Diastereomerically Pure Nucleoside-5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)s—Substrates for Synthesis of P-Chiral Derivatives of Nucleoside-5′-*O*-phosphorothioates

AGNIESZKA TOMASZEWSKA, PIOTR GUGA*, AND WOJCIECH J. STEC

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

ABSTRACT A method for stereocontrolled chemical synthesis of P-substituted nucleoside 5′-*O*-phosphorothioates has been elaborated. Selected 3′-*O*-acylated deoxyribonucleoside- and 2′,3′-*O,O*-diacylated ribonucleoside-5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)s were chromatographically separated into P-diastereomers. Their reaction with anions of phosphorus-containing acids was highly stereoselective (≥90%) and furnished corresponding P-chiral α-thiodiphosphates and their phosphonate analogs with satisfactory yield. *Chirality 23:237–244, 2011.*

KEY WORDS: stereoselectivity; P-diastereomers; oxathiaphospholane monomers; nucleoside thiophosphates; HPLC

INTRODUCTION

Nucleoside 5′-*O*-phosphates (NMPs), diphosphates (NDPs) and triphosphates (NTPs), as well as their dinucleoside polyphosphate analogs (e.g., 5′,5′-$N_{P_n}N$, n = 2–7) play an important role in a living cell serving as a source of energy or as regulatory factors in many metabolic processes. Some notable examples of involvement of NTPs in eukaryotic systems include developmental control,[1] signal transduction,[2] and tumor metastasis.[3] NTPs are widely used in biochemistry and molecular biology as substrates for the *in vitro* enzymatic synthesis of DNA and RNA. Natural and modified NTPs have also been used in DNA sequencing[4] and studies on the mode of action of numerous enzymes.[5,6]

All natural nucleoside phosphates and oligonucleotides are readily metabolized in the cell, therefore, more stable analogs are required for detailed studies on their mechanism of action. For that purpose, numerous analogs carrying modifications within nucleobases, ribose, deoxyribose, and phosphate moieties have been obtained.[7,8] Among these, the phosphorothioate nucleotides/oligonucleotides, in which one of the nonbridging phosphate oxygen atoms was substituted with a sulfur atom, were most extensively studied, both in *in vitro* and *in vivo* experiments.[9,10] Also, it should be noted that only recently phosphorothioation of DNA in bacteria has been discovered.[11]

In terms of chemistry, it should be emphasized that although the phosphorothioate group is isoelectronic with the natural phosphate moiety, this modification introduces important changes resulting from different steric requirements of the sulfur atom (P—S vs P—O bond length), different affinity toward metal ions ("soft" sulfur vs "hard" oxygen) and unsymmetrical negative charge distribution.[12] Moreover, although nucleoside 5′-*O*-phosphorothioates and symmetrical 5′,5′-$N_{PS}N$ are P-prochiral, the NTP and NDP analogs, with the nonbridging sulfur atom introduced into the α-phosphate group, are P-chiral species and if chemically synthesized, they consist of the practically equimolar mixture of P-diastereomers of either R_P or S_P absolute configuration. Given that nearly all biomolecules that recognize nucleoside 5′-polyphosphates and dinucleoside 5′,5′-polyphosphates are chiral and exist in pure stereochemical forms, their interactions may be significantly different for each P-diastereomer. Pure diastereomers can sometimes be obtained by enzymatic synthesis. For example, prochiral deoxyadenosine 5′-*O*-phosphorothioate can be stereospecifically converted in the presence of ATP with tandem adenylate kinase/pyruvate kinase into corresponding P-chiral α-thiodiphosphate and, finally, into α-thiotriphosphate.[13,14] Alternatively, in certain instances chromatographic separation of P-diastereomers can be applied.[15] However, the chromatography and the enzymatic methods are not general and suffer from severe limitations. Thus, a method for a stereocontrolled chemical synthesis of P-substituted nucleoside 5′-*O*-phosphorothioates is highly demanded. In this laboratory, the method for a stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s, based on chromatographically resolved P-diastereomers of 5′-*O*-DMT-nucleoside-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) monomers (3′-OTPs), has been developed.[16,17] On the other hand, because chromatographic separation of nucleoside-5′-*O*-oxathiaphospholane monomers, carrying typical protecting groups at the 3′- or 2′- and 3′-hydroxyl functions, could not be achieved, numerous P-chiral biophosphates were synthesized using unresolved monomers (e.g., **8**, Scheme 3).[18–20] Here, we report on the synthesis of selected 3′-*O*-acylated deoxyribonucleoside- and 2′,

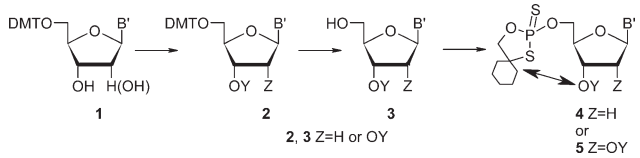
Additional Supplementary Material may be found in the online version of this article.

Dedicated to Professor Marian Mikołajczyk on the occasion of his 70th birthday.

Contract grant sponsor: Centre of Molecular and Macromolecular Studies; Contract grant number: statutory funds; Contract grant sponsor: Polish Ministry of Science and Higher Education; Contract grant number: 3 T09A 059 28 to WJS

*Correspondence to: Dr. Piotr Guga, Sienkiewicza 112, 90-363 Łódz, Poland. E-mail: pguga@bio.cbmm.lodz.pl

Received for publication 20 January 2010; Accepted 22 June 2010
DOI: 10.1002/chir.20905
Published online 6 October 2010 in Wiley Online Library (wileyonlinelibrary.com).



Scheme 1. Synthesis of 5′-OTPs of DNA (**4**) and RNA (**5**) series. Y = biphenyl-4-carbonyl, 1-adamantanecarbonyl, 2-naphthoyl or (−)-camphanoyl. B′ = nucleobase (*N,O*-protected if necessary). The two-head arrow shows expected interactions in **4** (or **5**) which substantially differentiate the energies of the most stable conformers of R_P and S_P-forms, and hence change their chromatographic mobility.

3′-*O,O*-diacylated ribonucleoside-5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)s (5′-OTPs, **4** and **5**, respectively, Scheme 1), which are chromatographically separable into P-diastereomers. Based on our earlier results on stereoretentive mode of DBU-assisted oxathiaphospholane ring opening process,[16] these new monomers were expected to be suitable for the stereocontrolled synthesis of P-chiral derivatives of nucleoside-5′-*O*-phosphorothioates.

EXPERIMENTAL SECTION

The nuclear magnetic resonance spectra (NMR) were recorded on a Bruker AC-200 instrument (200 MHz for ^{1}H) or on a Bruker DRX-500 (500 MHz for ^{1}H), with 85% H_3PO_4 used as an external standard for ^{31}P NMR. The FAB-MS spectra (13 keV, Cs^+) were recorded on a Finnigan MAT 95 spectrometer, in the positive and negative ions modes where $(M+H)^+$ and $(M-H)^-$ ions were observed, respectively. Calculated masses (calc.) refer to the parent compounds. Negative ion MALDI TOF mass spectra were recorded on a Voyager-Elite instrument (PerSeptive Biosystems Inc., Framingham, MA). HPLC analyses were done using a Gilson system consisting of two 306 pumps and a 151 UV/VIS detector. Anhydrous acetonitrile, 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) and anhydrous pyridine were supplied by Fluka, Merck and Aldrich, respectively. (1S)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl chloride (or (−)-camphanic chloride), 2-naphthoyl and biphenyl-4-carbonyl chlorides were supplied by FLUKA, 1-adamantanecarbonyl chloride by Aldrich, and DMT-Cl by Chemgenes. Crystalline phosphoric acid and *p*-toluenesulfonic acid monohydrate were bought from Aldrich. svPDE was obtained from Boehringer Mannheim GmbH, Germany. Nuclease P1 from *Penicillium citrinum* was purchased from Sigma. All silica gel media for chromatography were supplied by MERCK. Silica gel 60, 230–400 mesh, was used for routine chromatographic purification. For the open column separation of P-diastereomers, silica gel 60H (particles 5–40 μm) was employed. TLC silica gel 60 plates were used for routine analyses, whereas HP TLC silica gel 60 plates were used for assessment of chromatographic separability of P-diastereomers, all plates with UV F254 indicator. Note that useful technical suggestions have been published for closely related synthesis and separation of diastereomers of 3′-OTPs.[17] The following procedures describe synthesis of the intermediates **2**, **3** and oxathiaphospholane monomers **4**, **5** carrying the camphanoyl moiety/moieties. Those carrying 2-naphthoyl or biphenyl-4-carbonyl moieties can be obtained on a similar way using corresponding acyl chlorides.

Synthesis of Camphanoylated 5′-O-DMT-N-Protected Nucleosides 2—General Procedure

5′-*O*-DMT-*N*-protected deoxyribonucleoside or ribonucleoside (**1**; B′=Ade^{Bz}, Cyt^{Bz}, Gua^{iBu}, Thy or Ura), (2–4 mmol) was evaporated three times with anhydrous pirydine (with the exclusion of moisture), then dried in a dessicator under oil pomp vacuum for 24 h and dissolved in anhydrous pyridine (20 ml) under dry argon. To the solution, (−) camphanic acid chloride (20% molar excess, calculated over each hydroxyl group to be acylated) was added at room temperature. After the reaction was complete (ca. 5 h, TLC control, $CHCl_3$:MeOH, 9:1), the solvent was evaporated, the residue was dissolved in chloroform (2 ml), and applied on a silica gel column (25 × 3 cm). The column was eluted with a gradient of chloroform and methanol from 100:0 to 90:10 (v/v). Appropriate fractions (Rf 0.53, 0.78, 0.78, 0.81, 0.78, 0.85, and 0.86 were found for dG, dC, T, rA, rG, rC, and U derivatives, respectively, $CHCl_3$:MeOH, 9:1; Rf 0.88 was found for dA, $CHCl_3$:MeOH, 95:5) were combined and the solvent was evaporated under reduced pressure to give corresponding compounds **2** (Y = (−)-camphanoyl) in 55–90% yield, which were analyzed by FAB mass spectrometry: (1) deoxyribonucleoside series; Z = H; B′ = Ade^{Bz}, $C_{48}H_{47}N_5O_9$, calc. 837, $(M+H)^+$ 838, $(M-H)^-$ 836; B′ = Gua^{iBu}, $C_{45}H_{49}N_5O_{10}$, calc. 819, $(M+H)^+$ 820, $(M-H)^-$ 818; B′ = Cyt^{Bz}, $C_{47}H_{47}N_3O_{10}$, calc. 813, $(M+H)^+$ 814, $(M-H)^-$ 812; B′ = Thy, $C_{41}H_{44}N_2O_{10}$, calc. 724, $(M+H)^+$ 724, $(M-H)^-$ 723; (2) ribonucleoside series; Z = OY; B′ = Ade^{Bz}, $C_{58}H_{59}N_5O_{13}$, calc. 1033, $(M+H)^+$ 1034, $(M-H)^-$ 1032; B′ = Gua^{iBu}, $C_{55}H_{61}N_5O_{14}$, calc. 1015, $(M+H)^+$ 1016, $(M-H)^-$ 1014; B′ = Cyt^{Bz}, $C_{57}H_{59}N_3O_{14}$, calc. 1009, $(M+H)^+$ 1010, $(M-H)^-$ 1008; B′ = Ura, $C_{50}H_{54}N_2O_{14}$, calc. 906, $(M+H)^+$ 906, $(M-H)^-$ 905.

Removal of the DMT Group from 5′-O-DMT-N-Protected Nucleosides Carrying (−)-camphanoyl Moiety—Synthesis of Compounds 3

To the magnetically stirred solution of compound **2** (2–3 mmol) in a mixture $CHCl_3$:MeOH (9:1, 15 ml), *p*-toluenesulfonic acid monohydrate (3–4.5 mmol, 50% molar excess) was added at room temperature. After the reaction was complete (ca. 20 min, TLC control, $CHCl_3$:MeOH, 9:1), the solvent was evaporated, the residue was dissolved in chloroform (2 ml), and applied onto a silica gel column (25 × 3 cm). The column was eluted with a gradient of chloroform:methanol from 100:0 to 90:10, v/v). Appropriate fractions (camphanoylated deoxyribonucleosides, Rf 0.27–0.36, $CHCl_3$:MeOH, 95:5; camphanoylated ribonucleosides, Rf 0.26–0.48, $CHCl_3$:MeOH, 9:1) were combined and the solvent was evaporated under reduced pressure to give desired compounds **3** in ∼70–90% yields, further analyzed by FAB MS: (1) deoxyribonucleoside series; Z = H; B′ = Ade^{Bz}, $C_{27}H_{29}N_5O_7$, calc. 535, $(M+H)^+$ 536, $(M-H)^-$ 534; B′ = Gua^{iBu}, $C_{24}H_{31}N_5O_8$, calc. 517, $(M+H)^+$ 518, $(M-H)^-$ 516; B′ = Cyt^{Bz}, $C_{48}H_{47}N_5O_9$, calc. 511, $(M+H)^+$ 512, $(M-H)^-$ 510; B′ = Thy, $C_{20}H_{26}N_2O_8$, calc. 422, $(M+H)^+$ 423, $(M-H)^-$ 421; (2) ribonucleoside series; Z = OY; B′ = Ade^{Bz}, $C_{37}H_{41}N_5O_{11}$, calc. 731, $(M+H)^+$ 732, $(M-H)^-$ 730; B′ = Gua^{iBu}, $C_{34}H_{43}N_5O_{12}$, calc. 713, $(M+H)^+$ 714, $(M-H)^-$ 712; B′ = Cyt^{Bz}, $C_{36}H_{41}N_3O_{12}$, calc. 707, $(M+H)^+$ 708, $(M-H)^-$ 706; B′ = Ura $C_{29}H_{36}N_2O_{12}$, calc. 604, $(M+H)^+$ 605, $(M-H)^-$ 603.

Phosphitylation of 3 with 2-chloro-4,4-pentamethylene-1,3,2-oxathiaphospholane—Synthesis of Compounds 4 and 5

To the magnetically stirred solution of 1–3 mmol of **3** (dried overnight under high vacuum) and anhydrous *N,N*-diisopropylethylamine (10% molar excess) in anhydrous methylene chloride (20 ml), 2-chloro-4,4-pentamethylene-1,3,2-oxathiaphospholane[17] (10% molar excess) was added dropwise (under dry argon) at room temperature using a Gas-Tight Hamilton syringe. The reaction was complete in 5 h and then elemental sulfur (two-fold molar excess) was added. Stirring was continued for 24 h and excess sulfur was filtered off. After evaporation of the solvent, the residue was dissolved in chloroform (3 ml) and applied onto a silica gel column (25 × 3 cm). The column was eluted with a gradient of chloroform:methanol from 100:0 to 95:5, v/v). Appropriate fractions (Rf 0.54–0.83, $CHCl_3$:MeOH, 9:1) were combined, and the solvents were evaporated under reduced pressure to give desired compounds **4** or **5** in 55–75% yield. (1) deoxyribonucleoside series; **4**, Z = H; **4d**, B′ = Thy, FAB MS, $C_{34}H_{40}N_2O_9PS_2$, calc. 628, $(M+H)^+$ 629, $(M-H)^-$ 627; ^{31}P NMR, δ (ppm) 106.51, 106.13 (CD_3CN); **4f**, B′ = Gua^{iBu}, FAB MS, $C_{31}H_{42}N_5O_9PS_2$, calc. 723, $(M+H)^+$ 724, $(M-H)^-$ 722; ^{31}P NMR, δ (ppm) 108.24, 107.99 ($CDCl_3$); **4g**, B′ = Ade^{Bz}, FAB MS, $C_{34}H_{40}N_5O_8PS_2$, calc. 741, $(M+H)^+$ 742, $(M-H)^-$ 740; ^{31}P NMR, δ (ppm) 106.31, 106.29 ($CDCl_3$); **4h**, B′ = Cyt^{Bz}, FAB MS, $C_{33}H_{40}N_3O_9PS_2$, calc. 717, $(M+H)^+$ 718, $(M-H)^-$ 716; ^{31}P NMR, δ

TABLE 1. A list of the oxathiaphospholane monomers 4 and 5 separable into P-diastereomers

B′	Y	Z	Rf's or HPLC retention times
4a, Thy	Biphenyl-4-carbonyl	H	TLC 0.68, 0.60[a]
4b, Thy	2-naphthoyl	H	TLC 0.62, 0.51[a]; HPLC: 6.7, 7.5 min[b]
4c, Thy	1-adamantanecarbonyl	H	TLC 0.81, 0.76[c]
4d, Thy	(−)-camphanoyl	H	TLC 0.54, 0.49[d]
4e, $Gua^{iBu,\ DPC}$	(−)-camphanoyl	H	TLC 0.73, 0.70[d]
5a, Ura	(−)-camphanoyl	OY	TLC 0.74, 0.71[e]; HPLC: 6.9, 7.7 min[f]
5b, Ade^{Bz}	(−)-camphanoyl	OY	TLC 0.43, 0.40[e]; HPLC: 9.1, 10.1 min[f]
5d, Gua^{iBu}	(−)-camphanoyl	OY	HPLC: 15.0, 16.0 min[g]
5e, Ura	2-naphthoyl	OY	TLC 0.85, 0.82[d]
5f, Ade^{Bz}	2-naphthoyl	OY	TLC 0.56, 0.53[h]; HPLC: 5.6, 6.0 min[b]

[a]Ethyl acetate:butyl acetate:benzene 2:2:1, single development (sd) of the HPTLC plate.
[b]Ethyl acetate:hexane 52:48.
[c]Ethyl acetate:butyl acetate:benzene 3:2:1, double development (dd).
[d]Ethyl acetate:butyl acetate:benzene 2:2:1, dd.
[e]Ethyl acetate:butyl acetate:benzene 2:1:1, dd.
[f]Ethyl acetate:hexane 60:40.
[g]Ethyl acetate:hexane:methanol 52:45:3.
[h]Ethyl acetate:butyl acetate:benzene 1:2:2; sd.

(ppm) 106.74, 106.50 (CD_3CN); (2) ribonucleoside series; **5**, Z = OY; **5a**, B′ = Ura, FAB MS, $C_{36}H_{47}N_2O_{13}PS_2$, calc. 810, $(M+H)^+$ 811, $(M-H)^-$ 808; ^{31}P NMR, δ (ppm) 106.86, 106.47 (CD_3CN); ^{13}C NMR, δ (ppm) 177.70, 177.58, 166.54, 162.86, 149.93, 139.30, 103.19, 94.16, 90.51, 87.23, 80.37, 79.54, 78.72, 76.37, 73.59, 71.51, 70.70, 69.39, 66.11, 58.29, 54.83, 54.72, 54.32, 37.03, 36.73, 31.13, 30.75, 28.77, 28.65, 25.07, 23.79, 23.61, 16.82, 16.66, 16.39, 9.61 ($CDCl_3$). **5b**, B′ = Ade^{Bz}, FAB MS, $C_{44}H_{52}N_5O_{12}PS_2$, calc. 937, $(M+H)^+$ 938, $(M-H)^-$ 936; ^{31}P NMR, δ (ppm) 107.08, 107.02 ($CDCl_3$); ^{13}C NMR, δ (ppm) 177.55, 166.58, 166.37, 152.97, 151.43, 149.76, 141.29, 133.48, 132.81, 128.82, 127.84, 123.11, 90.61, 90.32, 86.01, 81.17, 79.55, 73.64, 71.46, 69.15, 66.27, 54.85, 54.73, 54.50, 54.39, 37.09, 36.54, 31.15, 30.80, 28.82, 28.63, 25.10, 23.77, 16.83, 16.69, 16.38, 9.57 ($CDCl_3$). **5c**, B′ = Cyt^{Bz}, FAB MS, $C_{43}H_{52}N_3O_{13}PS_2$, calc. 913, $(M+H)^+$ 914, $(M-H)^-$ 912; ^{31}P NMR, δ (ppm) 106.87, 106.44 (CD_3CN); ^{13}C NMR, δ (ppm) 176.81, 176.33, 165.11, 165.04, 161.51, 153.24, 132.01, 127.76, 126.38, 89.42, 89.17, 87.96, 79.00, 78.35, 73.24, 68.44, 68.20, 64.22, 53.67, 53.52, 53.01, 35.86, 35.47, 30.01, 29.52, 27.56, 23.88, 22.58, 22.38, 15.66, 15.44, 15.18, 8.43, 8.31 ($CDCl_3$). **5d**, B′ = Gua^{iBu}, FAB MS, $C_{41}H_{54}N_5O_{13}PS_2$, calc. 919, $(M+H)^+$ 920, $(M-H)^-$ 917; ^{31}P NMR, δ (ppm) 106.88, 106.52 (CD_3CN); ^{13}C NMR, δ (ppm) 178.85, 177.71, 166.87, 166.34, 155.29, 147.79, 138.21, 90.54, 90.34, 86.90, 86.49, 79.71, 76.37, 73.68, 73.13, 71.48, 71.18, 69.41, 69.30, 54.82, 54.70, 54.45, 54.29, 37.16, 36.90, 36.37, 31.25, 30.80, 28.78, 28.65, 25.01, 23.74, 23.60, 18.87, 16.74, 16.65, 16.44, 9.55 ($CDCl_3$).

Protection of 3′-O-camphanoyl-N^2-^{i}Bu-deoxyguanosine-5′-O-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) at the O6 Site with Diphenylcarbamoyl Chloride—Synthesis of Compound 4e

To a magnetically stirred solution of 3′-*O*-camphanoyl-N^2-^{i}Bu-deoxyguanosine-5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) (0.345 mmol, 250 mg) in pyridine (15 ml), *N,N*-diisopropylethylamine (0.1 ml, 0.51 mmol) and diphenylcarbamoyl chloride (Ph_2NCOCl, 0.16 g, 0.7 mmol) were added (under dry argon) at room temperature. The mixture was stirred for 2 h, concentrated, dissolved in chloroform (1.5 ml), and applied onto a silica gel column (25 × 3 cm). The column was eluted with chloroform-methanol (the methanol content 0–2%). Appropriate fractions were collected (Rf 0.87, $CHCl_3$:MeOH, 9:1) and evaporated under reduced pressure to give the product **4e** in ca. 80% yield. FAB MS: $C_{44}H_{51}N_6O_{10}PS_2$, calc. 918, $(M+H)^+$ 919, $(M-H)^-$ 917; ^{31}P NMR, δ (ppm) 106.68, 106.65 ($CDCl_3$); ^{13}C NMR, δ (ppm) 177.80, 175.01, 166.88, 156.14, 154.22, 151.94, 150.29, 142.76, 141.67, 129.07, 126.84, 121.63, 90.55, 85.11, 83.34, 79.47, 76.36, 75.75, 68.84, 54.76, 54.43, 36.00, 36.38, 36.11, 30.66, 28.81, 25.05, 23.56, 19.25, 19.18, 16.76, 16.69, 9.59 ($CDCl_3$).

Open Column Separation of Camphanoylated Diastereomers of 4 or 5

A 250–300 mg sample of a camphanoylated monomer **4** or **5** in 2 ml of appropriate eluent was applied onto a column (35 × 6 cm) containing 80–100 *g* silica gel (Merck 60H). The column was eluted with 1000–1500 ml of ethyl acetate-butyl acetate-benzene (2:2:1 v/v/v for the T and $dG^{iBu,\ DPC}$ derivatives, 2:1:1 v/v/v for the rA^{Bz} and U derivatives) and fractions of 4–5 ml were collected. TLC control of the eluate was performed on HP-TLC plates (double development, see Table 1). Appropriate fractions were combined and the solvent was evaporated under reduced pressure. Typically, the applied material was recovered in ca. 80% yield. The diastereomeric purity of resulting fractions was assessed by ^{31}P NMR spectroscopy.

Synthesis of Dinucleoside 3′,5′-phosphorothioates 7

To 5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) monomers **4d**, **5a**, **5b**, or **5f** (15–20 mg, 0.02 mmol, dried in a vacuum dessicator for 24 h) and 5′-*O*-DMT-thymidine (3 molar equivalents), 600 μl of dry acetonitrile and 5 μl of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 1.1 molar equivalent) were added under dry argon. After 2 h, the reaction was complete. The mixture was concentrated under reduced pressure and treated with concentrated ammonia solution (2 ml) for 2 h to remove the acyl group. For the derivatives of **5b** and **5f**, where the benzoyl group was used for base protection, heating for 15 h at 55°C was necessary. After removal of solvent, the DMT moiety was removed with 50% aqueous acetic acid for 1.5 h at room temperature, followed by evaporation under reduced pressure. The identity of resulting products **7** was confirmed in HPLC experiments by co-injection with corresponding deprotected dinucleotides obtained using standard phosphoramidite protocol with sulfurization of the P^{III} intermediate with 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent[21]). Conditions: C18 column, 250 × 4.6 mm, 5 μm; elution with a gradient of 0.1 M TEAB, pH 7.3 to 20% CH_3CN in 0.1 M TEAB over 20 min. The stereochemistry was determined by RP-HPLC either directly ($T_{PS}T$ from **4d**), or after enzymatic digestion with svPDE[22] and Nuclease P1[23] ($T_{PS}rA$ from **5a**, **5b**, or **5f**) (*vide infra* and Fig. 4S, Supporting Information). The HPLC conditions as above; the retention times: ca. 15.94 and 16.56 min for R_P and S_P diastereomers of $T_{PS}T$, and, assigned in this work, 18.04 and 18.84 min for R_P and S_P diastereomers of $T_{PS}rA$, respectively.

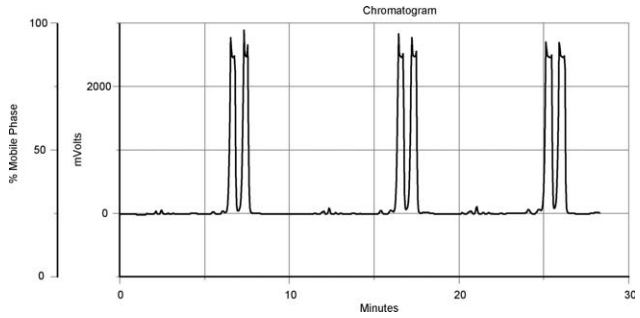


Fig. 1. Chromatogram from HPLC separation of 5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)-3′-*O*-(2-naphthoyl)-thymidine **4b**; consecutive profiles for separation of 3, 4, and 5 mg samples dissolved in 150–250 μl of chloroform (the detector overloaded). Conditions: a Hyperprep HS silica gel column 250 × 10 mm^2, 8 μm, mobile phase: ethyl acetate:hexane 52:48 v/v, isocratically, a flow rate 8 ml/min, retention times 6.7 and 7.5 min.

Hydrolysis of 7 with svPDE

The reaction mixture (20 μL) containing 25 mM Tris-Cl (pH 8.5), 5 mM $MgCl_2$, 1μL of the svPDE suspension, and dinucleoside 3′,5′-phosphorothioate **7** (0.2 OD) was incubated for 24 h at 37°C. Then, the sample was heat-denatured (for 1 min at 95°C) and analyzed by HPLC, similar conditions as above.

Hydrolysis of 7 with Nuclease P1

The reaction mixture (20 μL) containing 100 mM Tris-Cl (pH 7.2), 1 mM $ZnCl_2$, 2 μL of nuclease P1 suspension, and dinucleoside 3′,5′-phosphorothioate **7** (0.2 OD) was incubated for 24 h at 37°C. Then, the sample was heat-denatured (for 1 min at 95°C) and analyzed by HPLC, similar conditions as above.

Synthesis of Thymidine-5′-O-(α-thiodiphosphate)

5′-OTP-T monomer **4d** ("fast"/"slow" 86:14, 105 mg, 167 μmol) was mixed with anhydrous crystalline H_3PO_4 (49 mg, 501 μmol) and dried under high vacuum for 24 h. The reaction was carried in dry *N*-methylpyrrolidone (3 ml) in the presence of DBU (270 μl in 1 ml of acetonitrile; 1800 μmol, containing ca. 65 ppm of water as determined by Karl Fischer method) under dry argon for 2 h at room temperature. The product **9** was formed in ca. 32% yield (^{31}P NMR: δ (ppm, a few drops of d_6-benzene added for the lock of the instrument) 46.80, d, $^2J_{P\text{-}P}$ = 30 Hz, 46.64, d, $^2J_{P\text{-}P}$ = 30 Hz, for α-P atoms; δ (ppm) −6.66, d, $^2J_{P\text{-}P}$ = 30 Hz, −6.89, d, $^2J_{P\text{-}P}$ = 30 Hz, for β-P atoms). Despite of extensive drying of the substrates, the mixture contained 3′-*O*-camphanoyl-thymidine-5′-*O*-thiophosphate, i.e., the product of hydrolysis of **4d** (δ 50.21 ppm, 62%). A few OD units of compound **9** (carrying the camphanoyl residue) was isolated by RP-HPLC and analyzed by MALDI TOF MS ($C_{20}H_{28}N_2O_{13}P_2S$, calc. 598, $(M-H)^-$ 597). The remaining mixture was treated with equal volume of concentrated ammonium hydroxide to remove the base-labile protecting groups. The ammonia was removed under reduced pressure and a ^{31}P NMR spectrum was recorded in *N*-methylpyrrolidone solution with a few drops of d_6-benzene added. The ^{31}P NMR spectrum (proton decoupled, sweep width 64,000 Hz, time domain 65,536 points, acquisition time 0.51 s) contained the following resonances: δ (ppm) 42.41, d, $^2J_{P\text{-}P}$ = 37 Hz, 41.61, d, $^2J_{P\text{-}P}$ = 39 Hz, for α-P atoms; −5.53, d, $^2J_{P\text{-}P}$ = 37 Hz, −5.71, d, $^2J_{P\text{-}P}$ = 39 Hz, for β-P atoms; 46.79 for TMPS.

Synthesis of Thymidine-5′-O-(β,γ-methylene-α-thiotriphosphate) (11)

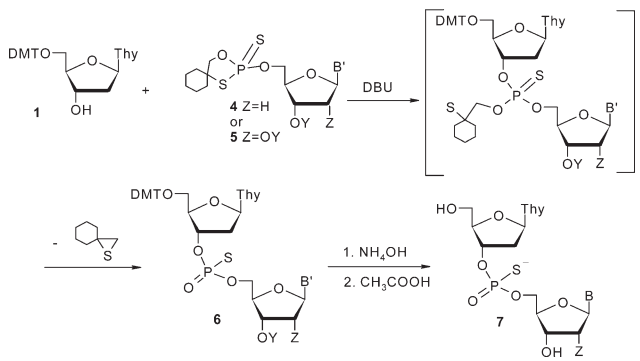
The monomer **4d** (fast/slow 1:10, 23 mg, 37 μmol) was mixed with anhydrous methylenediphosphonic acid (20 mg, 110 μmol) and dried under high vacuum for 24 h. The reaction was carried in dry acetonitrile (3 ml) in the presence of DBU (77 μl; 518 μmol, containing ca. 35 ppm of water as determined by Karl Fischer method) under dry argon for 4 h at room temperature. The ^{31}P NMR spectrum (proton decoupled, sweep width 7142 Hz, time domain 32 768 points, acquisition time 2.3 s, double zero-filling) contained resonances at δ 42.40 ppm (d, $^2J_{P\text{-}P}$ = 37 Hz) and 42.27 ppm (d, $^2J_{P\text{-}P}$ = 36 Hz) for α-P atoms, δ 12.35 ppm (d, $^2J_{P\text{-}P}$ = 3.5 Hz) and 12.21 ppm (d, $^2J_{P\text{-}P}$ = 3.5 Hz) for γ-P atoms, and δ 7.57 ppm (dd, $^2J_{P\text{-}P}$ = 36 Hz and $^2J_{P\text{-}P}$ = 3.5 Hz) for β-P atom of the main diastereomer (the resonances for the second diastereomer were too small to be precisely analyzed). Ca. 5% of the product **10** carrying the camphanoyl residue was isolated by RP-HPLC and analyzed by MALDI TOF MS ($C_{21}H_{31}N_2O_{15}P_3S$, calc. 676, $(M-H)^-$ 675). The remaining solution was concentrated to dryness and treated with concentrated ammonia (1.5 ml) for 1.5 h at room temperature. After evaporation, the residue was dissolved in water, and the product was isolated by means of ion exchange chromatography on Sephadex A-25 resin (pH 7, linear gradient 0.3 M to 1 M triethylammonium bicarbonate). The product **11** (7.8 mg, 43%) was analyzed by MALDI TOF MS ($C_{11}H_{19}N_2O_{12}P_3S$, calc. 496, $(M-H)^-$ 495).

RESULTS AND DISCUSSION

5′-OTPs of Deoxyribonucleoside Series

To obtain resolvable P-diastereomers of acylated monomers **4** and **5**, acyl chlorides possessing a bulky substituent were selected. It was assumed that a sterically demanding moiety in the 3′-position of the monomers would sterically interact with the oxathiaphospholane ring (the repulsion is depicted by a two-head arrow, Scheme 1) giving rise to differentiation of the chromatographic mobility of S_P and R_P forms. This idea arose from molecular modelling performed for a derivative of thymidine monomer **4a** carrying a biphenyl-4-carbonyl moiety at the 3′ position. Conformational search for the S_P and R_P diastereomers was performed with a Spartan'06 program using the MMFF force field method.[24] Five most stable conformers of each diastereomer were further optimized under the gas phase conditions with the Gaussian 03 program[25] using the *ab initio* HF/6-31G(d) method. Remarkably, the energies calculated for the most stable conformers of the S_P and R_P diastereomers differed by ca. 10 kcal/mol. Assuming that these most stable conformers remain predominant during interaction with particles of silica gel (which is achiral chromatography material) one may expect the formation of the diastereomeric monomer·silica complexes of the energies of sorption/desorption sufficiently different to allow for the separation of the P-diastereomers.

A general method of synthesis of the monomers **4** (Scheme 1, Y = acyl, Z = H) involves acylation of the 3′-OH group in 5′-*O*-DMT-*N*-protected deoxyribonucleosides (**1**, B′ = Thy, Ade^{Bz}, Cyt^{Bz}, or Gua^{iBu}) with an acyl chloride in pyridine (61–92% yield). The resulting acylated nucleosides **2** were detritylated with *p*-toluenesulfonic acid in a CH_2Cl_2-MeOH mixture (9:1) and crude **3** were purified by silica gel



Scheme 2. Reaction of the oxathiaphospholane monomers **4** or **5** with 5′-*O*-DMT-thymidine (**1**), leading to the corresponding protected dinucleoside phosphorothioates **6**, further converted into deprotected dinucleotides **7**.

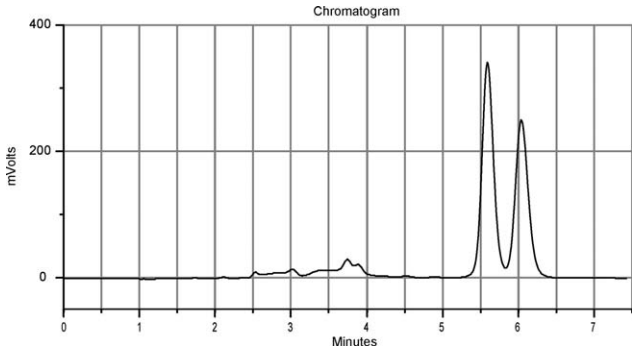
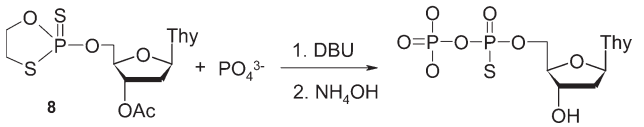


Fig. 2. Chromatogram from HPLC separation of 5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-di(2-naphthoyl)-N^6-benzoyl-adenosine **5f** (0.1 mg, analytical run). Conditions: Hyperprep HS silica gel column 250 × 10 mm, 8 μm, ethyl acetate:hexane 52:48 v/v, isocratically, flow rate 8 ml/min, retention times 5.6 and 6.0 min.



Scheme 3. Synthesis of thymidine 5′-*O*-(α-thiodiphosphate).

chromatography with 50–88% yield. Finally, phosphitylation with 2-chloro-4,4-pentamethylene-1,3,2-oxathiaphospholane in the presence of anhydrous *N,N*-diisopropylethylamine and elemental sulfur[16,17] furnished corresponding 5′-OTPs **4** with 57–75% yield (30% for 1-adamantanecarbonyl derivative, *vide infra*).

For the first selection, three 3′-*O*-acylated thymidine derivatives **2a–c** (B′ = Thy, Z = H) were obtained using biphenyl-4-carbonyl, 2-naphthoyl or 1-adamantanecarbonyl chlorides, respectively. They were further converted into corresponding monomers **4a–c**, and their structures were confirmed by FAB MS and ^{31}P NMR. The latter method revealed the presence of two closely located resonances of similar intensity at δ 105.7 ÷ 107.2 ppm (Δδ = 0.33 ÷ 0.59 ppm; $CDCl_3$ or CD_3CN), characteristic for the pairs of corresponding diastereomers. For the monomer carrying the adamantanecarbonyl moiety (**4c**, B′ = Thy), good HPTLC separation of the P-diastereomers was observed (Table 1), but the monomer was found to be unstable during chromatography (after rechromatography only 30% of the material was recovered). For biphenyl-4-carbonyl-(**4a**) and 2-naphthoyl-(**4b**) derivatives, the HPTLC showed even better separation of the P-diastereomers, however, very low solubility of these monomers in the mobile phase rendered the open-column preparative separation inefficient. Therefore, semipreparative HPLC on silica gel was employed and for **4b** baseline separation was achieved (Fig. 1) for sample loads of 3–5 mg per run.

The above observations prompted us for synthesis of 5′-OTPs derivatives of dC^{Bz} , dA^{Bz}, and dG^{iBu} carrying the 2-naphthoyl moiety at the 3′ position. The deoxycytidine monomer was obtained with good yield, but none of numerous developing systems used for HPTLC analyses allowed for separation of the P-diastereomers. Moreover, extremely poor solubility of naphthoylated intermediates **3** (B′ = Ade^{Bz} or Gua^{iBu}, Z = H) in non-nucleophilic solvents (required for the phosphitylation step) forced us to abandon the synthesis of the corresponding 5′-OTP derivatives. Thus, another acylating reagent, namely (-)-camphanoyl chloride, was used to obtain oxathiaphospholane monomers **4d–h** (B′ = Thy, $Gua^{iBu,DPC}$, Gua^{iBu}, Ade^{Bz}, Cyt^{Bz}, respectively, Y = (−)-camphanoyl). Their structures were confirmed by FAB MS and ^{31}P NMR. The 5′-OTP derivative of dG was prepared in two forms, i.e., with or without the diphenylcarbamoyl protecting moiety (DPC) at the O6 position.[16] Several attempts to separate compounds **4d–h** into fast- and slow-eluting P-diastereomers by silica gel column chromatography have been undertaken, but with a different degree of success. The OTP derivatives of dA^{Bz}, dC^{Bz}, and dG^{iBu} (**4f–h**, the last one without the DPC group at the O6 position in guanine) could not be separated. The separation was achieved for the thymidyl monomer **4d** (Table 1) and for $dG^{iBu,DPC}$ derivative **4e**. After optimization of a silica:monomer ratio, dimensions of the column and eluent composition, the fractions enriched in fast- and slow-eluting components (of diastereomeric ratio 7:1 to

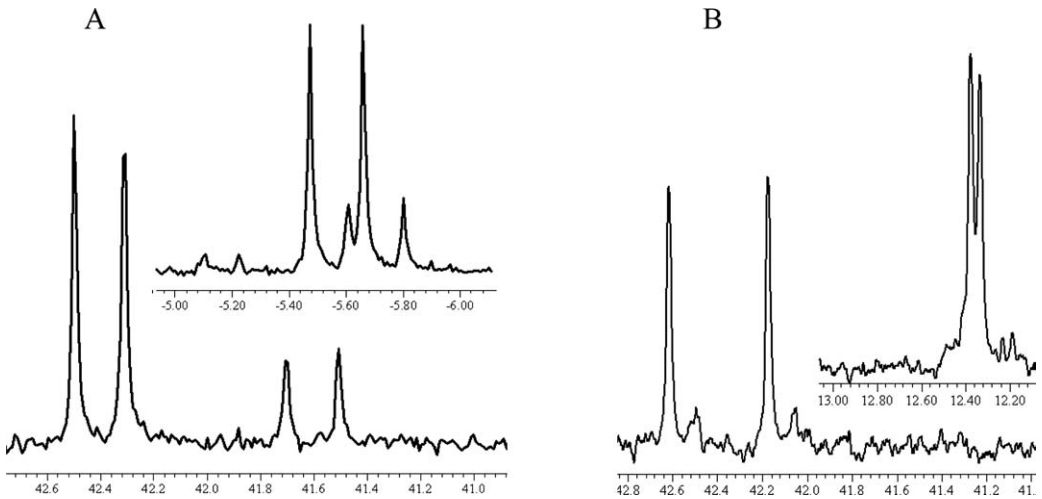
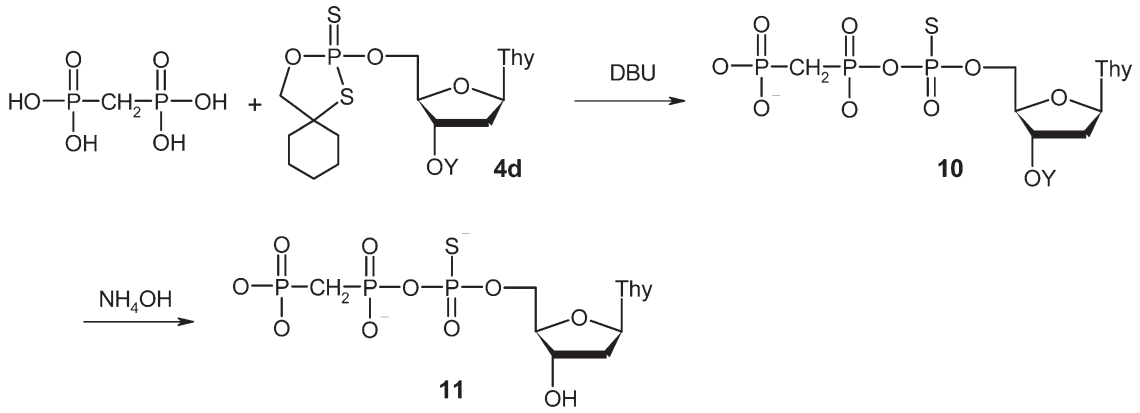


Fig. 3. (**A**) Two regions (δ 41 to 43 ppm, α-P atom; δδ−5 to −6 ppm, β-P atom) of the ^{31}P NMR spectrum (500 MHz, proton decoupled) recorded for crude TDPαS obtained in the reaction of **4d** (fast/slow 6:1) with H_3PO_4. (**B**) Two regions (δ 42 to 42.8 ppm, α-P atom; δ 12.0 to 12.6 ppm, γ-P atom) of the ^{31}P NMR spectrum (200 MHz, proton decoupled) recorded for crude **10** obtained in reaction of **4d** (fast/slow 1:10) with a methylenediphosphonate anion.



Scheme 4. Conversion of **4d** (diastereomerically enriched mixture slow/fast 10:1, Y = (−)-camphanoyl) into thymidine-5′-*O*-(β,γ -methylene-α-thiotriphosphate) **11**.

10:1) were collected, which after rechromatography provided diastereomerically pure species.

Fast-enriched fraction of **4d** (Scheme 2, B′ = Thy, Z = H; 65:35 diastereomeric composition by ^{31}P NMR) was used for the correlation between the relative chromatographic mobility of P-diastereomers and absolute configuration of the resulting condensation products. The sample was reacted with 5′-*O*-DMT-thymidine to yield the corresponding protected dinucleotide **6**, further converted into dinucleoside 3′,5′-phosphorothioate **7** (B = Thy, Z = H). Based on the literature data, under typical conditions of RP-HPLC (a C18 column, elution with a gradient of 0.1 M triethylammonium bicarbonate (TEAB) and acetonitrile), all 16 di(deoxyribonucleoside) phosphorothioates of R_P-configuration have shorter retention times (fast-eluting species) than their S_P-counterparts.[26,27] Since fast- and slow-eluting diastereomers of **4d** furnished slow- and fast-**7**, respectively, one can conclude that fast-**4d** and slow-**4d** yield the dinucleotides **7** of S_P and R_P absolute configuration, respectively. The stereoselectivity of condensation was not lower than 98%.

5′-OTPs of Ribonucleoside Series

Similar efforts were undertaken to obtain 5′-OTP-derivatives of appropriately protected 2′,3′-*O,O*-dicamphanoylated ribonucleosides (Scheme 1, **5a–d**, B′ = Ura, Ade^{Bz}, Cyt^{Bz}, Gua^{iBu}, respectively). The separation of P-diastereomers of **5a**, **5b**, and **5d** was achieved by means of HPLC on silica gel (see Supporting Information, Figs. 1S, 2S, 3S), while **5a** and **5b** could also be resolved using silica gel open-column chromatography. The corresponding derivative of cytidine (**5c**) could not be resolved, even by means of HPLC.

Amongst 2′,3′-*O,O*-dinaphthoylated monomers, the P-diastereomers of 5′-OTP derivative of adenosine (**5f**) were well separated using HPLC (Fig. 2). The analogous separation of naphthoylated uridine derivative **5e** was less efficient than that of camphanoylated **5a**.

To make the correlation between the chromatographic mobility of P-diastereomers of the 5′-OTPs of ribonucleoside series **5** (Scheme 2, Z = OY) and absolute configuration of corresponding condensation products, the monomers **5a** (slow, 100%), **5b** (two samples: slow, 100% and fast, 100%) and **5f** (fast, 100%) were condensed with 5′-*O*-DMT-thymidine. The products **6** (Z = OY) were deprotected to yield the corresponding dinucleoside phosphorothioates **7** (T_{PS}U or T_{PS}rA, Z = OH). Their enzymatic hydrolysis with S_P-specific Nuclease P1[23] and R_P-specific snake venom phosphodiesterase (svPDE)[22] (see Figs. 4S and 5S, Supporting Information) confirmed that slow-eluting isomers of **5a**, **5b**, and **5f** are the precursors of internucleotide bonds of R_P absolute configuration. This finding is analogous to the correlation performed for **4d** in the deoxyribonucleoside series. However, this correlation cannot be considered general, as even relatively small changes of the structure (e.g., lack of the pentamethylene substituent in the position 4 of the oxathiaphospholane ring) may reverse chromatographic mobility.[28] It should be noted that all the monomers **4** and **5** (with the exception of adamantanecarbonyl derivative **4c**) are stable for at least six months when stored in tightly closed vessels in a refrigerator.

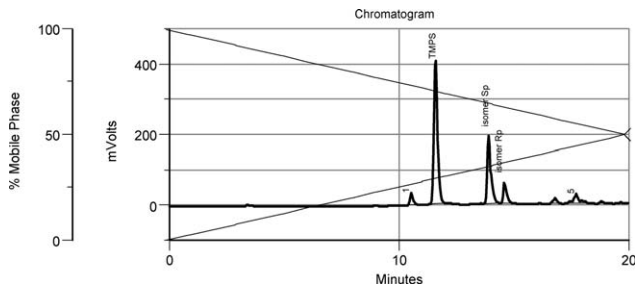


Fig. 4. Chromatogram from RP-HPLC analysis of crude TDPαS obtained in the reaction of **4d** (fast/slow 6:1) with H_3PO_4. Conditions: Alltima C18 column 250 × 4.6 mm, 5 μm, buffer A: 0.1 M TEAB, buffer B: 40% CH_3CN in 0.1 M TEAB, pH 7.5, gradient: 2.5% B/min., flow rate: 1 ml/min, UV detection at 260 nm.

Opening of the Oxathiaphospholane Ring with Anions of Phosphorus-Containing Acids

To date, the oxathiaphospholane method was used mostly for synthesis of stereodefined phosphorothiate analogs of DNA, where the electrophilic phosphorus centre in a 3′-*O*-oxathiaphospholane monomer was attacked by the 5′-OH group of the growing oligonucleotide. In an analogous reaction of 3′-OTP with fluoride anion a phosphorofluoridate derivative was formed, however, contrary to all so far known examples of 1,3,2-oxathiaphospholane ring opening condensations, the reaction proceeded with epimerization at P-atom.[29] As mentioned in the Introduction, NDPs and NTPs are biologically important compounds and their diastereomerically pure P-chiral analogs are useful tools in biochemi-

cal studies. Our earlier experiments[18] showed that 3′-*O*-acetyl-thymidine-5′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**8**, unseparated mixture of P-diastereomers) reacted with the phosphate and pyrophosphate anions to yield the corresponding 5′-*O*-(α-thiodiphosphate) (Scheme 3) and 5′-*O*-(α-thiotriphosphate), respectively.

To determine the stereochemistry of this type of reactions, diastereomerically enriched samples of **4d** were reacted with three anions of phosphorus-containing acids. In the first experiment, **4d** (containing 14% of the isomer slow) was reacted with anhydrous crystalline H_3PO_4 (three-fold molar excess) in the presence of DBU (11-fold molar excess) to form 3′-*O*-camphanoyl-thymidine-5′-*O*-(α-thiodiphosphate) (**9**). Dry *N*-methylpyrrolidone was used as a solvent because of very poor solubility of phosphoric acid in acetonitrile. The product was formed in ca. 32% yield (by ^{31}P NMR, Fig. 6S, Supporting Information), and contained ~ 20% of the minor isomer. The mixture was treated with concentrated ammonium hydroxide to remove the base-labile protecting groups. The ammonia was removed and for the resulting *N*-methylpyrrolidone solution of TDPαS (with a few drops of d_6-benzene added) a ^{31}P NMR spectrum was recorded. The resonances at δ 41 to 43 ppm for α-P atom and −5 to –6 ppm for β-P atom (Fig. 3A) indicate that the mixture contained the diastereomers in a ratio 79:21 indicating ≈90% stereoselectivity of the ring opening. The higher chemical shift for the α-P atom of the major isomer suggests that fast-**4d** yielded TDPαS of S_P configuration,[30] but because in Ludwig's article the reference spectra were recorded in H_2O/D_2O, additional RP-HPLC analysis was performed (Fig. 4), which confirmed that assignment.[30]

In next experiments, methylenediphosphonate (Scheme 4) and benzylphosphonate anions were used. The product of reaction of **4d** (diastereomerically enriched mixture slow/fast 10:1) with methylenediphosphonate anion, i.e., 3′-*O*-camphanoyl-thymidine-5′-*O*-(β,γ -methylene-α-thiotriphosphate) (**10**), was characterized by means of ^{31}P NMR and MALDI-TOF MS. The regions δ 42.0 to 42.8 ppm for α-P atom and δ 12 to 12.6 ppm for γ-P atom in a relevant ^{31}P NMR spectrum are presented in Figure 3B. In each region, the presence of two pairs of signals ($^2J_{P\text{-}P}$ = 36 Hz and) at 10:1 intensity ratio, i.e., identical to the diastereomeric composition of the substrate, indicates high stereoselectivity of the reaction. Compound **10** was deprotected with concentrated ammonia to yield thymidine-5′-*O*-(β,γ -methylene-α-thiotriphosphate) (**11**), further isolated on DEAE Sephadex A-25 (43% yield) and analyzed by MALDI-TOF MS.

Since virtually identical stereochemical outcome was observed for reaction of **4d** with benzylphosphonate anion ($PhCH_2PO_3^{2-}$, data not shown), one can conclude that monomers **4** can be used for highly stereoselective synthesis of P-chiral phosphorothioate analogs of nucleoside 5′-*O*-polyphosphates. Experiments on the use of monomers **5** as well as on the application of some other nucleophiles, e.g., pyrophosphate and zolendronate anion, are in progress.

CONCLUSION

Selected 3′-*O*-acylated deoxyribonucleoside- and 2′,3′-*O,O*-diacylated ribonucleoside-5′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)s, carrying bulky acyl substituent(s), can be separated into P-diastereomers either by open column chromatography or semi-preparative HPLC on silica gel columns. It was found that all investigated slow-eluting isomers of **4** and **5** are the precursors of internucleotide bonds of R_P absolute configuration. Compound **4d** was reacted with three anions of phosphorus-containing acid, furnishing thymidine 5′-*O*-(α-thiodiphosphate), thymidine-5′-*O*-(β,γ -methylene-α-thiotriphosphate) and thymidine-5′-*O*-(benzylphosphono-α-thiophosphate) in a highly stereoselective manner (≥90%).

ACKNOWLEDGMENTS

A scholarship founded by Prof. A. Legocki, the former President of Polish Academy of Sciences to A. Tomaszewska is gratefully acknowledged.

LITERATURE CITED

1. Biggs J, Hersperger E, Steeg PS, Liotta LA, Shearn A. A Drosophila gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. Cell 1990;63:933–940.
2. Bominaar AA, Molijin AC, Pestel M, Veron M, Van HPJ. Activation of G-proteins by receptor-stimulated nucleoside diphosphate kinase in *Dictyostelium*. EMBO J 1993;12:2275–2279.
3. Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, Sobel ME. Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 1988;80:200–204.
4. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–5467.
5. Eckstein F. Phosphorothioate analogues of nucleotides—tools for the investigation of biochemical processes. Angew Chem Int Ed Engl 1983;22:423–439.
6. Eckstein F. Nucleoside phosphorothioates. Annu Rev Biochem 1985;54: 367–402.
7. Micklefield J. Backbone modification of nucleic acids: synthesis, structure and therapeutic applications. Curr Med Chem 2001;8:1157–1179.
8. Corradini R, Sforza S, Tedeschi T, Marchelli R. Chirality as a tool in nucleic acid recognition: principles and relevance in biotechnology and in medicinal chemistry. Chirality 2007;19:269–294.
9. Stein CA, Krieg AM, editors. Applied antisense oligonucleotide technology. NewYork: Wiley; 1998.
10. Eckstein F. Phosphorothioate oligodeoxynucleotides: what is their origin and what is unique about them? Antisense Nucleic Acid Drug Dev 2000; 10:117–121.
11. Wang L, Chen S, Xu T, Taghizadeh K, Wishnok JS, Zhou X, You D, Deng Z, Dedon PC. Phosphorothioation of DNA in bacteria by dnd genes. Nat Chem Biol 2007;3:709–710.
12. Frey PA, Sammons RD. Bond order and charge localization in nucleoside phosphorothioates. Science 1985;228:541–545.
13. Sheu KF, Richard JP, Frey PA. Stereochemical courses of nucleotidyltransferase and phosphotransferase action. Uridine diphosphate glucose pyrophosphorylase, galactose-1-phosphate uridylyl transferase, adenylate kinase, and nucleoside diphosphate kinase. Biochemistry 1979;18:5548–5556.
14. Blatter WA, Knowles JR. Stereochemical course of phosphokinases. The use of adenosine [γ-(S)-^{16}O,^{17}O,^{18}O]triphosphate and the mechanistic consequences for the reactions catalyzed by glycerol kinase, hexokinase, pyruvate kinase, and acetate kinase. Biochemistry 1979;18:3927–3933.
15. Owczarek A, Kaczmarek R, Mikołajczyk B, Wasilewska E, Korczyński D, Baraniak J, Koziołkiewicz M, Stec WJ, Brenner C. Stereochemical analysis of diastereomeric 1,3-bis(adenosine-5′-*O*-phosphorothioyl)glycerols. Nucleosides Nucleotides Nucleic Acids 2003;22:797–799.
16. Stec WJ, Karwowski B, Boczkowska M, Guga P, Koziołkiewicz M, Sochacki M, Wieczorek M, Błaszczyk J. Deoxyribonucleoside 3′-*O*-(2-thio- and 2-oxo-"spiro"-4,4-pentamethylene-1,3,2-oxathiaphospholane)s: monomers for stereocontrolled synthesis of oligo(deoxyribonucleoside phosphorothioate)s and chimeric PS/PO oligonucleotides. J Am Chem Soc 1998;120:7156–7167.
17. Guga P, Stec WJ. Synthesis of phosphorothioate oligonucleotides with stereodefined phosphorothioate linkages. In: Beaucage SL, Bergstrom DE, Glick GD, Jones RA, editors. Current protocols in nucleic acid chemistry, Hoboken, NJ: Wiley; 2003. p 4.17.1–4.17.28.

18. Misiura K, Szymanowicz D, Stec WJ. Synthesis of nucleoside α-thiotriphosphates via an oxathiaphospholane approach. Organic Lett 2005;7: 2217–2220.
19. Misiura K, Szymanowicz D, Olesiak M, Stec WJ. DBU-assisted 1,3,2-oxathiaphospholane ring-opening condensation with selected O-, S-, N- and C-nucleophiles. Tetrahedron Lett 2004;45:4301–4305.
20. Okruszek A, Olesiak M, Balzarini J. The synthesis of nucleoside 5′-*O*-(1,1-dithiotriphosphates). J Med Chem 1994;37:3850–3854.
21. Iyer RP, Egan W, Regan JB, Beaucage SL. 3*H*-1,2-Benzodithiole-3-one 1,1-dioxide as an improved sulfurizing reagent in the solid-phase synthesis of oligodeoxyribonucleoside phosphorothioates. J Am Chem Soc 1990;112:1253–1254.
22. Eckstein F, Potter BVL, Connolly B. Synthesis and configurational analysis of a dinucleoside phosphate isotopically chiral at phosphorus. Stereochemical course of *Penicillium citrinum* nuclease P1 reaction. Biochemistry 1983;22:1369–1377.
23. Griffiths AD, Potter BVL, Eperon IC. Stereospecificity of nucleases towards phosphorothioate-substituted RNA: stereochemistry of transcription by T7 RNA polymerase. Nucleic Acids Res 1987;15:4145–4162.
24. Spartan'06 Wavefunction, Inc, 2006. Available at: www.wavefun.com.
25. Gaussian 03, Rev. D.01. Wallingford, CT: Gaussian, Inc.; 2004.
26. Stec WJ, Wilk A. Stereokontrollierte synthese von oligonucleosidphosphorothioaten. Angew Chem 1994;106:747–761.
27. Stec WJ, Wilk A. Stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s. Angew Chem Int Ed Engl 1994;33:709–722.
28. Stec WJ, Grajkowski A, Karwowski B, Koziołkiewicz M, Misiura K, Okruszek A, Wilk A, Guga P, Boczkowska M. Diastereomers of nucleoside 3′-*O*-(2-thio-1,3,2-oxathia(selena)phospholanes): building blocks for stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s. J Am Chem Soc 1995;117:12019–12029.
29. Misiura K, Szymanowicz D, Stec WJ. Synthesis and chemical and enzymatic reactivity of thymidine 3′-*O*- and 5′-*O*-phosphorofluoridothioates. Chem Commun 1998;515–516.
30. Ludwig J, Eckstein F. Rapid and efficient synthesis of nucleoside 5′-*O*-(1-thiotriphosphates), 5′-triphosphates and 2′,3′-cyclophosphorothioates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one. J Org Chem 1989; 54:631–635.