Synthesis, Structure−Activity Relationships, and Drug Resistance of β-D-3′-Fluoro-2′,3′-Unsaturated Nucleosides as Anti-HIV Agents

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Our recent studies demonstrated that D- and L-2′-fluoro-2′,3′-unsaturated nucleosides (D- and L-2′-F-d4Ns) display moderate to potent antiviral activities against HIV-1 and HBV. As an extension of these findings, β-D-3′-fluoro-2′,3′-unsaturated nucleosides were synthesized as potential antiviral agents. The key intermediate (2*S*)-5-(1,3-dioxolan)-1-benzoyloxy-3,3-difluoropentan-2-ol **6** was prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde **1**, which was converted to 5-*O*-benzoxy-D-2-deoxy-3,3-difluoropentofuranosyl acetate **7** by the ring-closure reaction under acidic conditions. The acetate **7** was condensed with silylated purine and pyrimidine bases, which produced the α and β isomers. The 3′,3′-difluoro nucleosides were then treated with *t*-BuOK to give the desired 3′-fluoro-unsaturated nucleosides. We studied the structure−activity relationships of D-3′-fluoro-2′,3′-unsaturated nucleosides against HIV-1 in human peripheral blood mononuclear cells, from which we found that the cytosine derivative **26** was the most potent among the synthesized compounds. To understand the mode of action and drug resistance profile, with particular regard to the role of fluorine, we performed the molecular modeling studies of the cytidine analogue D-3′F-d4C and found a good correlation between calculated relative binding energies and activity/resistance data. Our model also shows interactions of the 3′-fluorine and the 2′,3′ double bond, which can be correlated to the observed biological data. Differences between fluorine substitution at the 3′ and 2′ positions may account for the higher cross-resistance with lamivudine observed in the 2′-fluorinated series.

Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) have been the cornerstones of anti-HIV therapy.[1−4] Side effects and drug-resistant variants, however, have been the drawbacks of the existing regimens.[5−7] Approaches to address these problems include using drugs with different resistant profiles and mechanisms, as in the combination of 3TC, AZT, and indinavir.[8] It is now clear that in order to achieve the greatest reduction of viral load and delay the drug resistance, combinations of multiple-drug regimens are essential.[9] Therefore, there is need for new reverse transcriptase and protease inhibitors as well as new agents that exhibit different resistance profiles and different modes of action, thereby providing greater treatment options.

Since the discovery of 2′,3′-dideoxynucleosides (ddN) and 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4N), a number of 2′,3′-unsaturated nucleoside analogues have been identified as anti-HIV agents, such as D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-d4FC)[10] and its L-enantiomer (L-Fd4C).[11] It has also been found that the introduction of a fluorine atom into the 2′ position of nucleosides, and of purine nucleosides in particular, resulted in a stabilized glycosyl bond with good antiviral activity.[12−14] As a part of our drug discovery program for antiviral agents, we have recently reported the synthesis of D- and L-2′-fluoro-2′,3′-unsaturated nucleosides (D- and L-2′-F-d4Ns) and found that a number of compounds of these series displayed moderate to potent anti-HIV activity and anti hepatitis B virus (HBV) activities.[15−17] Among these, the adenine and hypoxanthine derivatives with D configurations exhibited potent anti-HIV-1 activities (EC_{50} = 0.04 and 0.5 μM, respectively) and showed favorable cross-resistance profiles with respect to the 2′,3′-dideoxy-3′-thiacytidine (3TC) resistant viral isolates. Furthermore, the cytosine and 5-fluorocytosine derivatives from the L series display potent HBV (EC_{50} = 0.002 and 0.004 μM, respectively)[16] as well as anti-HIV-1 activities without significant cytotoxicity. More recently, we described the structure−activity relationships of L-3′-fluoro-2′,3′-unsaturated nucleosides and found potent activity in the cytosine and 5-fluorocytosine derivatives (EC_{50} = 0.089 and 0.018 μM, respectively).[18b]

In view of these interesting biological results, it was of interest to introduce a fluorine in the 3′ position of 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4Ns) for the structure−activity relationships (SAR). Herein, we report a general synthetic method as well as the SAR studies of 3′-fluoro-2′,3′-unsaturated D-nucleosides (D-3′F-d4N) as anti-HIV agents. Thus far, there have been known several 3′F-substituted nucleosides, including D-3′F-d4C,[19,20] that have been synthesized from the corresponding nucleosides. The previously reported method is not amenable for the synthesis of a series of analogues for SAR studies.

Results and Discussion

Chemistry. As shown in Scheme 1, the starting material 2,3-*O*-isopropylidene-D-glyceraldehyde **1**, prepared from the oxidative cleavage of 1,2:5,6-di-*O*-iso-

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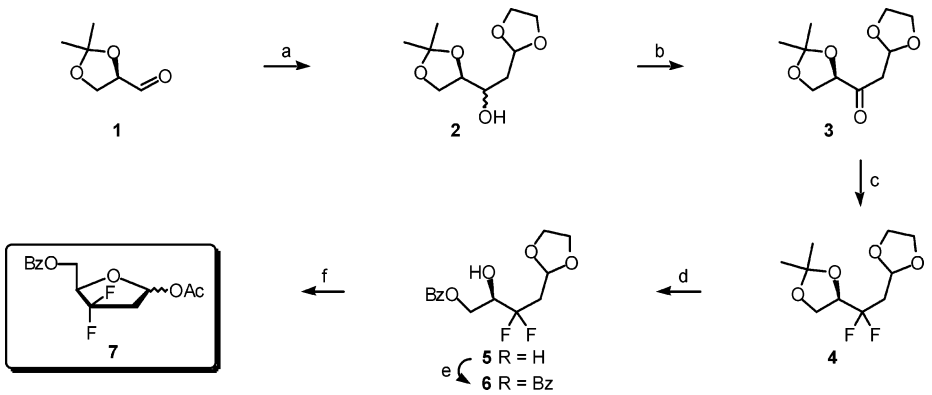
Scheme 1[a]



[a] Reagents and conditions: (a) (1,3-dioxolan-2-ylmethyl)magnesium bromide, THF, reflux; (b) DMSO, $(ClCO)_2$, TEA, dichloromethane; (c) DAST, dichloromethane, room temp; (d) 1:1 (5% HCl/dioxane), room temp; (e) BzCl, pyridine; (f) (1) 1.0 M HCl/ether, MeOH, (2) concentrated H_2SO_4, AcOH, Ac_2O, 15 min, room temp.

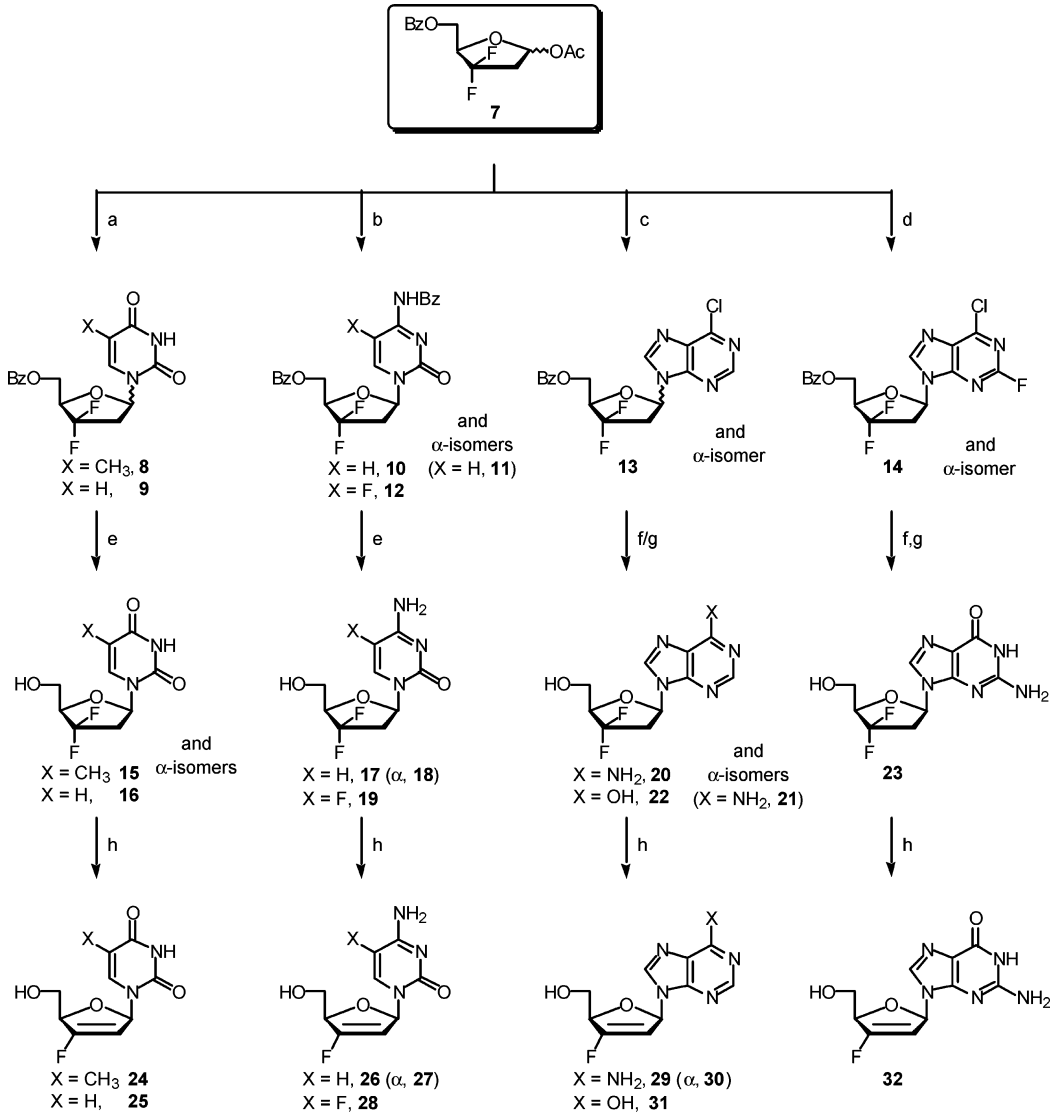
propylidene-D-mannitol, was reacted with (1,3-dioxolan-2-ylmethyl)magnesium bromide to give alcohol **2** in 94% yield, followed by Swern oxidation to give the intermediate **3** in 95% yield. The ketone **3** was treated with DAST to yield the difluorinated intermediate **4**. Selective deprotection of **4** gave a diol **5** in excellent yield, and subsequent benzoylation of the primary alcohol produced the key intermediate **6** in 85% yield. The intermediate **6** was then converted to epimeric acetates **7** by treatment with HCl/MeOH, followed by concentrated sulfuric acid and acetic anhydride in acetic acid. As shown in Scheme 2, condensation of acetates **7** with various persilylated pyrimidines and purines was carried out at room temperature in acetonitrile. Completion of the reaction with pyrimidines took 3−4 h in the presence of trimethylsilyltrifluoromethane sulfonate (TMSOTf) as the catalyst. In the case of purines, longer reaction times and/or temperatures of 50−60 °C were required to afford good yields of N^9-glycosylated products. The coupling reactions resulted in a mixture of α and β anomers in yields up to 90% (the ratio of β to α isomer ranged from 5:4 to 1:1). Thymine, uracil, protected cytosine, and 5-fluorocytosine derivatives were synthesized from the acetate **7** with the appropriate persilylated bases. Removal of the benzoyl group by treatment with saturated methanolic ammonia produced the desired free nucleosides **15**−**19**, followed by elimination of fluorine to afford the target nucleosides **24**−**28**. Similarly, acetate **7** was reacted with persilylated 6-chloropurine to give 5′-benzoyl-protected nucleosides **13** as a 1:1 anomeric mixture. Conversion of the compounds **13** to the adenine derivatives by ammonolysis in a steel bomb at 90−100 °C produced compound **20** and its α-isomer **21**. Compounds **13** were also converted to the inosine analogue **22** by refluxing with 2-mercaptoethanol and sodium methoxide in methanol. Treatment of **20**−**22** with potassium *tert*-butoxide in THF produced the 3′-fluoro-unsaturated nucleosides **29**−**31**. For the preparation of the guanine derivative, acetate **7** was condensed with persilylated 2-fluoro-6-chloropurine in acetonitrile to give protected nucleoside **14**. Compound **14** was then converted to the 6-amino-2-chloropurine derivative by treatment with ammonia in ethylene glycol dimethyl ether (DME), which was converted to the guanine derivative **23** by refluxing with 2-mercaptoethanol and sodium methoxide in methanol. Treatment of **23** with potassium *tert*-butoxide in THF gave the unsaturated 3′-fluoroguanine derivative **32**. Assignment of the structures of the newly synthesized nucleosides was based on NMR, mass, and UV spectroscopy.

Antiviral Activity. The anti-HIV-1 activity of the synthesized compounds was evaluated in peripheral blood mononuclear cells (PBMC), and the results are summarized in Table 1. The greatest activity was found in the cytidine analogue **26** (EC_{50} = 2.3 μM), in agreement with the reported data.[20] However, the D-cytosine and 5-fluorocytosine derivatives **26** and **28** are 100- to 500-fold less potent than their L-isomer **33** and **34**, previously reported by us.[18] In view of the fact that, according to our modeling studies (vide infra), the triphosphate of D-3′F-d4C binds favorably to the HIV-1 reverse transcriptase (RT), the reason for lower anti-HIV activity may be speculated to be the lower initial phosphorylation by deoxycytidine kinase. This may result in a lower level of triphosphate at the active site, thus the observed lower anti-HIV activity. To confirm the hypothesis, studies of intracellular phosphorylation of the corresponding nucleosides are required, which are beyond the scope of this paper. The adenine (**29**), 5-fluorocytosine (**28**), and hypoxanthine (**31**) derivatives also displayed moderate antiviral activity without significant cytotoxicity. In contrast, the L-adenine (**35**) and hypoxanthine derivatives were inactive.[18] The guanosine analogue **32** showed very weak antiviral activity, and the thymidine and uridine analogues **24** and **25** were inactive when tested up to 100 μM.

Although the cytosine derivative (D-3′F-d4C, **26**) was not exceptionally potent, in view of the clinical importance of the M184V mutation, which confers resistance to 3TC, it was of interest to evaluate D-3′F-d4C against the M184V mutant. From this study, we found that D-3′F-d4C was moderately cross-resistant (8.6-fold) with the M184V mutant (Table 2). This level of resistance is lower than what observed in the case of L-3′F-d4C.[18]

Chemical Stability. Fluorine substitution in the sugar is known to increase the chemical stability of nucleoside analogues, particularly in an acidic environment.[17] The inductive effect of the fluorine atom destabilizes the carbocation (or oxonium ion) intermediate of the acidic hydrolysis reaction (Scheme 3). To study the effect of 3′-fluorine substitution on the chemical stability, D-3′F-d4C (**26**) was treated with buffer solutions at pH 2.0, 7.0, and 11.0, and its stability was measured

Scheme 2[a]



[a] Reagents and conditions: (a) silylated thymine, TMSOTf, MeCN; (b) silylated N^4-Bz-cytosine derivatives, TMSOTf, MeCN; (c) silylated 6-chloropurine, TMSOTf, MeCN; (d) silylated 6-Cl-2-F-purine, TMSOTf, MeCN; (e) NH_3/MeOH, room temp; (f) NH_3/MeOH, 90 °C; (g) $HSCH_2CH_2OH$, sodium methoxide, MeOH, reflux; (h) *t*-BuOK, THF.

Table 1. In Vitro Anti-HIV-1 Activity and Toxicity of 3′-Fluoro-2′,3′-didehydro-2′,3′-deoxynucleosides

B	activity (μM) HIV-1 EC_{50} (PBM)	toxicity (μM) PBM	CEM	Vero
thymine **24**	>100	>100	>100	>100
uracil **25**	>100	>100	>100	82.6
cytosine **26**	2.3	>100	>100	>100
L-cytosine **33**[18b]	0.089	86.9	>100	>100
5-F-cytosine **28**	9.29	>100	>100	>100
L-5-F-cytosine **34**[18b]	0.018	>100	>100	>100
adenine **29**	6.55	>100	>100	>100
L-adenine **35**[18b]	>100	>100	>100	>100
hypoxanthine **31**	12.8	>100	77.66	>100
guanine **32**	44.9	>100	>100	75.5
D4C[25]	0.005	65		

by TLC every 15 min for the first hour, every 30 min for the following 3 h, every hour for the first 12 h, and then every 6 h. D-3′F-d4C was stable at pH 7.0 and 11.0 up to 7 days, but it slowly decomposed at pH 2.0, with a half-life of 48 h (Table 3). Its stability, however, was much improved ($t_{1/2}$ = 48 h) compared to the nonfluorinated compound (in our study we used the unnatural isomer L-d4C, synthesized in our laboratory for other purposes), which decomposes completely in less than 3 h with a half-life of 1 h. On the other hand, 2′F-d4C[17] showed indefinite stability, which is reasonable consid-

Table 2. In Vitro Anti-HIV-1 Activity of D-3′F-d4C against Wild Type (WT) and M184V Virus in Human PBM Cells and Correlation with the Calculated Energy of the Complex D-3′F-d4CTP/RT

	xxBRU (WT)		M184V		
compd	EC_{50} (μM)	E_{rel} [a] (kcal/mol)	EC_{50} (μM)	E_{rel} [b] (kcal/mol)	FI [a]
D-3′F-d4C (26)	2.3	53.6	19.7	33.6	8.6
L-3′F-d4C (33)[18]	0.089	51.4	2.4	28.3	27
3TC[24]	0.041	76.4	>50	−28.8	>1200
AZT[24]	0.0041	146.4	0.0027	107.0	0.66

[a] Fold increase. [b] Binding energy of inhibitor/TP minus binding energy of natural 2′-deoxynucleotide.

Scheme 3. Resistance to Hydrolysis of Fluorinated 2′,3′-Unsaturated Nucleosides

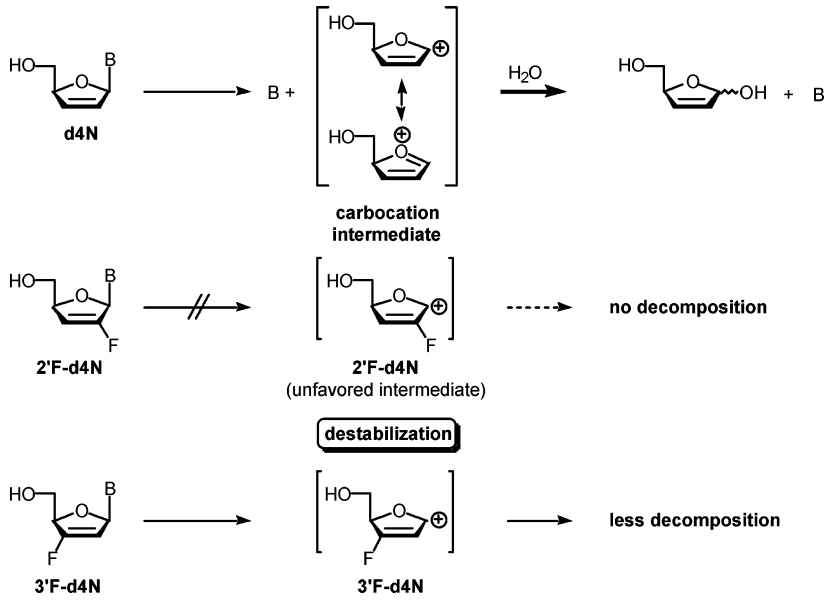
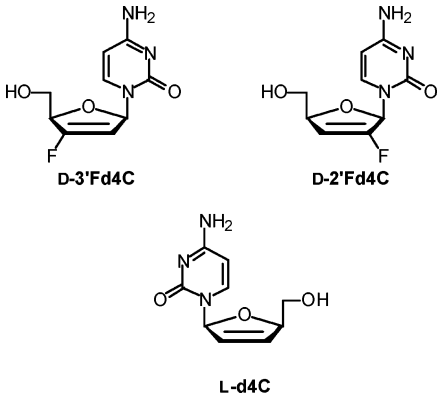


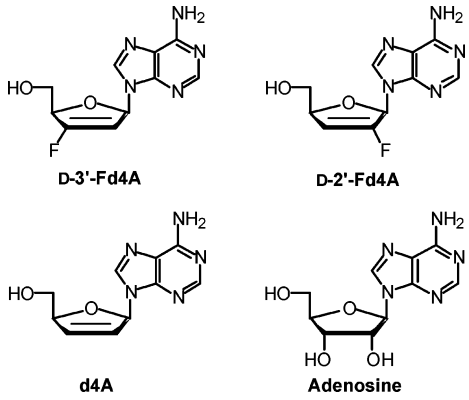
Table 3. Chemical Stability of 2′,3′-Unsaturated Cytidine Analogues at pH 2.0



compd	$t_{1/2}$ (h)
D-3′F-d4C (26)	48
D-2′F-d4C[16]	∞[a]
L-d4C	1

[a] No decomposition observed up to 7 days.

Table 4. Metabolic Stability of 2′,3′-Unsaturated Adenosine Analogues toward ADA[a]



compd	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($\mu M^{-1}/s^{-1}$)	$t_{1/2}$
D-3′F-d4A (29)	8.8	31.8	3.6	56 min
D-2′F-d4A[16]	23.0	NA[b]	NA[b]	42 min
d4A	30.0	NA[b]	NA[b]	3.1 days
adenosine[22]	24.5	76.4	3.1	0.5 min

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

ering the shorter distance between the 3′-fluorine and the anomeric carbon.

Metabolic Stability. A favorable effect of fluorine substitution may also provide an increased metabolic stability. Two important deactivating enzymes in nucleoside catabolism are cytidine deaminase and adenosine deaminase.[21] Because of the commercial unavailability of cytidine deaminase, only adenosine deaminase (ADA) was used in our study. The adenine derivative D-3′F-d4A (**29**) was compared to the 2′-fluorinated congener 2′F-d4A[17] and the natural substrate adenosine. As shown in Table 4, both fluorinated compounds are deaminated approximately 100-fold more slowly than adenosine. Analyzing the kinetic constants, it is interesting to note how D-3′F-d4A **29** can bind to adenosine deaminase with higher affinity than adenosine, as indicated by a 3-fold lower Michaelis−Menten constant K_M. The K_M values for 2′F-d4A and d4A are comparable to the K_M for adenosine. The lower deamination efficiency for D-3′F-d4A can be correlated to the lower turnover number k_{cat}. It is noteworthy that the experimental catalytic efficiency, k_{cat}/K_M, of D-3′F-d4A and adenosine is similar. These findings suggest that 3′-fluorination, analogous to 2′-fluorination,[17] is able to reduce, but not completely inhibit, metabolic deamination by ADA. Since d4A is deaminated very slowly by ADA ($t_{1/2}$ = 3.1 days), fluorine substitution seems to counteract the stabilizing effect of the double bond. This metabolic destabilizing effect of fluorine is in direct contrast to the chemical stability discussed in the previous section.

Molecular Modeling. To understand the mode of action of NRTIs, we have previously studied the molecular modeling of anti-HIV nucleosides from which we found a good correlation with relative binding energy between the HIV RT and the 5′-triphosphate of anti-HIV nucleosides.[23] When D-3′F-d4CTP is considered in

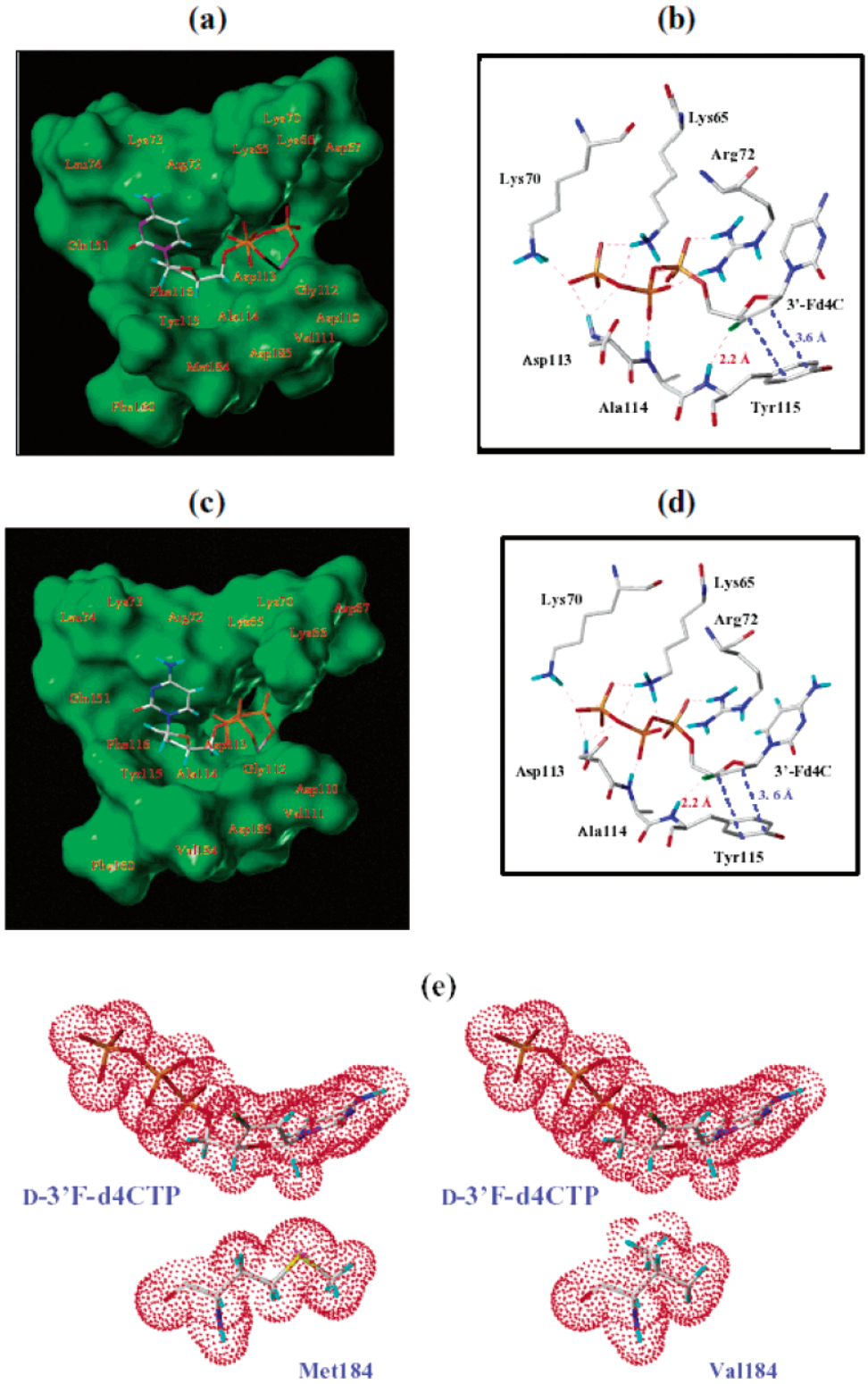


Figure 1. (a) D-3′F-d4CTP/RT_{WT} complex. (b) Binding mode of D-3′F-d4CTP at the active site of HIV-1 RT_{WT}. The 3′-F is stabilized by hydrogen-bonding to the −NH backbone of Tyr115. A p−p interaction is also possible between the 2′,3′ double bond and the aromatic ring of Tyr115. (c) D-3′F-d4CTP/RTM184V complex. (d) Similar interactions with Tyr115 are observed in the complex of D-3′F-d4CTP with the mutated enzyme. (e) The lack of steric hindrance between D-3′F-d4C and the bulky side chain of Val184 in M184V RT is consistent with the lack of complete cross-resistance with the 3TC-resistant mutant.

our model, the calculated relative binding energies of the complex with RT can be correlated with its antiviral activity against wild-type HIV-1 as well as with its lower activity in M184V mutants (Table 2). The calculated favorable relative binding energy with wild-type RT (E_{rel} = 53.6 kcal/mol) correlates qualitatively with antiviral activity, following the trend previously observed in the case of AZT and 3TC.[23a] Analogously, the D-3′F-d4CTP/RT_{M184V} complex, although less energetically favored, maintains a positive binding energy (E_{rel} = 33.6 kcal/mol), which is in agreement with the observed moderate decrease of activity with a 8.6-fold increase of resistance (Table 2). However, in the case of L-3′F-d4C, it shows higher cross-resistance with 3TC.[18b] As expected, our

model gives a qualitative but not a quantitative correlation with the experimental data. However, in accordance with our previous findings, the L-isomer **33** provides increased cross-resistance in comparison to the D-isomer **26**.

In the D-3′F-d4CTP/RT_{WT} (WT = wild type) complex (Figure 1a,b), the triphosphate is stabilized by hydrogen bonds with amino acids Lys65, Lys70, Arg72, Asp113, and Ala114. A hydrogen bond between the 3′-fluorine and Tyr115 is also detected. A $\pi-\pi$ interaction between the 2′,3′ double bond and the aromatic ring of Tyr115 also contributes to the binding. Similar stabilizing interactions had been reported in our previous study on the L-3′F-d4CTP/RT_{WT} complex, where the 3′-fluorine interacts via hydrogen bond with Asp185.[18b]

In the D-3′F-d4CTP/RT_{M184V} complex (Figure 1c,d), similar stabilizing interactions can be observed. Furthermore, substitution of methionine with the bulkier valine in the mutant enzyme (Figure 1e) does not cause any major steric clash with the nucleoside triphosphate, hence the absence of substantial cross-resistance. In contrast, in the L-3′F-d4CTP/RT_{M184V} complex, significant distortion of the binding was observed, due to the closer proximity of the 3′ position to Met184.[18b] Thus, substitution with valine results in unfavorable steric interactions and consequent loss of the stabilizing hydrogen bond between the 3′-fluorine and Asp185.[18b]

In conclusion, a general method for the synthesis of D-3′-fluorinated-2′,3′-unsaturated nucleosides has been developed and the structure−activity relationships of the synthesized nucleosides have been described. Among the synthesized nucleosides, D-3′F-d4C **26** showed the most potent anti-HIV activity and only partial cross-resistance with 3TC. Fluorine substitution at the carbohydrate moiety decreases the hydrolytic cleavage and increases the metabolic stability of these compounds. However, when compared to the other unsubstituted unsaturated nucleosides, fluorine substitution seems to have the opposite effect, reducing the metabolic stability to adenosine deaminase. These studies provide insights on the effect of fluorine substitution at the carbohydrate moiety on anti-HIV activity, resistance, and metabolic stability of nucleoside analogues.

Experimental Section

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Bruker AMX400 MHz spectrometer with tetramethylsilane as the internal reference; chemical shifts (δ) are reported in parts per million (ppm), and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), and m (multiplet). UV spectra were obtained on a Beckman DU 650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All reactions were monitored using thin-layer chromatography on Analtech 200 mm silica gel GF plates.

1-[(*S*)-2,2-Dimethyl-(1,3)-dioxolan-4-yl]-4-(1,3-dioxolan-2-yl)-2-ol (2). A solution of protected D-glyceraldehyde **1** (19.1 g, 147 mmol) in THF (100 mL) was added dropwise over 45 min to a solution of (1,3-dioxolan-2ylmethyl)magnesium bromide (0.5 M in THF, 325 mL, 162.5 mmol) at 60 °C. The resulting solution was stirred for 1 h more and then cooled to 0 °C and treated with saturated ammonium chloride aqueous solution. The reaction mixture was extracted thoroughly with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel column (1:2 EtOAc/hexanes) to yield the alcohol **2** (yellow oil, 30.1 g, 94%). ^{1}H NMR ($CDCl_3$) δ 1.34, 1.36, 1.40, 1.43 (s, 6H), 1.75−1.90 (m, 2H), 2.83 (d, J = 3.3 Hz, D_2O exchangeable 0.57H), 3.06 (br s, D_2O exchangeable, 0.43H), 3.75−4.11 (m, 8H), 5.07 (m, 1H); MS (FAB) *m/z* 218 (M^+).

1-[(*S*)-2,2-Dimethyl-(1,3)-dioxolan-4-yl]-4-(1,3-dioxolan-2-yl)-2-butanone (3). Oxalyl chloride (5.25 mL) was added dropwise to a solution of DMSO (8.58 mL) in dry dichloromethane (50 mL) at −78°C. After 5 min, **2** (12.0 g, 54.9 mmol) in dichloromethane was added dropwise to the solution over 5 min. After 15 min more, triethylamine (38.2 mL) was added and the reaction solution was allowed to warm to room temperature. The volatiles were removed under reduced pressure to yield a residue that was purified by flash silica gel column (1:4 EtOAc/hexanes) to give **3** (yellow oil, 11.4 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H), 1.45 (s, 3H), 2.95 (m, 2H), 3.83 (m, 2H), 3.95 (m, 2H), 4.03 (m, 1H), 4.15 (m, 1H), 4.42 (m, 1H), 5.27 (m, 1H); MS (FAB) *m/z* 216 (M^+).

1-[(*S*)-2,2-Dimethyl-(1,3)-dioxolan-4-yl]-2,2-difluoro-4-(1,3-dioxolan-2-yl)butane (4). A solution of **3** (8.5 g, 39.4 mmol) in dichloromethane (50 mL) was added dropwise to a solution of DAST (15 mL) in dry dichloromethane (50 mL) at 0 °C. The resulting solution was stirred for 24 h at room temperature. The reaction mixture was then poured into a saturated sodium bicarbonate solution at 0 °C and extracted with diethyl ether. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel column (1:9 EtOAc/hexanes) to give **4** (yellow oil, 4.8 g, 51.3%). ^{1}H NMR ($CDCl_3$) δ 1.340 (s, 3H), 1.424 (s, 3H), 2.23−2.41 (m, 2H), 3.85 (m, 2H), 3.97 (m, 2H), 4.09 (m, 2H), 4.24−4.33 (m, 1H), 5.121 (t, 1H); MS (FAB) *m/z* 238 (M^+).

3,3-Difluoro-5-(1,3-dioxolan-2-yl)-2*S*-pentane-1,2-diol (5). A 5% HCl aqueous solution (30 mL) was added to a solution of **4** (4.8 g, 20.2 mmol) in 1,4-dioxane (30 mL) at 0 °C. The resulting solution was stirred at room temperature overnight, then neutralized by sodium bicarbonate and concentrated under reduced pressure. The residue was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel column (3:2 EtOAc/hexanes) to yield **5** (colorless oil, 3.6 g, 90%). ^{1}H NMR ($CDCl_3$) δ 2.30−2.52 (m, 2H), 3.58 (d, J = 5.88 Hz, D_2O exchangeable, 1H), 3.83−4.10 (m, 7H), 5.09 (q, 1H); MS (FAB) *m/z* 198 (M^+).

1-Benzoyloxy-3,3-difluoro-5-(1,3-dioxolan-2-yl)-2*S*-pentan-2-ol (6). Benzoyl chloride (1.82 mL) was added dropwise to a solution of **5** (3.1 g) in dry pyridine (5 mL) at 0 °C. The resulting solution was stirred at room temperature for 1 h, then concentrated under reduced pressure and coevaporated with toluene to remove pyridine. The residue was dissolved in dichloromethane, washed with saturated sodium bicarbonate aqueous solution, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel column (1:2 EtOAc/hexanes) to yield **6** (yellow solid, 4.0 g, 85%). ^{1}H NMR ($CDCl_3$) δ 2.43 (m, 2H), 3.84 (m, 2H), 3.95 (m, 2H), 4.03 (m, 1H), 4.19 (m, 1H), 4.48 (m, 1H), 4.59 (m, 1H), 5.08 (m, 1H), 7.38 (m, 2H), 7.51 (m, 1H), 8.01 (m, 1H). Anal. Calcd for $C_{14}H_{16}F_2O_5$: C, 55.63; H, 5.34. Found: C, 55.70, H, 5.41.

1-Acetyl-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-D-ribofuranose (7). A solution of HCl in diethyl ether (1.0 M, 15 mL) was added to a solution of **6** (1.6 g, 5.3 mmol) in methanol (15 mL). The resulting solution was stirred and refluxed for 1 h. After cooling, the reaction solution was neutralized with sodium bicarbonate and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was dissolved in acetic anhydride (3 mL, 32.0 mmol)/glacial acetic acid (15 mL), the solution was cooled in an ice-cold water bath, and concentrated sulfuric acid (40 μL, 0.75 mmol) was added. The resulting solution was stirred

at room temperature for 30 min, then 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 brine (30 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel column (1:9 EtOAc/hexanes) to yield anomeric mixture (2:1 ratio) **7** (yellow oil, 1.5 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.02, 2.10 (s, 3H), 2.53−2.65 (m, 1H), 2.74−2.88 (m, 1H), 4.46−4.66 (m, 3H), 6.44, 6.40 (d, J = 5.8, 5.8 Hz, 1H), 7.38−7.51 (m, 2H), 7.57 (m, 1H), 7.98−8.12 (m, 2H); MS (FAB) *m/z* 301 (MH^+).

General Procedure for the Condensation of Acetates 7 with Heterocycles. A mixture of thymine (1.26 g, 10.0 mmol) and ammonium sulfate (67 mg, 0.505 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (30 mL) was refluxed for 4 h, and then the solvent was removed in vacuo at 30−35 °C. A solution of **7** (1.51 g, 5.0 mmol) in anhydrous acetonitrile (30 mL) was added to the residual oil, followed by trimethylsilyltrifluoromethane sulfonate (TMSOTf, 1.28 mL, 5.0 mmol). The resulting mixture was stirred at room temperature for 3 h and then poured into an ice-cold saturated solution of sodium bicarbonate (30 mL). The organic phase was separated, washed with brine (5 mL), dried over magnesium sulfate, filtered, and concentrated to a crude that was purified by a flash silica gel column (1:1 EtOAc/hexanes) to give **8** (1.65 g, 90%) as an epimeric mixture. All the pyrimidine nucleosides were synthesized analogously. The β isomers of the cytosine (**10**) and 5-fluorocytosine (**12**) derivatives could be purified by flash silica gel column chromatography (1:1 EtOAc/hexanes). In the case of purine nucleosides, slightly different conditions were needed in order to maximize the yield of N^9-glycosylated nucleosides. The full experimental details and characterization of the α-isomers **11**, **19**, and **28** are reported as an example in the pyrimidine series, and α-isomers **14**, **22**, and **31** are reported as examples in the purine series.

1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α/*β*-D-ribofuranosyl)thymine (8). Yellow oil; UV ($CHCl_3$) λ_{max} 256.0 nm. Anal. ($C_{17}H_{16}F_2N_2O_5$) C, H, N.

1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α/*β*-D-ribofuranosyl)uracil (9) was prepared from **7** on a 1.0 mmol scale in 88% yield by a similar procedure as described for **8**. Yellow oil; UV ($CHCl_3$) λ_{max} 256.5 nm. Anal. ($C_{16}H_{14}F_2N_2O_5$) C, H, N.

***N*^{4}-Benzoyl-1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)cytosine (10) and *N*^{4}-Benzoyl-1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α-D-ribofuranosyl)cytosine (11).** Compounds **10** and **11** were prepared from **7** on a 1.0 mmol scale in 43% and 31% yield, respectively, by a similar procedure as described for **8**. **10**: white solid; mp 166−167 °C (dec); $[\alpha]^{25}_D$ 80.50° (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} 259.5, 301.0 nm. Anal. ($C_{23}H_{19}F_2N_3O_5$) C, H, N. **11**: white solid; mp 177−178 °C; $[\alpha]^{26}_D$ −42.00° (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} 259.0, 299.5 nm. Anal. ($C_{23}H_{19}F_2N_3O_5$) C, H, N.

5-Fluoro-*N*^{4}-Benzoyl-1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)cytosine (12) was prepared from **7** on a 1.0 mmol scale in 45% yield by a similar procedure as described for **8**. White solid; mp 156−157°C; $[\alpha]^{25}_D$ 70.50° (*c* 0.50, MeOH); UV ($CHCl_3$) λ_{max} 325.0 nm. Anal. ($C_{23}H_{18}F_3N_3O_5$) C, H, N.

6-Chloro-9-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-α/*β*-D-ribofuranosyl)purine (13). A mixture of silylated 6-chloropurine [prepared from 6-chloropurine (1.04 g, 6.72 mmol) and HMDS (25 mL)], **7** (1.05 g, 3.5 mmol), and TMSOTf (0.90 mL, 3.5 mmol) in acetonitrile (30 mL) was stirred and refluxed for 5 h. After extractive workup analogous to that described in the general procedure above, purification by silica gel column chromatography (1:3 EtOAc/hexanes) gave **13** (1.18 g, 85%) as a yellow oil. UV ($CHCl_3$) λ_{max} 263.5 nm. Anal. ($C_{17}H_{13}ClF_2N_4O_3$) C, H, N.

6-Chloro-2-fluoro-9-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)purine (14). A mixture of silylated 2-fluoro-6-chloropurine [prepared from 2-fluoro-6-chloropurine (1.3 g, 7.6 mmol) and HMDS (25 mL)], **7** (1.2 g, 4.0 mmol), and TMSOTf (1.00 mL, 4.0 mmol) in acetonitrile (30 mL) was stirred and refluxed for 5 h. After extractive workup analogous to that described in the general procedure above, purification by silica gel column chromatography (1:3 EtOAc/hexanes) gave **14** (660 mg, 40%) as a yellow oil. $[\alpha]^{25}_D$ 10.11° (*c* 0.80, MeOH); UV ($CHCl_3$) λ_{max} 269.5 nm. Anal. ($C_{17}H_{12}ClF_3N_4O_3$·0.05 hexanes) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)thymine (15). Compound **8** (1.2 g, 3.3 mmol) was treated with saturated ammonia/methanol solution (30 mL). The reaction solution was stirred at room temperature for 10 h. After removal of solvent, the resulting β and α isomers were separated by flash silica gel chromatography (1:24 MeOH/$CHCl_3$) to afford, after trituration with ethanol, **15** (470 mg, 54%) as a white solid: mp 138−141 °C (dec); $[\alpha]^{27}_D$ 30.76 (*c* 0.33, $CHCl_3$); UV (H_2O) λ_{max} 264.0 nm (ϵ 11 400) (pH 2), 263.5 nm (ϵ 12 230) (pH 7), 264.0 nm (ϵ 9500) (pH 11) nm. Anal. ($C_{10}H_{12}F_2N_2O_4$·0.2 EtOH) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)uracil (16). Compound **9** (430 mg, 1.22 mmol) was treated with saturated ammonia/methanol solution (30 mL). The reaction solution was stirred at room temperature for 7 h. After removal of the solvent, the resulting β and α isomers were separated by flash silica gel chromatography (5% MeOH/CH_3Cl) to afford **16** (61 mg, 20%) as a white solid: mp 147−148°C; $[\alpha]^{24}_D$ 24.90 (*c* 1.24, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 7220) (pH 2), 259 nm (ϵ 7230) (pH 7), 258.0 nm (ϵ 5550) (pH 11). Anal. ($C_{10}H_{12}F_2N_2O_4$) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)cytosine (17). Compound **10** (1.3 g, 3.7 mmol) was treated with saturated ammonia/methanol solution. The reaction solution was stirred at room temperature for 10 h. After removal of the solvent, the resulting residue was purified by flash silica gel chromatography (10% MeOH/$CHCl_3$) to afford **17** (840 mg, 92%) as a white solid: mp 194−196 °C (dec); $[\alpha]^{25}_D$ 45.56° (*c* 1.00, 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). Anal. ($C_9H_{11}F_2N_3O_3$·0.2 MeOH) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-α-D-ribofuranosyl)cytosine (18). Compound **11** (140 mg, 0.31 mmol) was treated with a saturated ammonia/methanol solution. The reaction solution was stirred at room temperature for 4 h. After removal of the solvent, the resulting residue was purified by preparative TLC on silica gel (10% MeOH/$CHCl_3$) to afford **18** (63 mg, 83%) as a white solid: mp 188−190 °C (dec); $[\alpha]^{27}_D$ −29.24° (*c* 0.17, MeOH); UV (H_2O) λ_{max} 277.0 nm (ϵ 15 120) (pH 2), 268.5 nm (ϵ 10 930) (pH 7), 268.5 nm (ϵ 10 090) (pH 11). Anal. ($C_9H_{11}F_2N_3O_3$) C, H, N.

5-Fluoro-1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)-cytosine (19). Compound **12** (1.4 g, 3.8 mmol) was treated with a saturated ammonia/methanol solution. The reaction solution was stirred at room temperature for 10 h. After removal of the solvent, the resulting residue was purified by flash silica gel chromatography (10% MeOH/$CHCl_3$) to afford **19** (917 mg, 91%) as a white solid: mp 209−212 °C (dec); $[\alpha]^{27}_D$ 55.01 (*c* 0.52, MeOH); UV (H_2O) λ_{max} 283.0 nm (ϵ 10 100) (pH 2), 278.0 nm (ϵ 8800) (pH 7), 278.0 nm (ϵ 8500) (pH 11). Anal. ($C_9H_{10}N_3F_3O_3$) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)adenine (20) and 1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)adenine (21). A solution of **13** (1.5 g, 3.8 mmol) and saturated ammonia/methanol (40 mL) was heated at 90−100 °C in a steel bomb for 24 h. After the mixture was cooled to room temperature, the solvent was removed in vacuo, and the residue was purified by flash silica gel chromatography (4% MeOH/$CHCl_3$) to afford **20** (360 mg, 36%) and **21** (420 mg, 42%) as a white solids. **20**: mp 198−200 °C; $[\alpha]^{25}_D$ −17.55 (*c* 0.51, MeOH); UV (H_2O) λ_{max} 258.5 nm (ϵ 12900) (pH 11), 258.0 nm (ϵ 13100) (pH 7), 256.5 nm (ϵ 12 800) (pH 2). Anal. ($C_{10}H_{11}F_2N_5O_2$) C, H, N. **21**: mp 165−166 °C; $[\alpha]^{27}_D$ 36.70 (*c* 0.32, MeOH); UV (H_2O) λ_{max} 258.5 nm (ϵ 13 680) (pH 11), 256.5 nm (ϵ 13 350) (pH 7), 258.5 nm (ϵ 13 580) (pH 2). Anal. ($C_{10}H_{11}F_2N_5O_2$) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-*β*-D-ribofuranosyl)hypoxanthine (22). A mixture of **13** (1.5 g, 3.8 mmol), sodium methoxide (1 M solution in methanol) (12.5 mL, 12.5 mmol),

and 2-mercaptoethanol (0.9 mL, 12.5 mmol) in methanol (30 mL) was refluxed for 5 h under a nitrogen atmosphere. The reaction mixture was cooled, neutralized with glacial AcOH, and evaporated to dryness in vacuo. The residue was purified by flash silica gel chromatography (10% MeOH/$CHCl_3$) to afford, after trituration with CH_2Cl_2, **22** (310 mg, 30%) as a white solid: mp 166−168 °C; $[\alpha]^{25}_D$ −16.71 (*c* 0.35, MeOH); UV (H_2O) λ_{max} 252.5 nm (ϵ 22 800) (pH 11), 248.0 nm (ϵ 20 000) (pH 7), 251.5 nm (ϵ 21 000) (pH 2). Anal. ($C_{10}H_{10}F_2N_4O_2 \cdot 0.7H_2O$) C, H, N.

1-(2,3-Dideoxy-3,3-difluoro-β-D-ribofuranosyl)guanine (23). Dry ammonia was bubbled into a stirred solution of **14** (2.0 g, 4.8 mmol) in dry DME (50 mL) at room temperature for 6 h. The salt formed was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was purified by flash silica gel chromatography (2% MeOH/$CHCl_3$) to afford the 2-amino-6-chloropurine derivative. A mixture of 2-amino-6-chloropurine derivative, sodium methoxide (1.0 M solution in methanol) (16 mL, 16 mmol), and 2-mercaptoethanol (1.15 mL, 15.9 mmol) in methanol was refluxed overnight under a nitrogen atmosphere. The reaction mixture was cooled, neutralized with glacial AcOH, and evaporated to dryness in vacuo. The residue was purified by flash silica gel chromatography (10% MeOH/$CHCl_3$) to afford **23** (480 mg, 35%) as a white solid: mp >250°C; $[\alpha]^{27}_D$ −24.60 (*c* 0.15, DMF); UV (H_2O) λ_{max} 258.0 nm (ϵ 10 000) (pH 11), 253.0 nm (ϵ 11 000) (pH 7), 254 nm (ϵ 12 500) (pH 2). Anal. ($C_{10}H_{11}F_2N_5O_3$) C, H, N.

General Procedure for the Conversion of 3′,3′-Difluoro Nucleosides to 3′-Fluoro-Unsaturated Nucleosides. A solution of **15** (300 mg, 1.15 mmol) in dry THF was treated with *t*-BuOK (1 M in THF) (4.6 mL, 4.6 mmol) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred overnight and then neutralized with glacial AcOH and evaporated to dryness in vacuo. The residue was purified by flash silica gel chromatography (4% MeOH/$CHCl_3$) to afford **24** (83.5 mg, 30%) as a white solid.

1-(2,3-Dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)thymine (24). White solid; mp 144−146 °C (dec); $[\alpha]^{25}_D$ −30.38° (*c* 0.40, MeOH); UV (H_2O) λ_{max} 264.0 nm (ϵ 10 140) (pH 2), 264.5 nm (ϵ 10 200) (pH 7), 264.5 nm (ϵ 7910) (pH 11). Anal. ($C_{10}H_{11}FN_2O_4$) C, H, N.

1-(2,3-Dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)uracil (25). White solid; mp 136−138 °C; $[\alpha]^{27}_D$ −23.00° (*c* 0.48, MeOH); UV (H_2O) λ_{max} 258.0 nm (ϵ 12 780) (pH 2), 258.5 nm (ϵ 12 340) (pH 7), 258.5 nm (ϵ 9530) (pH 11). Anal. ($C_{10}H_{11}FN_2O_4 \cdot 0.47$ EtOH) C, H, N.

1-(2,3-Dideoxy-3-fluoro-β-D-glycero-pent-2-enofuranosyl)cytosine (26) was prepared from **17** on a 1.0 mmol scale by a similar procedure as described for **24**. White solid; mp 182−183 °C (dec); $[\alpha]^{25}_D$ −5.34° (*c* 0.39, 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). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

1-(2,3-Dideoxy-3-fluoro-α-D-glyceropent-2-enofuranosyl)cytosine (27) was prepared from **18** on a 1.0 mmol scale by a similar procedure as described for **24**. White solid; mp 132 °C (dec); $[\alpha]^{27}_D$ −177.70° (*c* 0.27, MeOH); UV (H_2O) λ_{max} 276.5 nm (ϵ 13 070) (pH 2), 268.0 nm (ϵ 8870) (pH 7), 268.0 nm (ϵ 8990) (pH 11). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

5-Fluoro-1-(2,3-dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)cytosine (28) was prepared from **19** on a 1.0 mmol scale by a similar procedure as described for **24.** White solid; mp 120−123 °C; $[\alpha]^{25}_D$ 28.20° (*c* 0.35, MeOH); UV (H_2O) λ_{max} 284.0 nm (ϵ 10 500) (pH 2), 277.5 nm (ϵ 8300) (pH 7), 277.5 nm (ϵ 8750) (pH 11). Anal. ($C_9H_9N_3F_2O_3$) C, H, N.

1-(2,3-Dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)adenine (29) was prepared from **20** on a 1.0 mmol scale by a similar procedure as described for **24**: mp 149−151°C; $[\alpha]^{25}_D$ −11.70 (*c* 0.378, MeOH); UV (H_2O) λ_{max} 258.5 nm (ϵ 12 900) (pH 2), 258.0 nm (ϵ 13 100) (pH 7), 257.5 nm (ϵ 13 400) (pH 11). Anal. ($C_{10}H_{10}FN_5O_2 \cdot 0.6$ MeOH) C, H, N.

1-(2,3-Dideoxy-3-fluoro-α-D-glyceropent-2-enofuranosyl)adenine (30) was prepared from **21** on a 1.0 mmol scale by a similar procedure as described for **24**: mp 170−172°C; $[\alpha]^{28}_D$ −142.00 (*c* 0.29, MeOH); UV (H_2O) λ_{max} 261.0 nm (ϵ 13 590) (pH 2), 259.0 nm (ϵ 15 090) (pH 7), 258.5 nm (ϵ 15 650) (pH 11). Anal. ($C_{10}H_{10}FN_5O_2$) C, H, N.

1-(2,3-Dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)hypoxanthine (31) was prepared from **22** on a 1.0 mmol scale by a similar procedure as described for **24**: mp 202 °C (dec); $[\alpha]^{25}_D$ −5.64 (*c* 0.434, MeOH); UV (H_2O) λ_{max} 247.5 nm (ϵ 11 500) (pH 2), 248.0 nm (ϵ 11 000) (pH 7), 253.5 nm (ϵ 12 800) (pH 11). Anal. ($C_{10}H_9FN_4O_2 \cdot 0.3H_2O$) C, H, N.

1-(2,3-Dideoxy-3-fluoro-β-D-glyceropent-2-enofuranosyl)guanine (32) was prepared from **23** on a 1.0 mmol scale by a similar procedure as described for **24**: mp >250°C; $[\alpha]^{27}_D$ −42.58 (*c* 0.81, MeOH); UV (H_2O) λ_{max} 259.0 nm (ϵ 18 500) (pH 2), 252.0 nm (ϵ 18 000) (pH 7), 250 nm (ϵ 19 000) (pH 11). Anal. ($C_{10}H_{10}FN_5O_3$) C, H, N.

Antiviral Assay. Human peripheral blood mononuclear (PBM) cells (obtained from the Atlanta Red Cross) were isolated by Ficoll−Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2−3 days prior to use. HIV-1_{LAI} obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infectious clones HIV-1_{xxBru} and HIV-$1_{M184Vpitt}$ were obtained from Dr. John Mellors (University of Pittsburgh). Infections were done in bulk for 1 h, either with 100 $TCID_{50}$/1 × 10^7 cells for a flask (T25) assay or with 200 $TCID_{50}$/6 × 10^5 cells per well for a 24-well plate assay. Cells were added to a plate or flask containing a 10-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat-inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/mL penicillin, 80 μg/mL streptomycin, 0.0008% DEAE-dextran, 0.045% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in humidified 5% CO_2/air at 37 °C for 5 days, and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12 000 rpm for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 μL of virus solubilization buffer (VSB) containing 0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.05 M Tris, pH 7.8. An amount of 10 μL of each sample was added to the 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 β counter (Meriden, CT). The RT results were expressed in counts per minute (cpm) per milliliter. The antiviral 50% effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) were determined from the concentration−response curve using the median effect method.[26]

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 the whole blood of healthy seronegative donors (HIV-1 and hepatitis B virus) by single-step Ficoll−Hypaque discontinuous gradient centrifugation. The 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 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.[25]

Adenosine Deaminase Study. Assays were performed at 25 °C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range 15−100 μM and with 0.15 units of adenosine deaminase (EC. 3.5.4.4. from calf intestinal mucosa, purchased from Sigma-Aldrich). The assays were monitored with a UV spectrometer at 265 nm. Initially, qualitative assays were performed with D-3′F-d4A **29** (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.[21] Initial hydrolysis rates for each substrate concentration were measured manually through graphical curve fitting of the concentration vs time data for the reaction of each substrate. From the Lineweaver−Burke plot of these initial rates, V_{max} (maximum velocity) and K_M (Michaelis−Menten constant) were obtained for each substrate and k_{cat} was also calculated with 0.15 unit of the enzyme (MW = 33 000) in 2 mL of buffer solution. The $t_{1/2}$ values of D-3′F-d4A **25** and adenosine were also measured at 20 μM with 0.15 unit of the enzyme.

Molecular Modeling Study. (a) Conformational Analysis. The initial conformation of d-3′F-d4C **26** was constructed by the builder module in Spartan 5.1.1 (Wavefunctions, Inc. Irvine, CA), and all calculations were performed on a Silicon Graphics O2 workstation. The initial conformations were cleaned up and geometry-optimized through quantum mechanical ab initio calculations using the RHF/3-21G* basis in Spartan 5.1.1.

(b) Binding Affinity Study for HIV-1 Reverse Transcriptase. All molecular modeling studies of the enzyme/substrate complexes were performed using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme/ligand complex was built on the basis of the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer/template duplex (PDB entry 1rtd).[25] A model of the NRTI binding site was built, which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template/primer) duplex. The conformationally optimized structure of D-3′F-d4CTP was used to define the initial Cartesian coordinates. The heterocyclic moiety of the (n + 1)th nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs under study, i.e., the adenine moiety that was in the original X-ray structure (1rtd)[27] was modified to guanine. The inhibitor triphosphates were manually docked at the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[27] Gästeiger−Hückel charges were given to the enzyme/ligand complex with formal charges (+2) to the two Mg atoms in the active site. Then, Kollman all-atom charges were loaded to the enzyme site using the biopolymer module in Sybyl. Fluorine parameters were obtained from literature[28] and MM2 parameters and were entered into 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 the energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme/inhibitor complexes were minimized by using Kollman all-atom force field until the iteration number reached 5000.

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Supporting Information Available: ^{1}H NMR and elemental analysis data for compounds **8**−**32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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