2′-Deoxynucleoside Dithiophosphates: Synthesis and Biological Studies[1]

Peter H. Seeberger, Eric Yau,† and Marvin H. Caruthers*

Contribution from the Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

Received September 9, 1994[⊗]

Abstract: Deoxyadenosine 5′- and 3′-dithiophosphate and thymidine 5′- and 3′-dithiophosphate were synthesized. These are the first examples of this class of compounds. Although several synthesis strategies were examined, the most successful involved coupling deoxynucleoside H-phosphonodithioates with 9−fluorenemethanol under oxidation conditions to generate deoxynucleoside 9-fluorenemethyl phosphorodithioates. Deprotection by treatment with concentrated ammonium hydroxide followed by HPLC purification yielded the deoxynucleoside dithiophosphates. These analogs showed modest inhibition of avian myeloblastosis virus reverse transcriptase. They failed to inhibit human immunodeficiency virus reverse transcriptase, alkaline phosphatase, T4 polynucleotide kinase, or DNA polymerase I Klenow fragment.

Introduction

Nucleotide and oligonucleotide analogs in which one or more of the bridging or nonbridging oxygens are replaced by sulfur have received much attention as potential enzyme inhibitors and/or antisense agents because this alteration in the phosphate moiety results in stability toward nucleases.[2−5] Due to the many potential biochemical applications of these derivatives, new synthetic routes for a variety of different sulfur containing analogs have been developed.[6]

Nucleoside phosphorothioates were first synthesized over 25 years ago.[7] They are now important biochemical tools for sequencing[8] and mutagenizing[9] DNA and for various mechanistic studies on nucleic acid enzymes.[2] These analogs also show inhibitory effects toward several enzymes, including alkaline phosphatase.[2] Recently Ludwig and Eckstein reported the synthesis of 2′-deoxynucleoside 5′-*O*-(1,1-dithiotriphosphates).[10] However, the thymidine derivative did not show any inhibitory effect on DNA polymerase Klenow fragment nor was it a substrate for this enzyme. Other nucleoside phosphorodithioate analogs of interest are the 2′,3′-cUMP[11] and especially 3′,5′-cAMP[12] because it is a competitive inhibitor of cAMP-dependent protein kinase. There are, however, no reports in the literature on the synthesis of compounds in which two of the nonbridging oxygens of a nucleoside monophosphate are replaced by sulfur (nucleoside-*O*-dithiophosphoric acid or nucleoside dithiophosphates). In this study, we report the synthesis of deoxynucleoside 5′- and 3′-dithiophosphates and biochemical studies on these analogs as potential inhibitors of viral reverse transcriptases, kinases, phosphatases, and polymerases.

Synthesis of Deoxynucleoside 5′ and 3′-Phosphorodithioates. The initial approach (Scheme 1) started with the fully protected deoxynucleoside 3′-phosphorodithioate triester (**1**) which can be prepared from commercially available *O*-[5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl] *O*-(β-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (**5**) by treatment first with 2,4-dichlorobenzylmercaptan and tetrazole and then, without isolation, a saturated sulfur solution.[13] Treatment of **1** with triethylamine in acetonitrile provided the diester **2** as expected. However further conversion of **2** to the monoester **3** proved unsuccessful even with 20 equiv of thiophenol and 20 equiv of triethylamine in refluxing dioxane or hot DMF (70 °C). An alternative deprotection strategy was also unsuccessful. Thus treatment of **1** with thiophenol (10 equiv) and triethylamine (10 equiv) in dioxane gave a mixture of **4** (11%) and **2** (73%). Since **2**, which could not be converted to **3**, was the major product, a different approach for the direct synthesis of **4** was investigated.

As indicated in Scheme 2, **4** was prepared in a one-pot reaction by treatment of **5** with H_2S and tetrazole followed by sulfur (94%). After removal of the 5′-dimethoxytrityl group with 80% acetic acid to yield **6**, several attempts to cleave the β-cyanoethyl group with sodium hydroxide or concentrated ammonium hydroxide at room temperature or 55 °C did not give satisfactory results. However, an acceptable result was obtained when **6** was treated with aqueous 0.4 M LiOH at reflux for 30 min.[14] Although this procedure was satisfactory for generating thymidine 3′-phosphorodithioate, the strong alkaline conditions required for removal of the β-cyanoethyl group would not be compatible with synthesis of the remaining deoxy- or ribonucleosides and also peptides having phosphorodithioate derivatized serine, threonine, or tyrosine.

As a consequence of these considerations, the synthetic route outlined in Scheme 3 was investigated. In a one-pot reaction, the properly protected deoxynucleoside (**8**−**13**) was reacted with the *in situ* formed tristriazolophosphine to give the deoxy-

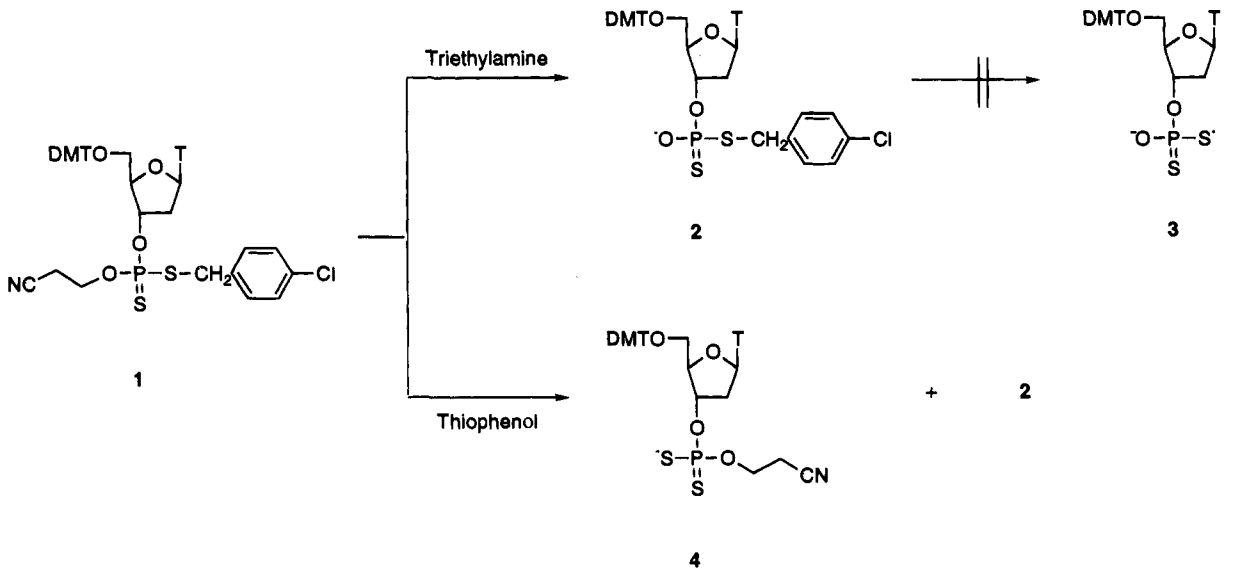
* To whom correspondence should be addressed.
† Current address: NeoRx, 410 West Harrison Street, Seattle, WA 98119.
⊗ Abstract published in *Advance ACS Abstracts*, January 1, 1995.

(1) This is paper 39 in a series on nucleotide chemistry. Paper 38 is Böhringer et al., *Tetrahedron Lett.* **1993**, *34*, 3723. This research was supported by the National Institutes of Health (Grant GM25680) and Amgen Inc. Abbreviations: DMT, 4,4′-dimethoxytrityl; B, purine or pyrimidine base; T, thymine; A, adenine; A^{Bz}, 6-*N*-benzoyladenine; NMM, *N*-methylmorpholine; TBDMS, *tert*-butyldimethylsilyl; TLC, thin layer chromatography; PAGE, polyacrylamide gel electrophoresis; TEAB, triethylammonium bicarbonate; HPLC, high performance liquid chromatography; DTT, dithiothreitol; TBAF, *tert*-butylammonium fluoride.

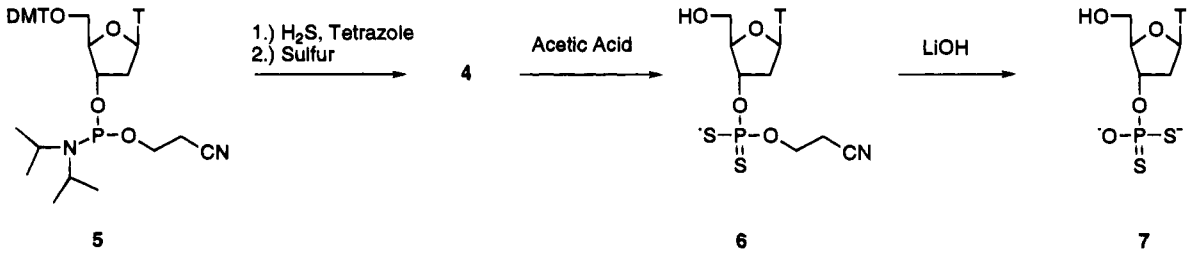
(2) Eckstein, F. *Ann. Rev. Biochem.* **1985**, *54*, 367.
(3) Caruthers, M.; Beaton, G.; Cummins, L.; Dellinger, D.; Graff, D.; Ma, Y.-X.; Marshall, W. S.; Sasmor, H.; Shankland, P.; Wu, J. V.; Yau, E. K. *Nucleosides Nucleotides* **1991**, *10*, 47.
(4) Nielsen, J.; Brill, W. K.-D.; Caruthers, M. H. *Tetrahedron Lett.* **1988**, *29*, 2911.
(5) Porritt, G. M.; Reese, C. B. *Tetrahedron Lett.* **1989**, *30*, 4713.
(6) Dahl, O. *Sulfur Reports* **1991**, *11*, 167.
(7) Eckstein, F. *J. Am. Chem. Soc.* **1966**, *88*, 4292.
(8) Gish, G.; Eckstein, F. *Science* **1988**, *240*, 1520.
(9) Sayers, J. R.; Schmidt, W.; Wendler, A.; Eckstein, F. *Nucleic Acids Res.* **1988**, *16*, 803.
(10) Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1991**, *56*, 1777.
(11) Eckstein, F. *J. Am.Chem. Soc.* **1970**, *92*, 4718.

0002-7863/95/1517-1472$09.00/0

Scheme 1



Scheme 2



nucleoside 3'-bistriazolophosphoramidite. This phosphoramidite was subjected to hydrogen sulfenolysis to generate the deoxynucleoside H-phosphonodithioate in 42–58% yield.[15] The main side product was the H-phosphonothioate. Purification of the deoxynucleoside H-phosphonodithioate (**14–19**) was affected by silica column chromatography to give each compound as its triethylamine salt (Table 1). These deoxynucleoside H-phosphonodithioates were stable indefinitely as solids at −40 °C and for several weeks in alkali at room temperature. However in acidic solutions they undergo complete oxidation/desulfurization in about 96 h at room temperature.

The direct oxidation of **14–19** with various oxidants to yield the deoxynucleoside dithiophosphates was unsuccessful. Iodine–water–pyridine, metachloroperbenzoic acid (MCPBA), *tert*-butylhydroperoxide, dimethyldioxirane, and sulfur/CS_2 solutions did not yield the desired product but instead mixtures of partially and fully desulfurized compounds.

Failure to directly oxidize **14–19**, under conditions compatible with nucleoside chemistry, was unexpected but further research using the oxidative coupling methodology[15] was more successful. This approach involved first the synthesis of an intermediate nucleoside phosphorodithioate diester having an alcohol protecting group on oxygen (**20–25**). A study of this system revealed that 9-fluorenemethanol was an ideal candidate as it could be introduced efficiently by oxidative coupling and then rapidly converted under mild conditions and high yield to the deoxynucleoside 5'- and 3'-dithiophosphates (**7** and **27–29**).

Oxidative coupling of the deoxynucleoside H-phosphonodithioates with 1.0 equiv of iodine in pyridine and 10 equiv of alcohol generated the *O*-deoxynucleosid-3'-yl and *O*-deoxynucleosid-5'-yl *O*-(9-fluorenemethyl) phosphorodithioates (**20–25**) as the main products. A two- or three-fold excess of 9-fluorenemethanol in the coupling reaction resulted in an incomplete reaction according to ^{31}P NMR. Under optimal conditions, compounds **20–25** were isolated by silica column chromatography in 40–60% yield (Table 2). The expected phosphorothioate (20%) and phosphate (7%) side products were also observed. When an excess of iodine was used during oxidation, the ^{31}P NMR showed an additional peak at 117.0 ppm which disappeared upon quenching with $Na_2S_2O_3$.

In contrast to the difficulties encountered in deprotecting **1**, **2**, and **6**, removal of blocking groups from **20–25** proved to be relatively straightforward. For the 5'-*O*-dimethoxytrityl protected products (**20** and **21**), this group was removed during workup following oxidative coupling. The base labile 9-fluorenemethyl group could then be removed successfully within seconds by treatment with piperidine or concentrated ammonium hydroxide. Unfortunately the 9-fluorenemethylphosphorodithioate diester proved to be labile to *tert*-butylammonium fluoride which meant that silyl protecting groups could not be used in this synthesis scheme. For example, treatment of **22** or **24** with 0.1 M TBAF/THF resulted in 80% loss of the dithiophosphate and recovery of the deoxynucleoside. In order to circumvent this problem and also simplify the deprotection scheme, a 3'-*O*-acetyl blocked deoxynucleoside was substituted for the corresponding silyl derivative. This alteration in strategy led to one step deprotection of **23** and **25** by treatment with concentrated ammonium hydroxide at 65 °C for 16 h as the *N*-benzoyl group on adenine, 9-fluorenemethyl, and acetyl were labile to this reagent (Table 3).

(12) Baraniak, J.; Stec, W. J. *J. Chem. Soc. Perkins Trans. 1* **1987**, 1645.
(13) Yau, E.; Ma, Y.-X.; Caruthers, M. H. *Tetrahedron Lett.* **1990**, *31*, 1953.
(14) Howard, F. B.; Miles, H. T. *Biochemistry* **1984**, *23*, 6723.
(15) Brill, W.; Yau, E.; Caruthers, M. H. *Tetrahedron Lett.* **1989**, *30*, 6621.

Scheme 3

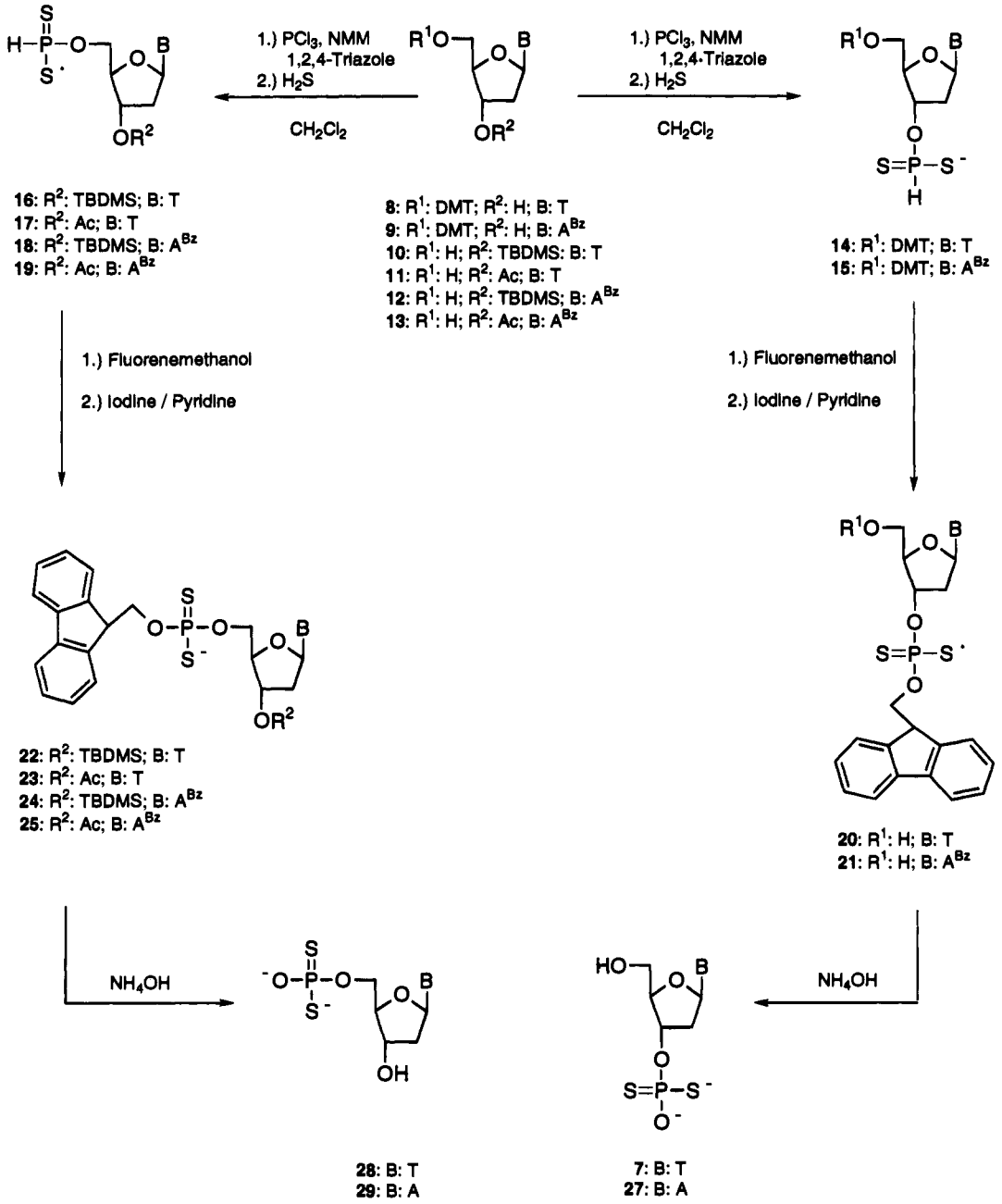


Table 1. Yields and Physicochemical Characteristics of **14**–**19**

compd	B	R^1	R^2	δ ^{31}P NMR ($CDCl_3$, ppm)	R_f system I	yield (%)
14	T	DMT		84.7	0.40	58.4
15	A^{Bz}	DMT		84.7	0.40	43.5
16	T		TBDMS	85.8	0.27	50.7
17	T		Ac	85.4	0.26	55.6
18	A^{Bz}		TBDMS	86.2	0.26	42.4
19	A^{Bz}		Ac	86.3	0.26	48.7

Table 2. Yields and Physicochemical Characteristics of **20**–**25**

compd	B	R^1	R^2	δ ^{31}P NMR ($CDCl_3$, ppm)	R_f system I	yield (%)
20	T	H		116.4	0.22	47.3
21	A^{Bz}	H		112.3	0.12	40.4
22	T		TBDMS	113.3	0.21	59.6
23	T		Ac	113.4	0.22	44.6
24	A^{Bz}		TBDMS	112.7	0.24	59.6
25	A^{Bz}		Ac	113.5	0.24	54.9

Stability Studies. The deoxynucleoside dithiophosphates were stable in dry form for at least eight weeks at −30 °C. As shown in Figure 1, deoxynucleoside dithiophosphates hydrolyze to the deoxynucleoside under acidic conditions. When monitored after 24 h, the hydrolysis occurred most rapidly at pH 3.5 (95%). Hydrolysis slowed significantly with increase in pH and at pH 8, none was observed. In a denaturing polyacrylamide gel with 20% cross-linking, the deoxynucleoside dithiophosphates migrated at a considerably slower rate than the deoxynucleoside monophosphate counterparts (presumably due to an increase in mass/charge ratio).

Enzyme Inhibition Studies. Deoxynucleoside dithiophosphates are potential inhibitors for several classes of enzymes such as viral reverse transcriptases, kinases, phosphatases, and polymerases. For *in vitro* studies with various members of these enzyme classes, deoxyadenosine 3′- and 5′-dithiophosphate and thymidine 5′-dithiophosphate were used. Reaction conditions, substrate and enzyme concentrations, were chosen so that each

Table 3. Yields and Physicochemical Characteristics of **7** and **27–29**

compd	δ ^{31}P NMR (D_2O, ppm)	t_R HPLC (min)	yield (%)
7	90.1	19.98	68.0
27	89.3	28.77	61.2
28	90.8	19.89	62.8
29	89.9	27.01	70.4

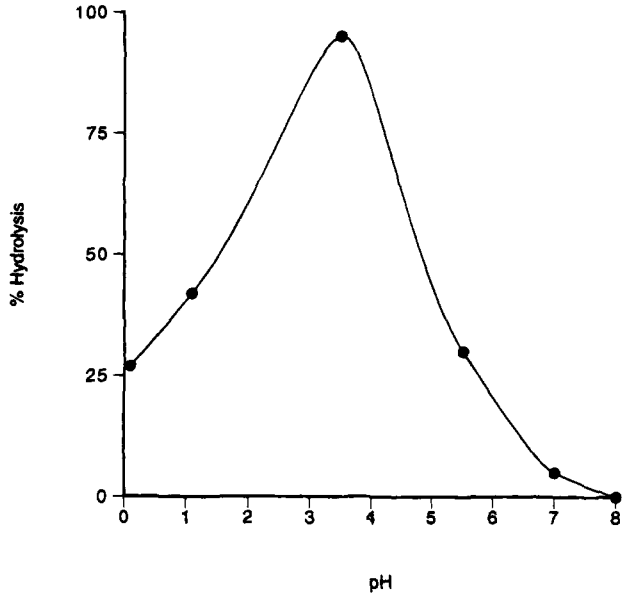


Figure 1. pH Dependent hydrolysis of compound **27** after 24 h.

enzyme was operating within its linear range. In order to study the influence of different metal ions, enzymic reactions were completed with both magnesium and manganese. Based upon Lewis acid–base considerations, sulfur (a soft Lewis base) and manganese (a soft Lewis acid) were expected to interact more strongly than sulfur and magnesium, a hard Lewis acid.

All three of the deoxynucleoside dithiophosphates that were tested (**27**, **28**, and **29**) showed some inhibitory activity against avian myeloblastosis virus reverse transcriptase (AMV RT) in a primer extension assay where 1 to 100 μM deoxynucleoside dithiophosphate was added (Figure 2). The IC 50s (the concentration of inhibitor required to decrease the activity of the enzyme by 50%) were 2 μM (**29**), 80 μM (**28**), and greater than 100 μM (**27**). With Mn^{2+} as the counter ion, the reaction rates decreased and the IC 50s remained in the same order with **29** requiring the lowest concentration for 50% inhibition (40 μM) and **27** the highest (greater than 100 μM). However these three deoxynucleoside dithiophosphates did not show inhibitory activity (1–100 μM) against human immunodeficiency virus reverse transcriptase (HIV RT), DNA polymerase I Klenow fragment, or alkaline phosphatase with either magnesium chloride or manganese chloride. Additionally, alkaline phosphatase did not hydrolyze **29** (no deoxynucleoside could be detected), whereas deoxyadenosine 5'-phosphate under identical conditions was completely dephosphorylated. T4 polynucleotide kinase also was not inhibited by any of the tested compounds (**27–29**), nor did it recognize compound **27** as a substrate. Thus in contrast to studies with nucleoside 5'-monophosphorothioates,[2,6] neither enzyme inhibition nor activity was observed with any of these analogs. These results are comparable to those of Ludwig and Eckstein where the deoxynucleoside 5'-*O*-(1,1-dithiotriphosphate) was inactive as a substrate for DNA polymerase Klenow fragment.[10] With the exception of AMV-RT, perhaps these observations (lack of inhibition and chemical reactivity) suggest that deoxynucleoside dithiophosphates have very low affinities for enzyme active sites. This may be due to

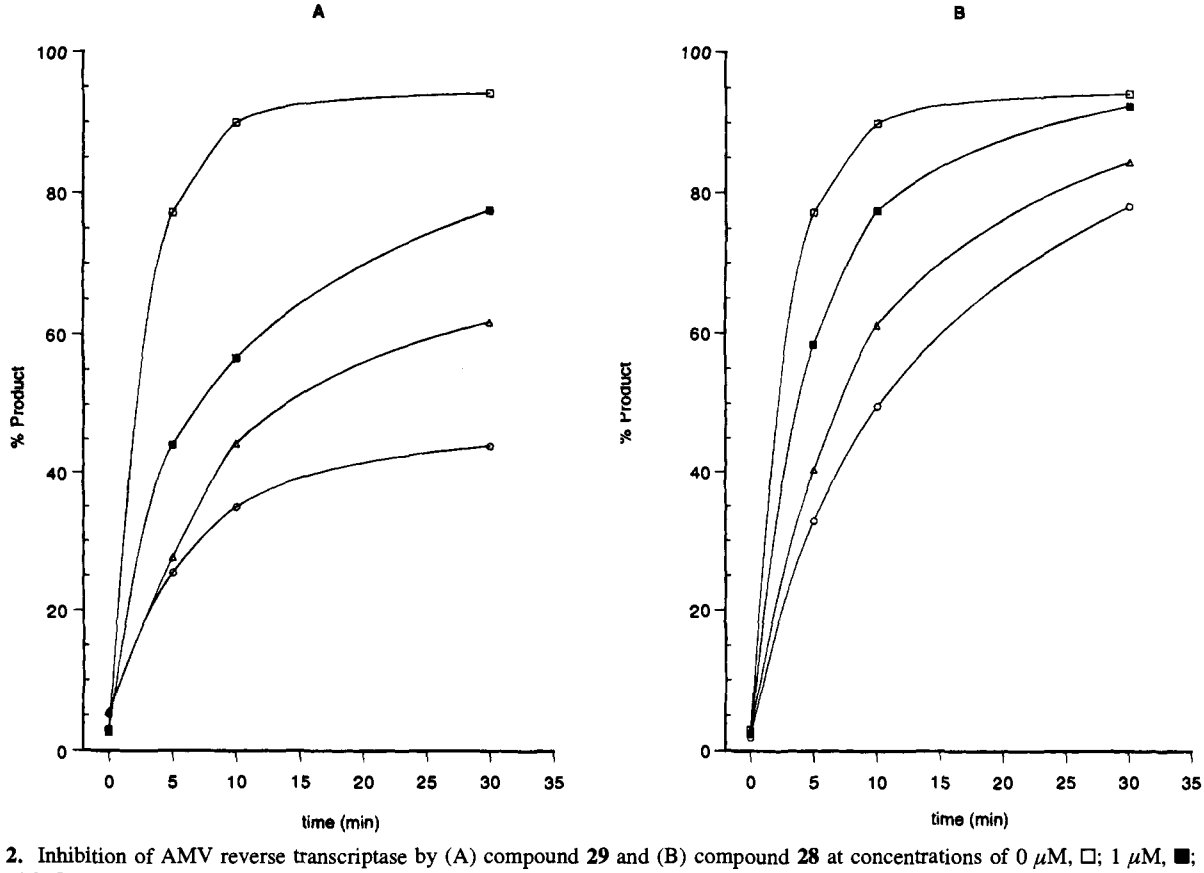


Figure 2. Inhibition of AMV reverse transcriptase by (A) compound **29** and (B) compound **28** at concentrations of 0 μM, □; 1 μM, ■; 10 μM, △; 100 μM, ○.

a steric effect as dithiophosphate is considerably larger than phosphate and might not fit into the active site of an enzyme. A lack of metal ion interaction with dithiophosphate does not appear to be a major contributing reason for the observed results. This is because manganese, which can substitute for magnesium in these enzymes, does not facilitate inhibition or enzyme reactivity with this analog.

Conclusions. This study constitutes the first report on the successful synthesis of deoxynucleoside monophosphates where two of the nonbridging oxygen atoms are replaced by sulfur. In contrast to expectations, these analogs did not demonstrate any inhibitory activity, even at 100 μM concentration, except against AMV RT. Relative to potential therapeutic uses of deoxyoligonucleotides having phosphorodithioate internucleotide linkages,[16,17] these results are encouraging. This is because the lack of inhibition or reactivity against several major classes of nucleic acid metabolizing enzymes suggests low toxicity for at least these potential breakdown products of deoxyoligonucleotides having the phosphorodithioate internucleotide linkage.

Experimental Section

Materials and Methods. Phosphorus trichloride, *N*-methylmorpholine, 1,2,4-triazole, 9-fluorenemethanol, iodine, and hydrogen sulfide were purchased from Aldrich Chemicals. Dry pyridine and dichloromethane (containing less than 0.005% water) were also purchased from Aldrich and used as supplied. Protected deoxyribonucleosides were purchased from Cruachem (Sterling, VA). 3′-*O*-Acetyl[18] and 3′-*O*-*tert*-butyldimethylsilyl[19] protected nucleosides were synthesized by published procedures. Ammonium hydroxide was obtained from Mallinckrodt. Ultrafree-MC (0.45 μm) centrifugal filters from Millipore (Bedford, MA) were used for filtration of deprotected deoxymononucleotides.

^{1}H NMR spectra were recorded on a Varian VXR-300S and a General Electric GE 300 in the solvent indicated. ^{31}P NMR spectra were recorded on a Varian VXR-300S spectrometer operating at 121.4 Hz, a Bruker AM-400 spectrometer operating at 162.0 Hz, and a General Electric GE 300 spectrometer with broad band decoupling referenced to 85% H_3PO_4 as an external standard.

TLC was performed on Kieselgel 60 Platten (Merck Darmstadt) eluted with either dichloromethane–ethylacetate–triethylamine–methanol (60:30:5:5, v/v/v/v) (system I) or dichloromethane–methanol (90:10, v/v) (system II) and DC-Folien PEI Cellulose F (Merck Darmstadt) eluted with *n*-propanol–water–ammonium hydroxide (55:35:10, v/v/v) and examined by UV light. Reverse-phase HPLC was performed with a Waters dual-pump 6000A system in combination with a Maxima 820 gradient controller and a Model 440 UV detector operating at 254 nm. Columns were packed with PRP-1 (5 μm, Hamilton) and eluted with 50 mM triethylammonium bicarbonate (TEAB), pH 7.5 (solvent system A), or 100 mM TEAB, pH 7.5 (solvent system B), containing a linear gradient of acetonitrile from 0–8% in 60 min.

DNA polymerase I (Klenow fragment), alkaline phosphatase from bovine liver, and T4 polynucleotide kinase were obtained from Boehringer Mannheim. Avian myeloblastosis virus reverse transcriptase (AMV RT) was purchased from Life Sciences Inc. (St. Petersburg, FL). Human immunodeficiency virus reverse transcriptase was used as described previously.[16] Deoxymononucleotides and *p*-nitrophenylphosphate were obtained from Sigma. α-^{32}P-ATP and γ-^{32}P-ATP were purchased from NEN (Boston, MA). Primer-template and other deoxyoligonucleotides were used as described previously.[16] Quantitation of radioactivity on gels analyzed by PAGE was with a Molecular Dynamics PhosphorImager. The alkaline phosphatase reactions were followed by use of a Molecular Devices V_{max} kinetic plate reader.

Synthesis of *O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-(β-Cyanoethyl) Phosphorodithioate (4). *O*-[5′-*O*-(4,4′-Dimethoxytrityl)-thymidin-3′-yl] *O*-(β-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (**5**; 1 mmol, 0.744 g) was dissolved in deoxygenated acetonitrile (10 mL), and H_2S bubbled through the solution for 6 min. Tetrazole (2.5 mmol, 0.175 g) in deoxygenated acetonitrile (5 mL) was then added, and the solution was stirred for 30 min with a constant stream of H_2S gas bubbling through it. Addition of sulfur (4.5 mL of a 0.5 M elemental sulfur in toluene–2,6-lutidine, 19:1, v/v) followed by stirring under argon at room temperature for 1 h converted the H-phosphonothioate to the phosphorodithioate diester (**4**). The reaction mixture was concentrated to a gum in vacuo, dissolved in ethyl acetate (50 mL), filtered through glass wool to remove excess sulfur, extracted consecutively with saturated aqueous bicarbonate and brine, and dried over magnesium sulfate. After removal of salt by filtration, the reaction products were concentrated to a glass, dissolved in dichloromethane–ethyl acetate–methanol–triethylamine (50:40:9:1, v/v/v/v), and fractionated by flash chromatography on silica gel to yield **4** as its triethylammonium salt (761 mg, 94%): ^{31}P NMR ($CDCl_3$) δ 113.3 ppm; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 7.3, 7.0 Hz, 9H, CH_3(TEA)), 2.41 (m, 1H, $H_{2'}$), 2.73 (m, 1H, $H_{2'}$), 3.19 (q, J = 7.3, 7.2, 7.2 Hz, 6H, N-CH_2), 3.49 (m, 2H, $H_{5'}$), 3.79 (s, 6H, OCH_3), 4.22 (m, 4H, CH_2 (β-cyanoethyl)), 4.40 (m, 1H, $H_{4'}$), 5.50 (m, 1H, $H_{3'}$), 6.46 (m, 1H, $H_{1'}$), 6.80–7.54 (m, 13H, DMT), 7.62 (s, 1H, H(6)), 8.75 (s, br, 1H, NH); MS (FAB-, glycerol) 708 (M^-).

Synthesis of (*O*-Thymidin-3′-yl) *O*-(β-Cyanoethyl) Phosphorodithioate (6). The 4,4′-dimethoxytrityl group was removed from **4** (348 mg g, 0.43 mmol) by treatment with 80% acetic acid (8 mL at room temperature for 20 min). The reaction mixture was diluted with water (20 mL), extracted with diethyl ether (3 × 20 mL), concentrated to a gum *in vacuo*, and purified on a DEAE-cellulose column using a linear gradient of triethylammonium bicarbonate. The major product corresponding to **6** was isolated in essentially quantitative yield (208 mg, 95%): ^{31}P NMR (CD_3OD) δ 114.7 ppm; ^{1}H NMR (D_2O) δ 1.15 (t, J = 7.4, 7.1 Hz, 9H, CH_3(TEA)), 1.78 (s, 3H, CH_3), 2.31 (m, 1H, $H_{2'}$), 2.52 (m, 1H, $H_{2'}$), 2.85 (t, J = 6.0, 3.5 Hz, 6H, N-CH_2), 2.98 (q, J = 7.3, 7.3, 7.1 Hz, 6H, N-CH_2), 3.85 (m, 2H, $H_{5'}$), 4.17 (m, 4H, CH_2(β-cyanoethyl), 4.27 (m, 1H, $H_{4'}$), 5.10 (m, 1H, $H_{3'}$), 6.32 (m, 1H, $H_{1'}$), 7.87 (s, 1H, H(6)); MS (FAB^-, glycerol) 408 (MH^+); MS (FAB^+, glycerol) 406 $(M - 1)^-$.

Synthesis of *O*-(Thymidin-3′-yl) Phosphorodithioate (7). *O*-(Thymidin-3′-yl) *O*-(β-cyanoethyl) phosphorodithioate (175 mg, 0.43 mmol) was refluxed in 0.4 M LiOH (6.5 mL) for 30 min, diluted to 1.5 L with water, and applied to a DEAE-Sephadex column (5 g, A25 Sephadex). Elution of the column with a linear gradient of triethylammonium bicarbonate (0–0.6 M; pH 8.0) separated the final product from minor impurities. Concentration of product fractions by lyopholization generated **7** as a hygroscopic solid. Compound **7** when isolated in this salt form decomposes slowly to thymidine in methanol and unbuffered water. Based upon analysis by TLC, **7** does not decompose when dissolved in phosphate buffered aqueous solutions (pH 7.4 and 8.0) at room temperature over 4 days. ^{31}P NMR (D_2O + NaOD, pH 10) δ 88.3 ppm; ^{1}H NMR (D_2O + NaOD, pH 10) δ 1.10 (t, J = 7.2, 7.0 Hz, 9H, CH_3(TEA)), 1.79 (s, 3H, CH_3), 2.46 (m, 2H, $H_{2'}$), 2.89 (q, J = 7.4, 7.2, 7.2 Hz, 6H, N-CH_2), 3.86 (m, 2H, $H_{5'}$), 4.20 (m, 1H, $H_{4'}$), 6.25 (t, J = 7.0, 6.6 Hz, 1H, $H_{1'}$), 7.53 (s, 1H, H(6)); MS (FAB^-, glycerol) 353 (M^-).

General Procedure for the Synthesis of *O*-(Deoxynucleosid-3′-yl) H-phosphonodithioate and *O*-(Deoxynucleosid-5′-yl) H-phosphonodithioate (14–19). Phosphorus trichloride (2.2 mL, 25 mmol), 1,2,4-triazole (5.75 g, 85 mmol), and *N*-methylmorpholine (54 mL, 0.25 mmol) were dissolved in anhydrous dichloromethane, and the solution stirred for 30 min at room temperature under argon. The mixture was cooled to 0 °C in an ice–water bath, and a solution of the protected deoxynucleoside (**8–13**, 5 mmol) in 75 mL of anhydrous dichloromethane was added dropwise. After complete addition, the reaction flask was removed from the ice–water bath, and the mixture was stirred at room temperature for 15 min. A steady stream of H_2S (dried over calcium oxide) was bubbled through the solution for 10 min. After the reaction mixture was purged with argon for 30 min to remove excess H_2S, it was washed with 5% aqueous bicarbonate (1 × 30 mL), the aqueous phase reextracted with 20 mL dichloromethane, and the combined organic phases were dried over magnesium sulfate. After solvent removal *in vacuo*, the crude product was purified by silica gel column chromatography (600 g) in dichloromethane–ethyl acetate–

(16) Marshall, W. S.; Caruthers, M. H. *Science* **1993**, *259*, 1564.
(17) Marshall, W. S.; Beaton, G.; Stein, C. A.; Matsukura, M.; Caruthers, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6265.
(18) Weber, H.; Khorana, H. G. *J. Mol. Biol.* **1972**, *72*, 219.
(19) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

methanol−triethylamine (60:30:5:5, v/v/v/v). The product containing fractions were combined, solvent was removed under high vacuum, and the appropriate product was isolated by precipitation from dichloromethane into pentane.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] H-Phosphonodithioate (14).** Yield 1.880 g (58.4%); TLC (system I) R_f 0.40; ^{31}P NMR ($CDCl_3$) δ 84.7 ppm; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, J = 7.3, 7.1 Hz, 9H, CH_3(TEA)), 2.3 (ddd, J = 13.2, 6.5, 3.6 Hz, 1H, $H_{2'}$), 2.6 (ddd, J = 13.5, 6.8, 6.8 Hz, 1H, $H_{2'}$), 3.1 (q, J = 7.3, 7.3, 7.2 Hz, 6H, N-CH_2), 3.33−3.47 (m, 1H, $H_{4'}$), 3.67−3.70 (m, 1H, $H_{5'}$), 3.73 (s, 6H, OCH_3), 4.3 (m, 1H, $H_{5'}$), 5.3 (ddd, J = 9.8, 5.1, 5.0 Hz, 1H, $H_{3'}$), 6.4 (dd, J = 6.4, 6.4 Hz, 1H, $H_{1'}$), 6.77−6.80 (m, 4H, DMT), 7.14−7.36 (m, 7H, DMT), 7.57 (s, 1H, H(6)), 8.68 (d, J = 552 Hz, 1H, P-H), 8.8−9.2 (br, 2H, NH); ^{13}C NMR ($CDCl_3$) 9.1 (CH_3 (TEA)), 11.8 (Me), 39.8 ($C_{2'}$), 46.4 (N-CH_2), 55.5 (OCH_3), 63.7 ($C_{5'}$), 76.0 ($C_{3'}$), 84.9 ($C_{1'}$), 85.4 ($C_{4'}$), 87.3 (DMT), 111.5 (C(4)), 113.6 (DMT), 127.4 (DMT), 128.3 (DMT), 128.5 (DMT), 130.5 (DMT), 135.7 (DMT), 135.8 (DMT), 136.0 (C(5)), 144.7 (DMT), 150.9 (C(2)), 159.0 (DMT), 164.5 (C(6)); MS (FAB$^-$, glycerol) 728 (M^- + Gly, 10), 639 (M^-, 65), 125 (100), 102 (100); MS (FAB$^+$, glycerol) 742 (M^+ + TEA, 40), 303 (100), 102 (100).

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-benzoyl-2′-deoxyadenosin-3′-yl] H-Phosphonodithioate (15).** Yield 1.640 g (43.5%); TLC (system I) R_f 0.40; ^{31}P NMR ($CDCl_3$) δ 84.7 ppm; ^{1}H ($CDCl_3$) δ 1.3 (t, J = 7.4, 7.1 Hz, 9H, CH_3(TEA)), 2.0 (s, 1H, $H_{2'}$), 2.85−2.92 (ddd, J = 14.7, 6.6, 6.1 Hz, 1H, $H_{2'}$), 3.10−3.17 (q, J = 7.4, 7.3, 7.1 Hz, 6H, N-CH_2), 3.36−3.39 (m, 2H, $H_{5'}$), 3.7 (s, 6H, OCH_3), 4.46 (m, 1H, $H_{4'}$), 5.4 (ddd, J = 9.8, 5.1, 5.0 Hz, 1H, $H_{3'}$), 6.53 (dd, J = 7.1, 6.6 Hz, 1H, $H_{1'}$), 6.70−6.73 (m, 4H, DMT), 7.11−7.20 (m, 7H, DMT), 7.24−7.43 (m, 7H, DMT), 7.50 (m, 1H), 7.92 (dd, J = 7.5, 1.3 Hz, 2H, Bz), 8.14 (s, 1H, H(8)), 8.6 (s, 1H, H(2)), 8.7 (d, J = 540 Hz, 1H, P-H); ^{13}C NMR ($CDCl_3$) 8.9 (CH_3 (TEA)), 38.2 ($C_{2'}$), 46.7 (N-CH_2), 55.5 (*O*-CH_3), 55.6 (*O*-CH_3), 64.0 ($C_{5'}$), 75.1 ($C_{3'}$); 85.7 ($C_{1'}$); 86.1 ($C_{4'}$), 86.7 (DMT), 113.6 (DMT), 125.3, 128.4, 128.7, 129.2, 130.7, 134.3 (DMT, Bz), 136.4 (DMT), 145.6 (DMT), 150.4 (C(4)), 152.4 (C(2)), 152.4 (C(6)), 159.1 (DMT), 166.6 (CO); MS (FAB$^+$, glycerol) 754 (M^+, 20), 369 (100), 303 (35), 102 (28).

***O*-[3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl] H-Phosphonodithioate (16).** Yield 1.136 g (50.7%); TLC (system I) R_f 0.27; ^{31}P NMR ($CDCl_3$) δ 85.8 ppm; ^{1}H NMR ($CDCl_3$) δ −0.11 (s, 6H, $SiMe_2$), 0.68 (s, 9H, tBu), 1.20 (t, J = 7.3, 7.1 Hz, 9H, CH_3(TEA)), 1.87 (d, J = 1.3 Hz, 3H, CH_3), 1.98−2.09 (m, 2H, $H_{2'}$), 2.81−2.92 (br, 2H, NH), 3.05−3.15 (q, J = 7.5, 7.3, 7.1 Hz, 6H, N-CH_2), 3.72 (m, 1H, $H_{4'}$), 3.85−4.03 (m, 2H, $H_{5'}$), 4.38 (ddd, J = 6.6, 3.4, 3.4 Hz, 1H, $H_{3'}$), 6.22 (dd, J = 8.0, 6.1 Hz, 1H, $H_{1'}$), 7.65 (s, 1H, H(6)), 8.53 (d, J = 543 Hz, 1H, P-H); ^{13}C NMR ($CDCl_3$) −4.5 ($SiMe_2$), 8.8 (CH_3(TEA)), 12.7 (Me), 18.0 (tBu), 25.9 (tBu), 41.0 ($C_{2'}$), 46.4 (N-CH_2), 64.0 ($C_{5'}$), 73.6 ($C_{3'}$), 85.0 ($C_{1'}$), 86.8 ($C_{4'}$), 111.4 (C(4)), 136.7 (C(5)), 151.2 (C(2)), 165.0 (C(6)); MS (FAB$^+$, glycerol) 554 (M^+ + TEA, 48), 452 (M^+, 41); MS (FAB$^-$, glycerol) 542 (M^- + TEA, 40), 451 (M^-,100).

***O*-(3′-*O*-Acetylthymidin-5′-yl) H-Phosphonodithioate (17).** Yield 1.338 g (55.6%); TLC (system I) R_f 0.26; ^{31}P NMR ($CDCl_3$) δ 85.4 ppm; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.3, 7.2 Hz, 9H, CH_3(TEA)), 1.94 (s, 3H, CH_3), 2.0 (s, 3H, CH_3(Ac)), 2.21−2.29 (m, 2H, $H_{2'}$), 3.18 (q, J = 7.4, 7.4, 7.1 Hz, 6H, N-CH_2), 3.45−3.71 (m, 1H, $H_{4'}$), 3.96−4.24 (m, 2H, $H_{5'}$), 5.31 (ddd, J = 9.7, 5.0, 5.0 Hz, 1H, $H_{3'}$), 6.36 (dd, J = 6.4, 6.4 Hz, 1H, $H_{1'}$), 7.81 (s, 1H, H(6)), 8.64 (d, J = 537 Hz, 1H, P-H), 10.01 (br, 1H, N-H); ^{13}C NMR ($CDCl_3$) 8.9 (CH_3(TEA)), 12.7 (CH_3), 21.3 (CH_3(Ac)), 37.6 ($C_{2'}$), 46.3 (N-CH_2), 64.6 ($C_{5'}$), 76.5 ($C_{3'}$), 83.9 ($C_{1'}$), 84.7 ($C_{4'}$), 112.1 (C(4)), 136.6 (C(5)), 151.2 (C(2)), 164.6 (C(6)), 170.7 (CO); MS (FAB$^-$, glycerol) 379 (M^-, 100), 169 (35); MS (FAB$^+$, glycerol) 303 (55), 239 (20), 197 (20).

***O*-[3′-*O*-(*tert*-Butyldimethylsilyl)-*N*-6-benzoyl-2′-deoxyadenosine-5′-yl] H-Phosphonodithioate (18).** Yield 1.171 g (42.4%); TLC (system I) R_f 0.26; ^{31}P NMR ($CDCl_3$) δ 86.2 ppm; ^{1}H NMR ($CDCl_3$) δ −0.02 (s, 6H, $SiMe_2$), 0.88 (s, 9H, tBu), 1.18 (t, J = 7.3, 7.1 Hz, 9H, CH_3(TEA)), 2.18−2.27 (ddd, J = 12.3, 6.6, 3.9 Hz, 1H, $H_{2'}$), 2.63 (ddd, J = 13.0, 6.6, 3.6 Hz, 1H, $H_{2'}$), 2.98 (q, J = 7.4, 7.3, 7.2 Hz, 6H, N-CH_2), 3.43−4.08 (m, 3H, $H_{4'}$/$H_{5'}$), 4.52 (ddd, J = 9.8, 5.2, 5.1 Hz, 1H, $H_{3'}$), 6.44 (dd, J = 7.1, 6.7 Hz, 1H, $H_{1'}$), 7.20−7.41 (m, 3H, Bz), 7.74 (dd, J = 7.6, 1.8 Hz, 2H, Bz), 8.41 (m, 1H, H(8)), 8.51 (d, J = 546 Hz, 1H, P-H), 8.76 (s, 1H, H(2)), 10.16 (br, 1H, NH); ^{13} C NMR (CD_3CN) −4.8 ($SiMe_2$), 8.8 (Me(TEA)), 18.1 (tBu), 25.8 (tBu), 41.3 ($C_{2'}$), 46.6 (N-CH_2), 64.2 ($C_{5'}$), 74.1 ($C_{3'}$), 84.5 ($C_{1'}$), 87.4 ($C_{4'}$), 124.7 (Bz), 129.1 (Bz), 129.2 (Bz), 132.8 (Bz), 134.3 (Bz), 143.2 (Bz), 150.2 (C(4)), 152.5 (C(2)), 152.6 (C(6)), 166.6 (CO); MS (FAB$^+$, glycerol) 656 (M^+ + TEA + Gly, 15), 553 (M^+ + Gly, 20), 461 (M^+, 30), 369 (100).

***O*-(3′-*O*-Acetyl-*N*-6-benzoyl-2′-deoxyadenosin-5′-yl) H-Phosphonodithioate (19).** Yield 1.446 g (48.7%); TLC (system I) R_f 0.26; ^{31}P NMR ($CDCl_3$) δ 86.3 ppm; ^{1}H NMR ($CDCl_3$): δ1.30 (t, J = 7.4, 7.2 Hz, 9H, CH_3(TEA)), 2.0 (s, 3H, CH_3), 2.41−2.48 (ddd, J = 12.1, 6.4, 4.0 Hz, 1H, $H_{2'}$), 2.78−2.96 (ddd, J = 12.8, 6.2, 3.7 Hz, 1H, $H_{2'}$), 3.15 (q, J = 7.3, 7.3, 7.2 Hz, 6H, N-CH_2), 3.70 (m, 2H, $H_{5'}$), 2.28 (m, 1H, $H_{4'}$), 5.45 (ddd, J = 9.7, 5.1, 5.1 Hz, 1H, $H_{3'}$), 6.60 (dd, J = 7.0, 6.6 Hz, 1H, $H_{1'}$), 7.20−7.62 (m, 3H, Bz), 7.88 (dd, J = 7.4, 2.0 Hz, 2H, Bz), 8.62 (s, 1H, H(8)), 8.63 (d, J = 558 Hz, 1H, P-H) 8.93 (s, 1H, H(2)), 9.20 (br, 1H, NH); ^{13}C NMR ($CDCl_3$) 8.7 (CH_3 (TEA)), 21.3 (CH_3), 38.9 ($C_{2'}$), 46.4 (N-CH_2), 65.2 ($C_{5'}$), 76.8 ($C_{3'}$), 84.1 ($C_{1'}$), 84.6 ($C_{4'}$), 123.4, 128.2, 129.1, 133.0, 134.0, 142.9 (Bz), 149.7 (C(4)), 152.3 (C(2)), 152.7 (C(6)), 165.5 (CO), 170.6 (CO(Ac)); MS (FAB$^+$, glycerol) 494 (M^+, 20), 240 (100), 136 (20); MS (FAB$^-$, glycerol) 492 (M^-, 55), 238 (100), 160 (15).

General Procedure for the Synthesis of *O*-(Deoxynucleosid-3′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate and *O*-(Deoxynucleosid-5′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (20−25). Appropriately protected 2′-deoxynucleosid-3′-yl H phosphonodithioate or 2′-deoxynucleosid-5′-yl H-phosphonodithioate (**14−19**, 1 mmol) and 9-fluorenemethanol (1.96 g, 10 mmol) were dissolved in anhydrous pyridine (10 mL). Iodine (0.1 M) in anhydrous pyridine was added dropwise until the dark color did not disappear (10 mL). The mixture was stirred for 5 min, 1.0 M aqueous $Na_2S_2O_3$ was added to quench the excess iodine, and solvent was removed by concentration in vacuum. The crude product was fractionated by silica gel flash column chromatography (500 g). Excess 9-fluorenemethanol was recovered by flushing the column with 1 L of hexane−ethyl acetate (90:10, v/v). The product containing fractions were combined and yielded the triethylamine salts of **20−25** as a white foam after the solvent was removed under high vacuum.

***O*-(Thymidin-3′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (20).** Yield 293 mg (47.3%); TLC (system II) R_f 0.22; ^{31}P NMR ($CDCl_3$) δ116.2 ppm; ^{1}H NMR (MeOH-D4): δ 1.30 (t, J = 7.6, 7.2 Hz, 30H, CH_3(TEA)), 1.80 (s, 3H, CH_3), 1.98−2.38 (m, 2H, $H_{2'}$), 3.19 (q, J =7.3, 7.1, 7.1 Hz, 24H, N-CH_2), 3.82 (m, 2H, $H_{4'}$/$H_{5'}$), 4.02−4.34 (m, 3H, $H_{5'}$/Fmol), 5.04 (m, 1H), 6.20 (m, 1H, $H_{1'}$), 7.07−7.40 (m, 4H, Fmol), 7.54−7.78 (m, 4H, Fmol); MS (FAB$^+$, glycerol) 102 (100); MS (FAB$^-$, glycerol) 517 (M^-, 100), 457 (31), 383 (69), 125 (45).

***O*-(6-*N*-Benzoyl-2′-deoxyadenosin-3′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (21).** Yield 600 mg (40.4%); TLC (system II) R_f 0.12; ^{31}P NMR ($CDCl3$): δ112.3 ppm; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, J = 7.4, 7.1 Hz, 9H, CH_3(TEA)), 2.4 (ddd, J = 12.2, 6.6, 3.7 Hz, 1H, $H_{2'}$), 2.65 (ddd, J = 13.1, 6.8, 3.5 Hz, 1H, $H_{2'}$), 3.05 (q, J = 7.3, 7.2, 7.1 Hz, 6H, N-CH_2), 3.30 (s, 1H, Fmol), 3.65 (m, 1H, $H_{5'}$), 4.02−4.35 (m, 3H, $H_{4'}$/$H_{5'}$/Fmol), 5.35 (ddd, J = 9.7, 5.1, 5.0 Hz, 1H, $H_{3'}$), 6.25 (dd, J = 7.0, 6.5 Hz, 1H, $H_{1'}$), 7.05−8.81 (m, 13H, Bz/Fmol); ^{13}C NMR ($CDCl_3$) 8.6 (CH_3 (TEA)), 39.2 ($C_{2'}$), 46.3 (N-CH_2), 62.5 ($C_{5'}$), 67.7 (Fmol), 71.8 ($C_{3'}$), 86.7 ($C_{1'}$), 87.6 ($C_{4'}$), 119.7, 123.7, 125.5, 125.6, 126.8, 126.9, 127.4, 127.5, 127.8, 128.7, 133.0, (Fmol, Bz), 141.0, 143.4, 144.3 (Fmol), 149.6 (C(4)), 150.7 (C(2)), 151.3 (C(6)), 165.8 (CO); MS (FAB$^-$, glycerol) 644 (M^-, 28), 437 (10); MS (FAB$^+$, glycerol) 738 (M^+ + glycerol, 40), 646 (M^+, 32).

***O*-[3′-*O*-(*tert*-Butyldimethylsilyl)thymidin-5′-yl] *O*-(9-Fluorenemethyl) Phosphorodithioate (22).** Yield 385 mg (59.6%); TLC (system II): R_f 0.21; ^{31}P NMR ($CDCl_3$) δ113.3 ppm; ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6H, $SiMe_2$), 0.85 (s, 9H, tBu), 1.34 (t, J = 7.0, 7.0 Hz, 9H, CH_3(TEA)), 1.83 (s, 3H, CH_3), 2.32−2.65 (m, 2H, $H_{2'}$), 3.19 (q, J = 7.4, 7.2, 7.2 Hz, 6H, N-CH_2), 4.0−4.35 (m, 6H, $H_{4'}$/$H_{5'}$/Fmol), 4.63 (ddd, J = 9.5, 6.3, 4.2 Hz, 1H, $H_{3'}$), 6.40 (dd, J = 7.0, 6.8 Hz, 1H, $H_{1'}$), 7.20−7.42 (m, 4H, Fmol), 7.58−7.84 (m, 4H, Fmol); ^{13}C NMR ($CDCl_3$) −5.4 ($SiMe_2$), 8.0 (CH_3 (TEA)), 11.4 (Me), 17.1 (tBu), 25.0 (tBu), 40.2 ($C_{2'}$), 46.0 (N-CH_2), 64.7 ($C_{5'}$), 67.2 (Fmol), 73.0 ($C_{3'}$), 84.5 ($C_{1'}$), 86.4 ($C_{4'}$), 110.4 (C(4)), 119.1, 123.8, 124.8, 126.2, 126.8, (Fmol), 136.2 (C(5)), 140.6 (Fmol), 143.8 (Fmol), 150.1 (Fmol), 163.8 (C(6));

MS (FAB$^+$, DTT/DTE) 685 (M$^+$ + TEA + K$^+$, 15), 223 (65), 179 (40), 131 (100); MS (FAB$^-$, DTT/DTE): 804 (M$^-$ + DTT + TEA), 10), 466 (15), 246 (15), 102 (100).

***O*-(3′-*O*-Acetylthymidin-5′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (23).** Yield 297 mg (44.6%); TLC (system II) R_f 0.22; ^{31}P NMR ($CDCl_3$) δ 113.4 ppm; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 7.1, 7.0 Hz, 9H, CH_3(TEA)), 1.81 (s, 3H, CH_3), 2.03 (s, 3H, CH_3(Ac)), 2.20 (m, 2H, $H_{2'}$), 3.13 (q, J = 7.3, 7.1, 7.1 Hz, 6H, N-CH_2), 3.92–4.21 (m, 5H, $H_{4'}$/$H_{5'}$/Fmol), 5.34 (ddd, J = 9.2, 5.0, 5.0 Hz, 1H, $H_{3'}$), 6.39 (dd, J = 6.8, 6.7 Hz, 1H, $H_{1'}$), 7.06–7.26 (m, 3H, Fmol), 7.56–7.74 (m, 3H, Fmol), 7.86 (s, 1H, H(6)), 8.21 (s, 2H, Fmol), 9.41–9.75 (br, 2H, NH); ^{13}C NMR ($CDCl_3$) 9.0 (CH_3 (TEA)), 12.6 (CH_3), 21.3 (CH_3 (Ac)), 37.6 ($C_{2'}$), 46.5 (N-CH_2), 65.9 ($C_{5'}$), 68.3 (Fmol), 76.5 ($C_{3'}$), 83.9 ($C_{1'}$), 84.8 ($C_{4'}$), 112.0 (C(4)), 120.1, 125.7, 127.2, 127.9 (Fmol), 136.8 (C(5)), 141.5, 144.5, 146.8 (Fmol), 151.1 (C(2)), 164.7 (C(6)), 170.8 (CO); MS (FAB$^+$, glycerol) 778 (M$^+$ + TEA, 40), 677 (M$^+$, 100), 573 (30), 395 (22), 102 (100); MS (FAB$^-$, glycerol) 331 (30), 101 (100).

***O*-[3′-O-(*tert*-Butyldimethylsilyl)-*N*-6-benzoyl-2′-deoxyadenosin-5′-yl] *O*-(9-Fluorenemethyl) Phosphorodithioate (24).** Yield 535 mg (59.6%); TLC (system II) R_f 0.24; ^{31}P NMR ($CDCl_3$) δ 112.7 ppm; ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6H, $SiMe_2$), 0.87 (s, 9H, tBu), 1.30 (t, J = 7.2, 7.0 Hz, 9H, CH_3(TEA)), 2.22–2.28 (ddd, J = 12.4, 6.7, 3.8 Hz, 1H, $H_{2'}$), 2.60–2.65 (ddd, J = 13.0, 6.6, 3.6 Hz, 1H, $H_{2'}$), 3.10 (q, J = 7.4 7.2, 7.2 Hz, 6H, N-CH_2), 3.40 (s, 1H, Fmol), 4.01–4.38 (m, 4H, $H_{4'}$/$H_{5'}$/Fmol), 4.62 (ddd, J = 9.4, 5.0, 4.9 Hz, 1H, $H_{3'}$), 6.53 (dd, J = 6.9, 6.6 Hz, 1H, $H_{1'}$), 7.08–7.93 (m, 11H, Bz, Fmol), 8.85 (s, 1H, Fmol), 9.07 (s, 1H, Fmol); ^{13}C NMR ($CDCl_3$) −4.5 ($SiMe_2$), 8.9 (CH_3 (TEA)), 18.2 (tBu), 25.9 (tBu), 44.3 ($C_{2'}$), 46.6 (N-CH_2), 63.9 ($C_{5'}$), 68.4 (Fmol), 74.2 ($C_{3'}$), 84.3 ($C_{1'}$), 88.2 ($C_{4'}$), 119.9, 121.2, 122.8, 125.8, 126.0, 127.1, 127.3, 128.3, 128.8, 129.1, 129.4 (Bz, Fmol), 141.4 (Fmol), 144.5 (Fmol), 148.3 (C(4)), 152.5 (C(2)), 152.7 (C(6)); MS (FAB$^+$, glycerol) 760 (M$^+$, 38); MS (FAB$^-$, glycerol) 850 (M$^-$ + TEA, 22), 758 (M$^-$, 100).

***O*-(3′-*O*-Acetyl-*N*-6-benzoyl-2′-deoxyadenosin-5′-yl) *O*-(9-Fluorenemethyl) Phosphorodithioate (25).** Yield 435 mg (54.9%); TLC (system II) R_f 0.24; ^{31}P NMR ($CDCl_3$) δ 113.5 ppm; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 7.2, 7.1 Hz, 9H, CH_3(TEA)), 2.03 (s, 3H, CH_3(Ac)), 2.41 (ddd, J = 12.2, 6.8, 3.7 Hz, 1H, $H_{2'}$), 2.82 (ddd, J = 12.8, 6.7, 3.9 Hz, 1H, $H_{2'}$), 3.17 (q, J = 7.5, 7.3, 7.2 Hz, 6H, N-CH_2), 4.02–4.20 (m, 5H, $H_{4'}$/$H_{5'}$/Fmol), 5.42 (ddd, J = 9.2, 5.0, 4.9 Hz, 1H, $H_{3'}$), 6.53 (dd, J = 6.8, 6.7 Hz, 1H, $H_{1'}$), 7.01–7.94 (m, 13H, Fmol/Bz); ^{13}C NMR ($CDCl_3$) 8.8 (CH_3 (TEA)), 21.3 (CH_3 (Ac)), 39.0 ($C_{2'}$), 46.6 (N-CH_2), 48.8 (Fmol), 66.0 ($C_{5'}$), 68.4 (Fmol), 84.4 ($C_{2'}$), 84.8 ($C_{4'}$), 120.0 (C(4)), 120.1, 123.0, 125.9, 126.0, 127.6, 128.4, 129.3, 133.8 (Bz, Fmol), 141.4 (Fmol), 144.7 (Fmol), 148.8 (C(4)), 152.3 (C(2)), 153.0 (C(6)), 170.6 (CO); MS (FAB$^-$, glycerol): 778 (M$^-$ + glycerol, 10), 686 (M$^-$, 100), 508 (35), 102 (100); MS (FAB$^+$, glycerol): 369 (10), 240 (100).

General Procedure for the Synthesis of *O*-(Deoxynucleosid-3′-yl) Phosphorodithioate and *O*-(Deoxynucleosid-5′-yl) Phosphorodithioate (7, and 27–29). Appropriately protected *O*-(2′-deoxynucleosid-3′-yl) *O*-(9-fluorenemethyl) phosphorodithioate or *O*-(2′-deoxynucleosid-5′-yl) *O*-(9-fluorenemethyl) phosphorodithioate (0.001 mmol) was dissolved in concentrated ammonium hydroxide (2 mL) and the sealed vial stored at 65 °C for 16 h. After cooling to room temperature, the solution was filtered through a 0.45 μm centrifugal microfilter to remove the dibenzofulvene. Solvent was then removed under vacuum and the residue taken up in 500 μL 50 mM TEAB (pH 7.5). The product was purified from the reaction mixture by HPLC using a linear gradient of 0–8% acetonitrile in 50 mM TEAB (system A) or 100 mM TEAB (system B) over 60 min.

***O*-(Thymidin-3′-yl) Phosphorodithioate (7).** Yield 3.70 mg (68.3%); ^{31}P NMR (D_2O) δ 90.1 ppm; MS (FAB$^+$, glycerol) 207 (20), 118 (100); MS (FAB$^-$, glycerol) 353 (M$^-$, 20), 219 (15), 127 (20); t_R = 19.98 (system A), 10.08 (system B).

***O*-(2′-Deoxyadenosin-3′-yl) Phosphorodithioate (27).** Yield 3.32 mg (61.2%); ^{31}P NMR (D_2O) δ 89.3 ppm; MS (FAB$^+$, glycerol) 465 (M$^+$ + TEA, 13), 364 (M$^+$, 20), 252 (10), 136 (100); MS (FAB$^-$, glycerol) 362 (M$^-$, 100), 227 (20), 134 (45), 111 (55); t_R = 28.77 (system A).

***O*-(Thymidin-5′-yl) Phosphorodithioate (28).** Yield 3.50 mg (62.8%); ^{31}P NMR (D_2O) δ 90.8 ppm; MS (FAB$^+$, glycerol) 207 (10), 118 (100); MS (FAB$^-$, glycerol) 353 (M$^-$, 15), 219 (14), 127 (20); t_R = 19.89 (system A), 9.46 (system B).

***O*-(2′-Deoxyadenosin-5′-yl) Phosphorodithioate (29).** Yield 3.92 mg (70.4%); ^{31}P NMR (D_2O) δ 89.9 ppm; MS (FAB$^+$, glycerol) 465 (M$^+$ + TEA, 30), 364 (M$^+$, 35), 252 (100); MS (FAB$^-$, glycerol) 362 (M$^-$, 55), 219 (15), 149 (38); t_R = 27.01 (system A).

Hydrolysis Studies. Compound **27** (0.5 mg) was dissolved in 500 μL of buffer solutions at the desired pH. The following buffer systems were used: pH 0.1, 1 N HCl; pH 1.1, 0.1 N HCl; pH 3, pH 5, and pH 7, 0.1 M citric acid and 0.25 M disodium phosphate; pH 8.0, Tris-HCl. Every 24 h, 5 μL aliquots were spotted onto a PEI Cellulose TLC plate and developed in *n*-propanol–water–concentrated ammonium hydroxide (55:35:10, v/v/v). Appropriate spots of deoxynucleoside and deoxynucleotide were eluted and analyzed spectrophotometrically at 260 nm.

Alkaline Phosphatase Substrate Studies. Reaction mixtures contained compound **29** at 4.5 mM, 10 units alkaline phosphatase, and buffer (Tris-HCl 50 mM, EDTA 0.1 mM, pH 8.5). During the course of the reaction (37 °C, 60 min), aliquots were removed and spotted on PEI-Cellulose TLC plates which were developed in *n*-propanol–water–concentrated ammonium hydroxide (55:35:10, v/v/v/v). Controls included reaction mixtures incubated without enzyme and also enzyme plus 5′-dAMP at 8.6 mM instead of compound **29**.

Enzyme Inhibition Studies. General Procedure. Solutions containing substrates, reaction buffers, enzyme, and compounds **27**, **28**, or **29** at 0, 1, 10, and 100 μM were incubated at 37 °C for 30 min. Aliquots were removed at specified time intervals, quenched with formaldehyde loading buffer, and analyzed by PAGE. The radioactivity on a gel was analyzed by placing the gel in a Molecular Dynamics PhosphorImager, recording radioactivity, and quantitating the results. The IC 50 values were determined by plotting the decrease in substrate versus time. The same reactions were carried out using 1 mM $MnCl_2$ in buffers instead of $MgCl_2$. Specific solution compositions for inhibition studies with each enzyme are as follows.

(i) **AMV RT.** The reaction mixture contained 5′-[^{32}P]-labeled primer-template complex[16] at 200 nM, dNTPs at 160 μM, reaction buffer (Tris 50 mM, $MgCl_2$ 10 mM, KCl 50 mM, DTT 5 mM, pH 8.3) and AMV RT at 0.5 units/μL.

(ii) **HIV RT.** The reaction was carried out with HIV RT at 2.4 μg/mL, 5′-[^{32}P]-labeled primer-template[16] at 200 nM, dNTPs at 160 μM and reaction buffer (Tris 50 mM, $MgCl_2$ 19 mM, NaCl 20 mM, DTT 5 mM, pH 8.0).

(iii) **T4 Polynucleotide Kinase.** The reaction contained a deoxyoligonucleotide 15mer (PRTI)[16] at 1 μM, [γ-^{32}P]-ATP at 0.5 μM, reaction buffer (Tris 50 mM, $MgCl_2$ 10 mM, NaCl 20 mM, DTT 5 mM, pH 8.0), and T4 polynucleotide kinase at 0.005 units/μL.

(Iv) **DNA polymerase I (Klenow Fragment).** The reaction mixtures contained 5′-[^{32}P]-labeled primer-template[16] at 200 nM, dNTPs at 160 μM, reaction buffer (KH_2PO_4, 50 mM; $MgCl_2$, 3 mM; 2-mercaptoethanol, 1 mM, pH 7.5) and 0.0001 units/μL Klenow fragment.

(v) **Alkaline Phosphatase.** The reactions were carried out in triplicate in a 72 well microtiter plate, and the absorbance was followed with a kinetic plate reader at 405 nm. The maximum velocity for the reaction in each well was determined using Softmax software (Molecular Devices, Menlo Park, CA). The reaction mixture contained *p*-nitrophenylphosphate at 220 μM, reaction buffer (diethanolamine, 1 M; $MgCl_2$, 0.5 mM, pH 9.8).

Acknowledgment. We thank Charles Greef and Darla Graff for advice on the biochemistry experiments, Chad Brautigam for purification of HIV RT, Dr. R. Barkley for the FAB mass spectral analysis, and Dr. G. Beaton for critical review of the manuscript.

Supplementary Material Available: Spectral data for the synthesis of compounds (51 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JA942995P