Synthesis of Oligo(deoxyribonucleoside phosphorodithioate)s by the Dithiaphospholane Approach†

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Received April 3, 1995®

A novel method of synthesis of oligo(deoxyribonucleoside phosphorodithioate)s (S_2-ODNs), based on the ring-opening condensation of nucleoside 3′-*O*-(2-thiono-1,3,2-dithiaphospholane)s with 5′-*O*-deprotected nucleosi(ti)des in the presence of a strong organic base such as DBU, is presented. The process has been adapted to the requirements of automated solid-phase oligonucleotide synthesis with a relatively short condensation step (5 min) and reasonable step-yield (>95%). *N*-Methylpyrrolidin-2-ylidenyl (Pya) was found to be the group of choice for the protection of reactive aminofunctions of nucleobases in nucleotide substrates. Sarcosine-containing linker (LCA CPG SAR) was employed due to its known resistance to cleavage by DBU. Several medium-size S_2-ODNs were prepared by this approach. Their identity and purity was confirmed by means of ^{31}P NMR, gel electrophoresis, and mass spectrometry. It has been demonstrated that, contrary to a recent report, S_2-ODNs are not degraded by DNaseI.

Introduction

Among several phosphate-modified analogues of oligonucleotides of special interest are oligo(deoxyribonucleoside phosphorodithioate)s (S_2-ODNs) of the general formula presented in Figure 1.

These analogues have both nonbridging phosphate oxygens replaced by sulfur, resulting in a 3′*O*-PS_2-*O*5′ phosphorodithioate bridge. Therefore, like natural oligomers, S_2-ODNs retain negative charge, are achiral at phosphorus, and have unaltered nucleoside composition. Earlier studies showed that S_2-ODNs form stable duplexes with complementary oligonucleotides,[1] and their hybrids with complementary mRNA are susceptible to RNaseH degradation.[1] According to some sources, S_2-ODNs are completely resistant to nuclease degradation,[1–3] although a recent report by Cohen *et al.* suggests they have limited stability in the presence of DNaseI.[4]

An important biochemical feature of S_2-ODNs is their inhibitory effect against viral reverse transcriptase, therefore hampering viral replication.[1,5] Further evaluation of S_2-ODNs as effective "antisense" agents against viral mRNA expression is in progress.[5] It is worthwhile to mention that S_2-ODNs show very little general toxicity to cells in culture.[6]

As is true for all potential therapeutics, the biological evaluation and promotion of S_2-ODNs depends upon their clean, efficient, and cost-effective manufacturing. Numerous efforts directed toward the synthesis of S_2-ODNs are summarized in a series of excellent reviews.[7–9] In this report, we present the new approach to the synthesis of S_2-ODNs.[10]

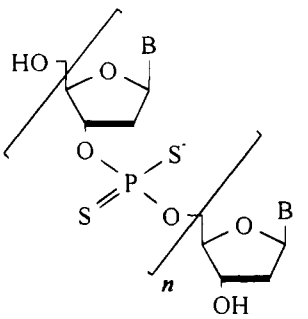

Figure 1. Schematic representation of oligo(deoxyribonucleoside phosphorodithioate) (S_2-ODN); n = number of repeating units; B = Ade, Gua, Cyt, Thy.

Results and Discussion

In the course of our studies on new methodology for the stereocontrolled synthesis of oligo(deoxyribonucleoside phosphorothioate)s (S-ODNs) with a predetermined sense of chirality at each phosphorothioate function, we have developed the oxathiaphospholane strategy, which relies upon the synthesis and separation of pure 5′-*O*-DMT-nucleoside 3′-*O*-(2-thiono-1,3,2-oxathiaphospholane)-s[11] into individual diastereomers. When these compounds are exposed to the appropriately protected 5′-OH

† Dedicated to Professor Tsujiaki Hata on the occasion of his 60th birthday.

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® Abstract published in *Advance ACS Abstracts,* September 15, 1995.

(1) Caruthers, M. H.; Beaton, G.; Cummins, L.; Dellinger, D.; Graff, D.; Ma, Yun-Xi; Marshall, W. S.; Sasmor, H.; Shankland, P.; VanWu, J.; Yau, E. K. *Nuclosides Nucleotides* **1991**, *10*, 47.

(2) Grandas, A.; Marshall, M. S.; Nielsen, J.; Caruthers, M. H. *Tetrahedron Lett.* **1989**, *30*, 543.

(3) Porritt, G. M.; Reese, C. B. *Tetrahedron Lett.* **1990**, *31*, 1319.

(4) Ghosh, M. K.; Gosh, K.; Dahl, O.; Cohen, J. S. *Nucleic Acids Res.* **1993**, *21*, 5761.

(5) Marshall, W. S.; Caruthers, M. H. *Science* **1993**, *259*, 1564.

(6) (a) Marshall, W. S.; Beaton, G.; Stein, C. A.; Matsukura, M.; Caruthers, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6265. (b) Tonkinson, J. L.; Guvakova, M.; Khaled, Z.; Lee, J.; Yakubov, L.; Marshall, W. S.; Caruthers, M. H.; Stein, C. A. *Antisense Res., Dev.* **1994**, *4*, 269.

(7) Dahl, O. *Sulfur Rep.* **1991**, *11*, 167.

(8) Beaton, G.; Dellinger, D.; Marshall, W. S.; Caruthers, M. H. In *Oligonucleotides and Analogues. A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford, 1991; pp 109–135.

(9) (a) Wiesler, W. T.; Marshall, W. S.; Caruthers, M. H. In *Methods in Molecular Biology: Oligonucleotide Synthesis*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; pp 191–206. (b) For the most recent paper see: Seeberger, P. H.; Caruthers, M. H. *Tetrahedron Lett.* **1995**, *36*, 695.

(10) For preliminary results see: Okruszek, A.; Sierzchała, A.; Sochacki, M.; Stec, W. J. *Tetrahedron Lett.* **1992**, 33, 7585.

(11) Stec, W. J.; Grajkowski, A.; Koziołkiewicz, M.; Uznański, B. *Nucleic Acids Res.* **1991**, *19*, 5883.

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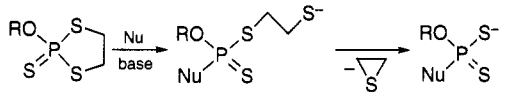
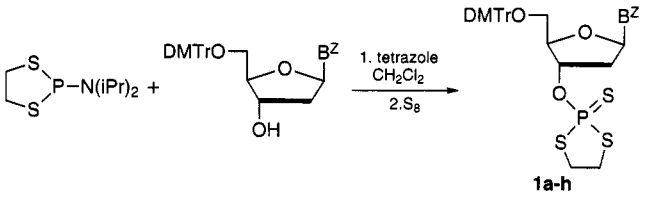
Scheme 1



Nu = OR, PPi

Scheme 2



1a: B = Thy
1b: B = Cyt, Z = Bz
1c: B = Cyt, Z = Pya
1d: B = Gua, Z = iBu
1e: B = Gua, Z = Pya
1f: B = Ade, Z = Bz
1g: B = Ade, Z = Bz_2
1h: B = Ade, Z = Pya

nucleosi(ti)de in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), they undergo oxathiaphospholane ring opening *via* chemoselective and stereospecific cleavage of the endocyclic PS bond, followed by elimination of ethylene sulfide and the formation of an internucleotide phosphorothioate linkage. This methodology was adapted to the requirements of solid-phase synthesis and allows the preparation of stereoregular oligo(nucleoside phosphorothioate)s with >98% stereospecificity for each coupling. The average coupling yield, measured by DMT-trityl cation assay, is higher than 95%. Preparative yields of S-ODN constructs depend on the oligonucleotide length and sequence.

As a natural extension of the aformentioned chemistry, we have adapted this methodology to the synthesis of S_2-ODNs by replacing nucleoside 3′-*O*-(2-thiono-1,3,2-oxathiaphospholane)s with corresponding nucleoside 3′-*O*-(2-thiono-1,3,2-dithiaphospholane)s.[10] In preliminary experiments we found that 2-*n*-butoxy-2-thiono-1,3,2-dithiaphospholane reacts with *n*-butanol in the presence of base catalysts, such as potassium *tert*-butoxide or tertiary amines [Et_3N, *N*-methylimidazole, 4-(dimethylamino)pyridine (DMAP), DBU], to give *O,O*-di-*n*-butyl-phosphorodithioate in 95% yield. Similarly, we have found that appropriately protected nucleoside 5′-*O*-(2-thiono-1,3,2-dithiaphospholane)s react with inorganic pyrophosphate under basic (DBU) catalysis to give nucleoside 5′-*O*-1,1-dithiotriphosphates.[12] These model reactions showed that 2-alkoxy-2-thiono-1,3,2-dithiaphospholanes can react with nucleophiles under basic catalysis to form a phosphorodithioate linkage (Scheme 1).

In order to evaluate this chemistry as a basis for a new method of formation of internucleotide phosphorodithioate bonds, we have synthesized base-protected (except thymine) 5′-*O*-DMT-deoxyribonucleoside 3′-*O*-(2-thiono-1,3,2-dithiaphospholane)s (**1a**−**h**) by reaction of the appropriate 3′-OH nucleoside with 2-(*N,N*-diisopropylamino)-1,3,2-dithiaphospholane[12] in the presence of 1*H*-tetrazole followed by oxidation with elemental sulfur (Scheme 2).

In addition to the standard base-protecting groups [benzoyl (Bz) for N^4 of cytosine and N^6 of adenine, isobutyryl (iBu) for N^2 of guanine], the *N*-methylpyrrolidin-2-ylidenyl group (Pya)[13] was applied to the aforementioned reactive amino groups in order to remove potentially reactive amide protons. In the case of adenine, a similar effect was achieved by introducing two benzoyl groups (Bz_2) at N^6. The compounds **1a-h** were purified by column chromatography on silica gel and were characterized by TLC, ^1H and ^{31}P NMR spectroscopy, mass spectrometry, and elemental analysis. Their ^{31}P NMR chemical shift values (124−125 ppm; CD_3CN) correlate well with those reported for 2-phenoxy-[14] (120.7 ppm; $CHCl_3$) and 2-β-naphthoxy-2-thiono-1,3,2-dithiaphospholane (118.74 ppm; C_6D_6).[15] They are stable for several months when stored as lyophilized powders at −20 °C in tightly closed vessels and are very soluble in solvents such as methylene chloride or acetonitrile.

The ability of **1** to form an internucleotide phosphorodithioate bond was first checked in solution using derivatives of thymidine. **1a** (0.3 mmol) was allowed to react in anhydrous CH_3CN with a 10% molar excess of 3′-*O*-acetylthymidine in the presence of an equimolar amount (0.3 mmol) of base catalyst. Of the organic bases mentioned above, DBU gave (by ^{31}P NMR) the fastest rate of reaction and the least byproducts. After removal of protecting groups by sequential treatment with 80% aqueous acetic acid and 25% aqueous ammonia, the product was isolated in 47% yield by ion exchange chromatography on DEAE Sephadex A-25 and identified as $T_{PS2}T$ (**2a**) by negative LSIMS mass spectrometry [found *m/z* 577.0 (M-H); calcd monoisotopic mass 578.09] and ^{31}P NMR chemical shift value (δ 113.8, D_2O).[7-9] The identity of **2a** was further confirmed by its quantitative conversion to thymidylyl(5′ → 3′)thymidine (T_PT) by treatment with butylene 1,2-oxide under conditions elaborated in our previous studies.[16] The oxidized product was identified by HPLC comparison with an authentic sample of T_PT prepared independently using standard phosphoramidite procedures.

Further studies of internucleotide phosphorodithioate bond formation with **1a-h** as the reactive precursors were performed using appropriately protected nucleosides bound to the standard solid support LCA CPG. The reactions were performed manually on a 1 μmol scale using syringes, standard DNA synthesis columns (ABI), and 30 μmol of **1** and 30 μmol of DBU. The manual synthesis protocol is presented as a supporting information.

After detritylation, cleavage from the support, and base deprotection with aqueous ammonia, the products (**2a-d**) were isolated by preparative HPLC. The $T_{PS2}T$ (**2a**) obtained using the solid phase method was identical to that prepared in solution by chromatographic (HPLC) and spectroscopic (^{31}P NMR, mass spectrometry) analyses. The structure of **2a**−**d** was confirmed by negative LSIMS mass spectrometry; for each compound the expected [M − H] ion was observed. The identity of **2b**−**d** was additionally confirmed by their conversion to the corresponding dinucleoside phosphates by treatment with butylene 1,2-oxide[16] and HPLC comparison of the products with authentic samples prepared using standard phosphoramidite chemistry. The role of the base protecting groups on the efficiency of coupling was determined by reacting several combinations of substrates **1a**−**h** with solid support bound nucleosides possessing different base protections (Scheme 3). The first base was attached to

(12) Okruszek, A.; Olesiak, M.; Balzarini, J. *J. Med. Chem.* **1994**, *37*, 3850.
(13) McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040.
(14) Revel, M.; Navech, J. *J. Bull. Soc. Chim. Fr.* **1973**, 1195.
(15) Błaszczyk, J.; Wieczorek, M. W.; Okruszek, A.; Olesiak, M.; Karwowski, B. *Heteroatom. Chem.* **1994**, *5*, 519.
(16) Okruszek, A.; Guga, P.; Stec, W. J. Unpublished results. Guga, P. Ph.D. *Thesis*, Łódź, 1991.

Scheme 3

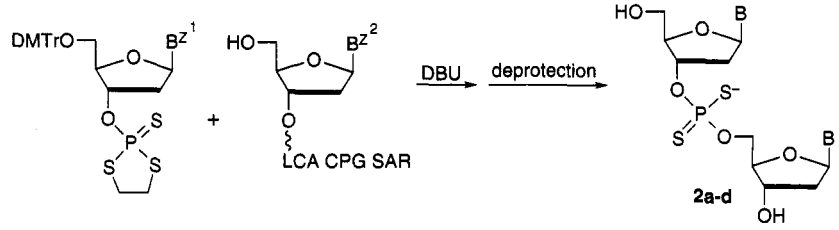


Table 1. Yields and Physicochemical Characteristics of Dinucleoside Phosphorodithioates 2a-d Synthesized According to Scheme 3

entry	compd	Z^1	Z^2	yield[a] (%)	HPLC[b] t_R (min)	molecular wt (Da) calcd[c]	molecular wt (Da) measured[d]
1	$T_{PS2}T$ (**2a**)			97.0	16.49	578.09	577.0
2		Bz	Bz	84.0			
3	$C_{PS2}C$ (**2b**)	Pya	Bz	98.0	11.86	548.08	547.1
4		Pya	Pya	98.6			
5	$G_{PS2}G$ (**2c**)	iBu	iBu	56.0	11.83	628.10	627.0
6		Pya	Pya	98.9			
7		Bz	Bz	68.0			
8		Bz	Bz_2	78.6			
9	$A_{PS2}A$ (**2d**)	Bz_2	Bz	73.0	14.80	596.11	595.0
10		Bz_2	Bz_2	86.5			
11		Pya	Pya	98.7			

[a] Calculated from integrated HPLC plot. [b] ODS Hypersil, 1.5 mL/min, 0–20% CH_3CN in 0.1 M TEAB (linear) in 20 min. [c] Monoisotopic mass corresponding to the neutral molecule with a hydrogen bonded to sulfur in the phosphorodithioate linkage. [d] Negative LSIMS mass spectrometry.

the CPG through a sarcosine-containing linker (LCA CPG SAR) which, according to the literature[17], does not decompose in the presence of DBU. The yields of di(nucleoside phosphorodithioate)s **2a–d** and their chromatographic characteristics are listed in Table 1.

An inspection of Table 1 clearly shows that the efficiency of internucleotide phosphorodithioate bond formation strongly depends upon full protection of the nucleobase's (Ade, Cyt, Gua) reactive amido groups in both reacting components. The best results were obtained when cyclic amidine groups (Pya)[13] were present in both reagents. Presumably, in the presence of DBU, free NH protons at sites with *N*-benzoyl or *N*-isobutyryl protection may lead to undesired side reactions. The introduction of a second benzoyl group at N^6 of adenine does not give sufficient protection, most likely due to its lability in the presence of an excess of DBU in the reaction medium.

The tedious procedure of manual synthesis promted us to adapt the synthetic protocol to an automated DNA synthesizer (ABI 391). The optimized protocol for automated synthesis of oligo(nucleoside phosphorodithioate)s is presented in Table 2.

The major difference between the automated and manual method is the mode of addition of the reagents into the reaction column containing the growing oligonucleotide bound to the solid support. In the automated protocol the solutions of dithiaphospholane and DBU are less concentrated and are added to the column sequentially, in small portions, followed by a 5 min wait (Table 2, entry 3). In the manual method, the reagents are premixed, introduced into the column in one portion, and are more concentrated. The protocol presented in Table 2 was employed for the automated synthesis of several

Table 2. Protocol for Automated Synthesis of Oligo(nucleoside phosphorodithioate)s by the Dithiaphospholane Approach (DNA Synthesizer ABI-391)

entry	step (reagent or solvent)	volume (mL)	time (s)
1	detritylation (3.5% DCA in CH_2Cl_2)	2.4	60
2	wash (CH_3CN)	2.5	50
3	condensation [0.1 M solution of dithiaphospholane **1** in CH_3CN (0.27 mL) + 0.5 M solution of DBU in CH_3CN (0.54 mL)]	0.81	300
4	wash (1% pyridine in CH_2Cl_2)	9.6	200
5	wash (CH_3CN)	2.5	50
6	capping [Ac_2O/2,6-lutidine/THF 1:1:8 (0.48 mL) + 0.25 M DMAP in THF (0.48 mL)]	0.96	200
7	wash (CH_3CN)	1.5	30

oligo(nucleoside phosphorodithioate)s (**3a–o**) with different sequences and lengths ranging from 8 to 20 bases. The results are presented in Table 3.

The syntheses were performed on a 1 μmol scale with all bases (except thymine) protected with amidine (Pya) groups, including the support-bound nucleosides. The crude product was cleaved from the support by overnight treatment with concentrated aqueous ammonia. For some sequences, especially those containing several dC residues, additional washing of the column with acetonitrile was necessary to ensure complete elution of the oligonucleotide. The removal of the amidine (Pya) groups was routinely accomplished by treatment of the crude product with concentrated aqueous ammonia containing 10% (w/v) ammonium acetate (NH_4OAc) for 48 h at 55 °C in a tightly capped tube. The addition of NH_4OAc is only necessary for sequences containing Pya-protected adenine or guanine residues.[13] The crude oligonucleotides containing a 5′-*O*-dimethoxytrityl protecting group were purified by RP HPLC, a method which easily separates full-length product from "acetyl capped" shorter oligomers which do not contain a 5′-*O*-DMT group. The dimethoxytrityl group was removed from the purified oligonucleotide by treatment with aqueous acetic acid. The product was then subjected to a final RP HPLC purification and, after evaporation, was lyophilized and stored at −20 °C under argon. The yields of the oligo(nucleoside phosphorodithioate)s were measured by UV spectroscopy at 260 nm and for the synthesized sequences (Table 3) were found to be in the range of 3–32%. These yields are somewhat lower than was expected from the "trityl-cation yields"[19] measured for each synthetic step, which were found to be within the 95–99% range for the syntheses summarized in Table 3.

The phosphorodithioate structure of the oligonucleotides both DMT-ON and DMT-OFF was confirmed by ^{31}P NMR. Two typical spectra are presented in Figure 2 which show the chemical shifts for the major products to be around δ 113–115,[7–9] with only minor (*ca.* 2–3%) phosphorothioate contamination (δ 56–57).

(17) Brown, T.; Pritchard, C. E.; Turner, G.; Salisbury, S. A. *J. Chem. Soc., Chem. Commun.* **1989**, 891.

(18) *CRC Handbook of Chemistry and Physics*, 71st ed.; Lide, D. R., Ed.; CRC Press: Boca Raton, FL, 1990; pp 1–7.

(19) Atkinson, T.; Smith, M. In *Oligonucleotide Synthesis. A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984; pp 35–81.

Table 3. S_2-ODNs Synthesized by the Dithiaphospholane Route

entry	S_2-ODN 3	base sequence	avg trityl cation yield (%)	total yield of HPLC purified product		molecular wt (Da)	
				OD_{260}[a]	%	calcd[c]	measured[b]
1	**3a**	5′CCC CCC CCC CCC CCC CCC C3′	97.4	23.5	13.3	6010.7	6008 ± 2.4
2	**3b**	AA GGG CTT CTT CCT TA	97.2	5.9	3.2		e
3	**3c**	CTC CAT GGT GCT CAC	98.3	17.2	10.3	4953.8	4952 ± 2.0
4	**3d**	CCC TGC TCC CCC CTG CCT CC	98.2	26.6	13.4	6512.1	6510 ± 2.6
5	**3e**	CGC GAA TTC GCG	99.1	20.5	14.3	3999.7	4000 ± 1.6
6	**3f**	AAA AAA AAA AAA	96.9	13.3	7.3	4049.9	4050 ± 1.6
7	**3g**	TTT TTT TTT TTT	99.2	39.0	31.8	3941.7	3941 ± 1.6
8	**3h**	CGC GCG CG	99.8	23.3	25.4	2636.4	2636 ± 1.1
9	**3i**	GCG CGC GC	98.1	15.8	17.2	2636.45[d]	5274 ± 2.1
10	**3j**	TAC GCC AAC AGC TCC	96.9	10.6	7.7	4931.7	4932 ± 2.0
11	**3k**	CCT ATA ATC C	96.6	4.2	4.6		e
12	**3l**	AAC GTT GAG GGG CAT	95.3	6.2	4.0	5106.8	5108 ± 2.0
13	**3m**	TGA ACA CGC CAT GTC	e	5.8	4.1		e
14	**3n**	CTG TAC CGC ACA AGT	e	8.5	5.9		e
15	**3o**	GGG GAG GGG GAG G	e	4.0	3.0		e

[a] Number of optical density (OD_{260}) units[32] of oligonucleotide obtained from the synthesis performed on a 1 μmol scale. [b] Negative electrospray ionization mass spectrometry. [c] The molecular weight corresponding to the neutral molecule with a hydrogen bonded to each sulfur in all the phosphorodithioate linkages was calculated using the average atomic masses teken from ref 18. The Table with subunit masses of phosphorodithioate nucleotides appears in the supporting information. [d] For the dimer molecular weight 5272.9 Da was calculated. [e] Not measured.

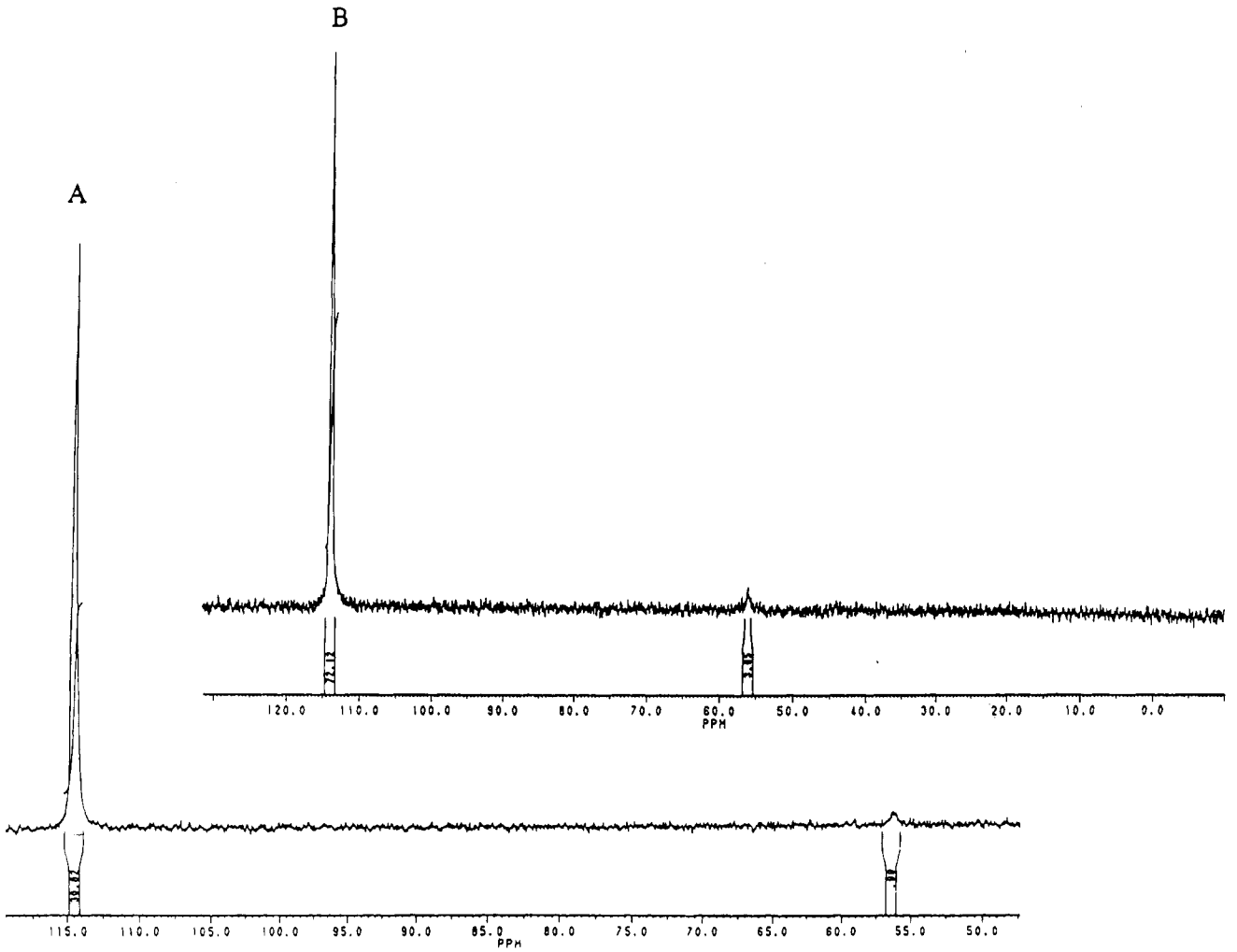


Figure 2. ^{31}P NMR spectra of oligo(nucleoside phosphorodithioate)s prepared on an automated DNA synthesizer: A, **3c** (D_2O, after DMT-ON purification); B, **3d** (D_2O, after DMT-OFF purification).

The purity of the products with regard to length homogeneity was checked by polyacrylamide gel electrophoresis (PAGE) under denaturating conditions. The oligo(nucleoside phosphorodithioate)s (**3a**–**o**) show somewhat lower PAGE mobility relative to phosphorothioate or phosphodiester oligomers of the same length and sequences. This phenomenon has been previously observed by other authors.[8,9,20] The phosphorodithioate bands on PAGE gels can be observed by UV-shadowing as described previously;[8,9,20] however, they also can be conveniently visualized with Stains-all to show violet bands, similar to those observed for S-ODNs. The stain-

(20) Bjergarde, K.; Dahl, O. *Nucleic Acids Res.* **1991**, *19*, 5843.

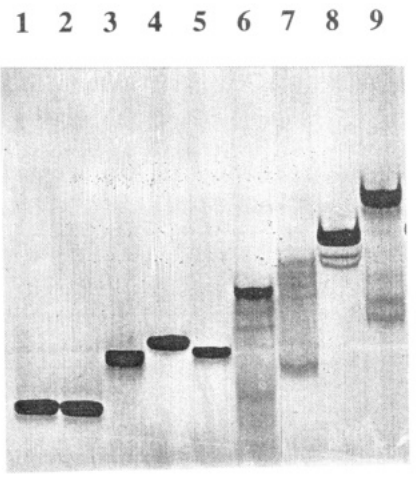


Figure 3. Denaturating polyacrylamide gel electrophoresis (PAGE) of HPLC-purified oligo(nucleoside phosphoroditioate)s **3** prepared by the automated dithiaphospholane approach (visualized by Stains-all). Lane 1: **3h**. Lane 2: **3i**. Lane 3: **3e**. Lane 4: **3g**. Lane 5: **3f**. Lane 6: **3c**. Lane 7: **3b**. Lane 8: **3a**. Lane 9: **3d**.

ed gel can be easily photographed and the bands can be quantified by laser scanning densitometry. The PAGE analysis of some of the oligo(nucleoside phosphorodithioate)s described in Table 3 is presented in Figure 3.

The PAGE analysis shows that, despite the double HPLC purification, longer oligomers (≥15) contain some impurities of shorter length. In one case (**3b**) no distinct product band could be found. Oligo(nucleoside phosphorodithioate)s can also be detected by autoradiography of the gel after their 5′-labeling with γ-^{32}P ATP and T4 polynucleotide kinase. The incorporation of [^{32}P], despite higher enzyme concentration and longer reaction time, was considerably lower than for the corresponding phosphate or phosphorothioate oligomers (see also Cohen *et al.*[4]).

The identity of S_2-ODNs synthesized by the dithiaphospholane method was confirmed by negative electrospray ionization mass spectrometry (ESI-MS) after their conversion into ammonium salts. The calculated and measured molecular weight values are listed in Table 3. In most cases, in addition to the molecular ion M, peaks corresponding to M − 16 and M − 32 were also observed in the ESI-MS, confirming the presence of some quantities of oligomers with one or two phosphorothioate linkages within the oligonucleotide chain, as previously seen in ^{31}P NMR spectra (see Figure 2). The presence of M, M − 16, M − 32, and M − 48 peaks is illustrated in the transformed ESI-MS spectrum of **3a** (Figure 4). For this sample, the peak corresponding to all phosphorodithioate products (6008 Da) accounts for *ca.* 74% of the aforementioned molecular ion family and corresponds to the presence of *ca.* 1.5% phosphoromonothioate contamination for each coupling-step.

In the case of self-complementary octamer **3i** (Table 3, entry 9) the mass corresponding to the duplex was observed (5274 ± 2.1 Da). The presence of Watson−Crick-type duplexes in ESI-MS spectra have been recently described by Bayer *et al.*[21] No duplex, however, was observed by this technique for isomeric (and also self-complementary) octamer **3h** (Table 3, entry 8) or self-complementary dodecamer **3e** (Table 3, entry 5).

The availability of highly purified preparations of S_2-ODNs prompted us to check the somewhat unexpected observation of Cohen *et al.*[4] on the high susceptibility of S_2-ODNs to hydrolysis in the presence of DNase I. Thus, each of three S_2-ODNs (**3d**, **3f**, and **3g**) after 5′-end labeling with γ-^{32}P ATP in the presence of T4 polynucleotide kinase was subjected to incubation with 250 units of DNase I in buffer identical to that employed by Cohen's group. After 2 h incubation at 37 °C no traces of hydrolysis were revealed by PAGE followed by autoradiography. Similarly, no hydrolysis was observed when oligo(nucleoside phosphorothioate)s (S-ODNs) with sequences identical to **3f** and **3g** were subjected to incubation with DNase I under identical conditions. Each of the S-ODNs was used as a stereorandom mixture of diastereomers due to the chirality of the internucleotide phosphorus atom (Mix) and as single diastereomers (all-R_P, all-S_P).[11] On the other hand, under the same experimental conditions, the phosphodiester analogues with the same base sequences as **3d**, **3f**, and **3g** were completely hydrolyzed within 30 min. The PAGE analyses of the products of incubation of a series of **3f** analogues with DNase I are presented in Figure 5.

The two aforementioned S_2-ODNs, **3f** and **3g**, were also checked for their ability to form stable duplexes with complementary oligodeoxyribonucleotides. The melting temperature (T_m) for a 2.4 μM solution of **3f/T$_{12}$-oxo** was found to be 27 °C, whereas for the **3g/(dA)$_{12}$-oxo** system only the upper part of the melting curve could be identified within the 2−80 °C temperature range employed (Figure 6).

Conclusions

Despite the fact that the first synthesis of oligo(nucleoside phosphorodithioate)s was published in 1988,[22] only a few laboratories have reported results on the biophysical and biological properties of these compounds. One possible reason may be the limited availability of these oligonucleotide analogues. In this report we present a novel method for the synthesis of S_2-ODNs, based upon the ring-opening condensation of nucleoside 3′-*O*-(2-thiono-1,3,2-dithiaphospholane)s with 5′-*O*-deprotected nucleosi(ti)des. This process requires the presence of a strong organic base such as DBU. The coupling time is relatively short (5 min), and the step-yield usually exceeds 95%. By automating this process, several medium-size S_2-ODNs were prepared, and their identity and purity was confirmed by ^{31}P NMR, gel electrophoresis, and mass spectrometry. Contrary to a recent report,[4] we have demonstrated that S_2-ODNs are not degraded by DNase I. It is worth mentioning that, following our preliminary paper,[10] Martin *et al.* successfully adapted the dithiaphospholane method to the synthesis of phosphorodithioate analogues of phospholipids.[23]

Experimental Section

Materials and Methods. The solvents were dried over calcium hydride and distilled before use. All reactions involving trivalent phosphorus compounds were performed under dry argon. Column chromatography and thin layer chromatography (TLC) were performed on 230−400 mesh silica gel and silica gel F254 plates, respectively (Merck). Chloroform−methanol (19:1, v/v) was used as the TLC developing system. Reversed-phase high performance liquid chromatography (RP HPLC) was performed on a LDC/Milton Roy system using an ODS Hypersil 5 μm, 4.7 × 300 mm column (Alltech) or a 7.0 × 305 mm Hamilton PRP-1 column. The elution system

(21) Bayer, E.; Bauer, T.; Schmeer, K.; Biecher, K.; Maier, M.; Gaus, H.-J. *Anal. Chem.* **1994**, *66*, 3858.

(22) Nielsen, J.; Brill, W. K.-D.; Caruthers, M. H. *Tetrahedron Lett.* **1988**, 29, 5517.

(23) Martin, S. F.; Wagman, A. S. *J. Org. Chem.* **1993**, 58, 5897.

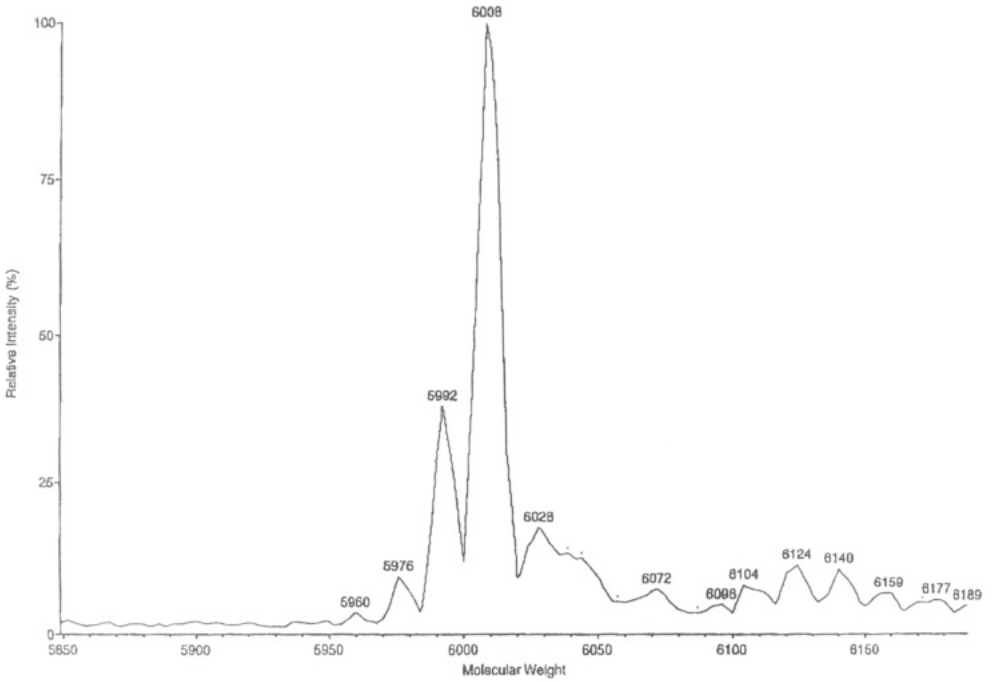


Figure 4. Electrospray ionization mass spectrum of **3a** after transformation by the Reconstruct program: 6008 (M, 73.8%), 5992 (M − 16, 22.3%), 5976 (M − 32, 3.4%), 5960 (M − 48, 0.5%).

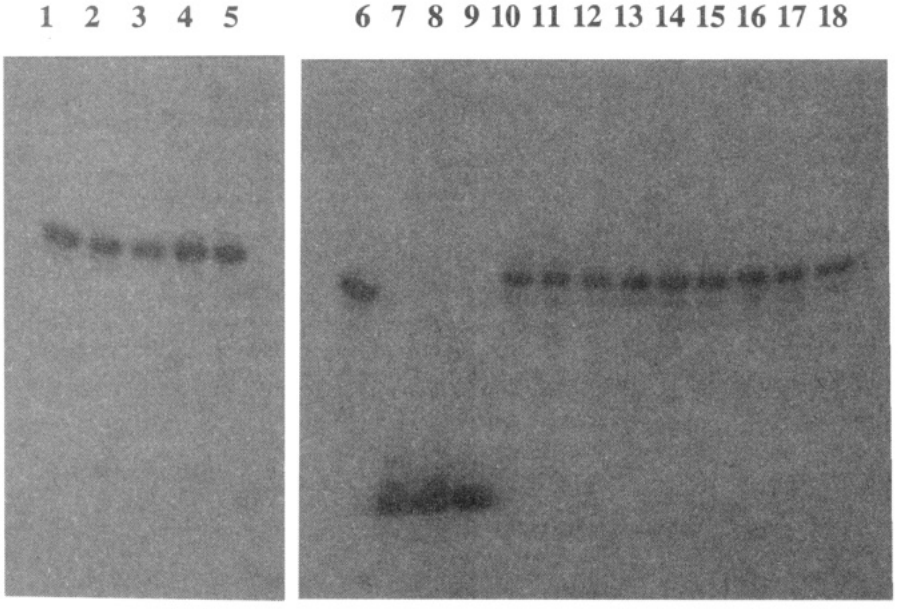


Figure 5. Autoradiogram of PAGE analysis of products of digestion of oligonucleotides of the sequence $(dA)_{12}$ with DNase I (250 units unless otherwise stated). Lane 1: **3f**, no enzyme added. Lane 2: **3f**, 50 units of DNase I, 30 min. Lane 3: **3f**, 50 units of DNase I, 2 h. Lane 4: **3f**, 30 min. Lane 5: **3f**, 2 h. Lane 6: **$(dA)_{12}$-oxo**, no enzyme added. Lane 7: **$(dA)_{12}$-oxo**, 15 min. Lane 8: **$(dA)_{12}$-oxo**, 30 min. Lane 9: **$(dA)_{12}$-oxo**, 2 h. Lane 10: **$(dA)_{12}$-thio** (Mix), no enzyme added. Lane 11: **$(dA)_{12}$-thio** (Mix), 30 min. Lane 12: **$(dA)_{12}$-thio** (Mix), 2 h. Lane 13: **$(dA)_{12}$-thio** (all-R_P), no enzyme added. Lane 14: **$(dA)_{12}$-thio** (all-R_P), 30 min. Lane 15: **$(dA)_{12}$-thio** (all-R_P), 2 h. Lane 16: **$(dA)_{12}$-thio** (all-S_P), no enzyme added. Lane 17: **$(dA)_{12}$-thio** (all-S_P), 30 min. Lane 18: **$(dA)_{12}$-thio** (all-S_P), 2 h.

involved a linear gradient of acetonitrile in 0.1 M triethylammonium bicarbonate (TEAB). Polyacrylamide gel electrophoresis (PAGE) was performed on 1 mm thick 20% polyacrylamide slab gels run under denaturating conditions (5 M urea) in 0.1 M Tris-borate (TBE) buffer (pH 8.0) containing 2.5 mM EDTA. The oligonucleotide products were visualized with Stains-all dye (Fluka) according to the manufacturer's protocol. UV spectra were recorded with a Uvikon 860 spectrometer (Kontron) and/or with a UV−vis 916 spectrometer (GBC). The thermal melting experiments (T_m) were performed at 260 nm with the latter instrument equipped with a Thermocell device in 10 mM cacodylate buffer (pH 7.4) containing 70 mM NaCl and 10 mM $MgCl_2$. The temperature was changed at a rate of 0.3 °C/min over a 2−80 °C range. ^1H NMR spectra were recorded in $CDCl_3$ with a Nicolet spectrometer operating at 360 MHz. ^{31}P NMR spectra were taken on a Bruker AC 200 instrument operating at 81 MHz (with broad band decoupling). Chemical shifts are given in ppm with respect to TMS (internal) for ^1H and 85% H_3PO_4 (external) for ^{31}P and are positive when downfield from the standard. LSIMS mass spectra were recorded on a Finnigan MAT 95 instrument equipped with a 13 keV Cs^+ gun, with glycerine matrix. The high-resolution fast atom bombardment mass spectra [HRMS (FAB)] were obtained on a VG ZAB-VSE double focusing spectrometer equipped with a 35 keV Cs^+ gun. Electrospray ionization mass spectrometry was performed on a PE-Sciex API I single quadrupole instrument. The solutions of S_2-ODNs in the ammonium ion form were infused at 4−5 μL/min into the ionspray interface of the spectrometer. The ionspray voltage was −3400 V, and the orifice voltage was set to −50

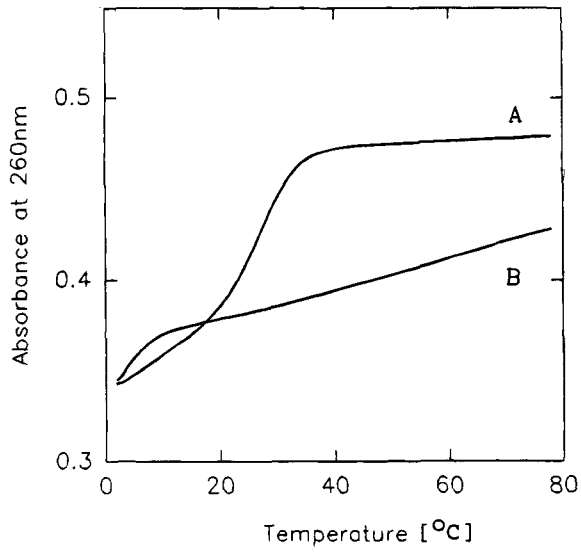


Figure 6. UV melting curves of oligo(nucleoside phosphorodithioate)s: (A) **3f/T_{12}-oxo**; (B) **3g/$(dA)_{12}$-oxo**.

V. Data were acquired in the multichannel averaging mode with 10 scans summed per spectrum. Raw data displaying multiple charges in the mass-to-charge dimension were transformed to a simple mass scale with the Reconstruct program. The masses were calculated from the *m/z* data using the Hypermass program, and the standard deviation was ±0.04%. Oligo(nucleoside phosphorodithioate)s were prepared on an Applied Biosystems (ABI) 391 DNA synthesizer using standard ABI columns. The same instrument was employed for the stereocontrolled synthesis of (all-R_P) and (all-S_P) S-ODNs using oxathiaphospholane chemistry.[11] Oligonucleotides containing normal phosphate linkages and/or S-ODNs prepared as a mixture of diastereomers were obtained on an ABI 380B DNA synthesizer using standard phosphoramidite chemistry.[24] For the S-ODN syntheses, a CH_3CN solution of bis(*O,O*-diisopropoxy phosphinothioyl)disulfide was used as the sulfurization agent.[25]

Deoxyribonucleosides were purchased from Pharma Waldhof. 1*H*-Tetrazole, 2,6-lutidine, potassium *tert*-butoxide, Et_3N, DMAP, DBU, and *N*-methylimidazole were obtained from Aldrich. *N*-Methylpyrrolidone was purchased from Merck. *N*-Methyl-2,2-diethoxypyrrolidine was prepared according to the published procedure.[26] Dichloroacetic acid (DCA) was obtained from Merck and freshly distilled before use. Di-*p*-methoxytrityl chloride (DMT Cl) was purchased from Janssen. Acetonitrile was obtained from Baker. Long-chain alkylamine controlled pore glass 500 Å (LCA CPG) and 3′-*O*-acetylthymidine were obtained from Sigma. Fmoc-sarcosine were purchased from Bachem.

2-Chloro-1,3,2-dithiaphospholane and 2-(*N,N*-diisopropylamino)-1,3,2-dithiaphospholane were prepared according to our previously published procedure.[12] N^4-Benzoyl-2′-deoxycytidine, N^2-isobutyryl-2′-deoxyguanosine, N^6-benzoyl-2′-deoxyadenosine, and N^6-dibenzoyl-2′-deoxyadenosine were prepared by the transient protection method.[27] The *N*-protected nucleosides and thymidine were 5′-*O*-dimethoxytritylated with DMT-Cl according to the procedure described by Khorana et al.[28] 5′-*O*-DMT-N^4-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxycytidine, 5′-*O*-DMT-N^6-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxyadenosine, and 5′-*O*-DMT-N^2-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxyguanosine were prepared as described by Caruthers *et al.*[13]

The 5′-*O*-DMT-2′-deoxyribonucleosides bearing the aforementioned *N*-protecting groups were succinylated by the standard procedure[29] and attached to sarcosinylated LCA CPG as described by Brown *et al.*[17] The following loadings of derivatized nucleoside were achieved (μmol/g): T, 31.9; N^4-Bz-dC, 34.0; N^4-Pya-dC, 48.0; N^2-iBu-dG, 30.9; N^2-Pya-dG, 23.8; N^6-Bz-dA, 34.8; N^6-Bz_2-dA, 25.1; and N^6-Pya-dA, 53.0.

Nucleoside 3′-*O*-(2-Thiono-1,3,2-dithiaphospholanes) 1a–h. Protected nucleoside [5′-*O*-DMT-thymidine, 5′-*O*-DMT-N^4-Bz-2′-deoxycytidine, 5′-*O*-DMT-N^4-Pya-2′-deoxycytidine, 5′-*O*-DMT-N^2-iBu-2′-deoxyguanosine, 5′-*O*-DMT-N^2-Pya-2′-deoxyguanosine, 5′-*O*-DMT-N^6-Bz-2′-deoxyadenosine, 5′-*O*-DMT-N^6-Bz_2-2′-deoxyadenosine, or 5′-*O*-DMT-N^6-Pya-2′-deoxyadenosine] (5.0 mmol) was mixed with tetrazole (0.37 g, 5.2 mmol), dried for 12 h under high vacuum, and dissolved in methylene chloride freshly distilled from CaH_2 (20 mL). Into the resulting solution stirred at room temperature was added by injection through a rubber septum 1.16 g (5.2 mmol) of 2-(*N,N*-diisopropylamino)-1,3,2-dithiaphospholane. Stirring at room temperature was continued until the TLC showed full disappearance of nucleoside substrate (*ca.* 1 h). Elemental sulfur (0.3 g), freshly dried under vacuum, was then added, and the mixture was stirred overnight at room temperature. The precipitate was filtered and washed with CH_2Cl_2 (5 mL) and the resulting filtrate evaporated. The residue was chromatographed (3 × 15 cm column) with chloroform/benzene 1:1 (0.5 L) as eluent, followed by a linear gradient of methanol in chloroform (0–3% v/v, 1 L) containing 0.1% of pyridine to give **1a–h** in the form of amorphous powders. When dried on a vacuum line and tightly closed under argon, the compounds **1a–h** do not decompose on storage at −20 °C for several months (TLC and ^{31}P NMR analysis). Their identity and purity was confirmed by high-resolution mass spectrometry, ^1H ($CDCl_3$) and ^{31}P NMR (CD_3CN), and elemental analysis.[30]

1a: yield 95%; TLC R_f 0.34; ^{31}P NMR δ 124.74; HRMS(FAB) calcd for $C_{33}H_{35}N_2O_7PS_3$ (M + Cs^+) 831.0398, found 831.0436. Anal. Calcd: H, 5.05; N, 4.01; P, 4.43; S, 13.77. Found: H, 5.26; N, 3.94; P, 4.25; S, 13.39.

1b: yield 80%; TLC R_f 0.32; ^{31}P NMR δ 124.70; HRMS(FAB) calcd for $C_{39}H_{38}N_3O_7PS_3$ (M + Cs^+) 920.0664, found 920.0698. Anal. Calcd: H, 4.86; N, 5.33; P, 3.93; S, 12.21. Found: H, 5.00; N, 5.57; P, 3.96; S, 12.01.

1c: yield 85%; TLC R_f 0.18; ^{31}P NMR δ 124.49; HRMS(FAB) calcd for $C_{37}H_{41}N_4O_6PS_3$ (M + Cs^+) 897.0980, found 897.1015. Anal. Calcd: H, 5.40; N, 7.32; P, 4.05; S, 12.58. Found: H, 5.59; N, 7.12; P, 4.04; S, 12.59.

1d: yield 74%; TLC R_f 0.17; ^{31}P NMR δ 124.96; HRMS(FAB) calcd for $C_{37}H_{40}N_5O_7PS_3$ (M + Cs^+) 926.0882, found 926.0922. Anal. Calcd: H, 5.08; N, 8.82; P, 3.90; S, 12.12. Found: H, 5.12; N, 8.63; P, 3.50; S, 11.80.

1e: yield 79%; TLC R_f 0.11; ^{31}P NMR δ 124.19; HRMS(FAB) calcd for $C_{38}H_{41}N_6O_6PS_3$ (M + Cs^+) 937.1042, found 937.1080. Anal. Calcd: H, 5.13; N, 10.44; P, 3.85; S, 11.95. Found: H, 5.21; N, 10.13; P, 3.97; S, 11.90.

1f: yield 50%; TLC R_f 0.37; ^{31}P NMR δ 124.36; HRMS(FAB) calcd for $C_{40}H_{38}N_5O_6PS_3$ (M + Cs^+) 944.0776, found 944.0819. Anal. Calcd: H, 4.71; N, 8.62; P, 3.81; S, 11.85. Found: H, 4.60; N, 8.32; P, 3.97; S, 11.96.

1g: yield 56%; TLC R_f 0.63; ^{31}P NMR δ 124.48; HRMS(FAB) calcd for $C_{47}H_{42}N_5O_7PS_3$ (M + Cs^+) 1048.1038, found 1048.1085. Anal. Calcd: H, 4.62; N, 7.64; P, 3.38; S, 10.50. Found: H, 4.76; N, 7.47; P, 3.37; S, 10.49.

1h: yield 83%; TLC R_f 0.21; ^{31}P NMR δ 124.27; HRMS(FAB) calcd for $C_{38}H_{41}N_6O_5PS_3$ (M + Cs^+) 921.1093, found 921.1135. Anal. Calcd: H, 5.23; N, 10.65; P, 3.93; S, 12.19. Found: H, 5.24; N, 10.24; P, 3.81; S, 11.94.

Reactions of 2-*n*-Butoxy-2-thiono-1,3,2-dithiaphospholane with *n*-Butanol in the Presence of Organic Bases. Into a solution containing 2.28 g (10 mmol) of 2-*n*-butoxy-2-thiono-1,3,2-dithiaphospholane [prepared from 2-chloro-1,3,2-dithiaphospholane and *n*-butanol in the presence of Et_3N and purified by column chromatography (benzene–hexane as eluent): thick colorless oil; yield 1.5 g (66%); TLC

(24) McBride, L.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, 24, 245.
(25) Stec, W. J.; Uznański, B.; Wilk, A.; Hirschbein, B. L.; Fearon, K. L.; Bergot, B. J. *Tetrahedron Lett.* **1993**, 34, 5317.
(26) Bredereck, H.; Effenberger, F.; Beyerlin, H. P. *Chem. Ber.* **1964**, 97, 3081.
(27) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, 104, 1316.
(28) Schaller, H.; Weiman, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, 85, 3821.
(29) Gait, M. J.; Singh, M.; Sheppard, R. C. *Nucl. Acids Res.* **1980**, 8, 1081.
(30) For compounds **1a–h** no good combustion analyses for carbon could be obtained—too low values were consistently obtained, most probably due to the presence of several tertiary carbon atoms in their molecules.

(benzene) R_f 0.59; ^{31}P NMR (C_6D_6) δ 120.30; LSIMS m/z 228, calcd monoisotopic mass 228] and 0.82 g (11 mmol) of *n*-butanol in 10 mL of anhydrous acetonitrile, was added at rt, with stirring, 1.12 g (10 mmol) of potassium *tert*-butoxide. Stirring was continued for 2 h at rt. The ^{31}P NMR analysis of the crude reaction mixture (CD_3CN) revealed the presence of a single peak at δ 115.5. After evaporation of the solvent the residue was treated with 10 mL of 2% HCl and extracted with chloroform (3 × 10 mL). The combined extracts were dried over anhyd $MgSO_4$ and evaporated to a thick colorless oil (2.3 g) which was identified as *O,O*-di-*n*-butylphosphorodithioic acid: yield 95%; ^{31}P NMR ($CDCl_3$) δ 86.4 [lit.[31] ^{31}P NMR (neat) δ 86]; negative LSIMS *m/z* 241 (M-H), calcd monoisotopic mass 242.

Essentially the same result was obtained when DBU was used as the base catalyst in place of potassium *tert*-butoxide. In the case of other catalysts such as Et_3N, DMAP, or *N*-methylimidazole, it was necessary to extend the reaction time to 12 h at rt in order to achieve full conversion of the dithiaphospholane substrate.

Reaction of 1a with 3′-*O*-Acetylthymidine in CH_3CN Solution. A 209.4 mg (0.3 mmol) portion of **1a** was combined with 93.8 mg (0.33 mmol) of 3′-*O*-acetylthymidine, dried for 6 h on a vacuum line, and dissolved in 2 mL of anhyd acetonitrile. Into this solution was injected 42.3 μL (0.3 mmol) of DBU through a septum, and the mixture was stirred at rt for 3 h and evaporated. The residue was treated at rt for 2 h with 80% aqueous acetic acid (3 mL) and, after evaporation, dissolved in 25% aqueous ammonia (5 mL). This solution was kept overnight at 55 °C in a tightly capped vial and evaporated. The residue was chromatographed on a DEAE Sephadex A-25 (200 mL) column and eluted with a linear gradient of 1 L of each 0.05 M and 1 M TEAB, collecting 20 mL fractions under UV control. The product-containing fractions were pooled and evaporated. The residue was evaporated twice with ethanol (2 × 100 mL) and dried on a vacuum line to give 95.7 mg (47%) of **2a** as an amorphous powder: ^{31}P NMR (D_2O) δ 113.8; negative LSIMS *m/z* 577.0 (M − H), calcd monoisotopic mass 578.09; RP HPLC t_R 16.49 min (ODS Hypersil, 1.5 mL/min, 0−20% CH_3CN in 20 min).

Reaction of 2a-d with Butylene 1,2-Oxide. The solution of **2a** (1 mg) in 50 μL of H_2O was diluted with 75 μL of ethanol. Into the resulting solution was added 25 μL of butylene 1,2-oxide, and the mixture was heated in an Eppendorf tube at 50 °C for 3 h. The product was analyzed by RP HPLC (ODS Hypersil, 1.5 mL/min, 0−20% CH_3CN in 20 min) and showed full disappearance of the substrate (t_R 16.49 min) and appearance of a peak at t_R 11.53 min, which coeluted with authentic T_PT.

The reactions of **2b−d** with butylene 1,2-oxide were performed as above to give the following results:

(a) **2b** was transformed to d(C_PC); t_R 7.90 min,

(b) **2c** was transformed to d(G_PG); t_R 8.32 min,

(c) **2d** was transformed to d(A_PA); t_R 10.76 min.

The Automated Synthesis of S_2-ODNs 3. For the synthesis of **3** on a 1 μmol scale the protocol described in Table 2 was followed. The oligonucleotides containing a 5′-*O*-dimethoxytrityl protecting group were cleaved from the support by 2 h elution with 15% aqueous NH_3 (2 mL), followed by a wash with acetonitrile (10 mL). The crude oligonucleotides, after evaporation, were dissolved in 25% aqueous ammonia (5 mL) containing NH_4OAc (10%, w/v) and heated for 48 h at 55 °C in a tightly closed tube. The resulting solution was evaporated, and the residue was purified by RP HPLC (DMT-ON step, ODS Hypersil, 1.5 mL/min, 0−40% CH_3CN in 40 min). Each product was collected as the major peak eluting between 30 and 40 min. The collected solution was evaporated, and traces of TEAB were removed by coevaporation with ethanol. The residue was dissolved in 50% aqueous acetic acid (0.5 mL), and after 40 min at rt, the acid was evaporated. The residue was purified by RP HPLC (DMT-OFF step) under identical conditions as those described above for DMT derivatives. The collected solution was evaporated and then coevaporated with ethanol (1 mL) in a Speed-vac (Savant) apparatus. The purified S_2-ODNs **3** were quantified by optical measurement at 260 nm. The identity and purity of **3** were checked by ^{31}P NMR (D_2O), gel electrophoresis (PAGE), and negative electrospray ionization mass spectrometry. For the latter measurements **3** were transformed into their ammonium salts.

Conversion of S_2-ODNs into Ammonium Salts. One OD_{260} unit of HPLC-purified **3** was dissolved in 40 μL of water and mixed with an equal volume of 10 M ammonium acetate. The mixture was vortexed and maintained for about 1 h before the product was precipitated with ethanol (0.8 mL). The sample was cooled at −20 °C for 1 h and centrifuged and the supernatant aspirated. The procedure was repeated, and the pellet of precipitated ammonium salt of oligonucleotide **3** was dissolved in 50 μL of deionized H_2O. Prior to infusion into the mass spectrometer, the solution was diluted 1/10 with methanol.

5′-End-^{32}P-Labeling of Oligonucleotides. The oligonucleotide (0.5−1.0 nmol) was dissolved in 20 μL of a buffer containing 10 mM Tris−Cl (pH 8.5), 10 mM $MgCl_2$, 7 mM β-mercaptoethanol, 15 μCi of ^{32}P-γ-ATP (Amersham), and T4 polynucleotide kinase (Amersham). For phosphorodithioates (**3d**, **3f**, **3g**) 15 units of kinase was used, whereas for phosphorothioates and/or oxo-analogues having the same sequence as **3d**, **3f**, **3g**, 5 units of enzyme was employed. The mixture was incubated at 37 °C for 4 h (phosphorodithioates) or 2 h (phosphorothioates, oxo-analogues) and then heated for 2 min at 95 °C in order to inactivate the enzyme.

Reaction with DNase I. Five μL of solution containing 5′-^{32}P-oligonucleotide (one of: **3d**, **3f**, **3g** or their monothio- or oxo-analogues prepared as above) was mixed with 35 μL of 50 mM sodium acetate buffer (pH 6.5) containing 10 mM $MgCl_2$ and 2 mM $CaCl_2$. Then, 250 units of DNase I (Serva) was added, and the mixture was incubated at 37 °C. After 15 min, 30 min, and 2 h, 10 μL aliquots were taken, heat-denaturated (2 min at 95 °C), evaporated in the Speed-vac, and dissolved in formamide containing xylenocyanol and bromophenol blue (7 μL). The samples were analyzed by PAGE, and the gels were autoradiographed.

Acknowledgment. We thank Dr. Maria Koziołkiewicz for the help in performing studies with DNase I, Mrs. Małgorzata Boczkowska for T_m measurements, Dr. Marek Sochacki for LSIMS analyses, Mrs. Anna Kobylańska for preparation of stereoregular S-ODNs, Mrs. Wiesława Goss and Mrs. Barbara Mikołajczyk for preparation of oxo-ODNs and Mix-S-ODNs, Mrs. Magdalena Olesiak for help in the preparation of protected nucleosides, and Mrs. Danuta Krajewska for excellent technical assistance. This project has been financially assisted by the State Committee for Scientific Research (KBN), Grant No. 6-6286-92-03.

Supporting Information Available: Protocol for the manual synthesis of S_2-ODNs by the dithiaphospholane approach and its application for the synthesis of short fragments containing T and C bases and a table containing phosphorodithioate nucleotide subunit masses for calculation of molecular weight of S_2-ODNs (4 pages). This material is contained in 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.

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(31) Moedritzer, K.; Van Vazer, J. R. *J. Inorg. Nucl. Chem.* **1963**, 25, 683.

(32) One OD_{260} unit is defined as the amount of oligonucleotide which, after being dissolved in 1 mL of water, gives the absorption *A* = 1 at 260 nm in a 1 cm cell.