Synthesis and Biological Evaluation of 2',3'-Dideoxy-L-pyrimidine Nucleosides as Potential Antiviral Agents against Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV)

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Various 2',3'-dideoxy-L-cytidine, 2',3'-dideoxy-L-uridine, and 3'-deoxy-L-thymidine analogues have been synthesized and evaluated in vitro as potential anti-HIV and anti-HBV agents. Coupling of 1-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose (1) with silylated derivatives of 5-fluorocytosine, cytosine, 5-fluorouracil, uracil, and thymine in the presence of ethylaluminum dichloride gave the corresponding nucleosides **2**, **3**, **4**, **5**, **10**, **11**, **12**, **16**, **17**, and **18** as a mixture of α- and β-anomers, which were then deblocked to yield the corresponding 2',3'-dideoxy-L-5-fluorocytidine derivatives, **6** and **7**, 2',3'-dideoxy-L-cytidine derivatives, **8** and **9**, 2',3'-dideoxy-β-L-fluorouridine (**13**), 2',3'-dideoxy-β-L-uridine (**14**), and 3'-deoxy-L-thymidine derivatives, **15** and **19**. Among these 2',3'-dideoxy-L-nucleoside analogues, 2',3'-dideoxy-β-L-5-fluorocytidine (**6**, β-L-FddC) was found to be the most active against HIV-1, which is approximately 3 and 4 times more active against HIV-1 in vitro than 2',3'-dideoxy-β-D-cytidine (ddC) and 2',3'-dideoxy-β-D-5-fluorocytidine (β-D-FddC) with ED_{50} values of 0.5, 1.5, and 2 μM, respectively. The dose-limiting toxicity of ddC is severe neuropathy which may be caused by the inhibition of the synthesis of mitochondrial DNA. ddC has an IC_{50} value of 0.022 μM against host mitochondrial DNA synthesis. Conversely, the IC_{50} values for β-L-FddC and β-L-ddC are >100 μM; therefore, neuropathy may not present itself to be a problem with β-L-FddC and β-L-ddC as chemotherapeutic agents. In addition, β-L-FddC and 2',3'-dideoxy-β-L-cytidine (**8**, β-L-ddC) demonstrated equally potent activity against HBV in vitro by having the same ED_{50} value of 0.01 μM. Both β-L-FddC and β-L-ddC, which have an "unnatural" L-configuration in the sugar moiety, are ~1000 and 280 times more potent, respectively, against HBV than the D-configuration β-D-FddC and ddC which have an ED_{50} values of 10 and 2.8 μM. In view of the potent antiviral activity of β-L-FddC against both HIV-1 and HBV and potent antiviral activity of β-L-ddC against HBV in vitro, their low cytotoxicity, and especially the negligible inhibitory effect on host mitochondrial DNA synthesis, β-L-FddC and β-L-ddC merit further development as potential anti-HIV and anti-HBV agents.

Since the identification of human immunodeficiency virus (HIV) as the etiological agent of acquired immunodeficiency syndrome (AIDS), considerable effort has been directed on the design and synthesis of compounds that would inhibit the replication of this and related viruses. Inhibition of the enzyme reverse transcriptase (RT), a key enzyme encoded by HIV and involved in its replication, has been widely studied. Among the HIV-RT inhibitors, 3'-azido-3'-deoxythymidine (Zidovudine, AZT),[1-3] 2',3'-dideoxycytidine (ddC),[4-6] and 2',3'-dideoxyinosine (ddI)[5,7,8] have been used clinically for the treatment of AIDS patients. It is of interest that the life cycle of hepatitis B virus (HBV), a causative agent of both an acute and chronic form of hepatitis which affects 300 million people worldwide,[9] also involves the enzyme reverse transcriptase in its DNA replication. In fact, at the present time there is no clinically useful drug for the treatment of HBV infection. Recently, ddC has been shown to be a potent inhibitor of the replication of both human immunodeficiency virus[10] and human hepatitis B virus[11,12] in vitro. However, long-term ddC usage causes delayed toxicity such as peripheral neuropathy in patients, which was suggested to be as a result of the depletion of mitochondrial DNA (mt DNA) in cells treated with ddC.[13]

L-Nucleosides, the enantiomers of natural D-nucleosides, were thought not to be recognized by normal cellular enzymes and, therefore, are not or very poorly metabolized in mice.[14] However, it was found that they interacted with bacterial polynucleotide phosphorylase and nucleolytic enzymes.[15,16] Recently, Spadari et al.[17] reported that L-thymidine is not recognized by human thymidine kinase but functions as specific substrates for the herpes simplex virus type 1 (HSV-1) viral enzyme and reduces HSV-1 multiplication in HeLa cells. Mansuri et al.[18] also reported that 2',3'-dideoxy-β-L-cytidine (β-L-ddC) showed activity against HIV in CEM cells. In addition, Belleau et al.[19] and Soudeyns et al.[20] reported the synthesis and anti-HIV-1 activity of 2',3'-dideoxy-3'-thiacytidine [BCH-189, (±)SddC], a new nucleoside analogue in which the 3'-carbon in the sugar moiety is substituted by sulfur. Doong et al.[21] first described that the racemic (±)SddC (BCH-189) demonstrated potent antiviral activity against HBV. Subsequently, Beach et al.[22] and Hoong et al.[23] reported the synthesis of (−)-2',3'-dideoxy-3'-thiacytidine [(−)SddC or (−)-3TC] and (−)-5-fluoro-2',3'-dideoxy-3'-thiacytidine [(−)FSddC or (−)-FTC] and their related enantiomers, respectively. Both (−)SddC [(−)-3TC] and (−)FSddC[(−)-FTC] were reported to show more potent antiviral activity against HIV and HBV and less cytotoxicity than their D-configuration counterparts.[24-27]

On the basis of these findings, various 2',3'-dideoxy-L-uridine, 3'-deoxy-L-thymidine, and 2',3'-dideoxy-L-cytidine analogues were synthesized and evaluated as potential anti-

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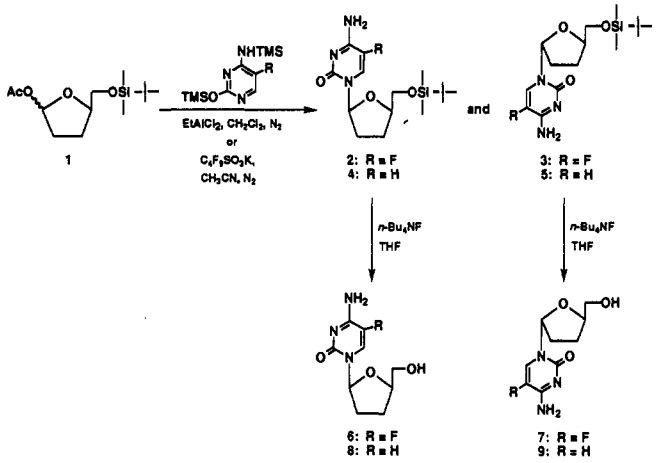
Scheme 1



HIV and anti-HBV agents. Among these compounds, 2',3'-dideoxy-β-L-5-fluorocytidine (β-L-FddC) showed more potent antiviral activity against HIV-1 and much more potent activity against HBV, and 2',3'-dideoxy-β-L-cytidine (β-L-ddC) showed much more potent antiviral activity against HBV than 2',3'-dideoxy-β-D-cytidine (ddC) with negligible inhibition to the host mitochondrial DNA synthesis. This report describes the synthesis and antiviral activity, especially the anti-HIV-1 and anti-HBV activities, of these compounds.

Chemistry

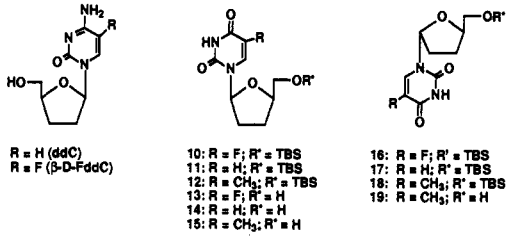
2',3'-Dideoxy-β-L-5-fluorocytidine (**6**, β-L-FddC) was synthesized by coupling of the silylated 5-fluorocytosine with 1-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose (**1**), which was prepared by the methodology of Okabe et al.[28] for the synthesis of the D-configuration counterpart with minor modifications, in the presence of either ethylaluminum dichloride ($EtAlCl_2$) or potassium nonafluoro-1-butanesulfonate ($C_4F_9SO_3K$) as the catalyst (Scheme 1). The ratio of the β-anomer (**2**) to α-anomer (**3**) varies depending on the catalyst used. When $EtAlCl_2$ was used as the catalyst, the total yield of **2** (β-anomer) and **3** (α-anomer) was 53% with a ratio of 3:2 in favor of the β-anomer, estimated by ^1H NMR spectra for the integration values of 6-H at δ 8.26 and 7.38 ppm, respectively, for both β- and α-anomers. However, with $C_4F_9SO_3K$ as the catalyst, the total yield of **2** (β-anomer) and **3** (α-anomer) was 60% with a ratio of 2:3 in favor of the α-anomer. Compounds **2** (β-anomer) and **3** (α-anomer) were separated by silica gel chromatography. Treatment[29] of compounds **2** and **3** with tetra-*n*-butylammonium fluoride in THF to afford the target compound **6** and the corresponding α-anomer, compound **7**. 2',3'-Dideoxy-β-L-cytidine (**8**, β-L-ddC) and 2',3'-dideoxy-α-L-cytidine (**9**, α-L-ddC) were synthesized by the same methodology as described for the synthesis of compounds **6** and **7**. Compounds **8** (β-L-ddC) and **9** (α-L-ddC) were also independently synthesized by Mansuri et al.;[18] however, no physical properties, spectroscopic data, and experimental details were reported.

Coupling of the siylated derivatives of 5-fluorouracil, uracil, and thymine with the acetate **1** in the presence of ethylaluminum dichloride ($EtAlCl_2$) gave the corresponding 5'-protected β-anomers **10–12** and the α-anomers **16–18**. Treatment[29] of compounds **10–12** and **18** with tetra-*n*-butylammonium fluoride in tetrahydrofuran (THF) afforded 2',3'-dideoxy-β-L-5-fluorouridine (**13**, β-L-FddU), 2',3'-dideoxy-β-L-uridine (**14**, β-L-ddU), 3'-deoxy-β-L-thymidine (**15**, β-L-dT), and 3'-deoxy-α-L-thymidine (**19**, α-L-dT), respectively.

Table 1. Proton NMR Chemical Shifts δ (ppm)

compound (abbr)	H-4' (anti)[a]	H-5' (syn)[a]
6 (β-L-FddC)[c]	4.01 (syn)	3.63 (anti)
7 (α-L-FddC)[c]	4.40 (anti)	3.35 (syn)
8 (β-L-ddC)[c]	3.99 (syn)	3.59 (anti)
9 (α-L-ddC)[c]	4.37 (anti)	3.38 (syn)
15 (β-L-dT)[c]	3.99 (syn)	3.60 (anti)
19 (α-L-dT)[c]	4.37 (anti)	3.38 (syn)
2 (TBS-β-L-FddC)[b]	4.18 (syn)	3.70 (H_A), 4.05 (H_B) (anti)
3 (TBS-α-L-FddC)[b]	4.40 (anti)	3.65 (syn)
10 (TBS-β-L-FddU)[b]	4.15 (syn)	3.70 (H_A), 4.05 (H_B) (anti)
16 (TBS-α-L-FddU)[b]	4.40 (anti)	3.58 (syn)
11 (TBS-β-L-ddU)[b]	4.10 (syn)	3.70 (H_A), 4.07 (H_B) (anti)
17 (TBS-α-L-ddU)[b]	4.43 (anti)	3.63 (syn)

[a] Stereochemistry relative to the base. [b] Spectra were recorded in $CDCl_3$; [c] in Me_2SO-d_6.



The assignment of the anomeric configuration of these nucleosides was made on the basis of characteristics proton NMR spectra. The H-4' protons of the α-anomers appear at a lower field than those of the β-anomers. Conversely, the H-5' protons of the α-anomers appear at a higher field than those of the β-anomers (Table 1). These shifts are attributed to the observation that protons at a syn-position relative to the base are more deshielded than those in anti-position to the base, which is consistent with the report by Okabe et al.[28] for the similar pyrimidine nucleosides. Furthermore, inspection of Table 1 reveals that the chemical shift values of the two H-5' protons in the molecules of 5'-*O*-(*tert*-butyldimethylsilyl)-substituted β-anomers **2**, **10**, and **11** are different with multiplet peaks each at 3.70 (5'-H_A) and 4.05 (5'-H_B) ppm; 3.70 (5'-H_A) and 4.05 (5'-H_B) ppm; and 3.70 (5'-H_A) and 4.07 (5'-H_B) ppm, respectively. However, the two protons of H-5' for the corresponding α-anomers **3**, **16**, and **17** show a doublet at 3.65, 3.58, and 3.63, respectively. The difference might be due to the asymmetric features of H-5' protons in β-anomers **2**, **10**, and **11**, which are located between the bulky groups of 5'-*O*-(*tert*-butyldimethylsilyl) and the base.

Since β-L-FddC (**6**) demonstrated potent antiviral activity against both HIV-1 and HBV, its D-configuration counterpart, 2',3'-dideoxy-β-D-5-fluorocytidine (β-D-FddC), was also synthesized for comparison by the previously reported methodology[30] with minor modifications.

Biological Evaluation

The synthesized compounds were tested for their antiviral activities in vitro, and the findings are shown in Table 2. Among the 2',3'-dideoxy-L-nucleoside analogues, 2',3'-dideoxy-β-L-5-fluorocytidine (**6**, β-L-FddC) was found to be most active against HIV-1. Compounds demonstrating significant anti-HIV-1 activity in terms of ED_{50} (μM) values with decreasing activity were β-L-FddC (0.5); ddC (1.5); β-D-FddC (2); β-L-ddC (5); α-L-FddC (6); α-L-ddC (>100); β-L-ddU (>100); β-L-FddU (>100); β-L-dT

Table 2. Evaluation of 2',3'-Dideoxy-L-pyrimidine Nucleosides Antiviral Activity against Human Immunodeficiency Virus (HIV-1) and Hepatitis B Virus (HBV), Effects on Mitochondrial DNA Synthesis, and Cycotoxicity against CEM Cells in Vitro

	ED_{50}[a] (μM)		IC_{50}[b] (μM)	
compound (abbr)	HIV-1[c]	HBV	mt DNA	CEM
ddC	1.5	2.8	0.022	28
β-D-FddC	2	10	ND	2
6 (β-L-FddC)	0.5	0.01	>100[d]	67
7 (α-L-FddC)	6	0.5	ND	>100[d]
8 (β-L-ddC)	5	0.01	>100[d]	70
9 (α-L-ddC)	>100[d]	5	ND	ND
13 (β-L-FddU)	>100[d]	>10[e]	ND[f]	>100[d]
14 (β-L-ddU)	>100[d,g]	>100[d]	ND	>100[d]
15 (β-L-dT)	>100[d]	>10[e]	ND	ND
19 (α-L-dT)	>100[d]	>10[e]	ND	ND

[a] ED_{50} values represent the drug concentration (μM) required to inhibit 50% of viral replication. [b] IC_{50} values represent the drug concentration (μM) required to inhibit 50% of the synthesis of the mitochondrial DNA (mt DNA) and the CEM cell growth. [c] The HIV-1 assays were performed using a viral multiplicity of 0.1 $TCID_{50}$/cell. [d] Compounds were tested up to 100 μM and found not to be active. [e] Compounds were tested up to 10 μM and found not to be active. [f] ND: not determined. [g] The HIV-1 assay for this compound was performed using a viral multiplicity of 0.01 $TCID_{50}$/cell.

(>100); and α-L-dT (>100). In addition, the compounds exhibiting activity against HBV in terms of ED_{50} values (μM) and decreasing antiviral activity were β-L-FddC and β-L-ddC (0.01); α-L-FddC (0.5); ddC (2.8); α-L-ddC (5); β-D-FddC (10); β-L-FddU (>10); β-L-dT (>10); α-L-dT (>10); and β-L-ddU (>100).

In comparison to 2',3'-dideoxy-β-D-cytidine (ddC), which is currently a clinically used drug for the treatment of AIDS, and to 2',3'-dideoxy-β-D-5-fluorocytidine (β-D-FddC), β-L-FddC is approximately 3 and 4 times more active against HIV-1 in vitro than ddC and β-D-FddC with ED_{50} values of 0.5, 1.5, and 2 μM, respectively. α-L-FddC also demonstrated significant anti-HIV-1 activity with an ED_{50} value of 6 μM. However, β-L-FddC (ED_{50}, 0.5 μM) is ~12 times more potent than α-L-FddC against HIV-1. Conversely, α-L-ddC was not active against HIV-1 at 100 μM. Although β-L-FddC is ~10 times more active against HIV-1 than β-L-ddC which has an ED_{50} value of 5 μM, β-L-FddC and β-L-ddC demonstrated equally potent activity against HBV in vitro with the same ED_{50} value of 0.01 μM. α-L-FddC was found to be ~10 times more active than α-L-ddC against HBV with respective ED_{50} values of 0.5 and 5 μM. Thus, β-L-FddC is ~50 times more active than α-L-FddC and β-L-ddC is ~500 times more active than α-L-ddC against HBV. Both β-L-FddC and β-L-ddC, which have an "unnatural" L-configuration in the sugar moiety, are ~1000 and 280 times more potent against HBV than β-D-FddC and ddC which have the respective ED_{50} values of 10 and 2.8 μM and a D-configuration. It is noteworthy that β-L-FddC is more potent than ddC against both HIV and HBV; however, β-L-ddC is less active against HIV-1 and more active against HBV than ddC. In addition, α-L-FddC is active against HIV-1 with an ED_{50} value of 6 μM. On the contrary, α-L-ddC showed no activity against HIV-1 at 100 μM. Replacement of the hydrogen with fluorine at the 5-position in the base of β-L-ddC and α-L-ddC produced β-L-FddC and α-L-FddC which are more potent against HIV-1 than β-L-ddC and α-L-ddC, respectively. Furthermore, these 2',3'-dideoxy nucleoside analogues such as β-L-FddC and β-L-ddC, which contain the dideoxyribofuranosyl moiety with the L-configuration as opposed to the naturally occurring D-configuration of nucleosides (i.e. 2'-deoxycytidine and thymidine), exhibited unexpected potent activities against HIV-1 and HBV.

The dose-limiting toxicity of ddC is severe neuropathy which may be caused by the inhibition of the synthesis of mitochondrial DNA. ddC has an IC_{50} value of 0.022 μM against mitochondrial host DNA synthesis. Conversely, the IC_{50} values for β-L-FddC and β-L-ddC are >100 μM; therefore, neuropathy may not present itself to be a problem with β-L-FddC and β-L-ddC as chemotherapeutic agents.

The compounds were tested in vitro for their cytotoxicity against L-1210, P388, S-180, and CCRF-CEM (CEM) cell lines. Only β-L-FddU showed some activity with IC_{50} values of 10, 10, and 60 μM against the corresponding L-1210, P388, and S-180 cell lines. All of the other compounds exhibited no significant cytotoxicity against L-1210, P388, and S-180 cell lines at a concentration up to 100 μM. However, ddC, β-L-ddC, and β-L-FddC exhibited only moderate cytotoxicity when tested against CEM cells with ID_{50} values of 28, 70, and 67 μM, respectively. Conversely, β-D-FddC was found to be ~34 times more cytotoxic to CEM cells than its L-configuration counterpart β-L-FddC, with an IC_{50} value of 2 μM.

None of the uracil L-nucleoside analogues showed any significant antiviral or anticancer activities in vitro.

These compounds also were tested against HSV-1 and HSV-2 in vitro and were found not to be active up to a concentration of 100 μM.

Although both (−)-2'-deoxy-3'-thiacytidine [(−)SddC or (−)-3TC] and (−)-5-fluoro-2'-deoxy-3'-thiacytidine [(−)-FSddC or (−)-FTC] showed potent antiviral activities against HIV and HBV, recently Boucher et al.[31] reported a high level of resistance in vitro to (−)SddC [(−)-3TC] due to one amino acid substitution at codon 184 location within the catalytic site of HIV-1 reverse transcriptase. In addition, Schinazi et al.[32] and Tisdale et al.[33] reported rapid in vitro selection of HIV-1 resistance to 3'-thiacytidine inhibitors due to a mutation in the YMMD region of reverse transcriptase. This had no effect on susceptibility to AZT or nevirapine and minimal effect on susceptibility to ddI and ddC. Since β-L-ddC and β-L-FddC are ddC analogues without any sulfur substitution in their sugar moiety, neither compound may have cross resistance with 3'-thiacytidine analogues.

In view of the potent antiviral activity of β-L-FddC against both HIV-1 and HBV, and potent antiviral activity of β-L-ddC against HBV in vitro, low cytotoxicity, and especially the negligible inhibitory effect on host mitochondrial DNA synthesis, β-L-FddC and β-L-ddC merit further development as potential anti-HIV and anti-HBV agents.

The mechanisms of action of β-L-FddC and β-L-ddC against HBV and HIV are currently under investigation.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz) NMR spectrometer or a Bruker WM-250 (250 MHz) spectrometer (compounds 6, 7, 8, 13, 15, and 19) with Me_4Si as the internal reference. Optical rotations were measured in a 1-dm cell with a Perkin-Elmer Model 241 polarimeter at 25 °C. The UV spectra were recorded on a Beckman-25 spectrophotometer. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols

of the elements, the analytical results for those elements were within ±0.4% of the theoretical value.

1-*O*-Acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose (1). This compound was prepared by the procedure of Okabe et al.[28] for the synthesis of the D-configuration counterpart with minor modifications and isolated as a viscous oil: $[\alpha]_D$ + 1.5° (*c* = 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.10 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.85–2.15 (m, 7 H, 2-H, 3-H and $COCH_3$), 3.50–3.65 (m, 2 H, 5-H), 4.00–4.30 (m, 1 H, 4-H), 6.20-6.30 (m, 1 H, anomeric H). Anal. ($C_{13}H_{26}O_4Si \cdot 0.35CH_2Cl_2$) C, H.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-β-L-ribofuranosyl]-5-fluorocytosine (2) and 1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-α-L-ribofuranosyl]-5-fluorocytosine (3). Method A. A mixture of 5-fluorocytosine (1.50 g, 11.6 mmol), 1,1,1,3,3,3-hexamethyldisilazane (10 mL), and ammonium sulfate (50 mg) was refluxed for 2 h and then cooled to room temperature. The mixture was concentrated in vacuo to give a residue, to which a solution of compound 1 (3.5 g, 13 mmol) in anhydrous methylene chloride (40 mL) was added, followed by the addition of a 1.8 M solution of ethylaluminum dichloride ($EtAlCl_2$) in toluene (8.3 mL, 15 mmol) over a period of 1 h at room temperature. The reaction mixture was stirred further for 40 min and then slowly poured into an ice-cooled mixture of methylene chloride and saturated sodium bicarbonate solution with stirring. The mixture was stirred for 10 min and filtered. The organic layer was washed with saturated sodium bicarbonate solution, brine, and water and then dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue was first purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1, v/v) to give 2.1 g (53%) of a mixture of β-anomer (2) and α-anomer (3) with a ratio of 3:2 (estimated by ^1H NMR spectra for the integration values of 6-H at δ 8.26 and 7.38 ppm, respectively, for β- and α-anomers). The mixture was separated by silica gel chromatography (CH_3COCH_3/CH_2Cl_2, 3:1, v/v) to afford 1.1 g (28%) of compound 2 (β-anomer) and 0.7 g (18%) of compound 3 (α-anomer).

Compound 2 was isolated as white solid: mp 174–176 °C; TLC R_f 0.33 (CH_2Cl_2/MeOH, 15:1, v/v); ^1H NMR ($CDCl_3$) δ 0.15 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.80–2.20 (m, 4 H, 2'-H and 3'-H), 3.70 (m, 1 H, 5'-H_A), 4.05 (m, 1 H, 5'-H_B), 4.18 (m, 1 H, 4'-H), 5.85–6.05 (m, 1 H, 1'-H), 7.30–7.70 (br s, 2 H, 4-NH_2, D_2O exchangeable), 8.10–8.20 (d, 1 H, 6-H). Anal. ($C_{15}H_{26}FN_3O_3Si \cdot 0.3H_2O$) C, H, N.

Compound 3 was isolated as a foam: TLC R_f 0.64 (CH_3COCH_3/CH_2Cl_2, 3:1, v/v); ^1H NMR ($CDCl_3$) δ 0.05 [s, 6 H, $Si(CH_3)_2$], 0.90 [s, 9 H, $SiC(CH_3)_3$], 1.85–2.65 (m, 4 H, 2'-H and 3'-H), 3.65 (m, 2 H, 5'-H), 4.30–4.50 (m, 1 H, 4'-H), 5.85–6.05 (m, 1 H, 1'-H), 7.38 (d, 1 H, 6-H), 7.20–7.80 (br s, 2 H, 4-NH_2, D_2O exchangeable).

Method B. To a mixture of compound 1 (3.5 g, 13 mmol), 5-fluorocytosine (1.5 g, 11.6 mmol), and potassium nonafluoro-1-butanesulfonate ($C_4F_9SO_3K$, 9.5 g, 28 mmol) in 150 mL of dry acetonitrile were added 1.6 mL of 1,1,1,3,3,3-hexamethyldisilazane and 4.5 mL of chlorotrimethylsilane with stirring under nitrogen. The reaction mixture was stirred at room temperature overnight and then slowly poured into an ice-cooled mixture of methylene chloride and saturated sodium bicarbonate solution. The product was isolated as described in method A to give 2.4 g (60%) of a mixture of β- and α-anomers (2:3), which were separated by silica gel column chromatography as previously mentioned to afford 0.8 g (20%) of 2 (β-anomer) and 1.3 g (32%) of 3 (α-anomer).

2',3'-Dideoxy-β-L-5-fluorocytidine (6). To a stirred solution of compound 2 (0.32 g, 0.93 mmol) in THF (18 mL) was added dropwise 3.7 mL of tetra-*n*-butylammonium fluoride (1 M solution in THF, 3.7 mmol) at ambient temperature. The reaction was complete after 30 min, and the solvent was evaporated in vacuo to dryness. The residue was dissolved in 15 mL of water and extracted with methylene chloride (2 × 10 mL). The water layer was evaporated with 5 g of silica gel to dryness, and the residue was chromatographed on a silica gel column (EtOAc/EtOH, 6:1, v/v) to afford 0.18 g (83%) of product as white crystals: mp 147–149 °C; TLC R_f 0.32 (EtOAc/EtOH, 4:1, v/v); $[\alpha]_D$ − 108° (*c* = 0.13, MeOH); UV (MeOH) λ_{max} 285 nm (ε 6481), λ_{min} 263 nm; UV (0.01 N HCl) λ_{max} 290 nm (ε 10 990), λ_{min} 250 nm; UV (0.01 N NaOH) λ_{max} 283 nm (ε 7984, λ_{min} 260 nm; ^1H NMR (Me_2SO-d_6) δ 1.76–2.28 (m, 4 H, 2'-H and 3'-H), 3.49–3.57 (m, 1 H, 5'-H_A), 3.69–3.77 (m, 1 H, 5'-H_B), 3.98–4.04 (m, 1 H, 4'-H), 5.11–5.15 (t, 1 H, 5'-OH, D_2O exchangeable), 5.85 (m, 1 H, 1'-H), 7.40 and 7.62 (2 s, 2 H, 4-NH_2, D_2O exchangeable), 8.26 (d, 1 H, 6-H, *J* = 7.4 Hz). Anal. ($C_9H_{12}FN_3O_3 \cdot 0.3H_2O$) C, H, N.

2',3'-Dideoxy-α-L-5-fluorocytidine (7). Compound 7 was synthesized from compound 3 by the same methodology as described for the synthesis of compound 6. Compound 7 was isolated as a foam: TLC R_f 0.48 (CH_2Cl_2/MeOH, 6:1, v/v); UV (MeOH) λ_{max} 285 nm, λ_{min} 264 nm; ^1H NMR (Me_2SO-d_6) δ 1.74–2.26 (m, 4 H, 2'-H and 3'-H), 3.33–3.38 (m, 2 H, 5'-H), 4.38–4.42 (m, 1 H, 4'-H), 4.78–4.83 (t, 1 H, 5'-OH, D_2O exchangeable), 5.91 (m, 1 H, 1'-H), 7.67 and 7.75 (2 s, 2 H, 4-NH_2, D_2O exchangeable), 7.77 (d, 1 H, 6-H, *J* = 6.9 Hz). Anal. ($C_9H_{12}FN_3O_3 \cdot 0.25H_2O \cdot 0.25CH_3OH$) C, H, N.

Compounds 8–19 were synthesized by similar methodology as described for the preparation of compounds 6 and 7.

2',3'-Dideoxy-β-L-cytidine (8). Isolated as white solid: mp 194–196 °C; TLC R_f 0.23 (EtOAc/EtOH, 2:1, v/v); $[\alpha]_D$ −90.3° (*c* = 0.14, MeOH); UV (MeOH) λ_{max} 270 nm (ε 6979), λ_{min} 248 nm; UV (0.01 N HCl) λ_{max} 282 nm (ε 11 965), λ_{min} 242 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ε 8340), λ_{min} 250 nm; ^1H NMR (Me_2SO-d_6) δ 1.74–2.24 (m, 4 H, 2'-H and 3'-H), 3.49–3.65 (m, 2 H, 5'-H), 3.98–4.04 (m, 1 H, 4'-H), 4.96–5.00 (t, 1 H, 5'-OH, D_2O exchangeable), 5.67 (d, 1 H, 5-H, *J* = 7.4 Hz), 5.91 (m, 1 H, 1'-H), 7.01–7.06 (br s, 2 H, 4-NH_2, D_2O exchangeable), 7.87–7.90 (d, 1 H, 6-H, *J* = 7.4 Hz). Anal. ($C_9H_{13}N_3O_3 \cdot 0.5CH_3OH$) C, H, N.

2',3'-Dideoxy-α-L-cytidine (9). Isolated as a glass: TLC R_f 0.25 (CH_2Cl_2/MeOH, 6:1, v/v); $[\alpha]_D$ +46.6° (*c* = 0.1, MeOH); UV (MeOH) λ_{max} 276 nm, λ_{min} 253 nm; ^1H NMR (Me_2SO-d_6) δ 1.70–2.30 (m, 4 H, 2'-H and 3'-H), 3.30–3.45 (m, 2 H, 5'-H), 4.30–4.45 (m, 1 H, 4'-H), 4.50–4.80 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.76 (d, 1 H, 5-H, *J* = 7.5 Hz) 5.90 (m, 1 H, 1'-H), 7.00–7.40 (br s, 2 H, 4-NH_2, D_2O exchangeable), 7.50 (d, 1 H, 6-H, *J* = 7.5 Hz). Anal. ($C_9H_{13}N_3O_3 \cdot 0.6CH_3OH$) C, H, N.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-β-L-ribofuranosyl]-5-fluorouracil (10). Isolated as white crystals: mp 133–135 °C; TLC R_f 0.40 (CH_2Cl_2/MeOH, 15:1, v/v); ^1H NMR ($CDCl_3$) δ 0.10 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.80–2.35 (m, 4 H, 2'-H and 3'-H), 3.70 (m, 1 H, 5'-H_A), 4.05 (m, 1 H, 5'-H_B), 4.15 (m, 1 H, 4'-H), 5.90–6.05 (m, 1 H, 1'-H), 8.10–8.20 (d, 1 H, 6-H), 9.30–9.50 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{15}H_{25}N_2O_4FSi \cdot 0.25H_2O$) C, H, N.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-β-L-ribofuranosyl]uracil (11). Isolated as a white foam: TLC R_f 0.50 (CH_2Cl_2/EtOAc, 1:1, v/v); ^1H NMR ($CDCl_3$) δ 0.08 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.90–2.22 (m, 4 H, 2'-H and 3'-H), 3.70 (m, 1 H, 5'-H_A), 4.07 (m, 1 H, 5'-H_B), 4.10 (m, 1 H, 4'-H), 5.65 (d, 1 H, 5-H), 6.10 (m, 1 H, 1'-H), 8.05 (d, 1 H, 6-H), 9.45 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{15}H_{26}N_2O_4Si \cdot 0.5CH_2Cl_2$) C, H, N.

2',3'-Dideoxy-β-L-5-fluorouridine (13). Isolated as white crystals: mp 108–110 °C; TLC R_f 0.29 (CH_2Cl_2/MeOH, 15:1, v/v); $[\alpha]_D$ −53° (*c* = 0.12, MeOH); UV (MeOH) λ_{max} 273 nm (ε 8199), λ_{min} 237 nm; UV (0.01 N HCl) λ_{max} 274 nm (ε 8987), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ε 7095), λ_{min} 249 nm; ^1H NMR (Me_2SO-d_6) δ 1.85–2.40 (m, 4 H, 2'-H and 3'-H), 3.55–3.75 (m, 2 H, 5'-H), 3.95–4.15 (m, 1 H, 4'-H), 5.10–5.25 (t, 1 H, 5'-OH, D_2O exchangeable), 5.90 (m, 1 H, 1'-H), 8.35 (d, 1 H, 6-H, *J* = 7.5 Hz), 11.6–11.7 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}FN_2O_4 \cdot 0.15H_2O$) C, H, N.

2',3'-Dideoxy-β-L-uridine (14). Isolated as a foam: $[\alpha]_D$ −28.0° (*c* = 0.1, MeOH); TLC R_f 0.60 (EtOAc/EtOH, 3:1, v/v); UV (MeOH) λ_{max} 264 nm (ε 7539), λ_{min} 234 nm; UV (0.01 N HCl) λ_{max} 264 nm (ε 8284), λ_{min} 234 nm; UV (0.01 N NaOH) λ_{max} 264 nm (ε 7911), λ_{min} 234 nm; ^1H NMR (Me_2SO-d_6) δ 1.80–2.05 (m, 4 H, 2'-H and 3'-H), 3.45–3.60 (m, 2 H, 5'-H), 3.85–4.05 (m, 1 H, 4'-H), 4.85–5.00 (t, 1 H, 5'-OH, D_2O exchangeable), 5.50 (d, 1 H, 5-H, *J* = 7.2 Hz), 5.85 (m, 1 H, 1'-H), 7.85 (d, 1 H, 6-H, *J* = 7.2 Hz), 11.1 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_9H_{12}N_2O_4$) C, H, N.

3'-Deoxy-β-L-thymidine (15). Isolated as white crystals: mp 148–149 °C; TLC R_f 0.47 (EtOAc); $[\alpha]_D$ −31.2° (*c* = 0.1, MeOH); UV (MeOH) λ_{max} 270 nm (ε 9321), λ_{min} 237 nm; UV (0.01 N HCl) λ_{max} 271 nm (ε 9515), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ε 8544), λ_{min} 238 nm; ^1H NMR (Me_2SO-d_6) δ 1.75 (s, 3 H, 5-CH_3), 1.81–1.89 (m, 2 H, 3'-H), 1.92–1.98 (m, 2 H, 2'-H_A), 2.17–

2.27 (m, 1 H, 2'-H_B), 3.47–3.71 (m, 2 H, 5'-H), 3.96–4.03 (m, 1 H, 4'-H), 5.03–5.07 (t, 1 H, 5'-OH, D_2O exchangeable), 5.92–5.96 (m, 1 H, 1'-H), 7.80 (s, 1 H, 6-H), 11.3 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{14}N_2O_4$) C, H, N.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-α-L-ribofuranosyl]-5-fluorouracil (16). Isolated as white solid: mp 105–107 °C; TLC R_f 0.37 (CH_2Cl_2/MeOH, 15:1, v/v); ^1H NMR ($CDCl_3$) δ 0.10 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.90–2.60 (m, 4 H, 2'-H and 3'-H), 3.58 (d, 2 H, 5'-H), 4.30–4.50 (m, 1 H, 4'-H), 5.90–6.05 (m, 1 H, 1'-H), 7.30–7.40 (d, 1 H, 6-H), 9.00–9.30 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{15}H_{25}N_2O_4FSi \cdot 0.25H_2O$) C, H, N.

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-dideoxy-α-L-ribofuranosyl]uracil (17). Isolated as a white foam: TLC R_f 0.38 (CH_2Cl_2/EtOAc, 1:1, v/v); ^1H NMR ($CDCl_3$) δ 0.08 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.85–2.45 (m, 4 H, 2'-H and 3'-H), 3.63 (m, 2 H, 5'-H), 4.43 (m, 1 H, 4'-H), 5.70 (d, 1 H, 5-H), 5.95–6.10 (m, 1 H, 1'-H), 7.40 (d, 1 H, 6-H), 9.50 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{15}H_{26}N_2O_4Si$) C, H, N.

3'-Deoxy-α-L-thymidine (19). Isolated as white crystals: mp 109–111 °C; TLC R_f 0.42 (EtOAc); $[\alpha]_D$ +17.7° (*c* = 0.1, MeOH); UV (MeOH) λ_{max} 270 nm (ε 9411), λ_{min} 237 nm; UV (0.01 N HCl) λ_{max} 272 nm (ε 9954), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 272 nm (ε 9592), λ_{min} 238 nm; ^1H NMR (Me_2SO-d_6) δ 1.76 (s, 3 H, 5-CH_3), 1.84–2.36 (m, 4 H, 2'-H and 3'-H), 3.22–3.29 (t, 2 H, 5'-H), 4.33–4.42 (m, 1 H, 4'-H), 4.77–4.81 (t, 1 H, 5'-OH, D_2O exchangeable), 5.98–6.02 (m, 1 H, 1'-H), 7.43 (s, 1 H, 6-H), 11.1 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{14}N_2O_4$) C, H, N.

Anticancer Assays. The synthesized compounds were evaluated in culture for their anticancer activity by growth inhibition studies using murine L1210 leukemia, P388, Sarcoma 180, and human CCRF-CEM T-lymphoblastic leukemia cells (CEM) as described. Murine L1210, P388, and S-180 cells were maintained as suspension cultures in Fisher's medium, and CEM cells were maintained as a suspension culture in Roswell Park Memorial Institute 1640 medium, both media supplemented with 10% horse serum, and all cells maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Under these conditions, the generation time for L1210, P388, S-180, and CCRF-CEM cells is approximately 12, 12, 18, and 20 h, respectively. Each compound was added at various concentrations to L1210, P388, S-180, and CEM cells (2×10^4 cells/mL) in their exponential phase of growth. The cell number of the drug-free cultures (control), as well as that of the cultures containing with the compounds, were determined after 3 days of growth. Concentrations of the drug that would result in killing 50% of the cell populations were determined from the plot generated by representing cell numbers corresponding to the individual drug concentrations.

Anti-HBV Assays. The biological activity of the present compounds was assessed as described by Doong et al.[21] The human hepatoma cell line carrying the HBV (designated 2.2.15), provided by Dr. G. Acs, was used in the study.[34] Six-day-old cultures were treated with varying concentrations of the drug in the culture medium (Minimum Essential Medium with Earl's salts and 10% fetal bovine serum). The drug was left in the culture medium for a period of 3 days, after which period the medium was aspirated and fresh medium containing the same concentration(s) of the drug was added. At the end of the subsequent 3-day period, the culture medium was harvested and processed to obtain the virions by the poly(ethylene glycol) precipitation method.[21] The viral DNA recovered from the secreted particles was subjected to Southern analysis. Inhibition of the viral DNA was determined from drug-treated versus control cultures not treated with the drug.

Mitochondrial DNA Assays. The effects of the various drug concentrations on mitochondrial DNA (mt DNA) was evaluated by the method described by Chen and Cheng.[13] CEM cells, treated with varying concentrations of the drug, were collected by centrifugation. After being washed with phosphate buffer saline, the cells were lysed by suspension in 10 mM Tris-HCl (pH 7.0) and repeated freeze–thaw cycles. The resulting cells were then subjected to RNase A treatment at a final enzyme concentration of 10 μg/mL, followed by proteinase K treatment (100 mg/mL) for 1 h. The DNA obtained from this procedure was then immobilized on nylon membrane after the addition of 0.8 volume of NaI and boiling for 10 min. Hybridization of the resulting DNA to a mt DNA specific probe was performed by following the method of Doong et al.[21] and by autoradiography. Quantitative estimates were obtained by using a scanning densitometer. The blots were stripped of the mt DNA probe and rehybridized to human Alu sequence probe to determine the amounts of DNA for normalization and estimation of absolute amounts of the mt DNA.

Anti-HIV-1 Assays. In a modification of a procedure by Mellors et al.,[35] the compounds were tested in drug susceptibility assays for determining their effectiveness against HIV-1 in MT-2 cells. Drug-mediated inhibition of virus-induced cell toxicity was measured by the A_{595} of MTT [3-(4,5-dimethylthiazol-2-yl)-2,3-diphenyltetrazolium bromide] (Sigma-M-2128). Triplicate wells of a 96-well tissue culture plate containing 1×10^4 MT-2 cells (AIDS-repository) per well were infected with HIV-1 (HTLV-IIIB Strain, R. C. Gallo) virus at a multiplicity of 0.1 $TCID_{50}$/cell. MT-2 cells in RPMI 1640 medium supplemented with 10% dialysized fetal bovine serum and 100 μg/mL Kanamycin were infected with virus and immediately added to serial dilution of the drug. After 5 days, 20 μL of MTT dye (2.5 mg/mL in PBS) was added per well. At the end of a 4-h incubation period, 150 μL of acidified 2-propanol with 2% NP-40 nonionic detergent was added per well. After the crystals of dye dissolved (usually 1–2 days), the plates are read on a microplate reader. Using this MTT-dye reduction method,[36] the percentage of protection can be calculated from the formula $[(a - b)/(c - b) \times 100]$ in which $a = A_{595}$ of drug-treated virus-infected wells, b is the A_{595} of no-drug infected cells, and c is the A_{595} of the no-drug uninfected cells. The ED_{50} was calculated from linear log 10 plots of percentage protection verses inhibition concentration.

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