Intimate Details of the Conformational Characteristics of Deoxyribodinucleoside Monophosphates in Aqueous Solution

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Abstract: The detailed conformational features and dynamics of the naturally occurring deoxyribodinucleoside monophosphates d-ApA, d-GpG, d-ApG, d-GpA, d-CpC, d-TpT, d-CpT, d-TpC, d-ApC, d-ApT, d-GpC, d-GpT, d-CpA, d-CpG, d-TpA, and d-TpG have been investigated at 20 °C and at higher temperatures in aqueous solution by nuclear magnetic resonance spectroscopy (NMR). Complete sets of NMR parameters were derived for each nucleotidyl unit by computer synthesis of spectra. A complete set of parameters was also obtained for all the constituent monomeric units at a concentration and ionization state comparable to that of the dimers. Even though the molecular framework of the dimers displays flexibility and they exist in solution as a mixture of conformers, the data lead to a number of discernible trends throughout the series. Thus, the pentose rings of the dimers and monomers exist as an equilibrium mixture of $^2E \rightleftarrows {}^3E$ conformers with significant preference for the ^2E pucker in all cases irrespective of the nature of the base and sequence. χ_1 was found to be always less than χ_2 and the C(4′)-C(5′) and C(5′)-O(5′) bonds form a stable conformational network in which $\psi_2 \simeq 60°$ and $\phi_2 \simeq 180°$. The C(3′)-O(3′) bond occupies a domain in which $\phi_1' \simeq 200°$. Except for d-TpT and d-TpC, the dimers form stacked arrays in which $\omega'\omega$ is in the g^-g^- domain. Elevation of temperature and consequent destacking have only minor effects on pentose conformation and that about ϕ_1'. However, it leads consistently to changes of ω' from g^- to t domains with little effect on ω, suggesting that torsional variation about ω' may be an important process for the unwinding of DNA during replication and transcription.

Recent nuclear magnetic resonance (NMR) studies of mono-, oligo-, and polyribonucleotides[1-6] and theoretical considerations[7-10] have revealed that ribonucleic acid components in aqueous solution exist as a mixture of conformers with a wide range of intramolecular order and molecular topology, primarily due to torsional variations about O(3′)–P(ω') and P–O(5′)(ω) bonds. Nevertheless, as a class they show a tendency to exist more in certain orientations than in others. These studies further revealed that conformational preferences about the various bonds are found to be strongly interdependent. Perturbation of the geometry along a key bond in the backbone causes a series of internal conformational adjustments; for example, stacking interaction accompanied by torsional variation about $\omega'\omega$ perturbs the magnitude of χ_{CN} which causes a $^2E \rightarrow {}^3E$ shift in the population of ribose conformers. This shift in ribose conformer equilibrium causes a change in the torsion about C(3′)-O(3′)(ϕ') from $\phi' \simeq 280°$ to $\phi' \simeq 200°$ domains. Changes in the conformational parameters of monoribonucleotides as they become part of oligo- and polymeric structures in aqueous solution were examined by Evans and Sarma.[3] They have concluded that the monoribonucleotides maintain their isolated conformations in the polymer when significant amounts of the polymer are in the destacked state. This conformational conservation breaks down as soon as the monoribonucleotides become integrated into the backbone framework of a base-stacked polyribonucleotide. The nucleotidyl units in a base-stacked oligomer and polymer exhibit backbone conformations significantly different from the individual conformations of the monomeric components in aqueous solution. The conformational principles so derived from the extensive studies of ribonucleic acid components have been utilized to obtain information about the solution conformational properties of t-RNA^{Phe}. This was done[3] under the assumption that the overall molecular shapes of yeast phenylalanine tRNA in crystals[11] and solution are the same[12-18] and that differences are expected to exist in certain domains in intimate details between the two states. It was projected[3] that in the fully base-stacked double helical regions of tRNA in *aqueous solution*, the sugar-base torsion will occupy the anti domain, the ribose ring a ^3E pucker, the C(3′)-O(3′), C(4′)–C(5′), C(5′)–O(5′), O(3′)-P, and O(5′)–P preferring domains around $\phi' \simeq 200°$, $\psi \simeq 60°$, $\phi \simeq 180°$, $\omega' \simeq 300°$, and $\omega \simeq 300°$. One would expect the unstacked regions (Kim and Sussman[13] indicate D16, D17, G20, U47, and A76 are unstacked) to display conformational freedom and flexibility in solution so that alternative conformers such as ^2E, $\psi \simeq 180°/300°$, become allowed. Because of the demonstrated[1-3] conformational interrelationships in aqueous solution among sugar–base torsion, C(3′)–O(3′) torsion, and $\omega\omega'$ rotations, Evans and Sarma[3] suggested that those fractional populations which exist in ^2E conformations in the unstacked region will also populate in domains in which $\omega'/\omega \simeq 180°/300°$, $300°/180°$, and $\phi' \simeq 280°$, and the values of χ_1, though still in the anti domain, are several degrees above the ones encountered in the base-stacked region.

Thus, these extensive NMR studies have revealed important information about the conformational features, properties, and dynamics of ribonucleic acid structures in aqueous solution. However, very little information of this sort is available about the *deoxyribo*nucleic acid structures. One is curious to know whether the simple substitution of a 2′-OH group by hydrogen in the sugar ring will have far-reaching repercussions with respect to the three-dimensional spatial configuration and whether such conformational effects may enable understanding of the profound differences in the physicochemical and biological properties between the ribo- and deoxyribonucleic acid structures. In the present work we attempt to provide a few answers to these questions.

Fang et al.[19] successfully analyzed the base proton and the H(1′), H(2′), and H(2″) regions of 3′-dAMP, 5′-dAMP, and d-ApA and reported that d-ApA has an anti,anti right-handed conformation with extensive base–base interaction. Detailed analyses of the proton NMR spectra of 5′-deoxyribomononucleotides and the dimers d-ApA and d-TpT were reported by various investigators.[20-24] Even though these studies provided important preliminary information about the aqueous solution conformational characteristics of deoxyribonucleic acid structures, further progress was hampered because of the difficulty in analyzing the complex NMR spectral pattern from the various deoxyribo dimers. We have overcome these difficulties by a combination of the use of high-frequency Fourier transform NMR and computer synthesis of spectra. Hence, in this paper we are able to present a systematic and comprehensive proton NMR study and conformational deductions for

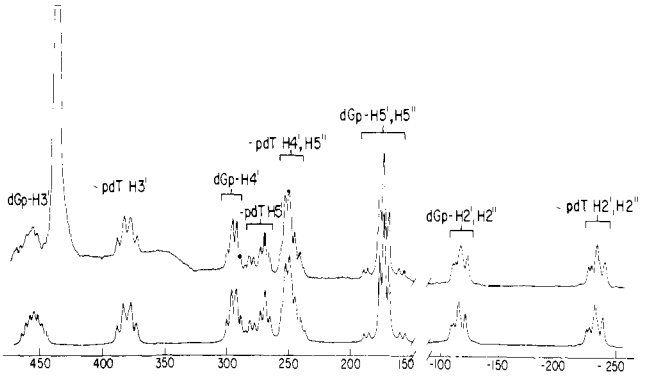

Figure 1. The 270-MHz ^{1}H NMR spectrum of d-GpC (top, 20 °C, 0.02 M, pD 7.4) and the corresponding line-shape simulation. The base and 1′ region of the spectra are not shown. The chemical shifts are expressed in hertz from internal Me_4NCl.

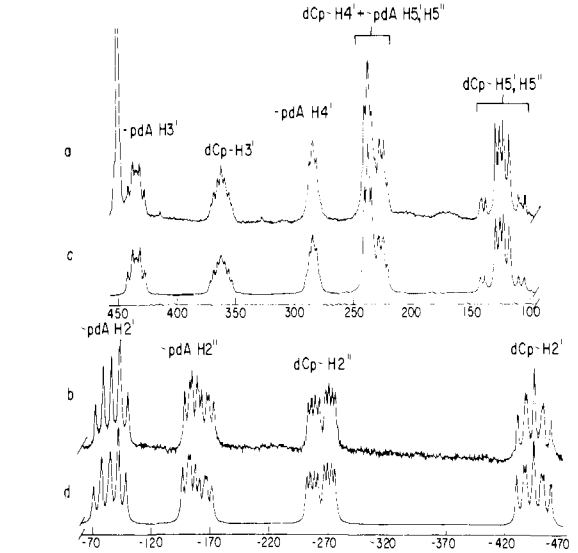

Figure 2. The 270-MHz ^{1}H NMR spectrum of d-CpA (a,b, 20 °C, 0.02 M, pD 7.4) and the corresponding line-shape simulation (c,d). The rest of the details are as described in Figure 1.

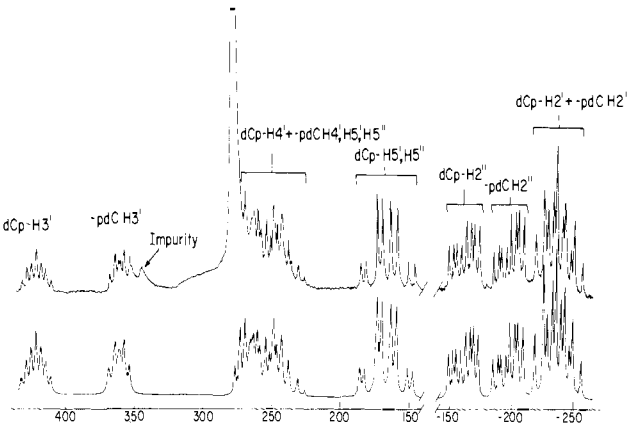

Figure 3. The 270-MHz ^{1}H NMR spectrum of d-CpC (80 °C, 0.02 M, pD 7.4) and the corresponding line-shape simulation. The rest of the details are as described in Figure 1.

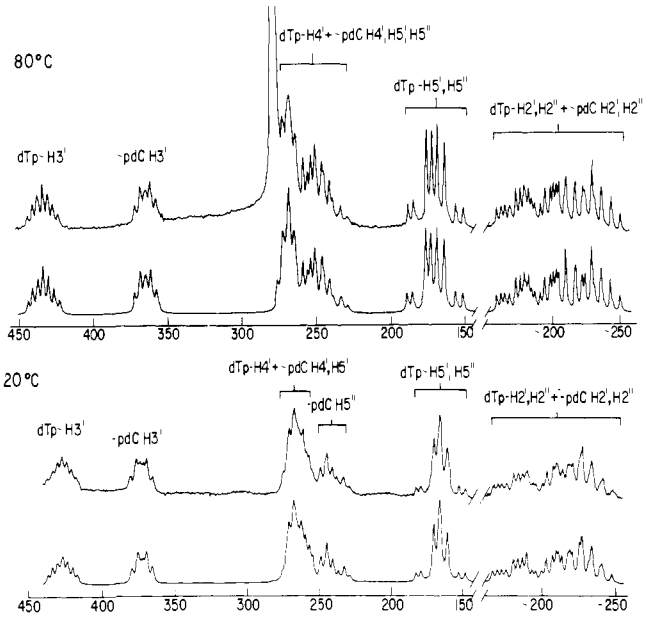

Figure 4. The 270-MHz ^{1}H NMR spectra of d-TpC (0.02 M, pD 7.4) at 20 and 80 °C. The line-shape simulations are on the bottom of the observed spectra. Rest of the details are as described in Figure 1.

all the naturally occurring purine-purine, pyrimidine-pyrimidine, purine-pyrimidine, and pyrimidine-purine deoxyribodinucleoside monophosphates.

Experimental Section

The 3′- and 5′-mononucleotides and dinucleoside monophosphates were purchased from Sigma Chemical Co. and Collaborative Research. All the samples were lyophilized three times from 99.8% D_2O and the final solutions were made up in 100% D_2O. The pD was measured with a Fisher Accument Model 320 pH meter (pD = meter reading + 0.4). Final concentrations ranged from 0.01 to 0.03 M and are sufficiently low to minimize intermolecular effects on the shifts.

Proton and phosphorus-31 spectra were recorded using a variety of spectrometers. Details of the instrumentations are given in Lee et al.[1] All the spectra were measured at 20 ± 1 °C and at elevated temperatures. Tetramethylammonium chloride (Me_4NCl) was used as an internal reference. In order to minimize the effects of difference in phosphate ionization state, spectra for 3′ and 5′ monomers were measured at pD 5.5 ± 0.1 and dimers at pD 7.4 ± 0.1. At these pD values the phosphate group has a single negative charge in both monomers and dimers. The spectra were analyzed using LAOCN III and the derived data are accurate ±0.005 ppm for chemical shifts and ±0.1 Hz for coupling constants.

Results and Discussion

Assignments. The assignments of the various resonances in the 16 deoxydinucleoside monophosphates, the 4 3′-deoxymononucleotides, and the 4 5′-deoxymononucleotides were made by (i) homonuclear and ^{31}P decouplings;[5,25] (ii) double checking of H–P couplings from ^{31}P spectra; (iii) comparison with the data for the corresponding ribo series;[1,2] and (iv) extensive computer line-shape simulations using LAOCN III. Elsewhere[24] we have illustrated the 40.48-MHz ^{31}P and 300-MHz ^{1}H observed and computer-simulated spectra of a dipurine deoxy system. In Figures 1 and 2 we illustrate sample 270-MHz ^{1}H spectra from purine-pyrimidine and pyrimidine-purine dimers, respectively. Figures 3 and 4 display sample spectra for the homodipyrimidine and heterodipyrimidine systems at high and low temperatures. The assignments of the 5′, 5″ protons of the 5′- and 3′-nucleotidyl units follow Lee et al.[1] An unambiguous assignment of the 2′ and 2″ protons is not possible without selective deuteration of 2′ or 2″. In assigning these protons we have followed the method of Davies and Danyluk,[22] i.e., $J_{1'2'}$ is larger than $J_{1'2''}$. In those cases when $\delta(H(2')) \simeq \delta(H(2''))$ assignment by this method is not possible because the simulation will yield only $J_{1'2'} + J_{1'2''}$. Unambiguous assignment of H(6) and H(5) in dipyrimidines to the 3′ and 5′ units is not possible without selective deuteration. In the present case tentative assignments of these

Table I. Chemical Shifts[a] of Deoxyribodinucleoside Monophosphates in D_2O[b]

Nucleotide		Temp, °C	Chemical shifts (δ), ppm 1′	2′	2″	3′	4′	5′	5″	H(2) (H(5),Me)[d]	H(8) (H(6))[d]
d-ApA	dAp-	27	2.862	−1.043	−0.759	1.598	1.017	0.525	0.525	4.630	4.770
	-pdA		3.044	−0.415	−0.661	1.601	1.057	0.956	0.934	4.790	5.070
d-ApA	dAp-	72	3.008	−0.852	−0.694	1.704	1.061	0.565	0.529	4.940	4.980
	-pdA		3.201	−0.398	−0.628	1.645	1.111	1.004	0.978	5.030	5.220
d-GpG	dGp-	80	2.924	−0.732	−0.668	1.643	0.999	0.548	0.513		4.631
	-pdG		3.044	−0.396	−0.658	1.567	0.940	0.948	0.903		4.799
d-ApG	dAp-	27	2.999	−0.870	0.620	1.615	1.007	0.531	0.531	4.992	4.947
	-pdG		2.869	−0.461	−0.735	1.566	1.044	0.969	0.929		4.778
d-ApG	dAp-	72	3.088	−0.700	−0.567	1.662	1.008	0.546	0.546	4.994	5.034
	-pdG		2.983	−0.433	−0.690	1.549	1.064	0.972	0.933		4.786
d-GpA	dGp-	20	2.677	−1.100	−0.864	1.567	0.974	0.508	0.488		4.529
	-pdA		3.005	−0.345	−0.626	1.650	1.070	0.940	0.927	4.795	5.167
d-GpA	dGp-	80	2.812	−0.865	−0.752	1.609	0.929	0.525	0.482		
	-pdA		3.243	−0.347	−0.584	1.579	0.963	0.934	0.924	4.944	5.148
d-CpC	dCp-	20	2.846	−0.866	−0.638	1.532	1.007	0.650	0.585	2.819	4.620
	-pdC		2.907	−0.896	−0.782	1.372	0.967	0.960	0.890	2.854	4.704
d-CpC	dCp-	80	3.043	−0.860	−0.599	1.562	1.007	0.655	0.585	2.852	4.604
	-pdC		3.103	−0.895	−0.734	1.340	0.974	0.944	0.888	2.878	4.688
d-TpT[c]	dTp-	18	3.024	−0.846	−0.626	1.574	0.984	0.634	0.584	−1.306	4.484
	-pdT		3.134	−0.826	−0.816	1.394	0.924	0.954	0.884	−1.296	4.504
d-TpT[c]	dTp-	65	3.034	−0.846	−0.636	1.594	0.984	0.634	0.584	−1.306	4.424
	-pdT		3.114	−0.826	−0.816	1.364	0.934	0.954	0.884	−1.286	4.474
d-CpT	dCp-	20	2.971	−0.760	−0.523	1.528	1.012	0.668	0.599	2.800	4.664
	-pdT		3.131	−0.744	−0.760	1.425	0.959	0.964	0.913	−1.299	4.530
d-CpT	dCp-	80	3.035	−0.866	−0.584	1.570	0.012	0.661	0.590	2.844	4.597
	-pdT		3.107	−0.811	−0.797	1.371	0.957	0.947	0.901	−1.271	4.470
d-TpC	dTp-	20	3.044	−0.829	−0.661	1.582	0.983	0.638	0.592	−1.302	4.497
	-pdC		3.111	−0.860	−0.750	1.383	0.997	0.973	0.891	2/922	4.790
d-TpC	dTp-	80	3.044	−0.800	−0.636	1.604	1.000	0.661	0.601	−1.284	4.414
	-pdC		3.098	−0.871	−0.718	1.350	0.991	0.959	0.894	2.937	4.750

[a] Shifts are given relative to internal Me_4NCl and are accurate to ±0.005 ppm. [b] pD 7.4; concentration = 0.01–0.03 M. [c] Data from ref 20. [d] Unambiguous assignments of base protons are not possible without selective deuteration studies.

protons were made from their shift trends compared to the ribo series in which Lee et al.[1] made unambiguous assignments. Hence necessary caution was exercised when we employed the shifts of H(5) and H(6) to make conformational deductions (vide infra).

The chemical shifts for the complete set of nonexchangeable protons in the homo dimers d-ApA, d-ApG, d-GpA, d-CpC, d-TpT, d-CpT, and d-TpC at 20 °C and at a high temperature (65–80 °C) are summarized in Table I. Data for d-GpG are given only at 80 °C because the room temperature spectrum could not be analyzed. A complete set of the chemical-shift data for the hetero dimers d-ApC, d-ApT, d-GpC, d-GpT, d-CpA, d-CpG, d-TpA, and d-TpG at 20 and 80 °C is summarized in Table II. The coupling constants for the homo and hetero dimers are listed in Tables III and IV.

Conformation of the Deoxyribofuranose Rings. Jardetzky[26,27] has demonstrated that the close contact between the two cis hydroxyl groups influences the ribofuranose ring conformation. It readily follows that the deoxyribose ring pucker will exhibit some conformational differences from that of the ribose ring. Solid-state data on ribo- and deoxyribonucleosides and nucleotides appear to support such a view. We have taken the reported crystal structures of all the nucleosides and mononucleotides compiled by Sundaralingam[28] and reclassified the sugar pucker in terms of the ribose and deoxyribose (Figure 5). It is found that 8 out of the total 23 deoxynucleosides and mononucleotides have ring conformations outside the normal 0–36° (^3E) and 144–180° (^2E) phase angle regions (Figure 5). This contrasts with the ribose ring for which only 1 out of the 59 total solid-state puckers lie outside the ^3E and ^2E regions. The data in Figure 5 indicate that the conformer potential energy well is most probably flatter for the deoxyribose ring at least in the ^2E range. For the ^3E range, there is presently too little x-ray data to draw the same conclusion, but there is no reason not to expect a similar trend.

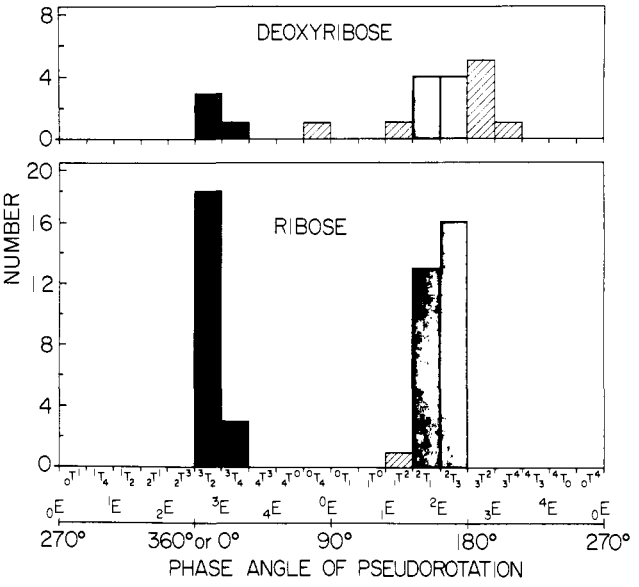

Figure 5. The number of solid state structures found for each of the possible sugar ring conformations in ribose and in deoxyribose nucleosides and mononucleotides.

Because of the wider conformational spread for the pentose ring in the deoxy system, translation of the time-averaged NMR coupling constants into conformer population appears to be less straightforward than in the case of the ribofuranose ring system where a simple $^2E \rightleftarrows {}^3E$ equilibrium is sufficient

Table II. Chemical Shifts[a] of Deoxyribonucleoside Monophosphates in D_2O[b]

Nucleotide		Temp, °C	Chemical shifts (δ), ppm								
			1′	2′	2″	3′	4′	5′	5″	H(2) (H(5),Me)[c]	H(8) (H(6))[c]
d-ApC	dAp-	27	3.204	−0.361	−0.436	1.704	1.145	0.685	0.633	4.980	5.094
	-pdC		3.023	−0.901	−0.786	1.369	0.970	1.026	0.914	2.686	4.624
d-ApC	dAp-	80	3.248	−0.339	−0.433	1.749	1.135	0.674	0.620	5.062	5.103
	-pdC		3.063	−0.929	−0.758	1.345	0.994	0.985	0.918	2.796	4.637
d-ApT	dAp-	27	3.187	−0.375	−0.375	1.694	1.136	0.703	0.646	4.961	5.086
	-pdT		3.003	−0.889	−0.889	1.380	0.915	1.030	0.914	−1.520	4.304
d-ApT	dAp	80	3.228	0.347	−0.404	1.748	1.138	0.683	0.629	5.051	5.093
	-pdT		3.042	−0.855	−0.831	1.370	0.951	0.963	0.922	−1.406	4.363
d-GpC	dGp-	20	2.999	−0.482	−0.429	1.682	1.087	0.643	0.612		4.787
	-pdC		3.082	−0.906	−0.809	1.381	0.961	1.016	0.918	2.603	4.574
d-GpC	dGp-	80	3.061	−0.404	−0.494	1.734	1.087	0.657	0.606		4.757
	-pdC		3.098	−0.904	−0.747	1.353	0.986	0.985	0.919	2.757	4.634
d-GpT	dGp-	20	3.019	−0.419	−0.437	1.684	1.090	0.656	0.620		4.846
	-pdT		3.076	−0.846	−0.868	1.410	0.922	1.015	0.917	−1.473	4.364
d-GpT	dGp-	80	3.057	−0.408	−0.470	1.740	1.148	0.657	0.607		4.740
	-pdT		3.083	−0.824	−0.815	1.382	0.963	1.023	0.924	−1.361	4.399
d-CpA	dCp-	20	2.815	−1.643	−0.978	1.342	0.837	0.485	0.429	2.751	4.333
	-pdA		3.245	−0.314	−0.586	1.609	1.052	0.878	0.878	4.984	5.264
d-CpA	dCp-	80	2.907	−1.271	−0.797	1.427	0.877	0.534	0.462	2.785	4.407
	-pdA		3.285	−0.329	−0.559	1.557	1.060	0.899	0.899	5.058	4.194
d-CpG	dCp-	20	2.895	−1.529	−0.865	1.407	0.880	0.457	0.457	2.673	4.416
	-pdG		3.026	−0.365	−0.683	1.580	1.005	0.889	0.889		4.852
d-CpG	dCp-	80	2.969	−1.202	−0.732	1.463	0.906	0.557	0.493	2.981	4.867
	-pdG		3.081	−0.374	−0.650	1.531	0.944	0.911	0.900		4.822
d-TpA	dTp-	20	2.782	−1.604	−1.029	1.405	0.835	0.463	0.463	−1.335	4.166
	-pdA		3.225	−0.304	−0.590	1.615	1.050	0.885	0.885	4.976	5.229
d-TpA	dTp-	80	2.869	−1.260	−0.870	1.466	0.862	0.530	0.474	−1.311	4.213
	-pdA		3.276	−0.325	−0.564	1.564	1.058	1.901	0.901	5.050	5.188
d-TpG	dTp-	20	2.883	−1.480	−0.926	1.456	0.881	0.475	0.475	−1.351	4.250
	-pdG		3.015	−0.365	−0.674	1.585	1.007	0.884	0.884		4.898
d-TpG	dTp-	80	2.937	−1.144	−0.783	1.521	0.883	0.549	0.502	−1.307	4.287
	-pdG		3.078	−0.381	−0.646	1.509	1.015	0.922	0.892		4.841

[a] Shifts are given relative to internal Me_4NCl and are accurate to ±0.005 ppm. [b] pD 7.4; concentration = 0.01–0.03 M [c] Unambiguous assignments of the base protons are not possible without selective deuteration studies.

to explain NMR data.[29] A quantitative method of treating the pentose coupling constants involves computation of the phase angle of pseudorotation (P) and the amplitude of pucker (τ) from $J_{1'2'} + J_{3'4'}$ and $J_{2'3'}$ using the procedure of Altona and Sundaralingam.[30] Recent criticisms[22,29,31] raise cogent questions about the validity of the AS[30] approach and indicate that changes in coupling constants within experimental error have a significant impact upon calculated values of P, τ, and ring conformer populations. We believe that the simplest way to treat the aqueous solution conformation of the deoxyribose ring is as an equilibrium blend of envelope conformations in the ^2E and ^3E domains. The ^2E domain contains $_1E$, ^2E, and $_3E$ conformations (Figure 5) with significant populations of ^2E, and the ^3E domain contains $_2E$, ^3E, and $_4E$ conformations with significant populations of ^3E, i.e.:

$$|_1E \rightleftarrows {}^2E \rightleftarrows {}_3E| \rightleftarrows |_2E \rightleftarrows {}^3E \rightleftarrows {}_4E|$$

These two domains will be called, for simplicity in description, ^2E and ^3E conformations. Evidence that an equilibrium between ^2E and ^3E types may be a true description of the system comes from an examination of the coupling constant data in Tables III and IV. Data available for 62 ribose units (data for room and high temperature) show that, in 59 units, (i) the magnitude of $J_{2'3'}$ remains constant at 6.5 ± 0.9 Hz and (ii) the magnitude of the sum $J_{1'2'} + J_{3'4'}$ remains constant at 10.8 ± 1 Hz. The observed deviation of 1 Hz in the sum is not large since the experimental error in obtaining the sum itself is about 0.5 Hz.

The magnitude of the corresponding sum in the ribo series is 9.5 Hz.[1] The observed difference of 1.3 Hz in the sum between the ribo and deoxyribo series is a reflection of the change in electronegativity effect resulting from the removal of the 2′-OH group. It is also possible that if significant amounts of $_4E$ and $_1E$ conformers contribute to the ^3E and ^2E types, respectively, an increase in the $J_{1'2'} + J_{3'4'}$ sum will result. This is quite unlikely because in the equilibrium:

$$|_1E \rightleftarrows {}^2E \rightleftarrows {}_3E| \rightleftarrows |_2E \rightleftarrows {}^3E \rightleftarrows {}_4E|$$

there is no reason to expect the contribution of $_1E$ to be greater than $_3E$ or $_4E$ to be greater than $_2E$. Under conditions in which populations of $_2E \simeq {}_4E$ and $_1E \simeq {}_3E$ no effect on the sum $J_{1'2'} + J_{3'4'}$ is expected. This is because the larger value of the sum in $_4E$ and $_1E$ is internally compensated by a corresponding smaller value of the sum in $_2E$ and $_3E$ conformers. To illustrate this point, in Table V are presented the projected J values for the various envelope conformations. These calculations were performed using the Karplus relation $J_{HH} = 10.5 \cos^2 \phi_{HH} - 1.2 \cos \phi_{HH}$, derived by Altona and Sundaralingam.[30] Alternatively, one may use any other Karplus relationships, references to which are given in Lee and Sarma.[32] No attempt is made to modify the Karplus equation for the deoxyribose ring system because one does not know with any reliability the effect of electronegativity contribution and the purpose of the data is only to drive home the concept that in the pentose ring the sum $J_{1'2'} + J_{3'4'}$ shall be constant for the above equilibrium under conditions in which $_2E \simeq {}_4E$ and $_1E \simeq {}_3E$. The theoretically projected data in Table V reveal that under these conditions the sum remains essentially constant (10.3–10.6 Hz). The experimental observation that out of 62 deoxyribose moieties examined, the sum $J_{1'2'} + J_{3'4'}$ in 59 of them has a constant value of 10.8 ± 1 Hz clearly substantiates our thesis that in the deoxy series the sugar ring exists an an equilibrium

Table III. Coupling Constants[a] for Deoxyribodinucleoside Monophosphates in D_2O[b]

Nucleotide		Temp, °C	Coupling constants, Hz													
			1′2′[d]	1′2″[d]	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	3′P	4′P	5′P	5″P	5′5″	5 6
d-ApA	dAp-	27	8.7	5.5	−14.0	5.6	2.3	2.4	3.3	3.3	5.6				−12.0	
	pdA		7.0	6.7	−13.9	6.6	4.1	4.0	2.5	2.8		2.7	3.8	3.6	−12.0	
d-ApA	dAp-	72	8.7	6.0	−14.6	6.2	2.5	2.2	3.4	4.6	6.2				−13.3	
	-pdA		6.9	7.1	−14.6	7.0	4.6	4.0	2.5	3.8		2.5	4.6	4.6	−12.6	
d-Gpg	dGp-	80	7.0	6.8	−13.8	7.0	3.4	3.0	3.6	4.6	5.6				−11.8	
	-pdG		6.5	6.6	−13.8	6.5	4.0	4.2	2.5	2.5		3.2	3.6	3.6	−11.4	
d-ApG	dAp-	27	8.9	5.7	−14.0	5.4	2.0	2.0		3.7	3.7	5.6				−12.0
	-pdG		7.1	6.3	−13.7	6.3	4.1	3.3	2.1	2.5		2.4	4.1	3.0	−11.8	
d-ApG	dAp-	72	8.5	5.9	−14.2	5.6	2.2	2.8	4.1	4.1	6.0				−12.0	
	-pdG		6.7	6.7	−13.8	6.7	4.0	4.0	1.9	4.4		2.2	4.5	4.6	−11.8	
d-GpA	dGp-	20	8.4	6.3	−14.2	5.4	2.8	3.6	3.4	3.6	7.0				−12.8	
	-pdA		7.0	6.4	−14.0	6.6	4.1	4.0	2.8	2.8		3.0	3.6	3.6	−11.8	
d-GpA	dGp-	80	8.2	6.3	−14.2	5.6	2.8	3.0	3.7	4.4	6.4				−12.6	
	-pdA		7.0	6.4	−14.0	6.6	4.1	3.4	4.1	4.5		3.2	4.5	5.2	−11.8	
d-CpC	dCp-	20	7.8	5.8	−13.6	6.6	3.5	3.6	3.6	3.6	7.0				−12.2	6.6
	-pdC		6.8	7.0	−13.6	6.3	4.2	3.5	3.2	3.2		2.0	4.4	4.8	−11.8	6.7
d-CpC	dCp-	80	7.0	6.2	−14.0	7.0	3.7	3.6	3.2	4.0	7.0				−12.2	7.0
	-pdC		6.9	6.5	−13.6	6.9	4.1	4.1	3.1	4.6		2.0	5.0	5.6	−11.8	7.3
d-TpT[c]	dTp-	18	7.2	6.1	−14.0	6.0	3.8	3.2	3.4	4.2	6.8				−12.6	
	-pdT		6.9	6.9	−14.1	5.8	5.8	4.0	2.5	4.0		1.6	4.0	4.0	−12.1	
d-TpT[c]	dTp-	65	7.8	6.3	−14.2	6.6	3.5	3.6	3.4	5.5	7.1				−12.3	
	-pdT		6.8	6.8	−14.2	5.4	5.4	3.7	2.5	5.0		1.6	4.0	5.0	−12.1	
d-CpT	dCp-	20	8.2	6.2	−13.8	6.0	3.9	3.6	3.8	3.8	7.6				−12.6	7.1
	-pdT		6.7	7.0	−13.8	6.5	3.8	3.5	3.2	3.6		2.4	4.8	4.8	−12.2	
d-CpT	dCp-	80	7.3	6.4	−14.1	6.9	4.1	3.4	3.0	4.1	7.6				−12.4	7.4
	-pdT		7.3	6.5	−13.8	7.3	3.2	4.0	3.2	3.4		2.0	4.6	4.6	−12.6	
d-TpC	dTp-	20	7.8	6.1	−14.2	6.4	3.1	2.9	3.3	5.0	7.4				−12.6	
	-pdC		6.8	6.5	−13.8	6.3	4.0	3.5	2.4	3.6		3.0	4.6	4.4	−11.6	8.0
d-TpC	dTp-	80	7.1	6.2	−14.4	7.1	3.3	3.5	3.3	5.0	7.4				−12.6	
	-pdC		7.1	6.3	−13.8	7.1	4.0	3.9	3.0	4.0		3.0	5.0	5.2	−11.8	7.8

[a] Coupling constants are accurate to ±0.1–0.2 Hz. [b] Solution conditions are the same as in Table I. [c] Data from ref 20. [d] When $\delta 2' \simeq \delta 2''$ only $J_{1'2'} + J_{1'2''}$ can be obtained.

blend of ^2E- and ^3E-type conformations with small but equal contribution from ${}_1E/{}_3E$ and ${}_2E/{}_4E$ conformers. The observation of the constancy in the sum in the entire series suggests that the puckering amplitude exhibits no base sequence dependence and remains relatively constant throughout the series of homo and hetero dimers.

Using the procedure adopted by Lee et al.[1] and Ezra et al.[2] we have computed the percentage population of ^3E-type conformers in the deoxy series employing the sum of 10.8 for $J_{1'2'} + J_{3'4'}$ and the observed magnitude of $J_{3'4'}$. The results are summarized in Tables VI and VII.

The data indicate that for both homo and hetero dimers the observed ^3E-type conformations range from 19 to 33% for the 3′-nucleotidyl residue and from 31 to 41% for the 5′-nucleotidyl unit at room temperature, i.e., the deoxy dimers in general show a clear preference to populate in ^2E-type conformations, the preference being in general larger for the 3′-nucleotidyl unit. In the case of homodipurines, i.e., d-ApA and D-GpG, dimerization (Table VI) has no influence on deoxyribo conformational distribution for the 3′ unit, but the 5′ unit undergoes a small (8–9%) increase in ^3E population. Comparison of the deoxyribose dimerization data for d-ApG and d-GpA indicates the influence of sequence; i.e., the adenine and guanine moieties at the 3′ end respectively cause a reduction and increase in ^3E population upon dimerization, but when the same units are in the 5′ end, guanine has no influence and the adenine ring causes an increase. For the homodipyrimidines d-CpC and d-TpT, dimerization contributes only to a mild increase in ^3E populations for the 5′ unit in d-TpT. Comparison of the deoxyribose dimerization data for the matched pairs d-CpT and d-TpC shows that a cytidine, whether at the 3′ or the 5′ end, has no influence, but thymidine at the 3′ end causes a reduction in ^3E population. The effect of sequence on deoxyribose conformational distribution becomes very clear on comparison of the pentose dimerization data on d-pupy[33] and d-pypu dimers. Data in Table VII for the d-pupy and d-pypu series show that on dimerization the ^3E population increases irrespective of whether the purine is on the 3′ end or the 5′ end; on the other hand, a pyrimidine at the 5′ end causes an increase and that at the 3′ end causes a reduction in ^3E ribose population. Comparison of the dimerization data for the matched pairs d-ApC/d-CpA, d-ApT/d-TpA, d-GpC/d-CpG, and d-GpT/d-T-G shows that transposing a pyrimidine from the 5′ end to the 3′ end can cause as much as 21% reduction in ^3E population upon dimerization.

The data in Tables VI and VII show that elevation of temperature causes minor perturbations in the $^2E \rightleftarrows {}^3E$ distribution in certain cases, but no discernible general trends are apparent. The lack of a strong conformational response on the part of the pentose ring in deoxy dimers to temperature changes should not be interpreted on the ground that in the deoxy dimers there are no stacking interactions because such interactions are postulated to be responsible for the observed temperature sensitivity of pentose conformation in ribo dimers.[1,2]

On the contrary, the dimerization shift data in Tables VIII and IX to be discussed later clearly show significant base-base stacking interactions. The present observations mean that the deoxyribose moieties of nucleotides intrinsically prefer to populate in ^2E-type conformations and that stacking interactions and consequent χ_{CN} changes are not sufficiently strong enough to overwhelm the intrinsic conformational proclivities of the deoxyribose moieties. EHT calculations on deoxyribose phosphate units[34] suggest that they prefer the ^2E conformation. Recent energy gradient molecular orbital calculations (personal communication to R.H.S. from Hashemi and Beckel,

Table IV. Coupling Constants[a] for Deoxyribodinucleoside Monophosphates in D_2O[b]

Nucleotide		Temp, °C	Coupling constants, Hz												
			1′2′[c]	1′2″[c]	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	2′P	4′P	5′P	5″P	5′5″	5 6
d-ApC	dAp-	27	7.4	6.1	−14.0	6.0	3.0	2.8	3.2	3.6	6.6				−12.8	
	-pdC		6.4	6.6	−14.1	6.8	4.6	4.0	3.6	4.0		4.0	4.0	4.0	−11.4	7.7
d-ApC	dAp-	80	7.4	6.3	−14.2	6.3	3.2	3.4	3.2	4.6	6.7				−12.8	
	-pdC		6.6	6.6	−14.1	7.0	4.5	4.0	4.2	4.4		2.0	5.0	5.0	−11.8	7.7
d-ApT	dAp-	27	6.4	6.4	−14.0	4.8	4.8	3.5	2.9	3.9	6.0				−12.8	
	-pdT		6.7	6.7	−14.0	5.9	5.9	4.4	2.4	3.0		2.5	3.2	3.3	−11.8	
d-ApT	dAp-	80	7.7	5.7	−14.2	5.8	3.4	2.8	3.2	4.2	7.2				−12.4	
	-pdT		6.8	6.4	−14.0	6.8	4.0	5.3	4.6	5.0		2.0	5.0	5.0	−11.8	
d-GpC	dGp-	20	7.0	6.5	−13.8	6.6	6.5	3.4	3.4	4.0	7.0				−13.0	
	-pdG		7.4	6.6	−13.6	7.4	3.4	4.0	3.0	3.6		1.0	4.0	4.0	−12.0	7.5
d-GpC	dGp-	80	6.8	6.1	−14.0	6.8	3.1	3.4	3.2	4.4	6.6				−11.8	
	-pdC		7.2	6.6	−14.0	7.2	4.2	4.0	3.3	4.3		1.8	4.7	5.1	−12.0	7.7
d-GpT	dGp-	20	7.7	5.4	−14.0	7.7	2.2	3.2	3.8	4.6	7.2				−13.1	
	-pdT		7.8	6.2	−14.0	7.8	3.1	4.8	2.6	3.2		2.2	3.9	4.0	−11.6	
d-GpT	dGp-	80	7.2	6.5	−14.0	7.2	3.2	3.6	3.8	4.6	6.8				−12.4	
	-pdT		6.6	6.4	−14.0	6.6	4.6	3.4	2.4	3.4		2.2	4.0	4.0	−11.4	
d-CpA	dCp-	20	8.1	6.3	−14.5	5.8	3.0	3.0	3.4	4.7	7.0				−12.6	7.1
	-pdA		6.8	6.4	−14.3	6.4	4.1	3.9	2.5	2.5		3.0	3.6	3.6	−11.8	
d-CpA	dCp-	80	7.0	6.0	−14.2	7.0	3.4	3.0	3.4	4.7	7.3				−12.6	7.5
	-pdA		6.8	6.6	−14.3	6.4	4.1	3.9	3.8	3.8		3.0	4.7	4.7	−11.8	
d-CpG	dCp-	20	7.0	6.1	−13.8	6.2	3.0	3.6	4.2	4.2	7.0				−12.8	7.1
	-pdG		6.4	6.6	−13.8	6.4	3.8	4.0	3.2	3.2		3.2	3.6	3.6	−11.8	
d-CpG	dCp-	80	7.1	6.1	−13.8	6.7	3.4	2.7	3.2	4.2	6.0					7.5
	-pdG		6.8	6.8	−13.8	6.8	4.0	4.0	3.8	4.5		3.2	4.5	4.9	−11.8	7.5
d-TpA	dTp-	20	8.2	6.2	−14.5	6.2	2.4	2.6	4.2	4.2	7.8				−12.8	
	-pdA		6.8	6.4	−13.9	6.4	4.2	3.9	2.5	2.5		3.0	3.6	3.6	−11.8	
d-TpA	dTp-	80	7.7	6.4	−14.5	6.5	2.9	3.6	3.2	4.4	7.0				−12.2	
	-pdA		6.6	6.6	−14.1	6.6	4.2	3.9	3.9	3.9		2.0	4.2	4.2	−11.8	
d-TpG	dTp-	20	9.2	5.8	−14.5	6.6	2.4	2.6	4.2	4.2	7.8				−12.8	
	-pdG		7.6	6.4	−13.8	6.6	4.0	3.8	3.2	3.2		3.0	3.6	3.6	3.6	−11.8
d-TpG	dTp-	80	7.6	6.0	−14.1	6.6	3.0	2.6	3.8	4.6	8.2				−12.4	
	-pdG		7.6	6.8	−13.8	6.6	4.0	3.1	3.0	3.0		3.0	3.4	3.4	−11.8	

[a] Coupling constants are accurate to ±0.1–0.2 Hz. [b] Solution conditions are the same as in Table I. [c] When $\delta 2' \simeq \delta 2''$ only $J_{1'2'} + J_{1'2''}$ can be obtained.

Table V. Calculated Coupling Constants in a Pentose Ring for Various Envelope Conformations (Hertz)

	$J_{1'2'}$	$J_{1'2''}$	$J_{2'3'}$	$J_{2''3'}$	$J_{3'4'}$
${}_2E$	0.2	5.0	4.9	11.0	8.2
${}^3E$	0.1	7.4	4.9	11.0	10.3
${}_4E$	2.4	9.1	7.0	9.0	10.3
${}_1E$	10.1	5.5	7.6	0.1	2.9
${}^2E$	10.1	5.5	5.5	0.1	0.2
${}_3E$	7.9	7.6	5.5	0.1	0.0

1976) clearly predict that for the deoxyribose ring the global minimum is at ${}^2E$ whereas ribose has two approximately equal minima along the pseudorotation path at ${}^2E$ and ${}^3E$.

Conformation about the C(4′)–C(5′) (ψ_1,ψ_2) and C(5′)–O(5′) (ϕ_2)[35] Bonds. Using expressions developed elsewhere[36] the population distribution of conformers about C(4′)–C(5′) and C(5′)–O(5′) bonds were computed; the computed populations of *gg* (ψ_1,ψ_2 = 60°) and *g′g′* (ϕ_2 = 180°) for monomeric components and dimers are compiled in Tables VI and VII. The data show that in monomers and dimers there is a general preference for the *gg* conformer. In all the dimers examined except d-ApC, the 5′-nucleotidyl unit shows a higher preference to exist in the *gg* state than does the 3′-nucleotidyl unit. The sequence has a profound and definite effect on the distribution of conformers about C(4′)-C(5′). The effect is such that movement of a given base from the 5′ end to the 3′ end invariably causes an increase in *gg* population. This is easily seen by comparing the values for the matched pairs d-ApC/d-CpA, d-ApT/d-TpA, d-GpC/d-CpG, and d-GpT/d-TpG. For example, moving thymine from the 3′ end in d-TpA to the 5′ end in d-ApT causes a staggering increase in *gg* population of over 30%. These are very real changes due to sequence as can be seen from the fact that 3′-dTMP and 5′-dTMP have extremely similar populations of *gg* conformers (Table VII).

Elevation of temperature and consequent destacking (vide infra) may cause reduction in the population of ψ_2 = 60° conformers because in the generally favored right-handed stack, the conformation about ψ_2 should be 60° and torsion from ψ_2 = 60° to ψ_2 = 180°/300° arrangements can cause destacking. Temperature-induced torsional variation about ψ_2 may also be an indication of changes in χ_2. Olson[37] has pointed out a conformational nexus between ψ and χ_{CN} and this has been experimentally demonstrated to be true by Sarma et al.[25] and Lee et al.[38] Yathindra and Sundaralingam[39] have published a series of papers on this. It is very difficult to disentangle the effect of χ_{CN} variation and destacking on ψ_2 because a change in χ_{CN} is expected when the dimer destacks[1,2] and it is generally safe to assume that a conspicuous decrease of ψ_2 = 60° conformers is an indication of destacking and χ_{CN} changes.[40]

For the dipurines (Table VI) elevation of temperature leads to a reduction in the density of ψ_2 = 60° conformers, the effect being very pronounced in the cases of d-GpA and d-ApG—20–30% reduction over the temperature range of 20–80 °C. In d-ApA the corresponding reduction is only about 10%. Obviously, for d-GpA and d-ApG, torsional variation about the C(4′)-C(5′) bond of the 5′ residue is an important mode of destacking and presumably such destacking causes changes in χ_2 (vide infra). Among the dipyrimidines ψ_2[41] of d-CpC is most sensitive to temperature effects and d-CpT the least

Table VI. Population Distribution of Conformers in Deoxyribodinucleoside Monophosphates and Their Components

Nucleotide		Temp, °C	Dimer: Sugar ring[a] % ^3E	Dimer: Backbone[b] K_{eq}[c]	Dimer: Backbone[b] % gg	Dimer: Backbone[b] % $g'g'$	Dimer: Backbone[b] θPH, deg	Monomer[d]: Sugar ring[a] % ^3E	Monomer[d]: Sugar ring[a] K_{eq}[c]	Monomer[d]: Backbone[b] % gg	Monomer[d]: Backbone[b] % $g'g'$	Monomer[d]: Backbone[b] θPH, deg
d-ApA	dAp-	27	22	0.3	73		±45	23	0.3	71		±38
	-pdA		37	0.6	87	85		28	0.4	63	70	
d-ApA	dAp-	72	20	0.3	59		±43					
	-pdA		37	0.6	76	76						
d-GpG	dGp-	80	28	0.4	57		±45					
	-pdG		39	0.6	90	86						
d-ApG	dAp-	27	19	0.2	65		±45	23	0.3	71		±38
	-pdG		31	0.4	94	86		32	0.5	63	67	
d-ApG	dAp-	72	26	0.4	57		±44					
	-pdG		37	0.6	76	76						
d-GpA	dGp-	20	33	0.5	69		±40	26	0.4	57		±38
	-pdA		37	0.6	84	86		28	0.4	63	70	
-d-GpA	dGp-	80	28	0.4	58		±42					
	-pdA		32	0.5	53	74						
d-CpC	dCp-	20	33	0.5	67		±40	35	0.5	58		±38
	-pdC		32	0.5	75	76		33	0.5	70	75	
d-CpC	dCp-	80	33	0.5	67		±40					
	-pdC		38	0.6	61	69						
d-TpT	dTp-	18	30	0.4	63		±41	33	0.5	57		±41
	-pdT		37	0.6	74	82		32	0.5	59	74	
d-TpT	dTp-	65	33	0.5	49		±39					
	-pdT		34	0.5	64	77						
d-CpT	dCp	20	33	0.5	63		±37	35	0.5	58		±38
	-pdT		32	0.5	71	74		32	0.5	59	74	
d-CpT	dCp-	80	31	0.4	68		±37					
	-pdT		37	0.6	73	76						
d-TpC	dTp-	20	27	0.4	56		±38	33	0.5	57		±41
	-pdC		32	0.5	79	77		33	0.5	70	75	
-TpC	dTp-	80	32	0.5	56		±38					
	-pdC		34	0.5	69	71						

[a] Computed by using $J_{1'2'} + J_{3'4'} = 10.8$ Hz for the dimers, and $J_{1'2'} + J_{3'4'} = 10.7$ Hz for the monomers. [b] Rotamer equations used: $gg = (13.7 - \Sigma)/9.7$; $g'g' = (25 - \Sigma')/20.8$. [c] $K_{eq}\ ^2E \rightleftarrows\ ^3E$. [d] Monomer data for solutions at pD 5.6.

sensitive. Among the hetero dimers (Table VII) the matched pairs d-GpT and d-TpG show little temperature sensitivity. For the remainder $\psi_2 = 60°$ conformers decrease with the matched pair d-ApT and d-TpA showing an overwhelming sensitivity. For example, in d-ApT elevation of temperature from 27 to 80 °C causes a 44% reduction in $\psi_2 = 60°$ conformers, clearly implicating that variation in ψ_2 is an important mode of destacking and χ_{CN} changes for d-ApT and d-TpA.

Comparison of the $\psi_2 = 60°$ conformers in the deoxyribo monomers and dimers shows that dimerization causes a definite increase (~15–20%) in their populations for the dipurines. For the dipyrimidines, the increase is a modest one (~5–10%). In the case of mixed dimers, except for d-ApC and d-GpC, dimerization causes a 10–30% increase in $\psi_2 \simeq 60°$ conformers. An increase in the populations of $\psi_2 = 60°$ conformers upon dimerization is in the correct direction for the formation of right-handed stacks. No common general conformational trends are apparent with elevation of temperature or upon dimerization for the C(4′)-C(5′) bond (ψ_1) of the 3′ unit. This is probably because this bond is not directly a part of the internucleotidyl linkage of a dimer.

Irrespective of the nature of the base and sequence all the deoxy dimers show a high preference (75–90%) for $\phi_2 = 180°$ ($g'g'$) conformer. Dimerization causes a 15–20% increase in their populations for the dipurines with no noticeable effect on the dipyrimidines. For the mixed series, there is an increase in $\phi_2 = 180°$ conformers upon dimerization, the effect being larger for the d-pypu (16–21%) than the d-pupy (7–15%) dimers. In general, elevation of temperature causes effects opposite of dimerization.

The data for the entire series (Tables VI and VII) indicate that the C(4′)–C(5′) and C(5′)-O(5′) bonds form a relatively stable conformational network where $\psi_2 = 60°/\phi_2 = 180°$ conformer populations vary from 92/86% in d-ApG to 71/74% in d-CpT. No correlation is present between $\psi_2 = 60°$ conformer populations and the conformational distribution in the pentose ring. This type of correlation has been obtained by Hruska[42] for nucleosides. Also, no correlation between $\psi_2 = 60°$ and $\phi_2 = 180°$ conformer populations[25] is found for the dimers.

Conformation about the C(3′)–O(3′) Bond (ϕ_1'). We have provided elsewhere in extenso discussion about the conformational possibilities about ϕ_1' in nucleic acid derivatives.[5] We have concluded there that the three-bond H-P coupling constants are best rationalized on the basis of an equilibrium between conformers in which the dihedral angle θHP (P-O-(3′)–C(3′)–H(3′)) has values in the neighborhood of ±35–45°. In the present series of dimers and the corresponding monomers the observed average value of θHP is ±~40°. This translates into an equilibrium system of conformers having ϕ_1' values of ~200 and ~280° which can be designated as the ϕ_-' and ϕ_+' domains for the C(3′)–O(3′) torsion. Lee and Sarma[43] have argued that detectable quantities of $^2E\phi_+'$ species will manifest in the four-bond coupling $J_{H(2')-P}$. In the deoxy series even though the favored mode of sugar pucker is ^2E, no such four-bond couplings were observed indicating that no detectable populations of $^2E\phi_+'$ conformers exist for the present system. Hence, it appears that for the deoxyribodinucleoside monophosphates torsion about C(3′)-O(3′) is restricted to a domain around $\phi_1' \simeq 200°$.

Table VII. Population Distribution of Conformers in Deoxyribodinucleoside Monophosphates and Their Components

Nucleotide		Temp, °C	Dimer: Sugar ring[a] % ^3E	K_{eq}[c]	Backbone[b] % gg	% $g'g'$	θPH, deg	Monomer[d]: Sugar ring[a] % ^3E	K_{eq}[c]	Backbone[b] % gg	% $g'g'$	θPH, deg
d-ApC												
	dAp-	27	26	0.4	71		±41	23	0.3	71		±38
	-pdC		37	0.6	63	82		33	0.5	70	75	
d-ApC	dAp-	80	31	0.5	61		±41					
	-pdC		37	0.6	53	72						
d-ApT	dAp-	27	32	0.5	71		±44	23	0.3	71		±38
	-pdT		41	0.7	86	89		32	0.5	59	74	
d-ApT	dAp-	80	26	0.4	65		±39					
	-pdT		49	1.0	42	72						
d-GpC	dGp-	20	31	0.5	65		±40	26	0.4	57		±38
	-pdC		37	0.6	73	82		33	0.5	70	75	
d-GpC	dGp-	80	31	0.5	63		±41					
	-pdC		37	0.6	63	73						
d-GpT	dGp-	20	30	0.4	55		±39	26	0.4	57		±38
	-pdT		44	0.8	81	82		32	0.5	59	74	
d-GpT	dGp-	80	33	0.5	55		±41					
	-pdT		31	0.5	81	82						
d-CpA	dCp-	20	28	0.4	58		±40	35	0.5	58		±38
	-pdA		36	0.6	90	86		28	0.4	63	70	
d-CpA	dCp-	80	28	0.4	58		±39					
	-pdA		36	0.6	63	75						
d-CpG	dCp-	20	33	0.5	55		±40	35	0.5	58		±38
	-pdG		37	0.6	75	86		32	0.5	63	67	
d-CpG	dCp-	80	25	0.3	65		±44					
	-pdG		37	0.6	56	75						
d-TpA	dTp-	20	24	0.3	55		±37	33	0.5	57		±41
	-pdA		36	0.6	90	86		28	0.4	63	70	
d-TpA	dTp-	80	33	0.5	63		±40					
	-pdA		36	0.6	61	80						
d-TpG	dTp-	20	24	0.3	55		±37	33	0.5	57		±41
	-pdG		35	0.5	75	86		32	0.5	63	67	
d-TpG	dTp-	80	24	0.3	55		±35					
	-pdG		29	0.4	79	88						

[a] Computed by using $J_{1'2'} + J_{3'4'} = 10.8$ Hz for the dimers and $J_{1'2'} + J_{3'4'}$ for the monomers. [b] Rotamer equations used: $gg = (13.7 - \Sigma)/9.7$; $g'g' = (25 - \Sigma')/20.8$. [c] $K_{eq}\ ^2E \rightleftarrows\ ^3E$. [d] Monomer data for solutions at pD 5.4.

Table VIII. Dimerization Shifts

Nucleotide		δ(monomer) − δ(dimer), ppm: 1′	2′	2″	3′	4′	5′	5″	H(2) (H(5),Me)	H(8) (H(6))
d-ApA	dAp-	0.323	0.657	0.298	0.150	0.143	0.157	0.111	0.227	0.270
	-pdA	0.229	0.018	0.085	−0.069	0.048	−0.056	−0.055	0.205	0.158
d-ApG	dAp-	0.186	0.484	0.159	0.133	0.153	0.151	0.105	−0.065	0.093
	-pdG	0.273	0.063	0.070	−0.048	0.014	−0.107	−0.072		0.122
d-GpA	dGp-	0.458	0.757	0.363	0.188	0.143	0.164	0.135		0.284
	-pdA	0.268	−0.052	0.050	−0.118	0.035	−0.040	−0.048	0.200	0.061
d-CpC	dCp-	0.258	0.038	0.052	0.036	0.027	0.030	0.022	0.081	0.08
	-pdC	0.224	0.022	0.042	−0.001	0.057	−0.049	−0.011	0.088	0.123
d-TpT	dTp-	0.103	0.076	−0.019	0.010	0.010	0.043	0.030	0.016	−0.012
	-pdT	0.030	0.01	0.000	0.001	0.067	−0.073	−0.003	0.028	0.066
d-CpT	dCp-	0.133	−0.068	−0.063	0.040	0.022	0.012	0.008	0.100	0.036
	-pdT	0.033	−0.072	−0.056	−0.010	0.032	−0.083	−0.032	0.031	0.040
d-TpC	dTp-	0.083	0.059	0.016	0.002	0.103	0.039	0.022	0.012	−0.025
	-pdC	0.020	−0.014	0.010	−0.012	0.022	−0.062	−0.012	0.020	0.037

Elevation of temperature and dimerization have very little influence on ϕ_1' rotations (Tables VI and VII) indicating that ϕ_1' in these dimers do not respond to stacking interactions and torsional variations about ψ_2. Insofar as stacking interactions are sensitive to $\omega'\omega$ torsions one must conclude, contrary to Olson and Flory,[44,45] that in the deoxyribodinucleoside monophosphates little correlation exists between ϕ_1' and $\omega'\omega$.

Conformation about the P–O(3′)(ω') and P–O(5′)(ω) Bonds. Torsional variation about $\omega'\omega$ can create a wide range of conformations such as stacked, skewed, and extended arrays.[10,28] A few of the important ones are illustrated in Figures 6–8. Contributions from *tt* orientations (Figure 8c) are not expected to be important for reasons outlined elsewhere.[46-48] Direct determination of the conformational preferences about $\omega'\omega$ from NMR data is difficult because of the lack of appropriate coupling constants. The conclusions have to be reached from

Table IX. Dimerization Shifts

Nucleotide		δ(monomer) − δ(dimer), ppm								
		1′	2′	2″	3′	4′	5′	5″	H(2) (H(5),Me)	H(8) (H(6))
d-ApC	dAp-	−0.019	−0.025	−0.025	0.044	0.015	−0.003	0.003	−0.123	−0.054
	-pdC	0.108	0.027	0.046	+0.002	0.054	−0.115	−0.035	0.256	0.203
d-ApT	dAp-	−0.002	−0.011	−0.086	0.054	0.024	−0.021	−0.010	−0.104	−0.046
	-pdT	0.161	0.073	0.073	0.015	0.076	−0.149	−0.033	0.252	0.266
d-GpC	dGp-	0.136	0.139	−0.072	0.073	0.030	0.029	0.011		0.026
	-pdC	0.049	0.032	0.069	−0.010	0.063	−0.105	−0.039	0.339	0.253
d-GpT	dGp-		/6	0.076	.071	0.027	0.016	0.003		−0.033
	-pdT	0.088	0.030	0.052	−0.015	0.069	−0.134	−0.036	0.205	0.206
d-CpA	dCp-	0.289	0.815	0.392	0.226	0.197	0.195	0.178	0.149	0.367
	-pdA	0.065	−0.083	0.01	−0.077	0.053	0.022	0.001	0.011	−0.036
d-CpG	dCp-	0.209	0.701	0.279	0.161	0.154	0.223	0.150	0.227	0.284
	-pdG	0.116	−0.033	0.018	−0.062	0.053	−0.027	−0.032		0.048
d-TpA	dTp-	0.345	0.834	0.384	0.179	0.159	0.214	0.151	0.045	0.306
	-pdA	0.048	−0.093	0.014	−0.083	0.055	0.015	−0.006	0.019	−0.001
d-TpG	dTp-	0.244	0.71	0.281	0.128	0.113	0.202	0.139	0.061	0.222
	-pdG	0.127	−0.033	0.009	−0.067	0.051	−0.022	−0.027		0.002

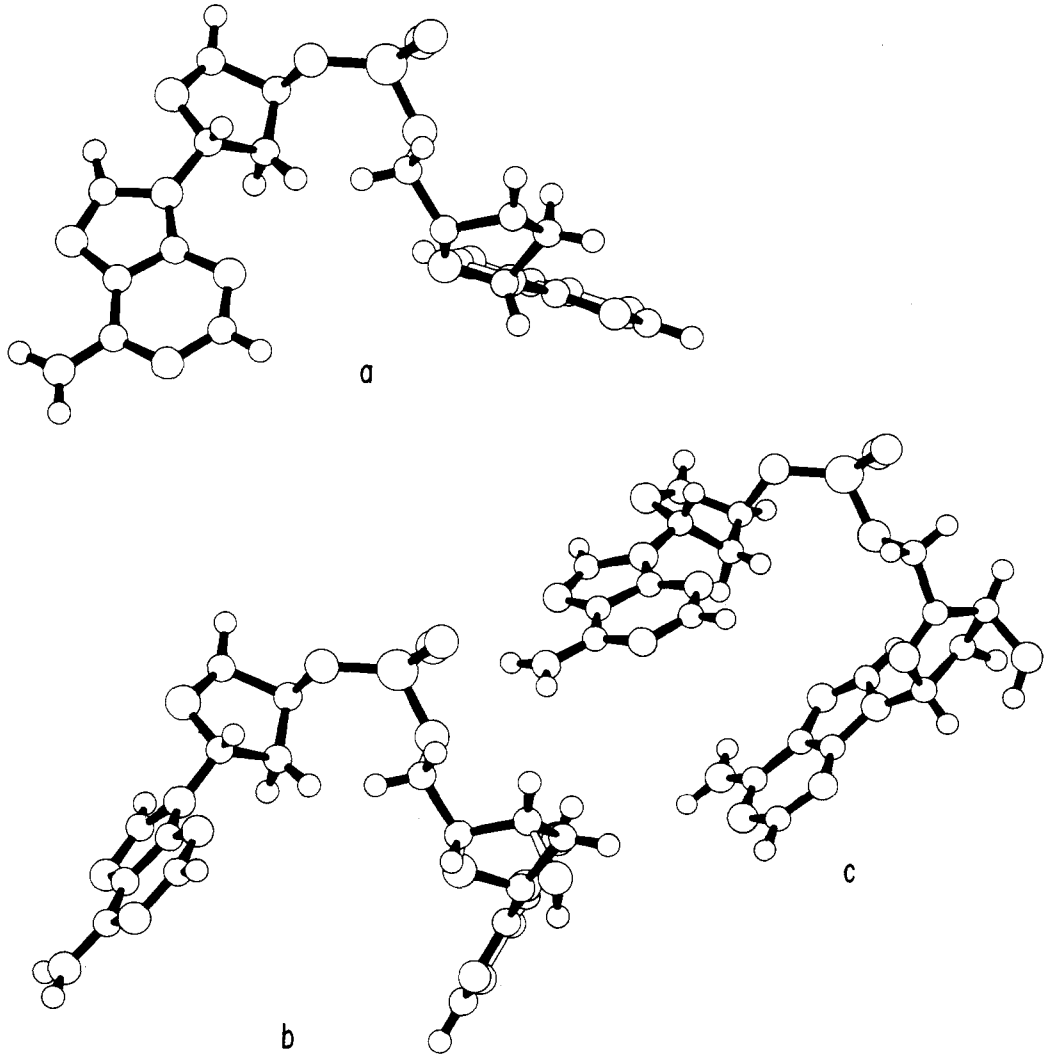

Figure 6. Perspectives of d-ApA conformations in g^-g^- domains. Certain atoms are omitted in this and the accompanying perspectives in Figures 7–9, for clarity. In all the perspectives (Figures 6–9) the pentose is ^2E, $\psi_1 = 60°$, $\phi_1' = 200°$, $\psi_2 = 60°$, $\phi_2 = 180°$. In a and b $\omega'/\omega = 300°/300°$ and in c $\omega'/\omega = 335°/240°$. In a $\chi_1 = \chi_2 = 0°$. In b χ_1 and χ_2 are, respectively, 50 and 100°. In c $\chi_1 = 10°$ and $\chi_2 = 100°$. The diagrams clearly reveal that overlap and stacking among the bases can be drastically altered by changes in χ_1, χ_2, ω', and ω.

chemical-shift trends of the various protons in the molecules *and hence are qualitative.* Before we present our chemical-shift arguments we wish to point out the following. (i) Because one cannot clearly separate the effect of various factors on chemical shift, no importance is attached to shift changes of magnitude of about 0.03 ppm or less. (ii) Because of the ambiguity in the assignment of H(6) and H(5) to the 3′ and 5′ units in dipyrimidines, shift trends of H(6) of both residues or H(5) of both

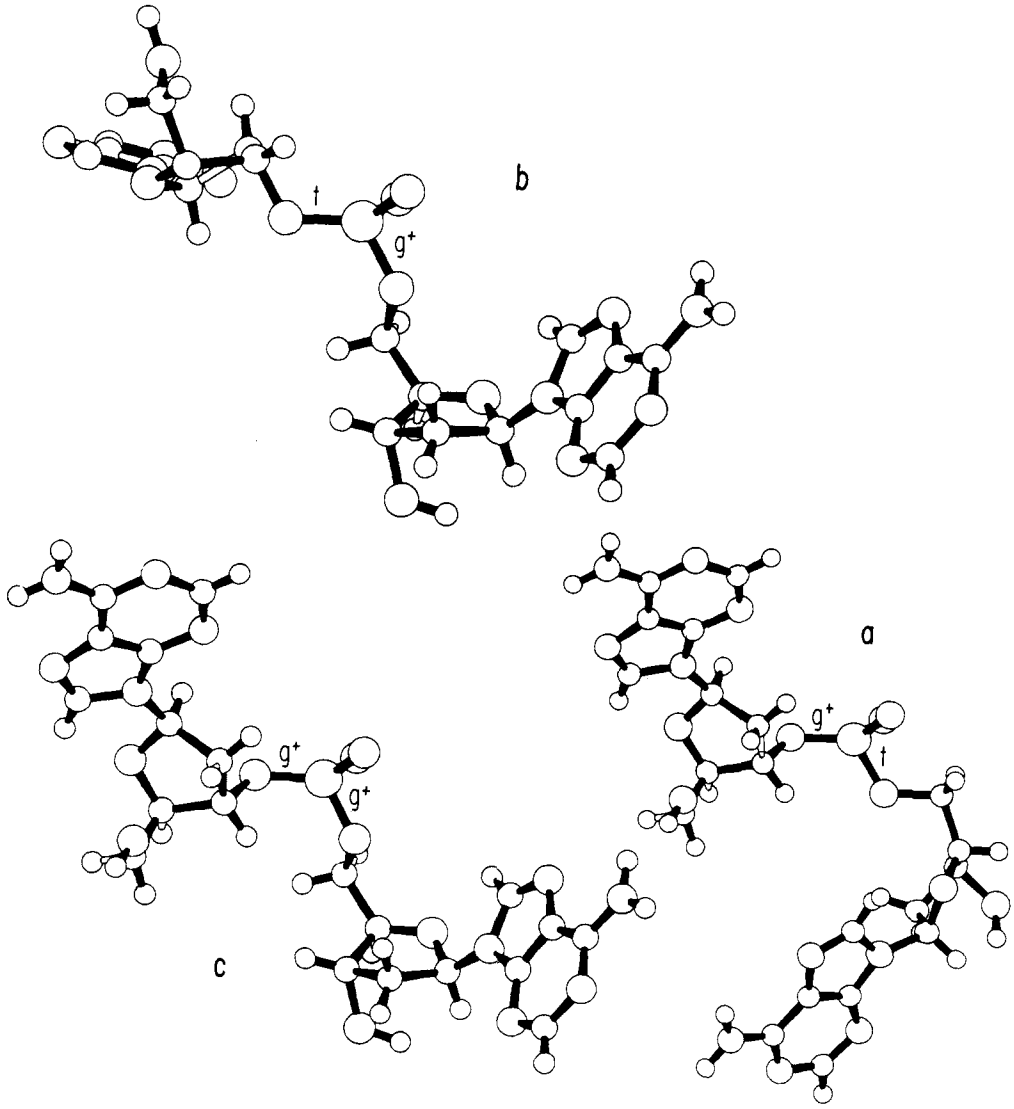

Figure 7. Perspectives of d-ApA conformations in the g^+g^+ ($\omega'/\omega = 60°/60°$), g^+t ($\omega'/\omega = 60°/180°$), and tg^+ ($\omega'/\omega = 180°/60°$) domains. In all cases $\chi_1 = \chi_2 = 0°$ and the remaining backbone is the same as in Figure 6. In the g^+t (a) and tg^+ (b) conformations overlap and stacking between the bases are not possible by varying χ_1 and χ_2. In the g^+g^+ conformer (c) stacking and overlap are possible. See ref 47.

residues are considered together. (iii) The chemical-shift arguments are developed under conditions in which the remaining backbone torsions conform to their preferred domain derived earlier, i.e., sugar pucker ^2E, $\phi_1' \simeq 200°$, $\phi_2 \simeq 180°$, $\psi_2 \simeq 60°$, and $\psi_1 \simeq 60°$. Below we provide our chemical-shift arguments to determine the conformational properties of the phosphodiester bonds in the four classes of deoxydinucleoside monophosphates.

(1) The Purine–Purines. Examination of the dimerization data (Table VIII) for the d-pupu dimers reveals that in each of the dimers in this class the protons of the 3′-nucleotidyl unit undergo a substantial upfield shift, the shift being largest for H(2′) and this is of the order of 0.8 to 0.5 ppm. The observed shifts of the protons of the dpu-residue cannot be entirely due to changes in χ_1 upon dimerization because the observed *relative* upfield shifts of H(1′), H(2′), and H(3′) are in total disagreement with those expected upon χ_{CN} variation. For example, in the purine system for ^2E pucker, a change in χ_{CN} from 60 to 0° will cause H(1′) and H(2′) to be shielded by 0.65 and 0.20 ppm, respectively, with no effect on δ(H(3′)).[49] This information along with the observation that substantial upfield shifts occur to the resonances in the 3′ residues of d-pupu and d-pypu dimers and that it does not happen in the d-pypy and d-pupy systems (Tables VIII and IX) suggest that the shifts originate from the ring current fields of the purine rings at the 5′ end.

Examination of the various perspectives in Figures 6-8 clearly reveals that among the various torsional possibilities about $\omega'\omega$, only those which lie in the g^-g^- domain[50] can generate strongly base-stacked structures by changes in χ_1 and χ_2 so that a 5′ base is positioned so as to cause upfield shifts of the 3′ residue, especially shifts of the observed magnitude for H(2′).[51] Several theoretical[52-56] calculations predict that g^-g^- is the global minimum. In the crystals GpC and ApU exhibit g^-g^- conformation and UpA alternate conformations.[57-59] Many of these calculations also indicate that the energy differences between g^-g^- and tg^- or g^-t are not sufficiently different suggesting that torsion about either ω' or ω is allowed. The recent work of Pullman et al.[60] on the effect of hydration upon the conformation of dimethyl phosphate anions shows that solvation will tend to increase the importance of tg or gt conformations. The solution data from 15 ribodinucleoside monophosphates[1,2] indicate that g^-g^- populations make a significant contribution to the conformational blend and in a majority of cases their population is exceeded only by the *combined* populations of tg^-, tg^+, g^-t, and g^+t conformers.[6]

In order to account for the observed upfield shifts of the 3′

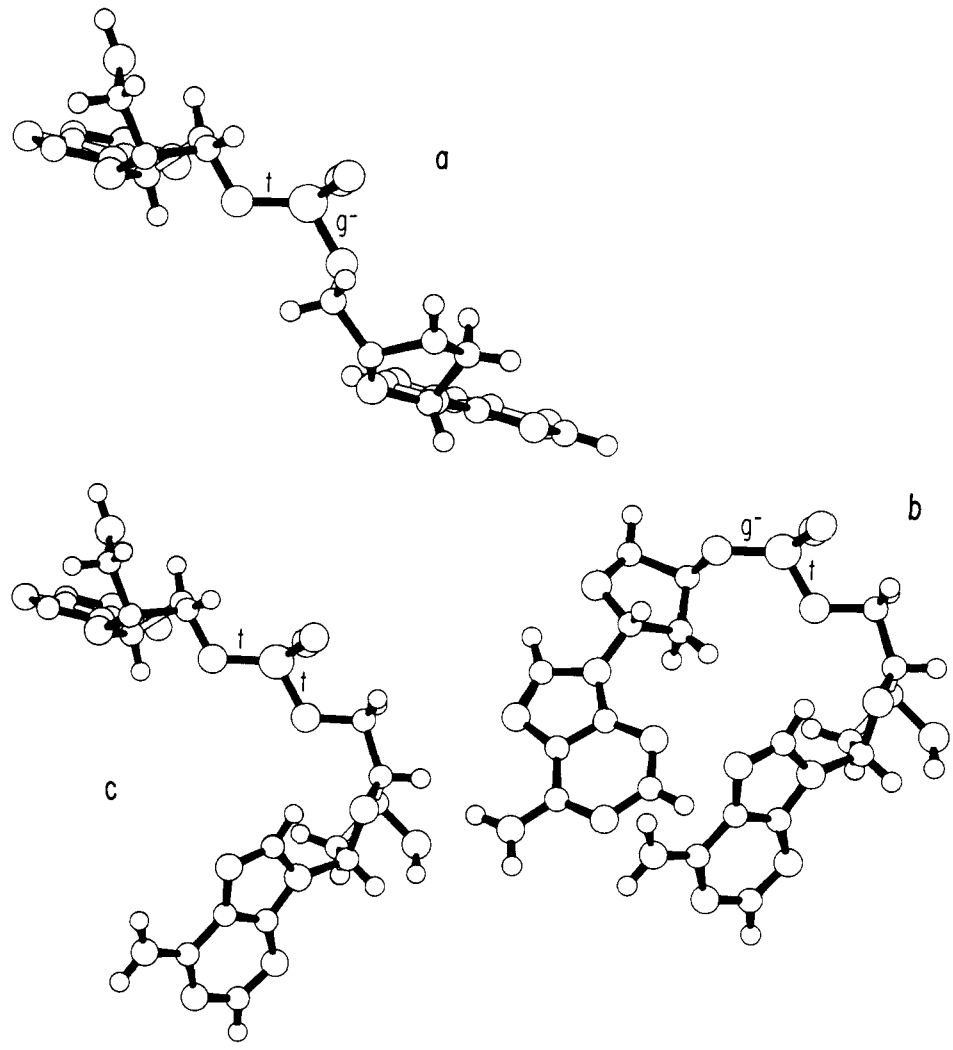

Figure 8. Perspectives of d-ApA conformations in the tg^- (ω'/ω = 180°/300°), gt (ω'/ω = 300°/180°), and tt (ω'/ω = 180°/180°) domains. In all cases $\chi_1 = \chi_2 = 0°$ and the remaining backbone is the same as in Figure 6. Overlap and stacking between the bases are not possible in any of the structures by varying χ_1 and χ_2; however see ref 51.

residue induced by the 5′-purine we have examined a range of conformers in the g^-g^- domain. A g^-g^- conformer in which ω'/ω have the classical values of 300°/300° and χ_1/χ_2 = 0°/0° generates a conformation in which the bases are in about perpendicular planes (Figure 6a). However, changing χ_1 to ~50° and χ_2 to ~100° generates a conformation in which the bases are in somewhat parallel planes (Figure 6b). However, the cylindrical coordinates z, ρ_5, and ρ_6[61] for the various protons of the 3′ residue in this conformation are such that the projected shieldings are *substantially* smaller than what is observed. For example, this conformation predicts no shielding of H(2) and a shielding of 0.26 ppm for H(2′) of the 3′ unit. Usually theory predicts shieldings larger than observed because it assumes 100% stacked arrays, and in reality one has a conformational blend of stacked, skewed, and extended arrays.[6] These observations, along with a belief that Giessner-Prettre et al.[61] calculations do not substantially underestimate the magnitude of ring current effects, enable us to rule out a g^-g^- conformer in which ω'/ω = 300°/300° as a serious candidate, i.e., Figures 6a and 6b.

Rotation of the phosphodiester and glycosyl bonds so that $\omega' \simeq 335°$, $\omega \simeq 240°$, $\chi_1 \simeq 10°$, and $\chi_2 \simeq 100°$ leads to a stacked array[62] in which the base planes are in approximately parallel planes (Figure 6c). A stereoview of this conformation is shown in Figure 9. The magnitude of z, ρ_5, and ρ_6 for the protons of the 3′ residue in this conformation with respect to the 5′-purine predicts substantial shielding.[61] (See the next section for methods of calculation of ρ_5, ρ_6, and z.) These values along with the projected and observed shieldings for the dAp-residue of d-ApA in this conformation are given in Table X. The data indicate a *general* agreement between projections and observations so far as the dimerization *trends* are concerned for δ(H(1′)), δ(H(2′)), δ(H(2″)), δ(H(2)), and δ(H(8)). One should not expect a clear agreement between the two sets of data for the following reasons. (a) The projections are based on 100% population of a static species while in reality the type of system investigated is expected to be conformationally pluralistic in aqueous solution.[6] (b) The calculations take into consideration only the ring current effects from the heterocycles whereas dimerization effects are the manifestation of effects on the chemical shifts of the protons on the 3′ residue due to addition of an adjacent residue which can cause changes in various torsion angles. For example, a change in χ_1 of dpu-upon dimerization will affect the magnitude of δ(H(1′)) and δ(H(2′)). In the 3′-mononucleotides one expects free rotation about O(3′)-P and depending upon ϕ', such a free rotation and the three oxygens attached to the ^{31}P nucleus will definitely influence δ(H(2′)), δ(H(2″)), δ(H(3′)), δ(H(4′)), and even δ(H(5′),H(5″)), of the monomer. Dimerization and formation of the phosphodiester linkage would introduce some constraint about O(3′)-P torsion and flexibility becomes limited to certain domains. And this conformational constraint is expected to

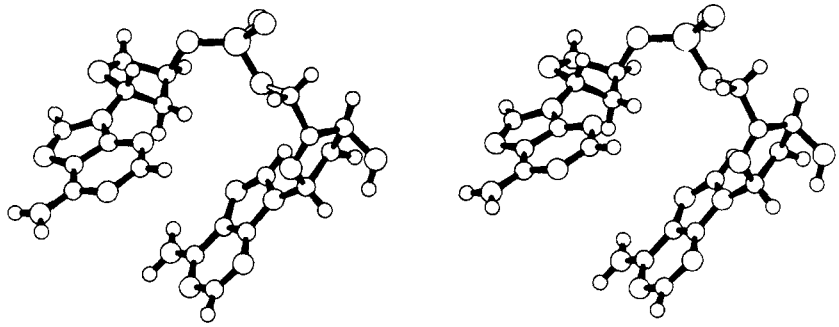

Figure 9. Stereoscopic drawing of d-ApA in a conformation shown in Figure 6c.

Table X. Cylindrical Coordinates ρ_5, ρ_6, and z in Å for the Various Protons and C(2) and C(8) of the 3′-Nucleotidyl Unit in d-ApA for the Conformation Shown in Figure 6c along with the projected and Observed Values for Shielding in ppm

Protons	z	ρ_5	ρ_6	Projected shielding	Obsd shielding
H(1′)	6.6	1.5	2.7	0.29	0.323
H(2′)	3.8	1.1	2.2	0.65	0.657
H(2″)	4.3	2.2	3.4	0.30	0.298
H(3′)	4.1	2.2	4.3	0.24	0.150
H(4′)	5.6	2.3	4.0	0.20	0.143
H(5′)	5.0	2.7	3.2	0.27	0.157
H(5″)	4.1	2.7	3.8	0.27	0.111
H(2)	5.7	6.2	5.8	0.00	0.227
H(8)	6.1	2.4	0.4	0.30	0.270
C(2)	5.8	5.5	4.9		
C(8)	6.1	2.8	0.8		

contribute to the shifts of the above protons and the projected values in Table X do not take this important source into consideration.

For the above reasons one should not use the data in Table X to quantitatively arrive at the population of the g^-g^- conformer from the ratio of the observed and theoretical shifts. The only reasonable conclusion that on may claim from the type of approach used here is that conformers in which $\omega' \simeq 335°$, $\omega \simeq 240°$, $\phi' \simeq 200°$, $\phi \simeq 180°$, $\psi_2 \simeq 60°$, and $\psi_1 \simeq 60°$ and deoxyribose units in ^2E pucker (Figure 6c and stereoview in Figure 9) makes a sizable contribution to the conformational blend of dipurine deoxydinucleoside monophosphates in aqueous solution. Insofar as a variety of conformers about ω'/ω can contribute to the blend, from the data in Table X one may qualitatively conclude that the population of the g^-g^- should be larger than that of any other conformer about $\omega'\omega$, i.e., for the d-pupu system the preferred conformer about $\omega'\omega$ is g^-g^-. This does not mean that the *combined* population of all the remaining conformers about $\omega'\omega$ is smaller or larger than that of g^-g^-.

The conclusion that sizable populations of g^-g^- conformers contribute to the blend is consistent with the dimerization data for the 5′ residue in the d-pupu system. Except for H(1′) (vide infra for reasons for this) dimerization has only a small influence on the chemical shift of the sugar protons. This is because in the g^-g^- conformer (Figures 6 and 9) the 5′ sugar lies outside the helix away from the strong shielding/deshielding realms of the 3′-purine, and the 3′ sugar lies inside the helix in the shielding region of 5′-purine. The 3′-purine in a g^-g^- stack is expected to affect the shift of the protons of the 5′ base. As can be seen from Table VIII for d-pupu systems dimerization causes upfield shifts of protons of 3′ and 5′ bases.

The chemical-shift trends of H(2′), H(2″), and H(3′) of the 3′ unit should provide information about the conformational proclivities about ω'. However, for the d-pupu system one cannot employ the shift trends of H(2′),H(2″) because one cannot separate the effects on δ(H(2′)),δH(2″)) due to torsional variation about ω' from that due to changes in ρ_5, ρ_6, and z as a result of χ_{CN} changes upon dimerization. We have argued in detail that a shift of ω' from t to g^- domains will be accompanied by the shielding of H(3′) of the 3′-nucleotidyl unit. This is particularly true for the present system because the preferred sugar pucker is ^2E and χ_{CN} variations in the anti domain have no effect[49] on δ(H(3′)). Data in Table VIII show that for the d-pupu dimers upon dimerization H(3′) of dpu- is shielded by 0.133–0.188 ppm and this is consistent with the transformation of a freely rotating O(3′)–P bond in the monomer to a relatively restricted one in the dimer and the restriction being in the g^- domain.

The chemical-shift trends of H(5′),H(5″) of the 5′ unit have been employed by Lee et al.[1] and Ezra et al.[2] to monitor the conformation about ω in ribodinucleoside monophosphates. They showed that the 2′-OH group of the 3′ residue will strongly perturb the magnitude of H(5′),H(5″) of the 5′ unit. This method obviously cannot be employed for the present system because they lack the crucial 2′-OH group. Nevertheless one should be able to obtain qualitative information about ω from the shift trends of H(5′),H(5″) of the 5′ unit because a change in ω from t to g^- domains essentially involves a change in the orientation of H(5′),H(5″) with respect to the *pro-S* and *pro-R* oxygens of the phosphate and hence should manifest in shift trends. For example when $\omega = 240°$ H(5′) and H(5″) of the 5′ residue will be subjected to identical magnitudes of deshielding by *pro-S* oxygen. This means that upon dimerization both these hydrogens will be deshielded relatively to the same extent. This is indeed what the data in Table VIII reveal for the d-pupu dimers.

Elevation of temperature in d-pupu dimers (Table I) causes substantial shifts of the protons of the 3′ residue to lower fields. The pioneering investigations of Ts'o and coworkers[63] and Danyluk and coworkers[64] have demonstrated that such downfield shifts arise from unstacking of the bases and consequent loss of ring current shieldings. Unstacking from the g^-g^- conformation (Figures 6c and 9) can result from torsional variation about ω', ω, ϕ_1', ϕ_2, and ψ_2.[65] Temperature data in Table VI show that for d-GpA and d-ApG as much as a 20–30% increase in unstacked arrays may result from variation about ψ_2 and ϕ_2, ψ_2 being the important one (vide supra). In d-ApA only ~10% of temperature-induced destacking could be accounted for by variation about ψ_2. The data in Table I show that elevation of temperature causes a *downfield shift* of δ(H(3′)) of the dpu- of d-pupu systems, the shift being 0.11 ppm for d-ApA and 0.045 ppm in d-ApG and d-GpA. The downfield direction of the shift[5] indicates torsional variation about ω' from g^- to t domains so that the populations of unstacked arrays in which $\omega'\omega = tg^-$ (Figure 8a) increase. The larger shift in d-ApA compared to d-GpA and d-ApG suggests that ω' variation from the g^- to the t domain is more dominant a mechanism for destacking for d-ApA than for d-GpA and

d-ApG. This may be due to the fact that in d-ApA destacking via ψ_2 variation is small compared to d-GpA and d-ApG. Equally interesting to observe is that in the matched pairs d-GpA and d-ApG the $\delta(H(5'))$, $\delta(H(5''))$ of the 5′ residue and hence ω display little sensitivity to temperature changes. This is unlike d-ApA (Table I).

(2) The Pyrimidine–Pyrimidines. Lack of aromatic systems with strong ring currents at the 5′ end makes it difficult to monitor $\omega'\omega$ preferences in d-pypy dimers. However some information can be derived from shift trends of certain interior protons. Thus, inspection of the dimerization data (Table VIII) for H(5′),H(5″) of the 5′ unit show a clear trend. It is seen that H(5′) is consistently deshielded considerably more than H(5″). This differential deshielding from the *pro-S* phosphate oxygen is clearly possible when ω occupies the g^- domain (e.g., $\omega \simeq$ 260–300°).

Dimerization shifts of H(2′), H(2″), and H(3′) of the 3′ residue in the pyrimidine system are independent of χ_1 changes[66] in the anti range and hence provide direct information about ω' because the chemical shifts of these protons should be sensitive to ω' variation. When ω' is changed, the orientation of H(2′), H(2″), and H(3′) with respect to the two phosphate oxygens changes. For example, in d-CpC, H(2′), H(2″), and H(3′) of d-Cp- undergo shielding (Table VIII), clearly suggesting a torsional event upon dimerization about ω' so that they are occupying domains away from the two oxygens; for example, $\omega' = g^-$. On the other hand in d-TpT and d-TpC (Table VIII) the H(2′) undergoes significant shielding with small effect on H(2″) and H(3′) and this is eminently consistent with a t domain for ω' in d-TpT and d-TpC. Data in Table I show that $\delta(H(2'))$, $\delta(H(2''))$, and $\delta(H(3'))$ of dpy- and $\delta(H(5'))$ and $\delta(H(5''))$ of -dpy in d-CpC, d-TpT, and d-TpC do not undergo meaningful changes with temperature implying that $\omega'\omega$ torsions in these dimers are insensitive to temperature. This is particularly true for d-TpT which displays a tg^- arrangement and this has also been reported for pdTpdT in its crystals.[67] Our conclusion that the preferred $\omega'\omega$ torsion for d-CpC is g^-g^- and that for d-TpT and dTpC is tg^- derives support from the dimerization shift for H(5) (Table VIII). Dimerization has no effect on $\delta(H(5))$ in d-TpT and dTpC as expected in a tg^- orientation, but in d-CpC dimerization has caused shielding of H(5). This is possible only in a g^-g^- orientation in which the bases can assume favorable χ_1 and χ_2 values so that the magnitude of ρ and z places H(5) in the shielding zone, however small the shielding domain in the pyrimidine system may be.[61] Upon dimerization d-CpT behaves very differently from other d-pypy dimers and also shows behavior very different from d-pupu, d-pypy, and d-pupy dimers. Dimerization causes approximately equal (~−0.065 ppm) deshielding of H(2′) and H(2″) of dCp- along with shielding of H(3′) and H(5) of dCp-. Equally strange is that elevation of temperature causes *shielding* of H(2′) and H(2″) of dCp-. Even though these changes are internally consistent (i.e., dimerization and elevation of temperature have opposite effects) we are unable to provide a credible rationalization for this exceptional and variant behavior of d-CpT. However we would like to point out that deshielding of equal magnitude of H(2′) and H(2″) of dCp- is possible if ω' fluctuates between ~210 and 90°.

(3) The Purine–Pyrimidines. Inspection of the dimerization data for the H(5′) and H(5″) of the 5′ unit shows a clear-cut trend in which H(5′) undergoes substantial deshielding with small effect on H(5″) (Table IX). The situation is similar to d-pypy dimers except that the shifts are substantial. The differential deshielding of H(5′) and H(5″) of the 5′ unit is an indication of the occupation of ω in the g^- domain ($\omega \simeq$ 260–300°) and the larger shifts are an indication of an increase in the population of g^- conformers in d-pupy dimers compared to d-pypy series. Rotational preferences about ω' in d-pupy dimers cannot be determined from shift trends of H(2′) and H(2″) of the 3′ residue due to complications from χ_{CN} variation. However, shift trends of H(3′) of dpu- are uniformly to the higher fields (Table IX) in all the d-pupy dimers as in the case for d-pupu dimers. This suggests the presence of g^- conformers for ω' in d-pupy systems. The observed difference in the dimerization shifts of H(3′) of the 3′ residue between d-pupu and d-pupy series may be due to the following: (a) a difference in the population of g^- conformers or (b) more likely differences in the local domains within the g^- conformation space for ω'. The conclusion that these dimers preferentially populate in g^-g^- domains is in eminent agreement with the observation that upon dimerization H(5) of -dpy undergoes shifts to higher fields by about 0.3 ppm. The $\delta(H(3'))$ data for the 3′ unit indicate that d-pypu behaves similarly to the d-pupu series to temperature effects, so far as ω' is concerned. Temperature has no effect on ω torsions in d-GpT. However, in d-GpC, d-ApC, and d-ApT the H(5′) of -dpy undergoes a shielding of 0.03 to 0.07 ppm (Table II). This can result from a torsional adjustment within the g^- domain (e.g., from $\omega \simeq$ 260° to $\omega \simeq$ 300°).

(4) The Pyrimidine–Purines. The d-pypu dimers contain a purine at the 5′ end and the dimerization data for the 3′ end (Table IX) show trends extremely similar to d-pupu dimers. This suggests that the favored conformers about $\omega'\omega$ for d-pypu dimers lie in the g^-g^- domain. Temperature data on H(3′) of the 3′ residue and H(5′),H(5″) of the 5′ residue suggest that temperature-induced unstacking is accompanied by a shift in population from $g^-g^- \rightarrow tg^-$. This is again similar to d-pupu dimers.

These studies indicate that d-pupu, d-pypu, and d-pupy deoxydinucleoside monophosphates prefer to populate in g^-g^- domains, a domain which permits stacking interaction between the bases. The degree of overlap and extent of interaction between the bases in the g^-g^- domain are determined by the magnitude of ω, ω', χ_1, and χ_2, when the rest of the torsion angles are kept constant (vide supra). In the d-pypy, series of d-CpC prefer the g^-g^- domain and the tg^- is preferred by d-TpT and d-TpC with no clear-cut decision on d-CpT. Temperature studies reveal that deoxydinucleoside monophosphates undergo torsional variation about ω' more readily than about ω. Even though it is theoretically possible for them to unstack by torsional variation about ω' and ω, they seem to show an exclusive tendency to do so by changes in ω'. This is certainly not true in crystalline tRNA which possesses a variety of $\omega'\omega$ combinations. In tRNA the turns at both the anticodon loop and the TψCG loop are negotiated via a change in ω from g^- to t, i.e. $\omega'\omega = g^-t$ at the turn.

NMR Data and B-DNA Model. Because the NMR methodology cannot provide direct information about χ_1, χ_2, ω', and ω, it is of interest to determine whether the dimerization data for d-ApA (Table VIII) are consistent with its assuming a conformation similar to a dimer segment in B-DNA. Using the presently determined conformation for the sugar rings, and that about ϕ', ϕ, and ψ, and by using the B-DNA torsions[68] for χ_1 (~65°), χ_2 (~80°), ω' (~260°), and ω (~300°) we have generated the x, y, z coordinates for the various atoms. These are used to generate the perspective in Figure 10 using ORTEP II. This is a B-DNA model for d-ApA. The same coordinates were used to calculate the coordinates of the center of the five- and six-membered rings of the adenine moiety of the 5′-nucleotidyl unit. Then the original x, y, z coordinates were used to generate a second set of x, y, z coordinates with the center of the rings as origin. Finally, the second set of x, y, z coordinates was used to generate the cylindrical coordinates, ρ_5, ρ_6, and z which were used to obtain the projected shielding patterns in the B-DNA model. The final data are summarized in Table XI. A comparison between the projected and observed shielding patterns in Tables X and XI clearly indicates that

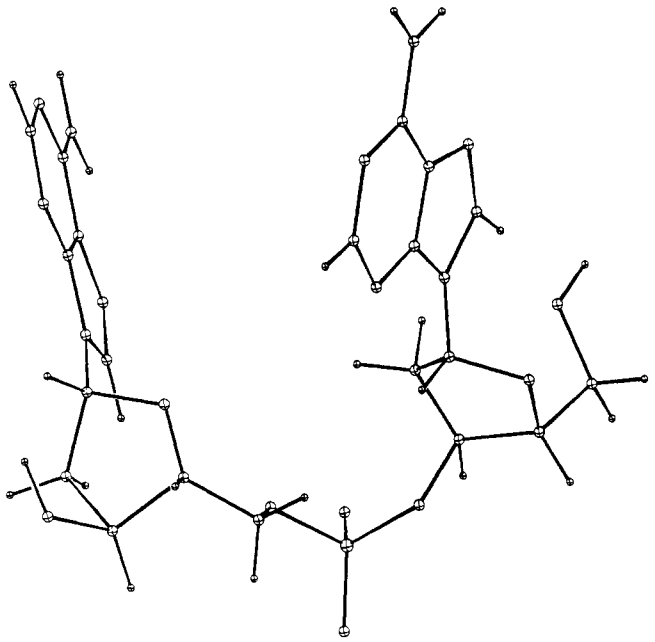

Figure 10. Perspective of d-ApA in B-DNA conformation drawn using ORTEP II. Both sugars are ^2E; $\chi_1 = 65°$, $\chi_1 = 60°$, $\phi_1' = 200°$, $\omega' = 65°$, $\omega = 80°$, $\phi_2 = 180°$, $\psi_2 = 60°$, and $\chi_2 = 80°$.

Table XI. Cylindrical Coordinates ρ_5, ρ_6, and z in Å for the Various Protons and C(2) and C(8) of the 3′-Nucleotidyl Unit in d-ApA for the B-DNA Model Shown in Figure 10 along with the Projected and Observed Values for Shielding in ppm

Protons	z	ρ_5	ρ_6	Projected shielding	Obsd shielding
H(1′)	5.6	3.5	5.1	0.08	0.323
H(2′)	5.4	1.8	3.9	0.24	0.657
H(2″)	4.3	2.2	4.2	0.24	0.298
H(3′)	5.3	4.6	6.7	0.00	0.150
H(4′)	7.3	5.0	7.0	0.00	0.143
H(5′)	8.8	3.7	5.8	0.00	0.157
H(5″)	7.9	4.6	6.6	0.00	0.111
H(2)	5.2	6.0	6.1	0.00	0.227
H(8)	7.2	0.6	2.6	0.21	0.270
C(2)	5.6	5.2	5.2		
C(8)	7.1	1.1	2.3		

the B-DNA model for d-ApA is considerably less satisfactory than the model in which $\chi_1 \simeq 10°$, $\chi_2 \simeq 100°$, $\omega' \simeq 335°$, and $\omega \simeq 240°$. The z value for C(8) and C(2) (Tables X and XI) indicates that there is a small tilt between the bases, the tilt being larger in the B-DNA model. However, these can be eliminated by small adjustments in χ_1, χ_2, ω', and ω.

Relative Orientation between Base and Sugar Ring. For reasons outlined elsewhere[1,2] throughout the previous discussions we have assumed that the collection of dimers examined here prefers the anti domain. Even though no satisfactory approach is now available for quantitative evaluation of χ_{CN} or syn/anti ratios, it is possible to determine relative differences in the magnitude of χ_1 and χ_2 as well as the dimerization induced changes of χ_{CN}. Inspection of data for d-CpC, d-TpT, d-CpT, and d-TpC (Table I) shows that H(1′) of the 3′ unit is always upfield relative to H(1′) of the 5′ residue. These observations, based on the arguments developed elsewhere,[1,2] suggest that $\chi_1 < \chi_2$ for the entire d-pypy series. A similar approach of comparing δ(H(1′)) of the 3′ and 5′ units in the same dimer in the d-pupu, d-pypu, and d-pupy series is not possible because of complications from ring current effects and the dependence of H(1′) chemical shifts on the chemical nature of the base. However, one may overcome these difficulties by comparing δ(H(1′)) in matched pairs d-ApC/d-CpA, d-ApT/d-TpA, d-GpC/d-CpG, and d-GpT/d-TpG. The data in Table II consistently show that in all the systems examined δ(H1′)) of the 3′ unit appears at a higher field than the same proton of the 5′ unit irrespective of whether the base is a purine and pyrimidine, provided the comparisons are limited to the same kind of base, i.e., in the system d-ApC/d-CpA, H(1′) of dAp- is at higher field than that of -dpA and similarly the H(1′) of dCp- is at a higher field than that of -pdC. These results imply that in all likelihood $\chi_1 < \chi_2$ for all deoxydinucleoside monophosphates.

Elevation of temperature causes significant shifts of H(1′) of both 3′ and 5′ units to lower fields in d-pupu dimers (Table I). As expected, dimerization (Table VIII) causes shifts to higher fields. For example, dimerization shifts H(1′) of dGp- by 0.458 ppm and that of -pdA by 0.268 ppm. Obviously one cannot use data from the 3′ unit to determine χ_{CN} variations because it is difficult to sort out the ring current influence from the 5′ base and that due to χ_2 changes. However, in a g^-g^- stacked array (Figures 6c and 9) H(1′) of the 5′ unit is not significantly influenced by the 3′-purine moiety. The significant shielding of H(1′) of the 5′ unit clearly indicates that upon dimerization χ_2 definitely changes and that a change in χ_{CN} is in a direction opposite to that induced by elevation of temperature. It is difficult to determine whether the observed shifts are due to a decrease or increase in χ_{CN} relative to the monomer without introducing boundaries to the anti domain. If the anti domain is restricted to the range of 0-60°, a shielding of H(1′) by 0.25 ppm indicates a reduction in χ_{CN} by about 20°, i.e., if the value in the monomer is ~60°, the value in the dimer is ~40° or if the value in the monomer is 40°, the corresponding value in the dimer is 20°, and so on.[46] However, if the size of the anti domain is expanded to the traditional anti domain ($\chi_{CN} \simeq \pm 90°$) the conclusion drastically changes. For example, a value of $\chi_{CN} \simeq 40°$ in the monomer and 90° in the dimer (an increase of 50°) will also result in a shielding of 0.25 ppm of H(1′). The shapes of δ(H(1′)) vs. χ_{CN} plots are such that an increase in χ_{CN} from 70 to 90° will also have the same effect.[46] Hence, the only reliable conclusion one may make from observed changes in δ(H(1′)) upon dimerization or elevation temperature is that they are *consistent* with changes in χ_{CN}. However, a lack of change in H(1′) does not necessarily mean no change in χ_{CN}![49,65]

Deoxyribo- and Ribonucleic Acid Structures. A Comparison. In the *deoxyribo*-3′- and -5′-mononucleotides, as well as in the dimers, irrespective of the nature of the base and sequence, the pentose ring shows a clear preference to exist in ^2E conformation. This is the case *in each and every naturally* occurring constituent of deoxyribonucleic acids. Furthermore, the conformational distribution of the pentose does not show any meaningful sensitivity to such strong intramolecular perturbations such as stacking/destacking interactions. From these one is reasonably sure to conclude that the conformation of the pentose moieties in hetero- and homodeoxyribopolynucleotides in aqueous solution resembles that in their constituents and that the pentose moieties in them will show a great deal of preference to the ^2E mode of pucker. We realize that in crystals both ^2E and ^3E DNAs have been reported. We do not have any evidence that there is any chance that a given sequence of DNA *in aqueous* solution could populate *predominantly* in the ^3E form.

This is in sharp contrast to the behavior of ribonucleic acid structures where in aqueous solution the kind of sugar pucker a system prefers is determined by the nature of the base and stacking interactions.[1-3] For example, the purine ribomononucleotides prefer ^2E pucker, the pyrimidines ^3E pucker. Stacking interactions cause a shift of $^2E \rightarrow {}^3E$ pucker so much so that in a fully base-stacked purineribopolynucleotide in

Table XII. Population of $\psi_2 = 60°/\phi_2 = 180°$ Conformers in Ribo- and Deoxyribodinucleoside Monophosphates[a]

Dimer	$\psi_2 = 60°/\phi_2 = 180°$ for Deoxyribo series	Ribo series
ApA	87/85	74/90
ApG	94/86	75/82
GpA	84/86	85/84
CpC	75/76	92/84
TpT(UpU)	74/82	77/73
CpT(CpU)	71/74	90/85
TpC(UpC)	79/77	87/83
ApC	63/82	93/85
ApT(ApU)	86/89	95/85
GpC	73/82	96/89
GpT(GpU)	81/82	92/82
CpA	90/86	90/84
CpG	75/86	85/85
TpA(UpA)	90/86	79/80
TpG(UpT)	75/86	80/81

[a] Data for the ribo series from ref 1 and 2.

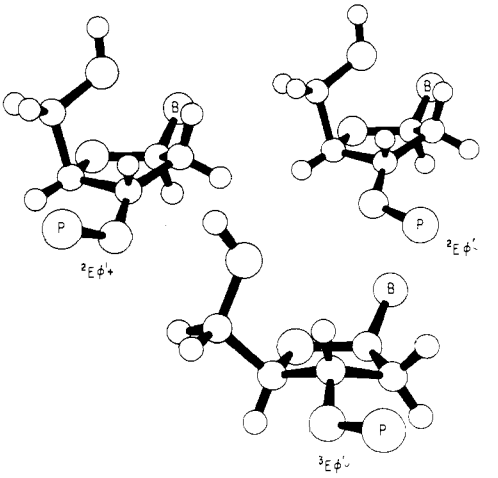

Figure 11. Conformational relationships among sugar pucker and C(3′)-O(3′) torsion.

aqueous solution the sugar moieties show outspoken preference for the ^3E mode and under conditions in which significant amounts of the polymer exist destacked the preferred pucker for the sugar is ^2E.[3]

The difference in the sugar pucker has profound implications with respect to the dimensions of polynucleotides.[69] The ^3E sugars in a nucleic acid produce a more compressed backbone than the ^2E rings. Lee and Sarma[43] have provided NMR evidence that the ^{31}P nucleotides in 3′,5′-nucleoside diphosphates are more apart from each other in the ^2E mode of pucker than they are when the sugar pucker is ^3E. (See Figure 7 in Lee and Sarma.[43])

In the ribo series Lee et al.[1] and Ezra et al.[2] have shown that torsional variation about ϕ_1' is coupled to the ribose conformation and is governed by the equilibrium:

$$^3E\phi_+' \rightleftharpoons {}^3E\phi_-' \rightleftarrows {}^2E\phi_+' \rightleftharpoons {}^2E\phi_-'$$

Conformationally compatible combinations are ^2E and ϕ_+' (Figure 11) on the one hand and ^3E and ϕ_-' (Figure 11) on the other. Combinations such as $^3E\phi_+'$ and $^2E\phi_-'$ (Figure 11), though accessible, are not stable and hence get converted into the favored $^3E\phi_-'$ and $^2E\phi_+'$ modes. In the deoxy series we find that the favored conformational coupling is between ^2E and ϕ_+' (Figure 11).

In both the ribo-[1,2] and deoxyribonucleic acid structures the C(4′)-C(5′) and C(5′)-O(5′) bonds form a stable conformational network in which $\psi_2 \simeq 60°$ and $\phi_2 \simeq 180°$ and this is the highly preferred orientation. In both systems elevation of temperature causes torsional variation about ψ_2 and ϕ_2. However, there are some distinct class differences which become obvious by examination of data in Table XII where we have retabulated the percent population of $\psi_2 = 60°$ and $\phi_2 = 180°$ conformers for both systems. The data show that in the ribose series the purine-pyrimidine dimers show the maximum preference for $\psi_2 = 60°/\phi_2 = 180°$ conformations. For the deoxy series the most preference is displayed by the dipurines which display the least preference for $\psi_2 = 60°/\phi_2 = 180°$ orientations in the ribose series. In the deoxy system the dipyrimidines display the least preference.

The majority of ribo-[1,2] and deoxyribodinucleoside monophosphates have a tendency to exist in stacked arrays in which $\omega'\omega$ lie in the g^-g^- domain. Because of the difference in the sugar pucker it is obligatory that there should be local differences between the two classes within the g^-g^- conformation space. An important observation in the present studies is that temperature-induced unstacking causes changes in ω' from g^- to t with little effect on ω. Hence, it is likely that the main torsional event at the onset of unwinding of DNA during replication and transcription is that about ω'. From the dimensions of random coil chains and helices of polynucleotides Olson[8] has concluded that the principal difference between the two is rotation about ω' in ribopolynucleotides. The present suggestion of torsional changes occurring during unwinding of DNA is similar to that made a number of years ago by Govil.[70] The idea that torsional flexibility resides primarily in the P-O bonds has also been proposed by Sundaralingam.[28]

In the ribo and deoxyribo series changes in $\omega'\omega$ are accompanied by destacking and χ_1,χ_2 changes. Such χ_1,χ_2 changes alter the mode of sugar pucker and torsion about C(3′)-O(3′) in the ribose series,[1,2] but no such changes are noticeable for the deoxy systems. This suggests that polyribonucleotides are engineered in such a way that their conformations are very sensitive to minor perturbations and that it is possible that they are capable of fulfilling their multifunctional biological roles as tRNA, mRNA, and rRNA because of this built-in potentiality in their constitution for conformational versatility. Even though the solution data project that deoxyribopolynucleotides may be conformationally less flexible than the ribopolymers, crystal data indicate that the deoxys exhibit greater flexibility in the sugar pucker. They are permitted both ^2E and ^3E pucker in the $\omega'\omega = g^-g^-$ domain. The former is sterically disflavored in the RNAs. Consequently in crystalline tRNA, for example, only a few of the approximately 80 residues have sugar pucker ^2E.

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