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Cyclization versus oligomerization of S_P- and R_P-5′-OH-N^4-benzoyl-2′-deoxycytidine-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane)s

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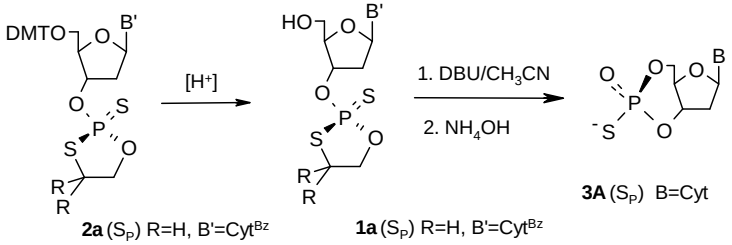
Dedicated to Professor David Shugar on the occasion of his 80th birthday

Abstract—The S_P-isomer of 5′-OH-N^4-benzoyl-2′-deoxycytidine-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) undergoes DBU-promoted intramolecular cyclization providing as a sole product S_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate. Unexpectedly, the R_P-counterpart yields a mixture of products consisting of R_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate and macrocyclic oligo(deoxycytidine phosphorothioate)s. The results of molecular modeling indicate that the dychotomy observed for the R_P substrate may result from remarkably higher energy of the corresponding transition states, caused by the presence of bulky 'spiro' pentamethylene substituent at the position C4 in the oxathiaphospholane ring.

1. Introduction

Nucleoside cyclic 3′,5′-*O*,*O*-phosphorothioates[1] (cNMPS, **3**, Scheme 1) are valuable tools for studying the mechanism of action of enzymes involved in the metabolism of cyclic nucleotides and are potent agonists or antagonists of the latter compounds. Due to assymetry of the phosphorus atom, cNMPS exist in the form of S_P and R_P diastereomers, which usually have markedly different biological properties.[1,2] The first reported synthesis of cNMPS involved cyclization of nucleoside 5′-*O*-(bis(4-nitrophenyl)phosphorothioate)s under treatment with *t*-BuOK in DMF, followed by hydrolytic removal of the remaining 4-nitrophenoxyl group. The resulting cNMPS were then chromatographically separated into individual diastereomers.[3] Reported to date stereocontrolled methods of synthesis of R_P- or S_P-cNMPS rely upon preparation of appropriately protected diastereomerically pure nucleoside cyclic 3′,5′-*O*,*O*-phosphoranilidates or phosphoranilidothioates and their stereoretentive conversion into corresponding



Scheme 1.

Keywords: Cyclic oligonucleotides; Phosphorothioate analogues of DNA; Oxathiaphospholane method.
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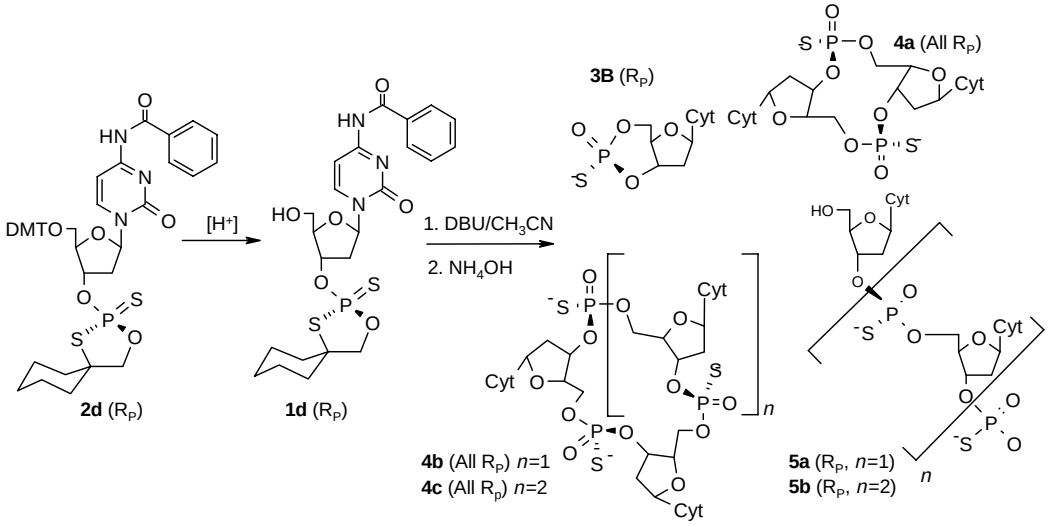


Scheme 2.

phosphorothioates using NaH/CS_2 or NaH/CO_2, respectively.[4] Here we describe highly efficient synthesis of S_P- and R_P-deoxycytidine cyclic 3′,5′-*O*-*O*-phosphorothioates (**3A** and **3B**, B = Cyt) in a stereospecific manner by oxathiaphospholane approach, as well as unexpected formation of stereodefined macrocyclic and linear oligonucleotides of general formula **4** and **5**, respectively (see Scheme 2). The macrocyclic oligo(deoxycytidine phosphorothioate)s **4** belong to the family of circular oligonucleotides, possessing interesting DNA, RNA, and protein binding properties and potential therapeutic applications.[5] To the best of our knowledge, the only two published examples of stereodefined macrocyclic phosphorothioate oligonucleotides were chimeric R_P-, and S_P-cyclic diribonucleotide c($G_{PS}G_{PO}$), which can be considered the analogues of cyclic diguanylic acid involved in regulatory system of cellulose synthesis in *Acetobacter xylinum*,[6] and R_P,R_P- and S_P,R_P-cyclic di(deoxycytidine phosphorothioate)s obtained by Battistini et al.[7]

2. Results and discussion

In the oxathiaphospholane method, which was originally designed for the stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s, chromatographically separated P-diastereomers of 5′-*O*-DMT-nucleoside-3′-*O*-(2-thio-1,3,2-oxathiaphospholane)s (**2a**,**b**, R = H) and their ring-substituted analogues (**2c**,**d**, R,R = –$(CH_2)_5$–) are used.[8] Their reaction with 5′-OH-nucleoside component (usually performed in CH_3CN solution in the presence of strong non-nucleophilic base, preferably DBU), proceeds according to the adjacent type mechanism, where pseudorotation of a P^V intermediate (such a process is marked with Ψ in Scheme 1S, Supplementary data) results in retention of configuration at the phosphorus atom.[8c,9] We anticipated that diastereomerically pure oxathiaphospholane substrates after acidolytic removal of the 5′-*O*-dimethoxytrityl protecting group may undergo a DBU-promoted intramolecular reaction in analogous stereospecific manner to give the desired cNMPS (see Scheme 1). Notably, intramolecular ring-opening reactions of oxathiaphospholanes were not reported in the literature, albeit it was known from our earlier work that structurally related N^6-benzoyl-2′-*O*-tetrahydropyranyl-adenosine-5′-*O*-(2-thio-1,3,2-dithiaphospholane) can be effectively transformed into adenosine cyclic 3′,5′-*O*,*O*-phosphorodithioate via intramolecular cyclization by treatment with *t*-BuOK in DMF solution, followed by removal of protecting groups.[4d] As model compounds we chose diastereomerically pure S_P- and R_P-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane)s (**1a** and **1b**, respectively, $B = Cyt^{Bz}$, R = H), which were prepared from chromatographically separated S_P- and R_P-5′-*O*-DMT-precursors **2a** and **2b**, by treatment with *p*-toluenesulfonic acid in methylene chloride. The reactions of **1a** and **1b** were performed on a 66 μmol scale by addition of equimolar amount of DBU into magnetically stirred solutions of the substrates in anhydrous acetonitrile. After 5 min at room temperature ^{31}P NMR spectra showed quantitative formation of single products. After ammoniacal deprotection these products were identified by ^{31}P NMR and HPLC comparison with genuine sample,[4d] as S_P- and R_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate (**3A** and **3B**). Thus, the reaction of intramolecular cyclization occurred with retention of configuration at phosphorus, most likely by the mentioned earlier adjacent mechanism.

The results obtained for **1a** and **1b** fulfilled our expectations, but for practical reasons we intended to use their ‘spiro’ analogues possessing 4,4-pentamethylene substituent in the oxathiaphospholane ring (**1c** and **1d**, $B = Cyt^{Bz}$, R,R = –$(CH_2)_5$–), which were known for considerably better chromatographic properties of their precursors **2c** and **2d** in respect to their separation into pure P-diastereoisomers.[8d] The reactions of diastereoisomerically pure S_P-**1c** and R_P-**1d** (obtained by detritylation of **2c** and **2d**, respectively) were performed exactly as for **1a**,**b**. While the S_P-substrate yielded the expected cyclic 3′,5′-*O*,*O*-phosphorothioate **3A** virtually quantitatively, the R_P-counterpart provided a mixture of products with several resonances (difficult for precise integration) within a range 55–57 ppm in a ^{31}P NMR spectrum. After ammoniacal deprotection the products were separated by RP HPLC and identified by MALDI-TOF MS (see Table 1). Approximate quantification of their distribution, expressed as percentage of the consumption of

Table 1. Identification and quantification of HPLC-separated products of cyclization/oligomerization of R_P-**1d** (DBU/CH_3CN procedure)

Compounds	HPLC t_R (min)[a]	Content (%)[b]	Molecular weight[c]	^{31}P NMR δ (ppm)
3B	10.61	41.8	304/305.3	55.23
4a	21.40	26.3	609/610.5	54.68
4b	26.39	20.1	915/915.8	55.76
4c	22.97	6.1	1222/1221.0	55.79
4d	23.45	1.7	1526/1526.3	ND[d]
4e	24.52	1.1	1833/1831.5	ND[d]
5a	14.27	1.7	627/628.5	ND[d]
5b	18.33	1.2	933/933.8	ND[d]

[a] HPLC was performed using Econosphere C_{18} (5 μ) 4.6×250 mm column (Alltech) with a linear gradient of acetonitrile in 0.1 M TEAB: 0–20 min—0.5%/min; 20–32 min—0.3%/min; flow 1 mL/min.
[b] Calculated from electronically integrated chromatograms (UV detection at 255 nm).
[c] Calculated/measured *m/z*; by MALDI-TOF MS, negative ion mode (Voyager™ Elite), calculated for free acids (Da).
[d] Not determined.

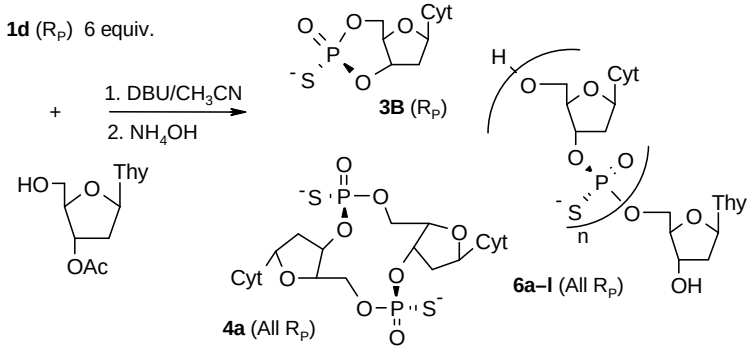
Table 2. HPLC-separation and identification of products of cyclization/oligomerization of R_P-**1d** in the presence of 3′-*O*-acetylthymidine

Compounds	HPLC t_R (min)[a]	Quantity of products[b]	Molecular weight	
			Measured[c]	Calculated[d]
3B	12.42	30.5	304.1	305.25
4a	30.84	15.1	610.0	610.50
6a ($n=1$)	26.00	13.0	546.9	547.48
6b ($n=2$)	31.44	18.0	852.3	852.73
6c ($n=3$)	35.30	7.9	1157.8	1157.98
6d ($n=4$)	38.44	16.1	1463.1	1463.20
6e ($n=5$)	41.12	3.9	1768.0	1768.49
6f ($n=6$)	43.33	3.0	2073.2	2073.70
6g ($n=7$)	45.26	2.1	2378.5	2378.95
6h ($n=8$)	47.01	1.5	2683.5	2684.20
6i ($n=9$)	48.61	1.1	2989.9	2989.45
6j ($n=10$)	50.22	1.0	3293.1	3294.70
6k ($n=11$)	51.82	1.0	3599.2	3599.95
6l ($n=12$)	53.20	0.8	3903.6	3905.20

[a] HPLC was performed using PTH C_{18} (5 μ) 2.1×220 mm column (Brownlee) with a linear gradient of acetonitrile in 0.1 M TEAB: 0–60 min—0.47%/min; flow 0.3 mL/min (UV detection at 255 nm).
[b] Given in optical density (measured at 260 nm).
[c] *m/z*; Measured by MALDI-TOF MS (Voyager™ Elite).
[d] Calculated for free acids (Da).

the starting material **1d**, was accomplished by integration of peaks in the HPLC profile. The inspection of the data (listed in Table 1) reveals that the main product of self-condensation of **1d** (41.8% conversion of the starting material) was R_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate (**3B**) as proved by MALDI-TOF MS, HPLC and ^{31}P NMR. In addition to **3B**, several oligomeric products were identified, including macrocyclic (**4a–e**) and linear oligo(deoxycytidine phosphorothioate)s (**5a**,**b**) (see Scheme 2). Obviously, expected highly favorable entropy of intramolecular cyclization did not assure full regioselectivity. The major macrocyclic products include those containing two (**4a**, 26.3% of total UV absorption), three (**4b**, 20.1%) and four deoxycytidine phosphorothioate units (**4c**, 6.1%). Minor products containing five (**4d**, 1.7%) and six units in macrocyclic ring (**4e**, 1.1%) were also identified. In fact, the formation of 'macrocyclic' fraction consumed over 55% of the oxathiaphospholane substrate. The formation of macrocyclic products **4** can be explained by conversion of substrate **1d** into corresponding linear oligomers (all with the oxathiaphospholane function at the 3′-end) followed by their inter- or intramolecular cyclization. In addition to the macrocyclic products, two minor linear products were isolated and identified by HPLC/MS as 3′-*O*-phosphorothioylated dinucleotide (**5a**, 1.7%) and trinucleotide (**5b**, 1.2%) (see Table 1 and Scheme 2). It seems reasonable to assume that the 3′-terminal oxathiaphospholane function was hydrolyzed with traces of moisture yielding inert phophorothioate group in **5a**,**b**. Similar results were obtained when a N^2-isobutyryl-deoxyguanosine 3′-*O*-(2-thio-'spiro'-4,4-pentamethylene-1,3,2-oxathiaphospholane) (a mixture of P-diastereoisomers ca. 1:1) was a substrate for DBU-promoted cyclization. After ammoniacal deprotection the obtained mixture was analyzed by ^{31}P NMR (in D_2O) showing the presence of four major products: S_P-deoxyguanosine cyclic 3′,5′-*O*,*O*-phosphorothioate[4c] (δ 52.8 ppm, 50% of total integral), its R_P-epimer (δ 54.6 ppm, 16%), deoxyguanosine analogue of 'dimeric' compound **4a** (δ 55.2 ppm, 12%), and deoxyguanosine 3′-*O*-phosphorothioate (δ 47.4 ppm, 5%). These products were separated on HPLC and their structures were confirmed by MALDI-TOF MS analysis.

The observed predominant formation of macrocyclic oligo(deoxycytidine phosphorothioate)s **4a–e** prompted us to perform the reaction in the presence of limited amount of 3′-*O*-acetylthymidine (1/6 mol equiv) acting as a '3′-end forming unit' to prevent 'macrocyclization' and facilitate the formation of linear products. The deprotected products were isolated by RP HPLC, quantified by UV absorption at 260 nm and identified by MALDI-TOF MS (Table 2, Scheme 3). It was found, that although the main product of the reaction was R_P-cyclic nucleotide **3B** (26.6% of total UV absorption) and among other prominent products



Scheme 3.

macrocyclic dimer **4a** was identified (13.1%), the major fraction of the products (60.3%) consisted of a mixture of linear phosphorothioate oligonucleotides **6a–l** ranging from 2 to 13 nucleotides in length and containing thymidine at the 3′-terminus. The trimer **6b** (15.7%), pentamer **6d** (14.0%), and dimer **6a** (11.3%) were most abundant, whereas each of those containing 8 or more nucleotides in the chain constituted less than 2% of the mixture of products.

The autoradiogram for PAGE analysis of compounds enzymatically radiolabeled with [^{32}P]phosphate group (Fig. 1) showed the expected pattern of bands of oligonucleotides **6a–l** and the lack of radioactive label in macrocyclic dinucleotide **4a**. The 5′-radiolabeled heptamer

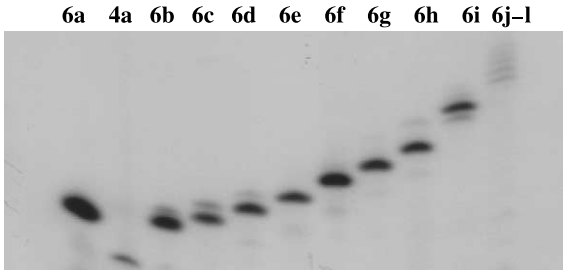
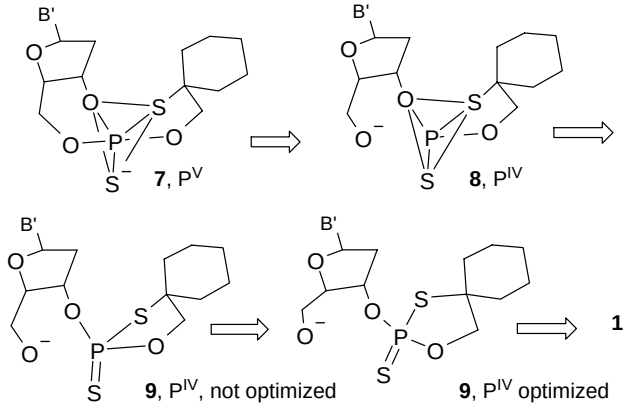


Figure 1. PAGE analysis (20% polyacrylamide/7 M urea) of HPLC-separated and ^{32}P-labeled products **6a–l** of DBU-promoted self-condensation of **1d** performed in the presence of ca. 1/6 mol equiv of 3′-*O*-acetylthymidine.



Scheme 4.

6f (0.1 OD_{260}) was treated with R_P-specific snake venom phosphodiesterase (svPDE)[10] and S_P-specific nuclease P1 (*n*P1).[11] It was found that **6f** was completely degraded on 10 min incubation with 50 μg of svPDE at 37 °C, whereas no degradation was observed after 2 h incubation of identical sample of **6f** with 1 μg of *n*P1 at room temperature. These results confirm that DBU-promoted self-condensation of R_P-oxathiaphospholane **1d** leads to homochiral oligomeric products **6a–l** with R_P-configuration at each internucleotide bond. Undoubtedly, the same absolute configuration must be assigned to phosphorus atoms in the macrocyclic compounds **4**. Notably, although it is known, that nucleases present in 50% human serum are able to hydrolyze phosphorothioate oligonucleotides of R_P-configuration,[12] using RP HPLC technique we found that both tested cyclic oligomers **4a** and **4b** were stable under these conditions for more than 24 h (data not shown).

Analysis of the mechanistic aspects of the condensation suggests that the observed intermolecular oligomerization or macrocyclization might successfully compete with entropically favored intramolecular cyclization for **1d** provided that the latter process was significantly slowed-down. This may happen due to higher energy barrier for the transition of **1d** to the corresponding P^V intermediate product **7** (Scheme 4). Conformational search (HyperChem 7.5 package, parameter set Amber99, HyperCube, Inc.) done for four P^V intermediates **7a–d**, derived from **1a–d**, respectively, showed that the pentacoordinate intermediates **7** adopt two basic structures with the 1,3,2-dioxaphosphorinane ring in either energetically favored chair conformation, or disfavored boat conformation. The numbering of atoms in **7**, a list of torsion angles varied during the search and the geometrical restraints imposed on bonds around P-atom in **7** are shown on Figure 2. Negative charge was assigned to the equatorial sulfur atom (S35). The obtained structures were analyzed in respect to the absolute configuration of the starting **1** and the relevant energies and torsion angles for those resulting from S_P-**1c** and R_P-**1d** are collected in Tables 1S and 2S, respectively (Supplementary data). Then, within the sets of the lowest energy conformers of **7a–b** (#1–33, in a range of 150.0–158.9 kcal/mol) and **7c–d** (#1–28, 157.0–164.4 kcal/mol, see Tables 1S and 2S) the apical bonds P–$O^{5'}$ were cleaved and geometries of the resulting molecules were optimized in two steps, that is, with geometrical restraints for P^V atom either kept (**8**) or

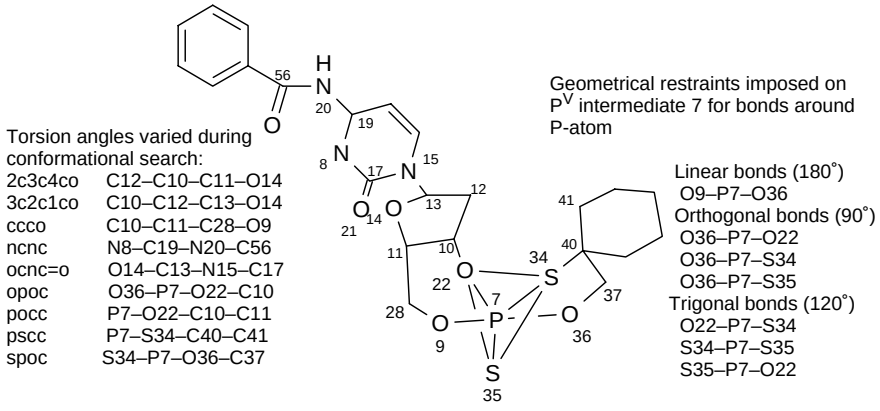


Figure 2. The numbering of atoms in **7**, a list of torsion angles varied during the conformational search and the geometrical restraints imposed on bonds around the P-atom.

released (**9**), giving on this 'retro' way information about relative energies of the structures **8** resembling corresponding transition states. Subsequent analysis of the profiles **1a**,**b**→**9**→**8a**,**b**→**7a**,**b** showed no significant differences in the energy barrier for both diastereomers. This finding corresponds with quantitative conversion of both P-diastereomeric substrates **1a** and **1b** into cyclic 3′,5′-*O*,*O*-phosphorothioates. Interestingly, among the profiles **1c**,**d**→**9**→**8c**,**d**→**7c**,**d** a very favorable profile was found for one of the chair conformers of **7c** (#5) with the energy barrier for its formation by 9.2 kcal/mol lower than for its **7d** counterpart (#4). The structures **7c**, #5 and **7d**, #4 are shown on Figures 1S and 2S, respectively, and related to them structures **8c**,**d** on Figure 3. Notably, in a case of **8d** we observed repulsion interactions along a narrow groove, indicated with the red arrow. This steric hindrance may result in higher energy barrier leading to the formation of **7d**. The calculated energy profiles for the intermediates formed from **1c** and **1d** during cyclization are shown on Figures 3S and 4S, respectively. Comparison of the lowest energy profiles for the cyclization **1c**,**d**→**9**→**8c**,**d**→**7c**,**d**, each ending with **7** adopting either the boat or chair conformations of the dioxaphosphorinane ring, is shown on Figure 4, while relevant energy values are collected in Tables 3S and 4S. Since the conformational search for R_P- and S_P-isomers of anionic **1** (it was assumed that DBU devoided the reacting substrate of 5′-OH proton) showed that for both pairs of diastereomers the energies of conformers suitable for cyclization are within 1 kcal/mol of those of the lowest energy (Tables 5S and 6S), apparently, a conformation of the substrate molecules is not a decisive factor in the problem under consideration.

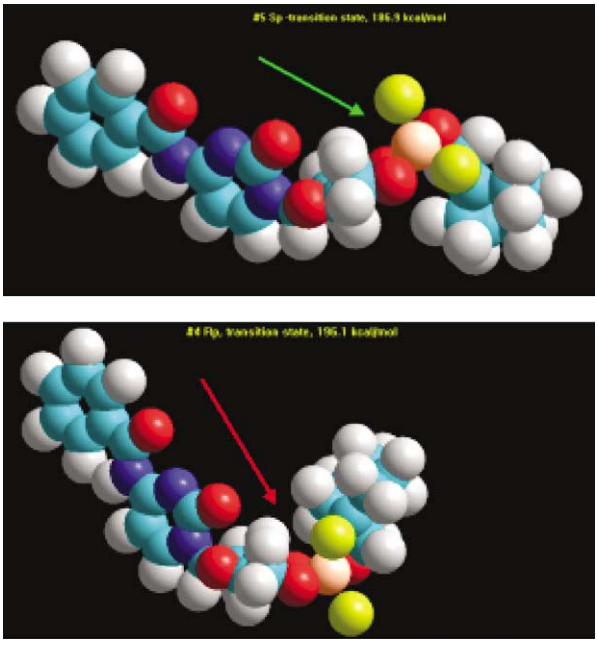


Figure 3. The calculated lowest energy barrier conformations of P^{IV}-intermediate **8c** (upper panel) and **8d** (bottom panel) formed during cyclization **1c**,**d**→**9**→**8c**,**d**→**7c**,**d** with the chair conformation of the 1,3,2-dioxaphosphorinane ring. The geometrical restraints present in **7c**,**d** were kept during geometry optimization. The green and red arrows indicate the grooves without and with repulsion interactions, respectively. Assignment of colors: hydrogen—white, carbon—blue, nitrogen—dark blue, oxygen—red, sulfur—yellow, phosphorus—pink.

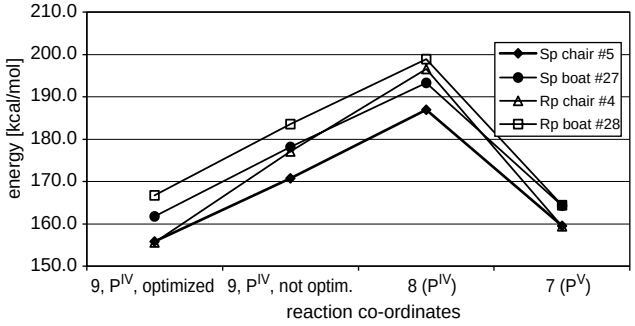


Figure 4. Comparison of the calculated profiles with the lowest energy barrier (for structures **8c**,**d** adopting either the chair or boat conformation of the dioxaphosphorinane ring) during the cyclization **1c**,**d**→**9**→**8c**,**d**→**7c**,**d**.

Although performed calculation does not reflect interactions with solvent molecules nor with DBU activator, these numbers suggest that despite of strong entropy factor facilitating intramolecular reaction, due to remarkably higher energy barrier **1**→**9**→**8** the cyclization of **1d** is slowed-down, compared to **1c**, and intermolecular reaction leads to linearized products.

3. Conclusions

The DBU-promoted intramolecular cyclization of diastereomerically pure S_P- and R_P-isomers of 5′-OH-N^4-benzoyl-2′-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) provides quantitatively corresponding S_P- and R_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate, respectively. The same applies to the S_P-isomer of 5′-OH-N^4-benzoyl-2′-deoxycytidine-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane), while its R_P-counterpart yields a mixture of products consisting of R_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate and macrocyclic oligo(deoxycytidine phosphorothioate)s. This difference can be explained in terms of steric hindrance (caused by bulky pentamethylene substituent) observed during formation of first pentacoordinate intermediate, which slows- down the intramolecular process and allows the intermolecular macrocyclization to compete. Similar dychotomy was observed for cyclization of deoxyguanosine derivatives. This approach is suitable for synthesis of stereodefined macrocyclic oligonucleotides—the analogues of cyclic diguanylic acid involved in regulatory system of cellulose synthesis in *A. xylinum*.

4. Experimental

4.1. General

4.1.1. 5′-*O*-DMT-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (2a,b, B=Cyt^{Bz}, R=H). The title compound was synthesized and separated into pure P-diastereomers (by ^{31}P NMR and TLC) as described.[8b]

4.1.2. 5′-*O*-DMT-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) (2c,d, B=Cyt^{Bz}, R=–(CH$_2$)$_5$–). The title compound was synthesized and separated into pure P-diastereomers (diastereomeric purity assessed by ^{31}P NMR and TLC) as described.[8d]

4.1.3. 5′-*O*-DMT-N^2-isobutyryl-deoxyguanosine 3′-*O*-(2-thio-'spiro'-4,4-pentamethylene-1,3,2-oxathiaphospholane). The title compound was synthesized as described.[8d]

4.1.4. S_P-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane)s (1a, B = Cyt^{Bz}, R = H). Into the solution of fast-5′-*O*-DMT-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (110 mg, 0.14 mmol) in methylene chloride (30 mL), *p*-toluenesulfonic acid monohydrate (120 mg, 0.66 mmol) was added with stirring at room temperature. The reaction progress was monitored by TLC (silica gel, chloroform/methanol 9:1 v/v, R_f substrate 0.78; R_f product 0.45). After 40 min, the reaction mixture was concentrated and the oil residue was applied on a silica gel column (20×100 mm, silica gel 60, 230–400 mesh, Merck). The column was eluted with a gradient of chloroform/2-propanol 100:0→50:50. Appropriate fractions were collected and evaporated to dryness to yield **1a** (35 mg, 0.075 mmol, 53%). δ ^{31}P NMR 104.11 ppm (CD_3CN). Compounds **1b**, **1c** and **1d** were obtained in analogous way.

4.1.5. The intramolecular cyclization reaction of 1a. Into the solution of fast-N^4-benzoyl-deoxycytidine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (31 mg, 0.066 mmol) in anhydrous acetonitrile (0.3 mL) equimolar amount of DBU (10 μL) was added. After 5 min at room temperature the reaction mixture was diluted with CD_3CN (0.3 mL) and ^{31}P NMR spectra showed quantitative formation of single product resonating at δ 51.5 ppm (CD_3CN). After removal of the benzoyl protecting groups (treatment with 30% aqueous ammonia at 55 °C for 16 h) the product was identified by ^{31}P NMR and HPLC comparison with genuine sample,[4d] as S_P-deoxycytidine cyclic 3′,5′-*O*,*O*-phosphorothioate (**3A**). Its structure was also confirmed by FAB MS (*m*/*z* 304.1 (negative ions); calculated M_W 305.25 (free acid)). The cyclization of **1b**, **1c** and **1d** was performed in analogous way. For **1b** and **1c** single products were observed, while for **1d** several resonances were found within a range 55–57 ppm.

4.1.6. The condensation reaction of 1d in the presence of 3′-*O*-acetylthymidine. A solution of **1d** (70 mg, 130 μmol) and 3′-*O*-acetylthymidine (6 mg, 21 μmol) in anhydrous acetonitrile (0.4 mL) was treated, with stirring at room temperature, with DBU (22 μL, 145 μmol). After 7 min the reaction mixture was diluted with CD_3CN (0.3 mL) and ^{31}P NMR showed several peaks within 56–58 ppm range. The deprotected products (treatment with 30% aqueous ammonia at 55 °C for 16 h) were isolated by preparative RP HPLC (PTH C_{18}, 5 μ, 2.1×220 mm column (Brownlee) with a linear gradient of acetonitrile in 0.1 M TEAB: 0–60 min—0.47%/min; flow 0.3 mL/min (UV detection at 255 nm)), quantified by UV absorption at 260 nm and identified by MALDI-TOF MS.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.12.022. Text containing detailed description of molecular modeling performed; Scheme 1S—mechanism of oxathiaphospholane ring-opening cyclization; Tables 1S, 2S—torsion angles found for 28 lowest energy conformers of P^V intermediate **7c**,**d** formed from **1c**,**d**; Figures 1S, 2S—the #4 and #5 conformations of P^V-intermediates **7**; Figures 3S, 4S—the energy profiles for intermediates formed from **1c**,**d** during cyclization **1**→**9**→**8**→**7**; Tables 3S, 4S—energies calculated for intermediates **8** and **9** leading to lowest energy conformers of **7c**,**d** derived from **1c**,**d**; Tables 5S, 6S—energies and torsion angles found in conformational search for ten lowest energy conformers of anionic form of **1c**,**d**.

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