 70.86 (dd, *J* = 31.5, 25.3 Hz), 60.81, 42.81 (d, *J* = 7.9 Hz), 40.62 (t, *J* = 20.6 Hz), 31.99, 23.05; Anal. Calcd for C₂₆H₂₆F₂O₃S₂: C, 63.91; H, 5.36; S, 13.13 Found: C, 64.51; H, 5.50; S, 13.20.

Benzoic acid 5-benzylsulfanyl-3,3-difluoro-tetrahydro-thiophen-2-ylmethyl ester (46). Triphenyl phosphine (322 mg, 1.23 mmol), iodine (260 mg, 1.02 mmol) and imidazole (111 mg, 1.64 mmol) were added to a solution of **45** (200 mg, 0.41 mmol) in a 2:1 mixture of dry toluene (6 ml) and CH₃CN (3 ml). The reaction mixture was stirred for 24h at 90°C after which time the solution was concentrated to dryness. The red wine residue was purified by column chromatography on silica gel (30:1 Hexane:EtOAc) to give 144 mg (0.38 mmol, 93% yield) of epimeric mixture **46** as a colorless syrup: ¹H NMR (CDCl₃) δ 8.11 (d, 2H, *J* = 8.1 Hz), 7.61 (t, 1H, *J* = 7.1 Hz), 7.48 (t, 2H, *J* = 7.12 Hz), 7.36 ~ 7.25 (m, 5H), 4.66 (dd, 1H, *J* = 11.7, 6.6 Hz), 4.51 (dd, 1H, *J* = 11.2, 6.1 Hz), 4.43 (t, 0.5H, *J* = 5.1 Hz), 4.36 (t, 0.5H, *J* = 7.6 Hz), 3.98 ~ 3.81 (m, 3H), 2.79 ~ 2.69 (m, 1H), 2.60 ~ 2.46 (m, 1H); Anal. Calcd for C₁₉H₁₈F₂O₂S₂: C, 59.98; H, 4.77; S, 16.86, Found: C, 60.31; H, 4.96; S, 16.51.

Benzoic acid 5-acetoxy-3,3-difluoro-tetrahydro-thiophen-2-ylmethyl ester (47). Mercury(II) acetate (6.74 g, 21.2 mmol) was added to a solution of **46** in glacial acetic

acid (20 ml) and the mixture was stirred for 24h at rt. The reaction mixture was diluted with CH₂Cl₂ and successively washed with water, saturated aqueous NaHCO₃ solution and 5% KCN solution. The organic layer was dried over MgSO₄, filtered and concentrated to give the crude, which was purified by column chromatography on silica gel (20:1 Hexane:EtOAc) to give 2.5 g (7.90 mmol, 82% yield) of **47** as a yellow oil: ¹H NMR (CDCl₃) δ 7.99 ~ 7.94 (m, 2H), 7.53 ~ 7.49 (m, 1H), 7.40 ~ 7.36 (m, 2H), 6.02, 6.02 ~ 5.55 (m, 1H), 4.64 (dd, 0.5H, *J* = 11.2, 6.6 Hz), 4.56 (dd, 0.5H, *J* = 11.7, 6.1 Hz), 4.45 (dd, 0.5H, *J* = 11.2, 6.1 Hz), 4.35 (dd, 0.5H, *J* = 11.7, 6.1 Hz), 4.02 ~ 3.98 (m, 0.5H), 3.95 ~ 3.86 (m, 0.5H), 2.87 ~ 2.63 (m, 0.5H), 2.03 (s, 3H), 1.99 (s, 3H); Anal. Calcd for C₁₄H₁₄F₂O₄S: C, 53.16; H, 4.46; S, 10.14, Found: C, 53.05; H, 4.41; S, 9.91.

General procedure for condensation reaction of the acetate **47** with pyrimidines. The preparation of cytosine derivatives **48** and **49** is representative.

***N*⁴-Benzoyl-1-[(1*S*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-*L*-ribofuranosyl] cytosine (48) and *N*⁴-benzoyl-1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-*L*-ribofuranosyl] cytosine (49).** A mixture of *N*⁴-benzoylcytosine (243 mg, 1.34 mmol) in a 1:1 mixture of 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 10 ml) and dry CH₃CN (10 ml) was heated under reflux for 4 h, then the solvent was removed *in vacuo* at 30-35 °C. To the residual oil, a solution of **47** (170 mg, 0.54 mmol) in anhydrous acetonitrile (10 ml) was added followed, upon cooling to rt, by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.12 ml, 0.64 mmol). The resulting mixture was stirred for 4h, then diluted to 50 ml with dichloromethane and poured into an ice-cold saturated solution of NaHCO₃ (25 ml). The organic phase was separated, washed with brine (5 ml), dried over MgSO₄, filtered and concentrated to a crude that was purified by flash column chromatography

(1:1 Hexane:EtOAc) to give 165 mg (0.38 mmol, 70% yield) of an epimeric mixture of **48** and **49** as a pale yellow oil: UV (MeOH) λ_{max} 258.5, 310.0; Anal. (C₉H₁₁F₂N₃O₂S) C, H, N, S.

N⁴-Benzoyl-1-[(1*S*, 4*S*)- 5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-5-fluorocytosine (50) and N⁴-Benzoyl-1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-5-fluorocytosine (51). See the general procedure for condensation reaction of the acetate **47** with pyrimidines. An epimeric mixture of the title compounds **50** and **51** was obtained on 2.53-mmol scale in 66% yield: UV(CHCl₃) λ_{max} 285.5 nm; Anal. (C₂₃H₁₈F₃N₃O₄S) C, H, N, S.

1-[(1*R*, 4*R*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-4-thio- β -L-ribofuranosyl] thymine (52) and 1-[(1*R*, 4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-4-thio- α -L-ribofuranosyl] thymine (53). See the general procedure for condensation reaction of the acetate **47** with pyrimidines. An epimeric mixture of the title compounds **12** and **13** was obtained on 0.63-mmol scale in 83% yield: UV (MeOH) λ_{max} 265.0; Anal. (C₁₇H₁₆F₂N₂O₄S· 0.2 EtOAc) C, H, N, S.

1-[(1*S*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]uracil (54) and 1-[(1*R*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]uracil (55). See the general procedure for condensation reaction of the acetate **47** with pyrimidines. After aqueous work-up, followed by a column chromatography on silica gel (20:1 CH₂Cl₂:MeOH), an epimeric mixture of the title compounds **54** and **55** (3.16-mmol scale, 86% yield) was used for the next step without further purification because of its instability under the normal silica gel column chromatography conditions.

General procedure for the deprotection reaction of the pyrimidine analogues **48** ~ **55** with methanolic ammonia. The preparation of cytosine derivatives **56** and **57** is representative.

(-)-1-[(1*S*, 4*S*)-2,3-Dideoxy-3,3-difluoro- β -L-ribofuranosyl]cytosine (56**) and (+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]cytosine (**57**).** A mixture of **48** (260 mg, 0.55 mmol) in saturated ammonia/methanol solution (25 ml) was stirred at rt for 4 h, and then concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (1:9 methanol/chloroform) to give 63 mg (0.24 mmol, 44% yield) of **56** and 58 mg (0.22 mmol, 40% yield) of **57** as white solids. For compound **56**: mp 178-180 °C; $[\alpha]^{24}_{\text{D}} -28.47^{\circ}$ (*c* 1.16, MeOH); UV λ_{max} 280.5 (ϵ 11,717) (pH 2), 272.0 (ϵ 7,424) (pH 7), 271.5 (ϵ 7,522) (pH 11); Anal. (C₉H₁₁F₂N₃O₂S) C, H, N, S. For compound **57**: white solid, mp 182-183°C; $[\alpha]^{27}_{\text{D}} 127.22^{\circ}$ (*c* 0.18, MeOH); UV λ_{max} 279.5 (ϵ 14,161) (pH 2), 270.5 (ϵ 7,226) (pH 7), 272.0 (ϵ 7,484) (pH 11); Anal. (C₉H₁₁F₂N₃O₂S) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-5-fluorocytosine (58**) and (+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-5-fluorocytosine (**59**).** See the general procedure for the deprotection reaction of the cytidine analogue **48** with methanolic ammonia. The title compounds **58** and **59** were obtained on 0.88-mmol scale in 53% and 42% yield, respectively: For the compound **58**; mp 165°C; $[\alpha]^{24}_{\text{D}} -36.08^{\circ}$ (*c* 0.53, CH₃OH); UV(H₂O) λ_{max} 289.5 nm (ϵ 13,098, pH 2). 280.5 nm (ϵ 7,704, pH 7), 283.0 nm (ϵ 7,986, pH 11); Anal. (C₉H₁₀F₃N₃O₂S) C, H, N, S. For the compound **59**; mp 163°C; $[\alpha]^{24}_{\text{D}} 109.0^{\circ}$ (*c* 0.44, CH₃OH); UV(H₂O) λ_{max} 291.0 nm (ϵ 16,485, pH 2). 282.0 nm (ϵ 10,618, pH 7), 282.0 nm (ϵ 11,956, pH11); Anal. (C₉H₁₀F₃N₃O₂S) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]thymine (**60**) and (+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]thymine (**61**). See the general procedure for the deprotection reaction of the cytidine analogue **48** with methanolic ammonia. The title compounds **60** and **61** were obtained on 0.89-mmol scale in 43% and 55% yield, respectively: For the compound **60**; White solid: mp 70-72 °C; $[\alpha]_{\text{D}}^{24}$ -14.87° (*c* 2.07, CH₃OH); UV (MeOH) λ_{max} 269.0 (ϵ 17719) (pH 2), 269.0 (ϵ 10580) (pH 7), 269.0 (ϵ 8914) (pH 11); Anal. (C₁₀H₁₂F₂N₂O₃S) C, H, N, S. For the compound **61**; White solid: mp 128-130 °C; $[\alpha]_{\text{D}}^{23}$ 102.77° (*c* 0.87, CH₃OH); UV (MeOH) λ_{max} 269.0 (ϵ 16714) (pH 2), 268.0 (ϵ 19960) (pH 7), 268.5 (ϵ 18266) (pH 11); Anal. (C₁₀H₁₂F₂N₂O₃S) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]uracil (**62**) and (+)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]uracil (**63**). See the general procedure for the deprotection reaction of the cytidine analogue **48** with methanolic ammonia. The title compounds **62** and **63** were obtained on 2.71-mmol scale in 42% and 56% yield, respectively: For the compound **62**; mp 212~213 °C; $[\alpha]_{\text{D}}^{25}$ -20.97° (*c* 0.22, MeOH); UV(H₂O) λ_{max} 263.5 nm (ϵ 24,316, pH 2), 263.5 nm (ϵ 22,971, pH 7), 263.5 nm (ϵ 18,253, pH 11); Anal. (C₉H₁₀F₂N₂O₃S) C, H, N, S. For the compound **63**; mp 132~133 °C; $[\alpha]_{\text{D}}^{23}$ 135.38° (*c* 0.2, MeOH); UV(H₂O) λ_{max} 263.5 nm (ϵ 21,707, pH 2), 263.5 nm (ϵ 24,787, pH 7), 263.5 nm (ϵ 21,540, pH 11); Anal. (C₉H₁₀F₂N₂O₃S) C, H, N, S.

General procedure for the elimination reaction of the pyrimidine analogues (**56**, **58**, **60** and **62**) with potassium *tert*-butoxide. The preparation of cytosine derivative **64** is representative.

(-)-1-[(1*S*, 4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]cytosine (64).

A mixture of **56** (47 mg, 0.18 mmol) and potassium *tert*-butoxide (1.0M solution in THF, 0.59 ml, 0.59 mmol) in a 1:6 mixture of anhydrous DMF (0.5 ml) and 1,4-dioxane (3 ml) was stirred for 3h at rt. Minimum amount of MeOH was added to the light yellow slurry and the resulting clear solution was filtered through a short silica gel pad washing with a 10:1 mixture of CH₂Cl₂ and MeOH. After concentration, the crude was purified by column chromatography on silica gel (9:1 CH₂Cl₂:MeOH) followed by preparative TLC (8:3:1 CHCl₃:Acetone:MeOH) to give 17 mg (0.07 mmol, 40% yield) of **64** along with the recovered starting material **56** (23 mg, 0.09 mmol, 50% yield) as white solids: mp 182-190 °C; $[\alpha]_D^{22}$ -236.45° (c 0.017, MeOH); UV $\lambda_{\max}$ 276.0 (ε 11,990) (pH 2), 267.5 (ε 8,010) (pH 7), 264.5 (ε 8,060) (pH 11); Anal. (C₉H₁₀FN₃O₂S•0.4H₂O) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-5-fluorocytosine (65). See the general procedure for the elimination reaction of the cytidine analogue **56** with potassium *tert*-butoxide. The reaction was complete after 5h, and no starting material was recovered. The title compound **65** was obtained on 0.25-mmol scale in 64% yield: mp 172-174 °C; $[\alpha]_D^{25}$ -238.35° (c 0.14, CH₃OH); UV(H₂O) $\lambda_{\max}$ 292.0 nm (ε 10,178, pH 2), 283.0 nm (ε 7,192, pH 7), 283.0 nm (ε 6,290, pH 11); Anal. (C₉H₉F₂N₃O₂S) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]thymine (66).

See the general procedure for the elimination reaction of the cytidine analogue **56** with potassium *tert*-butoxide. The reaction was complete after 72h at rt. The title compound **66** was obtained on 0.15-mmol scale in 53% yield: White solid: mp 188-190 °C (dec.);

$[\alpha]^{26}_D -72.63^\circ$ (*c* 0.29, CH₃COCH₃); UV (MeOH) $\lambda_{\max}$ 268.5 (ϵ 17,606) (pH 2), 269.0 (ϵ 10,094) (pH 7), 265.0 (ϵ 9,977) (pH 11); Anal. (C₁₀H₁₁FN₂O₃S) C, H, N, S.

(-)-1-[(1*S*, 4*S*)-2,3-dideoxy-2,3-dideoxy-3-fluoro- β -L-ribofuranosyl]uracil (67). See the general procedure for the elimination reaction of the cytidine analogue **56** with potassium *tert*-butoxide. The reaction was quenched after 24h at rt. After preparative TLC (25:3:1 CH₂Cl₂:Acetone:MeOH), the title compound **66** was obtained on 0.26-mmol scale in 36% yield along with the recovered starting material **56** (40% yield): mp 150~152 °C; $[\alpha]^{25}_D -143.10^\circ$ (*c* 0.19, MeOH); UV(H₂O) $\lambda_{\max}$ 265.0 nm (ϵ 19,598, pH 2), 266.0 nm (ϵ 18,721, pH 7), 265.0 nm (ϵ 15,542, pH 11); Anal. (C₉H₉FN₂O₃S) C, H, N, S.

9-[(1*S*/*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-6-

chloropurine (68). A mixture of 6-chloropurine (820 mg, 2.59 mmol), HMDS (10 ml) and anhydrous CH₃CN (10 ml) was refluxed for 6h. After cooling to rt, volatiles were evaporated under reduced pressure. To the residue, a solution of **47** in anhydrous CH₃CN (10 ml) was added, and the mixture was cooled to 0 °C, whereupon TMSOTf (0.56 ml, 3.11 mmol) was added in a dropwise fashion. The mixture was stirred for 3h at 0 °C, 24h at rt and then 8 h at 70 °C. The reaction mixture was poured into an aqueous NaHCO₃ solution and extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered and evaporated to give a yellow residue which was purified by column chromatography on silica gel (3:1 Hexane:EtOAc) to give 655 mg (1.59 mmol, 61% yield) of an inseparable anomeric mixture of **68** as a pale yellow oil: UV(CH₂Cl₂) $\lambda_{\max}$ 264.5 nm; Anal. (C₁₇H₁₃ClF₂N₄O₂S) C, H, N, S.

(-)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-adenine (69) and (-)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-adenine (70). An anomeric

mixture of compound **68** (427 mg, 1.04 mmol) in methanol saturated with dry ammonia was heated in a steel bomb at 100 °C for 12h. Solvent was evaporated and the residue was purified by preparative TLC (10:1 CH₂Cl₂:MeOH) to give the desired compounds **69** (100 mg, 0.35 mmol 34% yield) and **70** (130 mg, 0.45 mmol, 43% yield) as white solids. For the compound **69**; mp 212~213 °C; $[\alpha]_D^{25}$ -15.94° (c 0.10, CH₃OH); UV(H₂O) $\lambda_{\max}$ 257.5 nm (ϵ 19,957, pH 2), 259.5 nm (ϵ 20,464, pH 7), 259.5 nm (ϵ 20,500, pH11); Anal. (C₁₀H₁₁F₂N₅OS) C, H, N, S. For the compound **70**; mp 172~173 °C; $[\alpha]_D^{25}$ 92.65° (c 0.12, CH₃OH); UV(H₂O) $\lambda_{\max}$ 258.0 nm (ϵ 12,871, pH 2), 259.5 nm (ϵ 13,566, pH 7), 259.5 nm (ϵ 13,341, pH11); Anal. (C₁₀H₁₁F₂N₅OS) C, H, N, S.

(-)-1-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-hypoxanthine (71) and (+)-1-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl]-hypoxanthine (72). A mixture of compounds **68** (378 mg, 0.92 mmol), 2-mercaptoethanol (0.26 mL, 3.68 mmol) and NaOMe (204 mg, 3.77 mmol) in anhydrous MeOH (15 mL) was stirred at 60 °C for 12h. The resulting clear solution was cooled to rt, quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel. The filtrate was concentrated and purified by preparative TLC (10:1 CH₂Cl₂:MeOH) to give the desired compounds **71** (105 mg, 0.36 mmol, 39% yield) and **72** (120 mg, 0.41 mmol, 45% yield) as white solids: For the compound **71**; mp 223~224 °C; $[\alpha]_D^{25}$ -280.08° (c 0.15, CH₃OH); UV(H₂O) $\lambda_{\max}$ 249.5 nm (ϵ 21,279, pH 2), 249.0 nm (ϵ 22,067, pH 7), 253.5 nm (ϵ 22,800, pH11); Anal. (C₁₀H₁₀F₂N₄O₂S) C, H, N, S. For the compound **72**; mp 224~225 °C; $[\alpha]_D^{25}$ 104.60° (c 0.09, CH₃OH); UV(H₂O) $\lambda_{\max}$ 249.5 nm (ϵ 15,177, pH 2), 249.0 nm (ϵ 15,488, pH 7), 253.5 nm (ϵ 15,907, pH11); Anal. (C₁₀H₁₀F₂N₄O₂S) C, H, N, S.

(-)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-adenine (73).

A solution of **69** (70 mg, 0.24 mmol) in anhydrous 1,4-dioxane (7 ml) was treated with potassium *tert*-butoxide at 0°C, and the reaction mixture was stirred for 1 h at 0°C. Minimum amount of MeOH was added to the suspension to quench the reaction, and the resulting clear solution was filtered through a short silica gel pad and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (10:1 CH₂Cl₂:MeOH) followed by preparative TLC (10:1 CH₂Cl₂:MeOH) to give the desired product **73** (40 mg, 0.15 mmol, 63% yield) as an off-white solid: mp >200 °C; $[\alpha]_D^{22}$ -39.58° (c 0.10, CH₃OH); UV(H₂O) $\lambda_{\max}$ 260.0 nm (ϵ 10,497, pH 2), 259.5 nm (ϵ 7,322, pH 7), 259.0 nm (ϵ 11,275, pH 11); Anal. (C₁₀H₁₀FN₅OS) C, H, N, S.

(-)-1-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl]-hypoxanthine (74). A solution of **71** (50 mg, 0.17 mmol) in a 1:1 mixture of anhydrous 1,4-dioxane (2 ml) and DMF (2 ml) was treated with NaOMe (28 mg, 0.52 mmol) at 0 °C, and the resulting mixture was slowly warmed to rt over 5 h. The mixture was recooled to 0 °C, treated with potassium *tert*-butoxide (1.0M solution in THF, 0.26 ml, 0.26 mmol) and slowly warmed to rt over 8 h. Minimum amount of MeOH was added to the suspension to quench the reaction, and the resulting clear solution was filtered through a short silica gel pad and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (9:1 CH₂Cl₂:MeOH) followed by trituration with CH₂Cl₂ and Et₂O to give the desired product **74** (30 mg, 0.11 mmol, 65% yield) as an off-white solid: mp >200 °C; $[\alpha]_D^{23}$ -85.70° (c 0.10, CH₃OH); UV(H₂O) $\lambda_{\max}$ 244.0 nm (ϵ 24,252, pH 2), 249.0 nm (ϵ 21,430, pH 7), 253.5 nm (ϵ 22,487, pH 11); Anal. (C₁₀H₉FN₄O₂S) C, H, N, S.

9-[(1*S*/*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl]-6-chloro-2-fluoropurine (75). A mixture of 6-chloro-2-fluoropurine (409 mg, 2.37 mmol), HMDS (15 ml) and CH₃CN (15 ml) was heated under reflux for 4 h until a clear suspension was obtained. After cooling to rt, volatiles were evaporated under reduced pressure. To the off-white solid, a solution of **47** (500 mg, 1.58 mmol) in anhydrous CH₃CN (10 ml) was added, and the mixture was cooled to 0 °C, whereupon TMSOTf (0.34 ml, 1.90 mmol), was added dropwise. The mixture was stirred for 1 h at 0 °C, 24 h at rt and then 8 h at 50 °C. To a separatory funnel containing 100 ml of aqueous NaHCO₃ solution, the reaction mixture was slowly added. After diluting with CH₂Cl₂ and phase separation, the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, evaporated and purified by column chromatography on silica gel (4:1 hexane:EtOAc) to give 538 mg (1.25 mmol, 79% yield) of an inseparable anomeric mixture of **75** as a colorless foam : UV(CH₂Cl₂) λ_{max} 270.5 nm; Anal. (C₁₇H₁₂ClF₃N₄O₂S) C, H, N, S.

9-[(1*R*/*S*, 4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-L-ribofuranosyl]-2-amino-6-chloropurine (76). Anhydrous ammonia gas was bubbled into a solution of an anemic mixture of **75** (638 mg, 1.49 mmol) in dry dimethoxyethane (50 ml) at rt for 4h. After evaporation of volatiles, the residue was filtered through a short silica gel pad to remove inorganic salts, and the filtrate was concentrated and purified by column chromatography on silica gel (2:1 hexane:EtOAc) to give the desired product **76** (340 mg, 54% yield) as a white foam: UV(CH₂Cl₂) λ_{max} 303.5nm; Anal. (C₁₇H₁₄ClF₂N₅O₂S) C, H, N, S.

(-)-9-[(1*R*, 4*S*)-2,3-dideoxy-3,3-difluoro- β -L-ribofuranosyl] guanine (77) and (+)-9-[(1*S*, 4*S*)-2,3-dideoxy-3,3-difluoro- α -L-ribofuranosyl] guanine(78). A solution of **76**

(340 mg, 0.80 mmol) in anhydrous MeOH (15 ml) was treated with 2-mercaptoethanol (0.22 ml, 3.19 mmol) and NaOMe (177 mg, 3.27 mmol), and the resulting mixture was stirred for 12 h at 60 °C. After cooling to rt, 0.2 ml of AcOH was added. The volatiles were removed under reduced pressure, and the remaining inorganic salts were removed by filtering the residue through a short silica gel pad washing with 8:1 CH₂Cl₂:MeOH. The filtrate was concentrated to give a white solid, which was dissolved in a small amount of DMF and purified by column chromatography on silica gel (9:1 CH₂Cl₂:MeOH) followed by preparative TLC (9:1 CH₂Cl₂:MeOH) to give 88 mg (0.29 mmol, 36% yield) of **77** and 82 mg (0.27 mmol, 34% yield) of **78** as white solids. For compound **77**: mp >220 °C; $[\alpha]_D^{24}$ -18.82° (c 0.20, CH₃OH); UV(H₂O) $\lambda_{\max}$ 256.0 nm (ϵ 11,036, pH 2). 255.0 nm (ϵ 11,314, pH 7), 262.0 nm (ϵ 10,160, pH 11); Anal. (C₁₀H₁₁F₂N₅O₂S) C, H, N, S. For compound **78**: mp >220 °C; $[\alpha]_D^{24}$ 19.23° (c 0.13, CH₃OH); UV(H₂O) $\lambda_{\max}$ 255.5 nm (ϵ 15,240, pH 2), 254.5 nm (ϵ 16,807, pH 7), 261.0 nm (ϵ 14,725, pH 11); Anal. (C₁₀H₁₁F₂N₅O₂S) C, H, N, S.

(-)-9-[(1*R*, 4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro- β -L-ribofuranosyl] guanine (79**).**

A solution of **78** (27 mg, 0.09 mmol) in a 1:1 mixture of anhydrous 1,4-dioxane (2 ml) and DMF (2 ml) was treated with NaOMe (15 mg, 0.27 mmol) at 0 °C, and the resulting mixture was slowly warmed to rt over 2h. The mixture was recooled to 0 °C, treated with potassium tert-butoxide (1.0M solution in THF, 0.14 ml, 0.14 mmol) and slowly warmed to rt over 8h. Minimum amount of MeOH was added to the suspension to quench the reaction, and the resulting clear solution was filtered through a short silica gel pad and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (9:1 CH₂Cl₂:MeOH) followed by trituration with CH₂Cl₂ and Et₂O to give the

desired product **79** (12 mg, 0.04 mmol, 45% yield) as a white solid: mp >220 °C; $[\alpha]_D^{24}$ -66.87° (c 0.06, CH₃OH); UV(H₂O) λ_{max} 248.5 nm (ϵ 18,111, pH 2), 251.5 nm (ϵ 17,481, pH 7), 260.0 nm (ϵ 19,537, pH11); Anal. (C₁₀H₁₀FN₅O₂S) C, H, N, S.

Supplemental Material

Elemental Analysis Data

Cmpd No	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
48 & 49	C ₉ H ₁₁ F ₂ N ₃ O ₂ S	58.59	4.06	8.91	6.80	58.37	4.31	8.82	6.42
50 & 51	C ₂₃ H ₁₈ F ₃ N ₃ O ₄ S	56.44	3.71	8.58	6.55	56.77	4.05	8.86	6.49
52 & 53	C ₁₇ H ₁₆ F ₂ N ₂ O ₄ S•0.2EtOAc	53.45	4.43	7.00	8.02	53.28	4.40	6.93	7.69
56	C ₉ H ₁₁ F ₂ N ₃ O ₂ S	41.06	4.21	15.96	12.18	41.38	4.56	15.94	12.41
57	C ₉ H ₁₁ F ₂ N ₃ O ₂ S	41.06	4.21	15.96	12.18	41.43	4.43	15.99	11.88
58	C ₉ H ₁₀ F ₃ N ₃ O ₂ S	38.43	3.58	14.94	11.40	38.62	3.68	14.88	11.20
59	C ₉ H ₁₀ F ₃ N ₃ O ₂ S	38.43	3.58	14.94	11.40	38.47	3.62	14.91	11.19
60	C ₁₀ H ₁₂ F ₂ N ₂ O ₃ S	43.16	4.35	10.07	11.52	42.79	4.09	9.86	11.22
61	C ₁₀ H ₁₂ F ₂ N ₂ O ₃ S	43.16	4.35	10.07	11.52	43.48	4.26	10.03	11.41
62	C ₉ H ₁₀ F ₂ N ₂ O ₃ S	40.91	3.81	10.60	12.13	41.14	3.83	10.57	12.13
63	C ₉ H ₁₀ F ₂ N ₂ O ₃ S	40.91	3.81	10.60	12.13	41.10	3.84	10.51	12.15
64	C ₉ H ₁₀ FN ₃ O ₂ S•0.4H ₂ O	43.16	4.35	16.78	12.80	42.90	4.40	16.49	12.60
65	C ₉ H ₉ F ₂ N ₃ O ₂ S	41.38	3.47	16.08	12.27	41.47	3.73	15.70	11.99
66	C ₁₀ H ₁₁ FN ₂ O ₃ S	46.50	4.29	10.85	12.42	46.40	4.61	11.02	12.46
67	C ₉ H ₉ FN ₂ O ₃ S	44.26	3.71	11.47	13.13	44.37	4.02	11.12	13.37
68	C ₁₇ H ₁₃ ClF ₂ N ₄ O ₂ S	49.70	3.19	13.64	7.81	49.66	3.24	13.98	8.07
69	C ₁₀ H ₁₁ F ₂ N ₅ OS	41.81	3.86	24.38	11.16	42.01	3.99	23.98	10.89

70	C ₁₀ H ₁₁ F ₂ N ₅ OS	41.81	3.86	24.38	11.16	42.09	4.02	23.99	10.90
71	C ₁₀ H ₁₀ F ₂ N ₄ O ₂ S	41.66	3.50	19.44	11.12	41.37	3.74	19.76	11.30
72	C ₁₀ H ₁₀ F ₂ N ₄ O ₂ S	41.66	3.50	19.44	11.12	41.95	3.80	19.75	11.42
73	C ₁₀ H ₁₀ FN ₅ OS	44.94	3.77	26.20	12.00	45.23	3.63	26.19	12.34
74	C ₁₀ H ₉ FN ₄ O ₂ S	44.77	3.38	20.88	11.95	44.92	3.55	21.22	11.89
75	C ₁₇ H ₁₂ ClF ₃ N ₄ O ₂ S	47.62	2.82	13.07	7.48	47.76	3.09	12.75	7.14
76	C ₁₇ H ₁₄ ClF ₂ N ₅ O ₂ S	47.95	3.31	16.45	7.53	47.69	3.36	16.77	7.67
77	C ₁₀ H ₁₁ F ₂ N ₅ O ₂ S	39.60	3.66	23.09	10.57	39.58	4.04	23.35	10.42
78	C ₁₀ H ₁₁ F ₂ N ₅ O ₂ S	39.60	3.66	23.09	10.57	39.38	3.78	23.33	10.57
79	C ₁₀ H ₁₀ FN ₅ O ₂ S	42.40	3.56	24.72	11.32	42.68	3.60	24.39	11.17

Supplemental Material

¹H NMR Data^a

Cpd	H-1'	H-2'	H-4'	H-5'	Other protons
48/49	6.33 ~ 6.29 (m, 2H)	3.16 ~ 2.99 (m, 2H), 2.79 ~ 2.71 (m, 2H)	4.30 ~ 4.22 (m, 1H), 4.19 ~ 4.10 (m, 1H)	4.82 (dd, $J = 11.7$, 6.1 Hz, 1H), 4.70 ~ 4.65 (m, 2H), 4.49 (dd, $J = 11.7$, 6.1 Hz, 1H)	9.09 (br s, 2H), 8.44 (d, $J = 7.1$ Hz, 1H), 8.37 (d, $J = 7.6$ Hz, 1H), 8.02 (d, $J = 7.1$ Hz, 4H), 7.89 (d, $J = 7.6$ Hz, 4H), 7.60 ~ 7.45 (m, 14H)
50/51	6.43 ~ 6.36 (m, 1H), 6.31 ~ 6.23 (m, 1H)	3.03 ~ 2.54 (m, 4H)	4.78 ~ 4.44 (m, 6H)		8.06 ~ 7.99 (m, 4H), 7.88 ~ 7.85 (m, 4H), 7.66 ~ 7.45 (m, 12H), 5.88 (dd, $J = 48.3$, 3.1 Hz, 1H), 5.76 (ddd, $J = 48.3$, 9.7, 3.6 Hz, 1H)
52/53	6.44 ~ 6.37 (m, 2H)	3.11 ~ 2.93 (m, 2H), 2.75 ~ 2.60 (m, 2H)	4.35 ~ 4.27 (m, 1H), 4.17 ~ 4.08 (m, 1H)	4.82 (dd, 1H, $J = 11.7$, 5.1 Hz), 4.72 (dd, 1H, $J = 11.7$, 6.1 Hz), 4.67 (dd, 1H, $J = 11.2$, 5.6 Hz), 4.53 (dd, 1H, $J = 11.7$, 6.6 Hz)	8.08 ~ 8.06 (m, 4H), 7.64 ~ 7.48 (m, 8H), 2.07 (s, 3H), 2.00 (s, 3H)
56	6.27 (t, $J = 7.6$ Hz, 1H)	2.88 ~ 2.78 (m, 1H), 2.75 ~ 2.61 (m, 1H)	3.73 ~ 3.66 (m, 1H)	3.93 (dd, $J = 11.7$, 4.1 Hz, 1H), 3.78 (dd, $J =$	8.31 (d, $J = 7.6$ Hz, 1H), 5.91 (d, $J = 7.6$ Hz, 1H)

				11.7, 4.1 Hz, 1H)	
57	6.26 (dd, J = 8.1, 5.1 Hz, 1H)	3.05 ~ 2.92 (m, 1H), 2.74 ~ 2.63 (m, 1H)	4.08 ~ 4.00 (m, 1H)	3.87 (dd, J = 11.7, 4.6 Hz, 1H), 3.76 (dd, J = 11.2, 6.1 Hz, 1H)	8.04 (d, J = 7.6 Hz, 1H), 5.94 (d, J = 7.6 Hz, 1H)
58	6.21 (td, J = 7.6, 2.0 Hz, 1H)	2.85 ~ 2.74 (m, 1H), 2.66 ~ 2.52 (m, 1H)	3.65 ~ 3.59 (m, 1H)	3.94 (dd, J = 11.7, 4.1 Hz, 1H), 3.73 (dd, J = 11.7, 3.1 Hz, 1H)	8.45 (d, J = 6.6 Hz, 1H)
59	6.35 ~ 6.32 (m, 1H)	3.17 ~ 3.03 (m, 1H), 2.79 ~ 2.69 (m, 1H)	4.16 ~ 4.09 (m, 1H)	3.96 (dd, J = 11.7, 4.6 Hz, 1H), 3.88 (dd, J = 11.2, 5.6 Hz, 1H)	8.24 (d, J = 6.6 Hz, 1H)
60	6.28 (t, 1H, J = 8.1 Hz)	2.79 ~ 2.65 (m, 2H)	3.69 ~ 3.63 (m, 1H)	4.02 (dd, 1H, J = 11.7, 3.6 Hz), 3.79 ~ 3.76 (m, 1H)	8.19 (s, 1H), 1.90 (s, 3H)
61	5.96 (dd, 1H, J = 8.1, 5.1 Hz),	2.71 ~ 2.56 (m, 1H), 2.34 ~ 2.23 (m, 1H)	3.71 ~ 3.64 (m, 1H)	3.53 (dd, 1H, J = 11.7, 4.6 Hz), 3.46 (dd, 1H, J = 11.7, 6.1 Hz)	7.38 (s, 1H), 1.59 (s, 3H)
62	6.25 (t, J = 7.8 Hz, 1H)	2.91 ~ 2.70 (m, 2H)	3.76 ~ 3.70 (m, 1H)	3.99 (dd, J = 11.7, 3.9 Hz, 1H), 3.82 ~ 3.78 (m, 1H)	8.37 (d, J = 7.8 Hz, 1H), 5.76 (d, J = 7.8 Hz, 1H)
63	6.24 (dd, J = 7.8, 5.5 Hz, 1H)	2.83 (m), 2.65(m)	4.11 ~ 4.03 (m, 1H)	3.87 (dd, J = 11.7, 4.7 Hz, 1H), 3.77 (dd, J = 11.0, 6.3 Hz, 1H)	8.01 (d, J = 7.8 Hz, 1H), 5.75 (d, J = 7.8 Hz, 1H)
64	6.62 (br s, 1H)	5.45 (br s, 1H)	4.16 (br s,	3.87 (dd, J = 12.2, 3.6	8.01 (d, J = 7.6 Hz, 1H), 7.67

			1H)	Hz, 1H), 3.57 (dd, J = 12.2, 2.5 Hz, 1H)	(br s, 1H), 5.81 (d, J = 7.1 Hz, 1H)
65	6.61 (br s, 1H)	5.45 (br s, 1H)	4.18 (br s, 1H)	3.92 (dd, J = 12.2, 3.1 Hz, 1H), 3.55 (dd, J = 12.2, 2.0 Hz, 1H)	8.28 (d, J = 6.6 Hz, 1H)
66	6.68 ~ 6.65 (m, 1H)	5.60 ~ 5.58 (m, 1H)	4.32 ~ 4.31 (m, 1H)	4.07 ~ 4.02 (m, 1H), 3.78 ~ 3.72 (m, 1H)	8.04 (s, 1H), 1.76 (s, 3H)
67	6.68 (br s, 1H)	5.72 (br s, 1H)	4.29 (br s, 1H)	4.02 ~ 3.98 (m, 1H), 3.70 ~ 3.66 (m, 1H)	8.17 (d, J = 7.8 Hz, 1H), 5.56 (br s, 1H)
68	6.37 ~ 6.31 (m, 2H)	3.26 ~ 3.13 (m, 4H)	4.52 ~ 4.45 (m, 1H), 4.26 ~ 4.18 (m, 1H)	4.91 (dd, J = 11.0, 6.3 Hz, 1H), 4.79 ~ 4.74 (m, 2H), 4.57 (dd, J = 11.7, 6.3 Hz, 1H)	8.78 (s, 1H), 8.75 (s, 1H), 8.51 (br s, 2H), 8.05 (t, J = 7.8 Hz, 4H), 7.72 ~ 7.70 (m, 1H), 7.64 ~ 7.59 (m, 1H), 7.55 ~ 7.45 (m, 4H)
69	6.27 (t, J = 6.8 Hz, 1H)	3.29 ~ 3.20 (m, 1H), 3.10 ~ 3.05 (m, 1H)	3.88 ~ 3.84 (m, 1H)	4.05 (dd, J = 11.7, 4.9 Hz, 1H), 3.94 (dd, J = 11.7, 3.9 Hz, 1H)	8.48 (s, 1H), 8.21 (s, 1H)
70	6.29 (dd, J = 6.8, 4.9 Hz, 1H)	3.24 ~ 3.09 (m, 2H)	4.27 ~ 4.20 (m, 1H)	3.92 (dd, J = 11.7, 4.9 Hz, 1H), 3.82 (dd, J = 11.7, 6.8 Hz, 1H)	8.39 (s, 1H), 8.22 (s, 1H)
71	6.28 (t, J = 6.8 Hz, 1H)	3.26 ~ 3.05 (m, 2H)	3.89 ~ 3.83 (m, 1H)	4.02 (dd, J = 11.7, 5.9 Hz, 1H), 3.94 (dd, J = 11.7, 3.9 Hz, 1H)	8.49 (s, 1H), 8.07 (s, 1H)

72	6.31 (dd, $J = 7.8, 4.9$ Hz, 1H)	3.25 ~ 3.08 (m, 2H)	4.26 ~ 4.20 (m, 1H)	3.91 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.82 (dd, $J = 11.7, 5.9$ Hz, 1H)	8.33 (s, 1H), 8.07 (s, 1H)
73	6.73 (br s, 1H)	5.49 (s, 1H)	4.40 (br s, 1H)	4.06 (dd, $J = 11.7, 8.8$ Hz, 1H), 3.75 (dd, $J = 11.7, 2.0$ Hz, 1H)	8.51 (s, 1H), 8.20 (s, 1H)
74	6.73 (br s, 1H)	5.79 (br s, 1H)	4.38 (br s, 1H)	4.04 (dd, $J = 12.7, 3.9$ Hz, 1H), 3.73 (dd, $J = 12.7, 9.8$ Hz, 1H)	8.49 (s, 1H), 8.06 (s, 1H)
75	6.25 (dd, $J = 6.8, 3.9$ Hz, 1H), 6.22 (dd, $J = 7.8, 3.9$ Hz, 1H)	3.25 ~ 3.13 (m, 2H), 3.12 ~ 3.01 (m, 2H)	4.46 ~ 4.40 (m, 1H), 4.27 ~ 4.20 (m, 1H)	4.88 (dd, $J = 11.7, 6.8$ Hz, 1H), 4.75 ~ 4.71 (m, 2H), 4.54 (dd, $J = 11.7, 6.8$ Hz, 1H)	8.51 (s, 1H), 8.50 (s, 1H), 8.02 (t, $J = 6.8$ Hz, 4H), 7.60 (q, $J = 7.8$ Hz, 2H), 7.49 ~ 7.44 (m, 4H)
76	6.15 (t, $J = 6.8$ Hz, 1H), 6.07 (t, $J = 6.8$ Hz, 1H)	3.46 ~ 3.37 (m, 1H), 3.15 ~ 3.09 (m, 2H), 3.06 ~ 2.97 (m, 1H)	4.50 ~ 4.44 (m, 1H), 4.17 ~ 4.10 (m, 1H)	5.12 (dd, $J = 11.7, 6.8$ Hz, 1H), 4.88 (dd, $J = 11.7, 7.8$ Hz, 1H), 4.76 (dd, $J = 11.7, 5.9$ Hz, 1H), 4.54 (dd, $J = 11.7, 6.8$ Hz, 1H)	8.11 (s, 1H), 8.05 (t, $J = 6.8$ Hz, 4H), 8.0 (s, 1H), 7.61 (q, $J = 6.8$ Hz, 2H), 7.50 ~ 7.51 (m, 4H)
77	6.09 (t, $J = 7.8$ Hz, 1H)	3.22 ~ 3.11 (m, 1H), 3.09 ~ 2.98 (m, 1H)	3.85 ~ 3.77 (m, 1H)	4.00 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.91 (dd, $J = 12.7, 3.9$ Hz, 1H)	8.14 (s, 1H)

78	6.12 (t, $J = 7.8$ Hz, 1H)	3.14 ~ 3.06 (m, 2H)	4.25 ~ 4.18 (m, 1H)	3.91 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.80 (dd, $J = 11.7, 5.9$ Hz, 1H)	8.00 (s, 1H)
79	6.55 (t, $J = 3.9$ Hz, 1H)	5.73 (br s, 1H)	4.34 (br s, 1H)	4.01 (dd, $J = 11.7, 3.9$ Hz, 1H), 3.73 (dd, $J = 11.7, 2.9$ Hz, 1H)	8.14 (s, 1H)

^a CD₃OD;ppm;s, singlet; d, doublet; dd, double doublet; t, triplet, br s, broad singlet; m, multiplet; td, triple doublet; ddd, double double doublet.

CHAPTER 7

MOLECULAR MECHANISM OF DAPD/DXG AGAINST AZT- AND 3TC- DRUG RESISTANT MUTANTS: A MOLECULAR MODELING APPROACH¹

¹ Chong, Y., K. Borroto-Esoda, P. A. Furman, R. F. Schinazi and C. K. Chu. 2002, *Antivir. Chem. Chemother.* 13:115-128.

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Introduction

The reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) is a multifunctional enzyme, which catalyzes the conversion of viral genomic RNA into double-stranded proviral DNA. RT is an important target for antiviral chemotherapy because it is essential for the viral replication. Two classes of inhibitors are currently being used in the management of HIV infection. Nonnucleoside RT inhibitors (NNRTIs) bind directly to a hydrophobic pocket adjacent to the polymerase active site of RT. Nucleoside RT inhibitors (NRTIs) such as AZT and 3TC, which are being widely used in the treatment of HIV infection, act by competing with natural substrates at the HIV RT active site, leading to incorporation and termination of the DNA chain. Unlike most cellular DNA polymerases, RT exhibits lower fidelity and processivity than cellular DNA polymerases and lacks a 3'- to 5'- proofreading activity, resulting in a high mutation rate and drug-resistance.^{244,245,246,247,248} Anti-HIV therapy is limited due to a number of factors including development of viral drug resistance and significant host toxicity often observed following long-term exposure to antiviral drugs (Table 7.1).^{249,250} Consequently, efforts continue to identify potent antiviral agents effective against drug resistant virus. (-)- β -D-Dioxolane guanine (DXG) and its prodrug, 2,6-diaminopurine (DAPD), have been reported to be potent inhibitors of HIV-1.^{251,252,253} When recombinant or clinical variants of HIV-1 were used to assess the efficacy of this purine nucleoside analogue against drug-resistant HIV-1, it was observed that AZT-resistant and 3TC-resistant virus remained sensitive to DXG.^{8,254} These findings have particular importance because even though these two NRTIs (AZT and 3TC) are the most widely used combination therapy

regimen, RT can become multidrug-resistant under prolonged combination therapy.^{255,256,257} Therefore, the investigation of the characteristic binding patterns of DXG to the drug-resistant RTs might help us understand drug resistance mechanism, and, eventually, design more effective nucleoside reverse transcriptase inhibitors (NRTIs).

Table 7.1. Fold resistances in mutant HIV-1 RT associated with NRTI resistance

Mutation	Resistance Conferred (Fold Resistance) ^a
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)

^a Schinazi, R. F.; Larder, B. A.; Mellors, J. W. Mutations in retroviral genes associated with drug resistance. *International Antiviral News*, **2000**, 8, 129.

In the absence of structural data for NRTI-resistant RTs, various mechanisms have been proposed to explain the action of such mutations. These include selective alterations in NRTI binding and/or incorporation, template/primer (T/P) repositioning and NTP

mediated as well as phosphorolytic removal of an incorporated chain-terminating NRTI residue from the 3'-end of the nascent viral DNA.^{258,259} However, even though the phosphorolytic removal of AZT gains more support from other investigations,¹⁶ there has not been clear explanation about the detailed mechanism of the resistance at a molecular level.

Based on the X-ray structure of the covalently trapped catalytic complex of RT,²⁶⁰ Huang et al. have found that the point mutations sufficient for resistance (K65R, K70R, L74V, Q151M, M184I/V and T215Y/F) are all in the neighborhood of the incoming nucleotide. Therefore the primary mutations are likely to affect directly the position, stability, or reactivity of the bound analog itself or additionally the templating base or primer terminus. In other words, there might be significant differences in binding patterns and binding energies of nucleoside triphosphates (NRTIs as well as natural substrates) to wild-type (WT) with respect to the mutant enzymes. This idea prompted us to correlate the binding affinities of nucleoside analogs to the WT and mutant RT with the resistance conferred by mutations. A direct comparison of binding affinities of NRTIs to RT with their biological activity (EC_{50}) has not been thought to be possible because the efficiency of inhibition of HIV-1 replication by NRTIs is dependent on many factors including cellular uptake, phosphorylation by kinases and the efficiency of an analog binding to RT, and subsequent incorporation into the elongating viral DNA.²⁶¹ However, assuming that the mutation at RT does not affect any other events of NRTIs but its own structure and functions, it becomes possible to correlate the biological activity (EC_{50}) with the difference in the binding affinities of the NRTIs to mutant RT with respect to those to the WT RT. Furthermore, if we compare the binding energies of the NRTIs with those of the

corresponding natural substrates, we have more chance to correlate the antiviral activity (EC_{50}) with the events at the binding to the RT. For this purpose, we conducted molecular modeling studies on WT RT as well as RT mutated at specific residues. Three structurally different nucleotide inhibitors, AZTTP, 3TCTP and DXGTP (Figure 7.1), were chosen due to their distinct structural features as well as their unique drug resistance profiles.

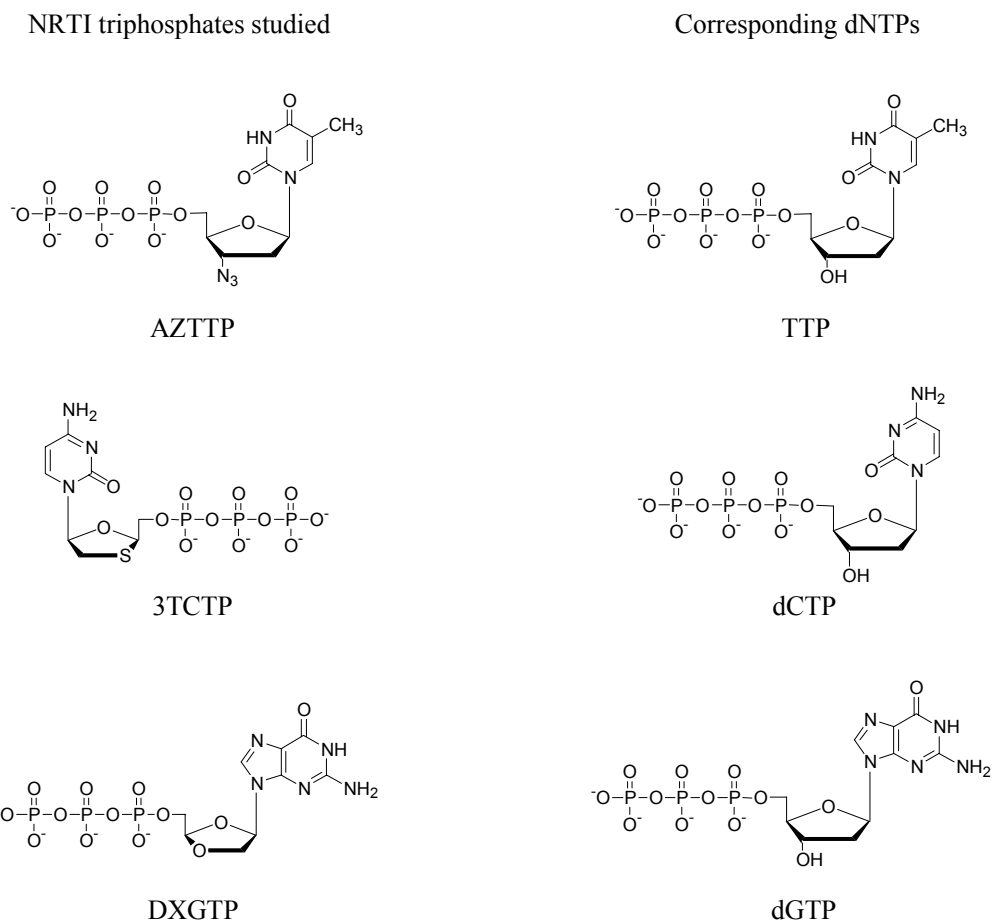


Figure 7.1. Structures of NRTI triphosphates studied and their corresponding natural dNTPs

The NRTI triphosphates were docked into the WT and mutant (M41L/D67N/K70R/T215Y, M184V, K65R, L74V and K65R/Q151M) RT active sites,

and then the resulting structures were minimized. The binding affinities of the examined structures toward HIV-1 RT were estimated by means of the binding energy differences (relative binding energy, E_{rel} , Table 7.2 ~ 7.4) between the NRTI triphosphate (DXGTP, AZTTP or 3TCTP)-RT complex and the corresponding natural substrate, 2'-deoxynucleoside triphosphates (dNTPs : dGTP, TTP or dCTP)-RT complex in the energy-minimized states. The relative binding energies of DXGTP, AZTTP and 3TCTP (E_{rel} , Table 7.2 ~ 7.4) were correlated with the anti-HIV activity of the nucleoside inhibitors, and the minimized structures of both WT and mutant enzymes bound to each inhibitor were analyzed to obtain the molecular mechanism of drug resistance conferred by mutations in RT.

Materials and Methods

All molecular modeling of the enzyme-substrate complexes was carried out using SYBYL 6.6 (Tripos Inc. St. Louis, MO).

HIV-1 RT structure (PDB entry 1rtd). HIV-1 RT is a heterodimer comprising of subunits of 66 and 51 kDa (p66 and p51). The overall shape of the p66 subunit has been likened to that of a right hand, with the major subdomains of the polymerase domain of p66 appropriately termed fingers, palm and thumb. The DNA polymerase catalytic aspartate residues (Asp110, Asp185, and Asp186) are in the palm subdomain, and the mutations associated with NRTI resistance occur primarily in the fingers and the palm subdomains of RT. Comparison of the structures of free RT with that of the RT-template/primer (RT-T/P) binary complex shows that the interaction of RT with

template-primer duplex induces a conformational rotation of the p66 thumb subdomain.^{262,263} Comparison of the structures of the RT-T/P binary complex with the RT*-T/P-dNTP ternary complex reveals that dNTP binding induces further conformational changes in RT.¹⁷ In particular, part of the fingers subdomain rotate inwards toward the palm subdomain and the polymerase active site, effectively clamping the dNTP into the active site region. Therefore in order for the enzyme-ligand complex to be catalytically active, there must be some conformational changes in RT leading to a tight binding of the substrate. The sugar 3'-OH of the dNTP projects into a small pocket formed by the side chains of D113, A114, Y115, F116 and Q151. Interestingly, the 3'-OH of the nucleotide forms hydrogen bonds with the backbone -NH of Y115 and triphosphate moiety of the dNTPs. However, since the NRTIs used in this study lack 3'-OH, the adjacent enzyme residues such as Arg65, Lys70 and Arg72 should be involved in stabilizing the bound nucleoside.

Model enzyme site. 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).¹⁷ Given the large size of the HIV-1 RT, calculations of the entire protein-ligand complex were not practical. Therefore, a model of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit. Hydrogen atoms were added, and the clipped residues (Lys1 and Pro243) were capped with acetyl groups. The template-primer duplex had to be truncated as well. Interestingly, 5:2 (template:primer, ratio of bases) duplex was not stable enough presumably because of the insufficient base stacking interaction to maintain the duplex structure. Thus, a 7:4 (template:primer) duplex, which

was shown to be stable enough to give a reliable result was used for the modeling studies.²⁶⁴ The missing 3'-OH group in G22 [the 3'-end (n^{th}) nucleotide in the primer strand] in the original X-ray coordinates (1rtd)¹⁷ was added, so that it could properly coordinate the adjacent Mg atom. Three clipped residues in template and primer strands [G3, C19(template)/ G9(primer)] were capped with methyl groups.

NRTIs. The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates (AZT)²⁶⁵ or conformational analysis (3TC).²¹ The X-ray coordinates of D-dioxolane cytosine²⁶⁶ were used as a template for construction of DXG structure, whose heterocyclic base moiety, cytosine, was modified to guanine before docking into the enzyme. The heterocyclic moiety of $n+1^{\text{th}}$ nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, in 3TCTP and DXGTP modeling, the adenine moiety which was in the original X-ray structure (1rtd)¹⁷ was modified to guanine and cytosine, respectively. The enzyme site and the inhibitor were merged to form a catalytic complex and then the NRTI triphosphate was situated in such an orientation that it could be paired with its complementary base in the template strand by adjusting the torsional angles to those found in the X-ray structure (1rtd: P_{α} -O5'-C5'-C4':-162.6°, O_{α} - P_{α} -O5'-C5':96.2°).¹⁷

Method. Gasteiger-Hückel charge^{267,268} 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 option in SYBYL 6.6 (Tripos Inc. St. Louis, MO). The mutated enzyme sites (M41L/D67N/K70R/T215Y, M184V, K65R, L74V and K65R/Q151M) were constructed by modifying the WT enzyme site by point mutation or a combination of the point mutations of the amino acid residues. In order to

eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed ('annealing' means minimization of a subset of a macromolecule to perform energy refinement (strain relaxation) on local regions of large structures. Detailed information can be found in the 'Biopolymer modeling manual', pp54-60, Tripos Bookshelf, version 6.6) until energy change from one iteration to the next was less than 0.05 kcal/mol (hot region: 6 Å , interesting region : 12 Å , Kollman-All-Atom Force Field, Kollman-All-Atom charge for enzyme and Gästeiger-Hückel charge for duplex). The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field until iteration number reached 5,000 or the energy change from one iteration to the next was less than 0.05 kcal/mol. The binding affinities of the examined structures toward HIV-1 RT was estimated by means of the relative binding energy (E_{rel} , Table 7.2 ~ 4) between the inhibitor triphosphate (DXGTP, AZTTP or 3TCTP)-RT complex and the corresponding natural substrate (dGTP, TTP or dCTP)-RT complex in the energy-minimized states.

Results

Calculation of Relative Binding Energy (E_{rel}). The binding affinities were estimated by means of the binding energy differences (relative binding energy, E_{rel}) between the NRTI triphosphate (DXGTP, AZTTP or 3TCTP)-RT complex and the corresponding natural substrate, 2'-deoxynucleoside triphosphates (dNTPs: dGTP, TTP or dCTP)-RT complex. The binding energy (E_{b}) of each nucleoside triphosphate was calculated by the Eq 1.

$$E_b = E(\text{ligand}) + E(\text{enzyme}) - E(\text{enzyme-inhibitor complex}) \dots\dots (\text{Eq 1})$$

Where, E_b = binding energy of the NRTI triphosphate or dNTP to RT

$E(\text{enzyme})$ = energy of RT-DNA duplex complex

$E(\text{ligand})$ = energy of NRTI triphosphate or dNTP

$E(\text{enzyme-ligand complex})$ = energy of RT-DNA duplex-NRTI triphosphate ternary complex or RT-DNA duplex-dNTP ternary complex

The relative binding energy (E_{rel}) of the NRTI triphosphate was calculated by the difference of the binding energy between NRTI triphosphate and the corresponding dNTP (Eq 2).

$$E_{\text{rel}} = E_b(\text{NRTI triphosphate}) - E_b(\text{dNTP}) \dots\dots (\text{Eq 2})$$

Therefore, a favorable binding affinity would be represented by a large positive E_{rel} and a poor binding NRTI triphosphate would have a small or negative E_{rel} .

The computational analyses indicated that the antiviral activity of the nucleoside RT inhibitors can be influenced by the binding affinity of their triphosphates to RT. In WT enzyme sites, DXGTP, AZTTP and 3TCTP showed favorable binding affinities toward RT (Table 7.2 ~ 4). The calculated relative binding energy (E_{rel}) for AZT to the AZT-resistant mutant (WT:146.4 and M41L/D67N/K70R/T215Y:73.4 kcal/mol) showed a good correlation with the drug resistance (Table 7.2). 3TC also showed a significant decrease in E_{rel} (Table 7.3) toward the 3TC-resistant mutant (WT:76.4 and M184V:-28.8 kcal/mol). Interestingly, DXG showed a linear correlation of E_{rel} with drug resistance. Both AZT and 3TC-resistant mutant strains (M41L/D67N/K70R/T215Y:8.0 and M184V:5.7, kcal/mol) maintain favorable affinities to DXG (Table 7.2 and 3), but the

mutants that confer resistance to DXG showed a significantly decreased E_{rel} (K65R:-37.1, L74V:0.2 and K65R/Q151M:-44.4 kcal/mol, Table 7.4).

Analysis of the minimized structure of RT-AZTTP complex.

The most widely used clinical therapeutic against the HIV-1 is the nucleoside analogue 3'-azido-3'-deoxythymidine (AZT), 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.²⁶⁹ However, prolonged clinical use of AZT monotherapy invariably results in the appearance of HIV-1 resistant to the drug (Table 7.1). The calculated relative binding energy (E_{rel}) of AZTTP toward RT showed that the E_{rel} could be correlated with the antiviral activity of AZT for WT RT and quadruple mutant resistant to AZT (M41L, D67N, K70R, T215Y) (Table 7.2).

WT RT-AZTTP complex (Figure 7.2a and 2b). On the basis of their crystal structure of WT RT/DNA/dNTP, Huang et al. have suggested that the "3'-OH pocket", lined by the side chains of Asp113, Tyr115, Phe116 and Gln151, and the peptide backbone between 113 and 115 can probably accommodate two or three water molecules in addition to the 3'-OH, and it clearly has room for the azido group of AZTTP.¹⁷ Many spectroscopic and kinetic studies showed that TTP and AZTTP have the same binding site as well as the same binding conformation when they are bound to RT, which supports the significance of the role of the pocket for AZTTP binding.^{270,271,272,273,274,275} Recent pre-steady-state kinetic analysis of WT RT²⁷⁶ showed that there is about two fold difference in binding affinity (K_d) between TTP and AZTTP, which can be correlated with our calculated relative binding energy (E_{rel}) (Table 7.2). The comparison of the binding modes showed

that there are two major differences. Different ring conformation between the natural substrate (TTP) and AZTTP allows an inward rotation of ϵ -amino group of Arg72 to the binding site (Figure 7.2a) to form a stable binding interaction between the enzyme and the triphosphate moiety of AZTTP.

Table 7.2. AZT- resistant RT : correlation of binding energy (E_{rel}) with activity (EC_{50})

	AZT			DXG		
	EC_{50}^a (μ M)	Fold Increase (EC_{50})	E_{rel}^b (kcal/mol)	EC_{50}^a (μ M)	Fold Increase (EC_{50})	E_{rel}^b (kcal/mol)
WT	0.0041	--	146.4	0.21	--	12.0
M41L/D67N/ K70R/T215Y	0.082	20	73.4	0.24	1.1	8.0

^a CMB Cells : Gu *et al.*, 1999

^b Relative binding energy (E_{rel}) = Binding energy of NRTI triphosphate – Binding energy of natural 2'-deoxynucleotide : Lee *et al.*, 2001

The bulky azido group of AZTTP, on the other hand, can be accommodated in the binding pocket without any steric conflict with neighboring enzyme residues and form hydrogen bonds with Tyr115 and Phe116 backbone –NH groups (Figure 7.2a). A combination of these two factors induces conformational changes in tertiary structure of the RT to form a tight complex by closing the gap between the fingers and palm domains (Figure 7.2b), which may be responsible for the difference in binding affinity as well as the potent anti-HIV activity of AZTTP.

The quadruple mutant (M41L/D67N/K70R/T215Y) RT-AZTTP complex (Figure 7.2c and Figure 7.2d). AZT resistance correlates with multiple mutations in RT, including M41L, D67N, K70R, L210W, T215Y/F and Q219K (Table 7.1 and 2).^{277,278,279} The extent of AZT resistance is related to the combination of mutations present. In general, two or more of these mutations in RT are normally needed to exhibit high-level AZT resistance.^{280,281} Examination of WT RT/DNA/dNTP ternary complex¹⁷ 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 WT RT for the incorporation of AZTTP into the DNA chain.^{33,282,283} 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, and this mechanism has been supported by other investigations.^{284,285,286,287,288} Recently, Hughes et al. used both structural and biochemical data to develop a model, which showed that ATP is most likely pyrophosphate donor in in vivo excision reaction and AZT resistant mutations serve to increase the affinity of RT for ATP so that, at physiological ATP concentrations, excision is reasonably efficient.⁴³ Their modeling study showed that the specificity of the excision reaction for an AZT-terminated primer is due to the structure of the region around the HIV-1 RT polymerase active site and its interaction with the azido group of AZT. Steric constraints involving the azido group cause the end of an AZT 5'-monophosphate-terminated primer to preferentially reside at the nucleotide binding site, which favors excision.⁴³

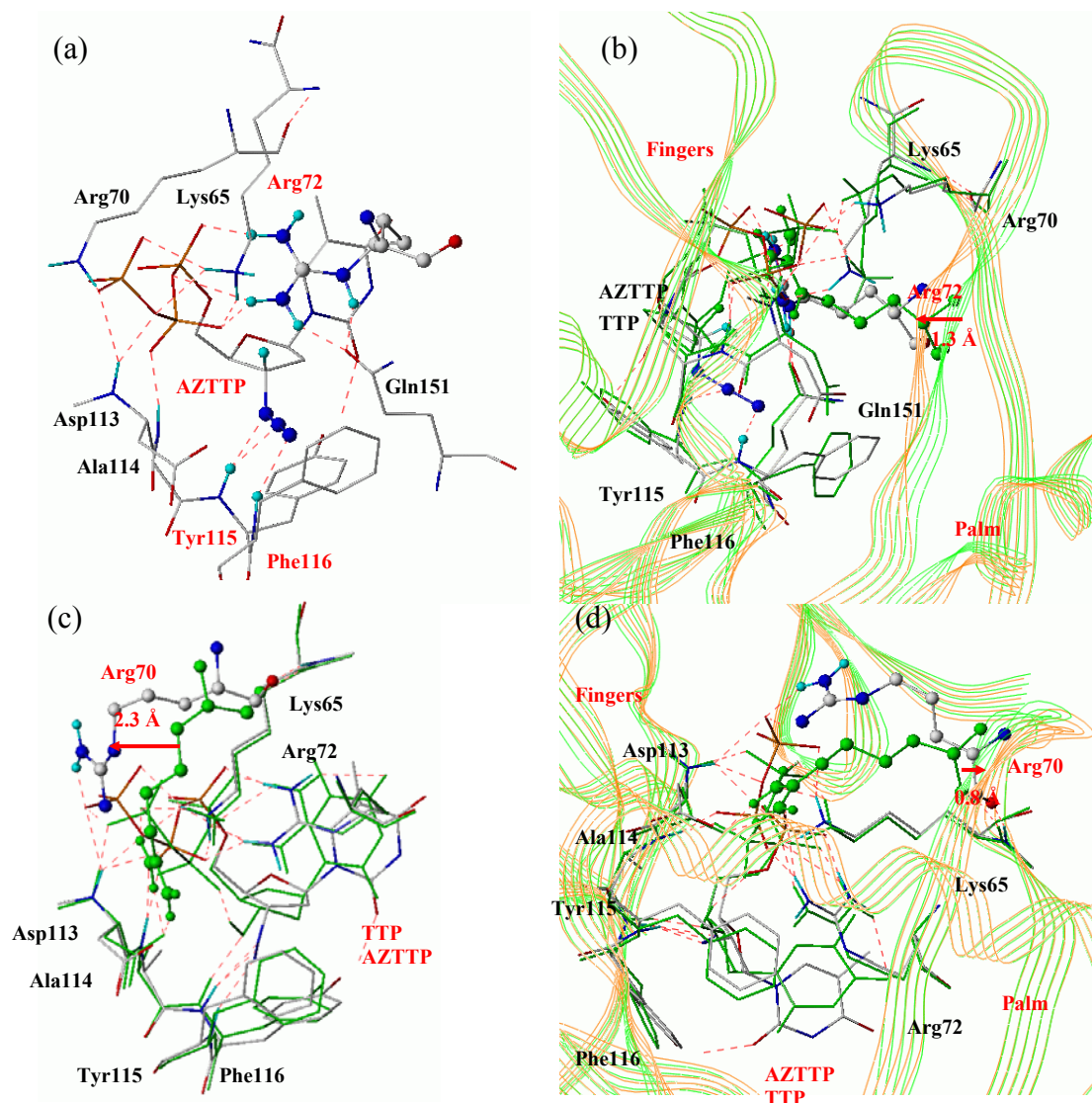


Figure 7.2. (a) Wild-type RT-AZTTP complex. Arg72 and 3'-azido group form extensive hydrogen bonds with triphosphate moiety and -NH backbone of Tyr115 and Phe116, respectively; (b) 3D binding mode of TTP (green) and AZTTP (atom types) to wild-type RT. Rotation of Arg72 induce movement of palm domain (1.3 Å) to fingers domain to form a tight binding state; (c) Superimposed structure of 4AZT mutant RT-TTP (green)/AZTTP (atom types) complex. Arg70 moves out of the binding site because of the extended conformation of triphosphate moiety in AZTTP complex; (d) 3D binding mode of TTP (green) and AZTTP (atom types) to 4AZT mutant RT. Movement of Arg70 induces opening of palm domain ~0.8 Å to form a loose binding state.

However, our result shows that the mutations still interfere with the ability of HIV-1 RT to incorporate AZTTP. The calculated binding energy of AZTTP toward the quadruple mutant (M41L/ D67N/ K70R/ T215Y) RT shows a sharp drop in comparison to the WT enzyme (Table 7.2). No significant change in either the binding conformation of AZTTP or the conformations of pocket residues (Asp113, Tyr115, Phe116 and Gln151) could be found, but a close comparison with TTP binding to this multiple mutant enzyme revealed an interesting change in the conformation of the enzyme-ligand complex mediated by the interaction between the triphosphate moiety of AZTTP and the Arg70 residue of RT. The lack of 3'-OH in AZTTP as well as the mutation K70R results in a shift of the entire triphosphate moiety outward from the binding site (0.8 Å). As a result, the Arg70, which interacts with the γ phosphate, shifts significantly (~ 3 Å) out of the binding pocket in order to maintain its hydrogen bonding to γ phosphate (Figure 7.2c). This movement of Arg70 exerts significant effects to the three dimensional structure of the enzyme-ligand (AZTTP) complex (Figure 7.2d): the hydrogen bond between Arg70 and Asp113 is not effective any more and the movement of Arg70 unfolds the palm domain resulting in a loose and ineffective binding mode. Therefore, a slight change caused by the binding of the AZTTP moiety was propagated to Arg70, resulting in a significant overall change in enzyme-ligand complex without deforming the "3'-OH pocket", which emulates essentially for the natural substrate (TTP) binding to the mutant RT.

Analysis of the minimized structure of RT-3TCTP complexes.

Among the nucleoside RT inhibitors currently used clinically, 3TC is the only one with the unnatural L-configuration. Most interestingly, 3TC and its corresponding 5-

fluorinated derivative, (-)-FTC, have been shown to be more potent than the D-isomers.^{28,289,290,291,292} The point mutation M184V/I confer high-level resistance to 3TC (Table 7.1). Met184 is located in the YMDD motif, which is highly conserved among retroviral RTs. In HIV-1 RT, this motif contains two of the three catalytic aspartates of the DNA polymerase active site. The M184V RT is more than 1,000-fold less efficient in incorporating 3TCTP into the nascent viral DNA compared with the wild-type enzyme (Table 7.1). The calculated relative binding energy (E_{rel}) was in good correlation with the antiviral activity of 3TC (Table 7.3). Particularly, the abortive binding mode to M184V mutant obtained after minimization is noteworthy.

Table 7.3. 3TC-resistant RT : correlation of binding energy (E_{rel}) with activity (EC_{50})

	3TC			DXG		
	EC_{50}^a (μ M)	Fold Increase (EC_{50})	E_{rel}^b (kcal/mol)	EC_{50}^a (μ M)	Fold Increase (EC_{50})	E_{rel}^b (kcal/mol)
WT	0.041	--	76.4	0.21	--	12.0
M184V	>50	>1,200	-28.8	0.44	2.1	5.7

^a CMB Cells : Gu *et al.*, 1999

^b Lee *et al.*, 2001

WT RT-3TCTP complex (Figure 7.3a). The mechanistic basis for the stereochemical selectivity of 3TC and (-)-FTC is not completely understood although a number of factors may clearly come into play. The differences in potency may be a combination of factors including uptake,^{28,47,48,293} transport,²⁹⁴ metabolic activation,^{28,47,48,295} incorporation,^{28,47,48,296} deamination,^{297,298} triphosphate binding to RT and repair.^{28,46,47,48}

A recent pre-steady-state kinetic analysis,²⁹⁹ to determine the maximum rate of incorporation (k_{pol}), nucleotide-binding affinity (K_d), and efficiency of incorporation (k_{pol}/K_d), for the D- and L- isomeric 3TC compounds as well as the corresponding 2',3'-dideoxy and natural nucleoside triphosphate into a primer-template complex using HIV-1 RT, provided a mechanistic basis for understanding the inhibition of RT by 3TC. The maximum rate of incorporation (k_{pol}), followed the decreasing order of dCTP > ddCTP > (+)-BCH-189 (D- counterpart of 3TC) > 3TC while the K_d values determined for the DNA/RNA primer-template followed the decreasing order of 3TC $\cong$ (+)-BCH-189 $\cong$ ddCTP > dCTP. The corresponding efficiency of incorporation followed the decreasing order of dCTP > ddCTP > (+)-BCH-189 > 3TC, which suggests that perturbations on the ribose ring of cytidine analogues (C $\rightarrow$ S) decrease the rate and efficiency of incorporation but enhance the binding affinity.

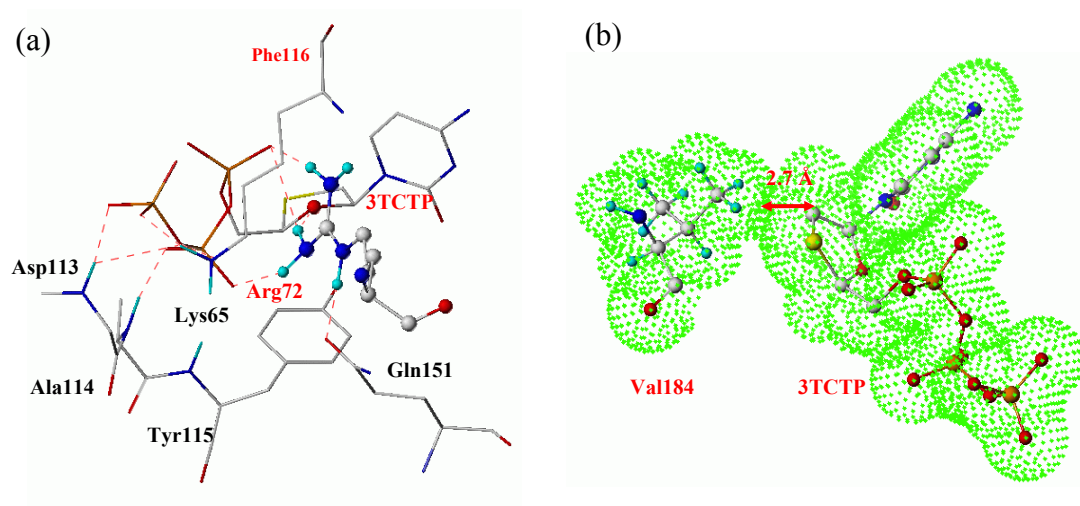


Figure 7.3. (a) Wild-type RT-3TCTP complex. Arg72 rotates down to the binding site to stabilize 3'-oxygen of thioxolane ring of 3TCTP by hydrogen bonding; (b) M184V mutant RT-3TCTP complex. After minimization. Val184 and 3TCTP sugar moiety are in too close contact (2.7 Å).

Our calculated relative binding energy indeed indicates a favorable binding of 3TCTP to WT RT compared to its corresponding natural substrate, dCTP (Table 7.3). Comparison of the minimized structure of RT/dCTP with RT/3TCTP complexes showed that Arg72 slides down to the inhibitor binding site to form a new network of hydrogen bonds by which the oxathiolane moiety of 3TCTP acquires further stabilization (Figure 7.3a). The conformational change of Arg72 results in an inward movement of palm domain toward fingers domain to form a tighter binding mode between 3TCTP and WT RT, which is consistent with the potent anti-HIV-1 activity of 3TC.

M184V RT-3TCTP complex (Figure 7.3b). On the basis of the crystal structure of WT RT/DNA/dNTP, Huang et al. have suggested that the side chain of either a valine or an isoleucine at position 184 would interfere with 3TCTP binding.¹⁷ However, in the absence of structures for 3TC-resistant RTs complexed with a template-primer and 3TCTP, the structural details of 3TC resistance have not yet been elucidated. Recently, Arnold and coworkers³⁰⁰ developed a model to explain the ability of 3TC-resistant mutant M184I to incorporate dNTPs but not 3TCTP. This model was based on a comparison of the crystal structure of the WT RT/DNA complex with the structure of M184I bound to the identical oligonucleotide substrate. Even though the crystal structure is lacking the inhibitor triphosphate (3TCTP), this model clearly shows that steric conflict between the oxathiolane ring of 3TCTP. The side chain of β -branched amino acids (Val, Ile, Thr) at position 184 perturbs the inhibitor binding, leading to a reduced efficiency of binding of 3TCTP. They proposed that this factor in combination with the repositioning of the template-primer^{301,302,303,304} could interfere with the formation of a catalytically competent closed complex between 3TCTP and M184I/V RT. Based on our calculation,

the binding of 3TCTP to M184V RT, which shows a sharp drop of relative binding energy (E_{rel}) compared to that to WT RT (Table 7.3), induces several changes: (a) an unminimized structure of the M184V RT/3TCTP complex shows significant steric clash between the β -sidechain of Val184 and the 3'-thio group of 3TCTP (2.2 Å apart). During minimization, this severe conflict is somewhat relieved but still, the distance between those two groups (2.7 Å) is not long enough to prevent unfavorable binding pattern for 3TCTP resulting from steric hindrance (Figure 7.3b); (b) Arg72, which played a critical role in stabilizing 3TCTP binding to WT RT, is not participating in hydrogen bonding to the sugar moiety of 3TCTP due to significant conformational change in 3TCTP during its escape from the steric conflict with the Val184. As Arg72 moves out of the binding site of the M184V RT/3TCTP complex, the fingers and palm domains become more separated to give a loose and abortive binding state; (c) Another serious steric conflict comes into play as Met184 mutates to Val184. As Arnold et al. suggested (53), the primer residue near the mutation site, G22 (the n^{th} residue in primer strand), is located at vicinity (0.8 ~ 1.6 Å) of Val184 resulting in a significant steric bulk. This unfavorably close contact between Val184 and primer chain results in repositioning of the template-primer duplex after minimization. A shift in the position of the primer would likely contribute to a misalignment of 3TCTP at the transition state and a decrease in the polymerization rate or processivity.

Analysis of the minimized structure of RT-DXGTP complex.

(-)- β -D-Dioxolane guanine (DXG) and its prodrug, 2,6-diaminopurine (DAPD), have been reported to be potent inhibitors of HIV-1.^{8,9,10} Antiviral assays performed in cell

culture systems determined that DXG had an EC₅₀ value of 0.046 and 0.085 μ M when evaluated against HIV-1_{IIIB} in cord blood mononuclear cells and MT-2 cells, respectively. These values indicate that DXG is approximately equipotent to 2',3'-dideoxy-3'-thiacytidine (3TC), but 5- to 10-fold less potent than AZT. When recombinant or clinical variants of HIV-1 were used to assess the efficacy of this purine nucleoside analogue against drug-resistant HIV-1, it was observed that AZT-resistant and 3TC-resistant virus remained sensitive to DXG.^{8,11} These findings have particular importance because even though AZT and 3TC are the most widely used combination therapy regimen, RT can become multidrug resistant under prolonged combination therapy.^{12,13,14} The site-directed mutation study showed that three point mutations (K65R, L74V and Q151M) and one combination mutation (K65R/Q151M) showed moderate (K65R, L74V and Q151M) to high (K65R/Q151M) resistance to DXG (Mewshaw *et al.*, 2002).³⁰⁵ These findings were reproduced in our study with a good correlation between the relative binding energy (E_{rel}) and the antiviral activity, in which E_{rel} of DXG to WT, AZT-resistant mutant (M41L,D67N,K70R,T215Y, Table 7.2) and M184V mutants (Table 7.3) were high enough but the binding energies significantly decreased to K65R, L74V and double mutant (K65/Q151M) (Table 7.4).

WT RT-DXGTP complex (Figure 7.4a). The relative binding energies (E_{rel}) of DXGTP to WT showed a favorable binding affinity toward the RT and consequently a good correlation with its biological activity (Table 7.2 ~ 4). A comparison of the binding modes of WT RT to DXGTP with its corresponding natural substrate, dGTP, showed a clear involvement of Arg72 in DXGTP binding at the active site (Figure 7.4a). In dGTP binding, Arg72 maintains its conformation, as shown in the X-ray structure of the RT*-

T/P-TTP ternary complex.¹⁷ However, when DXGTP binds to RT (Figure 7.4a), Arg72 slides down to the binding site without steric hindrance between the ϵ -amino and 3'-H of the sugar moiety which is lacking in DXGTP. As a result, the protruding ϵ -amino group forms a new network of hydrogen bonds with its neighboring residues, particularly with the DXGTP's dioxolane moiety and Gln151 (Figure 7.4a), which results in a tight binding of the inhibitor to RT. Consequently, this inward bending of Arg72 makes the fingers and palm subdomains closer to form a tight binding structure.

Table 7.4. DXG-resistant RTs : correlation of binding energy (E_{rel}) with activity (EC_{50})

	DXG		
	EC_{50} (μM) ^a	Fold Increase (EC_{50})	E_{rel} ^b (<i>kcal/mol</i>)
WT	2.19	--	12.0
K65R	14.0	6.4	-37.1
L74V	6.3	2.9	0.2
K65R/ Q151M	>50.0	22.8	-44.4

^a MT2 Cells : Mewshaw *et al.*, 2002

^b Lee *et al.*, 2001

The quadruple AZT mutant RT-DXGTP complex (Figure 7.4b). As shown in the minimized structure of the quadruple AZT mutant (M41L, D67N, K70R, and T215Y) RT-AZTTP complex (Figure 7.2c and 2d), the effect of these mutations did not affect the conformational change in 3'-OH binding pocket. Rather, mutation followed by conformational changes in adjacent residues and the different triphosphate binding mode

may be responsible for the changes in binding energy (Table 7.2). The favorable binding of DXGTP to the quadruple AZT mutant-RT can be explained by two factors. Mutations at 41 and 215 create small conformational changes around their positions. Therefore, several residues such as Glu40, Glu44, Lys46, Gly112 and Ser117 experience conformational changes, which propagate ultimately to the three catalytic residues Asp110, Asp185 and Asp186 through Asp110, Val111, Gly112 and Asp113 (Figure 7.4b). In addition, as Asp67 mutates to Asn67, three residues Asn67, Lys219 and Asp110 form a salt bridge, which shifts Asp110 1.2 Å out of the binding site (Figure 7.4b). Interestingly, this shift can not be found in the binding mode of either AZTTP, TTP, or dGTP to the same mutant enzyme. Therefore, the unique binding mode of DXGTP deserves more attention. Due to the lack of a 3'-OH in DXGTP sugar moiety, DXGTP fails to stabilize its β -phosphate by hydrogen bonding. As a result, an adjacent residue, Lys65, moves toward the hydrogen bonding distance to the β -phosphate. However, it should be noted that in AZTTP binding mode, the movement resides in Arg70 not in Lys65 (Figure 7.2c and 2d), which requires further explanation. Compared to the AZTTP binding, the interaction between Arg72 and the triphosphate moiety may be the critical factor in determining the residue which should move. In DXGTP binding, Arg72 rotates toward the triphosphate to form hydrogen bonds with both α and β phosphates. As a result, the triphosphate moiety has a more contracted conformation compared with that of AZTTP binding, and the γ phosphate does not move out significantly. The conformational change in Lys65 propagates to Asn67 through Arg70, Thr69, and Ser68. As the conformation of Asn67 changes, the adjacent residue Lys219 pulls down to form a stable salt bridge among Asn67, Lys219 and Asp110 (Figure 7.4b).

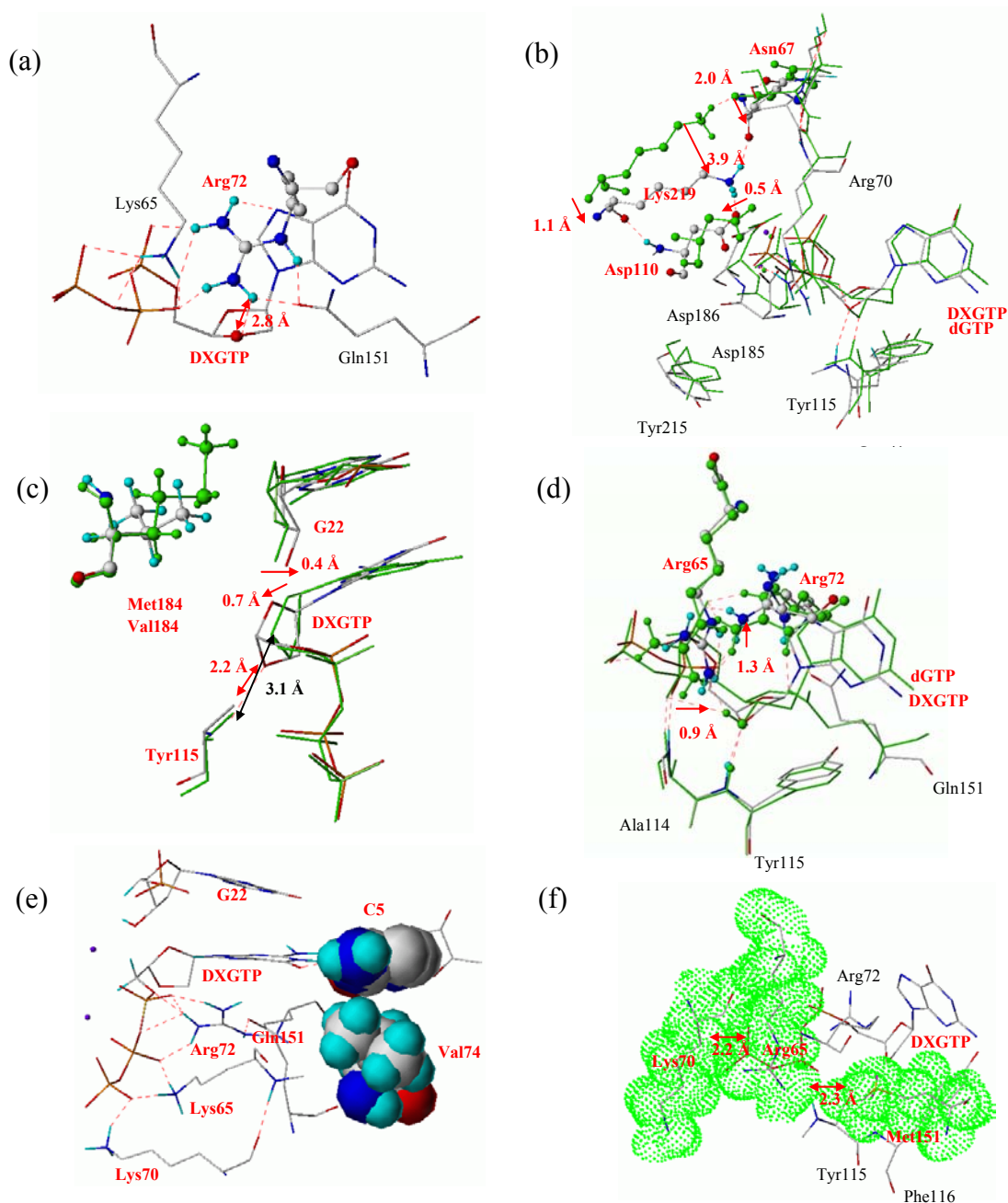


Figure 7.4. (a) Wild-type RT-DXGTP complex. Arg72 is hydrogen bonded to 3'-oxygen of dioxolane ring; (b) 4AZT mutant RT-dGTP (green)/ DXGTP (atom types) complex.; (c) Wild-type (green)/ M184V mutant RT (atom types)-DXGTP complex.; (d) K65R mutant RT-dGTP (green)/ DXGTP (atom types) complex.; (e) K65R/Q151M mutant RT/DXGTP complex.; (f) L74V mutant RT/DXGTP complex.

The combined effect of propagation of conformational change and salt bridge formation results in conformational changes in three catalytic aspartate residues (110, 185, and 186), which improves the chelation of the two Mg atoms. The two Mg^{2+} , one catalytic and the other nucleotide-binding are believed to facilitate an $\text{S}_{\text{N}}2$ nucleophilic attack by the 3'-OH of the primer terminus on the α -phosphorus of the incoming dNTP and to stabilize the negative charge in the transition state. Therefore, changes in conformation as well as binding mode of the three conserved Asp residues could contribute to an alignment of Mg atoms at the transition state and an increase in the polymerization rate or processivity. Another change followed by a salt bridge formation is the distance between fingers and palm domains. Fingers and palm domains move 1.1 Å and 0.4 Å toward each other to result in a decreased distance and therefore a tight binding state.

M184V RT-DXGTP complex (Figure 7.4c). M184V mutation, which confers a significant resistance to 3TC, does not significantly affect DXGTP binding. The high level resistance of this mutant to inhibition by 3TC may be due to the unnatural L-configuration. The alignment of the triphosphate and base would cause the oxathiolane ring of 3TC to project 1.5-2.0 Å further toward residue 184 than the ribose ring of nucleotides of natural D-configuration. However, in view of the fact that 3TC and DXG have similar pseudo-sugar moieties (oxathiolane and dioxolane, respectively) and 2',3'-dideoxy nucleosides with natural D-configuration such as ddC and ddI show some low level of cross-resistance to M184V mutant, it is not clear why DXG still maintains its anti-HIV-1 activity against M184V mutant strains. Our calculated relative binding energy (E_{rel}) suggests (Table 7.3) that DXGTP can maintain its favorable binding affinity toward

M184V mutant RT but with reduced degree, which can be correlated with 2.1 fold resistance. The minimized structure (Figure 7.4c) shows that there is enough distance (3.8 Å) between Val184 and DXGTP to avoid unfavorable steric conflict, and the distances between palm and fingers domains are exactly the same in DXGTP and dGTP binding. This favorable binding to DXGTP compared to dGTP comes from the template-primer repositioning followed by a change in the puckering of the dioxolane sugar ring. As methionine mutates to valine (M184V), the adjacent nucleotide in the primer chain (G22) experiences greater steric bulk (1.6 Å) with the mutated residue. Therefore, minimization shifts the entire template-primer about 0.4 Å out of the binding site, and this change propagates to the adjacent DXGTP to make a 0.7 Å movement of the sugar moiety down to the 3'-OH pocket (Figure 7.4c). The accompanying conformational change in the sugar moiety, from 3'-endo to 3'-exo, in combination with the reduced distance (2.2 Å) between 3'-O and backbone -NH group of Tyr115, enables a stabilizing hydrogen bonding between DXGTP and 3'-OH pocket (Figure 7.4c). Overall, the M184V mutation does not sterically hinder DXGTP binding, but enables a more stable binding of DXGTP through a new hydrogen bond between the 3'-oxygen of sugar moiety and the backbone -NH group of Tyr115.

K65R RT-DXGTP complex (Figure 7.4d). Several ddN resistance mutations arise in the flexible β 3- β 4 loop situated in the fingers domain of the p66 subunit of RT.¹³ Five residues, that form part of this loop (Ile63, Lys64, Lys65, Tyr71 and Arg72), are conserved in all retroviral RTs³⁸ and among those, two fingers residues make important contacts with the dNTP substrate. The ϵ -amino group of Lys65 and the guanidinium

group of Arg72 make salt bridges with the γ - and α - phosphates of the bound dNTP substrate, respectively. These interactions help positioning the dNTP for base-pairing with the complementary template residue as well as for catalysis of phosphodiester bond formation with the 3'-OH of the primer terminal nucleotide. While the available structural data have provided considerable information on the structure of RT as well as the relevant amino acid residues critical for catalysis, it has not provided the molecular mechanism how mutations on RT influence the dNTP binding, thereby the anti-HIV potency.

Our calculation indicates that DXGTP shows a large decrease in its relative binding energy (E_{rel}) to the mutant enzyme, K65R RT, compared to that of WT RT (Table 7.4), which is in good correlation with the increased resistance obtained with the K65R mutant. This decrease in binding energy can be explained by the outward rotation of Arg72 from the binding site in DXGTP binding mode (Figure 7.4d). This conformational change, initiated by the movement of Arg65 toward the binding site, results in the loss of hydrogen bonds between Arg72 and DXGTP dioxolane moiety and Gln151 (Figure 7.4d). The movement of Arg65 toward the binding site in DXGTP binding mode, as discussed above, can be explained by the extensive stabilization of triphosphate of DXGTP by ϵ -amino group of Arg65 because DXGTP is lacking the stabilizing interaction between 3'-OH of sugar moiety and β -phosphate. Therefore, for the sake of stabilization of triphosphate moiety of DXGTP, Arg65 slides into the binding site, and, as a result, the nearby Arg72 is pushed away from the binding site. As a consequence, the fingers and palm domains are departing from each other to give a less productive transition state, resulting in the DXGTP resistance.

L74V mutant RT-DXGTP complex (Figure 7.4e). In the structure of RT-DNA-dNTP,¹⁷ Leu74 is not in a position to directly affect dNTP binding. However, it does interact with the template nucleotide that is base paired to the incoming dNTP and is also proximal to Gln151, which is a key residue in the dNTP-binding pocket. The L74V mutation has been thought to reduce sensitivity to ddI and ddC by altering template position and/or by affecting the orientation of Arg72 and Gln151 sidechains.^{59,306} Leu74 upholds the template nucleotide by hydrophobic interactions so that the template nucleotide could be base paired with the incoming dNTP. Therefore, the mutation of this residue from leucine to valine results in the misalignment of the template residue for base pairing and a significant conformational change in adjacent residues such as Gln151 and Arg72 (Figure 7.4e). In DXGTP binding, the combined effect of the misalignment of base pairing and the conformational change in Arg72 results in the downward shift of the dioxolane ring and the conformational change in the triphosphate moiety, which is in good agreement with the decreased binding affinity (Table 7.4). However, in dGTP binding, Arg72 and the triphosphate moiety can maintain their conformation because the 3'-OH group in 2'-deoxyribose ring is involved in the stabilization of the β -phosphate group. As a result, the triphosphate moiety of DXGTP adopts a new conformation, which hampers the formation of optimal geometry for S_N2 type polymerization reaction: the distance between the nucleophile (3'-OH at the end of primer strand) and the reaction center (phosphorous atom at α phosphate moiety) is lengthened (3.66 Å, in WT the distance is 3.42 Å) and the angle among the nucleophile, reaction center and the leaving group (β and γ phosphates) (160.4°) deviates from that of optimal value (177.0° in WT) (Table 7.5).

Table 7.5. Comparison of geometry for S_N2 type nucleophilic attack.

	Wild-type		K65R		L74V		K65R/Q151M	
	DXG	dGTP	DXG	dGTP	DXG	dGTP	DXG	dGTP
Distance ^a (Å)	3.42	3.23	3.36	3.32	3.66	3.41	4.40	3.37
Angle ^b (degree)	177.0	174.9	177.3	176.0	160.4	173.6	158.8	176.4

^a Distance between the nucleophile (3'-oxygen of the terminal residue in primer strand) and the reaction center (α phosphorous atom in NRTI triphosphates)

^b Angle among the nucleophile, reaction center and the leaving group (β and γ phosphates in NRTI triphosphate)

Double mutant (K65R/Q151M) RT-DXGTP complex (Figure 7.4f). Gln151 interacts with residues that either directly interact with dNTP (e. g. Arg72, which hydrogen bonds with dNTP) or affect the position of important structural elements (e. g. the main chain carbonyl oxygen of residue 73 is hydrogen-bonded to Gln151, bridging the palm and fingers subdomains). The Q151M mutation might therefore engender multidrug resistance to AZT, d4T and ddC/ddI by inducing both direct and indirect changes at the dNTP-binding pocket (Table 7.1).³⁰⁷ A site-directed mutagenesis study found that a combination mutation of K65R and Q151M was fully resistant to DXGTP and this mutant exhibits a significant decrease in antiviral activity of DXGTP (Table 7.4). The decreased relative binding energy (E_{rel} , Table 7.4) and the minimized RT-DXGTP structure shows two important features which this mutation invokes at the dNTP binding site. The bulky Met151 pushes neighboring Arg65 away from the binding site (Figure 7.4f). In dGTP binding, the pushed Arg65 also rotates itself in order to prevent another

steric hindrance with the adjacent residue Lys70. However, in DXGTP binding, Arg65 is heavily involved in the stabilization of β -phosphate and can neither move out nor rotate to prevent the unfavorable steric hindrance with Met151 and Lys70, which results in a high-energy binding state (Figure 7.4f). Also, the geometry for S_N2 type polymerization reaction is significantly deformed as a result of conformational change in both Arg65 and the triphosphate moiety in DXGTP: the nucleophile and the reaction center are separated by 4.40 Å, and the angle around the reaction center (158.8°) deviates significantly from that of the WT (Table 7.5). Therefore, the incorporation of DXGTP into the growing DNA chain in K65R/Q151M mutant enzyme can be expected to be more difficult than in any other mutant enzymes studied.

Discussion

There have been numerous efforts to understand the molecular mechanism of resistance to nucleoside reverse transcriptase inhibitors conferred by mutations in HIV-1 RT. In the absence of the structural information of the mutant RT, several groups reported molecular modeling approaches to support their results. However, there has not been a comprehensive approach toward this goal. Even though the computational methods used in this study is far from ideal and the appropriate solvent effect was not taken into consideration, by constructing mutation sites by molecular modeling and comparing the relative binding energies (E_{rel}) of the NRTI triphosphates to RT, we determined that the antiviral activity of the nucleoside RT inhibitors can be influenced by the binding affinity of their triphosphates to RT.

The interactions between the NRTI triphosphates and adjacent amino acid residues (Lys65, Lys70, Arg72, Tyr115 and/or Gln151) played important roles in stabilizing the enzyme-inhibitor complex. Particularly, Arg72 was found to stabilize the dioxolane and oxathiolane sugar moiety through hydrogen bonding, which was responsible for favorable binding affinity of DXGTP to AZT- and 3TC-resistant mutants as well. The binding interactions of NRTI triphosphates (AZTTP, 3TCTP and DXGTP) with WT RT showed that NRTI triphosphates can bind to the enzyme more tightly than the corresponding dNTPs because of either participation of Arg72 in stabilizing dioxolane (DXGTP) or oxathiolane (3TCTP) ring, or formation of hydrogen bonding of azido group with the 3'-OH pocket (Tyr115 and Phe116) (AZTTP). In addition, our study provided a possible molecular mechanism for the resistance of RT toward antiviral nucleoside inhibitors. The rapid development of the significant 3TC-resistance by M184V RT could be explained by the steric hindrance between the Val184 and the unnatural L-oxathiolane moiety of 3TC, and this may be the general mechanism of the cross-resistance of M184V RT to many L-nucleoside RT inhibitors. AZTTP fails to stabilize its β -phosphate moiety due to the lack of 3'-hydroxy group, which allows an extended conformation of its triphosphate. The mutated residue, Arg70, moves out of the binding site interacting with the γ -phosphate, which results in a loose AZTTP-mutant RT (M41L/D67N/K70R/T215Y) complex. The minimized structures of DXGTP-mutant RTs gave us reasonable explanation for antiviral activity of DAPD, a prodrug of DXG, against the AZT- and 3TC-resistant mutants, where D-dioxolane sugar moiety of DXGTP provided interesting advantages. The natural D-configuration did not experience any significant steric hindrance with the Val184 in M184V RT, and the 3'-oxygen allowed a

stabilizing hydrogen bonding with the –NH backbone of Tyr115. In addition, the 3'-oxygen instead of 3'-CH₂ allowed the approach of Arg72 to stabilize the dioxolane ring moiety by hydrogen bonding. In AZT-resistant mutant RT, dioxolane ring induced the formation of a salt bridge among Asn67, Lys219 and Asp110 to result in a stable complex.

Interestingly, the conformational changes in the amino acid residues which are in close contacts to the NRTI triphosphates always affected the changes in the tertiary structures of enzyme-inhibitor complexes through either closing or opening the gap between the fingers and palm domains. Therefore, enzyme-inhibitor complexes with good binding affinity showed tight binding modes by closing the gap between the two domains, whereas weak inhibitors gave open and loose complexes.

Based on this preliminary information about the molecular mechanism conferred by mutations at HIV-1 RT, an exhaustive and a more computationally expensive treatment of this system is underway including the solvent (water) molecules and employing molecular dynamics simulations.

CHAPTER 8

UNDERSTANDING THE UNIQUE MECHANISM OF L-FMAU (CLEVUDINE) AGAINST HEPATITIS B VIRUS: MOLECULAR DYNAMICS STUDIES¹

¹ Chong, Y. and C. K. Chu. 2002. *Bioorg. Med. Chem. Lett.* 12:3459-3462.
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Introduction

In the last several years, active pursuit of effective anti-hepatitis B virus (HBV) agents has resulted in the identification of a number of potentially useful nucleoside analogs. Treatment with nucleosides has shown immediate clinical benefits such as reduced viral load, suppression of progression of liver disease, and induction of immunological clearance or seroconversion. L-FMAU (clevudine) was reported by Chu et al.³⁰⁸ as a potent antiviral agent against HBV (EC_{50} 0.1 μ M in HepG2 2.2.15 cells) as well as EBV, which has low cytotoxicities in a variety of cell lines including MT2, CEM, H1 and HepG2 2.2.15 and bone marrow progenitor cells. L-FMAU is metabolized in cells by the cellular thymidine kinase as well as deoxycytidine kinase to its monophosphate, and subsequently to the di- and triphosphate.³⁰⁹ L-FMAU is currently undergoing phase II clinical trials in patients who are chronically infected with HBV.

Hepadnaviruses replicate by a multistep mechanism that begins with reverse transcription of pregenomic RNA. The DNA synthesis is initiated by a primer which directly binds the first nucleotide (dGTP) of the DNA minus strand.³¹⁰ The DNA plus strand is then synthesized with the minus strand as a template to yield the mature, partially double-stranded DNA virus. Therefore, understanding how and which of these three distinct phases of hepadnaviral replication (priming, reverse transcription and DNA-dependent DNA synthesis) can be blocked by nucleoside analogues, can provide valuable information for the discovery of more potent and safe anti-HBV drugs. Even though L-FMAU is known to act specifically on viral DNA synthesis, and its triphosphate inhibits the HBV DNA synthesis in a dose-dependent manner³¹¹ without being incorporated into

the DNA or chain termination,³¹² the precise understanding of the mechanism of action of L-FMAU at the polymerase level has not been realized.

It is known that nucleoside inhibitors, in general, interfere with the viral polymerase activities by both competitive inhibition and incorporation to the viral DNA strands.³¹³ Most of the antiviral nucleoside analogs studied thus far, with the exception of ribavirin,³¹⁴ exerted their antiviral action through the inhibition of viral polymerase and their incorporation into the viral DNA. By using replicating cores extracted from congenitally infected ducks, the anti-HBV mechanism of 3TC-TP was determined to be the inhibition of virus replication by acting as a chain terminator of both the RT and DNA polymerase activities of the enzyme.³¹⁵ Also, even though entecavir and lobucavir are not obligate chain terminators of DNA synthesis by virtue of the OH group content of their sugar moieties, endogenous sequencing reactions conducted in replicative HBV nucleocapsids suggested that they may act as chain terminators by introducing enough structural distortion to preclude the enzyme from optimal interaction with the 3' end of the growing DNA chain.³¹⁶ It was found that L-FMAU-TP is not a substrate of EBV DNA polymerase, which suggests that the anti-EBV activity of L-FMAU may not be due to its incorporation into EBV DNA.³¹⁷

Therefore, of considerable mechanistic interest is how L-FMAU-TP can competitively inhibit the HBV polymerase,³¹¹ without being incorporated into the growing HBV-DNA chain. For this purpose, L-FMAU-TP/ HBV polymerase complex was constructed and simulated by molecular dynamics to obtain the structural as well as mechanistic information of L-FMAU-TP in a molecular level.

Methods

The three dimensional structural information of HBV polymerase is still not available. However, the recently published homology model of HBV polymerase using HIV-1 reverse transcriptase as a template³¹⁸ could be used for the construction of HBV polymerase·L-FMAU-TP complex. Therefore, the HBV polymerase domain was modeled by the composer module in Sybyl, version 6.7 (Tripos, Inc.) using the crystal structure of HIV-1 RT (PDB code 1RTD).³¹⁹ The two Mg^{2+} ions, thymidine triphosphate and template-primer duplex were located at the same position as the HIV-1 RT·DNA·dNTP complex structure (1RTD). The stability of the modeled HBV polymerase·DNA·thymidine triphosphate complex was confirmed by performing molecular mechanics energy minimization and molecular dynamics simulation by using the molecular graphics and simulation program MacroModel, version 7.0 (Schrödinger, Inc.). The complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient, Polak-Ribiere 1st derivative method was used for energy minimization. Molecular dynamics simulations on HBV polymerase·DNA·L-FMAU-TP was performed with MMFF94s in the presence of GB/SA continuum water model on a Silicon Graphics Octane2 workstation running the IRIX 6.5 operating system by heating from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. For simulation of the HBV polymerase·L-

FMAU-TP complex, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside 6 Å sphere from the L-FMAU-TP were allowed to move freely. The Monte Carlo conformational search of L-FMAU was performed in 5,000-step, in the presence of GB/SA water model using MM3 force field.

Results and Discussion

As was pointed out by Das et al.,³¹⁸ there were several interesting structural differences at the active sites between the template enzyme, HIV-1 reverse transcriptase (RT) and the final model of HBV polymerase. Among those differences, the most outstanding one on which we focused was Met171 in HBV polymerase which corresponds to Gln151 in RT (Figure 8.1). In RT, Gln151 is tightly bound to the neighboring residues such as Arg72 and Lys73 by hydrogen bonding and participates in the formation of the '3'-OH pocket'. Since this residue is directly involved in the stabilization of the bound nucleoside triphosphate, Q151M mutation causes multidrug resistance in RT.³²⁰ Therefore, of great structural interest was the role of Met171 at the active site of HBV polymerase (Figure 8.1). The crystal structure of L-FMAU was obtained from the Cambridge Structural Database (Figure 8.2a). In conformational point of view, L-FMAU is very characteristic since the gauche effects caused by the two electron withdrawing groups at the 2' (F) and 3' (OH) positions (Figure 8.2b) shift the equilibrium to the south (2'-endo) conformation (Figure 8.2c).

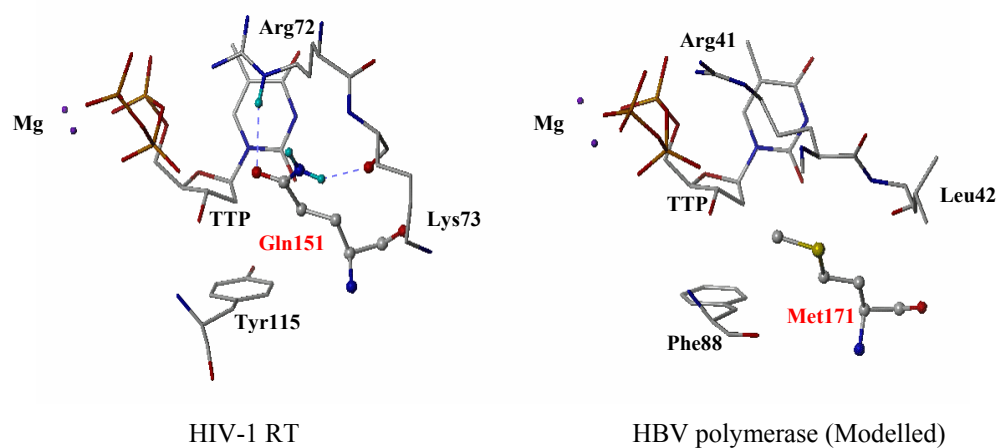


Figure 8.1. Comparison of the active site residues between the template (HIV-1 RT) and the modeled HBV polymerase.

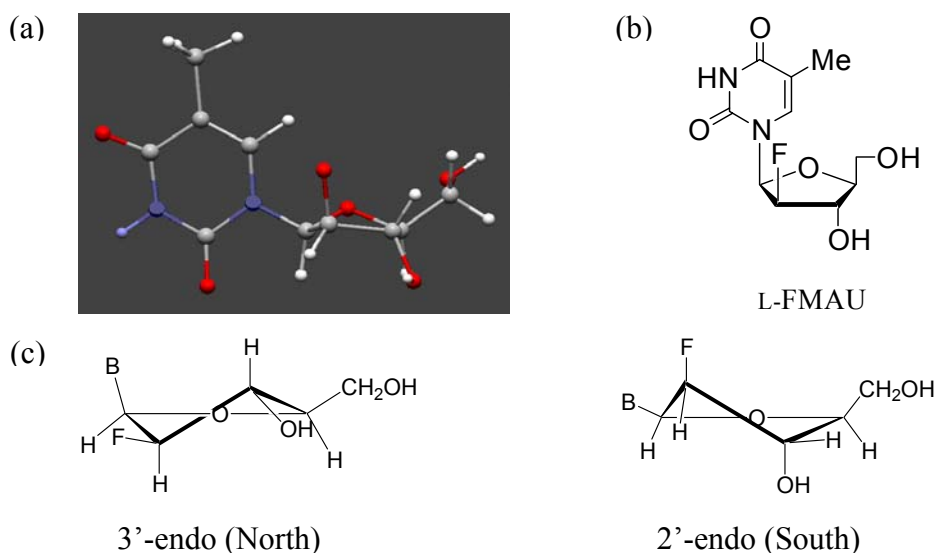


Figure 8.2. Structure of L-FMAU. (a) X-ray structure of L-FMAU. (b) L-FMAU has two electron withdrawing groups at 2' and 3' positions. (c) Gauche effect favors 2'-endo (south) conformation.

On the other hand, the ternary complex of RT, which was used as a template for the construction of the homology model of HBV polymerase, showed that although the DNA

appears to be predominantly in the B-form, the base pairs close to the polymerase active site have an A-like conformation with a widened minor groove.³¹⁹ It is important that in B-DNA the 2'-deoxysugars prefer a distinct south conformation, whereas in A-DNA the 2'-deoxysugars require puckering in the antipodal north conformation,³²¹ which disfavors the location of 2'-endo L-nucleoside at the active site of the polymerase. Docking the L-FMAU-TP at the active site of HBV polymerase was challenging because of the steric hindrance of the sugar moiety of L-FMAU with the 3'-end of the primer strand. Additionally, the 3'-OH group of L-FMAU was found to be in too close contact to the aromatic ring of Phe88 (Figure 8.3a). Therefore, the RT-like active site conformation may not be able to accommodate L-FMAU-TP. As the HBV polymerase has Met171, which is not involved in the specific interaction with the active site residues, the active site of HBV polymerase is not as tight as that of RT. Therefore, the conformational flexibility of HBV polymerase provided by Met171 might generate a favorable conformation to accommodate L-FMAU-TP (Figure 8.3b). The conformational space of the active site of HBV polymerase was investigated by simulating the complex by molecular dynamics to give a stable ternary complex which was free of steric hindrance among the 3'-end of the primer strand, L-FMAU-TP and the aromatic ring of Phe88 (Figure 8.3). The conformational adjustment initiated at Met171 propagated to the nearby Phe88 to give enough space for L-FMAU-TP (Figure 8.3b) to undergo the conformational change from 2'-endo to 3'-endo (Figure 8.4). As a result, the sugar moieties of L-FMAU-TP and 3'-terminal nucleoside of the primer strand could acquire enough space between each other (Figure 8.4 and 5). Even though the flexibility of the HBV polymerase gave a different active site conformation which could accommodate L-

FMAU-TP, its binding should take place through a conformational change in its own sugar ring. Since the 2'-endo conformation of FMAU is found in its X-ray structure and believed to be the most stable, the conformational change in L-FMAU-TP can be an unfavorable process with a loss of binding energy.

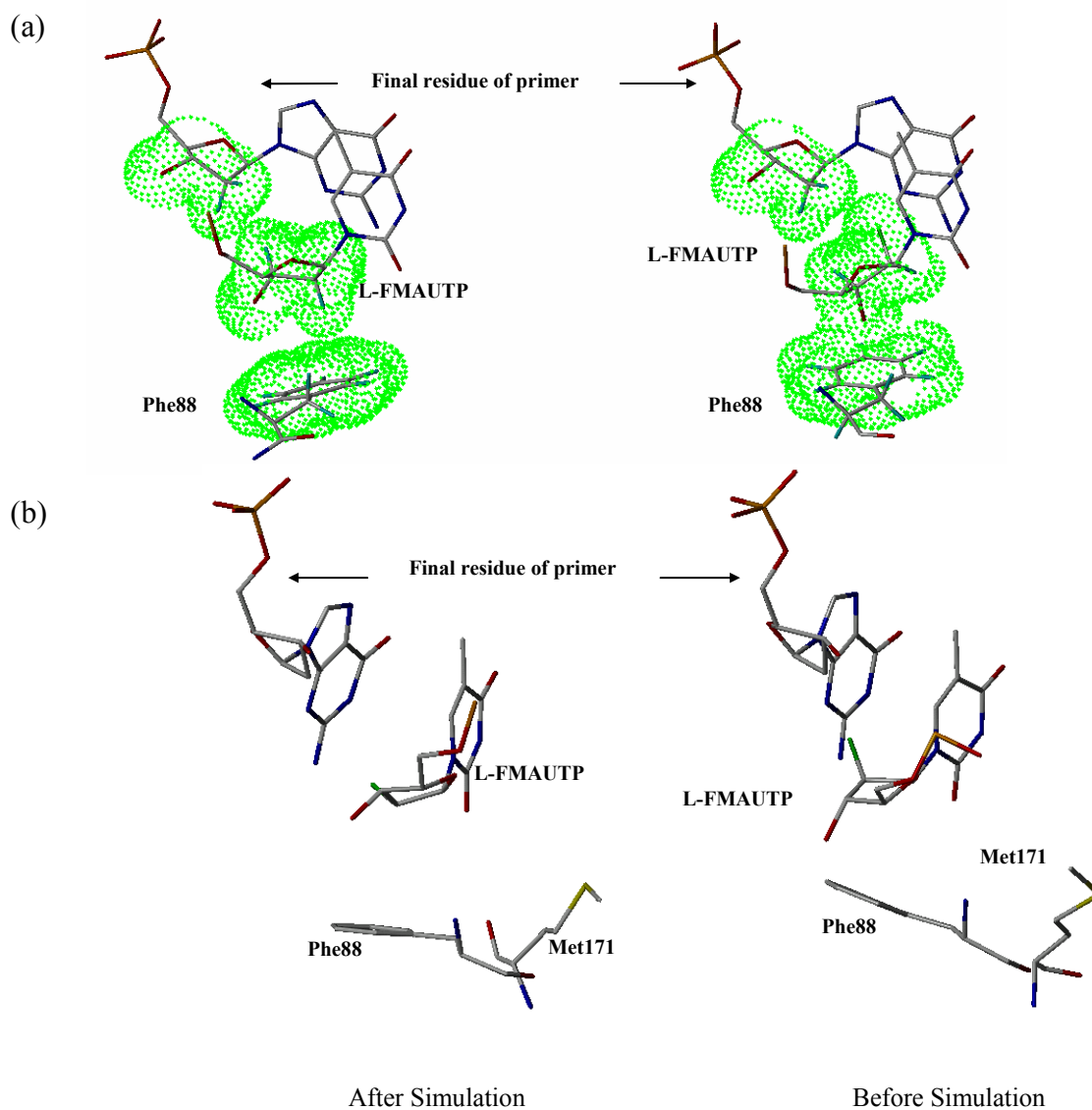


Figure 8.3. Before (right) and after (left) molecular dynamics simulation (a) The steric hindrance among the final primer residue, L-FMAU-TP and Phe88 is relieved after simulation. (b) The conformational change in Phe88 induced by Met171 provides enough space for L-FMAU-TP to adjust its conformation.

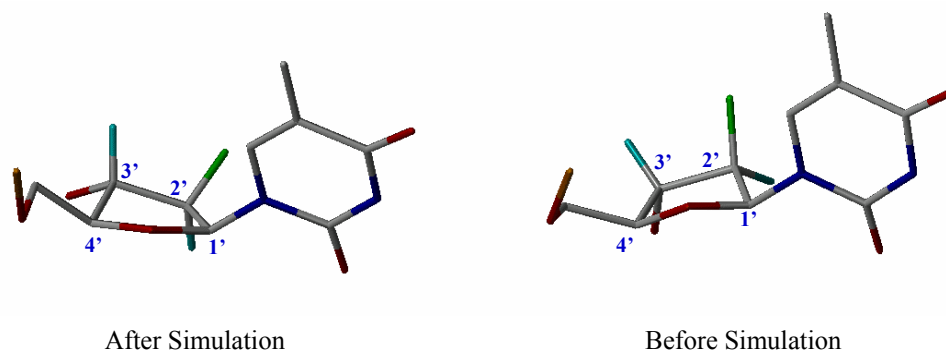


Figure 8.4. Conformational change of L-FMAU-TP from 2'-endo (right) to 3'-endo (left) after molecular dynamics simulation.

The relative stabilities of 2'-endo and 3'-endo conformations of L-FMAU were compared by a Monte Carlo conformational search, which found the 2'-endo conformation as a global minimum (-120.93 Kcal/mol) with 45 hits. However, the 3'-endo conformer was found as stable as the global minimum conformer (-120.86 Kcal/mol) of 2'-endo with 37 hits, which suggests that L-FMAU-TP can adjust its conformation to bind to the active site of HBV polymerase without significant loss of energy.

On the other hand, the conformational change in L-FMAU-TP resulted in an interesting change in the polymerization geometry of the growing viral DNA chain (Figure 8.6). Since this polymerization should take place by the S_N2 -type attack of the 3'-OH group at the 3'-end of the primer strand on the α -phosphorous atom of the nucleoside triphosphate, the distance and relative orientation between the two atoms are critical. As can be found in many X-ray structures of the protein including the DNA duplex and the nucleoside triphosphate substrate, the optimum distance between the nucleophile (3'-OH at the primer end) and the reaction center (α -phosphorous of the nucleoside triphosphate) is around 3.5 Å.³¹⁹

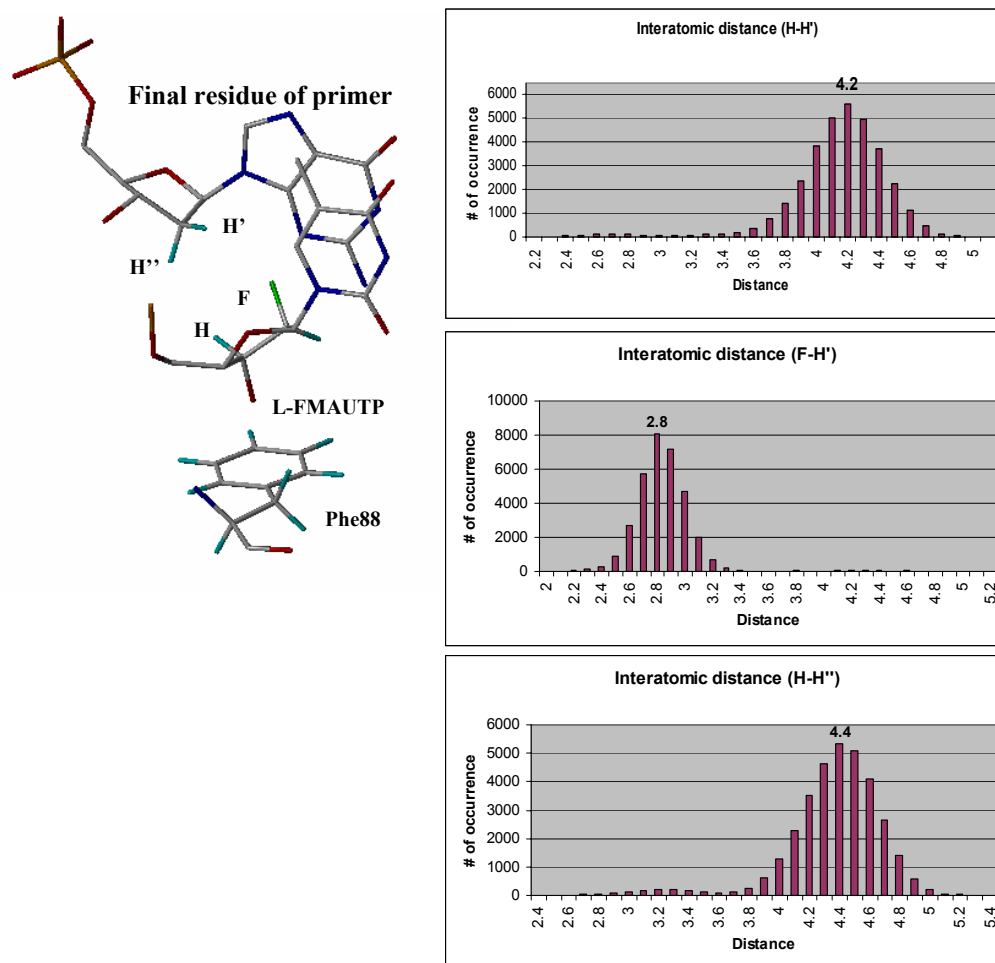


Figure 8.5. The monitored interatomic distances (H-H'', H-H' and H'-F) shows that the conformational change in L-FMAU-TP provided enough space between the final primer residue and L-FMAU-TP. Among the triphosphate part of L-FMAU-TP, only α -phosphorous atom is shown for clarity.

In L-FMAU-TP binding to the active site of HBV polymerase, however, as the sugar moiety of L-FMAU-TP separates from the 3'-end of the primer strand by the conformational change, its α -phosphate atom also moves out from the 3'-OH group of the primer end by more than 2 Å (5.7 Å) (Figure 8.6). Therefore, the incorporation of L-FMAU-TP to the growing viral DNA chain to act as a chain terminator would not take

place easily because of this deformed polymerization geometry, which may be the reason that L-FMAU is not incorporated to the HBV DNA.

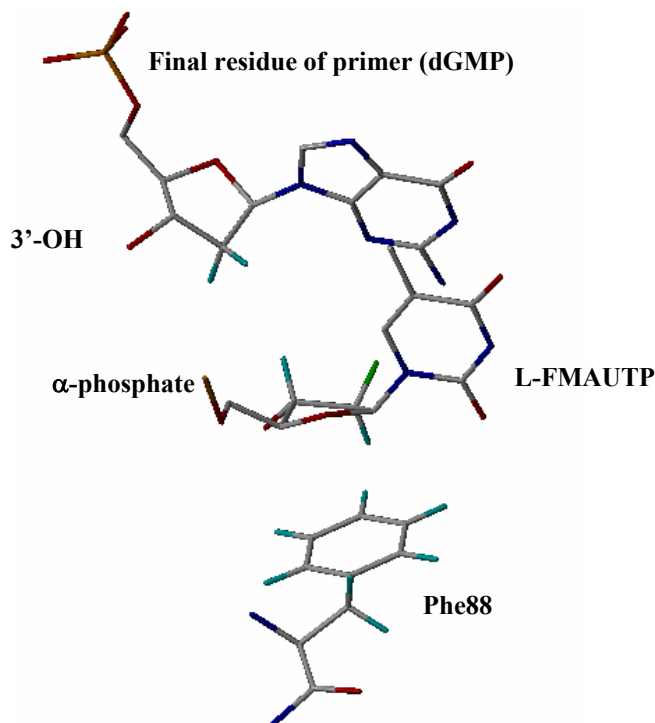


Figure 8.6. The conformational change in L-FMAU-TP separates its α -phosphorous atom from the 3'-OH group in the final primer residue (dGMP), which deforms the normal polymerization geometry (3.5 Å) for the viral chain elongation. Among the triphosphate part of L-FMAU-TP, only α -phosphorous atom was shown for clarity.

In summary, because of the unique conformation of L-FMAU, the binding of its triphosphate to the active site of HBV polymerase should take place with conformational changes at the enzyme active site as well as the L-FMAU-TP itself. During this conformational change, however, the nucleophile and the reaction center for the S_N2 -type DNA chain elongation separate from each other to give a deformed polymerization geometry. Taken together, by the molecular dynamics simulation study of HBV-

polymerase·DNA·L-FMAU-TP complex, we have demonstrated that L-FMAU-TP can act as a competitive inhibitor by binding to the active site of HBV polymerase, but not act as a substrate because the binding of L-FMAU-TP to the active site of HBV polymerase cannot take place without the conformational adjustment, which prevents L-FMAU-TP from being incorporated into the growing viral DNA chain. This result may suggest that L-FMAU-TP occupies the catalytic site of the polymerase, thereby inhibits the priming of the HBV DNA chain elongation.

CHAPTER 9

MOLECULAR MECHANISM OF DIOXOLANE NUCLEOSIDES AGAINST 3TC RESISTANT HIV M184V MUTANT

Introduction

Concerns about the development of viral resistance to 3TC^{322,323} have prompted the discovery of structurally related nucleosides with antiviral activity against HIV isolates containing common 3TC resistance mutations. Since the discovery of D-dioxolane guanosine and its prodrug, D-dioxolane 2,6-diaminopurine (DAPD), which are active against AZT- as well as 3TC-resistant mutants,^{324,325} nucleosides which have dioxolane sugar moiety have been targeted in the pursuit of new drug candidates for anti-HIV therapy. Among the series of the dioxolane nucleosides, thymidine, 5-fluorocytidine and guanidine analogues (Figure 9.1) showed potent anti-HIV activity against 3TC-resistant mutant RT (Table 9.1).

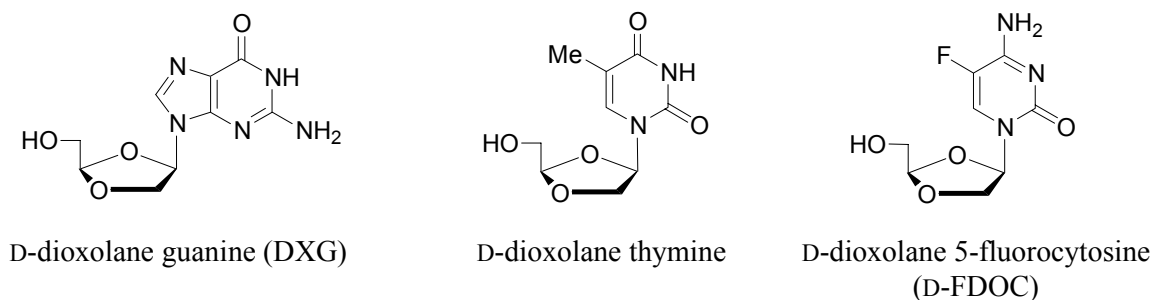


Figure 9.1. D-dioxolane nucleosides active against M184V HIV-1 RT.

In this context, it is of great interest to understand the characteristic binding modes of these D-dioxolane nucleosides against 3TC-resistant mutant RT. For this purpose, various D-dioxolane nucleoside triphosphates (thymine, 5-fluorocytosine and guanosine derivatives), which are active against M184V RT were docked into the active site of the computer-generated M184V RT, and the resulting complexes were energy-minimized.³²⁶

Molecular modeling studies indicate that, unlike other nucleosides resistant to M184V RT, the D-dioxolane nucleosides are able to escape the stress of Val184 by adjusting its conformation with no energy cost. Also, the 3'-oxygen atom of D-dioxolane sugar moiety is critical in locating the nucleoside triphosphate away from Val184 by interacting with the active site residues of RT.

Table 9.1. Antiviral activities of 3TC and D-dioxolane nucleosides against wild-type and 3TC-resistant RT (M184V).

	3TC		D-Dioxolane T		DXG		D-FDOC	
	EC ₅₀ ^a	FI	EC ₅₀ ^b	FI	EC ₅₀ ^a	FI	EC ₅₀ ^b	FI
WT	0.041	--	0.200	--	0.21	--	0.045	--
M184V	>50	>1,200	0.088	0.44	0.44	2.1	0.030	0.67

^a. CMB Cells (Gu et al. *Antimicrob. Agents Chemother.* **1999**, 43, 2376-2382)

^b. PBM Cells (Schinazi, *Personal Communication*)

Materials and Methods

All molecular modeling of the enzyme-substrate complexes was carried out using SYBYL 6.6 (Tripos Inc. St. Louis, MO), and the Monte Carlo conformational search³²⁷ of the D-dioxolane thymine was performed by Macromodel 7.0 (Schrödinger, Inc). The enzyme site of the enzyme-ligand complex was constructed as previously reported.³²⁶

M184V HIV-1 RT. The crystal structure of the covalently trapped catalytic complex of

HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd)³²⁸ was used as a template to generate the three dimensional structure of the M184V RT. The residue Met184 was mutated to Val184 in the biopolymer module in Sybyl 6.7. Hydrogen atoms were added to the mutated residue and the side chain was fixed. Gasteiger-Hückel charge^{329,330} and Kollman-All-Atom charge^{331,332} were loaded to the TTP and M184V RT, respectively, and the residues inside 6 Å from TTP were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol.³²⁶ The annealed M184V RT-TTP complex was fully minimized by using the Koll-All-Atom force field until the iteration number reached 5,000.

NRTIs. The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates of D-dioxolane cytosine³³³ or conformational analysis (3TC).³³⁴ The heterocyclic base was appropriately modified before docking into the enzyme. Also, the heterocyclic moiety of n+1th nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, in 3TCTP and DXGTP modeling, the adenine moiety which was in the original X-ray structure (1rtd)³²⁸ was modified to guanine and cytosine, respectively. The enzyme site and the inhibitor were merged to form a catalytic complex and then the NRTI triphosphate was situated in such an orientation that it could be paired with its complementary base in the template strand by adjusting the torsional angles to those found in the X-ray structure.³²⁸

Method. Gasteiger-Hückel charge^{329,330} was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges^{331,332} were loaded to enzyme site from the biopolymer option in SYBYL 6.6

(Tripos Inc. St. Louis, MO). Fluorine parameters were obtained from the literatures^{335,336} 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 mutated residues were annealed³²⁶ until energy change from one iteration to the next was less than 0.05 kcal/mol (hot region: 6 Å , interesting region : 12 Å). The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field³³⁷ until iteration number reached 5,000. The binding affinities of the examined structures toward HIV-1 RT was estimated by means of the relative binding energy (E_{rel} , Table 9.1) between the inhibitor triphosphate (3TCTP, D-dioxolane thymidine triphosphate, D-dioxolane 5-fluorocytidine triphosphate, or DXGTP)-RT complex and the corresponding natural substrate (dGTP, TTP or dCTP)-RT complex in the energy-minimized states.^{326,334}

Monte Carlo conformational search. The Monte Carlo conformational search of D-dioxolane thymine was performed in 5,000-step, in the presence of GB/SA water model using MM3 force field (Macromodel).⁷⁴

Results

M184V HIV-1 RT vs 3TC-TP. The computer-generated three dimensional structure of M184V RT has almost no difference from the wild-type (WT) RT even at the active site because, by simply rotating its side chain to the open NRTI binding site, the mutated

residue, Val184, can acquire stable conformation. As a result, the deoxyribose moiety of TTP is in close proximity to Val184 (3.3 Å) but not enough to be sterically hindered (Figure 9.2a). However, even though the natural substrate such as TTP can manage to fit into the active site without unfavorable steric hindrance, it is obvious that the approach of the sterically demanding Val184 into the NRTI binding site significantly limits the conformation of the sugar moiety of the NRTI, which can be confirmed by the binding mode of 3TC triphosphate (Figure 9.2b). For the sake of proper base pairing with the complementary base in the template strand, the unnatural L-configuration of 3TC locates its oxathiolane sugar moiety at the upper open space of the NRTI binding site in the WT RT, where Met184 contributes to the binding of 3TCTP by an extended van der Waals contact to the oxathiolane sugar moiety of 3TCTP (Figure 9.2b). However, in M184V RT, 3TCTP cannot be accommodated into the binding site, which is already occupied by the side chain of Val184 (Figure 9.2b).

M184V HIV-1 RT vs D-dioxolane nucleoside-TP. Our molecular modeling study indicated that D-dioxolane nucleoside triphosphates-RT complexes maintain the favorable binding modes through the interaction of its 3'-oxygen with active site residues such as Arg72 or Tyr115 depending on the dioxolane sugar conformation (3'-endo or 3'-exo) (Figure 9.3). The calculated relative binding energies, based on the minimized energy of enzyme-ligand complex, free enzyme and ligand,^{326,334} showed that the D-dioxolane nucleoside triphosphates can bind to the WT and M184V RT with the same efficiency (Table 9.2).

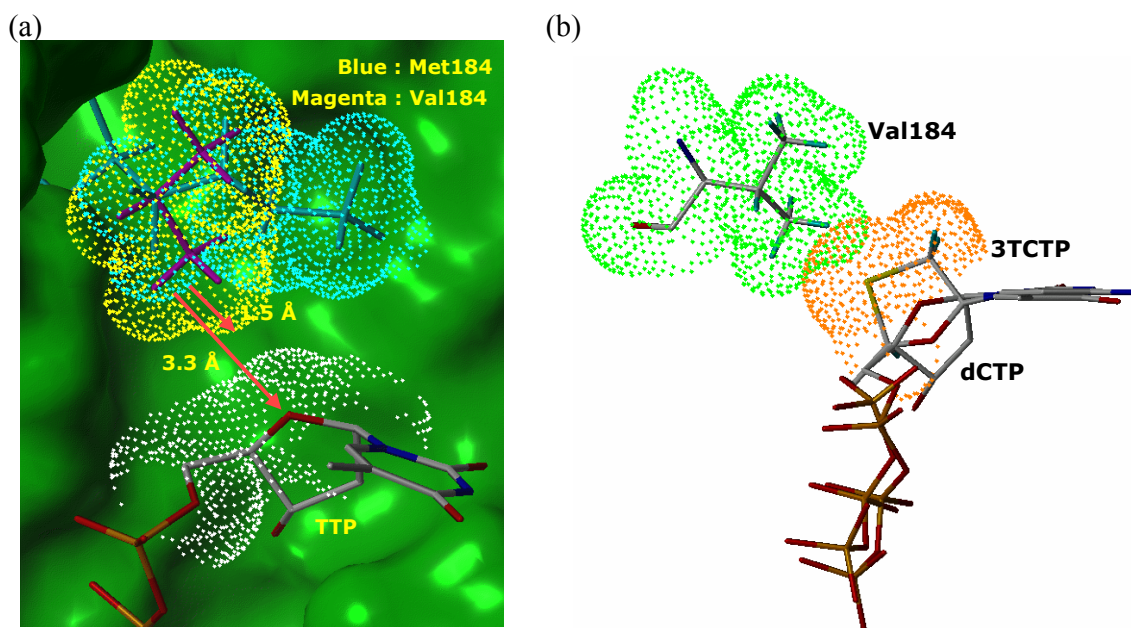


Figure 9.2. (a) Val184 protrudes (~ 1.5 Å) its bulky side chain into the binding site of the nucleoside triphosphate of HIV-1 RT. Natural substrates, such as TTP, manage to bind to the active site of M184V RT. (b) L-nucleoside, such as 3TC, locates its sugar moiety at the upper open space of the NRTI binding site, which is already occupied by Val184. Therefore, in M184V RT, 3TCTP cannot be accommodated into the binding site.

Table 9.2. Fold increases (FI) and the calculated relative binding energies (E_{rel}) of 3TC and D-dioxolane nucleosides against 3TC-resistant RT (M184V).

	3TC		D-Dioxolane T		DXG		D-FDOC	
	FI ^a	E_{rel}^b (kcal/mol)	FI ^a	E_{rel}^b (kcal/mol)	FI ^a	E_{rel}^b (kcal/mol)	FI ^a	E_{rel}^b (kcal/mol)
WT	--	76.4	--	16.91	--	12.05	--	10.83
M184V	>1,200	-28.8	0.44	20.20	2.1	5.67	0.67	35.56

^a. Fold Increase

^b. Relative binding energy = Binding energy of NRTI triphosphate to RT – Binding energy of dNTP to RT

In WT RT, the energetically more stable 3'-exo conformation of D-dioxolane sugar moiety, which can be found in the crystal structure of D-dioxolane cytosine,³³³ locates its 3'-oxygen atom within the hydrogen bonding distance with the amide backbone of Tyr115 (Figure 9.3a). On the other hand, the 3'-endo conformation pulls Arg72 into the nucleoside triphosphate binding site by the interaction between the 3'-oxygen and ϵ -side chain of Arg72 (Figure 9.3a). It is interesting that, even though every dioxolane nucleoside triphosphates was constructed based on the same crystal structure of dioxolane cytosine, the purine nucleoside DXG prefers 3'-exo (Figure 9.3a) but the pyrimidines such as dioxolane thymine and 5-fluorocytosine (Figure 9.3a) are minimized to have 3'-endo conformation. In M184V RT, thymidine and guanosine analogues readily changed their conformations in order to cope with the steric pressure on the active site by the bulky side chain of Val184 (Figure 9.3b). However, the 5-fluorocytosine derivative maintained its conformation in M184V RT by virtue of another hydrogen bonding between its 5-fluorine atom and Arg72 (Figure 9.3b). Therefore, dioxolane 5-fluorocytosine derivative is strongly biased to 3'-endo conformer. In either way, the contribution of 3'-oxygen atom by interaction with the enzyme residues (Tyr115 and Arg72) enables the dioxolane sugar moiety to stay away from Val184 (Figure 9.3c). The conformational search of D-dioxolane thymine by Monte Carlo method in 5,000 steps⁷⁴ found the global minimum as 3'-endo sugar conformation with 28 hits, but the 3'-exo conformation (26 hits) was only 0.4 kcal/mol less stable than the global minimum (Figure 9.4).

Therefore, the dioxolane sugar moiety is flexible enough to change its conformation depending on its environment to cope with the steric pressure conferred by the bulky side

chain of Val184, which can explain the maintenance of its anti-HIV activity against the M184V mutant.

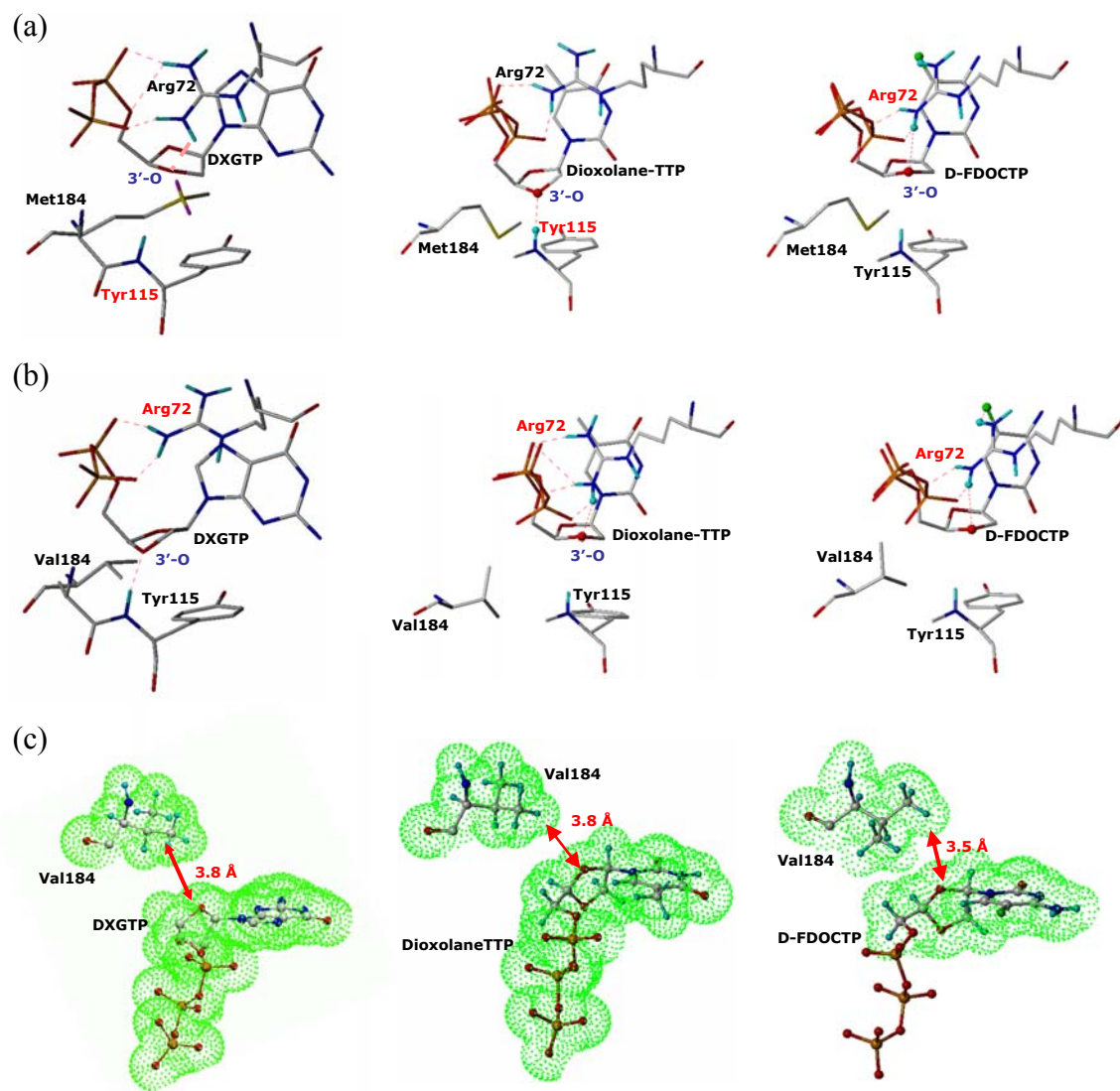


Figure 9.3. D-dioxolane nucleoside triphosphates-RT complexes maintain the favorable binding modes through the interaction of its 3'-oxygen with active site residues such as Arg72 or Tyr115 depending on the dioxolane sugar conformation (3'-endo or 3'-exo) ((a) WT RT, (b) M184V RT). (c) The contribution of 3'-oxygen atom by interaction with the enzyme residues (Tyr115 and Arg72) enables the dioxolane sugar moiety to stay away from Val184.

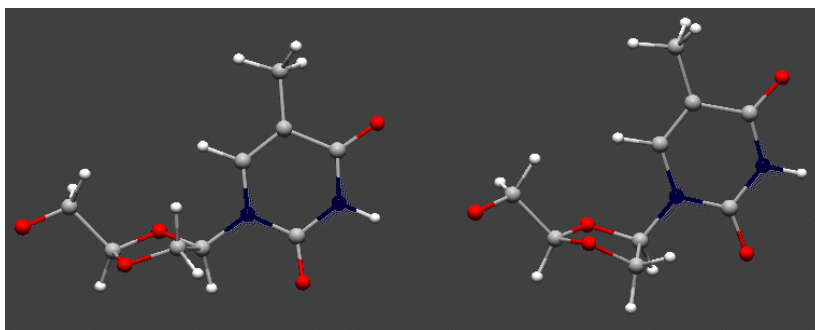


Figure 9.4. Conformational search of D-dioxolane thymine by Monte Carlo method in 5,000 steps found the global minimum as 3'-endo sugar conformation (a), but the 3'-exo conformation (b) was only 0.4 kcal/mol less stable than the global minimum.

Discussion

Among enzymes involved in the synthesis of nucleotides and DNA, some exceptions have been found to the rule that only one of the enantiomeric forms of chiral molecules may bind effectively at the catalytic sites of enzymes, displaying biological activity. These exceptions include: herpes virus-1 TKs (HSV-1 TK),^{338,339} cellular dCK,³⁴⁰ mitochondrial deoxyguanosine kinases (dGK),³⁴¹ and HIV-1 reverse transcriptase.^{342,343} Recent findings have indicated that several β -L-nucleoside analogues exhibit potent antiviral activity against both human HIV and HBV replication, accompanied by low host cellular toxicity when compared to their corresponding natural β -D-counterparts.^{344,345,346,347} In order for L-nucleosides to be active against HIV-1 RT, their L-ribose moieties should bind to the enzyme active site in an opposite orientation with respect to the D-enantiomer.³³⁴ The three dimensional structure of the catalytically competent HIV-1 RT shows that the active site is wide enough to accommodate the L-configured sugar

moiety.³²⁸ The computer-generated, energy-minimized model of HIV-1 RT complexed with 3TCTP clearly shows that the 3TCTP and TTP nucleoside ring configurations are stereochemically opposite, so that alignment of the triphosphates and bases would cause the nucleoside ring of the β -L-inhibitor to project 1.5-2.0 Å further toward residue 184 than would the deoxyribose ring of TTP. As a result, in WT RT, the Met184 and the β -L-oxathiolane moiety of 3TC are in a very stable extended van der Waals contact. Therefore, the most significant difference between the interactions of wild-type HIV-1 RT and the 3TC-resistant mutant HIV-1 RT (M184V RT) with deoxyribose and β -L-oxathiolane nucleoside inhibitors would be a very close contact between the C γ 2 methyl of Val and the protruding oxathiolane ring. This unfavorably close contact (1.8 Å in M184V vs. 3.3 Å in wild-type, Figure 9.2a) would result in severe steric hindrance. In this context, it is noteworthy that, to date, no L-configured nucleoside has been reported to have antiviral activity against the M184V RT.

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. The D-2'-fluoro-4'-thio-2',3'-unsaturated nucleoside is a good example in this category because the cytidine nucleoside, potent against WT RT, shows high cross-resistance to M184V RT.³⁴⁸ In contrast, the 4'-oxa derivative of the D-2'-fluoro-2',3'-unsaturated nucleoside, is known to maintain its antiviral activity against M184V RT. The energy-minimized structure of the D-2'-fluoro-4'-thio cytidine/RT complex shows that, in spite of the same conformation of D-2'-fluoro-4'-thio-2',3'-unsaturated cytidine as that of its 4'-oxa derivative, the steric clash with Val184 caused by the longer bond length of C-S bond as well as the larger van der

Van der Waals radius of the 4'-sulfur atom result in a deformation of the active site.³⁴⁸ Therefore, of great structural interest was how the D-dioxolane nucleosides escape from the steric hindrance with the bulky side chain of Val184 in M184V RT. In every minimized structure of D-dioxolane nucleoside triphosphate/RT complexes, the 3'-oxygen atom of D-dioxolane sugar moiety was always found to be in specific interaction with the nearby enzyme residues regardless of its conformation (Figure 9.3), and the conformational analysis showed that the interconversion of the dioxolane's conformation from one to another can take place without significant loss of energy (Figure 4). The specific interaction between dioxolane sugar moiety and the enzyme residues effectively holds the nucleoside triphosphate out of the clashing distance with Val184 (Figure 9.3(c)). In this context, it is interesting that, in the crystal structure of TTP bound to the WT RT, the 3'-OH of TTP is in specific interaction with its own triphosphate moiety as well as the nearby enzyme residue Tyr115 (Figure 9.2a).³²⁸ As Tyr115 is located at the other side of the active site of RT from Val184, it is possible that the 2'-deoxy sugar moiety of TTP can stay away from Val184 by virtue of the interaction between 3'-OH and Tyr115. Also, AZT, which has the same conformation as TTP, does not experience any cross-resistance to M184V RT.³⁴⁹ In our previous study, we have demonstrated that the 3'-azido group of AZT is in the right position to interact with Tyr115 (Figure 9.5).³²⁶ On the other hand, moderate cross-resistance of ddI and ddC to M184V RT,³⁵⁰ which do not have any functionality on their sugar ring to interact with the enzyme residues in a specific manner supports our claim.

In summary, our molecular modeling study unequivocally indicates that the functional 3'-oxygen atom of the dioxolane sugar moiety mimics the 3'-OH group of the natural substrate to hold the nucleoside triphosphate away from Val184.

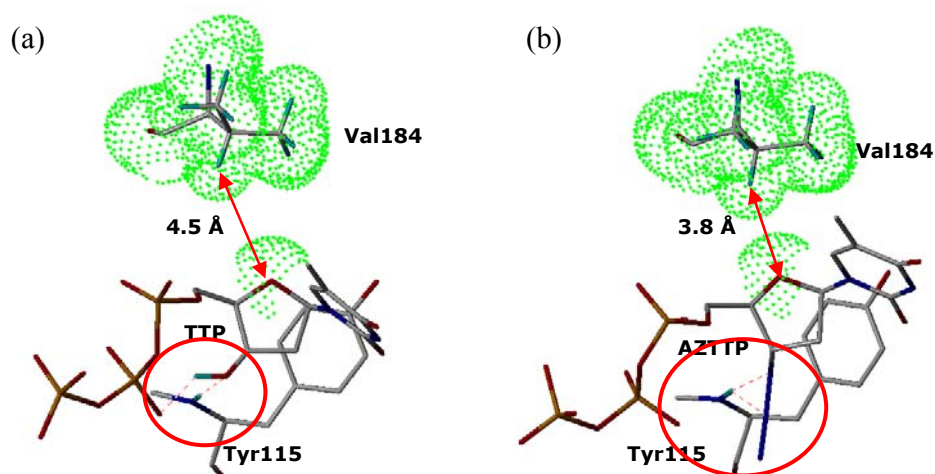


Figure 9.5. AZT (b), which has the same conformation as TTP (a), does not experience any resistance to M184V RT. The 3'-azido group of AZT is in the right position to interact with Tyr115 to hold the nucleoside triphosphate away from Val184.

CHAPTER 10

MECHANISM OF ANTIVIRAL ACTIVITIES OF 3'-SUBSTITUTED L- NUCLEOSIDES AGAINST 3TC-RESISTANT HBV POLYMERASE

Introduction

Hepatitis B virus (HBV) is the leading cause of chronic hepatitis throughout the world. Despite the availability of a safe and effective vaccine, the prevalence of HBV-related infection and development of hepatocellular carcinoma have not declined significantly, and the treatment with antiviral agents remains the only therapeutic option available to individuals chronically infected with HBV.

Alpha interferon (IFN), lamivudine (3TC) and recently approved adefovir dipivoxil (ADV),^{351,352} are the only drugs currently approved for the treatment of chronic hepatitis B in most countries. Unfortunately, the rate of response to IFN is low, and drug-associated side effects are significant.^{353,354} Clinicians can now choose to treat with lamivudine, a well-tolerated, orally available drug that has minimal side effects.³⁵⁵ As with IFN, however, a complete antiviral response, as assessed by HBe seroconversion, is seen in only a minority of patients after 1 year of treatment.³⁵⁶

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 severely 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,^{356,357,358} which is usually associated with mutations in the HBV DNA polymerase gene.³⁵⁹ 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) 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.^{358,360,361,362,363,364}

The emergence of HBV drug resistance highlights the need for development of alternative therapies that suppress HBV replication *in vivo* more effectively. A more potent antiviral agent, for example, should suppress HBV replication and also slow the emergence of drug-resistant variants. Therapies that would also suppress the replication of 3TC-resistant HBV are needed. In this regard, the unnatural L-nucleosides attract special attention because they have proven their specific antiviral activities against HBV.³⁶⁵ More interestingly, several L-nucleosides (L-dT,³⁶⁶ L-Fd4C,³⁶⁷ L-FMAU³⁶⁸ and L-3'Fd4³⁶⁹) show antiviral activity against 3TC-resistant HBV polymerase. As L-nucleosides show poor antiviral activity against 3TC-resistant HIV-1, the structural difference between the HBV polymerase and HIV-1 RT is warranted to investigate. For this purpose, the active site of the homology-modeled three dimensional structure of the HBV polymerase^{370,371} was compared with that of HIV-1 RT.

Materials and Methods

All molecular modeling of the enzyme-substrate complexes was carried out using SYBYL 6.6 (Tripos Inc. St. Louis, MO). A three-dimensional homology model of the catalytic core of HBV polymerase was constructed via extrapolation from HIV-1 reverse transcriptase structures.^{370,371}

Homology modeling of the HBV polymerase. The crystal structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd)³⁷² was used as a template to generate the three dimensional structure of the HBV polymerase (Composer module in Sybyl 6.7).^{370,371} The two Mg²⁺ ions, thymidine triphosphate and template-primer duplex were located at the same position as the HIV-1 RT·DNA·dNTP complex structure (1RTD).³⁷²

The stability of the modeled HBV polymerase·DNA·thymidine triphosphate complex was confirmed by performing molecular mechanics energy minimization and molecular dynamics simulation by using the molecular graphics and simulation program MacroModel, version 7.0 (Schrödinger, Inc.).³⁷¹

The complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient, Polak-Ribiere 1st derivative method was used for energy minimization. Molecular dynamics simulations on HBV polymerase·DNA·TTP was performed with MMFF94s in the presence of GB/SA continuum water model on a Silicon Graphics Octane2 workstation running the IRIX 6.5 operating system by heating from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms.

After molecular dynamics simulation, the HBV polymerase·DNA·TTP ternary complex was fully minimized until there was no significant movement in atomic coordinates (MMFF94s force field, GB/SA water, conjugate gradient and Polak-Ribiere 1st derivative

method) to give the final three dimensional structure of the HBV polymerase complexed with the DNA duplex and the natural substrate, TTP.³⁷¹

3TC-resistant HIV-1 RT and HBV polymerase. The crystal structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd)³⁷² was used as a template to generate the three dimensional structure of the M184V RT. The residue Met184 was mutated to Val184 in the biopolymer module in Sybyl 6.7. Hydrogen atoms were added to the mutated residue and the side chain was fixed. Gästeiger-Hückel charge^{373,374} and Kollman-All-Atom charge^{375,376} were loaded to the TTP and M184V RT, respectively, and the residues inside 6 Å from TTP were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol.³⁷⁷ The annealed M184V RT-TTP complex was fully minimized by using the Kollman-All-Atom force field until the iteration number reached 5,000. In HBV polymerase, the residue 204 was mutated from methionine to isoleucine, and the same treatment was done to generate the 3TC-resistant M204I mutant enzyme.

NRTIs. The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates (AZT³⁷⁸ and ddC³⁷⁹) or conformational analysis³⁸⁰ of the modified crystal structures (3TC from FTC³⁸¹/ L-d4FC and L-3'Fd4C from d4C³⁸²). The heterocyclic base was appropriately modified before docking into the enzyme. Also, the heterocyclic moiety of n+1th nucleotide in template overhang was modified to the base complementary to the incoming NRTIs.³⁷⁷

Method. Gästeiger-Hückel charge^{373,374} was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges^{375,376} were loaded to enzyme site from the biopolymer option in SYBYL 6.6

(Tripos Inc. St. Louis, MO). Fluorine parameters were obtained from the literature^{383,384} 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 mutated residues were annealed³⁷⁷ until energy change from one iteration to the next was less than 0.05 kcal/mol (hot region: 6 Å , interesting region : 12 Å). The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field³⁸⁵ until iteration number reached 5,000. The binding affinities of the examined structures toward HIV-1 RT and HBV polymerase was estimated by means of the relative binding energy (E_{rel} , Table 10.1) between the inhibitor triphosphate-RT complex and the corresponding natural substrate (dGTP, TTP or dCTP)-RT complex in the energy-minimized states.^{377,380}

Results

HIV-1 RT vs HBV polymerase. In order for L-nucleosides to be active against HIV-1 RT, their L-ribose should bind to the enzyme active site with an opposite orientation with respect to the D-enantiomer,³⁸⁰ and the three dimensional structure of the catalytically competent HIV-1 RT shows an open space at the active site wide enough to accommodate the L-configured sugar moiety (Figure 10.1b).³⁷² The homology-modeled active site of HBV polymerase complexed with 3TCTP also shows that its active site is open to L-nucleosides, particularly the position where the 3'-substituent of the L-ribose projects is wider and deeper than RT (Figure 10.1a).^{370,371} However, that space is not

loose enough to accommodate a bulky 3'-substituent such as the 3'-azido group of L-AZT (Figure 10.2). Therefore, even though a number of L-configured nucleoside triphosphates including L-ddC,³⁸⁶ L-FMAU³⁸⁷ and L-dT³⁸⁸ show favorable binding affinity to the wild type HBV polymerase, L-AZT³⁸⁹ does not fit in the active site of HBV polymerase (Table 10.1).

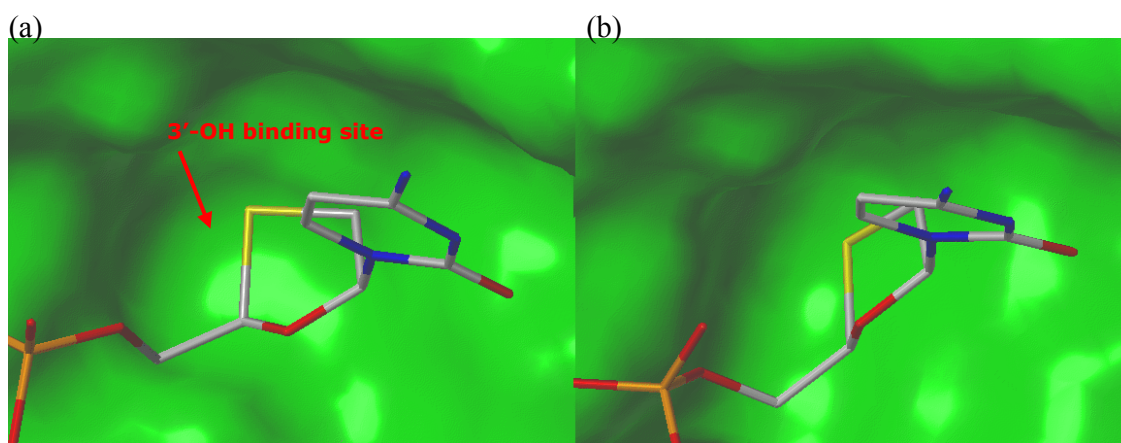


Figure 10.1. 3TCTP complexed with HBV polymerase (a) and HIV-1 RT (b). Wide and deep 3'-OH binding site can be seen in the HBV polymerase complex (a).

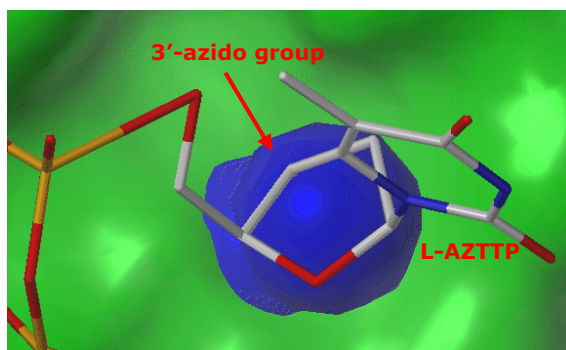


Figure 10.2. 3'-OH binding site is not big enough to accommodate the bulky 3'-azido group of L-AZT. 3'-Azido group is embedded.

Table 10.1. Anti-HBV activity (EC₅₀) and calculated relative binding energy (E_{rel}) of various L-nucleosides.

	3TC	L-AZT	L-ddC	L-d4FC	L-3'-Fd4C	L-FMAU
EC ₅₀ (μM)	0.01 ^a	>10 ^b	0.01 ^c	0.002 ^d	0.22 ^e	0.1 ^f
E _{rel} (kcal/mol) ^g	25.2	-15.6	28.5	37.6	8.8	36.6

^a. Jarvis, B.; Faulds, D. *Drugs* **1999**, 58, 101-141.

^b. Faraj, A.; Alaoui, M. A. E.; Pavia, G.; Gosselin, G.; Imbach, J-L. *Nucleosides Nucleotides*, **1997**, 16, 1287-1290.

^c. Lin, T. S.; Luo, M. Z.; Liu, M. C.; Pai, S. B.; Dutschman, G. E.; Cheng, Y. C. *Biochem. Pharm.* **1994**, 47, 171-174.

^d. Lin, T. S.; Luo, M. A.; Liu, M. C.; Zhu, Y. L.; Gullen, E.; Dutschman, G. E.; Cheng, Y. C. *J. Med. Chem.* **1996**, 39, 1757-1759.

^e. EC₉₀

^f. Chu, C. K.; Ma, T-W.; Shanmuganathan, K.; Wang, C-G.; Xiang, Y-J.; Pai, S. B.; Yao, G-Q.; Sommadossi, J-P.; Cheng, Y-C. *Antimicrob. Agents Chemother.* **1995**, 39, 979-981.

^g. Relative binding energy = Binding energy of NRTI triphosphate to RT – Binding energy of dNTP to RT

M184V HIV-1 RT vs M204I HBV polymerase. Unlike HIV-1 RT, the bulky side chain of Val204 in the M204V HBV polymerase/3TC triphosphate complex is not in close proximity to the oxathiolane sugar moiety of 3TC triphosphate to cause the unfavorable steric hindrance. However, the calculated relative binding energy of the M204V HBV polymerase/3TCTP complex is very low, which indicates that the mutation results in the

deformation of the enzyme/ligand complex (Table 10.2). Particularly, the mutant enzyme/3TCTP complex has markedly low electrostatic energy.

Table 10.2. Fold increase (FI) and the relative binding energy of 3TC and L-3'-Fd4C against the wild-type and M204V HBV polymerase.^a

	3TC			L-3'-Fd4C		
	EC ₉₀	FI	E _{rel}	EC ₉₀	FI	E _{rel}
WT	0.05	--	25.2	0.22	--	8.8
M204V	>10	>200	-110.9	2.3	10.5	46.3

^a. Schinazi, R. F. Personal Communication.

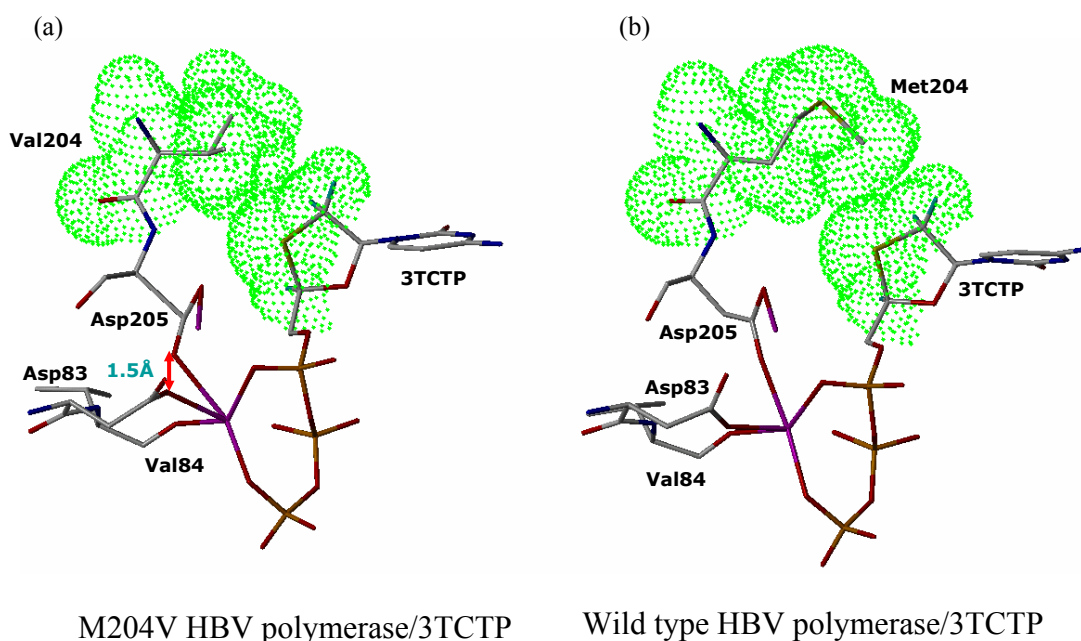


Figure 10.3. Minimized structures of HBV polymerase complexed with 3TCTP: Mutant (M204V) structure (a) in comparison to the wild type (b) shows a large movement of the 3TCTP toward Val204, which accompanies a concurrent disruption of the coordination geometry of the two Mg atoms. The unfavorable close contact (1.5 Å) between two negatively charged aspartic acid residues (Asp83 and Asp205) results in repulsive electrostatic interaction and thereby poor binding energy.

The analysis of the minimized mutant structure in comparison to the wild type shows a large movement of the 3TCTP toward Val204, which accompanies a concurrent disruption of the coordination geometry of the two Mg atoms (Figure 10.3). The unfavorable close contact (1.5 Å) between two negatively charged aspartic acid residues (Asp83 and Asp205) results in repulsive electrostatic interaction and thereby poor binding energy (Figure 10.3a). As the M204V mutation reduces the stabilization of the oxathiolane moiety of 3TCTP through van der Waals interaction, the movement of 3TCTP toward its closest residue, Val204, is conceivable, which results in the disruption of the active site conformation. Therefore, loss of the stabilizing van der Waals contact in M204V HBV polymerase leaves highly unfavorable environment for 3TCTP to bind at the active site. The binding mode of L-3'-Fd4CTP to the active site of M204V HBV polymerase (Figure 10.4a) shows another example in which the mutated Val204 does not interfere with the binding of the L-nucleoside triphosphate by the projection of the bulky side chain of valine into the active site of the polymerase. Rather, it is obvious that the direct effect of the mutation is the loss of the stabilizing van der Waals contact not the steric hindrance. However, in this case, the 3'-fluorine atom attached to the 2',3'-double bond easily compensates for the slight loss of the van der Waals contact by forming a hydrogen bond with the amide backbone of the active site residue, Asp205 (Figure 10.4a). The calculated relative binding energy of M204V HBV polymerase complexed with L-3'-Fd4CTP was much higher than the wild type enzyme complex (Table 10.2). Particularly, the electrostatic energy showed large difference between the mutant and the wild type enzyme complexes, which indicates the contribution of the newly formed hydrogen bond to the total binding energy of the nucleoside triphosphate.

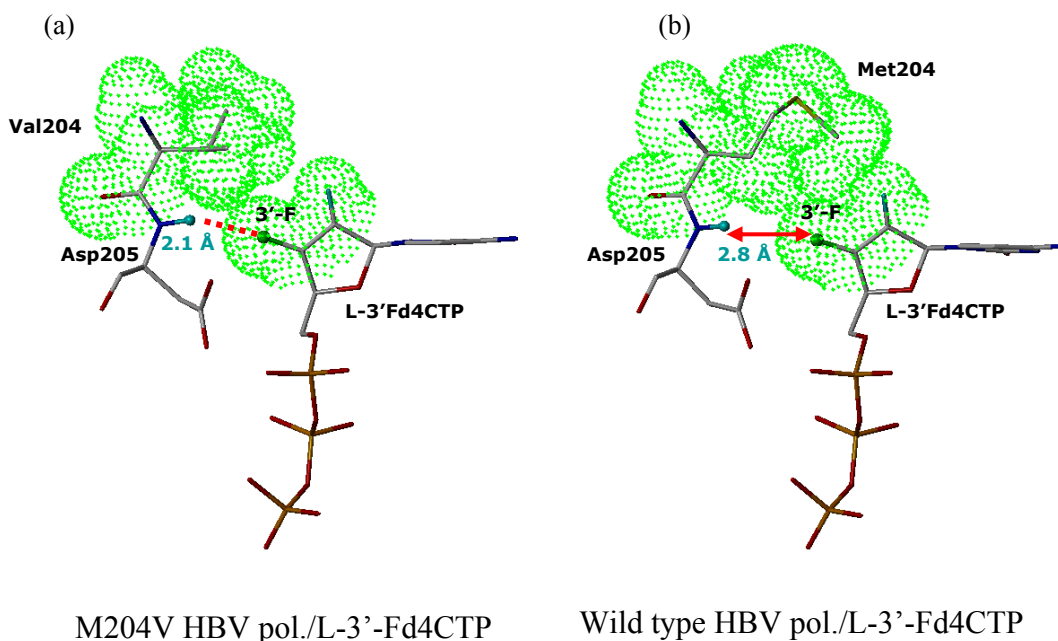


Figure 10.4. The binding mode of L-3'-Fd4CTP to the active site of HBV polymerase: (a) M204V HBV polymerase/ L-3'-Fd4CTP complex. The mutated Val204 does not interfere with the binding of the L-nucleoside triphosphate by the projection of the bulky side chain of valine into the active site of the polymerase. The 3'-fluorine atom attached to the 2',3'-double bond easily compensates for the slight loss of the van der Waals contact by forming a hydrogen bond with the amide backbone of the active site residue, Asp205. (b) Wild type HBV polymerase/ L-3'-Fd4CTP complex. The direct effect of the mutation is the loss of the stabilizing van der Waals contact not the steric hindrance. The wild type HBV polymerase complex shows an extensive hydrophobic interaction between Met204 and the sugar moiety of L-3'-Fd4CTP.

Discussion

HBV's genome is small and composed of circular, partially double-stranded DNA.³⁹⁰ The viral genome is very compact and contains four overlapping open-reading frames (ORFs)

in the negative strand,³⁹¹ among which ORF *P* covers 75% of the viral genome and encodes for the viral DNA polymerase/ reverse transcriptase. The inhibition of HBV replication by nucleoside analogs results from the recognition of nucleoside analog triphosphates by the RNA-dependent DNA polymerase as well as DNA-dependent DNA polymerase of HBV. The product of ORF *P* shares similarity with the HIV-1 reverse transcriptase (RT) in that it has both reverse transcriptase and RNase H activities. Amino acid sequence alignment of the hepadnaviruses *P* proteins with those of the retroviruses indicates a significant homology within the functional domains, for example, the YMDD motif within the catalytic site of reverse transcriptase.³⁹² Considering the similarities between HIV-1 RT and HBV polymerase, it is not surprising that many compounds showing anti-HIV activity are also effective against HBV. However, in spite of the lack of the three-dimensional structural information of the HBV polymerase, at least two studies provide interesting evidences that the active site of the HBV polymerase has an arrangement of its amino acid residues to accommodate wide variety of the unnatural L-nucleoside triphosphates. From the kinetic analysis of the incorporation of D- and L- β -dCTP, β -ddCTP and β -ddFCTP catalyzed by HIV-1 RT, Maga and coworkers suggested that the hydroxyl group at the 3'-position of the L-sugar moiety makes an unfavorable steric hindrance with residues in the RT nucleotide binding site, since the affinity increases from the L-2'-deoxy- to the L-2',3'-dideoxynucleoside triphosphate analogues for HIV-1 RT.³⁹³ On the other hand, through an extensive structure-activity analysis, the 3'-OH group of the β -L-2'-deoxyribose of the β -L-2'-deoxynucleoside series was suggested to confer unique specificity for anti-HBV activity.³⁸⁸ From these studies, it is clear that the active site of the HBV polymerase has an open space wide enough to

accommodate the 3'-substituted β -L-2'-deoxynucleoside. The high anti-HBV activity of two β -L-2'-deoxynucleosides which have 3'-OH groups (L-FMAU³⁹⁴ and L-dT³⁹⁵), which do not show significant antiviral activity against HIV-1 RT, strongly supports this idea. Our modeled active site of HBV polymerase complexed with 3TCTP also shows that there is an enough space at the 3'-position of the L-nucleoside (Figure 10.1a). However, it is interesting to note that the active site of HBV polymerase is not wide enough to accommodate a bulky 3'-substituent such as the 3'-azido group of L-AZT (Figure 10.2),³⁸⁹ which shows no antiviral activity against HBV.

Related to the structural difference between the active sites of HIV-1 RT and HBV polymerase, of greater interest is the mechanism of 3TC-resistance. The computer-generated, energy-minimized model of HIV-1 RT complexed with 3TCTP clearly shows that the 3TCTP and TTP nucleoside ring configurations are enantiomeric, so that alignment of the triphosphates and bases would cause the oxathiolane ring of the β -L-inhibitor such as 3TC to project 1.5-2.0 Å further toward residue 184 than would the ribose ring of TTP (Figure 10.5). As a result, in WT RT, Met184 and the β -L-oxathiolane moiety of 3TC are in a very stable extended van der Waals contact. Therefore, the most significant difference between the interactions of wild-type HIV-1 RT and the 3TC-resistant mutant HIV-1 RT (M184V RT) with ribose and β -L-oxathiolane nucleoside inhibitors would be a very close contact between the C γ 2 methyl of Val and the protruding oxathiolane ring. This unfavorably close contact (1.8 Å in M184V vs. 3.3 Å in wild-type) would result in severe steric hindrance (Figure 10.5). In this context, it is noteworthy that, to date, no L-configured nucleoside has been reported to have antiviral activity against the M184V RT. In view of the structural similarity between HIV-1 RT

and HBV polymerase, Das *et al.* also suggested that the steric hindrance between the mutant amino acid side chain and lamivudine could account for the resistance phenotype.³⁷⁰ Specifically, steric conflict between the C γ 2-methyl group of Ile or Val at position 204 in HBV polymerase and the sulfur atom in the oxathiolane ring was proposed to account for the resistance observed upon M204I/V mutation.³⁷⁰ Therefore, according to this mechanism, as was with M184V HIV-1 RT, there would be no chance for any L-configured nucleosides to be active against the mutant HBV polymerase whose Met204 is mutated to the sterically demanding isoleucine or valine.

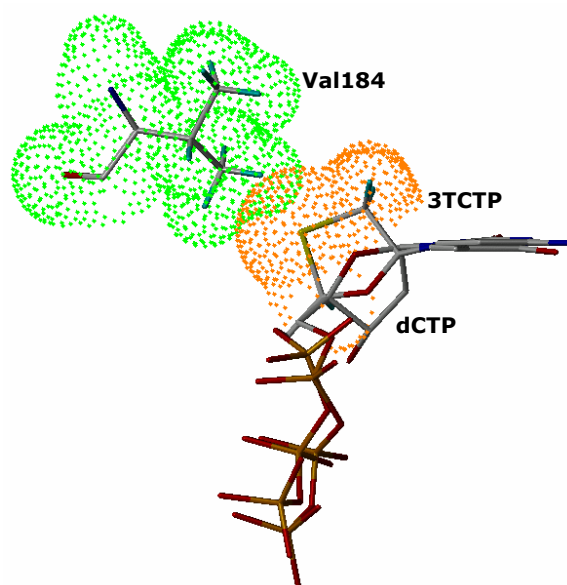


Figure 10.5. L-nucleoside, such as 3TC, locates its sugar moiety at the upper open space of the NRTI binding site, which is already occupied by Val184. Therefore, in M184V RT, 3TCTP cannot be accommodated into the binding site.

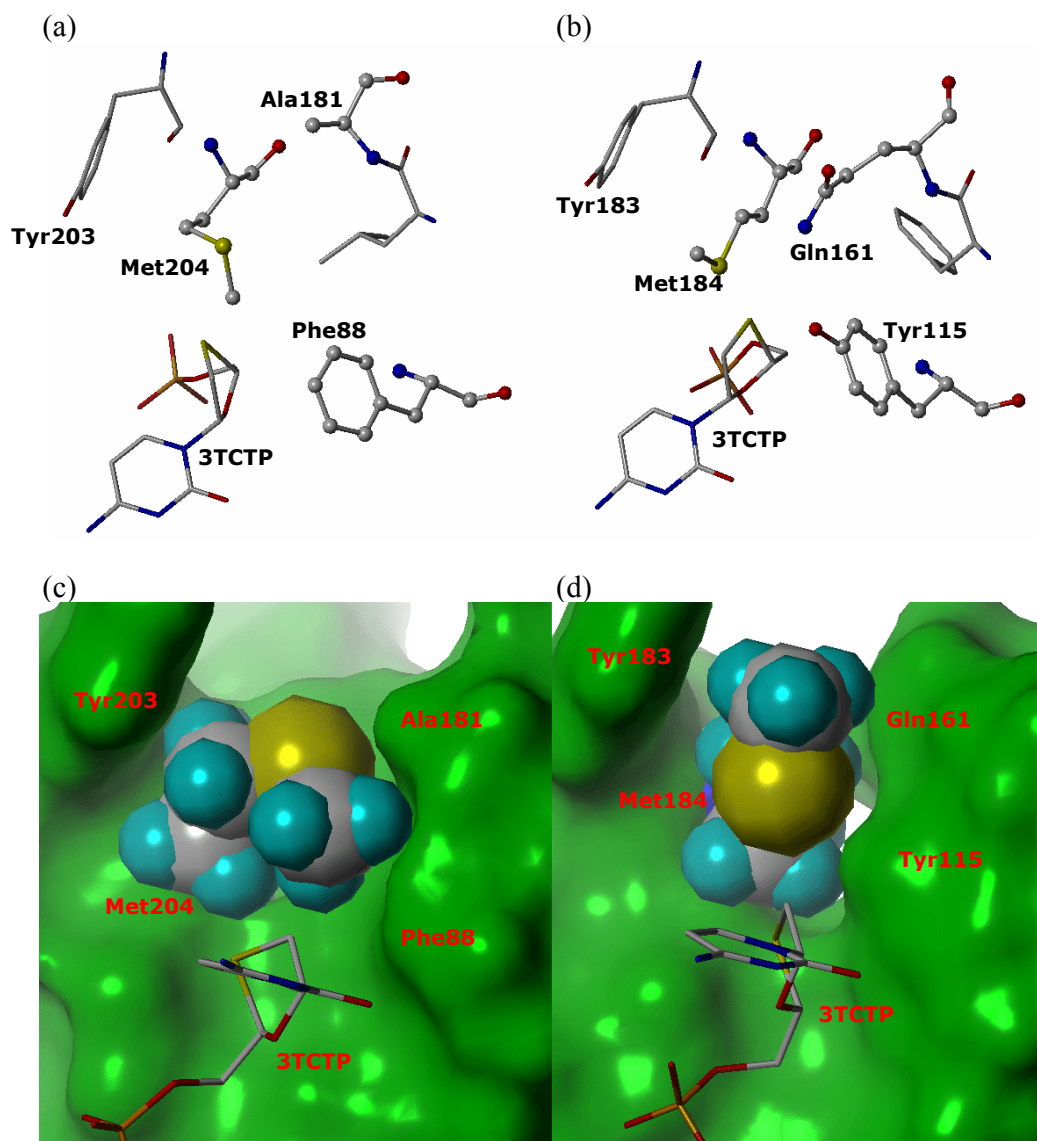


Figure 10.6. In HIV-1 RT (b and d), the residue Met184 is located inside a narrow pocket composed of Gln161, Tyr115, Pro157 and Tyr183. Therefore, Met184 has a linear conformation along the pocket. HBV polymerase has a much bigger pocket composed of Ala181, Phe88, Pro177 and Tyr203 (a and c). Particularly, compared with Gln161 in RT (b), the short side chain of Ala181 (a) broadens the pocket. As a result, Met204 in HBV polymerase can adopt a flexible conformation which can wrap around the sugar moiety of 3TCTP by extensive van der Waals interaction.

However, the high antiviral activities of several L-nucleosides such as L-dT,³⁶⁶ L-d4FC,³⁶⁷ L-FMAU³⁶⁸ and L-3'-Fd4C³⁶⁹ against M204V HBV polymerase do not allow this generalization. In order to address this challenging question, computer-generated M184V HIV-1 RT and M204V HBV polymerase complexed with a DNA duplex as well as a natural substrate (TTP) were energy-minimized under the same conditions. In HIV-1 RT, the residue Met184 is located inside a narrow pocket composed of Gln161, Tyr115, Pro157 and Tyr183 (Figure 10.6b). Therefore, Met184 has a linear conformation along the pocket (Figure 10.6b and 6d). However, HBV polymerase has a much bigger pocket composed of Ala91, Phe88, Pro177 and Tyr203 (Figure 10.6a and 6c). Particularly, compared with Gln161 in RT, the short side chain of Ala91 broadens the pocket. As a result, Met204 in HBV polymerase can adopt a flexible conformation which can wrap around the sugar moiety of 3TCTP by extensive van der Waals interaction (Figure 10.3b). Upon mutation to valine, the narrow pocket where Met184 resides in RT strongly limits the conformation of the side chain of Val184. Under the given circumstances, projection of the side chain into the nucleoside binding site, which does not allow binding of L-nucleoside triphosphate, is the lowest energy conformation of Val184 (Figure 10.7b). However, by virtue of the loose environment around the residue 204, the bulky side chain of Val204 of M204V HBV polymerase can stay away from the nucleoside binding site to avoid the unfavorable steric hindrance with the incoming L-nucleoside triphosphate (Figure 10.7a). Additionally, the bulky side chain of Leu180 in HBV polymerase prevents the rotation of the side chain of Val204 into the active site (Figure 10.7c). Therefore, it is not likely that the 3TC-resistance in M204V HBV polymerase happens in the same way as in HIV-1 RT. Our molecular modeling study of M204V

HBV polymerase/3TCTP complex shows that, instead of imposing steric pressure into the nucleoside binding site, the mutation from methionine to valine followed by the loss of van der Waals interaction is believed to destabilize the enzyme/3TCTP complex. Not unexpectedly, in the relatively wide active site of HBV polymerase (the closest enzyme residue from 3TCTP is Met204, which is 3.5Å away), the nonspecific van der Waals interaction between the enzyme residue, particularly Met204 and the sugar moiety of the L-configured nucleoside triphosphate is critical for stabilizing the enzyme/nucleoside complex. Therefore, nucleosides, which are able to specifically interact with enzyme residues, would have benefits in coping with the loss of the nonspecific hydrophobic interaction caused by mutation. Specifically, the substitution at the 3'-position would provide more chance to interact with the enzyme residues as the active site of the HBV polymerase has an open space composed of the highly conserved secondary structure (YMDD motif) where the 3'-substituents of the L-nucleosides project. In this regard, it is interesting to note that many L-nucleosides which have 3'-substituents such as L-dT,³⁶⁶ L-FMAU³⁶⁸ and L-3'-Fd4C³⁶⁹ show antiviral activity against the 3TC-resistant mutant HBV polymerase.

As an example, in M204V HBV polymerase/L-3'-Fd4CTP complex (Figure 10.4), the mutation also results in the loss of the van der Waals contact between Met204 and the 2',3'-double bond of the sugar moiety. However, a slight movement of the nucleoside triphosphate toward Val204 followed by a formation of the hydrogen bonding between the 3'-F atom and amide backbone of Asp205 can readily compensate for the loss of van der Waals contact.

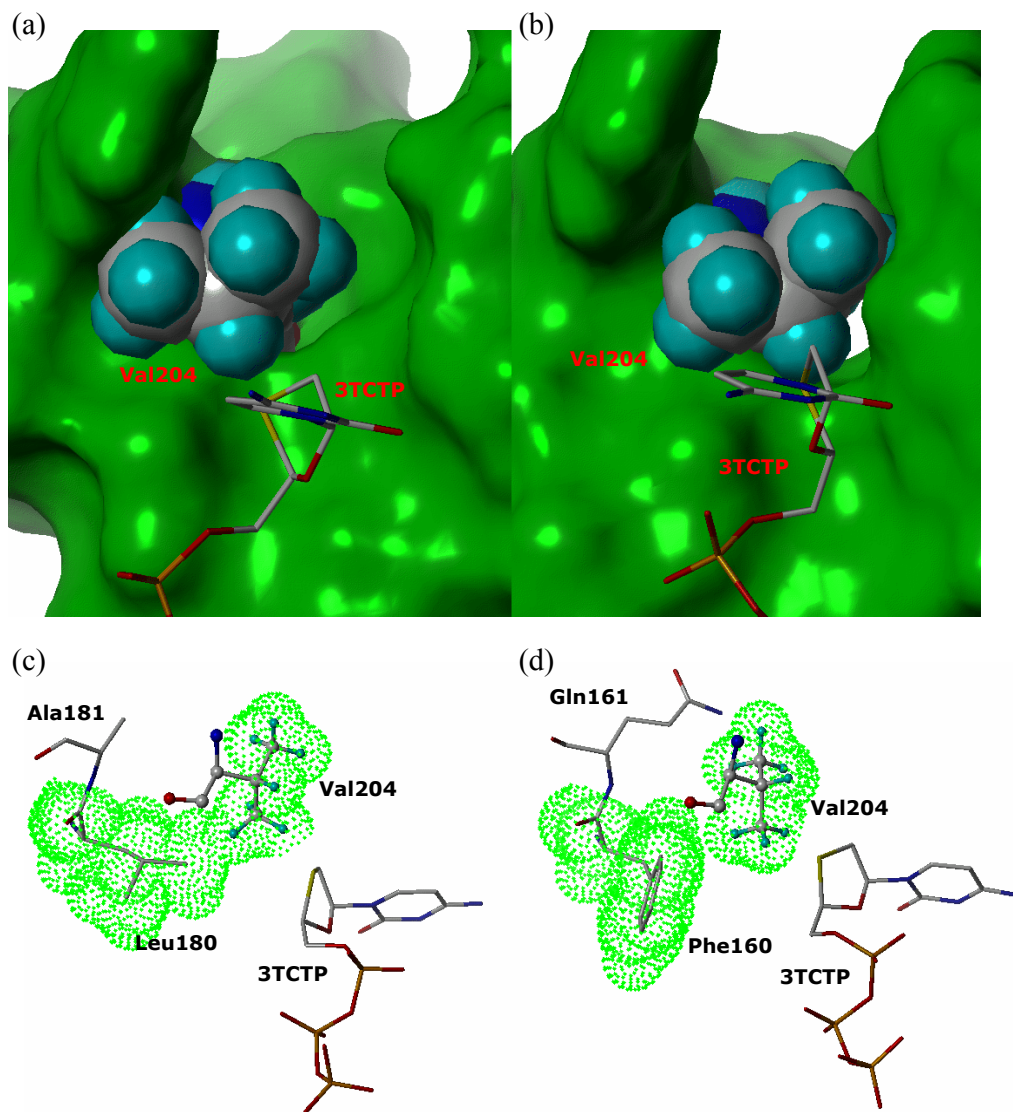


Figure 10.7. In M184V RT (b), the narrow pocket where the residue 184 resides strongly limits the conformation of the side chain of Val184. Under the given circumstances, projection of the side chain into the nucleoside binding site, which does not allow binding of L-nucleoside triphosphate, is the lowest energy conformation of Val184 (b and d). However, by virtue of the loose environment around the residue 204 (a), the bulky side chain of Val204 of M204V HBV polymerase can rotate itself to stay away from the nucleoside binding site (a). Additionally, the bulky side chain of Leu180 in HBV polymerase prevents the rotation of the side chain of Val204 into the active site (c).

In summary, the comparison of the active sites of the HIV-1 RT and the homology-modeled HBV polymerase shows that the active sites of both enzymes are open to L-nucleosides, but the position where the 3'-substituent of the L-ribose projects in HBV polymerase is wider and deeper than RT, which enables the HBV polymerase to accommodate various 3'-substituted L-nucleosides. However, it is unlikely that the two enzymes have the same mechanism of 3TC-resistance. The analysis of the minimized structure of M204V HBV polymerase/3TCTP complex shows that a loss of the van der Waals contact around the oxathiolane sugar moiety of 3TCTP results in the disruption of the active site. Therefore, upon mutation, nucleosides which can be stabilized by the additional specific interaction with enzyme residues may be able to circumvent the destabilization caused by the loss of the hydrophobic interaction. Specifically, the substitution at the 3'-position would be beneficial as the active site of the HBV polymerase has an open binding site composed of the highly conserved motif (YMDD) where the 3'-substituents of the L-nucleosides project. As an example, our study shows that the 3'-fluorine atom contributes for the antiviral activity of L-3'-Fd4CTP against M204 HBV polymerase by readily compensating for the loss of the van der Waals interaction around the 2',3'-double bond through a formation of a hydrogen bond.

CHAPTER 11

EFFECTS OF FLUORINE SUBSTITUTION OF CYTOSINE ANALOGS ON THE BINDING AFFINITY TO HIV-1 REVERSE TRANSCRIPTASE¹

¹Chong, Y. H. Choo and C. K. Chu, Submitted to *Bioorg. Med. Chem. Lett.* 7/2/03

Introduction

The addition of a fluorine at the 5-position of the pyrimidine ring has been found to alter the kinetic parameters as well as antiviral potency of the corresponding nucleoside analogues.³⁹⁶ Studies have shown that modifying the 5-position of the cytosine ring with fluorine may reduce toxicity and in some cases increase the antiviral potency.³⁹⁷ In the previous kinetics studies, the 5-fluoro substitution of the triphosphates of (-)- β -L-2',3'-dideoxy-3'-thiacytidine (3TC) and (+)- β -D-2',3'-didehydro-2',3'-dideoxycytidine (d4C) caused a higher overall efficiency of incorporation compared to their unfluorinated counterparts during DNA-directed synthesis (Table 11.1), suggesting the possible role of 5-fluoro substituent in the binding of the nucleoside triphosphate at the active site of HIV-1 reverse transcriptase (RT).³⁹⁶ However, it is noteworthy that, while the 5-fluoro substitution of D-d4CTP resulted in remarkable increase in both maximum rates of incorporation (k_{pol}) and equilibrium binding constant (K_d), FTCTP's marginally increased efficiency of incorporation is the result of the increased rate of incorporation (k_{pol}) instead of the increased stability of the enzyme-ligand complex (K_d) (Table 11.1). Also, in contrast to the rarely negative effect of a 5-fluoro substitution, the 2'-fluoro substitution of D-d4FCTP caused a 13.5-fold decrease in the efficiency of incorporation and two-fold increase in K_d during DNA-directed incorporation (Table 11.1). Therefore, even though the fluorine substitution apparently alters the kinetic behaviors of the nucleoside triphosphates, in terms of the binding affinity, it is clear that the effect of fluorine substitution of the nucleoside triphosphate is highly dependent on the substitution position as well as the conformation of the sugar moiety.

Table 11.1. Efficiency of cytidine analog incorporation into a DNA primer directed by a DNA template.³⁹⁶

Compound	k_{pol}	K_d	Efficiency ^a
3TCTP	0.019	15	0.0013
FTCTP	0.039	12	0.0033
D-d4CTP	0.16	18	0.0086
D-d4FCTP	0.95	31	0.031
D-2'F-d4CTP	0.15	64	0.0023

^a k_{pol}/K_d

Thus, it is of interest to investigate the molecular mechanism by which this subtle substitution with the highly electronegative fluorine atom affects the binding mode of the nucleoside triphosphate analogues to the active site of RT.

For this purpose, three structurally different fluorinated nucleoside triphosphates such as FTCTP, D-dioxolane 5-FCTP and D-2'F-d4-5FCTP (Fig. 11.1) were docked into the active site of HIV-1 RT. After molecular dynamics simulation followed by exhaustive energy minimization, the resulting enzyme-inhibitor complexes were analyzed to provide a dynamic picture of the active site residues of RT which discriminates subtle conformational differences of the incoming nucleoside triphosphate analogues.

Since the change in binding mode of cytidine analogs in response to the isosteric 5-fluorination should influence electrostatic, not steric effects, the analysis was focused on the electronic effect of the fluorine atom.

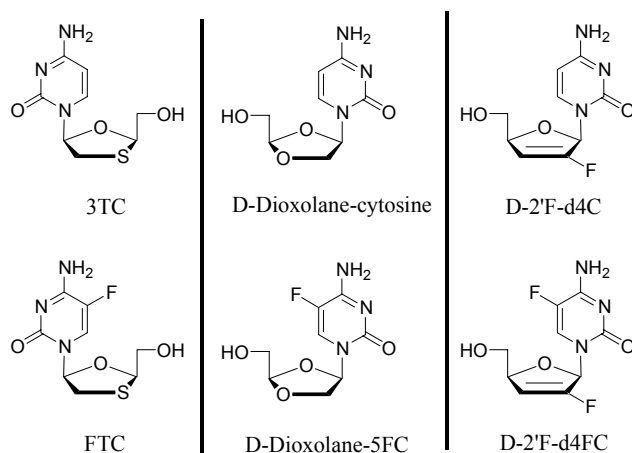


Figure 11.1. 5-Fluoro cytidine analogues studied.

Materials and Methods

All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos, Inc.) and MacroModel 7.0 (Schrödinger, Inc.). As was reported in our previous molecular modeling study of HIV-1 RT,³⁹⁸ 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).³⁹⁹ The initial Cartesian coordinates for each inhibitor were generated based on the X-ray coordinates (D-dioxolane cytidine⁴⁰⁰ and D-d4C) or conformational analysis (FTC).⁴⁰¹ Each RT·DNA·nucleoside triphosphate complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient, Polak-Ribiere 1st derivative method, was used for energy minimization. Molecular dynamics simulations on RT·DNA·nucleoside-TP

complex was performed with MMFF94s in the presence of GB/SA continuum water model on a Silicon Graphics Octane2 workstation running the IRIX 6.5 operating system by heating from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. For simulation of the RT·nucleoside-TP complex, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside 6 Å sphere from the bound nucleoside-TP were allowed to move freely.

Results and Discussion

Fluorine can often be used to isosterically replace a hydroxyl group because of its similar polarity to oxygen and the ability to be a hydrogen bond acceptor. Fluorine is also the third smallest element and can serve as a replacement for hydrogen on a methylene unit without a large steric change, possibly endowing novel electrostatic interactions to the modified compound such as hydrogen bonding interactions at the enzyme active site. The changes of the binding affinities of the cytidine analogs in response to 5-fluorination may be attributable to an interaction with Arg72 in the RT active site because the Arg72 is the only amino acid within 5 Å of the anticipated position of the 5-fluorine (similar to the position of the methyl unit of thymidine in the crystal structure of RT bound to TTP and primer/template) (Fig.11.2).³⁹⁹

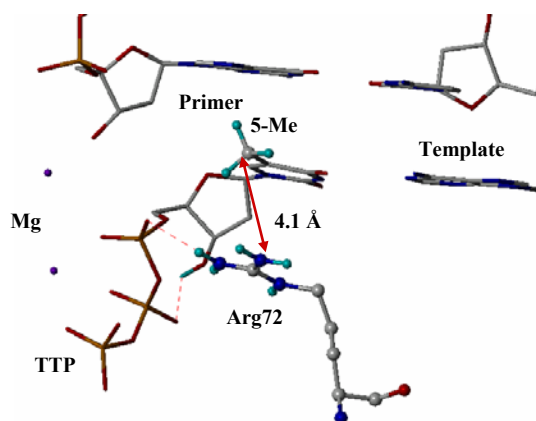


Figure 11.2. Crystal structure of the active site of RT.

The presence of three nitrogens in Arg72, one of which interacts with the triphosphate moiety of TTP by a hydrogen bond along with the fluorine's ability to be a hydrogen bond acceptor, may allow Arg72 to have an additional contact with the base moiety of the nucleoside analogue at the active site of RT. However, after molecular dynamics simulation, RT-FTCTP complex maintained almost the same conformation as that of RT-3TCTP because Arg72 was found to be interacting not with 5-fluorine atom but with the α -phosphate as well as 4'-oxygen atom in the oxathiolane sugar moiety (Fig. 11.3). By virtue of the unnatural L-sugar conformation, the 4'-oxygen atoms in both 3TCTP and FTCTP are located in close proximity to Arg72. Therefore, energetically, RT-FTCTP and RT-3TCTP were indistinguishable, which is consistent with the similar equilibrium binding constants of 3TCTP and FTCTP (Table 11.1). In spite of the lack of the kinetic data for the incorporation of D-dioxolane cytosine and the corresponding 5-fluoro analogue, the molecular dynamics study expects that the D-dioxolane 5-FC would be energetically favored over its cytosine analogue mainly due to the electrostatic interaction between 5-fluorine atom and ϵ -amino group of Arg72, which shows significant

conformational difference compared with the binding mode of D-dioxolane cytosine (Fig. 11.4). Unlike 3TC/FTC, the natural D-conformation of D-dioxolane nucleoside does not allow the interaction between the 3'-oxygen atom in the dioxolane sugar ring with Arg72, which enables Arg72 to readily donate hydrogen bonding to the fluorine atom at 5-position of the cytosine base (Fig. 11.4).

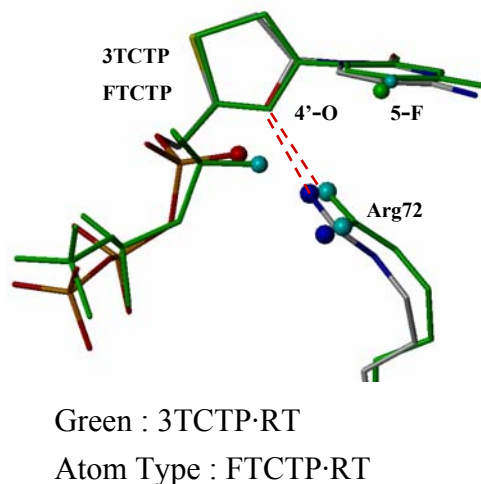


Figure 11.3. RT· FTCTP vs RT· 3TCTP.

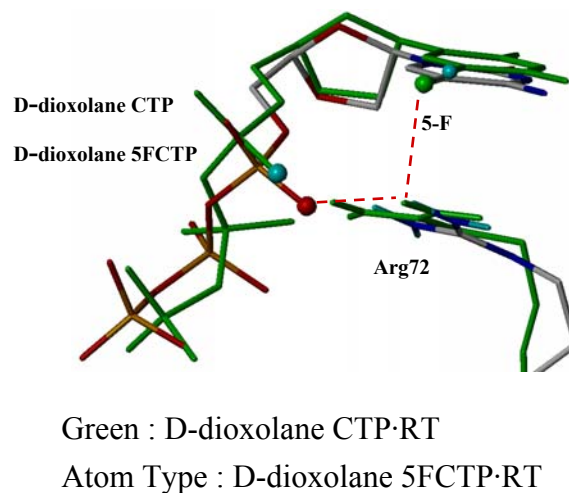


Figure 11.4. RT·D-dioxolane cytosine vs RT·D-dioxolane 5FC.

In the case of D-2'F-d4FC, Arg72 is involved in the electrostatic interaction with the 2'F substituent, instead of the fluorine atom at the 5-position. Therefore, compared with the 3TC/FTC binding, Arg72 is located deep inside of the active site (Fig. 11.5).

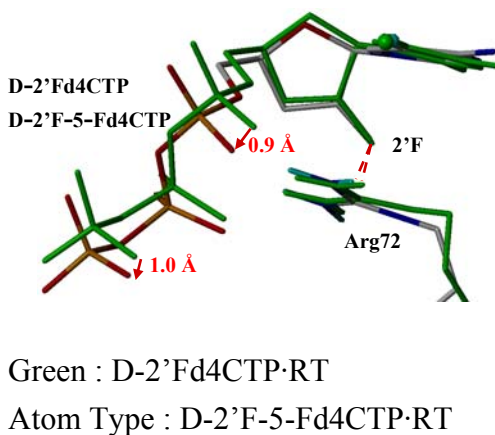
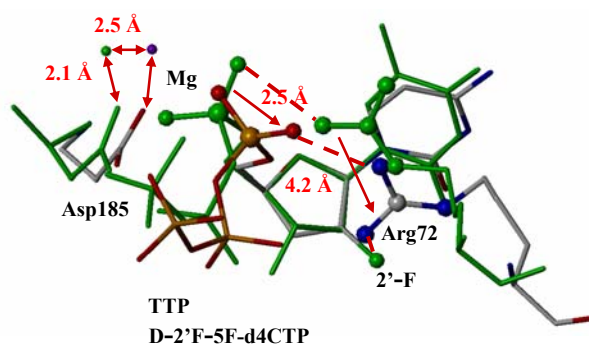


Figure 11.5. RT· D-2'Fd4CTP vs RT· D-2'F-d4FC.

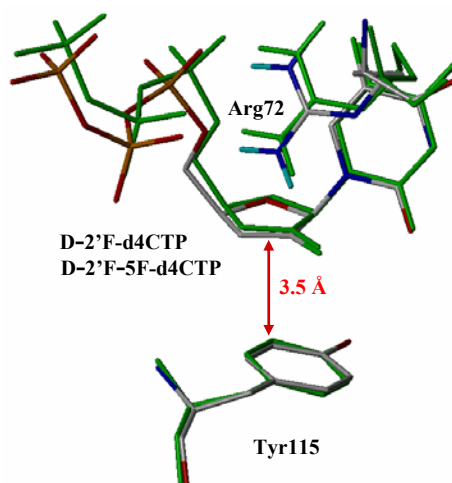
As a result, the conformation of D-2'F-d4C/D-2'F-d4FC triphosphate is quite different from that of 3TC/FTC triphosphate in which the α phosphate is hydrogen bonded to Arg72. As a result of this conformational change, one of the two Mg atoms shifts outside of the YMDD motif, resulting in a distorted binding mode (Fig. 11.6) and thereby reduced binding affinity to the RT. However, the unfavorable effect of 2'-fluorine substitution at the 2',3'-unsaturated nucleosides can be readily compensated by the hydrophobic interaction of the 2',3'-double bond with Tyr115 which forms a bottom of the so called '3'-OH pocket' (Fig. 11.7). Tyr115 is known to exclude the binding of RNA nucleotides by sterically disallowing the presence of a 2'-hydroxyl and this has lead it to be called the "steric gate" of RT.⁴⁰²



Green : TTP/ RT

Atom Type : D-2'F-d4CTP·RT

Figure 11.6. Conformational change at the active site.



Green : D-2'F-d4CTP·RT

Atom Type : D-2'F-5F-d4CTP·RT

Figure 11.7. Hydrophobic interaction.

However, according to our modeling studies, the Tyr115 is positively contributing for the binding of 2'-F-d4 compound through π - π interaction between the aromatic ring of the Tyr115 residue and the double bond of the fluorovinyl moiety of 2'-F-d4N nucleosides

(~3.4 Å) (Fig. 11.7). In our previous study,⁴⁰³ this kind of interaction was found in almost every d4 nucleosides, and the electronegative 2'-fluoro substitution strengthens this interaction. In addition, the previously noted increased stability imparted to nucleoside analogs by 2'-substitutions⁴⁰⁴ may outweigh the negative effects.

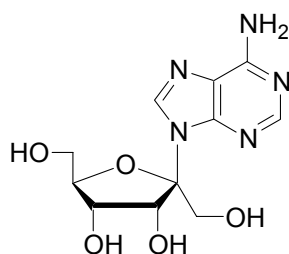
In summary, by molecular dynamics studies, we have found that formation of a hydrogen bond between fluorine-substituted nucleoside analogues and Arg72 may provide additional favorable contact within the active site of RT. However, as Arg72 is heavily involved in the stabilization of the triphosphate moiety of the bound nucleoside triphosphate, its interaction with the 5-fluorine atom on the cytosine ring was observed only in a limited case (D-dioxolane-5FCTP).

CHAPTER 12

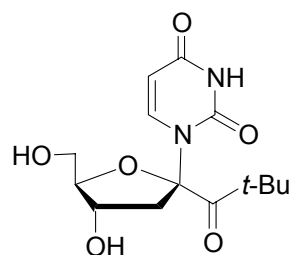
ATTEMPTED SYNTHESIS OF 1'-FLUORO RIBONUCLEOSIDE

Introduction

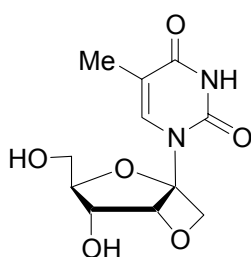
Even though several 1'-substituted nucleoside analogues such as the antibiotic angustmycin C,⁴⁰⁵ 2'-deoxy-1'-pivaloyluridine⁴⁰⁶ and the conformationally locked 1'-branched nucleoside derivative⁴⁰⁷ (Figure 12.1) proved their biological importance, syntheses of nucleoside analogues substituted at the anomeric 1'-position are not as common as syntheses of 2'- and 3'-substituted nucleoside analogues.



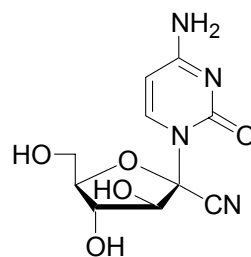
Angustmycin C



2'-deoxy-1'-pivaloyluridine



(3'-endo)-locked 1'-branched thymidine



1'-Cyano-arabinofuranosyl cytosine

Figure 12.1. Examples of biologically important 1'-substituted nucleosides.

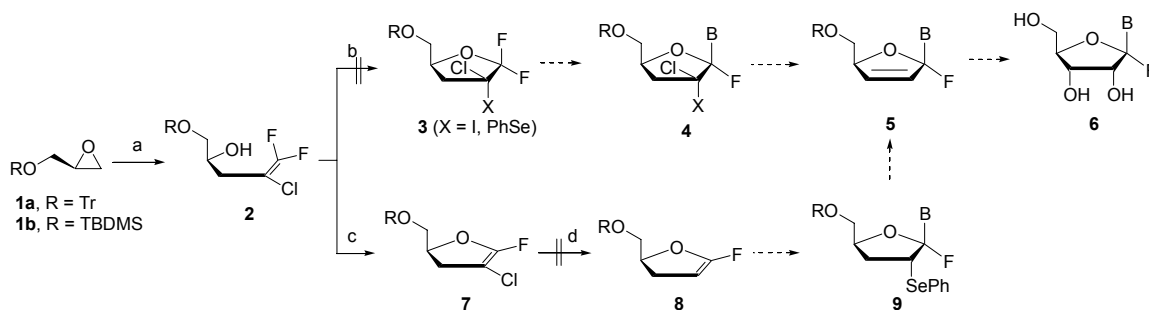
Furthermore, most efforts in this area have been driven to the syntheses of 1'-branched nucleosides which have both a heterocyclic base and a carbon substituent at the anomeric position of ribose or 2-deoxy-ribose.⁴⁰⁸ Because of their resistance to hydrolysis, those C-

glycosides are expected to be stable mimics for natural *O*-glycosides exhibiting biological activity.⁴⁰⁹ Moreover, nucleoside with an electron withdrawing group at the 1'-position, 2'-ara-1'-cyano cytidine analogue (Figure 12.1), was found to be stable against chemical as well as enzymatic hydrolysis to exhibit potent antitumor activity.⁴¹⁰ Therefore, in view of achieving stability of ribonucleoside against hydrolytic cleavage of the glycosyl bond, it is of interest to introduce other electronegative group such as fluorine atom at the 1'-position. The introduction of fluorine atom into an organic molecule has been widely carried out in the fields of medicinal chemistry, because it causes a dramatic enhancement in biological activity. The change is mainly due to the high electronegativity of the fluorine atom, the strong carbon-fluorine bond and increased lipophilicity. However, to our knowledge, preparation of a quaternary carbon center substituted with oxygen, nitrogen and fluorine atom at the same time has never been reported. Herein, we report our extensive search for the synthetic method to prepare the hereto unknown 1'-fluoro ribonucleoside.

Results and Discussion

Even though available synthetic methods allow the introduction of various nucleobases, there are considerable limitations in the diversity of the anomeric substitution. This can be attributed to the fact that these methods necessitates starting with sugar precursors, which have to be converted through a lengthy reaction sequences to the requisite 1-fluoro substituted derivatives before being condensed with nucleobases. Thus, we have been

exploring the stereoselective synthesis of the target nucleoside by addition of selenium electrophile to 1-fluoro furanoid glycal. After condensation, the 2'-phenylseleno group was believed to undergo facile oxidative elimination to afford the corresponding d4 nucleoside, which can be easily converted to the 1-fluoro ribonucleoside by dihydroxylation (Scheme 12.1).⁴¹¹

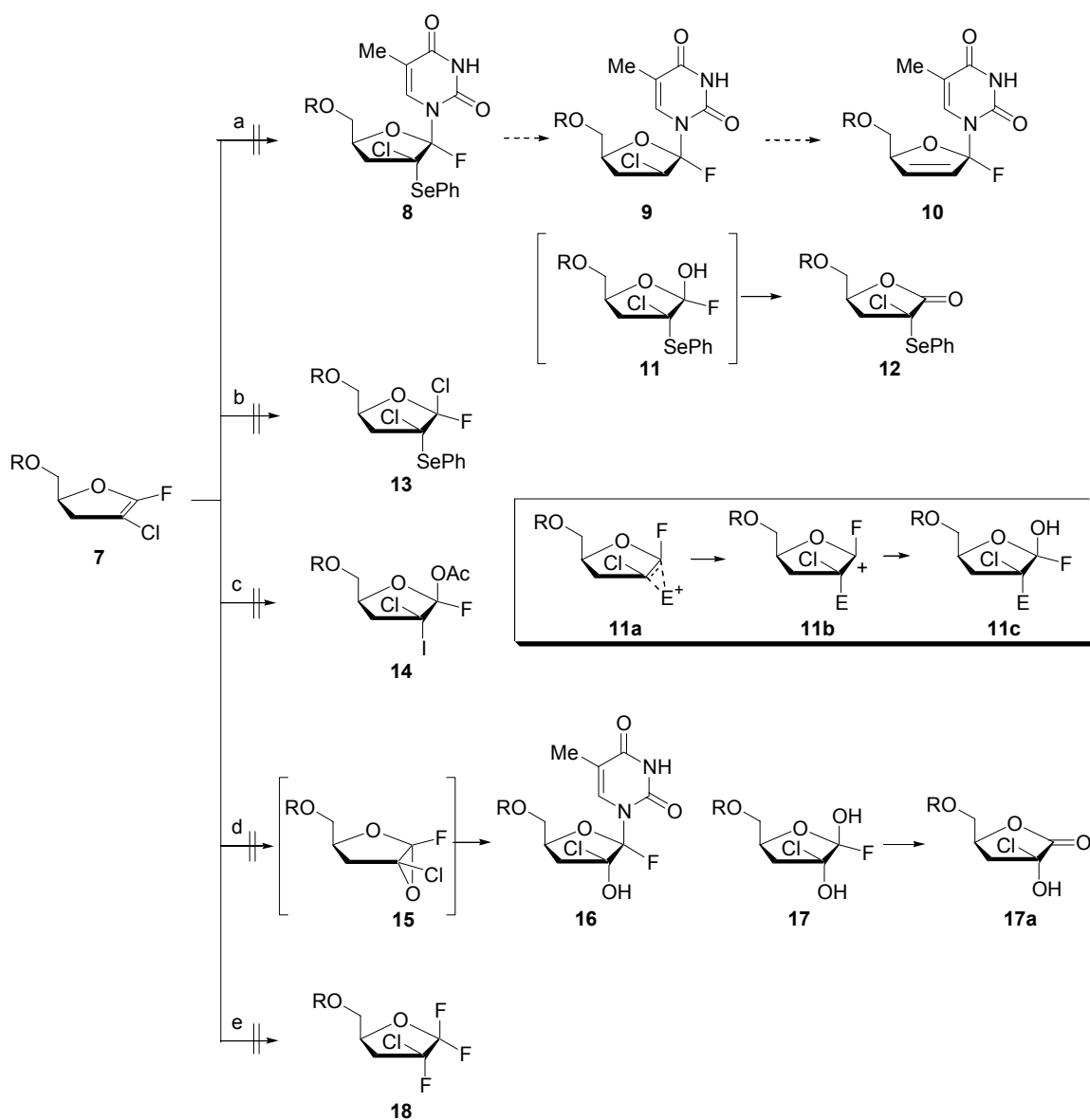


^aKeys: (a) 2-Bromo-2-chloro-1,1,1-trifluoroethane, nBuLi, Et₂O, -78°C; **1**, BF₃·OEt₂, (b) i) I₂, NaHCO₃, CH₂Cl₂, ii) PhSeCl, AgOTf, Et₂O, iii) NaH, Et₂O; PhSeCl, AgOTf, (c) xs. NaH, THF, reflux, (d) i) ^tBuLi, Et₂O, -78°C, ii) nBu₃SnH, AIBN, toluene, reflux, iii) nBuLi, Et₂O, -78°C, iv) Zn, AcOH, heat.

Scheme 12.1. Preparation of the key intermediate **7**.

The preparation of the key intermediate, 1-fluoro-2-chloro furanoid glycal **7** was reported by Coe et al.,⁴¹² but their procedure necessitated the use of 1-chloro-2,2,2-trifluoroethane gas, which is not commercially available for laboratory use. However, this problem was solved by use of 1-bromo-1-chloro-2,2,2-trifluoroethane, which provided the desired 1-chloro-2,2-difluorovinyl lithium by the treatment with nBuLi (Scheme 12.1). To the THF solution of 1-chloro-2,2-difluorovinyl lithium, thus obtained, the commercially available (*S*)-(-)-glycidyl trityl ether **1a** was added followed by dropwise addition of BF₃·OEt₂ to give difluorochlorovinyl alcohol **2**. Iodoetherification of **2** to 1,1-difluorofuranosine **3** did not proceed at all, but the treatment of **2** with excess NaH in refluxing THF provided the

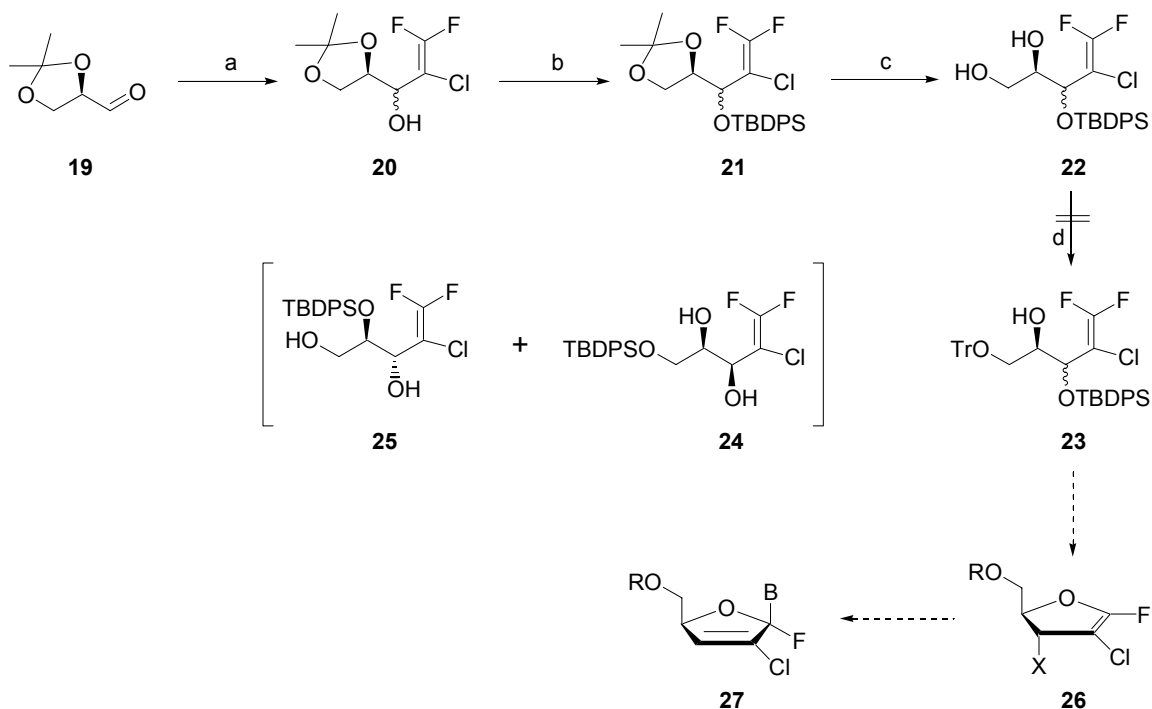
key intermediate **7** in moderate yield (48%). In order to reduce the chlorine atom substituted at 2-position of the furanoid glycal **7**, a number of different reduction conditions were tested without success.^{413,414,415} As the chlorine atom can be selectively reduced after condensation with heterocyclic base, various electrophilic addition to the 1,2-double bond of the glycal **7** were performed (Scheme 12.2). When **7** was reacted with PhSeCl and persilylated thymine in the presence of AgOTf,⁴¹⁶ TLC analysis of the reaction mixture showed complete conversion of **7** to a product, which was assumed to be a mixture of the adducts **8**. However, this product appeared to be rather unstable, giving a complex mixture upon attempted aqueous work-up followed by purification by silica gel column chromatography, from which only **12** was isolated after hydrolysis. The formation of **12** can be explained, at least in part, by assuming an α -anion-stabilizing effect of the selenium atom,⁴¹⁷ which facilitates elimination of thymine from the initially formed adduct. Chlorophenylselenylation⁴¹⁸ as well as acetoxyiodination⁴¹⁹ of **7**, by using PhSeCl and AgOTf in Et₂O, and NIS and AcOH in THF, respectively, again formed a mixture of unstable adducts, which decomposed to **12** during purification. The observed formation of the lactone derivative **12** indicated that the oxonium ion is highly susceptible to nucleophilic attack, and consequently water had served as a nucleophile instead of the weak nucleophiles such as persilylated thymine, chlorine and acetate anion. By the same token, 1,2-epoxy-1-fluoro-2-chloro furanoside **15**, obtained from **7** by the treatment with dimethyldioxirane,⁴²⁰ was also transformed to the corresponding lactone **17a** instead of the desired nucleoside analogue **16** upon reaction with the persilylated thymine in the presence of TMSOTf.⁴²¹ The attempted difluorination of **7** with PhTeF₅ in CH₂Cl₂ resulted in the formation of complex mixtures.⁴²²



^aKeys: (a) i) Thymine, HMDS/CH₃CN, reflux; **7**, PhSeCl, AgOTf (*TMSOTf*, *SnCl*₄, *HgCl*₂), Et₂O (CH₃CN, CH₂Cl₂), ii) NaH, Thymine, THF, 0°C; **7**, PhSeCl, HgCl₂, CH₃CN, (b) PhSeCl, AgOTf, Et₂O, (c) NIS, AcOH, THF, -20°C to rt, (d) Dimethyldioxirane, acetone, rt; silylated thymine, TMSOTf, CH₃CN, (e) PhTeF₅, CH₂Cl₂

Scheme 12.2. Electrophilic substitution reaction of the key intermediate **7**.

As electrophilic addition to the furanoid glycal **7** continuously resulted in the formation of the lactone derivative, a S_N2'-type nucleophilic addition of the heterocyclic base to the glycal with a leaving group substituted at 3-position **26** was conceivable (Scheme 12.3).



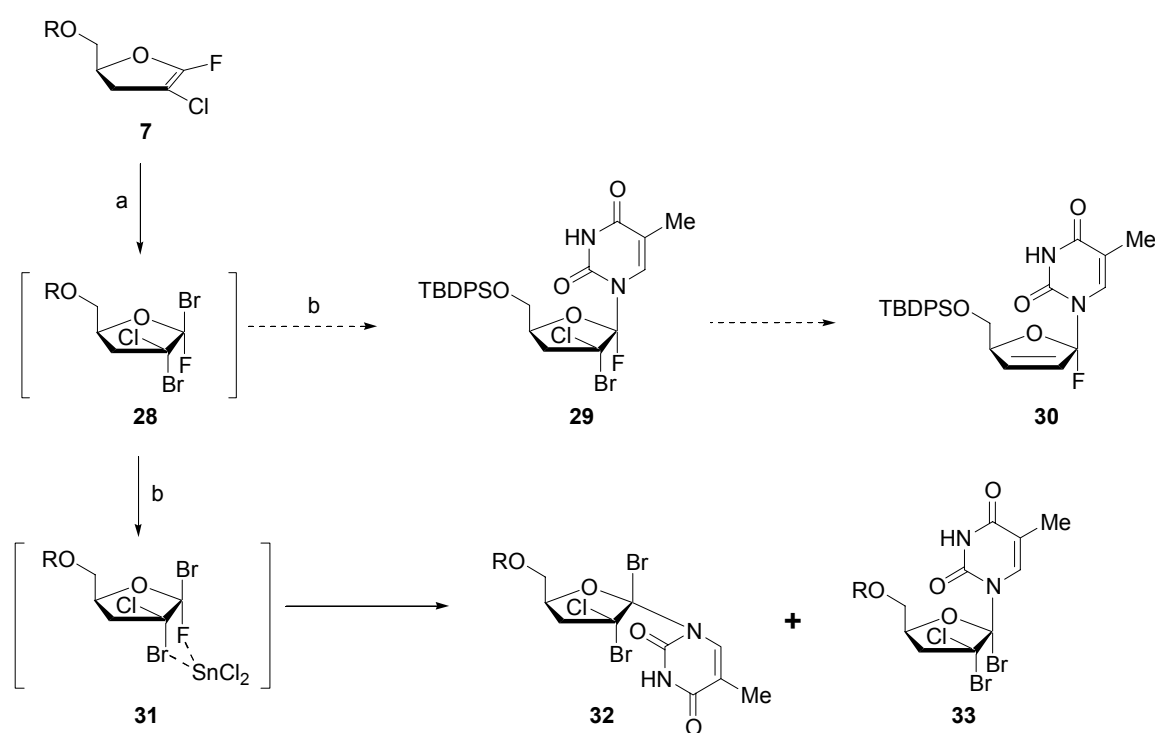
^aKeys: (a) 2-Bromo-2-chloro-1,1,1-trifluoroethane, nBuLi, Et₂O, -78°C; **19**, BF₃·OEt₂, (b) TBDPSCl, Imidazole, TEA, CH₂Cl₂, (c) p-TsOH, MeOH, (d) TrCl, DMAP, DMF

Scheme 12.3. Attempted preparation of 3-substituted furanoid glycol **26**.

Thus, the isopropylidene glycerinaldehydes **19** was reacted with 1-chloro-2,2-difluorovinyl lithium to provide a diastereomeric mixture **20**, which was protected with TBDPS group to give **21**. After deprotection of the isopropylidene group of **21**, the resulting primary hydroxyl group was attempted to be selectively protected by trityl group. However, under the reaction conditions, instead of the monotritylation, the TBDPS group migrated into the neighboring primary as well as secondary hydroxyl group to give a mixture of **24** and **25**. Interestingly, this migration was highly stereoselective to give diastereomerically pure **24** and **25**. Unfortunately, attempts to fully

deprotect **24** and **25** to the corresponding triol by using TBAF in THF resulted in decomposition of the starting material.

In contrast to the unsuccessful difluorination, dibromination of **7** by using bromine in CH_2Cl_2 underwent smoothly to give an unstable mixed-halide **28**, which was condensed with persilylated thymine in the presence of SnCl_4 in refluxing CH_3CN to give an anomeric mixture of a nucleoside analogue, **32** and **33** (Scheme 12.4).⁴²³



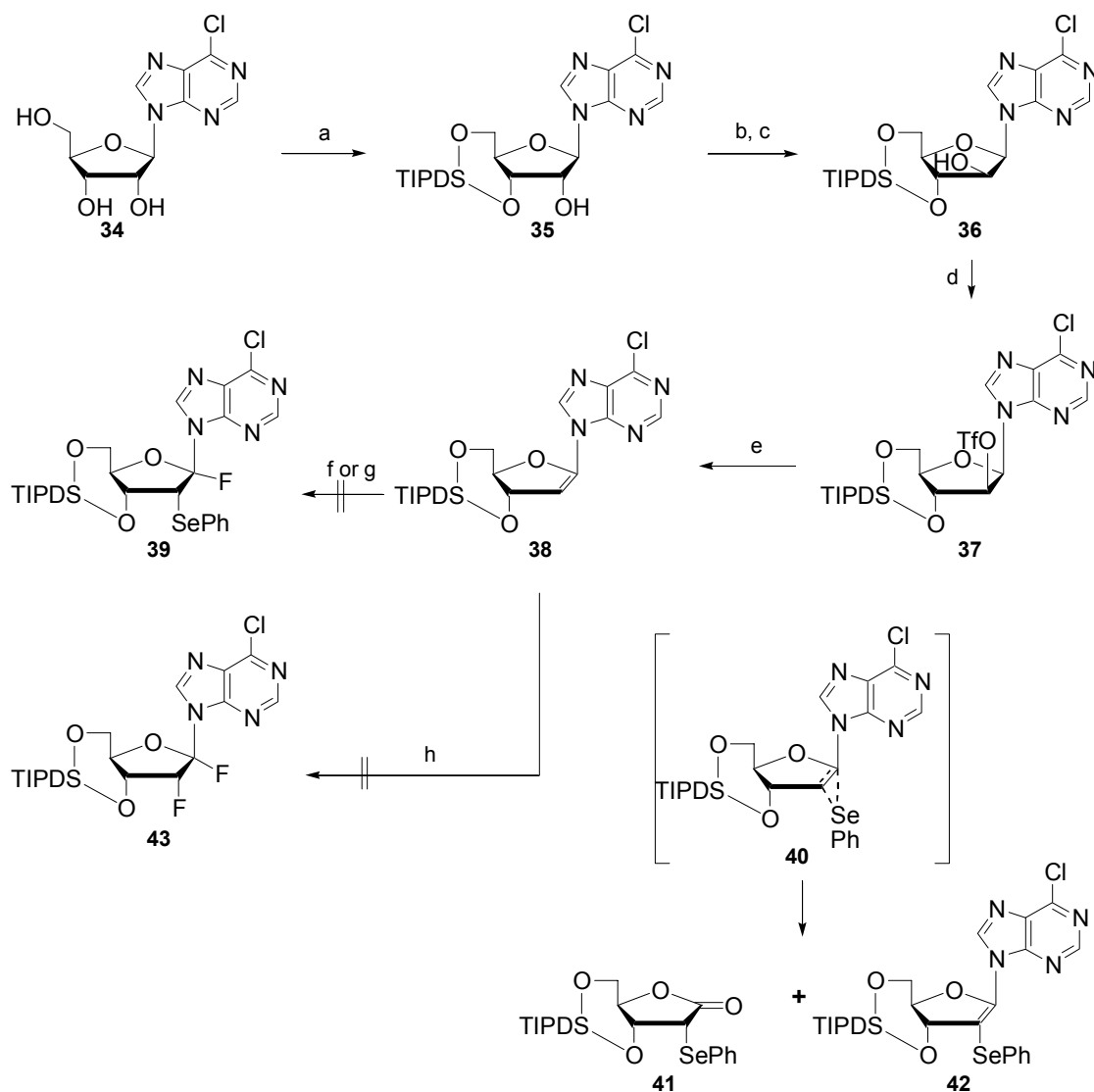
^aKeys: (a) Br_2 , CH_2Cl_2 , 0°C , (b) Silylated thymine, SnCl_4 (*TMSOTf*), CH_3CN , 80°C

Scheme 12.4. Condensation of the mixed halide **28** with persilylated thymine.

^1H NMR of **32** and **33** show no 1' and 2' protons. However, to our disappointment, the ^{13}C NMR showed no fluorine coupling, which suggested that, instead of C-Br bond, the

stronger C-F bond was cleaved by SnCl₄ during the condensation reaction. Presumably, tin chloride chelates neighboring bromide and fluoride to activate the anomeric fluoride instead of bromide (**31**). To circumvent this unexpected loss of fluorine during condensation, other Lewis acid such as TMSOTf was used, but the starting material decomposed under the reaction conditions.

Introduction of fluorine atom to the 1',2'-unsaturated nucleoside can be another way to synthesize the target compound. Thus, 6-chloropurine riboside **34** was converted to the 1',2'-unsaturated 6-chloropurine analogue **38** according to the known procedure by Kittaka et al. (Scheme 12.5).⁴²⁴ However, fluorophenylselenylation⁴²⁵ of **38** unequivocally resulted in a formation of a mixture of a lactone derivative **41** and 2'-phenylseleno-1',2'-unsaturated 6-chloropurine derivative **42**, which indicated that the addition of the phenylselenium and fluorine does not occur at the same time, but the oxonium ion formed after addition of phenylselenide is highly susceptible to hydrolysis presumably due to the low nucleophilicity of the fluorine atom. α -Fluorination of the 2'-keto uridine **45** prepared from uridine **43** in two steps⁴²⁶ was tested (Scheme 12.6). Thus, after deprotonation of the 1'-proton of **45** with LiHMDS in THF at -78°C, the resulting lithium enolate **46** was treated with two different fluorinating agents, *N*-fluorobenzenesulfonimide⁴²⁷ and Selectfluor^{®428}. However, the nucleophilic fluorination did not proceed presumably because of the low electrophilicity of the fluorinating agents. The lithium enolate **46** was then trapped as silyl enol ether by treatment with TMSCl or TBDMSCl (Scheme 12.7). TMS-enol ether **46**, however, was not stable enough to be isolated or be used for the next step without purification.

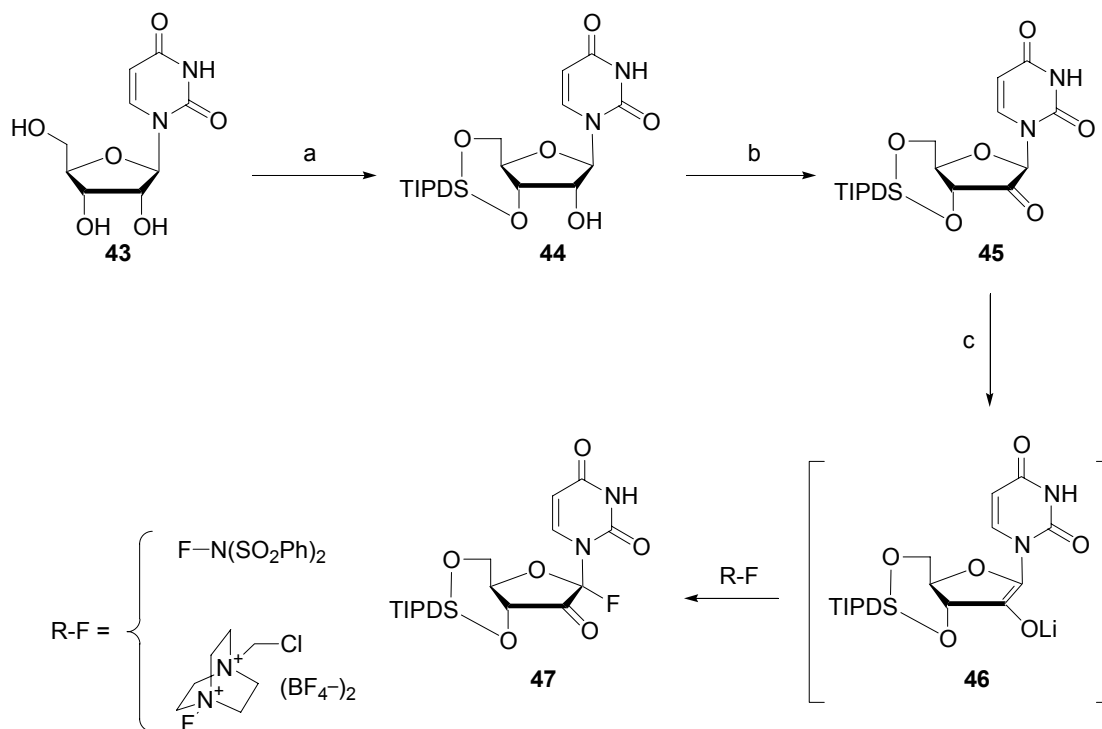


^aKeys: (a) TIPDSCl₂, Pyr, 0°C, (b) DMSO, Tf₂O, CH₂Cl₂, -78°C; TEA, (c) NaBH₄, MeOH, (d) Tf₂O, DMAP, TEA, CH₂Cl₂, (e) DBU, DMF, (f) PhSeCl, AgF, CH₃CN, (g) PhSeBr, AgF, CH₂Cl₂, sonication, (h) XeF₂, CH₂Cl₂

Scheme 12.5. Attempted fluorination of 1',2'-unsaturated purine nucleoside **38**.

The crude ¹H NMR shows 3:2 ratio of the 2'-keto uridine **45** and the corresponding TMS-enol ether **46**. Thus, the enolate **46** was trapped as a TBDMS-enol ether **47**, which was stable enough to be purified by silica gel column chromatography. When the TBDMS-enol ether **47** was reacted with *N*-fluoropyridinium salt⁴²⁹ in refluxing CH₂Cl₂

for 12h, no fluorination at 1'-position was observed, but a furan derivative **48** was formed in good yield after loss of 4'-proton followed by elimination.

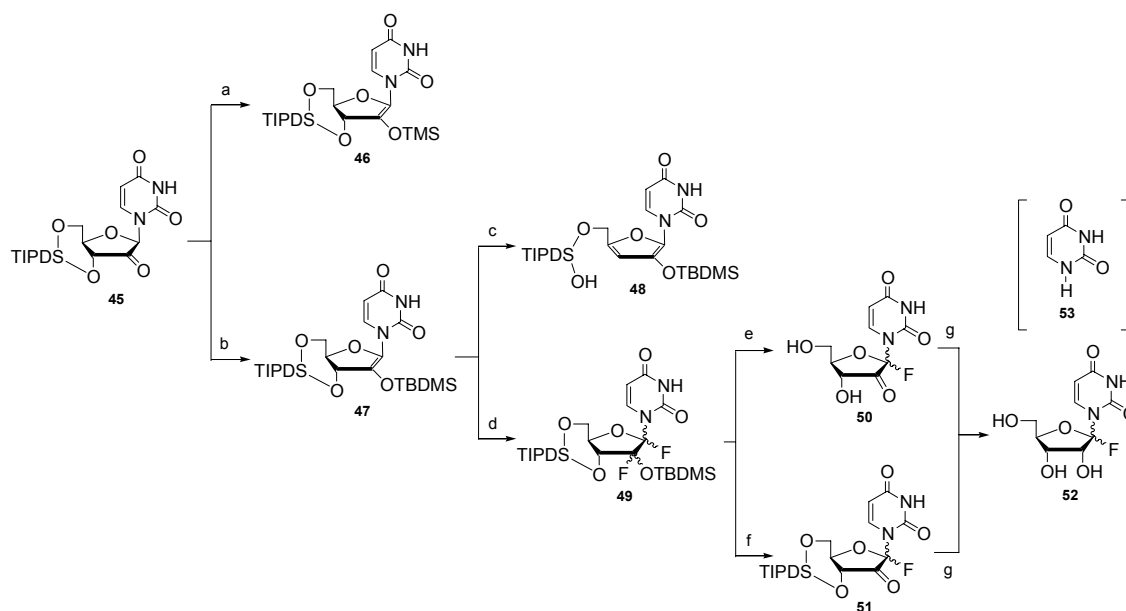


^aKeys: (a) TIPDSCl₂, Pyr, 0°C, (b) PDC, Ac₂O, CH₂Cl₂, reflux, (c) LiHMDS, THF, -78°C

Scheme 12.6. Attempted fluorination of 2'-keto uridine **45**.

However, when compound **47** was treated with more powerful fluorine source, XeF₂,⁴³⁰ under mild conditions, a difluorinated compound **49** was obtained as a complex mixtures. Three different isomers of **49** were separable by column chromatography, and they showed characteristic splitting patterns of vicinal difluoro derivatives in ¹³C NMR, but their absolute stereochemistry remains to be determined. It is interesting that Si-O bond was not cleaved to give 1'-fluoro 2'-keto uridine analogue under the reaction conditions.

Thus, selective deprotection of the 2'-TBDMS ether to give the corresponding 2'-keto moiety was attempted under various acidic conditions,⁴³¹ but it remained unchanged. Instead, 5'-monodeprotection was observed. When TBAF was used for deprotection of the silyl protection groups, after a few minutes a very polar compound, which is believed to be compound **50** was detected on TLC. However, this compound quickly decomposed upon longer reaction time or concentration for isolation to give a deglycosylation product, uracil **53**. Deprotection reaction under mild acidic conditions such as TBAF and AcOH in THF or 0.2 N HCl and HF·TEA in THF preceded slowly and the reaction product remained stable for some time, but it was not isolatable. Whenever the reaction mixture was concentrated for isolation, the compound quickly decomposed to uracil.



^aKeys: (a) LiHMDS, THF, -78°C; TMSCl, (b) LiHMDS, THF, -78°C; TBDMSCl, (c) N-fluoro-2,4,6-trimethylpyridinium triflate, CH₂Cl₂, (d) XeF₂, CH₂Cl₂, (e) i) TBAF, THF (CH₃CN), ii) TBAF, AcOH, THF, iii) 0.2N HCl, HF·TEA, (f) i) PPTS, MeOH, ii) p-TsOH, MeOH, iii) TFA, THF/CH₂Cl₂, (g) i) NaBH₄, MeOH, ii) NaBH(OAc)₃, AcOH, CH₃CN.

Scheme 12.7. Fluorination of silyl enol ether **47**.

As the compound **50** was believed to be unstable under basic conditions, it was directly reduced to the final compound **52** without isolation. Thus, compound **49** was dissolved in 5% AcOH in CH₃CN and treated with TBAF, and the reaction was monitored by TLC. After stirring for 2 hours at room temperature, the reaction mixture was cooled to 0°C and treated with NaBH(OAc)₃. The analysis of TLC after 10 minutes showed clear change, but upon filtration of the reaction mixture through a short silica gel pad, the reaction mixture quickly decomposed to uracil. Several different deprotection conditions of silyl ethers are undergoing, but, at this point, it is not clear if the final compound itself is not stable or it is not stable under the reaction conditions.

In summary, various reaction conditions have been tested to synthesize the hereto unknown 1'-fluoro ribonucleoside. In electrophilic addition to 1-fluoro furanoid glycal as well as 1',2'-unsaturated nucleoside, lactone derivatives usually formed after hydrolysis. Fluorination of TBDMS-enol ether of 2'-keto uridine analogue by using XeF₂ as a fluorine source provided a promising intermediate, but the final conversion to the target nucleoside was hampered by basic reaction conditions.

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 ¹H NMR and 100MHz for ¹³C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q

(quartet), m (multiplet), or br s (broad singlet). 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 silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(-)-1-(Triphenylmethyloxy)-4-chloro-5,5-difluoro-pent-4-en-2-ol (2) A solution of 2-bromo-2-chloro-1,1,1-trifluoroethane (0.17 ml, 1.58 mmol) in dry ether (15 ml) was treated with nBuLi (2.5M solution in hexane, 1.58 ml, 3.95 mmol) at -78°C and the mixture was stirred for 1h at that temperature. Solid (S)-(-)-glycidyl trityl ether (500 mg, 1.58 mmol) was added to the reaction mixture, which was then treated with BF₃·OEt₂ (0.2 ml, 1.58 mmol) in a dropwise fashion. The reaction mixture was slowly warmed to rt over 3h and then quenched with aq. NaHCO₃ solution. The aqueous phase was extracted three times with ether and the combined organic layers were dried over MgSO₄, filtered and concentrated to give a pale yellow syrup, which was purified by column chromatography on silica gel (10:1 Hexane:Ether) to give 540 mg (1.30 mmol, 82% yield) of **2** as a colorless oil.: [α]_D²⁴ -7.45° (c 0.21, CHCl₃); ¹H NMR (CDCl₃) δ 7.56-7.31 (m, 15H), 4.16-4.05 (br s, 1H), 3.38-3.32 (m, 1H), 3.28-3.22 (m, 1H), 2.66-2.53 (m, 2H), 2.48-2.40 (m, 1H); ¹³C NMR (CDCl₃) δ 155.0 (t, *J* = 286.7 Hz), 144.0, 129.0, 128.3, 127.6, 89.7 (dd, *J* = 44.1, 20.1 Hz), 87.2, 68.3, 66.9, 34.6; Anal. Calcd for C₂₄H₂₁ClF₂O₂·0.3Hexane: C, 70.31; H, 5.76 Found: C, 70.49; H, 5.39

(+)-tert-Butyl-(4-chloro-5-fluoro-2,3-dihydro-furan-2-ylmethoxy)-diphenyl-silane (7)
A mixture of **2** (440 mg, 1.06 mmol) and NaH (60% in mineral oil, 300 mg, 7.42 mmol)

in anhydrous THF (15 ml) was heated under reflux for 8h. The reaction mixture was cooled and ice was carefully added. The organic layer and ether extracts (4x20ml) of the aqueous layer were combined, dried over MgSO₄ and the solvent was removed to leave an oil, which was purified by column chromatography on silica gel (100:1 Hexane:Ether) to give 200 mg (0.51 mmol, 48% yield) of **7** as a colorless syrup.: $[\alpha]_D^{24}$ 52.9° (c 0.46, CHCl₃); ¹H NMR (CDCl₃) δ 7.60-7.24 (m, 15H), 4.82-4.75 (m, 1H), 3.41-3.38 (m, 1H), 3.35-3.29 (m, 1H), 2.92-2.84 (m, 1H), 2.72-2.65 (m, 1H); ¹³C NMR (CDCl₃) δ 155.5 (d, 262.5 Hz), 144.0, 128.5, 128.2, 127.8, 87.0, 78.2, 74.2 (d, *J* = 19.5 Hz), 65.9, 34.1: Anal. Calcd for C₂₄H₂₀ClFO₂: C, 73.00; H, 5.11 Found: C, 73.20; H, 5.13

5-(*tert*-Butyldiphenylsilyloxymethyl)-3-chloro-3-phenylselanyl-dihydro-furan-2-one (12) A solution of furanoid glycal **7** (200 mg, 0.51 mmol) in dry ether (5 ml) at rt, kept under argon and protected from light, was treated with PhSeCl (194 mg, 1.01 mmol). After 5 min, the silylated thymine (160 mg, 1.27 mmol) and AgOTf (260 mg, 1.01 mmol) were added. The reaction mixture was stirred for 5h at rt. Aqueous NaHCO₃ solution was added and the aqueous phase was extracted three times with ether. The combined organic layers were dried over MgSO₄, filtered and concentrated to give a yellow oil, which was purified by column chromatography to give 135 mg of **12** as a yellow syrup: ¹H NMR (CDCl₃) δ 7.77-7.30 (m, 10H), 4.53-4.47 (m, 1H), 3.89 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.69 (dd, *J* = 11.7, 3.9 Hz, 1H), 2.97 (dd, *J* = 13.7, 9.8 Hz, 1H), 2.72 (dd, *J* = 13.7, 5.9 Hz, 1H), 1.03 (s, 9H).

5-(*tert*-Butyldiphenylsilyloxymethyl)-3-chloro-3-hydroxy-dihydro-furan-2-one (17a) To a stirred solution of **7** (100 mg, 0.26 mmol) in 5 ml acetone was added a solution of 0.06M dimethyldioxirane in acetone (4.7 ml, 0.28 mmol) at rt. After 5min,

removal of the solvent on a rotary evaporator gave colorless oil, which was used for the next step without purification. To the freshly prepared silylated thymine (97 mg, 0.77 mmol), a solution of the epoxide prepared above in dry CH₃CN (10 ml) was added. Upon cooling to -40°C, TMSOTf (0.06 ml, 0.31 mmol) was added dropwise, and the reaction mixture was warmed to rt. After stirring for 2h at rt, aq. NaHCO₃ was added, and the aqueous phase was extracted three times with EtOAc, dried over Na₂SO₄, filtered and concentrated to give an oil, which was purified by column chromatography on silica gel (4:1 Hexane:EtOAc) to give **17a** as a yellow oil.: ¹H NMR (CD₃COCD₃) δ 7.73-7.69 (m, 4H), 7.49-7.42 (m, 6H), 5.08-5.06 (m, 1H), 4.01 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.80 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.22 (dd, *J* = 18.5, 7.8 Hz, 1H), 2.98 (dd, *J* = 18.5, 2.0 Hz, 1H), 1.03 (s, 9H); ¹³C NMR (CD₃COCD₃) δ 168.9, 144.3, 136.0, 130.1, 128.1, 115.6, 78.9, 64.4, 19.1.

2-Chloro-1-(2,2-dimethyl-[1,3]dioxolan-4-yl)-3,3-difluoro-prop-2-en-1-ol (20) A solution of 2-bromo-2-chloro-1,1,1-trifluoroethane (0.91 ml, 4.61 mmol) in dry ether (15 ml) was treated with nBuLi (2.5M solution in hexane, 4.6 ml, 11.5 mmol) at -78°C and the mixture was stirred for 1h at that temperature. A solution of glyceraldehydes isopropylidene (500 mg, 3.84 mmol) in ether (5 ml) was added to the reaction mixture, which was then treated with BF₃·OEt₂ (0.54 ml, 4.23 mmol) in a dropwise fashion. The reaction mixture was slowly warmed to rt over 3h and then quenched with aq. NaHCO₃ solution. The aqueous phase was extracted three times with ether and the combined organic layers were dried over MgSO₄, filtered and concentrated to give a pale yellow syrup, which was purified by column chromatography on silica gel (6:1 Hexane:EtOAc) to give 200 mg (0.87 mmol, 23% yield) of **20** as a yellow oil.: For 1:1 mixture of

diastereomers; ^1H NMR (CD_3COCD_3) δ 4.96 (d, $J = 4.9$ Hz, 1H), 4.80 (d, $J = 4.9$ Hz, 1H), 4.50-4.45 (m, 1H), 4.39-4.34 (m, 1H), 4.28 (q, $J = 6.8$ Hz, 1H), 4.15-4.09 (m, 2H), 4.05 (dd, $J = 11.7, 6.8$ Hz, 1H), 4.02 (dd, $J = 8.8, 6.8$ Hz, 1H), 3.73 (dd, $J = 8.8, 6.8$ Hz, 1H), 1.37 (s, 3H), 1.33 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 154.7 (t, $J = 286.7$ Hz), 154.6 (t, $J = 287.7$ Hz), 109.9, 109.5, 94.8 (dd, $J = 39.3, 14.4$ Hz), 93.2 (dd, $J = 39.3, 16.3$ Hz), 77.5, 75.0, 68.6, 68.3, 66.8, 65.5, 26.5, 26.4, 24.9, 24.7; Anal. Calcd for $\text{C}_8\text{H}_{11}\text{ClF}_2\text{O}_3$: C, 42.03; H, 4.85 Found: C, 41.85; H, 4.77.

3-(*tert*-Butyldiphenylsilyloxy)-4-chloro-5,5-difluoro-pent-4-ene-1,2-diol (22) A solution of **20** (252 mg, 1.10 mmol) and imidazole (263 mg, 3.86 mmol) in dry CH_2Cl_2 (40 ml) was treated with TBDPSCl (0.42 ml, 1.65 mmol) at 0°C and the reaction mixture was stirred for 24h at rt. The reaction mixture was diluted with CH_2Cl_2 , washed with water twice, washed with brine, dried over MgSO_4 , filtered and concentrated to give **21**. Compound **21** was dissolved in MeOH and treated with catalytic amount of p-TsOH and the mixture was stirred for 2h at rt. TEA (1 ml) was added to the reaction mixture and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel to give **22** (265 mg, 0.62 mmol, 56% yield) as a colorless syrup.: For 1:1 mixture of diastereomers; ^1H NMR (CD_3COCD_3) δ 7.82-7.71 (m, 8H), 7.50-7.41 (m, 12H), 4.67-4.66 (m, 1H), 4.61-4.60 (m, 1H), 4.21 (d, $J = 4.9$ Hz, 1H), 4.12 (d, $J = 5.9$ Hz, 1H), 3.94-3.88 (m, 3H), 3.85-3.78 (m, 1H), 3.76-3.69 (m, 3H), 3.49-3.44 (m, 1H), 1.11 (s, 9H), 1.10 (s, 9H); Anal. Calcd for $\text{C}_{21}\text{H}_{25}\text{ClF}_2\text{O}_3\text{Si}$: C, 59.08; H, 5.90 Found: C, 59.42; H, 5.82.

1-(*tert*-Butyldiphenylsilyloxy)-4-chloro-5,5-difluoro-pent-4-ene-2,3-diol (24) and 2-(*tert*-Butyldiphenylsilyloxy)-4-chloro-5,5-difluoro-pent-4-ene-1,3-diol (25) A

solution of **22** (265 mg, 0.62 mmol) and DMAP (227 mg, 1.86 mmol) in dry DMF (15 ml) was treated with TrCl (173 mg, 0.62 mmol), and the mixture was stirred for 24h at rt. The reaction mixture was diluted with EtOAc and washed with water and brine, dried over Na₂SO₄, filtered and concentrated to give a yellow residue, which was purified by column chromatography on silica gel (10:1 Hexane:EtOAc) to give **24** and **25** as syrups.: For compound **24**; ¹H NMR (CD₃COCD₃) δ 7.76-7.72 (m, 4H), 7.47-7.44 (m, 6H), 4.74-4.73 (m, 1H), 4.69 (d, *J* = 4.9 Hz, 1H), 4.27 (d, *J* = 4.9 Hz, 1H), 3.92-3.88 (m, 1H), 3.82 (dd, *J* = 10.7, 4.9 Hz, 1H), 3.71 (dd, *J* = 10.7, 4.9 Hz, 1H), 1.05 (s, 9H); For compound **25**; ¹H NMR (CD₃COCD₃) δ 7.75-7.71 (m, 4H), 7.50-7.42 (m, 6H), 4.60 (dt, *J* = 8.8, 2.0 Hz, 1H), 4.14 (d, *J* = 5.9 Hz, 1H), 3.92-3.87 (m, 1H), 3.85-3.79 (m, 1H), 3.77-3.75 (m, 1H), 3.74-3.70 (m, 1H), 1.10 (s, 9H).

1-[5-*O*-(*tert*-butyldimethylsilanyloxymethyl)-1,2-dibromo-2-chloro-2,3-dideoxy- α -D-ribofuranosyl]-thymine (32) and 1-[5-*O*-(*tert*-butyldimethylsilanyloxymethyl)-1,2-dibromo-2-chloro-2,3-dideoxy- β -D-ribofuranosyl]-thymine (33) A solution of **7** (76 mg, 0.19 mmol) in CH₂Cl₂ was treated with bromine dropwise at 0°C until fail yellow color persisted. The reaction mixture was diluted with CH₂Cl₂, washed with water, saturated Na₂S₂O₃ solution and brine, dried over MgSO₄, filtered and concentrated to give a colorless oil, which was briefly filtered through a short silica gel column washing with a 80:1 Hexane:Ether. The filtrate was concentrated and used for the next step without further purifications. Compound **28** obtained above (107 mg, 0.19 mmol) was dissolved in dry CH₃CN (10 ml) and, to this mixture, freshly prepared persilylated thymine (122 mg, 0.97 mmol) and SnCl₄ (0.03 ml, 0.10 mmol) were added. The resulting mixture was stirred for 0.5h at 80°C. Aqueous NaHCO₃ solution was added and the

reaction mixture was extracted three times with EtOAc. The combined organic layers was washed with brine, dried over MgSO₄, filtered and concentrated to give a colorless oil, which was purified by column chromatography on silica gel (4:1 Hexane:EtOAc) to give 20 mg (0.04 mmol, 21% yield) of **32** and 41 mg (0.08 mmol, 42% yield) of **33** as white solids.: For compound **32**; ¹H NMR (CD₃COCD₃) δ 7.76-7.67 (m, 4H), 7.56-7.43 (m, 6H), 7.26 (s, 1H), 4.97-4.91 (m, 1H), 4.09 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.95 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.31 (dd, *J* = 13.7, 4.9 Hz, 1H), 3.10 (dd, *J* = 13.7, 9.8 Hz, 1H), 1.80 (s, 3H), 1.05 (s, 9H); Anal. Calcd for C₂₆H₂₉Br₂ClN₂O₄Si: C, 47.54; H, 4.45; N, 4.26 Found: C, 47.92; H, 4.22; N, 3.86. For compound **33**; ¹H NMR (CD₃COCD₃) δ 7.78-7.66 (m, 4H), 7.53-7.41 (m, 6H), 5.03-4.92 (m, 1H), 4.14 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.97 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.31 (dd, *J* = 14.6, 4.9 Hz, 1H), 3.14 (dd, *J* = 14.6, 9.8 Hz, 1H), 1.82 (s, 3H), 1.05 (s, 9H); ¹³C NMR (CD₃COCD₃) δ 164.0, 160.7, 149.7, 142.5, 135.7, 132.8, 130.3, 128.2, 109.4, 82.2, 68.6, 63.0, 46.7, 26.5, 19.2, 11.7. Anal. Calcd for C₂₆H₂₉Br₂ClN₂O₄Si: C, 47.54; H, 4.45; N, 4.26 Found: C, 47.15; H, 4.23; N, 4.55.

5,5,7,7-Tetraisopropyl-3-phenylselanyl-tetrahydro-1,4,6,8-tetraoxa-5,7-disilacyclopentacycloocten-2-one (41) and **6-Chloro-9-[2-deoxy-2-phenylseleno-3,5-*O*-(tetraisopropylidisiloxan-1,3-diyl)-D-erythro-pent-1-enofuranosyl]purine (42)** After AgF (79 mg, 0.63 mmol) in CH₃CN (10 ml) was stirred for 10 min, compound **38** (97 mg, 0.19 mmol) was introduced. PhSeCl (120 mg, 0.63 mmol) was added to the reaction mixture and stirred for 10 min at rt. The dark orange slurry was filtered through a short silica gel pad washing with EtOAc, and then the filtrate was concentrated and purified by column chromatography on silica gel (20:1 Hexane:EtOAc) to give **41** and **42** as pale yellow oil.: For compound **41**; ¹H NMR (CD₃COCD₃) δ 7.69-7.68 (m, 2H), 7.37-7.34 (m,

1H), 7.32-7.29 (m, 2H), 4.69 (t, $J = 7.8$ Hz, 1H), 4.35-4.30 (m, 1H), 4.13 (d, $J = 6.8$ Hz, 1H), 4.10 (dd, $J = 12.7, 2.9$ Hz, 1H), 4.02 (dd, $J = 12.7, 4.9$ Hz, 1H), 1.08-1.06 (m, 24H); ^{13}C NMR (CD_3COCD_3) δ 172.1, 136.7, 129.4, 129.2, 126.0, 82.6, 71.2, 61.6, 44.5, 17.7, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.9, 13.5, 13.2, 13.0; For compound **42**; UV(CH_2Cl_2) λ_{max} 265.5 nm; ^1H NMR (CD_3COCD_3) δ 8.79 (s, 1H), 8.24 (s, 1H), 7.41-7.40 (m, 2H), 7.20-7.16 (m, 3H), 5.39 (d, $J = 3.9$ Hz, 1H), 4.86-4.81 (m, 1H), 4.36 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.96 (t, $J = 11.7$ Hz, 1H), 1.10-1.01 (m, 24H); ^{13}C NMR (CD_3COCD_3) δ 153.0, 151.7, 148.8, 144.1, 130.5, 130.4, 129.3, 127.1, 96.3, 89.2, 80.2, 63.6, 17.5, 17.4, 17.3, 17.0, 16.9, 16.8, 16.8, 13.6, 13.4, 13.1, 12.4.

1-[3-(*tert*-Butyldimethylsilanyloxy)-5,5,7,7-tetraisopropyl-9,9a-dihydro-3aH-1,4,6,8-tetraoxa-5,7-disila-cyclopentacycloocten-2-yl]-1H-pyrimidine-2,4-dione (47)

Compound **45** (500 mg, 1.03 mmol) was dissolved in dry THF (30 ml) and cooled to -78°C under nitrogen. To this was added LiHMDS (1.0M in THF, 2.06 ml, 2.06 mmol), and the solution was stirred for 2 hrs. A solution of TBDMSCl (311 mg, 2.06 mmol) in THF (5 ml) was added, the solution was stirred for 4 hrs and allowed to warm to rt overnight. The reaction mixture was diluted with ether, washed with water, dried over Na_2SO_4 , filtered and concentrated to give a yellow suspension, which was briefly filtered through a short silica gel pad washing with a 3:1 Hexane:EtOAc. The filtrate was concentrated to give 480 mg (0.80 mmol, 78% yield) of **47** as a pale yellow syrup.: $[\alpha]_{\text{D}}^{26}$ 6.48° (c 0.35, CHCl_3); UV(CHCl_3) λ_{max} 245.0 nm; ^1H NMR (CD_3COCD_3) δ 10.31 (s, 1H), 7.47 (d, $J = 7.8$ Hz, 1H), 5.72 (d, $J = 8.8$ Hz, 1H), 5.20 (d, $J = 4.9$ Hz, 1H), 4.39-4.35 (m, 1H), 4.10 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.82 (t, $J = 11.7$ Hz, 1H), 1.12-1.05 (m, 24H), 0.88 (s, 9H), 0.17 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 168.2, 154.1,

149.7, 138.8, 133.4, 108.1, 90.0, 82.2, 69.2, 30.5, 22.6, 22.4, 22.4, 22.3, 22.2, 22.1, 22.0, 21.9, 19.0, 18.7, 18.4, 18.1, 0.8, 0.0; Anal. Calcd for C₂₇H₅₀N₂O₇Si₃: C, 54.14; H, 8.41; N, 4.68 Found: C, 53.91; H, 8.52; N, 4.59.

1-[3-(*tert*-Butyldimethylsilanyloxy)-2,3-difluoro-5,5,7,7-tetraisopropyl-tetrahydro-1,4,6,8-tetraoxa-5,7-disila-cyclopentacycloocten-2-yl]-1H-pyrimidine-2,4-dione (49)

A solution of **47** (354 mg, 0.59 mmol) in dry CH₂Cl₂ (15 ml) was treated with XeF₂ (330 mg, 1.95 mmol), and the mixture was stirred for 30 min at rt. The reaction mixture was diluted with CH₂Cl₂ and washed with water followed by brine. After drying over Na₂SO₄, the mixture was filtered and the filtrate was concentrated to give a pale yellow oil, which was purified by column chromatography on silica gel (6:1 Hexane:EtOAc) to give 120mg (0.19 mmol, 32% yield), 100 mg (0.16 mmol, 27% yield) and 50 mg (0.08 mmol, 14% yield) of isomers.: For the first isomer; UV(CHCl₃) λ_{max} 252.0 nm; ¹H NMR (CD₃COCD₃) δ 10.18 (br s, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 5.69 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.97 (dd, *J* = 13.7, 6.8 Hz, 1H), 4.20-4.09 (m, 3H), 1.14-1.06 (m, 24H), 0.82 (s, 9H), 0.24 (s, 3H), 0.16 (s, 3H); ¹³C NMR (CD₃COCD₃) δ 166.5, 152.9, 142.9 (d, *J* = 15.3 Hz), 119.8 (dd, *J* = 255.1, 25.9 Hz), 117.5 (dd, *J* = 248.4, 25.9 Hz), 106.1, 86.3, 79.7 (d, *J* = 20.1 Hz), 66.7, 29.1, 21.1, 20.9, 20.9, 20.9, 20.7, 20.6, 20.6, 20.5, 17.4, 17.0, 16.8, 16.6, 0.7, 0.2; Anal. Calcd for C₂₇H₅₀F₂N₂O₇Si₃: C, 50.91; H, 7.91; N, 4.40 Found: C, 51.29; H, 7.99; N, 4.11. For the second isomer; UV(CHCl₃) λ_{max} 253.5 nm; ¹H NMR (CD₃COCD₃) δ 9.99 (br s, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 5.54 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.38 (ddd, *J* = 19.5, 9.8, 3.9 Hz, 1H), 4.25-4.22 (m, 1H), 3.99-3.97 (m, 2H), 0.99-0.92 (m, 24H), 0.81 (s, 9H), 0.11 (s, 3H), 0.00 (s, 3H); ¹³C NMR (CD₃COCD₃) δ 162.6, 148.3, 140.5 (d, *J* = 7.7 Hz), 114.3 (dd, *J* = 250.3, 33.6 Hz), 113.5 (dd, *J* = 203.3, 33.6 Hz),

102.0, 81.5, 73.1 (dd, $J = 19.1, 2.9$ Hz), 61.5, 25.5, 17.1, 17.0, 16.9, 16.9, 16.9, 16.8, 16.6, 16.6, 13.6, 13.5, 13.2, 13.1, 12.8, 12.6; Anal. Calcd for $C_{27}H_{50}F_2N_2O_7Si_3$: C, 50.91; H, 7.91; N, 4.40 Found: C, 51.11; H, 8.14; N, 4.15. For the third isomer; UV($CHCl_3$) λ_{max} 253.0 nm; ^1H NMR (CD_3COCD_3) δ 9.97 (br s, 1H), 7.67 (d, $J = 8.8$ Hz, 1H), 5.43 (dd, $J = 7.8, 2.0$ Hz, 1H), 4.52-4.47 (m, 1H), 4.05-3.90 (m, 3H), 0.96-0.91 (m, 24H), 0.67 (s, 9H), 0.11 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 166.6, 152.3 (d, $J = 2.9$ Hz), 143.8, 118.3 (dd, $J = 257.9, 26.9$ Hz), 115.4 (dd, $J = 257.0, 33.6$ Hz), 105.2, 84.9, 74.5 (d, $J = 27.8$ Hz), 63.2, 29.0, 20.8, 20.7, 20.6, 20.6, 20.3, 20.3, 20.3, 20.1, 17.2, 17.2, 16.9, 16.6, 16.5, 16.5. Anal. Calcd for $C_{27}H_{50}F_2N_2O_7Si_3$: C, 50.91; H, 7.91; N, 4.40 Found: C, 51.11; H, 7.85; N, 4.33.

CHAPTER 13

CONCLUSION

Nucleoside analogues continue to be important drug regimens as part of antiviral therapy. However, because of the drug toxicity, and the lack of a durable response due to resistance, there is clearly a need for new compounds to cope with these drawbacks. For this purpose, we developed novel nucleoside analogues and several protocols for the molecular modeling studies which can provide a plausible mechanism of antiviral drug resistance conferred by mutation in HIV-1 reverse transcriptase (RT) as well as HBV polymerase.

Nucleoside-resistant isolates have been identified in patients receiving antiretroviral nucleoside drugs. The different resistance phenotypes seem to correlate with different sets of mutations in reverse transcriptase (RT), and the effect of individual set of mutations on resistance to a specific NRTI can only be presumed by kinetic studies and building up the enzyme active site by molecular modeling studies. However, the understanding how mutations affect RT structure and function, and the ensuing loss of potent antiviral activities of certain NRTIs have not been demonstrated in conjunction with their binding modes, which would provide invaluable insight into the design of more effective NRTIs active against the mutant RTs. Chapter 1 discusses our recent efforts to assess the structural adjustment resulting from mutations and the accompanying energetic consequences based on the assumption that mutation may either deform the active site conformation through structural realignment or destabilize inhibitor binding.

The importance of carbocyclic nucleosides as antiviral agents, as well as the potential advantage of fluorine substitution, prompted us to combine these two features into novel carbocyclic 4'-fluoro-2',3'-dideoxy nucleosides. In Chapter 2, we have developed a synthetic method stereoselectively introducing tertiary fluoride group and a *de novo*

synthesis of key intermediates, α - and β -cyclopentanol. Condensation of these intermediates with protected purine and pyrimidine bases, followed by ammonolysis and deprotection, readily provided optically pure D- and L- carbocyclic 4'-fluoro-2',3'-dideoxynucleosides. The convenience of our approach lies in the possibility of obtaining a number of different analogues by the reaction of a unique key intermediate with several heterocyclic bases. Unfortunately, however, the synthesized nucleosides did not show significant anti-HIV activity.

The use of L-carbohydrates and related nucleosides in medicinal application has greatly increased due to their potent biological activity and lower toxicity compared to their D-counterpart. For these reasons, modified nucleosides in the unnatural L-configuration have become an important synthetic target, which requires ready access to L-carbohydrates, especially L-ribose and its derivatives. However, the high cost of L-ribose and 2-deoxy-L-erythro-pentose (2-deoxy-L-ribose) has hampered their use as starting material for the synthesis of L-nucleosides in large quantities. In Chapter 3, we have devised a short, five-step synthesis of 2-deoxy-L-ribose from an inexpensive and readily available starting material L-arabinose. After survey of available methods for radical deoxygenation, we have modified and improved a commonly used procedure to provide 2-deoxy-L-ribose in higher yield from L-arabinose, which can be readily adapted to the large-scale synthesis

As 2',3'-didehydro-2',3'-dideoxy-2'-fluoronucleosides have exhibited interesting antiviral effects against HIV-1 as well as HBV, it was of interest to synthesize the isosterically substituted 4'-thionucleosides in which 4'-oxygen is replaced by a sulfur atom. In Chapter 4, in order to study structure-activity relationships, various pyrimidine

and purine nucleosides were synthesized from the key intermediate (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio- β -D-ribofuranoside. The antiviral activity of the synthesized compounds were evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells, among which cytidine, 5-fluorocytidine, adenosine and 2-fluoroadenosine showed moderate to potent anti-HIV activities (EC_{50} 1.3, 11.6, 8.1 and 1.2 μ M, respectively). It is noteworthy that 2-fluoroadenosine analogue showed antiviral potency as well as high cytotoxicity (IC_{50} 1.5, 1.1 and 7.6 μ M for PBM, CEM and Vero, respectively) whereas no other compound showed cytotoxicity up to 100 μ M. The cytidine and 5-fluorocytidine analogues showed significantly decreased antiviral activity against the clinically important lamivudine-resistant variants (HIV-1_{M184V}), whereas the corresponding D-2'-Fd4 nucleosides showed limited cross-resistance. Molecular modeling studies demonstrated that the larger van der Waals radius as well as the close proximity to Met184 of the 4'-sulfur atom D-2'-F-4'-Sd4C may be the reasons for the decreased antiviral potency of synthesized 4'-thio nucleosides against the lamivudine-resistant variants (HIV-1_{M184V}).

On the other hand, as antiviral nucleosides containing a 2',3'-unsaturated sugar moiety with 2'-fluoro substitution are endowed with increased stabilization of the glycosidic bond, it was of another interest to investigate the influence of the fluorine atom at the 3' position. In Chapter 5, various pyrimidine and purine L-3'-fluoro-2',3'-unsaturated nucleosides were synthesized from their precursors, L-3',3'-difluoro-2',3'-dideoxy nucleosides, by elimination of hydrogen fluoride. In the L-3',3'-difluoro-2',3'-dideoxy nucleoside series, cytidine and 5-fluorocytidine analogues showed modest antiviral activity (EC_{50} 11.5 and 8.8 μ M, respectively) when evaluated against HIV-1 in human

peripheral blood mononuclear (PBM) cells. In the 2',3'-unsaturated series, L-3'-fluoro-2',3'-didehydro-2',3'-dideoxycytidine and 5-fluorocytidine showed highly potent antiviral activity (EC_{50} 0.089 and 0.018 μ M, respectively) without significant cytotoxicity. The guanosine analogue showed only marginal anti-HIV activity with some cytotoxicity (EC_{50} 38.5 μ M, and IC_{50} 17.4, 58.4, 36.5 μ M in PBM, CEM and Vero cells, respectively). The cytidine and 5-fluorocytidine analogues, however, showed significantly decreased antiviral activity against the clinically important lamivudine-resistant variants (HIV-1_{M184V}). Molecular modeling studies demonstrated that the 3'-fluoro atom of the L-3'-fluoro-2',3'-unsaturated nucleoside is within the hydrogen bonding distance with the amide backbone of Asp185, which favors the binding of the nucleoside triphosphate to the wild type RT. This favorable binding mode, however, cannot be maintained when the triphosphate of 3'-fluoro 2',3'-unsaturated nucleoside binds to the active site of M184V RT because the bulky side chain of Val184 occupies the space needed for the nucleotide. The biological results suggest that, in addition to the sugar conformation, the base moiety may also play a role in their interaction with the M184V RT.

Since the discovery of d4T as an anti-HIV drug, there have been numerous attempts to substitute the 2',3'-unsaturated sugar moiety with various functionalities. Among those, 3'-fluoro substituted 2',3'-unsaturated nucleosides proved to be extremely potent against HIV-1 without significant cytotoxicity. For this reason, the chemistry and biology of the isosterically substituted 4'-thio-2',3'-unsaturated nucleosides in which 4'-oxygen is replaced by a sulfur atom have been extensively explored. However, the combination of the 4'-sulfur atom with the 3'-fluorine atom within the 2',3'-unsaturated sugar moiety has

never been reported presumably because of the synthetic difficulty. In Chapter 6, various D-2',3'-unsaturated 3'-fluoro-4'-thio pyrimidine and purine nucleosides were synthesized for the structure-activity relationship study. Interestingly, in contrast to the rarely negative effect of fluorine substitution at the 2',3'-unsaturated double bond of the sugar moiety, the synthesized D-2',3'-unsaturated 3'-fluoro-4'-thionucleosides did not show any significant antiviral activity against HIV-1. Molecular modeling study shows that the 3'-fluorine atom of the D-2',3'-unsaturated nucleoside triphosphates experiences unfavorable electrostatic interaction with its own triphosphate moiety to result in the decreased binding affinity to HIV-1 RT, which may be one of the reasons for the insensitivity of HIV-1 RT to these compounds.

In Chapter 7, in order to understand molecular mechanism of antiviral drug resistance of HIV-1 reverse transcriptase (RT) as well as potent antiviral activity of DAPD (prodrug of DXG) against drug-resistant RTs, molecular modeling studies of three structurally distinct nucleoside RT inhibitor (NRTI) triphosphates (AZTTP, 3TCTP and DXGTP) complexed with the wild-type (WT) and mutated RT were conducted. The computational analyses indicated that the antiviral activity and the calculated relative binding energy of the RT inhibitor triphosphates can be correlated, and the minimized structures gave information on the molecular mechanism of drug resistance conferred by mutations. The interactions between the NRTI triphosphates and adjacent amino acid residues (Lys65, Lys70, Arg72, Tyr115 and/or Gln151) played important roles in stabilizing the enzyme-inhibitor complex. Particularly, Arg72 was found to stabilize the dioxolane and oxathiolane sugar moiety through hydrogen bonding, which was responsible for favorable binding affinity of DXGTP to AZT- as well as 3TC-resistant mutants. The

conformational changes in these amino acid residues caused by mutation always affected the changes in the tertiary structures of enzyme-inhibitor complexes through either closing or opening the gap between the fingers and palm domains. The enzyme-inhibitor complexes with good binding affinity showed tight binding modes by closing the gap between the two domains, whereas weak inhibitors gave open and loose complexes.

Even though L-FMAU is known to act specifically on viral DNA synthesis, and its triphosphate inhibits the HBV DNA synthesis in a dose-dependent manner without being incorporated into the DNA or chain termination, the precise understanding of the mechanism of action of L-FMAU at the polymerase level has not been realized. For this purpose, In Chapter 8, L-FMAU-TP/ HBV polymerase complex was constructed and simulated by molecular dynamics to obtain the structural as well as mechanistic information of L-FMAU-TP in a molecular level, which demonstrated that L-FMAU-TP can act as a competitive inhibitor by binding to the active site of HBV polymerase, but not act as a substrate because the binding of L-FMAU-TP to the active site of HBV polymerase cannot take place without the conformational adjustment, which prevents L-FMAU-TP from being incorporated into the growing viral DNA chain.

The mutability and resultant adaptability of HIV-1 reverse transcriptase (RT) present a major challenge to the design of the effective antiviral strategies because many initially potent drugs lose efficacy over time. Even though there is an urgent need for a comprehensive understanding of the molecular mechanism of anti-HIV drug resistance by mutant RTs, the unavailability of the structural information of the mutant RTs has prevented from investigations. In Chapter 9, the active site of the 3TC-resistant (M184V) RT is constructed by computational method, which clearly shows that the side chain of

Val184 occupies the binding site for the nucleoside triphosphates. Therefore, the distance between the side chain of Val184 and the sugar moiety of the nucleoside triphosphate must be closely related to the cross-resistance by M184V RT. The natural substrates, 2'-deoxyribo nucleoside triphosphates, escape from the steric stress from the bulky side chain of Val184 by virtue of the D-sugar conformation as well as the interaction of its 3'-OH group with Tyr115, which locates the nucleoside triphosphate out of the clashing distance from Val184. Similarly, the energy-minimized structures of various D-dioxolane nucleoside triphosphates (TP)/ RT complexes indicate that the D-dioxolane sugar moiety acquires enough distance from Val184 due to the specific interaction of its 3'-oxygen atom with the nearby enzyme residues such as Tyr115 and Arg72.

The emergence of HBV drug resistance highlights the need for development of alternative therapies that suppress the replication of 3TC-resistant variants as well as the HBV replication *in vivo* more effectively. In Chapter 10, the comparison of the active sites of the HIV-1 RT and the homology-modeled HBV polymerase shows that the active sites of both enzymes are open to L-nucleosides, but the position where the 3'-substituent of the L-ribose projects in HBV polymerase is wider and deeper than RT, which enables the HBV polymerase to accommodate various 3'-substituted L-nucleosides. However, the analysis of the minimized structure of M204V HBV polymerase/3TCTP complex shows that, instead of the steric stress by Val204, a loss of the van der Waals contact around the oxathiolane sugar moiety of 3TCTP caused by the mutation results in the disruption of the active site. The nonspecific van der Waals interaction between the enzyme residue, particularly Met204, and the sugar moiety of the L-configured nucleoside triphosphate is critical for stabilizing the enzyme/nucleoside

complex, and the loss of the hydrophobic interaction caused by mutation significantly destabilizes the complex. Therefore, nucleosides which are stabilized by additional specific interaction with the enzyme residues can have more chances to circumvent the destabilization by the loss of hydrophobic interaction conferred by mutation. Specifically, the substitution at the 3'-position would be beneficial as the HBV polymerase has wide open space composed of the highly conserved motif (YMDD) where the 3'-substituents of the L-nucleosides project. As an example, our study shows that the 3'-fluorine atom contributes for the antiviral activity of L-3'-Fd4CTP against M204 HBV polymerase by readily compensating for the loss of the van der Waals interaction around the 2',3'-double bond through a formation of a hydrogen bond to the amide backbone of Asp205.

In Chapter 11, in order to find out the possible role of fluorine substituent in binding of the fluorinated cytidine analogues at the active site of HIV-1 RT, binding modes of several 5-fluoro cytidine analogues such as FTC, D-dioxolane 5-FC and D-2'-F-d4-5FC to the active site of HIV-1 reverse transcriptase were compared by molecular dynamics studies. By molecular dynamics studies, we have found that formation of a hydrogen bond between fluorine-substituted nucleoside analogues and Arg72 may provide additional favorable contact within the active site of RT. However, as Arg72 is heavily involved in the stabilization of the triphosphate moiety of the bound nucleoside triphosphate, its interaction with the 5-fluorine atom on the cytosine ring was observed only in a limited case (D-dioxolane-5FCTP).

Most efforts in 1'-substituted nucleoside area have been driven to the syntheses of 1'-branched nucleosides which have both a heterocyclic base and a carbon substituent at the anomeric position of ribose or 2-deoxy-ribose. Because of their resistance to hydrolysis,

those *C*-glycosides are expected to be stable mimics for natural *O*-glycosides exhibiting biological activity. Moreover, nucleoside with an electron withdrawing group at the 1'-position, 2'-ara-1'-cyano cytidine analogue, was found to be stable against chemical as well as enzymatic hydrolysis to exhibit potent antitumor activity. Therefore, in view of achieving stability of ribonucleoside against hydrolytic cleavage of the glycosyl bond, it is of interest to introduce other electronegative group such as fluorine atom at the 1'-position. However, to our knowledge, preparation of a quaternary carbon center substituted with oxygen, nitrogen and fluorine atom at the same time has never been reported. Chapter 12 describes our extensive search for the synthetic method to prepare the hereto unknown 1'-fluoro ribonucleoside.

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