L-2′,3′-Didehydro-2′,3′-dideoxy-3′-fluoronucleosides: Synthesis, Anti-HIV Activity, Chemical and Enzymatic Stability, and Mechanism of Resistance

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Received January 16, 2003

As antiviral nucleosides containing a 2′,3′-unsaturated sugar moiety with 2′-fluoro substitution are endowed with increased stabilization of the glycosyl bond, it was of interest to investigate the influence of the fluorine atom at the 3′-position. Various pyrimidine and purine L-3′-fluoro-2′,3′-unsaturated nucleosides were synthesized from their precursors, L-3′,3′-difluoro-2′,3′-dideoxy nucleosides, by elimination of hydrogen fluoride. In the L-3′,3′-difluoro-2′,3′-dideoxy nucleoside series, cytidine **16** and 5-fluorocytidine **18** analogues showed modest antiviral activity (EC_{50} 11.5 and 8.8 μM, respectively) when evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells. In the 2′,3′-unsaturated series, L-3′-fluoro-2′,3′-didehydro-2′,3′-dideoxycytidine **24** and 5-fluorocytidine **26** showed highly potent antiviral activity (EC_{50} 0.089 and 0.018 μM, respectively) without significant cytotoxicity. The guanosine analogue **48** showed only marginal anti-HIV activity with some cytotoxicity (EC_{50} 38.5 μM, and IC_{50} 17.4, 58.4, 36.5 μM in PBM, CEM, and Vero cells, respectively). The cytidine **24** and 5-fluorocytidine **26** analogues, however, showed significantly decreased antiviral activity against the clinically important lamivudine-resistant variants (HIV-1_{M184V}). Molecular modeling studies demonstrated that the 3′-fluoro atom of the L-3′-fluoro-2′,3′-unsaturated nucleoside is within the hydrogen bonding distance with the amide backbone of Asp185, which favors the binding of the nucleoside triphosphate to the wild-type RT. This favorable binding mode, however, cannot be maintained when the triphosphate of 3′-fluoro 2′,3′-unsaturated nucleoside binds to the active site of M184V RT because the bulky side chain of Val184 occupies the space needed for the nucleotide. The biological results suggest that, in addition to the sugar conformation, the base moiety may also play a role in their interaction with the M184V RT.

Introduction

A number of fluorine-substituted nucleoside analogues have demonstrated their potent antiviral activity as well as favorable chemical and pharmacological properties by virtue of the small but highly electronegative nature of the fluorine atom, which is also capable of participating in hydrogen bonding. Thus, fluorination at the 5-position of the pyrimidine base[1] as well as at the 2′- and/or 3′-position of the sugar moiety of the nucleoside analogue have been extensively studied in the pursuit of safe, effective and chemically stable antiviral agents.[2,3] Among those, the most interesting antiviral activities were found when a fluorine was substituted in 2′,3′-dideoxy-2′,3′-didehydro nucleosides. Both the 5-fluorocytidine analogue[1c,e] and the 2′-fluoro analogue[2b−d] showed potent anti-HIV activity without significant cytotoxicity. It is interesting to note that the antiviral activities of nucleosides containing the 2′,3′-unsaturated sugar moiety with a 2′-fluoro substitution have increased stabilization of the glycosyl bond. As this stabilization of the glycosyl bond might result from the destabilization of the oxonium ion resulting from the cleavage of the glycosyl bond by the highly electronegative fluorine atom (Scheme 4), it was of interest to investigate the influence of the fluorine atom at the 3′-position. In view of the fact that the L-isomers tend to have potent antiviral activity with reduced toxicity in comparison to their D-counterparts, in a recent communication, we reported the stereoselective synthesis of the β-L-3′-F-d4C which showed potent anti-HIV activity (EC_{50} 0.03 μM) in PBM cells.[4] By using a similar synthetic strategy, a series of L-nucleosides were synthesized from the key intermediate, (*4S*)-1-*O*-acetyl-5-*O*-benzoyl-2,3-dideoxy-2,2-difluoro-β-D-ribofuranoside (**7**), and their antiviral activities were evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells. The cytidine **24** and 5-fluorocytidine **26** analogues were also evaluated against a lamivudine-resistant HIV-1 RT (HIV-1_{M184V}).

Results and Discussion

Chemistry. Synthesis of the nucleoside analogues with a fluorine atom at the 3′-position of a 2′,3′-unsaturated sugar ring has been hampered by the lack of an efficient method for introducing the fluorovinyl group at the 3′-position. The only synthetic method known to date employs oxidation followed by difluorination of the 3′-OH group at the nucleoside level.[5] Elimination of HF by sodium methoxide afforded the desired 3′-fluoro-2′,3′-unsaturated thymidine analogue. However, for the extensive structure−activity relation-

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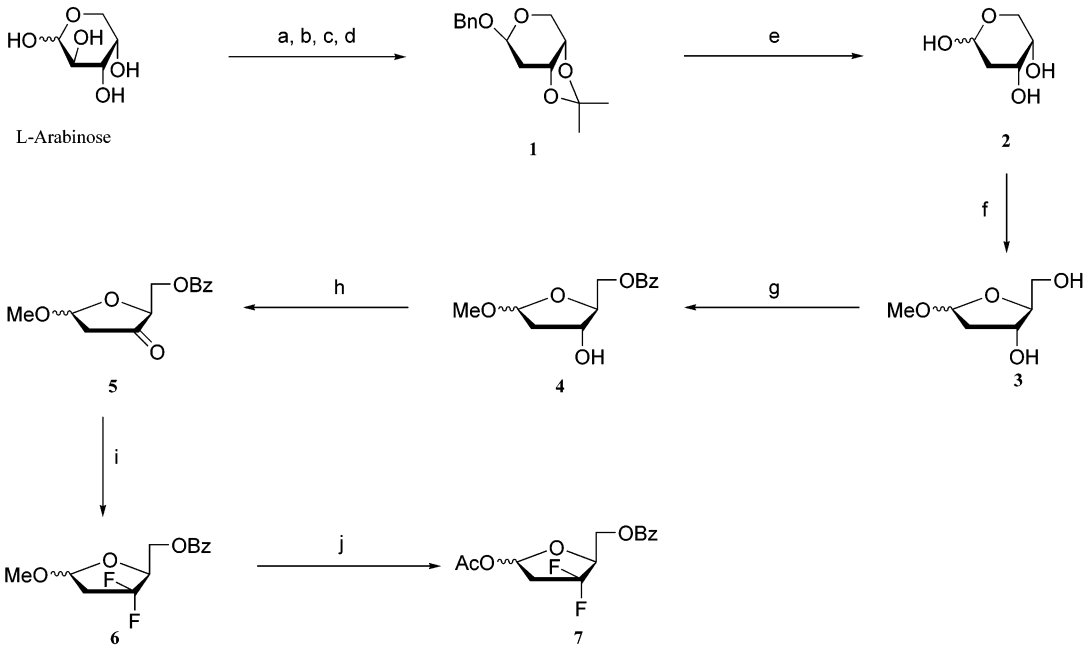
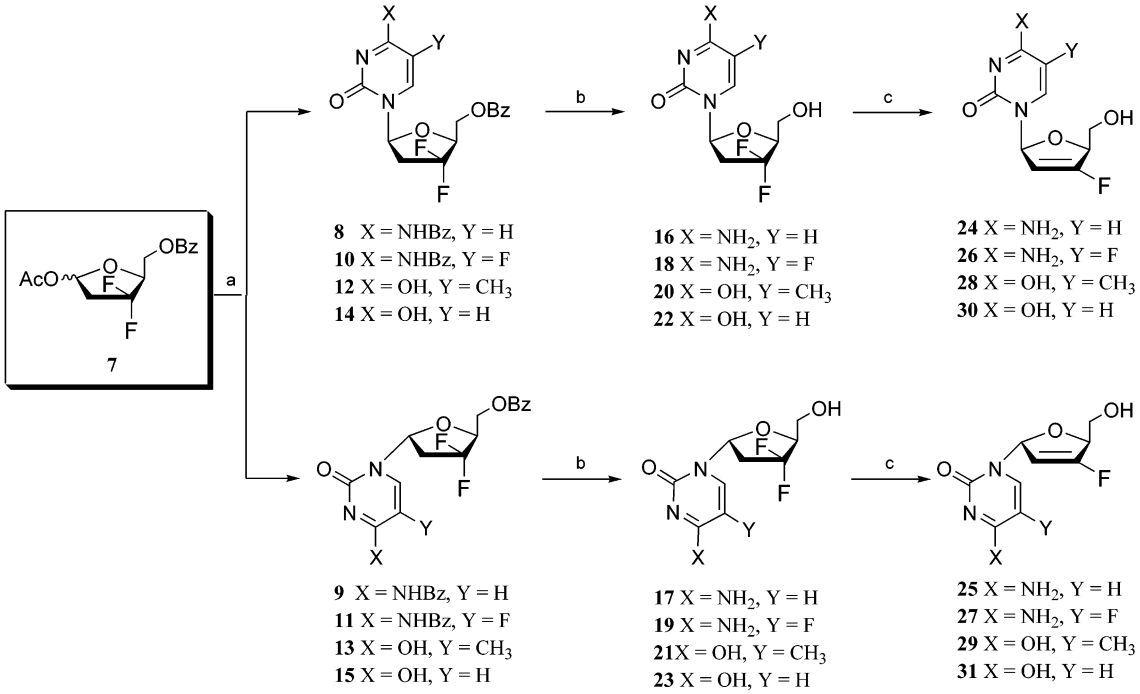
10.1021/jm0300274 CCC: $25.00
Published on Web 06/24/2003

Scheme 1. Synthesis of the Key Intermediate **7**[a]



[a] Keys: (a) BnOH, HCl (g); (b) 2,2-dimethoxypropane, *p*-TsOH, acetone; (c) $CSCl_2$, PhOH, pyr, CH_2Cl_2; (d) $^nBu_3SnH$, AIBN, toluene, reflux; (e) 4% TFA, 40 °C; (f) HCl, MeOH; (g) BzCl, pyr; (h) PDC, Ac_2O, CH_2Cl_2; (i) DAST, CH_2Cl_2, reflux; (j) Ac_2O, H_2SO_4, AcOH.

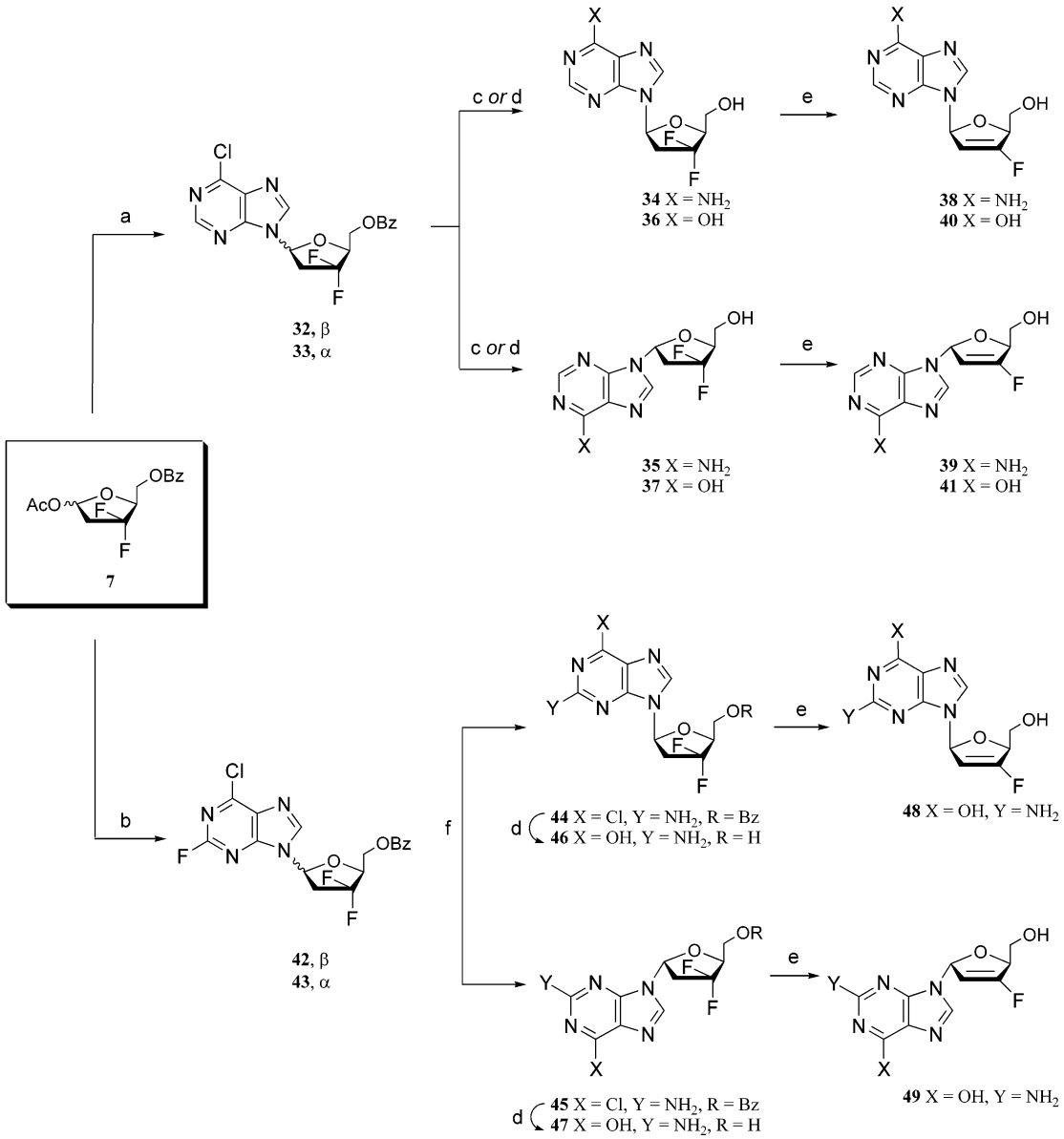
Scheme 2. Synthesis of the Pyrimidine Nucleosides[a]



[a] Keys: (a) HMDS, CH_3CN, pyrimidines, TMSOTf, (b) NH_3, MeOH, rt, (c) NaOMe, DMF (or 2:1 DMF:dioxane), rt.

ship study, a more general synthetic method such as the introduction of the 3,3-difluoro functionality before condensation with the heterocyclic base needed to be developed. In addition, the preparation of the key intermediate **7** required improvement from our previous report due to the high cost of L-xylose as well as lengthy synthetic steps. Therefore, 2-deoxyribose, which can be prepared from inexpensive L-arabinose in large quantity and high yield,[6] was methylated followed by benzoylation to give the 5-*O*-benzoyl methyl furanoside **4**. Pyridinium dichromate-mediated oxidation of compound **4** followed by difluorination with DAST provided the intermediate **6**, which was transglycosylated to the key intermediate (4*S*)-1-*O*-acetyl-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-D-ribofuranoside (**7**) in high yield (55% yield overall from **4**, Scheme 1) by treatment with acetic anhydride and acetic acid in the presence of sulfuric acid. Condensation of **7** with various pyrimidine heterocyclic bases under Vorbrüggen conditions gave α/β mixtures of the corresponding pyrimidine analogues **8**−**15** in 40−60% yield (Scheme 2). The stereochemistry of condensation products has been established by NOE experiments, where clear correlations between protons $H_{1'}$ and $H_{4'}$ were unequivocally observed for β-anomers.[4]

Scheme 3. Synthesis of the Purine Nucleosides[a]

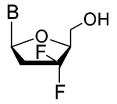


[a] Keys: (a) HMDS, $(NH_4)_2SO_4$, 6-chloropurine; TMSOTf, CH_3CN, (b) HMDS, $(NH_4)_2SO_4$, 2-fluoro-6-chloropurine; TMSOTf, CH_3CN, (c) NH_4OH, 60 °C, (d) $HSCH_2CH_2OH$, NaOMe, 60 °C, (e) NaOMe, DMF, rt, (f) NH_3 bubbling, DME, rt.

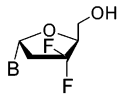
The ^1H NMR data of the condensation products are summarized in Table 5. The protecting groups on the heterocyclic base and the 5′-position of the sugar moiety were deblocked by methanolic ammonia to give the corresponding 2′,3′-dideoxy-3′,3′-difluoro pyrimidine nucleosides **16**−**23** (Scheme 2), which underwent smooth elimination to the final 3′-fluoro-2′,3′-unsaturated pyrimidine nucleosides **24**−**29** by treatment with NaOMe in DMF. However, the instability of the uridine analogues (**30** and **31**) under the reaction conditions resulted in decomposition, which was circumvented by using a 2:1 mixture of DMF and dioxane to give the desired compounds (**30** and **31**) in moderate yields (54% and 41%, respectively).

For the preparation of the purine nucleosides, the key intermediate **7** was condensed with 6-chloropurine and 2-fluoro-6-chloropurine to give α/β mixtures of the corresponding nucleosides **32/33** and **42/43** in 47% and 78% yield, respectively (Scheme 3). To achieve a clean and high-yield conversion, the temperature of the reaction mixture had to be carefully controlled in such a manner that, after addition of TMSOTf at 0 °C, the reaction mixture was stirred for 6 h at room temperature and then for another 2 h at 60 °C. Amination of the mixture of **32** and **33** by treatment with ammonium hydroxide at 60 °C in a steel bomb gave the adenosine analogue (**34** and **35**), whereas hydrolysis in the presence of sodium methoxide and 2-mercaptoethanol in refluxing methanol gave the inosine analogues (**36** and **37**). Dry ammonia gas was bubbled into a solution of **42** and **43** in ethylene glycol dimethyl ether (DME) at room temperature for 16 h to give the 2-amino-6-chloropurine derivatives (**44** and **45)** in 29% and 28% yield, respectively, which were readily separated by silica gel column chromatography. The 2-amino-6-chloropurine derivatives (**44** and **45)**, thus obtained, were hydrolyzed by using NaOMe and 2-mercaptoethanol in refluxing methanol to give 2′,3′-dideoxy-3′,3′-difluoroguanosine derivatives (**46** and **47**) in 51% and 80% yield, respectively. The 2′,3′-dideoxy-3′,3′-difluoro-

Table 1. Anti-HIV-1 Activity of L-2′-Deoxy-3′,3′-difluoronucleosides



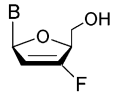
16, 18, 20, 22, 34, 36, 46



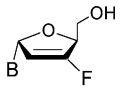
17, 19, 21, 23, 35, 37, 47

compd	base	activity (EC_{50}, μM)	cytotoxicity (IC_{50}, μM) PBM	CEM	Vero
16	cytosine	11.5	>100	>100	>100
17	cytosine	>100	>100	>100	>100
18	5-F-cytosine	8.8	>100	>100	>100
19	5-F-cytosine	>100	>100	>100	>100
20	thymine	>100	>100	>100	>100
21	thymine	>100	>100	>100	>100
22	uracil	>100	>100	>100	>100
23	uracil	>100	>100	>100	>100
34	adenine	>100	>100	>100	>100
35	adenine	>100	>100	>100	>100
36	hypoxanthine	>100	>100	>100	>100
37	hypoxanthine	>100	>100	>100	>100
46	guanine	>100	>100	>100	>100
47	guanine	>100	>100	>100	>100

Table 2. Anti-HIV-1 Activity of L-2′,3′-Dideoxy-2′,3′-didehydro-3′-fluoronucleosides



24, 26, 28, 30, 38, 40, 48



25, 27, 29, 31, 39, 49

compd	base	activity (EC_{50}, μM)	cytotoxicity (IC_{50}, μM) PBM	CEM	Vero
24	cytosine	0.089	86.9	>100	>100
25	cytosine	>100	>100	>100	>100
26	5-F-cytosine	0.018	>100	>100	>100
27	5-F-cytosine	30.0	>100	>100	>100
28	thymine	>100	>100	>100	>100
29	thymine	>100	>100	>100	>100
30	uracil	>100	>100	30.1	>100
31	uracil	>100	>100	>100	>100
38	adenine	>100	>100	>100	>100
39	adenine	>100	>100	>100	>100
40	hypoxanthine	>100	>100	>100	>100
48	guanine	38.5	17.4	58.4	36.5
49	guanine	>100	>100	>100	>100

purine nucleosides (**34**−**37** and **46**−**47**) were treated with NaOMe in DMF to give the final 3′-fluoro-2′,3′-unsaturated nucleosides (**38**−**41** and **48**−**49**). However, these nucleosides slowly decomposed during purification (column chromatography on silica gel followed by fractional crystallization in EtOH) resulting in low yields (33−46%).

Structure−Activity Relationships. Using AZT as a positive control, the EC_{50} values of the synthesized nucleosides were evaluated against HIV-1 in human PBM cells in vitro, and the results are summarized in Tables 1 and 2. In the 3′,3′-difluoro series (Table 1), only the cytidine **16** (EC_{50} 11.5 μM) and 5-fluorocytidine **18** (EC_{50} 8.8 μM) analogues showed moderate antiviral activity. However, the 2′,3′-unsaturated nucleosides (Table 2) showed more potent anti-HIV-1 activity in comparison to the corresponding 3′,3′-difluoro analogues, among which cytidine **24** (EC_{50} 0.089 μM), 5-fluorocytidine **26** (EC_{50} 0.018 μM), and guanosine **48** (EC_{50} 38.5 μM) showed moderate to potent antiviral activities. Even though the cytidine analogue **24** showed minimal cytotoxicity in PBM cells (IC_{50} 86.9 μM), the selectivity index (SI, 976) is very high. No other synthesized nucleoside showed significant cytotoxicity up to 100 μM except the 2′,3′-unsaturated uridine **30** (IC_{50} 30.1 μM for CEM) and guanosine **48** (IC_{50} 17.4, 58.4, and 36.5 μM in PBM, CEM, and Vero cells, respectively) analogues.

Table 3. Activity of Selected Nucleosides against Lamivudine-Resistant Virus (HIV-1_{M184V}) in Human PBM Cells

compound	xxBRU (EC_{50}, μM)	M184V EC_{50}, μM	M184V EC_{90}, μM	FI[a]
24 (β-cytidine)	0.023	2.4	15.7	104
26 (β-5F-cytidine)	0.009	10.9	67.4	1211
L-d4C	0.17	2.9	28.2	17
AZT	0.01	0.003	0.025	0.3
3TC	0.035	>100	>100	>2857

[a] FI is the fold increase (EC_{50} HIV-1_{M184V}/EC_{50} HIV-1_{xxBRU}). [b] Not available.

Table 4. Comparison of the Calculated Energy Required for the Dissociation of the Glycosidic Bond of the Protonated Cytidine Nucleosides with Their Stabilities at pH 2 Buffer Solution

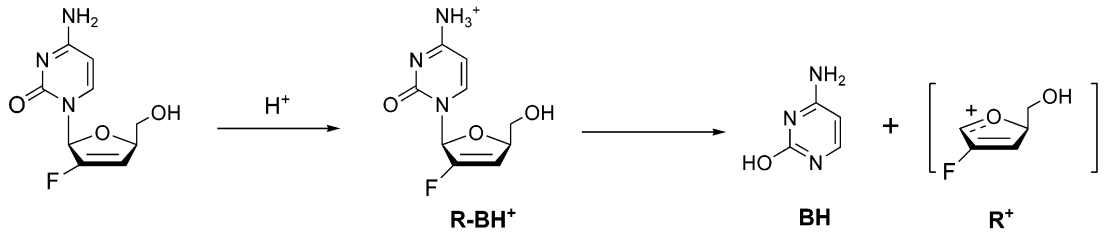
compound	$t_{1/2}$ (h)[a]	ΔE_{calcd},[b] (kcal/mol)
D-2′-Fd4C[2c]	>72	57.55
L-3′-Fd4C (**24**)	48	50.71
L-d4C	1	40.56

[a] Half-life at pH 2 buffer solution. [b] Calculated energy required for dissociation of the glycosidic bond, $\Delta E_{calcd} = E(R^+) + E(BH) - E(R{-}BH^+)$ (Figure 1).

Antiviral Activity against Lamivudine-Resistant (HIV-1_{M184V}) Mutant Strain. Single point mutation at residue 184 (M184V) of the reverse transcriptase (RT) in HIV causes high-level resistance to lamivudine (3TC) and contributes to the failure of anti-AIDS therapy.[7,8] Therefore, to discover novel drug candidates active against the M184V mutant HIV-1 RT, the cytosine (**24**), 5-fluorocytosine analogues (**26**), and L-d4C 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 3). Unfortunately, the results of the study suggests that the 3′-fluoro-2′,3′-unsaturated nucleosides are significantly cross-resistant to the M184V mutant. Interestingly, the 5-fluorocytosine analogue (**26**) was 11.6-fold more cross-resistant than the cytosine analogue (**24**), suggesting that in addition to the sugar conformation, the base moiety also plays a role. Compared with L-d4C, L-3′Fd4C (**24**) showed more potent anti-HIV activity against wild-type RT. However, L-d4C and L-3′Fd4C (**24**) showed similar anti-HIV activity against M184V RT. Therefore, it is apparent that the 3′-fluorine substituent of L-3′Fd4C (**24**) can be specifically recognized by the wild-type RT, but not by M184V RT (vide infra for molecular modeling studies).

Chemical Stability. It is conceivable that the 2′-fluoro-2′,3′-unsaturated compound, whose fluorine atom is closer to the glycosyl bond than the 3′-fluoro analogue, is more effective in stabilizing the nucleoside in an acidic environment. To prove this hypothesis, D-2′-Fd4C,[2c] L-3′-Fd4C **24**, and L-d4C[1e] were treated in a pH 2 buffer solution, and their stabilities were measured by TLC every 10 min (initial 3 h) to 6 h (Table 4). As expected, L-d4C started decomposition to the corresponding sugar moiety and aglycon (cytosine), marking its half-life at 1 h. Compared with D-2′-Fd4C, which showed indefinite

Scheme 4. Dissociation of the Glycosyl Bond of 2′,3′-Unsaturated Nucleosides



stability under acidic buffer solution, L-3′-Fd4C **24** slowly decomposed, but its half-life (48 h) was much longer than that for L-d4C.

In many cases, the stability of the nucleoside analogue, particularly the stability of the glycosyl bond, is an important factor governing the biological activity as well as the therapeutic usefulness of the nucleoside drug candidate. Therefore, for the purpose of designing novel nucleoside antiviral agents, an indirect method of measuring the stability of the glycosyl bond was of interest. For this purpose, we tried to evaluate the energy required for the dissociation of the glycosyl bond as a result of a protonation under acidic conditions (Scheme 4). The energy of each chemical species was determined by quantum mechanical (RHF, 6-31G*) method, and the energy required for the bond dissociation reaction (ΔE) was calculated to find a potential correlation with the chemical stability of the nucleoside analogues. As shown in Table 4, the stability of the resulting oxonium ion (Scheme 4) correlates with the energy required for the bond dissociation. The fluorine substitution at the 2′- or 3′-position destabilizes the corresponding oxonium ion species, and the destabilization was greater in the 2′-F substitution because of the shorter distance between the oxonium ion center and the fluorine atom.

Molecular Modeling. The triphosphate of the quantum mechanically geometry-optimized L-3′-Fd4C was docked into the active site of HIV-1 RT, and the resulting complex was minimized[9] to give a characteristic binding mode of this nucleoside triphosphate, in which the 3′-fluorine atom is strongly interacting with the amide backbone of Asp185 by hydrogen bonding (Figure 1a). The large binding pocket of the HIV-1 RT, which accommodates the unnatural L-configured nucleosides such as 3TC,[10] L-d4FC,[1e] and L-2′-Fd4C,[2b] was found to provide enough space for L-3′-Fd4CTP to bind at the active site without any steric hindrance (Figure 1b). This favorable binding mode, however, cannot be maintained when the triphosphate of the synthesized 3′-fluoro 2′,3′-unsaturated nucleoside binds to the active site of M184V RT because the bulky side chain of Val184 occupies the space needed for binding and pushes the 3′-fluorine atom of L-3′-Fd4CTP away from the amide backbone of Asp185, out of hydrogen bonding distance, resulting in poor binding energy and thereby cross-resistance (Figure 1c).

Metabolic Stability. The metabolic stability toward deaminases such as cytidine deaminase and adenosine deaminase is another measure for the successful therapeutic candidates because, from a therapeutic point of view, along with nucleoside phosphorylases, these are the two major deactivating enzymes of the nucleoside catabolism pathway.[11] Due to the unavailability of the commercial cytidine deaminase, only the adenosine deaminase (ADA) binding efficiencies and deamination kinetics studies of the adenosine analogue **38** were conducted.[12] As expected, the unnatural L-configured adenine derivative **38** was found not to be deaminated by the enantioselective adenosine deaminase ensuring the metabolic stability of the unnatural L-nucleosides against the enzyme. As other L-cytidine analogues,[13] it is unlikely that the L-cytidine derivatives with 3′-F substitution are substrates for cytidine deaminase.

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

Experimental Section

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz for ^{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.

Methyl 2-Deoxy-L-erythropentofuranoside (3). Methanolic HCl (1%, 50.4 mL made by adding 1.7 mL of AcCl to100 mL of MeOH) was added to a solution of **2**[6] (25.2 g, 0.19 mol) in MeOH (250 mL). The reaction mixture was stirred at room temperature for 25 min and neutralized with Amberlite IRA 400 (OH^-) resin. After filtration followed by evaporation of the volatiles, the residual syrup was used for the next step without further purifications.

Methyl 5-*O*-Benzoyl-2-deoxy-β-L-erythropentofuranoside (4). Methyl 2-deoxy-L-ribofuranoside **3** (1.1 g, 7.46 mmol)

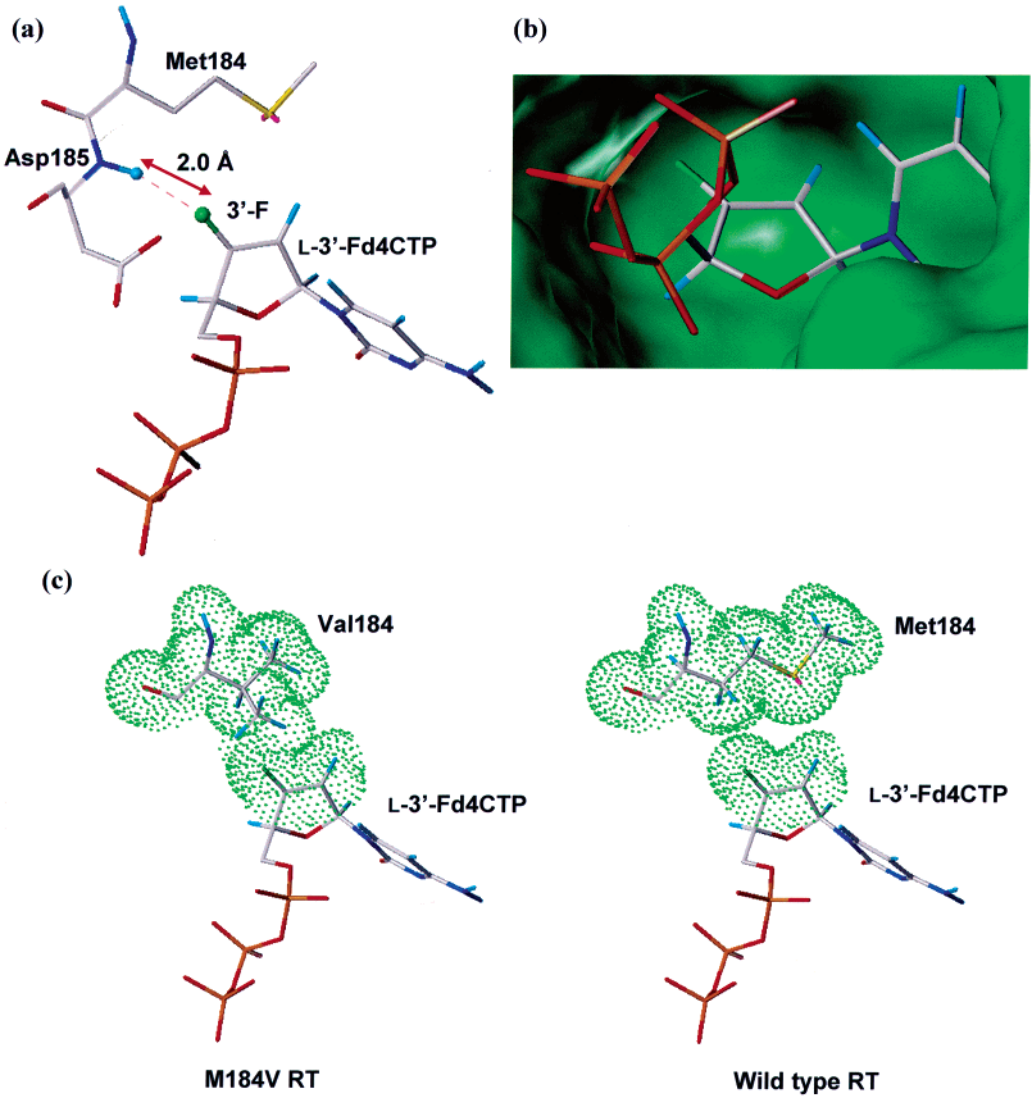


Figure 1. (a) Binding mode of L-3′-Fd4CTP at the active site of HIV-1 RT. 3′-F is hydrogen bonded to the amide backbone of Asp185. (b) Binding site of HIV-1 RT, deep and spacious enough to accommodate the unnatural L-nucleoside triphosphates. (c) Steric hindrance of L-3′-Fd4CTP with the bulky side chain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

was dissolved in dry pyridine (10 mL), cooled to 0 °C, and treated with BzCl. The mixture was stirred at room temperature for 10 h and then diluted with cold water. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were washed twice with aqueous $NaHCO_3$ solution, 2 N HCl, and water. The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a crude, which was purified by column chromatography on silica gel (hexane:EtOAc = 2:1) to give 1.35 g (5.37 mmol, 72% yield) of **4** as a pale yellow oil (1:1 epimeric mixture): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08−8.07 (m, 2H), 8.03−8.01 (m, 2H), 7.60−7.56 (m, 2H), 7.47−7.43 (m, 4H), 5.15 (d, J = 4.5 Hz, 1H), 5.11 (dd, J = 5.3, 1.5 Hz, 1H), 4.57 (td, J = 6.8, 4.8 Hz, 1H), 4.43−4.36 (m, 4H), 4.26 (d, J = 6.1 Hz, 1H), 4.20 (q, J = 5.2 Hz, 1H), 3.41 (s, 3H), 3.32 (s, 3H), 2.32 (ddd, J = 13.3, 6.8, 1.5 Hz, 1H), 2.22−2.10 (m, 3H); HRMS (FAB) obsd, m/z 253.1083, calcd for $C_{13}H_{17}O_5$, m/z 253.1076 (M + H$^+$). Anal. ($C_{13}H_{16}O_5$) C, H.

Methyl 5-*O*-Benzoyl-2-deoxy-*β*-L-glycero-pentofuranosid-3-ulose (5). Chromic anhydride (2.73 g, 27.30 mmol) was added to a stirred mixture of anhydrous pyridine (4.5 mL, 55.64 mmol) and anhydrous dichloromethane (50 mL) at room temperature, and stirring was continued at room temperature for 15 min. A solution of **4** (1.72 g, 6.82 mmol) in anhydrous dichloromethane (20 mL) was then added, followed immediately by acetic anhydride (2.6 mL, 27.51 mmol). After 15 min, the dark brown solution was treated with ethyl acetate (400 mL), and the resulting mixture was filtered through a TLC-grade silica gel pad, which was washed with ethyl acetate (one portion of 500 mL). The filtrate was concentrated under reduced pressure and coevaporated with toluene (3 × 100 mL), and solvents were removed in vacuo to give 1.50 g (88%) of **5** as an orange oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.12−8.10 (m, 4H), 7.63−7.58 (m, 2H), 7.49−7.45 (m, 4H), 5.41 (t, J = 5.0 Hz, 2H), 4.80 (dd, J = 12.1, 2.7 Hz, 1H), 4.69 (dd, J = 11.7, 3.2 Hz, 1H), 4.51−4.47 (m, 2H), 4.44−4.42 (m, 1H), 4.31 (t, J = 3.0 Hz, 1H), 3.50 (s, 3H), 3.43 (s, 3H), 2.85 (dd, J = 18.3, 5.7 Hz, 1H), 2.71 (ddd, J = 18.2, 5.4, 0.6 Hz, 1H), 2.56 (d, J = 12.6 Hz, 1H), 2.52 (d, J = 12.4 Hz, 1H); HRMS (FAB) obsd, m/z 251.0920, calcd for $C_{13}H_{15}O_5$, m/z 251.0919 (M + H$^+$). Anal. ($C_{13}H_{14}O_5$·0.07$C_6H_5CH_3$) C, H.

Methyl 5-*O*-Benzoyl-2-dideoxy-3,3-difluoro-3-deoxo-*β*-L-glyceropentofuranosid-3-ulose (6). (Diethylamino)sulfur trifluoride (3.0 mL, 22.71 mmol) was added to a solution of **5** (1.40 g, 5.59 mmol) in anhydrous dichloromethane (25 mL), and the reaction mixture was refluxed for 36 h. The resulting solution was then carefully poured into an ice-cooled saturated solution of sodium bicarbonate (50 mL) and extracted with diethyl ether (4 × 100 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to a crude oil that was purified by silica gel flash column chromatography (1:9 ethyl acetate/hexanes) to give 1.01 g (66%) of **6** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.90−

7.18(m, 4H), 5.10(m, 1H), 4.54−4.29(m, 3H), 3.31, 3.29(s, 3H), 3.28 (s, 3H), 2.67−2.21(m, 2H); HRMS (FAB) obsd, *m/z* 273.0950, calcd for $C_{13}H_{15}F_2O_4$, *m/z* 273.0938 ($M + H^+$). Anal. ($C_{13}H_{14}F_2O_4$) C, H.

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

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

(−)-*N*^{4}-Benzoyl-1-[(1*S*,4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]cytosine (8) and (+)-*N*^{4}-Benzoyl-1-[(1*R*,4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]cytosine (9). A mixture of N^4-benzoylcytosine (225 mg, 1.05 mmol) and ammonium sulfate (7 mg, 0.053 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was heated under reflux for 4 h, and then the solvent was removed in vacuo at 30−35 °C. To the residual oil, a solution of **7** (210 mg, 0.70 mmol) in anhydrous acetonitrile (10 mL) was added followed, upon cooling to 0 °C, by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.19 mL, 1.05 mmol). The resulting mixture was stirred at room temperature overnight, diluted to 50 mL with dichloromethane, and poured into an ice-cold saturated solution of $NaHCO_3$ (25 mL). The organic phase was separated, washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated to a crude that was purified by flash column chromatography (3:97 MeOH/$CHCl_3$) to give 80 mg of **9** (25%) as the first eluted product and 100 mg of **8** (31%) as the second eluted product. Both isomers are colorless oils, which become white solids upon trituration with diethyl ether and a drop of methanol. For the compound **8**; white solid, mp 166−167 °C (dec); $[\alpha]^{24}_D$ −89.75° (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} 259.5 nm, 301.0 nm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.48, 165.91, 162.58, 154.55, 143.35, 133.59, 133.31, 129.63, 129.07, 128.60, 127.53, 125.71 (t, J_{C-F} = 253.6 Hz), 96.90, 84.39 (dd, J_{C-F} = 6.6, 3.6 Hz), 79.80 (dd, J_{C-F} = 31.5, 25.1 Hz), 60.77 (d, J_{C-F} = 4.8 Hz), 41.69 (t, J_{C-F} = 23.7 Hz); HRMS (FAB) obsd, *m/z* 456.1377, calcd for $C_{23}H_{20}F_2N_3O_5$, *m/z* 456.1371 (MH^+). Anal. ($C_{23}H_{19}F_2N_3O_5$) C, H, N. For the compound **9**: white solid, mp 176−178 °C (dec); $[\alpha]^{24}_D$ 42.61° (*c* 1.19, $CHCl_3$); UV (MeOH) λ_{max} 259.0 nm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.54, 165.80, 162.77, 154.62, 143.48, 133.51, 133.19, 129.58, 128.94, 128.60, 127.58, 126.51 (dd, J_{C-F} = 255.7, 249.3 Hz), 96.71, 86.83 (dd, J_{C-F} = 8.1, 2.0 Hz), 80.78 (dd, J_{C-F} = 31.7, 25.2 Hz), 61.47 (t, J_{C-F} = 5.3 Hz), 41.14 (t, J_{C-F} = 24.0 Hz); HRMS (FAB) obsd, *m/z* 456.1374, calcd for $C_{23}H_{20}F_2N_3O_5$, *m/z* 456.1371 (MH^+). Anal. ($C_{23}H_{19}F_2N_3O_5$) C, H, N.

(−)-*N*^{4}-Benzoyl-1-[(1*S*,4*S*)- 5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]-5-fluorocytosine (10) and (+)-*N*^{4}-Benzoyl-1-[(1*R*,4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]-5-fluorocytosine (11). See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **10** and **11** were obtained on 1.67-mmol scale in 59% yield: For the compound **10**; $[\alpha]^{28}_D$ −67.91° (*c* 0.87, CH_2Cl_2); UV ($CHCl_3$) λ_{max} 326.5 nm; ^{13}C NMR ($CDCl_3$) δ 165.85, 152.25 (d, J = 18.4 Hz), 146.90, 140.06 (d, J = 240.6 Hz), 135.44, 133.60, 133.19, 129.90, 129.55, 129.01, 128.61, 128.34, 125.51 (t, J = 253.9 Hz), 124.09, 123.75, 82.69, 79.50 (dd, J = 31.4, 24.9 Hz), 60.58 (d, J = 4.1 Hz), 40.80 (t, J = 23.8 Hz); Anal. ($C_{23}H_{18}F_3N_3O_5$) C, H, N.; For the compound **11**; $[\alpha]^{27}_D$ 41.49° (*c* 0.15, $CHCl_3$);); UV ($CHCl_3$) λ_{max} 327.0 nm; ^{13}C NMR ($CDCl_3$) δ 165.80, 152.49 (d, J = 16.5 Hz), 146.91, 139.98 (d, J = 240.2 Hz), 135.53, 133.63, 133.23, 129.94, 129.62, 129.05, 128.68, 128.40, 126.35 (dd, J = 257.7, 249.5 Hz), 124.53, 124.23, 85.60, 80.79 (dd, J = 31.1, 25.1 Hz), 61.42 (d, J = 5.6 Hz), 40.80 (t, J = 24.3 Hz); Anal. ($C_{23}H_{18}F_3N_3O_5 \cdot 0.2C_6H_{14}$) C, H, N.

***N*^{4}-Benzoyl-1-[(1*S*,4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]thymine (12) and *N*^{4}-Benzoyl-1-[(1*R*,4*S*)-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]thymine (13).** See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **12** and **13** were obtained on 0.62-mmol scale in 53% yield: UV (MeOH) λ_{max} 263.5 nm; ^{13}C NMR ($CDCl_3$, 100 MHz) (*major isomer*) δ 165.87, 163.78, 150.25, 135.05, 133.47, 126.67 (dd, J_{C-F} = 255.7, 251.1 Hz), 111.71, 84.84 (t, J_{C-F} = 5.9 Hz), 80.15 (dd, J_{C-F} = 31.3, 24.5 Hz), 61.51 (dd, J_{C-F} = 6.7, 4.1 Hz), 40.34 (t, J_{C-F} = 24.3 Hz), 12.59; (*minor isomer*) δ 165.84, 163.45, 150.25, 133.82, 133.59, 125.91 (dd, J_{C-F} = 255.1, 252.3 Hz), 112.39, 81.18 (t, J_{C-F} = 5.9 Hz), 79.11 (dd, J_{C-F} = 31.6, 24.6 Hz), 60.88 (dd, J_{C-F} = 6.1, 3.1 Hz), 40.47 (t, J_{C-F} = 23.9 Hz), 12.32; HRMS (FAB) obsd, *m/z* 367.1122, calcd for $C_{17}H_{17}F_2N_2O_5$, *m/z* 367.1106 (MH^+). Anal. ($C_{17}H_{16}F_2N_2O_5 \cdot 0.03CHCl_3$) C, H, N.

1-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]uracil (14) and 1-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]uracil (15). See the general procedure for condensation reaction of the acetate **7** with pyrimidines. The title compounds **14** and **15** were obtained on 1.67-mmol scale in 35% yield: UV ($CHCl_3$) λ_{max} 256.5 nm; Anal. ($C_{16}H_{14}F_2N_2O_5$) C, H, N.

General procedure for the deprotection reaction of the pyrimidine analogures **8**−**1**5 with methanolic ammonia. The preparation of cytosine derivative **16** is representative.

(−)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl]cytosine (16). A mixture of **8** (70 mg, 0.15 mmol) in saturated ammonia/methanol solution (10 mL) was stirred at room temperature for 4 h, and then concentrated to dryness under reduced pressure. The residue was purified by preparative TLC (1:9 methanol/chloroform) to give 38 mg of **16** (100%) as a white solid: mp 194−196 °C (dec); $[\alpha]^{24}_D$ −51.89° (*c* 1.15, MeOH); UV (H_2O) λ_{max} 276.5 nm (ε 18 160, pH 2), 268.0 nm (ε 13 280, pH 7), 268.5 nm (ε 13 580, pH 11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.19, 158.46, 142.62, 128.71 (dd, J_{C-F} = 255.1, 247.4 Hz), 97.04, 84.73 (dd, J_{C-F} = 6.7, 4.9 Hz), 83.74 (dd, J_{C-F} = 29.5, 25.0 Hz), 60.73 (t, J_{C-F} = 4.8 Hz), 42.14 (t, J_{C-F} = 23.4 Hz), 12.29; HRMS (FAB) obsd, *m/z* 248.0850, calcd for $C_9H_{12}F_2N_3O_3$, *m/z* 248.0847 (MH^+). Anal. ($C_9H_{11}F_2N_3O_3 \cdot 0.1H_2O$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl]cytosine (17). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compound **17** was obtained on 0.09-mmol scale in 100% yield: White solid, mp 198−200 (dec); $[\alpha]^{25}_D$ 28.23° (*c* 0.27, MeOH); UV (H_2O) λ_{max} 277.5 nm (ε 15 010, pH 2), 269.0 nm (ε 10 130, pH 7), 269.0 nm (ε 10 150, pH 11); ^{13}C NMR (CD_3OD, 100 MHz) δ 168.37, 158.54, 142.57, 129.28 (dd, J_{C-F} = 254.3, 247.2 Hz), 96.48, 87.75 (dd, J_{C-F} = 8.7, 3.6 Hz), 84.93 (dd, J_{C-F} = 29.3, 25.3 Hz), 61.24 (t, J_{C-F} = 5.4 Hz), 42.63 (t, J_{C-F} = 23.9 Hz), 12.29; HRMS (FAB) obsd, *m/z* 248.0829, calcd for $C_9H_{12}F_2N_3O_3$, *m/z* 248.0847 (MH^+). Anal. ($C_9H_{11}F_2N_3O_3$) C, H, N.

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

the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compounds **18** and **19** were obtained on 1.29-mmol scale in 47% and 43% yield, respectively: For the compound **18**; $[\alpha]^{25}_D$ −54.71° (*c* 0.99, CH_3OH); UV (H_2O) λ_{max} 283.5 nm (ϵ 10 050, pH 2), 278.0 nm (ϵ 8430, pH 7), 278.0 nm (ϵ 8350, pH 11); ^{13}C NMR (CD_3OD) δ 150.30 (d, J = 14.1 Hz), 146.82, 129.14 (d, J = 241.0 Hz), 118.67 (dd, J = 255.9, 247.1 Hz), 116.73 (d, J = 33.3 Hz), 74.93 (dd, J = 7.8, 3.5 Hz), 73.82 (dd, J = 29.6, 25.1 Hz), 50.69 (t, J = 4.9 Hz), 32.02 (t, J = 23.4 Hz); HRMS (FAB) obsd, *m*/*z* 266.08, calcd for $C_9H_{11}F_3N_3O_3$, *m*/*z* 266.07 $(M + H)^+$; Anal. ($C_9H_{10}F_3N_3O_3$) C, H, N. For the compound **19**; mp 159−160 °C; $[\alpha]^{25}_D$ 19.68° (*c* 0.23, CH_3OH);); UV (H_2O) λ_{max} 284.0 nm (ϵ 7494, pH 2), 278.5 nm (ϵ 5816, pH 7), 278.5 nm (ϵ 6030, pH 11); ^{13}C NMR (CD_3OD) δ 159.86 (d, J = 14.0 Hz), 156.28, 138.51 (d, J = 242.3 Hz), 128.65 (dd, J = 255.1, 247.2 Hz), 126.20 (d, J = 34.1 Hz), 87.27 (d, J = 5.5 Hz), 84.44 (dd, J = 29.0, 25.5 Hz), 60.69 (t, J = 5.3 Hz), 42.02 (t, J = 24.1 Hz); Anal. ($C_9H_{10}F_3N_3O_3$) C, H, N.

(−)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl]thymine (20) and (−)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl]thymine (21). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compounds **20** and **21** were obtained on 0.38 mmol scale in 55% and 45% yield, respectively: For the compound **20**; White solid: mp 140−148 °C (dec); $[\alpha]^{22}_D$ −31.27° (*c* 0.21, $CHCl_3$); UV (H_2O) λ_{max} 264.0 nm (ϵ 11 700, pH 2), 263.5 nm (ϵ 12 170, pH 7), 264.0 nm (ϵ 8660, pH 11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.59, 150.36, 135.40, 127.26 (dd, J_{C-F} = 255.8, 246.7 Hz), 110.20, 80.97 (dd, J_{C-F} = 28.5, 24.4 Hz), 80.32 (dd, J_{C-F} = 7.1, 4.1 Hz), 58.50, 38.54 (t, J_{C-F} = 23.6 Hz), 12.29; HRMS (FAB) obsd, *m*/*z* 263.0849, calcd for $C_{10}H_{13}F_2N_2O_4$, *m*/*z* 263.0843 (MH^+). Anal. ($C_{10}H_{12}F_2N_2O_4 \cdot 0.14\ Et_2O$) C, H, N. For the compound **21**; White solid: mp 145−148 °C (dec); $[\alpha]^{24}_D$ −26.50° (*c* 0.15, $CHCl_3$); UV (H_2O) λ_{max} 265.5 nm (ϵ 12 820, pH 2), 263.5 nm (ϵ 12 590, pH 7), 264.0 nm (ϵ 10 030, pH 11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.77, 150.38, 136.32, 127.77 (dd, J_{C-F} = 252.3, 249.2 Hz), 109.82, 83.47 (t, J_{C-F} = 6.0 Hz), 81.75 (dd, J_{C-F} = 28.5, 24.3 Hz), 58.76, 38.57 (t, J_{C-F} = 23.6 Hz), 12.17; HRMS (FAB) obsd, *m*/*z* 263.0835, calcd for $C_{10}H_{13}F_2N_2O_4$, *m*/*z* 263.0843 (MH^+). Anal. ($C_{10}H_{12}F_2N_2O_4 \cdot 0.5MeOH$) C, H, N.

(−)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl]uracil (22) and (−)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl]uracil (23). See the general procedure for the deprotection reaction of the cytidine analogue **8** with methanolic ammonia. The title compounds **22** and **23** were obtained on 0.57-mmol scale in 42% and 56% yield, respectively: For the compound **22**; mp 138−140 °C; $[\alpha]^{26}_D$ −25.86° (*c* 0.21, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 8725, pH 2), 259.0 nm (ϵ 9145, pH 7), 259.0 nm (ϵ 6638, pH 11); ^{13}C NMR (CD_3OD) δ 165.92, 152.01, 141.79, 128.09 (dd, J = 255.5, 247.4 Hz), 103.35, 83.20 (dd, J = 29.6, 24.7 Hz), 83.08 (dd, J = 8.0, 4.3 Hz), 60.21 (t, J = 4.7 Hz), 40.87 (t, J = 23.6 Hz); Anal. ($C_9H_{10}F_2N_2O_4$) C, H, N. For the compound **23**; mp 194−196 °C; $[\alpha]^{25}_D$ −5.3° (*c* 0.32, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 8833, pH 2), 259.0 nm (ϵ 9150, pH 7), 259.0 nm (ϵ 6629, pH 11); ^{13}C NMR (CD_3OD) δ 166.58, 152.42, 142.50, 129.14 (dd, J = 254.4, 247.5 Hz), 103.23, 86.95 (dd, J = 8.1, 4.0 Hz), 84.78 (dd, J = 29.2, 25.2 Hz), 61.09 (t, J = 5.4 Hz), 41.82 (t, J = 24.2 Hz); Anal. ($C_9H_{10}F_2N_2O_4$) C, H, N.

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

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]cytosine (24). A mixture of **16** (30 mg, 0.12 mmol) and sodium methoxide (50 mg, 0.37 mmol) in anhydrous DMF (5 mL) was stirred at room temperature overnight and then quenched with 1 mL of a saturated aqueous solution of ammonium chloride. Evaporation of volatiles in vacuo gave a crude, which was purified by a short silica gel flash column chromatography ($CHCl_3$ to 1:15 MeOH/$CHCl_3$) to yield 15 mg (54%) of **24** as a white solid.: mp 182−183 °C (dec); $[\alpha]^{22}_D$ 6.67° (*c* 0.54, MeOH); UV (H_2O) λ_{max} 276.0 nm (ϵ 11 990, pH 2), 267.5 nm (ϵ 8010, pH 7), 264.5 nm (ϵ 8060, pH 11); ^{13}C NMR (CD_3OD, 100 MHz) δ 168.31, 163.40 (d, J_{C-F} = 284.8 Hz), 159.05, 144.05, 102.34 (d, J_{C-F} = 9.7 Hz), 95.68, 88.42 (d, J_{C-F} = 14.9 Hz), 81.96 (d, J_{C-F} = 24.7 Hz), 61.82 (d, J_{C-F} = 2.2 Hz); HRMS (FAB) obsd, *m*/*z* 228.0778, calcd for $C_9H_{11}FN_3O_3$, *m*/*z* 228.0784 (M + H+). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-α-L-ribofuranosyl]cytosine (25). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **25** was obtained on 0.06-mmol scale in 51% yield: White solid: mp 135 °C (dec); $[\alpha]^{24}_D$ 169.67° (*c* 1.17, MeOH); UV (H_2O) λ_{max} 276.5 nm (ϵ 14 140, pH 2), 268.0 nm (ϵ 9510, pH 7), 268.5 nm (ϵ 9660, pH 11); ^{13}C NMR (CD_3OD, 100 MHz) δ 167.67, 163.00 (d, J_{C-F} = 285.1 Hz), 158.31, 142.36, 101.93 (d, J_{C-F} =10.0 Hz), 96.82, 89.55 (d, J_{C-F} = 15.1 Hz), 81.80 (d, J_{C-F} = 24.9 Hz), 61.88 (d, J_{C-F} = 2.2 Hz); HRMS (FAB) obsd, *m*/*z* 228.0773, calcd for $C_9H_{11}FN_3O_3$, *m*/*z* 228.0784 (MH+). Anal. ($C_9H_{10}FN_3O_3 \cdot 0.82MeOH$) C, H, N.

(−)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]-5-fluorocytosine (26). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **26** was obtained on 0.12-mmol scale in 95% yield: mp 124 °C; $[\alpha]^{25}_D$ −25.47° (*c* 0.10, CH_3OH); UV (H_2O) λ_{max} 284.0 nm (ϵ 10 057, pH 2), 277.5 nm (ϵ 8191, pH 7), 277.5 nm (ϵ 8519, pH 11); ^{13}C NMR (CD_3OD) δ 162.99 (d, J = 285.1 Hz), 159.91 (d, J = 14.2 Hz), 156.91, 138.37 (d, J = 242.0 Hz), 127.44 (d, J = 33.0 Hz), 101.83 (d, J = 10.0 Hz), 88.32 (d, J = 15.0 Hz), 81.51 (d, J = 24.8 Hz), 61.08; Anal. ($C_9H_9F_2N_3O_3$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-α-L-ribofuranosyl]-5-fluorocytosine (27). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **27** was obtained on 0.15-mmol scale in 94% yield: mp 146 °C (dec); $[\alpha]^{26}_D$ 143.04° (*c* 0.13, CH_3OH); UV (H_2O) λ_{max} 284.0 nm (ϵ 7837, pH 2), 277.5 nm (ϵ 6280, pH 7), 278.0 nm (ϵ 6033, pH 11); ^{13}C NMR (CD_3OD) δ 163.23 (d, J = 281.8 Hz), 159.89 (d, J = 14.0 Hz), 156.77, 138.77 (d, J = 243.7 Hz), 126.25 (d, J = 31.8 Hz), 101.75 (d, J = 10.1 Hz), 90.06 (d, J = 15.2 Hz), 81.86 (d, J = 25.2 Hz), 61.86; Anal. ($C_9H_9F_2N_3O_3$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]thymine (28). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **28** was obtained on 0.11-mmol scale in 54% yield: White solid: mp 146−148 °C (dec); $[\alpha]^{22}_D$ 32.19° (*c* 0.20, $CHCl_3$); UV (H_2O) λ_{max} 264.0 nm (ϵ 10 080, pH 2), 264.5 nm (ϵ 10 130, pH 7), 264.5 nm (ϵ 7710, pH 11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.01, 161.54 (d, J_{C-F} = 287.8 Hz), 150.50, 136.85, 110.83, 99.64 (d, J_{C-F} = 10.2 Hz), 85.65 (d, J_{C-F} = 14.8 Hz), 79.98 (d, J_{C-F} = 24.5 Hz), 60.56 (d, J_{C-F} = 1.8 Hz), 12.28; HRMS (FAB) obsd, *m*/*z* 243.0789, calcd for $C_{10}H_{12}FN_2O_4$, *m*/*z* 243.0781 (MH^+). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

(−)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]thymine (29). See the general procedure for the elimination reaction of the cytidine analogue **16** with sodium methoxide. The title compound **29** was obtained on 0.10-mmol scale in 51% yield White solid: mp 146−148 °C (dec); $[\alpha]^{22}_D$ −18.68° (*c* 0.22, CH_3OH); UV (H_2O) λ_{max} 264.0 nm (ϵ 10 075, pH 2), 264.5 nm (ϵ 10 130, pH 7), 264.5 nm (ϵ 7708, pH 11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.61, 161.53 (d, J_{C-F} = 289.0 Hz), 150.83, 134.66, 111.96, 99.99 (d, J_{C-F} = 10.1 Hz), 86.97 (d, J_{C-F} = 14.9 Hz), 80.25 (d, J_{C-F} = 24.6 Hz), 61.42, 12.56; HRMS (FAB) obsd, *m*/*z* 243.0773, calcd for $C_{10}H_{12}FN_2O_4$, *m*/*z* 243.0781 (MH^+). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-dideoxy-3-fluoro-β-L-ribofuranosyl]uracil (30). A solution of **22** (56 mg, 0.23 mmol) in a 1:2 mixture of anhydrous dioxane (1 mL) and DMF (2 mL) was treated with sodium methoxide (49 mg, 0.90 mmol) at room temperature, and the resulting mixture was stirred at 50−55 °C for 6 h. After dilution with MeOH, the mixture was filtered through a short silica gel pad washing with 10:1 CH_2Cl_2:MeOH. The filtrate was evaporated to dryness under

reduced pressure, and the residue was purified by a short silica gel flash column chromatography (20:1 CH_2Cl_2/MeOH) to yield 20 mg (0.09 mmol, 54% yield) of **30** as a white solid: mp 92−94 °C; $[\alpha]^{22}_D$ 11.74° (*c* 0.61, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 26 681, pH 2), 258.5 nm (ϵ 15 591, pH 7), 259.0 nm (ϵ 11 073, pH 11); ^{13}C NMR (CD_3OD) δ 166.27, 163.31 (d, J = 285.4 Hz), 152.56, 143.16, 102.59, 101.19 (d, J = 10.8 Hz), 87.13 (d, J = 15.2 Hz), 81.53 (d, J = 24.5 Hz), 61.25 (d, J = 2.6 Hz); Anal. ($C_9H_9FN_2O_4$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-α-L-ribofuranosyl]uracil (31). See the procedure for the preparation of the uridine analogue **30** by elimination reaction of **22** with sodium methoxide. The title compound **31** was obtained on 0.36-mmol scale in 41% yield: mp 88−90 °C; $[\alpha]^{23}_D$ 184.6° (*c* 0.30, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 28 099, pH 2), 258.5 nm (ϵ 15 700, pH 7), 259.5 nm (ϵ 12 669, pH 11); ^{13}C NMR (CD_3OD) δ 166.11, 163.41 (d, J = 285.7 Hz), 152.31, 142.09, 103.34, 101.30 (d, J = 10.8 Hz), 88.77 (d, J = 15.4 Hz), 81.91 (d, J = 25.2 Hz), 61.77; Anal. ($C_9H_9FN_2O_4 \cdot 0.2C_2H_5OC_2H_5$) C, H, N.

9-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]-6-chloropurine (32) and 9-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]-6-chloropurine (33). A mixture of 6-chloropurine (1.03 g, 6.66 mmol), ammonium sulfate (44 mg, 0.33 mmol), and HMDS (20 mL) was refluxed for 6 h. After being cooled to room temperature, volatiles were evaporated under reduced pressure. To the residue, a solution of **7** in anhydrous CH_3CN was added, and the mixture was cooled to 0 °C, whereupon TMSOTf was added dropwise. The mixture was stirred for 8h at 0 °C, 10 h at room temperature, and then 3 h at 60 °C. The reaction mixture was poured into an aqueous $NaHCO_3$ solution and extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a yellow residue which was purified by column chromatography on silica gel (1:2 hexane:EtOAc) to give 610 mg (1.55 mmol, 47% yield) of a inseparable mixture of **32** and **33** as a pale yellow oil: UV (CH_2Cl_2) λ_{max} 266.6 nm; HRMS (FAB) obsd, *m*/*z* 395.0723, calcd for $C_{17}H_{18}ClF_2N_4O_3$, *m*/*z* 395.0719 ($M + H^+$); Anal. ($C_{17}H_{13}ClF_2N_4O_3 \cdot 0.3H_2O$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl]adenine (34) and (−)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl]adenine (35). A mixture of compound **32** and compound **33** (0.1 g, 0.25 mmol) was treated with aqueous ammonia in a steel bomb at 60 °C for 8 h. Solvent was evaporated, and the residue was purified by column chromatography (20:1 CH_2Cl_2:MeOH) to give the desired compounds **34** (95 mg, 0.35 mmol 42% yield) and **35** (100 mg, 0.37 mmol, 44% yield) as white solids. For the compound **34**; mp 198−200 °C; $[\alpha]^{25}_D$ 18.21° (*c* 0.45, CH_3OH); UV (H_2O) λ_{max} 256.0 nm (ϵ 12 797, pH 2), 258.0 nm (ϵ 13 143, pH 7), 258.5 nm (ϵ 12 876, pH 11); ^{13}C NMR (CD_3OD) δ 160.70, 157.58, 153.86, 150.21, 141.17, 128.22 (t, J = 257.6 Hz), 120.60, 83.81 (dd, J = 29.2, 24.6 Hz), 83.35 (d, J = 7.7 Hz), 60.96 (t, J = 5.7 Hz), 40.90 (t, J = 24.1 Hz); Anal. ($C_{10}H_{11}F_2N_5O_2$) C, H, N.

For the compound **35**; mp 152−154 °C; $[\alpha]^{24}_D$ −36.67° (*c* 0.47, CH_3OH); UV (H_2O) λ_{max} 256.0 nm (ϵ 14 121, pH 2), 258.0 nm (ϵ 14 226, pH 7), 258.5 nm (ϵ 14 598, pH 11); ^{13}C NMR (CD_3OD) δ 157.40, 154.03, 150.38, 140.79, 128.88 (t, J = 252.1 Hz), 120.61, 84.13 (t, J = 6.0 Hz), 83.71 (dd, J = 29.3, 24.7 Hz), 60.36 (dd, J = 6.8, 3.1 Hz), 41.15 (t, J = 25.0 Hz); Anal. ($C_{10}H_{11}F_2N_5O_2$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl]hypoxanthine (36) and (−)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl]hypoxanthine (37). A mixture of compounds **32** and **33** (0.222 g, 0.56 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.16 mL, 2.25 mmol) and NaOMe (0.125 g, 2.31 mmol) at 60 °C for 6 h. The resulting mixture was quenched with 0.1 mL of glacial AcOH, concentrated, and filtered through a short pad of silica gel. The filtrate was concentrated and purified by column chromatography (10:1 CH_2Cl_2:MeOH) to give the desired compounds **36** (0.06 g, 0.22 mmol, 39% yield) and **37** (0.056 g, 0.21 mmol, 38% yield) as white solids: For the compound **36**; mp 166−168 °C; $[\alpha]^{24}_D$ 5.82° (*c* 0.21, CH_3OH); UV (H_2O) λ_{max} 252.5 nm (ϵ 18 306, pH 2), 249.0 nm (ϵ 19 825, pH 7), 257.5 nm (ϵ 20 988, pH 11); ^{13}C NMR (CD_3OD) δ 158.85, 149.75 (dd, J = 276.7, 264.3 Hz), 147.10, 140.36, 125.74, 111.57, 83.80 (t, J = 24.6 Hz), 83.00 (d, J = 6.0 Hz), 60.68, 41.25 (t, J = 24.5 Hz); Anal. ($C_{10}H_{10}F_2N_4O_3$) C, H, N. For the compound **37**; mp 103−105 °C; $[\alpha]^{25}_D$ −39.39° (*c* 0.323, CH_3OH); UV (H_2O) λ_{max} 250.0 nm (ϵ 19 258, pH 2), 250.0 nm (ϵ 19 480, pH 7), 258.0 nm (ϵ 22 384, pH 11); ^{13}C NMR (CD_3OD) δ 159.28, 150.84 (t, J = 227.0 Hz), 147.33, 140.76, 126.48, 109.46, 84.90 (d, J = 5.8 Hz), 84.15 (dd, J = 29.0, 24.6 Hz), 60.71 (t, J = 4.0 Hz), 41.83 (t, J = 24.9 Hz); Anal. ($C_{10}H_{10}F_2N_4O_3$) C, H, N.

(−)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]adenine (38). A solution of **34** (30 mg, 0.11 mmol) in a 2:1 mixture of anhydrous dioxane (1.5 mL) and DMF (0.7 mL) was treated with NaOMe (18 mg, 0.33 mmol) at 0 °C, and the resulting mixture was stirred for 15 h at room temperature. The crude was filtered through a short silica gel pad, and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (10:1 CH_2Cl_2:MeOH) to give the desired product **38** (20 mg, 0.08 mmol, 73% yield) as a white solid: mp 148 °C (dec); $[\alpha]^{25}_D$ −11.78° (*c* 0.18, CH_3OH); UV (H_2O) λ_{max} 258.0 nm (ϵ 11 310, pH 2), 258.5 nm (ϵ 12 814, pH 7), 258.5 nm (ϵ 13 283, pH 11); ^{13}C NMR (CD_3OD) δ 163.23 (d, J = 285.1 Hz), 157.42, 153.84, 150.30, 141.56, 120.21, 101.33 (d, J = 11.3 Hz), 86.39 (d, J = 15.5 Hz), 82.16 (d, J = 24.7 Hz), 61.64; Anal. ($C_{10}H_{10}FN_5O_2$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-α-L-ribofuranosyl]adenine (39). See the procedure for the preparation of the adenosine analogue **38** by elimination reaction of **34** with sodium methoxide. The title compound **39** was obtained on 0.16-mmol scale in 62% yield: mp 146 °C (dec); $[\alpha]^{24}_D$ 86.06° (*c* 0.66, CH_3OH); UV (H_2O) λ_{max} 258.5 nm (ϵ 10 076, pH 2), 258.5 nm (ϵ 11 042, pH 7), 258.5 nm (ϵ 10 812, pH 11); ^{13}C NMR (CD_3OD) δ 163.70 (d, J = 285.5 Hz), 157.36, 154.08, 150.41, 140.64, 120.41, 101.10 (d, J = 11.4 Hz), 87.19 (d, J = 15.7 Hz), 81.88 (d, J = 25.2 Hz), 61.66; Anal. ($C_{10}H_{10}FN_5O_2$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]hypoxanthine (40). A solution of **36** (52 mg, 0.19 mmol) in a 2:1 mixture of anhydrous dioxane (1 mL) and DMF (0.5 mL) was treated with NaOMe (31 mg, 0.57 mmol) at 0 °C, and the resulting mixture was stirred for 12 h at room temperature. A mixture of NaOMe (31 mg, 0.57 mmol) in 0.5 mL of DMF was added to the mixture, and the mixture was stirred for 12 h at room temperature. The crude was filtered through a short silica gel pad, and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (10:1 CH_2Cl_2:MeOH) to give the desired product **40** (15 mg, 0.06 mmol, 32% yield) as a white solid and the starting material **36** (17 mg, 0.06 mmol) recovered: mp 200 °C (dec); $[\alpha]^{24}_D$ 6.74° (*c* 0.17, CH_3OH); UV (H_2O) λ_{max} 247.5 nm (ϵ 6346, pH 2), 248.5 nm (ϵ 6505, pH 7), 256.5 nm (ϵ 11 296, pH 11); ^{13}C NMR (CD_3OD) δ 160.65 (d, J = 271.7 Hz), 150.03, 147.00, 140.93, 125.25, 118.01, 101.19 (d, J = 11.3 Hz), 86.26 (d, J = 15.1 Hz), 82.20 (d, J = 24.6 Hz), 61.45; Anal. ($C_{10}H_9FN_4O_3$) C, H, N.

9-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]-6-chloro-2-fluoropurine (42) and 9-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]-6-chloro-2-fluoropurine (43). A mixture of 6-chloro-2-fluoropurine (1.26 g, 7.33 mmol), ammonium sulfate (220 mg, 1.67 mmol), and HMDS (40 mL) was heated under reflux for 4 h until a clear suspension was obtained. After being cooled to room temperature, volatiles were evaporated under reduced pressure. To the off-white solid, a solution of **7** (1 g, 3.33 mmol) in anhydrous CH_3CN was added, and the mixture was cooled to 0 °C, whereupon TMSOTf (0.96 mL, 5.33 mmol) was added dropwise. The mixture was stirred for 1 h at 0 °C, 24 h at room temperature and then 3 h at 60 °C. To a separatory funnel containing 100 mL of aqueous $NaHCO_3$ solution was slowly added the reaction mixture. After dilution with CH_2Cl_2 and phase separation, the aqueous layer was extracted three times

with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, evaporated, and purified by column chromatography on silica gel (4:1 hexane:EtOAc) to give 1.07 g (2.6 mmol, 78% yield) of an inseparable mixture of **42** and **43** as a pale yellow foam: UV (CH_2Cl_2) λ_{max} 269.5 nm; Anal. ($C_{17}H_{12}ClF_3N_4O_3 \cdot 0.04C_6H_{14}$) C, H, N.

(+)-1-[(1*R*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl]-2-amino-6-chloropurine (44) and (+)-1-[(1*S*,4*S*)-5-*O*-Benzoyl-2,3-dideoxy-3,3-difluoro-α-L-ribofuranosyl]-2-amino-6-chloropurine (45). Anhydrous ammonia was bubbled into a solution of a mixture of **42** and **43** (152 mg, 0.37 mmol) in dry dimethoxyethane (20 mL) at room temperature for 6 h. After evaporation of volatiles, the residue was filtered through a short silica gel pad to remove inorganic salts, and the filtrate was concentrated and purified by column chromatography on silica gel (3:1 hexane:EtOAc) to give the desired products **44** (43 mg, 29% yield) and **45** (42 mg, 28% yield) as white foams: For the compound **44**; $[\alpha]^{23}_D$ 10.18° (*c* 0.17, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 300.0 nm; ^{13}C NMR ($CDCl_3$) δ 165.89, 159.11, 153.09, 151.73, 140.33, 133.35, 129.61, 129.15, 128.42, 126.83 (t, J = 253.8 Hz), 125.59, 82.40 (t, J = 5.3 Hz), 79.47 (dd, J = 31.1, 24.1 Hz), 61.05 (d, J = 7.3 Hz), 39.51 (t, J = 25.2 Hz); Anal. ($C_{17}H_{14}ClF_2N_5O_3$) C, H, N. For the compound **45**; $[\alpha]^{22}_D$ 6.27° (*c* 2.011, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 300.0 nm; ^{13}C NMR ($CDCl_3$) δ 165.92, 159.16, 153.07, 151.64, 139.74, 133.38, 129.53, 129.10, 128.43, 126.19 (dd, J = 256.4, 251.2 Hz), 125.33, 81.24 (t, J = 5.8 Hz), 79.41 (dd, J = 31.3, 24.3 Hz), 61.37 (t, J = 4.3 Hz), 39.60 (t, J = 24.4 Hz); Anal. ($C_{17}H_{14}ClF_2N_5O_3$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-3,3-difluoro-β-L-ribofuranosyl] guanine (46). A solution of **44** (166 mg, 0.41 mmol) in anhydrous MeOH (20 mL) was treated with 2-mercaptoethanol (0.11 mL, 1.62 mmol) and NaOMe (90 mg, 1.66 mmol), and the resulting mixture was stirred for 18 h at 60 °C. After the mixture was cooled to room temperature, 0.2 mL of AcOH was added. The volatiles were removed under reduced pressure, and the remaining inorganic salts were removed by filtering the residue through a short silica gel pad washing with 8:1 CH_2Cl_2:MeOH. The filtrate was concentrated to give a white solid, which was dissolved in a small amount of DMF and purified by column chromatography on silica gel (8:1 CH_2Cl_2:MeOH) to give 60 mg (0.21 mmol, 51% yield) of **46** as a white solid: mp > 300 °C; $[\alpha]^{25}_D$ 25.49° (*c* 0.189, DMF); UV (H_2O) λ_{max} 253.5 nm (ϵ 12 628, pH 2), 251.5 nm (ϵ 11 901, pH 7), 258.0 nm (ϵ 10 140, pH 11); ^{13}C NMR (DMSO-d_6) δ 160.30, 157.48, 154.57, 138.49, 130.93 (dd, J = 255.4, 246.4 Hz), 120.09, 84.83 (t, J = 28.2 Hz), 82.80 (t, J = 4.1 Hz), 62.36, 42.17 (t, J = 23.7 Hz); Anal. ($C_{10}H_{11}F_2N_5O_3$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-3,3-difluoro-α-L-ribofuranosyl] guanine(47). See the procedure for the preparation of the guanosine analogue **46** from **44** by hydrolysis reaction using 2-mercaptoethanol and sodium methoxide. The title compound **47** was obtained on 0.27-mmol scale in 80% yield: mp > 250 °C; $[\alpha]^{24}_D$ 24.40° (*c* 0.157, DMF); UV (H_2O) λ_{max} 254.0 nm (ϵ 14 309, pH 2), 254.0 nm (ϵ 14 464, pH 7), 258.0 nm (ϵ 12 693, pH 11); ^{13}C NMR (CD_3OD) δ 165.97, 161.12 (dd, J = 272.3, 246.3 Hz), 152.70, 137.79, 128.81, 117.96, 83.56 (dd, J = 29.5, 24.9 Hz), 82.55 (d, J = 8.9 Hz), 60.84 (t, J = 5.5 Hz), 40.83 (t, J = 24.1 Hz); Anal. ($C_{10}H_{11}F_2N_5O_3 \cdot 0.9H_2O$) C, H, N.

(+)-1-[(1*R*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-β-L-ribofuranosyl]guanine (48). A solution of **46** (110 mg, 0.38 mmol) in anhydrous DMF (1.5 mL) was treated with NaOMe (83 mg, 1.53 mmol), and the resulting mixture was stirred for 24 h at room temperature. Two more equivalents (41 mg) of sodium methoxide was added, and the mixture was stirred for 12 h at room temperature. The mixture was filtered through a short silica gel pad washing with 7:1 CH_2Cl_2:MeOH. The filtrate was evaporated to dryness, and the residue was dissolved in a small amount of DMF. The undissolved solid was filtered off, and the filtrate was evaporated to dryness again. The residue was dissolved in a small amount of DMF and purified by column chromatography on silica gel (CH_2Cl_2:MeOH = 8:1) to give a pale yellow gel, which was trituated with CH_2Cl_2 and Et_2O to give the desired product **48** (35 mg, 0.13 mmol, 46% yield) as an off-white solid: mp > 250 °C; $[\alpha]^{24}_D$ 44.25° (*c* 0.677, CH_3OH); UV (H_2O) λ_{max} 248.5 nm (ϵ 18 111, pH 2), 251.5 nm (ϵ 17 481, pH 7), 260.0 nm (ϵ 19 537, pH 11); ^{13}C NMR (CD_3OD) δ 163.45 (d, J = 285.0 Hz), 159.35, 149.12, 137.41, 117.96 (d, J = 10.7 Hz), 109.70, 101.27 (d, J = 11.4 Hz), 86.87 (d, J = 15.7 Hz), 81.73 (d, J = 25.1 Hz), 61.67; Anal. ($C_{10}H_{10}FN_5O_3 \cdot 0.5H_2O$) C, H, N.

(+)-1-[(1*S*,4*S*)-2,3-Dideoxy-2,3-didehydro-3-fluoro-α-L-ribofuranosyl]guanine (49). See the procedure for the preparation of the guanosine analogue **48** from **46** by elimination reaction using sodium methoxide in DMF. The title compound **49** was obtained on 0.10-mmol scale in 33% yield : mp 200 °C (dec); $[\alpha]^{23}_D$ 21.75° (*c* 0.15, DMF); UV (H_2O) λ_{max} 253.0 nm (ϵ 20 778, pH 2), 249.0 nm (ϵ 11 259, pH 7), 266.5 nm (ϵ 9759, pH 11); ^{13}C NMR (CD_3OD) δ 164.97, 162.14, 160.71 (d, J = 215.84 Hz), 156.00, 138.86, 118.03, 101.48 (d, J = 11.0 Hz), 86.17 (d, J = 15.3 Hz), 82.42 (d, J = 24.6 Hz), 62.15; Anal. ($C_{10}H_{10}FN_5O_3$) C, H, N.

Stability Studies. Energy required for the cleavage of the glycosyl bond: The initial conformations of L-d4C, L-β-2′-Fd4C, and L-β-3′-Fd4C **24** were constructed by builder module in Spartan 5.1.1 (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/6-31G* basis in Spartan 5.1.1. Proton (H^+) was added to the O^2 atom of the cytidine analogues to build $R-BH^+$(Scheme 4), which was again geometry-optimized by using the same basis function (RHF/6-31G*). The aglycon (BH) and oxonium ion of the sugar moiety (R^+) were also independently optimized to give the corresponding minimized energies. The dissociation enthalpy of the glycosyl bond (Table 4) was calculated by using the minimized energies: [$\Delta E_{calcd} = E(R^+) + E(BH) - E(R-BH^+)$].

Molecular Modeling Study. Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling of the enzyme−substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme−ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer−template duplex (PDB entry 1rtd).[14] A model of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template−primer) duplex. The geometry-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of n + 1th nucleotide in the template strand overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd)[14] 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.[14] 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 the enzyme site from the biopolymer module in Sybyl. Fluorine parameters were obtained from the literature[15,16] 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 Study. Assays were performed at 25 °C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range of 15−100 μM and with 0.15 unit of adenosine deaminase (EC 3.5.4.4 from calf intestinal mucosa). The assays were monitored with a UV spectrometer at 265 nm. Initially, the qualitative assays were performed with D-β-2′-Fd4A **38** (200 μM) in the presence of 0.24 unit of adenosine deaminase for 120 min to determine whether it is

a substrate of this enzyme. The concentration (c_t) of each substrate at a certain time (t) was calculated from the absorbance (A_t) at that time (t), where it was assumed that the total change of absorbance ($A_0 - A_\infty$) was directly related to the disappearance of the substrate.[17]

Antiviral Assay. Human peripheral blood mononuclear (PBM) cells (obtained from Atlanta Red Cross) were isolated by Ficoll-Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2−3 days prior to use. HIV-1_{LAI} obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infectious clones HIV-1_{xxBru} and HIV-$1_{M184Vpitt}$ were obtained from Dr. John Mellors (University of Pittsburgh). Infections were done in bulk for 1 h, either with 100 $TCID_{50}/1 \times 10^7$ 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. 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 and/or 3TC were used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in a humidified 5% 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 virus solubilization buffer (VSB) containing 0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.05 M Tris, pH 7.8. 10 μL of each sample were added to 75 μL RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M $MgCl_2$, 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.[18]

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.[18]

Acknowledgment. This research was supported by the U.S. Public Health Service Grant (AI32351 and AI41890) from the National Institutes of Health and the Department of Veterans Affairs.

Supporting Information Available: ^{1}H NMR data for compounds **8**−**40** and **42**−**49**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0300274