CONFORMATIONAL STUDIES ON DIRIBONUCLEOSIDE MONOPHOSPHATE ANALOGS CONTAINING A NONISOSTERIC, ISOPOLAR, PHOSPHONATE-BASED INTERNUCLEOTIDE LINKAGE

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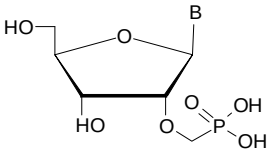
Two types of (2′-5′) and (3′-5′) isomers of phosphonate analogs of diribonucleotides derived from 2′-, 3′- or 5′-*O*-phosphonomethyl ribonucleosides, that differ in the position of methylene group in the phosphonate internucleotide linkage, have been studied in aqueous solution by NMR techniques in order to compare their basic structural features with those of the natural (3′-5′) diribonucleotides. Despite the additional methylene group in the ribose–phosphate backbone, dinucleoside phosphonates are structurally and conformationally very similar to their natural counterparts.

Key words: Conformation; Diribonucleotide analogs; Phosphonates.

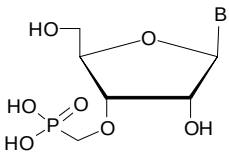
During the past decade many intriguing modifications of the internucleotide linkage of oligonucleotides have been proposed, with the ultimate goal being to prepare compounds with enhanced metabolic stability and cellular uptake, and with equal or better hybridization properties (for review see ref.[1]). Virtually all modifications of the internucleotide linkage preserve the number of atoms in the sugar–phosphate backbone. This conservation of isostericity is a common starting point in the design of a modified internucleotide linkage. Surprisingly, very little attention has been devoted to an enzymatically and chemically stable, isopolar, isosteric phosphonate bond as a potential candidate for the internucleotide linkage[2], although nucleotide analogs containing a CH_2 group instead of a 3′- or 5′-oxygen atom have been known for more than 30 years[3,4] and even a crystal structure exists[5].

A novel type of enzymatically stable, isopolar but nonisosteric nucleotide derivatives **1**, **2** and **3a** has been prepared[6]. These derivatives contain a methanephosphonic acid residue attached to either the 2′-, 3′- or 5′-hydroxyl group of the nucleoside by an ether

bond. Conservation of the 5′-oxygen atom in the molecule of the analog, and the possibility of free rotation around the P–C–O bond, seems to be important for the interaction of these compounds with various enzymes (e.g., inhibition of 5′-nucleotidase and phosphomonoesterase[7], RNA-polymerase[8], uridine kinase of leukemic cells[9], etc.). In a previous NMR study[10] on monomethyl esters of 5′-*O*-phosphonylmethyl ribonucleosides **3b**, these compounds in solution were found to be conformationally similar to that of natural 5′-nucleotides.



1



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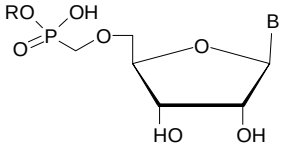
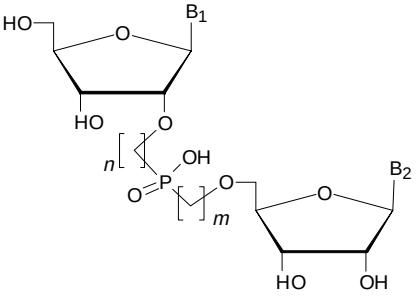
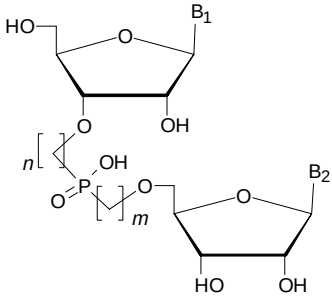


3a, R = H
3b, R = CH_3



4a, $n = 1$, $m = 0$; N_1p_c-N_2 (2′-5′)
4b, $n = 0$, $m = 1$; N_1p_c-N_2 (2′-5′)



5a, $n = 1$, $m = 0$; N_1p_c-N_2 (3′-5′)
5b, $n = 0$, $m = 1$; N_1p_c-N_2 (3′-5′)

On the basis of these results, two types of 2′-5′ and 3′-5′ isomers (N_1p_c-N_2 **4a**, **5a** and N_1-p_cN_2 **4b**, **5b**) of phosphonate analogs of diribonucleotides, differing in the position of the methylene bridge in the modified internucleotide linkage (O3′(2′)–CH_2–P–O5′, N_1p_c-N_2; O3′(2′)–P–CH_2–O5′, N_1-p_cN_2), have been synthesized[11]. Since these dimers are extremely stable against cleavage by snake venom exonuclease and ribonucleases, they could be also used as promising constructs in longer oligonucleotides, providing that this nonisosteric but conformationally flexible block with P–C–O grouping mimics the natural diribonucleotide unit.

The capability of base-stacking is essential to evaluate the prospective use of these nonisosteric units. Conformational analysis and comparison of structural features of the smallest oligomers represented by dimers could help answer this question.

The circular dichroism spectra of dipurine [R-R] (Ap_c-A (2′-5′), Ap_c-A (3′-5′), A-p_cA (3′-5′)), dipyrimidine [Y-Y] (Up_c-U (2′-5′), Up_c-U (3′-5′), Cp_c-C (2′-5′), Cp_c-C (3′-5′), U-p_cU (2′-5′), U-p_cU (3′-5′)), purine-pyrimidine [R-Y] (Ap_c-U (2′-5′), Ap_c-U (3′-5′), A-p_cU (2′-5′), A-p_cU (3′-5′), G-p_cC (2′-5′), G-p_cC (3′-5′)) and pyrimidine-purine [Y-R] (Up_c-A (2′-5′), Up_c-A (3′-5′), U-p_cA (2′-5′), C-p_cG (2′-5′), C-p_cG (3′-5′)) phosphonate analogs of diribonucleotides revealed[12] the strongest intramolecular base-stacking interactions for Ap_c-A (2′-5′), U-p_cU (2′-5′), U-p_cU (3′-5′), A-p_cU (3′-5′), G-p_cC (2′-5′), G-p_cC (3′-5′), Up_c-A (2′-5′) and U-p_cA (2′-5′). In this paper we present a comprehensive ^{1}H NMR study and conformational analysis of these modified diribonucleotides (exhibiting strongest base-stacking) in terms of pseudorotational parameters for the ribose rings and exocyclic backbone rotamers.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 500.13 MHz on a Bruker AM-500 spectrometer. All experiments were run at 21 °C. In order to minimize the residual HDO signal, the dinucleotides were lyophilized from deuterium oxide (Aldrich, 99.8% D) three times. The phosphate buffer, pH 7.2, containing 0.1 mM EDTA to remove paramagnetic ions, was lyophilized in the same way. The final lyophilized buffer, pD 7.4, was dissolved in deuterium oxide (Aldrich, 99.95% D) to yield 0.1 M solution. Buffered solutions of lyophilized dimers, with sodium disilapentanesulfonate added as an internal standard, were adjusted to a concentration of 0.05 mol/l. All manipulations of lyophilized dinucleotides and buffer were performed under a dry, inert (N_2) atmosphere.

^{1}H signal assignments were made based on chemical shifts, observed multiplicities, and decoupling experiments. Chemical shifts (δ) and spin-coupling constants (J) for the sugar protons of each nucleotidyl unit were determined with an accuracy of 0.003 ppm and 0.1 Hz, respectively, by spectral simulation-iteration procedures using the LAOCOON3 program[13]. Definitions of torsion angles and conformational nomenclature conform to the IUPAC/IUB convention for nucleic acids[14,15].

RESULTS AND DISCUSSION

Assignments

Due to the presence of a methylene group in the modified internucleotide linkage, only protons of one nucleotidyl residue in each diribonucleotide exhibit scalar spin–spin (J) interaction with the phosphorus nucleus. This fact, together with decoupling experiments, allowed unambiguous assignment of ribose proton signals for each nucleotidyl unit. According to Remin and Shugar[16] the H5′ and H5" proton signals were assigned such that $\delta_{H5'} > \delta_{H5"}$, where H5′ refers to the proton *gauche* to H4′ and O4′ (γ^+ conformation). Unambiguous stereochemical assignments of the PCH_2 proton signals could not be made; the lower field doublet of doublets (dd) is denoted as PCH_a and the higher field as PCH_b.

Chemical Shifts and Coupling Constants

The proton chemical shifts and coupling constants of the studied phosphonate analogs of diribonucleotides, refined by spectral simulation, are summarized in Table I and Table II, respectively. The chemical shift data show that, in all cases except for Y-R dimers (IUPAC-IUB nomenclature, R = purine, Y = pyrimidine), the H-2′ and H-3′ protons of the 2′- and 3′-nucleotidyl units always resonate at lower field than the same protons in the 5′-nucleotidyl units. Also, the chemical shift difference between the H-5′ and H-5" proton signals ($\Delta_{5'5"} = \delta_{5'} - \delta_{5"}$) is greater in the 5′-nucleotidyl moieties than in the 2′- or 3′-nucleotidyl moieties. The difference is smallest for G-p_cC(2′-5′). The signals of the remaining ribose protons, H1′ and H4′, do not exhibit any discernable trends. The resonances of the base protons of uridine residues in the uridine-uridine dimers show a pronounced shift towards higher field when one uridine residue is replaced by the adenosine moiety, regardless of the type of internucleotide linkage ((2′-5′) or (3′-5′)) and the position of the methylene group in that linkage.

Interesting trends are also revealed for coupling constants of the ribose protons (Table II). For each nucleotidyl unit, $J_{1'2'}$ is smaller than $J_{3'4'}$ except for the 2′-adenosine residue in the Ap_c-A (2′-5′). In Y-R and R-Y dimers, the $J_{1'2'}$ of purine nucleotides are larger than that of pyrimidine nucleotides. On the other hand, the coupling constants $J_{3'4'}$ exhibit the reverse relations in those dimers. The $J_{2'3'}$ are nearly constant (within 0.8 Hz) across the whole series. Similarly, no significant variations exist for either $J_{2'P}$, $J_{3'P}$ or $J_{4'P}$.The magnitude of scalar coupling of the H-4′ proton to the H-5′ is smaller than the magnitude of scalar coupling between H-4′ and H-5" protons in all nucleotidyl units, with an exception of the Ap_c-A (2′-5′) dimer, where $J_{4'5'}$ and $J_{4'5''}$ are the same within experimental errors for each adenosine residue, respectively.

Modified dinucleotides exhibiting $J_{2'P}$ or $J_{3'P}$ couplings exhibit neither $J_{4'P}$, $J_{5'P}$ nor $J_{5''P}$ and vice versa, due to an extra methylene group in the internucleotide linkage. There are only two dinucleotides in the series showing $J_{4'P}$, $J_{5'P}$ and $J_{5''P}$ couplings. The sum $J_{5'P} + J_{5''P}$ ($\Sigma' J_{5'P} + J_{5''P}$) for the Up_c-A (2′-5′) is much larger than for the Ap_c-A (2′-5′). However, the value of Σ' for the latter dimer is the same[17] as for natural ApA (3′-5′).

Conformations of Ribofuranose Rings

Conformations of the furanose rings can be elucidated by analyzing the vicinal coupling constants of the ribose protons J_{ij} in terms of pseudorotation parameters[18] of two conformers undergoing interconversion and their relative populations in equilibrium. One of the conformers belongs to the *N* region and the other is from the *S* region of the pseudorotation pathway. Therefore, the experimental NMR coupling constants represent time-averaged couplings, that are related to the couplings of these two conformers ($^N J_{ij}$, $^S J_{ij}$) and their relative populations (X_N, X_S) by the expressions[19]

TABLE I

^{1}H NMR chemical shifts (δ, ppm) of phosphonate analogs of diribonucleoside monophosphates

Compound		Chemical shifts									
		H1′	H2′	H3′	H4′	H5′	H5′′	H2(H5)	H8(H6)	P-CH_a	P-CH_b
U-p_cA(2′-5′)	U	5.806	4.708	4.184	4.011	3.852	3.727	5.609	7.665	3.840	3.769
	p_cA	6.012	4.699	4.434	4.261	3.935	3.719	8.138	8.394		
U-p_cU(3′-5′)	U	5.805	4.397	4.616	4.266	3.935	3.823	5.766[a]	7.914[b]	3.819	3.774
	p_cU	5.841	4.270	4.276	3.960	3.966	3.772	5.901[a]	8.110[b]		
U-p_cU(2′-5′)	U	5.948	4.860	4.340	4.096	3.870	3.770	5.821[a]	7.804[b]	3.820	3.732
	p_cU	5.887	4.277	4.256	4.161	3.899	3.733	5.876[a]	8.070[b]		
A-p_cU(3′-5′)	A	5.993	4.677	4.768	4.386	4.007	3.882	8.090	8.254	3.819	3.793
	p_cU	5.508	4.136	4.145	4.111	3.967	3.733	5.611	7.846		
G-p_cC(2′-5′)	G	5.955	5.176	4.749	4.164	3.880	3.762	–	7.923	3.801	3.665
	p_cC	5.810	4.137	4.173	4.101	3.799	3.677	5.619	8.015		
G-p_cC(3′-5′)	G	5.807	4.663	4.800	4.325	3.937	3.843	–	7.941	3.808	3.702
	p_cC	5.761	4.183	4.165	4.153	3.971	3.786	5.763	7.890		
Up_c-A(2′-5′)	Up_c	5.323	4.289	4.049	3.953	3.854	3.716	5.590	7.651	3.896	3.751
	A	5.994	4.510	4.732	4.274	4.133	3.836	8.105	8.321		
Ap_c-A(2′-5′)	Ap_c	5.970	4.573	4.585	4.268	3.823	3.778	7.853[a]	8.065[a]	4.043	3.791
	A	5.788	4.296	4.330	4.204	4.319	4.142	7.857[a]	8.134[a]		

[a,b] Assignments in the same column may be reversed for given dimer.

TABLE II
^{1}H NMR coupling constants ($J_{i,j}$, Hz) of phosphonate analogs of diribonucleoside monophosphates

Compound		*J*													
		1′,2′	2′,3′	3′,4′	4′,5′	4′,5″	5′,5″	2′,P	3′,P	4′,P	5′,P	5″,P	5,6	P,CH_a	P,CH_b	$^{gem}CH_{ab}$
U-p_cA(2′-5′)	U	3.3	5.2	6.8	2.3	4.0	–12.8	8.7					8.2	9.0	9.4	–13.2
	p_cA	4.9	4.9	5.5	2.5	5.4	–11.0									
U-p_cU(3′-5′)	U	3.5	5.1	6.4	2.6	4.0	–13.0		8.8				8.1	8.0	10.6	–13.2
	p_cU	2.9	5.2	6.4	2.8	3.2	–11.4						8.1			
U-p_cU(2′-5′)	U	4.3	5.4	5.6	2.8	4.3	–12.8	8.8					8.1	8.2	9.4	–13.3
	p_cU	3.6	5.2	5.4	2.5	3.5	–11.2						8.1			
A-p_cU(3′-5′)	A	3.4	4.8	6.4	2.5	3.5	–13.2		8.6					7.6	10.7	–13.2
	p_cU	2.8	5.0	6.8	2.7	3.2	–11.4						8.0			
G-p_cC(2′-5′)	G	4.0	5.6	6.0	2.7	4.3	–12.8	8.4						9.0	10.3	–12.7
	p_cC	2.9	4.9	6.8	2.5	2.3	–11.0						7.4			
G-p_cC(3′-5′)	G	4.1	5.0	5.6	2.8	4.0	–13.0		8.6					8.8	10.1	–13.1
	p_cC	2.2	5.1	6.4	1.9	3.1	–11.0						7.5			
Up_c-A(2′-5′)	Up_c	2.5	5.0	7.7	2.6	3.8	–13.0						8.1			
	A	5.1	5.2	5.4	2.5	4.3	–12.2			2.5	4.3	5.0		8.2	10.9	–13.2
Ap_c-A(2′-5′)	Ap_c	6.5	5.2	3.0	2.7	2.9	–13.0									
	A	3.8	5.0	5.5	2.5	2.5	–12.1			2.5	3.0	3.2		8.6	11.0	–13.0

$$_{\exp}J_{ij} = X_N\,{}^{N}J_{ij} + X_S\,{}^{S}J_{ij} \ , \qquad (1)$$

where $X_N + X_S = 1$.

Vicinal proton–proton coupling constants J_{ij} of the individual conformers can be expressed as a function of the proton–proton dihedral angles Φ_{ij} using a Karplus equation[20]

$$J_{ij} = A \cos^2 \Phi_{ij} + B \cos \Phi_{ij} + C \qquad (2)$$

with[18,21] $A = 10.0$, $B = -1.2$, $C = 0$.

The torsion angles τ_i in an approximately equilatelar 5-membered ring are determined by the pseudorotational parameters[18,19], phase angle P and puckering amplitude τ_m

$$\tau_i = \tau_m \cos (P + (i - 2)144)) \ , \qquad (3)$$

where $i = 0, 1, 2, 3, 4$.

The dihedral angles, Φ_{ij}, can be then related to the pseudorotational angles by a simple geometrical relations[21]

$$\Phi_{1'2'} = \tau_1 + 120°$$

$$\Phi_{2'3'} = \tau_2$$

$$\Phi_{3'4'} = \tau_3 - 120° \ . \qquad (4)$$

Using Eqs (*1*)–(*4*) all conformations for τ_m values between 35 and 45° in 1° steps and all P values in 3° steps were computed. From such data base the possible fits of pairs between $(J_{1'2'} + J_{3'4'})$ and $J_{2'3'}$ were searched and printed.

We also used the more complex treatment of Altona's group which accounts for differences in electronegativity of the substituents. The vicinal proton–proton coupling constants J_{ij} of the individual conformers are expressed as a function of the torsion (dihedral) angles Φ_{ij} with a generalized Karplus equation[2]

$$J_{ij} = P_1 \cos^2 \Phi_{ij} + P_2 \cos \Phi_{ij} + P_3 + \Sigma\, \Delta\chi_k \{P_4 + P_5 \cos^2 (\zeta_k \Phi_{ij} + P_6 |\Delta\chi_k|)\} \ , \qquad (2a)$$

where P_1–P_6 are empirically determined parameters (P_1 = 13.24, P_2 = –0.91, P_3 = 0, P_4 = 0.53, P_5 = –2.41, P_6 = 15.5°), Φ_{ij} is the proton–proton torsion angle in a given H_i–C–C–H_j fragment, $\Delta\chi_k$ is the difference in the Huggins electronegativity between substituent and hydrogen in the fragment, and ζ_k = +1 or –1 accounts for the orientation of the substituent.

For β-D-ribofuranose systems, the torsion angles Φ_{ij} are related to the pseudorotation parameters, phase angle P and puckering amplitude τ_m, via equations[23]

$$\Phi_{1'2'} = 123.3° + 1.102\tau_m \cos(P - 144°)$$

$$\Phi_{2'3'} = 0.2° + 1.090\tau_m \cos(P)$$

$$\Phi_{3'4'} = -124.9° + 1.095\tau_m \cos(P + 144°) \quad . \qquad (4a)$$

Equations (*1*), (*2a*) and (*4a*) were used to deduce the pseudorotation parameters P^N, P^S, τ_m^N , τ_m^S , and the relative population X_N for the two state conformational model of the furanose rings in the modified dinucleotides. As there are only three observable coupling constants ($J_{1'2'}$, $J_{2'3'}$, $J_{3'4'}$) to calculate five parameters, some constraints have to be introduced in the calculation procedure. In the first step, an approximate value of the X_N was estimated using $X_N + X_S = 1$ and Eq. (*5*) (ref.[24]).

$$X_S/X_N = J_{1'2'}/J_{3'4'} \qquad (5)$$

The values of P^N and P^S were then calculated by an iterative least-squares computer program starting from P^N = 18° and P^S = 162°, while the puckering amplitudes τ_m^N and τ_m^S ($\tau_m^N = \tau_m^S = 36°$) and the relative population X_N were kept fixed. The justification of the equality τ_m^N and τ_m^S has been discussed previously[21,25]. The phase amplitudes P^N = 18° and P^S = 162° correspond to the pure ^3E and ^2E pseudorotation conformations, respectively, which were used to describe conformational properties of the ribofuranose rings in the natural (3′-5′) dinucleotides[17,26]. In the second step, the τ_m^N and τ_m^S were iterated while the phase angles P^N and P^S and the X_N were kept fixed at values determined in the first step. Next, the phase angles and the X_N were iterated while the puckering amplitudes were kept constant. Then, the puckering amplitudes and the X_N were iterated and the phase angles were kept constant. These last two steps were repeated until a minimum in the root mean square difference between observed and calculated coupling constants was reached.

The values of the pseudorotation parameters and the relative populations X_N determined by both procedures are given in Table III. Since the precision of the coupling constants is ±0.1 Hz, it is evident that the simplified method[21] is largely sufficient to describe the conformational equlibria of ribonucleotides and dinucleotides.

TABLE III
Ribofuranose ring conformational properties of phosphonate analogs of diribonucleoside monophosphates

Compound		Pseudorotation parameters[a] and conformational populations[b]										
		Simplified method[21]					Method of Altona[22,45]					
		τ_m^N	P^N	P^S	X_N	$K_{eq}{}^c$	τ_m^N	P^N	τ_m^S	P^S	X_N	$K_{eq}{}^c$
U-p_cA(2′-5′)	U	41	18	162	0.68	0.47	43	38	35	189	0.69	0.45
	p_cA	43	21	159	0.53	0.89	44	22	40	144	0.46	1.17
U-p_cU(3′-5′)	U	41	15	165	0.65	0.54	37	30	40	180	0.68	0.47
	p_cU	39	9	171	0.69	0.45	34	11	36	171	0.72	0.39
U-p_cU(2′-5′)	U	39	18	162	0.57	0.75	36	36	38	171	0.58	0.72
	p_cU	39	5	175	0.60	0.67	34	7	35	171	0.60	0.67
A-p_cU(3′-5′)	A	42	12	168	0.66	0.53	40	23	38	171	0.66	0.52
	p_cU	40	12	168	0.71	0.40	37	16	36	171	0.74	0.35
G-p_cC(2′-5′)	G	39	21	159	0.61	0.65	35	27	36	153	0.61	0.64
	p_cC	41	12	168	0.70	0.42	38	18	38	171	0.73	0.37
G-p_cC(3′-5′)	G	41	12	168	0.58	0.73	38	20	38	162	0.57	0.75
	p_cC	39	–3	183	0.75	0.34	35	–5	34	189	0.78	0.28
Up_c-A(2′-5′)	Up_c	42	18	162	0.76	0.31	38	22	40	153	0.82	0.22
	A	42	24	156	0.52	0.94	41	45	42	162	0.53	0.89
Ap_c-A(2′-5′)	Ap_c	39	12	168	0.31	2.22	36	39	38	162	0.24	3.17
	A	40	6	174	0.59	0.68	36	14	37	171	0.59	0.69

[a] In °; [b] in mole fractions; [c] $K_{eq} = X_S/X_N$.

The X_N values show that nearly all nucleotidyl units exist predominantly in the *N*-type conformation. Only the p_cA fragment of the U-p_cA (2′-5′) and the Ap_c fragment of the Ap_c-A (2′-5′) exhibit a preference for the *S*-type conformation. For a given dimer, the *N*-type conformer is more populated in the 5′-nucleotide residue than in the 2′- or 3′-nucleotide residue except for the U-p_cA (2′-5′) and the Up_c-A (2′-5′). The nucleotidyl units of natural (3′-5′) diribonucleotides are predominantly in the *N*-type conformation, as shown by Danyluk et al.[17,26].

The puckering amplitudes and the phase angles do not show any characteristics common to all nucleotide units of the studied dimers with modified internucleotide linkages. There are always some exceptions from the relations exhibited by the majority of the dimers. However, all values of the puckering amplitudes are within the same range of 34–42° as found for the β-D-furanoside fragments of natural nucleosides and nucleotides[25].

In the N_1-p_cN_2 type dimers interesting relations exist between τ_m^N, τ_m^S and P^N of the 5′-nucleotide fragments and τ_m^N, τ_m^S and P^N of the 2′- or 3′-nucleotide fragments. The *N*-conformers of the 5′-fragments exhibit smaller phase angles than the *N*-conformers of the 2′- or 3′-fragments. On the other hand, the phase angles of the *S*-conformers do not show any unique relation for those fragments. The puckering amplitudes τ_m^N and τ_m^S retain the same relation as the phase angles P^N only for the N_1-p_cN_2 dimers with (3′-5′) linkage. Although in the N_1-p_cN_2(2′-5′) dimers the values of the τ_m^N and τ_m^S for the 5′-fragments can be bigger or smaller than the values of corresponding parameters for the 2′-fragments, both τ_m^N and τ_m^S always exhibit the same relation in a given dimer.

For the U-p_cA (2′-5′) and the Up_c-A (2′-5′) there is a clear distinction between pseudorotation parameters of the corresponding nucleotidyl fragments as evident from data in Table III. One can speculate that the conformational difference of these dimers is due to the different position of the methylene group in their interunit linkages.

Backbone Conformations

The backbone of the natural polynucleotide chain with the 3′-5′ phosphodiester internucleotide linkages is made up of a repeating unit of six bonds in the order P_i–O5′–C5′–C4′–C3′–O3′–P_{i+1}. Its conformation is determined by the sets of six torsion angles[15,28] α, β, γ, δ, ε and ζ for each unit about the respective backbone bonds. The α and ζ torsion angles cannot be established from vicinal coupling constants.

By analogy we then define the backbone torsion angles for the diribonucleotides with phosphonate interunit linkages as indicated in Fig. 1. The convention introduced by Klyne and Prelog[29] for the signs of the torsion angles is strictly followed through the paper. The definition of torsions angles allows for direct comparison of the backbone conformational properties of dimers with natural and modified interunit linkages.

Populations of three classical rotamers β^+, β^t and β^- for the torsion angle β(C4′–C5′–O5′–P) can be established from two proton–phosphorus vicinal coupling constants $J_{5'P}$ and

$J_{5''P}$. Several parameterizations of the Karplus equation have been proposed[28–33] for the H–C–O–P fragment. In this work, the parameterization of Mooren et al.[33] was used, leading to the *trans* proton–phosphorus coupling, J_t = 23.0 Hz, and the *gauche* proton–phosphorus coupling J_g = 2.5 Hz. The rotamer populations β^+, β^t, β^- were then calculated[34] from Eqs (*6*)

$$\beta^+ = (J_{5''P} - J_g)/(J_t - J_g)$$

$$\beta^t = ((J_t + J_g) - (J_{5'P} + J_{5''P}))/(J_t - J_g)$$

$$\beta^- = (J_{5'P} - J_g)/(J_t - J_g) \qquad (6)$$

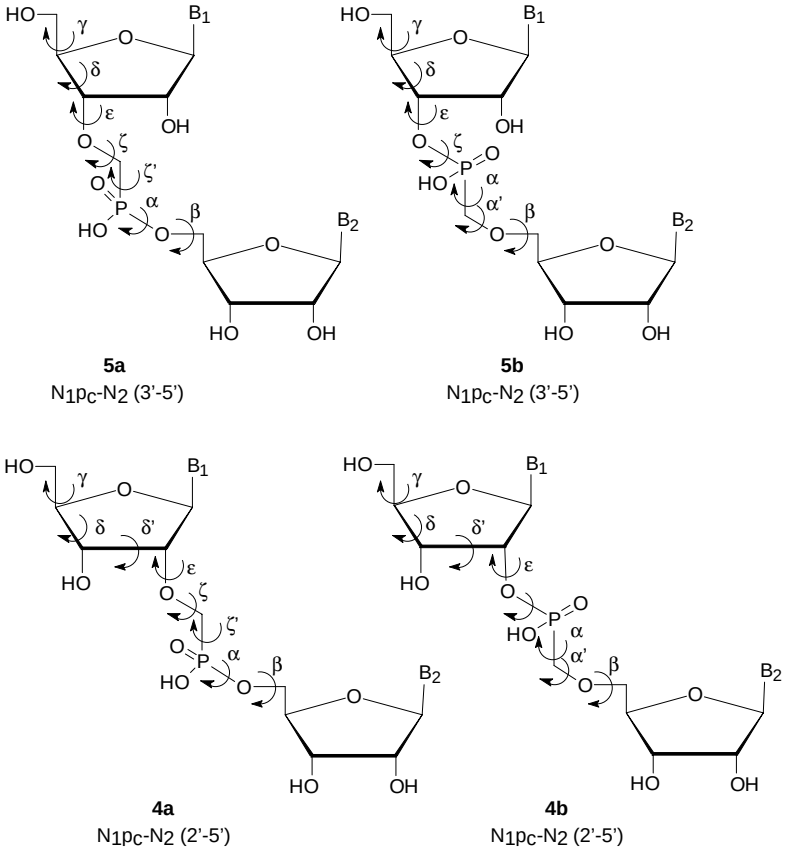


FIG. 1
Definition of the backbone torsion angles α, α′, β, γ, δ, δ′, ε, ε′, ζ and ζ′ for dimers N_1p_c-N_2 and N_1-p_cN_2

and the values are given in Table IV. It is obvious from Eqs (*6*) that calculated values of the β^t do not depend on the assignment of H-5′ and H-5" signals. Both the U-p_cA (2′-5′) and the Up_c-A (2′-5′) exhibit a high preference for the β^t conformation. For the latter, population of β^t is even higher (+4%) than for the natural UpA dimer, while for the former, it is slightly lower (–3%) in comparison to the natural UpA (ref.[26]).

Magnitudes of the long-range coupling constant $J_{4'P}$ provide information about the conformational distribution simultaneously around both C5′–O5′ and C4′–C5′ bonds. Inspection of a Dreiding model reveals that simultaneous existence of β^t and γ^+ conformations leads to the planar *W*-conformation of the fragment H4′–C4′–C5′–O5′–P. This conformation corresponds[35] to maximum $J_{4'P}$ of 2.7 Hz, which is very close to our $J_{4'P}$ values 2.5 Hz for the N_1p_c-N_2 type dimers (Table II). However, Altona[28] and Sarma et al.[36] have determined for the *W*-conformation the $J_{4'P}$ values of 3.3 Hz and 2.8 Hz, respectively.

Populations of γ^+, γ^t and γ^- conformers about the C4′–C5′ bond were calculated from vicinal coupling constants $J_{4'5'}$ and $J_{4'5''}$ using Eqs (*7*) with a *trans* proton–proton coupling constant[28] J_t of 11.5 Hz and a *gauche* proton–proton coupling constant J_g of 1.8 Hz.

$$\gamma^+ = ((J_t + J_g) - (J_{4'5'} + J_{4'5''}))/(J_t - J_g)$$

$$\gamma^t = (J_{4'5''} - J_g)/(J_t - J_g)$$

$$\gamma^- = (J_{4'5'} - J_g)/(J_t - J_g) \quad . \qquad (7)$$

The values for all nucleotidyl units are given in Table IV. The data reveal that, in all dimers there is a distinct preference for the γ^+ conformation. The degree of preference is higher for the 5′-fragments than for 2′- or 3′-fragments except in Y-R dimers. In Y-Y and R-Y dimers both isomers (2′-5′) and (3′-5′) exhibit almost the same γ^+ population. With exception of the Ap_c-A (2′-5′) dimer, populations of the γ^+ rotamer in diribonucleotides with modified phosphonate linkages are lower than corresponding γ^+ populations in the natural diribonucleotides[17,26].

Backbone torsion angles δ (O3′–C3′–C4′–C5′) and δ' (O2′–C2′–C3′–C4′) are related to phase angle *P* and puckering amplitude τ_m by Eq. (*8*)

$$\delta = 120.6 + 1.1\tau_m \cos(P + 145.2)$$

$$\delta' = 119.8 + 1.09\tau_m \cos(P) \qquad (8)$$

or using simple geometrical relations they can be expressed as[31] Eq. (*8a*)

$$\delta = 120 - \tau_3$$

$$\delta' = 120 + \tau_2 \; . \qquad (8a)$$

TABLE IV
Rotamer populations and torsion angles about the backbone bonds for the phosphonate analogs of diribonucleoside monophosphates

Compound		β(C4′-C5′-O5′-P)[a]			γ(C3′-C4′-C5′-O5′)[a]			δ(O3′-C3′-C4′-C5′)[b]		δ′(O2′-C2′C3′C4′)[b]	
		β^+	β^t	β^-	γ^+	γ^t	γ^-	$^N\delta$	$^S\delta$	$^N\delta'$	$^S\delta'$
U-p_cA(2′-5′)	U				0.72	0.23	0.05	73.5	155.3	156.8	82.1
	p_cA				0.56	0.37	0.07	73.7	135.1	164.2	84.5
U-p_cU(3′-5′)	U				0.69	0.23	0.08	79.6	156.7		
	p_cU				0.75	0.14	0.11	86.1	149.2		
U-p_cU(2′-5′)	U				0.64	0.26	0.10	81.2	150.8	151.6	78.9
	p_cU				0.75	0.18	0.07	87.4	148.4	156.7	82.1
A-p_cU(3′-5′)	A				0.75	0.18	0.07	77.9	150.8		
	p_cU				0.76	0.14	0.10	82.2	149.2		
G-p_cC(2′-5′)	G				0.65	0.26	0.09	83.0	139.3	153.3	84.8
	p_cC				0.88	0.05	0.07	81.2	150.8	158.8	78.9
G-p_cC(3′-5′)	G				0.67	0.23	0.10	80.7	145.9		
	p_cC				0.86	0.13	0.01	90.7	154.3		
Up_c-A(2′-5′)	Up_c				0.71	0.21	0.08	79.7	141.4	158.5	81.0
	A	0.12	0.77	0.09	0.67	0.26	0.07	76.7	148.5	151.2	76.3
Ap_c-A(2′-5′)	Ap_c				0.79	0.11	0.10	81.1	145.9	150.3	80.4
	A	0.03	0.94	0.03	0.86	0.07	0.07	83.6	150.0	157.9	80.0

[a] In mole fractions. [b] In °.

The values of δ and δ′ were calculated for both *N* conformer and *S* conformer of each corresponding nucleotidyl unit and are also given in Table IV.

Conformational properties about the C3′–O3′ and C2′–O2′ bonds of the respective isomers can be deduced from the magnitudes of $J_{3'P}$ and $J_{2'P}$, respectively. It is obvious that only limited information about conformations around these bonds can be gained from the respective coupling constants. The Karplus equation[33] for the fragment H–C–O–P affords, in principle, a pair of the dihedral angles $\pm\Phi_{HP}$ for each constant $J_{3'P}$ and $J_{2'P}$, respectively, corresponding to the backbone torsion angles ε and ε′, respectively. However, X-ray crystal structure investigations of the 3′- or 2′-nucleotides[17,37,38] do not show the presence of the ε^+ rotamer, while the ε^- rotamer has been found about the C2′–O2′ bond[38]. Theoretical calculations on the natural diribonucleotides or the polynucleotides[17,39–43] do not support the existence of the ε^+ rotamer either. A two state conformational model around the backbone C3′–O3′ bond was proposed[44] with nonconventional rotamers ε^t ($\varepsilon \approx 210°$) and ε^- ($\varepsilon \approx 330°$). It is reasonable to assume that the ε^+ rotamer does not occur in either 2′-5′ or 3′-5′ isomers of diribonucleotides with the phosphonate interunit linkages. Using the generalized Karplus equation for proton–phosphorus coupling constants[33], the values of the observed $J_{3'P}$ and $J_{2'P}$ constants (Table II) yield a value of the $\varepsilon \approx 216°$, corresponding to virtual exclusive γ^- of the phosphate groups. We therefore conclude that these diribonucleoside phosphonates assume a nearly pure γ^- conformation for both the ε and ε′ angles, as has been observed for natural dinucleoside phosphates[17,26,44].

Base Stacking

A method for the calculation of the fractions of base-stacked forms of the natural (3′-5′) diribonucleoside monophosphates using ribose $J_{1'2'}$ and $J_{3'4'}$ coupling constants has been proposed by Altona[45]. The method is based upon two assumptions. First, the ribofuranose rings of both 3′- and 5′-nucleotidyl fragments exhibit a preference for the *N*-type conformations. Second, in unstacked forms the transmission of conformations between 3′- and 5′-fragments is relatively insignificant.

The conformational transmission is certainly even less important in unstacked forms of diribonucleotides with both (3′-5′) and (2′-5′) phosphonate interunit linkages. As X_N data in Table III reveal, nucleotidyl units of all dimers except U-p_cU (2′-5′) and A-p_cA (2′-5′) show a preference for the *N*-type conformers. For dimers with both nucleotidyl fragments exhibiting a preference for the *N*-type conformations, the fraction of the base stacking p(*S*) was then calculated[17,45] from the experimental coupling constants for the nucleotidyl fragments of dimers and the corresponding monomers[10,17,26,46]. The p(*S*) values obtained by this method for the 3′- and 5′-bound nucleoside show, however, such large spread that their values do not provide credible results, although they indicate considerable stacking. We have therefore not included them in this paper.

CONCLUSIONS

The introduction of an additional CH_2 group in the sugar–phosphate backbone of the dinucleoside phosphates changes little its conformation. This appears to be independent of the position of the methylene group, i.e., on the 2-′, 3′- or 5′-side. The resistance of the studied type of phosphonate linkage to various nucleases along with the fact that conformational properties appear to be greatly similar to those of dinucleoside monophosphates make these analogs suitable candidates for further use in oligonucleotide chemistry.

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