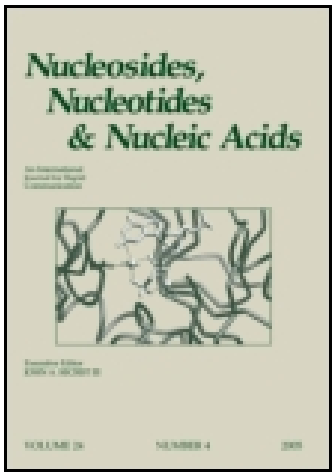

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^{13}C-^{31}P Couplings in the Study of Conformational Properties of Some Diastereomeric Nucleoside-3′-Thiophosphate Derivatives

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^{13}C-^{31}P COUPLINGS IN THE STUDY OF CONFORMATIONAL PROPERTIES OF SOME DIASTEREOMERIC NUCLEOSIDE-3′-THIOPHOSPHATE DERIVATIVES

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▫ *Synthesis and stereochemical characterization of enantiomerically pure nucleoside-3′-phosphorothioate esters and salts are reported. Vicinal carbon phosphorus couplings reflect different predominance of the ε conformation in the isomeric (Rp and Sp) esters, while for the salts the ε^t conformation prevails in both stereoisomers. The influence of solvent and temperature on the conformational preferences is also described.*

Keywords Modified Nucleotides, ^{1}H, ^{13}C, ^{31}P NMR ^{13}C-^{31}P Coupling Constants, Solution Conformation

INTRODUCTION

It is now generally recognized that a correlation exists between the stereochemical properties and the biological activity of modified oligonucleotides. The structural and biochemical consequences of diastereoisomerism have been extensively studied by Steck and coworkers.[1] In particular, the role of the configuration of the phosphorothioate group in the conformational preferences was described by Cosstick and Eckstein.[2] In most cases, the NMR methods proved to be extremely useful in correlating molecular conformation with biological activity.

Introduction of various modifications at the phosphorus atom results in chiral phosphate derivatives (Rp and Sp configuration). Conformational preferences about the C3′–O3′ bond differ for the Rp and Sp stereoisomers, which are best reflected by the systematic differences of the vicinal carbon–phosphorus coupling values ($\Delta J = {}^{3}J_{C4',P} - {}^{3}J_{C2',P}$) found for the diastereomers.[3–9] The assessment of

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conformational equilibrium inferred from these data together with the results from NOE and TROESY experiments provided the configurational assignment of some diastereomeric nucleotide derivatives.

The ΔJ values are influenced also by factors other than the absolute configuration. Thus the nature and size of the pendant groups at the phosphorus atom, the presence of the 5′-protecting group, the aromatic solvent effect, and changes in temperature all affect the conformational properties of the modified nucleotides.

In the cases studied by us, the conformational equilibrium about the C3′–O3′ bond was primarily affected by two competing interactions of the phosphorus substituents with the nucleobase and the C5′-ODMT-protected sugar unit.

1. Similar to the stacking interaction of two nucleobases in the dinucleotides, a phenyl ring in the side-chain of a P-modified nucleotide is also prone to a stacking-like interaction with the nucleobase.[6] The steric arrangements of the stacked states, depending also on the nature of the other P-substituents, differ for the stereoisomers.
2. Sizeable substituents at the phosphorus aim at avoiding the steric proximity with the sugar unit and the C5′-protecting group. As a result, the predominant conformation notably differs in the stereoisomers, as reflected by their ΔJ values.[7]

In earlier papers we described the NMR characterization of some modified nucleotides, where one of the substituents at the phosphorus was S-Me. One of those isomer pairs (**3a**, **3b**)[9] was synthesized from salts **2a** and **2b**. In this article, we compare the conformational properties of diastereomeric **2a**, **2b** with **3a**, **3b** and consider the effect of the introduction of a methyl group to the sulfur atom on the chemical shifts and coupling constant values. We also reported for **3a** and **3b** that the temperature dependence of the ΔJ values differed from that of some P-modified parent compounds studied earlier. This difference, according to our supposition, was connected with the altered stacking-like interactions. It seemed reasonable, therefore, to observe the temperature-dependent changes of the ΔJ values also in **2a** and **2b**, where one of the steric factors was absent and only the stacking-like interaction remained. To our knowledge, this is the first case when results of NMR experiments were reported for conformational characterization of diastereomeric nucleotide derivatives in ionic states.

EXPERIMENTAL

Materials

N^4-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine and 2-(4-nitrophenyl) ethanol were purchased from Pharma Waldhof GmbH and Fluka, respectively. Pyridine

was distilled from P_2O_5 and stored over molecular sieves. Pivaloyl chloride was freshly distilled before use.

Chromatography

For silica gel column chromatographic separations Kieselgel 60 (particle size: 0.04–0.63 mm) was used. Solvent systems applied were the following: (A): EtOAc-MeOH (20%)-Et_3N (1%), (B): EtOAc-MeOH (5%)-Et_3N (1%), (C): EtOAc-Et_3N (1%).

MS Spectrometry

ESI mass spectra were recorded with a Perkin Elmer SCIEX, API 2000 tandem mass spectrometer equipped with electrospray ion source both in positive and negative ion mode.

NMR Spectroscopy

High-resolution ^1H, ^{13}C, and ^{31}P magnetic resonance experiments were carried out on a Varian Unity Inova 400 NMR spectrometer equipped with a 5-mm probe in $CDCl_3$ and C_6D_6 solutions. The ^1H NMR spectra were acquired with 64 scans, 4 KHz spectral width and 32 K data points, giving a digital resolution of 0.125 Hz. The ^{13}C spectra were accumulated with 2000–7000 scans using a spectral width of ca 14 KHz, 64 K data points, and WALTZ decoupling. The FIDs were zero-filled to 128 K, giving a digital resolution of 0.11 Hz. The proton resonances were assigned with the help of homonuclear decoupling experiments. The assignment of the carbon resonances were inferred from two-dimensional heterocorrelated experiments by a program incorporated in the Varian software package. The chemical shifts are expressed on the δ scale relative to internal TMS. The ^{13}C-^{31}P–coupling constant values were directly determined from the proton-decoupled ^{13}C spectra and given in Hz. Temperature-dependent ^1H, ^{13}C, and ^{31}P experiments were run in the 17–65°C range in C_6D_6 solutions.

(R_P)- and (S_P)-N^4-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine-3′-*O*-monothio-phosphate-*O*-(2-(4-nitrophenyl)ethyl) ester triethylammonium salts (2a and 2b). Coupling of N^4-benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine-3′-H-phosphonate DBU salt[10] (**1**)/4.88 g, 5.75 mmol with 2-(4-nitrophenyl)ethanol/0.84 g, 5.0 mmol followed by sulfurization of the H-phosphonate diester intermediates was carried out according to the literature.[5,11] The crude P-diastereomeric mixture of the nucleoside-3′-thiophosphate-*O*-esters obtained was dissolved in CH_2Cl_2-Et_3N (1%)/6.0 mL and applied to a silica gel column (320 g) packed in system (C). It was first eluted with a linear gradient of systems (B) → (A) 800–800 mL, finally with system (A) 500 mL. The appropriate fractions were combined and evaporated to give the required P-diastereomerically pure R_P and S_P isomers (**2a** and **2b**) as pale yellow solid foams, which were then dissolved in EtOAc and added dropwise to tenfold volumes of

cold hexane. The white precipitates thus obtained were filtered and dried in vacuo over paraffin shavings. Thus we isolated 2.31 g = 2.33 mmol of (R_P)-N^4-benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine-3′-*O*-monothiophosphate-*O*-(2-(4-nitrophenyl)ethyl) ester triethyl-ammonium salt (**2a**) as white powder. Yield: 46.7%, R_f (A): 0.20. ESI MS m/z (%): 102.0 (100) $[M + H]^+$ (triethylamine). For the free acid: 879.2 (14) $[M + H]^+$, 901.0 (12) $[M + Na]^+$, $C_{45}H_{43}N_4O_{11}PS$ requires 878.23. The pure, higher moving S_P isomer (**2b**) was also obtained in the same form. Yield: 1.60 g = 1.62 mmol (32.4%), R_f (A): 0.24. Its MS data were identical with those of **2a**. The absolute configurations of **2a** and **2b** follow from those of **3a** and **3b**, studied earlier.[9]

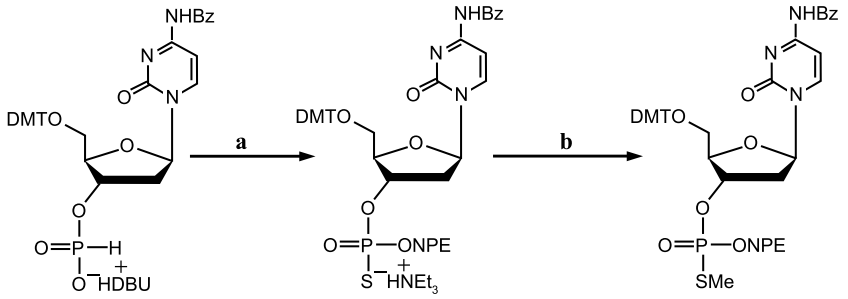
(R_P)- and (S_P)- N^4-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine-3′-*O*-monothio-phosphate-*O*-(2-(4-nitrophenyl)ethyl)-S-methyl triesters (3a and 3b). **2a** 33 mg, 33.3 μmol was dissolved in dry pyridine 3 mL then MeI 21 μL, 335 μmol was added and the mixture was stirred overnight at ambient temperature. Since TLC indicated incomplete reaction the next morning additional MeI 11 μL, 176 μmol and Et_3N 28 μL, 202 μmol were added and the stirring was continued for further 6 h. Although the mixture still contained some starting material, it was worked up on the following way: CH_2Cl_2 12 mL was added and the diluted solution was washed with satd. aq. $NaHCO_3$ 4 mL and brine 4 mL. The organic phase was separated, dried with Na_2SO_4, filtered, and evaporated to dryness. Pyridine traces were removed by repeated evaporations with toluene 2 × 10 mL and the residue was purified by silica gel column chromatography using linear gradient of systems (C) → (B) 200–200 mL as eluent. The pure fractions were combined and evaporated to give **3a** as semisolid residue. Yield: 18.3 mg = 20.3 μmol (61%), R_f (B): 0.33, ESI MS m/z (%): 893.2 (9) $[M + H]^+$, 915.2 (100) $[M + Na]^+$, 931.0 (10) $[M + K]^+$, $C_{46}H_{45}N_4O_{11}PS$ requires 892.24. Starting from **2b** the corresponding pure S_P S-methyl triester (**3b**) was prepared and isolated in the same way. Yield: 19.5 mg = 21.6 μmol (65%), R_f (B): 0.36. Its MS data were essentially identical with those of **3a**.

RESULTS AND DISCUSSION

Synthesis

N^4-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine-3′-H-phosphonate-1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) salt (**1**) was prepared from the protected nucleoside (N^4-Bz-5′-DMT-dC) according to the literature.[10]

Coupling of **1** with 2-(4-nitrophenyl)ethanol (see Scheme 1) was accomplished applying standard H-phosphonate nucleotide synthesis protocol[11] resulting in the corresponding N^4-benzoyl-5′-dimethoxytrityl-2′-deoxycytidine-3′-H-phosphonate-*O*-(2-(4-nitrophenyl)ethyl) esters as a P-diastereomeric mixture. It was subsequently oxidized with elemental sulfur to give the chemically much more



SCHEME 1 **a**, i. 2-(4-Nitrophenyl)ethanol, pivaloyl chloride, pyridine, 25°C, 30 min. ii. Sulfur, pyridine-CS_2, 25°C, 16 h. iii. Silica gel chromatography. **b**, i. MeI, pyridine, Et_3N, 25°C, 24 h. ii. Silica gel chromatography. **Scheme**: Synthesis of N^4-Bz-5′-DMT-dC-3′-monothiophosphate-*O*-(*p*-nitrophenylethyl)-S-methyl triesters.

stable N^4-benzoyl-5′-dimethoxytrityl-2′deoxycytidine-3′-*O*-monothiophosphate-*O*-(2-(4-nitrophenyl)ethyl) esters (**2a** and **2b**). Separation of the P-isomers by silica gel column chromatography was carried out at this stage providing the pure R_P (**2a**) and S_P (**2b**) diastereomers in good overall yield. Probably due to electron withdrawing effect of the 2-(4-nitrophenyl)ethyl group, the S-methylations, even with large excess of MeI, proceeded slowly and gave the required S-methyl triesters (**3a** and **3b**) with significantly lower yields compared to analogous cases when there were no electron-withdrawing substituents on the thiophosphate moiety.[5,9]

NMR Study

Earlier, the configurational determination of the P-isomers was based on the phosphorus chemical shifts. Inspection of Table 1 reveals that these data hardly differ in the stereoisomers, thus stereochemical assignment cannot be based on them. Similarly, minor differences were observed between the isomers in the other parameters collected in the table, such as carbon chemical shifts and proton-phosphorus couplings. Larger deviations were observed when comparing the esters (**3a**, **3b**) and salts (**2a**, **2b**), since the above parameters reflect the changes of the

TABLE 1 Selected NMR Parameters Reflecting Changes of Electrondensity Around the Phosphorus[a]

	δ_P (ppm)[b]	$\delta_{C3'}$ (ppm)[c]	δ_{OCH2} (ppm)[c]	$^3J_{H3',P}$ (Hz)	$^3J_{CH2,P}$ (Hz)	$^2J_{OCH2,P}$ (Hz)
2a	57.93	75.95	65.73	8.6	8.2	5.6
2b	58.20	75.64	65.78	8.5	8.3	5.5
3a	29.75	77.46	67.18	8.0	7.4	6.1
3b	29.77	77.66	67.16	7.8	7.5	5.9

[a]In $CDCl_3$ solutions.
[b]Relative to external H_3PO_4.
[c]Relative to internal TMS.

TABLE 2 ^{1}H Chemical Shifts (δppm)[a] and Proton–Proton Couplings (J, Hz) of Diastereomers **2–3a,b**

	2a	**2b**	**3a**	**3b**
H1′	6.28 (6.5 + 5.9)	6.28 (6.3 + 5.9)	6.32 (6.8 + 6.1)	6.29 (6.9 + 6.0)
H2′α	2.93 (−14.0 + 5.9 + 3.1)	2.89 (−14.0 + 5.9 + 3.1)	2.93 (−14.3 + 6.1 + 3.0)	2.92 (−14.3 + 6.0 + 2.9)
H2′β	2.28 (−14.0 + 6.5 + 6.3)	2.26 (−14.0 + 6.3 + 6.2)	2.34 (−14.3 + 6.8 + 6.1)	2.33 (−14.3 + 6.9 + 6.1 + 1.7)
H3′	5.25 (6.3 + 3.1 + 2.9 + 8.6)	5.15 (6.2 + 3.1 + 3.4 + 8.5)	5.18 (6.1 + 3.0 + 3.1 + 8.0)	5.14 (6.1 + 2.9 + 3.0 + 7.8)
H4′	4.37 (2.9 + 3.4 + 3.4)	4.45 (3.4 + 3.2 + 3.2)	4.38 (3.1 + 3.4 + 3.4)	4.39 (3.0 + 3.4 + 3.2)
H5′	3.35 (3.4)	3.47 (3.2)	3.43 (−10.6 + 3.4)	3.45 (−10.7 + 3.4)
			3.49 (−10.6 + 3.4)	3.48 (−10.7 + 3.2)
Ph-CH_2	3.08 (m)	3.05 (m)	3.13 (m)	3.13 (m)
OCH_2	4.16 (m)	4.18 (m)	4.33 (m)	4.34 (m)
C5-H	7.30 (7.5)	7.30 (7.5)	7.25 (7.6)	7.26 (7.5)
C6-H	8.16 (7.5)	8.18 (7.5)	8.13 (7.6)	8.14 (7.5)
S-Me	—	—	2.12 (15.5)	2.18 (15.4)

[a]In $CDCl_3$, relative to TMS.

electron density around the phosphorus. In agreement with literature data,[12,13] the increased electron density of the molecules in ionic states results in mighty increase in the phosphorus chemical shift and smaller, but still characteristic changes in the other NMR parameters of Table 1. Other spectral parameters, which were found to be characteristic for the conformational properties of the sugar ring and for the configuration at the phosphorus, are listed in Tables 2 and 3.

The proton–proton coupling constants are known to provide information about the preferred conformation of the sugar ring (S and N, see Figure 1). From the sum of the H2′α couplings, with the help of an empirical expression[14] the percentage of the S-type (C2′-endo) conformer can be estimated. We have found for all of our modified 5′-protected mono- and dinucleotides that in solution the S-type sugar conformation is predominant. The S-character had the largest value for mononucleotides where stacking-like interactions were absent (94–99%).[4] For all

TABLE 3 ^{13}C Chemical Shifts (δppm) of the Pertinent Carbons and ^{13}C-^{31}P Couplings (J, Hz) of Diastereomers **2–3a,b**[a]

	2a	**2b**	**3a**	**3b**
C1′	87.60	87.63	87.08	87.35
C2′	41.29 (3.4)	40.91 (3.1)	40.90 (3.1)	40.61 (5.1)
C3′	75.95 (broad)	75.64 (broad)	77.46 (5.6)	77.66 (5.5)
C4′	85.90 (6.6)	86.13 (7.1)	85.19 (7.2)	85.65 (5.3)
C5′	63.43	63.37	62.73	62.79
S-CH_3	—	—	12.58 (4.8)	12.60 (4.8)
Ph-CH_2	36.86 (8.2)	36.91 (8.3)	36.52 (7.4)	36.51 (7.5)
O-CH_2	65.73 (5.6)	65.58 (5.5)	67.18 (6.1)	67.16 (5.9)
ΔJ	3.2	4.0	4.1	0.2

[a]In $CDCl_3$ solutions, at 30°C.

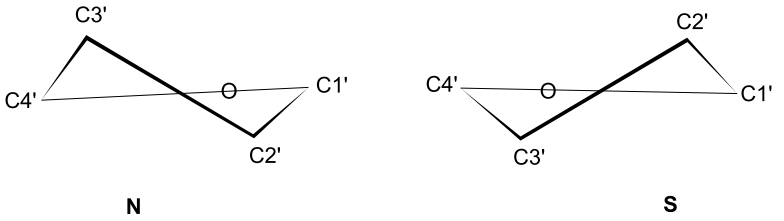


FIGURE 1 Equilibrium between the two extreme sugar puckers, north and south.

four molecules presented here, the calculated percentage of S-conformations are definitely lower (74–79%) and are close to those of the natural dinucleotides.[15] The lower value indicates that the conformational equilibrium is somewhat biased toward the N-puckered form. The conformational distribution of the pentose, however, does not show any meaningful changes on going from ionic to ester-type molecules. Furthermore, no significant differences can be seen in the proton parameters of the stereoisomers (Table 2).

Conformational preferences about the various bonds in nucleotides and deoxynucleotides are known to be interdependent,[16] consequently, the conformational variations in the sugar ring pucker may influence the torsional preferences about the C3′–O3′ bond. These characteristics can be inferred from the proton-decoupled ^{13}C spectra of the compounds (Table 3).

Applying a Karplus-type relationship, Lankhorst and coworkers[15] have demonstrated for ribonucleotides and deoxyribonucleotides that the vicinal ^{13}C-^{31}P couplings reflect the conformational preferences about the C3′–O3′ bond. In phosphate-modified nucleotides, the difference of the $^{3}J_{C4',P}$ and $^{3}J_{C2',P}$ vicinal coupling values (ΔJ parameter) was introduced[3] to provide information about the preferred ε^{t} (trans) and ε^{-} (gauche-) conformations (Figure 2). Accordingly, large positive ΔJ value refers to prevalent ε^{t} conformation, while an increase of the ε^{-} conformation in the conformational equilibrium reduces the ΔJ values. Negative ΔJ values are indicative of the preferred ε^{-} conformation about the C3′–O3′ bond. It was found for all diastereomer pairs studied by us that different chirality at the

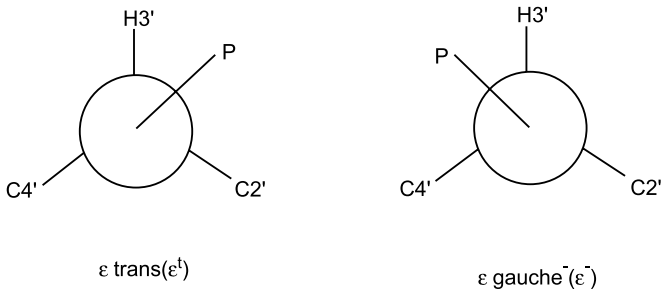


FIGURE 2 Newman projection about the C3′–O3′ bond.

phosphorus was always accompanied by characteristic conformational differences about the C3′–O3′ bond, as reflected by their ΔJ values.[3–9]

In our earlier studies, the largest differences in the ΔJ values of the diastereomers were found for mononucleotides with S-Me and Se-Me substituents (ΔΔJ = 5.2 and 7.0 Hz, respectively), where the other substituent on the phosphorus was a methyl group, thus stacking, or stacking-like, interactions could not be formed.[4] On the contrary, we supposed for **3a** and **3b** that the phenyl ring in the side chain may take part in a stacking-like interaction with the nucleobase.[9] As a result, the restricted rotation about the C3′–O3′ bond is reflected by a smaller but still characteristic ΔJ difference in the diastereomers (ΔΔJ = 3.9 Hz in $CDCl_3$ solution). These conformational differences in the isomers were sufficient to identify the absolute configuration at the phosphorus by T-ROESY experiments.[9] Since **3a** and **3b** were derived from **2a** and **2b**, respectively, considering also that the CIP priorities of the functional groups have not changed (S^- > nucleoside 3′-O > $O(CH_2)_2pNO_2PH$ > = O), the configuration of this latter isomeric pair directly follows from the esters of known configurations (**2a** = R_p, **2b** = S_p).

In salts **2a** and **2b** one interaction, namely that of the S-Me group with the protected sugar unit, is absent and solely the stacking interaction remains. In the absence of the conformational perturbance caused by the different configuration of the S-Me group, the extent of the stacking in the isomers **2a** and **2b** becomes rather similar and differs from that of the corresponding esters (Figure 3). As a result, the preferred conformation about the C3′–O3′ bond is predominantly ε^t in both isomers, as reflected by their ΔJ values (3.2 and 4.0 Hz, respectively, in $CDCl_3$ solution). This steric arrangement can be correlated with that of the natural dinucleotides for which the vicinal couplings at room temperature reflect the predominancy of the ε^t conformation (Figure 4). The ΔJ value (3.3 Hz) calculated from the respective vicinal ^{13}C-^{31}P couplings of 2′-deoxycytidilyl(3′ → 5′)-2′-deoxycytidine (CpC)[15] is in good agreement with that of **2a** and **2b**.

Other similarities can be found in the temperature dependence of **2a** and **2b** when compared with the natural dinucleotides, where higher temperature induced destacking and detachment of the interacting nucleobases. As a result, the vicinal

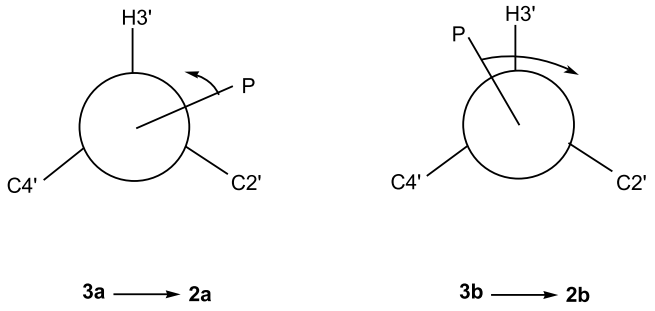


FIGURE 3 Changes in conformational preferences on going from esters **3a**,**3b** to salts **2a**,**2b**.

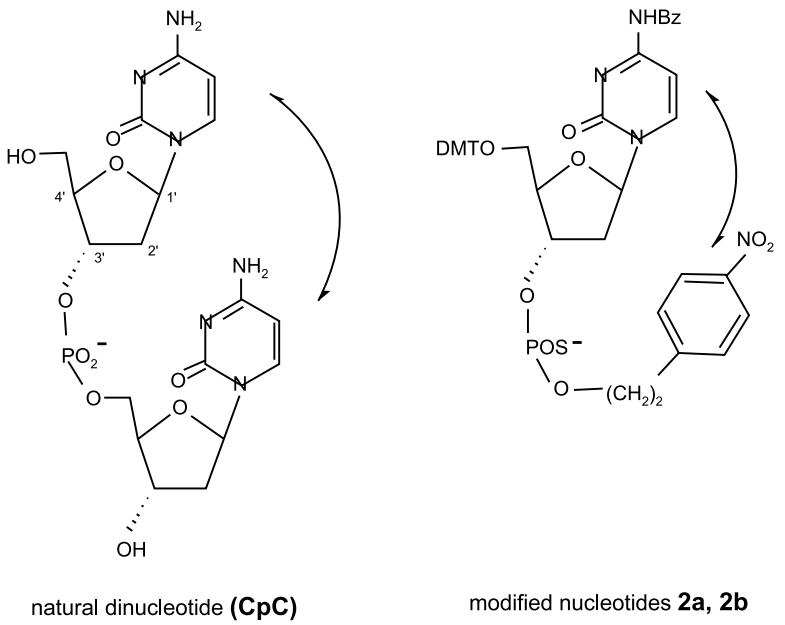


FIGURE 4 Schematic representation of base–base and base–phenyl overlaps in the stacked states.

^{13}C-^{31}P couplings reflected an increase of the ε^- conformer population.[15] According to our calculations, the decrease in ΔJ of CpC is 1.8 Hz in 79°C temperature range.

Due to their tendency to decompose at elevated temperatures, the temperature-dependent measurements of compounds **2a**, **2b**, **3a**, and **3b** were accomplished in deuterobenzene solutions. The aromatic solvent effect and change of temperature are both known to influence the conformational preferences; consequently, the ΔJ values. The solvation in benzene enhanced the extent to which the nucleobase and the phenyl ring in the side-chain overlap. The changes in the ΔJ values demonstrate that the more extensive overlap in C_6D_6 solution induces

TABLE 4 Temperature Dependence of ΔJ Values[a]

	17°C	30°C	45°C	60°C
2a	4.6	4.5	4.3	4.0
2b	3.1[b]	3.2	2.5	2.1
	25°C	35°C	50°C	65°C
3a	5.3	5.1	4.8	4.4
3b	1.0[b]	1.1	1.0	1.0

[a]In C_6D_6, in Hz.
[b]Calculated from broad signals.

opposite shift in the conformational equilibrium of the isomers **2a** and **2b** relative to their ΔJ values in $CDCl_3$ (Table 4).

Elevation of temperature over the 17–65°C temperature range and consequent destacking caused increase of population in the ε^- conformer of **2a** and **2b**, and a similar trend was found for **3a** (Table 4). The temperature-induced behavior is in line with that found in natural dinucleotides and differs from that observed in some modified derivatives studied earlier, where the change of ΔJ values reflected shifts toward the ε^t conformation.[6,7] **3b** presents here the only exception, where the ΔJ values remained practically invariant over the temperature range. This insensitivity to temperature changes reflects that in this stereoisomer the steric control imposed on the $\varepsilon^- - \varepsilon^t$ equilibrium by the S-Me group may have a more significant role than the tendency to destacking.

The above temperature dependence of compounds **2a**, **2b**, and **3a** in comparison with other modifed nucleotides[6,7] may be attributable to numerous factors. However, the type of the nucleobases cannot be as significant as we supposed earlier,[9] since in mononucleotides **2a**, **2b**, and **3a** the heating caused the same, anticlockwise rotation about the C3′–O3′ bond as in the natural dinucleotides. Furthermore, the presence of the bulky 5′-ODMT group cannot be responsible for the different temperature dependence, either, since it was present in all of the cases we studied earlier. An important structural feature of the previously studied thiophosphoric-acid-O,O,O-triester derivatives[6,7] was that the double-bonded heteroatom was a more voluminous and less electronegative sulfur than the oxygen atom in the present compounds and the natural dinucleotides. In order to demonstrate such heteroatom effects, a more systematic examination is in progress.

CONCLUSION

The present study shows that salts **2a** and **2b** have a comparable distribution of ε conformers. Comparison of diagnostic NMR data of **2a** and **2b** with those of unmodified CpC demonstrated that neither $P\text{-}O^- \rightarrow P\text{-}S^-$ exchange nor the presence of a p-nitrophenyl-ethyl moiety instead of a second nucleoside unit may not markedly affect the conformational preferences about the C3′–O3′ bond. As a consequence, the predominant conformations are ε^t in both stereoisomers. For **2a** and **2b**, the change in the preferred ε conformation upon elevation of temperature also resembles that found for unmodified dinucleotides. One can thus anticipate that replacement of a second nucleoside moiety to a side-chain carrying a phenyl group does not induce substantial changes in the stacking ability. Contrary to this, our study here has shown that methylation at the sulfur causes sizeable differences in the conformational equilibrium of **3a** and **3b**, as reflected by their ΔJ values. This can be interpreted by the stacked states, which differ for the stereoisomers. Differences are also evident in the temperature-dependent conformational changes of the two isomeric esters, which can be attributed to the significant role of the S-Me substituent on the stereochemical constraints.

REFERENCES

1. Steck, W.J.; Wilk, A. Stereocontrolled synthesis of oligo-nucleoside-phosphorothioates. Angew. Chem., Int. Ed. Engl. **1994**, 33, 709–722.
2. Cosstick, R.; Eckstein, F. Synthesis of D(CG)octamers containing alternating phosphoro-thioate linkages. Effect of the phosphorothioate group on the B-Z transition. Biochemistry **1985**, 24, 3630–3638.
3. Gács-Baitz, E.; Tömösközi, I.; Kajtár-Peredy, M. Configurational determination and conformational properties of diastereomeric nucleoside-phosphorothioates. Tetrahedron: Asymmetry **1996**, 7, 2447–2452.
4. Gács-Baitz, E.; Wozniak, L.A.; Kajtár-Peredy, M. Conformational properties of diastereomeric 5′-ODMT-2′deoxythymidine 3′-O-(S-methyl-methanephosphono-thiolate)s and (Se-methyl-methanephophono-selenolate)s in solution as studied by NMR methods. Chirality **2000**, 12, 675–680.
5. Machytka, D.; Sági, G.; Kajtár-Peredy, M.; Gács-Baitz, E. Synthesis and NMR characterization of diastereomeric C_{PSMe} derivatives. Nucleosides Nucleotides **2000**, 19, 903–915.
6. Kajtár-Peredy, M.; Tömösközi, I.; Gács-Baitz, E. Synthesis and stereochemical characterization of diastereomeric nucleoside-phosphorothioselenoates by NMR methods. Nucleosides Nucleotides Nucleic Acids **2001**, 20, 1615–1623.
7. Gács-Baitz, E.; Kajtár-Peredy, M. Solution structure of diastereomeric thymidine 3′-O(N,N-diisopropyl-*O*-β-cyanoethyl-phosphoramidothioate)s by NMR spectroscopy. Chirality **2002**, 14, 814–818.
8. Machytka, D.; Gács-Baitz, E.; Tegyey, Z. Extension of the applicability of ΔJ values for the configurational assignment of diastereomeric phosphate-modified dideoxy-nucleotides. Nucleosides Nucleotides **1998**, 17, 2311–2322.
9. Gács-Baitz, E.; Kajtár-Peredy, M.; Sági, G. NMR study of some diastereomeric nucleoside-3′-thiophosphate derivatives in solution. Anal. Bioanal. Chem. **2004**, 379, 56–59.
10. Froehler, B.C.; Mateucci, M.D. Synthesis of DNA via deoxynucleoside H-phosphonate intermediates. Nucleic Acids Res. **1986**, 14, 5399–5407.
11. Seela, F.; Kretschmer, U. Stereochemistry of oxidation of diastereoisomeric d(TpA) phosphonates with sulphur and iodine[^{18}O] water. J. Chem. Soc., Chem. Commun. **1990**, 1154–1159.
12. Gorenstein, D.G.Phosphorus-31 NMR: Principles and Applications. Academic Press: Orlando, FL, 1984; 7–36.
13. Glemarec, C.; Reynolds, R.C.; Crooks, P.A.; Maddry, J.A.; Akhtar, M.S.; Montgomery, J.A.; Secrist, J.A., III; Chattopadhyaya, J. Conformational studies of thymidine dimers containing sulfonate and sulfonamide linkages by NMR spectroscopy. Tetrahedron **1993**, 49, 2287–2298.
14. Wijmenga, S.S.; Mooren, M.M.V.; Hilbers, C.W.NMR of Nucleic Acids, from Spectrum to Structures. Oxford University Press: Oxford, 1993; 266–267.
15. Lankhorst, P.P.; Haasnoot, C.A.G.; Erkelens, C.; Westerink, H.P.; van der Marel, G.A.; van Boom, J.H.; Altona, C. Carbon-13 NMR in conformational analysis of nucleic acid fragments. The torsion angle distribution about the C3′–O3′ bond in DNA constituents. Nucleic Acids Res. **1985**, 13, 927–942.
16. Cheng, D.M.; Sarma, R.H. Intimate details of the conformational characteristics of deoxyribonucleoside monophosphates in aqueous solution. J. Am. Chem. Soc. **1977**, 99, 7333–7348.