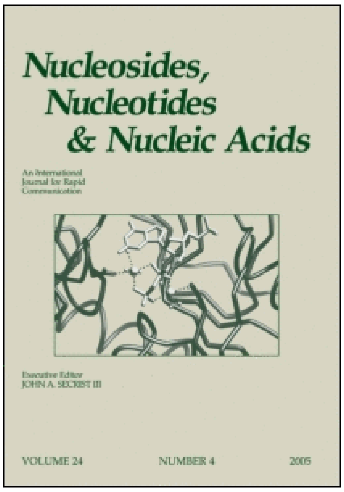

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Phosphonoformate Esters of Anti-HIV Nucleosides: 3'-Azido-3'-deoxythymidine and 2',3'-Dideoxycytidine Derivatives Containing a Small 5'-(O-Alkoxycarbonylphosphinyl) or 5'-(O-Cholesterylcarbonylphosphinyl) Substituent

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SUMMARY: A convenient general method of synthesis of 5'-O-(alkoxycarbonyl)phosphonate esters of 2',3'-dideoxyribonucleosides is presented, using the 5'-O-(methoxycarbonyl)phosphinyl, 5'-O-(ethoxycarbonyl)phosphinyl, and 5'-O-(cholesterylcarbonyl)phosphinyl derivatives of 3'-azido-3'-deoxythymidine (AZT) and the 5'-O-(ethoxycarbonyl)phosphinyl derivative of 2',3'-dideoxycytidine (ddC) as examples. Reaction of trimethyl phosphonoformate, triethyl phosphonoformate, or dimethyl cholesterylcarbonylphosphonate with phosphorus pentachloride in carbon tetrachloride, followed by direct condensation of the resulting phosphonyl chloride with the nucleoside, gave the fully esterified phosphonoformate derivatives, which on treatment with sodium iodide in tetrahydrofuran underwent selective cleavage of the P-OMe or P-OEt groups, leaving the carboxylate esters intact. The resulting products were converted from sodium salts to ammonium salts by ion-exchange chromatography.

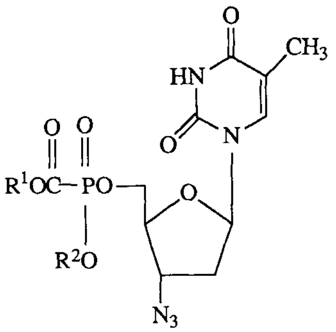
Inhibitors of the enzyme reverse transcriptase are currently the only major class of drugs approved for clinical treatment of human acquired immunodeficiency syndrome (AIDS).[1] Human immunodeficiency virus type 1 (HIV-1), the infectious cause of AIDS, is a retrovirus.[2] Reverse transcriptase is carried into the cell by the virion during infection. The role of the enzyme in the lifecycle of the virus is to synthesize a double-stranded DNA copy of the viral RNA template, so that integration of the viral genome into host DNA can ultimately occur. Because reverse transcriptase is required for viral replication in infected cells, inhibition of this enzyme is one of the most effective therapeutic strategies against AIDS.[3]

Major classes of known reverse transcriptase inhibitors include (a) molecules that bind to the enzyme itself (e.g., suramin, triphenylmethane dyes, tungstoantimonates), (b) molecules that bind to the RNA template (e.g., antisense oligonucleotides), and (c) molecules that are substrate analogues (e.g., 2',3'-dideoxyribonucleosides, as the 5'-triphosphates) or product analogues (e.g., phosphonoformic acid).[4] The agents used most extensively in AIDS treatment up to now have been of the third type, with 3'-azido-3'-deoxythymidine (AZT) being the first to reach clinical trial,[5,6] followed by 2',3'-dideoxycytidine (ddC)[7] and 2',3'-dideoxyinosine (ddI).[8] These 2',3'-dideoxyribonucleosides (ddN's) undergo intracellular conversion to 5'-triphosphates (ddNTP's) that can serve as analogues of natural 2'-deoxyribonucleoside 5'-triphosphates.[9] Absence of a free 3'-hydroxyl group in the sugar moiety of the ddNTP results in DNA chain termination,[10] with the result that reverse transcriptase is prevented from making a DNA copy of the viral genome. Phosphonoformic acid (PFA) is considered a *product analogue* in the context of reverse transcriptase inhibition because of its structural resemblance to pyrophosphate,[11] one molecule of which is formed each time the enzyme catalyzes addition of a nucleotide to a nascent DNA chain. PFA is of interest as an antiviral agent not only because of its effect on HIV replication but also because of its activity against herpes[12] and cytomegalovirus[13] infections, which are major causes of morbidity and mortality in immunocompromised AIDS patients.[14]

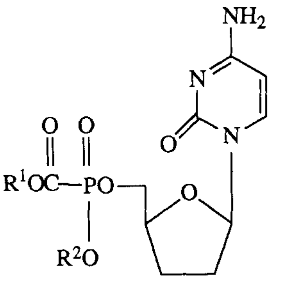
Site-directed mutagenesis experiments have suggested that substrate and product analogues probably bind to adjacent but non-identical sites in the complex between reverse transcriptase and the RNA template.[15] The possibility therefore exists for synergistic interaction between a substrate analogue (e.g., a ddNTP) and a product analogue (e.g., PFA). Indeed, synergistic inhibition of HIV replication in cells has been observed with combinations of AZT and PFA, as well as synergistic inhibition of isolated reverse transcriptase with combinations of AZTTP and PFA.[16-18] These considerations suggested to us that PFA esters of 2',3'-dideoxyribonucleosides might be of interest as anti-HIV agents because of their potential ability to form two synergistically active drugs

-- one a substrate analogue, the other a product analogue -- as a result of intracellular ester metabolism. In essence these esters can be viewed as prodrugs containing *a masking group which is itself a drug.*

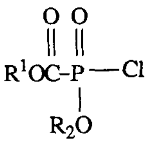
Two types of PFA-dideoxyribonucleoside adducts are possible in principle, depending on whether attachment of PFA to the 5'-hydroxyl group of the nucleoside is via the phosphonate or carboxylate moiety. This paper reports the synthesis of adducts of the first type, in the form of the AZT derivatives **1-3** and the ddC derivative **4**. Compounds **1**, **2**, and **4** were isolated as stable, crystalline, water-soluble ammonium salts; compound **3** was obtained as the free acid. Although simple PFA esters[19] and PFA esters of nucleosides have been described previously,[20-22] compounds **1-4** are, to our knowledge, the first examples of PFA derivatives of anti-HIV 2',3'-dideoxyribonucleosides.[23] The lipophilic cholesteryl analogue **3** is potentially of interest as a macrophage-targeted AZT and PFA prodrug.[24]



1 : $R^1 = Me, R^2 = NH_4$
2 : $R^1 = Et, R^2 = NH_4$
3 : R^1 = Cholesteryl, $R^2 = H$
6 : $R^1 = R^2 = Me$
9 : $R^1 = R^2 = Et$
10 : R^1 = Cholesteryl, $R^2 = Me$



4 : $R^1 = Et, R^2 = NH_4$
11 : $R^1 = R^2 = Et$



5 : $R^1 = R^2 = Me$
7 : $R^1 = R^2 = Et$
8 : R^1 = Cholesteryl, $R^2 = Me$

Repeated attempts to prepare the chloro diester **5** from trimethyl phosphonoformate by the method of Petrov and coworkers[25] with the intent of phosphorylating 2',3'-dideoxyribonucleosides in a separate step were unsuccessful. However, when trimethyl phosphonoformate was treated with one equivalent of PCl_5 in CCl_4 at 50°C, and the crude product after removal of volatile materials was treated directly with AZT in DMF solution, a good yield of 3'-azido-3'-deoxy-5'-O-[P-methoxy-P-(methoxycarbonyl)phosphinyl]thymidine (**6**) was obtained. The phosphorylation was carried out initially at -50°C, with subsequent warming to room temperature. The phosphorylated nucleoside was purified by silica gel flash chromatography and obtained as a TLC-homogeneous product pure enough to use in the next step (see below) without further purification. The same reaction sequence starting with triethyl phosphonoformate and dimethyl cholesterylcarbonylphosphonate afforded intermediates **7** and **8**, which were converted directly to 3'-azido-3'-deoxy-5'-O-[P-ethoxy-P-(ethoxycarbonyl)phosphinyl]thymidine (**9**) and 3'-azido-3'-deoxy-5'-O-[P-(cholesterylcarbonyl)phosphinyl-P-methoxy]thymidine (**10**), respectively. The reaction of AZT was slower with **7** and **8** than with **5**, requiring 3 h at 85°C instead of 1.5 h at 50°C to reach completion. Ease of chloride displacement from phosphorus in this reaction appears to depend on the size of the alkoxy group. The presence of two methoxy groups in **6** was evident from the ^{1}H-NMR spectrum, which contained a singlet at δ 3.8 (3H, C-OCH_3) and a doublet at δ 3.9 (3H, P-OCH_3). Two ethoxy groups were likewise discernible in the spectrum of **9**, but were less easily identified because they gave complex multiplets at δ 1.4 (C-OCH_2CH_3, P-OCH_2CH_3) and δ 4.3 (C-OCH_2CH_3, P-OCH_2CH_3), the latter of which also contained peaks corresponding to the $C_{3'}$, $C_{4'}$, and $C_{5'}$ sugar protons.

Treatment of **6** with NaI in dry THF solution at room temperature for 3 h resulted in selective O-methyl cleavage on phosphorus[19] and formation of a Na salt which was converted conveniently into **1** during anion exchange chromatographic purification on N,N-diethylaminoethylcellulose (DEAE-cellulose) with NH_4HCO_3 as the eluent. Freeze-drying of appropriate fractions afforded **1** as a colorless solid with the expected ^{1}H NMR spectral properties, including notably the loss of δ 3.9 doublet corresponding to the P-OCH_3 group. The ^{31}P NMR spectrum (H_2O, 121.3 MHz) contained a singlet at δ -5.05 consistent with the ROC(=O)P(=O)OR' group. Similar treatment of **9** afforded **2** in 60% yield. More time was required to cleave **9** (20 h) than **6** (3 h), as expected from the greater nucleophilicity of iodide ion toward methyl esters than ethyl esters. Demethylation of the cholesteryl derivative **10** to form **3** also required 20 h of reaction with NaI, probably because the P-OMe group is more sterically hindered in **10** than in **6**. Compound **2** moved further than compound **1** on silica gel TLC plates, indicating greater lipophilicity, and had an ^{1}H NMR spectrum consistent with loss of only the P-OCH_2CH_3 group. Variation of the alkyl chain length in these C-esters is expected to have an effect on their water-lipid partition coefficient and hence their ability to be taken up into cells. As expected, **3** was much more lipophilic than **1** or **2**, and possessed the typical tendency of amphipathic compounds to form micelles. For this reason we found it necessary with **3** to use CD_3OD rather than D_2O to obtain acceptable NMR spectra.

Treatment of 2',3'-dideoxycytidine *in situ* with **7** as described above for the one-pot reaction of AZT, followed by cleavage of the P-OEt ester with NaI in THF (room temperature, 20 h), afforded **4**. Isolation of the intermediate diethyl ester **11** was dispensed with, since TLC showed the reaction to be complete and the product to be quite pure. Purification of the product from the NaI cleavage reaction by DEAE-cellulose chromatography, followed by preparative HPLC on C_{18} silica gel with 5% MeCN in 0.01 M NH_4OAc as the eluent, gave **4** in 29% overall yield. The ^1H NMR spectrum of **4** was consistent with loss of one ethoxy group relative to **11**, and the IR spectrum confirmed that the carboxyl group of the PFA moiety was still esterified.

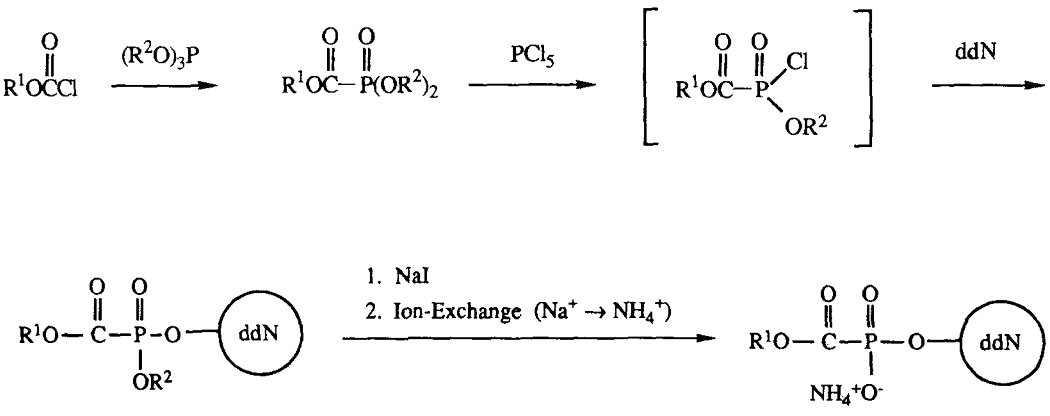
Interestingly, microchemical analysis of **2** and **4** indicated the presence of less than one mole of ammonia, while that of **3** revealed that the compound was actually the free acid rather than the ammonium salt even though NH_4OAc buffer had been used for preparative HPLC purification. Since all the other properties of these compounds were perfectly consistent with their assigned structures, it appears that variable amounts of ammonia were lost during freeze-drying. These results are compatible with the very low acidity of the P-OH proton, which in the case of **3** allowed the ammonium salt to be converted entirely to the free acid during lyophilization. In compound **4** the presence of a basic 4-amino group on the cytosine ring may also promote loss of ammonia under high vacuum.

The method of synthesis described in this paper is quite general (Scheme 1), and can be extended to the preparation of PFA conjugates of other antiretroviral nucleosides. The ability to selectively cleave methyl esters from the phosphonyl moiety with NaI is useful, because a wide variety of alkoxycarbonylmethylphosphonyl chlorides should be accessible from alkylchloroformates on successive treatment with $P(OMe)_3$ (Arbuzov reaction) and PCl_5. The scope of this method is currently under investigation in our laboratory as a route to 2',3'-dideoxyribonucleoside derivatives of PFA.

Experimental Section

^1H NMR spectra were obtained at 60 MHz on a Varian Model EM360 spectrometer and at 300 MHz on a Varian Model XL300 instrument, using Me_4Si as the reference. We thank Dr. Jonathan Lee, of the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, for his help in obtaining the 300 MHz spectra. TLC was on Whatman MK6F and Baker 250F silica gel plates containing a fluorescent indicator. Spots were visualized in a viewing chamber under 254-nm UV light. Column chromatography was on Baker 3405 (60-200 mesh), Baker "Flash" (40 mm) silica gel, or Whatman DE52 pre-swollen DEAE-cellulose. Melting points were determined on a Fisher-Johns hot-stage apparatus and are not corrected. Solvents were

Scheme 1



ddN = 2',3'-dideoxynucleoside (e.g., AZT, ddC)

WI) and Sigma (St. Louis, MO). AZT was synthesized from thymidine by the method of Rao and Reese.[26]

3'-Azido-3'-deoxy-5'[*P*-hydroxy-*P*-(methoxycarbonyl)phosphinyl]thymidine, Ammonium Salt (1). PCl_5 (1.2 g, 5.5 mmol) was added to a solution of trimethyl phosphonoformate (1 g, 6 mmol) in CCl_4 (50 mL), and the suspension was warmed to 50°C and stirred for 1.5 h. The reaction mixture was evaporated to dryness under reduced pressure, the residue (containing **5**) was cooled to -50°C, and a solution of AZT (0.45 g, 1.7 mmol) in dry DMF (10 mL), also pre-cooled to -50°C, was added. After 1 h of stirring at -50°C, the mixture was allowed to come to room temperature and concentrated to dryness under reduced pressure. Flash chromatography of the residue on silica gel (98:2 $CHCl_3$-MeOH) gave **6** as a colorless gum (241 mg, 35%) which was pure enough to use directly in the next step (see below); TLC: R_f 0.42 (silica gel, 95:5 CH_2Cl_2-MeOH); ^1H NMR ($CDCl_3$, 60 MHz) δ 1.9 (s, 3H, 5-CH_3), 2.4 (m, 2H, $C_{2'}$-H), 3.8 (s, 3H, C-OCH_3), 3.9 (d, J = 10 Hz, 3H, P-OCH_3), 4.1 (m, 1H, $C_{3'}$-H), 4.4 (m, 3H, $C_{4'}$-H, $C_{5'}$-H), 6.2 (t, 1H, $C_{1'}$-H), 7.3 (broad s, 1H, C_6-H), 9.8 (broad s, 1H, NH).

NaI (23 mg, 0.15 mmol) was added to a stirred solution of **6** (73 mg, 0.17 mmol) in anhydrous THF (1 mL) and the solution was left to stir under N_2 at room temperature. After 3 h, the reaction mixture was filtered, and the product was washed with anhydrous Et_2O and dried in vacuo; yield 63 mg. Chromatography on a C_{18} silica gel column (10 cm x 1 cm) with MeCN as the eluent afforded gave a pale-yellow solid (50 mg) which was taken up in water and applied onto a DEAE-cellulose column (HCO_3^- form). The product was eluted with 0.05 M NH_4HCO_3, and TLC-homogeneous fractions were pooled and freeze-dried to a white solid (27 mg, 35%); mp 109-110°C; TLC: R_f 0.21 (silica gel, 3:1 CH_2Cl_2-MeOH); ^1H NMR (D_2O, 300 MHz) δ 1.8 (t, 3H, 5-CH_3), 2.4 (t, 2H, $C_{2'}$-H), 3.7 (s, 3H, C-OCH_3), 4.1 (m, 3H, $C_{3'}$-H, $C_{5'}$-H), 4.4 (m, 1H, $C_{4'}$-H), 6.2 (t, 1H, $C_{1'}$-H), 7.6 (s, 1H, C_6-H); ^{31}P NMR (D_2O, 121.4 MHz) δ -5.05 (external reference: 1% H_3PO_4). Anal. ($C_{12}H_{16}N_5O_8P \cdot NH_3 \cdot 1.25H_2O$) Calcd: C, 33.60; H, 5.01; N, 19.60; P, 7.23. Found: C, 33.62; H, 4.88; N, 19.77; P, 7.20.

3'-Azido-3'-deoxy-5'-O-[*P*-ethoxy-*P*-(ethoxycarbonyl)phosphinyl]thymidine (9). Triethyl phosphonoformate (0.68 g, 3.2 mmol) was added to a suspension of PCl_5 (0.65 g, 3 mmol) in CCl_4 (20 mL) at 77°C, and the mixture was stirred for 3 h. The reaction mixture was cooled and concentrated to dryness with the aid of a rotary evaporator and vacuum pump. The oily residue (containing **7**) was redissolved in DMF (2 mL) cooled to -50°C in a dry ice-actone mixture, a pre-cooled (-50°C) solution of AZT (0.27 g, 1 mmol) in dry DMF (3 mL) was added, and the reaction mixture was allowed to come to room temperature and stirred for 20 h. The resulting solution was concentrated to dryness under reduced pressure, and the residue was purified by flash chromatography on silica gel (98:2 CH_2Cl_2-MeOH) to obtain the triester as a colorless gum (0.32 g, 75%); TLC: R_f 0.25 (96:4 CH_2Cl_2-MeOH) ; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.4 (m, 6H, C-$OCH_2\mathit{CH_3}$ and P-$OCH_2\mathit{CH_3}$), 1.9 (s, 3H, 5-CH_3), 2.2 (m, 2H, $C_{2'}$-H), 4.3 (m, 8H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H, C-$O\mathit{CH_2}CH_3$, and P-$O\mathit{CH_2}CH_3$), 6.2 (t, 1H, $C_{1'}$-H), 7.4 (s, 1H, 6-H). Anal. ($C_{15}H_{22}N_5O_8P$) Calcd: C, 41.76; H, 5.10; N, 16.24; P, 7.19. Found: C, 41.80; H, 5.31; N, 15.97; P, 7.50.

3'-Azido-3'-deoxy-5'-O-[*P*-(ethoxycarbonyl)-*P*-hydroxyphosphinyl]thymidine, Ammonium Salt (2). NaI (30 mg, 0.24 mmol) was added to a solution of **9** (73 mg, 0.17 mmol) in anhydrous THF (2 mL), and the solution was stirred at room temperature for 20 h. After evaporation of the solvent under reduced pressure, the residue was applied onto a DEAE-cellulose column (HCO_3^- form). and the column was eluted successively with distilled H_2O and 0.05 M NH_4HCO_3. Fractions of the latter eluent containing the product were pooled and freeze-dried to obtain a colorless solid (42 mg, 60% yield); mp 119-120°C; TLC: R_f 0.32 (3:1 CH_2Cl_2-MeOH); HPLC: 11.0 min as compared with 8.0 min for AZT (C_{18} silica gel, 0.01 M NH_4OAc, pH 6.6, with 5% MeCN, 1.0 mL/min); IR (KBr): ν 2120 (N_3), 1710 (ester C=O) cm^{-1}; NMR

(D_2O, 300 MHz) δ 1.2 (t, 3H, C-OCH_2CH_3), 1.8 (s, 3H, 5-CH_3), 2.4 (m, 2H, $C_{2'}$-H), 3.95 (m, 1H, $C_{3'}$-H), 4.2 (m, 4H, $C_{5'}$-H, C-OCH_2CH_3), 4.4 (m, 1H, $C_{4'}$-H), 6.2 (t, 1H, $C_{1'}$-H), 7.6 (s, 1H, 6-H). Anal. ($C_{13}H_{18}N_5O_8P\cdot0.9NH_3\cdot1.25H_2O$) Calcd: C, 35.47; H, 5.09; N, 18.78; P, 7.04. Found: C, 35.47; H, 5.16; N, 18.57; P, 7.25.

2',3'-Dideoxy-5'-[P-(ethoxycarbonyl)-P-hydroxyphosphinyl]cytidine, Ammonium Salt (4). Triethyl phosphonoformate (0.3 g, 1.4 mmol) and PCl_5 (0.3 g, 1.4 mmol) were heated in CCl_4 (15 mL) at the reflux temperature for 3 h, and the reaction mixture was cooled to room temperature and concentrated to dryness under reduced pressure. The residue (containing **7**) was taken up in DMF (3 mL), the solution was cooled to -50°C, and 2',3'-dideoxycytidine (0.1 g, 0.47 mmol) was added under a dry N_2 atmosphere. The reaction mixture was allowed to come to room temperature, and after 2 h the solvent was removed in vacuo. The residue was taken up directly in anhydrous THF (4 mL) containing NaI (65 mg, 0.43 mmol), and the mixture was stirred at room temperature for 20 h. After evaporation of the reaction mixture to dryness, the residue was aplied onto a DEAE-cellulose column (HCO_3^- form), and the column was eluted first with water and then with 0.05 M NH_4HCO_3. Appropriate fractions were pooled and freeze-dried to obtain a white solid (0.1 g) which was further purified by preparative HPLC on C_{18} silica gel, with 5% MeCN in 0.01 M NH_4OAc as the eluent. Freeze-drying of the principal fraction afforded the product as a white solid (50 mg, 29% overall yield based on 2',3'-dideoxycytidine); mp 148-150°C; TLC: R_f 0.1 (2:1 CH_2Cl_2-MeOH); IR (KBr): ν 1720 (ester C=O) cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.2 (t, 3H, OCH_2CH_3), 1.8-2.4 (m, 4H, $C_{2'}$-H and $C_{3'}$-H), 4.0-4.3 (m, 5H, $C_{4'}$-H, $C_{5'}$-H, and OCH_2CH_3), 6.0 (m, 2H, $C_{1'}$-H and C_5-H), 8.0 (d, 1H, J = 7 Hz, C_6-H). Anal. ($C_{11}H_{18}N_3O_6\cdot0.4NH_3\cdot1.25H_2O$) Calcd: C, 38.26; H, 5.77; N, 12.65; P, 8.23. Found: C, 38.17; H, 5.73; N, 12.66; P, 8.03.

Dimethyl Cholesterylcarbonylphosphonate. Solid cholesteryl chloroformate (4 g, 0.9 mmol) was added slowly to refluxing trimethyl phosphite (7 mL), and refluxing was continued for 30 min. The reaction mixture was allowed to cool to room temperature, the product was dissolved in CH_2Cl_2, and the solution was concentrated to dryness under reduced pressure to obtain a white solid. Purification by silica gel flash chromatography (95:5 CH_2Cl_2-MeOH) afforded the desired triester as a coloress solid (2.8 g, 60% yield); mp 100 °C. Anal. Calcd for $C_{30}H_{51}O_5P$: C, 68.96; H, 9.77; P, 5.93. Found: C, 69.17; H, 9.88; P, 5.85.

3'-Azido-3'-deoxy-5'-O-(P-cholesterylcarbonyl-P-methoxyphosphinyl)thymidine (10). A mixture of dimethyl cholesterylcarbonylphosphonate (1.64 g, 3.14 mmol) and PCl_5 (654 mg, 3.13 mmol) in CCl_4 (20 mL) was refluxed for 3 h, and concentrated to dryness under reduced pressure. The residue was taken up in dry DMF (4 mL) and the solution was kept in a dry ice-acetone bath (-50° C) and stirred while adding a solution of AZT (268 mg, 1 mmol) in dry DMF (1 mL). When addition was complete, the reaction mixture was allowed to come to room temperature and left to stir overnight. The solvent was evaporated under high vacuum and the residue purified by silica gel flash chromatography (98:2 CH_2Cl_2-MeOH) to obtain a colorless gum (295 mg, 39% yield). After one more purification by flash chromatography the product was a pale-yellow solid; mp 100 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.5-3.0 (m, 44H, cholesteryl CH_3, CH_2, CH), 3.85 (d, J = 10 Hz, 3H, P-OCH_3), 4.1 (m, 1H, $C_{3'}$-H), 4.35 (m, 3H, $C_{4'}$-H, $C_{5'}$-H), 4.8 (m, 1H, cholesteryl CH-O), 5.3 (m, 1H, cholesteryl CH=), 6.2 (t, 1H, $C_{1'}$-H), 7.35 (broad s, C_6-H), 8.4 (broad s, NH) Anal. Calcd for $C_{39}H_{60}N_5O_8P \cdot 2H_2O$: C, 59.00; H, 8.12; N, 8.82; P, 3.90. Found: C, 59.11; H, 7.75; N, 9.10; P, 3.49.

3'-Azido-3'-deoxy-5'-O-(P-cholesterylcarbonyl-P-hydroxyphosphinyl)thymidine (3). A mixture of the methyl ester **10** (422 mg, 0.55 mmol) and NaI (92 mg, 0.60 mmol) in dry THF (5 mL) was stirred at room temperature for 20 h, and the solvent was evaporated under reduced pressure. Purification of the residue by silica gel flash chromatogaphy (85:15:3 CH_2Cl_2-MeOH-AcOH) afforded a colorless solid (267 mg, 58% yield based on the empirical formula); mp 210 °C (dec); ^1H NMR (CD_3OD, 60 MHz) δ 0.5-2.5 (m, 44H, cholesteryl CH_3, CH_2, CH), 4.2 (m, 4H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 4.5 (m, 1H, cholesteryl CH-O), 5.25 (m, 1H, cholesteryl CH=), 6.2 (t, 1H, $C_{1'}$-H), 7.8 (1H, s, C_6-H) Anal. Calcd for $C_{38}H_{58}N_5O_8P \cdot CH_3CO_2H \cdot 2H_2O$: C, 57.21; H, 7.86; N, 8.34; P, 3.69. Found: C, 56.95; H, 7.52; N, 7.92; P, 3.70.

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References

1. For a recent overview of human immunodeficiency viruses and the pathogenesis of AIDS, see: Levy, J.A. *J. Am. Med. Assoc.* **1989**, *261*, 2997.
2. For a comprehensive discussion of retroviruses, see: Varmus, H. *Science* **1988**, *240*, 1427.
3. Yarchoan, R.; Broder, S. *Pharmacol. Ther.* **1989**, *40*, 329.
4. Sarin, P.S. *Ann. Rev. Pharmacol.* **1988**, *28*, 411.
5. Yarchoan, R.; Klecker, R.W.; Weinhold, K.J.; Markham, P.D.; Lyerly, H.K.; Durack, D.T.; Gelmann, E.; Lehrman, S.N.; Blum, R.M.; Barry, D.W.; Shearer, G.M.; Fischl,

M.S.; Mitsuya, H.; Gallo, R.C.; Collins, J.M.; Bolognesi, D.P.; Myers, C.E.; Broder, S. Lancet **1986**, *i*, 575.

6. Fischl, M.A.; Richman, D.D.; Grieco, M.,H.; Gottlieb, M.S.; Volberding, P.A.; Laskin, O.L..; Leedom, J.M.; Groopman, J.E.; Mildvan D.; Schooley, R.T.; Jackson, G.G.; Durack, D.T.; King, D.; The AZT Collaborative Working Group. *N. Engl. J. Med.* **1987**, *317*, 185.
7. Yarchoan, R.; Perno, C.F.; Thomas, R.V.; Klecker, R.W.; Allain, J.P. Wills, R.J.; McAtee, N.; Fischl, M.A.; Dubinsky, R.; McNeely, M.C.; Mitsuya, H.; Pluda, J.M.; Lawley, T.J.; Leuther, M. Safai, B.; Collins, J.M.; Myers, C.E.; Broder, S. *Lancet* **1988**, *i*, 76.
8. Yarchoan, R.; Mitsuya, H.; Thomas, R.V.; Pluda, J.M.; Hartman, N.R.; Perno, C.-F.; Marczyk, K.S.; Allan, J.-P.; Johns, D.G.; Broder, S. *Science* **1989**, *245*, 412.
9. Cheng, Y.C.; Dutschman, G.E.; Bastow, K.F.; Sarngadharan, M.G.; Ting, R.Y.C. *J. Biol. Chem.* **1987**, *262*, 2187.
10. For what may be one of the earliest recorded suggestions of the possible therapeutic utility of 2',3'-dideoxynucleosides as DNA chain terminators, see: Robins, M.J.; Robins, R.K. *J. Am. Chem. Soc.* **1964**, *86*, 3585.
11. Vrang, L.; Oberg, B. *Antimicrob. Agents Chemother.* **1986**, *29*, 867.
12. Helgstrand, E.; Eriksson, B.; Johansson, N.G.; Lannero, B.; Larsson, A.; Misiorny, A.; Noren, J.O.; Sjoberg, B.; Stenberg, K.; Stening, G; Stridh, S.; Oberg, B.; Alenius, S.; Philipson, L. *Science* **1978**, *201*, 819.
13. Ringden, O.; Wilczek, H.; Lonnqvist, B.; Gahrton, G.; Wahren, B.; Lernestedt, J.-O. *Lancet* **1985**, *i*, 1503.
14. DeVita, V.T., Jr.;Broder, S.; Fauci, A.S.; Kovacs, J.A.; Chabner, B.A. *Ann. Intern. Med.* **1987**, *106*, 568.
15. Larder, B.A.; Purifoy, D.J.M.; Powell, K.L.; Darby, G. *Nature* (London) **1987**, *327*, 716.
16. Eriksson, B.F.H.; Schinazi, R.F. *Antimicrob. Agents Chemother.* **1989**, *33*, 663.
17. Koshida, R.; Vrang, L.; Gilljam, G.; Harmenberg, J.; Oberg, B.; Wahren, B. *Antimicrob. Agents Chemother.* **1989**, *33*, 778.
18. Ruprecht, R.M.; Rohrer, L.; Moelling, K.; Chou, T.-C. Manuscript in preparation.
19. Noren, J.A.; Helgstrand, E.; Johansson, N.G.; Misiorny, A.; Stening, G. *J. Med. Chem.* **1983**, *26*, 264.

20. Vaghefi, M.M.; McKernan, P.A.; Robins, R.K. *J. Med. Chem.* **1986**, *29*, 1389.

21. Griengl, H.; Hayden, W.; Penn, G.; De Clercq, E.; Rosenwirth, B. *J. Med. Chem.* **1988**, *31*, 1831.

22. Lambert, R.W.; Martin, J.A.; Thomas, G.J.; Duncan, I.B.; Hall, M.J.; Heimer, E.P. *J. Med. Chem.*, **1989**, *32*, 367.

23. For a recent example of macrophage-targeted AZT prodrugs containing a lipophilic phosphoglyceride moiety rather than cholesterol, see: Hostetler, K.Y.; Stuhmiller, L.M.; Lenting, H.B.M.; van den Bosch, H; Richman, D.D. *J. Biol. Chem.* **1990**, *265*, 6112.

24. Preliminary biological activity data for compound **2** were first presented at the Third Annual Meeting of the NIH National Cooperative Drug Discovery Groups for the Treatment of AIDS, Washington, D.C., May 15-18, 1990; see also: Rosowsky, A.; Saha, J.; Fazely, F.; Koch, J.; Ruprecht, R.M. *Biochem. Biophys. Res. Commun.*, **1990**, *172*, 288

25. Petrov, K.A.; Maklyaev, F.L.; Korshunov, M.A. *J. Gen. Chem. USSR (Engl. Transl.)* **1959**, *29*, 304.

26. Rao, T.S.; Reese, C.B. *J. Chem. Soc., Chem. Commun.* **1989**, 997.

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