# Potential Prodrug Derivatives of 2',3'-Didehydro-2',3'-dideoxynucleosides. Preparations and Antiviral Activities

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The preparations and antiviral activities of a series (4–17) of potential prodrug forms of the antivirals 2',3'-didehydro-2',3'-dideoxyadenosine (D4A) and 2',3'-didehydro-2',3'-dideoxycytosine (D4C) are reported. The 5'-phenyl- and 5'-methylphosphonates (4, 6, 8, and 10) and their phosphonothionate congeners (5, 7, 9, and 11), with the exception of 10, were inactive in vitro against HIV-1 and HIV-2. However, the 5'-phenyl, 5'-methyl, and 5'-(3'-thymidyl) phosphate diesters (12–17) demonstrated inhibition of the cytopathic effect of HIV-1 and HIV-2 ($EC_{50} \approx 1$–60 μM) and cytotoxicities ($CC_{50} \approx 35$–200 μM) at concentration levels comparable to those of their parent compounds, D4A and D4C. This strongly suggests that the diesters are hydrolyzed to the nucleosides D4A and D4C and/or their 5'-monophosphates. The facile hydrolysis of 12 and 13 to these products was demonstrated in a medium containing 10% fetal calf serum. The molecules can serve as ready prodrug sources of the free nucleosides and their 5'-monophosphates. Evidently, the phosphonates and phosphonothionates are not similarly cleaved, nor are they phosphorylated to form antivirally active or cytotoxic products. The importance of intracellular formation of these products in the activation of 12–17 is less clear. Potential prodrugs 4–17 are all stable in aqueous solution for hours with the exception of 14. Conjugates 4–17 showed no activity against a series of DNA and RNA viruses.

In the search for 2'-deoxynucleoside analogues with anti-HIV activity, the 2',3'-dideoxynucleoside derivatives, generalized by structure 1, have emerged as molecules with
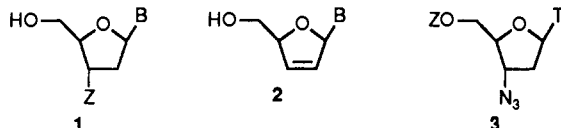
high activities and often very favorable selectivity indexes.[1] In clinical use is the widely prescribed therapeutic agent AZT (1, Z = N_3, B = thymin-1-yl), as well as 2',3'-dideoxycytidine (ddC, 1, Z = H, B = cytosin-1-yl) and the recently approved 2',3'-dideoxyinosine (ddI, 1, Z = H, B = hypoxant-9-yl). Two unsaturated analogues that show very promising activity and selectivity are 2',3'-didehydro-3'-deoxythymidine (commonly called D4T, 2, B = thymin-1-yl)[2] and its cytidine counterpart, 2',3'-didehydro-2',3'-dideoxycytidine (D4C, 2, B = cytosin-1-yl).[2a,b,c,3] Analogues D4C and D4T are less toxic than AZT.[1,2a,4] in cell culture.[5] D4T has already been subjected to clinical trials.[6]

Derivatives of active drugs are often synthesized as prodrugs. Some of the goals of this approach are to improve drug transport, to increase drug stability, to reduce toxicity, and to overcome drug resistance. A large number of prodrug derivatives of AZT have been prepared, usually by derivatization of AZT at the 5'-O position (structure 3). The obvious drug-design rationale for 3 is that the conjugate will be converted by hydrolysis or enzyme action to AZT itself or its 5'-monophosphate. Very recent examples of derivatives that do indeed display in vitro anti-HIV activity include 5'-(alkylamido ether phospholipid) conjugates,[7] the 5'-methylphosphonic acid,[8] the CF_3CH_2 and CCl_3CH_2 5'-trialkyl phosphates,[9] a 5'-dimyristoylphosphatidyl derivative,[10] the 5'-monophosphite,[8] a 5'-monophosphate diglyceride (phosphatic acid conjugate),[11] a sugar n-$C_{16}H_{33}$ 5'-phosphate triester,[12] the 5'-[(1,4-dihydro-1-methyl-3-pyridinyl)carbonyl] ester,[13] and the 5'-phosphonomethoxy derivative.[8,14] The latter is in fact a metabolite analogue rather than a prodrug. In addition AZT and ddA have been joined through the 5'

positions to form a phosphate diester with anti-HIV activity.[15] Analogous diester conjugates with ddA and ddI[16]

(1) De Clercq, E. New Acquisitions in the Development of Anti-HIV Agents. *Antiviral Res.* 1989, *12*, 1–20. For a review of the chemistry and biological activities of 2',3'-dideoxynucleosides, including the 2',3'-didehydro analogues, see: Herdewijn, P.; De Clercq, E. Dideoxynucleoside Analogues as Inhibitors of HIV Replication. In *Design of Anti-AIDS Drugs*: De Clercq, E., Ed.; Elsevier: Amsterdam; 1990; pp 141–174.

(2) (a) Baba, J.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Desmyter, J.; Vandeputte, M. Both 2',3'-Dideoxythymidine and its 2',3'-Unsaturated Derivative (2',3'-Dideoxythymidinene) are Potent and Selective Inhibitors of Human Immunodeficiency Virus Replication in Vitro. *Biochem. Biophys. Res. Commun.* 1987, *142*, 128–134. (b) Hamamoto, Y.; Nakashima, H.; Matsui, T.; Matsuda, A.; Ueda, T.; Yamamoto, N. Inhibitory Effect of 2',3'-Didehydro-2',3'-dideoxynucleosides on Infectivity, Cytopathic Effects, and Replication of Human Immunodeficiency Virus. *Antimicrob. Agents Chemother.* 1987, *31*, 907–910. (c) Balzarini, J.; Kang, G.-J.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. The Anti-HTLV-III (Anti-HIV) and Cytotoxic Activity of 2',3'-Didehydro-2',3'-dideoxyribonucleosides: A Comparison with Their Parental 2',3'-Dideoxyribonucleosides. *Mol. Pharmacol.* 1987, *32*, 162–167. (d) Lin, T.-S.; Schinazi, R. F.; Chen, M. S.; Kinney-Thomas, E.; Prusoff, W. H. Antiviral Activity of 2',3'-Dideoxycytidin-2'-ene (2',3'-Dideoxy-2',3'-didehydrocytidine) Against Human Immunodeficiency Virus In Vitro. *Biochem. Pharmacol.* 1987, *36*, 311–316.

(3) (a) Balzarini, J.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Cooney, D. A.; Kang, G.-J.; Dalal, M.; Johns, D. G.; Broder, S. Potent and Selective Anti-HTLV-III/LAV Activity of 2',3'-Dideoxycytidinene, the 2',3'-Unsaturated Derivative of 2',3'-Dideoxycytidine. *Biochem. Biophys. Res. Commun.* 1986, *140*, 735–742. (b) Lin, T.-S.; Chen, M. S.; McLaren, C.; Gao, Y.-S.; Ghazzouli, I.; Prusoff, W. H. Synthesis and Antiviral Activity of Various 3'-Azido, 3'-Amino, 2',3'-Unsaturated and 2',3'-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides Against Retroviruses. *J. Med. Chem.* 1987, *30*, 440–444. (c) Lin, T.-S.; Schinazi, R. F.; Prusoff, W. H. Potent and Selective In Vitro Activity of 3'-Deoxythymidine-2'-ene (3'-Deoxy-2',3'-didehydrothymidine) Against Human Immunodeficiency Virus. *Biochem. Pharmacol.* 1987, *36*, 2713–2718.

(4) Reduced toxicity of D4T compared to AZT toward bone marrow is of particular note. Mansuri, M. M.; Starrett, J. E., Jr.; Ghazzouli, I., Hitchcock, M. J. M.; Sterzycki, R. Z.; Brankovan, V.; Lin, T.-S.; August, E. M.; Prusoff, W. H.; Sommadossi, J.-P.; Martin, J. C. 1-(2,3-Dideoxy-β-glycero-pent-2-enofuranosyl)thymine. A Highly Potent and Selective Anti-HIV Agent. *J. Med. Chem.* 1989, *32*, 461–466.

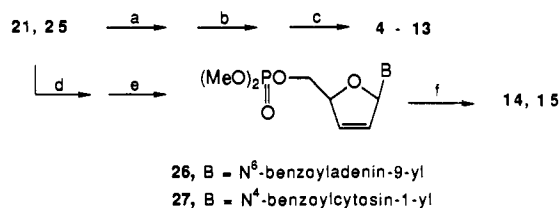§ Katholieke Universiteit Leuven.
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and with another molecule of AZT[16] also showed activity. By contrast a conjugate involving the derivatization of the 5'-hydroxyls of two AZT to form the neutral methylphosphonate was inactive[17] as were the diethyl, diisopropyl, and diisobutyl 5'-phosphate triesters.[18] A few active prodrug conjugates of ddC also have been prepared.[19]

(5) Balzarini, J.; Herdewijn, P.; De Clercq, E. Differential Patterns of Intracellular Metabolism of 2',3'-Didehydro-2',3'-dideoxythymidine and 3'-Azido-2',3'-dideoxythymidine, Two Potent Anti-Human Immunodeficiency Virus Compounds. *J. Biol. Chem.* 1989, *264*, 6127–6133.

(6) Reported in De Clercq, E. Targets and Strategies for the Antiviral Chemotherapy of AIDS. *Trends Pharmacol. Sci.* 1990, *11*, 198–205.

(7) Piantadosi, C.; Marasco, C. J., Jr.; Morris-Natschke, S. L.; Meyer, K. L.; Gumus, F.; Surles, J. R.; Ishaq, K. S.; Kucera, L. S.; Iyer, N.; Wallen, C. A.; Piantadosi, S.; Modest, E. J. Synthesis and Evaluation of Novel Ether Lipid Nucleoside Conjugates for Anti-HIV-1 Activity. *J. Med. Chem.* 1991, *34*, 1408–1414.

(8) Tarusova, N. B.; Khorlin, A. A.; Kraeveskii, A. A.; Korneeva, M. N.; Nosik, D. N.; Kruglov, I. V.; Galegov, G. A.; Bibilashvili, R. Sh. Inhibition of HIV Reproduction in Cell Cultures by 5'-Phosphonates of 3'-Azido-2',3'-dideoxynucleosides. *Mol. Biol. (Moscow)* 1989, *23*, 1716–1724; *Chem. Abstr.* 1990, *112*, 91229b.

(9) McGuigan, C.; O'Conner, T. J.; Nicholls, S. R.; Nickson, C.; Kinchington, D. Synthesis and Anti-HIV Activity of Some Novel Substituted Dialkyl Phosphate Derivatives of AZT and dd Cyd. *Antiviral Chem. Chemother.* 1990, *1*, 355–360.

(10) Hostetler, K. Y.; Carson, D. A.; Richman, D. D. Phosphatidylazidothymidine. Mechanism of Antiretroviral Action in CEM Cells. *J. Biol. Chem.* 1991, *266*, 11714–11717.

(11) Steim, J. M.; Neto, C. C.; Sarin, P. S.; Sun, D. K.; Sehgal, R. K.; Turcotte, J. G. Lipid Conjugates of Antiviral Agents I. Azidothymidine-Monophosphate-Diglyceride: Anti-HIV Activity, Physical Properties, and Interaction with Plasma Proteins. *Biochem. Biophys. Res. Commun.* 1990, *171*, 451–457.

(12) Henin, Y.; Gouyette, C.; Schwartz, O.; Debouzy, J.-C.; Neumann, J.-M.; Huynh-Dinh, T. Lipophilic Glycosyl Phosphotriester Derivatives of AZT: Synthesis, NMR, Transmembrane Transport Study, and Antiviral Activity. *J. Med. Chem.* 1991, *34*, 1830–1837.

(13) Gogu, S. R.; Aggarwal, S. K.; Rangan, S. R. S.; Agrawal, K. C. A Pro-Drug of Zidovudine with Enhanced Efficacy Against Human Immunodeficiency Virus. *Biochem. Biophys. Res. Commun.* 1989, *160*, 656–661. Torrence, P. F.; Kinjo, J.; Lesiak, K.; Balzarini, J.; De Clercq, E. AIDS Dementia: Synthesis and Properties of a Derivative of 3'-Azido-3'-deoxythymidine (AZT) that May Become Locked in the Central Nervous System. *FEBS Lett.* 1988, *234*, 135–140.

(14) Jie, J.; Van Aerschot, A.; Balzarini, J.; Janssen, G.; Busson, R.; Hoogmartens, J.; De Clercq, E.; Herdewijn, P. 5'-O-Phosphonmethyl-2',3'-dideoxynucleosides: Synthesis and Anti-HIV Activity. *J. Med. Chem.* 1990, *33*, 2481–2487.

(15) Saksena, A. K.; Girijavallabhan, V. M.; Ganguly, A. K. Preparation of Carbocyclic Nucleoside-Containing Nucleoside Phosphate and Phosphonate Dimers and Trimers as Antiviral Agents. PCT Int. Appl. WO 90 06,319. *Chem. Abstr.* 1991, *114*, 7091m.

(16) Busso, M.; Mian, A. M.; Hahn, E. F.; Resnick, L. Nucleotide Dimers Supress HIV Expression in Vitro. *AIDS Res. Hum. Retroviruses* 1988, *4*, 449–455.

(17) Puech, F.; Gosselin, G.; Balzarini, J.; Good, S. S.; Rideout, J. L.; De Clercq, E.; Imbach, J.-L. Synthesis and Biological Evaluation of Dinucleoside Methylphosphonates of 3'-Azido-3'-deoxythymidine and 2',3'-Dideoxycytidine. *Antiviral Res.* 1990, *14*, 11–24.

(18) McGuigan, C.; Nicholls, S. R.; O'Conner, T. J.; Kinchington, D. Synthesis of Some Novel Dialkyl Phosphate Derivatives of 3'-Modified Nucleosides as Potential Anti-AIDS Drugs. *Antiviral Chem. Chemother.* 1990, *1*, 25–33.

(19) See e.g. ref 9 and the following: Magnani, M.; Bianchi, M.; Rossi, L.; Stocchi, V. Human Red Blood Cells as Bioreactors for the Release of 2',3'-Dideoxycytidine, an Inhibitor of HIV Infectivity. *Biochem. Biophys. Res. Commun.* 1989, *164*, 446–452.

Scheme I[a]

18, 19, 20, 21, B = N[6]-benzoyladenin-9-yl
22, 23, 24, 25, B = N[4]-benzoylcytosin-1-yl
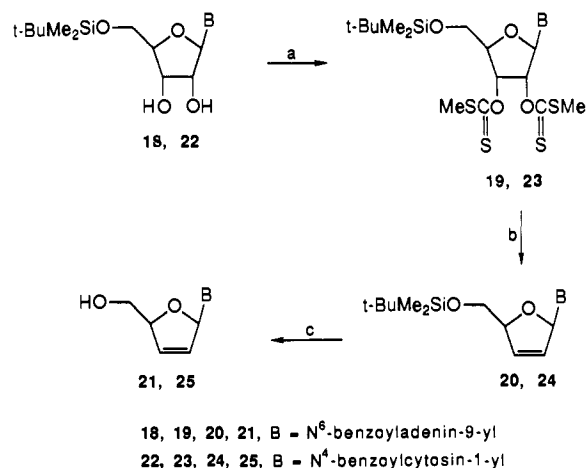
[a] (a) NaH/imidazole/CS₂/MeI; (b) AIBN/Bu₃SnH; (c) TBAF.

Scheme II[a]

26, B = N[6]-benzoyladenin-9-yl
27, B = N[4]-benzoylcytosin-1-yl

[a] (a) ZP(O)Cl₂/1,2,4-triazole/Et₃N; (b) H₂O/Et₃N; (c) NH₄OH; (d) i-Pr₂NP(OMe)₂/1H-tetrazole; (e) H₂O/I₂; (f) MeOH/NH₃.
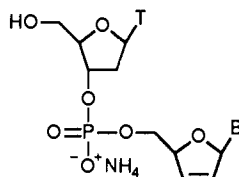
The 2',3'-didehydro-2',3'-dideoxynucleosides, however, have not been converted to a large number of potential prodrugs. The 5'-methyl phosphate diester of D4T was reported as a derivative successfully introduced transdermally through rat skin with the assistance of 3% 1-menthol.[20] The preparation of the 5'-[(1,4-dihydro-1-methyl-3-pyridinyl)carbonyl] ester of D4T has been reported as a potential brain–blood barrier transport system.[21] Recently, the phosphonate isosteres of the D4T and D4A 5'-monophosphates were found to exhibit potent anti-HIV activity comparable to that of D4T.[22]

In the present paper we report the synthesis and antiviral activities of a series of derivatives of D4A and D4C. These include the methyl- and phenylphosphonates and phosphonothioates (4–11), the 5'-methyl and 5'-phenyl phosphate diesters (12–15), and two 3',5'-phosphate diester conjugates involving thymidine (16 and 17). Potentially, hydrolysis of 4–17, intra- or extracellularly, will give D4A or D4C. The phosphate diesters also are plausible intracellular sources of the 5'-monophosphates of D4A or D4C, the initial metabolites in their activation, which might even arise as products of phosphodiesterase activity. The 5'-phenyl- and 5'-methylphosphonate hydrolysis products (and their thiono analogues) from 4–11 appear to be less likely candidates as antiviral precursors (although they conceivably could be further phosphorylated) unless they

(20) Kawaguchi, T.; Hasegawa, T.; Endoh, H.; Seki, T.; Juni, K. Saneyoshi, M. Percutaneous Penetration of 2',3'-Didehydro-3'-deoxythymidine (D4T) and its Ester Prodrugs Through Rat Skin. *Drug Delivery Syst.* 1991, *6*, 57–60.

(21) Palomino, E.; Kessel, D.; Horwitz, J. P. A Dihydropyridine Carrier System for Sustained Deliver of 2',3'-Dideoxynucleosides to the Brain. *J. Med. Chem.* 1989, *32*, 622–625.

(22) Kim, C. U.; Luh, B. Y.; Martin, J. C. Regiospecific and Highly Stereoselective Electrophilic Addition to Furanoid Glycals: Synthesis of Phosphonate Nucleotide Analogues with Potent Activity against HIV. *J. Org. Chem.* 1991, *56*, 2642–2647.
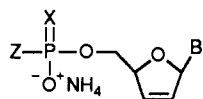
4: Z = Me, X = O, B = adenin-9-yl
5: Z = Me, X = S, B = adenin-9-yl
6: Z = Ph, X = O, B = adenin-9-yl
7: Z = Ph, X = S, B = adenin-9-yl
8: Z = Me, X = O, B = cytosin-1-yl
9: Z = Me, X = S, B = cytosin-1-yl
10: Z = Ph, X = O, B = cytosin-1-yl
11: Z = Ph, X = S, B = cytosin-1-yl
12: Z = PhO, X = O, B = adenin-9-yl
13: Z = PhO, X = O, B = cytosin-1-yl
14: Z = MeO, X = O, B = adenin-9-yl
15: Z = MeO, X = O, B = cytosin-1-yl
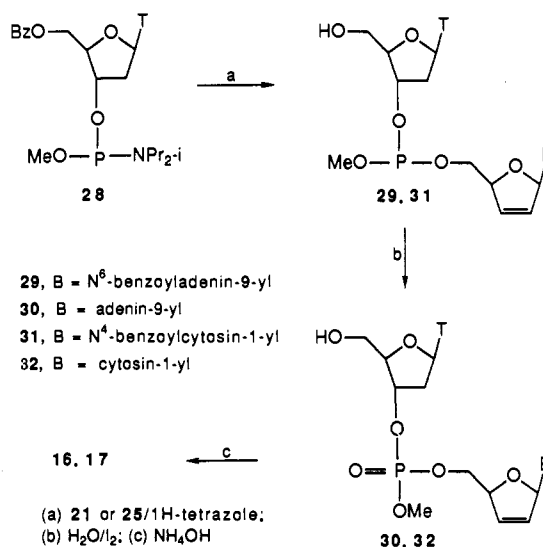
16: B = adenin-9-yl
17: B = cytosin-1-yl

Scheme III^a

29, B = N^6-benzoyladenin-9-yl
30, B = adenin-9-yl
31, B = N^4-benzoylcytosin-1-yl
32, B = cytosin-1-yl

16, 17

(a) 21 or 25/1H-tetrazole;
(b) H_2O/I_2; (c) NH₄OH

can be subsequently hydrolyzed to D4A or D4C. It can be hoped that modification of D4A, which does not display potent anti-HIV activity,[2,3] may improve its activity and that the derivatization of D4C will decrease its inherent instability in solution[2c,23] and perhaps also raise its selectivity index.

Results and Discussion

Chemistry. N^6-Benzoyl-2',3'-didehydro-2',3'-dideoxyadenosine (21) and N^4-benzoyl-2',3'-didehydro-2',3'-dideoxycytidine (25) were prepared following the procedure of Chu et al.[24] We chose to employ the N-benzoylated derivatives rather than the unprotected nucleosides[24] to avoid reaction of the amino groups on the nucleobases in subsequent derivatization of the 5'-hydroxyls. (See Scheme I.) Thus, N^6-benzoyl-5'-O-silyl-protected adenosine (18) was reacted with carbon disulfide in presence of sodium hydride and imidazole and then alkylated in situ with methyl iodide to form the bisxanthate 19 in 80% yield. Compound 19, on treatment with tri-n-butyltin hydride and AIBN in refluxing toluene, afforded 20 in 91% yield. Deprotection of the silyl ether functionality of 20 by TBAF resulted in 21 in 87% yield. The same procedure was applied to the cytidine derivative 22 to give 24, which on deprotection with TBAF yielded 25 (88%).

Methyl- and phenylphosphonic dichloride, and the corresponding phosphonothioic dichlorides, were converted to their bistriazolides on treatment with 1,2,4-triazole and triethylamine, which then were reacted in situ with the protected nucleoside analogues 21 and 25 (Scheme II). Hydrolysis of the resulting 5'-derivatives, followed by debenzoylation with NH₄OH, gave phosphonates and phosphonothioates 4–11. Analogously, phenyl phosphorodichloridate, PhOP(O)Cl₂, was converted to its triazolide and ultimately to phosphates 12 and 13. Derivatives 4–13 were isolated as the *ammonium* salts by chromatography on Sephadex A-25 in 64–83% yields. Methyl phosphates 14 and 15 could not be prepared by this procedure. Direct reaction of MeOP(O)Cl₂ with 21 or 25 in presence of triethylamine or pyridine was also unsuccessful. However, 14 and 15 were prepared (Scheme II) following the phosphite triester procedure of Caruthers et al.[25] Thus, re-

action of dimethyl N,N-diethylphosphoramidite with 21 in presence of 1H-tetrazole afforded the phosphite (δ ^{31}P = 141.6) which was then oxidized by I_2/H_2O to give 26, isolated in 80% yield by column chromatography. Partial deprotection of 26 by MeOH/NH₃ resulted in 14 (83%).

The same procedure was extended to cytidine derivative 25 to prepare 15 (84%) via 27 (Scheme II). Nucleosides 21 and 25 were converted to nucleoside diesters 16 and 17 by the same approach. Reaction of methyl tetraisopropylphosphorodiamidite with 5'-benzoylthymidine formed 28 in 85% yield. Treatment of 28 with 21 and 25 in presence of 1H-tetrazole gave phosphites 29 and 31, which were oxidized (I_2/H_2O) and deprotected to afford 16 and 17 in 84 and 82% yield, respectively, based on 28 (Scheme III). All of 4–17 were isolated as the *ammonium salts*.

The 2',3'-didehydro-2',3'-dideoxynucleotide derivatives, 4–17, were characterized by ^{1}H and ^{13}C NMR spectral data as well as by mass spectrometry and/or quantitative elemental analysis. Our assignments of chemical shifts follow those of Robins et al.[26] based on their experiments with deuterio-2',3'-unsaturated adenosine that defined the following general chemical shift trend: H-1' < H-3' < H-2' < H-4' < H-5', H-5" and C-3' < C-2' < C-1' < C-4' < C-5'.

The inherent instability of 2',3'-didehydro-2',3'-dideoxynucleosides toward cleavage of the sugar–nucleobase bond and anomerization of that stereocenter is well known.[2c,23] In the present work, only diester 14 showed unusual instability. It was about 50% base cleaved in 12 h in D_2O in an NMR tube at room temperature as estimated by proton NMR. By contrast diester 12 underwent only about 5% base cleavage in 15 h, and diester 16 was completely stable for 15 h under the same conditions. None of the other conjugates showed any tendency toward decomposition in D_2O. These results suggest that the stability of such conjugates is dependent on structure. The use of biologically active phosphate diesters in place of the 2'3'-didehydro-2',3'-dideoxynucleoside itself may increase the lifetimes of these antivirals after in vivo administration.

Biological Activity. The ammonium salts of the above conjugates of D4C and D4A (4-17) were evaluated for their inhibitory effect on the replication of a series of DNA and RNA viruses, including herpes simplex virus type 1 (HS-

(23) Kawaguchi, T.; Ishikawa, K.; Seki, T.; Juni, K.; Fukushima, S.; Nakano, M. Studies on 2',3'-Dideoxy-2',3'-didehydropyrimidine Nucleosides. II. N₄-Benzoyl-2',3'-dideoxy-2',3'-didehydrocytidine as a Prodrug of 2',3'-Dideoxy-2',3'-didehydrocytidine (DDCN). *Chem. Pharm. Bull.* **1989**, 37, 2547–2549.

(24) Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. General Syntheses of 2',3'-Dideoxynucleosides and 2',3'-Didehydro-2',3'-dideoxynucleosides. *J. Org. Chem.* **1989**, 54, 2217–2225.

(25) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. In situ Activation of Bis-Dialkylaminophosphines—A New Method for Synthesizing Deoxyoligonucleotides on Polymer Supports. *Nucleic Acids Res.* **1984**, 12, 4051–4069.

(26) Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. A Mild Conversion of Vicinal Diols to Alkenes. Efficient Transformation of Ribonucleosides into 2'-Ene and 2',3'-Dideoxynucleosides. *Tetrahedron Lett.* **1984**, 25, 367–370.

Table I. Anti-HIV Activity of D4C and D4A Derivatives in MT-4 Cells

compd	EC$_{50}^a$ (μM)		CC$_{50}^b$ (μM)
	HIV-1	HIV-2	
4	>500		>500
5	>500	>500	>500
6	>500	>500	>500
7	>500	>500	>500
8	>500		>500
9	>500	>500	>500
10	111 ± 5	136 ± 8	>500
11	>500	>500	>500
12	24 ± 14	37 ± 19	157 ± 42
13	1.9 ± 0.3	1.5 ± 0.7	35 ± 8.5
14	38 ± 0.5	58 ± 17	168 ± 16
15	3.1 ± 0.48	1.7 ± 0.77	27 ± 17
16	26 ± 15	29 ± 18	198 ± 26
17	1.8 ± 0.30	1.6 ± 0.77	28 ± 5.7
D4A	41 ± 33	72 ± 23	≥100
D4C	0.91 ± 0.9	0.69 ± 0.4	30 ± 4.8

^{a} 50% effective concentration, or concentration required to protect 50% of HIV-infected MT-4 cells against viral cytopathicity. ^{b} 50% cytotoxic concentration, or concentration required to reduce the number of viable uninfected MT-4 cells by 50%.

V-1), thymidine kinase deficient (TK$^-$) HSV-1, herpes simplex virus type 2 (HSV-2), vaccinia virus, and vesicular stomatitis virus (VSV) in E$_6$SM cells; parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie virus type B4, and Semliki forest virus in Vero cells; and VSV, Coxsackie virus type B4, and poliovirus type 1 in HeLa cells. The test compounds were neither cytotoxic in these cell cultures nor active against any of the viruses tested. Also, except for compound 10, none of the 5'-methyl- and 5'-phenylphosphonate and phosphonothionate derivatives of D4A and D4C (4-11) was active in protecting MT-4 cells against the cytopathic effect of HIV-1 or HIV-2 (Table I). These observations indicate that the phosphonates and phosphonothionates are either not taken up by the cells or not phosphorylated to their corresponding 5'-diphosphate derivatives (the presumably active form of these compounds). From our data it is also clear that the phosphonate moiety is not cleaved extra- or intracellularly from the parental nucleoside ((D4C or D4A). Otherwise, some anti-HIV activity should have been observed.

In contrast, the phosphate diester derivatives of D4A (compounds 12 and 14) and D4C (compounds 13 and 15) proved inhibitory to the cytopathicity of HIV-1 and HIV-2 in MT-4 cells at concentrations comparable to those of the parent compounds, D4C and D4A (Table I). Also, the cytotoxic activities of these derivatives (12 and 14, on the one hand, and 13 and 15, on the other) were similar to those observed for D4A and D4C, respectively. Essentially the same behavior was shown by the 3',5'-diester conjugates of thymidine with D4A (compound 16) or D4C (compound 17).

To obtain better insight into the presumed hydrolytic activation of the phosphodiester derivatives, compounds 12 and 13 (1 mM concentrations) were incubated in the presence of RPMI-1640 culture medium containing 10% fetal calf serum. The solution was monitored over time by HPLC (Partisil SAX-10) for release of nucleoside 5'-monophosphate and free nucleoside. After 5 h 12 had produced the 5'-monophosphate, D4AMP (28% based on starting 12), and the free nucleoside, D4A (9%). These products increased in 23 h to 38% D4AMP and 41% D4A. Prodrug 13 yielded 60% of the monophosphate (D4CMP) and 18% of the free nucleoside (D4C) in 5 h, yields which changed to 38% (monophosphate) and 61% (D4C) after 23 h. Thus 13 was essentially totally consumed in 23 h.

These results show that 12 and 13 are rapidly hydrolyzed in serum-containing medium. Furthermore, the 5'-monophosphates are formed more rapidly than the nucleosides in the serum-containing medium; the 5-monophosphates are subsequently further hydrolyzed to the nucleosides. It is also clear that phosphodiester 13 is more rapidly hydrolyzed than is 12. Recall that these compounds are stable in D$_2$O (vide supra).

When CEM cells (7 × 10^5 cells/mL) also were added to the incubation mixture containing 10% serum, the rates of conversion of 12 and 13 to their hydrolysis products were not markedly altered. In the absence of double-labeling experiments, definitive conclusions about the degree of uptake and intracellular hydrolysis of 12 and 13, at the concentration of CEM cells employed, cannot be made. However, derivatives 12 and 13 in serum-containing medium clearly *can* function as prodrugs of the antiviral nucleosides which are released before their introduction into cells. (Presumably, extracellular nucleoside monophosphate, which would be an active prodrug form of the triphosphate, is not taken into cells.) This view is consistent as well with the similarities in the activities of D4C and D4A in culture medium to those of their nucleoside counterparts as shown in Table I. It is likely that the inactivities of the 5'-phosphonates and 5'-phosphonothioates result from their hydrolytic stabilities, enzymic or nonenzymic.

Summary

A series of potential prodrug derivatives of the 2',3'-didehydro-2',3'-dideoxynucleosides D4A and D4C were prepared in the form of the 5'-phenyl- and 5'-methylphosphonates (4, 6, 8, and 10), the corresponding phosphonothionates (5, 7, 9, and 11), and the 5'-methyl, 5'-phenyl, and 5'-(3'-thyminyl) phosphate diesters (12-17). These molecules were generally stable toward sugar-nucleobase bond cleavage in aqueous solution for several hours with the possible exception of 14. In contrast to the phosphonates and phosphonothionates (except for 10), the 5'-phosphate diesters showed in vitro anti-HIV activities and cytotoxicities comparable to those of D4A and D4C. This suggests that the active compounds are cleaved under the test conditions to release the parent nucleosides or 5'-monophosphates. Indeed, 12 and 13 were efficiently cleaved to the nucleosides D4A and their monophosphates in serum containing medium. The inactivities of the 5'-phosphonate and 5'-phosphonothioate analogues presumably are a result of their comparative stabilities toward release of nucleoside and 5'-monophosphate. 4-17 were inactive against a series of DNA and RNA viruses.

Experimental Section

Melting points were recorded on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were taken on a Varian XL-300 spectrometer at 300 MHz, and ^{13}C NMR spectra were obtained at 75.5 MHz. The carbons of the phenyl group are designated as C-1'', C-2'', etc.; app denotes apparent. Coupling constants given are phosphorus-carbon values; listed J values in the ^{1}H NMR spectral data refer to proton-proton couplings unless otherwise stated. ^{31}P NMR spectra were recorded at 121.3 MHz. UV absorption spectra were taken with a Hewlett-Packard 8452A diode-array spectrophotometer. Mass spectra were determined on Finnigan MAT 95 mass spectrometer. Analytical TLC was performed on Merck 0.2-mm layer silica gel 60 F$_{245}$. Column chromatography employed Merck silica gel 60, 230-400 mesh. Sephadex A-25 (HCO$_3^-$ form) was used for ion-exchange chromatography. CH$_3$P(S)Cl$_2$, CH$_3$P(O)Cl$_2$, PhP(S)Cl$_2$, PhP(O)Cl$_2$, and PhOP(O)Cl$_2$ were purchased from Aldrich Chemical Co. Anhydrous solvents were obtained as follows: acetonitrile by successive distillations first from P$_4$O$_{10}$ and then from CaH$_2$; benzene and toluene, distillation from sodium; dichloromethane,

distillation from P_4O_{10}; tetrahydrofuran, distillation from sodium/benzophenone; dimethylformamide and pyridine, distillation from CaH_2.

Antiviral Assays. The origin of the following virus stocks was described previously: herpes simplex virus (HSV) type 1 (HSV-1, strain KOS), HSV-2 (strain G), thymidine kinase-deficient (TK^-) HSV-1 (strain B2006),[27] vaccinia virus, vesicular stomatitis virus (VSV), Coxsackie virus type B4, poliovirus type 1 and Sindbis virus,[28] reovirus type 1 (ATCC VR-230), parainfluenza virus type 3 (ATCC VR-93), and Semliki forest virus (ATCC VR-67) [ATCC: American Type Culture Collection (Rockville, MD)]. The inhibitory effects of the test compounds on the virus replication were monitored by microscopic examination of virus-induced cytopathicity in cell cultures.[27,28]

Anti-HIV Assays. Anti-HIV activity was determined using an HIV cytopathicity assay in human T-lymphocyte MT-4 cells, as described previously.[29,30] Briefly, MT-4 cells, subcultured 1 day before the start of the experiment, were adjusted to 5×10^5 cells/mL and infected with HIV-1 (strain HTVLz-III$_B$) or HIV-2 (strain ROD at 400 CCID$_{50}$/mL (1 CCID$_{50}$ being the infective dose for 50% of the cell cultures). Then, 100 μL of the infected cell suspension were transferred to wells of a microtiter plate containing 100 μL of varying dilutions of the test compounds. After 5 days of incubation at 37 °C, the number of viable cells was determined microscopically with an hematocytometer following the trypan blue exclusion procedure.

N^6-**Benzoyl-5'-O-($tert$-butyldimethylsilyl)-2',3'-bis-O-[(methylthio)thiocarbonyl]adenosine (19).** To a stirred solution of N^6-benzoyl 5'-O-($tert$-butyldimethylsilyl)adenosine (18) (5.90 g, 12.2 mmol) and imidazole (350 mg) in THF (100 mL) was added sodium hydride (2.04 g of 60% reagent in mineral oil, 51.1 mmol) at 0 °C under argon atmosphere. After 45 min CS$_2$ (4.85 g, 63.9 mmol) was added, and the reaction was continued for 30 min. Then methyl iodide (6.90 g, 48.7 mmol) was added, and after 30 min the reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL) and washed with cold H$_2$O (2 × 50 mL). The organic layer was dried (MgSO$_4$) and evaporated to give a yellow gum. Purification of the crude product by column chromatography on silica gel eluting with 0–2% MeOH in CHCl$_3$ gave 19 as a foam (6.47 g, 80%): UV (MeOH) λ_{max} 224, 280 nm; ^{1}H NMR (CDCl$_3$) δ −0.01 (6 H, s, SiMe$_2$), 0.98 (9 H, s, SiBu-t) 2.54 (3 H, SCH$_3$), 2.64 (3 H, s, SCH$_3$), 3.99 (1 H, dd, J = 11.4, 2.1 Hz, 5''-H), 4.09 (1 H, dd, J = 11.4, 2.1 Hz, 5'-H), 4.58 (1 H, m, 4'-H), 6.44 (1 H, dd, J = 5.4, 1.8 Hz, 3'-H), 6.55 (1 H, dd, J = 7.2, 5.4 Hz, 2'-H), 6.71 (1 H, d, J = 7.2 Hz, 1'-H), 7.50–7.63 (3 H, m, C$_6$H$_5$), 8.02 (2 H, m, C$_6$H$_5$), 8.43 (1 H, s, 8-H), 8.84 (1 H, s, 2-H), 9.02 (1 H, s, NH); MS (CI) m/z 666.137570; C$_{27}$H$_{35}$N$_5$O$_5$S$_4$Si requires 666.136864.

N^6-**Benzoyl-5'-O-($tert$-butyldimethylsilyl)-2',3'-didehydro-2',3'-dideoxyadenosine (20).** A solution of tri-n-butyltin hydride (10.7 g, 36.9 mmol) in dry toluene (100 mL) was dropwise added to a refluxing solution of compound 19 (6.0 g, 9.2 mmol) and azobisisobutyronitrile (1.0 g) in toluene under an argon atmosphere over a period of 1 h. The reaction mixture was refluxed for 1 h and then cooled to room temperature. The solvent was removed, and the residue was chromatographed on silica gel eluting with 0–2% MeOH in CHCl$_3$. Appropriate fractions were combined and evaporated to give the title compound as a colorless foam (3.78 g, 91%): UV (MeOH) λ_{max} 234, 260, 280 nm; ^{1}H NMR

(CDCl$_3$) δ 0.05 (3 H, s, SiMe), 0.06 (3 H, s, SiMe), 0.89 (9 H, s, SiBu-t), 3.85 (2 H, m, 5'-H), 5.02 (1 H, m, 4'-H), 6.08 (1 H, m, 2'-H), 6.45 (1 H, app dt, J = 6.1, 1.5 Hz, 3'-H), 7.18 (1 H, m, 1'-H), 7.47–7.63 (3 H, m, C$_6$H$_5$), 8.02 (2 H, d, J = 7.3 Hz, C$_6$H$_5$), 8.30 (1 H, s, 8-H), 8.83 (1 H, s, 2-H), 9.15 (1 H, s, NH); MS (EI) m/z 394.132060; C$_{23}$H$_{29}$N$_5$O$_3$Si requires 394.133543.

N^6-**Benzoyl-2',3'-didehydro-2',3'-dideoxyadenosine (21).** To a solution of 20 (4.51 g, 10.0 mmol) in THF (50 mL) at 0 °C was added a 1 M solution of tetra-n-butylammonium fluoride in THF (12 mL, 12.0 mmol). The reaction mixture was stirred at room temperature for 1 h and then concentrated. The resulting yellow syrupy residue was purified by column chromatography on silica gel using 0–4% MeOH in CHCl$_3$ as eluent. Product 21 was obtained as a white solid (2.93 g, 87%): mp 98–99 °C; UV (MeOH) λ_{max} 232, 260, 280 nm; ^{1}H NMR (DMSO-d_6) δ 3.59 (2 H, m, 5'-H), 4.93 (1 H, m, 4'-H), 4.98 (1 H, t, J = 5.4 Hz, 5'-OH), 6.21 (1 H, m, 2'-H), 6.53 (1 H, app dt, J = 6.0, 1.8 Hz, 3'-H), 7.10 (1 H, m, 1'-H), 7.54 (2 H, app t, J = 7.5 Hz, C$_6$H$_5$), 7.64 (1 H, app t, J = 7.5 Hz, C$_6$H$_5$), 8.04 (2 H, app d, J = 7.5 Hz, C$_6$H$_5$), 8.49 (1 H, s, 8-H), 8.76 (1 H, s, 2-H), 11.19 (1 H, s, NH); ^{13}C NMR (CDCl$_3$) δ 62.58 (C-5'), 87.99 (C-4'), 88.30 (C-1'), 122.10 (C-5), 124.32 (C-2'), 127.78 (C-3''), 128.11 (C-2''), 132.12 (C-4''), 133.38 (C-1''), 134.84 (C-3'), 141.79 (C-8), 148.62 (C-4), 150.69 (C-6), 152.14 (C-2), 164.98 (C=O); MS (EI) m/z 319 (M-18), (FAB) 338 (M + 1). Anal. (C$_{17}$H$_{15}$N$_5$O$_3$) C, H, N.

N^4-**Benzoyl-5'-O-($tert$-butyldimethylsilyl)-2',3'-bis-O-[(methylthio)thiocarbonyl]cytidine (23).** N^4-Benzoyl-5'-O-($tert$-butyldimethylsilyl)cytidine (22) (2.34 g, 5.1 mmol) was converted to 23 (2.14 g, 60%) by following the procedure described for 19: UV (MeOH) λ_{max} 216, 276 nm; ^{1}H NMR (CDCl$_3$) δ 0.19 (6 H, s, SiMe$_2$), 0.98 (9 H, s, SiBu-t), 2.56 (3 H, s, SMe), 2.60 (3 H, s, SMe), 3.98 (1 H, dd, J = 11.5, 2.0 Hz, 5''-H), 4.05 (1 H, dd, J = 11.5, 1.8 Hz, 5'-H), 4.50 (1 H, m, 4'-H), 6.15 (1 H, dd, J = 6.8, 5.6 Hz, 2'-H), 6.27 (1 H, dd, J = 5.6, 2.0 Hz, 3'-H), 6.75 (1 H, d, J = 6.8 Hz, 1'-H), 7.47–7.65 (4 H, m, C$_6$H$_5$, 5-H), 7.92 (2 H, d, J = 7.3 Hz, C$_6$H$_5$), 8.29 (1 H, d, J = 8.0 Hz, 6-H), 8.82 (1 H, br s, NH); MS (CI) m/z 642.125019; C$_{26}$H$_{35}$O$_6$SiS$_4$ requires 642.125631.

N^4-**Benzoyl-5'-O-($tert$-butyldimethylsilyl)-2',3'-didehydro-2',3'-dideoxycytidine (24).** A refluxing solution of 23 (6.0 g, 9.4 mmol) and azobisisobutyronitrile (800 mg) in toluene was treated with a solution of tri-n-butyltin hydride (10.9 g, 37.4 mmol) in toluene as described for the preparation of 20. Workup of the reaction mixture and chromatography of the crude product over silica gel using CHCl$_3$–MeOH (99:1) yielded compound 24 as a colorless solid (2.60 g, 65%); UV (MeOH) λ_{max} 260, 304 nm; ^{1}H NMR (CDCl$_3$) δ 0.09 (3 H, s, SiMe), 0.11 (3 H, s, SiMe), 0.91 (9 H, s, SiBu-t), 3.87 (1 H, dd, J = 11.7, 2.6 Hz, 5''-H), 4.00 (1 H, dd, J = 11.7, 2.7 Hz, 5'-H), 4.98 (1 H, m, 4'-H), 6.03 (1 H, m, 2'-H), 6.18 (1 H, app dt, J = 6.0, 1.6 Hz, 3'-H), 7.04 (1 H, m, 1'-H), 7.35–7.63 (4 H, m, C$_6$H$_5$ and 5-H), 7.88–7.93 (2 H, m, C$_6$H$_5$), 8.38 (1 H, d, J = 7.5 Hz, 6-H), 8.77 (1 H, br s, NH); MS (CI) m/z 426.198305; C$_{22}$H$_{29}$N$_3$O$_4$Si requires 428.200560.

N^4-**Benzoyl-2',3'-didehydro-2',3'-dideoxycytidine (25).** Compound 24 (2.0 g, 4.7 mmol) was deprotected with a 1 M solution of tetra-n-butylammonium fluoride (5.5 mL, 5.5 mmol) in THF (20 mL) as described for the preparation of compound 21. Chromatography of the crude product on silica gel using CHCl$_3$–MeOH (0–5% MeOH) gave 25 as a colorless solid (1.29 g, 88%): mp >300 °C; UV (MeOH) λ_{max} 260, 304 nm; ^{1}H NMR (DMSO-d_6) δ 3.62 (2 H, m, 5'-H), 4.87 (1 H, m, 4'-H), 5.04 (1 H, t, J = 5.0 Hz, 5'-OH), 5.96 (1 H, m, 2'-H), 6.40 (1 H, m, 3'-H), 6.90 (1 H, m, 1'-H), 7.25–7.63 (4 H, m, C$_6$H$_5$, 5-H), 7.98 (2 H, m, C$_6$H$_5$), 8.26 (1 H, d, J = 7.2 Hz, 6-H), 11.25 (1 H, s, NH); ^{13}C NMR (DMSO-d_6) δ 62.29 (C-5'), 88.07 (C-4'), 90.80 (C-1'), 96.33 (C-5), 126.21 (C-2'), 128.42 (C-3''), 128.48 (C-2''), 132.73 (C-4''), 133.12 (C-1''), 134.67 (C-3'), 145.78 (C-6), 154.80 (C-2), 163.20 (C=O), 167.32 (C-4); MS (EI) m/z 313 (M$^+$). Anal. (C$_{16}$H$_{15}$N$_3$O$_4$) C, H, N.

General Procedure for the Preparation of 5'-Phosphonates and 5'-Phosphonothionates of 2',3'-Didehydro-2',3'-dideoxyadenosine and 2',3'-Didehydro-2',3'-dideoxycytidine. To a stirred solution of dichloride, RP(O)Cl$_2$ or RP((S)Cl$_2$ (0.64 mmol), in dry THF (10 mL) were added 1,2,4-triazole (1.28 mmol) and Et$_3$N (1.47 mmol) at room temperature. After 30 min a solution of N-benzoyl-2',3'-didehydro-2',3'-dideoxynucleoside (0.64 mmol)

(27) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative Efficacy of Antiherpes Drugs Against Different Strains of Herpes Simplex Virus. *J. Infect. Dis.* **1980**, *141*, 563–574.

(28) De Clercq, E.; Luczak, M.; Reepmeyer, J. C.; Kirk, K. L.; Cohen, L. A. Fluoroimidazoles as Antiviral Agents and Inhibitors of Polynucleotide Biosynthesis. *Life Sci.* **1975**, *17*, 187–194.

(29) Balzarini, J.; Pauwels, R.; Baba, M.; Robins, M. J.; Zou, R.; Herdewijn, P.; De Clercq, E. The 2',3'-Dideoxyriboside of 2,6-Diaminopurine Selectively Inhibits Human Immunodeficiency Virus (HIV) Replication in Vitro. *Biochem. Biophys. Res. Commun.* **1987**, *145*, 269–276.

(30) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. Marked in vivo Antiretrovirus Activity of 9-(2-Phosphonylmethoxyethyl)adenine, a Selective Anti-Human Immunideficiency Virus Agent. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332–336.

and *N*-methylimidazole (2.06 mmol) was added, and the reaction mixture was stirred for 45 min. The salt was removed by filtration; H₂O (10 mL) and Et₃N (1.0 mL) were added. After 10 min the reaction mixture was concentrated, and NH₄OH (10 mL, 30%) was added, and the solution was stirred overnight at room temperature. The reaction mixture was concentrated, applied to a Sephadex A-25 column, and eluted with an H₂O–0.1 M NH₄HCO₃ gradient. Appropriate fractions were combined and evaporated to give the pure product as the ammonium salt. Yields are based on starting material, 21 or 25.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-methylphosphonate (4): yield 64%; UV (H₂O) λ_{max} 260 nm; ¹H NMR (D₂O) δ 1.07 (3 H, d, J_{HP} = 16.5 Hz, PCH₃), 3.99 (2 H, m, 5′-H), 5.25 (1 H, m, 4′-H), 6.27 (1 H, app dt, J = 6.0, 1.8 Hz, 2′-H), 6.60 (1 H, app dt, J = 6.0 Hz, 1.8 Hz, 3′-H), 7.07 (1 H, m, 1′-H), 8.20 (1 H, s, 8-H), 8.30 (1 H, s, 2-H); ³¹P NMR (D₂O) δ 27.79; MS (FAB) *m/z* 312 (M + 1, free acid). Anal. (C₁₁H₁₇O₄P·2H₂O) C, H, N.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-methylphosphonothionate (5): yield 80%; UV (H₂O) λ_{max} 260 nm; ¹H NMR (D₂O) δ 1.49 (0.4 × 3 H, d, J_{HP} = 14.5 Hz, PCH₃), 1.50 (0.6 × 3 H, d, J_{HP} = 14.5 Hz, PCH₃), 4.01 (2 H, m, 5′-H), 5.18 (1 H, m, 4′-H), 6.19 (1 H, ddd, J = 6.0, 1.8, 1.5 Hz, 2′-H), 6.52 (1 H, app dt, J = 6.0, 1.7 Hz, 3′-H), 6.90 (1 H, m, 1′-H), 8.06 (0.4 H, s, 8-H), 8.16 (0.6 H, s, 8-H), 8.17 (0.4 H, s, 2-H), 8.28 (0.6 H, s, 2-H); ³¹P NMR (D₂O) δ (area) 77.57 (0.4), 77.95 (0.6); ¹³C NMR (D₂O) δ (italicized carbons are assigned to the minor isomer) 24.04 (d, J = 104.6 Hz, CH₃), 24.10 (d, J = 104.9 Hz, *CH₃*), 65.35 (d, J = 6.0 Hz, C-5′), 65.49 (d, J = 6.0 Hz, *C-5′*), 88.14 (d, J = 9.0 Hz, *C-4′*), 88.20 (d, J = 8.7 Hz, C-4′), 89.63 (C-1′), 125.99 (*C-2′*), 126.03 (C-2′), 135.80 (*C-3′*), 135.87 (*C-3′*), 142.06 (C-8), 142.31 (*C-8*), 149.81 (C-4), 153.38 (*C-2*), 153.90 (C-2), 156.65 (C-6) 159.47 (*C-6*); MS(FAB) *m/z* 344 (M⁺), 328 (M⁺ + 1 for free acid). Anal. (C₁₁H₁₇N₆O₃PS·H₂O) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-phenylphosphonate (6): yield 70%; UV (H₂O) λ_{max} 260 nm; ¹H NMR (D₂O) δ 3.69 (1 H, app dt, J = 11.4 Hz, $J_{HP} \approx J_{HH} \approx$ 5.4 Hz, 5″-H), 3.95 (1 H, ddd, J = 11.4 Hz, J_{HP} = 3.2, 2.4 Hz, 5′-H), 5.13 (1 H, m, 4′-H), 6.13 (1 H, app dt, J = 6.0, 1.8 Hz, 2′-H), 6.48 (1 H, app dt, J = 6.0, 1.7 Hz, 3′-H), 6.77 (1 H, m, 1′-H), 6.96 (2 H, app dt, J = 7.5, 3.6 Hz, C₆H₅), 7.14 (1 H, m, C₆H₅), 7.25 (2 H, app dd, J = 12.0, 7.5 Hz, C₆H₅), 7.87 (1 H, s, 8-H), 8.06 (1 H, s, 2-H); ³¹P NMR (D₂O) δ 16.28; ¹³C NMR (D₂O) δ 67.11 (d, J = 4.5 Hz, C-5′), 88.27 (d, J = 8.7 Hz, C-4′), 89.60 (C-1′), 120.00 (C-5), 125.80 (C-2′), 128.85 (d, J = 14.0 Hz, C-2″), 131.88 (d, J = 6.8 Hz, C-3″), 131.94 (d, J = 2.6 Hz, C-4″), 135.47 (C-3′), 141.57 (C-8), 149.56 (C-4), 153.42 (C-2), 156.46 (C-6); MS (FAB) *m/z* 390 (M + 1 for free acid). Anal. (C₁₆H₁₉N₆O₄P·1.5H₂O) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-phenylphosphonothionate (7): yield 78%; UV (H₂O) λ_{max} 260 nm; ¹H NMR (D₂O) δ 3.35–4.10 (2 H, m, 5′-H), 5.12 (1 H, m, 4′-H), 6.19 (1 H, m, 2′-H), 6.53 (1 H, m, 3′-H), 6.91 (1 H, m, 1′-H), 7.00 (2 H, m, C₆H₅), 7.20 (1 H, m, C₆H₅), 7.45 (2 H, m, C₆H₅), 7.95 (0.6 H, s, 8-H), 8.00 (0.6 H, s, 2-H), 8.17 (0.4 H, s, 8-H), 8.19 (0.4 H, s, 2-H); ³¹P NMR (D₂O) δ 68.13 (0.6), 70.07 (0.4); MS (FAB) *m/z* 390 (M + 1, free acid). Anal. (C₁₆H₁₉N₆O₃PS·H₂O) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxycytidine 5′-methylphosphonate (8): yield 68%; UV (H₂O) λ_{max} 228, 270 nm; ¹H NMR (D₂O) δ 1.19 (3 H, d, J_{HP} = 16.5 Hz, PCH₃), 3.99 (2 H, m, 5′-H), 5.08 (1 H, m, 4′-H), 5.96 (1 H, ddd, J = 6.0, 2.1, 1.5 Hz, 2′-H), 6.03 (1 H, d, J = 7.5 Hz, 5-H), 6.45 (1 H, app dt, J = 6.0, 1.8 Hz, 3′-H), 6.96 (1 H, m, 1′-H), 7.80 (1 H, d, J = 7.5 Hz, 6-H); ³¹P NMR (D₂O) δ 27.64; MS (FAB) *m/z* 288 (M + 1, free acid). Anal. (C₁₀H₁₇N₄O₅P·H₂O) C, H; N: calcd, 17.30; found, 16.77.

2′,3′-Didehydro-2′,3′-dideoxycytidine 5′-methylphosphonothionate (9): yield 82%; UV (H₂O) λ_{max} 230, 270 nm; ¹H NMR (D₂O) δ 1.62 (0.4 × 3 H, d, J_{HP} = 14.7 Hz, PCH₃), 1.63 (0.6 × 3 H, d, J_{HP} = 14.4 Hz, PCH₃), 4.09 (2 H, m, 5′-H), 5.13 (1 H, m, 4′-H), 5.97 (1 H, m, 2′-H), 6.04 (0.4 H, d, J = 7.5 Hz, 5-H), 6.05 (0.6 H, d, J = 7.5 Hz, 5-H), 6.45 (1 H, app dt, J = 6.3, 1.7 Hz, 3′-H), 6.96 (1 H, m, 1′-H), 7.83 (0.4 H, d, J = 7.5 Hz, 6-H), 7.85 (0.6 H, d, J = 7.5 Hz, 6-H); ³¹P NMR (D₂O) δ 77.32 (0.4), 77.66 (0.6); ¹³C (D₂O) δ (italicized carbons are assigned to the minor isomer) 23.96 (d, J = 103.4 Hz, CH₃), 24.02 (d, J = 103.5 Hz, *CH₃*), 65.58 (d, J = 6.0 Hz, C-5′), 65.75 (d, J = 5.9 Hz, *C-5′*), 87.39 (d, J = 8.8 Hz, *C-4′*), 87.45 (d, J = 8.7 Hz, C-4′), 92.37 (C-1′), 97.55 (*C-5*), 97.63 (C-5), 126.99 (*C-2′*), 127.04 (C-2′), 135.57 (C-3′), 135.63

(*C-3′*), 144.73 (C-6), 158.17 (C-2), 166.69 (C-4); MS (FAB) *m/z* 320 (M⁺), 304 (M⁺ + 1 for free acid). Anal. (C₁₀H₁₇N₄O₄PS·H₂O) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxycytidine 5′-phenylphosphonate (10): yield 83%; UV (H₂O) λ_{max} 218, 234, 270 nm; ¹H NMR (D₂O) δ 3.85 (1 H, m, 5″-H), 3.99 (1 H, m, 5′-H), 5.05 (1 H, m, 4′-H), 5.62 (1 H, d, J = 7.8 Hz, 5-H), 5.88 (1 H, m, 2′-H), 6.40 (1 H, m, 3′-H), 6.85 (1 H, m, 1′-H), 7.35 –7.70 (6 H, m, 6-H and C₆H₅); ³¹P NMR (D₂O) δ 15.95; ¹³C (D₂O) δ 66.50 (d, J = 5.0 Hz, C-5′), 87.48 (d, J = 8.6 Hz, C-4′), 92.34 (C-1′), 97.28 (C-5), 126.74 (C-2′), 129.65 (d, J = 13.8 Hz, C-2″), 132.35 (d, J = 9.3 Hz, C-3″), 132.67 (d, J = 2.8 Hz, C-4″), 135.65 (C-3′), 144.31 (C-6), 157.97 (C-2), 166.45 (C-4), C-1″ could not be observed; MS (FAB) *m/z* 368 (M⁺), 351 (M⁺ + 1 for free acid). Anal. (C₁₅H₁₉N₄O₅P) C, H, P; N: calcd, 14.42; found, 13.98.

2′,3′-Didehydro-2′,3′-dideoxycytidine 5′-phenylphosphonothionate (11): yield 81%; UV (H₂O) λ_{max} 232, 272 nm; ¹H NMR (D₂O) δ 4.04 (2 H, m, 5′-H), 5.11 (1 H, m, 4′-H), 5.45 (0.47 H, d, J = 7.5 Hz, 5-H), 5.50 (0.53 H, d, J = 7.5 Hz, 5-H), 5.93 (1 H, m, 2′-H), 6.44 (1 H, m, 3′-H), 6.88 (1 H, m, 1′-H), 7.37–7.55 (4 H, m, C₆H₅), 7.64–7.76 (2 H, m, H-6, C₆H₅); ³¹P NMR (D₂O) δ 68.35 (0.47), 69.27 (0.53); ¹³C (D₂O) δ (italicized carbon denotes the lower intensity peak) 65.43 (d, J = 6.0 Hz, C-5′), 66.06 (d, J = 6.0 Hz, *C-5′*), 86.56 (d, J = 8.4 Hz, C-4′), 86.68 (d, J = 8.8 Hz, C-4′), 91.64 (C-1′), 96.55 (*C-5*), 96.61 (C-5), 126.11 (*C-2′*), 126.25 (C-2′), 128.88 (d, J = 13.8 Hz, *C-2″*), 128.91 (d, J = 13.9 Hz, C-2″), 130.58 (d, J = 11.2 Hz, C-3″), 130.73 (d, J = 10.9 Hz, *C-3″*), 131.88 (d, J = 3.5 Hz, *C-4″*), 131.92 (d, J = 3.1 Hz, C-4″), 134.71 (*C-3′*), 134.84 (C-3′), 138.33 (d, J = 139.0 Hz, C-1″), 138.61 (d, J = 138.5 Hz, *C-1″*), 143.46 (*C-6*), 143.51 (C-6), 157.82 (C-2), 166.10 (*C-4*), 166.16 (C-4); MS (FAB) *m/z* 366 (M + 1 for free acid). Anal. (C₁₅H₁₉N₄O₄PS·H₂O) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-Phenyl Phosphate (12). Et₃N (157 mg, 217 μL, 1.56 mmol) was added to a stirred solution of phenyl phosphorodichloridate (144 mg, 101 μL, 0.68 mmol) and 1,2,4-triazole (94 mg, 1.36 mmol) in THF (10 mL) under argon. The mixture was stirred for 45 min and then a solution of *N*⁶-benzoyl-2′,3′-didehydro-2′,3′-dideoxyadenosine (230 mg, 0.68 mmol) and *N*-methylimidazole (179 mg, 174 μL, 2.18 mmol) in THF (5 mL) was added. After 2 h the salt was filtered off, and H₂O (10 mL) and then Et₃N (1 mL) were added and stirred for 2 h. The volatile materials were removed, and the residue was dissolved in concentrated NH₄OH (10 mL, 30%) and stirred overnight. The product was purified by column chromatography on Sephadex A-25 eluting with an H₂O–NH₄HCO₃ gradient (0–0.1 M NH₄HCO₃) as eluent. Evaporation of the appropriate fractions gave compound 12 as a white solid (195 mg, 70%): UV (MeOH) λ_{max} 260 nm; ¹H NMR (CD₃OD) δ 4.05–4.18 (2 H, m, 5′-H), 5.10 (1 H, m, 4′-H), 6.12 (1 H, app dt, J = 6.0, 1.8 Hz, 2′-H), 6.49 (1 H, app dt, J = 6.0, 1.5 Hz, 3′-H), 6.90 (1 H, m, 1′-H), 7.00–7.10 (5 H, m, C₆H₅), 8.20 (1 H, s, 8-H), 8.29 (1 H, s, 2-H); ³¹P NMR (CD₃OD) δ 4.35; ¹³C NMR (CD₃OD) δ 67.60 (d, J = 5.7 Hz, C-5′), 87.78 (d, J = 9.4 Hz, C-4′), 89.53 (C-1′), 119.95 (C-5), 121.12 (d, J = 4.7 Hz, C-2″), 124.11 (C-2′), 126.56 (C-4″), 129.94 (C-3″), 135.34 (C-3′), 141.36 (C-8), 153.29 (C-1″), 153.92 (C-4), 154.01 (C-6), 156.95 (C-2); MS (FAB) *m/z* 406 (M⁺), 390 (M⁺ + 1 for free acid). Anal. (C₁₆H₁₉N₆O₅·2H₂O) C, H, N, P.

2,3′-Didehydro-2′,3′-dideoxycytidine 5′-Phenyl Phosphate (13). Compound 13 (78%) was prepared from *N*⁴-benzoyl-2′,3′-didehydro-2′,3′-dideoxycytidine by following the procedure described for compound 12: UV (MeOH) λ_{max} 274 nm; ¹H NMR (CD₃OD) δ 4.16 (2 H, m, 5′-H), 5.02 (1 H, m, 4′-H), 5.80 (1 H, d, J = 7.8 Hz, 5-H), 5.88 (1 H, ddd, J = 6.0, 2.1, 1.5 Hz, 2′-H), 6.40 (1 H, app dt, J = 6.0, 1.8 Hz, 3′-H), 6.96 (1 H, m, 1′-H), 6.98–7.26 (5 H, m, C₆H₅), 7.93 (1 H, d, J = 7.8 Hz, 6-H); ³¹P NMR (CD₃OD) δ 4.49; ¹³C (CD₃OD) δ 67.19 (d, J = 5.8 Hz, C-5′), 87.49 (d, J = 9.3 Hz, C-4′), 91.97 (C-1′), 95.96 (C-5), 121.47 (d, J = 4.6 Hz, C-2″), 124.60 (C-2′), 127.05 (C-4″), 130.13 (C-3″), 135.77 (C-3′), 145.49 (C-6), 153.64 (C-2), 153.94 (d, J = 6.9 Hz, C-1″), 163.95 (C-4); MS (FAB) *m/z* 366.084625 (M⁺ + 1 for free acid); C₁₅H₁₆N₃O₆P + 1 requires 366.085499.

N⁶-Benzoyl-2′,3′-didehydro-2′,3′-dideoxyadenosine 5′-Dimethyl Phosphate (26). Dimethyl *N,N*-diethylphosphoramidite (264 mg, 1.60 mmol) was added to a stirred solution of *N*⁶-benzoyl-2′,3′-didehydro-2′,3′-dideoxyadenosine (21) (450 mg, 1.33 mmol) and 1*H*-tetrazole (47 mg, 0.67 mmol) in CH₂Cl₂–pyridine

(20 mL/2 mL) at room temperature under argon. After 3 h the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (2 × 20 mL), H_2O (1 × 20 mL), and saturated NaCl (1 × 20 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a foam. TLC showed total disappearance of starting material, and ^{31}P NMR showed only one peak at δ 141.16. The product was dissolved in THF-pyridine (15 mL/2 mL) and oxidized by I_2/H_2O. The iodine solution was slowly added at −45 °C until a yellow color remained. The reaction mixture was allowed to warm up to −25 °C over 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1% $Na_2S_2O_3$ (2 × 20 mL) and then saturated NaCl solution (1 × 30 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a pale yellow foam. The product was purified by column chromatography on silica gel eluting with 0–3% MeOH in $CHCl_3$. The product was obtained as a colorless foam (480 mg, 80%): UV (MeOH) λ_{max} 232, 258, 280 nm; ^1H NMR (CDCl_3) δ 3.69 (3 H, d, J_{HP} = 11.1 Hz, POCH_3), 3.73 (3 H, d, J_{HP} = 11.1 Hz, POCH_3), 4.25 (2 H, m, 5′-H), 5.16 (1 H, m, 4′-H), 6.18 (1 H, poorly resolved ddd, 2′-H), 6.47 (1 H, app dt, J = 6.3, 1.7 Hz, 3′-H), 7.20 (1 H, m, 1′-H), 7.46–7.63 (3 H, m, C_6H_5), 8.02 (2 H, app dd, J = 7.0, 1.5 Hz, C_6H_5), 8.22 (1 H, s, 8-H), 8.78 (1 H, s, 2-H), 9.32 (1 H, br s, NH); ^{31}P NMR (CDCl_3) δ 1.66; ^{13}C NMR (CDCl_3) δ 54.24 (d, J = 6.0 Hz, OCH_3), 54.26 (d, J = 6.0 Hz, OCH_3), 67.10 (d, J = 5.5 Hz, C-5′), 85.27 (d, J = 7.7 Hz, C-4′), 88.06 (C-1′), 123.02 (C-5), 126.07 (C-2′), 127.68 (C-3″), 128.35 (C-2″), 132.32 (C-4″), 132.91 (C-3′), 133.31 (C-1″), 141.12 (C-8), 149.34 (C-4), 151.52 (C-2), 152.33 (C-6), 164.75 (C=O); MS (EI) m/z 446 (M + 1). Anal. (C_{19}-$H_{20}N_5O_6P \cdot 0.5H_2O$) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxyadenosine 5′-Methyl Phosphate (14). Methanolic ammonia (15 mL) was added to compound **26** (200 mg, 0.45 mmol) and stirred at room temperature. The reaction was continued for 48 h. Every 12 h, solvent was removed, and fresh MeOH/NH_3 was added. The product was purified by column chromatography on Sephadex A-25 eluting with an H_2O–0.1 M NH_4HCO_3 gradient to give **14** as a colorless solid (128 mg, 83%): UV (MeOH) λ_{max} 260 nm; ^1H NMR (D_2O) δ 3.26 (3 H, d, J = 10.8 Hz, POCH_3), 3.98 (2 H, m, 5′-H), 5.21 (1 H, m, 4′-H), 6.25 (1 H, ddd, J = 6.0, 2.1, 1.5 Hz, 2′-H), 6.58 (1 H, app dt, J = 6.0, 1.7 Hz, 3′-H), 7.04 (1 H, m, 1′-H), 8.22 (1 H, s, 8-H), 8.25 (1 H, s, H-2); ^{31}P NMR (D_2O) δ 1.99; MS (FAB) m/z 328 (M + 1 for free acid). Anal. ($C_{11}H_{17}N_6O_5P \cdot H_2O$) C, H, N, P.

N^4-Benzoyl-2′,3′-didehydro-2′,3′-dideoxycytidine 5′-Dimethyl Phosphate (27). Treatment of N^4-benzoyl-2′,3′-didehydro-2′,3′-dideoxycytidine (500 mg, 1.59 mmol) with tetrazole (56 mg, 0.78 mmol) and dimethyl N,N-diethylphosphoramidite (316 mg, 1.91 mmol), following the procedure described for compound **26**, gave the triester as a yellowish foam. The ^{31}P NMR spectrum showed only one peak at δ 141.57. The triester was oxidized to phosphate **27** as described for the preparation of **26**. Chromatography on silica gel eluting with 0–4% MeOH in $CHCl_3$ gave the title compound as a colorless foam (460 mg, 81%): UV (MeOH) λ_{max} 260, 304 nm; ^1H NMR (CDCl_3) δ 3.78 (3 H, d, J_{HP} = 11.1 Hz, POCH_3), 3.79 (3 H, d, J_{HP} = 11.1 Hz, POCH_3), 4.30 (2 H, m, 5′-H), 5.13 (1 H, m, 4′-H), 6.13 (1 H, ddd, J = 6.0, 2.4, 1.5 Hz, 2′-H), 6.30 (1 H, app dt, J = 6.0, 1.7 Hz, 3′-H), 7.07 (1 H, m, 1′-H), 7.48–7.65 (4 H, m, 5-H and C_6H_5), 7.90 (2 H, app d, J = 7.3 Hz, C_6H_5), 8.07 (1 H, app d, J = 7.5 Hz, 6-H), 8.68 (1 H, br s, NH); ^{31}P NMR (CDCl_3) δ 1.93; ^{13}C NMR (CDCl_3) δ 54.44 (d, J = 5.8 Hz, OCH_3), 54.47 (d, J = 5.8 Hz, OCH_3), 67.21 (d, J = 5.4 Hz, C-5′), 85.30 (d, J = 7.8 Hz, C-4′), 91.65 (C-1′), 96.88 (C-5), 127.55 (C-3″), 128.01 (C-2′), 128.67 (C-2″), 131.77 (C-4″), 132.52 (C-1″), 132.87 (C-3′), 144.56 (C-6), 154.87 (C-2), 162.38 (C=O), 166.92 (C-4); MS (FAB) m/z 422 (M + 1). Anal. ($C_{18}H_{20}N_3O_7$-$P \cdot H_2O$) C, H, N, P.

2′,3′-Didehydro-2′,3′-dideoxycytidine 5′-Methyl Phosphate (15). Compound **27** (250 mg, 0.59 mmol) was converted to phosphodiester **15** (160 mg, 84%) by following the procedure described for **14**. UV (H_2O) λ_{max} 230, 270 nm; ^1H NMR (D_2O) δ 3.48 (3 H, d, J_{HP} = 10.8 Hz, POCH_3), 4.01 (2 H, m, 5′-H), 5.10 (1 H, m, 4′-H), 5.99 (1 H, ddd, J = 6.0, 2.4, 1.5 Hz, 2′-H), 6.05 (1 H, d, J = 7.8 Hz, 5-H), 6.48 (1 H, app dt, J = 6.0, 1.8 Hz, 3′-H), 6.99 (1 H, m, 1′-H), 7.81 (1 H, d, J = 7.8 Hz, 6-H); ^{31}P NMR (D_2O) δ 1.83; ^{13}C (D_2O) δ 53.43 (d, J = 5.8 Hz, OCH_3), 66.56 (d, J = 5.5 Hz, C-5′), 86.57 (d, J = 8.8 Hz, C-4′), 91.61 (C-1′), 96.85 (C-5),

126.34 (C-2′), 134.70 (C-3′), 143.79 (C-6), 157.80 (C-2), 166.27 (C-4); MS (FAB) m/z 321 (M + 1), 304 (M + 1, for free acid). Anal. ($C_{10}H_{17}N_4O_8P \cdot 0.5H_2O$) C, H, N, P.

5′-Benzoylthymidine 3′-(Methyl tetraisopropyl-phosphorodiamidite) (28). Methyl N,N-diisopropylphosphoramidite (288 mg, 1.10 mmol) was added to a stirred solution of 5′-benzoylthymidine (346 mg, 1.00 mmol) and 1H-tetrazole (35 mg, 0.50 mmol) in CH_2Cl_2-pyridine (15 mL/3 mL) at room temperature under argon. The reaction mixture was stirred for 2 h when TLC showed the disappearance of starting material. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ (1 × 30 mL), and then saturated NaCl (1 × 30 mL). The organic layer was dried ($MgSO_4$) and evaporated to give an oil which was dissolved in hexane. At −78 °C compound **28** was precipitated as a colorless solid (429 mg, 85%); ^1H NMR (CDCl_3) δ 1.38 (9 H, d, J = 6.6 Hz, NCHMe_2), 1.39 (3 H, d, J = 6.6 Hz, NCHMe_2), 1.65 (0.5 × 3 H, d, J = 1.2 Hz, 5-CH_3), 1.67 (0.5 × 3 H, d, J = 1.2 Hz, 5-CH_3), 2.17 (1 H, m, 2′-H), 2.57 (1 H, m, 2′-H), 3.37 (0.5 × 3 H, d, J_{HP} = 13.2 Hz, POCH_3), 3.39 (0.5 × 3 H, d, J_{HP} = 13.2 Hz, POCH_3), 3.53–3.67 (1 H, m, 4′-H), 4.32–4.43 (1 H, m, 3′-H), 4.46–4.71 (2 H, m, 5′-H), 6.31 (1 H, m, 1′-H), 7.23 (0.5 × 1 H, unresolved q, H-6), 7.25 (1 H, unresolved q, H-6), 7.45–8.05 (5 H, m, C_6H_5); ^{31}P NMR (CDCl_3) δ 150.14, 150.57 (near-equal intensities).

Phosphate Triester (30). A solution of **21** (275 mg, 0.88 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of phosphoramidite **28** (447 mg, 0.88 mmol) and 1H-tetrazole (84 mg, 1.2 mmol) in CH_2Cl_2 (10 mL) at room temperature under argon. TLC after 2 h showed complete disappearance of **28**. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (2 × 30 mL) and H_2O (1 × 50 mL). The organic extract was dried ($MgSO_4$) and evaporated to give a pale yellow foam. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2-EtOAc:Et_3N (50:40:10). Phosphite **29** was obtained as a colorless foam (500 mg, 78%). The ^{31}P NMR spectrum showed peaks at δ 140.59 and 140.80. Compound **29** (300 mg) was oxidized by I_2/H_2O by following the procedure described for compound **26** to give phosphate **30**. Chromatography on silica gel eluting with 4% MeOH in $CHCl_3$ gave **30** as a colorless foam (260 mg, 83%): UV (MeOH) λ_{max} 230, 260, 276 nm; ^1H NMR (CDCl_3) δ (italicized protons are assigned to the thymidyl moiety) 1.54 (0.4 × 3 H, d, J = 1.0 Hz, 5-*CH_3*), 1.56 (0.6 × 3 H, d, J = 1.0 Hz, 5-*CH_3*), 2.15 (1 H, m, 2′-*H*), 2.59 (1 H, m, 2′-*H*), 3.65 (0.6 × 3 H, d, J_{HP} = 11.4 Hz, POCH_3), 3.68 (0.4 × 3 H, d, J_{HP} = 11.4 Hz, POCH_3), 4.29 (2 H, m, 5′-H), 4.44–4.65 (3 H, m, 4′-H, 5′-*H*), 5.08 (1 H, m, 3′-*H*), 5.19 (1 H, m, 4′-H), 6.22 (1 H, m, 2′-H), 6.32 (1 H, m, 1′-H), 6.45 (1 H, m, 3′-H), 7.07 (0.4 × 1 H, unresolved q, 6-*H*), 7.10 (0.6 × 1 H, unresolved q, 6-*H*), 7.21 (1 H, m, 1′-H), 7.39–7.61 (6 H, m, C_6H_5), 7.91–8.04 (4 H, m, C_6H_5), 8.22 (0.4 × 1 H, s, 8-H), 8.25 (0.6 × 1 H, s, 8-H), 8.82 (0.6 × 1 H, s, 2-H), 8.83 (0.4 × 1 H, s, 2-H), 8.99 (0.4 × 1 H, s, NH), 9.23 (0.6 × 1 H, s, NH), 9.57 (0.6 × 1 H, s, N*H*), 9.59 (0.4 × 1 H, s, N*H*); ^{31}P NMR (CDCl_3) δ 0.13 (0.4), −0.29 (0.6); MS (FAB) m/z 760 (M + 1). Anal. ($C_{35}H_{34}N_7O_{11}P \cdot H_2O$) C, H, N, P.

Phosphate Diester 16. Compound **30** (180 mg, 0.24 mmol) was dissolved in concentrated NH_4OH (10 mL, 30%) and stirred overnight at room temperature. The reaction mixture was concentrated, dissolved in H_2O (10 mL), and then applied to a Sephadex A-25 column. Gradient elution with H_2O–NH_4HCO_3 (0–0.8 M) gave diester **16** as a colorless solid (104 mg, 84%): UV (H_2O) λ_{max} 260 nm; ^1H NMR (D_2O) δ (italicized protons are assigned to the thymidyl moiety) 1.16 (1 H, m, 2′-*H*), 1.82 (4 H, m, 2′-*H*, 5-*CH_3*), 3.55 (2 H, m, 5′-*H*), 3.87 (1 H, m, 4′-*H*), 3.99 (2 H, m, 5′-H), 4.41 (1 H, m, 3′-*H*), 5.17 (1 H, m, 4′-H), 5.82 (1 H, dd, J = 8.1 Hz, 6.0 Hz, 1′-*H*), 6.20 (1 H, unresolved dt, J = 6.0 Hz, 2′-H), 6.56 (1 H, unresolved dt, J = 6.0 Hz, 3′-H), 6.88 (1 H, m, 1′-H), 7.20 (1 H, br s, 6-*H*), 7.98 (1 H, s, 8-H), 8.16 (1 H, s, 2-H); ^{31}P NMR (D_2O) δ −0.82; ^{13}C NMR (D_2O) δ (italicized carbons are assigned to the thymidyl moiety) 12.51 (5-*CH_3*), 37.53 (*C*-2′), 61.85 (*C*-5′), 66.54 (d, J = 5.0 Hz, C-5′), 76.26 (d, J = 5.6 Hz, C-3′), 85.31 (*C*-1′), 86.43 (d, J = 7.7 Hz, C-4′), 87.34 (d, J = 10.6 Hz, C-4′), 88.73 (C-1′), 112.00 (*C*-5), 118.88 (C-5), 125.30 (C-2′), 135.07 (C-3′), 137.30 (C-6), 141.47 (C-8), 149.27 (C-4), 151.70 (*C*-2), 153.27 (C-6), 155.93 (C-2), 166.81 (C-4); MS (FAB) m/z 538 (M + 1). Anal. ($C_{20}H_{27}N_8O_9P \cdot 1.5H_2O$) C, H, N, P.

Phosphate Triester 32. Treatment of compound 25 (400 mg, 1.28 mmol) with phosphoramidite 28 (650 mg, 1.28 mmol) and 1*H*-tetrazole (127 mg, 1.8 mmol) following the procedure described for 29 gave triester 31 (725 mg, 79%) as a colorless foam after chromatography (eluent CH_2Cl_2–Et_3N–MeOH, 96:3:1). The ^{31}P NMR showed peaks at δ 140.80, 140.91. Compound 31 (350 mg, 0.49 mmol) was converted to 32 (278 mg, 80%) by oxidation with I_2/H_2O following the procedure described for the preparation of compound 26: UV (MeOH) λ_{max} 230, 262, 304, nm; ^{1}H NMR ($CDCl_3$) δ (italicized protons are assigned to the thymidyl moiety) 1.26 (0.7 × 3 H, br s, 5-C*H*$_3$), 1.73 (0.3 × 3 H, br s, 5-C*H*$_3$), 2.27 (1 H, m, 2′-*H*), 2.64 (1 H, m, 2′-*H*), 3.80 (0.7 × 3 H, d, J_{HP} = 11.4 Hz, POC*H*$_3$), 3.81 (0.3 × 3 H, d, J_{HP} = 11.4 Hz, POC*H*$_3$), 4.30 (2 H, m, 5′-*H*), 4.48–4.72 (4 H, m, 3′-*H*, 4′-*H*, 5′-*H*), 5.13 (1 H, m, 4′-*H*), 6.16 (1 H, m, 2′-*H*), 6.25–6.31 (2 H, m, 1′-*H*, 3′-*H*), 7.05 (0.3 × 1 H, m, 1′-*H*), 7.16 (0.7 × 1 H, m, 1′-*H*), 7.40–7.62 (7 H, m, C_6H_5, 5-H), 7.87 (1 H, m, 6-*H*), 7.99 (4 H, m, C_6H_5), 8.66 (0.7 × 1 H, br s, NHCO), 8.72 (0.3 × 1 H, NHCO), 8.83 (1 H, br s, NH); ^{31}P NMR ($CDCl_3$) δ 0.06; MS (FAB) *m/z* 736 (M + 1), 737 (M + 2). Anal. ($C_{34}H_{34}N_5O_{12}P\cdot0.5H_2O$) H, N, P; C: calcd, 54.84; found, 54.20.

Phosphate Diester 17. Compound 32 (240 mg, 0.35 mmol) was deprotected by following the procedure described for the preparation of 16 to give 17 (145 mg, 82%) as a colorless solid: UV (H_2O) λ_{max} 268 nm; ^{1}H NMR (D_2O) δ (italicized protons are assigned to the thymidyl moiety) 1.90 (3 H, br s, 5-C*H*$_3$), 2.18 (1 H, quintet, 2′-H), 2.43 (1 H, ddd, *J* = 14.1, 6.3, 3.0 Hz, 2′-H), 3.70 (1 H, dd, *J* = 12.6, 4.8 Hz, 5″-H), 3.78 (1 H, dd, *J* = 12.6, 3.6 Hz, 5′-H), 3.98–4.14 (3 H, m, 4′-*H*, 5′-H), 4.65 (1 H, m, 3′-H), 5.10 (1 H, m, 4′-H), 5.98 (1 H, app dt, *J* = 6.0, 1.8 Hz, 2′-H), 6.03 (1 H, d, *J* = 7.5 Hz, 5-H), 6.20 (1 H, t, *J* = 6.3 Hz, 1′-*H*), 6.48 (1 H, app dt, *J* = 6.0, 1.7 Hz, 3′-H), 6.96 (1 H, m, 1′-H), 7.62 (1 H, m, 6-*H*), 7.79 (1 H, d, *J* = 7.5 Hz, 6-H); ^{31}P NMR (D_2O) δ –0.60; ^{13}C NMR(D_2O) δ (thymidyl carbons are italicized) 12.44 (5-C*H*$_3$), 38.43 (d, *J* = 3.3 Hz, *C*-2′), 61.83 (*C*-5′), 66.77 (d, *J* = 5.4 Hz, C-5′), 75.77 (d, *J* = 5.3 Hz, C-3′), 85.82 (*C*-1′), 86.36 (d, *J* = 6.4 Hz, *C*-4′), 86.55 (d, *J* = 9.3 Hz, C-4′), 91.67 (C-1′), 96.66 (C-5), 112.20 (*C*-5), 126.45 (*C*-2′), 134.68 (C-3′), 138.07 (*C*-6), 143.96 (C-6), 152.17 (*C*-2), 157.83 (C-2), 166.24 (*C*-4), 167.10 (C-4); MS (FAB) *m/z* 530 (M$^+$), 514 (M + 1, for free acid), 513 (M$^+$, free acid). Anal. ($C_{19}H_{27}N_6$-$O_{10}P\cdot H_2O$) C, H, N, P.

The Calanolides, a Novel HIV-Inhibitory Class of Coumarin Derivatives from the Tropical Rainforest Tree, *Calophyllum lanigerum*[1]

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Eight new coumarin compounds (1–8) were isolated by anti-HIV bioassay-guided fractionation of an extract of *Calophyllum lanigerum*. The structures of calanolide A (1), 12-acetoxycalanolide A (2), 12-methoxycalanolide A (3), calanolide B (4), 12-methoxycalanolide B (5), calanolide C (6) and related derivatives 7 and 8 were solved by extensive spectroscopic analyses, particularly HMQC, HMBC, and difference NOE NMR experiments. The absolute stereochemistry of calanolide A (1) and calanolide B (4) was established by a modified Mosher's method. Calanolides A (1) and B (4) were completely protective against HIV-1 replication and cytopathicity (EC$_{50}$ values of 0.1 μM and 0.4 μM, respectively), but were inactive against HIV-2. Some of the related compounds also showed evidence of anti-HIV-1 activity. Studies with purified bacterial recombinant reverse transcriptases (RT) revealed that the calanolides are HIV-1 specific RT inhibitors. Moreover, calanolide A was active not only against the AZT-resistant G-9106 strain of HIV-1 but also against the pyridinone-resistant A17 strain. This was of particular interest since the A17 virus is highly resistant to previously known HIV-1 specific, non-nucleoside RT inhibitors (e.g., TIBO; BI-RG-587; L693,593) which comprise a structurally diverse but apparently common pharmacologic class. The calanolides represent a substantial departure from the known class and therefore provide a novel new anti-HIV chemotype for drug development.

Introduction

The National Cancer Institute is actively acquiring and screening extracts from diverse plant, marine, and microbial sources for anti-HIV activity.[2] Stemming from these efforts, HIV-inhibitory compounds have thus far been isolated and identified from plants in the families Euphorbiaceae,[3,4] Ancistrocladaceae,[5] Combretaceae,[6] and Piperaceae.[7] In this continuing program, an organic ex-

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(1) Part 7 in the series HIV Inhibitory Natural Products. For part 6, see ref 7.
(2) Boyd, M. R. Strategies for the identification of new agents for the treatment of AIDS: A national program to facilitate the discovery and preclinical development of new drug candidates for clinical evaluation. In *AIDS Etiology, Diagnosis, Treatment and Prevention*; DeVita, V. T., Jr., Hellman, S., Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1988; pp 305–319.
(3) Gustafson, K. R.; Munro, M. H. G.; Blunt, J. W.; Cardellina, J. H., II; McMahon, J. B.; Gulakowski, R. J.; Cragg, G. M.; Cox, P. A.; Brinen, L. S.; Clardy, J.; Boyd, M. R. HIV inhibitory natural products. 3. Diterpenes from *Homalanthus acuminatus* and *Chrysobalanus icaco*. *Tetrahedron* 1991, *47*, 4547–4554.