Synthesis and Biological Evaluation of 2′,3′-Didehydro-2′,3′-dideoxy-5-fluorocytidine (D4FC) Analogues: Discovery of Carbocyclic Nucleoside Triphosphates with Potent Inhibitory Activity against HIV-1 Reverse Transcriptase[1]

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The discovery of a novel cytosine nucleoside, β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-D4FC), as a potent antihuman immunodeficiency virus (HIV) agent led us to synthesize a series of analogues and derivatives of β-D-D4FC that could be more selective and also possess increased glycosidic bond stability. The synthesized D-D4FC analogues were evaluated for anti-HIV-1 activity, anticancer activity, and cytotoxicity in various cells. The biological data demonstrated that the 5-substitution of β-D-D4FC with bromine (**6c**) and iodine (**6d**) resulted in the loss of antiviral activity, and the α-D anomer (**7a**) of D-D4FC was also devoid of activity. The 5-fluorouracil analogues (**6b** and **7b**) of D-D4FC were less potent and more cytotoxic than the parent compound, whereas the β-L-D4FU (**11**) showed both potent anti-HIV-1 activity and cytotoxicity. N^4- and 5′-*O*-acyl derivatives (**17**, **15a**−**c**) of β-D-D4FC exhibited comparable antiviral activity to β-D-D4FC. In contrast, the N^4-isopropyl derivative (**20**) of β-D-D4FC was not active against HIV-1, even at 100 μM. The carbocyclic analogues (**26a,b**) of D4FC demonstrated weak activity against HIV-1 and no toxicity in various cells. The triphosphates (**27a,b**) of the carbocyclic nucleosides demonstrated potent inhibitory activity against recombinant HIV-1 reverse transcriptase at submicromolar concentrations. Of the compounds tested as potential anticancer agents, β-D-, α-D-, and β-L-D4FU (**6b**, **7b**, **11**) showed inhibitory activity against rat glioma and modest activity against human lung carcinoma, lymphoblastoid, and skin melanoma cells.

Introduction

In search of new therapies against human immunodeficiency virus (HIV), certain 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4N) have emerged as effective antiviral agents. In this series of nucleosides, 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T)[2−4] has been already approved for the treatment of HIV infections. Recently, our group reported the antiviral spectrum of another d4-nucleoside, β-D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-D4FC).[5,6] This compound exhibited potent anti-HIV activity in vitro with a median effective concentration, EC_{50}, of 0.05 μM. The selectivity of D-D4FC was comparable to some of the most effective anti-HIV agents. In contrast to the toxic β-L-enantiomer,[7] β-D-D4FC is not toxic in various cells even at 100 μM and has a therapeutic index of over 1000. Furthermore, β-D-D4FC demonstrated no cross-resistance with all approved anti-HIV agents, showing a promising biological profile as an anti-HIV drug candidate.[8] However, β-D-D4FC is not stable under acidic conditions, since the compound is degraded to 5-fluorocytosine, a product of glycosidic bond cleavage.[6] Although it is stable under neutral and basic conditions, this acidic instability can be limiting when the compound is administered orally without protection from gastric acid. Thus, a stabilized form of D-D4FC should be developed. Furthermore, the optimization and variation of the lead compound may result in a superior drug candidate and add to our understanding of structure−activity relationships for these d4 analogues.

Therefore, as a part of our continuing efforts in the synthesis of nucleosides as antiviral agents, a series of D-D4FC analogues and derivatives were synthesized and evaluated in vitro for anti-HIV-1 activity and cytotoxicity in various cells. Cytotoxic compounds were also evaluated as potential anticancer agents in vitro.

Results and Discussion

Chemistry. β-D-D4FC (**6a**) and its analogues, including anomeric isomer α-D-D4FC (**7a**), uracil analogues β- and α-D-D4FU (**6b** and **7b**), and 5-halogen analogues β-D-D4BrC (**6c**) and β-D-D4IC (**6d**), were synthesized by the coupling reaction of the corresponding silylated nucleic base with 1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-(phenylselenenyl)-β-ribofuranose (**1**).[9] In the presence of TMSOTf, the coupling reaction between silylated 5-halogenated cytosine and the acetate **1** afforded the β-D-nucleosides **2a,c,d** exclusively in moderate to excellent yields. In comparison, the coupling reaction between silylated 5-fluorocytosine and

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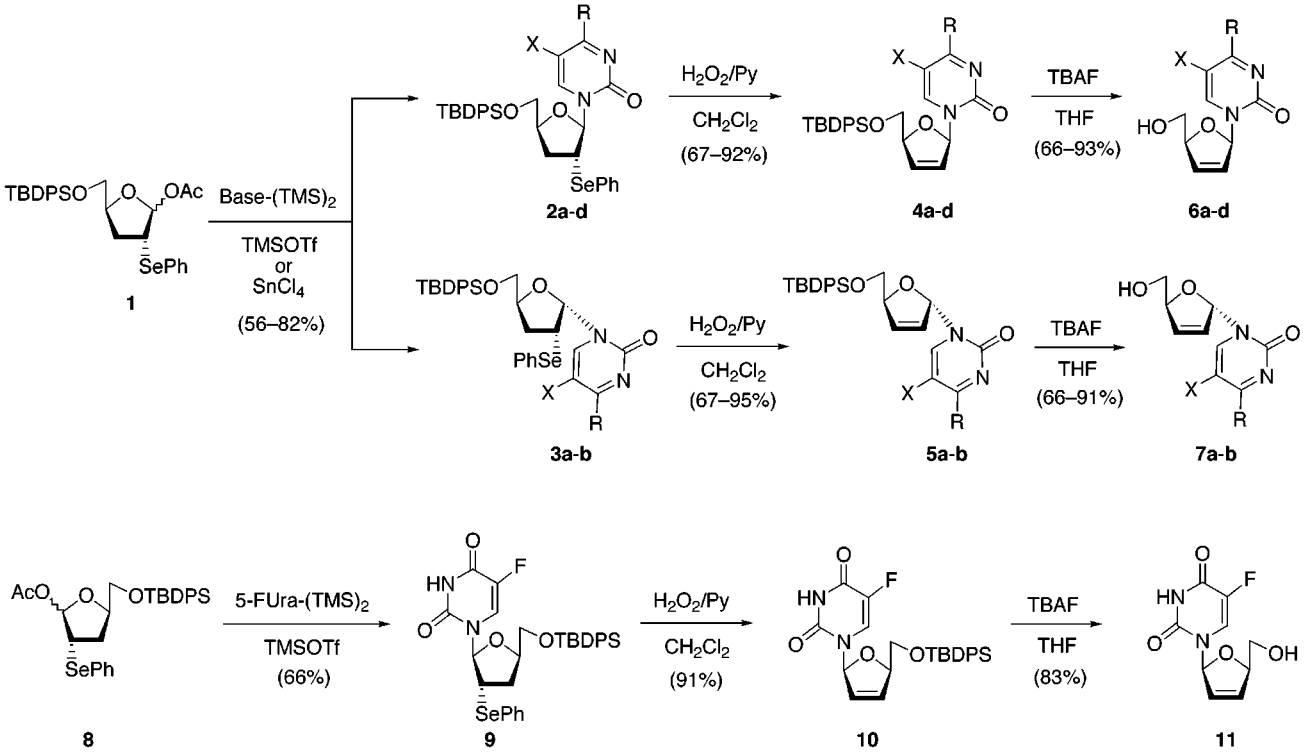
Scheme 1[a]



[a] **a**: X = F, R = NH_2. **b**: X = F, R = OH. **c**: X = Br, R = NH_2. **d**: X = I, R = NH_2.

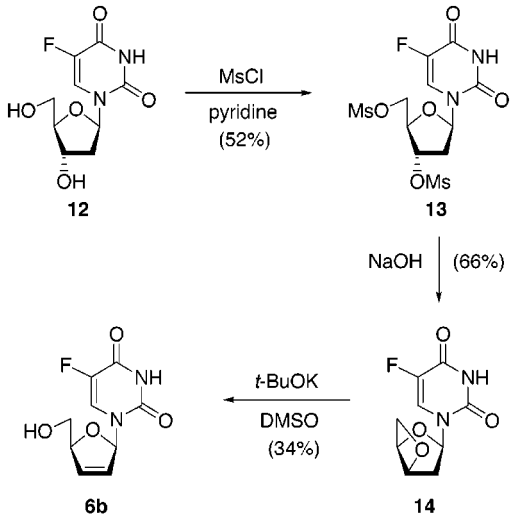
acetate **1** in the presence of tin(IV) chloride gave a small amount of α-isomer **3a** (~3%), together with the major *β*-isomer **2a**. The two anomers were separated by flash chromatography and recrystallized from CH_2Cl_2 and MeOH. In contrast, the coupling reaction between acetate **1** and silylated 5-fluorouracil resulted in an inseparable mixture of *β*- and α-D-isomers (**2b**/**3b**) in a roughly 1:1 ratio, in the presence of $SnCl_4$. All of these selenenyl nucleosides **2** and **3** were subjected to oxidative elimination by H_2O_2 in CH_2Cl_2 with a catalytic amount of pyridine, giving the 5′-*O*-protected 2′,3′-didehydro-2′,3′-dideoxynucleosides **4a**−**d** and **5a,b** in good to excellent yields. The deprotection of these compounds **4a**−**d** and **5a,b** by TBAF in THF afforded, after purification or separation either by column or thin-layer chromatography, the d4-nucleosides **6a**−**d**, **7a,b**, respectively (Scheme 1).

In a similar fashion, the *β*-L-isomer of D4FU was synthesized from 5-fluorouracil and an L-lactol acetate **8**[10] which was prepared by a similar procedure as for the D-acetate **1**. In the presence of TMSOTf, the coupling of **8** with silylated 5-fluorouracil gave exclusively the *β*-L-seleno nucleoside **9**. The same oxidation and deprotection as for the D-analogue offered *β*-L-D4FU (**11**) (Scheme 1).

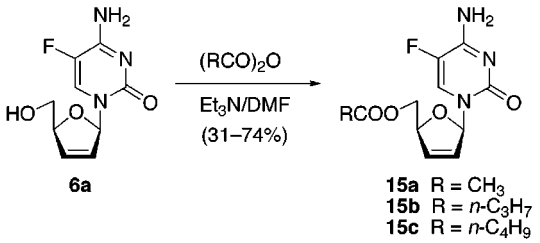
β-D-D4FU was also prepared from *β*-D-2′-deoxy-5-fluorouridine (**12**), by adapting Horwitz's methodology for preparing 2′,3′-didehydro-2′,3′-dideoxythymidine.[11] Thus, compound **12**, after mesylation, cyclization, and *β*-elimination as depicted in Scheme 2, afforded *β*-D-D4FU (**6b**) in a pure *β*-form.

5′-*O*-Acyl *β*-D-D4FC derivatives **15a**−**c** were synthesized from *β*-D-D4FC (**6a**) by a simple acylation reaction with anhydrides (Scheme 3), while the acetylation at the N^4-amino group necessitated the protection of the 5′-hydroxyl group. A silyl-protected D4FC precursor (**4a**)

Scheme 2



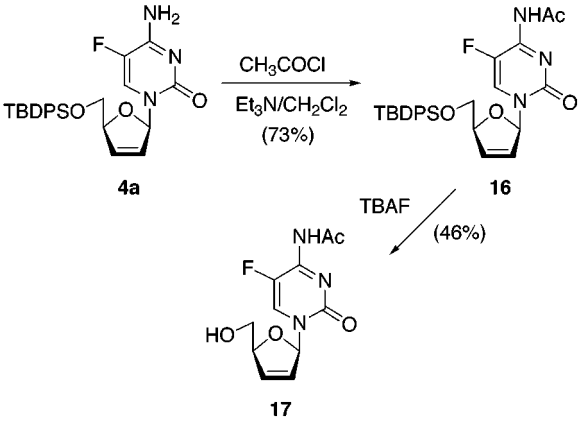
Scheme 3



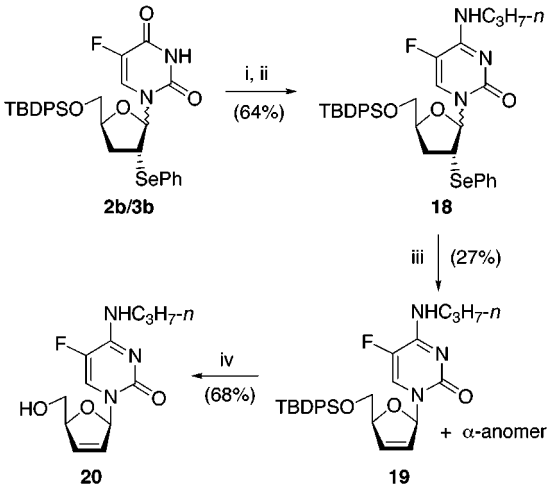
was treated with acetyl chloride followed by deprotection of the silyl group, affording the N^4-acetyl D4FC derivative **17** (Scheme 4).

The preparation of N^4-isopropyl D4FC derivative **20** was achieved by a transformation of a uracil nucleoside through a triazole intermediate, which is a widely used

Scheme 4



Scheme 5[a]



[a] Reagents: (i) 4-chlorophenyl dichlorophosphate/1,2,4-triazole/pyridine; (ii) *i*-$C_3H_7NH_2$/1,4-dioxane; (iii) H_2O_2/pyridine/CH_2Cl_2 and separation; (iv) TBAF/THF.

method for conversion of uracil to cytosine in pyrimidine nucleoside chemistry.[12,13] The α/β-mixture of the protected selenouracil nucleosides **2b**/**3b** was treated with 4-chlorophenyl dichlorophosphate and triazole, followed by substitution with isopropylamine, giving an α/β-mixture of N^4-isopropyl nucleosides **18**. Oxidative elimination of the α/β-mixture afforded the d4-nucleosides as a mixture and allowed the separation of the β-isomer **19** from the remaining isomeric mixture. After deprotection of **19**, the N^4-isopropyl β-D-D4FC derivative **20** was obtained (Scheme 5).

The carbocyclic analogues of D4FC were synthesized by the coupling reaction of the diacetate **24** with nucleic bases. Diacetate **24** was obtained by a multistep synthesis starting from cyclopentenecarboxylate **21**, which was prepared from diallylmalonate diethyl ester by the published method.[14,15] Reduction of **21** with $LiAlH_4$ produced cyclopentenemethanol **22**. The latter, after epoxidation using a published procedure,[16] furnished the *cis*-epoxide **23** as a racemic mixture. The epoxide **23** was opened with phenylselenide generated in situ, giving a seleno intermediate. After acetylation and oxidative elimination, the seleno intermediate afforded the racemic diacetate **24**. In the presence of tetrakis(triphenylphosphine)palladium and NaH, the coupling reaction between diacetate **24** and the nucleic bases (5-fluorocytosine, N^4-acetyl-5-fluorocytosine,[17] N^4-acetylcytosine) gave, after deprotection, the desired racemic carbocyclic nucleosides **26a,b**. By phosphorylation[18] and purification using fast preparative liquid chromatography (FPLC), the corresponding racemic carbocyclic nucleoside triphosphates **27a,b** were obtained (Scheme 6).

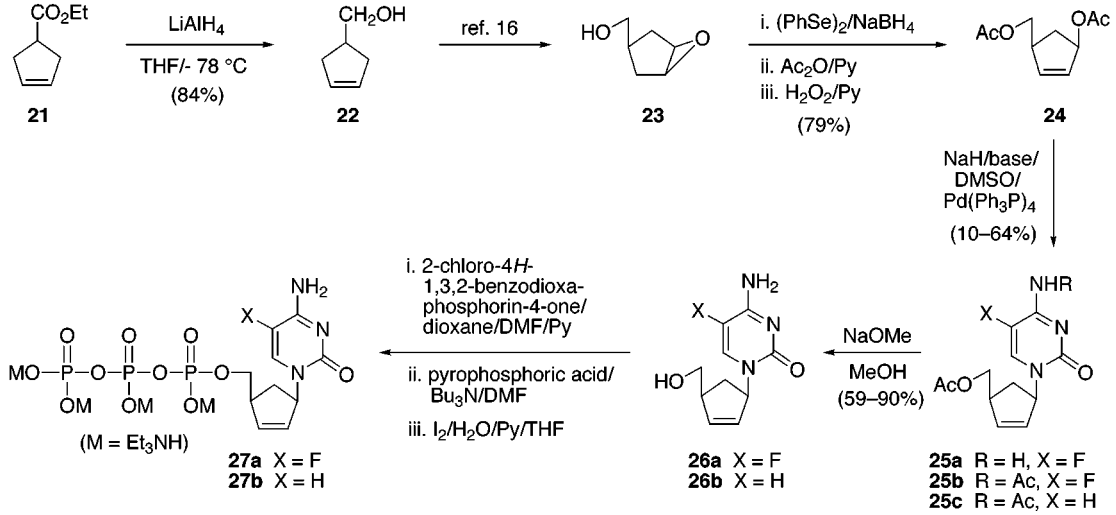
Biology. The anti-HIV-1 activity (EC_{50} and EC_{90}) and growth inhibition (IC_{50}) of the synthesized nucleosides were evaluated in primary human peripheral blood mononuclear (PBM) cells acutely infected with HIV-1_{LAI} and in uninfected cells including human PBM and rapidly dividing Vero cells.[19] The results are shown in Table 1. In comparison to the potent β-D-D4FC (**6a**), the 5-substitutions with bromine and iodine (**6c,d**) resulted in the loss of anti-HIV activity. As expected, the change of the geometry from β to α (**7a**) also caused loss of activity. The 5′-*O*- and N^4-acyl β-D-D4FC derivatives (**15a**−**c** and **17**) exhibited potent antiviral activity (EC_{50} = 0.056−1.04 μM) comparable to the parent compound β-D-D4FC. In contrast, the N^4-isopropyl β-D-D4FC derivative (**20**) was devoid of antiviral activity even at 100 μM. The uracil analogues β-D- and α-D-D4FU (**6b** and **7b**) showed less potent anti-HIV activity and more toxicity than β-D-D4FC, whereas β-L-D4FU (**11**) demonstrated both potent antiviral activity (EC_{50} = 0.26 μM) and cytotoxicity in various cells (median inhibitory concentration, IC_{50} = 2.3−32.0 μM). The carbocyclic analogues (±)-C-D4FC (**26a**) and (±)-C-D4C (**26b**) showed weak antiviral activity (EC_{50} = 8.2 and 9.0 μM, respectively) and no cytotoxicity in different cells.

The potent antiviral activity of the acyl prodrugs of β-D-D4FC was probably due to enzymatic digestion of the labile ester and amide compounds to the parent compound. In contrast, the N^4-alkyl group is relatively more difficult to remove, resulting in inactivity in vitro.

We considered that the weak antiviral activity of the two carbocyclic analogues was probably due to their poor phosphorylation in primary human lymphocytes. Thus, the two carbocyclic nucleosides **26a,b** were chemically transformed to their 5′-triphosphates **27a,b** and were evaluated against recombinant HIV-1 reverse transcriptase (RT). Interestingly, both triphosphates **27a,b** exhibited potent inhibitory activity against HIV-1 RT (IC_{50} = 0.40 and 0.38 μM, respectively). The potency of the triphosphate of the parent compound, β-D-D4FC-TP, was of the same order, whereas 3TC-TP was markedly less potent (Table 2). This confirms the hypothesis that the phosphorylation is the rate-limiting step resulting in modest antiviral activity. In contrast to the low activity of the carbocyclic nucleosides **26a,b** against HIV, their triphosphates **27a,b** demonstrated potent inhibitory activity against HIV-1 RT. We hypothesize that the change of the oxygen to carbon in the sugar causes the electronic and steric alteration of the nucleosides, resulting in poor substrate specificity of the carbocyclic nucleosides for the cellular kinases.

Although the replacement of the oxygen with carbon in D-D4FC results in a major difference in the phosphorylation kinetics by enzymes, the finding that the carbocyclic nucleoside triphosphates **27a,b** are inhibitors of HIV-1 RT suggests a similar mechanism for their antiviral activity. It would be expected that the carbocyclic nucleotides **27a,b** may also act as competitive inhibitors and/or chain terminators, like other 2′,3′-didehydro-2′,3′-dideoxynucleosides. On the other hand,

Scheme 6[a]



[a] For clarity, only one enantiomer of each racemate is shown.

Table 1. Anti-HIV-1 and Anticancer Activity and Cytotoxicity of the D-D4FC Analogues and Derivatives

		anti-HIV-1 activity in PBM cells		cytotoxicity in normal cells IC_{50} (μM)[a]		anticancer activity IC_{50} (μM)[b]							
code	compound	EC_{50} (μM)	EC_{90} (μM)	PBM cells	Vero cells	CEM	HepG2	PC-3	SK-MEL-28	SK-MES-1	MCF-7	LNCaP	9L
6a	β-D-D4FC	0.046	0.77	>100	>100	>100[a]	>100	>10	>100	>100	>100	>100	ND
	β-L-D4FC[7]	0.034	0.16	12.9	9.4	0.46[a]	118[a]	>100	>100	99.1	82.8	55.2	ND
6c	β-D-D4BrC	>10	>10	91.6	>100	42.5[a]	>100	>100	>100	>100	>100	>100	ND
6d	β-D-D4IC	>10	>10	98.3	>100	107[a]	>100	>100	>100	>100	>100	>100	ND
7a	α-D-D4FC	20.0[c]	70.0[c]	>100	>100	>100[a]	>100	>100	>100	>100	>100	>100	>100
6b	β-D-D4FU	7.9	23	32.2	7.9	9.1[a]	59.3	63.0	21.7	4.2	54.1	67.8	1.2
7b	α-D-D4FU	7.5	23	47.4	3.0	11.4[a]	37.1	>100	9.8	10.0	67.3	85.8	2.4
11	β-L-D4FU	0.26	3.0	2.3	32.0[b]	12.9	35.4	83.4	7.45	4.6	15.8	57.2	1.9
15a	β-D-5′-*O*-Ac-D4FC	0.13	0.40	>100[b]	>100[b]	>100	>100	>100	>100	>100	ND	ND	ND
15b	β-D-5′-*O*-Bu-D4FC	0.15	0.61	>100	>100	>100[a]	>100	>100	>100	>100	>100	>100	>100
15c	β-D-5′-*O*-Va-D4FC	0.056	0.34	>100	>100	124[a]	>100	>100	>100	>100	>100	>100	ND
17	β-D-N^4-Ac-D4FC	1.0	6.0	>100[b]	103[b]	14.7	>100	>100	108	84.6	62.9	>100	ND
20	β-D-N^4-*i*Pr-D4FC	>100	>100	34.7[b]	>100	>100[a]	>100	>100	>100	>100	>100	>100	ND
26a	(±)-C-D4FC	8.2	71	>100	>100	>100[a]	ND	ND	ND	ND	ND	ND	ND
26b	(±)-C-D4C	9.0	59.9	>100	>100	>100[a]	ND	ND	ND	ND	ND	ND	ND
	5-fluorouracil[d]	ND	ND	5.0	6.81	90.5	37.9	>100	4.5	13.1	41.0	>100	1.0
	cycloheximide[d]	ND	ND	2.1	1.2	0.12	2.5	3.5	1.0	2.7	1.5	1.2	0.2
	AZT	0.002	0.02	>100	29.0	30.9	ND	ND	>10	>100	>10	ND	ND

[a] Cell count. [b] Denotes MTT endpoint. [c] The result obtained may be caused by trace amounts of β-D-D4FC. [d] Known cytotoxic agent; ND, not determined.

Table 2. Inhibitory Activity by the Carbocyclic Nucleoside Triphosphates of Recombinant HIV-1 RT Compared to 3TC-TP

compound	IC_{50} (μM)	IC_{90} (μM)
(±)-C-D4FCTP (**27a**)	0.40	4.0
(±)-C-D4CTP (**27b**)	0.38	3.4
(+)-β-D-D4FCTP	0.27	2.2
3TC-TP	3.6	29.8

the replacement of the oxygen by carbon led to the more acid-stable carbocyclic nucleosides. Considering the structural similarity between β-D-D4FC and the carbocyclic analogues and the potent antiviral activity of the carbocyclic nucleoside triphosphates, the carbocyclic nucleotides have potential as antiviral drugs. Hence, the resolution of the racemic carbocyclic nucleosides is underway in order to determine which enantiomer possesses the superior antiviral profile.

Most of the D-D4FC analogues and derivatives were further evaluated for inhibitory effect in a panel of cancer cells, including human lymphoblastoid (CEM), human liver hepatocellular carcinoma (HepG2), human prostate carcinoma (PC-3 and LNCaP), human skin melanoma (SK-MEL-28), human lung squamous cell carcinoma (SK-MES-1), breast adenocarcinoma (MCF-7), and rat glioma (9L) cells. Among the compounds evaluated, the three isomers of D4FU, namely β-D-, α-D-, and β-L-D4FU (**6b, 7b, 11**), showed inhibitory activity against 9L (IC_{50} = 1.2−2.4 μM) and modest activity against SK-MES-1 (IC_{50} = 4.2−10.0 μM), CEM (IC_{50} = 9.1−12.9 μM), and SK-MEL-28 (IC_{50} = 7.5−21.7 μM) cells, whereas all the other compounds showed no significant inhibition against all tested cancer cells (Table 1).

In summary, a series of D-D4FC analogues and derivatives were synthesized and evaluated for anti-HIV-1 and anticancer activity and cytotoxicity against various cells. The study revealed that N^4- and 5′-*O*-acyl derivatives of β-D-D4FC had potent antiviral activity comparable to that of β-D-D4FC. All other analogues were significantly less potent than the parent compound. β-L-D4FU also showed potent anti-HIV-1 activity

(albeit lower than that of β-D-D4FC) and cytotoxicity. Only D4FU (β-D, α-D, β-L) exhibited some modest anticancer activity against rat glioma (9L), human lung carcinoma (SK-MES-1), human lymphoblastoid (CEM), and human skin melanoma (SK-MEL-28) cells, which could be related to the lability of the glycosidic bond, releasing the well-known anticancer agent 5-fluorouracil. Although the carbocyclic analogues of D4FC showed modest activity against HIV-1, their triphosphates were potent inhibitors of recombinant HIV-1 RT. The resolution and preparation of the phosphate prodrugs of these carbocyclic nucleosides and their testing against various polymerases are ongoing.

Experimental Section

All reagents were used as received unless stated otherwise. Anhydrous solvents were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Melting points (mp) were determined on an Electrothermal digit melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity Plus 400 spectrometer at room temperature and reported in ppm downfield from internal tetramethylsilane. Mass spectra were recorded at either the Emory University Mass Spectrometry Center (Atlanta, GA) or the VA Medical Center (Atlanta, GA). Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Analytic TLC was performed on Whatman LK6F silica gel plates and preparative TLC on Whatman PK5F silica gel plates. HPLC identification was conducted with a Hewlett-Packard 1050, and FPLC separation was performed in a Pharmacia FPLC system. All final recrystallizations generally resulted in over 80% recovery of product.

β-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-5-fluoro-2′-(phenylselenenyl)cytidine (2a). 5-Fluorocytosine (3.044 g, 23.58 mmol), $(NH_4)_2SO_4$ (312 mg, 2.36 mmol), and hexamethyldisilazane (50 mL) were mixed and heated under reflux for 2 h in an argon atmosphere. After removal of the solvent by evaporation under reduced pressure, the residue was treated with a solution of 1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-(phenylselenenyl)-α- and β-D-*erythro*-pentofuranose (**1**)[9] (10.43 g, 18.86 mmol) in dry 1,2-dichloroethane (40 mL) in an argon atmosphere. The reaction mixture was cooled to 5 °C, and TMSOTf (3.56 mL, 19.65 mmol) was added. After stirring at 5 °C for 15 min, the reaction mixture was stirred at room temperature for another 15 min. Then the mixture was poured into a mixture of EtOAc and saturated $NaHCO_3$ aqueous solution with stirring. The organic layer was separated, washed with saturated $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography over silica gel eluting with CH_2Cl_2/MeOH (99:1 to 96:4) to give **2a** (9.665 g, 82%) as a white solid: mp 163−164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 6.4 Hz, 1H, H-6), 7.67−7.62 (m, 6H, arom.), 7.47−7.25 (m, 9H, arom.), 6.90 (bs, 1H, NH), 6.15−6.14 (m, 1H, H-1′), 5.40 (bs, 1H, NH), 4.32−4.30 (m, 1H, H-4′), 4.12−4.08 (m, 1H, H-5′a), 3.84−3.83 (m, 1H, H-2′), 3.65 (dd, *J* = 2.4 & 11.2 Hz, 1H, H-5′b), 2.45−2.42, 2.01−1.98 (2m, 2H, H-3), 1.08 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 157.0, 156.8, 153.3, 137.3, 135.6, 135.5, 135.4, 134.9, 132.6, 132.3, 130.1, 130.0, 129.2, 128.3, 127.9, 127.4, 125.5, 125.2, 91.0, 80.2, 64.8, 45.4, 32.3, 26.9, 19.2.

α-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-5-fluoro-2′-(phenylselenenyl)cytidine (3a). To the freshly prepared silylated 5-fluorocytosine (48.83 mmol) (prepared according to the procedure described for **2a**) were added dry CH_2Cl_2 (100 mL) and $SnCl_4$ (52.90 mL, 1 M solution in CH_2Cl_2, 52.90 mmol). After being stirred at room temperature for 15 min, the solution was transferred to a solution of **1** (22.50 g, 40.69 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C by cannulation over a period of 20 min. The reaction mixture was stirred at 0 °C for 2 h, and then a solution of NH_4OH (28%, 20 mL) was added dropwise. After stirring at room temperature for 20 min, the resulting precipitates were filtered through a Celite pad and rinsed with hot $CHCl_3$ (4 × 150 mL). The combined filtrates were concentrated by evaporation. The residue was purified by flash chromatography over silica gel eluting with CH_2Cl_2/MeOH (99:1 to 95:5) and recrystallization from MeOH to give **3a** (0.51 g, 2%) (and **2a**: 18.50 g, 73%) as white foams: R_f 0.37 ($CHCl_3$/MeOH, 95:5); mp 93−94 °C; ^{1}H NMR ($CDCl_3$) δ 7.67−7.60 (m, 6H, arom.), 7.46−7.37 (m, 6H, arom.), 7.29−7.22 (m, 3H, arom.), 7.12 (d, *J* = 6.0 Hz, 1H, H-6), 5.94 (d, *J* = 6.0 Hz, 1H, H-1′), 5.80 (bs, 1H, NH), 4.48−4.45 (m, 1H, H-4′), 3.93−3.90 (m, 1H, H-2′), 3.72 (d, *J* = 4.4 Hz, 2H, H-5′), 2.48−2.43 (m, 1H, H-3′a), 2.15−2.07 (m, 1H, H-3′b), 1.05 (s, 9H, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 157.5, 153.5, 137.4, 135.61, 135.58, 135.49, 135.0, 133.2, 133.1, 129.7, 129.2, 128.3, 127.7, 127.2, 125.6, 125.3, 94.5, 80.7, 65.5, 42.9, 34.1, 26.8, 19.2.

β/α-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-5-fluoro-2′-(phenylselenenyl)uridine (2b/3b). In an analogous manner to the preparation of **3a**, the title compounds **2b/3b** were obtained from 5-fluorouracil and the acetate **1** (56% yield): white foam; mp 68−69 °C; ^{1}H NMR ($CDCl_3$) δ 8.24, 7.74 (2d, *J* = 6.0 Hz, 1H, H-6), 7.66−7.58 (m, 6H, arom.), 7.47−7.26 (m, 9H, arom.), 6.14−6.12 (m, 1H, H-1′), 4.60−3.60 (m, 4H, H-4′, H-5′, H-2′), 2.70−2.09 (m, 2H, H-3′), 1.11, 1.06 (2s, 9H, *t*-Bu).

β-D-5-Bromo-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-dideoxy-2′-(phenylselenenyl)cytidine (2c). In an analogous manner to the preparation of **2a**, the title compound **2c** was obtained from silylated 5-bromocytosine and the acetate **1** (74% yield): white foam; mp 78−79 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (bs, 1H, NH), 7.84 (s, 1H, H-6), 7.66−7.62 (m, 6H, arom.), 7.48−7.23 (m, 9H, arom.), 6.19 (d, *J* = 6.0 Hz, 1H, H-1′), 5.95 (bs, 1H, NH), 4.30−4.27 (m, 1H, H-4′), 4.04 (dd, *J* = 2.4 & 11.2 Hz, 1H, H-5′a), 3.81 (q, *J* = 6.8 Hz, 1H, H-2′), 3.66 (dd, *J* = 2.4 & 11.2 Hz, 1H, H-5′b), 2.42 (dt, *J* = 12.8 & 6.0 Hz, 1H, H-3′a), 2.08 (dt, *J* = 12 & 6 Hz, 1H, H-3′b), 1.06 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 161.4, 154.1, 140.9, 135.8, 135.6, 135.4, 132.8, 132.5, 130.1, 130.0, 129.2, 128.3, 127.9, 127.8, 127.1, 90.9, 79.4, 65.3, 44.6, 32.9, 27.1, 19.4.

β-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-2′-(phenylselenenyl)-5-iodocytidine (2d). In an analogous manner to the preparation of **2a**, the title compound **2d** was obtained from 5-iodocytosine and the acetate **1** (63% yield): white foam; mp 91−92 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (bs, 1H, NH), 7.81 (s, 1H, H-6), 7.67−7.61 (m, 6H, arom.), 7.48−7.23 (m, 9H, arom.), 6.19 (d, *J* = 6.8 Hz, 1H, H-1′), 5.85 (bs, 1H, NH), 4.30−4.25 (m, 1H, H-4′), 4.01 (dd, *J* = 11.2 & 2.4 Hz, 1H, H-5′a), 3.78 (q, *J* = 6.8 Hz, 1H, H-2′), 3.67 (dd, *J* = 11.2 & 3.2 Hz, 1H, H-5′b), 2.42 (dt, *J* = 12.8 & 6.4 Hz, 1H, H-3′a), 2.14−2.05 (m, 1H, H-3′b), 1.12 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 163.1, 154.5, 146.2, 135.7, 135.6, 135.4, 132.9, 132.5, 130.0, 139.9, 129.2, 128.3, 127.9, 127.7, 127.1, 90.8, 79.2, 65.5, 44.4, 33.0, 27.2, 19.5.

β-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (4a). Into a solution of **2a** (15.47 g, 24.87 mmol) in CH_2Cl_2 (150 mL) containing 5 drops of pyridine at 0 °C was added a solution of H_2O_2 (15.5 mL of 30% solution) dropwise over a period of 15 min. After stirring at 0 °C for 20 min and at room temperature for 30 min, the reaction solution was diluted with $CHCl_3$ (200 mL), washed with H_2O, saturated $NaHCO_3$ solution, and H_2O, dried (Na_2SO_4), filtered, and concentrated. The residue, after purification by chromatography over silica gel eluted with CH_2Cl_2−MeOH (96:4), gave **4a** (9.907 g, 86%) as a pale-yellow foam: mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (d, *J* = 6.4 Hz, 1H, H-6), 7.66−7.65 (m, 4H, arom.), 7.47−7.37 (m, 6H, arom.), 7.00−6.99 (m, 1H, H-1′), 6.50 (bs, 1H, NH), 6.12 (d, *J* = 6.0 Hz, 1H, H-3′), 5.98 (d, *J* = 4.8 Hz, 1H, H-2′), 5.35 (bs, 1H, NH), 4.89 (bs, 1H, H-4′), 4.00 (dd, *J* = 3.2 & 11.6 Hz, 1H, H-5′a), 3.81 (dd, *J* = 3.6 & 12.4 Hz, 1H, H-5′b), 1.06 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 157.3, 157.1, 153.6, 137.5, 135.6, 135,4, 135.1, 133.3, 132.8, 132.6, 130.1, 130.0, 127.9, 127.8, 127.4, 126.1, 125.8, 91.3, 87.3, 65.1, 26.9, 19.2.

β/α-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluorouridine (4b/5b). In an analogous manner to the preparation of **4a**, the title compounds **4b/5b** were

prepared from **2b/3b** (67% yield): white foam; ^{1}H NMR ($CDCl_3$) δ 9.32 (bs, 1H, NH), 7.73, 7.16 (2d, J = 6.0 & 5.2 Hz, 1H, H-6), 7.68−7.64 (m, 4H, arom.), 7.48−7.38 (m, 6H, arom.), 7.04−6.99 (m, 1H, H-1′), 6.40, 6.26 (ddt, J = 6.0, 6.0 & 1.6 Hz, 1H, H-3′), 5.92, 5.88 (2d, J = 6.0 Hz, 1H, H-2′), 5.16−5.11, 4.92−4.88 (m, 1H, H-4′), 3.97, 3.83, 3.77 (3 dd, J = 2.4, 12.0 & 4.0 Hz, 2H, H-5′), 1.08, 1.06 (2s, 9H, *t*-Bu).

β-D-5-Bromo-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxycytidine (4c). In an analogous manner to the preparation of **4a**, the title compound **4c** was prepared from **2c** (92% yield): light-yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1H, H-6), 7.67−7.64 (m, 4H, arom.), 7.47−7.37 (m, 6H, arom.), 6.92−6.91 (m, 1H, H-1′), 6.16 (d, J = 6.0 Hz, 1H, H-3′), 6.04 (d, J = 6.0 Hz, 1H, H-2′), 5.79 (bs, 1H, NH), 4.95 (bs, 1H, H-4′), 3.97 (dd, J = 12 & 3.2 Hz, 1H, H-5′a), 3.83 (dd, J = 12 & 3.2 Hz, 1H, H-5′b), 1.07 (s, 9H, *t*-Bu).

β-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-iodocytidine (4d). In an analogous manner to the preparation of **4a**, the title compound **4d** was prepared from **2d** (73% yield): yellow solid; mp > 200 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1H, H-6), 7.67−7.64 (m, 4H, arom.), 7.47−7.37 (m, 6H, arom.), 6.90−6.89 (m, 1H, H-1′), 6.19 (d, J = 6.0 Hz, 1H, H-3′), 6.05 (d, J = 6.0 Hz, 1H, H-2′), 5.80 (bs, 1H, NH), 4.96 (bs, 1H, H-4′), 3.95 (dd, J = 12 & 3.6 Hz, 1H, H-5′a), 3.84 (dd, J = 12 & 3.6 Hz, 1H, H-5′b), 1.08 (s, 9H, *t*-Bu).

α-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (5a). In an analogous manner to the preparation of **4a**, the title compound **5a** was prepared from **3a** (95% yield): white foam; mp 80−81 °C; ^{1}H NMR ($CDCl_3$) δ 7.68−7.66 (m, 4H, arom.), 7.46−7.38 (m, 6H, arom.), 7.21 (d, J = 6.0 Hz, 1H, H-6), 7.02−7.01 (m, 1H, H-1′), 6.28 (dt, J = 6.0 & 1.6 Hz, 1H, H-3′), 6.01 (d, J = 4.2 Hz, 1H, H-2′), 5.65 (bs, 1H, NH), 5.15−5.10 (m, 1H, H-4′), 3.80−3.75 (m, 2H, H-5′), 1.06 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 157.8, 157.7, 153.9, 137.8, 135.5, 135.4, 133.4, 133.1, 133.0, 129.8, 127.7, 127.4, 124.9, 124.5, 92.1, 87.6, 65.6, 26.7, 19.2.

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorocytidine (6a). To a solution of **4a** (9.7 g, 20.86 mmol) in THF (200 mL) was added a solution of tetrabutylammonium fluoride (1 M solution in THF, 20.86 mL, 20.86 mmol). The reaction mixture was stirred at room temperature for 1.5 h, and then the solvent was removed under reduced pressure. The residue, after purification by chromatography over silica gel eluted with CH_2Cl_2−MeOH (9:1), afforded **6a** (4.392 g, 93%) as a white solid: mp 175−176 °C; ^{1}H NMR (DMSO-d_6) δ 8.04 (d, J = 7.6 Hz, 1H, H-6), 7.77 (bs, 1H, NH), 7.55 (bs, 1H, NH), 6.82 (bs, 1H, H-1′), 6.32 (d, J = 7.6 Hz, 1H, H-3′), 5.88 (d, J = 6.0 Hz, 1H, H-2′), 5.11 (t, J = 6.0 Hz, 1H, OH), 4.78 (bs, 1H, H-4′), 3.62−3.61 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 157.7, 157.5, 153.7, 137.2, 134.8, 134.4, 126.7, 126.1, 125.8, 90.1, 87.3, 62.1; MS (ESI) *m/e* 228 (MH^+). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorouridine (6b) and α-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorouridine (7b). In an analogous manner to the preparation of **6a**, the title compounds **6b** and **7b** were prepared from **4b/5b** and purified by preparative TLC (eluting with $CHCl_3$/MeOH, 12:1). **6b** (41% yield): white solid; R_f 0.36 ($CHCl_3$/MeOH, 90:10); mp 129−130 °C; ^{1}H NMR (DMSO-d_6) δ 8.04 (d, J = 7.2 Hz, 1H, H-6), 6.81 (bs, 1H, H-1′), 6.36 (d, J = 6.4 Hz, 1H, H-3′), 5.88 (d, J = 4.8 Hz, 1H, H-2′), 4.77 (bs, 1H, H-4′), 3.62−3.58 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 158.9, 158.7, 150.7, 141.2, 138.9, 135.0, 126.1, 124.8, 124.5, 89.5, 87.4, 62.0; MS (FAB) *m/e* 229 (MH^+). Anal. ($C_9H_9FN_2O_4$) C, H, N. **7b** (25%): white sticky solid; R_f 0.32 ($CHCl_3$/MeOH, 90:10); ^{1}H NMR (DMSO-d_6) δ 7.44 (d, J = 7.2 Hz, 1H, H-6), 6.84 (d, J = 4.8 Hz, 1H, H-1′), 6.38 (d, J = 6.0 Hz, 1H, H-3′), 5.88 (d, J = 6.0 Hz, 1H, H-2′), 5.05 (bs, 1H, H-4′), 3.48−3.40 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 159.1, 158.9, 150.7, 141.8, 139.4, 135.2, 125.5, 123.9, 123.6, 90.2, 87.7, 63.2; MS (FAB) *m/e* 229 (MH^+). Anal. ($C_9H_9FN_2O_4$) C, H, N.

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorouridine (**6b**) was also prepared from 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (**14**). A mixture of *t*-BuOK (332 mg, 2.9 mmol) and **14** (319 mg, 1.4 mmol) in dry DMSO (15 mL) was stirred at room temperature for 2 h. The reaction mixture was neutralized by addition of dilute AcOH and then concentrated under reduced pressure. The orange-colored residue was extracted by hot acetone, and the extraction was filtered and concentrated. The residue was crystallized from CH_2Cl_2/MeOH/hexane providing **6b** (108 mg, 34%) as colorless crystals. The physical and spectroscopic characteristics of the compound prepared by this method were identical to that of the compound described above.

β-D-5-Bromo-2′,3′-didehydro-2′,3′-dideoxycytidine (6c). In an analogous manner to the preparation of **6a**, the title compound **6c** was prepared from **4c** (84% yield): white solid; mp > 200 °C; ^{1}H NMR (DMSO-d_6) δ 8.22 (s, 1H, H-6), 7.90 (bs, 1H. NH), 7.04 (bs, 1H, NH), 6.84−6.82 (m, 1H, H-1′), 6.33 (dt, J = 6.0 & 2.0 Hz, 1H, H-3′), 5.90 (d, J = 5.2 Hz, 1H, H-2′), 5.11 (t, J = 5.2 Hz, 1H, OH), 4.80 (bs, 1H, H-4′), 3.61−3.59 (m, 2H, H-5′); MS (ESI) *m/e* 288 (MH^+). Anal. ($C_9H_{10}BrN_3O_3$·0.5CH_3OH) H, N; C: calcd, 37.52; found, 37.96. HRMS (FAB) calcd for ($C_9H_{11}BrN_3O_3$): 287.9984, found, 287.9988.

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-iodocytidine (6d). In an analogous manner to the preparation of **6a**, the title compound **6d** was prepared from **4d** (86% yield): white solid; mp > 200 °C; ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H, H-6), 7.85 (bs, 1H. NH), 6.82 (d, J = 2.0 Hz, 1H, H-1′), 6.65 (bs, 1H, NH), 6.33 (d, J = 6.0 Hz, 1H, H-3′), 5.90 (d, J = 4.2 Hz, 1H, H-2′), 5.09 (t, J = 4.0 Hz, 1H, OH), 4.79 (bs, 1H, H-4′), 3.59−3.58 (m, 2H, H-5′); MS (ESI) *m/e* 336 (MH^+). Anal. ($C_9H_{10}IN_3O_3$) C, H, N.

α-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorocytidine (7a). In an analogous manner to the preparation of **6a**, the title compound **7a** was prepared from **5a** (91% yield): white solid; mp 128−130 °C; ^{1}H NMR (DMSO-d_6) δ 7.80, 7.54 (2 bs, 2H, NH_2), 7.48 (d, J = 6.8 Hz, 1H, H-6), 6.84−6.83 (m, 1H, H-1′), 6.34 (dt, J = 6.0 & 2.0 Hz, 1H, H-3′), 5.88 (d, J = 6.0 Hz, 1H, H-2′), 5.07−5.04 (m, 1H, H-4′), 4.83 (t, J = 5.2 Hz, OH), 3.46−3.39 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 157.6, 157.5, 153.5, 137.5, 135.1, 134.6, 126.2, 125.3, 124.9, 90.8, 87.6, 63.3; MS (FAB) *m/e* 228 (MH^+). Anal. ($C_9H_{10}FN_3O_3$) C, H, N.

β-L-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-5-fluoro-2′-(phenylselenenyl)uridine (9). In an analogous manner to the preparation of **2a**, the title compound **9** was prepared from 5-fluorouracil and the acetate **8**[10] (66% yield): white foam; mp 69−70 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (bs, NH), 7.74 (d, J = 6.0 Hz, 1H, H-6), 7.66−7.58 (m, 6H, arom.), 7.49−7.26 (m, 9H, arom.), 6.13−6.11 (m, 1H, H-1′), 4.25−4.23 (m, 1H, H-4′), 4.05−4.03 (m, 1H, H-2′), 3.75−3.71, 3.67−3.63 (m, 2H, H-5′), 2.49−2.45, 2.15−2.07 (2m, 2H, H-3′), 1.11 (s, 9H, *t*-Bu).

β-L-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluorouridine (10). In an analogous manner to the preparation of **4a**, the title compound **10** was prepared from **9** (91% yield): white foam; mp 64−66 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (bs, 1H, NH), 7.72 (d, J = 6.0 Hz, 1H, H-6), 7.66−7.63 (m, 4H, arom.), 7.46−7.38 (m, 6H, arom.), 6.98−6.95 (m, 1H, H-1′), 6.27−6.26 (m, 1H, H-3′), 5.88−5.86 (m, 1H, H-2′), 4.89 (bs, 1H, H-4′), 3.97 (dd, J = 3.6 & 12.0 Hz, 1H, H-5′a), 3.83 (dd, J = 3.6 & 12.0 Hz, 1H, H-5b), 1.08 (s, 9H, *t*-Bu).

β-L-2′,3′-Didehydro-2′,3′-dideoxy-5-fluorouridine (11). In an analogous manner to the preparation of **6a**, the title compound **11** was prepared from **10** (83% yield): white solid; mp 130−131 °C; ^{1}H NMR (DMSO-d_6) δ 11.86 (bs, 1H, NH), 8.18 (d, J = 7.2 Hz, 1H, H-6), 6.80 (bs, 1H, H-1′), 6.39 (d, J = 5.6 Hz, 1H, H-3′), 5.91 (d, J = 5.6 Hz, 1H, H-2′), 5.16 (t, J = 6.0 Hz, 1H, OH), 4.81 (bs, 1H, H-4′), 3.65−3.64 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 157.3, 157.0, 149.4, 140.8, 138.5, 135.3, 125.8, 125.5, 125.2, 89.4, 87.5, 61.8; MS (FAB) *m/e* 229 (MH^+). Anal. ($C_9H_9FN_2O_4$) C, H, N.

β-D-2′-Deoxy-3′,5′-di-*O*-mesyl-5-fluorouridine (13). To a solution of β-D-2′-deoxy-5-fluorouridine (**12**; 1.23 g, 5 mmol) in dry pyridine (10 mL) at −10 °C was added methanesulfonyl chloride (1.317 g, 11.5 mmol, 0.9 mL) dropwise. The reaction mixture was stored in a refrigerator overnight. After addition of water (0.5 mL), the mixture was poured into a 500 mL of ice−water and stirred for 1 h. The precipitates were filtered and chromatographed on silica gel eluting with CH_2Cl_2/MeOH

(96:4) providing **13** (1.109 g, 52%) as a pale-yellow solid: mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 11.98 (bs, 1H, NH), 8.00 (d, J = 7.6 Hz, 1H, H-6), 6.19 (t, J = 6.8 Hz, 1H, H-1′), 5.31−5.27 (m, 1H, H-3′), 4.49−4.46 (m, 2H, H-5′), 4.38−4.36 (m, 1H, H-4′), 3.33 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 2.51−2.50 (m, 2H, H-2′).

1-(2-Deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (14). A solution of **13** (1.0 g, 2.49 mmol) and NaOH (312 mg, 7.8 mmol) in water (50 mL) was heated under reflux for 2 h. The reaction mixture was neutralized to pH 7 by dilute hydrochloric acid and evaporated under reduced pressure. The residue was extracted with hot acetone, and the mixture was filtered. After concentration of the filtrate, the residue was crystallized from EtOH to give **14** (374 mg, 66%) as brownish-yellow crystals: mp 200−201 °C; ^{1}H NMR (DMSO-d_6) δ 11.91 (bs, 1H, NH), 8.40 (d, J = 7.2 Hz, 1H, H-6), 6.50 (d, J = 8.8 Hz, 1H, H-1′), 5.48 (t, J = 4.4 Hz, 1H, H-4′), 4.89 (bs, 1H, H-3′), 4.69 (q, J = 4.4 Hz, 1H, H-5′a), 4.05 (d, J = 10.4 Hz, 1H, H-5′b), 2.59−2.41 (m, 2H, H-2′).

β-D-5′-*O*-Acetyl-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (15a). To a solution of **6a** (50 mg, 0.22 mmol), DMAP (5 mg), and Et_3N (40 mg, 0.39 mmol) in DMF (3 mL) at 0 °C was added Ac_2O (24 mg, 0.22 mmol). The reaction solution was stirred at 0 °C to room temperature overnight. After removal of the solvent by evaporation, the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (98:2) to give **15a** (18 mg, 31%) as a white powder: R_f 0.75 ($CHCl_3$/MeOH, 75:25); mp > 200 °C; ^{1}H NMR (DMSO-d_6) δ 7.89, 7.63 (2s, 2H, NH_2), 7.58 (d, J = 6.8 Hz, 1H, H-6), 6.84−6.83 (m, 1H, H-1′), 6.37−6.36 (m, 1H, H-3′), 5.99−5.97 (m, 1H, H-2′), 4.97 (bs, 1H, H-4′), 4.26−4.14 (m, 2H, H-5′), 2.01 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 170.0, 157.7, 157.6, 153.6, 137.2, 134.8, 133.3, 127.1, 125.4, 125.0, 90.3, 83.7, 64.6, 20.5; MS (FAB) *m/e* 270 (MH^+). Anal. ($C_{11}H_{12}FN_3O_4 \cdot H_2O$) C, H, N.

β-D-5′-*O*-Butyryl-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (15b). In an analogous manner to the preparation of **15a**, the title compound **15b** was prepared from **6a** and butyric anhydride (74% yield): white solid; mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (d, J = 6.0 Hz, 1H, H-6), 7.03 (bs, 1H, H-1′), 7.28, 6.40 (2bs, 2H, NH_2), 6.23−6.22 (m, 1H, H-3′), 6.00−5.99 (m, 1H, H-2′), 5.10 (bs, 1H, H-4′), 4.56−4.52, 4.25−4.22 (2m, 2H, H-5′), 2.32 (t, J = 7.6 Hz, 2H, CH_2), 1.69−1.64 (m, 2H, CH_2), 0.95 (t, J = 7.6 Hz, 3H, CH_3); MS (FAB) *m/e* 298 (MH^+). Anal. ($C_{13}H_{16}FN_3O_4$) C, H, N.

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluoro-5′-*O*-valerylcytidine (15c). In an analogous manner to the preparation of **15a**, the title compound **15c** was prepared from **6a** and valeric anhydride (74% yield): white solid; mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (d, J = 6.0 Hz, 1H, H-6), 7.02 (bs, 1H, H-1′), 7.28, 6.90 (2bs, 2H, NH_2), 6.25−6.24 (m, 1H, H-3′), 6.00−5.99 (m, 1H, H-2′), 5.11 (bs, 1H, H-4′), 4.57−4.53, 4.24−4.21 (2m, 2H, H-5′), 2.34 (t, J = 7.6 Hz, 2H, CH_2), 1.63−1.60 (m, 2H, CH_2), 1.37−1.32 (m, 2H, CH_2), 0.92 (t, J = 7.6 Hz, 3H, CH_3); MS (FAB) *m/e* 312 (MH^+). Anal. ($C_{14}H_{18}FN_3O_4$) C, H, N.

β-D-*N*^{4}-Acetyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (16). To a solution of **4a** (465 mg, 1 mmol), DMAP (5 mg), and Et_3N (405 mg, 4 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added acetyl chloride (236 mg, 3 mmol) dropwise. The reaction mixture was stirred at 0 °C to room temperature in an argon atmosphere overnight. The solvent was then evaporated, and the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (99:1) to give **16** (373 mg, 73%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 6.0 Hz, 1H, H-6), 7.67−7.61 (m, 4H, arom.), 7.48−7.38 (m, 6H, arom.), 6.93−6.92 (m, 1H, H-1′), 6.14 (d, J = 6.0 Hz, 1H, H-3′), 6.06 (d, J = 6.0 Hz, 1H, H-2′), 4.95 (bs, 1H, H-4′), 4.06 (dd, J = 3.6 & 12.0 Hz, 1H, H-5a), 3.85 (dd, J = 3.6 & 12.0 Hz, 1H, H-5b), 2.66 (s, 3H, CH_3), 1.06 (s, 9H, *t*-Bu).

β-D-*N*^{4}-Acetyl-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (17). To a solution of **16** (390 mg, 0.77 mmol) in THF (5 mL) was added TBAF (1 M solution in THF, 0.77 mmol). The reaction mixture was stirred at room temperature for 3 h. The solvent was then evaporated, and the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (95:5) to give **17** (96 mg, 46%) as a yellow solid: mp 106−108 °C; ^{1}H NMR (DMSO-d_6) δ 10.60 (s, 1H, NH), 8.50 (d, J = 6.0 Hz, 1H, H-6), 6.82 (bs, 1H, H-1′), 6.39 (d, J = 6.0 Hz, 1H, H-3′), 5.98 (d, J = 6.0 Hz, 1H, H-2′), 5.19 (t, J = 4.8 Hz, 1H, OH), 4.89 (bs, 1H, H-4′), 3.68−3.65 (m, 2H, H-5′), 2.23 (s, 3H, CH_3); MS (FAB) *m/e* 270 (MH^+). Anal. ($C_{11}H_{12}FN_3O_4 \cdot H_2O$) H, N; C: calcd, 46.00; found, 46.38.

β/α-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-5-fluoro-*N*^{4}-isopropyl-2′-(phenylselenenyl)cytidine (18). To a solution of **2b**/**3b** (500 mg, 0.8 mmol) in pyridine at 0 °C were added 4-chlorophenyl dichlorophosphate (295 mg, 1.2 mmol) and 1,2,4-triazole (166 mg, 2.4 mmol). The reaction mixture was stirred at room temperature in an argon atmosphere for 3 days. After removal of the solvent by evaporation, the residue was treated with CH_2Cl_2 (25 mL). The organic phase was washed with water and saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The residue was dried under vacuum and dissolved in 1,4-dioxane (5 mL). Then isopropylamine (236 mg, 4 mmol) was added, and the reaction mixture was stirred at room temperature in an argon atmosphere overnight. After removal of the solvent, the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (98:2) to give **18** (340 mg, 64%) as a light-yellow foam: mp 64−65 °C; ^{1}H NMR ($CDCl_3$) δ 8.25−8.15, 7.85−7.80 (m, 1H, H-6), 7.67−7.26 (m, 15H, arom.), 6.20−6.05 (m, 1H, H-1′), 5.05−5.00 (m, 1H, NH), 4.60−3.50 (m, 5H, H-4′, H-5′, H-2′, CH), 2.70−2.00 (m, 2H, H-3′), 1.40−1.20 (m, 6H, 2 CH_3), 1.11−0.99 (m, 9H, *t*-Bu).

β-D-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-fluoro-*N*^{4}-isopropylcytidine (19). In an analogous manner to the preparation of **4a**, the title compound **19** was prepared from **18** (27% yield): colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 6.0 Hz, 1H, H-6), 7.66−7.64 (m, 4H, arom.), 7.45−7.39 (m, 6H, arom.), 7.06 (bs, 1H, H-1′), 6.13 (d, J = 6.0 Hz, 1H, H-3′), 5.96 (d, J = 6.0 Hz, 1H, H-2′), 5.05 (d, 1H, NH), 4.87 (bs, 1H, H-4′), 4.60−4.46 (m, 1H, CH), 3.97 (dd, J = 2.4 & 12.0 Hz, 1H, H-5a), 3.81 (dd, J = 3.2 & 11.2 Hz, 1H, H-5b), 1.26, 1.24 (2d, J = 3.2 Hz, 6H, 2 CH_3), 1.07 (s, 9H, *t*-Bu).

β-D-2′,3′-Didehydro-2′,3′-dideoxy-5-fluoro-*N*^{4}-isopropylcytidine (20). In an analogous manner to the preparation of **6a**, the title compound **20** was prepared from **19** (68% yield): white solid; mp > 200 °C; ^{1}H NMR (DMSO-d_6) δ 7.98 (d, J = 7.6 Hz, 1H, H-6), 7.84 (d, J = 8.4 Hz, 1H, NH), 6.83 (bs, 1H, H-1′), 6.31 (d, J = 6.0 Hz, 1H, H-3′), 5.87 (d, J = 4.0 Hz, 1H, H-2′), 5.06 (t, J = 4.8 Hz, 1H, OH), 4.77 (bs, 1H, H-4′), 4.24−4.18 (m, 1H, CH), 3.62−3.60 (m, 2H, H-5′), 1.15−1.13 (m, 6H, 2 CH_3); ^{13}C NMR (DMSO-d_6) δ 154.3, 154.2, 153.6, 137.3, 134.9, 134.3, 126.7, 125.0, 124.7, 90.1, 87.2, 62.0, 41.4, 21.8; MS (FAB) *m/e* 270 (MH^+). Anal. ($C_{12}H_{16}FN_3O_3$) C, H, N.

(±)-1-(Hydroxymethyl)-3-cyclopentene (22).[20] To a cold (−78 °C) solution of (±)-3-cyclopentenecarboxylic acid ethyl ester (**21**;[14,15] 7 g, 50 mmol) in dry THF (150 mL) was added $LiAlH_4$ (1 M solution in THF, 25 mL, 25 mmol), and the reaction solution was stirred at −78 °C in an argon atmosphere for 4 h. Then the reaction solution was allowed to warm to 0 °C, and 2.5 mL of water, 2.5 mL of 15% NaOH, and 7.5 mL of water were added sequentially. After warming to room temperature, the mixture was filtered through a Celite pad, and the Celite was washed with hot EtOAc. The combined filtrates were washed with 0.1 N NaOH and brine, dried ($MgSO_4$), filtered, concentrated, and dried in vacuo to give **22** (4.294 g, 84%) as a pale-yellow liquid: ^{1}H NMR ($CDCl_3$) δ 5.68 (s, 2H, 2 CH=CH), 3.57 (d, J = 6.0 Hz, 2H, CH_2OH), 2.54−2.48 (m, 3H, CH + CH_2), 2.15−2.10 (m, 2 H, CH_2).

***cis*-(±)-1,2-Epoxy-4-(hydroxymethyl)cyclopentane (23).** The title compound was prepared according to the literature method.[16]

***cis*-(±)-3-Acetoxy-5-(acetoxymethyl)cyclopentene (24).** To a solution of diphenyl diselenenide (2.70 g, 8.65 mmol) in anhydrous EtOH (100 mL) at room temperature was added $NaBH_4$ in portions. The solution was stirred until the yellow solution turned colorless, and then a solution of **23** (1.70 g, 14.4 mmol) in anhydrous THF (10 mL) was added. The

reaction solution was heated under reflux for 1 h in a nitrogen atmosphere, and the solvent was then evaporated in vacuo. To the residue were added EtOAc (80 mL) and water (30 mL). The organic phase was separated, washed with brine, dried ($MgSO_4$), filtered, concentrated, and dried in vacuo. The resulting (±)-1-hydroxy-4-(hydroxymethyl)-2-(phenylselenenyl)-cyclopentane (light-yellow oil) was used for the next reaction directly without further purification. To the crude product obtained above were added anhydrous CH_2Cl_2 (60 mL), Et_3N (30 mL, 216 mmol), and DMAP (50 mg). The resulting solution was cooled to 0 °C, and Ac_2O (14.7 g, 144 mmol) was added dropwise. After stirring at room temperature in an argon atmosphere overnight, evaporation of the solvent provided (±)-1-acetoxy-4-(acetoxymethyl)-2-(phenylselenenyl)cyclopentane as a light-yellow oil. To a cold (0 °C) solution of this oil in CH_2Cl_2 (50 mL) containing 3 drops of pyridine was added 30% H_2O_2 solution (20 mL) over a period of 5 min. After stirring at 0 °C for 30 min and at room temperature for another 30 min, the reaction mixture was diluted by addition of CH_2Cl_2 (50 mL). The organic phase was separated, washed with water, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated by evaporation in vacuo. The residue was purified by flash chromatography on silica gel eluting with 0−10% EtOAc in hexane to give **24** (2.254 g, 79%) as a pale-brown liquid: ^{1}H NMR ($CDCl_3$) δ 6.01−6.00, 5.92−5.90 (2m, 2H, CH=CH), 5.66−5.64 (m, 1H, H-3), 4.04 (d, J = 6.8 Hz, 2H, CH_2O), 2.98−2.92 (m, 1H, H-5), 2.53−2.46 (m, 1H, H-4a), 2.08, 2.04 (2s, 6H, 2 CH_3), 1.60−1.54 (m, 2H, H-4b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.9 (2s, 2 C=O), 137.0, 131.4 (2d, CH=CH), 79.2 (d, C-3), 67.4 (t, CH_2O), 43.7 (d, C-5), 33.4 (t, C-4), 21.3, 20.9 (2q, 2 CH_3).

***cis*-(±)-1-[4-(Acetoxymethyl)-2-cyclopenten-1-yl]-4-amino-5-fluoro-2(1*H*)-pyrimidinone (25a).** A suspension of 5-fluorocytosine (258 mg, 2 mmol) and NaH (58 mg, 2.4 mmol) in anhydrous DMSO (15 mL) was heated in a prewarmed oil bath at 70 °C for 30 min. The resulting solution was cooled to room temperature, and $Pd(PPh_3)_4$ (73 mg, 0.063 mmol) and a solution of **24** (298 mg, 1.5 mmol) in anhydrous THF (2 mL) were added. The reaction mixture was stirred at 70 °C in an argon atmosphere for 3 days. After removal of the solvent by evaporation in vacuo, the residue was treated with CH_2Cl_2 (50 mL). The mixture was filtered through a Celite pad, and the Celite was washed with CH_2Cl_2. The combined filtrates were concentrated, and the residue was purified by flash chromatography on silica gel eluting with 0−5% MeOH in CH_2Cl_2 to give **25a** (40 mg, 10%) as a light-brown solid. Recrystallization from MeOH/CH_2Cl_2/hexane provided the product as a white powder: R_f 0.56 ($CHCl_3$/MeOH, 95:5); mp 182−184 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (d, J = 6.0 Hz, 1H, H-6), 6.18−6.16 (m, 1H, H-3′), 5.83−5.81 (m, 1H, H-2′), 5.73−5.71 (m, 1H, H-1′), 4.23−4.21, 4.08−4.04 (2m, 2H, CH_2O), 3.14−3.12 (m, 1H, H-4′), 2.92−2.84, 1.41−1.35 (2m, 2H, CH_2), 2.08 (s, 3H, CH_3).

***cis*-(±)-4-Acetamido-1-[4-(acetoxymethyl)-2-cyclopenten-1-yl]-5-fluoro-2(1*H*)-pyrimidinone (25b).** In an analogous manner to the preparation of **25a**, the title compound **25b** was prepared from **24** and N^4-acetyl-5-fluorocytosine[17] (64% yield): brown oil; ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 1H, H-6), 6.19−6.17 (m, 1H, H-3′), 5.82−5.79 (m, 1H, H-2′), 5.75−5.73 (m, 1H, H-1′), 4.22−4.19, 4.07−4.03 (2m, 2H, CH_2O), 3.15−3.12 (m, 1H, H-4′), 2.92−2.88, 1.45−1.42 (2m, 2H, CH_2), 2.65, 2.07 (2s, 2 × 3H, 2 CH_3).

***cis*-(±)-4-Acetamido-1-[4-(acetoxymethyl)-2-cyclopenten-1-yl]-2(1*H*)-pyrimidinone (25c).** In an analogous manner to the preparation of **25a**, the title compound **25c** was prepared from **24** and N^4-acetylcytosine (27% yield): white powder; R_f 0.53 ($CHCl_3$/MeOH, 95:5); mp 169.5−171.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (bs, 1H, NH), 7.72 (d, J = 6.8 Hz, 1H, H-6), 7.39 (d, J = 6.8 Hz, 1H, H-5), 6.19−6.17 (m, 1H, H-3′), 5.86−5.81 (m, 1H, H-2′), 5.77−5.75 (m, 1H, H-1′), 4.17−4.13, 4.07−4.02 (2m, 2H, CH_2O), 3.18−3.10 (m, 1H, H-4′), 2.96−2.88, 1.43−1.37 (2m, 2H, CH_2), 2.27, 2.06 (2s, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$) δ 170.8, 162.0, 155.6, 145.3, 139.2, 130.0, 96.8, 66.3, 62.8, 44.2, 34.7, 25.0, 20.9.

***cis*-(±)-4-Amino-5-fluoro-1-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-2(1*H*)-pyrimidinone (26a).** NaOMe (0.5 M solution in MeOH, 0.5 mL) was added to a flask containing **25a** (33 mg, 0.12 mmol) at room temperature. The solution was stirred at room temperature for 1 h, and the solvent was then evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with 5−10% MeOH in CH_2Cl_2 to give **26a** (17 mg, 61%) as a light-brown solid. Recrystallization from MeOH/CH_2Cl_2/hexane provided the product as a white powder: mp 205.5−206.0 °C; ^{1}H NMR (DMSO-d_6) δ 7.66 (d, J = 6.0 Hz, 1H, H-6), 7.60, 7.40 (2bs, 2H, NH_2), 6.06−6.05 (m, 1H, H-3′), 5.68−5.65 (m, 1H, H-2′), 5.53−5.50 (m, 1H, H-1′), 4.77−4.75 (m, 1H, H-4′), 3.50−3.48, 3.41−3.37 (2m, 2H, CH_2O), 2.79−2.77, 1.34−1.27 (2m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 157.1, 156.9, 154.0, 139.2, 136.9, 134.6, 130.2, 126.8, 126.7, 63.5, 61.3, 47.2, 33.3; MS (FAB) *m/e* 226 (MH^+). Anal. ($C_{10}H_{12}FN_3O_2$) C, H, N.

In an analogous manner to the above procedure, the title compound **26a** was also prepared from **25b** (59% yield).

***cis*-(±)-4-Amino-1-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-2(1*H*)-pyrimidinone (26b).** In an analogous manner to the preparation of **26a**, the title compound **26b** was prepared from **25c** (90% yield): white solid; mp 200−201 °C; ^{1}H NMR (DMSO-d_6) δ 7.40 (d, J = 7.2 Hz, 1H, H-6), 7.03, 6.95 (2bs, 2H, NH_2), 6.07−6.05 (m, 1H, H-3′), 5.67 (d, J = 7.2 Hz, 1H, H-5), 5.65−5.64 (m, 1H, H-2′), 5.55−5.52 (m, 1H, H-1′), 4.71−4.68 (m, 1H, H-4′), 3.43−3.36 (m, 2H, CH_2O), 2.78−2.76, 1.24−1.18 (2m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 165.5, 155.8, 142.2, 138.6, 130.5, 93.7, 63.9, 60.8, 47.3, 34.0; MS (FAB) *m/e* 208 (MH^+). Anal. ($C_{10}H_{13}N_3O_2$) H, N; C: calcd, 57.96; found, 57.35. HRMS (FAB) calcd for ($C_{10}H_{14}N_3O_2$): 208.1086, found 208.1088.

***cis*-(±)-4-Amino-5-fluoro-1-[4-(triphosphonooxymethyl)-2-cyclopenten-1-yl]-2(1*H*)-pyrimidinone, Triethylammonium Salt (27a).** To a solution of **26a** (10 mg) in anhydrous DMF (0.3 mL) and pyridine (0.1 mL) was added a 1 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one in anhydrous 1,4-dioxane (0.05 mL). The resulting solution was stirred at room temperature for 15 min. A solution of 1 M pyrophosphoric acid−Bu_3N in anhydrous DMF (0.12 mL) and Bu_3N (0.05 mL) were then added sequentially. After the mixture was stirred at room temperature for another 15 min, a solution of I_2/H_2O/pyridine/THF was added to the above solution dropwise until the iodine color persisted (about 0.5 mL), and the mixture was concentrated by evaporation in vacuo. The residue was dissolved in water (2 mL), washed with CH_2Cl_2 (3 × 1 mL), filtered, and purified by FPLC (column, HiLoad 26/10 Q Sepharose Fast Flow; buffer A, 0.01 M Et_3NHCO_3; buffer B, 0.7 M Et_3NHCO_3; flow rate, 10 mL/min; gradient, starting from 90% A and 10% B, changing to 100% B from 4 to 64 min, keeping this ratio till 70 min; monitor, UV-M). Collection and lyophilization of the appropriate fractions afforded **27a** as a colorless syrup. The triphosphate was characterized by HPLC analysis by comparison of the retention time and enzyme assays (see below) to commercially available triphosphates of nucleotides, using the following conditions: column, 100 × 4.6 mm Rainin Hydropore SAX ionic exchange; buffer A, 10 mM $NH_4H_2PO_4$ in 10% MeOH/H_2O (pH 5.5); buffer B, 125 mM $NH_4H_2PO_4$ in 10% MeOH/H_2O (pH 5.5); flow rate, 1.0 mL/min; gradient, increasing B from 0% at the beginning to 100% at 25 min. The retention time for the triphosphate was 11.9 min. MS (FAB) *m/e* 464 ($[M - H]^+$).

***cis*-(±)-4-Amino-1-[4-(triphosphonooxymethyl)-2-cyclopenten-1-yl]-2(1*H*)-pyrimidinone, Triethylammonium Salt (27b).** In an analogous manner to the preparation of **27a**, the title compound **27b** was prepared from **26b**. HPLC (same conditions as above) retention time: 12.1 min. MS (FAB) *m/e* 446 ($[M - H]^+$).

Antiviral and Cytotoxicity Assays. Anti-HIV-1 activity of the compounds was determined in PBM cells as described previously.[19] Stock solutions (40 mM) of the new compounds were prepared in sterile DMSO and then diluted to the desired concentration in growth medium. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantitated on day 6

after infection by a reverse transcriptase assay using $(rA)_n \cdot (dT)_{12-18}$ as template-primer. The DMSO present in the diluted solution (<0.1%) had no effect on the virus yield. The toxicity of the compounds was assessed in human PBM and Vero cells, as described previously.[19] The antiviral EC_{50}, EC_{90}, and cytotoxicity IC_{50} were obtained from the concentration−response curve using the median effective method described previously.[21,22] The anticancer activity of the compounds was assessed in CEM (human lymphoblastoid), LNCaP (human metastatic prostate adenocarcinoma), MCF-7 (human breast adenocarcinoma), SK-MES-1 (human lung carcinoma), SK-MES-28 (human skin melanoma), PC-3 (human prostate adenocarcinoma), HepG2 (human hepatocellular carcinoma), and 9L (rat glioma) cells. Appropriate numbers of cells were cultured with the drug for a specific number of days in 96-well plates (LNCaP and SK-MES-1: 5 days; CEM, MCF-7, SK-MES-28, HepG2, and 9L: 4 days; PC-3: 3 days). 5-Fluorouracil and cycloheximide were included as positive cytotoxic controls, and untreated cells exposed to solvent were included as negative controls. After incubation, actively metabolizing cells were quantified using the CellTiter 96 Cell Proliferation Assay (MTT, Promega, Madison, WI), as described by the manufacturer.

Effect of the Carbocyclic Nucleoside Triphosphates against HIV-1 Reverse Transcriptase. Extension assays were performed using a $r(I)_n \cdot (dC)_{12-18}$ homopolymer template-primer (Pharmacia, Piscataway, NJ) and the HIV-1 heterodimer p66/51 RT (Biotechnology General, Rehovot, Israel). The standard reaction mixture (100 μL) contained 100 μM Tris hydrochloride (pH 8.0), 50 μM KCl, 2 μM $MgCl_2$, 0.05 units/mL $r(I)_n \cdot (dC)_{12-18}$, 5 μM DTT, 100 μg/mL bovine serum albumin, and 1 μM [^{3}H]dCTP (23 Ci/mmol). 3TC-TP (0.001−50 μM) was the positive control. Compounds were incubated for 1 h at 37 °C in the reaction mixture with 1 unit of HIV-1 RT. The reaction was stopped with the addition of an equal volume of cold 10% TCA/0.05% sodium pyrophosphate and incubated for 30 min at 4 °C. The precipitated nucleic acids were harvested onto fiberglass filter paper using a Packard manual harvester (Meriden, CT). The radiolabel uptake in counts per minute (cpm) was determined using a Packard 9600 Direct Beta counter.

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