Conformational Transmission in Four- and Five-Coordinated Phosphorus Compounds. Solvent Effects on the $C_{4'}-C_{5'}$ Conformation in 5'-Phosphorylated Model Nucleosides

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Abstract: The $C_{4'}-C_{5'}$ conformation of the $5'-P^{IV}$ model compounds **1–3** and the $5'-P^V$ TBP model compounds **4–6** has been determined in various solvents with 300- and 500-MHz ^1H NMR. It is found that lowering the solvent polarity results in a substantial increase in the g⁻ populations for the model compounds **1, 2, 4**, and **5** (e.g., for **1** in D_2O, $x(g^-) = 0.00$; CCl_4, $x(g^-) = 0.23$). This effect can be attributed to an enhanced electrostatic repulsion between the charge densities on O_5 and the endocyclic oxygen(s) at lower solvent polarities. This conclusion is supported by the experimental finding that the $5'-P^{IV}$ system **3** and the $5'-P^V$ TBP system **6**, in which the endocyclic oxygen(s) are replaced by $C(H_2)$, do not show a $C_{4'}-C_{5'}$ conformational change when the medium polarity is changed. Furthermore, it is found that the $5'-P^{IV}$ nucleotides **7** and **8**, which are made soluble in apolar solvents by triesterification of the phosphorus, show also increased g⁻ populations upon lowering the solvent polarity. The present results confirm our earlier proposal that enhanced charge repulsion between $O_{5'}$ and $O_{1'}$ in mononucleotides drives a rotation around the $C_{4'}-C_{5'}$ bond toward g⁻.

Recently we performed a 300-MHz ^1H NMR conformational analysis study on a set of 5'-phosphorylated tetrahydrofurfuryl compounds in which it was demonstrated that an increase in coordination from $5'-P^{IV}$ to a $5'-P^V$ trigonal bipyramid (TBP) will be transmitted into a substantial preference for the gauche(−) (g⁻) conformation around the $C_{4'}-C_{5'}$ bond, if the tetrahydrofurfuryl group is located in the axis of the TBP.[1] It was argued that the $C_{4'}-C_{5'}$ conformational change which occurs upon going from $5'-P^{IV}$ to $5'-P^V$ TBP is brought about via enhanced charge repulsion between $O_{5'}$ in the axial position and $O_{1'}$. This is in good agreement with the experimental finding that substitution of $O_{1'}$ by $C(H_2)$ leads to identical $C_{4'}-C_{5'}$ conformations for $5'-P^{IV}$ and $5'-P^V$ TBP. Considering the 5'-phosphorylated tetrahydrofurfuryls as simplified models for the sugar–phosphate constituents of nucleic acids, it was suggested that specific conformational changes in DNA (e.g., the isomerization of right-handed B DNA into left-handed Z DNA) therefore may be achieved by a coordinational transition from $5'-P^{IV}$ into $5'-P^V$ TBP in the helix backbone.[2,3] In order to investigate the impact of charge repulsion between $O_{5'}$ and $O_{1'}$ on $C_{4'}-C_{5'}$ conformational changes in phosphorylated (bio)molecules in general, we selected the $5'-P^{IV}$ and $5'-P^V$ TBP models **1–8** (Chart I) for a $C_{4'}-C_{5'}$ conformational analysis in relation to the polarity of the solvent. Since lowering the medium polarity will enhance the electrostatic repulsion between $O_{5'}$ and the endocyclic oxygen(s), it is expected that the $C_{4'}-C_{5'}$ conformation in **1, 2, 4, 5, 7**, and **8** can be also modulated via the solvent. On the other hand, the $C_{4'}-C_{5'}$ conformation of **3** and **6**, in which $O_{1'}$ is replaced by $C(H_2)$, should be invariant upon changing the medium polarity. An additional interesting aspect of the modified nucleotides **7** and **8**, which are made soluble in apolar media by triesterification of the phosphorus, is the possibility to investigate the impact of the solvent polarity on the conformation of the ribose ring. In addition, the syn ⇌ anti equilibrium of the adenine base in **7** could be determined in various solvents, on the basis of NOE measurements.

Experimental Section

Spectroscopy. ^1H NMR spectra were run in the FT mode at 300 MHz on a Bruker CXP-300 spectrometer[4] and at 500 MHz on a Bruker WM-500 spectrometer.[5] Both instruments are interfaced with an AS-

Chart I. Model Compounds Studied in This Work
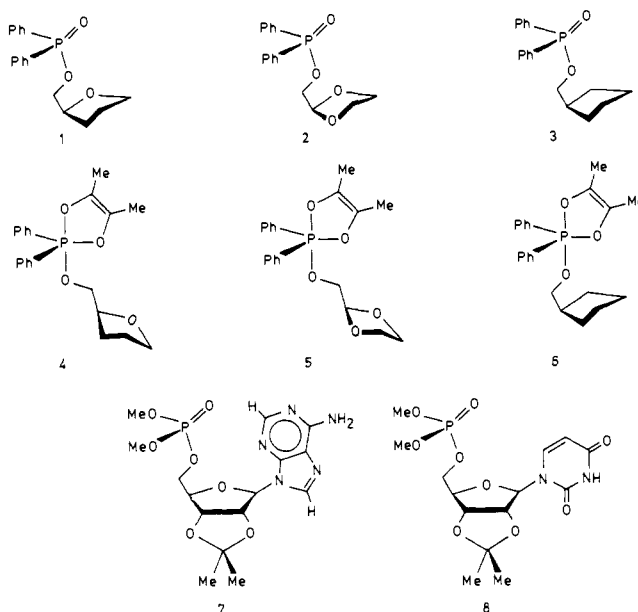
PECT 2000 computer. A standard computer simulation–iteration procedure[6] was employed to obtain accurate values for spin–spin coupling constants and chemical shifts. All NOE measurements were conducted at 300 MHz. Reported relative enhancements are averaged over at least four independent spectra. ^{31}P NMR spectra were run in the FT mode at 36.4 MHz on a Bruker HX-90 spectrometer with a Digilab FT-NMR-3 pulsing accessory. ^{31}P chemical shifts are related to 85% H_3PO_4 as external standard.

Synthesis. The model compounds **1, 3, 4**, and **6** were prepared according to procedures described in ref 1.

(1,3-Dioxolyl-2-methyleneoxy)diphenylphosphine. A solution of chlorodiphenylphosphine (50 mmol, 11.03 g) in 30 mL of anhydrous *n*-hexane was added over 30 min to a cooled (0 °C) and stirred solution of 2-(hydroxymethylene)-1,3-dioxolane[7] (50 mmol, 5.20 g) and *N,N*-dimethylaniline (50 mmol, 6.05 g) in 80 mL of anhydrous *n*-hexane. After completion of the addition, the reaction mixture was refluxed for 2 h. The precipitated *N,N*-dimethylaniline hydrochloride was removed by filtration and washed with 20 mL of anhydrous *n*-hexane. After

(1) Koole, L. H.; Lanters, E. J.; Buck, H. M. *J. Am. Chem. Soc.* **1984**, *106*, 5451.
(2) Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1980**, *99*, 181.
(3) van Lier, J. J. C.; Smits, M. T.; Buck, H. M. *Eur. J. Biochem.* **1983**, *132*, 55.
(4) NMR facility at the University of Technology, Eindhoven.

(5) Dutch National 500-MHz ^1H NMR facility, Nijmegen.
(6) PANIC program: Copyright, Bruker Spectrospin AG, Switzerland.
(7) Fraser-Reid, B.; Anderson, R. C.; Hicks, D. R.; Walker, D. L. *Can. J. Chem.* **1977**, *55*, 3993.

Table I. [$H_{4'}$–$C_{4'}$–$C_{5'}$–$H_{5'(5'')}$] Torsion Angles (ϕ) in the Rotamers around the $C_{4'}$–$C_{5'}$ Bond, and the Corresponding Calculated Proton–Proton Coupling Constants

conform	ϕ-[$H_{4'}$–$C_{4'}$–$C_{5'}$–$H_{5'}$], deg	$J_{H4'H5'}$, Hz X = C(H₂), Y = C(H₂)	X = O, Y = O	X = O, Y = C(H₂)		$J_{H4'H5'}$, Hz X = C(H₂), Y = C(H₂)	X = O, Y = O	X = O, Y = C(H₂)
g⁺	−60	1.9	1.8	2.8	60	1.9	1.8	0.9
gᵗ	60	4.1	1.8	3.1	180	11.5	9.8	10.7
g⁻	180	11.5	9.8	10.7	−60	4.1	1.8	5.0

removal of the solvent, the oily residue was distilled in vacuo to yield the desired product as a colorless oil (5.76 g, 40%), bp 148–152 °C (0.01 mmHg); ¹H NMR (CDCl₃) δ 3.5–3.9 (6 H, m, dioxolane H, H₅', H₅''), 4.8 (1 H, t, H₄'), 6.7–7.3 (10 H, m, aromatic H); ³¹P NMR (CDCl₃) δ 117.
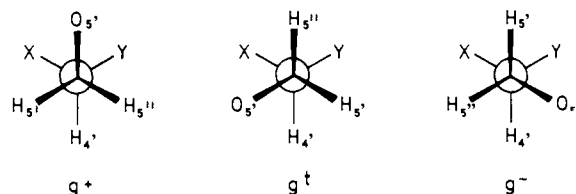
(1,3-Dioxolyl-2-methyleneoxy)diphenylphosphine Oxide (2). An ozone–oxygen (15:85) stream was bubbled through a solution of (1,3-dioxolyl-2-methyleneoxy)diphenylphosphine (2 g, 7 mmol) in 15 mL of anhydrous dichloromethane at −78 °C, until a blue color of excess ozone was apparent.[8] The reaction vessel was then sparged with dry nitrogen to remove the excess ozone. Meanwhile, the solution was carefully warmed to room temperature. Evolution of singlet oxygen could be observed between −40 and 0 °C. Evaporation of the solvent afforded 2 as a colorless oil: ¹H NMR (CDCl₃) δ 3.5–3.9 (6 H, m, dioxolane H, H₅', H₅''), 4.7 (1 H, t, H₄'), 6.65–7.35 (10 H, m, aromatic H); ³¹P NMR (CDCl₃) δ 33.

2,2-Diphenyl-2-(1,3-dioxolyl-2-methyleneoxy)-4,5-dimethyl-1,3,2-dioxaphosphol-4-ene (5). This compound was prepared by the addition of 1 equiv of freshly distilled butanedione to a solution of (1,3-dioxolyl-2-methyleneoxy)diphenylphosphine in a 5-mm NMR sample tube. The pentacoordinated phosphorus structure was identified by ³¹P NMR: ¹H NMR (CDCl₃) δ 2.0 (6 H, s, CH₃), 3.6–4.0 (6 H, m, dioxolane H, H₅', H₅''), 4.7 (1 H, t, H₄'), 6.7–7.5 (10 H, m, aromatic H); ³¹P NMR (CDCl₃) δ−26.

Dimethoxy-*N*,*N*-(dimethylamino)phosphine. Phosphorus trichloride (0.5 mol, 69 g) was added over 30 min to trimethyl phosphite (1 mol, 124 g) that was kept at 60 °C in a 1000-mL round-bottom flask. Then, the reaction mixture was cooled to 0 °C and diluted with 500 mL of sodium-dried diethyl ether. Dimethylamine (3 mol, 135 g) was bubbled through the reaction mixture. After filtration of the dimethylamine hydrochloride, evaporation of the diethyl ether yielded a yellowish oil, which was distilled twice at 45 mmHg through a 20-cm Vigreux column to afford 46 g (22%) of the desired product: bp 51–52 °C at 45 mmHg; ¹H NMR (C₆D₆) δ 2.63 (6 H, d, N(CH₃)₂, J_{PNCH} = 8.8 Hz), 3.42 (6 H, d, OCH₃, J_{POCH} = 12.0 Hz); ³¹P NMR (C₆D₆) δ 148.

2′,3′-*O*-Isopropylideneadenosine 5′-Dimethylphosphite. A magnetically stirred solution of 2′,3′-*O*-isopropylideneadenosine[9] (6.51 mmol, 2.00 g) in 30 mL of dry 1,4-dioxane was kept at 85 °C. A solution of dimethoxy-*N*,*N*-(dimethylamino)phosphine (11.20 mmol, 1.53 g) in 10 mL of dry 1,4-dioxane was added over 3 h. After completion of the addition, thin-layer chromatography (TLC), using dry methyl ethyl ketone (MEK) as eluent, indicated the presence of a product (R_f = 0.52) along with unreacted 2′,3′-*O*-isopropylideneadenosine (R_f = 0.21). The reaction mixture was kept at 85 °C and stirred for 15 h. Then TLC proved the conversion to be complete. Evaporation of the solvent afforded a viscous, colorless oil that was separated on a Woelm silica gel column by using dry MEK as eluent. Pure 2′,3′-*O*-isopropylideneadenosine 5′-dimethylphosphite was obtained as a white crystalline material in 79% yield: mp 164–165 °C; ¹H NMR (CDCl₃) δ 1.41 (3 H, s, CH₃ isopropylidene), 1.64 (3 H, s, CH₃ isopropylidene), 3.47 (6 H, d, OCH₃, J_{POCH} = 10.8 Hz), 4.00 (2 H, m, H₅', H₅''), 4.48 (1 H, m, H₄'), 5.05 (1 H, dd, H₃'), 5.39 (1 H, dd, H₂'), 6.19 (3 H, m, H₁', NH₂), 8.04 (1 H, s, H₈), 8.36 (1 H, s, H₂); ³¹P NMR (CDCl₃) δ 141. Anal. Calcd for C₁₅H₂₂N₅O₆P: C, 45.11; H, 5.55; N, 17.54. Found: C, 44.95; H, 5.68; N, 18.06.

2′,3′-*O*-Isopropylideneadenosine 5′-Dimethylphosphate (7). 2′,3′-*O*-Isopropylideneadenosine 5′-dimethylphosphite (1.13 mmol, 450 mg) was dissolved in 25 mL of anhydrous dichloromethane, and an ozone–oxygen (15:85) stream was bubbled through. After 35 min, the passage of the ozone–oxygen mixture was stopped, as TLC using MEK as eluent indicated complete conversion into a product with R_f = 0.27. Evaporation of the dichloromethane yielded 7 as a hygroscopic white solid in 96% yield. ¹H NMR (CDCl₃) δ 1.40 (3 H, s, CH₃ isopropylidene), 1.62 (3 H, s, CH₃ isopropylidene), (6 H, d, OCH₃, J_{POCH} = 11.0 Hz), 4.28 (2 H, m, H₅'/H₅''), 4.51 (1 H, m, H₄'), 5.13 (1 H, dd, H₃'), 5.46 (1 H, dd, H₂'), 6.19 (1 H, d, H₁'), 6.42 (2 H, br s, NH₂), 8.08 (1 H, s, H₈), 8.48

(8) Thompson, Q. E. *J. Am. Chem. Soc.* **1961**, *83*, 845.
(9) Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *36*, 2315.

Figure 1. Newman projections of the rotamers around the $C_{4'}$–$C_{5'}$ bond.

(1 H, s, H₂); ³¹P NMR (CDCl₃) δ 1. Anal. Calcd for C₁₅H₂₂N₅O₇P: C, 43.37; H, 5.34; N, 16.87. Found: C, 43.41; H, 5.44; N, 17.09.

2′,3′-*O*-Isopropylideneuridine 5′-Dimethylphosphite. A solution of dimethoxy-*N*,*N*-(dimethylamino)phosphine (9.3 mmol, 1.28 g) in 5 mL of anhydrous 1,4-dioxane was added over 3 h to a stirred and heated (80 °C) suspension of 2′,3′-*O*-isopropylideneuridine[9] (5.5 mmol, 1.55 g) in 15 mL of anhydrous 1,4-dioxane. After completion of the addition, the reaction mixture was stirred for 2 h at room temperature. At this point, TLC using MEK as eluent indicated the presence of the desired product (R_f = 0.66), along with unreacted 2′,3′-*O*-isopropylideneuridine (R_f = 0.51). Evaporation of the solvent and trituration with anhydrous dichloromethane afforded a colorless oil that was separated on a Woelm silica column, using MEK as eluent. Pure 2′,3′-*O*-isopropylideneuridine 5′-dimethylphosphite was obtained as a colorless viscous oil in approximately 30% yield: ¹H NMR (acetone-d₆) δ 1.31 (3 H, s, CH₃ isopropylidene), 1.50 (3 H, s, CH₃ isopropylidene), 3.48 (6 H, d, OCH₃, J_{POCH} = 10.6 Hz), 4.00 (2 H, m, H₅'/H₅''), 4.24 (1 H, m, H₄'), 4.86 (1 H, dd, H₃'), 5.01 (1 H, dd, H₂'), 5.65 (1 H, d, H₅), 5.87 (1 H, d, H₁'), 7.70 (1 H, d, H₆), 8.05 (1 H, br s, NH); ³¹P NMR (acetone-d₆) δ 145.

2′,3′-*O*-Isopropylideneuridine 5′-Dimethylphosphate (8). An ozone–oxygen (15:85) stream was passed through a solution of 2′,3′-*O*-isopropylideneuridine 5′-dimethylphosphite in 15 mL of anhydrous dichloromethane. After 45 min, TLC using MEK as eluent indicated complete conversion into a product with R_f = 0.35. Evaporation of the solvent afforded 8 as a colorless glass: ¹H NMR (acetone-d₆) δ 1.32 (3 H, s, CH₃ isopropylidene), 1.51 (3 H, s, CH₃ isopropylidene), 3.73 (6 H, d, OCH₃, J_{POCH} = 11.1 Hz), 4.56 (3 H, m, H₄', H₅', H₅''), 4.90 (1 H, dd, H₃'), 5.10 (1 H, dd, H₂'), 5.64 (1 H, d, H₅), 5.83 (1 H, d, H₁'), 7.68 (1 H, d, H₆); ³¹P NMR (acetone-d₆) δ 7.

Results and Discussion

$C_{4'}$–$C_{5'}$ Conformational Analysis of 1-6. In solution, rapid interconversion between the staggered rotamers gauche(+) (g⁺), gauche(trans) (gᵗ), and g⁻ (Figure 1) yields weighted time-averaged coupling constants $J_{H4'H5'}$ and $J_{H4'H5''}$ which are related to the individual rotamers and their populations $x(g^+)$, $x(g^t)$, and $x(g^-)$

$$J_{H4'H5'(5'')} =$$
$$x(g^+)J^{g^+}_{H4'H5'(5'')} + x(g^t)J^{g^t}_{H4'H5'(5'')} + x(g^-)J^{g^-}_{H4'H5'(5'')}$$

with $x(g^+)$ + $x(g^t)$ + $x(g^-)$ = 1. The rotamer populations can be solved with the generalized Karplus equation introduced by Altona et al.[10,11] Table I lists the calculated coupling constants

(10) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
(11) In this generalized equation, the standard Karplus relation is extended with a correction term which accounts for the influence of electronegative substituents on ³J_{HH}:

$$^3J_{HH} =$$
$$13.22 \cos^2 \phi - 0.99 \cos \phi + \sum\{0.87 - 2.46 \cos^2 (\xi_i\phi + 19.9|\Delta\chi_i|)\}\Delta\chi_i$$

ϕ is the proton–proton torsion angle, $\Delta\chi_i$ is the difference in electronegativity between the substituent and hydrogen according to the electronegativity scale of Huggins, and ξ_i is a substituent orientation parameter.

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and the corresponding proton–proton torsion angles. The experimental coupling constants $J_{H_4'H_5'}$ and $J_{H_4'H_5''}$ and the $C_{4'}$–$C_{5'}$ rotamer populations for 1–6 are listed in Table II. The data on 1 and 2 indicate that the $C_{4'}$–$C_{5'}$ conformation in these species is dominated by the gauche effect,[12] i.e., the predominant preference of $O_{5'}$ to adopt a gauche arrangement with respect to the endocyclic oxygen(s). The gauche effect results in pronounced populations of g^+ and g^t for 1, whereas 2 shows a unique preference for g^+, in which $O_{5'}$ is oriented gauche to both endocyclic oxygen atoms. The gauche effect is absent in model compound 3, which shows a clear preference for the symmetric rotamers g^t and g^- over g^+. The $5'$-P^V TBP model compound 4 shows greater g^- populations with respect to the $5'$-P^{IV} counterpart 1 in all solvents (e.g., in acetone-d_6 for 4, $x(g^-) = 0.32$; 1, $x(g^-) = 0.17$). As was shown previously,[1] this difference is essentially due to the conformational transmission effect which occurs upon the coordinational increase from $5'$-P^{IV} to $5'$-P^V TBP. In 4, however, the conformational transmission is obscured by pseudorotation around the pentacoordinated phosphorus. Pseudorotation involves rapid interchange of $O_{5'}$ between one axial site (that is associated with a pronounced preference for g^-) and two equatorial sites (leading to a $C_{4'}$–$C_{5'}$ conformation in which g^+ and g^t are dominant). The $5'$-P^V TBP model compound 5 shows increased populations of the symmetric rotamers g^t and g^- with respect to 2. This result can be interpreted in terms of enhanced electrostatic repulsion between $O_{5'}$ and both oxygen atoms of the dioxolane ring. Furthermore, the results show that lowering the solvent polarity leads to a $C_{4'}$–$C_{5'}$ conformational change from g^+ and g^t toward g^- for 1 and 4 and from g^+ toward g^t and g^- for 2 and 5. On the other hand, no $C_{4'}$–$C_{5'}$ conformational change upon lowering the polarity of the medium is found for the cyclopentane derivatives 3 and 6. In Figure 2, the g^- populations for 1–6 are plotted as a function of the solvent polarity parameter E_T, which is based on the position of electronic spectra peaks of pyridinium-N-phenolbetaine in various solvents.[13] These findings strongly support our earlier proposal that enhanced electrostatic repulsion between the charge densities on $O_{5'}$ and $O_{1'}$ in $5'$-phosphorylated tetrahydrofurfuryl systems drives a rotation around the $C_{4'}$–$C_{5'}$ linkage toward g^-. Apparently, this conformational transmission can be accomplished in two ways: (i) by a coordinational transition from $5'$-P^{IV} into $5'$-P^V TBP in such a way that $O_{5'}$ becomes located in the axis of the TBP or (ii) by lowering the polarity of the solvent, both for $5'$-P^{IV} and $5'$-P^V TBP systems.

Conformational Analysis of 7 and 8

$C_{4'}$–$C_{5'}$ Conformation. The spin–spin coupling constants, measured on the modified nucleotides 7 and 8, are collected in Table III. The assignments of the various resonances were based on detailed computer simulations of the spectral patterns between 4.1 and 6.5 ppm (Figure 3). The assignment of $H_{5'}$ and $H_{5''}$ was accomplished according to Altona[14] and is found to agree with the Remin and Shugar rule[15] for all solvents. The $C_{4'}$–$C_{5'}$ rotamer populations (Table III) were calculated from $J_{H_4'H_5'}$ and $J_{H_4'H_5''}$, now using a set of statistically determined nucleic acid torsion angles.[16] In Figure 4, the g^- populations of 7 and 8 are plotted as a function of the medium polarity. Evidently, lowering the medium polarity causes a marked increase of the g^- population around $C_{4'}$–$C_{5'}$, which is fully consistent with the results obtained on 1, 2, 4, and 5.

Ribose Conformation. Information concerning the conformation of the ribose ring in 7 and 8 can be obtained from $J_{H_1'H_2'}$, $J_{H_2'H_3'}$, and $J_{H_3'H_4'}$. From the experimental values (Table III), it can be
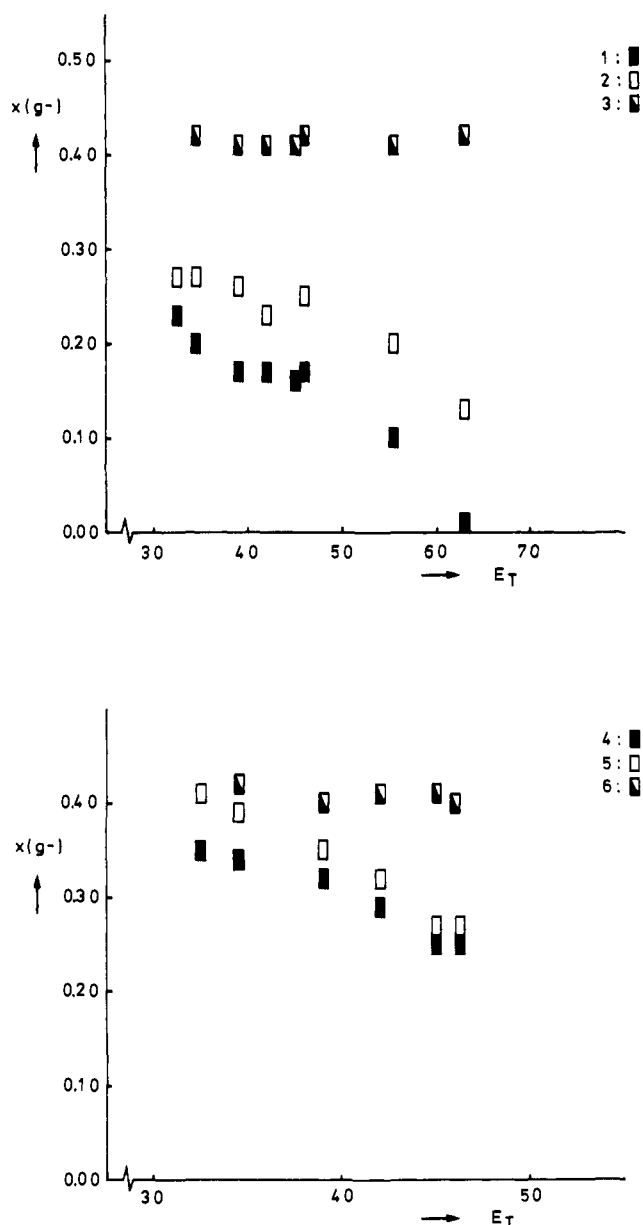

Figure 2. Variation of the g^- population of 1, 2, and 3 (upper graph) and 4, 5, and 6 (lower graph) with the solvent polarity.

concluded that the conformation of the ribose ring does not vary to a great extent over the entire solvent polarity range. The PSEUROT subroutine[17] was used to analyze the ribose conformations in terms of a rapid equilibrium between two puckered ring forms, although application of this method is seriously hampered by the fact that only three spin–spin couplings between the ring protons are available.[17] For this reason, it was assumed that the puckering amplitudes for both ring forms in 7 are equal to $32°$, i.e., the puckering amplitude for ring puckered $2',3'$-O-isopropylidene-adenosine in the solid state.[18] It follows that the ribose ring in 7 is involved in a rapid equilibrium between a $C_{1'}$ endo form (I, see Figure 5) with $P_I = 323°$, $\psi_{m,I} = 32°$, and a $C_{4'}$ endo form (II) with $P_{II} = 224°$, $\psi_{m,II} = 32°$. The distribution over I and II is found to vary slightly for the various solvents (Table IV). A minor discrepancy is observed for D_2O, because a small preference for form II is found. A similar approach was followed in the conformational analysis of the ribose ring in 8. Application of the PSEUROT method indicates a conformational equilibrium

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Table II. $J_{H_{4'}H_{5'(5'')}}$ Values and the Corresponding Rotamer Populations around $C_{4'}$–$C_{5'}$ for **1–6** in Different Solvents

compd	solvent	$J_{H_{4'}H_{5'}}$, Hz	$J_{H_{4'}H_{5'(5'')}}$, Hz	$x(g^+)$	$x(g^t)$	$x(g^-)$
	CCl_4	4.7	5.6	0.39	0.38	0.23
	C_6D_6	4.5	5.7	0.40	0.40	0.20
	$CDCl_3$	4.3	6.0	0.42	0.41	0.17
	$(CD_3)_2CO$	4.3	5.8	0.40	0.43	0.17
1	$(CD_3)_2SO$	4.2	5.9	0.40	0.44	0.16
	CD_3CN	4.2	5.7	0.41	0.42	0.17
	CD_3OD	3.7	6.0	0.42	0.48	0.10
	D_2O	3.0	6.3	0.45	0.55	0.00
	CCl_4	4.0	4.0	0.46	0.27	0.27
	C_6D_6	4.0	4.0	0.46	0.27	0.27
	$CDCl_3$	3.9	3.9	0.48	0.26	0.26
2	$(CD_3)_2CO$	3.6	3.6	0.54	0.23	0.23
	CD_3CN	3.8	3.8	0.50	0.25	0.25
	CD_3OD	3.4	3.4	0.60	0.20	0.20
	D_2O	2.8	2.8	0.74	0.13	0.13
	C_6D_6	6.9	6.9	0.16	0.42	0.42
	$CDCl_3$	6.7	6.7	0.18	0.41	0.41
	$(CD_3)_2CO$	6.7	6.7	0.18	0.41	0.41
3	$(CD_3)_2SO$	6.7	6.7	0.18	0.41	0.41
	CD_3CN	6.9	6.9	0.16	0.42	0.42
	CD_3OD	6.7	6.7	0.18	0.41	0.41
	D_2O	6.7	6.7	0.18	0.41	0.41
	CCl_4	5.8	5.5	0.31	0.32	0.37
	C_6D_6	5.7	5.5	0.32	0.32	0.36
	$CDCl_3$	5.4	5.7	0.32	0.36	0.32
4	$(CD_3)_2CO$	5.4	5.6	0.33	0.35	0.32
	$(CD_3)_2SO$	4.8	5.8	0.36	0.40	0.24
	CD_3CN	4.8	5.9	0.35	0.41	0.24
	CCl_4	5.1	5.1	0.18	0.41	0.41
	C_6D_6	4.9	4.9	0.22	0.39	0.39
	$CDCl_3$	4.6	4.6	0.30	0.35	0.35
5	$(CD_3)_2CO$	4.2	4.2	0.40	0.30	0.30
	$(CD_3)_2SO$	4.0	4.0	0.46	0.27	0.27
	CD_3CN	4.0	4.0	0.46	0.27	0.27
	C_6D_6	6.9	6.9	0.16	0.42	0.42
	$CDCl_3$	6.6	6.6	0.20	0.40	0.40
6	$(CD_3)_2CO$	6.7	6.7	0.18	0.41	0.41
	$(CD_3)_2SO$	6.7	6.7	0.18	0.41	0.41
	CD_3CN	6.6	6.6	0.20	0.40	0.40
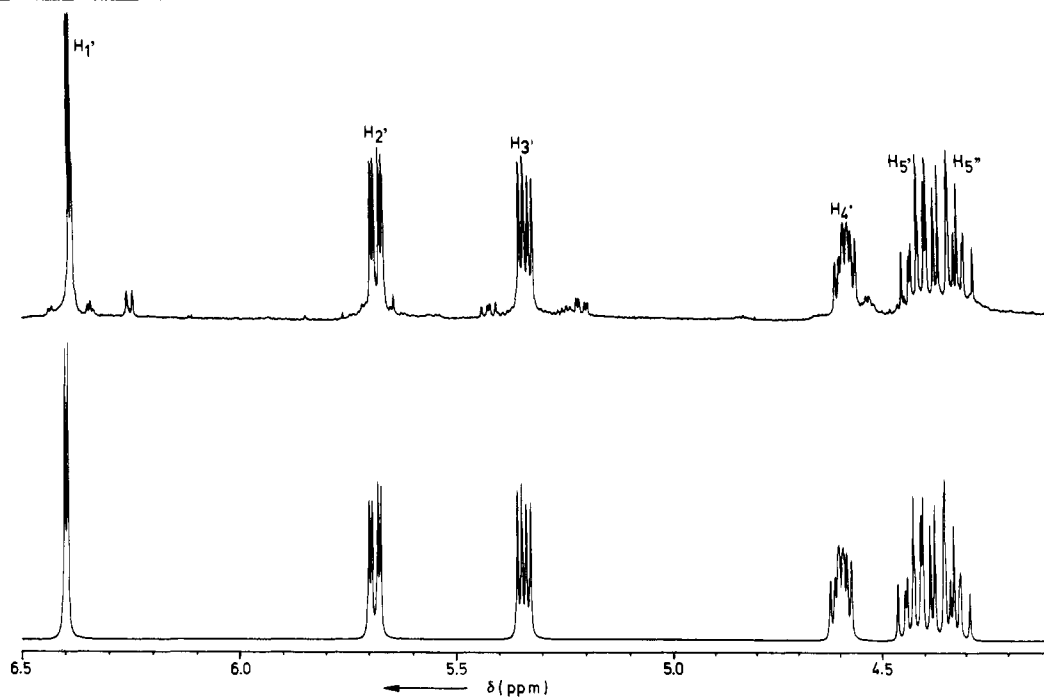
Figure 3. Experimental (upper trace) and computer-simulated (lower trace) 300-MHz ^{1}H NMR spectrum of **7** in acetone-d_6.

between a ring form III with $P_{III} = 323°$, $\psi_{m,III} = 32°$, and a ring form IV with $P_{IV} = 221°$, $\psi_{m,IV} = 32°$. The distribution over III and IV in the different media is summarized in Table IV. In-

terestingly, the ribose ring forms II and IV closely resemble the ribose conformations as determined by X-ray diffraction for $2',3'$-O-isopropylideneadenosine (P = 215°, ψ_m = 32°) and

Table III. Spectral Parameters and the Rotamer Populations for **7** and **8** in Different Solvents

solvent	E_T	$J_{H_1'H_2'}$	$J_{H_2'H_3'}$	$J_{H_3'H_4'}$	$J_{H_4'H_5'}$	$J_{H_4'H_5''}$	$J_{H_5'H_5''}$	$J_{PH_5'}$	$J_{PH_5''}$	$J_{PH_4'}$	$\Delta\delta$, ppm	$x(g^+)$	$x(g^t)$	$x(g^-)$
						Compound **7**								
C_6D_6	34.5	1.9	6.3	3.1	5.3	5.9	−11.0	7.0	7.3		0.094	0.25	0.41	0.34
$CDCl_3$	39.1	2.2	6.3	3.1	4.9	5.5	−11.0	6.5	6.8		0.069	0.33	0.38	0.29
$(CD_3)_2CO$	42.2	2.2	6.2	3.2	5.3	6.3	−11.0	6.7	7.3	0.6	0.082	0.21	0.45	0.34
$(CD_3)_2SO$	45.0	2.3	6.2	3.2	4.9	7.0	−11.1	6.3	7.0		0.078	0.18	0.54	0.28
CD_3CN	46.0	2.2	6.3	3.1	4.7	6.3	−11.1	6.3	6.9		0.050	0.26	0.48	0.26
CH_3CH_2OD	51.9	2.2	6.3	3.2	4.9	6.2	−11.0	6.6	7.1		0.052	0.26	0.45	0.29
CD_3OD	55.5	2.3	6.2	3.2	4.7	6.2	−11.1	6.5	6.8		0.035	0.28	0.46	0.26
D_2O/dioxane[a]	59.5	2.5	6.2	2.9	3.7	5.1	−11.4	5.5	6.0	1.7	0.018	0.48	0.38	0.14
D_2O	63.1	2.3	6.1	2.6	3.2	5.3	−11.5	5.5	5.2		0.022	0.50	0.42	0.08
						Compound **8**								
C_6D_6		1.9	6.6	3.6	5.2	5.5	−10.0	7.0	7.3	0.5	0.004	0.31	0.36	0.33
$CDCl_3$		2.2	6.4	3.7	3.9	5.4	−11.1	6.7	7.0	1.5	0.037	0.44	0.39	0.17
$(CD_3)_2CO$		2.2	6.4	3.6	4.1	6.6	−11.3	6.2	6.7	0.7	0.025	0.29	0.52	0.19
$(CD_3)_2SO$		2.0	6.4	3.7	4.4	6.7	−10.9	6.4	7.3	0.2	0.056	0.25	0.53	0.22
CD_3CN		2.3	6.5	3.9	4.2	6.2	−11.1	6.3	6.9	0.9	0.038	0.33	0.47	0.20
CH_3CH_2OD		2.0	6.5	3.7	4.4	6.3	−11.0	7.2	7.2	0.4	0.010	0.29	0.48	0.23
CD_3OD		2.0	6.5	3.6	3.8	6.6	−10.8	6.5	7.4	0.3	0.010	0.32	0.53	0.15
D_2O/dioxane[a]		2.3	6.4	3.6	3.4	5.7	−11.3	5.8	6.5	1.5	0.045	0.44	0.45	0.11
D_2O		2.3	6.3	3.4	3.3	5.7	−11.4	5.9	6.5	1.9	0.039	0.45	0.45	0.10

[a] D_2O/dioxane mixture 84:16 (v/v).

Figure 5. Ribose ring forms I and II.

Table IV. Populations of the Ribose Pucker Forms II (Compound **7**) and IV (Compound **8**) in Different Solvents

solvent	mol fraction of form II	mol fraction of form IV
C_6D_6	0.43	0.36
$CDCl_3$	0.47	0.37
$(CD_3)_2CO$	0.46	0.38
$(CD_3)_2SO$	0.46	0.35
CD_3CN	0.46	0.35
CH_3CH_2OD	0.46	0.35
CD_3OD	0.46	0.37
D_2O/dioxane[a]	0.53	0.39
D_2O	0.55	0.42

[a] D_2O/dioxane mixture 84:16 (v/v).

Table V. Nuclear Overhauser Enhancements of H_8, Obtained by Selective Irradiation of $H_{1'}$, $H_{2'}$, and $H_{3'}$ in Different Solvents, and the Corresponding Syn Populations

solvent	$f_8\{1'\}$	$f_8\{2'\}$	$f_8\{3'\}$	P_{syn}
C_6D_6	0.10	0.00	−0.02	1.00
$CDCl_3$	0.15	0.03	0.05	0.94
$(CD_3)_2SO$	0.12	0.04	0.01	0.95
CD_3CN	0.15	0.01	−0.01	1.00
CD_3OD	0.11	0.02	0.02	0.96
D_2O	0.25	0.14	0.03	0.92

Figure 4. Variation of the g^- population of **7** and **8** with the solvent polarity.

2',3'-*O*-isopropylideneuridine[19] (P = 216°, ψ_m = 22°).

C_1–N_9 Conformation of 7. The conformation around $C_{1'}$–N_9

can be described in terms of a syn $\rightleftharpoons$ anti equilibrium of the adenine base, on the basis of the relative Overhauser enhancements of the H_8 signal, obtained by selective irradiation of $H_{1'}$, $H_{2'}$, and $H_{3'}$. Following Gueron et al.[20] who derived a qualitative expression for the syn population of nucleosides and nucleotides, we derived

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(20) Tran-Dinh, S.; Guschlbauer, W.; Gueron, M. *J. Am. Chem. Soc.* **1972**, *94*, 7903.

an adapted expression, which is suitable for 7.[21] Application of this formula indicates a marked preference for the syn conformation in C_6D_6, $CDCl_3$, $(CD_3)_2SO$, CD_3CN, CD_3OD, and D_2O (Table V). This illustrates the well-known fact that the syn $\rightleftarrows$ anti distribution is shifted in favor of syn for 2',3'-bridged nucleosides and nucleotides, in comparison with their unmodified counterparts. It should be mentioned that the C_1–N_1 conformation in **8** could not be determined by NOE measurements, due to a near coincidence of the resonances of $H_{1'}$ and H_5 in the high-resolution ^1H NMR spectra.

Concluding Remarks

A marked increase of the g⁻ population around the C_4–C_5 bond is found for the 5'-P^{IV} and 5'-P^V TBP models **1, 2, 4**, and **5**, and the 5'-P^{IV} modified nucleotides **7** and **8**, upon lowering the solvent polarity. This effect can be explained on the basis of an enhanced charge repulsion between $O_{5'}$ and the endocyclic oxygen(s) at lower polarities. This is strongly supported by the experimental finding that the model systems **4** and **6** do not show a C_4–C_5 conformational change when the polarity of the solvent is varied. The present results are in line with our earlier proposal that the en-

hanced repulsion between $O_{5'}$ and $O_{1'}$, triggered via a coordinational transition from 5'-P^{IV} into 5'-P^V TBP, drives a rotation around C_4–C_5 toward g⁻. Extended conformational analyses on the 5'-P^{IV} modified nucleotides **7** and **8** indicate that the ribose conformation can be best described as a two-state equilibrium between two puckered ring forms. The distribution over these forms varies slightly with the solvent polarity. A pronounced preference for syn orientation of the adenine base in **7** is found.

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(21) This equation is based on the minimal distances between H_8 and $H_{1'}$, $H_{2'}$, and $H_{3'}$ in forms 1 and 11. From $r_{H_8-H_{1'},min} = 2.58$ Å, $r_{H_8-H_{2'},min} = 1.84$ Å, $r_{H_8-H_{3'},min} = 1.94$ Å, it follows that $(r_{H_8-H_{2'},min})^6 \approx (r_{H_8-H_{3'},min})^6 \approx {}^1/_8(r_{H_8-H_{1'},min})^6$. This leads to $P_{syn} = 8f_8\{1'\}/(8f_8\{1'\} + f_8\{2'\} + f_8\{3'\})$.

Stereochemistry of the Carbon-Skeleton Rearrangements Dependent on Coenzyme B_{12}. MNDO Quantum Chemical Calculations

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Abstract: Vitamin B_{12} acts as a cofactor in the enzyme-catalyzed carbon-skeleton rearrangements of methylmalonyl-coenzyme A to succinyl-coenzyme A, methylaspartate to glutamate, and methylitaconate to methylene glutarate. The stereochemistry of these isomerizations will be discussed on the basis of anionic enzyme-stabilized cyclopropane intermediates. With the help of MNDO calculations, energy profiles are constructed for the three ring-closure reactions. Following the reaction path, charge distribution and migration in the substrates are monitored, as well as the evolution of the coefficients of the atomic orbitals in the HOMO of the cyclopropane intermediates. Large charge migration will force the electron density at the carbon that undergoes inversion of configuration in the methylaspartate isomerization, in a direction opposite to the glycyl group. Orbital inversion on the adjacent glycyl carbon prevents the electron density to flow back, which is reflected in the antibonding character of the bond between these two carbons in the HOMO. On the other hand, retention of configuration in the methylmalonyl-coenzyme A rearrangement is attended with a smaller charge migration and a bonding character of the corresponding bond in the HOMO. Inversion of configuration is suggested for the methylitaconate isomerization.

Vitamin B_{12} has been shown to act as an obligatory enzyme cofactor, to effect a remarkable series of 11 rearrangement reactions. They consist of the carbon-skeleton rearrangements and the hydroxyl and the amine migrations, according to the bond that is broken during the reaction.[1] In this study special attention is given to the carbon-skeleton rearrangements, i.e., the isomerization of L-methylmalonyl-coenzyme A to succinyl-coenzyme A, *threo*-β-methylaspartate to L-glutamate, and β-methylitaconate

to α-methyleneglutarate, where hydrogens (for the sake of clarity deuterons are used in Figure 1) and a carbon-centered group R migrate in an intramolecular [1,2] shift. Under enzymatic conditions the hydrogen (deuteron in Figure 1) is transferred via the 5'-methylene group of vitamin B_{12}[2] and migrates in methylmalonyl-coenzyme A with retention of configuration[3] (i.e., the incoming hydrogen and the leaving group R occupy the same position). The migration in methylaspartate occurs with inversion

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