Notes

The Synthesis of Nucleoside 5′-*O*-(1,1-Dithiotriphosphates)[†,1]

Andrzej Okruszek* and Magdalena Olesiak

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, Lodz 90-363, Poland

Jan Balzarini

Rega Institute for Medical Research, Catholic University Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received May 9, 1994[⊗]

Appropriately protected nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholanes) react with inorganic pyrophosphate in the presence of a strong base catalyst (DBU) to give nucleoside 5′-*O*-(1,1-dithiotriphosphates) **1a–g**. The latter compounds, including an AZT analogue, show modest antivirial activity against HIV-1 and HIV-2 replication in CEM cells. The AZT and deoxyadenosine derivatives were found to be inhibitors of HIV reverse transcriptase.

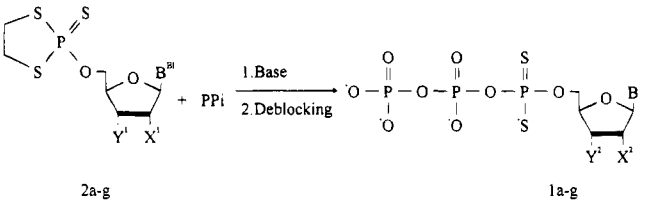
Introduction

Nucleoside phosphorothioate analogues of nucleotides and oligonucleotides, introduced originally by Eckstein,[2] have found wide application in both biochemistry[3] and molecular biology.[4] Among many classes of compounds studied, the sulfur analogues of nucleoside 5′-*O*-triphosphates (NTP) play a very special role due to the importance of their parent nucleotides as substrates for polymerases (2′-deoxy series) and as key components (cofactors) of several enzymatic systems involving phosphoryl transfer (ATP, GTP). The vast majority of the synthetic effort was devoted to the preparation of phosphorothioate analogues of NTP, containing only one sulfur atom attached to one of the phosphorus atoms of the triphosphate chain.[3,5] Doubly sulfur-substituted NTP's were recently synthesized by Ludwig and Eckstein,[6,7] mostly as 1,3- and 1,2-dithiotriphosphate analogues. These authors have also described the preparation of thymidine and guanosine 5′-*O*-(1,1-dithiotriphosphate) by the reaction of the corresponding 5′-*O*-(thiocyclotriphosphates) with lithium sulfide in DMF solution.[6] Although the yields were low (13–22%), the compounds were the first published examples of 1,1-phosphorodithioate analogues of NTP. The thymidine analogue was found not to be a substrate for the Klenow DNA polymerase.[6]

Other examples of members among the nucleoside phosphorodithioate family are the cyclic analogue of 2′,3′-cUMP described in 1970 by Eckstein[8] and that of 3′,5′-cAMP prepared by Baraniak and Stec.[9] The latter compound was found to have unique properties as a cAMP antagonist, being a competitive inhibitor of cAMP-dependent protein kinase.[10] Recently a considerable synthetic effort is being observed in several laboratories to develop efficient methods of preparation of oligonucleotide phosphorodithioates as potential "antisense" modulators of gene expression.[11–13]

In this paper, we present our approach to the synthesis of nucleoside 5′-*O*-(1,1-dithiotriphosphates) **1**

Scheme 1



which utilizes base-catalyzed nucleophilic substitution at a tetracoordinated phosphorothioyl center. This approach is based on our earlier observations regarding the reactivity of intermediates containing the 2-oxo-1,3,2-oxathiaphospholane ring system,[14] further developed by Stec *et al.*,[15] by introducing nucleoside 3′-*O*-(2-thio-1,3,2-oxathiaphospholane) synthons in their method of synthesis of oligo(deoxyribonucleoside phosphorothioates). We assumed that reaction of appropriately protected nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholanes) **2**, containing one more sulfur atoms in the five-membered phospholane ring, with inorganic pyrophosphate in the presence of a base catalyst should lead, after deblocking and spontaneous elimination of ethylene sulfide, to the desired nucleoside 5′-*O*-(1,1-dithiotriphosphates) **1** (Scheme 1).

Results and Discussion

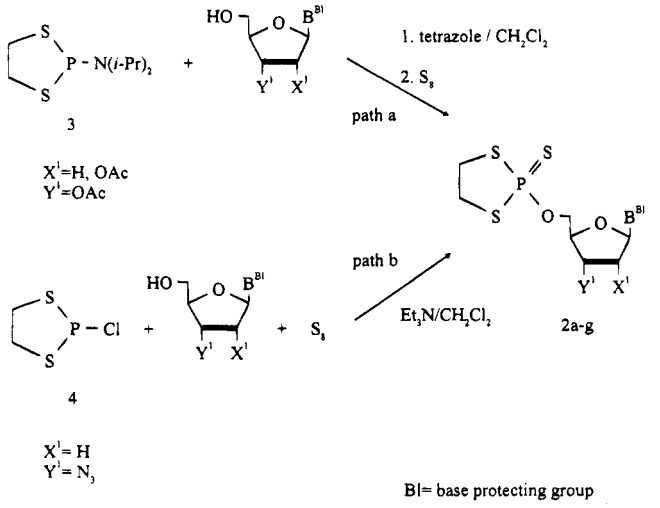
To check this hypothesis, we prepared a series of compounds **2a–f**, by reaction of appropriately protected nucleosides with 2-(*N*,*N*-diisopropylamino)-1,3,2-dithiaphospholane (**3**) in the presence of tetrazole as a catalyst (Scheme 2, path a). The 3′- and (if present) 2′-hydroxyl functions were blocked with acetyl groups, whereas the N^2-amino function of guanine was protected with an isobutyryl residue. The N^4-amino group of cytosine and the N^6-amino group of adenine (only in the deoxy series) were benzoylated. The resulting nucleoside 5′-*O*-(1,3,2-dithiaphospholanes) were, without isolation, oxidized

[†] This article is dedicated to Professor Fritz Eckstein on the occasion of his 60th birthday.

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1994.

0022-2623/94/1837-3850$04.50/0

Scheme 2



with elemental sulfur to give nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholanes) **2a–f**.

The 3′-deoxy-3′-azidothymidine (AZT) derivative **2g** was prepared in a slightly modified way, using 2-chloro-1,3,2-dithiaphospholane (**4**)[16] in the presence of an excess of elemental sulfur and triethylamine for introduction of the 2-thio-1,3,2-dithiaphospholane function[17] (Scheme 2, path b). Such a strategy was chosen in order to minimize possible side reactions between the compounds containing an azido group (AZT and its further derivatives) and the reactive P^{III} substrates and/or intermediates (Staudinger reaction[18,19]). The phosphorochloridites (*e.g.*, **4**) are known to be much less reactive toward organic azides than phosphoroamidites (*e.g.*, **3**). The relative inertness of **4** toward addition of elemental sulfur allows condensation of **4** with AZT in the presence of an excess of S_8, thus allowing preferential thioylation of the P^{III} intermediate.

The crude products **2** were purified by column chromatography on silica gel and, after drying on a vacuum line, isolated in the form of amorphous powder. The yields and physicochemical characteristics of **2a–g** are presented in Table 1. The observed ^{31}P NMR chemical shifts of **2** are fully consistent with their 2-alkoxy-2-thio-1,3,2-dithiaphospholane structure.[21] All compounds gave correct elemental analyses. Their ^1H NMR spectra (not shown) as well as mass spectra are in accord with the presumed structure. For **2g**, the characteristic IR band at 2110 cm^{-1} (N_3, asymmetric stretching) was observed.[22]

The reactions of **2a–g** with inorganic pyrophosphate (PPi) were performed on a 0.25 mmol scale with 100% molar excess of PPi and monitored by ^{31}P NMR. Two forms of pyrophosphate were checked, bis(tri-*n*-butylammonium)pyrophosphate[5] and tris(tetra-*n*-butylammonium)pyrophosphate;[23] the latter form appeared to be superior and gave better yields of 1,1-dithiotriphosphates **1a–g**. Four base catalysts were tried, triethylamine, *N*-methylimidazole, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and potassium *tert*-butoxide. The latter was used in DMF, where in other cases acetonitrile was employed as a reaction medium. The experiments have shown that for the reaction of **2a–g** with PPi thecatalyst of choice is DBU, used in a 1:1 mole ratio with respect to pyrophosphate. The reaction in the presence of triethylamine is too slow, whereas with *t*-BuOK more byproducts were observed.

The reaction between **2** and PPi has to be performed with absolute exclusion of moisture. Thus, the two components (1:2 mole ratio) were dried on a vacuum line for several hours, and the flask was filled with dry argon through the rubber septum. Then, anhydrous acetonitrile (2 mL/1 mmol of **2**) and dry DBU were syringed through the septum, and the reaction mixture was stirred magnetically at room temperature. The ^{31}P NMR analysis of the crude reaction mixture after 10–12 h revealed full disappearance of substrate **2** and the appearance of signals at 98–101 (doublet), −7–−10 (doublet), and −23–−25 ppm (double doublet) in a 1–1–1 ratio, which could be tentatively assigned to the phosphorus atoms P^1, P^3, and P^2, respectively, of triphosphate **1**. In addition, the signal at *ca.* −5 ppm corresponding to an excess of pyrophosphate was observed as were the signals at *ca.* 2, 68, and 116 ppm, which were due to the reaction byproducts.

After completion of the reaction, the crude mixture was treated with 25% aqueous ammonia in order to remove protecting groups. In the case where benzoyl or isobutyryl groups were used for base protection, heating for 15 h at 55 °C was necessary, whereas removal of acetyl residues, protecting 3′- and 2′-hydroxyl groups of the sugar moiety, was performed at room temperature during 12 h. The products **1a–g** were isolated and purified by ion exchange chromatography on a DEAE-Sephadex A-25 column with aqueous triethylammonium bicarbonate (TEAB) as eluent. The purity of the products was checked by ^{31}P NMR spectroscopy and HPLC. The yields and physicochemical characteristics of **1a–g** are listed in Table 2.

The structural assignment of **1a–g** was made on the basis of their ^{31}P NMR spectra. All of the spectra show for P^1 a doublet at *ca.* 100 ppm, a chemical shift characteristic for the phosphodiester type phosphorodithioate moiety.[9,11–13] The chemical shifts of P^2 and P^3 correspond to those of a normal NTP. The $^2J_{PP}$ coupling constant pattern is also characteristic for the proposed structure. The ^{31}P NMR data for **1d** are in full agreement with those reported by Eckstein[6] for thymidine 5′-*O*-(1,1-dithiotriphosphate). The identity of **1a–g** was further confirmed by negative LSIMS mass spectroscopy. All compounds gave molecular ions consistent with the proposed structure. In the IR spectrum

Table 1. Yields and Physicochemical Characteristics of **2a–g**

compd	formula	B	Bl	X^1	Y^1	anal.	^{31}P NMR in CD_3CN, δ (ppm)	TLC R_f	yield (%)
2a	$C_{21}H_{22}N_5O_5S_3P$	ade	Bz	H	OAc	C,H,N	125.38	0.70	72
2b	$C_{20}H_{22}N_{13}O_6S_3P$	cyt	Bz	H	OAc	C,H,N	125.50	0.66	80
2c	$C_{18}H_{24}N_5O_6S_3P$	gua	*i*-Bu	H	OAc	C,H,N	125.52	0.58	32
2d	$C_{14}H_{19}N_2O_6S_3P$	thy	–	H	OAc	C,H,N	125.63	0.59	50
2e	$C_{16}H_{20}N_5O_6S_3P$	ade	–	OAc	OAc	C,H,N	125.70	0.48	98
2f	$C_{20}H_{26}N_5O_8S_3P$	gua	*i*-Bu	OAc	OAc	C,H,N	125.91	0.46	42
2g	$C_{12}H_{16}N_5O_4S_3P$	thy	–	H	N_3	C,H,N	125.75	0.75	48

Table 2. Yield and Physicochemical Characteristics of **1a–g**

compd	B	X^2	Y^2	HPLC t_R (min)	^{31}P NMR in D_2O, δ (ppm)			coupling constants (Hz)		yield (%)
					P^1	P^2	P^3	$J_{P^1P^2}$	$J_{P^2P^3}$	
1a	ade	H	OH	11.51	98.4 (d)	−25.0 (q)	−9.5 (d)	32.8	16.9	12
1b	cyt	H	OH	7.98	100.2 (d)	−23.5 (q)	−9.0 (d)	33.7	18.5	22
1c	gua	H	OH	10.31	100.5 (d)	−23.8 (q)	−8.3 (d)	33.4	20.1	10
1d[a]	thy	H	OH	11.15	100.1 (d)	−23.7 (q)	−9.4 (d)	38.8	15.6	17
1e	ade	OH	OH	11.03	99.1 (d)	−23.9 (q)	−7.5 (d)	42.9	20.2	14
1f	gua	OH	OH	10.69	100.2 (d)	−24.1 (q)	−10.1 (d)	34.9	18.9	15
1g	thy	H	N_3	12.43	100.3 (d)	−24.1 (q)	−10.5 (d)	34.3	19.0	26

[a] Lit.[6] ^{31}P NMR (D_2O) δ 99.7 (d, P^1), −22.96 (q, P^2), −7.09 (d, P^3); $J_{P^1P^2}$ = 34.25 Hz, $J_{P^2P^3}$ = 20.25 Hz.

Table 3. EC_{50} Values (μg/mL) for **1a–g**

compd	HIV-1 (III_B)	HIV-2 (ROD)
1a	43	50
1b	97	123
1c	>50	>50
1d	≥50	≥50
1e	42	116
1f	>50	>50
1g	0.5	*a*

[a] The compound **1g** has not been tested for HIV-2 inhibition.

of **1g**, the characteristic band at 2110 cm^{-1} (N_3, asymmetric stretching) was observed.[22]

Although the yields of **1a–g** were relatively low, they are comparable with those reported by Eckstein.[6] Several attempts were made to improve the effectiveness of the procedure by changing the solvent for condensation (DMF) or the base catalyst (potassium *tert*-butoxide, triethylamine), but in no case were better results achieved. Apparently, strong bases used to activate the pyrophosphate ion by increasing its nucleophilicity are themselves nucleophiles and attack the phosphorus atom, giving some unidentified byproducts and thus decreasing the yield of the desired triphosphate analogue.

The successful preparation of **2e** and **1e** starting from 2′,3′-*O*,*O*-diacetyladenosine clearly shows that for procedures presented in this paper N^6-benzoylation of adenine is not essential. On the other hand, the failure to isolate the product of reaction of 2′,3′-*O*,*O*-diacetylguanosine with **3** followed by sulfurization (data not shown) strongly calls for the necessity of protection of reactive groups of guanine. Further studies on the application of nucleoside 5′-*O*- and 3′-*O*-(2-thio-1,3,2-dithiaphospholanes) for the synthesis of nucleoside phosphorodithioates, including oligonucleotide phosphorodithioate analogues, are underway.[24]

The nucleoside 5′-*O*-(1,1-dithiotriphosphates) **1a–g** were tested for their ability to inhibit HIV-1 (III_B) and HIV-2 (ROD) in CEM cells. Prior to testing, the compounds were transformed into their Na^+ salts by passing through an ion exchange resin (Dowex 50Wx2, Na^+ form). The EC_{50} (50% effective concentration) values are listed in Table 3. None of the natural nucleoside derivatives (**1a–f**) was endowed with a marked anti-HIV activity.

Compound **1a** emerged as the most potent inhibitor of HIV-1 and HIV-2 replication in CEM cells (EC_{50} = 42–50 μg/mL). The compounds **1a–f** proved toxic for CEM cells at a concentration of 250 μg/mL. Thus, antiviral selectivity (ratio toxic dose/active dose) for them was invariably well below 10. The EC_{50} value of 0.5 μg/mL obtained for **1g** in an anti-HIV-1 test may suggest its relatively strong antiviral activity. Such a conclusion should, however, be treated with great precaution, taking into account the fact that the parent nucleoside (AZT) shows *ca.* 100-fold higher anti-HIV activity,[25] so even small (<1%) extracellular degradation of **1g**, leading to release of AZT, can have a great influence on the obtained results. The compound **1g** was not toxic for CEM cells at a concentration of 100 μg/mL.

The triphosphates **1a–g** were also tested as potential inhibitors of HIV-1 reverse transcriptase. The compounds **1a–f** were evaluated in the presence of primed 16S/26S rRNA as the heteropolymeric template and [^{3}H]-dATP as the competing substrate. The deoxyadenosine derivative **1a** was found to inhibit the enzyme by 50% at 50 μg/mL. The compounds **1b–f** were not inhibitory toward HIV-1 reverse transcriptase at concentrations within the range of 200–250 μg/mL. The compound **1g** was evaluated in the presence of poly(rA)/$(dT)_{12-18}$ as template and [*methyl*-^{3}H]dTTP as competing substrate. The IC_{50} (50% inhibitory concentration) value of 0.94 μM was obtained, which means that **1g** has *ca.* 100-fold higher inhibitory properties than **1a** (using only a slightly different substrate–template system) although much lower than AZT triphosphate, for which an IC_{50} of 0.003 μM was found under the same testing conditions (poly(rA)/$(dT)_{12-18}$).

Experimental Section

Materials and Methods. The unprotected nucleosides were purchased from Pharma Waldhof. 2′,3′-*O*,*O*-Diacetyladenosine, 2′,3′-*O*,*O*-diacetylguanosine, and AZT were obtained from Sigma. N^6-Benzoyl-2′-deoxyadenosine, N^4-isobutyryl-2′-deoxyguanosine, and N^2-isobutyrylguanosine were synthesized by the transient protection method.[26] These compounds were reacted with 4,4′-dimethoxytriphenylmethyl chloride by standard procedures,[27] and the 5′-*O*-DMT derivatives were isolated by column chromatography on silica gel. The 3′-*O*-acetyl- and 2′,3′-*O*,*O*-diacetyl-protected nucleosides were prepared by reaction of 5′-*O*-DMT derivatives with acetic anhydride and subsequent removal of the dimethoxytrityl group with *p*-toluenesulfonic acid. Tris(tetra-*n*-butylammonium)pyrophosphate was synthesized according to published procedure.[23] DMF and tri-*n*-butylamine were purchased from Fluka. 1,2-Ethanedithiol was obtained from Aldrich. Acetonitrile (HPLC grade), benzene, and methylene chloride were purchased from Baker. Elemental sulfur (sublimed) and pyridine were obtained from Merck Darmstadt. Triethylamine, diisopropylamine, and tetrazole were purchased from Janssen. The solvents were dried over calcium hydride and distilled before use. All reactions involving trivalent phosphorus compounds were performed under dry argon.

NMR spectra were recorded on a Bruker AC200 spectrometer operating at 200.113 MHz for ^{1}H NMR and at 81.01 MHz (with broad band decoupling) for ^{31}P NMR. Chemical shifts are given in ppm with respect to TMS (internal standard) for ^{1}H NMR and 85% H_3PO_4 (external standard) for ^{31}P NMR. Chemical shifts are positive when downfield from the standard. The spectra of crude reaction mixtures were recorded by addition of CD_3CN to the reaction solution. Negative

LSIMS mass spectra were recorded on a Finnigan MAT 95 spectrometer with a Cs^+ gun operating at 13 keV (glycerin matrix). HR EI mass spectra were obtained with the same instrument at 70 eV ionizing energy with PFK as an internal standard (resolution 10 000). UV spectra were recorded on a Uvikon 860 spectrometer (Kontron Instruments AG), and IR spectra were obtained on a Specord M80 spectrometer (Carl Zeiss Jena) in KBr discs.

TLC was performed on Kieselgel 60F 254 plates (Merck Darmstadt) developed with chloroform/methanol (9:1, v/v). Reverse-phase HPLC was performed with a LDC Milton Roy instrument equipped with a Spectromonitor III and CI-10B integrator. Columns were packed with ODS Hypersil (5 μm; Shandon Southern) and eluted with 0.1 M triethylammonium bicarbonate, pH 7.5, with a linear gradient of acetonitrile from 0% to 14% in 20 min.

The methodology of the anti-HIV assays has been described previously.[28] HIV-induced giant cell formation in CEM cultures was used as the antiviral parameter. Cytotoxicity was established by trypan blue dye staining of the cell cultures.[28] The HIV-1 reverse transcriptase assays using 16S/26S rRNA as the template and [^{3}H]dATP as the radiolabeled competing substrate were carried out as described previously.[29] The tests with poly(rA)/$(dT)_{12-18}$ as template and [*methyl*-^{3}H]dTTP as competing substrate were performed as described previously.[30]

2-Chloro-1,3,2-dithiaphospholane (4). This compound was prepared from phosphorus trichloride and 1,2-ethanedithiol as described by Peake *et al.*[16] (method A): colorless liquid; yield 67%; bp 82–4 °C/1 mmHg; ^{31}P NMR δ 170.7 (C_6D_6) (lit.[16] bp 100 °C/10 mmHg; ^{31}P NMR δ 179.2 (neat)).

2-(*N*,*N*-Diisopropylamino)-1,3,2-dithiaphospholane (3). The chlorophosphite **4** (12.7 g, 0.08 mol) was dissolved in 100 mL of benzene, and to this solution was added dropwise, with stirring and external cooling at 5–10 °C, a solution of diisopropylamine (16.2 g, 0.16 mol) in benzene (50 mL). Stirring was continued for 2 h at room temperature, and amine hydrochloride was filtered off and washed with benzene (2 × 30 mL). The combined filtrates were evaporated, and the residue was distilled at 14 mmHg to yield 15.2 g (90%) of product **3** as a colorless liquid: bp 115 °C; ^{31}P NMR δ 95.1 (C_6D_6); ^{1}H NMR (C_6D_6) 1.08 (d) CH_3, $^3J_{HH}$ = 6.79 Hz), 3.35 (m, CH, $^3J_{PH}$ = 10.25 Hz), 2.55–2.71 and 2.88–3.03 (2 m, CH_2); HR MS for $C_8H_{18}NPS_2$ calcd *m/z* 223.0618, found 223.0596 (deviation 2.2 mmu).

Nucleoside 5′-*O*-(2-Thio-1,3,2-dithiaphospholanes) 2a–f (Path a). One of the protected nucleosides (N^6-benzoyl-3′-*O*-acetyl-2′-deoxycytidine, N^2-isobutyryl-3′-*O*-acetyl-2′-deoxyguanosine, 3′-*O*-acetylthymidine, 2′,3′-*O*,*O*-diacetyladenosine, N^2-isobutyryl-2′,3′-*O*,*O*-diacetylguanosine) (5.0 mmol) was mixed with 0.37 g (5.2 mmol) of tetrazole, dried for 3 h on a vacuum line, and dissolved in anhydrous CH_2Cl_2 (5 mL). To this solution was added **3** (1.16 g, 5.2 mmol) by injection through a septum with stirring at room temperature, and stirring at this temperature was continued for 3 h. Elemental sulfur (0.2 g) was then added, the mixture was stirred overnight at room temperature and filtered, and the filtrate was evaporated. The residue was chromatographed on a column (3 × 15 cm) filled with silica gel (Kieselgel 60, 230–400 mesh; Merck Darmstadt) and eluted with hexane–chloroform–methanol under TLC control. Fractions containing product were pooled and evaporated. Drying on a vacuum line gave **2a–f** in the form of an amorphous white powder. Yields, analytical data, and physicochemical parameters are summarized in Table 1.

3′-Azido-3′-deoxythymidine-5′-*O*-(2-thio-1,3,2-dithiaphospholane) (2g) (Path b). AZT (0.160 g, 0.6 mmol) was mixed with elemental sulfur (0.3 g), dried for 3 h on a vacuum line, and suspended in anhydrous CH_2Cl_2 (10 mL). Into this suspension was added, with stirring and external cooling to 0 °C, triethylamine (0.43 mL, 0.6 mmol) followed by a solution of **4** (0.095 g, 0.6 mmol) in 2 mL of CH_2Cl_2. Stirring was continued for 48 h. The resulting mixture was filtered, concentrated, and chromatographed on a column (3 × 15 cm) filled with silica gel (Kieselgel 60, 230–400 mesh; Merck Darmstadt) using chloroform–ethyl acetate (gradient from 2:1 to 1:1) as eluent under TLC control. Fractions containing product were pooled and evaporated to give **2g** (0.121, 48%) as a white, amorphous powder. Analytical and physicochemical data are listed in Table 1.

Nucleoside 5′-*O*-(1,1-Dithiotriphosphates) 1a–g. One of the protected nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholanes) **2a–g** (0.25 mmol) and tris(tetra-*n*-butylammonium)-pyrophosphate (0.45 g, 0.5 mmol) were combined in a 10 mL flask and dried over P_2O_5 under vacuum for 4 h. The flask was filled with dry argon and closed with a rubber septum, and a solution of 76 mg (0.5 mmol) of DBU in 0.5 mL of anhydrous CH_3CN was injected through the septum. The mixture was stirred for 12 h at room temperature and evaporated. For those compounds possessing acyl-protecting groups (reactions starting from **2a–f**), the residue was dissolved in 3 mL of 25% aqueous NH_3 and tightly stoppered. Ammoniolysis was carried out for 15 h at 55 °C for **2a–c,f** and for 12 h at room temperature for **2d,e**. Each of the ammonia solutions was then evaporated to dryness.

The crude product was dissolved in water (5 mL), and the resulting solution, after filtration, was applied to a DEAE-Sephadex A-25 column (2 × 30 cm). Chromatography was performed with a linear gradient of 1 L each 0.05 M and 1 M TEAB, collecting 20 mL fractions. The purine derivatives (**1a,c,e,f**) eluted between 0.7 and 0.8 M buffer and the pyrimidine derivatives between 0.55 and 0.65 M buffer. Fractions containing product were pooled and evaporated to dryness on a rotary evaporator, and the residue was coevaporated twice with ethanol (50 mL) to remove traces of buffer. The products were finally lyophilized in high vacuum. The yields and physicochemical characteristics of **1a–g** are summarized in Table 2.

Acknowledgments. The authors are indebted to Prof. Wojciech J. Stec for his interest in this work and helpful discussion. We thank Mrs. Danuta Krajewska for excellent technical assistance in chemical synthesis, Dr. Marek Soochacki for measuring and interpretation of LSIMS spectra, and Mrs. Ann Absillis and Mrs. Lizette van Berckelaer for assistance with the biological assays. This work was supported in part by the Belgian Geconcerteerde Onderzoeksacties (Project No. 90/94-2).

References

(1) This work was presented in part as a poster at the 35th IUPAC Congress, Beijing, China, August 15–20, 1993.
(2) Eckstein, F. Nucleoside Phosphorothioates. *J. Am. Chem. Soc.* **1966**, *88*, 4292–4294.
(3) Eckstein, F. Nucleoside Phosphorothioates. *Annu. Rev. Biochem.* **1985**, *54*, 367–402.
(4) Eckstein, F.; Gish, G. Phosphorothioates in Molecular Biology. *TIBS* **1989**, *14*, 97–100.
(5) Ludwig, J.; Eckstein, F. Rapid and Efficient Synthesis of Nucleoside 5′-O-(1-Thiotriphosphates), 5′-Triphosphates and 2′,3′-Cyclophosphorothioates Using 2-Chloro-4H-1,3,2-benzodioxaphosphorin-4-one. *J. Org. Chem.* **1989**, *54*, 631–635.
(6) Ludwig, J.; Eckstein, F. Synthesis of Nucleoside 5′-O-(1,3-Dithiotriphosphates) and 5′-O-(1,1-Dithiotriphosphates). *J. Org. Chem.* **1991**, *56*, 1777–1783.
(7) Ludwig, J.; Eckstein, F. Stereospecific Synthesis of Guanosine 5′-O-(1,2-Dithiotriphosphates). *J. Org. Chem.* **1991**, *56*, 5860–5865.
(8) Eckstein, F. Nucleoside Phosphorothioates. *J. Am. Chem. Soc.* **1970**, *92*, 4718–4723.
(9) Baraniak, J.; Stec, W. J. Ribonucleoside Cyclic 3′,5′-Phosphoramidates: Synthesis, Stereochemistry and Conversion into Ribonucleoside Cyclic 3′,5′-Phosphorothioates and -[^{18}O]Phosphates. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1645–1656.
(10) Parker Botelho, L. H.; Webster, L. C.; Rothermel, J. D.; Baraniak, J.; Stec, W. J. Inhibition of cAMP-dependent Protein Kinase by Adenosine Cyclic 3′,5′-Phosphorodithioate, a Second cAMP Antagonist. *J. Biol. Chem.* **1988**, *263*, 5301–5305.
(11) Dahl, O. Preparation of Nucleoside Phosphorothioates, Phosphorodithioates and Related Compounds. *Sulfur Rep.* **1991**, *11*, 167–192.

(12) Beaton, G.; Dellinger, D.; Marshall, W. S.; Caruthers, M. H. Synthesis of Oligonucleotide Phosphorodithioates. In *Oligonucleotides and Analogues. A Practical Approach*; Eckstein, F., Ed.; JRL Press: Oxford, 1991; pp 109–135.
(13) Wiesler, W. T.; Marshall, W. S.; Caruthers, M. H. Synthesis and Purification of Phosphorodithioate DNA. In *Methods in Molecular Biology, Vol. 20: Protocols for Nucleotides and Analogs*; Agrawal, S., Ed.; Humana Press Inc.: Totowa, NJ, 1993; pp 191–206.
(14) Okruszek, S.; Guga, P.; Stec, W. J. Novel Approach to the Synthesis of Isotopomeric Monoalkyl [^{16}O,^{17}O,^{18}O]Phosphates. The Stereospecific One-pot Conversion of (Rp)-Thymidine 3′-(4-Nitrophenyl)-Phosphorothioate into (Rp)-Thymidine 3′-[^{16}O,^{17}O, ^{18}O]Phosphate. *J. Chem. Soc., Chem. Commun.* **1987**, 594–595.
(15) Stec, W. J.; Grajkowski, A.; Koziołkiewicz, M.; Uznański, B. Novel Route to Oligo(Deoxyribonucleoside Phosphorothioates). Stereocontrolled Synthesis of P-Chiral Oligo(Deoxyribonucleoside Phosphorothioates). *Nucleic Acids Res.* **1991**, *19*, 5883–5888.
(16) Peake, S. C.; Fild, M.; Schmutzler, R.; Harris, R. K.; Nichols, J. M.; Rees, R. G. Preparation and Nuclear Magnetic Resonance Spectral Analysis of Some 1,3,2-Dithiaphospholanes. *J. Chem. Soc., Perkin Trans. 2* **1972**, 380–385.
(17) Martin, S. F.; Wagman, A. S. General and Efficient Route to Phosphorodithioate Analogues of Naturally Occurring Lipids. *J. Org. Chem.* **1993**, *58*, 5897–5899.
(18) Staudinger, H.; Meyer, J. Über neue organische Phosphorverbindungen III. Phosphinmethylenederivate und Phosphinimine. (New Organophosphorus Compounds III. Phosphinmethylene Derivatives and Phosphinimines.) *Helv. Chim. Acta* **1919**, *2*, 635–646.
(19) Kabachnik, M. I.; Giliarov, W. A. Imidates of alkylphosphoric acids. Trialkyl-N-phenylimidophosphates. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1956**, 790–797.
(20) Mosby, W. L.; Silva, M. L. Naphthaquinone Chemistry, Part IV. The Reactions of Phosphines with Vicinal Bisazides. *J. Chem. Soc.* **1965**, 1003–1012.
(21) For 2-phenoxy-2-thio-1,3,2-dithiaphospholane, ^{31}P NMR δ 120.7 ($CHCl_3$) was reported; see: Revel, M.; Navech, J. Heterocycles contenant du phosphore. XVI. Calcul du l'angle de torsion de la liaison C_4–C_5 dans des series de dioxa-, diaza-, et ditiaphospholanes-1,3,2. (Phosphorus-containing heterocycles. XVI. Calculation of the torsional angle of the C4–C5 bond in dioxa-, diaza-, and dithia-1,3,2-phospholanes). *Bull. Soc. Chim. Fr.* **1973**, 1195–1199.
(22) For several side-chain analogues of AZT, the values of 2102–2118 cm^{-1} for N_3 absorption were reported; see: Hiebl, J.; Zbiral, E.; Balzarini, J.; De Clercq, E. Side-Chain Derivatives of Biologically Active Nucleosides. 1. Side-Chain Analogs of 3′-Azido-3′-deoxythymidine (AZT). *J. Med. Chem.* **1992**, *35*, 3016–3023.
(23) Dixit, V. M.; Laskovics, F. M.; Noall, W. I.; Poulter, C. D. Tris-(tetra-n-butylammonium)Hydrogen Pyrophosphate. A New Reagent for the Preparation of Allylic Pyrophosphate Esters. *J. Org. Chem.* **1981**, *46*, 1967–1969.
(24) For preliminary results, see: Okruszek, A.; Sierzchala, A.; Sochacki, M.; Stec, W. J. The Synthesis of Di- and Oligo-(deoxyribonucleoside phosphorodithioates) by Dithiaphospholane Method. *Tetrahedron Lett.* **1992**, *33*, 7585–7588.
(25) For AZT, the EC_{50} value of 0.003 μM was reported; see: Balzarini, J.; Karlsson, A.; Perez-Perez, M.-J.; Vrang, L.; Walbers, J.; Zhang, H.; Öberg, B.; Vandamme, A.-M.; Camarasa, M.-J.; De Clercq, E. HIV-1-Specific Reverse Transcriptase Inhibitors Show Differential Activity against HIV-1 Mutant Strains Containing Different Amino Acid Substitutions in the Reverse Transcriptase. *Virology* **1993**, *192*, 246–253.
(26) Ti, G. S.; Gaffney, B. L.; Jones, R. A. Transient Protection: Efficient One-Flask Synthesis of Protected Deoxynucleosides. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.
(27) Jones, R. A. Preparation of Protected Deoxyribonucleosides. In *Oligonucleotide Synthesis. A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984; pp 23–34.
(28) Balzarini, J.; Naesens, L.; Slachmnylders, J.; Niphnis, H.; Rosenberd, I.; Holy, A.; Schellebens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)Adenine (PMEA) Effectively Inhibits Retrovirus Replication *in vitro* and Simian Immunodeficiency Virus Infection in Rhesus Monkey. *AIDS* **1991**, *5*, 21–28.
(29) Balzarini, J.; Perez-Perez, M.-J.; San-Felix, A.; Camarasa, M.-J.; Bathurst, I. C.; Barr, P. J.; De Clercq, E. Kinetics of Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcriptase by the Novel HIV-1-Specific Nucleoside Analogue [2′,5′-bis-O-(tert-Butyl-dimethylsilyl)-β-D-Ribofuranosyl]-3′-Spiro-5″-[4″-Amino-1″,2″-Oxathiole-2′,2′-Dioxide]Thymine (TSAO-T). *J. Biol. Chem.* **1992**, *267*, 11831–11838.
(30) Balzarini, J.; Herdewijn, P.; Pauwels, R.; Broder, S.; De Clercq, E.; α,β- and β,γ-Methylene 5′-Phosphonate Derivatives of 3′-Azido-2′,3′-dideoxythymidine-5′-triphosphate. Correlation Between Affinity for Reverse Transcriptase Susceptibility to Hydrolysis by Phosphodiesterases and Anti-Retrovirus Activity. *Biochem. Pharmacol.* **1988**, *37*, 2395–2403.