5′-O-Phosphonomethyl-2′,3′-dideoxynucleosides: Synthesis and Anti-HIV Activity

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5′-O-Phosphonomethylation of different pyrimidine 2′,3′-dideoxynucleosides was accomplished by reaction of the latter with diethyl [(*p*-tolylsulfonyl)oxy]methanephosphonate (1) in the presence of sodium hydride. The base-phosphonomethylated (**15–19**) and sugar-phosphonomethylated (**8–12**) derivatives could be readily distinguished by ^1H and ^{13}C NMR and MS analysis. Protection of the uracil or thymine residue with a N^3-benzoyl group failed to prevent base modification. However, O^4-methyl-protected 2′,3′-dideoxyuridine readily afforded the 5′-O-phosphonomethylated derivative **12**, which was converted to both the 2′,3′-dideoxyuridine analogue **27** and the 2′,3′-dideoxycytidine counterpart **29**. The 5′-*O*-phosphonomethyl derivatives of 3′-deoxythymidine (**23**), 2′,3′-dideoxyuridine (**27**), 2′,3′-dideoxycytidine (**29**), 3′-*O*-methylthymidine (**26**), and 3′-amino-3′-deoxythymidine (**28**) did not show an appreciable anti-HIV activity in MT-4 cells. In contrast, the 5′-*O*-phosphonomethyl derivatives of 3′-deoxy-3′-fluorothymidine (**24**) and 3′-azido-3′-deoxythymidine (**25**) inhibited HIV-1 cytopathogenicity by 50% at a concentration of approximately 1 μM.

Introduction

Since the discovery of the broad spectrum anti-DNA virus activity of HPMPA [(*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine] by De Clercq and co-workers,[1] phosphonylated nucleoside analogues have attracted considerable interest. Among the phosphonylated acyclic nucleoside analogues, HPMPC [(*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine] is a highly promising agent for the treatment of cytomegalovirus infections[2] and so is PMEA [9-[2-phosphonomethoxy)ethyl]adenine] for the treatment of retrovirus infections.[3] The oxygen atom of the phosphonomethoxy group seems to be imperative since phosphonate analogues missing this linkage show much less activity.[4–6]

Phosphonate analogues of dideoxynucleosides,[7] including azidothymidine (AZT),[8,9] have been reported, but these compounds lack the anti-HIV activity of the parent nucleosides, although the interatomic distance between the base part and the phosphorus atom is respected. These results can be explained either by lack of cellular uptake or inefficient intracellular phosphorylation to the corresponding triphosphate derivatives, should this be necessary for antiviral activity.

As some phosphonomethoxy derivatives of acyclic nucleosides (i.e. PMEA) show significant anti-retrovirus activity, we focused our efforts on the synthesis of phosphonomethoxy analogues of various dideoxynucleosides which have proved to be effective anti-HIV agents.

Chemistry

All 5′-*O*-methanephosphonic acid derivatives were synthesized according to the procedure originally reported by Holy.[10] This means reaction of diethyl [(*p*-tolylsulfonyl)oxy]methanephosphonate (1), prepared by starting from formaldehyde,[10,11] with the respective 2′,3′-dideoxynucleosides in the presence of sodium hydride, affording the diethyl 5′-*O*-methanephosphonates, followed by cleavage of the phosphonic ester function.

3′-Deoxythymidine (ddThd, **2**), 2′,3′-dideoxyuridine (ddUrd, **3**), and 2′,3′-dideoxycytidine (ddCyd) were prepared from the corresponding 2′-deoxynucleosides according to a well established procedure.[12] However, the benzoyl group was used instead of the acetyl group for protection of the 5′-O-position since selective 5′-O-benzoylation is more straightforward than selective 5′-O-acetylation.[13] 3′-Deoxy-3′-fluorothymidine (FddThd, **4**),[14] 3′-azido-3′-deoxythymidine (AzddThd, **5**),[15] and 3′-*O*-methylthymidine (**6**)[14,16] were prepared according to published procedures. Reaction of 5′-O-benzoylated **3** with 1-methylimidazole in the presence of phosphorous oxychloride followed by treatment with triethylamine and methanol according to the procedure of Matsuda et al.,[17] and 5′-deprotection with a mixture of concentrated aqueous ammonia and methanol, afforded the base methylated 2′,3′-dideoxyuridine (**7**) in 40% yield. N^3-Benzoyl-3′-deoxy-3′-fluorothymidine (**20**) was prepared in 93% yield by reaction of 5′-O-tritylated **4** with benzoyl chloride in the presence of diisopropylethylamine, followed by detritylation with *p*-toluenesulfonic acid in $CHCl_3$–MeOH (4:1).

The synthesis of the 5′-*O*-methanephosphonates is depicted in Figure 1. By following Holy's procedure, using a molar ratio of ddThd, the tosylate **1** and sodium hydride of 1:1:2, the reaction was found to take place mainly on

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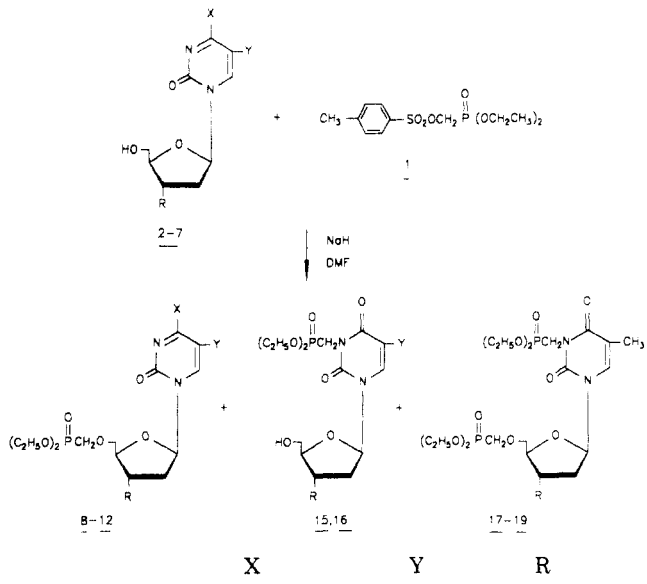

	X	Y	R
2	OH	CH_3	H
3	OH	H	H
4	OH	CH_3	F
5	OH	CH_3	N_3
6	OH	CH_3	OCH_3
7	OCH_3	H	H
8	OH	CH_3	H
9	OH	CH_3	F
10	OH	CH_3	N_3
11	OH	CH_3	OCH_3
12	OCH_3/OC_2H_5	H	H
15	-	CH_3	H
16	-	H	H
17	-	-	H
18	-	-	N_3
19	-	-	OCH_3

Figure 1.

the base moiety to yield **15** as the major product. With an excess of **1** and sodium hydride (3 equiv each), 33% of the sugar phosphonomethylated dideoxynucleoside **8** was isolated, together with 6% of bis-phosphonylated product **17**.

Both nucleoside derivatives **8** and **15** can be easily distinguished from each other by NMR, MS, and UV spectra. The mass spectrum of compound **15** reveals the presence of the base moiety with a [(diethylphosphono)methyl]-methyl group (*m/e* 276), but a phosphonomethylated sugar moiety as in **8** (*m/e* 251) is not detectable. UV spectrometric analysis shows the maximum absorption at higher wavelength (270 nm) for **15** compared to **8** (268 nm). ^{13}C NMR analysis shows distinctive differences for both compounds. For compound **15**, the resonance of C-5′ appears at 62.4 ppm at the expected position for C-5′ unsubstituted nucleosides, while for compound **8**, the C-5′ signal has moved downfield to 74.1 ppm and is split into a doublet by phosphorus ($J_{P,C}$ = 7.4 Hz). Furthermore, the chemical shifts and coupling constants of the methylene groups α to the phosphorus are distinctively different. In the ^1H NMR, this signal of **8** (3.88 ppm, $J_{P,H}$ = 7.7 Hz) is found at higher field and has a smaller coupling constant than the analogous signal for the base-phosphonomethylated product **15** (4.41 ppm, $J_{P,H}$ = 12.3 Hz). In the ^{13}C NMR, this signal of **8** is found at 65.3 ppm with a coupling constant of $J_{P,C}$ = 166 Hz, while that of **15** is at 36.1 ppm with a coupling constant of $J_{P,C}$ = 156.3 Hz. From these UV and NMR data the phosphonomethyl group of **15** is assumed to be at N^3. The NMR spectra for the bis-phosphonomethylated nucleoside **17** show both α-methylene signals.

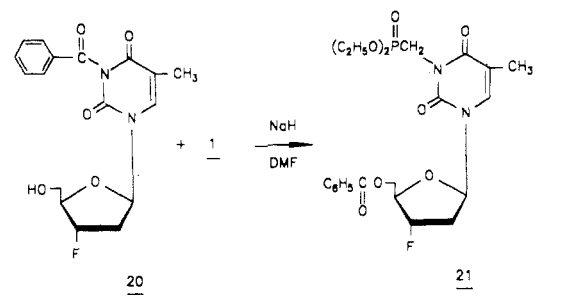

Figure 2.

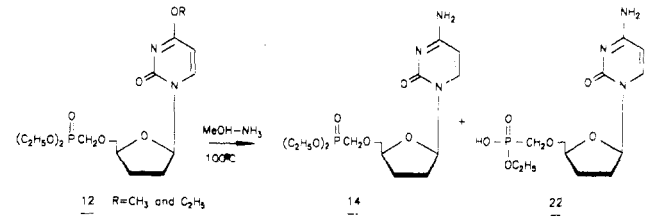

Figure 3.

The 5′-*O*-methanephosphonates of **4**, **5**, and **6** were synthesized by the same procedure affording **9**, **10**, and **11** in 76%, 50%, and 56% yield, respectively. The corresponding 5′-*O*,N^3-bismethanephosphonates **18** and **19** were also isolated in 14% and 6% yield, respectively. All 5′-*O*-methanephosphonates and 5′-*O*,N^3-bismethanephosphonates share the same spectral characteristics with the phosphonates (**8** and **17**) from ddThd.

Protection of the thymidine analogues with a N^3-benzoyl group to improve the yield of the desired 5′-*O*-methanephosphonates was unsuccessful. When N^3-benzoyl-3′-fluoro-3′-deoxythymidine (**20**) was reacted with **1**, a benzoyl migration to the 5′-position occurred with the formation of 5′-*O*-benzoyl-N^3-[(diethylphosphono)methyl]-3′-fluoro-3′-deoxythymide (**21**) as the major isolated product (Figure 2).

Whereas a change of the ratio of the reagents altered the site of phosphonomethylation for ddThd, phosphonomethylation of ddUrd always afforded N^3-[(diethylphosphono)methyl]-2′,3′-dideoxyuridine (**16**) as the major product, no matter how much of the tosylate **1** and of sodium hydride were used (1:1.2:3 or 1:3:3). Increasing the amount of **1** and NaH resulted only in a higher yield of **16** and lesser recovery of the starting material **3**. Therefore, it seemed mandatory to protect the base moiety of ddUrd.

Reaction of **7** with the tosylate **1** afforded the 5′-*O*-(diethylphosphono)methyl derivative **12** in 44% yield, while no base-phosphonomethylated product could be detected. However, according to NMR, the methyl group protecting the base had been largely replaced by an ethyl group. This could be the result of a nucleophilic exchange with ethanolate, formed under the basic NaH conditions. The methyl and ethyl base-protected derivatives were difficult to separate and the mixture was used as such for the hydrolysis, affording **27** as an anomeric mixture, as will be explained below.

Reaction of ddCyd with **1** gave a complex mixture under the reaction conditions described. Therefore, the phosphonate **14** was obtained from the uridine analogue **12**. As shown in Figure 3, treatment of **12** with ammonia-saturated methanol at 100 °C[18] afforded a mixture of the

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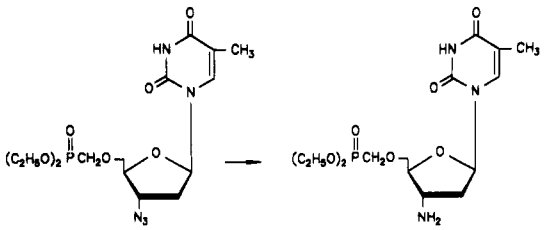
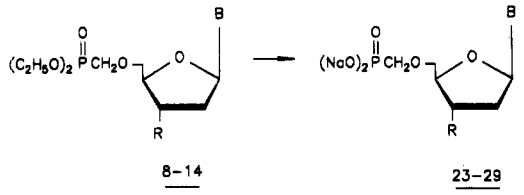


Figure 4.



	B	R
23	Thy	H
24	Thy	F
25	Thy	N_3
26	Thy	OCH_3
27	Ura	H
28	Thy	NH_2
29	Cyt	H

Figure 5.

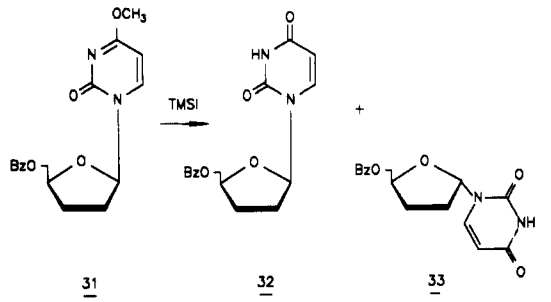


Figure 6.

monoester **22** and the diester **14** in a ratio of 4:1, respectively.

The phosphonate **13** was prepared from its 3'-azido counterpart **10** (Figure 4) by reduction of the azido group with triphenylphosphine followed by treatment with aqueous ammonia.[19]

Deesterification of the phosphonates to the corresponding phosphonic acids **23–29** was achieved by reaction with trimethylsilyl iodide (TMSI) in DMF[10] or, alternatively, with trimethylsilyl chloride in the presence of sodium iodide in acetonitrile[20] (Figure 5). Because of the low solubility in acetonitrile, the phosphonic monoester of ddCyd (**22**) could be hydrolyzed only by the first method (trimethylsilyl iodide in DMF). All hydrolyzed products were purified by ion-exchange chromatography and HPLC.

As mentioned before, the phosphonate **27** proved to be a mixture of the α and β dideoxyuridine derivatives, as shown by NMR. Reaction of the base-protected dideoxyuridine analogue **31** with TMSI likewise afforded **32** and **33** as an anomeric mixture (Figure 6). The structure of the latter two compounds was firmly established by UV, MS, and NMR analysis.

The triphosphate analogue **30** (Figure 7) was prepared from **24** following the procedure of Hoard and Ott[21] with

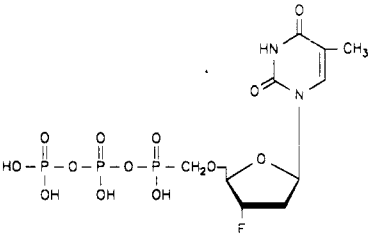


Figure 7.

Table I. Potency and Selectivity of 5'-Phosphonomethyl Derivatives of 2',3'-Dideoxynucleosides as Inhibitors of HIV-1 Replication in MT-4 Cells

compound	EC_{50},[a] μM	CC_{50},[b] μM	SI[c]
22	≥500	>500	-
23	500	>500	>1
24	0.90 ± 0.25	17.3 ± 16	19
25	1.28 ± 0.14	161 ± 40	125
26	500	>500	>1
27	90	>500	>5
28	≥500	>500	≥1
29	≥500	>500	≥1
30	100	>500	>5
FddThd	0.001[d]	0.197[d]	197
AzddThd	0.003	4.8	1600

[a] Effective concentration, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV-1. [b] Cytotoxic concentration, required to reduce the viability of normal uninfected MT-4 cells by 50%. [c] Selectivity index, or ratio of CC_{50} to EC_{50}. [d] Data taken from ref 28.

1,1'-carbonyldiimidazole and pyridinium pyrophosphate and was isolated as the sodium salt.

Antiviral Activity

The 5'-triphosphate derivative of 3'-amino-3'-deoxythymidine (AmddThd) is one of the compounds endowed with a strong affinity for the reverse transcriptase.[22] However, AmddThd by itself does not inhibit HIV replication in vitro at subtoxic concentrations.[23,24] Moreover it displays a high toxicity to human cells.[25] The reason for its inactivity against HIV is still unclear. Although structurally related to the very active 3'-azido-3-deoxythymidine (AzddThd) and 3'-deoxy-3'-fluorothymidine (FddThd), several 2',3'-dideoxynucleosides are devoid of any marked anti-HIV activity (i.e. 2',3'-dideoxyuridine[26]). A detailed study revealed that ddUrd is not recognized as a substrate for cytosol dThd kinase and not significantly phosphorylated to its 5'-triphosphate catabolite.[27] It was anticipated that introduction of a 5'-phosphonomethyl moiety in the 2',3'-dideoxynucleosides would circumvent the first activation step and lead to compounds with high anti-HIV activity.

This premise was not borne out for the 5'-phosphonates of ddUrd (**27**), ddThd (**23**), 3'-O-methylthymidine (**26**), and

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Table II. Inhibitory Effects of Compound **30** and Other 5′-Triphosphate Derivatives on MLV- and HIV-Associated Reverse Transcriptase Activity

compound	50% inhibitory concentration, μM	
	MLV	HIV
α,β-methylene AzddTTP	>200[a]	4.6 ± 1.1[a]
β,γ-methylene AzddTTP	156[a]	7.9 ± 0.2[a]
AzddTTP	1.15 ± 0.4[a]	0.023 ± 0.004[a]
FddTTP	35.6 ± 12	0.33 ± 0.28
30	-	322 ± 60

[a] Data taken from ref 29.

AmddThd (**28**) which did not achieve any inhibition of the cytopathogenic effect of HIV. Only **27** was endowed with marginal activity, while its parent compound ddUrd is less effective[26,27] (Table I).

The phosphonomethyl derivatives of FddThd (**24**) was AzddThd (**25**) were the only compounds that proved to be active against HIV (EC_{50} ca. 1 μM). However, the compounds were about 1000-fold less active in the MT-4 cell system than the parent compounds. This could be explained by a reduction in their cellular uptake, lower phosphorylation to their 5′-triphosphate derivatives or diminished affinity of their active (diphosphorylated) forms for the target enzyme (reverse transcriptase). In fact, the diphosphate derivative of 5′-*O*-(phosphonomethyl)-3′-deoxy-3′-fluorothymidine (**24**) showed a 1000-fold reduction in its affinity for HIV reverse transcriptase, as compared to the triphosphate of FddThd). A similar observation has been made for the α,β- and β,γ-methylene 5′-phosphonate derivatives of AzddThd triphosphate, which also show a 200-fold reduction in affinity for the HIV reverse transcriptase compared to AzddThd triphosphate[29] (Table II). Likewise, compound **30** had no inhibitory effect on the murine leukemia virus (MLV) associated reverse transcriptase, although it should be mentioned that the triphosphate of FddThd itself (FddTTP) has a rather low affinity for this reverse transcriptase, as has been observed before.[30]

The complete lack of activity and of toxicity of the ddCyd phosphonate **29**, which contrasts with the potent anti-HIV activity (and cytotoxicity) of the parent compound ddCyd, could be explained by an inefficient intracellular phosphorylation of the compound in MT-4 cells. Thymine nucleosides are known to be better phosphorylated in MT-4 cells than cytosine nucleosides. Likewise, the monoethyl ester **22** was devoid of any activity. In fact, enzymatic tests showed that the compound was not hydrolyzed by phosphodiesterase (data not shown). This is in contrast with a previous report, where methylene phosphonates of dideoxynucleosides were shown to be deesterified with the phosphodiesterase of *Crotalus atrox*.[7]

Experimental Section

All methods and reagents were essentially as previously described.[14,31] Electron-impact mass spectra were obtained by direct insertion on an AEI MS-12 mass spectrometer at 8-kV accelerating voltage, 100-μA trap current, and 70-eV ionization energy; B = base fragment, S = sugar fragment, P = (diethylphosphono)methyl fragment. (Diethylamino)ethyl cellulose (DEAE) was used for ion-exchange chromatography and a triethylammonium bicarbonate (TEAB) gradient (pH 7.5) was used for elution. Reversed-phase HPLC was performed on a C18 (0.15 9m) column and a UV detector was used to monitor the eluent. Conversion of the phosphonic acids to their sodium salts was done by passing a solution through a column filled with CG-50-I (Na^+) resin.

1-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-4-methoxypyrimidin-2(1*H*)-one (7). 5′-*O*-Benzoyl-2′,3′-dideoxyuridine[12] (3.2 g, 10 mmol) in 50 mL of anhydrous acetonitrile was added to a mixture of 2.8 mL (30 mmol) of phosphoryl chloride and 8 mL (100 mmol) of 1-methylimidazole in 200 mL of anhydrous acetonitrile at 0 °C. The reaction mixture was stirred at room temperature for 2 h. After the mixture was cooled in an ice bath, 13.5 mL of triethylamine and 50 mL of methanol were added. The mixture was stirred overnight at room temperature and evaporated. The residue was dissolved in chloroform, washed twice with a 5% aqueous solution of sodium bicarbonate, dried, evaporated, and purified by silica gel column chromatography eluting with chloroform–acetone (98:2). The 5′-*O*-benzoylated 7 was treated with 30 mL of ammonia (33%) in 170 mL of methanol for 30 h at room temperature. The reaction mixture was evaporated and coevaporated with ethanol. Purification by silica gel column chromatography (60 g) eluting with chloroform followed by chloroform–methanol (97:3 and 95:5), yielded 900 mg (4 mmol, 40%) of the title product: UV (MeOH) λ_{max} 276 nm; ^1H NMR (CD_3OD) δ 1.93–2.44 (m, H-2′, H-2″, H-3′, H-3″), 3.33–3.77 (m, H-4′, H-5′, H-5″), 3.90 (s, OCH_3), 4.80 (br, OH), 6.02 (d, J = 7.9 Hz, H-5), 6.06 (m, H-1′), 8.45 (d, J = 7.9 Hz, H-6) ppm; ^{13}C NMR (CD_3OD) δ 25.4 (C-3′), 34.4 (C-2′), 54.8 (OCH_3), 63.4 (C-5′), 84.2 (C-4′), 89.2 (C-1′), 96.2 (C-5), 145.1 (C-6), 158.3 (C-2), 173.6 (C-4) ppm.

***N*^{3}-Benzoyl-3′-deoxy-3′-fluorothymidine (20).** 5′-*O*-Monomethoxytrityl-3′-deoxy-3′-fluorothymidine[5] (0.86 g, 1.61 mmol) was coevaporated twice with anhydrous pyridine and dissolved in 35 mL of dry pyridine. To this solution was added 0.5 mL (3 mmol) of *N*,*N*-diisopropylethylamine and 0.5 mL (4 mmol) of benzoyl chloride. The mixture was allowed to react at 4 °C overnight. After the pyridine was partially removed in vacuo, the reaction mixture was quenched with a 5% aqueous sodium bicarbonate solution and extracted with ether. The ether solution was dried (Na_2SO_4) and evaporated to give crude N^3-benzoyl-5′-*O*-monomethoxytrityl-3′-deoxy-3′-fluorothymidine. The crude product was treated at room temperature with a solution of 3.8 g (20 mmol) *p*-toluenesulfonic acid monohydrate (3.8 g, 20 mmol) in chloroform (80 mL) and methanol (20 mL). After stirring for 15 min, the mixture was neutralized with a saturated aqueous solution of sodium bicarbonate to pH 8 and evaporated. The residue was dissolved in ethyl acetate and washed with brine. After purification on silica gel (40 g) which was eluted with chloroform followed by chloroform–methanol (97:3), the title product was obtained in 93% yield (0.52 g, 1.5 mmol): UV (MeOH) λ_{max} 253 nm; ^1H NMR ($CDCl_3$) δ 1.92 (s, CH_3), 2.0–2.8 (m, H-2′, H-2″), 3.80 (m, H-5′, H-5″), 4.28 (dm, $J_{F,H}$ = 27 Hz, H-4′), 5.23 (dm, $J_{F,H}$ = 54 Hz, H-3′), 6.25 (dd, H-1′), 7.35–8.0 (m, arom-H and H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 12.4 (CH_3), 38.1 ($J_{F,C}$ = 20.7 Hz, C-2′), 62.0 ($J_{F,C}$ = 12.2 Hz, C-5′), 85.4 ($J_{F,C}$ = 24.2 Hz, C-4′), 86.5 (C-1′), 94.2 ($J_{F,C}$ = 177 Hz, C-3′), 111.0 (C-5), 129.0, 130.2, 131.4, 134.9 (arom-C), 136.4 (C-6), 149.3 (C-2), 162.7 (C-4), 168.7 (CO) ppm.

5′-*O*-[(Diethylphosphono)methyl]-3′-deoxythymidine (8) and *N*^{3},5′-*O*-Bis[(diethylphosphono)methyl]-3′-deoxythymidine (17). A mixture of 1 g (4.4 mmol) of 3′-deoxythymidine **2**[12] and 320 mg (13.2 mmol) of sodium hydride in 45 mL of anhydrous DMF was stirred under N_2 for 30 min. To this suspension was added 4.26 g (13.2 mmol) of diethyl [(*p*-tolylsulfonyl)oxy]methanephosphonate **1**,[10] dissolved in 2 mL of anhydrous DMF. The reaction mixture was stirred at room temperature for 65 h, neutralized with 0.7 mL (13 mmol) of acetic acid, and evaporated in vacuo. The residual oil was partitioned between chloroform (100 mL) and water (25 mL). The organic layer was separated, dried and evaporated. Purification by column chromatography ($CHCl_3$–MeOH 99:1) followed by preparative TLC ($CHCl_3$–MeOH 95:5) yielded 540 mg (1.44 mmol, 33%) of 5′-*O*-[(diethylphosphono)methyl]-3′-deoxythymidine (**8**) as an oil: R_f 0.42, ($CHCl_3$–MeOH 9:1); UV (MeOH) λ_{max} 268 nm; MS (*m*/*e*) 376 (M^+), 251 (S + $P^•$, 100), 125 ($B^•$); ^1H NMR ($CDCl_3$) δ 1.32

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(t, J = 7.0 Hz, $POCH_2CH_3$), 1.95 (s, CH_3), 1.95–2.4 (m, H-2', H-2'', H-3', H-3''), 3.75–4.02 (m, H-4', H-5', H-5''), 3.88 (d, J = 7.7 Hz, OCH_2P), 4.18 (dq, J = 8.3 and 7.0 Hz, $POCH_2CH_3$), 6.11 (dd, H-1'), 7.56 (s, H-6), 9.1 (br, NH); ^{13}C NMR ($CDCl_3$) δ 12.3 (CH_3), 16.3 ($J_{P,C}$ = 6.3 Hz, $POCH_2CH_3$), 25.5 (C-3'), 32.0 (C-2'), 62.1 ($J_{P,C}$ = 9.8 Hz, $POCH_2CH_3$), 65.3 ($J_{P,C}$ = 166.0 Hz, OCH_2P), 74.1 ($J_{P,C}$ = 7.4 Hz, C-5'), 79.2 (C-4'), 85.6 (C-1'), 110.3 (C-5), 135.6 (C-6), 150.3 (C-2), 163.9 (C-4) ppm.

Likewise, 130 mg (0.25 mmol, 5.7%) of the title product **17** was isolated: R_f 0.49 ($CHCl_3$–MeOH 9:1); UV (MeOH) λ_{max} 270 nm; MS (m/e) 526 (M^+), 277 (B + P + 2H$^+$), 276 (B + P + 1 H$^+$), 251 (S + P$^+$, 100); ^1H NMR ($CDCl_3$) δ 1.32 (t, $POCH_2CH_3$), 1.98 (s, CH_3), 1.9–2.45 (m, H-2', H-2'', H-3', H-3''), 3.88 (d, J = 7.7 Hz, OCH_2P), 3.75–4.02 (m, H-4', H-5', H-5''), 4.18 (dq, $POCH_2CH_3$), 4.41 (d, J = 12.5 Hz, NCH_2P), 6.10 (dd, H-1'), 7.50 (s, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 13.1 (CH_3), 16.2 and 16.6 ($J_{P,C}$ = 6.1 Hz, 2 × $POCH_2CH_3$), 25.2 (C-3'), 32.3 (C-2'), 36.3 ($J_{P,C}$ = 155 Hz, NCH_2P), 62.3 and 62.5 ($J_{P,C}$ = 6.1 Hz, 2 × $POCH_2CH_3$), 65.8 ($J_{P,C}$ = 166.7 Hz, OCH_2P), 73.9 ($J_{P,C}$ = 8.6 Hz, C-5'), 79.6 (C-4'), 86.7 (C-1'), 109.2 (C-5), 133.9 (C-6), 150.2 (C-2), 162.5 (C-4) ppm.

N^3-[(Diethylphosphono)methyl]-3'-deoxythymidine (15). A mixture of 0.45 g (2 mmol) of **2**[12] and 0.65 g (2 mmol) of the tosylate **1**[10] in DMF was stirred in the presence of 96 mg (4 mmol) NaH at room temperature for 65 h. The reaction mixture was worked up in the same manner as described for **8**, yielding 120 mg (0.32 mmol, 16%) of **15**: R_f 0.35 ($CHCl_3$–MeOH 9:1), UV (MeOH) λ_{max} 270 nm; MS (m/e) 376 (M^+), 276 (B + P + H$^+$, 100), 101 (S$^+$); ^1H NMR ($CDCl_3$) δ 1.32 (t, J = 7.0 Hz $POCH_2CH_3$), 1.92 (s, CH_3), 1.95–2.5 (m, H-2', H-2'', H-3', H-3''), 3.62–4.02 (m, H-4', H-5', H-5''), 4.18 (dq, $POCH_2CH_3$), 4.41 (d, J = 12.3 Hz, NCH_2P), 6.10 (dd, 1 H, H-1'), 7.77 (s, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 12.8 (CH_3), 16.5 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 24.7 (C-3'), 32.3 (C-2'), 36.1 ($J_{P,C}$ = 156.3 Hz, NCH_2P), 62.4 (C-5'), 62.7 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 81.6 (C-4'), 87.0 (C-1'), 108.7 (C-5), 134.5 (C-6), 150.1 (C-2), 162.5 (C-4) ppm.

An amount of 160 mg (0.71 mmol, 35%) of the starting material **2** was recovered.

N^3-[(Diethylphosphono)methyl]-2',3'-dideoxyuridine (16). To the mixture of 160 mg (0.5 mmol) of **3** and 36 mg (1.5 mmol) of NaH in 5 mL of anhydrous DMF was added 483 mg (1.5 mmol) of **1**. The reaction mixture was stirred at room temperature for 65 h and worked up as described for **8**. The crude product was purified on silica gel ($CHCl_3$–MeOH 98:2, 96:4) followed by preparative TLC ($CHCl_3$–MeOH 95:5), which gave 52 mg (0.15 mmol, yield 29%) of the title compound: UV (MeOH) λ_{max} 264 nm; MS (m/e) 362 (M^+); ^1H NMR ($CDCl_3$) δ 1.33 (t, J = 7.0 Hz, $POCH_2CH_3$), 1.8–2.5 (m, H-2', H-2'', H-3', H-3''), 4.18 (dq, J = 8.4 and 7.0 Hz, $POCH_2CH_3$), 4.0–4.3 (m, H-4', H-5', H-5'', $POCH_2CH_3$), 4.39 (d, J = 12.7 Hz, NCH_2P), 5.74 (d, J = 8.2 Hz, H-5), 6.05 (dd, H-1'), 7.91 (d, J = 8.2 Hz, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 16.1 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 24.5 (C-3'), 32.8 (C-2'), 35.5 ($J_{P,C}$ = 155 Hz, NCH_2P), 62.6 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 62.8 (J = 8.5 Hz, C-5'), 81.9 (C-4'), 87.3 (C-1'), 100.5 (C-5), 138.6 (C-6), 150.3 (C-2), 161.8 (C-4) ppm.

5'-O-[(Diethylphosphono)methyl]-3'-deoxy-3'-fluorothymidine (9). A mixture of 0.98 g (4.02 mmol) of **4**[14] and 3.62 g (11.6 mmol) of **1**[10] in 35 mL of anhydrous DMF was stirred in the presence of 340 mg (14 mmol) of sodium hydride for 3 days at room temperature. The reaction mixture was neutralized with acetic acid and evaporated. The residue was purified by column chromatography ($CHCl_3$–MeOH 98:2 and 95:5), followed by preparative TLC ($CHCl_3$–acetone 8:2), yielding 1.2 g (3 mmol, 76%) of the title compound as an oil: UV (MeOH) λ_{max} 266 nm; MS (m/e) 394 (M^+); ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7.0 Hz, $POCH_2CH_3$); 1.96 (s, CH_3), 2.02–2.64 (m, H-2', H-2''), 3.75–4.05 (m, H-5', H-5'', OCH_2P), 4.20 (dq, J = 8.4 and 7.0 Hz, $POCH_2CH_3$), 4.37 (dm, $J_{F,H}$ = 27.2 Hz, H-4'), 5.27 (dm, $J_{F,H}$ = 54.0 Hz, H-3'), 6.44 (dd, H-1'), 7.49 (s, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 12.2 (CH_3), 16.3 ($J_{P,C}$ = 4.9 Hz, $POCH_2CH_3$), 38.0 ($J_{F,C}$ = 22.0 Hz, C-2'), 62.3 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 65.5 ($J_{P,C}$ = 168.4 Hz, OCH_2P), 72.7 (dd, J = 9.8 and 8.0 Hz, C-5'), 83.3 ($J_{F,C}$ = 25.6 Hz, C-4'), 84.6 (C-1'), 94.0 ($J_{F,C}$ = 177 Hz, C-3'), 111.4 (C-5), 135.1 (C-6), 150.3 (C-2), 163.5 (C-4) ppm.

5'-O-[(Diethylphosphono)methyl]-3'-azido-3'-deoxythymidine (10) and N^3,5'-O-Bis[(diethylphosphono)methyl]-3'-azido-3'-deoxythymidine (18). A mixture of 1 g (3.75 mmol) of 3'-azido-3'-deoxythymidine,[15] 3.62 g (11.62 mmol) of the tosylate **1**, and 340 mg (14.1 mmol) of sodium hydride in 40 mL of anhydrous DMF was stirred for 3 days at room temperature. The reaction mixture was neutralized with acetic acid, evaporated, and diluted with 150 mL of $CHCl_3$. The solution was washed with H_2O (25 mL), dried, and evaporated. The title compound **10** was obtained in pure form (740 mg, 1.78 mmol, 50% yield) after column chromatographic purification ($CHCl_3$–MeOH 99:1): UV (MeOH) λ_{max} 266 nm; MS (m/e) 417 (M^+); ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, $POCH_2CH_3$), 1.96 (s, CH_3), 2.36 (m, H-2', H-2''), 3.8–4.52 (m, OCH_2P, H-3', H-4', H-5', H-5''), 4.22 (dq, J = 8.4 and 7.0 Hz, $POCH_2CH_3$), 6.24 (t, J = 6.5 Hz, H-1'), 7.47 (s, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 12.2 (CH_3), 16.3 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 37.3 (C-2'), 60.2 (C-3'), 62.3 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 65.5 ($J_{P,C}$ = 166 Hz, OCH_2P), 72.2 ($J_{P,C}$ = 8.5 Hz, C-5'), 82.8 (C-4'), 84.5 (C-1'), 111.2 (C-5), 135.3 (C-6), 150.2 C-2), 163.7 (C-4) ppm.

N^3,5'-O-bis[(diethylphosphono)methyl]-3'-azido-3'-deoxythymidine (**18**) was obtained in 14% yield (300 mg, 0.53 mmol) as the major side compound: UV (MeOH) λ_{max} 267 nm; MS (m/e): 567 (M^+); ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, $POCH_2CH_3$, OCH_2CH_3), 1.99 (s, CH_3), 2.25–2.60 (m, H-2', H-2''), 3.75–4.05 (m, OCH_2P, H-3', H-4', H-5', H-5'', $POCH_2CH_3$), 4.33 (d, J = 12.3 Hz, NCH_2P), 6.18 (t, J = 6.2 Hz, H-1), 7.43 (s, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 13.0 (CH_3), 16.1 and 16.3 ($J_{P,C}$ = 4.8 Hz, 2 × $POCH_2CH_3$), 35.0 ($J_{P,C}$ = 155 Hz, NCH_2P), 37.5 (C-2'), 60.2 (C-3'), 61.2 and 62.4 ($J_{P,C}$ = 4.9 Hz, 2 × $POCH_2CH_3$), 65.5 ($J_{P,C}$ = 167 Hz, OCH_2P), 72.0 ($J_{P,C}$ = 9.7 Hz, C-5'), 82.8 (C-4'), 85.4 (C-1'), 111.2 (C-5), 133.4 (C-6), 150.1 (C-2), 162.3 (C-4) ppm.

5'-O-[(Diethylphosphono)methyl]-3'-O-methylthymidine (11) and 5',N^3-Bis[(diethylphosphono)methyl]-3'-O-methylthymidine (19). A mixture of 0.64 g (2.5 mmol) of 3'-O-methylthymidine[16] and 0.18 g (7.5 mmol) of sodium hydride was stirred in 23 mL of DMF. After 30 min, 2.0 g (6.25 mmol) of the tosylate **1** in 2 mL of DMF was added, and the reaction mixture was stirred at room temperature for 65 h. The mixture was evaporated to dryness, and the residue was partitioned between 15 mL of water and 50 mL of ethyl acetate. The water phase was extracted three times with 50 mL of ethyl acetate. The organic phase was dried and evaporated. The crude product was purified by silica gel (50 g) column chromatography (elution with chloroform–methanol gradient (99:1 to 97:3)) followed by preparative TLC, eluted with chloroform–methanol (95:5). The title product **11** (562 mg, 1.38 mmol, yield 56%) was obtained as an oil: R_f 0.45 ($CHCl_3$–MeOH 9:1); UV (MeOH) λ_{max} 267 nm; MS (m/e) 406; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.2 Hz, $POCH_2CH_3$), 1.96 (s, CH_3), 2.10–2.35 (m, H-2', H-2''), 3.35 (s, OCH_3), 3.7–4.5 (m, H-3', H-4', H-5', H-5'', OCH_2P, $POCH_2CH_3$), 6.30 (dd, H-1'), 7.50 (s, H-6), 9.64 (br, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 12.2 (CH_3), 16.3 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 36.7 (C-2'), 56.6 (OCH_3), 62.2 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 65.4 ($J_{P,C}$ = 167.2 Hz, OCH_2P), 73.2 ($J_{P,C}$ = 9.8 Hz, C-5'), 80.9 (C-3'), 83.1 (C-4'), 84.7 (C-1'), 110.9 (C-5), 135.4 (C-6), 150.3 (C-2), 163.9 (C-4) ppm.

Some 5',N^3-bis[(diethylphosphono)methyl]-3'-O-methylthymidine (**19**) (80 mg, 0.14 mmol, yield 5.6%) was also isolated: R_f 0.58 ($CHCl_3$–MeOH 9:1); UV (MeOH) λ_{max} 267 nm; ^1H NMR ($CDCl_3$) δ 1.32 and 1.35 (t, J = 7.0 Hz, 2 × $POCH_2CH_3$), 1.98 (s, 5-CH_3), 2.34–2.57 (m, H-2', H-2''), 3.34 (s, 3'-OCH_3), 3.86 (d, $J_{P,H}$ = 8.8 Hz, OCH_2P), 3.68–4.10 (m, H-3', H-4', H-5', H-5''), 4.18 (m, $POCH_2CH_3$), 4.41 (d, $J_{P,H}$ = 12.5 Hz, NCH_2P), 6.32 (dd, H-1'), 7.51 (s, 6-H) ppm; ^{13}C NMR ($CDCl_3$) δ 13.0 (CH_3), 16.0 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 16.3 ($J_{P,C}$ = 4.9 Hz, $POCH_2CH_3$), 36.2 ($J_{P,C}$ = 165.0 Hz, NCH_2P), 37.0 (C-2'), 56.7 (OCH_3), 62.1 ($J_{P,C}$ = 4.9 Hz, $POCH_2CH_3$), 62.4 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 65.5 ($J_{P,C}$ = 167.2 Hz, OCH_2P), 72.7 ($J_{P,C}$ = 8.6 Hz, C-5'), 80.8 (C-3'), 83.2 (C-4'), 85.7 (C-1'), 109.8 (C-5), 133.2 (C-6), 150.1 (C-2), 162.4 (C-4) ppm.

5'-O-[(Diethylphosphono)methyl]-O^4-alkyl-2',3'-dideoxyuridine (12). A mixture of 0.90 g (4 mmol) of **7**, 400 mg (10 mmol) of sodium hydride and 3.22 g (10 mmol) of the tosylate **1** in 40 mL of anhydrous DMF was stirred at room temperature for 3 days. The reaction mixture was neutralized with acetic acid and evaporated. The residue was dissolved in 150 mL of $CHCl_3$ and washed with 50 mL of H_2O. The organic layer was dried, evaporated, and purified on silica gel by eluting with $CHCl_3$–MeOH (98:2), yielding 720 mg (46–48% yield) of a mixture of 5'-O-[(diethylphosphono)methyl]-O^4-methyl-2',3'-dideoxyuridine and 5'-O-[(diethylphosphono)methyl]-O^4-ethyl-2',3'-dideoxyuridine:

UV (MeOH) λ_{max} 276 nm; MS (*m/e*) 390 (M^+), 376 (M^+); ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, $POCH_2CH_3$ and OCH_2CH_3), 2.1–2.7 (m, H-2′, H-2″, H-3′, H-3″), 3.67 (d, J = 8.6 Hz, OCH_2P), 3.60–4.05 (m, OCH_3, H-4′, H-5′, H-5″), 4.19 (dq, J = 8.1 and 7.0 Hz, $POCH_2CH_3$), 4.42 (q, J = 7.0 Hz, OCH_2CH_3), 5.94 (d, J = 7.4 Hz, H-5), 6.09 (dd, H-1′), 8.23 (d, J = 7.4 Hz, H-6) ppm.

5′-*O*-[(Diethylphosphono)methyl]-3′-amino-3′-deoxythymidine (13). To a solution of 285 mg (0.68 mmol) of **10** in 2 mL of pyridine was added 290 mg (1.1 mmol) of triphenylphosphine. After 1 h of stirring at room temperature, 1 mL of 33% NH_4OH was added. The reaction mixture was stirred overnight and evaporated. After column chromatographic purification on 20 g of silica gel (elution with a gradient of chloroform–methanol (95:5 to 8:2)), the title compound **13** (220 mg, 0.56 mmol, yield 82%) was obtained as an oil: UV (MeOH) λ_{max} 268 nm; MS (*m/e*) 391 (M^+); ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 8.1 Hz, 2 × $POCH_2CH_3$), 1.94 (d, J = 1.1 Hz, CH_3), 2.23 (m, H-2′, H-2″), 3.61–3.95 (m, H-3′, H-4′, H-5′, H-5″, OCH_2P), 4.18 (dq, $J_{P,C}$ = 8.1 Hz, 2 × $POCH_2CH_3$), 6.25 (t, J = 6.1 Hz, H-1′), 7.51 (d, J = 1.1 Hz, H-6) ppm. ^{13}C NMR ($CDCl_3$) δ 12.3 (CH_3), 16.3 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 41.0 (C-2′), 51.1 (C-3′), 62.3 ($J_{P,C}$ = 5.3 Hz, $POCH_2CH_3$), 65.3 ($J_{P,C}$ = 166.0 Hz, OCH_2P), 72.1 ($J_{P,C}$ = 8.6 Hz, C-5′), 84.2 (C-1′), 85.5 (C-4′), 110.6 (C-5), 135.6 (C-6), 150.4 (C-2), 163.9 (C-4) ppm.

N^3-[(Diethylphosphono)methyl]-5′-*O*-benzoyl-3′-deoxy-3′-fluorothymidine (21). A mixture of 148 mg (0.42 mmol) of N^3-benzoyl-3′-deoxy-3′-fluorothymidine (**20**), 410 mg (1.27 mmol) of the tosylate **1** and 15 mg (0.62 mmol) of NaH was stirred for 65 h at room temperature. The reaction mixture was neutralized with acetic acid and evaporated. Purification on silica gel ($CHCl_3$–MeOH 99:1) yielded 100 mg (0.2 mmol, yield 48%) of **21**: UV (MeOH) λ_{max} 268 nm; MS (*m/e*) 498 (M^+); ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.0 Hz, $POCH_2CH_3$), 1.71 (s, CH_3), 2.1–2.7 (m, H-2′, H-2″), 4.05–4.35 (m, $POCH_2CH_3$), 4.42 (d, J = 12.2 Hz, NCH_2P), 4.62 (m, H-5′, H-5″), 5.18 (dm, $J_{F,H}$ = 9.8 Hz, H-4′), 5.37 (dm, $J_{F,H}$ = 53 Hz, H-3′), 6.42 (dd, H-1′), 7.3–8.3 (m, arom-H, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 12.8 (CH_3), 16.1 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 35.9 ($J_{P,C}$ = 150.1 Hz, NCH_2P), 38.9 ($J_{F,C}$ = 33 Hz, C-2′), 62.5 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 63.7 (br, C-5′), 82.6 ($J_{F,C}$ = 25.6 Hz, C-4′), 86.1 (C-1′), 93.4 ($J_{F,C}$ = 180.7 Hz, C-3′), 110.3 (C-5), 133.6 (C-6), 149.7 (C-2), 162.1 (C-4), 128.7, 129.3 and 132.6 (arom-C) ppm.

5′-*O*-[(Diethylphosphono)methyl]-2′,3′-dideoxycytidine (14) and 5′-*O*-[(Ethylphosphono)methyl]-2′,3′-dideoxycytidine (22). An amount of 320 mg of the phosphonate **12** was dissolved in 50 mL of anhydrous MeOH saturated with NH_3 and was heated in a pressure bottle at 100 °C overnight. The reaction mixture was evaporated and purified by column chromatography eluting with $CHCl_3$–MeOH (9:1) and $CHCl_3$–MeOH–Et_3N (9:1:0.3), affording 60 mg (0.17 mmol) of the phosphonate **14**: R_f 0.64 ($CHCl_3$–MeOH 1:1); MS (*m/e*) 361 (M^+); UV (MeOH) λ_{max} 273 nm; ^1H NMR ($CDCl_3$) δ 1.33 (t, J = 7.0 Hz, $POCH_2CH_3$), 1.97–2.65 (m, H-2′, H-2″, H-3′, H-3″), 3.72–4.09 (m, H-4′, H-5′, H-5″), 4.10 (d, J = 8.4 Hz, OCH_2P), 4.25 (dq, J = 8.3 and 7.0 Hz, $POCH_2CH_3$), 6.12 (m, H-5, H-1′), 8.01 (d, J = 7.5 Hz, H-6) ppm; ^{13}C NMR ($CDCl_3$) δ 16.9 (J = 6.1 Hz, $POCH_2CH_3$), 25.9 (C-3′), 33.0 (C-2′), 64.9 (J = 163.6 Hz, OCH_2P), 65.3 (J = 7.0 Hz, $POCH_2CH_3$), 75.0 (J = 12.2 Hz, C-5′), 81.8 (C-4′), 88.2 (C-1′), 96.8 (C-5), 143.0 (C-6), 158.5 (C-2), 167.1 (C-4) ppm.

The major product (R_f 0.15, $CHCl_3$–MeOH 1:1) was identified as the monoester **22** (205 mg, yield 68%) after conversion to its sodium salt: UV (MeOH) λ_{max} 274 nm, ϵ = 7400; ^1H NMR (D_2O) δ 1.29 (t, J = 7.0 Hz, $POCH_2CH_3$), 1.98–2.64 (m, H-2′, H-2″, H-3′, H-3″), 3.65–3.95 (OCH_2P, H-5′, H-5″), 4.01 (dq, J = 8.4 and 7.0 Hz, $POCH_2CH_3$), 4.30 (m, H-4′), 6.10 (m, H-1′, H-5), 8.03 (d, J = 7.5 Hz, H-6) ppm; ^{13}C NMR (D_2O) δ 16.9 ($J_{P,C}$ = 6.1 Hz, $POCH_2CH_3$), 25.9 (C-3′), 33.0 (C-2′), 62.2 ($J_{P,C}$ = 4.9 Hz, $POCH_2CH_3$), 67.2 ($J_{P,C}$ = 158.7 Hz, OCH_2P), 74.1 ($J_{P,C}$ = 12.2 Hz, C-5′), 81.6 (C-4′), 87.9 (C-1′), 96.7 (C-5), 142.6 (C-6), 158.4 (C-2), 167.0 (C-4) ppm.

Disodium 5′-*O*-(Phosphonomethyl)-3′-deoxythymidine (23). Nitrogen was bubbled through a solution of 330 mg (0.76 mmol) of **8** in 15 mL of anhydrous DMF for 5 min, after which 0.8 mL (6.3 mmol) of trimethylsilyl iodide was added. The reaction mixture was stirred in the dark at room temperature overnight, and after cooling on an ice bath the reaction was quenched with anhydrous methanol. After addition of 1 mL of triethylamine the mixture was evaporated and the residue was dissolved in water, which was washed with chloroform. The crude product was purified by ion-exchange chromatography (2 × 20 cm, DEAE cellulose) by eluting with a TEAB gradient (0.0–0.15 M). The combined fractions containing the desired product were further purified by reversed-phase HPLC (C18, ethanol–H_2O 3:97) and subsequently converted into the sodium salt, affording 216 mg (0.59 mmol, 68% yield) of the title compound **23**: UV (H_2O) λ_{max} 269 nm, ϵ = 10,300; ^1H NMR (D_2O) δ 1.82 (s, CH_3), 1.9–2.5 (m, H-2′, H-2″, H-3′, H-3″), 3.50 (d, J = 8.3 Hz, OCH_2P), 3.55–3.75 (m, H-4′, H-5′, H-5″), 6.04 (dd, H-1′), 7.44 (s, H-6) ppm; ^{13}C NMR (D_2O) δ 12.5 (CH_3), 26.6 (C-3′), 31.2 (C-2′), 70.2 ($J_{P,C}$ = 150.1 Hz, OCH_2P), 75.1 ($J_{P,C}$ = 10.9 Hz, C-5′), 80.6 (C-4′), 86.6 (C-1′), 111.9 (C-5), 137.9 (C-6), 153.4 (C-2), 168.4 (C-4) ppm. Anal. ($C_{11}H_{15}N_2O_7PNa_2 \cdot 2H_2O$) C, H, N.

Disodium 5′-*O*-(Phosphonomethyl)-3′-deoxy-3′-fluorothymidine (24). Nitrogen was bubbled for 5 min into a solution of 350 mg (0.9 mmol) of 5′-*O*-[(diethylphosphono)methyl]-3′-deoxy-3′-fluorothymidine (**9**) and 405 mg (2.7 mmol) of sodium iodide in 20 mL of anhydrous acetonitrile, after which 0.34 mL (2.7 mmol) of trimethylsilyl chloride was added through a syringe. After the reaction mixture was stirred in the dark under nitrogen and at room temperature for 15 h, anhydrous methanol was added and the mixture was evaporated to dryness. The residue was dissolved in water and washed with chloroform. The water phase was purified by ion-exchange chromatography (2 × 20 cm) on DEAE cellulose, which was eluted with a TEAB gradient (0.0–0.2 M). The triethylammonium salt of the title compound was further purified by HPLC (silica gel (15 9m), 100% ethanol), and converted into the sodium salt, yielding 130 mg (0.34 mmol, 38%) of the title product **24**: UV (MeOH) λ_{max} 267 nm, ϵ = 8700; ^1H NMR (D_2O) δ 1.97 (s, CH_3), 2.2–2.8 (m, H-2′, H-2″), 3.58 (d, J = 7.9 Hz, OCH_2P), 3.85 (m, H-5′, H-5″), 4.54 (dt, $J_{F,H}$ = 26.8 Hz, H-4′), 5.48 (dm, $J_{F,H}$ = 54.7 Hz, H-3′), 6.35 (dd, H-1′), 7.66 (s, H-6) ppm; ^{13}C NMR (D_2O) δ 12.8 (CH_3), 37.6 ($J_{C,F}$ = 20.8 Hz, C-2′), 70.5 ($J_{P,C}$ = 148.9 Hz, OCH_2P), 72.6 (dd, C-5′), 85.0 ($J_{F,C}$ = 23.4 Hz, C-4′), 86.3 (C-1′), 95.6 ($J_{F,C}$ = 174.6 Hz, C-3′), 112.8 (C-5), 137.9 (C-6), 154.3 (C-2), 169.1 (C-4) ppm. Anal. ($C_{11}H_{14}N_2O_7PNa_2 \cdot H_2O \cdot CH_3OH$) C, H, N.

Disodium 5′-*O*-(Phosphonomethyl)-3′-azido-3′-deoxythymidine (25). Nitrogen was bubbled through a solution of 0.57 g (1.37 mmol) of 5′-*O*-[(diethylphosphono)methyl]-3′-azido-3′-deoxythymidine (**10**) and 0.47 g (3.15 mmol) of sodium iodide in 20 mL of anhydrous acetonitrile and 4 mL of dichloromethane for 5 min, after which 0.4 mL (3.15 mmol) of trimethylsilyl chloride was added through a syringe. After stirring overnight under nitrogen at room temperature in the dark, the reaction mixture was quenched with 18 mL of 0.3 M TEAB buffer. After evaporation to dryness, the residue was dissolved in water and washed with chloroform. The crude product was purified by ion-exchange chromatography as for **25**. The fractions containing the desired compound were combined and further purified by HPLC (silica gel (15 9m), EtOH–H_2O 98:2) followed by conversion into the sodium salt. This afforded 126 mg (0.31 mmol, 28% yield) of disodium 5′-*O*-(phosphonomethyl)-3′-azido-3′-deoxythymidine (**25**): IR 2120 cm^{-1} (N_3); UV (MeOH) λ_{max} 268 nm, ϵ = 9400; ^1H NMR (D_2O) δ 1.98 (s, CH_3), 2.59 (m, H-2′), 3.68 (d, J = 8.3 Hz, OCH_2P), 3.88 (m, H-5′, H-5″), 4.24 (m, H-4′), 4.49 (m, H-3′), 6.29 (t, J = 6.8 Hz, H-1′), 7.65 (s, H-6) ppm; ^{13}C NMR (D_2O) δ 12.5 (CH_3), 36.7 (C-2′), 61.5 (C-3′), 69.8 ($J_{P,C}$ = 150.2 Hz, OCH_2P), 73.3 ($J_{P,C}$ = 9.7 Hz, C-5′), 83.6 (C-4′), 86.0 (C-1′), 112.6 (C-5), 138.4 (C-6), 152.5 (C-2), 167.4 (C-4) ppm. Anal. ($C_{11}H_{14}N_5O_7PNa_2 \cdot 2H_2O$) C, H, N.

Disodium 5′-*O*-(Phosphonomethyl)-3′-*O*-methylthymidine (26). To a solution of 562 mg (1.38 mmol) of **11** in 25 mL of anhydrous DMF was added 1.18 mL (8.3 mmol) of trimethylsilyl iodide under nitrogen. The reaction mixture was stirred at room temperature overnight in the dark and quenched with methanol. TEAB buffer (10 mL, 0.2 M) was added, and the mixture was evaporated to dryness. The residue was dissolved in water and washed with chloroform. The crude product was purified by ion-exchange chromatography (0.0–0.1 M TEAB buffer) followed by purification on reversed-phase HPLC (ethanol–water 3:97). After conversion into the sodium salt 120 mg (0.3 mmol, 37% yield) of the title product **26** was obtained: UV (H_2O) λ_{max} 267

nm, ϵ = 10100; ^{1}H NMR (D_2O) δ 1.90 (s, CH_3), 2.38 (m, H-2', H-2''), 3.41 (s, OCH_3), 3.55 (J = 8.8 Hz, OCH_2P), 3.78 (m, H-5'), 4.17 (m, H-3', H-4'), 6.23 (t, J = 6.6 Hz, H-1'), 7.53 (s, H-6) ppm; ^{13}C NMR (D_2O) δ 12.5 (CH_3), 36.0 (C-2'), 57.2 (OCH_3), 70.2 ($J_{P,C}$ = 151.9 Hz, OCH_2P), 73.4 ($J_{P,C}$ = 9.2 Hz, C-5'), 83.4 (C-4'), 85.9 (C-1'), 112.3 (C-5), 137.6 (C-6), 154.3 (C-2), 169.5 (C-4) ppm. Anal. ($C_{12}H_{17}N_2O_8PNa_2 \cdot 5H_2O$) C, H, N.

Disodium 5'-O-(Phosphonomethyl)-2',3'-dideoxyuridine (27). To a solution of 370 mg (1 mmol) of **12** in 20 mL of anhydrous DMF was added 2.85 mL (20 mmol) of trimethylsilyl iodide under nitrogen. The reaction mixture was stirred at room temperature overnight in the dark, quenched with methanol and evaporated to dryness. The residue was dissolved in water and washed with chloroform. The crude product was purified as for the synthesis of **27**. After conversion into the sodium salt, 110 mg (yield 30.7%) of the title product **27** was obtained, which was a mixture of the α and β anomers as shown by NMR analysis: UV (H_2O) λ_{max} 263 nm, ϵ = 9600; ^{1}H NMR (D_2O) δ 1.62–2.47 (m, H-2', H-2'', H-3', H-3''), 3.49 (d, J = 7.5 Hz, OCH_2P), 3.50–3.80 (m, H-4', H-5', H-5''), 5.80 (d, J = 7.8 Hz, H-5), 5.78* (d, J = 7.9 Hz, H-5), 6.07 (m, H-1'), 7.61* (d, J = 7.9 Hz, H-6), 7.72 (d, J = 7.9 Hz H-6) ppm; ^{13}C NMR (D_2O) δ 25.9 (C-3'), 31.2 (C-2'), 31.4* (C-2'), 69.7 ($J_{P,C}$ = 148.9 Hz, OCH_2P), 74.5 ($J_{P,C}$ = 9.8 Hz, C-5'), 74.8* ($J_{P,C}$ = 9.8 Hz, C-5'), 80.4 (C-4'), 86.7 (C-1'), 87.8* (C-1'), 102.2* (C-5), 102.4 (C-5), 141.4* (C-6), 141.6 (C-6), 154.9 (C-2), 170.6 (C-4) ppm. Anal. ($C_{10}H_{13}N_2O_7PNa_2 \cdot 2.5H_2O$) C, H, N; N: calcd, 7.09; found, 6.59.

Disodium 5'-O-(Phosphonomethyl)-3'-amino-3'-deoxythymidine (28). To a solution of 340 mg (0.87 mmol) of **13** in 18 mL of anhydrous DMF was added 1.28 mL (9 mmol) of trimethylsilyl iodide under nitrogen. The reaction mixture was stirred at room temperature overnight in the dark, quenched with methanol, and evaporated to dryness. The residue was dissolved in water and washed with chloroform. The crude product was purified by ion-exchange chromatography (TEAB gradient, 0.0–0.2 M), followed by purification on reversed phase HPLC (3% EtOH–H_2O). After conversion into the sodium salt, 120 mg (0.32 mmol, 37% yield) of the title compound **28** was obtained: UV (H_2O) λ_{max} 267 nm (ϵ = 9500); ^{1}H NMR (D_2O) δ 1.88 (s, CH_3), 2.36 (m, H-2', H-2''), 3.45–3.80 (m, H-3', OCH_2P, J = 8.2 Hz), 3.84 (m, H-5', H-5''), 3.97 (m, H-4'), 6.21 (t, J = 6.1 Hz, H-1'), 7.50 (s, H-6) ppm; ^{13}C NMR (D_2O) δ 12.5 (CH_3), 38.9 (C-2'), 51.9 (C-3'), 70.4 ($J_{P,C}$ = 149.9 Hz, OCH_2P), 73.4 ($J_{P,C}$ = 9.8 Hz, C-5'), 85.0 (C-4'), 85.7 (C-1'), 112.4 (C-5), 138.3 (C-6), 153.0 (C-2), 168.0 (C-4) ppm. Anal. ($C_{11}H_{16}N_3O_7PNa_2 \cdot 2H_2O$) C, H, N.

Disodium 5'-O-(Phosphonomethyl)-2',3'-dideoxycytidine (29). The sodium salt of 5'-O-[(ethylphosphono)methyl]-2',3'-dideoxycytidine (**22**) (180 mg, 0.53 mmol) was dried over phosphorus pentoxide in vacuum and coevaporated with anhydrous DMF. After the residue was dissolved in 10 mL of DMF, 0.16 mL (1.1 mmol) of iodotrimethylsilane was added under nitrogen. The reaction mixture was stirred overnight in the dark, another 0.1 mL of iodotrimethylsilane was added and the mixture was further stirred for 20 h. Dry methanol was added at 0 °C, followed by 10 mL of 0.4 M TEAB buffer. Workup and chromatography on DEAE cellulose afforded 40 mg (0.12 mmol) of recovered monoester **22**. The triethylammonium salt of the phosphonic acid was converted into the sodium salt and dried over P_2O_5 in vacuo, yielding 200 mg of the title product **29**. UV analysis (MeOH) showed λ_{max} 272 nm (ϵ = 3900) which indicated that the product was not pure. Further purification was done by reversed-phase HPLC on C18 (ethanol–water 1:9), affording 80 mg (0.23 mmol, yield 45%) of **30**: UV (H_2O) λ_{max} 272 nm (ϵ = 9000); ^{1}H NMR (D_2O) δ 1.60–2.60 (m, H-2', H-2'', H-3', H-3''), 3.56 (d, J = 8.8 Hz, OCH_2P), 3.80 (m, H-5', H-5''), 4.36 (m, H-4'), 6.05 (m, H-1', H-5), 7.87 (d, J = 7.5 Hz, H-6) ppm; ^{13}C NMR (D_2O) δ 26.1 (C-3'), 32.1 (C-2'), 70.5 ($J_{P,C}$ = 151.4 Hz, OCH_2P), 74.9 ($J_{P,C}$ = 9.7 Hz, C-5'), 80.9 (C-4'), 87.4 (C-1'), 96.6 (C-5), 142.3 (C-6), 158.0 (C-2), 166.5 (C-4) ppm. Anal. ($C_{10}H_{14}N_3O_6PNa_2 \cdot 2.5H_2O$) C, H, N.

Sodium Salt of 5'-O-(Triphosphomethyl)-3'-deoxy-3'-fluorothymidine (30). Disodium 5'-O-(phosphonomethyl)-3'-deoxy-3'-fluorothymidine (**24**) (78 mg, 0.2 mmol) was converted to its pyridinium salt by Dowex-50W X-8 cation-exchange resin, followed by addition of 95 μL (0.4 mmol) of tributylamine. After coevaporation with anhydrous pyridine and with DMF, the tributylammonium salt was dissolved in 1 mL of DMF, and 162 mg (1 mmol) of 1,1'-carbonyldiimidazole was added. After 5 h of stirring at room temperature, 50 μL of anhydrous methanol was added, and the solution was stirred further for 20 min. Tributylammonium pyrophosphate (1 mmol) dissolved in 10 mL of DMF was added dropwise with stirring. The mixture was stirred overnight at room temperature and centrifugated. The white precipitate was washed with another 5 mL of DMF. The combined DMF fractions were evaporated to dryness. The residue was dissolved in water, neutralized with ammonia (33%) to pH 8, and washed with diethyl ether. The water phase was concentrated and chromatographed on DEAE cellulose, which was eluted with water, followed with a linear gradient of TEAB (0.1–0.4 M). The title product (55 mg) was obtained after conversion into the sodium salt, UV (H_2O) λ_{max} 266 nm.

Antiviral Assay Procedures. HIV-1 (strain HTLV-III_B) was prepared from the culture supernatant of persistently HTLV-III_B-infected MT-4 cells. The antiviral activity assays[32] were based on the protection of MT-4 cells against the cytopathogenicity of HIV-1. They were run in parallel with the cytotoxicity assays aimed at establishing the toxicity of the compounds for uninfected MT-4 cells. Inhibitory effects of **30** and of the AzddThd derivatives on the activity of MLV- and HIV-associated reverse transcriptases were determined according to Balzarini et al.[29]

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