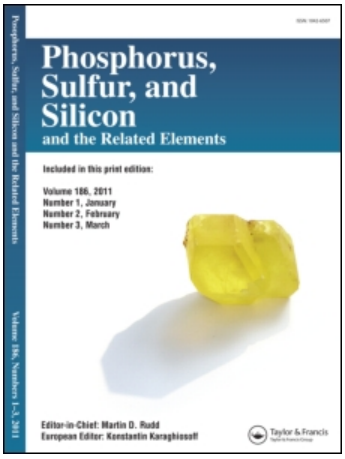

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Studies of Asymmetric Induction in the Synthesis of Dinucleoside Phosphorothioates from 2-Oxo-1,3,2-dithiaphospholane Nucleoside Derivatives

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Studies of Asymmetric Induction in the Synthesis of Dinucleoside Phosphorothioates from 2-Oxo-1,3,2-dithiaphospholane Nucleoside Derivatives

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The nucleoside derivatives, containing at 3′-O or 5′-O-position 2-oxo-1,3,2-dithiaphospholane function were found to react in the presence of DBU with a hydroxyl group of appropriately protected nucleosides to yield, after deprotection, dinucleoside phosphorothioates in moderate yields. An asymmetric induction connected with the formation of new chiral phosphorothioate center has been examined.

Keywords Asymmetric induction; DBU assistance; dinucleoside phosphorothioates; dithiaphospholane ring cleavage

INTRODUCTION

Chemically modified oligonucleotides have recently found wide application in biochemistry and molecular biology as indispensible tools for studying interactions of nucleic acids with other biomolecules[1,2] and as potential therapeutics in antisense/antigene or ribozyme strategies.[3–5]

Among oligonucleotide analogues most widely used for such studies are those modified within the internucleotide phosphate group, and in particular, phosphorothioate oligonucleotides [oligo(nucleoside phosphorothioate)s] in which one of the nonbridging oxygen atoms is substituted by sulfur.[6] Phosphorothioate oligonucleotides are the most promising candidates as antisense therapeutics against several viral and cancer diseases, as indicated by their use in numerous clinical

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Dedicated to Professor Marian Mikołajczyk, CBMiM PAN in Łódź, Poland, on the occasion of his 70th birthday.

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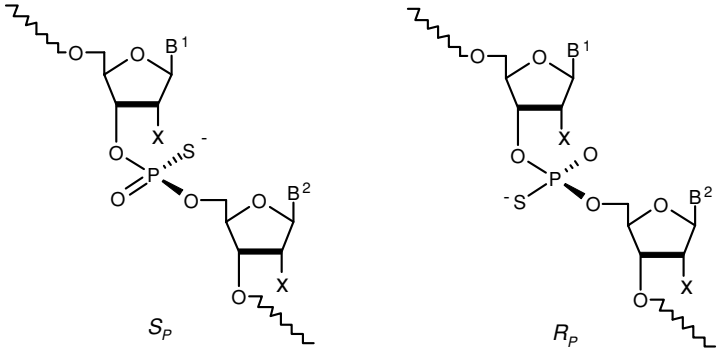


FIGURE 1 Schematic representation of oligo(deoxyribonucleoside phosphorothioate)s (X=H) and oligo(ribonucleoside phosphorothioate)s (X=OH) with opposite configuration (S_P or R_P) of internucleotide bond.

trials[7] and by FDA approval of phosphorothioate 22-mer (Vitravene) for treatment of CMV retinitis in AIDS patients (August 1998). Unfortunately, no other oligonucleotides have been approved as antisense drugs, in spite of numerous clinical trials.

The first automated synthesis of oligo(deoxyribonucleoside phosphorothioate)s was accomplished by Stec et al.[8] using a modified phosphoramidite approach with a stepwise sulfurization of intermediate phosphite linkage. An alternative approach was proposed by Froehler,[9] who modified the H-phosphonate method by introducing final one-step sulfurization with a solution of elemental sulfur. The modifications of the phosphotriester method found only limited application for the synthesis of oligo(deoxyribonucleoside phosphorothioate)s.[10] In contrast to DNA analogues, the synthesis of oligo(ribonucleoside phosphorothioate)s was described only in a few cases, usually by the H-phosphonate method.[11,12]

An intrinsic property of oligo(nucleoside phosphorothioate)s is the formation of a new center of chirality at each internucleotide phosphorus, leading to R_P and S_P diastereomers (see Figure 1). With an increasing length of oligonucleotide, the number of diastereomers (m) grows exponentially ($m = 2^n$ for n phosphorothioate bonds).

As an important consequence of aforementioned polydiastereomerism of oligo(nucleoside phosphorothioate)s, stereodifferentiated interactions could occur between particular diastereomers and other chiral biomolecules such as DNA, RNA, proteins, carbohydrates, or lipids. This could in turn lead to stereodifferentiated uptake, cellular trafficking, stability in biological media, pharmacokinetics, and

toxicity. As a result, particular diastereomers may have different therapeutic properties when applied in antisense, antigene, or ribozyme strategies.[13]

The synthesis of oligo(deoxyribonucleoside phosphorothioate)s by the most commonly used solid support phosphoramidite/sulfurization approach leads to a more or less random mixture of all possible diastereomers. The detailed studies have shown, however, that every individual coupling step occurs with some stereoselectivity, and the formation of phosphorothioate center with R_P configuration is preferred (usually 52–62% of R_P).[14]

The enzymatic template-directed processes, utilizing DNA or RNA polymerases of different origin and [S_P]-nucleoside 5′-*O*-α-thiotriphosphates as substrates, have been employed for the stereocontrolled synthesis of several phosphorothioate analogues of DNA[15,16] or RNA.[17] An important limitation of this methodology is that it can only be applied for the synthesis of [*All*-R_P] diastereomers of phosphorothioate DNA or RNA fragments.

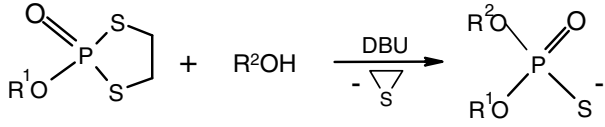
Fully stereocontrolled chemical synthesis of oligo(deoxyribonucleoside phosphorothioate)s was introduced by Stec et al., based on the stereospecific reaction of appropriately protected and previously separated into diastereomers nucleoside-3′-*O*-(2-thio-1,3,2-oxathiaphospholane)s with 5′ -oxygen of nucleosides or growing oligonucleotide chain.[18–21] This methodology, usually referred to as the oxathiaphospholane approach, allows us to accomplish a solid-state automated synthesis of phosphorothioate analogues of oligodeoxyribonucleotides with a predetermined sense of chirality at each internucleotide phosphorus. The oxathiaphospholane methodology found some application for the stereocontrolled synthesis of short oligo(ribonucleoside phosphorothioate)s containing either 3′,5′−[22] or 2′,5′-internucleotide linkages.[23]

Several attempts of stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s were undertaken by other authors, however with very limited success. By application of the phosphotriester approach, with separated into diastereomers nucleoside 3′-*O*-(*S*-alkyl-*O*-*p*-nitrophenyl)-phosphorothioates as precursors, Leśnikowski and Jaworska were able to synthesize diastereomerically pure phosphorothioate analogues of trithymidine[24] and triuridine.[25] However, the method could not be employed for solid-support synthesis. In alternative studies, Just and coworkers used as substrates nucleoside 3′-*O*-cyclic phosphoramidites based on α-D-*xylo*-furanose[26] and L- or D-tryptophan[27] as chiral auxiliaries, whereas Iyer et al. employed nucleoside 3′-*O*-bicyclic oxazaphospholidines derived from enantiomers of

prolinol.[28] However, in all cases, only dinucleoside phosphorothioates could be synthesized in a stereocontrolled way. An improved method was reported by Wilk et al., who were able to prepare selected stereoregular oligo(nucleoside phosphorothioate)s of longer nucleotide sequence by using nucleoside 3′-*O*-(N-acyl-1,3,2-oxazaphospholane)s as starting material.[29]

RESULTS AND DISCUSSION

All aforementioned methods involved the use of nucleoside derivatives with cyclic P-chiral precursors of phosphorothioate moiety, often containing additional chiral auxiliary, which had to be separated into individual P-diastereomers prior to coupling reaction. In this article, we wish to report the results of our studies on the application of nucleoside derivatives of type **1**, containing P-prochiral 2-oxo-1,3,2-dithiaphospholane function as a precursor of dinucleotide analogues **2**, containing internucleotide phosphorothioate linkage (Scheme 1). All reactions were performed in solution, which is typical to introductory studies on the dinucleotide stage. Compounds **1** are close analogues of nucleoside-3′-*O*-(2-thio-1,3,2-dithiaphospholane)s, which were developed by us as substrates for the solid-state synthesis of oligo(nucleoside phosphorodithioate)s (dithiaphospholane method).[30]



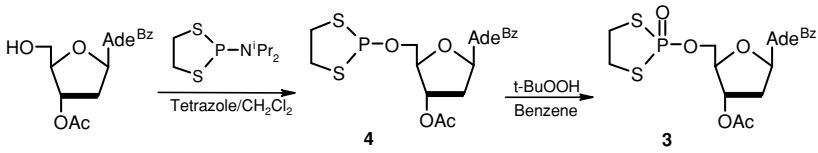
1 **2**

SCHEME 1 The proposed synthesis of phosphorothioate diesters by modified dithiaphospholane approach. R^1 and R^2 can be appropriately protected nucleoside derivatives.

It was anticipated that in the reaction shown in Scheme 1, with R^1O and R^2O being nucleoside residues, a stereoselective formation of internucleotide phosphorothioate linkage can occur due to the presence of two nucleoside sugar residues as an "internal" chiral auxiliaries. The potential of ribose moiety to induce the preferential formation of dinucleoside H-phosphonates of R_P-configuration (up to 85% of R_P) from P-prochiral appropriately protected nucleoside-3′-*O*-H-phosphonates was demonstrated by Almer et al.[12]

The aforementioned hypothesis has been verified using model substrate **3** bearing 2-oxo-1,3,2-dithiaphospholane residue in 5′-*O*-position

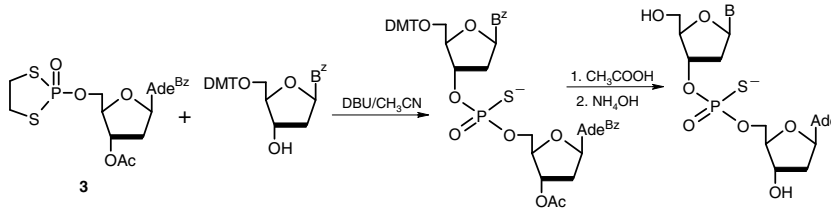
of N^6-benzoyl-3′-*O*-acetyldeoxyadenosine. Compound **3** was synthesized as depicted in Scheme 2 in the reaction of appropriately protected nucleoside with 2-*N*, *N*-diisopropylamino-1,3,2-dithiaphospholane in the presence of 1H-tetrazole.



SCHEME 2 The synthesis of N^6-benzoyl-3′-*O*-acetyldeoxyadenosine-5′-*O*-(2-oxo-1,3,2-dithia-phospholane) **3**.

The P^{III} intermediate **4** was isolated by column chromatography in 91% yield (^{31}P NMR: δ 150.62 ppm, C_6D_6) and was further oxidized with *tert*-butyl hydroperoxide (5–6 M solution in decane) to give **3**, which was isolated in 87% yield as white solid by precipitation from *n*-hexane (^{31}P NMR: δ 80.45 ppm, C_6D_6). The presence of minor unidentified contamination was also detected (3–10%; δ 20.9 ppm), which however could not be removed due to decomposition of **3** during chromatography on silicagel column. Samples of **3** prepared as above are relatively stable when stored at –20°C under argon (^{31}P NMR assay).

The dithiaphospholane **3** was reacted with equimolar amounts of nucleosides containing free 3′-OH group in anhydrous acetonitrile solution, in the presence of equimolar amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). This compound was previously selected as optimal activator of alcohols in their reactions with nucleoside oxathia- and dithiaphospholanes.[17–22,29,30] The reactions were performed on 0.1 mmol scale, at room temperature, under ^{31}P NMR control (Scheme 3).



SCHEME 3 The synthesis of dinucleoside phosphorothioates from dithiaphospholane **3**.

After 30 min, full disappearance of the signal of substrate **3** was observed with simultaneous formation of signals corresponding to R_P and S_P diastereomers of dinucleoside phosphorothioates (55–57 ppm range). After evaporation of solvent, the deprotection procedure was applied. The treatment with 80% aq acetic acid (2 h, r.t.) removed 5′-O-dimethoxyl group (DMT), whereas incubation in 30% aq ammonia (12 h, 55°C) led to hydrolytic removal of all acyl groups. Dinucleoside phosphorothioates (a mixture of R_P and S_P diastereomers) were identified as major components of the reaction mixture by spectroscopic (^{31}P NMR chemical shifts) and chromatographic (RP HPLC retention times) comparison with original samples prepared independently by phosphoramidite method.[14] Thus, the signal of R_P isomer appears for all dinucleoside phosphorothioates at higher frequencies in ^{31}P NMR (D_2O solution), and this isomer shows higher chromatographic mobility under conditions of reversed phase HPLC. The reaction of dithiaphospholane **3** with 3′ -OH nucleosides gave desired dinucleoside phosphorothioates in ca. 50% yield as determined by integration of NMR spectra and HPLC profiles, and by measurements of UV absorption of dinucleotides isolated by preparative HPLC (selected cases). These measurements also allowed us to determine their R_P/S_P diastereomeric ratio for each dinucleotide product. The average values of R_P/S_P ratios taken from both NMR and HPLC measurements are listed in Table I.

The analysis of the data collected in Table I reveals that in all examined cases, a preferential formation of R_P isomer was observed for deprotected dinucleotides; however its excess over S_P isomer was not

TABLE I Stereochemical Analysis of Dinucleoside Phosphorothioates Formed in Reactions of Dithiaphospholane 3 with 3′-OH Nucleosides Shown in Scheme 3

B	Z	R_P/S_P (%)[a]	δ^{31}P NMR R_P/S_P (ppm)	RP HPLC[d] retention time (min) R_P/S_P
Ade	N^6-Bz	65/35	56.80/56.91[b] 56.16/55.30[c]	19.57/21.65
Cyt	N^4-Bz	62/38	56.82/57.22[b] 56.25/55.58[c]	17.44/18.46
Gua	N^2-^{i}Bu	55/45	56.96/57.06[b] 56.00/55.00[c]	17.70/19.12
Thy	H	65/35	56.91/57.21[b] 56.14/55.45[c]	20.17/21.40

[a]Average values for ^{31}P NMR and HPLC measurements.

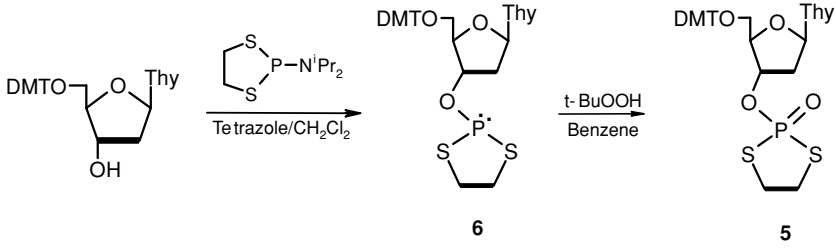
[b]CH_3CN/CD_3CN solution; crude reaction mixture before removal of protecting groups.

[c]D_2O solution; fully deprotected dinucleotides.

[d]Linear gradient of acetonitrile in 0,1 M triethylammonium bicarbonate (1%/min); fully deprotected dinucleoside phosphorothioates.

extremely prevailing (from 55/45% to 62/38%). The R_P/S_P ratios were also measured from integrated ^{31}P NMR spectra of crude reaction mixtures before removal of protecting groups (CH_3CN/CD_3CN solution). The values agreed well with those obtained for deprotected products, however in this case the NMR signal resulting from predominant R_P isomer was found at lower resonance frequency (see Table I). The observed "reversal" of relative position of R_P/S_P isomers in ^{31}P NMR spectra of protected dinucleoside phosphorothioates is probably caused by the presence of 5′-*O*-dimethoxytrityl group.

In order to increase the asymmetric induction, further experiments were performed with substrates bearing 2-oxo-1,3,2-dithiaphospholane function in 3′-*O*-position of nucleoside. The 5′-*O*-dimethoxytritylthymidine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) (**5**), which was chosen as model substrate, was synthesized by phosphitylation of 5′-*O*-protected nucleoside as shown in Scheme 4.

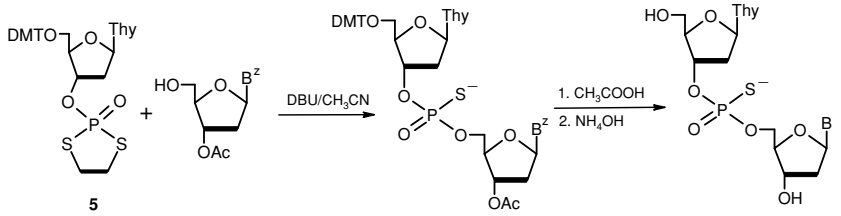


SCHEME 4 The synthesis of 5′-*O*-dimethoxytritylthymidine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) **5**.

The intermediate P^{III} dithiaphospholane **6** (isolated quantitatively by column chromatography, ^{31}P NMR: δ 150.66 ppm, CD_3CN) was further oxidized with *tert*-butyl hydroperoxide to give product **5**, which was isolated by precipitation from *n*-hexane as white solid (^{31}P NMR: δ 79.47 ppm, C_6D_6; yield 84%).

The reactions of dihiaphospholane **5** with equimolar amounts of nucleosides containing free 5′-OH group were performed again in anhydrous acetonitrile solution in the presence of equimolar amount of DBU, exactly as described above for reactions of compound **3** (0.1 mmol scale, 30 min at room temperature, ^{31}P NMR control, see Scheme 5).

After 30 min, full disappearance of the signal of substrate **5** was observed with the formation of R_P and S_P diastereomers of dinucleoside phosphorothioates (55–57 ppm range). After removal of all protecting groups (procedure as described above), dinucleoside phosphorothioates (a mixture of R_P and S_P diastereomers) were identified as major



SCHEME 5 The synthesis of dinucleoside phosphorothioates from dithiaphospholane **5**.

components of the reaction mixture by spectroscopic (^{31}P NMR chemical shifts) and chromatographic (RP HPLC retention times) comparison with original samples prepared independently by the phosphoramidite method.[14] As expected, the signal of R_P isomer appeared for all dinucleoside phosphorothioates at higher frequencies in ^{31}P NMR (D_2O solution), and this isomer showed higher chromatographic mobility under conditions of RP HPLC. Despite of using strictly anhydrous conditions, only ca. 50% yield of desired dinucleoside phosphorothioates was observed (^{31}P NMR spectra and HPLC analysis). These measurements also allowed us to determine their R_P/S_P diastereomeric ratio for each dinucleotide product. The average values of R_P/S_P ratios taken from both NMR and HPLC measurements are listed in Table II.

An inspection of Table II shows that the shift of dithiaphospholane ring from 5′-*O*-position (in **3**) to 3′-*O*-position (in **5**) reversed the outcome of asymmetric reaction. Now, a preferential formation of S_P isomer was observed (72% in the case of deoxyguanosine derivative). However, for

TABLE II Stereochemical Analysis of Dinucleoside Phosphorothioates Formed in Reactions of Ditiaphospholane 5 with 5′-OH Nucleosides

B	Z	R_P/S_P (%)[a]	δ^{31}P NMR[b] R_P/S_P (ppm)	RP HPLC[c] retention time (min) R_P/S_P
Ade	N^6-Bz	38.5/61.5	56.99/57.28	19.03/19.82
Cyt	N^4-Bz	41/59	59.62/59.71	17.51/18.34
Gua	N^{2-i}Bu	28/72	57.29/57.33	17,28/18,19
Thy	H	44/56	56.55/56.86	19.61/20.18

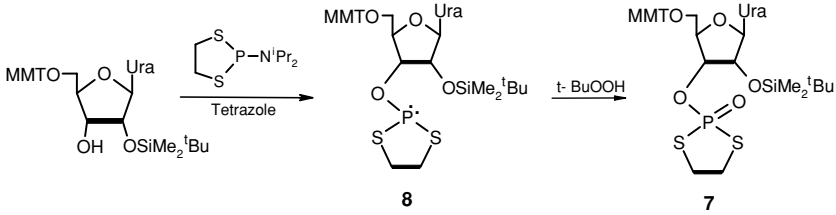
[a]Average values for ^{31}P NMR and HPLC measurements.

[b]D_2O solution; fully deprotected dinucleotides.

[c]Linear gradient of acetonitrile in 0,1 M triethylammonium bicarbonate (1%/min); fully deprotected dinucleoside phosphorothioates.

other nucleoside substrates, the asymmetric induction was lower (56–61.5% of S_P isomer).

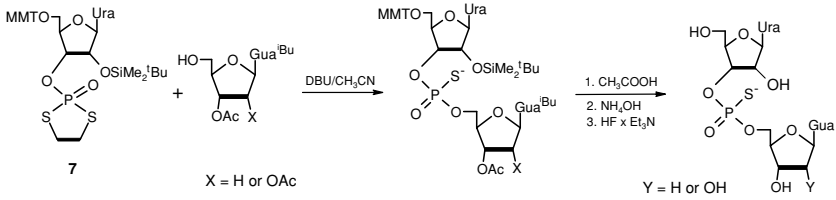
In the search for improvement of asymmetric induction, 5′-*O*-monomethoxytrityluridine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) **7** was synthesized, bearing in 2′-*O*-position *tert*-butyldimethylsilyl substituent. It was hoped that the presence of a bulky 2′-*O*-group would increase the chances for stereoselective formation of internucleotide phosphorothioate linkage. Thus, commercially available 2′-*O*-substituted uridine was phosphitylated in 3′-*O*-position in the manner described for thymidine derivative (Scheme 6).



SCHEME 6 The synthesis of 5′-*O*-monomethoxytrityl-2′-*O*-*tert*-butyldimethylsilyluridine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) (**7**).

The intermediate trivalent phosphorus dithiaphospholane **8** was isolated chromatographically in 93% yield (^{31}P NMR: δ 158.85 ppm, CD_3CN) and oxidized with *tert*-butyl hydroperoxide to give desired product **7**, which was isolated by precipitation from *n*-hexane as white solid (^{31}P NMR: δ 82.38 ppm, CD_3CN; yield 79.4%).

The efficiency of dithiaphospholane **7** as stereoselective precursor of phosphorothioate linkage was tested by its reaction with 5′-OH derivatives of guanosine and deoxyguanosine (Scheme 7).



SCHEME 7 The synthesis of dinucleoside phosphorothioates from dithiaphospholane **7**.

Due to lower reactivity of sterically hindered substrate **7**, the reactions were carried out for 2 h, with 30% molar excess of protected guanine nucleosides. For removal of dinucleotide protecting groups, in

TABLE III Stereochemical Analysis of Dinucleoside Phosphorothioates Formed in Reactions of Ditiaphospholane 7 with Guanine Nucleosides (Scheme 7)

X	Y	R_P/S_P (%)[a]	δ^{31}P NMR[b] R_P/S_P (ppm)	RP HPLC[c] retention time (min) R_P/S_P
H	H	35/65	58.28/58.43	13.11/13.55
OAc	OH	46.5/53.5	58.06/58.22	12.41/12.86

[a]Average values for ^{31}P NMR and HPLC measurements.

[b]CH_3CN/CD_3CN solution; crude reaction mixture before removal of protecting groups.

[c]Linear gradient of acetonitrile in 0,1 M triethylammonium bicarbonate (1%/min); fully deprotected dinucleotides.

addition to standard acid and ammonia treatment, the product was reacted with triethylammonium hydrogen fluoride (cleavage of silyl ether). The results are listed in Table III.

Unfortunately, even the increase of steric hindrance in dithiaphospholane substrate did not enhance the extent of asymmetric induction, which led to the preponderant formation (65%) of S_P isomer of resulting phosphorothioate for deoxyguanosine derivative. In the case of protected guanosine derivative, almost equal proportion of isomers was observed (53.5% S_P).

In conclusion, appropriately protected nucleosides containing 2-oxo-1,3,2-dithiaphospholane function were found to react with nucleosides containing free hydroxyl group in anhydrous acetonitrile, in the presence of equimolar amount of DBU, with the formation (after deprotection) of dinucleoside phosphorothioates. The products were formed in moderate yields (ca. 50%), which resulted mainly from the difficulty in obtaining completely anhydrous reaction conditions. Due to the fact that initial dithiaphospholane substrates were prochiral, and in the reaction a new phosphorothioate chiral center was formed, there was hope for the asymmetric induction and the formation of products highly enriched in one of the P-isomers. The induction could be caused by "internal" chiral auxiliaries present in nucleoside sugar moieties. Unfortunately, the experiments performed with substrates containing 2-oxo-1,3,2-dithiaphospholane function either in 5′-*O*- or in 3′-*O*-position of deoksyribonucleoside, or even in 3′-*O*-position of 2′-*O*-bulky substituted ribonucleoside, did not generate the formation of dinucleoside phosphorothiotes highly enriched in one of P-diastereomers. The highest observed enrichment was 72/28 S_P/R_P ratio (Table II).

EXPERIMENTAL

TLC was performed on Kieselgel $60F_{254}$ (Merck) with UV (254 nm) detection. Flash column chromatography was run on silica gel 230–400 mesh (Merck). Reverse phase high performance liquid chromatography (RP HPLC) was made with Gilson chromatograph (model 306) using analytical column 4.6 × 250 mm packed with ODS Hypersil, 5μ (Alltech). The columns were eluted with 0.1 M aqueous triethylammonium bicarbonate (TEAB), pH 7.5, supplemented by linear gradient of acetonitrile from 0 to 20% in 20 min. ^{31}P NMR spectra were recorded on a Bruker AC 200 spectrometer (200.113 MHz for ^{1}H) and referenced to 85% H_3PO_4 used as an external standard. Mass spectra were recorded on Finnigan MAT 95. Dinucleoside phosphorothioate syntheses were performed by modified phosphoramidite method (sulfurisation with S-TETRA) on an automatic ABI 394 DNA Synthesizer on 1 μ mol scale.

The analytical grade solvents were purchased from Aldrich and freshly distilled from CaH_2. Analytical grade phosphorus trichloride, diisopropylamine, 1,2-ethanedithiol, triethylamine, tetrazole, and DBU were purchased from Aldrich. Nucleosides were purchased from Pharma Waldhof.

N,N-Diisopropylaminophosphorodichloridite

Into a solution of freshly distilled phosphorus trichloride (67 g, 0.49 mol) in anhydrous benzene/hexane (1:1, 500 mL), a solution of dry diisopropylamine (99 g, 0.98 mole) in benzene (100 mL) was added dropwise, with mechanical stirring and cooling at 4°C. The solution was then stirred for 1 h at r.t., filtered, and the precipitate was washed with benzene/hexane (1:1, 250 mL). The filtrate was evaporated, and the residue was distilled at 0.4 mmHg to yield 78.5 g (79.3%) of colorless liquid product boiling at 53–5°C, ^{31}P NMR: δ 169.74 ppm ($CDCl_3$). Lit.[31] bp 56–9°C/0.5 mmHg, ^{31}P NMR: δ 169.0 ppm (C_6D_6).

2-*N,N*-Disopropylamino-1,3,2-dithiaphospholane

Into a solution of *N, N*-diisopropylaminophosphorodichloridite (78.5 g, 0.39 mol) in anhydrous benzene/hexane (1:1, 900 mL), a solution of 1,2-ethanedithiol (36.5 g, 0.39 mole) and triethylamine (78.5, 0.78 mole) in 200 mL of benzene was added dropwise, with mechanical stirring and cooling at 4°C. After 1 h stirring at r.t., the amine hydrochloride was filtered off and washed with benzene/hexane (1:1, 400 mL). The combined filtrates were evaporated, and the residue was distilled at

0.1 mmHg yielding 79.5 g (91.7%) of colorless liquid boiling at 115°C, ^{31}P NMR: δ 94.61 ppm ($CDCl_3$). HR MS m/z 223.0596 Da (calcd m/z 223.0618 Da).

5′-*O*-Dimethoxytritylthymidine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) 5

The mixture of 5′-*O*-dimethoxytritylthymidine (1.0 g, 1.84 mmol) and tetrazole (126 mg, 1.84 mmol) was dried on a vacuum line for 12 h. The mixture was dissolved under argon in 20 mL of anhydrous methylene chloride, and 410 mg (1.84 mol) of 2-*N*, *N*-disopropylamino-1,3,2-dithiaphospholane was added with stirring at r.t. Stirring was continued overnight, and after evaporation, the trivalent phosphorus product **6** was isolated by flash column chromatography using chloroform with a gradient of methanol (0–3%) as eluent (white powder, 1.21 g, ^{31}P NMR: δ 150.66 ppm, CD_3CN). The product **6** was dissolved in benzene (10 mL) and 360 μl of 5–6 M decane solution of *tert*-butyl hydroperoxide was added dropwise at r.t. After 1 h the resulting solution was added dropwise at 0°C to the vigorously stirred *n*-hexane (50 mL). The final product **5** was obtained by centrifugation of precipitate and its drying in vacuo (white powder, 1.04 g, 84%, ^{31}P NMR: δ 79.47 ppm, C_6D_6). The compound **5** was stored under argon at –20°C.

Basically the same procedure was applied for the synthesis of 2-oxo-1,3,2-dithiaphospholane compounds **3** and **7**.

The Synthesis of Dinucleoside Phosphorothioates from Dithiaphospholane 5: General Procedure

The mixture of 5′-*O*-dimethoxytritylthymidine-3′-*O*-(2-oxo-1,3,2-dithiaphospholane) **5** (70 mg, 0.1 mmol) and 3′-*O*-acetyl-N-protected-2′-deoxyribonucleoside (0.1 mmol) was dried on a vacuum line for 12 h. Into this mixture a solution of DBU (15 mg, 0.1 mmol) in anhydrous acetonitrile (1.5 mL) was added with stirring. After 30 min at r.t., the mixture was analyzed by ^{31}P NMR and evaporated. The residue was dissolved in 80% aq acetic acid (1 mL). After 2 h at r.t., the solution was evaporated. The residue was dissolved in 1 mL of acetonitrile and 2 mL of 30% aq ammonia was added. The flask was closely capped and kept at 55°C for 12 h. The solution was evaporated and the residue was analyzed by RP HPLC and ^{31}P NMR.

Basically the same procedure was applied for reactions of compounds **3** and **7** with appropriate nucleoside derivatives.

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