# Synthesis, Anti-HIV Activity, and Molecular Mechanism of Drug Resistance of L-2′,3′-Didehydro-2′,3′-dideoxy-2′-fluoro-4′-thionucleosides

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

## Introduction

A number of L-nucleosides such as 3TC,[1,2] FTC,[3] L-FMAU,[4] L-d4FC,[5] and L-dT[6] have been discovered as potent antiviral agents. Particularly, 2′,3′-unsaturated nucleosides such as L-d4C,[5] L-d4FC,[5] and L-d4A[7] have drawn our attention because of their high antiviral potency. A combination of the unnatural L-configuration and the 2′,3′-unsaturated sugar moiety with 2′-fluoro substitution was worth exploring, since the 2′-fluoro moiety can stabilize the glycosidic bond. We have recently discovered 2′,3′-unsaturated L-2′-fluoronucleosides (β-L-2′-F-d4Ns) as potent antiviral agents against HIV-1 and hepatitis B virus (HBV).[8] Thus, to expand our efforts to discover more potent and less toxic antiviral agents, it was of interest to study the effect of isosteric replacement of 4′-oxygen by a sulfur atom. While there have been numerous efforts to modify the sugar as well as the heterocyclic bases of natural nucleosides for the purpose of developing new antiviral agents, 2′,3′-unsaturated 4′-thionucleosides have not been well investigated because of the synthetic difficulties.[9] In a recent communication,[10] we reported a stereoselective synthesis of L-2′,3′-didehydro-2′,3′-dideoxy-2′-fluoro-4′-thiocytidine (β-L-2′-F-4′-S-d4C), which showed potent anti-HIV activity ($EC_{50}$ = 0.12 μM) in human peripheral blood monomuclear (PBM) cells without cytotoxicity up to 100 μM. In this article, various pyrimidine and purine nucleosides were synthesized for the structure−activity relationship study. The cytosine derivative **17** (β-L-2′-F-4′-S-d4C) was also evaluated against a 3TC-resistant variant (HIV-$1_{M184V}$). To understand the molecular basis of the antiviral activity as well as the cross-resistance by the mutant RT strain (HIV-$1_{M184V}$), molecular modeling studies were conducted using the published crystal structure of HIV-1 reverse transcriptase.[11,12] Herein, we report the full accounts of the synthesis and biological evaluation of the titled nucleosides along with their molecular modeling studies.

## Results and Discussion

**Chemistry.** Several synthetic strategies were explored for the synthesis of our target nucleosides: (1) introduction of fluorine at the 2′-carbon by the modified Horner−Emmons reaction using a fluorinated phosphonate reagent, which has been successfully used by our group for syntheses of several fluorinated nucleosides,[13,14] (2) introduction of sulfur at the 4′-carbon by double inversion,[15] and (3) introduction of a phenylselenyl group at the 2′-carbon, resulting in β-selectivity during condensation with heterocyclic bases as well as generation of a double bond to give d4 nucleosides.[16] (*R*)-2-Fluorobutenolide **2** was prepared from 2,3-*O*-isopropylidene-L-glyceraldehyde **1** in three steps by a known method (Scheme 1).[13] Stereoselective hydrogenation of (*R*)-2-fluorobutenolide **2** by using 5% Pd/C smoothly occurred to give β-2-fluorolactone **3** in 95% yield. Hydrolysis of β-2-fluorolactone **3** using NaOH in aqueous EtOH followed by methylation of the resulting

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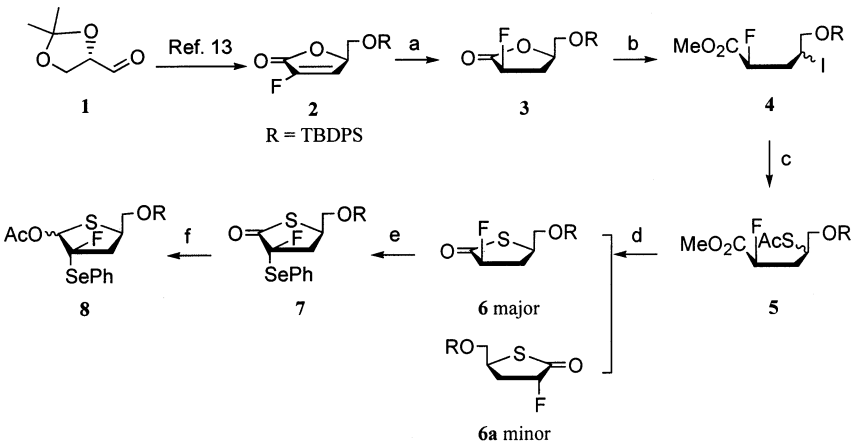
**Scheme 1**[a]

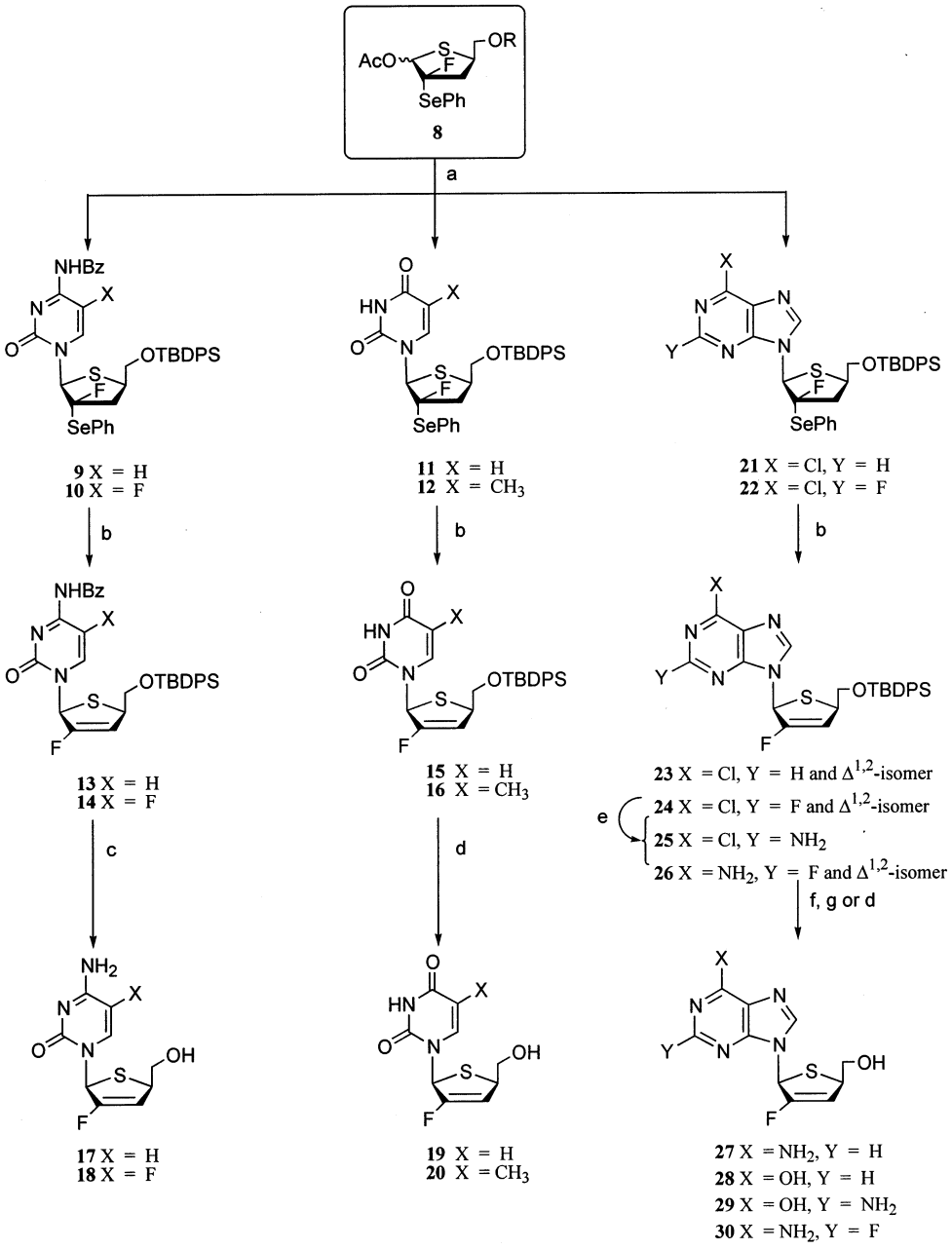



[a] (a) $H_2$, Pd/C, EtOAc; (b) (i) NaOH, EtOH, (ii) dimethyl sulfate, DMSO, (iii) $I_2$, $Ph_3P$, imidazole, toluene; (c) KSAc, DMF; (d) (i) DIBAL-H, toluene, −78 °C, (ii) DMSO, $Ac_2O$; (e) LiHMDS, TMSCl, PhSeBr, −78 °C; (f) (i) DIBAL-H, toluene, −78 °C, (ii) $Ac_2O$, TEA, $CH_2Cl_2$.

carboxylic acid gave a hydroxymethyl ester, 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 iodo esters **4** in 82% overall yield. No significant epimerization during saponification followed by methylation was found when the hydroxymethyl ester was treated with basic conditions to give the starting compound 2-fluorobutenolide **3**. The spectroscopic data ($^1$H and $^{13}$C NMR) of this compound matched the data of the starting compound. Iodination at high temperature and long reaction time, however, resulted in partial epimerization at C4. After optimization, the epimerization could be minimized by treatment of the hydroxymethyl ester under Mitsunobu conditions at 60 °C for 4 h. The inseparable epimeric mixture of iodo esters **4** was converted to an epimeric mixture of thiolacetates **5** in 91% yield by nucleophilic substitution with potassium thiolacetate in DMF. Reductive cyclization of the thiolacetates **5** using DIBAL-H in toluene at −78 °C followed by Moffatt-type oxidation provided the corresponding thiolactone **6** in 54% yield as well as the separable minor epimer **6a**. The thiolatone **6** was treated with LiHMDS and TMSCl followed by PhSeBr to give the phenylselenylthiolatone **7** stereoselectively (Scheme 1). Presumably, the phenylselenyl group approached the α-face of the TMS-enol ether plane predominantly because the *β*-face of the plane is sterically hindered by the bulky 5-*O*-TBDPS group, resulting in highly stereoselective addition**.** DIBAL reduction of the 2-fluoro-2-phenylselenothiolactone **7** followed by acetylation using $Ac_2O$ and triethylamine in $CH_2Cl_2$ gave the key intermediate **8** in 83% yield. The acetate **8** was then condensed with various pyrimidine heterocyclic bases under Vorbrüggen conditions to give the corresponding pyrimidine analogues **9**−**12** in 39−59% yield (Scheme 2). During the condensation, the *β*-anomer was obtained exclusively by virtue of the bulky α-phenylselenyl group.[16] Oxidation of the phenylselenyl group by mCPBA at −78 °C followed by treatment with pyridine gave the corresponding syn-eliminated 2′,3′-unsaturated compounds **13**−**16** in moderate to good yields (68−80%). The desired cytidine and 5-fluorocytidine analogues **17** and **18** were obtained by successive treatment with TBAF in THF and methanolic ammonia. On the other hand, the TBDPS protecting groups at 5′-positions of the sugar moieties of compounds **15** and **16** were removed by treatment with TBAF in THF to give the desired 2′-fluoro-4′-thio-2′,3′-unsaturated uracil and thymine nucleosides **19** and **20**, respectively.

To synthesize the purine analogues **27**−**30**, the key intermediate **8** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give the corresponding nucleosides **21** and **22** in 73% and 67% yield, respectively (Scheme 2). The 6-chloropurine derivative **21** was treated with mCPBA followed by pyridine to give a syn-eliminated inseparable mixture of 2′,3′-unsaturated nucleoside **23** and its $\Delta^{1,2}$-isomer in 86% yield (3:1 determined by $^1$H NMR). Under the same reaction conditions, the 2-fluoro-6-chloropurine derivative **22** also gave an inseparable mixture of the syn-eliminated product **24** and its $\Delta^{1,2}$-isomer in 71% yield (2:1 determined by $^1$H NMR). A mixture of compound **23**, its $\Delta^{1,2}$-isomer, and methanolic ammonia was heated at 100 °C in a steel bomb to give the corresponding adenosine analogue, which was deprotected by TBAF in THF to give the 2′-fluoro-4′-thio-2′,3′-unsaturated adenosine **27** in 57% yield. The $\Delta^{1,2}$-isomer, however, was unstable under the reaction conditions and decomposed. The 2′,3′-unsaturated 6-chloropurine derivative **23** was treated with sodium methoxide and 2-mercaptoethanol in refluxing methanol to give a hypoxanthine derivative, which was converted to the final 2′-fluoro-4′-thio-2′,3′-unsaturated inosine derivative **28** in 81% yield. On the other hand, a solution of **24** and its $\Delta^{1,2}$-isomer in ethylene glycol dimethyl ether (DME) was bubbled with dry ammonia at room temperature for 16 h to give the 2-amino-6-chloropurine **25** and 2-fluoro-6-aminopurine derivative **26** in 49% and 28% yield, respectively. The $\Delta^{1,2}$-isomer of **24**, however, decomposed under the reaction as well as under purification conditions presumably because of its instability. The 2-amino-6-chloropurine derivative **25** was hydrolyzed by using sodium methoxide and 2-mercaptoethanol in refluxing methanol to give the final 2′,3′-unsaturated 2′-fluoro-4′-thioguanosine **29** in 61% yield. The 2′,3′-unsaturated 2′-fluoro-4′-thio-2-fluoroadenosine **30** was obtained by deprotection of the TBDPS group of the 2-fluoro-6-aminopurine derivative **26** by TBAF in THF in 70% yield.

**Scheme 2**[a]




[a] (a) HMDS, $CH_3CN$, pyrimidines or purines; TMSOTf, room temp, (b) mCPBA, −78 °C; pyridine, room temp; (c) (i) TBAF, THF, (ii) $NH_3$, MeOH, room temp; (d) TBAF, THF, room temp; (e) $NH_3$ bubbling, DME, room temp; (f) (i) $NH_3$, MeOH, 80 °C, (ii) TBAF, THF, room temp; (g) (i) $HSCH_2CH_2OH$, NaOMe, 60 °C, (ii) TBAF, THF, room temp.

**Anti-HIV Activity.** The synthesized pyrimidine (**17**−**20**) and purine (**27**−**30**) nucleosides were evaluated for their anti-HIV activity and cytotoxicity in vitro, and results are summarized in Table 1. The anti-HIV activity was evaluated in PBM cells infected with HIV-1, and AZT was included as a positive control.[17] The cytotoxicity of the synthesized nucleosides was also assessed in human PBM, CEM, and Vero cells. Among these nucleosides, two pyrimidine nucleosides, cytidine **17** ($EC_{50}$ = 0.12 $\mu$M) and 5-fluorocytidine **18** ($EC_{50}$ = 0.15 $\mu$M), showed the most potent anti-HIV-1 activity, and the purine nucleosides, adenosine **27** ($EC_{50}$ = 1.7 $\mu$M), inosine **28** ($EC_{50}$ = 15.5 $\mu$M), guanosine **29** ($EC_{50}$ = 43.5 $\mu$M) and 2-fluoroadenosine **30** ($EC_{50}$ = 11.5 $\mu$M), also showed moderate antiviral activity whereas significant cytotoxicity was observed in 2-fluoroadenosine **30** ($IC_{50}$ = 13.0, 10.4, and 66.1 $\mu$M for PBM, CEM, and Vero cells, respectively). However, all other synthesized nucleosides showed no significant cytotoxicity. Despite the replacement of an oxygen atom ($\beta$-L-2′-F-d4N) by a sulfur atom ($\beta$-L-2′-F-4′-S-d4N), the anti-HIV activity was generally maintained.

**Antiviral Activity against Lamivudine (3TC)-Resistant (HIV-$1_{M184V}$) Mutant Strain.** Although 3TC is the most commonly used nucleoside in combination therapy for HIV-1 infection,[18] 3TC monotherapy results in the selection of a 3TC-resistant viral variant, resulting in prompt rebound in plasma viral load.[19] High-level resistance to 3TC is conferred by a single mutation at codon 184 (M184V) in the catalytic domain of HIV-1 RT, and the M184V substitution increases the 50% inhibitory concentration of 3TC at least 1000-fold.[20,21] This

**Table 1.** Activity of L-2′,3′-Didehydro-2′,3′-dideoxy-2′-fluoro-4′-thionucleosides

| compd | configuration and base | anti-HIV-1 activity ($EC_{50}$, $\mu$M) PBM | cytotoxicity ($IC_{50}$, $\mu$M) PBM | CEM | Vero |
|---|---|---|---|---|---|
| **17** | L-cytosine | 0.12 | >100 | >100 | >100 |
| **18** | L-5-F-cytosine | 0.15 | >100 | >100 | >100 |
| **19** | L-uracil | >100 | >100 | >100 | >100 |
| **20** | L-thymine | >100 | >100 | >100 | >100 |
| **27** | L-adenine | 1.7 | >100 | >100 | >100 |
| **28** | L-hypoxanthine | 15.5 | >100 | >100 | >100 |
| **29** | L-guanine | 43.5 | >100 | 41.5 | 66.4 |
| **30** | L-2-F-adenine | 11.5 | 13.0 | 10.4 | 66.1 |
| AZT | | 0.004 | >100 | 29.0 | 14.3 |

**Table 2.** Correlation of Relative Binding Energy Difference ($\Delta E_{Rel}$) with Fold Increase (FI)

| compd | WT (xxBRU) activity ($EC_{90}$, $\mu$M) | WT (xxBRU) $E_{rel}$[a] (kcal/mol) | M184V activity ($EC_{90}$, $\mu$M) | M184V $E_{rel}$[a] (kcal/mol) | FI[b] | $\Delta E_{rel}$[c] (kcal/mol) |
|---|---|---|---|---|---|---|
| $\beta$-L-2′-F-4′-S-d4C **17** | 1.4 | 86.0 | >100 | −21.5 | >100 | 107.5 |
| $\beta$-L-2′-F-d4C | 2.1 | 36.8 | >100 | −70.0 | >100 | 106.8 |
| $\beta$-D-2′-F-d4C | 8.0 | 44.3 | 1.8 | −6.5 | 0.2 | 50.8 |

[a] Relative binding energy ($E_{rel}$) is the binding energy of nucleoside triphosphate minus the binding energy of dCTP. [b] $EC_{90}$(HIV-$1_{M184V}$)/$EC_{90}$(HIV-$1_{xxBRU}$). [c] Relative binding energy difference: $\Delta E_{rel} = E_{rel}(WT) - E_{rel}(M184V)$.

viral resistance to 3TC prompted the discovery of nucleosides with antiviral activity against HIV-1 isolates containing common 3TC resistance mutation. It is noteworthy that the recently U.S. FDA approved anti-HIV drug, tenofovir, showed unique activity against AZT- as well as 3TC-resistant mutant HIV strains.[22] In view of this resistance issue, antiviral activity of the cytosine analogue (**17**) was evaluated against the lamivudine-resistant mutant strain (HIV-$1_{M184V}$) along with other 2′,3′-unsaturated nucleosides such as $\beta$-L-2′-F-d4C[8] and $\beta$-D-2′-F-d4C[23] (Table 2). Among these nucleosides, only $\beta$-D-2′-F-d4C[23] showed antiviral activity against the M184V mutant, whereas its L-congener ($\beta$-L-2′-F-d4C)[8] and the 4′-sulfur-containing nucleoside ($\beta$-L-2′-F-4′-S-d4C **17**) were significantly cross-resistant. From this study, it is apparent that the unnatural L-sugar configuration of 2′-fluoro-2′,3′-unsaturated nucleosides cannot be accommodated by the mutation at the codon 184 of HIV-1 RT. Therefore, it was of interest to investigate the molecular basis of drug resistance by M184V mutant to various 2′-F-2′,3′-unsaturated nucleosides (vide infra).

**Molecular Modeling Studies.** To understand the molecular basis of the antiviral activity and resistance profiles of the two types of nucleosides (2′-F-4′-S-d4N vs 2′-F-d4N) as well as the role of the isosterically substituted sulfur atom, we conducted molecular modeling studies of the representative cytidine analogues (2′-F-d4C and 2′-F-4′-S-d4C). Our molecular modeling studies mainly focused on the interactions of the nucleoside triphosphates with the viral polymerase HIV-1 RT. The crystal structure of HIV-1 RT, complexed with thymidine triphosphate and DNA duplex reported by Huang et al.,[11] enabled us to perform molecular modeling studies of the triphosphates of several nucleoside reverse transcriptase inhibitors (NRTIs). Previously, we qualitatively demonstrated that the calculated binding affinity of NRTIs correlates with anti-HIV activity.[12,24] In our current studies, quantum mechanical ab initio calculations at the level of RHF/3-21G* using Spartan 5.1.1 (Wavefunctions, Inc.) were performed on the cytidine analogues ($\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C), and the geometry-optimized structures were compared (Figure 1a). The two geometry-optimized structures were nicely superimposed on each other except for positions of the 4′-oxygen and 4′-sulfur. Because the sulfur atom has a longer van der Waals radius than the oxygen atom, the 4′-sulfur was positioned slightly outside of the 4′-oxygen when two cytidine analogues were superimposed. However, the distance between the heterocyclic base and the 5′-hydroxy group, which has been considered as the key factor[25,26] in binding of the nucleosides to the nucleoside kinases, is similar in the two classes, as shown in Figure 1a. From this result, we may assume that both $\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C may be potentially the substrates of nucleoside kinases, such as 2′-deoxycytidine kinase. After the rate-limiting initial phosphorylation of the synthesized nucleosides, their triphosphates could be readily synthesized by cellular nucleotide kinases.[27] Recently it was also reported that L-nucleoside diphosphates are phosphorylated by 3-phosphoglycerate kinase.[28] The triphosphates of geometry-optimized $\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C, $\beta$-L-2′-F-4′-S-d4CTP and $\beta$-L-2′-F-d4CTP were manually docked into the truncated catalytic site of HIV-1 RT (Lys1−Pro243 in p66 subunit), and the resulting enzyme−inhibitor complexes were energy-minimized using the Kollman all-atom force field.[29] Both minimized structures showed that the triphosphates of $\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C were bound tightly to the active site of HIV-1 RT (parts 1c and 1d of Figure 1). In addition to the structural similarity, which was shown in Figure 1a, their binding modes to the HIV-1 RT were also similar in several aspects. Arg72 at the active site of HIV-1 RT plays a key role in binding the triphosphates of $\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C. The guanidinium moiety of Arg72 approaches the active site in order to establish multiple hydrogen bonds with the triphosphates of $\beta$-L-2′-F-4′-S-d4CTP and $\beta$-L-2′-F-d4CTP, resulting in stabilization of the nucleoside triphosphates. The backbone amides, which are part of

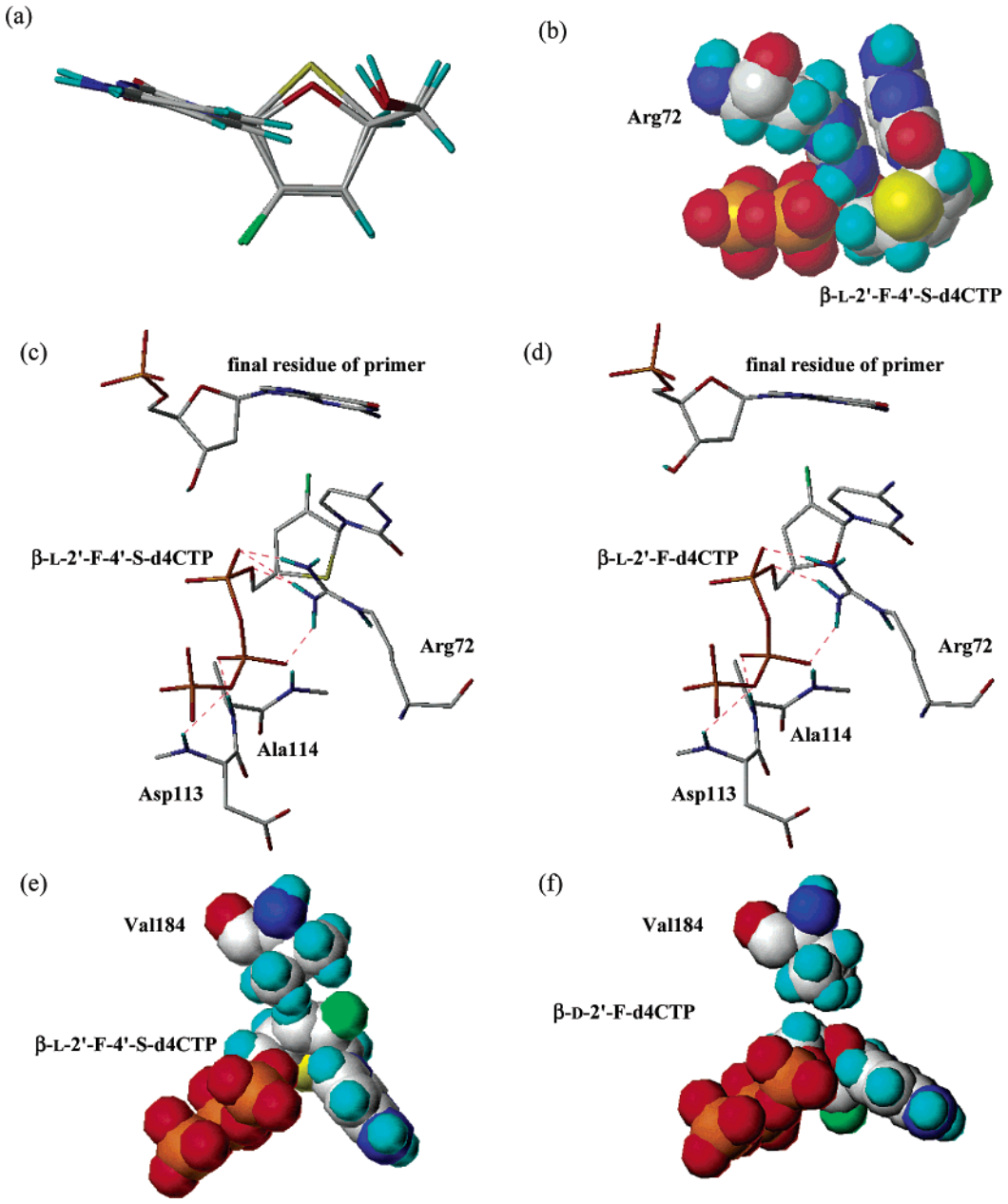



**Figure 1.** (a) Superimposed structures of *β*-L-2′-F-d4C and *β*-L-2′-F-4′-S-d4C. (b) CPK structure showing interaction of *β*-L-2′-F-4′-S-d4CTP with Arg72. (c) Minimized structure of *β*-L-2′-F-4′-S-d4CTP after docking to the active site of HIV-1 reverse transcriptase. (d) Minimized structure of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

3′-OH pocket residues (Asp113 and Ala114), also stabilize the triphosphates through the hydrogen bonding interaction. Even though the 4′-sulfur atom in *β*-L-2′-F-4′-S-d4C **17** is closer to Arg72 compared to the 4′-oxygen in *β*-L-2′-F-d4C, there was no destabilizing steric hindrance between the 4′-sulfur atom and the guanidinium moiety of Arg72, which allowed stable orientation of *β*-L-2′-F-4′-S-d4C **17** at the active site (Figure 1b). The conformational analysis and binding affinity studies indicate that *β*-L-2′-F-4′-S-d4C **17** and *β*-L-2′-F-d4C have similar structures, and the substitution of a sulfur atom is well tolerated at the polymerase level, which could explain their similar antiviral potency in the wild-type RT. However, the M184V mutation in HIV-1 RT causes a serious problem in positioning the L-configured nucleoside triphosphates at the active site because the branched methyl groups of Val184 tend to occupy the space where the sugar moiety of L-2′-F-2′,3′-unsaturated nucleosides projects. The resulting steric hindrance destabilized the L-2′-F-2′,3′-unsaturated nucleoside triphosphate−RT complex (Figure 1e), which can be confirmed by the highly decreased relative binding energies of L-nucleosides (Table 2). On the other hand, the structure of the *β*-D-2′-F-d4CTP−RT complex does not show any significant steric crash between Val184 and the sugar moiety of the nucleoside triphosphate because the natural D-configured sugar moiety was located on the opposite side (Figure 1f). The potent anti-HIV-$1_{M184V}$ activity of *β*-D-2′-F-d4C supports this concept.

In summary, we have developed an efficient synthetic methodology for *β*-L-2′,3′-didehydro-2′,3′-dideoxy-2′-

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

## Experimental Section

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

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

**(2*R*,4*R*/*S*)-5-*tert*-Butyldiphenylsilyloxy-2-fluoro-4-iodopentanoic Acid Methyl Ester (4).** A mixture of compound **3** (12.2 g, 32.6 mmol) in 5% aqueous EtOH was treated with solid NaOH (1.44 g, 36.0 mmol) at room temperature for 2 h. The resulting mixture was concentrated and coevaporated two times with 250 mL of toluene to dryness. The crude carboxylate sodium salt was dissolved in 15 mL of DMSO and treated with dimethyl sulfate (3.71 mL, 39.2 mmol) at 0 °C. After addition, the ice bath was removed. The reaction mixture was stirred for 1 h and then poured into ice-cooled water (500 mL) and extracted with ethyl ether (3 × 200 mL). The combined organic layer was washed with water (3 × 200 mL), dried over $MgSO_4$, and concentrated to dryness. The crude methyl ester was treated with $I_2$ (14.9 g, 58.7 mmol), imidazole (6.67 g, 98.0 mmol), and $Ph_3P$ (17.1 g, 65.3 mmol) in toluene (300 mL) at 60 °C for 4 h. Aqueous $NaHCO_3$ (200 mL) was added to the resulting mixture, and iodine was added portionwise until the iodine color persisted, indicating the absence of remaining $Ph_3P$. Aqueous $Na_2S_2O_3$ was added dropwise until the iodine color disappeared, indicating the absence of the remaining iodine. The resulting mixture was poured to a separatory funnel, diluted with 300 mL of toluene, washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography with 7% EtOAc in hexanes to give compounds **4** (13.7 g, 26.6 mmol, 82% yield) as a pale-yellow oil: $^1$H NMR ($CDCl_3$) for the major δ 7.75−7.35 (m, 10H), 5.10 (ddd, *J* = 48.4, 7.5, 5.2 Hz, 1H), 4.33−4.18 (m, 1H), 3.95−3.78 (m 2H), 3.82 (s, 3H) 2.80−2.40 (m, 2H), 1.10 (s, 9H); $^1$H NMR ($CDCl_3$) for the minor δ 7.75−7.35 (m, 10H), 5.18 (ddd, *J* = 49.2, 10.8, 2.3 Hz, 1H), 4.33−4.18 (m, 1H), 3.95−3.78 (m 2H), 3.83 (s, 3H), 2.45−2.10 (m, 2H), 1.09 (s, 9H); HRMS (FAB) obsd, *m*/*z* 515.0972, calcd for $C_{22}H_{29}FIO_3Si$, *m*/*z* 515.0915 $(M + H)^+$. Anal. ($C_{22}H_{28}FIO_3Si{\cdot}0.05C_6H_{14}$) C, H.

**(2*R*,4*S*/*R*)-4-Acetylsulfanyl-5-*tert*-butyldiphenylsilyloxy-2-fluoropentanoic Acid Methyl Ester (5).** A solution of compounds **4** (13.7 g, 26.6 mmol) in 20 mL of DMF was treated with solid KSAc (6.08 g, 53.2 mmol) at room temperature for 8 h. The resulting mixture was diluted with EtOAc (500 mL), washed with water (2 × 200 mL), dried over $MgSO_4$, filtered, concentrated and purified by column chromatography with 10% EtOAc in hexanes to give products **5** (11.14 g, 24.1 mmol, 91% yield) as a red-brown oil: $^1$H NMR ($CDCl_3$) for the major δ 7.67−7.35 (m, 10H), 5.12−4.92 (m, 1H), 3.93−3.68 (m, 3H), 3.81 (s, 3H), 2.44−2.10 (m, 2H), 2.32 (s, 3H), 1.057 (s, 9H); $^1$H NMR ($CDCl_3$) for the minor δ 7.67−7.35 (m, 10H), 5.12−4.92 (m, 1H), 3.93−3.68 (m, 3H), 3.81 (s, 3H), 2.44−2.10 (m, 2H), 2.30 (s, 3H), 1.062 (s, 9H); HRMS (FAB) obsd, *m*/*z* 463.1789, calcd for $C_{24}H_{32}FO_4SSi$, *m*/*z* 463.1775 $(M + H)^+$. Anal. ($C_{24}H_{31}FO_4SSi$) C, H, S.

**(2*R*,4*R*)-(−)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-thiobutyrolactone (6).** A solution of compounds **5** (11.1 g, 24.1 mmol) in toluene (200 mL) was treated with 53 mL of 1 M DIBAL-H in hexane at −78 °C for 1 h. The reaction was quenched with 12 mL of MeOH and warmed to room temperature for 1 h, and aqueous $NaHCO_3$ (23 mL) and EtOAc (200 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to dryness. The crude thiolactol was treated with $Ac_2O$ (34 mL) and DMSO (35 mL) at room temperature for 24 h. The reaction mixture was poured into a separatory funnel containing ice-cooled water (300 mL) and extracted with ethyl ether (3 × 300 mL). The combined organic layer was washed with water (3 × 300 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography with 5% $Et_2O$ in hexanes to give the products **6** (5.08 g, 13.1 mmol, 54% yield) and **6a** (0.85 g, 2.2 mmol, 9% yield) as a yellow oil. For **6**: $[\alpha]^{25}_D$ −29.4° (*c* 1.18, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 7.68−7.37 (m, 10H), 5.07 (ddd, *J* = 50.5, 10.2, 6.9 MHz, 1H), 3.96−3.78 (m, 3H), 2.72−2.62 (m, 1H), 2.18− 2.07 (m, 1H), 1.07 (s,9H); $^{13}$C NMR ($CDCl_3$) δ 200.70 (d, *J* = 17.4 MHz), 135.50, 132.66, 132.58, 129.99, 127.85, 93.26 (d, *J* = 196.9 MHz), 66.61, 43.97 (d, *J* = 7.1 MHz), 32.58 (d, *J* = 19.6 MHz), 26.68, 19.21. Anal. ($C_{21}H_{25}FO_2SSi$) C, H, S. For **6a**: $[\alpha]^{25}_D$ 50.7° (*c* 1.3, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 7.66−7.40 (m, 10H), 5.18 (dt, *J* = 44.4, 7.0 Hz, 1H), 4.02 (quint, *J* = 5.0 Hz), 3.86 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.84 (dd, *J* = 10.8, 5.0 Hz, 1H), 2.88−2.78 (m, 2H), 1.07 (s, 9H). Anal. ($C_{21}H_{25}FO_2SSi$) C, H, S.

**(2*S*,4*R*)-(−)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl-γ-thiobutyrolactone (7).** To a solution of compound **6** (5.08 g, 13.1 mmol) in THF (60 mL), 15.7 mL of 1 M LiHMDS in THF was added slowly at −78 °C, and the reaction mixture was stirred at the same temperature for 1 h. TMSCl (2.16 mL, 17 mmol) was added dropwise to the reaction mixture, and the mixture was warmed to room temperature. The resulting mixture was stirred at room temperature for 30 min and cooled to −78 °C. A solution of PhSeBr (4.69 g, 19.6 mmol) in THF (20 mL) was rapidly added, and the mixture was stirred at −78 °C for 1 h. The mixture was diluted with ethyl ether (300 mL), washed with water (4 × 100 mL), dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography with 3% $Et_2O$ in hexanes to give the desired product **7** (5.28 g, 9.69 mmol, 74% yield) as a pale-yellow syrup: $[\alpha]^{24}_D$ −56.4° (*c* 0.542, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 7.70−7.35 (m, 15H), 3.97−3.77 (m, 3H), 2.49 (dd, *J* = 13.3, 4.5 Hz, 1H), 2.22 (td, *J* = 14.4, 10.5 Hz, 1H), 1.05 (s, 9H); $^{13}$C NMR ($CDCl_3$) δ 196.34 (d, *J* = 22.7 Hz), 137.03, 135.53, 132.63, 132.50, 130.03, 129.39, 127.87, 124.79, 105.13 (d, *J* = 260.6

Hz), 65.67, 44.59 (d, $J = 2.9$ Hz), 39.13 (d, $J = 21.4$ Hz), 26.70, 19.21; HRMS (FAB) obsd, *m/z* 545.0873, calcd for $C_{27}H_{30}FO_2SSeSi$, *m/z* 545.0885 $(M + H)^+$. Anal. ($C_{27}H_{29}FO_2SSeSi \cdot 0.01H_2O$) C, H, S.

**(1*S*/*R*,2*S*,4*R*)-1-*O*-Acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranoside (8).** A solution of compound **7** (4.98 g, 9.14 mmol) in toluene (100 mL) was treated with 18.3 mL of 1 M DIBAL-H in hexane at −78 °C for 1 h. The reaction was quenched with 4 mL of MeOH, the mixture was warmed to room temperature for 1 h, and aqueous $NaHCO_3$ (8 mL) and EtOAc (100 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to dryness. A solution of the crude thiolactol in $CH_2Cl_2$ (100 mL) was treated with $Ac_2O$ (2.6 mL, 28 mmol), TEA (3.8 mL, 28 mmol), and a catalytic amount of 4-DMAP at room temperature for 3 h. The resulting mixture was concentrated and purified by silica gel column chromatography with 3% $Et_2O$ in hexanes to give the acetate **8** (4.48 g, 7.61 mmol, 83% yield) as a pale-yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.70−7.32 (m, 15H), 6.03, 5.99 (d & s, $J = 7.6$ Hz, 1H), 3.76−3.60 (m, 3H), 2.60−2.53 (m, 1H), 2.41−2.31 (m, 1H), 2.13, 2.01 (2s, 3H), 1.04, 0.99 (2s, 9H). Anal. ($C_{29}H_{28}FO_3SSeSi$) C, H, S.

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

**(−)-*N*$^4$-Benzoyl-1-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]cytosine (9).** A mixture of $N^4$-benzoylcytosine (0.548 g, 2.55 mmol) in HMDS (15 mL) and $CH_3CN$ (15 mL) was refluxed for 5 h. After removal of the solvent using a vacuum pump, a solution of the acetate **8** (0.500 g, 0.849 mmol) in 15 mL of $CH_3CN$ was added to the reaction flask containing the silylated $N^4$-benzoylcytosine, and then TMSOTf (0.31 mL, 1.7 mmol) was added dropwise at room temperature. After 16 h, the reaction was quenched with 1 mL of saturated $NaHCO_3$ and the resulting mixture was concentrated to one-fifth of the volume. The crude mixture was diluted with 100 mL of $CH_2Cl_2$, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 30% EtOAc in hexanes to give cytosine derivative **9** (0.300 g, 0.404 mmol, 48% yield) as a foam: $[\alpha]^{24}_D$ −168.38° (*c* 0.486, $CH_2Cl_2$); UV(MeOH) $\lambda_{max}$ 308 nm. Anal. ($C_{38}H_{38}FN_3O_3SSeSi$) C, H, N, S.

**(−)-*N*$^4$-Benzoyl-1-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (10).** See the general procedure for the condensation reaction of the acetate **8** with pyrimidines. The title compound **10** was obtained in 59% yield (0.849 mmol): $[\alpha]^{24}_D$ −209.1° (*c* 0.693, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 332.5 nm; FABMS *m/z* 762 $(M + H)^+$. Anal. ($C_{38}H_{37}F_2N_3O_3SSeSi$) C, H, N, S.

**(−)-1-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]uracil (11).** See the general procedure for the condensation reaction of the acetate **8** with pyrimidines. The title compound **11** was obtained in 48% yield (0.849 mmol): $[\alpha]^{23}_D$ −129.2° (*c* 0.578, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 262.5 nm. Anal. ($C_{31}H_{33}FN_2O_3SSeSi$) C, H, N, S.

**(−)-1-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]thymine (12).** See the general procedure forthe condensation reaction of the acetate **8** with pyrimidines. The title compound **12** was obtained in 38% yield (0.849 mmol): $[\alpha]^{26}_D$ −130.6° (*c* 0.464, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ ($CH_2Cl_2$) 266.0 nm; FABMS *m/z* 655 $(M + H)^+$. Anal. ($C_{32}H_{35}FN_2O_3SSeSi$) C, H, N, S.

**General Procedure for Syn Elimination Reaction Using mCPBA to give 2′,3′-Unsaturated Nucleosides.** The preparation of cytosine derivative **13** is representative.

**(+)-*N*$^4$-Benzoyl-1-[(1*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]cytosine (13).** To a solution of compound **9** (0.169 g, 0.228 mmol) in 5 mL of $CH_2Cl_2$, a solution of mCPBA (57−86%, 69 mg) in 5 mL of $CH_2Cl_2$ was added at −78 °C, and the mixture was stirred at −78 °C for 30 min. Pyridine (0.07 mL, 0.9 mmol) was then added, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 50 mL of $CH_2Cl_2$, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 30% EtOAc in hexanes to give the eliminated product **13** (0.107 g, 0.183 mmol, 80% yield) as a foam: $[\alpha]^{24}_D$ 128.81° (*c* 0.515, $CH_2Cl_2$); UV(MeOH) $\lambda_{max}$ 308 nm. Anal. ($C_{32}H_{32}FN_3O_3SSi \cdot 0.1C_6H_{14}$) C, H, N, S.

**(+)-*N*$^4$-Benzoyl-1-[(1*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (14).** See the general procedure for the syn elimination reaction. The title compound **14** was obtained in 73% yield (0.493 mmol): $[\alpha]^{26}_D$ 152.58° (*c* 0.485, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 331.0 nm; FABMS *m/z* 604 $(M + H)^+$. Anal. ($C_{32}H_{31}FN_3O_3SSi$) C, H, N, S.

**(+)-1-[(1*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]uracil (15).** See the general procedure for the syn elimination reaction. The title compound **15** was obtained in 68% yield (0.411 mmol): $[\alpha]^{26}_D$ 77.7° (*c* 0.492, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 262.5 nm; HRMS (FAB) obsd, *m/z* 483.1572, calcd for $C_{25}H_{28}FN_2O_3SSi$, *m/z* 483.1574 $(M + H)^+$. Anal. ($C_{25}H_{27}FN_2O_3SSi$) C, H, N, S.

**(+)-1-[(1*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]thymine (16).** See the general procedure for the syn elimination reaction. The title compound **16** was obtained in 76% yield (0.326 mmol): $[\alpha]^{26}_D$ 50.7° (*c* 0.530, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 266.0 nm; HRMS (FAB) obsd, *m/z* 497.1725, calcd for $C_{26}H_{30}FN_2O_3SSi$, *m/z* 497.1730 $(M + H)^+$. Anal. ($C_{26}H_{29}FN_2O_3SSi$) C, H, N, S.

**General Procedure for Successive Deprotections of Protected Unsaturated Nucleosides.** The preparation of cytidine analogue **17** is representative.

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

**(+)-1-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-5-fluorocytosine (18).** See the general procedure for successive deprotections. The title compound **18** was obtained in 88% yield (0.356 mmol): mp 189−190 °C; $[\alpha]^{24}_D$ 169.2° (*c* 0.159, MeOH); UV($H_2O$) $\lambda_{max}$ 286.0 nm ($\epsilon$ 8600, pH 2). 282.0 nm ($\epsilon$ 7500, pH 7), 282.0 nm ($\epsilon$ 6900, pH 11). Anal. ($C_9H_9F_2N_3O_2S$) C, H, N, S.

**General Procedure for Desilylation Reaction of *tert*-Butyldiphenyl Ether by Using TBAF.** The preparation of uridine analogue **19** from compound **15** is representative.

**(+)-1-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]uracil (19).** A solution of compound **15** (0.135 g, 0.280 mmol) in THF (10 mL) was treated with 0.33 mL of 1 M TBAF in THF at room temperature for 2 h. The mixture was concentrated and purified by silica gel column chromatography with 3% MeOH in $CHCl_3$ to give the desired product **19** (0.063 g, 0.258 mmol, 92% yield) as a white solid: mp 184−185 °C; $[\alpha]^{24}_D$ 132.9° (*c* 0.103, MeOH); UV($H_2O$) $\lambda_{max}$ 263.0 nm ($\epsilon$ 9100, pH 2). 263.0 nm ($\epsilon$ 9700, pH 7), 263.5 nm ($\epsilon$ 7800, pH 11). Anal. ($C_9H_9FN_2O_3S$) C, H, N, S.

**(+)-1-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]thymine (20).** See the general procedure for desilylation reaction and trituation with $Et_2O$. The

title compound **20** was obtained in 91% yield (0.248 mmol): mp 174−176 °C; $[\alpha]^{24}_D$ 50.4° (*c* 0.180, MeOH); UV($H_2O$) $\lambda_{max}$ 268.5 nm ($\epsilon$ 5700, pH 2), 268.5 nm ($\epsilon$ 5500, pH 7), 269.0 nm ($\epsilon$ 4500, pH 11). Anal. ($C_{10}H_{11}FN_2O_3S \cdot 0.35Et_2O$) C, H, N, S.

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

**(−)-9-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-6-chloropurine (21).** A mixture of 6-chloropurine (0.789 g, 5.10 mmol) and ammonium sulfate (0.112 g, 0.85 mmol) in 30 mL of HMDS was refluxed for 5 h. HMDS was evaporated to give a yellow solid. To this flask containing the silylated 6-chloropurine, a solution of the acetate **8** (1.0 g, 1.70 mmol) in 1,2-dichloroethane (30 mL) was added. The resulting slurry was cooled to −25 °C and TMSOTf (0.62 mL, 3.40 mmol) was added dropwise at −25 °C. The reaction mixture was stirred for 3 h at −25 to −10 °C, for 8 h at room temperature, and for 5 h at 40 °C. The resulting mixture was diluted with 300 mL of $CH_2Cl_2$, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 11% EtOAc in hexanes to give compound **21** (0.847 g, 1.24 mmol, 73% yield) as a pale-yellow foam: $[\alpha]^{24}_D$ −90.0° (*c* 1.287, $CHCl_3$); UV($CH_2Cl_2$) $\lambda_{max}$ 264.5 nm. Anal. ($C_{32}H_{32}ClFN_4OSSeSi$) C, H, N, S.

**(−)-9-[(1*S*,2*S*,4*R*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-L-ribofuranosyl]-6-chloro-2-fluoropurine (22).** See the general procedure for condensation reaction of the acetate **8** with purines. The title compound **22** was obtained on in 67% yield (1.70 mmol): $[\alpha]^{24}_D$ −86.5° (*c* 1.44, $CHCl_3$); UV($CHCl_3$) $\lambda_{max}$ 266.0 nm. Anal. ($C_{32}H_{31}ClF_2N_4OSSeSi$) C, H, N, S.

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

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

**(+)-2-Amino-9-[(1*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-6-chloropurine (25), 6-Amino-9-[(1*S*,4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoropurine (26) and 6-Amino-9-[(4*R*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoropurine ($\Delta^{1,2}$-Isomer).** Dry ammonia gas was bubbled into a stirred solution of a mixture of compound **24** and its $\Delta^{1,2}$-isomer (0.430 g, 0.79 mmol) in 1,2-dimethoxyethane (25 mL) at room temperature 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 **25** (0.209 g, 0.387 mmol, 49% yield) and a mixture of compound **26** and its $\Delta^{1,2}$-isomer (0.116 g, 0.221 mmol, 28% yield). For compound **25**: $[\alpha]^{24}_D$ 74.0° (*c* 0.236, $CH_2Cl_2$); UV($CH_2Cl_2$) $\lambda_{max}$ 300.0 nm. Anal. ($C_{26}H_{27}ClFN_5OSSi \cdot 0.3EtOAc$) C, H, N, S. For a mixture of compounds **26** and $\Delta^{1,2}$-isomer: UV($CHCl_3$) $\lambda_{max}$ 261.0 nm. $^1$H NMR: see Supporting Information.

**(−)-9-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]adenine (27).** A mixture of compound **23** and its $\Delta^{1,2}$-isomer (0.241 g, 0.46 mmol) was treated with methanolic ammonia at 80 °C for 12 h using a steel bomb. Solvent was evaporated, and the residue was dried under vacuum for 3 h. A solution of the dried crude product in 30 mL of THF was treated with 0.5 mL of 1 M TBAF in THF at room temperature for 3 h. The resulting mixture was concentrated and purified by preparative TLC chromatography with 5−10% MeOH in $CH_2Cl_2$ to give the desired compound **27** (0.070 g, 0.26 mmol, 57% yield) as a white solid: mp 192−194 °C; $[\alpha]^{26}_D$ −38.1° (*c* 0.165, MeOH); UV($H_2O$) $\lambda_{max}$ 258.0 nm ($\epsilon$ 9500, pH 2), 259.0 nm ($\epsilon$ 8900, pH 7), 259.5 nm ($\epsilon$ 8600, pH 11); HRMS (FAB) obsd, *m*/*z* 268.0669, calcd for $C_{10}H_{11}FN_5OS$, *m*/*z* 268.0668 $(M + H)^+$. Anal. ($C_{10}H_{10}FN_5OS$) C, H, N, S.

**(−)-9-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]hypoxanthine (28).** A mixture of compound **23** and its $\Delta^{1,2}$-isomer (0.216 g, 0.41 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.12 mL, 1.65 mmol) and NaOMe (0.091 g, 1.69 mmol) at 60 °C for 24 h. The resulting mixture was quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel with 4% MeOH in $CH_2Cl_2$. The filtrate was concentrated to dryness. The crude product was treated with 0.84 mL of 1 M TBAF in THF at room temperature for 3 h. The reaction mixture was concentrated and purified by preparative TLC chromatography with 7% MeOH in $CH_2Cl_2$ to give the desired compound **28** (0.089 g, 0.33 mmol, 81% yield) as a white solid: mp 143−145 °C (dec); $[\alpha]^{23}_D$ −23.7° (*c* 0.324, 7:1 $CH_2Cl_2$/MeOH); UV($H_2O$) $\lambda_{max}$ 246.5 nm ($\epsilon$ 12 000, pH 2). 248.0 nm ($\epsilon$ 10 600, pH 7), 253.5 nm ($\epsilon$ 10 900, pH 11); HRMS (FAB) obsd, *m*/*z* 269.0517, calcd for $C_{10}H_9FN_4O_2S$, *m*/*z* 269.0509 $(M + H)^+$. Anal. ($C_{10}H_9FN_4O_2S$) C, H, N, S.

**(+)-9-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]guanosine (29).** See the procedure for the preparation of compound **28**. The title compound **29** was obtained in 61% yield (0.16 mmol): mp 210 °C (dec); $[\alpha]^{25}_D$ 75.2° (*c* 0.15, DMSO); UV($H_2O$) $\lambda_{max}$ 256.0 nm ($\epsilon$ 11 100, pH 2). 255.0 nm ($\epsilon$ 12 500, pH 7), 266.0 nm ($\epsilon$ 10 700, pH 11). Anal. ($C_{10}H_{10}FN_5O_2S \cdot 0.2H_2O$) C, H, N, S.

**(−)-9-[(1*S*,4*R*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-L-ribofuranosyl]-2-fluoroadenosine (30).** See the general procedure of desilylation and trituation with $Et_2O$. The title compound **30** was obtained in 70% yield (0.20 mmol): mp >300 °C; $[\alpha]^{26}_D$ −17.8° (*c* 0.200, MeOH); UV($H_2O$) $\lambda_{max}$ 260.5 nm ($\epsilon$ 12 700, pH 2). 260.5 nm ($\epsilon$ 12 700, pH 7), 261.5 nm ($\epsilon$ 12 200, pH 11). Anal. ($C_{10}H_9F_2N_5OS$) C, H, N, S.

**Antiviral Assay.** Human peripheral blood mononuclear (PBM) cells (obtained from Atlanta Red Cross) were isolated by Ficoll−Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2−3 days prior to use. HIV-$1_{LA}$I obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular 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 $TCID_{50}/1 \times 10^7$ cells for a flask (T25) assay or with 200 $TCID_{50}/6 \times 10^5$ cells/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. The assay medium was RPMI-1640 supplemented with heat-inactivated 16% fetal bovine serum, 1.6 mM l-glutamine, 80 IU/mL penicillin, 80 μg/mL streptomycin, 0.0008% DEAE-dextran, 0.045% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in a humidified 5% $CO_2$/air at 37 °C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12 000 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 M

dithiothreitol, 0.006 mg/mL poly(rA)$_n$oligo(dT)$_{12-18}$, 96 $\mu$g/mL dATP, and 1 $\mu$M 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 $\mu$L 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 $\beta$-counter (Meriden, CT). The RT results were expressed in counts per minute (CPM) per milliliter. The antiviral 50% effective concentration ($EC_{50}$) and 90% effective concentration ($EC_{90}$) were determined from the concentration−response curve using the median effect method.[30]

**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 in 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 a density of $5 \times 10^3$, $2.5 \times 10^3$, and $5 \times 10^4$ 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 a humidified 5% $CO_2$/air at 37 °C. At the end of incubation, MTT tetrazolium dye solution (Cell titer 96, Promega, Madison, WI) was added to each well and incubated overnight. The reaction was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570 nm using an ELISA plate reader (Bio-tek instruments, Inc., Winooski, VT, model EL 312e). The 50% inhibition concentration ($IC_{50}$) was determined from the concentration−response curve using the median effect method.[30]

**Molecular Modeling Studies. (a) Conformational Analysis.** The initial conformations of $\beta$-L-2′-F-4′-S-d4C **17** and $\beta$-L-2′-F-d4C were constructed by the builder module in Spartan 5.1.1 (Wavefunctions, Inc. Irvine, CA), and all calculations were performed on a Silicon Graphics O2 workstation. The initial conformations were cleaned up and geometry-optimized through quantum mechanical ab initio calculation using the RHF/3-21G* basis in Spartan 5.1.1.

**(b) Binding Affinity 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 geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of $(n + 1)$th nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety in the original X-ray structure (1rtd) was modified to guanine. The inhibitor triphosphates were manually docked into the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure. The Gästeiger−Hückel charge was given to the enzyme−ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman all-atom charges were loaded onto enzyme site from the biopolymer module in Sybyl 6.7. Fluorine parameters were obtained from the literature[31,32] and MM2 parameters and put into the parameter files. To eliminate local strains resulting from merging inhibitors, residues within 6 Å from the merged inhibitors and mutated residues were annealed until the 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:** Tables of $^1$H NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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