Stereoselective Synthesis and Antiviral Activity of D-2′,3′-Didehydro-2′,3′-dideoxy-2′-fluoro-4′-thionucleosides

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As 2′,3′-didehydro-2′,3′-dideoxy-2′-fluoronucleosides have exhibited interesting antiviral effects against HIV-1 as well as HBV, it is of interest to synthesize the isosterically substituted 4′-thionucleosides in which 4′-oxygen is replaced by a sulfur atom. To study structure−activity relationships, various pyrimidine and purine nucleosides were synthesized from the key intermediate (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranoside **8**, which was prepared from the 2,3-*O*-isopropylidene-D-glyceraldehyde **1** in 13 steps. The antiviral activity of the synthesized compounds were evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells, among which cytidine **17**, 5-fluorocytidine **18**, adenosine **24**, and 2-fluoroadenosine **32** showed moderate to potent anti-HIV activities (EC$_{50}$ 1.3, 11.6, 8.1, and 1.2 μM, respectively). It is noteworthy that 2-fluoroadenosine analogue **32** showed antiviral potency as well as high cytotoxicity (IC$_{50}$ 1.5, 1.1, and 7.6 μM for PBM, CEM, and Vero, respectively) whereas no other compound showed cytotoxicity up to 100 μM. The cytidine **17** and 5-fluorocytidine **18** analogues showed significantly decreased antiviral activity against the clinically important lamivudine-resistant variants (HIV-1$_{M184V}$), whereas the corresponding D-2′-Fd4 nucleosides showed limited cross-resistance. Molecular modeling studies demonstrated that the larger van der Waals radius as well as the close proximity to Met184 of the 4′-sulfur atom of D-2′-F-4′-Sd4C (**17**) may be the reasons for the decreased antiviral potency of synthesized 4′-thio nucleosides against the lamivudine-resistant variants (HIV-1$_{M184V}$).

Introduction

Nucleoside analogue reverse transcriptase inhibitors continue to be important drug regimens as part of highly active antiretroviral therapy (HAART). However, because of the drug toxicity[1,2] and the lack of a durable response due to resistance,[3] there is clearly a need for new compounds to cope with these drawbacks. Particularly, compounds with activity against HIV isolates that are resistant to currently available therapies as well as agents with beneficial pharmacokinetic profiles that allow infrequent dosing are under active investigation.

The past decade has witnessed the emergence of a class of 2′,3′-unsaturated nucleosides such as d4T,[4,5] L-d4C,[6,7] L-d4FC,[6,7] and abacavir[8,9] as interesting therapeutic candidates for anti-HIV therapy because of their potent antiviral activity. The importance of this class of compounds has been confirmed after a recent study which showed that the instability of the purine analogues can be remarkably improved by the isosteric replacement of 2′-hydrogen by fluorine atom.[10] Therefore, agents containing the 2′,3′-unsaturated sugar moiety with 2′-fluoro substitution has become one of the rational targets in search for safe, effective, and chemically stable antiviral agents. Thus, it was of interest to synthesize the nucleosides with isosteric replacement of 4′-oxygen by sulfur atom. The first example of 4′-thionucleosides was reported in 1964 by Reist et al., who synthesized the 4′-thio counterpart of naturally occurring adenosine.[11] Since then, several classes of 4′-thionucleosides have been reported,[12] including 9-(4-thio-D-xylofuranosyl)adenine,[13] 9-(4-thio-D-arabinofuranosyl)adenine,[13] and 4′-thio-araC.[14] However, the difficulty of synthesizing optically pure 4′-thionucleosides has impaired additional syntheses of these analogues, and only a few examples have been known until recently.[15] Moreover, biologically interesting 2′-deoxy-4′-thionucleosides had not been synthesized until Walker[16] and Secrist[17] independently reported the syntheses of pyrimidine 2′-deoxy-4′-thionucleosides in 1991, which were followed by an alternative synthesis of 2′-deoxy-4′-thionucleosides using the Sharpless asymmetric epoxidation,[18] synthesis of 4′-thio-2′,3′-dideoxynucleosides,[19] and the syntheses of 4′-thioarabinonucleosides[20] as well as 2′-modified 2′-deoxy-4′-thiocytidines.[21] The synthesized 4′-thio-2′-deoxy, 4′-thio-2′,3′-dideoxy and 4′-thioarabino nucleosides have shown to have potent anti-herpes, anti-HIV, and anti-cytomegalovirus activities, respectively, and some analogues, especially 4′-thiothymidine and 2′-deoxy-4′-thiocytidine, have exhibited potent cytotoxicity.[16−19] Interestingly, the 4′-thionucleosides are usually resistant to hydrolytic cleavage of glycosyl linkage catalyzed by nucleoside phosphorylase,[22] which is one of the advantages of 4′-

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**Scheme 1.** Synthesis of the Key Intermediate **8**[a]
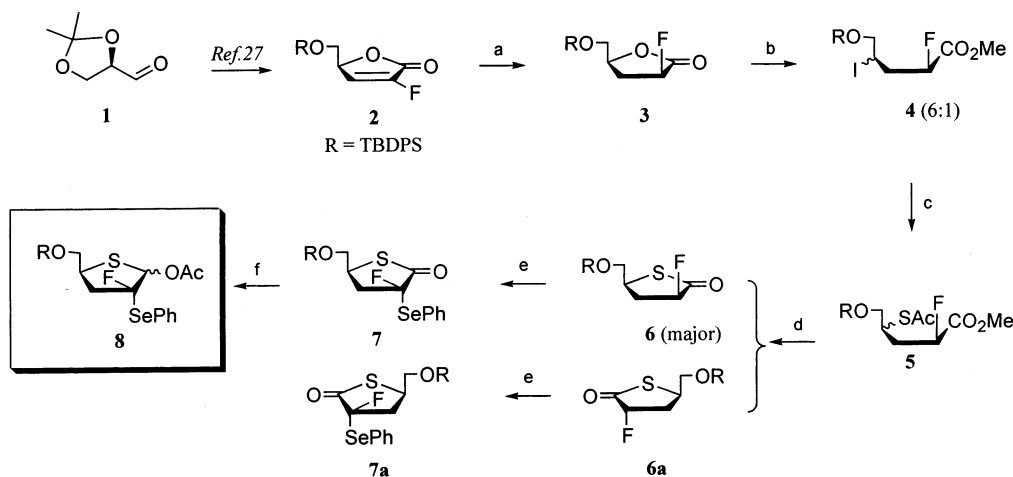
[a] Keys (a) H_2, Pd/C, EtOAc, (b) (i) NaOH, EtOH, (ii) dimethyl sulfate, DMSO, (iii) I_2, Ph_3P, imidazole, toluene, (c) KSAc, DMF, (d) (i) DIBAL-H, toluene, −78 °C, (ii) DMSO, Ac_2O, (e) LiHMDS, TMSCl, PhSeBr, −78 °C, (f) (i) DIBAL-H, toluene, −78 °C, (ii) Ac_2O, TEA, CH_2Cl_2.

thionucleosides compared with several metabolically unstable "4'-oxy" antiviral agents which are substrates for nucleoside phosphorylase.[23] Even though 4'-thionucleosides have recently received considerable attention as potential antiviral agents, their 2',3'-unsaturated analogues have not been well investigated probably because of the synthetic difficulties. A report by Young et al., however, reemphasized the importance of this class of nucleosides, in which L-4'-thio-d4C analogues showed marked anti-HBV as well and anti-HIV activity.[24] In a recent communication, we reported the stereoselective synthesis of the β-L-2'-F-4'-Sd4C which showed potent anti-HIV activity (EC$_{50}$ 0.12 μM) in human peripheral blood monomuclear (PBM) cells.[25] By using the same synthetic strategy, a series of D-counterparts were synthesized for the structure−activity relationship studies. Various pyrimidine and purine nucleosides were synthesized from the key intermediate, (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranoside **8**, which was prepared from the 2,3-*O*-isopropylidene-D-glyceraldehyde **1** in 13 steps. The antiviral activities of the synthesized compounds were evaluated against HIV-1 in human PBM cells. The antiviral activity of cytidine **17** and 5-fluorocytidine **18** analogues were also examined against the clinically important lamivudine-resistant variants (HIV-1$_{M184V}$). To understand the reduced antiviral activity of the synthesized β-D-2'-F-4'-Sd4N compared with β-D-2'-Fd4N[10] and the cross-resistance of the lamivudine-resistant variants (HIV-1$_{M184V}$) to β-D-2'-F-4'-Sd4C (**17**) and β-D-2'-F-4'-Sd4FC (**18**), molecular modeling studies including a quantum mechanical geometry optimization of β-D-2'-F-4'-Sd4C (**17**) as well as energy-minimization of HIV-1 RT/β-D-2'-F-4'-Sd4C (**17**) complex were performed. Herein, we report the full accounts of the synthesis, biological evaluation, and molecular modeling studies of the titled nucleosides.
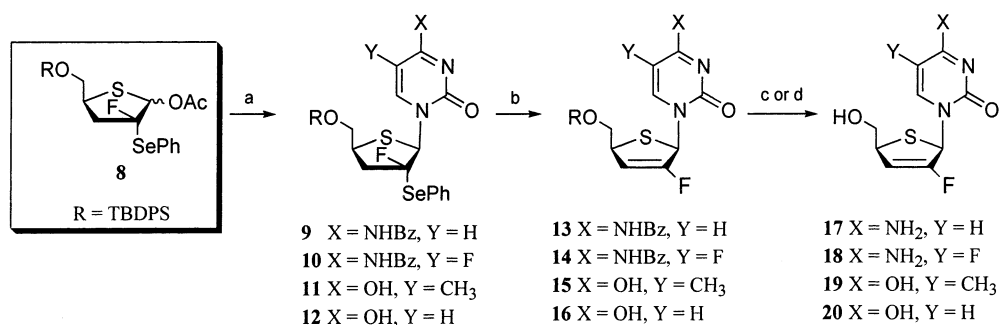
Results and Discussion

Chemistry. Introduction of a sulfur atom at the 4'-position of a sugar ring has been hampered by the laborious synthetic procedures. The synthetic methods known to date employ either nucleophilic attack of dimesylate by disulfide anion,[20,21] ring closure of dithioacetal,[20] or reductive cyclization of thioacetic acid ester.[19] 2-Fluoro-2-buten-4-olide **2**, which was used as the key intermediate for the synthesis of 2'-fluoro-2',3'-unsaturated nucleosides,[10] can also be used for this purpose because the butenolide ring can be converted into the appropriate intermediates (dimesylate, dithioacetal, and thioacetic acid ester) for any of those three reactions after some manipulations. The attempted syntheses of these intermediates from the 2-fuoro-2-buten-4-olide **2**, however, gave very complicated mixtures presumably because of the instability of fluorovinyl moiety. The transformation was possible only after saturation of the butenolide **2** by catalytic hydrogenation. Therefore, 2-fluoro-γ-butyrolactone **3** was used as a platform for the generation of the key intermediate (2*R*,4*S*)-1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranoside **8** (Scheme 1). (*R*)-2-Fluorobutenolide **2** was prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde **1** in three steps by a known method (Scheme 1).[26] (*S*)-2-Fluorobutenolide **2** was hydrogenated to β-2-fluorolactone **3** by treatment with 5% Pd/C under H_2 in a quantitative yield. The ^{1}H NMR of the crude compound **3** showed no contamination by the epimer at C2. The lactone **3** was converted to iodo ester **4** in three consecutive steps. Hydrolysis using NaOH in aqueous EtOH followed by methylation of the corresponding carboxylic acid gave hydroxy methyl ester **3a** (Scheme 2), which was treated with iodine, triphenylphosphine, and imidazole in toluene at 60 °C for 4 h to give a 6:1 epimeric mixture of the iodo ester **4** in 83% overall yield (Scheme 1). To confirm which carbon center has epimerized, the hydroxyl methyl ester **3a** was subjected to reclosure of the ring to give the starting 2-fluorobutenolide **3** under basic conditions (Scheme 2). The spectroscopic data (^{1}H and ^{13}C NMR) of this compound matched that of the starting compound, which suggested that there was no significant epimerization during saponification followed by methylation. Iodination under several different reaction conditions, however, confirmed that high temperature and longer reaction time resulted in partial

Scheme 2. Epimerization at C4 during Iodination[a]

R = TBDPS
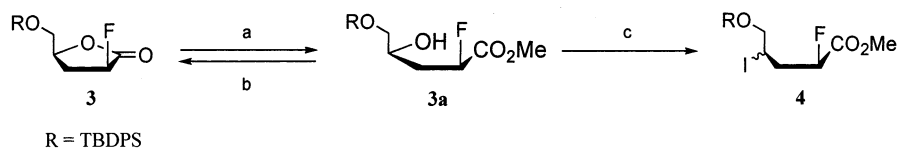
[a] Keys: (a) (i) NaOH, EtOH, (ii) dimethyl sulfate, DMSO, (b) pyridine, rt, (c) I_2, Ph_3P, imidazole, toluene.

Scheme 3. Synthesis of the Pyrimidine Nucleosides **17−20**[a]

R = TBDPS

9 X = NHBz, Y = H	**13** X = NHBz, Y = H	**17** X = NH_2, Y = H
10 X = NHBz, Y = F	**14** X = NHBz, Y = F	**18** X = NH_2, Y = F
11 X = OH, Y = CH_3	**15** X = OH, Y = CH_3	**19** X = OH, Y = CH_3
12 X = OH, Y = H	**16** X = OH, Y = H	**20** X = OH, Y = H

[a] Keys: (a) HMDS, CH_3CN, pyrimidines; TMSOTf, (b) mCPBA, −78 °C; pyridine, rt, (c) (i) TBAF, THF, (ii) NH_3, MEOH, rt, (d) TBAF, THF.

epimerization at C4. Treatment of the hydroxyl methyl ester **3a** under Mitsunobu conditions at 60 °C for 4 h was found to be the optimum conditions to minimize the epimerization. The epimeric mixture of iodo ester **4** was subjected to nucleophilic attack by potassium thioacetate in DMF to give an epimeric mixture of thioacetates **5**. DIBAL-mediated reduction of the thioacetates **5** followed by Moffat-type oxidation provided the corresponding thiolactone **6** and **6a**, in 54% yield. At this stage, the two epimers could be separated by column chromatography on silica gel. During the ensuing phenylselenylation, the C2 position was sp[2]-hybridized by trapping the enolate as silyl enol ether. The phenylselenyl group approached the least hindered α face of the silyl enol ether to give the corresponding enantiomers **7** and **7a** (Scheme 1). The optical rotation values of these two compounds were matched with opposite signs {for **7**; $[\alpha]^{24}_D$ 54.0° (c 0.606, $CHCl_3$) and for **7a**; $[\alpha]^{24}_D$ −56.4° (c 0.542, $CHCl_3$)}, which finally confirmed the epimerization at C4 during iodination under Mitsunobu conditions. The epimerized product **7a**, therefore, can be recycled as the key intermediate for the synthesis of L-antipodes. DIBAL reduction of the 2-fluoro-2-phenylselenothiolactone **7** followed by acetylation using Ac_2O and triethylamine in CH_2Cl_2 gave the key intermediate (2R,4S)-1-O-acetyl-5-O-(tert-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranoside **8** in 90% yield (Scheme 1). Condensation of **8** with various pyrimidine heterocyclic bases under Vorbrüggen conditions gave the corresponding pyrimidine analogues **9−12** in 40−60% yield (Scheme 2). The condensation gave the β-anomer exclusively by virtue of the bulky α-phenylselenyl group.[27] Oxidation of the phenylselenide group by mCPBA at low temperature followed by treatment with pyridine gave the corresponding syn-eliminated 2′,3′-unsaturated compounds **13−16** in high yields (70−80%) (Scheme 3). The protecting groups on the heterocyclic base and the 5′ position of the sugar moiety were sequentially deblocked by the treatment with TBAF in THF and methanolic

ammonia to give the desired 2′-fluoro-4′-thio-2′,3′-unsaturated pyrimidine nucleosides **17−20** (Scheme 3).

The synthesis of purine analogues (**24**, **25**, **30**, and **32**) needed more careful treatments (Scheme 4). The key intermediate **8** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give the corresponding nucleosides **21** and **26** in 65% and 66% yield, respectively. To achieve a clean and high-yield conversion, the temperature of the reaction mixture had to be carefully controlled in such a manner that, after addition of TMSOTf at 0 °C, the reaction mixture was stirred for 6 h at room temperature and then for 2 h at 60 °C. The 6-chloropurine derivative **21** was syn-eliminated by successive treatment with mCPBA and pyridine to give an inseparable mixture of 2′,3′-unsaturated nucleoside **22** and its $\Delta^{1,2}$-isomer **23** in 81% yield (3:1 determined by ^{1}H NMR). Amination of the mixture of **22** and **23** by treatment with methanolic ammonia at 100 °C in a steel bomb gave the adenosine analogue, which was deprotected by TBAF in THF to give 2′-fluoro-4′-thio-2′,3′-unsaturated adenosine **24** in 61% yield. The $\Delta^{1,2}$-isomer **23**, however, did not survive under reaction conditions and silica gel column chromatography due to its instability. On the other hand, the 2′,3′-unsaturated 6-chloropurine derivative **22** was hydrolyzed in the presence of sodium methoxide and 2-mercaptoethanol in refluxing methanol to give the hypoxanthine derivative, which was converted to the desired 2′-fluoro-4′-thio-2′,3′-unsaturated inosine **25** in 46% yield (yield is not optimized). The 2-fluoro-6-chloropurine derivative **26** was also treated with mCPBA followed by pyridine to give an inseparable mixture of the syn-eliminated product **27** and its $\Delta^{1,2}$-isomer **28** in 71% yield. Dry ammonia gas was bubbled into a solution of **27** and **28** in ethylene glycol dimethyl ether (DME) at room temperature for 16 h to give the 2-amino-6-chloropurine derivative **29** and 2-fluoro-6-aminopurine derivative **31** in 45% and 25% yield, respectively, which were readily separated by silica gel column chromatography. Unfortunately, the $\Delta^{1,2}$-isomer **28** was not stable enough to survive the reaction and purification conditions. The

Scheme 4. Synthesis of the Purine Nucelosides **24−25**, **30**, and **32**[a]
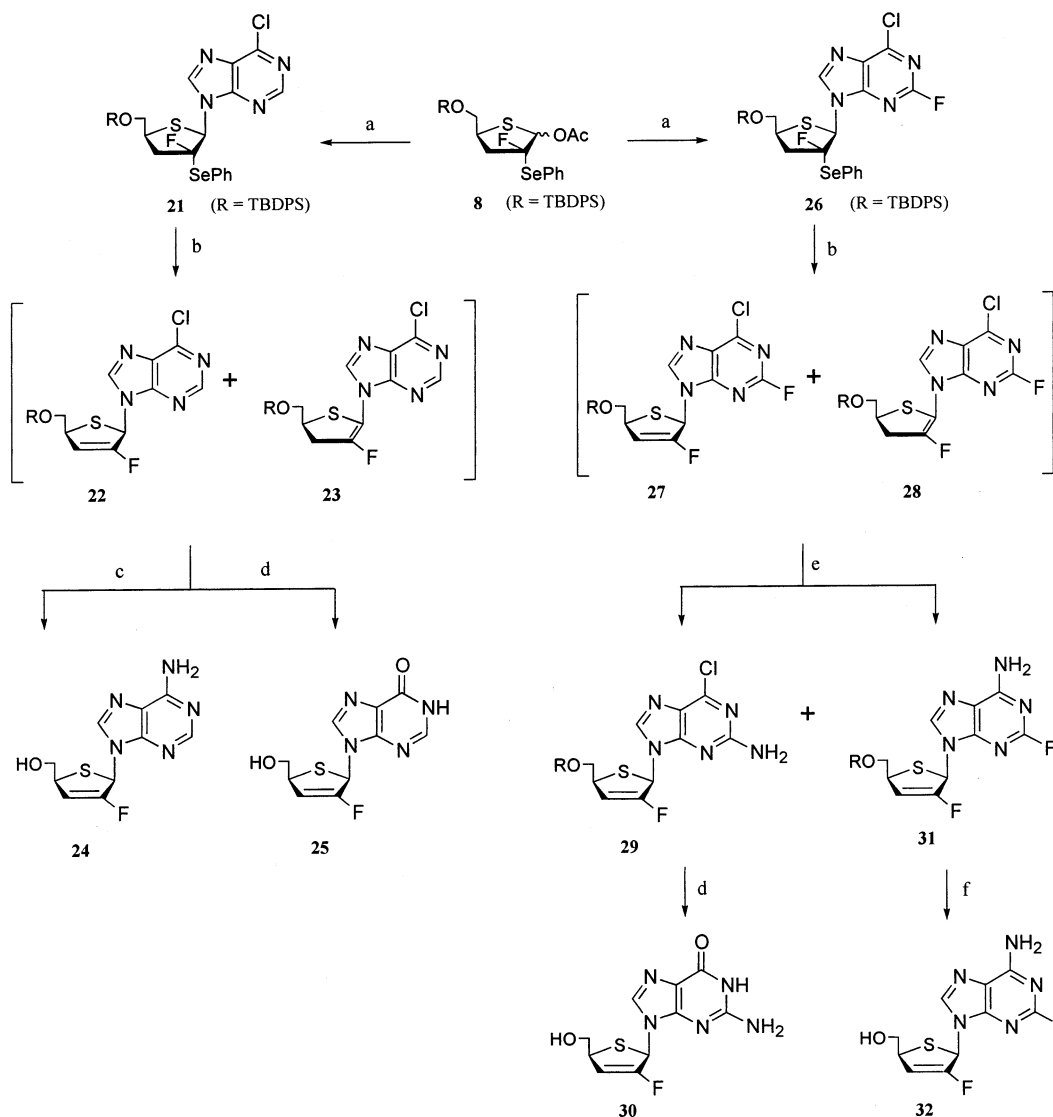
[a] Keys: (a) HMDS, $(NH_4)_2SO_4$, purines; TMSOTf, (b) mCPBA, −78 °C; pyridine, rt, (c) (i) NH_3, MeOH, 80 °C, (ii) TBAF, THF, (d) (i) $HSCH_2CH_2OH$, NaOH, 60 °C, (ii) TBAF, THF, (e) NH_3 bubbling, DME, rt, (f) TBAF, THF.

2-amino-6-chloropurine derivative **29**, thus obtained, was hydrolyzed by using sodium methoxide and 2-mercaptoethanol in refluxing methanol to give the final 2'-fluoro-4'-thio-2',3'-unsaturated guanosine **30** in 61% yield. The 2-fluoro-6-aminopurine derivative **31**, on the other hand, was deprotected by TBAF in THF to afford the 2'-fluoro-4'-thio-2',3'-unsaturated 2-fluoroadenosine **32** in 70% yield.

Structure−Activity Relationships. The synthesized pyrimidine (**17−20**) and purine (**24**, **25**, **30**, and **32**) nucleosides were evaluated against HIV-1 in human PBM cells in vitro, and AZT was included as a positive control. The results are summarized in Table 1. Among the tested nucleosides, two pyrimidine nucleosides, cytidine **17** (EC$_{50}$ 1.3 μM) and 5-fluorocytidine **18** (EC$_{50}$ 11.6 μM), and all purine nucleosides synthesized, adenosine **24** (EC$_{50}$ 8.1 μM), inosine **25** (EC$_{50}$ 43.6 μM), guanosine **30** (EC$_{50}$ 80.5 μM), and 2-fluoroadenosine **32** (EC$_{50}$ 1.2 μM), showed moderate to potent antiviral activities. It is noteworthy that 2-fluoroadenosine analogue **32** showed antiviral potency as well as high cytotoxicity (IC$_{50}$ 1.5, 1.1, and 7.6 μM for PBM, CEM,

Table 1. Anti-HIV Activity and Cytotoxicity of
D-2',3'-Didehydro-2',3'-dideoxy-2'-fluoro-4'-thionucleosides

compound	activity (EC$_{50}$, μM)	cytotoxicity (IC$_{50}$, μM)		
	HIV-1	PBM	CEM	Vero
17	1.3	>100	>100	>100
18	11.6	>100	>100	>100
19	92.3	>100	>100	53.0
20	>100	>100	>100	>100
24	8.1	>100	>100	>100
25	43.6	>100	>100	>100
30	80.5	>100	>100	>100
32	1.2	1.5	1.1	7.6
AZT	0.004	>100	14.3	28.0

and Vero, respectively) whereas no other compound showed cytotoxicity up to 100 μM. Compared with the β-D-2'-Fd4N nucleosides,[10] there was a general trend of antiviral activities throughout the series, where cytidine, 5-fluorocytidine, and adenosine analogues were most potent and almost all purine nucleosides showed moderate to potent activities. However, the β-D-2'-Fd4N nucleosides[10] were consistently more potent than their 4'-thio congeners, which suggests that the two types of

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

compound	xxBRU (EC$_{90}$, μM)	M184V (EC$_{90}$, μM)	FI[a]
17	5.0	$\approx$125	25
β-D-2′-F−d4FC[b]	9.9	34.7	3.5
AZT	0.01	0.003	0.3
3TC	0.08	535	6688

[a] FI is the fold increase (EC$_{90}$ HIV-1$_{M184V}$/EC$_{90}$ HIV-1$_{xxBRU}$). [b] Reference 11b: Lee et al. *J. Med. Chem.* **2002**, *45*, 1313−1320.

nucleosides may have similar structural features and may be recognized in the same fashion at the kinase level, but the way they interact with the viral polymerase (HIV-1 reverse transcriptase) must be somewhat different (vide infra for molecular modeling studies).

Antiviral Activity against Lamivudine-Resistant (HIV-1$_{M184V}$) Mutant Strain. Lamivudine (3TC, (−)-β-2′,3′-dideoxy-3′-thiacytidine) is an important component of the highly active antiretroviral therapy (HAART). However, single mutation at residue 184 (M184V) of the reverse transcriptase (RT) in HIV causes high-level resistance to 3TC and contributes to the failure of anti-AIDS combination therapy.[28,29] Therefore, there is an urgent need to discover new drug candidates active against the M184V mutant HIV-1 RT. The cytosine (**17**) and 5-fluorocytosine analogues (**18**), along with two positive controls, AZT and 3TC, were evaluated against the lamivudine-resistant mutant strain (HIV-1$_{M184V}$) in human PBM cells in vitro (Table 2). Compared with the 4′-oxygen congeners such as β-D-2′-Fd4FC,[10] the β-D-2′-F-4′-Sd4C (**17**) showed significantly reduced anti-HIV activity against HIV-1$_{M184V}$ (Table 2), which suggests the isosteric substitution of 4′-oxygen with 4′-sulfur made the β-D-2′-F-4′-Sd4N cross-resistant to the M184V mutation in HIV-1 RT (vide infra for molecular modeling studies).

Molecular Modeling. Since the conformational difference between the nucleoside analogues is one of the critical factors in determining their antiviral activity, it was of interest to compare the conformations of β-D-2′-Fd4N and the corresponding 4′-thio congeners. For this purpose, a quantum mechanical calculation (RHF/3-21G*) was performed on cytidine analogues (β-D-2′-Fd4C[10] and β-D-2′-F-4′-Sd4C **17**), and the geometry-optimized structures were compared (Figure 1a). The two structures were superimposable except for the 4′-positions; the strain inside the 4′-thio sugar ring caused by the longer C−S bond length resulted in movement of the 4′-sulfur atom by 0.5 Å down to the plane formed by C1′, C2′, C3′, and C4′. Another reason for this out-of-plane movement of the 4′-sulfur atom is that, unlike 4′-oxygen, the 4′-sulfur atom cannot form a hydrogen bond with 5′-OH due to the lack of the gauche effect. However, the critically important configurations of the N1 and C5′ atoms of a nucleoside are in good accordance, which may imply the similar tolerance to the two types of nucleosides by the kinases. On the other hand, when the triphosphates of β-D-2′-Fd4C[10] and β-D-2′-F-4′-Sd4C **17** bind to the active site of HIV-1 reverse transcriptase,[30] the isosteric replacement of 4′-oxygen with the 4′-sulfur atom exerts effects on the interaction between the nucleoside triphosphates and the active site residues, particularly Tyr115 and Met184 (Figure 1, parts b and c). The energy-minimized structures[31] of β-D-2′-Fd4C and β-D-2′-F-4′-Sd4C **17** bound to HIV-1 RT

shows that nucleoside inhibitors are located in a well-defined binding pocket formed by Arg72, Met184, and 3′-OH pocket residues (Asp113, Tyr115, Phe116, and Gln151) (Figure 1b). The role of Arg72 is notable because, as it moves into the binding pocket, it stabilizes the bound nucleotide through hydrogen bonding with the triphosphate moiety as well as nonspecific hydrophobic interaction with the heterocyclic moiety of the nucleoside triphosphate (Figure 1b). An additional characteristic binding mode of these d4-nucleotides is the possible $\pi-\pi$ interaction[32] between C2′−C3′ fluorovinyl moiety of the nucleotide and the aromatic ring of nearby Tyr115, which could be one reason for the high antiviral activity of 2′,3′-unsaturated nucleosides[4−9] (Figure 1b). Fluorine substitution at C2′ position would make the C2′-C3′ double bond electron poor and, thus, increase the $\pi-\pi$ interaction with the electron-rich aromatic ring of Tyr115. Despite almost the same binding mode of β-D-2′-Fd4C and β-D-2′-F-4′-Sd4C **17**, the decreased antiviral activity of β-D-2′-F-4′-Sd4C can be explained by the decrease in $\pi-\pi$ interaction. The replacement of electronegative oxygen by sulfur depolarizes charges inside the sugar ring in β-D-2′-F-4′-Sd4C **17**, which may result in reduced $\pi-\pi$ interaction between the fluorovinyl moiety and aromatic ring of Tyr115. Because of its out-of-plane location and large van der Waals radius, the 4′-sulfur atom in the β-D-2′-F-4′-Sd4N sugar moiety is in close contact to Met184 (Figure 1c). As a result, the mutation of Met184 to Val184, which has a bulky side chain, results in a significant steric hindrance between the 4′-sulfur atom in the β-D-2′-F-4′-Sd4N sugar moiety and the side chain of Val184, which might be one of the reasons for the high cross-resistance of HIV-1$_{M184V}$ to β-D-2′-F-4′-Sd4C (**17**) and β-D-2′-F-4′-Sd4FC (**18**) (Table 2, Figure 1c).

Metabolic Stability. In an attempt to understand metabolic stability of the adenosine analogue **24**, adenosine deaminase (ADA) binding efficiencies and deamination kinetics studies were performed.[33] As shown in Table 3, the adenine derivative **24** was found to be tightly bound (K_M = 18.3 μM) to the mammalian adenosine deaminase (from calf intestinal mucosa, EC.3.5.4.4), but the turnover number (k_{cat} = 0.94 s^{-1}) was very low, which indicates that even though the adenine derivative **24** can strongly bind to ADA, it can barely be catalyzed by ADA to the corresponding inosine derivative. It is noteworthy that, compared with other substrates of ADA, β-D-2′-F-4′-Sd4A **24** has very low K_M and k_{cat} values, and the catalytic efficiency (k_{cat}/K_M) is as low as that of β-FddA.[34] From a therapeutic point of view, this metabolic stability of the adenosine analogue[24] has important implications because adenosine deaminase is one of the major deactivating enzymes of the purine catabolism pathway.[35]

In summary, we have developed an efficient synthesis of optically pure β-D-2′-F-4′-Sd4 nucleosides. The isosteric replacement of 4′-oxygen in β-D-2′-Fd4 nucleosides with 4′-sulfur caused substantial changes in the binding mode of β-D-2′-F-4′-Sd4 to the active site of HIV-1 RT, resulting in reduced in vitro anti-HIV-1 activity. The lamivudine-resistant mutant strain (HIV-1$_{M184V}$) showed significant cross-resistance to the β-D-2′-F-4′-Sd4 nucleosides, which could be explained by the larger van
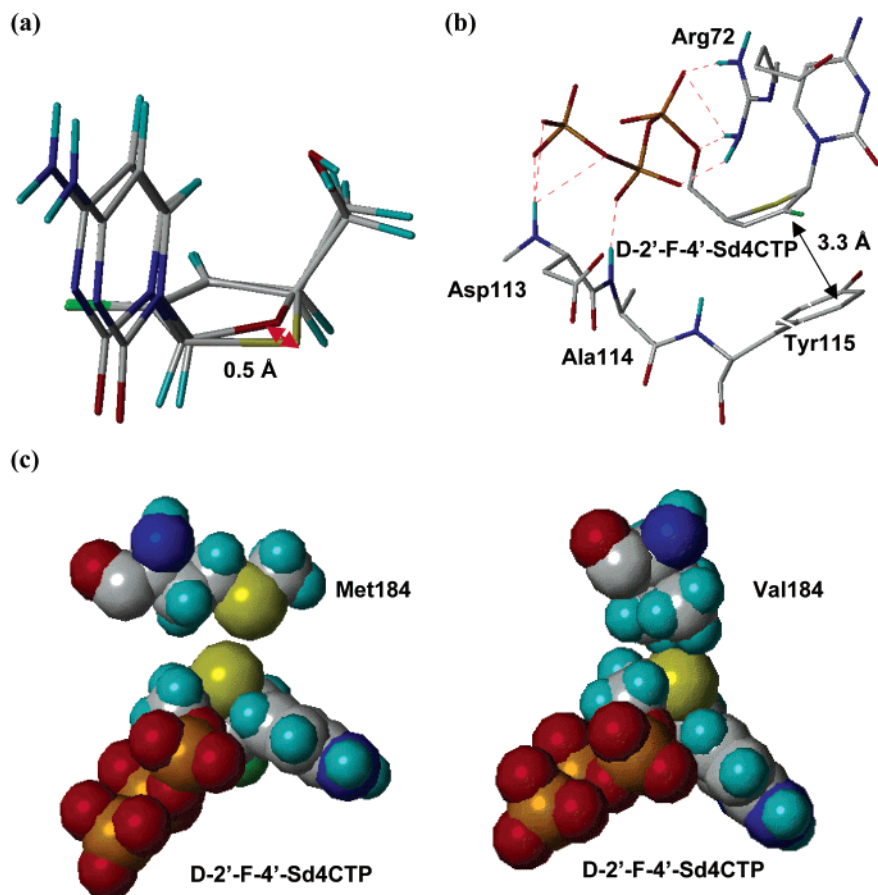
(a)

(b)

(c)

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

Table 3. Kinetic Constants for Adenosine Analogues as Substrates of ADA[a]

compound	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (μM^{-1} s^{-1})	$t_{1/2}$ (min)
D-2′-F-4′-Sd4A (**24**)	18.3	0.94	0.05	33
D-2′-F-d4A[28]	23.0	NA[b]	NA[b]	42
adenosine	24.5	76.4	3.1	0.5

[a] Kinetic data were obtained spectrophotometrically at 25 °C by following the decrease in absorbance at 265 nm. [b] Not available.

der Waals radius as well as the close proximity to Met184 of the 4′-sulfur atom.

Experimental Section

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

(2*S*,4*S*)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-butyrolactone (3). To a solution of lactone **2** (14.8 g, 39.9

mmol) in 200 mL of EtOAc was added 2.0 g of Pd/C (5% w/w) under H$_2$ atmosphere, and the mixture was stirred for 3 h. After filtration of the reaction mixture through a Celite pad, the filtrate was concentrated and purified by silica gel column chromatography with 5% EtOAc in hexanes to give compound **3** (13.9 g, 37.5 mmol, 94% yield) as a white solid: mp 87−89 °C; [α]^{24}$_D$ 15.5° (*c* 0.85, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 7.70−7.35 (m, 10H), 5.26 (dt, J = 51.2, 8.8 Hz, 1H), 4.54−4.47 (m, 1H), 3.93 (dd, J = 11.4, 2.3 Hz, 1H), 3.71 (dd, J = 11.4, 3.6 Hz, 1H), 2.65 (ddt, J = 13.1, 8.4, 6.6 Hz, 1H), 2.54 (ddt, J = 24.2, 13.2, 9.0 Hz, 1H), 1.04 (s, 9H); ^{13}C NMR (CDCl$_3$) δ 171.30 (d, J = 21.3 Hz), 135.59, 135.30, 132.52, 132.20, 129.76, 129.35, 127.82, 127.63, 89.30 (d, J = 191.0 Hz), 76.24 (d, J = 6.1 Hz), 63.73, 30.14 (d, J = 20.0 Hz), 26.58, 19.12; Anal. (C$_{21}$H$_{25}$FO$_3$-Si) C, H.

(2*S*,4*S*/*R*)-5-*tert*-Butyldiphenylsilyloxy-2-fluoro-4-iodopentanoic Acid Methyl Ester (4). A mixture of compound **3** (7.49 g, 20.1 mmol) in 5% aqueous EtOH was treated with solid NaOH (0.885 g, 22.1 mmol) at 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 (2.29 mL, 24.2 mmol) at 0 °C. After addition, the ice-bath was removed. The reaction mixture was stirred for 1 h and then poured 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$_2$ (7.65 g, 30.1 mmol), imidazole (4.11 g, 60.4 mmol), and Ph$_3$P (10.55 g, 40.2 mmol) in toluene (300 mL) at 60 °C for 4 h. Aqueous NaHCO$_3$ (200 mL) was added to the resulting mixture, and iodine was added portionwise until the iodine color persisted, to remove residual Ph$_3$P. Aqueous Na$_2$S$_2$O$_3$ was added dropwise until the iodine color disappeared, to remove residual iodine. The resulting mixture was

poured to a separatory funnel, diluted with 300 mL of toluene, washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography with 7% EtOAc in hexanes to give compounds **4** (7.52 g, 14.6 mmol, 73% yield) as pale yellow oil: ^{1}H NMR ($CDCl_3$) for major δ 7.71–7.33 (m, 10H), 5.08 (ddd, $J = 47.9$, 7.2, 5.0 Hz, 1H), 4.31–4.17 (m, 1H), 3.96–3.76 (m, 2H), 3.79 (s, 3H) 2.75–2.08 (m, 2H), 1.10 (s, 9H), for minor δ 7.71–7.33 (m, 10H), 5.16 (ddd, $J = 48.2$, 10.3, 2.1 Hz, 1H), 4.31–4.17 (m, 1H), 3.96–3.76 (m 2H), 3.81 (s, 3H), 2.75–2.08 (m, 2H), 1.08 (s, 9H); HRMS (FAB) obsd, *m/z* 515.0945, calcd for $C_{22}H_{29}FIO_3Si$, *m/z* 515.0915 (M + H)$^+$; Anal. ($C_{22}H_{28}FIO_3Si\cdot0.2C_6H_{14}$) C, H.

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

(2S,4S)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-thiobutyrolactone (6) and (2S,4R)-(−)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-γ-thiobutyrolactone (6a). A solution of compounds **5** (9.2 g, 19.9 mmol) in toluene (200 mL) was treated with 43.7 mL of 1 M DIBAL-H in hexane at −78 °C for 1 h. The reaction was quenched with 9.6 mL of MeOH and warmed to room temperature for 1 h, and aq $NaHCO_3$ (19 mL) and EtOAc (200 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to driness. The crude thiolactol was treated with Ac_2O (19 mL) and DMSO (20 mL) at room temperature for 24 h. The reaction mixture was poured to 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 product **6** (4.2 g, 10.8 mmol, 54% yield) and **6a** (0.7 g, 1.8 mmol, 9% yield) as yellow oil: For **6**: [α]$^{25}_D$ 28.2° (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68–7.37 (m, 10H), 5.07 (ddd, $J = 50.5$, 10.2, 6.9 Hz, 1H), 3.96–3.78 (m, 3H), 2.70–2.62 (m, 1H), 2.18–2.05 (m, 1H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 200.69 (d, $J = 18.0$ Hz), 135.53, 132.68, 132.60, 130.01, 127.86, 93.27 (d, $J = 197.0$ Hz), 66.64, 43.98 (d, $J = 7.0$ Hz), 32.61 (d, $J = 19.4$ Hz), 26.69, 19.23; Anal. ($C_{21}H_{25}FO_2SSi$) C, H, S, For **6a**: [α]$^{27}_D$ −46.7° (*c* 0.5, $CHCl_3$); ^{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.

(2R,4S)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl-γ-thiobutyrolactone (7). To a solution of compound **6** (4.74 g, 12.2 mmol) in THF (60 mL) was added 14.7 mL of 1 M LiHMDS in THF slowly at −78 °C, and the reaction mixture was stirred at the same temperature for 1 h. TMSCl (2.01 mL, 15.9 mmol) was added dropwise to the reaction mixture, and the mixture was allowed to warm to room temperature. The resulting mixture was stirred at room temperature for 30 min and cooled to −78 °C. A solution of PhSeBr (4.37 g, 18.3 mmol) in THF (20 mL) was rapidly added, and the mixture was stirred at −78 °C for 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 desired product **7** (4.77 g, 8.76 mmol, 72% yield) as a pale yellow syrup: [α]$^{24}_D$ 54.0° (*c* 0.606, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68–7.35 (m, 15H), 3.96–3.89 (m, 1H), 3.87 (dd. $J = 10.2$, 5.1 Hz, 1H), 3.80 (dd, $J = 10.1$, 7.1 Hz, 1H),

2.49 (dd, $J = 13.4$, 4.0 Hz, 1H), 2.22 (td, $J = 13.5$, 10.5 Hz, 1H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 196.32 (d, $J = 23.1$ Hz), 137.01, 135.52, 132.63, 132.50, 130.02, 129.99, 129.38, 127.87, 124.78, 105.12 (d, $J = 260.6$ Hz), 65.66, 44.58 (d, $J = 2.9$ Hz), 39.13 (d, $J = 21.4$ Hz), 26.69, 19.20; Anal. ($C_{27}H_{29}FO_2SSeSi$) C, H, S.

(2S,4R)-(+)-4-*tert*-Butyldiphenylsilyloxymethyl-2-fluoro-2-phenylselenyl-γ-thiobutyrolactone (7a). See the previous procedure for reaction of the compound **7** with phenylselenyl bromide. The title compound **7a** was obtained on 9.69-mmol scale in 74% yield as a pale yellow syrup: [α]$^{24}_D$ −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. ($C_{27}H_{29}FO_2SSeSi\cdot0.15CHCl_3$) C, H, S.

(1R/S,2R,4S)-1-*O*-Acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranoside (8). A solution of compounds **7** (4.77 g, 8.76 mmol) in toluene (100 mL) was treated with 17.5 mL of 1 M DIBAL-H in hexane at −78 °C for 1 h. The reaction was quenched with 4 mL of MeOH and warmed to room temperature for 1 h and aq $NaHCO_3$ (8 mL) and EtOAc (100 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to dryness. A solution of the crude thiolactol in CH_2Cl_2 (100 mL) was treated with Ac_2O (2.48 mL, 26.3 mmol), TEA (3.66 mL, 26.3 mmol), and 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** (4.4 g, 7.48 mmol, 85% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.70–7.32 (m, 15H), 6.03, 5.99 (d and 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-[(1R,2R,4S)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranosyl]cytosine (9). A mixture of *N*[4]-benzoylcytosine (0.505 g, 2.35 mmol) in HMDS (15 mL) and CH_3CN (15 mL) was heated under reflux for 5 h. After removal of solvent by using a vacuum pump, a solution of the acetate **8** (0.460 g, 0.786 mmol) in 15 mL of CH_3CN was added to the reaction flask containing the silylated *N*[4]-benzoylcytosine, and then TMSOTf (0.28 mL, 1.4 mmol) was added dropwise at room temperature. After 16 h, the reaction was quenched with 1 mL of sat. $NaHCO_3$ and the resulting mixture was concentrated. The crude mixture was diluted with 100 mL of CH_2Cl_2, washed with aq $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 30% EtOAc in hexanes to give cytosine derivative **9** (0.269 g, 0.362 mmol, 46% yield) as a foam: [α]$^{24}_D$ 179.9° (*c* 0.50, CH_2Cl_2); UV(MeOH) λ_{max} 308 nm; ^{13}C NMR ($CDCl_3$) δ 161.94, 155.42, 147.17, 136.96, 135.60, 135.54, 133.21, 132.83, 129.96, 129.92, 129.41, 129.20, 129.03, 127.78,0.127.60, 125.73, 106.39 (d, $J = 248.8$ Hz), 96.87, 66.74 (d, $J = 17.6$ Hz), 66.29, 47.21, 42.39 (d, $J = 22.2$ Hz), 26.73, 19.15; Anal. ($C_{38}H_{38}FN_3O_3SSeSi$) C, H, N, S.

(+)-*N*[4]-Benzoyl-1-[(1R,2R,4S)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranosyl]-5-fluorocytosine (10). See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **10** was obtained on 0.850-mmol scale in 52% yield: [α]$^{24}_D$ 202.6° (*c* 0.410, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 332.5 nm; ^{13}C NMR ($CDCl_3$) δ 151.88 (d $J = 19.2$ Hz), 147.15, 139.01 (d, $J = 237.9$ Hz), 136.78, 135.90, 135.49, 135.47, 133.00, 132.71, 130.01, 129.96, 129.91, 129.51, 129.18, 128.24, 127.76, 126.85 (d, $J = 34.4$ Hz), 124.74, 105.60 (d, $J = 247.5$ Hz), 67.01 (d, $J = 18.6$), 66.43, 47.19, 41.64 (d, $J = 21.0$ Hz),

26.68, 19.09; HRMS (FAB) obsd, m/z 762, calcd for $C_{38}H_{38}F_2N_3O_3$-SSeSi, m/z 762.15 (M + H)$^+$; Anal. ($C_{38}H_{37}F_2N_3O_3SSeSi$) C, H, N, S.

(+)-1-[(1R,2R,4S)-5-O-($tert$-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-$β$-D-ribofuranosyl]thymine (11). See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **11** was obtained on 0.850-mmol scale in 45% yield: $[α]^{25}_D$ 137.8° (c 0.42, CH$_2$Cl$_2$); UV(CH$_2$Cl$_2$) $λ_{max}$ (CH$_2$Cl$_2$) 266.0 nm; ^{13}C NMR (CDCl$_3$) δ 163.25, 150.93, 137.22 (d, J = 6.1 Hz), 136.88, 135.48, 132.94, 132.90, 129.87, 129.46, 129.15, 127.74, 125.16, 110.67, 106.15 (d, J = 247.03 Hz), 66.76, 65.85 (d, J = 18.3 Hz), 46.79, 42.04 (d, J = 21.0 Hz), 26.70, 19.17, 12.59; HRMS (FAB) obsd, m/z 655, calcd for $C_{32}H_{36}FN_2O_3SSeSi$, m/z 655.1287 (M + H)$^+$; Anal. ($C_{32}H_{35}FN_2O_3SSeSi·0.4H_2O$) C, H, N, S.

(+)-1-[(1R,2R,4S)-5-O-($tert$-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-$β$-D-ribofuranosyl]uracil (12). See the general procedure for condensation reaction of the acetate **8** with pyrimidines. The title compound **12** was obtained on 0.766-mmol scale in 53% yield: $[α]^{23}_D$ 136.5° (c 0.36, CH$_2$Cl$_2$); UV(CH$_2$Cl$_2$) $λ_{max}$ 262.5 nm; ^{13}C NMR (CDCl$_3$) δ 162.77, 150.77, 141.80 (d, J = 5.0 hz), 136.94, 135.58, 135.51, 132.80, 132.77, 129.93, 129.61, 129.26, 127.79, 125.12, 106.24 (d, J = 248.7 Hz), 102.31, 66.06, 65.99 (d, J = 14.6 Hz), 46.86, 41.58 (d, J = 21.8 Hz), 26.75, 19.18; Anal. ($C_{31}H_{33}FN_2O_3$-SSeSi) 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-[(1R,4S)-5-O-($tert$-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]cytosine (13). To a solution of compound **9** (0.229 g, 0.310 mmol) in 5 mL of CH$_2$Cl$_2$ was added a solution of mCPBA (57–86%, 69 mg) in 5 mL of CH$_2$Cl$_2$ 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$_2$Cl$_2$, washed with aq NaHCO$_3$, dried over MgSO$_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 40% EtOAc in hexanes to give eliminated product **13** (0.107 g, 0.225 mmol, 72% yield) as a foam: $[α]^{25}_D$ −140.2° (c 0.366, CH$_2$Cl$_2$); UV(MeOH) $λ_{max}$ 308 nm; ^{13}C NMR (CDCl$_3$) δ 171.09, 166.78, 162.14, 155.06, 155.04 (d, J = 280.8 Hz), 144.49, 135.58, 135.50, 133.19, 132.96, 132.76, 132.43, 130.11, 128.97, 127.89, 127.58, 111.29 (d, J = 16.6 Hz), 98.40, 67.75, 61.91 (d, J = 23.6 Hz), 46.83 (d, J = 7.2 Hz), 26.82, 19.24; Anal. ($C_{32}H_{32}FN_3O_3SSi$) C, H, N, S.

(−)-N^4-Benzoyl-1-[(1R,4S)-5-O-($tert$-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]-5-fluorocytosine (14). See the general procedure for syn-elimination reaction. The title compound **14** was obtained on 0.465-mmol scale in 69% yield: $[α]^{25}_D$ −150.5° (c 0.345, CH$_2$-Cl$_2$); UV(CH$_2$Cl$_2$) $λ_{max}$ 331.0 nm; ^{13}C NMR (CDCl$_3$) δ 152.88 (d, J = 281.3 Hz), 152.08 (d, J = 19.1 Hz), 147.13, 140.42 (d, J = 241.0 Hz), 135.86, 135.59, 135.50, 133.17, 132.59, 132.39, 130.18, 130.15, 130.11, 128.33, 127.93, 124.05 (d, J = 36.2 Hz), 111.24 (d, J = 16.5 Hz), 67.77, 61.85 (d, J = 23.54 hz), 47.05 (d, J = 7.1 Hz), 26.79, 19.23; FAB-MS obsd, m/z 604, calcd for $C_{32}H_{32}F_3N_3O_3SSi$, m/z 604.1823 (M + H)$^+$; Anal. ($C_{32}H_{31}F_2N_3O_3$-SSi) C, H, N, S.

(−)-1-[(lR,4S)-5-O-($tert$-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]thymine (15). See the general procedure for the syn-elimination reaction. The title compound **15** was obtained on 0.375-mmol scale in 69% yield: $[α]^{26}_D$ −43.2° (c 0.34, CH$_2$-Cl$_2$) $λ_{max}$ 266.0 nm; ^{13}C NMR (CDCl$_3$) δ 163.47, 154.97 (d, J = 281.2 Hz), 150.78, 135.52, 135.43, 134.52, 132.79, 132.51, 130.03, 127.84, 112.65, 110.45 (d, J = 16.8 Hz), 68.23, 60.49 (d, J = 23.7 Hz), 46.74 (d, J = 7.1 Hz), 26.74, 19.23, 12.54; FAB-MS obsd, m/z 497, calcd for $C_{26}H_{30}F N_2O_3SSi$, m/z 497.1652 (M + H)$^+$; Anal. ($C_{26}H_{29}FN_2O_3SSi·0.6H_2O$) C, H, N, S.

(−)-1-[(1R,4S)-5-O-($tert$-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]uracil (16).

See the general procedure for syn-elimination reaction. The title compound **16** was obtained on 0.391-mmol scale in 58% yield: $[α]^{25}_D$ −72.1° (c 0.35, CH$_2$Cl$_2$); UV(CH$_2$Cl$_2$) $λ_{max}$ 262.5 nm; ^{13}C NMR (CDCl$_3$) δ 162.78, 154.79 (d, J = 280.9 Hz), 150.59, 139.55, 135.59, 135.49, 132.79, 132.37, 130.13, 130.10, 127.92, 127.90, 110.70 (d, J = 16.9 Hz), 104.01, 67.33, 60.74 (d, J = 23.7 Hz), 46.88 (d, J = 7.2 Hz), 26.87, 19.31; FAB-MS obsd, m/z 483, calcd for $C_{25}H_{28}FN_2O_3SSi$, m/z 483.1496 (M + H)$^+$; Anal. ($C_{25}H_{27}FN_2O_3SSi$) C, H, N, S.

General Procedure for Two Successive Deprotections of Protected Unsaturated Nucleosides. This is representative of the preparation of cytidine analogues **17** and **18**.

(−)-1-[(1R,4S)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]cytosine (17). A solution of the protected cytidine **13** (0.135 g, 0.225 mmol) in 15 mL of THF was treated with 0.31 mL of 1 M TBAF in THF for 2 h. The mixture was concentrated and filtered through a short pad of silica gel. After the filtrate was concentrated, without further purification, the crude product was treated with methanolic ammonia at room temperature for 30 h. After removal of solvent, the residue was purified by silica gel column chromatography with 5% MeOH in CH$_2$Cl$_2$ to give cytidine analogue **17** (0.048 g, 0.186 mmol, 84% yield) as a white solid: mp 89–91 °C (dec); $[α]^{24}_D$ −208.3° (c 0.44, MeOH); UV(H$_2$O) $λ_{max}$ 279.5 nm ($ε$ 19900, pH 2). 272.0 nm ($ε$ 15900, pH 7), 272.5 nm ($ε$ 16200, pH 11); Anal. ($C_9H_{10}FN_3O_2S$ 0.2H$_2$O) C, H, N, S.

(−)-1-[(1R,4S)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]-5-fluorocytosine (18). See the general procedure for two successive deprotections. The title compound **18** was obtained on 0.296-mmol scale in 90% yield: mp 187–189 °C; $[α]^{25}_D$ −171.3° (c 0.42, MeOH); UV(H$_2$O) $λ_{max}$ 286.0 nm ($ε$ 8600, pH 2). 282.0 nm ($ε$ 7500, pH 7), 282.0 nm ($ε$ 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 thymidine analogue **19** is representative.

(−)-1-[(1R,4S)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]thymine (19). A solution of compound **15** (0.119 g, 0.235 mmol) in THF (10 mL) was treated with 0.25 mL of 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 CH$_2$Cl$_2$ to give the desired product **19** (0.055 g, 0.206 mmol, 88% yield) as a white solid: mp 174–176 °C; $[α]^{25}_D$ −46.5° (c 0.29, MeOH); UV(H$_2$O) $λ_{max}$ 268.5 nm ($ε$ 5700, pH 2). 268.5 nm ($ε$ 5500, pH 7), 269.0 nm ($ε$ 4500, pH 11); Anal. ($C_{10}H_{11}FN_2O_3S$ 0.1Et$_2$O) C, H, N, S.

(−)-1-[(1R,4S)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-$β$-D-ribofuranosyl]uracil (20). See the general procedure for the desilylation reaction. The title compound **20** was obtained on 0.193-mmol scale in 89% yield: mp 184–185 °C; $[α]^{25}_D$ −136.4° (c 0.47, MeOH); UV(H$_2$O) $λ_{max}$ 263.0 nm ($ε$ 9100, pH 2). 263.0 nm ($ε$ 9700, pH 7), 263.5 nm ($ε$ 7800, pH 11); Anal. ($C_9H_9FN_2O_3S·0.3MeOH$) C, H, N, S.

General Procedure for Condensation Reaction of the Acetate 8 with Purines. The preparation of 6-chloropurine derivative **21** is representative.

(+)-9-[(1R,2R,4S)-5-O-($tert$-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-$β$-D-ribofuranosyl]-6-chloropurine (21). A mixture of 6-chloropurine (0.947 g, 6.12 mmol) and ammonium sulfate (0.135 g, 1.02 mmol) in 60 mL of HMDS was refluxed for 5 h. HMDS was evaporated to give a yellow solid. To this flask containing the silylated 6-chloropurine was added a solution of the acetate **8** (1.2 g, 2.04 mmol) in 1,2-dichloroethane (30 mL). The resulting slurry was cooled to −25 °C, and TMSOTf (0.74 mL, 4.08 mmol) was added dropwise at −25 °C. The reaction mixture was stirred for 3 h at −25 → −10 °C, for 8 h at room temperature, and for 5 h at 40 °C. The resulting mixture was diluted with 300 mL of CH$_2$Cl$_2$, washed with aq NaHCO$_3$, dried over MgSO$_4$, filtered, and concentrated. The crude product was purified by silica gel column chromatography with 11% EtOAc in hexanes to give compound **21** (1.01 g, 1.48 mmol, 73% yield) as a pale yellow foam: $[α]^{22}_D$ 75.4° (c 1.08, CHCl$_3$); UV(CH$_2$Cl$_2$) $λ_{max}$ 264.5 nm; ^{13}C NMR (CDCl$_3$) δ 151.84, 150.87, 144.98 (d, J =

5.7 Hz), 136.32, 135.60, 135.53, 132.85, 132.76, 131.21, 130.02, 129.96, 129.44, 128.99, 128.79, 127.82, 124.34, 105.10 (d, $J =$ 248.1 Hz), 66.64, 65.68 (d, $J = 20.3$ Hz), 47.33, 41.68 (d, $J =$ 20.7 Hz), 26.74, 19.17; Anal. ($C_{32}H_{32}ClFN_4OSSeSi$) C, H, N, S.

(+)-9-[(1*R*,2*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-fluoro-2-phenylselenyl-4-thio-β-D-ribofuranosyl]-6-chloro-2-fluoropurine (26). See the general procedure for condensation reaction of the acetate **8** with purines. The title compound **26** was obtained on 1.70-mmol scale in 67% yield: $[\alpha]^{23}_D$ 81.0° (*c* 1.37, CHCl₃); UV(CHCl₃) λ_{max} 270.0 nm; ¹³C NMR (CDCl₃) δ 157.00 (d, $J = 220.8$ Hz), 153.53 (d, $J =$ 17.1 Hz), 152.4 (d, $J = 17.3$ Hz), 145.53, 136.26, 135.59, 135.52, 132.80, 132.70, 130.03, 129.97, 129.79 (d, $J = 4.9$ Hz), 129.49, 129.00, 127.83, 124.19, 104.99 (d, $J = 248.5$ Hz), 66.53, 65.90 (d, $J = 20.7$ Hz), 47.36, 41.48 (d, $J = 20.7$ Hz), 26.75, 19.17; Anal. ($C_{32}H_{31}ClF_2N_4OSSeSi\cdot0.2hexane$) C, H, N, S.

9-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-6-chloropurine (22) and 9-[(4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-6-chloropurine (23). See the general procedure for syn-elimination reaction. A mixture of title compounds **22** and **23** (3:1) were obtained on 1.48-mmol scale in 86% yield as a pale yellow foam: UV(CH₂Cl₂) λ_{max} 264.5 nm; Anal. ($C_{26}H_{26}ClFN_4OSSi$) C, H, N, S.

9-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-6-chloro-2-fluoropurine (27) and 9-[(4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-1,2-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-6-chloro-2-fluoropurine (28). See the general procedure for syn-elimination reaction. A mixture of title compounds **27** and **28** were obtained on 1.14-mmol scale in 71% yield as a pale yellow foam: UV(CH₂Cl₂) λ_{max} 266.0 nm; Anal. ($C_{26}H_{25}ClF_2N_4OSSi$) C, H, N, S.

(−)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]adenine (24). A mixture of compound **22** and compound **23** (0.342 g, 0.65 mmol) was treated with methanolic ammonia at 80 °C for 12 h using steel bomb. Solvent was evaporated, and the residue was dried under vacuum for 3 h. A solution of the dried crude product in 30 mL of THF was treated with 0.3 mL of 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 CH₂Cl₂ to give the desired compound **24** (0.099 g, 0.37 mmol 57% yield) as a white solid: mp 190−191 °C; $[\alpha]^{25}_D$ −33.7° (*c* 0.164, 7:1 CH₂Cl₂:MeOH); UV(H₂O) λ_{max} 257.5 nm (ε 9,600, pH 2). 259.0 nm (ε 9,500, pH 7), 259.0 nm (ε 9,800, pH 11); ¹³C NMR (MeOH-d_4) δ 157.43, 156.31 (d, $J =$ 277.9 Hz), 153.98, 150.51, 141.22, 120.10, 111.65 (d, $J = 17.1$ Hz), 65.63, 60.38 (d, $J = 24.7$ Hz), 49.73 (d, $J = 7.7$ Hz); Anal. ($C_{10}H_{10}FN_5OS$) C, H, N, S.

(+)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]hypoxanthine (25). A mixture of compound **22** and compound **23** (0.323 g, 0.62 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.17 mL, 2.46 mmol) and NaOMe (0.136 g, 2.52 mmol) at 60 °C for 24 h. The resulting mixture was quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel with 4% MeOH in CH₂Cl₂. The filtrate was concentrated to driness. The crude product was treated with 0.68 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 CH₂Cl₂ to give the desired compound **25** (0.135 g, 0.50 mmol, 81% yield) as a white solid: mp 143−145 °C (dec); $[\alpha]^{27}_D$ 21.5° (*c* 0.132, 7:1, CH₂Cl₂:MeOH); UV(H₂O) λ_{max} 247.5 nm (ε 10200, pH 2). 248.0 nm (ε 10000, pH 7), 253.5 nm (ε 10700, pH 11); ¹³C NMR (MeOH-d_4) δ 158.85, 156.22 (d, $J = 248.4$ Hz), 150.04, 147.13, 140.66, 125.29, 111.77 (d, $J = 17.0$ Hz), 65.50, 60.51 (d, $J = 24.8$ Hz), 49.83 (d, $J = 7.3$ Hz); Anal. ($C_{10}H_9FN_4O_2S$) C, H, N, S.

(−)-2-Amino-9-[(1*R*,4*S*)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofura-

nosyl]-6-chloropurine (29) and 6-amino-9-[(1*R*,4*S*)-5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-2-fluoropurine (31). Dry ammonia gas was bubbled into a stirred solution of a mixture of compounds **27** and **28** (0.430 g, 0.79 mmol) in 1,2-dimethoxyethane (25 mL) at 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 **29** (0.190 g, 0.352 mmol, 45% yield) and a mixture of compound **31** and its $\Delta^{1,2}$-isomer (0.116 g, 0.22 mmol, 28% yield): for compound **29**; $[\alpha]^{24}_D$ −70.4° (*c* 0.40, CH₂Cl₂); UV(CH₂Cl₂) λ_{max} 303.5 nm; ¹³C NMR (CDCl₃) δ 159.11, 154.78 (d, $J = 281.2$ Hz), 153.48, 151.49, 139.92, 135.57, 135.48, 132.76, 132.48, 130.12, 130.09, 127.91, 127.88, 125.30, 109.95 (d, $J = 16.5$ Hz), 68.01, 58.33 (d, $J =$ 24.0 Hz), 47.46 (d, $J = 7.1$ Hz), 26.76, 19.19; Anal. ($C_{26}H_{27}$-ClFN₅OSSi) C, H, N, S: a mixture of compound **31** and its $\Delta^{1,2}$-isomer; UV(CHCl₃) λ_{max} 261.0 nm.

(−)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]guanosine (30). See the procedure for the preparation of compound **25**. The title compound **30** was obtained on 0.29-mmol scale in 61% yield: mp 210 °C (dec); $[\alpha]^{25}_D$ −73.7° (*c* 0.152, DMSO); UV(H₂O) λ_{max} 254.0 nm (ε 11,600, pH 2). 255.0 nm (ε 12,400, pH 7), 265.0 nm (ε 12,-500, pH 11); ¹³C NMR (DMSO-d_6) δ 160.12, 157.49 (d, $J =$ 278.1 Hz), 157.44, 154.47, 138.61, 119.83, 114.34 (d, $J = 17.0$ Hz), 67.90, 60.99 (d, $J = 24.5$ Hz), 51.73 (d, $J = 7.6$ Hz), 44.06 (d, $J = 9.1$ Hz); Anal. ($C_{10}H_{10}FN_5O_2S$) C, H, N, S.

(+)-9-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-2-fluoro-4-thio-β-D-ribofuranosyl]-2-fluoroadenosine (32). See the general procedure of desilylation. The title compound **32** was obtained on 0.22-mmol scale in 70% yield: mp >300 °C; $[\alpha]^{26}_D$ 17.1° (*c* 0.15, MeOH); UV(H₂O) λ_{max} 261.0 nm (ε 12700, pH 2). 261.0 nm (ε 12900, pH 7), 260.5 nm (ε 13100, pH 11); ¹³C NMR (MeOH-d_4) δ 160.82 (d, $J = 269.8$ Hz), 159.67, 159.02, 155.19, 152.65, 141.63, 118.69, 111.99 (d, $J = 17.1$ Hz), 65.96 (d, $J =$ 1.8 Hz), 60.77 (d, $J = 24.3$ Hz), 50.04; Anal. ($C_{10}H_9F_2N_5OS$) C, H, N, S.

Molecular Modeling Study. (a) Conformational analysis: The initial conformations of d-β-2′-F−d4C and D-β-2′-F-4′-S−d4C **17** were constructed by builder module in Spartan 5.1.1 (Wave functions, Inc. Irvine, CA), and all calculations were performed on a Silicon Graphics O2 workstation. The initial conformations were cleaned up and geometry-optimized through quantum mechanical ab initio calculations using RHF/3-21G* basis in Spartan 5.1.1. (b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling of the enzyme−substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme−ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd).[30] A model of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template−primer) duplex. The geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of $n + 1$th nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd)[30] 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.[30] Gästeiger−Hückel charge was given to the enzyme−ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to enzyme site from the biopolymer module in Sybyl. Fluorine parameters were obtained from the literatures[36,37] and MM2 parameters and put to the parameter files. To eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05 kcal/

mol. The annealed enzyme−inhibitor complexes were minimized by using Kollman-All-Atom Force Field until iteration number reached 5000.

Adenosine Deaminase Kinetics Study. Assays were performed at 25 °C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range of 15−100 μM and with 0.15 units of adenosine deaminase (EC 3.5.4.4. from calf intestinal mucosa). The assays were monitored with a UV spectrometer at 265 nm. Initially, the qualitative assays were performed with d-β-2′-F-4′-S−d4A **24** (200 μM) in the presence of 0.24 units of adenosine deaminase for 120 min to determine whether it is a substrate of this enzyme. The concentration (c_t) of each substrate at a certain time (t) was calculated from the absorbance (A_t) at that time (t), where it was assumed that the total change of absorbance ($A_0 - A_\infty$) was directly related to the disappearance of the substrate.[35] Initial hydrolysis rates for each substrate concentration were measured manually through graphical curve fitting of the concentration vs time data for reaction of each substrate. From Lineweaver−Burke plot of these initial rates, V_{max} (maximum velocity) and K_M (Michaelis constant) were obtained for each substrate and k_{cat} was also calculated with 0.15 units of the enzyme (MW 33000) in 2 mL of buffer solution. The $t_{1/2}$ of D-β-2′-F-4′-S−d4A **24** and adenosine were also measured at 20 μM with 0.15 unit of the enzyme.

Antiviral Assay. Human peripheral blood mononuclear (PBM) cells (obtained from Atlanta Red Cross) were isolated by Ficoll−Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2−3 days prior to use. HIV-1$_{LAI}$ obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infetious 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/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 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 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. Ten microliters 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 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 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.[38]

Cytotoxicity Assays. The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD) and Vero (African green monkey kidney) cells. PBM cells were obtained from whole blood of healthy seronegative donors

(HIV-1 and hepatitis B virus) by single-step Ficoll−Hypaque discontinuous gradient centrifugation. Log phase Vero, CEM, and PHA-stimulated human PBM cells were seeded at a density of 5 × 10^3, 2.5 × 10^3, and 5 × 10^4 cells/well, respectively. All of the cells were plated in 96-well cell culture plates containing 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.[38]

Supporting Information Available: ^{1}H NMR and elemental analysis data for compounds **9**−**22**, **24**−**27**, **29**−**30**, and **32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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