Efficient One-Pot Synthesis of 2′-Deoxyribonucleoside 3′-*O*- and 5′-*O*-Phosphorodithioates

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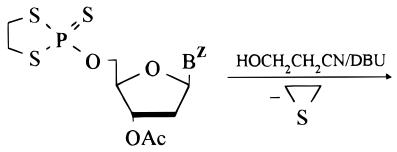
Introduction

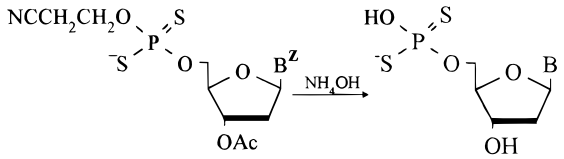
Although *O*,*O*-dialkyl esters of phosphorodithioic acid and their salts are well known and have found broad application as rubber and engine oil antioxidants[1] and important substrates in pesticide industry,[2] nucleoside and oligonucleoside phosphorodithioates became available only recently. The first known examples were uridine cyclic 2′,3′-phosphorodithioate[3a] and adenosine cyclic 3′,5′-phosphorodithioate.[3b] The latter compound had shown interesting properties as an antagonist of cAMP-dependent protein kinase.[4] The first dinucleoside 3′,5′-phosphorodithioates bearing both sulfur atoms in nonbridging positions were described in 1988.[5] Soon afterwords, several methods of synthesis of oligonucleotides bearing in internucleotide positions phosphorodithioate moieties were developed,[6] some of them following the theory that phosphorodithioate analogues of oligonucleotides strongly inhibit reverse transcriptase.[7] In this vein, phosphorodithioate oligonucleotides with sulfur replacing oxygen in both the bridging and nonbridging positions have been synthesized.[8] Interestingly, *O*-monoesters of phosphorodithioic acid bearing a nucleoside ligand were not known, and only recently, Caruthers *et al.*[9] described the synthesis of 3′-*O*- and 5′-*O*-phosphorodithioate derivatives of thymidine and deoxyadenosine, in which two of the nonbridging oxygen atoms of a nucleoside monophosphate were replaced by sulfur. The synthesis involved a multistep procedure, with two chromatographic purifications of intermediates, and provided final products in 11−19% yields after isolation by preparative HPLC.

We report here a one-pot synthetic procedure allowing preparation of the 3′-*O*- and 5′-*O*-phosphorodithioate derivatives of all four deoxyribonucleosides (**1a**−**d** and **2a**−**d**, respectively) in reasonable preparative yields, starting from the corresponding protected nucleoside 3′-*O*- (**3a**−**d**)[10] and 5′-*O*-(2-thio-1,3,2-dithiaphospholane)s (**4a**−**d**).[11]

Scheme 1



4a, B^Z = B = Thy
4b, B^Z = Cyt^{Bz}
4c, B^Z = Ade^{Bz}
4d, B^Z = Gua^{iBu}



5a-d **2a-d**

Results and Discussion

The method of synthesis of *O*,*O*-dialkyl esters of phosphorodithioic acid, developed in this laboratory, relies upon phosphitylation of the corresponding alcohol with 2-(*N*,*N*-diisopropylamino)- or 2-chloro-1,3,2-dithiaphospholane followed by sulfurization of intermediary 2-alkoxy-1,3,2-dithiaphospholane with elemental sulfur. The resulting 2-alkoxy-2-thio-1,3,2-dithiaphospholane can be isolated in satisfactory preparative yield. DBU-assisted reaction of the latter compound with another alcohol gives intermediate *O*,*O*-dialkyl-*S*-(*β*-mercaptoethyl)phosphorodithioate, which undergoes fast elimination of ethylene sulfide, leaving the desired product (Scheme 1). According to this reaction sequence, numerous derivatives of phosphorodithioic acid were obtained, including *O*,*O*-dibutyl phosphorodithioate,[10a] oligo(deoxyribonucleoside phosphorodithioate)s,[10b] nucleoside cyclic 3′,5′-phosphorodithioates,[12] and phosphorodithioate analogues of phospholipids.[13] In addition to dialkyl esters, polyphosphates bearing phosphorodithioate moieties such as nucleoside 5′-*O*-(1,1-dithiotriphosphates) were obtained in the reaction of the corresponding nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholanes) with inorganic pyrophosphate.[11] In light of the aforementioned experience, it seemed feasible that a synthesis of nucleoside monoesters of phosphorodithioic acid (otherwise prepared with difficulty by literature method)[9] may be accomplished if corresponding the nucleoside 5′-*O*- or 3′-*O*-(2-thio-1,3,2-dithiaphospholane) was subjected to a base-catalyzed hydrolytic ring-opening process. Therefore, in our preliminary experiments we have attempted to transform **3** into **1** (and/or **4** into **2**) by reacting it in acetonitrile solution with an excess of aqueous sodium hydroxide and/or aqueous ammonia. Unfortunately, the ^{31}P NMR analysis of the crude product revealed that the reaction led to a complex mixture of phosphorus-containing products (not shown).

Since the direct hydrolysis of dithiaphospholane derivatives did not yield the desired product, we turned our

(1) Emsley, J.; Hall, D. *The Chemistry of Phosphorus*; Harper and Row, Ltd.: London, 1976; p 12.
(2) Eto, M. *Organophosphorus Pesticides: Organic and Biological Chemistry*; CRC Press, Inc.: Cleveland, 1974; p 27.
(3) (a) Eckstein, F. *J. Am. Chem. Soc.* **1970**, *92*, 4718. (b) Baraniak, J.; Stec, W. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1645.
(4) Parker-Botelho, L. H.; Webster, L. C.; Rothermel, J. D.; Baraniak, J.; Stec, W. J. *J. Biol.Chem.* **1988**, *263*, 5301.
(5) Nielsen, J.; Brill, W. K.-D.; Caruthers, M. *Tetrahedron Lett.* **1988**, *29*, 2911.
(6) (a) Dahl, O. *Sulfur Rep.* **1991**, *11*, 167. (b) Wiesler, W. T.; Marshall, W. S.; Caruthers, M. H. In *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; p 191.
(7) Marshall, W. S.; Caruthers, M. H. *Science* **1993**, *259*, 1564.
(8) Cosstick, R.; Vyle, J. S. *Nucleic Acids Res.* **1990**, *18*, 829.
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(13) Martin, S. F.; Wagman, A. S. *J. Org. Chem.* **1993**, *58*, 5897.

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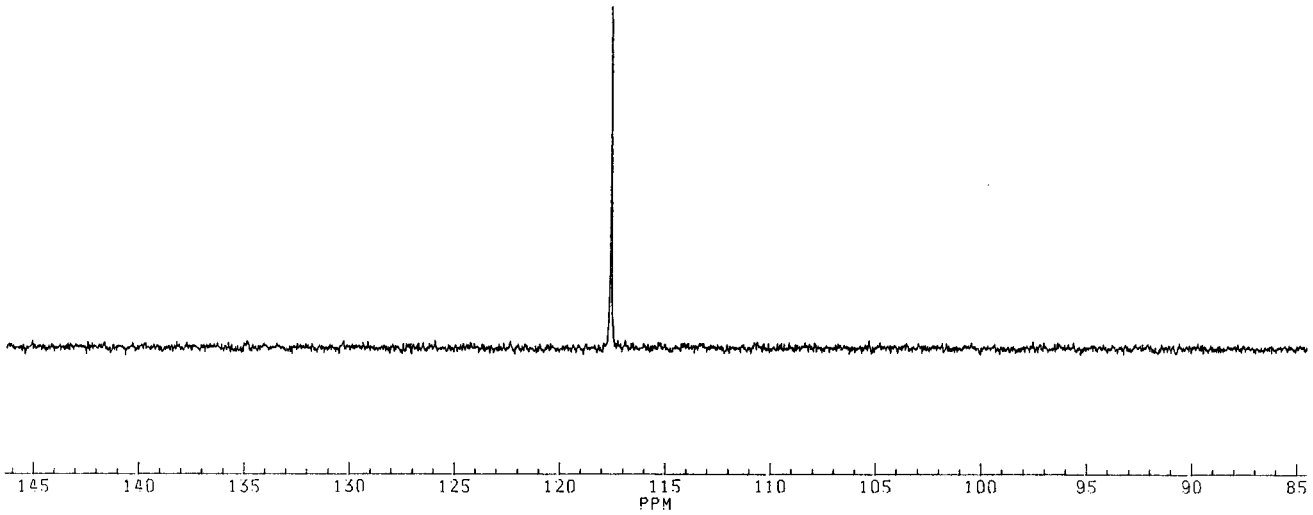


Figure 1. ^{31}P NMR spectrum of the crude product of reaction of **4b** with $HOCH_2CH_2CN$/DBU.

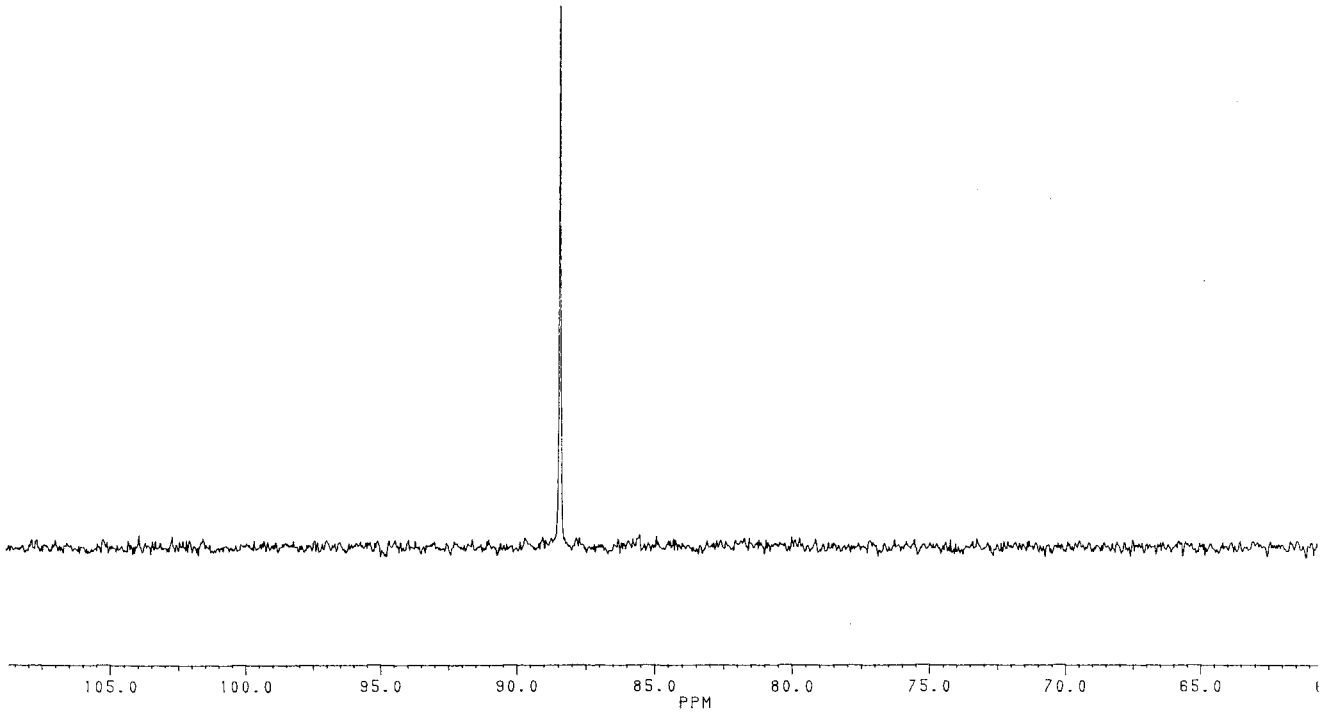


Figure 2. ^{31}P NMR spectrum of crude **2b** (after ammonia deprotection).

attention toward a two-step procedure with intermediate protection of the oxygen atom of the phosphorodithioate group by means of an easily removable alkyl residue, taking advantage of our earlier observation on the clean reaction of nucleoside 3′-*O*-(2-thio-1,3,2-dithiaphospholane)s with alcohols.[10a] Thus, N^4-B^z-deoxycytidine 3′-*O*-acetyl-5′-*O*-(2-thio-1,3,2-dithiaphospholane) (**4b**)[11] in acetonitrile was reacted at room temperature with 3-hydroxypropionitrile in the presence of an equimolar amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Examination of the reaction mixture after 10 min by ^{31}P NMR revealed full conversion of **4b** into a single organophosphorus product with a chemical shift at δ 117.6 ppm (see Figure 1) characteristic for *O*,*O*-dialkyl phosphorodithioates,[6,7,10] which on this basis was tentatively identified as *O*-[(3′-*O*-acetyl)N^4-B^z-deoxycytidine-5′-yl] *O*-(β-cyanoethyl phosphorodithioate) (**5b**).[14] The crude reaction mixture was then treated overnight at 55 °C with an excess of 15% aqueous ammonia. Since the only organophosphorus compound in the crude reaction mixture had a ^{31}P NMR chemical shift δ 88.4 ppm (see Figure 2), we assumed that ammonia treatment removed 3′-*O*-acetyl, N^4-benzoyl, and *O*-(β-cyanoethyl) protecting groups to give deoxycytidine 5′-*O*-phosphorodithioate (**2b**)[15] as a single product. The crude **2b** was purified by means of ion-exchange chromatography on Sephadex A25 and isolated in 52.5% yield. Its structure was confirmed by FAB mass spectrometry and its purity checked by HPLC. The same procedure was applied to the corresponding base-protected (except thymidine) nucleoside 3′-*O*-acetyl-5′-*O*-(2-thio-1,3,2-dithiaphospholane)s **4a**,**c**,**d**[11] (see Scheme 1) leading to 5′-*O*-phosphorodithioate derivatives of thymidine (**2a**), deoxyadenosine (**2c**), and deoxyguanosine (**2d**). The overnight ammonia treatment at 55 °C was

(14) For *O*-(thymidin-3′-yl) *O*-(β-cyanoethyl)phosphorodithioate a ^{31}P NMR chemical shift δ 114.7 ppm (CD_3CN) was reported.[9]

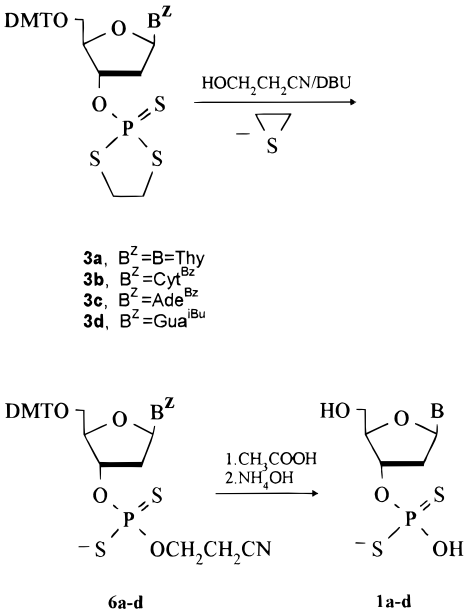
(15) For **2c** the ^{31}P NMR chemical shift δ 89.9 ppm (D_2O) was reported.[9]

Table 1. Yields and Physicochemical Characteristics of Nucleoside 3′-*O*- and 5′-*O*-Phosphorodithioates and the Intermediate *O*-(*β*-Cyanoethyl) Diesters

	nucleoside phosporodithiohate						intermediate diester	
substrate compd no.	compd no.	yield (%)	δ ^{31}P NMR (D_2O) ppm	HPLC t_R (min)	mol wt calcd[a]	mol wt measd[b]	compd no.	δ ^{31}P NMR (CD_3CN) ppm
3a	**1a**	52.0	90.3	6.88	354.3	353	**6a**	116.6
3b	**1b**	52.5	90.3	4.73	339.3	338	**6b**	117.4
3c	**1c**	49.8	90.4	7.30	363.4	362	**6c**	117.2
3d	**1d**	49.7	90.6	6.80	379.3	378	**6d**	116.9
4a	**2a**	52.0	89.2	5.77	354.3	353	**5a**	116.9
4b	**2b**	51.0	88.6	4.07	339.3	338	**5b**	116.9
4c	**2c**	53.0	88.8	6.83	363.4	362	**5c**	116.8
4d	**2d**	51.5	88.7	5.65	379.4	378	**5d**	117.1

[a] Molecular weight calculated for a fully protonated acid form. [b] Negative FAB MS (M−H) ions.

Scheme 2



necessary for removal of *N*-benzoyl and *N*-isobutyryl base-protecting groups and 3′-*O*-acetyl and *O*-(*β*-cyanoethyl) functions. The yields and physicochemical characteristics of **2a**−**d** as well as ^{31}P NMR chemical shifts of intermediates **5a**−**d** are listed in Table 1.

A set of nucleoside 3′-*O*-phosphorodithioates **1a**−**d** have been prepared by basically the same synthetic approach starting from 3′-*O*-(2-thio-1,3,2-dithiaphospholane) derivatives of 5′-*O*-DMT-deoxyribonucleosides with acyl-protected reactive amino groups at nucleobases **3a**−**d** (Scheme 2). The presence of the acid-labile dimethoxytrityl group (DMT) and the reported instability of phosphorodithioates **1a** and **1c** in acidic media[9] prompted us to apply the modified deprotection procedure including the treatment of intermediate *O*-nucleoside *O*-(*β*-cyanoethyl)phosphorodithioates **6a**−**d** with 80% aqueous acetic acid prior to treatment with ammonia (Scheme 2). The yields and physicochemical characteristics of **1a**−**d** as well as ^{31}P NMR chemical shifts of intermediate **6a**−**d** are presented in Table 1.

The data listed in Table 1 reveal that the yields of isolated nucleoside phosphorodithioates **1a**−**d** and **2a**−**d** are in the range of 50−53%. This is somewhat lower than could be expected from the appearance of the ^{31}P NMR spectra of crude products, where only in the case of syntheses leading to **1a**−**d** some minor phosphoro-organic byproducts could be seen, most probably as a result of acid treatment of intermediates **6a**−**d**. It has to be concluded that the partial loss of products, both

Table 2. Anti-HIV-1 and -HIV-2 Activity and Cytotoxic Properties of Selected Nucleoside 3′-*O*- and 5′-*O*-Phosphorodithioates in Human T-Lymphocyte (CEM/O) Cells

	EC_{50}[a] (*μ*g/mL)		
compd	HIV-1	HIV-2	CC_{50}[b] (*μ*g/mL)
1b	>40	>40	84 ± 13
2a	>40	>40	54 ± 11
2b	>40	>40	100 ± 21
2d	>40	>40	19 ± 4.2

[a] 50% effective concentration or concentration required to protect CEM cell against the cytopathogenicity of HIV by 50%. [b] 50% cytotoxic concentration or concentration required to reduce CEM cell viability by 50%.

1a−**d** and **2a**−**d**, had to occur during final isolation on a Sephadex column, which is a lengthy procedure followed by evaporation of large quantities of aqueous eluate. Indeed, the phosphorodithioates **1a**−**d** and **2a**−**d** are quite unstable and decompose readily on storage at room temperature. Their high instability at neutral and especially at low pH was previously reported by other authors.[9]

The nucleoside 3′-*O*-phosphorodithioates **1a**−**d** were found to undergo alkylation at one of the sulfur atoms by treatment with 5′-bromo-5′-deoxyribonucleosides. The resulting dinucleoside (O3′→S5′) phosphorodithioates are the first examples of a new class of dinucleotide analogues possessing the internucleotide phosphorodithioate linkage with one of the sulfur atoms in a 5′-bridging position.[16]

The selected nucleoside phosphorodithioates (**1b**, **2a**,**b**,**d**) were tested for their potential antiviral and/or cytotoxic activity at the Rega Institute, Leuven. The results, which are listed in Table 2, clearly indicate that neither compound was active against HIV-1 or HIV-2 at subtoxic concentration. The observed lack of antiviral activity of **1** and **2** is in accordance with earlier studies by Caruthers *et al.*,[9] who found that none of the phosphorodithioates **1a**, **1c**, **2a**, or **2c** are inhibitors of HIV reverse transcriptase.

A procedure similar to that described above was performed with support-bound oligodeoxyribonucleotide T_{10} containing 2-thio-1,3,2-dithiaphospholane residue attached to the terminal 5′-OH group.[17] Its treatment with 3-hydroxypropionitrile/DBU followed by ammonia

(16) Okruszek, A.; Olesiak, M.; Stec, W. J. *Phosphorus, Sulfur Silicon* **1996**, *111*, 81.

(17) The introduction of dithiaphospholane residue was accomplished by treatment of support-bound decathymidine with (*N*,*N*-diisopropylamino)-1,3,2-dithiaphospholane in the presence of tetrazole followed by sulfurization with S-TETRA.[18]

(18) Stec, W. J.; Uznański, B.; Wilk, A.; Hirschbein, B. L.; Fearon, K. L.; Bergot, B. J. *Tetrahedron Lett.* **1993**, *34*, 5317.

deprotection allowed us to obtain the oligonucleotide T_{10} containing the 5′-terminal phosphorodithioate function. Further development of this method for 5′-labeling of oligonucleotides is in progress.

Experimental Section

Materials and Methods. The solvents were dried over calcium hydride and distilled before use. 3-Hydroxypropionitrile and DBU (Aldrich) were freshly distilled over calcium hydride. High-performance liquid chromatography (RP HPLC) was performed on a LDC/Milton Roy system using ODS Hypersil 5 μm, 4.7 × 300 mm column (Alltech). The elution system involved a linear gradient of acetonitrile (Baker) in 0.1 M triethylammonium bicarbonate (TEAB). UV spectra were recorded with a UV−vis 916 spectrometer (GBC). ^{31}P NMR spectra were taken on a Bruker AC 200 instrument operating at 81 MHz with broadband decoupling. Chemical shifts are given in ppm with respect to external 85% H_3PO_4. FAB mass spectra were recorded on a Finnigan MAT 95 instrument equipped with a 13 keV Cs^+ gun and a glycerine matrix.

Deoxyribonucleosides were purchased from Pharma-Waldhof. Base-protected (except thymidine) nucleoside 5′-*O*-DMT-3′-*O*-(2-thio-1,3,2-dithiaphospholane)s **2a**−**d** and nucleoside 3′-*O*-acetyl-5′-*O*-(2-thio-1,3,2-dithiaphospholane)s **4a**−**d** were prepared according to our previously published procedures.[10,11]

Nucleoside 3′-*O*-Phosphorodithioates (1a−d). 5′-*O*-DMT-base-protected (except thymidine) nucleoside 3′-*O*-(2-thio-1,3,2-dithiaphospholane) (**3a**, **b**, **c**, or **d**) (1 mmol) was dried overnight at high vacuum and dissolved under argon in 5 mL of dry acetonitrile. Into this solution was added 341 μL of 3-hydroxypropionitrile (355 mg, 5 mmol), with stirring at room temperature, by injection through a rubber septum, followed by 149 μL (152 mg, 1 mmol) of DBU. A small exothermic effect was observed, and the reaction mixture was stirred for 15 min at room temperature. At this point, the ^{31}P NMR control showed full disappearance of the substrate and quantitative formation of *O*-(β-cyanoethyl) phosphorodithioate diester (see Table 1). The solvent was evaporated, and the residue was dissolved in 15 mL of 80% aqueous acetic acid and left for 5 h at room temperature. The acid was then evaporated, and the residue was dissolved in 15 mL of 15% aqueous ammonia and left overnight at 55 °C (water bath) in a tightly closed vessel. The ammonia was evaporated, and the residue was dissolved in 0.01 M TEAB (20 mL), filtered, and applied on a top of a column filled with Sephadex A25 (4 × 25 cm). The column was eluted with a linear gradient of 0.01 and 0.5 M TEAB (pH 8.0, 1 L/1 L). The product-containing fractions (UV detection at 260 nm) were pooled and evaporated (bath temperature, 30 °C). The residue was evaporated twice with ethanol (2 × 50 mL).

Nucleoside 3′-*O*-phosphorodithioates **1a**−**d** (Et_3NH^+ salts) were obtained in the form of an amorphous white powder after drying at high vacuum. They were stable when stored at −80 °C under argon as checked by ^{31}P NMR and HPLC. The yields and physicochemical data of **1a**−**d** are given in Table 1.

Nucleoside 5′-*O*-Phosphorodithioates 2a−d. 3′-*O*-Acetyl-base-protected (except thymidine) nucleoside 5′-*O*-(2-thio-1,3,2-dithiaphospholane) (**4a**, **b**, **c**, or **d**) (1 mmol) was reacted with 3′-hydroxypropionitrile (5 mmol) and DBU (1 mmol) as described above for **3a**−**d**.

After complete disappearance of the substrate (^{31}P NMR control), 15 mL of 15% aqueous ammonia was added, and the solution was left overnight at 55 °C (water bath) in a tightly closed vessel. The products **2a**−**d** were isolated by ion-exchange chromatography as described above for **1a**−**d**.

The nucleoside 5′-*O*-phosphorodithioates (Et_3NH^+ salts) were obtained in the form of amorphous white powders after drying at high vacuum. They were stable when stored at −80 °C under argon as checked by ^{31}P NMR and HPLC. The yields and physicochemical characteristics are given in Table 1.

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