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

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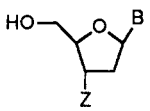
The preparations and antiviral activities of a series (4–17) of potential prodrug forms of the antivirals 2',3'-didehydro-2',3'-dideoxyadenosine (D4A) and 2',3'-didehydro-2',3'-dideoxycytosine (D4C) are reported. The 5'-phenyl- and 5'-methylphosphonates (4, 6, 8, and 10) and their phosphonothionate congeners (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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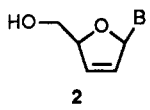
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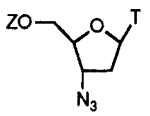
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high activities and often very favorable selectivity indexes.[1] In clinical use is the widely prescribed therapeutic agent AZT (1, Z = N_3, B = thymin-1-yl), as well as 2',3'-dideoxycytidine (ddC, 1, Z = H, B = cytosin-1-yl) and the recently approved 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]

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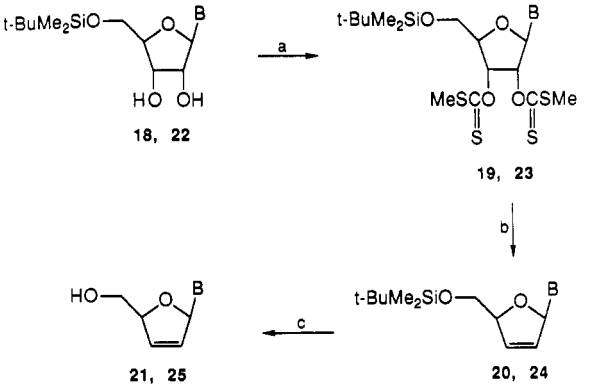
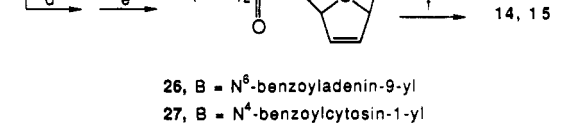
and with another molecule of AZT[16] also showed activity. By contrast a conjugate involving the derivatization of the 5'-hydroxyls of two AZT to form the neutral methylphosphonate was inactive[17] as were the diethyl, diisopropyl, and diisobutyl 5'-phosphate triesters.[18] A few active prodrug conjugates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

Scheme I[a]



[a] (a) NaH/imidazole/CS_2/MeI; (b) AIBN/Bu_3SnH; (c) TBAF.

Scheme II[a]



[a] (a) $ZP(O)Cl_2$/1,2,4-triazole/Et_3N; (b) H_2O/Et_3N; (c) NH_4OH; (d) i-$Pr_2NP(OMe)_2$/1H-tetrazole; (e) H_2O/I_2; (f) $MeOH/NH_3$.

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 phosphonothionates (**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

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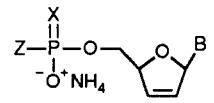
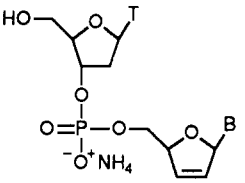


4: Z = Me, X = O, B = adenin-9-yl
5: Z = Me, X = S, B = adenin-9-yl
6: Z = Ph, X = O, B = adenin-9-yl
7: Z = Ph, X = S, B = adenin-9-yl
8: Z = Me, X = O, B = cytosin-1-yl
9: Z = Me, X = S, B = cytosin-1-yl
10: Z = Ph, X = O, B = cytosin-1-yl
11: Z = Ph, X = S, B = cytosin-1-yl
12: Z = PhO, X = O, B = 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

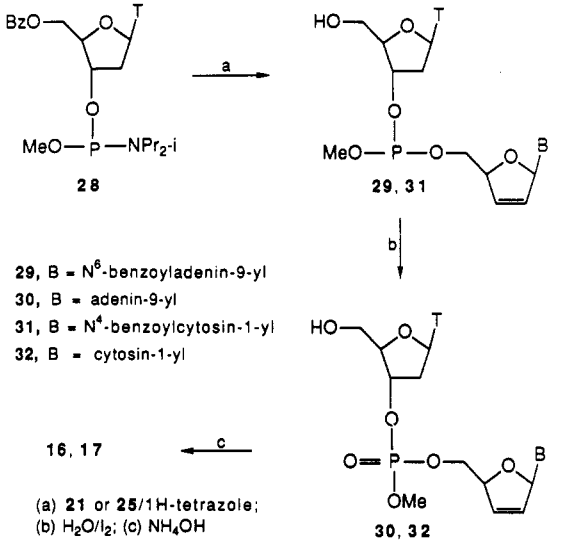
can be subsequently hydrolyzed to D4A or D4C. It can be hoped that modification of D4A, which does not display potent anti-HIV activity,[2,3] may improve its activity and that the derivatization of D4C will decrease its inherent instability in solution[2c,23] and perhaps also raise its selectivity index.

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 NH_4OH, gave phosphonates and phosphonothioates **4–11**. Analogously, phenyl phosphorodichloridate, $PhOP(O)Cl_2$, 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 $MeOP(O)Cl_2$ 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, reaction of dimethyl *N,N*-diethylphosphoramidite with **21** in presence of 1*H*-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 $MeOH/NH_3$ resulted in **14** (83%).

Scheme III[a]



(a) **21** or **25**/1H-tetrazole; (b) H_2O/I_2; (c) NH_4OH

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 1*H*-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 ^1H 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-

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

compd	EC_{50}[a] (μM) HIV-1	HIV-2	CC_{50}[b] (μM)
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_6SM 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_2O (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. ^1H 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 ^1H 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. $CH_3P(S)Cl_2$, $CH_3P(O)Cl_2$, $PhP(S)Cl_2$, $PhP(O)Cl_2$, and $PhOP(O)Cl_2$ were purchased from Aldrich Chemical Co. Anhydrous solvents were obtained as follows: acetonitrile by successive distillations first from P_4O_{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 $HTVLz\text{-}III_B$) 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_2Cl_2 (100 mL) and washed with cold H_2O (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; ^1H 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_6H_5), 8.02 (2 H, m, C_6H_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; $C_{27}H_{35}N_5O_5S_4Si$ 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; ^1H 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_6H_5), 8.02 (2 H, d, *J* = 7.3 Hz, C_6H_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; $C_{23}H_{29}N_5O_3Si$ 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; ^1H 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_6H_5), 7.64 (1 H, app t, *J* = 7.5 Hz, C_6H_5), 8.04 (2 H, app d, *J* = 7.5 Hz, C_6H_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. ($C_{17}H_{15}N_5O_3$) 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; ^1H 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_6H_5, 5-H), 7.92 (2 H, d, *J* = 7.3 Hz, C_6H_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; $C_{26}H_{35}O_6SiS_4$ 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; ^1H 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_6H_5 and 5-H), 7.88–7.93 (2 H, m, C_6H_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; $C_{22}H_{29}N_3O_4Si$ 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; ^1H 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_6H_5, 5-H), 7.98 (2 H, m, C_6H_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. ($C_{16}H_{15}N_3O_4$) 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_3N (1.47 mmol) at room temperature. After 30 min a solution of *N*-benzoyl-2′,3′-didehydro-2′,3′-dideoxynucleoside (0.64 mmol)

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and *N*-methylimidazole (2.06 mmol) was added, and the reaction mixture was stirred for 45 min. The salt was removed by filtration; H_2O (10 mL) and Et_3N (1.0 mL) were added. After 10 min the reaction mixture was concentrated, and NH_4OH (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 H_2O–0.1 M NH_4HCO_3 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 (H_2O) λ_{max} 260 nm; ^1H NMR (D_2O) δ 1.07 (3 H, d, J_{HP} = 16.5 Hz, PCH_3), 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); ^{31}P NMR (D_2O) δ 27.79; MS (FAB) m/z 312 (M + 1, free acid). Anal. ($C_{11}H_{17}O_4P\cdot 2H_2O$) C, H, N.

2',3'-Didehydro-2',3'-dideoxyadenosine 5'-methylphosphonothionate (5): yield 80%; UV (H_2O) λ_{max} 260 nm; ^1H NMR (D_2O) δ 1.49 (0.4 × 3 H, d, J_{HP} = 14.5 Hz, PCH_3), 1.50 (0.6 × 3 H, d, J_{HP} = 14.5 Hz, PCH_3), 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); ^{31}P NMR (D_2O) δ (area) 77.57 (0.4), 77.95 (0.6); ^{13}C NMR (D_2O) δ (italicized carbons are assigned to the minor isomer) 24.04 (d, J = 104.6 Hz, CH_3), 24.10 (d, J = 104.9 Hz, CH_3), 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. ($C_{11}H_{17}N_6O_3PS\cdot H_2O$) C, H, N, P.

2',3'-Didehydro-2',3'-dideoxyadenosine 5'-phenylphosphonate (6): yield 70%; UV (H_2O) λ_{max} 260 nm; ^1H NMR (D_2O) 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, C_6H_5), 7.14 (1 H, m, C_6H_5), 7.25 (2 H, app dd, J = 12.0, 7.5 Hz, C_6H_5), 7.87 (1 H, s, 8-H), 8.06 (1 H, s, 2-H); ^{31}P NMR (D_2O) δ 16.28; ^{13}C NMR (D_2O) 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. ($C_{16}H_{19}N_6O_4P\cdot 1.5H_2O$) C, H, N, P.

2',3'-Didehydro-2',3'-dideoxyadenosine 5'-phenylphosphonothionate (7): yield 78%; UV (H_2O) λ_{max} 260 nm; ^1H NMR (D_2O) 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, C_6H_5), 7.20 (1 H, m, C_6H_5), 7.45 (2 H, m, C_6H_5), 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); ^{31}P NMR (D_2O) δ 68.13 (0.6), 70.07 (0.4); MS (FAB) m/z 390 (M + 1, free acid). Anal. ($C_{16}H_{19}N_6O_3PS\cdot H_2O$) C, H, N, P.

2',3'-Didehydro-2',3'-dideoxycytidine 5'-methylphosphonate (8): yield 68%; UV (H_2O) λ_{max} 228, 270 nm; ^1H NMR (D_2O) δ 1.19 (3 H, d, J_{HP} = 16.5 Hz, PCH_3), 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 Hz, 1.8 Hz, 3'-H), 6.96 (1 H, m, 1'-H), 7.80 (1 H, d, J = 7.5 Hz, 6-H); ^{31}P NMR (D_2O) δ 27.64; MS (FAB) m/z 288 (M + 1, free acid). Anal. ($C_{10}H_{17}N_4O_5P\cdot H_2O$) C, H; N: calcd, 17.30; found, 16.77.

2',3'-Didehydro-2',3'-dideoxycytidine 5'-methylphosphonothionate (9): yield 82%; UV (H_2O) λ_{max} 230, 270 nm; ^1H NMR (D_2O) δ 1.62 (0.4 × 3 H, d, J_{HP} = 14.7 Hz, PCH_3), 1.63 (0.6 × 3 H, d, J_{HP} = 14.4 Hz, PCH_3), 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); ^{31}P NMR (D_2O) δ 77.32 (0.4), 77.66 (0.6); ^{13}C (D_2O) δ (italicized carbons are assigned to the minor isomer) 23.96 (d, J = 103.4 Hz, CH_3), 24.02 (d, J = 103.5 Hz, CH_3), 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. ($C_{10}H_{17}N_4O_4PS\cdot H_2O$) C, H, N, P.

2',3'-Didehydro-2',3'-dideoxycytidine 5'-phenylphosphonate (10): yield 83%; UV (H_2O) λ_{max} 218, 234, 270 nm; ^1H NMR (D_2O) 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 C_6H_5); ^{31}P NMR (D_2O) δ 15.95; ^{13}C (D_2O) 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. ($C_{15}H_{19}N_4O_5P$) C, H, P; N: calcd, 14.42; found, 13.98.

2',3'-Didehydro-2',3'-dideoxycytidine 5'-phenylphosphonothionate (11): yield 81%; UV (H_2O) λ_{max} 232, 272 nm; ^1H NMR (D_2O) 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, C_6H_5), 7.64–7.76 (2 H, m, H-6, C_6H_5); ^{31}P NMR (D_2O) δ 68.35 (0.47), 69.27 (0.53); ^{13}C (D_2O) 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. ($C_{15}H_{19}N_4O_4PS\cdot H_2O$) C, H, N, P.

2',3'-Didehydro-2',3'-dideoxyadenosine 5'-Phenyl Phosphate (12). Et_3N (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 H_2O (10 mL) and then Et_3N (1 mL) were added and stirred for 2 h. The volatile materials were removed, and the residue was dissolved in concentrated NH_4OH (10 mL, 30%) and stirred overnight. The product was purified by column chromatography on Sephadex A-25 eluting with an H_2O–NH_4HCO_3 gradient (0–0.1 M NH_4HCO_3) 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 (CD_3OD) 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, C_6H_5), 8.20 (1 H, s, 8-H), 8.29 (1 H, s, 2-H); ^{31}P NMR (CD_3OD) δ 4.35; ^{13}C NMR (CD_3OD) 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. ($C_{16}H_{19}N_6O_5\cdot 2H_2O$) 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 (CD_3OD) 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, C_6H_5), 7.93 (1 H, d, J = 7.8 Hz, 6-H); ^{31}P NMR (CD_3OD) δ 4.49; ^{13}C (CD_3OD) 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); $C_{15}H_{16}N_3O_6P$ + 1 requires 366.085499.

N^6-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 CH_2Cl_2–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. ($C_{19}H_{20}N_5O_6P·0.5H_2O$) 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. ($C_{11}H_{17}N_6O_5P·H_2O$) C, H, N, P.

N⁴-Benzoyl-2′,3′-didehydro-2′,3′-dideoxycytidine 5′-Dimethyl Phosphate (27). Treatment of N⁴-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, 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. ($C_{18}H_{20}N_3O_7P·H_2O$) 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. ($C_{10}H_{17}N_4O_6P·0.5H_2O$) C, H, N, P.

5′-Benzoylthymidine 3′-(Methyl tetraisopropylphosphorodiamidite) (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 1*H*-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 1*H*-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-*C*H_3), 1.56 (0.6 × 3 H, d, J = 1.0 Hz, 5-*C*H_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. ($C_{35}H_{34}N_7O_{11}P·H_2O$) 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-*C*H_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-*C*H_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. ($C_{20}H_{27}N_8O_9P·1.5H_2O$) 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; ^1H 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, $POCH_3$), 3.81 (0.3 × 3 H, d, J_{HP} = 11.4 Hz, $POCH_3$), 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. ($C_{34}H_{34}N_5O_{12}P·0.5H_2O$) 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; ^1H 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. ($C_{19}H_{27}N_6O_{10}P·H_2O$) C, H, N, P.

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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.

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