Synthesis, Structure−Activity Relationships, and Mechanism of Drug Resistance of D- and L-β-3′-Fluoro-2′,3′-unsaturated-4′-thionucleosides as Anti-HIV Agents

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Various D- and L-2′,3′-unsaturated 3′-fluoro-4′-thionucleosides (D- and L-3′F-4′Sd4Ns) were synthesized for the studies of structure−activity relationships. The synthesized D-2′,3′-unsaturated 3′-fluoro-4′-thionucleosides did not show any significant antiviral activity against HIV-1, while unnatural L-nucleosides such as cytosine **34** (EC_{50} = 0.13 μM; EC_{90} = 1.7 μM) and 5-fluorocytosine **35** (EC_{50} = 0.031 μM; EC_{90} = 0.35 μM) derivatives exhibited potent anti-HIV activity without significant toxicity. Molecular modeling study shows that the 3′-fluorine atom of the D-2′,3′-unsaturated cytidine triphosphate (D-3′F-4′Sd4CTP) experiences unfavorable electrostatic interaction with its own triphosphate moiety, resulting in the decreased binding affinity to wild-type HIV-1 reverse transcriptase (RT), which may be one of the reasons for the insensitivity of HIV-1 RT to these compounds. On the other hand, L-3′F-4′Sd4CTP binds to the active site of wild-type HIV-1 RT without steric hindrance and there is a possible hydrogen bonding between the 3′-fluorine atom and Asp185, which correlates with its potent anti-HIV activity. However, L-3′F-4′Sd4C **34** and L-3′F-4′Sd4FC **35** showed high cross-resistance to 3TC-resistant mutant (M184V) RT. Like other unnatural L-nucleosides, the unfavorable steric hindrance of the sugar moiety of L-3′F-4′Sd4CTP with the side chain of Val184 explains its significant cross-resistance to the M184V mutant.

Introduction

There have been considerable interest in the modification of nucleosides with a fluorine atom in the sugar moiety because of the strong electronegativity of a fluorine atom, which can influence the overall electronic property of a nucleoside, resulting in the increased chemical stability of fluorinated nucleosides.[1] 4′-Thionucleosides with isosteric replacement of the 4′-oxygen by a sulfur atom have been synthesized and biologically evaluated as antiviral and antitumor agents[2−6] because 4′-thionucleosides are known to be resistant to hydrolytic cleavage of a glycosyl linkage catalyzed by nucleoside phosphorylases,[7] which deactivates several antiviral nucleosides.[8] However, among the 4′-thionucleosides, only 2′,3′-unsaturated nucleoside analogues showed potent antiviral activity against HIV.[5,6] Recently, L-2′,3′-unsaturated 3′-fluoronucleosides have been reported to exhibit potent antiviral activity against HIV-1 without significant cytotoxicity.[9] Thus, it was of interest to synthesize their corresponding 4′-thionucleosides.

Introduction of a fluorine atom into the 3′-position of the 2′,3′-unsaturated sugar moiety has always been challenging. There are limited examples to introduce the 3′-fluorine in 2′,3′-unsaturated nucleosides.[9,10] In this study, by adaptation of the synthetic protocol, which was used in the synthesis of the L-2′,3′-unsaturated 3′-fluorocytidine analogue,[9] various D- and L-2′,3′-unsaturated 3′-fluoro-4′-thionucleosides were prepared and their antiviral activity was evaluated against HIV in human peripheral blood mononuclear (PBM) cells. To understand the structure−activity relationships and the mechanism of the cross-resistance against 3TC-resistant (M184V) reverse transcriptase (RT), the binding modes of D- and L-2′,3′-unsaturated 3′-fluoro-4′-thiocytidine triphosphates to the wild type as well as the 3TC-resistant (M184V) RT were investigated by molecular modeling studies. Herein, we report the synthesis, biological evaluation, and molecular modeling studies of the D- and L-4′-thionucleosides.

Results and Discussion

Chemistry. Enantioselective synthesis of 3′-fluoro-2′,3′-unsaturated nucleosides is extremely limited by the lack of synthetic methodologies to introduce a fluorine atom at the 3′-position of the 2′,3′ double bond. In this study, the method in the synthesis of L-3′-fluoro-2′,3′-unsaturated nucleosides,[9] which introduced a 2′,3′ double bond by elimination of hydrogen fluoride (HF) from a 2′,3′-dideoxy-3′,3′-difluoronucleoside, was adapted to the synthesis of their corresponding 4′-thionucleosides.

To synthesize the target compounds, L-β-3′-fluoro-2′,3′-didehydro-2′,3′-dideoxy-4′-thio-nucleosides (L-3′F-4′Sd4Ns), a versatile carbohydrate precursor **8** was used as a key intermediate, which was obtained from commercially available 2-deoxy-D-ribose (Scheme 1). The conversion of 2-deoxy-D-ribose to its ketone **3** was accomplished in three steps in 70% overall yield by the reported method.[11] Treatment of the ketone **3** with

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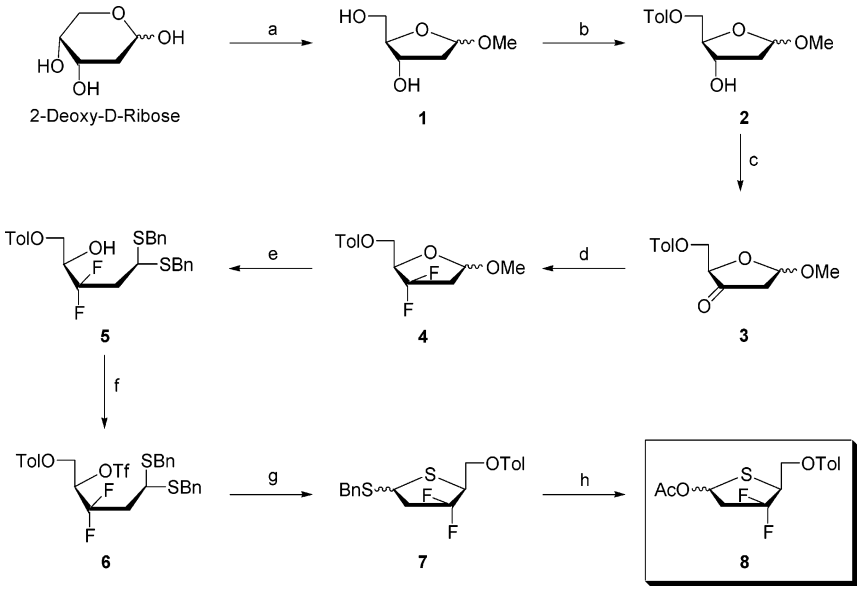
Scheme 1. Preparation of the Key Intermediate **8**[a]



[a] Reagents and conditions: (a) H_2SO_4, MeOH, 0 °C to room temp; (b) Tol-Cl, pyr, 0−5 °C; (c) PDC, CH_2Cl_2, room temp; (d) DAST, CH_2Cl_2; (e) BnSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, reflux; (f) Tf_2O, 2,6-lutidine, CH_2Cl_2; (g) $BaCO_3$, TBAI, CH_3CN, heating; (h) $Hg(OAc)_2$, Ac_2O, AcOH.

(diethylamino)sulfur trifluoride (DAST) afforded the difluorinated intermediate **4** in 55% yield.[12] The ring-opening reaction with benzyl mercaptan (BnSH) was catalyzed by 0.5 equiv of $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at 40 °C to give the thioacetal **5** in 94% yield.[13] The 4-OH was converted to the corresponding triflate **6**, which was cyclized to thiosugar **7** in 78% yield. The cyclization at C-4 could also be accomplished under Mitsunobu conditions with triphenylphosphine, iodine, and imidazole.[14] Transglycosylation of compound **7** by the treatment with mercury(II) acetate in acetic acid provided the key intermediate **8** in 72% yield.[15]

A series of pyrimidine and purine nucleosides were prepared by coupling the key intermediate **8** with various silyl-protected bases in the presence of TMSOTf (Scheme 2). Uracil and thymine nucleosides, **9** and **10**, were obtained in 67−71% yield as inseparable α/β mixtures. The anomeric mixtures, **9** and **10**, were treated with NH_3/MeOH at room temperature to give free nucleosides **15**−**18**, which were readily separated by silica gel column chromatography (α/β = 1:1, 90% yield). Similarly, condensation of **8** with silylated N^4-isobutyrylcytosine and N^4-benzoyl-5-fluorocytosine in dry acetonitrile gave protected cytosine derivatives **11**/**12** and 5-fluorocytosine derivatives **13**/**14**, respectively. These anomers were readily separated by silica gel column chromatography. Deprotection of individual anomers by ammonolysis using saturated methanolic ammonia at room temperature afforded the free nucleosides **19**−**22**.

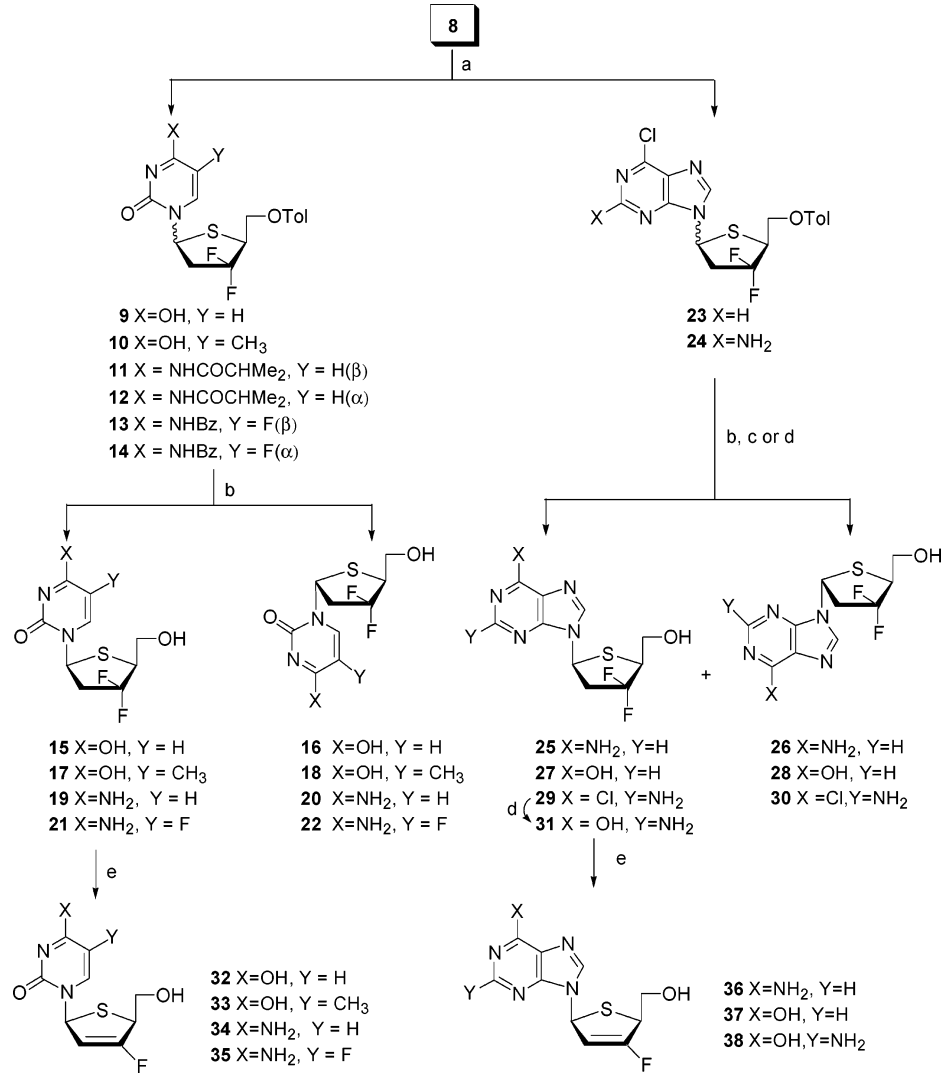
Condensation of **8** with silylated 6-chloropurine in dry acetonitrile at 80 °C for 24 h gave inseparable 6-chloropurine nucleosides **23**. After treatment with methanolic ammonia in a steel bomb at 100 °C for 24 h, anomeric mixtures of adenine derivatives (**25**/**26**) were separated on a silica gel column. The hypoxanthine derivatives (**27**/**28**) were also obtained from 6-chloropurine nucleosides in 79% yield by refluxing with sodium methoxide and 2-mercaptoethanol in MeOH. 5′-Toluoyl-protected 2-amino-6-chloropurine derivatives (**24**) were also obtained by the condensation of intermediate **8** with the silyl-protected 2-amino-6-chloropurine in 1,4-dioxane at 100 °C for 12 h. After removal of the 5′-toluoyl protecting group, 2-amino-6-chloropurine derivatives **29**/**30**, were isolated by silica gel column chromatography. The β-isomer **29** was then further transformed to the guanine derivative **31** by heating under reflux in the presence of 2-mercaptoethanol and sodium methoxide in 60% yield.

The stereochemistry of these anomers was determined on the basis of nuclear Overhauser effect (NOE) experiments of the thymine derivatives (**17**/**18**). NOE (3%) was observed between the 6-H and 4′-H in the α-thymine derivative **18** when the 6-H was irradiated, whereas no NOE was observed between the corresponding protons in β-analogue **17**. This assignment was also supported by lower field chemical shifts of the 4′-H (**18**, α-form) compared to that of the 4′-H (**17**, β-form) because of deshielding effects by heterocyclic bases.

The β-difluoronucleosides (**15**, **17**, **19**, **21**, **25**, **27**, and **31**) were converted to the target unsaturated nucleosides (**32**−**38**) by treatment with potassium *tert*-butoxide in THF. A careful control of the reaction was critical because a longer reaction time usually resulted in decomposition of the starting material and poor yield. Unfortunately, the reaction did not proceed to completion, and only the adenine derivative **36** could be separated from its starting material **25** with a normal silica gel column chromatography. In the other derivatives, final products could not be separated from their corresponding difluoro starting materials by silica gel column chromatography. Several other methods of separation such as silver nitrate−silica gel column chromatography and chiral auxiliaries were tried without success. Therefore, after the reaction mixture was stirred for 3−5 h at room temperature, the reaction was stopped and, after purification, the recovered starting material was recycled.

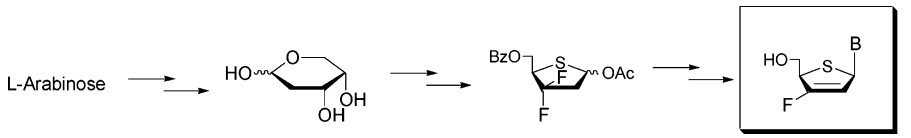
The D-isomers (D-3′F-4′S-d4Ns) were also synthesized under similar reaction conditions starting from the opposite enantiomer, 2-deoxy-L-riboside (Scheme 3). The key intermediate **39**, the enantiomer of **8**, was prepared

Scheme 2. Synthesis of the Pyrimidine and Purine Nucleosides[a]



[a] Reagents and conditions: (a) BSA, pyrimidines or purines, CH_3CN or 1,4-dioxane, TMSOTf, heat; (b) NH_3/MeOH, room temp; (c) NH_3/MeOH, steel bomb, 100 °C; (d) 2-mercaptoethanol, NaOMe/MeOH, reflux; (e) ^{t}BuOK/THF.

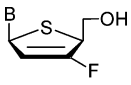
Scheme 3. Synthesis of d-3′F-4′Sd4 Nucleosides



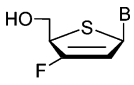
from 2-deoxy-L-ribose, which can be prepared from L-arabinose in large quantity and high yield in six steps.[16] After coupling of **39** with the appropriately protected pyrimidine and purine bases, the final D-2′,3′-unsaturated 3′-fluoro-4′-thionucleosides (**40**−**46**) were obtained using the same procedure for L-isomers. During syntheses of the final D-isomers (**40**−**46**), several reaction conditions for the HF elimination were tried to achieve completion of the reaction in a single operation. The best result was obtained using a combination of NaOMe and ^{t}BuOK in DMF at 0°C, which afforded 2′,3′-unsaturated nucleosides with no remaining starting material and marginal decomposition. Presumably, deprotonation of acidic protons such as the proton of the 5′-hydroxy group and the proton of an amide in the base moiety with 2.2 equiv of a weaker base, NaOMe, followed by the HF elimination by a stronger base, ^{t}BuOK, at low temperature proceeds in a more controlled manner.

Structure−**Activity Relationships.** The antiviral activity of the synthesized D- and L-nucleosides (**32**−**38** and **40**−**46**) was evaluated against HIV-1 in human PBM cells in vitro, and the results are summarized in Table 1. Among the tested nucleosides, L-cytosine **34**

Table 1. Anti-HIV Activity of D- and L-β-3′-Fluoro-2′,3′-unsaturated-4′-thionucleosides



32 - 38



40 - 46

compd	config	base	HIV (PBM) EC_{50} (μM)	HIV (PBM) EC_{90} (μM)	toxicity, IC_{50} (μM) PBM	CEM	Vero
32	L	uracil	>100	>100	>100	>100	>100
33	L	thymine	>100	>100	>100	≥100	>100
34	L	cytosine	0.13	1.7	>100	>100	>100
35	L	5-F-cytosine	0.031	0.35	>100	>100	88.1
36	L	adenine	14.9	57.0	>100	>100	>100
37	L	hypoxanthine	>100	>100	>100	>100	>100
38	L	guanine	43.9	>100	>100	>100	>100
40	D	uracil	>100	>100	>100	>100	>100
41	D	thymine	>100	>100	>100	>100	>100
42	D	cytosine	>100	>100	>100	>100	>100
43	D	5-F-cytosine	>100	>100	>100	>100	>100
44	D	adenine	>100	>100	>100	>100	>100
45	D	hypoxanthine	>100	>100	>100	>100	>100
46	D	guanine	23.4	>100	>100	>100	>100
AZT			0.004	0.01	>100	14.3	29.0

(EC_{50} = 0.13 μM), L-5-fluorocytosine **35** (EC_{50} = 0.031 μM), L-adenine **36** (EC_{50} = 14.9 μM), and L-guanine **38** (EC_{50} = 43.9 μM) derivatives showed moderate to potent antiviral activity. Only the L-5-fluorocytosine derivative **35** showed marginal cytotoxicity (IC_{50} = 88.1 μM) in Vero cell, whereas no other compounds showed any significant cytotoxicity up to 100 μM. Like L-β-3′Fd4N nucleosides,[9] there was a general trend of antiviral activity throughout the series, in which the cytosine and 5-fluorocytosine derivatives exhibited the most potent anti-HIV activity, which suggests that the two types of nucleosides may have similar structural features and can be recognized by the cellular kinases as well as viral polymerases. However, the synthesized D-2′,3′-unsaturated 3′-fluoro-4′-thionucleosides did not show any significant antiviral activity against HIV-1. Only D-guanosine analogue **46** showed marginal anti-HIV activity (EC_{50} = 23.4 μM) (vide infra for molecular modeling studies)

Antiviral Activity against Lamivudine-Resistant (HIV-1_{M184V}) Mutant Strain. Treatment with 3TC [lamivudine, (−)-β-2′,3′-dideoxy-3′-thiacytidine] rapidly induces resistance in HIV-infected individuals. The resistant isolates showed reduced susceptibility to lamivudine, and genotypic analysis showed that the resistance was due to specific substitution mutation in HIV-1 RT at codon 184 within the YMDD motif.[17] Therefore, recent studies on anti-HIV nucleoside analogues have been focused on the discovery of new drug candidates active against the drug-resistant HIV strains, which resulted in the recent approval of tenoforvir disoproxil by the FDA.[18] To access the anti-HIV activity, the most potent compounds from our studies, the cytosine **34** and 5-fluorocytosine **35** derivatives 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 2). The results indicated that both cytosine **34** and 5-fluorocytosine **35** derivatives are not active against 3TC-resistant mutant (M184V) while AZT is still active against the mutant. Molecular modeling studies were conducted to explain the drug resistance of 3′-fluoro-4′-thionucleosides toward 3TC-resistant mutant RT (vide infra).

Table 2. Activity of Selected Nucleosides against Lamivudine-Resistant Virus (HIV-1_{M184V}) in Human PBM Cells

compd	WT (xxBRU),[a] EC_{90} (μM)	M184V,[b] EC_{90} (μM)	FI[c]
34 (L-cytosine)	1.7	>100	>58.8
35 (L-5-F-cytosine)	0.35	>100	>285.7
AZT	0.01	0.003	0.3
3TC	0.08	535	6688

[a] WT: wild type (drug-sensitive virus). [b] M184V: drug-resistant virus against 3TC. [c] FI: fold increase [EC_{90}(HIV$_{M184V}$)/EC_{90}(HIV-1_{xxBRU})].

Molecular Modeling. To investigate the characteristic binding modes of D-3′F-4′Sd4C and L-3′F-4′Sd4C as well as the mechanism of L-3′F-4′Sd4C's high cross-resistance to 3TC-resistant mutant (M184V) RT, molecular modeling studies were performed by using the wild-type RT (PDB entry 1rtd)[19] as well as the computer-generated M184V HIV-1 RT complexed with D- and L-3′F-4′Sd4C triphosphates (D- and L-3′F-4′Sd4CTPs). The geometrically optimized conformation of L-3′F-4′Sd4C, which was obtained by a quantum mechanical calculation (RHF/6-31G**),[6b,c] was docked into the active site of HIV-1 RT, and the resulting complex was minimized[20] until there was no significant change in atomic coordinates. As other unnatural L-nucleosides,[6c] L-3′F-4′Sd4CTP binds well in the active site of the wild-type RT without any steric hindrance with the neighboring enzyme residues, particularly the most adjacent residue Met184 (Figure 1a). Interestingly, the 3′-fluorine atom is within the hydrogen-bonding distance with the amide backbone of Asp185, even though the distance (~2.2 Å) between the hydrogen-bonding donor (amide backbone of Asp185) and the acceptor (3′F) is longer than the normal distance (Figure 1a). However, because of the long distance between the hydrogen-bonding pair and relatively weak hydrogen-bonding ability of the fluorine atom,[21] the contribution of this interaction to the binding affinity of the L-3′F-4′Sd4CTP to the active site of RT is expected to be insignificant. In the M184V RT•L-3′F-4′Sd4CTP complex, the binding pocket of the L-3′F-4′Sd4CTP tends to overlap with the bulky side chain of Val184, which provides steric hindrance when the L-3′F-4′Sd4CTP binds to the M184V RT (Figure 1b). To circumvent this abortive binding mode, the M184V RT•L-3′F-4′Sd4CTP complex had to undergo significant conformational change that resulted in a large decrease in the calculated relative binding energy, reflecting the high cross-resistance of L-3′F-4′Sd4C to M184V RT.

It is interesting to find that D-3′F-4′Sd4C **42** is not active against HIV-1 in view of the fact that D-2′F-4′Sd4C is active against HIV-1 with an EC_{50} of 1.3 μM.[6b] The quantum mechanically geometrically optimized structures of D-3′F-4′Sd4C and D-2′F-4′Sd4C show almost the same conformations, which implies that the 3′-fluorine plays a role in the inactivity of D-3′F-4′Sd4C to HIV-1. In our molecular modeling studies, the 3′-fluorine in D-3′F-4′Sd4CTP is located very close to the β-phosphate of its own triphosphate moiety when it is bound to the active site of HIV-1 RT (Figure 1c). The two negatively charged moieties strongly repulse each other to force a conformational change in the sugar moiety. However, the possible steric hindrance between the bulky 4′-sulfur atom of the sugar and Met184 does

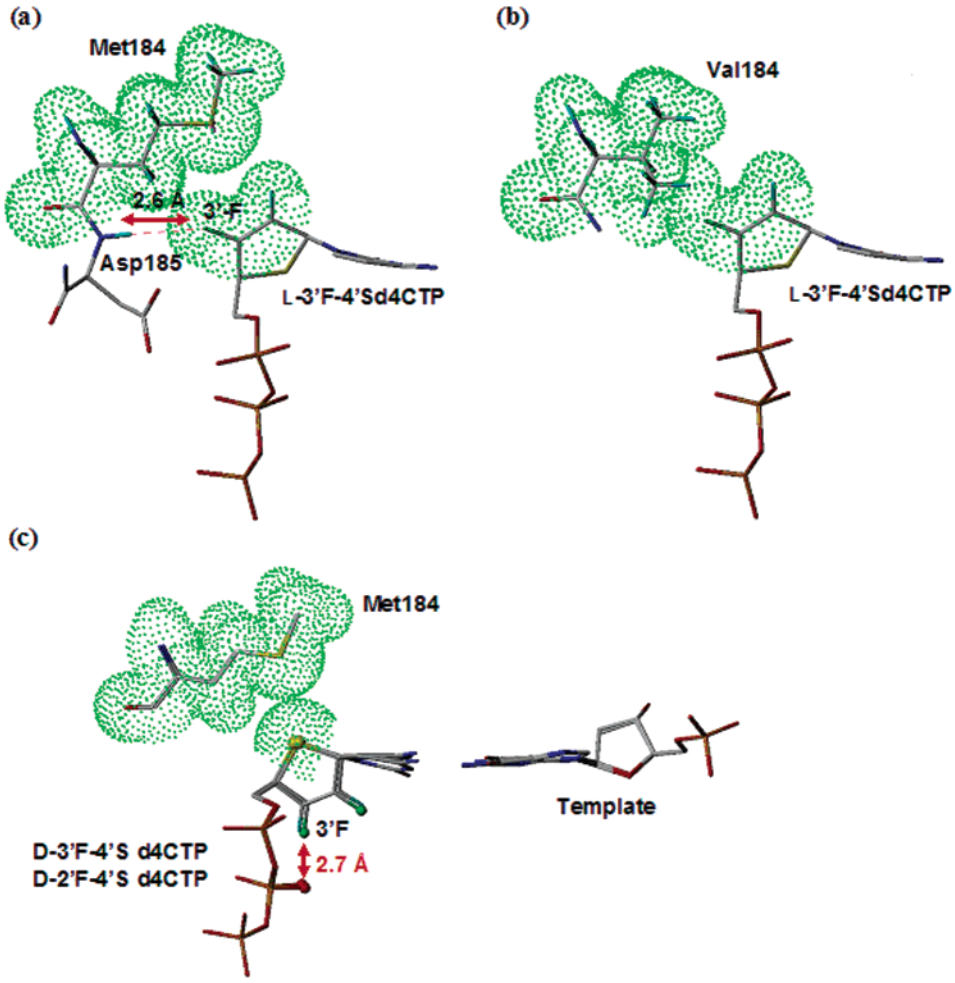


Figure 1. (a) L-3′F-4′Sd4CTP binds to the active site of HIV-1 RT without significant unfavorable steric hindrance with the adjacent enzyme residues, particularly Met184. 3′-F is hydrogen-bonded to the −NH backbone of Asp185. (b) L-3′F-4′Sd4CTP experiences strong steric hindrance with the bulky side chain of Val184 in M184V RT. (c) 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.

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 (Figure 1c). Therefore, under the assumption that D-3′F-4′Sd4C is phosphorylated to the corresponding triphosphate with the same efficiency as its L-congener, our molecular modeling study demonstrates that the repulsive electrostatic interaction between the 3′-fluorine atom and the triphosphate moiety distorts the binding mode of D-3′F-4′Sd4CTP to result in the loss of its binding affinity to HIV-1 RT and thereby its inactivity against HIV-1.

In summary, various D- and L-3′F-4′Sd4 nucleosides were prepared and their anti-HIV activity was evaluated. Among the synthesized L-nucleosides, L-cytosine (**34**, EC_{50} = 0.13 μM) and L-5-fluorocytosine (**35**, EC_{50} = 0.031 μM) derivatives showed potent anti-HIV activity against the wild-type HIV-1 RT. However, they failed to show antiviral activity against the 3TC-resistant mutant (M184V) RT. The synthesized D-isomers did not show any significant anti-HIV activity presumably because of the decreased binding affinity of the corresponding triphosphates caused by the unfavorable electrostatic interaction between the 3′-fluorine and its own triphosphate moiety, resulting in the distruption of the base-pairing.

Experimental Section

Melting points were determined on a Mel-temp II apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (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. Silica gel G (TLC grade, >440 mesh) was used for vacuum column chromatography as well as for flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

Methyl-2-deoxy-5-*O*-toluoyl-D-furan-3-ulose (3). Pyridinium dichromate (159 g, 0.42 mol) and powder molecular sieves (4 Å, 150 g) were added to a solution of **2** (75 g, 0.30 mol) in dry dichloromethane (1.5 L), and the mixture was mechanically stirred at room temperature for 4 h. The suspen-

sion was then filtered through a silica gel pad (eluting with EtOAc). After concentration, the resulting residue was purified by silica gel column chromatography (hexanes/EtOAc, 20:1 to 10:1) to give the ketone **3** (57 g, 0.22 mol, 73% yield) as a colorless syrup: R_f = 0.59 (hexanes/EtOAc, 3:1); ^{1}H NMR ($CDCl_3$) δ 7.97−7.23 (m, 4H, Ar−H), 5.38 (m, 1H, H-1), 4.80−4.28 (m, 3H, H-4, H-5), 3.49, 3.38 (2 × s, 3H, OCH_3), 2.88−2.49 (m, 2H, H-2), 2.41 (s, 3H, $PhCH_3$). Anal. ($C_{14}H_{16}O_5$) C, H.

1-*O*-Methyl-2,3-dideoxy-3,3-difluoro-5-*O*-toluoyl-D-furanoside (4). DAST (75 mL, 0.57 mol) was added to a solution of ketone **3** (50 g, 0.19 mol) in CH_2Cl_2 (500 mL). The mixture was stirred under N_2 at room temperature for 24 h. The reaction mixture was poured into cold saturated aqueous $NaCO_3$ solution (1 L) and extracted with CH_2Cl_2 (3 × 500 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The remaining residue was purified by silica gel column chromatography (hexanes/EtOAc, 20:1 to 10:1) to give compound **4** (30 g, 0.105 mol, 55% yield) as a colorless syrup and recycle starting material **3** (15 g, 0.057 mol): R_f = 0.75 (hexanes/EtOAc, 3:1); ^{1}H NMR ($CDCl_3$) δ 7.90−7.18 (m, 4H, Ar−H), 5.10 (m, 1H, H-1), 4.54−4.29 (m, 3H, H-4, H-5), 3.31, 3.29 (2 × s, 3H, OCH_3), 2.67−2.21 (m, 2H, H-2), 2.30 (s, 3H, $PhCH_3$). Anal. ($C_{14}H_{16}F_2O_4$) C, H.

2,3-Dideoxy-3,3-difluoro-5-*O*-toluoyl-D-ribose Dibenzyl Dithioacetal (5). Benzyl mercaptan (47 mL, 0.4 mol) was added to a solution of compound **4** (28.7 g, 0.100 mol) and boron trifluoride etherate (6.3 mL, 0.05 mol) in CH_2Cl_2 (200 mL), and the mixture was stirred at 40 °C for 2 h. The mixture was diluted with saturated aqueous $NaHCO_3$ solution (150 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was dissolved in EtOAc (300 mL), washed with brine (200 mL), dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography to give dithioacetal **5** (47 g, 0.094 mol, 94% yield) as a colorless syrup: R_f = 0.70 (hexanes/EtOAc, 3:1); $[\alpha]^{22}_D$ +15.4 (*c* 2.9, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.94−7.04 (m, 14H, Ar−H), 4.44 (dd, *J* = 11.9, 2.5 Hz, 1H, H-5a), 4.35 (dd, *J* = 11.9, 7.0 Hz, 1H, H-5b), 3.84−3.70 (m, 6H, H-1, H-4, 2 × CH_2Ph), 2.93 (d, *J* = 6.0 Hz, 1H, OH), 2.68−2.42 (m, 5H, H-2, $PhCH_3$); MS (ESI) *m*/*z* 503 (MH^+). Anal. ($C_{27}H_{28}F_2O_3S_2$) C, H.

Benzyl-2,3-dideoxy-3,3-difluoro-1,4-dithio-5-*O*-toluoyl-L-furanoside (7). Trifluoromethanesulfonic anhydride (18 mL, 108 mmol) was slowly added to a solution of compound **5** (45 g, 90 mmol) and 2,6-lutidine (16.3 mL, 140 mmol) in CH_2Cl_2 (200 mL) over 10 min at −10 °C. After being stirred for an additional 45 min at 0 °C, the reaction mixture was quenched by adding MeOH (10 mL). After removal of the solvents, the residue was purified by vacuum silica gel column chromatography to afford compound **6** (50 g, R_f = 0.59 (hexanes/EtOAc, 5:1)) as a colorless syrup, which was dissolved in anhydrous acetonitrile (200 mL). $BaCO_3$ (23 g, 118 mmol) and *n*-TBAI (44 g, 118 mmol) were added, and the mixture was stirred for 3 h at 50−60 °C. After cooling to room temperature, the reaction mixture was filtered and concentrated to give a residue, which was participated between water and EtOAc. After phase separation, the organic phase was dried over Na_2SO_4, filtered, concentrated, and purified by vacuum silica gel column chromatography to afford compound **7** (27.6 g, 70 mmol, 78% yield from compound **5**) as a colorless syrup: R_f = 0.66 (hexanes/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$) δ 7.97−7.22 (m, 9H, Ar−H), 4.63−3.82 (m, 6H, H-1, H-4, H-5, CH_2Ph), 2.49−2.40 (m, 5H, H-2, $PhCH_3$); MS (ESI) *m*/*z* 259 (M-OCOPhMe). Anal. ($C_{20}H_{20}F_2O_2S_2$) C, H.

1-*O*-Acetyl-2,3-dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-L-furanose (8). Compound **7** (25 g, 63.4 mmol) was treated with a mixture of acetic acid (80 mL), acetic anhydride (20 mL), and mercuric acetate (30 g, 94 mmol) at room temperature for 24 h. The mixture was filtered through a Celite pad, and the filtrate was diluted with EtOAc, which was washed sequentially with water, saturated aqueous $NaHCO_3$ solution, and 5% NaCN aqueous solution, dried over Na_2SO_4, and concentrated. Column chromatography of the crude product in hexanes/EtOAc (30:1 to 10:1) gave the key intermediate **8** (15 g, 45.6 mmol, 72% yield) as a colorless syrup: R_f = 0.46 (hexanes/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$) δ 7.94−7.20 (m, 4H, Ar−H), 6.07−6.00 (m, 1H, H-1), 4.68−4.03 (m, 3H, H-4, H-5), 2.87−2.71 (m, 2H, H-2), 2.37 (s, 3H, $PhCH_3$), 2.06, 2.01 (2 × s, 3H, OAc). Anal. ($C_{15}H_{16}F_2O_2S$) C, H.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio- 5-*O*-toluoyl-α/β-L-ribofuranosyl]uracil (9). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA, 4.4 mL, 18 mmol) was added at room temperature to a mixture of compound **8** (1.65 g, 4.99 mmol) and uracil (730 mg, 6.5 mmol) in anhydrous acetonitrile (40 mL), and the resulting mixture was stirred under argon for 2 h at 50−60 °C to form a clear solution. After cooling to room temperature, TMSOTf (1 mL, 5.5 mmol) was added and the mixture was refluxed for 3 h under argon atmosphere. The reaction mixture was cooled to room temperature and subsequently quenched with saturated aqueous $NaHCO_3$ solution (20 mL) and stirred until the evolution of CO_2 ceased. The resulting mixture was diluted with EtOAc (150 mL), washed with brine (2 × 100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 30:1) to give compound **9** (α/β = 1, 1.28 g, 3.35 mmol, 67% yield) as a white foam: R_f = 0.56 (CH_2Cl_2/MeOH, 15:1); ^{1}H NMR ($CDCl_3$) δ 8.58 (br, 1H, NH), 7.93−7.29 (m, 5H, H-6 and Ar−H), 6.34−6.28 (m, 1H, H-1′), 5.85−5.63(m, 1H, H-5), 4.82−4.47 (m, 2H, H-5′), 4.26−4.06 (m, 1H, H-4′), 3.09−2.59 (m, 2H, H-2′), 2.44 (s, 3H, $PhCH_3$). Anal. ($C_{17}H_{16}F_2N_2O_4S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-α/β-L-ribofuranosyl]thymine (10). By use of the same procedure as above, compound **8** (1.65 g, 4.99 mmol) was condensed with thymine (820 mg, 6.5 mmol) to produce compound **10** (α/β = 1, 1.4 g, 3.53 mmol, 71% yield) as a white foam: R_f = 0.63 (CH_2Cl_2/MeOH, 15:1). ^{1}H NMR ($CDCl_3$) δ 10.34, 10.31 (2 × s, 1H, NH), 7.91−7.20 (m, 5H, H-6 and Ar−H), 6.36 (m, 1H, H-1′), 4.78−4.00 (m, 3H, H-4′, H-5′), 3.03−2.55 (m, 2H, H-2′), 2.39 (s, 3H, $PhCH_3$), 1.94, 1.68 (2 × s, 3H, 5-CH_3). Anal. ($C_{18}H_{18}F_2N_2O_4S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-β-L-ribofuranosyl)-*N*^{4}-isobutylcytosine (11) and 1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-α-L-ribofuranosyl]-*N*^{4}-isobutylcytosine (12). By use of the same procedure as described for compound **9**, compound **8** (1.65 g, 4.99 mmol) was condensed with N^4-isobutyrylcytosine (1.2 g, 6.6 mmol) to give compound **11** (β anomer, 0.74 g, 1.64 mmol, 33% yield) and compound **12** (α anomer, 0.79 g, 1.75 mmol, 35% yield) as white foams.

Compound 11: R_f = 0.33 (hexanes/EtOAc, 1:2); $[\alpha]^{23}_D$ −57.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.03 (s, 1H, NH), 8.36 (d, *J* = 7.5 Hz, 1H, H-6), 7.89 (d, *J* = 8.2 Hz, 2H, Ar−H), 7.41 (d, *J* = 7.5 Hz, 1H, H-5), 7.23 (d, *J* = 8.2 Hz, 2H, Ar−H), 6.31 (dd, *J* = 7.4, 3.3 Hz, 1H, H-1′), 4.79 (dd, *J* = 11.8, 6.1 Hz, 1H, H-5a′), 4.63 (dd, *J* = 11.8, 4.1 Hz, 1H, H-5b′), 4.09 (m, 1H, H-4′), 3.17−2.65 (m, 3H, H-2′ and C(O)CHMe$_2$), 2.40 (s, 3H, $ArCH_3$), 1.19 (d, *J* = 4.2 Hz, 6H, 2 × CH_3). Anal. ($C_{21}H_{23}F_2N_3O_4S$) C, H, N.

Compound 12: R_f = 0.45 (hexanes/EtOAc, 1:2); $[\alpha]^{24}_D$ −46.8 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.00 (s, 1H, NH), 8.31 (d, *J* = 7.5 Hz, 1H, H-6), 7.89 (d, *J* = 8.1 Hz, 2H, Ar−H), 7.51 (d, *J* = 7.5 Hz, 1H, H-5), 7.24 (d, *J* = 8.1 Hz, 2H, Ar−H), 6.31 (dd, *J* = 7.5, 3.3 Hz, 1H, H-1′), 4.66 (dd, *J* = 11.8, 5.7 Hz, 1H, H-5a′), 4.46 (dd, *J* = 11.8, 6.1 Hz, 1H, H-5b′), 4.25 (m, 1H, H-4′), 3.10−2.66 (m, 3H, H-2′ and C(O)CHMe$_2$), 2.40 (s, 3H, $ArCH_3$), 1.19 (d, *J* = 4.2 Hz, 6H, 2 × CH_3). Anal. ($C_{21}H_{23}F_2N_3O_4S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-β-L-ribofuranosyl]-*N*^{4}-benzoyl-5-fluorocytosine (13) and 1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-5-*O*-toluoyl-α-L-ribofuranosyl)-*N*^{4}-benzoyl-5-fluorocytosine (14). By use of the same procedure as described for compound **9**, compound **8** (1.65 g, 4.99 mmol) was condensed with N^4-benzoyl-5-fluorocytosine (1.54 g, 6.6 mmol) to give compound **13** (β anomer, 0.81 g, 1.61 mmol, 32% yield) and compound **14** (α anomer, 0.83 g, 1.65 mmol, 33% yield) as white solids.

Compound 13: R_f = 0.58 (hexanes/EtOAc, 1:1); mp 134−136 °C; $[\alpha]^{25}_D$ −32.2 (*c* 1.7, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 13.07 (br, 1H, NH), 8.31−7.27 (m, 10H, H-6 and Ar−H), 6.28 (m, 1H, H-1′), 4.81 (dd, *J* = 11.9, 5.8 Hz, 1H, H-5a′), 4.67 (dd, *J* =

11.9, 6.5 Hz, 1H, H-5b′), 4.13 (m, 1H, H-4′), 3.05−2.67 (m, 2H, H-2′), 2.43 (s, 3H, $ArCH_3$). Anal. ($C_{24}H_{20}F_3N_3O_4S$) C, H, N.

Compound 14: $R_f = 0.79$ (hexanes/EtOAc, 1:1); mp 95−97 °C; $[\alpha]^{24}_D$ −70.3 (*c* 0.8, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 13.06 (br, 1H, NH), 8.44−7.26 (m, 10H, H-6 and Ar−H), 6.31 (m, 1H, H-1′), 4.66 (dd, $J = 11.7$, 5.5 Hz, 1H, H-5a′), 4.48 (dd, $J = 11.7$, 5.8 Hz, 1H, H-5b′), 4.24 (m, 1H, H-4′), 3.11−2.66 (m, 2H, H-2′), 2.42 (s, 3H, $ArCH_3$). Anal. ($C_{24}H_{20}F_3N_3O_4S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]uracil (15) and 1-[(1*S*,4*S*)-2,3-Deoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]uracil (16). Compound **9** (α/β) (1.15 g, 3.01 mmol) was treated with saturated methanolic ammonia solution (20 mL) at room temperature for 8 h. Upon completion of the reaction, the solvent was removed under reduced pressure. The resulting syrup was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 50:1) to afford pure compound **15** (β anomer, 350 mg, 1.32 mmol, 44% yield) and compound **16** (α anomer, 363 mg, 1.37 mmol, 46% yield) in 90% overall yield.

Compound 15: $R_f = 0.58$ (CH_2Cl_2/MeOH, 9:1); mp 210−212 °C; $[\alpha]^{22}_D$ +25.4 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max} = 262$ nm ($\epsilon = 10\,805$); MS (ESI) *m*/*z* 265 (MH^+). Anal. ($C_9H_{10}F_2N_2O_3S$) C, H, N.

Compound 16: $R_f = 0.55$ (CH_2Cl_2/MeOH, 9:1); foam; $[\alpha]^{24}_D$ −117.3 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max} = 262$ nm ($\epsilon = 10\,487$); MS (ESI) *m*/*z* 265 (MH^+). Anal. ($C_9H_{10}F_2N_2O_3S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]thymine (17) and 1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]thymine (18). Conversion of **10** (α/β) (1.20 g, 3.03 mmol) to **17/18** was accomplished using the same procedure as described above. The resulting syrup was purified on a silica gel column (CH_2Cl_2/MeOH, 50:1) to afford pure compound **17** (β anomer, 370 mg, 1.33 mmol, 44% yield) and compound **18** (α anomer, 380 mg, 1.37 mmol, 45% yield) as white solids in 90% overall yield.

Compound 17: $R_f = 0.25$ (CH_2Cl_2/MeOH, 15:1); mp 151−153 °C; $[\alpha]^{25}_D$ +16.5 (*c* 2.2, MeOH); UV (MeOH) $\lambda_{max} = 268$ nm ($\epsilon = 8500$); MS (ESI) *m*/*z* 279 (MH^+). Anal. ($C_{10}H_{12}F_2N_2O_3S\cdot 0.9H_2O$) C, H, N.

Compound 18: $R_f = 0.17$ (CH_2Cl_2/MeOH, 15:1); mp 124−126 °C; $[\alpha]^{26}_D$ −90.4 (*c* 1.8, MeOH); UV (MeOH) $\lambda_{max} = 267.5$ nm ($\epsilon = 8400$); MS (ESI) *m*/*z* 279 (MH^+). Anal. ($C_{10}H_{12}F_2N_2O_3S\cdot 0.9H_2O$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]cytosine (19). Conversion of **11** (690 mg, 1.53 mol) to **19** was accomplished using the same procedure as described for **15/16**. The obtained residue was purified by flash silica gel column chromatography with 9% MeOH in CH_2Cl_2 to give **19** (343 mg, 1.30 mmol, 85% yield) as a white solid: $R_f = 0.15$ (CH_2Cl_2/MeOH, 10:1); mp >200 °C; $[\alpha]^{23}_D$ +35.5 (*c* 1, MeOH); UV (MeOH) $\lambda_{max} = 273.5$ nm ($\epsilon = 8057$); MS (ESI) *m*/*z* 264 (MH^+). Anal. ($C_9H_{11}F_2N_3O_2S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]cytosine (20). Conversion of **12** (750 mg, 1.66 mmol) to **20** was accomplished using the same procedure as described for **15/16**. The obtained residue was purified by a flash silica gel column chromatography with 9% MeOH in CH_2Cl_2 to give **20** (379 mg, 1.44 mmol, 87% yield) as a white solid: $R_f = 0.15$ (CH_2Cl_2/MeOH, 10:1); mp 198−201 °C; $[\alpha]^{24}_D$ −118.8 (*c* 0.9, MeOH); UV (MeOH) $\lambda_{max} = 273$ nm ($\epsilon = 7101$); MS (ESI) *m*/*z* 264 (MH^+). Anal. ($C_9H_{11}F_2N_3O_2S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl)-5-fluorocytosine (21). Conversion of **13** (740 mg, 1.47 mmol) to **21** was accomplished using the same procedure as described for **15/16**. The obtained residue was purified by flash silica gel column chromatography with 9% MeOH in CH_2Cl_2 to give **21** (364 mg, 1.29 mmol, 88% yield) as a white foam: $R_f = 0.32$ (CH_2Cl_2/MeOH, 5:1); $[\alpha]^{24}_D$ +21.4 (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max} = 242$ nm ($\epsilon = 7281$), 283 nm ($\epsilon = 6362$); MS (ESI) *m*/*z* 282 (MH^+). Anal. ($C_9H_{10}F_3N_3O_2S$) C, H, N.

1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]-5-fluorocytosine (22). Conversion of **14** (740 mg, 1.47 mmol) to **22** was accomplished using the same procedure as described for **15/16**. The obtained residue was purified by flash silica gel column chromatography with 9% MeOH in CH_2Cl_2 to give **22** (372 mg, 1.32 mmol, 90% yield) as a white foam: $R_f = 0.32$ (CH_2Cl_2/MeOH, 5:1); $[\alpha]^{24}_D$ −103.7 (*c* 1, MeOH); UV (MeOH) $\lambda_{max} = 241$ nm ($\epsilon = 6886$), 283 nm ($\epsilon = 5896$); MS (ESI) *m*/*z* 282 (MH^+). Anal. ($C_9H_{10}F_3N_3O_2S\cdot 0.2H_2O$) C, H, N.

6-Chloro-9-[(1*S*,4*S*)-2,3-dideoxy-3,3-difluoro-5-*O*-toluoyl-4-thio-α/β-L-ribofuranosyl]purine (23). By use of the same procedure as described for compound **9**, compound **8** (3.3 g, 10 mmol) was condensed with 6-chloropurine (2.0 g, 13 mmol) for 24 h to obtain compound **23** (2.4 g, 5.6 mmol, 56% yield) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.74, 8.71 (2 × s, 1H, H-2), 8.50 (s, 1H, H-8), 7.90−7.20 (m, 4H, Ar−H), 6.32 (m, 1H, H-1′), 4.84−4.20 (m, 3H, H-4′, H-5′), 3.20−3.14 (m, 2H, H-2′), 2.39, 2.38 (2 × s, 3H, $ArCH_3$). Anal. ($C_{18}H_{15}ClF_2N_4O_2S$) C, H, N.

2-Amino-6-chloro-9-[(1*S*,4*S*)-2,3-dideoxy-3,3-difluoro-5-*O*-toluoyl-4-thio-α/β-L-ribofuranosyl]purine (24). By use of the same procedure as described for compound **9**, compound **8** (3.3 g, 10 mmol) was condensed with 2-amino-6-chloropurine (2.2 g, 13 mmol) in anhydrous 1,4-dioxane (60 mL) for 12 h at 100 °C to produce compound **24** (1.76 g, 4.00 mmol, 40% yield) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.09, 7.95 (2 × s, 1H, H-8), 7.92−7.22 (m, 4H, Ar−H), 6.13, 6.03 (m, 1H, H-1′), 5.40, 5.17 (2 × br, 2H, NH_2), 5.07−4.47 (m, 3H, H-4′, H-5′), 3.42−3.05 (m, 2H, H-2′), 2.40, 2.39 (2 × s, 3H, $ArCH_3$). Anal. ($C_{18}H_{16}ClF_2N_5O_2S$) C, H, N.

9-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-furanosyl]adenine (25) and 9-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]adenine (26). A solution of **23** (1.0 g, 2.35 mmol) in methanolic ammonia (60 mL) was heated at 100 °C for 24 h in a steel bomb. After the mixture was cooled, the solvent was removed under reduced pressure and the residue was purified by a silica gel column chromatography (1% MeOH in CH_2Cl_2) to yield **25** (β anomer, 280 mg, 0.975 mmol, 41% yield) and **26** (α anomer, 290 mg, 1.01 mmol, 43% yield) as white solids with 84% overall yield.

Compound 25: $R_f = 0.43$ (CH_2Cl_2/MeOH, 10:1); mp >200 °C; $[\alpha]^{23}_D$ +13.5 (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max} = 259.5$ nm ($\epsilon = 9300$); MS (ESI) *m*/*z* 288 (MH^+). Anal. ($C_{10}H_{11}F_2N_5OS$) C, H, N.

Compound 26: $R_f = 0.33$ (CH_2Cl_2/MeOH, 10:1); mp 187−191 °C; $[\alpha]^{24}_D$ −83.7 (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max} = 260$ nm ($\epsilon = 8930$); MS (ESI) *m*/*z* 288 (MH^+). Anal. ($C_{10}H_{11}F_2N_5OS$) C, H, N.

9-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]hypoxanthine (27) and 9-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-α-L-furanosyl]hypoxanthine (28). To a solution of **23** (1.1 g, 2.59 mmol) in MeOH (20 mL), $NaOCH_3$ (840 mg) and 2-mercaptoethanol (900 mg) were added. The mixture was refluxed for 12 h, and the solvent was removed under reduced pressure. The syrupy residue was purified by silica gel column chromatography (3% MeOH in CH_2Cl_2) to yield **27** (β anomer, 295 mg, 1.02 mmol, 39% yield) and compound **28** (α anomer, 300 mg, 1.04 mmol, 40% yield) as white solids in 80% overall yield.

Compound 27: $R_f = 0.71$ (CH_2Cl_2/MeOH, 3:1); mp >200 °C; $[\alpha]^{24}_D$ +19.7 (*c* 0.25, MeOH); UV (MeOH) $\lambda_{max} = 246.5$ nm ($\epsilon = 14\,219$); MS (ESI) *m*/*z* 289 (MH^+). Anal. ($C_{10}H_{10}F_2N_4O_2S$) C, H, N.

Compound 28: $R_f = 0.57$ (CH_2Cl_2/MeOH, 3:1); mp >200 °C; $[\alpha]^{23}_D$ −111.8 (*c* 0.14, MeOH); UV (MeOH) $\lambda_{max} = 247$ nm ($\epsilon = 10\,912$); MS (ESI) *m*/*z* 289 (MH^+). Anal. ($C_{10}H_{10}F_2N_4O_2S$) C, H, N.

2-Amino-6-chloro-9-[(1*S*,4*S*)-2,3-dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]purine (29) and 2-Amino-6-chloro-9-[(1*S*,4*S*)-2,3-dideoxy-3,3-difluoro-4-thio-α-L-ribofuranosyl]purine (30). Conversion of **24** (1.5 g, 3.41 mmol) to **29**/**30** was performed using the same procedure as described for **15**/**16**. The obtained residue was purified by flash silica gel column chromatography with 9% MeOH in CH_2Cl_2 to afford pure compound **29** (β anomer, 475 mg, 1.47 mmol, 43% yield) and compound **30** (α anomer, 480 mg, 1.49 mmol, 44% yield) as white solids in 87% overall yield.

Compound 29: R_f = 0.25 (CH_2Cl_2/MeOH, 15:1); mp >200 °C; $[\alpha]^{24}_D$ +18.6 (*c* 0.5, MeOH); UV (MeOH) λ_{max} = 309.5 nm (ϵ = 9430). Anal. ($C_{10}H_{10}ClF_2N_5OS$) C, H, N.

Compound 30: R_f = 0.17 (CH_2Cl_2/MeOH, 15:1); mp 183−185 °C; $[\alpha]^{25}_D$ −74.2 (*c* 1.5, MeOH); UV (MeOH) λ_{max} = 309.5 nm (ϵ = 9169). Anal. ($C_{10}H_{10}ClF_2N_5OS$) C, H, N.

9-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-4-thio-β-L-ribofuranosyl]guanine (31). To a solution of **29** (400 mg, 1.24 mmol) in MeOH (20 mL), $NaOCH_3$ (420 mg) and 2-mercaptoethanol (450 mg) were added. The mixture was refluxed for 12 h, and the solvent was removed under reduced pressure. The syrupy residue was purified by silica gel column chromatography with 3% MeOH in CH_2Cl_2 to yield **31** (226 mg, 0.745 mmol, 60% yield) as a white solid: R_f = 0.71 (CH_2Cl_2/MeOH, 3:1); mp >200 °C; $[\alpha]^{25}_D$ −8.5 (*c* 0.3, MeOH); UV (MeOH) λ_{max} = 255.5 nm (ϵ = 10 105); MS (ESI) *m*/*z* 304 (MH^+). Anal. ($C_{10}H_{11}F_2N_5O_2S\cdot 1.4H_2O$) C, H, N.

General Procedure for Elimination Reactions. β-Difluoronucleosides (**15**, **17**, **19**, **21**, **25**, **27**, and **31**) were suspended or dissolved in anhydrous THF, and then 2−3 equiv of 1 M potassium *tert*-butoxide solution in THF was added at 0 °C. After being stirred at 0 °C for 12 h, the slurry suspension was quenched by adding MeOH. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography with 8% MeOH in CH_2Cl_2. The whole process was repeated several times to get the pure final compounds (**32**−**38**) in 30−40% yield.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-dideoxy-3-fluoro-4-thio-β-L-ribofuranosyl]uracil (32): foam; $[\alpha]^{22}_D$ +135.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} = 263.5 nm (ϵ = 9357); MS (ESI) *m*/*z* 245 (MH^+). Anal. ($C_9H_9FN_2O_3S$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]thymine (33): mp 195−197 °C; $[\alpha]^{23}_D$ +77.3 (*c* 0.4, MeOH); UV (MeOH) λ_{max} = 269 nm (ϵ = 7025); MS (ESI) *m*/*z* 259 (MH^+). Anal. ($C_{10}H_{11}FN_2O_3S$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]cytosine (34): mp 192−195 °C; $[\alpha]^{23}_D$ +225.1 (*c* 0.5, MeOH); UV (MeOH) λ_{max} = 275.5 nm (ϵ = 6825); MS (ESI) *m*/*z* 244 (MH^+). Anal. ($C_9H_{10}FN_3O_2S$) C, H, N.

(+)-1-[(1*S*,*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (35): mp 174−176 °C; $[\alpha]^{24}_D$ +223.1 (*c* 0.22, MeOH); UV (MeOH) λ_{max} = 284.5 nm (ϵ = 9247), 240.5 nm (ϵ = 10 500); MS (ESI) *m*/*z* 262 (MH^+). Anal. ($C_9H_9F_2N_3O_2S$) C, H, N.

(+)-9-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]adenine (36): mp >200 °C; $[\alpha]^{24}_D$ +32.2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} = 259.5 nm (ϵ = 8703); MS (ESI) *m*/*z* 268 (MH^+). Anal. ($C_{10}H_{10}FN_5OS$) C, H, N.

(+)-9-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]hypoxanthine (37): mp >200 °C; $[\alpha]^{23}_D$ +29.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} = 246.5 nm (ϵ = 8917); MS (ESI) *m*/*z* 269 (MH^+). Anal. ($C_{10}H_9FN_4O_2S\cdot 0.5H_2O$) C, H, N.

(+)-9-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-L-ribofuranosyl]guanine (38): mp >200 °C; $[\alpha]^{25}_D$ +64.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} = 256 nm (ϵ = 10 169); MS (ESI) *m*/*z* 284 (MH^+). Anal. ($C_{10}H_{10}FN_5O_2S\cdot H_2O$) C, H, N.

Benzoic acid 5-acetoxy-3,3-difluorotetrahydrothiophen-2-ylmethyl ester (39): ^{1}H NMR ($CDCl_3$) 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. ($C_{14}H_{14}F_2O_4S$) C, H.

(−)-1-[(1*R*,4*R*)-2,3-Dideoxy-2,3-dideoxy-3-fluoro-4-thio-β-D-ribofuranosyl]uracil (40): mp 150−152 °C; $[\alpha]^{25}_D$ −133.1 ° (*c* 0.19, MeOH); UV (H_2O) λ_{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. ($C_9H_9FN_2O_3S$) C, H, N.

(−)-1-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]thymine (41): mp 188−190 °C (dec); $[\alpha]^{26}_D$ −72.6° (*c* 0.29, CH_3COCH_3); UV (MeOH) λ_{max} = 268.5 nm (ϵ = 17 606) (pH 2), 269.0 nm (ϵ = 10 094) (pH 7), 265.0 nm (ϵ = 9977) (pH 11). Anal. ($C_{10}H_{11}FN_2O_3S$) C, H, N.

(−)-1-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]cytosine (42): mp 182−190 °C; $[\alpha]^{22}_D$ −230.5° (*c* 0.17, MeOH); UV λ_{max} = 276.0 nm (ϵ = 11 990) (pH 2), 267.5 nm (ϵ = 8010) (pH 7), 264.5 nm (ϵ = 8060) (pH 11). Anal. ($C_9H_{10}FN_3O_2S\cdot 0.4H_2O$) C, H, N.

(−)-1-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]-5-fluorocytosine (43): mp 172−174 °C; $[\alpha]^{25}_D$ −228.4° (*c* 0.14, CH_3OH); UV (H_2O) λ_{max} = 292.0 nm (ϵ = 10 178, pH 2), 283.0 nm (ϵ = 7192, pH 7), 283.0 nm (ϵ = 6290, pH 11). Anal. ($C_9H_9F_2N_3O_2S$) C, H, N.

(−)-9-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]adenine (44): mp >200 °C; $[\alpha]^{22}_D$ −34.6° (*c* 0.10, CH_3OH); UV (H_2O) λ_{max} = 260.0 nm (ϵ = 10 497, pH 2), 259.5 nm (ϵ = 7322, pH 7), 259.0 nm (ϵ = 11 275, pH 11). Anal. ($C_{10}H_{10}FN_5OS$) C, H, N.

(−)-9-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]hypoxanthine (45): mp >200 °C; $[\alpha]^{23}_D$ −27.7° (*c* 0.10, CH_3OH); UV (H_2O) λ_{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. ($C_{10}H_9FN_4O_2S$) C, H, N.

(−)-9-[(1*R*,4*R*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-4-thio-β-D-ribofuranosyl]guanine (46): mp >220 °C; $[\alpha]^{24}_D$ −66.9° (*c* 0.06, 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, pH 11). Anal. ($C_{10}H_{10}FN_5O_2S$) C, H, N.

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 1 h, either with 100 $TCID_{50}$/1 × 10^7 cells for a flask (T25) assay or with 200 $TCID_{50}$/6 × 10^5 cells per well for a 24-well plate assay. Cells were added to a plate or flask containing a 10-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/mL penicillin, 80 μg/mL streptomycin, 0.0008% DEAE-dextran, 0.045% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in humidified 5% CO_2−air at 37 °C for 5 days, and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12000 rpm for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 μL of 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. An amount of 10 μL of each sample was added to 75 μL of RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M $MgCl_2$, 0.006 mg/mL poly(rA)$_n$ oligo(dT)$_{12-18}$, 96 μg/mL dATP, and 1 μM of 0.08 mCi/mL ^{3}H-thymidine triphosphate (Moravek Biochemicals, Brea, CA)) and incubated at 37 °C for 2 h. The reaction was stopped by the addition of 100 μL of 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 β counter (Meriden, CT). The RT results were expressed in counts per minute (cpm) per milliliter. The antiviral 50% effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) were determined from the concentration−response curve using the median effect method.[22]

Cytotoxicity Assays. The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells and 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 the 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 densities 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 10-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 humidified 5% CO_2−air at 37°C. At the end of incubation, MTT tetrazolium dye solution (Cell titer 96, Promega, Madison, WI) was added to each well and incubated overnight. The reaction was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570 nm using an ELISA plate reader (Bio-tek instruments, Inc., Winooski, VT, model EL 312e). The 50% inhibition concentration (IC_{50}) was determined from the concentration−response curve using the median effect method.[22]

Molecular Modeling Study. (a) Conformational Analysis. The initial conformations of D- and l-3′F-4′Sd4C were constructed by a builder module in Spartan 5.1.1 (Wavefunctions, Inc., Irvine, CA). The initial conformations were cleaned up and geometrically optimized through quantum mechanical ab initio calculations using the RHF/6-31G** 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 on the basis of the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer−template duplex (PDB entry 1rtd). A model of the NRTI binding site was constructed that consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template−primer) duplex. The geometrically optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of the $(n + 1)$th nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety that was in the original X-ray structure (1rtd) 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. Gästeiger−Hückel charge was given to the enzyme−ligand complex with formal charges (+2) on two Mg atoms in the active site. Then, Kollman all-atom charges were loaded onto the enzyme site from the biopolymer module in Sybyl. 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 the Kollman all-atom force field until the iteration number reached 5000.

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Supporting Information Available: ^1H and ^{13}C NMR data for compounds **15**−**22** and **25**−**38**. This material is available free of charge via the Internet at http://pubs.acs.org.

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