Structure−Activity Relationships of 2′-Fluoro-2′,3′-unsaturated D-Nucleosides as Anti-HIV-1 Agents

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We studied the structure−activity relationships of a series of 2′-fluoro-2′,3′-unsaturated D-nucleosides against HIV-1 in human peripheral blood mononuclear (PBM) cells. The target compounds **10**−**21** and **28**−**33** were prepared by N-glycosylation of the acetate **4**, which was readily prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde in five steps. Among the newly synthesized nucleosides, 2-amino-6-chloropurine (**11**), adenine (**14**), inosine (**16**), guanine (**18**), 2,6-diaminopurine (**20**), and 5-fluorocytosine (**30**) derivatives were found to exhibit interesting anti-HIV activities with EC_{50} values of 4.3, 0.44, 1.0, 2.6, 3.0, and 0.82 μM, respectively. The implications for drug resistance of the titled nucleosides with respect to lamivudine-resistant variants (M184V) were also examined, and no significant cross-resistance with the variants was observed with the D-series.

Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) continue to be the cornerstone of anti-HIV (human immunodeficiency virus) therapy. Side effects of the existing regimens, however, have been one of the drawbacks.[1−4] Additionally, the efficacy of the NRTIs has been compromised by the development of resistant variants.[5−7] Approaches to address these problems include using drugs with different resistance profiles and mechanisms, such as a combination therapy of 3TC, AZT, and indinavir.[8] Indeed, it has become increasingly clear that in order to achieve the greatest benefit in terms of reduction of viral load and delay of resistance, a combination of multiple-drug regimens is required.[9] Therefore, there is a need for new RT inhibitors with high potency and different resistance profiles, thereby providing a greater choice of drug combinations.

Since the discovery of 2′,3′-dideoxy (ddN)- and 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4N), including 2′,3′-dideoxycytidine (ddC),[10,11] 2′,3′-dideoxyinosine (ddI),[11−16] 2′,3′-didehydro-3′ deoxythymidine (d4T),[17,18] and abacavir[19,20] as antiviral agents, a number of modified dideoxynucleoside analogues have been synthesized and identified as anti-HIV agents, such as L-2′,3′-didehydro-2′,3′-dideoxycytidine (β-L-d4C), and its 5-fluoro congener (β-L-Fd4C).[21] It has also been found that the introduction of a fluorine atom into the 2′-position of nucleosides in general, and of purine nucleosides in particular, results in the stabilization of the glycosyl bond and confers interesting biological activity as exemplified by 2′-β-fluoro-2′,3′-dideoxyadenosine (F-ddA).[22−24] So far, there have been limited reports on the synthesis of pyrimidine nucleosides possessing a 2′-fluorinated ene-type moiety (uracil, cytosine, and thymine derivatives).[25−27] However, relatively little effort has been devoted to the synthesis of purine analogues with 2′-fluorinated ene-type modification, presumably due to the difficulty to access the corresponding purine nucleosides. Recently, we have reported the synthesis and anti-HIV and anti-HBV (hepatitis B virus) activities of 2′-fluoro-2′,3′-unsaturated L-nucleosides. Of significance was that β-L-2′-F-d4C, β-L-2′-F-d4FC, and β-L-2′-F-d4A exhibited potent activities against HIV-1 and HBV.[28] Encouraged by these promising biological results of L-enantiomers, it was of interest to synthesize hitherto unknown purine D-nucleosides for the study of the structure−activity relationship. Herein, we report the synthesis and anti-HIV activities as well as the activity against a lamivudine-resistant mutant. It is critical to discover antiviral agents that can demonstrate effective antiviral activity against these resistant viruses (M184V mutant) for combination chemotherapy since lamivudine is currently widely used in the treatment of HIV infection.

Results and Discussion

Chemistry. Following previously described methodologies,[28−30] we have synthesized 2′-vinyl fluoride containing D-nucleoside analogues (Scheme 1). 2,3-*O*-Isopropylidene-D-glyceraldehyde, prepared by oxidative cleavage of 1,2:5,6-di-*O*-isopropylidene-D-mannitol, was reacted with triethyl α-fluorophosphonoacetate to give alkene **1**, mainly as the *E*-isomer,[32] along with a small amount of *Z*-isomer, which was subsequently transformed to the 2-fluorobutenolide intermediate **2**.[31−33] Selective reduction of **2** to lactol **3** by DIBAL-H in CH_2Cl_2 followed by treatment with acetic anhydride and triethylamine gave the key intermediate **4**. Condensation of acetate **4** with various trimethylsilylated purines and pyrimidines was carried out at room temperature in 1,2-dichloroethane (DCE). Completion of the reaction took ca. 1 h in the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf) as a catalyst. The

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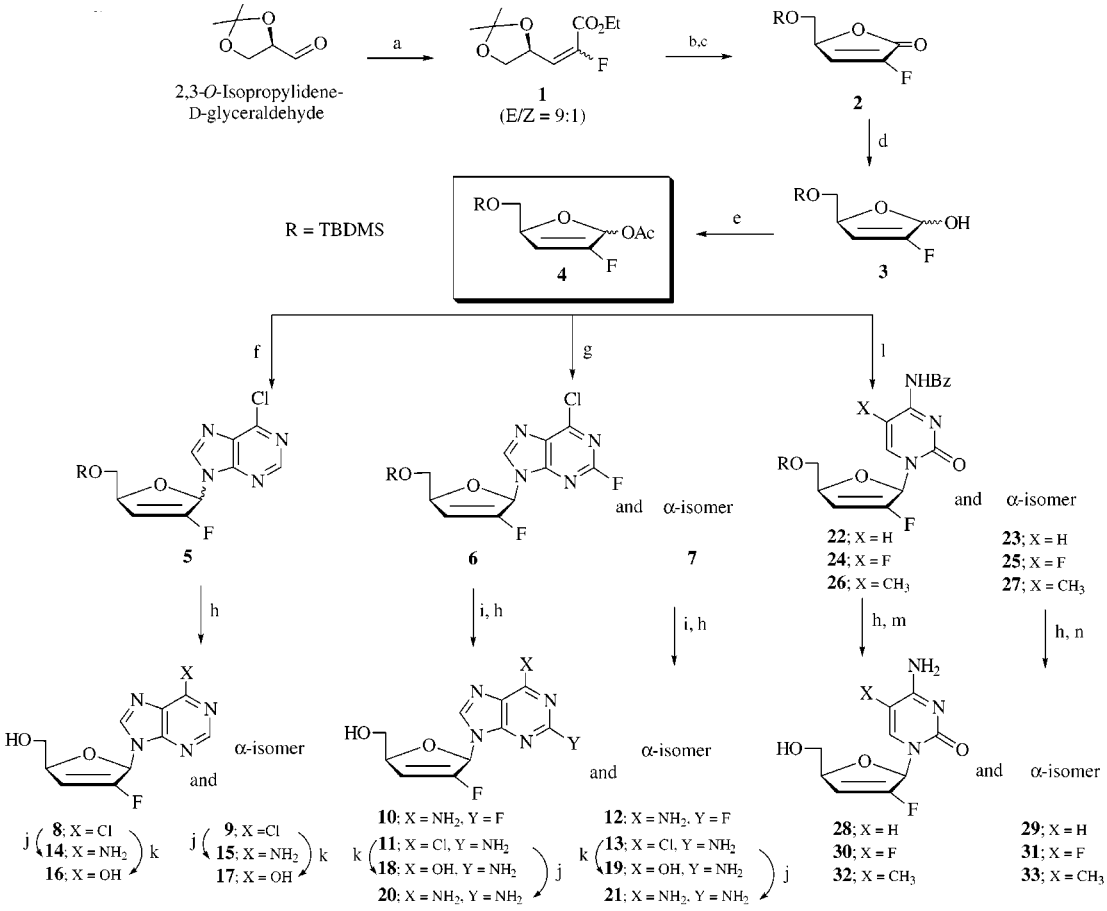
Scheme 1[a]



[a] Reagents: (a) $(EtO)_2P(O)CHFCO_2Et$, NaHMDS, THF, −78 °C; (b) *c*-HCl, EtOH; (c) TBDMSCl, imidazole, CH_2Cl_2; (d) DIBAL-H, CH_2Cl_2, −78 °C; (e) Ac_2O, TEA, CH_2Cl_2; (f) silylated 6-Cl-purine, TMSOTf, DCE; (g) silylated 6-Cl-2-F-purine, TMSOTf, DCE; (h) TBAF, CH_3CN; (i) NH_3/DME; (j) NH_3/MeOH, 90 °C; (k) $HSCH_2CH_2OH$, NaOMe, reflux; (l) silylated N^4-Bz-cytosine derivatives, TMSOTf, DCE; (m) NH_3/MeOH.

coupling reactions provided a mixture of α- and *β*-anomers in 50−58% yield. For instance, acetate **4** was reacted with silylated 6-chloropurine to give 5′-*tert*-butyldimethylsiyl protected nucleosides **5** as an anomeric mixture. Deprotection of **5** with tetrabutylammonium fluoride (TBAF) in acetonitrile facilitated the separation of isomers on silica gel (3% MeOH/CH_2Cl_2) to provide the precursors **8** (*β*-isomer) and **9** (α-isomer) for adenine and hypoxanthine derivatives. Acetonitrile gave smoother conversion and better yields than tetrahydrofuran (THF) during deprotections of the silyl groups. Conversion of the *β*-6-chloropurine derivative **8** to the *β*-adenine derivative by ammonolysis in a steel bomb at 90−100 °C produced **14** in 90% yield. Compound **8** was also converted to the *β*-inosine analogue **16** by refluxing with mercaptoethanol and sodium methoxide in methanol. For the preparation of 2,6-disubstituted purine nucleosides, the acetate **4** was condensed with silylated 2-fluoro-6 chloropurine in DCE to give silyl protected nucleosides **6** and **7**, which were readily separated by column chromatography (12% EtOAc/hexanes). Compound **6** was then converted to the 2-fluoroadenine derivative **10** and the 6-amino-2-chloropurine derivative **11** by treatment with ammonia in ethylene glycol dimethyl ether (DME) followed by TBAF in acetonitrile. The guanine derivative **18** was readily prepared from compound **11** analogously to the inosine analogue **16**. Compound **11** was also further converted to the 2,6-diaminopurine derivative **20** by ammonolysis. The α-purine nucleosides **12**, **13**, **15**, **17**, **19**, and **21** were prepared analogously to their corresponding *β*-isomers. Similarly, protected cytosines and their 5-substituted congeners, **22**−**27**, were synthesized by N-glycosylation of the key intermediate **4** with appropriate N^4-benzoylated bases. Removal of the *tert*-butyldimethylsiyl group of compounds **22**−**27**, followed by ammonolysis in the presence of methanolic ammonia, afforded the desired free nucleosides **28**−**33**, as described in Scheme 1. Assignment of the structures of the newly synthesized nucleosides was based on the comparison of the chemical, physiological, and optical data to those of the corresponding L-series.[28]

All the spectral properties of the newly synthesized compounds (Table 1) were in good agreement with those published for their corresponding L-enantiomers.

Antiviral Activity. Anti-HIV activities of the synthesized nucleoside analogues were determined in human peripheral blood mononuclear (PBM) cells acutely infected with HIV-1 and compared to those of AZT and FTC. The cytosine derivative **28**, which has been published by several laboratories,[25−27] was also included to compare the antiviral activities in different cell lines. Results in Table 2 indicate the median antiviral potency and growth inhibition of the newly synthesized compounds expressed as EC_{50} and IC_{50}, respectively. It was found that D-2′-fluorinated unsaturated nucleosides (D-

Table 1. Physical Properties of the Synthesized Nucleosides

no.	mp °C (solv)[a]	$[\alpha]_D$, deg	formula	anal.
8	128−130 (B)	+40.79 (*c* 0.18, MeOH)	$C_{10}H_8FClN_4O_2 \cdot 0.1EtOH$	C, H, N
9	157−159 (A)	−162.32 (*c* 0.13, MeOH)	$C_{10}H_8FClN_4O_2$	C, H, N
10	188−190 (C)	+56.62 (*c* 0.13, $CHCl_3$)	$C_{10}H_9F_2N_5O_2$	C, H, N
11	157−160 (dec, B)	−10.99 (*c* 0.17, MeOH)	$C_{10}H_9FClN_5O_2$	C, H, N
12	183−185 (dec, C)	−154.01 (*c* 0.19, MeOH)	$C_{10}H_9F_2N_5O_2$	C, H, N
13	148−150 (C)	−134.78 (*c* 0.12, MeOH)	$C_{10}H_9FClN_5O_2 \cdot 0.3H_2O$	C, H, N
14	>191 (dec, C)	+53.91 (*c* 0.12, MeOH)	$C_{10}H_{10}FN_5O_2$	C, H, N
15	173−174 (B)	−163.83 (*c* 0.34, MeOH)	$C_{10}H_{10}FN_5O_2$	C, H, N
16	129−131 (dec, B)	+52.77 (*c* 0.27, MeOH)	$C_{10}H_{10}FN_4O_3$	C, H, N
17	>202 (dec, B)	−159.85 (*c* 0.19, MeOH)	$C_{10}H_{10}FN_4O_3$	C, H, N
18	198−201 (B)	−24.64 (*c* 0.02, DMF)	$C_{10}H_9FN_5O_3$	C, H, N
19	>212 (dec, B)	−57.16 (*c* 0.09, DMF)	$C_{10}H_9FN_5O_3 \cdot 0.3H_2O$	C, H, N
20	146−148 (B)	+7.18 (*c* 0.23, MeOH)	$C_{10}H_{11}FN_6O_2 \cdot 0.1CH_2Cl_2$	C, H, N
21	184−186 (B)	−102.18 (*c* 0.11, MeOH)	$C_{10}H_{11}FN_6O_2 \cdot 0.4H_2O$	C, H, N
28	172−174(D)	+21.94 (*c* 0.68, MeOH)	$C_9H_{10}FN_3O_3$	C, H, N
29	182−183 (B)	−157.86 (*c* 0.30, MeOH)	$C_9H_{10}FN_3O_3$	C, H, N
30	201−203 (D)	+34.35 (*c* 0.15, MeOH)	$C_9H_9F_2N_3O_3$	C, H, N
31	158−161 (B)	−153.51 (*c* 0.11, MeOH)	$C_9H_9F_2N_3O_3$	C, H, N
32	179−182 (dec, B)	+39.65 (*c* 0.33, MeOH)	$C_{10}H_{12}FN_3O_3$	C, H, N
33	180−182 (B)	−169.31 (*c* 0.37, MeOH)	$C_{10}H_{12}FN_3O_3$	C, H, N

[a] Solvents: A, EtOAc−hexanes; B, CH_2Cl_2−MeOH; C, MeOH; D, hexanes−MeOH−CH_2Cl_2.

Table 2. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentration of D-2′-F-d4Ns in Human PBM Cells and Cytotoxicity in Vero and CEM Cells

		EC_{50} (μM)		IC_{50} (μM)	
compd	B	HIV-1	PBM cells	Vero cells	CEM cells
10	2-F-adenine (β)	>100	>100	>100	>100
11	6-Cl-2-NH_2-purine (β)	4.3	98.8	>100	>100
12	2-F-adenine (α)	3.97	41.2	16.1	28.3
13	6-Cl-2-NH_2-purine (α)	≥75	77.4	>100	30.2
14	adenine (β)	0.44	68.3	>100	57.6
15	adenine (α)	>100	>100	>100	>100
16	hypoxanthine (β)	1.0	92.7	87.7	>100
17	hypoxanthine (α)	>100	>100	>100	>100
18	guanine (β)	2.6	>100	>100	>100
19	guanine (α)	18.3	>100	>100	>100
20	2,6-di-NH_2-purine (β)	3.0	>100	>100	>100
21	2,6-di-NH_2-purine (α)	>100	>100	>100	>100
28	cytosine (β)[25−27]	2.6	7.3	>100	35.3
29	cytosine (α)	>100	>100	>100	>100
30	5-F-cytosine (β)	0.82	>100	>100	>100
31	5-F-cytosine (α)	>100	>100	>100	>100
32	5-Me-cytosine (β)	>100	>100	>100	>100
33	5-Me-cytosine (α)	>100	>100	>100	>100
AZT		0.004	>100	29.0	14.3

2′-F-d4N) had significant anti-HIV-1 activities. The toxicity of these nucleosides has been assessed in human PBM, Vero, and CEM cells (Table 2). No significant toxicity was detected for most of the evaluated compounds. Within the class of β-D-2′-F-d4N, purine derivatives (adenine, hypoxanthine, 2-fluoroadenine, 6-chloro-2-aminopurine, 2,6-diaminopurine, and guanine) showed moderate to potent anti-HIV-1 activities, including two α-anomers, and generally they were more potent than their L-counterparts.[28] The adenine derivative **14** exhibited 10-fold greater potency than its corresponding L-enantiomer (EC_{50} 0.44 μM vs 4.7 μM). One probable explanation is that these D-purine derivatives may be better substrates for nucleoside kinases than their corresponding L-nucleosides.[34] Particularly, in the case of β-guanine and its 6-chloro-congener, the D-series (**18** and **11**) was found to exhibit moderate anti-HIV activity (EC_{50} 2.6 and 4.3 μM, respectively), while the L-nucleosides showed no antiviral activity against HIV-1 (>100 μM).[28] Surprisingly, α-2′-F-adenine **12** displayed potent anti-HIV activity (EC_{50} 3.97 μM), whereas its β-isomer **10** was inactive. These data, not fully explainable at this stage, were confirmed by repeated biological essays and stereochemical assignments of the two isomers. Furthermore, other examples where α-nucleosides have shown anti-HIV activity have been reported.[28,35] Interestingly, it was found that in the D-series several purine derivatives, such as the adenine **14** and hypoxanthine **16**, show significant antiviral activity whereas, in the corresponding L-series,[29] the pyrimidine derivatives such as the cytosine and 5-fluorocytosine are the most potent. The anti-HIV activity and cytotoxicity of cytosine derivative **28** were in accordance with the reported data.[25−27] In the case of the 5-fluorocytosine derivative **30**, however, the L-enantiomer exhibited higher antiviral potency than the newly synthesized D-counterpart (0.17 vs 0.82 μM). It is interesting to note that 5-fluoro substitution on the cytosine base moiety retained potent antiviral activity compared to the parent derivative. In contrast, the 5-methylcytosine derivatives showed no antiviral activity.

Although both 3TC and FTC showed potent antiviral activities against HIV and HBV, several investigators have reported rapid emergence of resistant viruses, in vitro as well as in clinic, characterized by a mutation at the 184 M codon (ATG) of the reverse transcriptase (RT).[36−39] However, this mutation had no effects on the susceptibility toward AZT or nevirapine and minimal effects on susceptibility to ddI and ddC. Huang et al. have described the structure of HIV-1 RT complexed with thymidine triphosphate and its implications in drug resistance, and they suggested a correlation between mutations and the chemical structure of the inhibitor.[40] The M184V mutant can influence both the triphosphate of the inhibitor as well as the primer terminus in the active site. It is reported that interference with valine at 184 is enhanced by the oxathiolane ring with respect to the 2′-deoxynucleoside triphosphate, which may account for the decreased antiviral activity of 3TC. In view of these findings, it was anticipated that β-D-2′-F-d4Ns may not be significantly affected by point mutation at M184 by the sugar moiety. Preliminary biological evaluations suggest this is indeed the case.

Table 3. Activity of Selected β-D-2′-F-d4Ns against M184V Pitt Virus in Human PBM Cells

		EC_{50}		
compd	B	XxBRU Pitt[a] (μM)	M184V Pitt[b] (μM)	FI[c]
14	adenine	0.46	0.8	1
16	hypoxanthine	2.8	2.5	1
18	guanine	4.4	11.2	3
30	5-F-cytosine	3.1	9.61	3
AZT		0.005	0.004	1
(−)-FTC		0.0046	21.5	4674

[a] (−)-FTC-sensitive isolates. [b] (−)-FTC resistant isolates. [c] FI (fold increase) EC_{50} = EC_{50} data resistant virus/EC_{50} data from xxBRU Pitt virus.

Table 4. Kinetic Parameters of the Adenosine Analogues with Mammalian Adenosine Deaminase Relative to Adenosine

compd	K_M (M)	$t_{1/2}$
14	2.3×10^{-5}	41.6 min
β-L-2′-F−d4A[28]	NS[a]	ND[b]
adenosine	5×10^{-6}	25 s

[a] β-L-2′-F−d4A is not a substrate of adenosine deaminase. [b] Not determined.

The anti-HIV-1 activity of selected β-D-2′-F-d4Ns was confirmed in human PBM cell lines with different virus strains including an HIV-1 strain resistant to 3TC/FTC (M184V Pitt.) as shown in Table 3. AZT, lamivudine, and (−)-FTC (emtricitabine) were included as positive controls. The decreasing order of potency of the selected nucleosides in xxBRU Pitt.-infected human PBM cells was β-D-adenine **14** (0.46 μM) > β-D-inosine **16** (2.8 μM) > β-D-5-F-cytosine **30** (3.1 μM) > β-D-guanine **18** (4.4 μM). As expected, high levels of cross-resistance with M184V isolates were evident with lamivudine and (−)-FTC, not with AZT. However, no significant cross-resistance was found for the newly synthesized compounds.

In an attempt to understand metabolic properties, the adenine derivative **14**, which showed a favorable resistance profile, was studied with respect to adenosine deaminase, along with the previously reported enantiomer β-L-2′-F-d4A[28] and adenosine. As shown in Table 4, the adenine derivative **14** was found to be a substrate for mammalian adenosine deaminase (from calf intestinal mucosa, EC. 3.5.4.4.), while its L-enantiomer was not converted to the corresponding inosine derivative. Compound **14** is deaminated more effectively than the nonfluorinated analogue D-d4A (K_M 30 μM, $t_{1/2}$ 3.1 days).[34] Compared to adenosine, however, the D-isomer **14** is deaminated about 100 times slower. This property of **14** may be favorable as a therapeutic agent, since adenosine deaminase is one of the major deactivating enzymes in vivo. As for chemical stability, as expected, compound **14** has a half-life of ca. 3 days at pH 2.0 and is stable at pH 7.4 and 11.0, whereas D-d4A has been reported to have a half-life of less than 1 day at pH 7.0.[41]

In summary, from the structure−activity relationships of 2′-fluoro-2′,3′-unsaturated D-nucleosides, it was found that the β-D-2′-F-d4N series exhibited moderate to potent anti-HIV-1 activities with high selectivity in human PBM cells. Preliminary cross-resistance studies indicated that no significant cross-resistance with 3TC/FTC resistant virus (M184V) was detected. In view of these interesting preliminary antiviral data, additional biological studies are warranted.

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 400 MHz spectrometer with tetramethylsilane as the internal reference. 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.

(*S*)-(−)-4-[(*tert*-Butyldimethylsilyloxy)methyl]-2-fluoro-2-buten-4-olide (2). A solution of triethyl 2-fluorophosphonoacetate (40.5 g, 167.5 mmol) in THF (70 mL) was cooled to −78 °C, and sodium bis(trimethylsilyl)amide (1.0 M solution in THF, 167.5 mL, 167.5 mmol) was added dropwise. The mixture was kept for 30 min at −78 °C, and then a solution of D-(*R*)-glyceraldehyde acetonide (19.79 g, 152 mmol) in THF (70 mL) was added. After being stirred for 1 h at −78 °C, the reaction mixture was treated with aqueous NH_4Cl and extracted with ether. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel to give a mixture of *E*-**1** and *Z*-**1** (9:1 by ^1H NMR) as a yellowish oil (34.4 g, 97.1%).

A solution of *E*/*Z*-**1** (10.2 g, 46.7 mmol) in 60 mL of anhydrous EtOH was treated with 15 mL of concentrated HCl and stirred at room temperature for 2.5 h. The solvent was removed in vacuo and the residue coevaporated with toluene (3 × 200 mL) to give a lactone intermediate, along with uncyclized ester intermediate. The resulting yellowish syrup was used as such for the next reaction without further purification. *tert*-Butyldimethylsilyl chloride (17.5 g, 116.5 mmol) and imidazole (8.0 g, 117 mmol) were added to a solution of hydrolyzed products in CH_2Cl_2 (150 mL), and the mixture was reacted overnight at room temperature. The resulting mixture was treated with ice and diluted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and concentrated to dryness. Purification on silica gel (4% EtOAc/hexanes) provided 18.89 g (70.5% from D-(*R*)-glyceraldehyde acetonide) of a crystalline solid **2**: mp 48−50 °C; $[\alpha]^{27}_D$ −105.9 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{10}H_{19}FO_3Si$: C, 53.63; H, 7.77. Found: C, 53.68; H, 7.82.

1-Acetyl-4-[(*tert*-butyldimethylsilyloxy)methyl]-2-fluoro-2-buten-4-olide (4). 2-Fluorobutenolide **2** (10.47 g, 42.5 mmol) was dissolved in 150 mL of CH_2Cl_2 under a nitrogen atmosphere, and then the mixture was cooled to −78 °C and treated with 1.0 M solution of DIBAL-H in CH_2Cl_2 (63.6 mL, 63.6 mmol). The resulting mixture was reacted for 2 h at −78 °C. The cold mixture was treated with dilute nitric acid, washed with water, and dried (Na_2SO_4). Evaporation of the solvent gave lactol intermediate **3** (anomeric mixture) as a pale yellowish oil (8.6 g, crude yield 82%), which was used as such for the next step.

To a solution of the lactol **3** (5.18 g, 20.85 mmol) and triethylamine (11.6 mL, 83.4 mmol) in CH_2Cl_2 (40 mL) was added acetic anhydride (7.9 mL, 83.4 mmol) at 0 °C, and the resulting mixture was kept for 2 h at room temperature. The reaction mixture was concentrated under vacuo and purified by column chromatography (6.5% EtOAc/hexanes) to give **4** (4.79 g, 79.1%) as a yellowish oil.

General Procedure for Condensation of Acetate 4 with Heterocycles. A mixture of 6-chloropurine (1.04 g, 6.72 mmol), hexamethyldisilazane (25 mL), and ammonium sulfate (catalytic amount) was refluxed for 4 h under a nitrogen atmosphere. The clear solution obtained was concentrated in vacuo, and the residue was dissolved in DCE (5 mL) and reacted with a solution of **4** (1.50 g, 5.17 mmol) in DCE (40 mL), followed by trimethylsilyl triflate (1.3 mL, 6.72 mmol) at 0 °C. After the mixture was stirred for 1 h at room temperature under a nitrogen atmosphere, the reaction solution was poured into an ice-cold saturated $NaHCO_3$ solution (20 mL) and stirred for 15 min. The organic layer was washed with water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue

was separated by silica gel column chromatography (17% EtOAc/hexanes) to give anomeric mixture **5** (β:α = 1:1.3, 1.15 g, 58.1%) as a syrup.

6-Chloro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-D-*glycero*-pent-2-enofuranosyl]purine (5): ^{1}H NMR ($CDCl_3$) δ 0.09−0.11 (m, 4 × CH_3), 0.91, 0.92 (2s, *t*-Bu), 3.85 (m, H-5′), 5.19 (ps t, J = 4.4 Hz, H-4′), 5.02 (m, H-4′), 5.78, 5.85 (2s, H-3′), 6.93 (t, J = 4.8 Hz, H-1′), 7.01 (m, H-1′), 8.21, 8.60 (2s, H-2), 8.78, 8.79 (2s, H-8); UV ($CHCl_3$) λ_{max} 263.5 nm. Anal. Calcd for $C_{16}H_{22}FClN_4O_2Si$: C, 49.93; H, 5.76; Cl, 9.21; N, 14.56. Found: C, 50.04; H, 5.73; Cl, 9.33; N, 14.45.

6-Chloro-2-fluoro-9-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]purine (6) and Its α-Isomer (7). A mixture of silylated 2-fluoro-6 chloropurine [prepared from 1.3 g (7.6 mmol) of 2-fluoro-6-chloropurine and 20 mL of HMDS], **4** (1.7 g, 5.85 mmol), and TMSOTf (1.5 mL, 5.85 mmol) in DCE (40 mL) was stirred at room temperature overnight. After extractive work-up, purification by silica gel column chromatography (12% EtOAc/hexanes) gave β-anomer **6** (718 mg, 30.4%) as a white foam and α-anomer **7** (473.7 mg, 20.1%) as a yellowish syrup. **6**: UV (MeOH) λ_{max} 268.5 nm. Anal. Calcd for $C_{16}H_{21}ClF_2N_4O_2Si$·0.1acetone: C, 47.90; H, 5.33; N, 13.71. Found: C, 48.02; H, 5.30; N, 13.41. **7**: UV (MeOH) λ_{max} 268.0 nm. Anal. Calcd for $C_{16}H_{21}F_2ClN_4O_2Si$·0.6 acetone: C, 48.84; H, 5.66; N, 12.80. Found: C, 48.89; H, 5.60; N, 12.53.

6-Chloro-9-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)purine (8) and Its α-Isomer (9). A solution of **5** (800 mg, 2.96 mmol) in CH_3CN (20 mL) was treated with TBAF (1 M solution in THF) (3.8 mL, 3.8 mmol) and stirred for 30 min at room temperature. After evaporation of the solvent, the residue was purified by column chromatography (3% MeOH/CH_2Cl_2) to obtain β-anomer **8** (283.6 mg, 35.4%) as a white solid and α-anomer **9** (341.3 mg, 42.6%) as a white solid. **8**: UV (MeOH) λ_{max} 262.5 nm. Anal. ($C_{16}H_8ClFN_4O_2$·0.1EtOH). **9**: UV (MeOH) λ_{max} 262.5 nm. Anal. ($C_{16}H_8ClFN_4O_2$) C, H, N.

2-Fluoro-6-amino-9-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)purine (10) and 2-Amino-6-chloro-9-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)-purine (11). Dry ammonia was bubbled into a stirred solution of **6** (330 mg, 0.82 mmol) in dry DME (35 mL) at room temperature overnight. The salts formed were removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was purified by preparative TLC (20∼30% EtOAc/hexanes) to give two compounds, 5′-protected 2-fluoro-6-aminopurine derivative [86 mg, 27.4%: ^{1}H NMR ($CDCl_3$) δ 0.084, 0.09 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.80−3.90 (m, H-5′), 4.96 (s, H-4′), 5.73 (s, H-3′), 5.86 (m, OH), 6.81 (s, H-1′), 8.19 (s, H-8); UV (MeOH) λ_{max} 268.5 nm. Anal. Calcd for $C_{16}H_{23}F_2N_5O_2Si$: C, 50.11; H, 6.05; N, 18.26. Found: C, 50.18; H, 6.05; N, 18.24] as a white solid and 5′-protected 2-amino-6-chloropurine derivative [141.7 mg, 43.2%: ^{1}H NMR ($CDCl_3$) δ 0.072, 0.078 (2s, 2 × CH_3), 0.92, (s, *t*-Bu), 3.81 (m, H-5′), 4.95 (m, H-4′), 5.12 (s, NH_2), 5.75 (s, H-3′), 6.78 (s, H-1′), 8.14 (s, H-8); UV (MeOH) λ_{max} 307.5 nm. Anal. Calcd for $C_{16}H_{23}FClN_5O_2Si$: C, 48.05; H, 5.80; N, 17.51. Found: C, 48.26; H, 5.88; N, 17.51] as a white solid.

These two isolated isomers were desilylated to give free nucleosides **10** and **11** by similar methodology described for compounds **8** and **9**. After evaporation of the solvent, the residues were purified by column chromatography to give **10** (36.3 mg, 89.9%, 9% MeOH/CH_2Cl_2) as crystals and **11** (110.4 mg, 92%, 5% MeOH/CH_2Cl_2) as a white solid. **10**: UV (H_2O) λ_{max} 260.5 nm (ϵ 11200) (pH 7), 261.0 nm (ϵ 9800) (pH 2), 261.0 nm (ϵ 10000) (pH 11). Anal. ($C_{10}H_9F_2N_5O_2$) C, H, N. **11**: UV (H_2O) λ_{max} 305.5 nm (ϵ 9800) (pH 7), 308.0 nm (ϵ 9800) (pH 2), 305.0 nm (ϵ 5400) (pH 11). Anal. ($C_{10}H_9ClFN_5O_2$) C, H, N.

2-Fluoro-6-amino-9-(2,3-dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)purine (12) and 2-Amino-6-chloro-9-(2,3-dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)-purine (13). Dry ammonia was bubbled into a stirred solution of **7** (454.6 mg, 1.23 mmol) in dry DME (40 mL) at room temperature for 6 h. The salts formed were removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (25−50% EtOAc/hexanes) to give two compounds, 5′-protected 2-fluoro-6-aminopurine derivative [130 mg, 27.5%: ^{1}H NMR ($CDCl_3$) δ 0.08, 0.093 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.65−3.76 (m, H-5′), 5.13 (ps t, J = 4.4, 4.8 Hz, H-4′), 5.80 (s, H-3′), 6.76 (s, H-1′), 7.84 (s, H-8); UV (MeOH) λ_{max} 268.5 nm. Anal. Calcd for $C_{16}H_{23}F_2N_5O_2Si$: C, 50.11; H, 6.05; N, 18.26. Found: C, 50.23; H, 5.99; N, 18.18] as a white solid and 5′-protected 2-amino-6-chloropurine derivative [199.3 mg, 44.3%: ^{1}H NMR ($CDCl_3$) δ 0.082, 0.093 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.73−3.84 (m, H-5′), 5.09 (m, H-4′), 5.12 (s, NH_2), 5.80 (s, H-3′), 6.74 (s, H-1′), 7.84 (s, H-8); UV (MeOH) λ_{max} 307.5 nm. Anal. Calcd for $C_{16}H_{23}FClN_5O_2Si$: C, 48.05; H, 5.80; N, 17.51. Found: C, 48.30; H, 5.72; N, 17.23] as a white solid.

These two isolated isomers were desilylated to give free nucleosides **12** and **13** by the same methodology described for compounds **8** and **9**. After evaporation of the solvent, the residues were purified by column chromatography to give **12** (36.3 mg, 89.9%, 9% MeOH/CH_2Cl_2) as a white solid and **13** (110.4 mg, 92%, 5% MeOH/CH_2Cl_2) as a white solid. **12**: UV (H_2O) λ_{max} 260.5 nm (ϵ 11200) (pH 7), 261.0 nm (ϵ 9800) (pH 2), 261.0 nm (ϵ 10000) (pH 11). Anal. ($C_{10}H_9F_2N_5O$) C, H, N. **13**: UV (H_2O) λ_{max} 305.5 nm (ϵ 9800) (pH 7), 308.0 nm (ϵ 9800) (pH 2), 305.0 nm (ϵ 5400) (pH 11). Anal. ($C_{10}H_9ClFN_5O_2$·0.3H_2O) C, H, N.

9-(2,3-Dideoxy-2-fluoro-β-D-gycero-pent-2-enofuranosyl)adenine (14). A solution of **8** (75.5 mg, 0.28 mmol) and saturated NH_3/MeOH (50 mL) was heated at 90−100 °C in a steel bomb for 36 h. After cooling to room temperature, the solvent was removed under vacuum, and the residue was purified by column chromatography using 6% MeOH/CH_2Cl_2 as eluent to give **14** (48.1 mg, 68.4%) as a white solid: UV (H_2O) λ_{max} 259.0 nm (ϵ 22500) (pH 7), 258.0 nm (ϵ 23900) (pH 2), 259.0 nm (ϵ 21400) (pH 11). Anal. ($C_{10}H_{10}FN_5O_2$) C, H, N.

9-(2,3-Dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)adenine (15). Compound **15** was prepared from **7** on a 0.29 mmol scale by the same procedure for its β-isomer **14**. Purification by column chromatography using 6% MeOH/CH_2Cl_2 as eluent afforded **15** (72 mg, 78%) as a white solid: UV (H_2O) λ_{max} 258.5.0 nm (ϵ 24200) (pH 7), 256.5 nm (ϵ 25300) (pH 2), 258.0 nm (ϵ 27100) (pH 11). Anal. ($C_{10}H_{10}FN_5O_2$) C, H, N.

9-(2,3-Dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)hypoxanthine (16). A mixture of **8** (84.4 mg, 0.31 mmol), NaOMe (1 M solution in MeOH) (2.18 mL, 1.25 mmol), and 2-mercaptoethanol (0.09 mL, 1.25 mmol) in MeOH (20 mL) was refluxed for 7 h under a nitrogen atmosphere. The reaction mixture was cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue was purified by silica gel column chromatography (11% MeOH/$CHCl_3$) to afford **16** (61 mg, 78%) as a white solid: UV (H_2O) λ_{max} 247.5 nm (ϵ 20000) (pH 7), 247.5 nm (ϵ 19600) (pH 2), 252.5 nm (ϵ 22900) (pH 11). Anal. ($C_{10}H_9FN_4O_3$) C, H, N.

9-(2,3-Dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)hypoxanthine (17). Compound **17** was prepared from **9** on a 0.30 mmol scale by the same procedure used for its β-isomer **16**. Purification by silica gel column chromatography (10% MeOH/CH_2Cl_2) afforded **17** (53.8 mg, 71.1%) as a white solid: UV (H_2O) λ_{max} 248.5 nm (ϵ 24200) (pH 7), 247.5 nm (ϵ 25200) (pH 2), 253.0 nm (ϵ 27100) (pH 11). Anal. ($C_{10}H_9FN_4O_3$) C, H, N.

9-(2,3-Dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)guanine (18). A mixture of **11** (75.6 mg, 0.27 mmol), 2-mercaptoethanol (0.07 mL, 1.06 mmol), and 1 N NaOMe (1.06 mL, 1.06 mmol) in MeOH (10 mL) was refluxed for 9 h under a nitrogen atmosphere. The mixture was cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. Purification on silica gel (12% MeOH/CH_2Cl_2) and recrystallization from hot EtOH afforded **18** (43.4 mg, 60.2%) as a white solid: UV (H_2O) λ_{max} 252.0 nm (ϵ 9600) (pH 7), 249.5 nm (ϵ 7400) (pH 2), 263.5 nm (ϵ 6600) (pH 11). Anal. ($C_{10}H_{10}FN_5O_3$) C, H, N.

9-(2,3-Dideoxy-2-fluoro-α-D-gycero-pent-2-enofuranosyl)guanine (19). Compound **19** was prepared from **10** on a 0.26 mmol scale following the same procedure used for its β-isomer **18**. Purification on silica gel using 12% MeOH/CH_2Cl_2 as eluent gave 52.1 mg (75%) of **19** as white solid: UV (H_2O) λ_{max} 252.0 nm (ϵ 9600) (pH 7), 249.5 nm (ϵ 7400) (pH 2), 263.5 nm (ϵ 6600) (pH 11). Anal. ($C_{10}H_{10}FN_5O_3 \cdot 0.3\ H_2O$) C, H, N.

2,6-Diamino-9-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)purine (20). A mixture of **11** (42 mg, 0.147 mmol) and saturated NH_3/MeOH (20 mL) was heated at 90−100 °C in a steel bomb for 36 h. After cooling to room temperature, the solvent was removed under vacuum, and the residue was purified by column chromatography using 10% MeOH/CH_2Cl_2 as eluent to give **20** (30.5 mg, 78.2%) as a white solid: UV (H_2O) λ_{max} 279.5 nm (ϵ 9300) (pH 7), 288.5 nm (ϵ 9700) (pH 2), 279 nm (ϵ 9700) (pH 11). Anal. ($C_{10}H_{11}FN_6O_2 \cdot 0.1CH_2Cl_2$) C, H, N.

2,6-Diamino-9-(2,3-dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)purine (21). Compound **21** was prepared from **13** on a 0.158 mmol scale following the same procedure used for its β-isomer **20**. Purification on silica gel using 10% MeOH/CH_2Cl_2 as eluent gave 33 mg (78.5%) of **21** as white solid: UV (H_2O) λ_{max} 279 nm (ϵ 8000) (pH 7), 290 nm (ϵ 11600) (pH 2), 279 nm (ϵ 11100) (pH 11). Anal. ($C_{10}H_{11}FN_6O_2 \cdot 0.4H_2O$) C, H, N.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2 enofuranosyl]-N^4-benzoylcytosine (22) and Its α-Isomer (23). Silylated N^4-benzoylcytosine [prepared from 1.56 g (7.24 mmol) of N^4-benzoylcytosine and 30 mL of HMDS], **2** (1.5 g, 5.17 mmol), and TMSOTf (1.4 mL, 1.4 mmol) in dry DCE (20 mL) were reacted for 1 h at room temperature under a nitrogen atmosphere. After extractive workup, isolation by silica gel column chromatography (30% EtOAc/hexanes) afforded β-anomer **22** (1.10 g, 47.8%) and α-anomer **23** (0.87 g, 38.1%) as white solids. **22**: ^{1}H NMR ($CDCl_3$) δ 0.12, 0.13 (2s, 2 × CH_3), 0.94, (s, *t*-Bu), 3.80−3.97 (m, H-5′), 4.94 (m, H-4′), 5.61 (s, H-3′), 7.12 (m, H-1′), 7.50−7.92 (m, H−Bz), 7.91 (d, J = 6.4 Hz, H-5), 8.41 (d, J = 5.6 Hz, H-6), 8.72 (s, NH); UV (MeOH) λ_{max} 302.0 nm. Anal. Calcd for $C_{22}H_{28}FN_3O_4Si$: C, 59.30; H, 6.33; N, 9.43. Found: C, 59.39; H, 6.37; N, 9.42. **23**: ^{1}H NMR ($CDCl_3$) δ 0.08, 0.09 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.70−3.82 (m, H-5′), 5.05 (m, H-4′), 5.75 (s, H-3′), 7.08 (m, H-1′), 7.51−7.90 (m, 7H, H-5, H 6, H−Bz); UV (MeOH) λ_{max} 302.0 nm. Anal. Calcd for $C_{22}H_{28}FN_3O_4Si$: C, 59.30; H, 6.33; N, 9.43. Found: C, 59.14; H, 6.19; N, 9.31.

5-Fluoro-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]-N^4-benzoylcytosine (24) and Its α-Isomer (25). Silylated N^4-benzoyl-5 fluorocytosine [prepared from 822.1 mg (3.52 mmol) of N^4-benzoyl-5-fluorocytosine and 20 mL of HMDS], **2** (787 mg, 2.71 mmol), and TMSOTf (0.5 mL, 2.73 mmol) in dry DCE (20 mL) were reacted for 1.5 h at room temperature under a nitrogen atmosphere. After extractive workup, isolation by silica gel column chromatography (12−25% EtOAc/hexanes) afforded β-anomer **24** (0.55 g, 44.4%) and α-anomer **25** (0.39 g, 31.5%) as white solids. **24**: ^{1}H NMR ($CDCl_3$) δ 0.145, 0.151 (2s, 2 × CH_3), 0.94, (s, *t*-Bu), 3.81−4.01 (m, H-5′), 4.92 (m, H-4′), 5.62 (s, H-3′), 7.93 (m, H-1′), 7.44−7.58 (m, 4H, H−Bz), 8.29 8.32 (m, 2H, H-6, H−Bz); UV ($CHCl_3$) λ_{max} 325.0 nm. Anal. Calcd for $C_{22}H_{27}F_2N_3O_4Si$: C, 57.00; H, 5.87; N, 9.06. Found: C, 56.95; H, 5.83; N, 8.96. **25**: ^{1}H NMR ($CDCl_3$) δ 0.08, 0.09 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.70−3.80 (m, H-5′), 5.30 (m, H-4′), 5.77 (s, H-3′), 6.92 (m, H-1′), 7.31 (d J = 7.8 Hz, H-5), 7.44−8.31 (m, H−Ph); UV ($CHCl_3$) λ_{max} 325.5 nm. Anal. Calcd for $C_{22}H_{27}F_2N_3O_4Si$: C, 57.00; H, 5.87; N, 9.06. Found C, 56.87; H, 5.86; N, 9.09.

5-Methyl-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl]-N^4-benzoylcytosine (26) and Its α-Isomer (27). Silylated N^4-benzoyl-5 methylcytosine [prepared from 806 mg (3.50 mmol) of N^4-benzoyl-5-methylcytosine and 25 mL of HMDS], **2** (780 mg, 2.69 mmol), and TMSOTf (0.68 mL, 3.50 mmol) in dry DCE (25 mL) were reacted for 5 h at room temperature under a nitrogen atmosphere. After extractive workup, isolation by silica gel column chromatography (12.5% EtOAc/cyclohexane) afforded β-anomer **26** (0.54 g, 43.2%) and α-anomer **27** (0.48 g, 38.6%) as white solids. **26**: ^{1}H NMR ($CDCl_3$) δ 0.11, 0.12 (2s, 2 × CH_3), 0.93, (s, *t*-Bu), 3.80−3.89 (m, H-5′), 4.87 (m, H-4′), 5.68 (s, H-3′), 6.94 (m, H-1′), 7.43−7.55 (m, 4H, H−Bz), 8.31 8.33 (m, 2H, H-6, H−Bz); UV ($CHCl_3$) λ_{max} 326.0 nm. Anal. Calcd for $C_{23}H_{30}FN_3O_4Si$: C, 60.11; H, 6.58; N, 9.14. Found: C, 59.92; H, 6.69; N, 8.94. **27**: ^{1}H NMR ($CDCl_3$) δ 0.08, 0.09 (2s, 2 × CH_3), 0.91, (s, *t*-Bu), 3.70−3.80 (m, H-5′), 5.03 (m, H-4′), 5.76 (m, H-3′), 7.11 (m, H-1′), 7.43−7.56 (m, 4H, H−Bz), 8.31−8.33 (m, 2H, H-6, H−Bz), 13.15 (s, NH); UV ($CHCl_3$) λ_{max} 326.5 nm. Anal. Calcd for $C_{23}H_{30}FN_3O_4Si$: C, 60.11; H, 6.58; N, 9.14. Found: C, 60.13; H, 6.53; N, 9.11.

1-(2,3-Dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (28). A solution of **22** (418,8 mg, 0.94 mmol) in dry CH_3CN (20 mL) was treated with TBAF (1 M solution in THF, 1.4 mL, 1.4 mmol) and stirred for 1 h at room temperature. After evaporation of the solvent, the resulting residue was purified by preparative TLC (2.5% MeOH/CH_2Cl_2) to give the N^4-benzoylcytosine intermediate (256.0 mg, 82.2%, Anal. Calcd for $C_{16}H_{14}FN_3O_4$: C, 58.01; H, 4.26; N, 12.68. Found: C, 57.88; H, 4.27; N, 12.99) as a white solid, which was treated with saturated methanolic ammonia solution (20 mL). The reaction mixture was allowed to stir at room temperature until TLC showed the disappearance of the starting material (8 h). The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (10% MeOH/CH_2Cl_2) to give **28** (144.6 mg, 83%) as a solid, which was crystalized from hexanes−MeOH−CH_2Cl_2: UV (H_2O) λ_{max} 266.0 nm (ϵ 7500) (pH 7), 276.0 nm (ϵ 11100) (pH 2), 266.5 nm (ϵ 9500) (pH 11). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

1-(2,3-Dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)cytosine (29). Compound **29** was prepared from **23** on a 0.87 mmol scale in two steps by the method described for compound **28**. Flash column chromatography (silica gel, 12% MeOH/CH_2Cl_2) gave 119.8 mg (60.2% from compound **23**) of **29** as a white solid: UV (H_2O) λ_{max} 266.0 nm (ϵ 7500) (pH 7), 276.0 nm (ϵ 12600) (pH 2), 267.0 nm (ϵ 8900) (pH 11). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

5-Fluoro-1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (30). Compound **30** was prepared from **24** on a 0.50 mmol scale in two steps by the method described for compound **28**. Flash column chromatography (silica gel, 10% MeOH/CH_2Cl_2) and subsequent recrystallization (hexanes−MeOH−CH_2Cl_2) gave 71.1 mg (58.6% from compound **24**) of **30** as a white solid: UV (H_2O) λ_{max} 277.5 nm (ϵ 9800) (pH 7), 282.0 nm (ϵ 12600) (pH 2), 277.0 nm (ϵ 8900) (pH 11). Anal. ($C_9H_9F_2N_3O_3$) C, H, N.

5-Fluoro-1-(2,3-dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)cytosine (31). Compound **31** was prepared from **25** on a 0.64 mmol scale in two steps by the method described for compound **28**. Flash chromatography (silica gel, 11% MeOH/CH_2Cl_2) and subsequent recrystallization (MeOH−CH_2Cl_2-hexanes) gave 93.7 mg (59.7% from compound **25**) of **31** as a white solid: UV (H_2O) λ_{max} 277.0 nm (ϵ 8800) (pH 7), 282.5 nm (ϵ 11400) (pH 2), 276.0 nm (ϵ 7800) (pH 11). Anal. ($C_9H_9F_2N_3O_3$) C, H, N.

5-Methyl-1-(2,3-dideoxy-2-fluoro-β-D-*glycero*-pent-2-enofuranosyl)cytosine (32). Compound **32** was prepared from **26** on a 0.47 mmol scale in two steps by the method described for compound **28**. Flash chromatography (silica gel, 10% MeOH/CH_2Cl_2) and subsequent trituration with EtOAc gave 69.5 mg (61.3% from compound **26**) of **32** as a white solid: UV (H_2O) λ_{max} 274.5 nm (ϵ 7000) (pH 7), 282.5 nm (ϵ 9600) (pH 2), 274.5 nm (ϵ 6000) (pH 11). Anal. ($C_{10}H_{12}FN_3O_3$) C, H, N.

5-Methyl-1-(2,3-dideoxy-2-fluoro-α-D-*glycero*-pent-2-enofuranosyl)cytosine (33). Compound **33** was prepared from **27** on a 0.40 mmol scale in two steps by the method described for compound **28**. Flash chromatography (silica gel, 10% MeOH/CH_2Cl_2) and subsequent trituration with EtOAc gave 61.3 mg (63.5% from compound 27) of **33** as a white

solid: UV (H_2O) λ_{max} 274.0 nm (ϵ 6900) (pH 7), 283.0 nm (ϵ 11300) (pH 2), 274.5 nm (ϵ 6800) (pH 11). Anal. ($C_{10}H_{12}FN_3O_3$) C, H, N.

Adenosine Deaminase Assay.[24a] Assays were performed at 25 °C in phosphate buffer solution (pH 7.4) with substrate concentrations in the range of 15−200 μM and with 0.15−0.24 unit of adenosine deaminase (EC 3.5.4.4. from calf intestinal mucosa) depending on the substrate. The assays were monitored with a UV spectrometer at 265 nm, and every reaction medium was checked by TLC or UV spectrometry to ensure that the sole reaction product was the deaminated substrate. Initially, the qualitative assays were performed with D-β-2′F-d4A **14** and its l-enantiomer[29] (200 μM) in the presence of 0.24 unit of adenosine deaminase for 120 min to determine whether they were substrates of this enzyme. L-β-2′F-d4A was further observed for 3 days by monitoring on TLC in the same conditions. From the Michaelis−Menten equation, V_{max} (maximal velocity) and K_M (the Michaelis constant) were obtained for compound **14** and adenosine (15 and 159 μM) with 0.15 unit of the enzyme. The $t_{1/2}$ values of D-β-2′F-d4A **14** and adenosine were also measured at 0.15 μM with 0.15 unit of the enzyme.

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References

(1) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Phil, D.; Lusinoff-Lehrman, S. The AZT collaborative working group. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.* **1987**, *317*, 192−197.

(2) Strarnes, M. C.; Cheng, Y.-C. Cellular metabolism of 2′,3′-dideoxycytidine, a compound active against human immunodeficiency virus in vitro. *J. Biol. Chem.* **1987**, *262*, 988−991.

(3) Lambert, J. S.; Seidlin, M.; Reichman, R. C.; Plank, C. S.; Laverty, M.; Morse, G. D.; Knupp, C.; McLaren, C.; Pettineli, C.; Valentine, F. T.; Dolin, R. 2′,3′-Dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex. *N. Engl. J. Med.* **1990**, *322*, 1333−1340.

(4) Mansuri, M. M.; Hitchcock, M. J. M.; Starrett, J. E.; Sterzycki, R. Z.; Martin, J. C. Comparison of in vitro biological properties and mouse toxicities of three thymidine analogues active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **1990**, *34*, 637−641.

(5) Larder, B. A.; Darby, G.; Richman, D. D. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **1989**, *243*, 452−454.

(6) St Clair, M. H.; Martin, J. L.; Tudor-Williams, G.; Bach, M. C.; Vavro, C. L.; King, D. M.; Kelham, P.; Kemp, S. D.; Larder, B. A. Resistant to ddI and selectivity to AZT-induced by a mutation in HIV-1 reverse transctiptase. *Science* **1991**, *253*, 1557−1559.

(7) Richman, D.; Shih, C. K.; Lowy, I.; Rose, J.; Prodanovich, P.; Goff, S.; Griffin, J. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11241−11245.

(8) Gulick, R. M.; Mellers, J. W.; Havlir, D.; Eron, J. J.; Gonzalez, C.; McMahon, D.; Richman, D. D.; Valentine, F. T.; Jonas, L.; Emini, E. A.; Chodakewitz, J. A. Treatment, with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiviral therapy. *N. Engl. J. Med.* **1997**, *337*, 734−739.

(9) De Clercq, E. In Search of a selective antiviral chemotherapy. *Clin. Microbiol. Rev.* **1997**, *10*, 674−693.

(10) Yarchoan, R.; Perno, C. F.; Thomas, R. V.; Klecker, R. W.; Allain, R. P.; Wills, R. J.; McAtee, N.; Fishl, M. A.; Dubinsky, R.; McNeely, M. C.; Mitsuya, H.; Pluda, J. M.; Lawley, T. J.; Leuther, M.; Safai, B.; Collins, J. M.; Myers, C. E.; Broder, S. Phase I studies of 2′,3′ dideoxycytidine in human immunodeficiency virus infection as a single agent and alternating with zidovudine (AZT). *Lancet* **1988**, *1*, 76−81.

(11) Balzarini, J.; Cooney, D. A.; Dalad, M.; Kang, G.-J.; Cupp, J. E.; De Clerq, E.; Broder, S.; Johns, D. G. 2′,3′-Dideoxycytidine; regulation of its metabolism and antiretroviral potency by natural pyrimidine nucleosides inhibitors of pyrimidine nucleoside synthesis. *Pharmacology* **1987**, *32*, 798−806.

(12) Dahlberg, J. E.; Mitsuya, H.; Blam, S. B.; Broder, S.; Aaronson, S. A. Broad spectrum antiviral activity of 2′,3′-dideoxynucleosides. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2469−2473.

(13) Mitsuya, H.; Broder, S. Inhibition of the in vitro infectivity and cytopathic effect of human T lymphotrophic virus type III/lymphadenopathy associated virus in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *82*, 1911−1915.

(14) Yarchoan, R.; Mitsuya, H.; Thomas, R. V.; Pluda, J. M.; Hartman, N. R.; Perno, C. F.; Marczyk, K. S.; Allain, J. P.; Johns, D. G.; Broder, S. In vivo activity against HIV and favorable toxicity profile of 2′,3′-dideoxyinosine. *Science* **1989**, *245*, 412−415.

(15) Yarchoan, R.; Mitsuya, H.; Broder, S. Clinical and basic advances in the retroviral therapy of human immunodeficiency virus infection. *Am. J. Med.* **1989**, *87*, 191−200.

(16) Faulds, D.; Brogden, R. N. Didanosine: A review of its antiviral activity, pharmacokinetic properties and therapeutic potential in human immunodeficiency virus infection. *Drugs* **1992**, *44* (1), 94−116.

(17) Hamamoto, Y.; Yamamoto, N.; Matsui, T.; Matsuda, A.; Ueda, T.; Yamamoto, N. Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects and replication of human immunodeficiency virus. *Antimicrob. Agents Chemother.* **1987**, *31*, 907−910.

(18) Lin, T.-S.; Schinazi, R. F.; Prusoff, W. H. Potent and selective in vitro activity of 3′ deoxyadenosine, deoxythymidine-2′-ene (3′-deoxy-2′,3′-didehydrothymidine) against human immunodeficiency virus in vitro. *Biochem. Pharmacol.* **1987**, *36*, 2713−2718.

(19) Daluge, S. M.; Good, S. S.; Faletto, M. B.; Miller, W. H.; St. Clair, M. H.; Boone, L. R.; Tisdale, M.; Parry, N. R.; Reardon, J. E.; Dornsife, R. E.; Averet, D. R.; Krenitsky, T. A. 1592U89, A novel carbocyclic nucleoside analogue with potent, selective anti-human immunodeficiency virus activity. *Antimicrob. Agents Chemother.* **1997**, *41*, 1082−1093.

(20) Tisdale, M.; Alnadaf, T.; Cousens, D. Combination of mutations in human immunodeficiency virus type 1 reverse transcriptase required for resistance to the carbocyclic nucleoside 1592U89. *Antimicrob. Agents Chemother.* **1997**, *41*, 1094−1098.

(21) (a) Gosselin, G.; Schinazi, R. F.; Sommadossi, J.-P.; Mathe, C.; Bergogone, M. C.; Aubertin, A.-M.; Kirn, A.; Imbach, J.-L. Anti-human immunodeficiency viruses activities of the β-L-enantiomer of 2′,3′-dideoxycytidine and its 5-fluoro derivative in vitro. *Antimicrob. Agents Chemother.* **1994**, *38*, 1292−1297. (b) Lin, T. S.; Luo, M. Z.; Liu, M. C.; Zhu, Y. L.; Gullen, E.; Dutchman, G. E.; Cheng, Y.-C. Design and synthesis of 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L−d4C) and of 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C), two exceptionally potent inhibitors of hepatitis B virus (HBV) and potent inhibitors of human immunodeficiency virus (HIV) in vitro. *J. Med. Chem.* **1996**, *39*, 1757−1759.

(22) Watanabe, K. A.; Su, T.-L.; Lein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J. Nucleosides. 123. Synthesis of antiviral nucleosides: 5-Substituted-1-(2-deoxy-2 halogeno-β-D-arabinofuranosyl)cytosines and -uracils. Some structure−activity relationships. *J. Med. Chem.* **1983**, *26*, 152−156.

(23) (a) Chu, C. K.; Ma, T. W.; Shanmuganathan, K.; Wang, C. G.; Xiang, Y. J.; Pai, S. B.; Yao, G. Q.; Sommadossi, J.-P.; Cheng, Y.-C. Use of 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil as a novel antiviral agents for hepatitis B virus and epstein barr virus. *Antimicrob. Agents Chemother.* **1995**, *39*, 979−981. (b) Ma, T. W.; Pai, S. B.; Zhu, Y. L.; Lin, J. S.; Shamuganathan, K.; Du, J. F.; Wang, C.; Kim, H. B.; Newton, M. G.; Cheng, Y.-C.; Chu, C. K. Structure−activity relationships of 1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)pyrimidine nucleosides as anti-Hepatitis B virus agents. *J. Med. Chem.* **1996**, *39*, 2835−2843.

(24) (a) Marquez, V. E.; Tseng, C. K.-H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. Acid-stable 2′-fluoro purine dideoxynucleosides as active agents against HIV. *J. Med. Chem.* **1990**, *33*, 978−985. (b) Ruxrungtham, K.; Boone, E.; Ford, H., Jr.; Driscoll, J. S.; Davey, R. T.; Lane, H. C. Potent activity of 2′-β-fluoro-2′,3′ dideoxyadenosine against human immunodeficiency virus type 1 infection in hu PBM-SCID mice. *Antimicrob. Agents Chemother.* **1996**, *40*, 2369−2374.

(25) Sterzycki, R. Z.; Ghazzouli, I.; Brankovan, V.; Martin, J. C.; Mansuri, M. M. Synthesis and anti HIV activity of several 2′-fluoro-containing pyrimidine nucleosides. *J. Med. Chem.* **1990**, *33*, 2150−2157.

(26) Martin, J. A.; Bushnell, D. J.; Duncan, I. B.; Dunsdon, S. J.; Hall, M. J.; Machin, P. J.; Merret, J. H.; Parkes, K. E. B.; Roberts, N. A.; Thomas, G. J.; Galpin, S. A.; Kinchington, D. Synthesis and antiviral activity of monofluoro and difluoro analogs of pyrimidine deoxyribonucleosides against human immunodeficiency virus (HIV-1). *J. Med. Chem.* **1990**, *33*, 2137−2145.

(27) Koshida, R.; Cox, S.; Harmenberg, J.; Gilljam, G.; Wahren, B. Structure−activity relationships of fluorinated nucleoside analogs and their synergestic effect in combination with phosphonoformate against human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1989**, *33*, 2083−2088.
(28) Lee, K.; Choi, Y.; Gullen, E.; Schlueter-Wurtz, S.; Schinazi, R.; Cheng, Y.-C.; Chu, C. K. Synthesis and anti-HIV and anti-HBV activities of 2′-fluoro-2′,3′-unsaturated L-nucleosides. *J. Med. Chem.* **1999**, *42*, 1320−1328.
(29) Wilson, L. J.; Hager, M. W.; El-Kattan, Y. A.; Liotta, D. C. Nitrogen glycosylation reactions involving pyrimidine and purine nucleoside bases with furanoside sugars. *Synthesis* **1995**, 1465−1479.
(30) Sujino, K.; Yoshida, T.; Sugimura, H. Facile synthesis of 2′,3′-unsaturated nucleosides from 2-deoxyribose. *Tetrahedron Lett.* **1996**, *37*, 6133−6136.
(31) Morikawa, T.; Sasaki, H.; Mori, K.; Shiro, M.; Taguchi, T. Simmons-Smith reactions of fluoroallyl alcohol derivatives. *Chem. Pharm. Bull.* **1992**, *40*, 3189−3193.
(32) Häfele, B.; Jäger, V. A short synthesis of (*S*)-5-hydroxy-2-penten-4-olide from D-mannitol via 2,3-O-isopropylidene-D-glyceraldehyde. *Liebigs Ann. Chem.* **1987**, 85−87.
(33) Choi, Y.; Lee, K.; Hong, J. H.; Schinazi, R. F.; Chu, C. K. Synthesis and anti-HIV activity of L-2′-fluoro-2′,3′-unsaturated purine nucleosides. *Tetrahedron Lett.* **1998**, *39*, 4437−4440.
(34) Pélicano, H.; Pierra, C.; Eriksson, S.; Gosselin, G.; Imbach, J.-L.; Maury, G. Enzymatic properties of the unnatural β-L-enantiomers of 2′,3′-dideoxyadenosine and 2′,3′-didehydro-2′,3′-dideoxyadenosine. *J. Med. Chem.* **1997**, *40*, 3969−3973.
(35) (a) Chu, C. K.; Ma, L.; Olgen, S.; Pierra, C.; Du, J.; Gumina, G.; Gullen, E.; Cheng, Y.-C.; Schinazi, R. F. Synthesis and antiviral activity of oxaselenolane nucleosides. *J. Med. Chem.* **2000**, *43*, 3906−3912.
(36) Schinazi, R. F.; Lloyd, R. M., Jr.; Nguyen, M. H.; Cannon, D. L.; McMillan, A.; Ilksoy, N.; Chu, C. K.; Liotta, D. C.; Bazmi, H. Z.; Mellors, J. W. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine Nucleosides. *Antimicrob. Agents Chemother.* **1993**, *37*, 875−881.
(37) Boucher, C. A. B.; Gu, Z.; Cammack, N.; Schipper, P., Gao, Q.; Rouse, P.; Schurrman, R.; Cameronm J. M.; Wainberg, M. A. High level resistance in vitro to the nucleoside analogue 2′ deoxy-3′-thiacytidine (3TC) is due to one amino acid change substitution at codon 184 located within the catalytic site of HIV-1 reverse transcriptase. *IXth International Conference on AIDS*, Berlin, 1993, I, *242*, Abstract PO-A26-0645.
(38) Tisdale, M.; Kemp, S. D.; Parry, N. R.; Larder, B. A. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3′-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5653−5656.
(39) Wainberg, M. A.; Hsu, M.; Gu, Z.; Borkow, G.; Parniak, M. A. Effectiveness of 3TC in HIV clinical trials may be due in part to the M184 substitution in 3TC-resistant HIV-1 reverse transcriptase. *AIDS* **1996**, *10*, S3−S10 (Suppl 5).
(40) Huang, H.; Chopra, R.; Verdine, G.; Harrison, S. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase; implications for drug resistance. *Science* **1998**, *282*, 1669−1675.
(41) Balzarini, J.; Kang, G.-J.; Dalal, M.; Herdewijn, P.; De Clerq, E.; Broder, S.; Johns, D. G. The anti-HTLV−III (anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides: a comparison with their parental 2′,3′-dideoxyribonucleosides. *Mol. Pharm.* **1987**, *32*, 162−167.

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