The Spatial Configuration of a Left-Handed Base Stacked Dinucleoside Monophosphate

M. M. Dhingra, R. H. Sarma,*[1] S. Uesugi, and M. Ikehara*

Contribution from the Institute of Bimolecular Stereodynamics, State University of New York at Albany, Albany, New York 12222, and Faculty of Pharmaceutical Sciences, Osaka University, 133-1 Yamadakami Suita, Osaka, Japan. *Received December 16, 1977*

Abstract: Detailed 270-MHz nuclear magnetic resonance studies of the dinucleoside monophosphate of 8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine A^spA^s, and the component monomers A^sp and pA^s, were undertaken. A complete set of NMR parameters was derived for each nucleotidyl unit by simulation–iteration methods. The data indicate that the arabinose ring of the monomers exists as an equilibrium blend of $^2E \rightleftarrows {}^3E$ with bias for ^2E pucker. Upon dimerization, the population of ^3E pucker increases. In the monomers the C4′-C5′ bond shows a distinct preference for gauche-trans and trans-gauche conformations while the conformation about the C5′-O5′ bond is gauche′-gauche′. However, in the dimer A^spA^S, the network containing C4′-C5′ and C5′-O5′ bonds prefers the classically stable gauche-gauche and gauche′-gauche′ conformation. The C3′-O3′ bond exist as a conformation blend of $\phi_1' \simeq 194$ and 286° in which 194° is coupled to ^3E sugar pucker and 286° to ^2E pucker. The cyclization of the base with the sugar residue introduces constraint on χ_{CN} and the magnitude is about 120°. Temperature and dimerization data indicate that despite cyclization a certain amount of flexibility is accessible to the base–sugar units. A search in the $\omega'\omega$ conformation space to account for the dimerization data indicated that $\omega' \simeq 265 \pm 5°$ and $\omega \simeq 280 \pm 5°$. The nature of the base–base stacking was found to be left-handed in A^spA^s.

Introduction

A plethora of NMR studies on mono-, oligo-, and polyribonucleotides[2-7] and theoretical calculations[8-12] have established the coupling of the glycosidic torsion (χ_{CN}) with the various other backbone torsional angles. A variation in χ_{CN} which results due to base destacking and consequent change in ω' and ω alters the mode of sugar pucker and the torsion about the C3′-O3′ bond. In other words, it means that the information about the experimentally indeterminable torsion angles ω' and ω can be extracted if the glycosidic torsion is tied down to a fixed value and the remaining torsion angles to their experimental values. This is done by delineating the geometry of base stack which in turn will provide information about ω' and ω. In addition the interrelationship between χ_{CN} and the sense of base stack can be explored.

To investigate the influence of χ_{CN} on the backbone conformation, Ikehara et al.[13-16] have synthesized a number of 8-cyclonucleosides and their dimers having fixed glycosidic torsion angles. The magnitude of glycosidic torsion depends whether the cyclization involves the 2′, 3′, or 5′ carbon atom. The fixed torsion angle about the glycosidic bond in a 8,2′-*S*-cycloadenosine (8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine, A^s) residue is 122°, 75° in 8,3′-*S*-cycloadenosine and 45° in 8,5-*S*-cycloadenosine.[17] The unusual CD spectrum[14a] observed for the dimer A^spA^s as well as preliminary partial chemical shift data[14b] have been interpreted in terms of a left-handed base stacking arrangement. The conformational energy minimization calculations[18] for A^spA^s suggests that the conformation about the phosphodiester bond is in the classical g^-g^- domain ($\omega' = 286°$ and $\omega = 279°$) and the sense of base stack is left-handed. The reported magnitude of ϕ_1' (170°) corresponding to the minimum energy conformer seems to be rather unusual.

Thus the available data suggest that the molecule A^spA^s may have unusual conformational properties and hence we have undertaken a detailed study of the spatial configuration of this molecule in aqueous solution. Since it has been established that the glycosidic torsion and the phosphodiester torsion angles[4,5] are interrelated, it would be interesting to determine the conformational status about the phosphodiester bonds and its effect on the overall topology of the molecule in a system in which χ_{CN} is fixed. This can be accomplished by a method recently advocated by Cheng and Sarma[19] using the dimerization shift data and the ring current field theory.[20]

In this paper we report the complete analysis of proton magnetic resonance spectra of the dimer A^spA^s and its constituent monomers. The structure of the molecule, the numbering of the atoms, and the conformational nomenclature for the angles of interest are shown in Figure 1. The experimentally determined sugar conformation, ψ_1, ψ_2, ϕ_2, and ϕ_1' and $\chi_{CN} = 120°$ have been used to generate a model which satisfies the observed dimerization data and this gives the ω' and ω torsion angles.

Experimental Section

The 8,2′-*S*-cyclonucleoside 5′-phosphate, 3′-phosphate, and the dimer were synthesized in one of our laboratories. The synthetic details have been reported elsewhere.[14,21,22] All samples were lyophilized three times from 99.8% D_2O and the final solutions were made in 100% D_2O. Final concentrations ranged from 0.01 to 0.02 M and are sufficiently low to minimize the intermolecular effects on the chemical shifts. The pD (pH + 0.4) was measured with a Fisher Accument Model 320 pH meter and were 7.5 and 5.5 for the dimer and monomers, respectively.

Proton magnetic resonance spectra were recorded on a Bruker 270-MHz spectrometer. All the spectra were measured at 20 ± 1 and at 70 ± 1 °C. Tetramethylammonium chloride (TMA) was used as an internal calibrant. The spectra were analyzed using LAOCN III and the NMR parameters are accurate to ±0.005 ppm for chemical shifts and ±0.1 Hz for coupling constants.

Results and Discussion

Assignment of the Spectral Lines. The assignment of the sugar proton resonances (e.g., 1′, 2′, 3′) of the two monomers and the dimer was made by homonuclear decoupling experiments and finally confirmed by the line shape analysis using LAOCN III. Some of the observed and simulated spectra are given in Figures 2 and 3.

The assignment of the base proton H(2) for the 3′ and 5′ nucleotidyl units was made by comparing the shift with the corresponding proton in the monomer, considering the ring current effect and from the model building. The assignment of the 5′ and 5″ protons of the 3′ and 5′ nucleotidyl units of the dimer is made by comparison with the monomers. However, the unambiguous assignment of 5′ and 5″ protons of a nu-

0002-7863/78/1500-4669$01.00/0

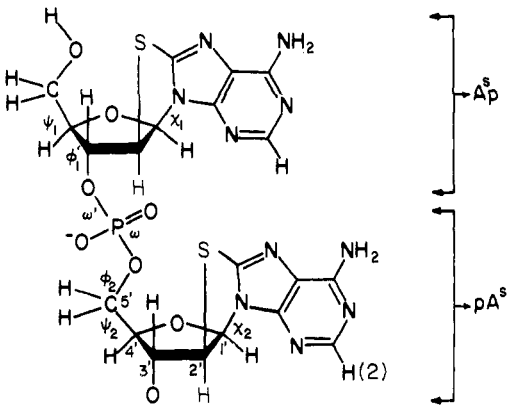

Figure 1. The structure of $A^{s}pA^{S}$, the numbering of the atoms, and the conformational nomenclature for the angles of interest.

cleotidyl unit is not possible. We have assumed that the low-field lines in the ABX-type spectrum arise from H5′ and the high field are due to H5″. The observed chemical shifts and coupling constants are given in Tables I and II.

Conformation of Arabinose Ring. The sugar ring conformation in naturally occurring nucleic acids has been treated as an equilibrium blend of ^2E and ^3E conformers;[4] the population of the ^2E and ^3E conformers is normally computed[23] using the sum $J_{1'2'} + J_{3'4'}$ which is expected to remain constant for this equilibrium since it involves for either sugar pucker a gauche and a trans coupling constant. On the other hand, for the arabinose ring which differs from the ribose in the configuration of 2′-carbon atom, this sum of $J_{1'2'} + J_{3'4'}$ cannot be used to compute the equilibrium populations of ^2E and ^3E conformers. This is due to the fact that the sum $J_{1'2'} + J_{3'4'}$ is variant because ^2E sugar pucker has both gauche couplings while ^3E pucker will have a gauche and a trans coupling. Hence the sum for the ^3E pucker is going to be larger than that for ^2E pucker. Therefore, the displacement of the equilibrium will not entail the constancy of this sum. However, an increment in the sum will be indicative of the increased population of ^3E conformer. The coupling constant $J_{1'2'}$ in either of the puckers is a gauche coupling and hence is restricted to a small range. On the other hand, the magnitude of $J_{3'4'}$ will have a rather large range because in ^2E conformation, $J_{3'4'}$ is expected to be close to 0 ($\phi_{3'4'} \simeq 90°$). While in the ^3E conformer, this coupling constant, being trans, will have a large magnitude, say $\simeq 10$ Hz. A pure conformational state $^3E/^2E$ will be manifested in the large/small value of $J_{3'4'}$, respectively, without any significant change in $J_{1'2'}$. The observed value of $J_{3'4'}$ (Table II) for the monomers suggests that the arabinose ring exists in an equilibrium blend of $^2E \rightleftarrows {}^3E$ with a bias toward ^2E. The existence of equilibrium is confirmed from the increase in $J_{3'4'}$ (Table II) as well as in the sum $J_{1'2'} + J_{3'4'}$ (Table III) on dimerization while $J_{1'2'}$ remains approximately constant. The increase in $J_{3'4'}$ indicates that on dimerization the population of ^3E pucker increases and is very close to 50% compared to approximately 30% in the monomers. The existence of equilibrium between $^2E \rightleftarrows {}^3E$ and the observations of the sensitivity of sugar coupling constants to temperature (Table II) suggest the flexibility of the arabinose ring even under the constraint of cyclization between the base and the sugar ring involving the 2′-carbon atom and the sulfur atom of the adenine base. Since the sugar conformation is interrelated to χ_{CN}, it is apparent that upon dimerization some relative changes in χ_{CN}[4] occur and this is addressed later. This flexibility observed for the fused ring system is contrary to what has been reported for 2′,O^2-cyclouridine[24] and 2′,O^2-cyclocytidine,[25] which are arabinose nucleosides, containing oxygen bridge.

Conformation about C4′–C5′ (ψ_1,ψ_2) and C5′–O5′ (ϕ_2). The conformational preference about C4′–C5′ and C5′–O5′ bonds is manifested in the sum of exocyclic coupling constants $^3J_{H-H}$ and $^3J_{H-P}$, respectively. The populations of the conformers can be estimated using the recent modified expressions developed by Lee and Sarma.[26] The computed populations for the monomer and the dimer are given in Table III. The data in Table III reveal that in monomers the C4′–C5′ bond shows a distinct preference for the gt/tg conformation while the conformation about the C5′–O5′ bond is g′g′. A comparison of the complete molecular framework of the monomer, especially of 5′-phosphate, i.e., high anti ($^2E \rightleftarrows {}^3E$) g/t–g′g′, with that of 5′-AMP anti ($^2E \rightleftarrows {}^3E$) gg–g′g′ suggests that sulfur substitution and cyclization bring about reduction in the gg population. The occurrence of the g/t–g′g′ conformation in the 5′-phosphate monomer may be necessary to overcome the repulsive interaction between the lone pairs on sulfur and the negatively charged phosphate group. A similar situation was noticed in the 8-aza analogues[27] of 5′-AMP and 5′-GMP which show preference for (syn $\rightleftarrows$ anti) ($^2E \rightleftarrows {}^3E$) g/t–g′g′ conformations. The observation of identical conformational parameters (Table III) for 5′ and 3′ phosphates makes one believe that it is not only the repulsive interaction between the lone pair of sulfur atom and the negatively charged phosphate group that is important, but also the interaction between 5′ oxygen and lone pairs on sulfur is responsible for the C4′–C5′ bond showing g/t preferences.

The data for the dimer (Table III) indicate that dimerization brings about a dramatic change in the conformation about the C4′–C5′ bond of the 5′ nucleotidyl unit. The population of gg conformer increases from 24% to 100% while there is a slight increase about 5% for the 3′ nucleotidyl unit. The conformation about C5′–O5′ is still g′g′ but registers an increase of about 18% on dimerization. This conformational change over from g/t–g′g′ in the monomer to gg–g′g′ in the dimer must be accompanied by some other conformational events. It is quite likely that the glycosidic torsion χ_{CN} may have somewhat of a different value in the monomer and the dimer. This point will be highlighted in the discussion of dimerization shifts data. The elevation of temperature does not seem to affect very much the preferences of conformation about C4′–C5′ and C5′–O5′ bonds. The increase of temperature brings about a 22% reduction in the population of gg and a 4% reduction in the population of g′g′ for the dimer while there is no noticeable change for the monomers.

Conformation about C3′–O3′ Bond (ϕ_1'). There are three distinct rotamers possible about this bond; these are the P–O3′ bond trans to C3′–C4′ ($\phi_1' = 180°$, ϕ_-' domain), the P–O3′ bond trans to C3′–C2′ ($\phi_1' = 300°$, ϕ_+' domain), and P–O3′ gauche to both C3′–C4′ and C3′–C2′ (t, $\phi_1' = 60°$). The conformational behavior about this bond is manifested in the $J_{H3'-P3'}$ coupling constant. The H–C3′–O3′–P dihedral angle can be computed from the Karplus equation, i.e., $^3J_{HP} = 18.1 \cos^2\theta\,HP - 4.8 \cos\theta\,HP$ using the observed $J_{H3'-P3'}$ and yields $\theta = \pm 39$ and $\pm 46°$ (Table III) as allowable values for the monomer and the dimer, respectively. These values could be either in ϕ_-' [$\phi_1' = 201$ (monomer) and 194° (dimer)] or in ϕ_+' [$\phi_1' = 279$ (monomer) and 286° (dimer)] domains. Evidence has been presented[4] for the coupling between the sugar pucker and the torsion angle about the C3′–O3′ bond. The ϕ_+' orientation of the 3′-phosphate favors ^2E while ^3E sugar pucker is favored by ϕ_-'. Arguments have been presented elsewhere[28] to exclude the t conformation from this equilibrium. Essentially the equilibrium is between $\phi_+' \rightleftarrows \phi_-'$ conformers. There is no direct method of finding the preference for a particular conformer. However, it is reasonable to conclude from the conformational interrelationships between sugar pucker and ϕ' that in the dimer, for those fractional populations in which sugar pucker is ^3E, $\phi_1' \simeq 194°$, and for those in which sugar pucker is ^2E, $\phi_1' \simeq 286°$.

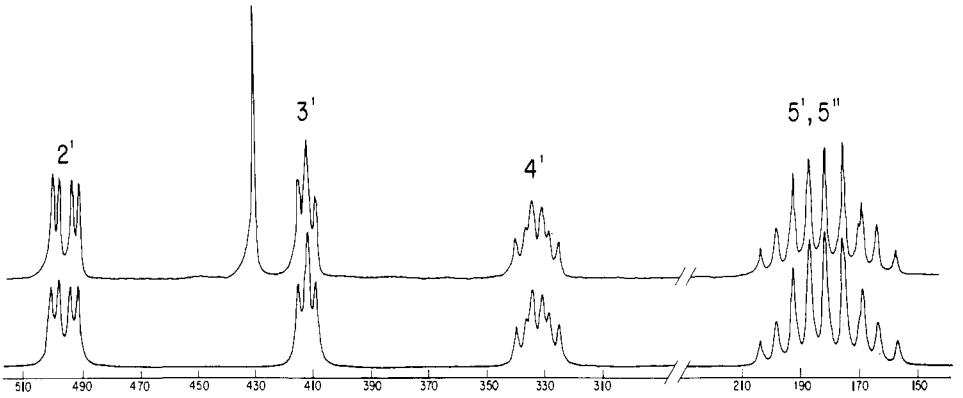

Figure 2. The observed (top) and computer-simulated (bottom) 270-MHz ^{1}H NMR spectra of 8,2'-*S*-cyclonucleoside 5'-phosphate. The chemical shifts are in hertz downfield from internal tetramethylammonium chloride. H1' proton is not shown.

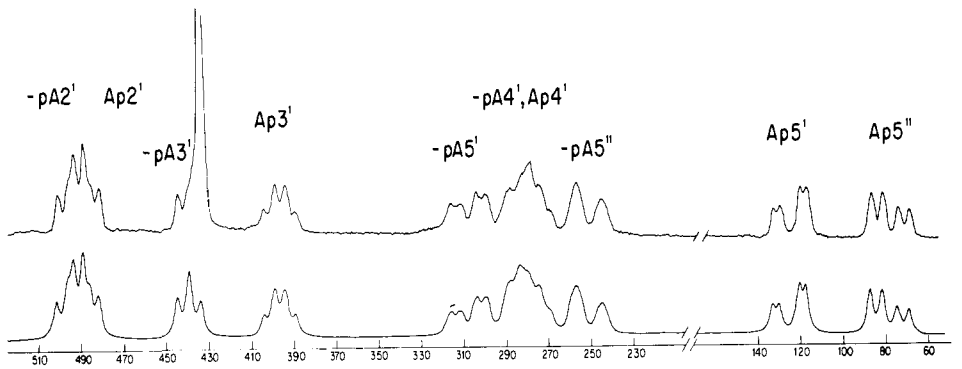

Figure 3. The observed (top) and computer-simulated (bottom) 270-MHz ^{1}H NMR spectra of A^{s}pA^s. The chemical shifts are in hertz downfield from internal tetramethylammonium chloride. H1' protons are not shown.

Table I. Proton Chemical Shifts in Cyclonucleotides

nucleotide		temp, °C	chemical shifts,[a] ppm 1'	2'	3'	4'	5'	5"	2
A^{s}p		20	3.431	1.904	1.680	1.211	0.565	0.439	4.852
		70	3.511	2.028	1.710	1.249	0.558	0.429	4.989
pA^s		20	3.452	1.839	1.528	1.233	0.707	0.633	4.892
		70	3.493	1.839	1.528	1.232	0.724	0.650	4.963
A^{s}pA^s	A^{s}p	20	2.861	1.789	1.452	1.006	0.464	0.295	4.948
	pA^s		3.278	1.815	1.611	1.048	1.121	0.918	4.544
	A^{s}p	70	3.024	1.799	1.494	1.037	0.422	0.263	5.004
	pA^s		3.368	1.841	1.588	1.118	1.074	0.879	4.677

[a] Downfield with respect to TMA.

Table II. Proton–Proton Coupling Constants in Cyclonucleotides

nucleotide		temp, °C	coupling constants, Hz 1'2'	2'3'	3'4'	4'5'	4'5"	3'p	4'p	5'p	5"p	5'5"
A^{s}p		20	6.8	2.8	4.1	3.7	6.1	7.5				−12.9
		70	6.8	2.5	3.5	4.6	6.2	7.3				−12.6
pA^s		20	6.7	2.7	3.3	5.3	6.1			5.8	6.9	−11.5
		70	6.7	2.7	3.2	5.3	6.2			5.8	6.5	−11.6
A^{s}pA^s	A^{s}p	20	7.4	4.0	5.0	3.5	5.8	6.0	1.5			−12.8
	pA^s		7.5	5.0	6.3	1.9	2.1		2.0	5.0	3.5	−12.0
	A^{s}p	70	7.0	3.2	5.0	3.9	5.6	5.7				−12.3
	pA^s		6.8	3.8	6.3	2.2	3.9		2.0	5.5	3.8	−12.4

Glycosidic Torsion (χ_{CN}). The glycosidic torsion in naturally occurring nucleic acid components is usually found to have two ranges, $\chi_{CN} = 0 \pm 90$ and $180 \pm 90°$, referred to as anti and syn, respectively. The cyclization of the base with the sugar fixes this torsion and its magnitude depends on the carbon atom of the sugar which is involved in the cyclization (vide supra). This cyclization is supposed to impart rigidity to the structural framework. In A^{s}pA^s, the cyclization between S(8) of adenine base and carbon 2' of the sugar fixes this angle around 120°. In fact a value of 122° has been reported for 8,2'-*S*-cycloadenosine 3',5'-cyclic phosphate.[17] The range of 110–130° for χ_{CN} is usually referred to as high anti. So far there is no direct experimental determination of χ_{CN} in solution for molecule of this type. However, the sensitivity of the arabinose coupling constant to stacking interactions and hence to χ_{CN} can be used as an index of the relative change in χ_{CN}.

Table III. Population Distribution of Conformers in Cyclonucleotides

		dimer					monomer			
		arabinose ring		backbone			arabinose ring		backbone	
nucleotide		temp, °C	$J_{1'2'} + J_{3'4'}$	% gg[a]	% g'g'[b]	θPH	$J_{1'2'} + J_{3'4'}$	% gg	% g'g'	θPH
A^sp		20					10.9	40		±39
		70					10.3	30		±40
pA^s		20					10.0	24	60	
		70					9.9	23	61	
A^spA^s	A^sp	20	12.4	45		±46				
	pA^s		13.8	100	79					
	A^sp	70	12.0	43		±47				
	pA^s		13.1	78	75					

[a] Computed using the equation gg = (13.7 − Σ)/9.7, Σ = $J_{4'5'} + J_{4'5''}$. [b] Computed using the equation g'g' = (25.0 − Σ')/20.8, Σ' = $J_{5'p} + J_{5''p}$.

Table IV. Dimerization Shifts

			δ(monomer) − δ(dimer), ppm						
nucleotide		temp, °C	1'	2'	3'	4'	5'	5''	2
A^spA^s	Ap^s	20	0.570	0.215	0.227	0.205	0.101	0.145	−0.095
	pA^s		0.174	0.024	−0.083	0.187	−0.414	−0.285	0.347
A^spA^s	Ap^s	70	0.487	0.229	0.216	0.212	0.135	0.166	−0.015
	pA^s		0.125	0.00	−0.061	0.114	−0.350	−0.299	0.285

Table V. The Cylindrical Coordinates[a] ρ_5, ρ_6, and z (Å) for the Various Protons of the 5' Unit in the Dimer along with the Projected and Observed Values for Shielding (ppm)

Protons	ρ_5	ρ_6	z	projected shielding	obsd shielding
H-1'	4.9	3.3	2.5	0.11[b]	0.57[b]
H-2'	3.2	2.5	2.9	0.33	0.215
H-3'	2.5	0.8	5.1	0.47	0.227
H-4'	4.6	3.7	5.3	0.15	0.205
H-5'	5.0	3.5	7.1	0.08	0.101
H-5''	3.4	2.2	7.3	0.13	0.145
H-2	8.9	6.8	2.8	−0.03	−0.095

[a] The torsional angles used are ψ = 60°, ϕ = 180°, C3'-endo, ϕ_1' = 194°, ω' = 265°, ω = 280°, χ_{CN} = 120°. [b] If the assignments for the H1' of the 3'- and 5'-nucleotidyl units were reversed, a great improvement will result in the agreement between projected and observed shieldings of H1'. This has not been done for the following reasons: (1) Very careful homonuclear decoupling experiments agree with the present assignments. (2) The present assignment for H1' is same as proposed by Uesugi et al.[14b] (3) During the LAOCN III simulation, the present as well as the reverse assignments were used. The present ones gave a better fit. (4) A reversal of the assignment for H1', while it provides a better agreement between projected and observed shifts for the 5' unit, becomes adverse with respect to the 3' unit. We have explained the discrepancy between the projected and observed shifts for H1' in the text.

The increase in the population of ^{3}E and the shift from g/t-g'g' to gg-g'g' from monomer to dimer suggests that the glycosidic torsion in the dimer may have a slightly different value than that in the monomer and this will reflect in the chemical shift of H(1') as discussed in the next section.

Conformation about P-O3' (ω') and P-O5' (ω). It has been shown that if individual nucleotidyl units maintain their preferred conformations, the dimer still has the flexibility to exist in a variety of extended and stacked conformations.[29,30] This flexibility is attributed to the free rotations possible about the phosphodiester bonds. Single crystal structural studies[30] and theoretical calculations[31,32] for dinucleoside monophosphates have demonstrated the existence of the conformations with ω' and ω lying in the g^-g^- and g^+g^+ stacked domains. The chemical shift trends of 5' and 5'' protons have been utilized to distinguish between these stacks.[4] The dimerization shifts, i.e., the shift difference between a particular proton in the monomer and that in the dimer, is also utilized to probe the base-base interactions and hence ω' and ω (vide supra).

Since in A^spA^s the χ_{CN} is fixed to $\simeq$120°, the base-base stacking interaction will be governed mainly by ω' and ω. The dimerization shift data in Table IV indicate that all the arabinose protons of the 3' moiety are shifted upfield, the shift being maximum for H(1'). The base proton H(2) experiences a small amount of deshielding. On the other hand, 1', 2', and 4' protons of the 5' unit are shifted upfield while 3', 5', and 5'' are shifted downfield and the base proton H(2) is in a zone of maximum shielding. This pattern of the dimerization shifts is found to be inconsistent with a right-handed base stack usually encountered in naturally occurring oligonucleotides.[4]

A search of ω' and ω in the g^-g^- domain was made to produce a geometry of the dimer which would explain the observed dimerization shift data. For this purpose, the experimentally determined torsion angles ψ_2 (60°), ϕ_2 (180°), and ϕ_1' (194°) were used; the arabinose ring was kept in the ^{3}E conformation and χ_{CN} was kept at 120°. ω' and ω were varied in intervals of 5° to calculate atomic coordinates. The Cartesian coordinates so obtained for a particular set of ω' and ω were then transformed into cylindrical coordinates ρ and z. The cylindrical coordinates were translated into the projected shielding using the ring current field curves of Giessner-Prettre et al.[20] It was observed that the geometry of the dimer corresponding to ω' = 265 ± 5° and ω = 280 ± 5° could reasonably reproduce the observed shieldings for all the protons of the 5' unit except for H(1'). The cylindrical coordinates and the projected and observed shieldings are given in Table V. This set of ω' and ω could also reproduce the maximum shielding for H(2) of the 5' unit. The geometry of the dimer generated is shown in Figure 4a. The magnitudes of the projected shielding in Table V are not identical with those observed. This is entirely expected because of the approximations in the ring current calculations as well as the fact that the calculations neglect contributions to shieldings from parts other than the aromatic systems. We have emphasized elsewhere,[19,33] that calculations of this type provide only information about general trends. For example, the inconsistency in the observed and calculated shielding for H(1') could be possibly due to the following factors: The change of χ_{CN} (vide supra) in the dimer with respect to monomer could be partly responsible for this large upfield shift.[34,35] Further, a change in χ_{CN} will automatically change the orientation of the lone pairs on sulfur atom with respect to the geometry of H(1'). This may have a shielding effect.

Table VI. Experimental Torsional Angles for ApA and $A^sp A^s$ (deg)

torsion angle	ApA[a]	$A^sp A^s$
χ_1	25	120
ψ_1	60	60
ϕ_2	180	180
ϕ_1'	206	194
ω'	285	265
ω	290	280
ψ_2	60	60
χ_2	50	120

[a] Reference 38.

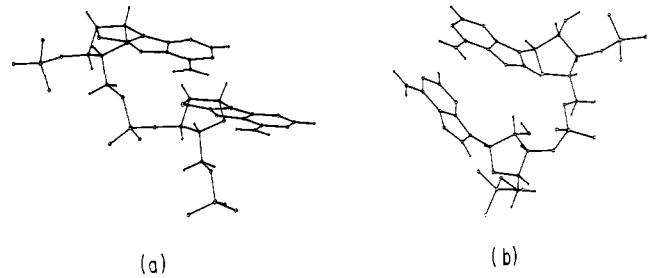

Figure 4. (a) The stacked spatial configuration of $A^sp A^s$ in aqueous solution. The ribose of both the units is ^3E; the various torsion angles are $\chi_1 = 120°, \psi_1 = 60°, \phi_1' = 194°, \phi_2 = 180°, \omega' = 265°, \omega = 280°, \psi_2 = 60°, \chi_2 = 120°$. (b) The stacked spatial configuration of 3′5′ ApA in aqueous solution; the ribose of both the units is ^3E; the various torsion angles are $\chi_1 = 25°, \psi_1 = 60°, \phi_1' = 206°, \phi_2 = 180°, \omega' = 285°, \omega = 290°, \psi_2 = 60°, \chi_2 = 50°$.

Figure 4a shows that the five-membered ring of the 3′ base covers the six-membered ring of the 5′ base and there is significant base–base overlap. In contrast, this base–base overlap in right-handed stacks (observed for dinucleoside monophosphate with χ_{CN} in the anti range, Figure 4b) positions the six-membered ring of the 3′ unit opposite to the five-membered ring of the 5′ unit. The major difference is that the rotation of the 5′ nucleotidyl unit is changed from a counterclockwise (right-handed) to a clockwise (left-handed) fashion in order to accommodate the change of χ_{CN} and to maintain the maximum overlap. It is probably important that the conformation about C4′–C5′ (ψ_2) has to be nearly 100% gg to allow ω' and ω to assume a certain set of values to change the sense of base stacking because the molecule has limited flexibility about χ_{CN}. It is thus obvious that the nature of the base–base stacking is left-handed in $A^sp A^s$. In order to determine quantitatively the sense of the base stack we have computed the following structural criteria: Z = the mean distance between adjacent base planes, Λ = the mean angle between neighboring bases, θ = the cylindrical rotation about the base stacking axis describing the angular displacement of neighboring bases, and η = the angle between the base stacking axis and the helix axis. The observed values were $Z = 3.4$ Å, $\Lambda = 8°$, $\theta = -27°$, and $\eta = 30°$. The important observation is that the ratio θ/Z is negative and such negative ratio is a criteria for left-handed base stacks.[36,37] The base stacking parameters are also in agreement with the theoretical projections of Fujii et al.[18] The final set of backbone conformational angles for the preferred geometry of $A^sp A^s$ is $\psi_2 = 60°$, $\phi_2 = 180°$, $\phi_1' = 194°$, $\omega' = 265$, $\omega = 280°$ and 3′-endo for both arabinose units. These conformational angles, except for ϕ_1', are in good agreement with those predicted by theoretical calculations.[18]

The elevation of temperature reduces the magnitude of the dimerization shifts for H(1′) and H(2). The percentage reduction of dimerization shifts (Table IV) for $A^sp A^s$ is much less compared to that observed for ApA for the same temperature range.[4] This indicates that $A^sp A^s$ at 70 °C is still strongly stacked and is comparatively more stable to thermal changes. The reason for this greater thermal stability is still an open question. This could possibly be because of the limited flexibility about the glycosidic torsion.

In Table VI a summary of the conformational properties of ApA and $A^sp A^S$ is given. The data (Table VI) as well as inspection of Figures 4a and 4b clearly reveal that the major difference between ApA and $A^sp A^s$ is in the magnitude of χ_{CN} with small differences in the magnitudes of ϕ_1', ω', and ω. These differences lead to the existence of ApA in a right-handed stack and $A^sp A^s$ in a left-handed stack.

There is a heated theoretical controversy over the helical organization of the backbone of polycyclonucleotides. Yathindra and Sundaralingam[39] maintain that the backbone in polycyclonucleotides forms left-handed helices while Olson[40] maintains it to be right-handed. Both groups agree that the sense of base stack is left-handed as has been experimentally shown to be true in this paper. The experimental data in the present work alone are insufficient to settle the controversy with respect to the helical organization of the backbone of polycyclonucleotides.

References and Notes

(1) To whom correspondence should be addressed at the State University of New York at Albany.
(2) (a) D. B. Davies and S. S. Danyluk, *Biochemistry*, **13**, 4417 (1974); (b) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1968).
(3) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997 (1969).
(4) C. H. Lee, F. S. Ezra, N. S. Kondo, R. H. Sarma, and S. S. Danyluk, *Biochemistry*, **15**, 3627 (1976).
(5) F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R. H. Sarma, *Biochemistry*, **16**, 1977 (1977).
(6) F. E. Evans and R. H. Sarma, *Nature (London)*, **263**, 5 (1976).
(7) H. Singh, M. H. Herbut, C. H. Lee, and R. H. Sarma, *Biopolymers*, **15**, 2167 (1976).
(8) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 297 (1973).
(9) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 2075 (1973).
(10) D. Perahia, B. Pullman, and A. Saran, *Biochim. Biophys. Acta*, **353**, 16 (1974).
(11) R. Tewari, R. K. Nanda, and G. Govil, *Biopolymers*, **13**, 2015 (1974).
(12) W. K. Olson, *Biopolymers*, **14**, 1775 (1975).
(13) M. Ikehara, *Acc. Chem. Res.*, **2**, 47 (1969).
(14) (a) M. Ikehara, S. Uesugi, and M. Yasumoto, *J. Am. Chem. Soc.*, **92**, 4735 (1970); (b) S. Uesugi, M. Yasumoto, M. Ikehara, K. N. Fang, and P. O. P. Ts'o, *ibid.*, **94**, 548 (1972).
(15) (a) M. Ikehara, S. Uesugi, and J. Yano, *Nature (London), New Biol.*, **240**, 16 (1972); (b) *J. Am. Chem. Soc.*, **96**, 4966 (1974).
(16) S. Uesugi, J. Yano, E. Yano, and M. Ikehara, *J. Am. Chem. Soc.*, **99**, 2313 (1977).
(17) K. Tomita, M. Yoneda, T. Fujiwara, and M. Ikehara, *Acta Crystallogr., Sect. A*, **28**, S45 (1972).
(18) S. Fujii and K. Tomita, *Nucleic Acids Res.*, **3**, 1973 (1976).
(19) D. Cheng and R. H. Sarma, *J. Am. Chem. Soc.*, **99**, 7333 (1977).
(20) C. Giessner-Prettre, B. Pullman, P. N. Borer, K. S/ Kan, and P. O. P. Ts'o, *Biopolymers*, **15**, 2277 (1976).
(21) M. Ikehara and S. Uesugi, *Tetrahedron*, **28**, 3687 (1972).
(22) M. Ikehara, T. Nagura, and E. Ohtsuka, *Nucleic Acids Res.*, **2**, 1345 (1975).
(23) F. E. Evans and R. H. Sarma, *J. Biol. Chem.*, **249**, 4754 (1974).
(24) B. P. Cross and T. Schleich, *Biopolymers*, **12**, 2381 (1973).
(25) C. H. Lee and R. H. Sarma, *J. Am. Chem. Soc.*, **98**, 3541 (1976).
(26) C. H. Lee and R. H. Sarma, *Biochemistry*, **15**, 697 (1976).
(27) C. H. Lee, F. E. Evans, and R. H. Sarma, *J. Biol. Chem.*, **250**, 1290 (1975).
(28) C. H. Lee and R. H. Sarma, *J. Am. Chem. Soc.*, **97**, 1225 (1975).
(29) M. Sundaralingam, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 417 (1973).
(30) S. H. Kim, H. M. Berman, N. C. Seeman, and M. Newton, *Acta Crystallogr., Sect. B*, **29**, 703 (1973).
(31) S. B. Broyde, R. M. Wartell, D. Stellman, B. Hingerty, and R. Langridge, *Biopolymers*, **14**, 1597 (1975).
(32) N. Yatindra and M. Sundaralingam, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3325 (1974).
(33) F. E. Evans and R. H. Sarma, *Biopolymers*, **13**, 2117 (1974).
(34) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **65**, 189 (1977).
(35) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **65**, 171 (1977).
(36) W. K. Olson, *Nucleic Acids Res.*, **2**, 2055 (1975).
(37) W. K. Olson, *Biopolymers*, **15**, 859 (1976).
(38) M. M. Dhingra and R. H. Sarma, *Nature (London)*, in press.
(39) N. Ythindra and M. Sundaralingam, *Nucleic Acids Res.*, **3**, 729 (1976).
(40) W. K. Olson, *J. Am. Chem. Soc.*, **98**, 537 (1976).