Conformational Study of a Dinucleoside Monophosphate in Aqueous Solution using the Lanthanide Probe Method

By **Carlos F. G. C. Geraldes,*** Department of Chemistry, University of Coimbra, 3000 Coimbra, Portugal
Robert J. P. Williams, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR

The conformation of the metal-bound dinucleoside monophosphate adenylyl-3'→5'-adenosine (ApA) in aqueous solution at different pH values and temperatures has been studied using the lanthanide probe method. The conformational analysis, based on a mixture of different conformations in fast exchange within the n.m.r. time scale, agrees well with the results from coupling constants, proton dimerization shifts, and *ab initio* molecular orbital calculations, obtained for the metal-free system. The unstacking process of ApA depends on the temperature and the state of protonation of the adenine bases. In particular the conformational populations about the phosphodiester bonds for the stacked and unstacked forms of ApA show that this part of the molecule has a noticeable preference for the gauche forms *gg* when the adenine bases are unprotonated, and for the *gt,tg* forms in the protonated species. A combination of proton dimerization shift data and lanthanide data indicate a two-state equilibrium for the unstacking process of ApA.

RIBODINUCLEOSIDE monophosphates are the simplest models for the study of the conformational properties of ribonucleic acids. Although the most comprehensive structural information about the intrinsic conformational preferences of the polynucleotide chain has so far been obtained from *X*-ray crystallographic studies,[1-6] only physical studies in solution can assess the degree of conformational rigidity preserved by these systems in a medium which resembles more closely the true biological situation. The structural information available from solution studies, namely from n.m.r. spectroscopy, is however less definitive than in the crystalline state.

The objective of the present work is an extensive ^1H n.m.r. study of the conformation of adenylyl-3'→5'-adenosine (ApA) in aqueous solution using the lanthanide probe method, together with chemical shift, coupling constants, and nuclear Overhauser effect (NOE) studies, as neither is a self-sufficient conformational method for torsionally flexible molecules in solution.[7-9] The effect of temperature and pH on the conformation of ApA is studied, using shift data for all non-exchangeable protons and relaxation data for most of them. Figure 1 depicts the chemical bonds of ApA, labelled conventionally [10] on its framework and the description of the corresponding torsion angles and conformer designations also follows Sundaralingam's convention.[10]

A large number of n.m.r. studies on nucleic acid constituents have already been reported.[11] Conformations of 3'→5' dinucleoside monophosphates in solution have extensively been studied by high-field n.m.r. techniques, such as proton dimerization shifts, ^{31}P chemical shifts, ^1H–^1H, ^{31}P–^1H, and ^{31}P–^{13}C vicinal coupling constants and NOE.[12-25] These recent studies have however not yet reached general agreement on some aspects of the intramolecular stacking process, especially in respect to the conformational state about the ϕ', ω, ω', and χ torsion angles.[15-25]

The lanthanide probe method [26] has been previously applied to conformational studies of various 3'→5' dinucleoside monophosphates in D_2O.[27,28] The study of ApA [27] was based only on shift data for some protons and the 'single family of conformations' derived from their analysis is not consistent with vicinal spin-coupling constants and NOE data.[15,22] Moreover, that study was carried out at a very acidic pH, thus preventing the analysis of the intramolecular base-stacking process. Therefore in this work the lanthanide-induced shift and relaxation data obtained in various solution conditions are analysed in terms of a conformational equilibrium.[28,29]

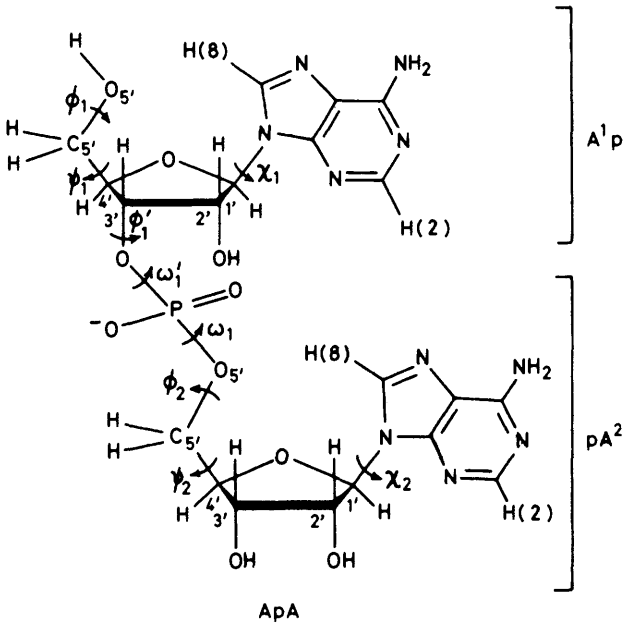


FIGURE 1 Chemical structure, torsion angle notation, and numbering scheme of adenylyl-(3'→5')-adenosine (ApA). The structure is divided in two parts: the A¹p or 3'-nucleotide part and the pA² or 5'-nucleotide part

The shifts of n.m.r. resonances induced by lanthanide ion binding to a specific site on a substrate molecule may result from a through-space dipolar interaction (pseudo-contact shift) and/or a direct delocalization of unpaired electron density from the metal to the nuclei (contact

shift). The pseudo-contact shifts are related to the average dynamic structure of the lanthanide–substrate complex [26] [equation (1) where D_z is a ligand field term,

$$\frac{\Delta\nu}{\nu} = \frac{g^2\beta^2 J(J+1)(2J-1)(2J+3)}{60(KT)^2} D_z \left\langle \frac{3\cos^2\theta_i - 1}{r_i^3} \right\rangle \quad (1)$$

r_i the distance between nucleus i and the metal ion, and θ_i the angle between the metal–nucleus i vector and the principal axis of symmetry of the ligand field]. This equation assumes effective axial symmetry for the complex. By expressing the ligand-induced shifts within a given metal ion–nucleotide complex as ratios, the equation is further simplified to yield the necessary geometrical information.[26]

The contributions of bound Gd^{III} ions, used as relaxation probes, to the measured spin–spin relaxation times (T_{2M}), and the spin–lattice relaxation times (T_{1M}) of the nuclei of metal-bound nucleotides, given by the Solomon–Bloembergen equations,[26] can be calculated as a set of ratios, and give directly the relative distances r of those nuclei to the probe ion in the complexes, if it is assumed that the scalar part of the relaxation equations can be neglected, a condition found to apply for protons [equation (2)].[26]

$$\frac{(1/T_{jM})_i}{(1/T_{jM})_0} = \frac{r_i^{-6}}{r_0^{-6}} \; (j = 1,2) \quad (2)$$

EXPERIMENTAL

ApA was obtained from Sigma Chemical Co. 99.8% D_2O (Norsk Hydro) was used as solvent. NaOD and DCl were obtained from Ciba. The lanthanide(III) oxides were purchased from Koch–Light. The lanthanide(III) chloride solutions were prepared as previously described.[26] ApA was lyophilised from D_2O and then dissolved in either 99.8% D_2O or lanthanide chloride solutions. Final pH adjustments were made using NaOD and DCl.

The ^1H n.m.r. spectra were measured on a 270 MHz Bruker spectrometer operating in the Fourier transform mode using a Nicolet Technology 1085 computer. Proton shifts are given in p.p.m. from sodium 3-trimethylsilyl-[2,2,3,3-$^2H_4$]propionate (TSS) used as an internal standard. The proton spin–lattice relaxation times (T_1) were measured using pulsed Fourier transform techniques (180°–τ–90° sequence).

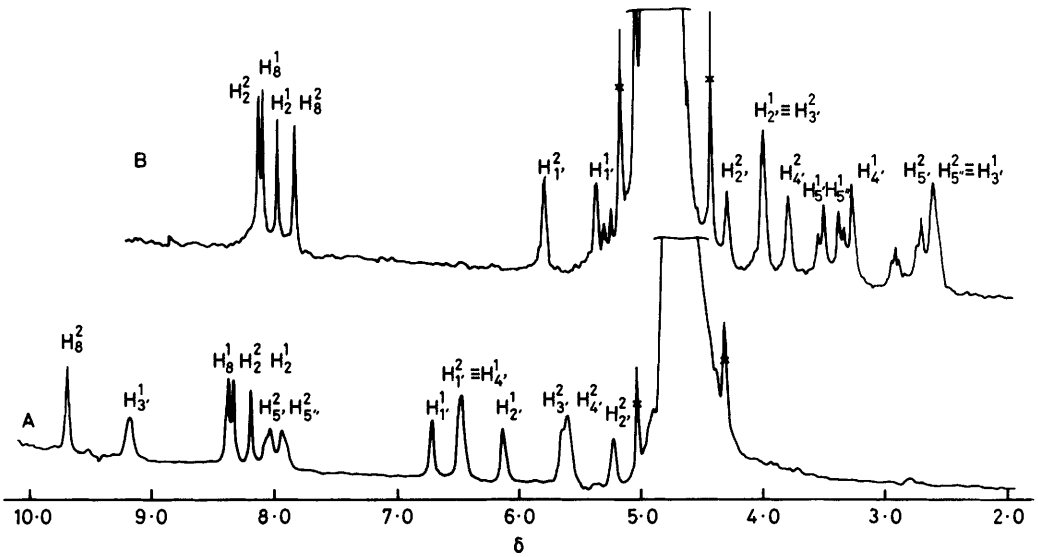


FIGURE 2 270 MHz Fourier transform ^1H n.m.r. spectrum of ApA (6mM, pH 4.3, T 298 K) in the presence of lanthanide ions: (A) $[Pr^{3+}]$ 0.47M; (B) $[Eu^{3+}]$ 0.40M

RESULTS

The ^1H n.m.r. spectrum of ApA in D_2O has been completely assigned.[15,16] Therefore it is possible to study in detail the perturbations induced by addition of different lanthanide(III) cations to ApA in different conditions of temperature and pH.

A, *Shift Studies*.—The conformation of ApA was studied by using the shift probes Eu^{III} and Pr^{III} as aqueous ions at different temperatures and pH values (pH ≤ 5.8, due to hydrolysis of the aqueous ions at higher pH values). Addition of the probes causes the proton resonances of ApA to be shifted to different extents (Figure 2). As usual, the signs of the Pr^{III}- and Eu^{III}-induced shifts on each proton are opposite, due to opposite signs of the D_z terms for these ions in the shift equation.[26] The resultant spectra are sharp, indicating that, in all the experimental conditions used, the exchange between the free and bound nucleotide is fast in the n.m.r. time-scale. From the n.m.r. titrations of ApA with the shift probes, binding curves for all protons were obtained (Figure 3). These curves were fitted to an association constant for formation of 1 : 1 weak complexes between the lanthanide(III) ions and ApA, whose values were K 3.5 l mol^{-1} for both lanthanides at pH 4.3, whereas a value of K 4.0 l mol^{-1} was obtained for Eu^{III} binding at pH 1.8.[27]

The paramagnetic contributions to the observed shifts were obtained by subtracting the diamagnetic shifts induced by La^{III}, and the shift ratios, normalized to H_2^5', were

verified as practically independent of the lanthanide ion concentration. These shift ratios, extrapolated to zero metal concentration, for all the protons and at various temperature and pH conditions, are shown in Table 1. We found that the Eu^{III} and Pr^{III} shift ratios are nearly the same in all experimental conditions used. This is illustrated in Table 1 for pH 4.3 and 298 K. Although for a few protons the differences of the Eu^{III} : Pr^{III} shift ratios are just outside the experimental errors, the usual assumptions that the proton paramagnetic shifts are of pseudo-contact origin and that the ApA–lanthanide complexes have effective axial magnetic symmetry is still a good one.[7,26]

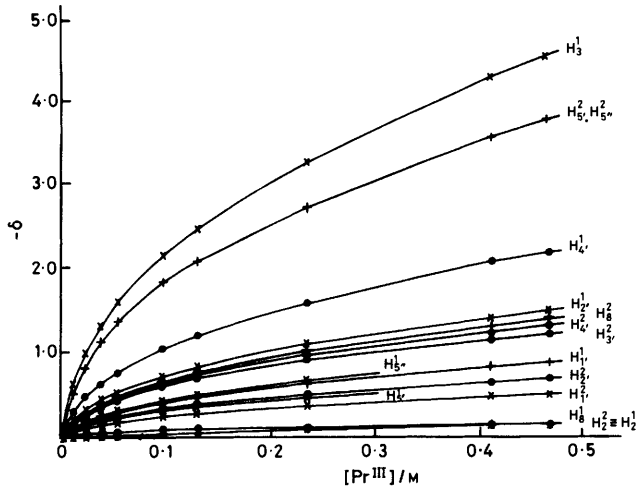


FIGURE 3 Titration curves for ApA protons in the presence of Pr^{3+} (pH 4.3; T 298 K); δ values are ^1H paramagnetic shifts

The effect of temperature on the ApA proton shift ratios with Eu^{III} was also studied at different pH values. This is illustrated in Table 1 and Figure 4 for pH 2.2 and 5.8.

It will be useful to compare the shift ratios for the protons of each nucleotidyl unit of ApA with the same ratios obtained for the protons of the corresponding mononucleotides: Ap-(3′-part of ApA) with 3′-AMP (shift ratios normalized to $H_{3'}$) and -pA (5′-part of ApA) with 5′-AMP (shift ratios normalized to $H_{5'}$). This is shown in Table 2.

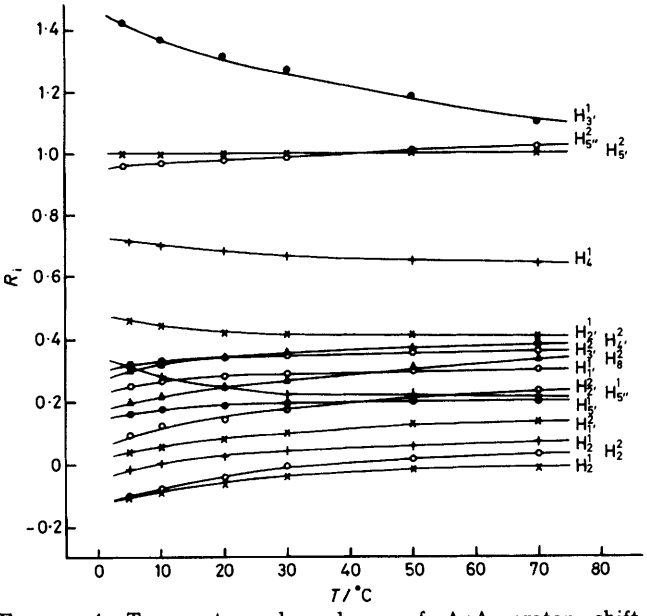


FIGURE 4 Temperature dependence of ApA proton shift ratio (relative to $H^2_{5'} \equiv 1.00$) with Eu^{3+} (pH 5.8; $[Eu^{3+}]$ 20mM)

B, *Relaxation Studies*.—Proton T_1 measurement were carried out in the absence and presence of Gd^{III}. Table 3 shows the diamagnetic spin–lattice relaxation times (T_{1_0}) for some ApA protons, the corresponding values (T_{1_P}) in the presence of the indicated Gd^{III} concentrations (in the range 10^{-5}—10^{-4}M) and the ratios R_i (relative to $H_{5'} = 80$) of the measured paramagnetic contributions to T_1 values (T_{1M}).[26] From these relaxation ratios the distance ratios of the ApA protons to the metal ion in the complexes can easily be calculated. The relaxation experiments require much lower Gd^{III} concentrations than those used in a shift titration with Eu^{III} or Pr^{III}.[26] Therefore we could obtain ApA T_{1M} values at a pH nearer to neutrality (pH 6.5) than for the shift ratios (pH 5.8) because the Gd^{III} concentrations required for the relaxation experiments are low enough to prevent the hydrolysis of the aqueous ion at pH 6.5. However we found no differences in ApA T_{1M} ratios at both pH values.

TABLE 1

Proton shift ratios for ApA (relative to $H_{5'}$ of -pA moiety) with different lanthanide(III) ions in different conditions; [ApA] 6mM

pH	Metal	T/K	Moiety	Shift ratio for proton [c]							
				$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	H_8	H_2
4.3 [a]	Eu	298	-pA	0.12	0.16	0.33	0.33	1.00	1.00	0.33	0.02
			Ap-	0.31	0.38	1.29	0.64	0.17	0.25	0.10	0.02
	Pr	298	-pA	0.14	0.19	0.34	0.37	1.00	1.02	0.36	0.02
			Ap-	0.22	0.40	1.16	0.58	0.17	0.21	0.05	0.02
2.2 [a]	Eu	298	-pA	0.12	0.20	0.30	0.31	1.00	1.03	0.30	0.02
			Ap-	0.34	0.41	1.29	0.70	0.18	0.23	0.10	0.00
		323	-pA	0.12	0.24	0.42	0.40	1.00	1.02	0.32	0.04
			Ap-	0.35	0·42	1.25	0.69	0.20	0.25	0.10	0.01
5.8 [b]	Eu	277	-pA	0.04	0.09	0.31	0.30	1.00	0.96	0.20	−0.10
			Ap-	0.25	0.46	1.42	0.71	0.16	0.32	−0.02	−0.11
		298	-pA	0.08	0.14	0.34	0.34	1.00	0.98	0.25	−0.04
			Ap-	0.28	0.42	1.32	0.68	0.18	0.25	0.02	−0.07
		343	-pA	0.14	0.22	0.36	0.38	1.00	1.03	0.34	0.03
			Ap-	0.30	0.41	1.15	0.65	0.21	0.22	0.07	−0.02

[a] Shift ratios extrapolated to zero metal concentration, obtained from metal titrations. [b] Data at constant Eu^{3+} concentration (20mM). [c] Experimental errors are typically ±0.02.

TABLE 2

Comparison of the Eu^{III} shift ratios[a] of the adenylyl units of ApA with the corresponding mononucleotides 3′-AMP and 5′-AMP

Nucleotide	pH	T/K	Shift ratio for proton $H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	H_8	H_2
Ap–	2.2	298	0.27	0.33	1.00	0.56	0.14	0.18	0.08	0.00
	5.8	277	0.18	0.32	1.00	0.50	0.11	0.23	−0.01	−0.08
	5.8	298	0.21	0.32	1.00	0.52	0.14	0.19	0.02	−0.05
	5.8	343	0.26	0.36	1.00	0.57	0.18	0.19	0.06	−0.02
3′-AMP	2.2	298	0.19	0.24	1.00	0.49	0.13	0.17	0.04	−0.01
	7.6	298	0.18	0.22	1.00	0.49	0.15		0.02	0.00
–pA	2.2	298	0.12	0.20	0.30	0.31	1.00	1.03	0.30	0.02
	5.8	277	0.04	0.09	0.31	0.30	1.00	0.96	0.20	−0.10
	5.8	298	0.08	0.14	0.34	0.34	1.00	0.98	0.25	−0.04
	5.8	343	0.14	0.22	0.36	0.38	1.00	1.03	0.34	0.03
5′-AMP	2.2	298	0.08	0.24	0.38	0.32	1.00	1.00	0.31	−0.02
	7.6	298	0.08	0.29	0.37	0.31	1.00	1.00	0.29	−0.02

[a] Shift ratios for Ap– and 3′-AMP relative to $H_{3'}$; for –pA and 5′-AMP relative to $H_{5'}$.

TABLE 3

Proton relaxation ratios (relative to $H^2_{5'} \equiv 80$) for ApA at different temperatures and pH values. T_1 was measured in seconds; [ApA] 6mM; [Gd^{III}] 3.2×10^{-5}M; reference compound is sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSS)

pH	T/K	Parameter	Value for proton[b] H^2_8	H^2_2	$H^2_{1'}$	$H^2_{3'}$	$H^2_{5',5''}$	H^1_8	H^1_2	$H^1_{1'}$	$H^1_{5',5''}$	Reference compound
2.2	298	T_{10}	0.90	3.47	1.45	0.67	0.30	1.36	3.04	1.30	0.22	2.17
		T_{1p}	0.11	0.61	0.41	0.17	0.03	0.31	0.60	0.21	0.10	1.45
		R_i	21	3	4	11	80	6	3	10	14	
6.5	277	T_{10}	0.39	2.01	1.03	a	0.27	0.72	1.70	0.82	a	1.30
		T_{1p}	0.06	0.56	0.19	a	0.02	0.16	0.33	0.14	a	1.10
		R_i	23	2	6	a	80	8	4	10	a	
6.5	343	T_{10}	2.97	7.30	4.11	a	0.40	2.88	7.31	2.93	a	3.61
		T_{1p}	0.18	0.77	0.53	a	0.05	0.33	1.25	0.33	a	2.29
		R_i	20	4	6	a	80	10	2	10	a	

[a] Not observed. [b] Experimental errors in distance ratios are typically ±0.02.

TABLE 4

Comparison of the proton relaxation ratios,[a] obtained from Gd^{III} T_1 measurements, of the adenylyl units of ApA with the corresponding mononucleotides

Nucleotide	pH	T/K	R_{H_8}	R_{H_2}	$R_{H_{1'}}$	$R_{H_{3'}}$	$R_{H_{2'}}$	$R_{H_{4'}}$	$R_{H_{5'},5''}$
Ap–	2.2	298	6	3	10	b	b	b	14
	6.5	277	8	4	10	b	b	b	b
	6.5	343	10	2	10	b	b	b	b
3′-AMP	2.2	298	4	2	10	15	66	18	14
–pA	2.2	298	53	8	10	b	28	b	200
	6.5	277	35	3	10	b	b	b	133
	6.5	343	33	7	10	b	b	b	133
5′-AMP	2.2	298	75	7	10	12	22	30	156

[a] Ratios relative to the same $H_{1'}$ proton of the dimer residues and the corresponding monomers: $R_{H_i} = (T_{1M^{-1}})_{H_i}/(T_{1M^{-1}})_{H_{1'}}$. [b] Not observed.

It will again be useful to compare the relaxation ratios of each nucleotidyl unit of ApA with the relaxation ratios obtained for the corresponding mononucleotides (Table 4).

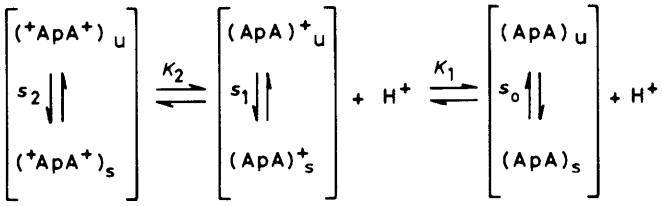
DISCUSSION

It has been shown that ApA has two protonation sites, besides the phosphate diester group, which are the N-1 atoms of the adenine bases. Studies of the effect of ionization upon the base–base stacking properties of ApA using spectrophotometric methods [30] at 298 K and 0.1 allowed the proposal of the Scheme where s and u represent respectively the stacked and unstacked forms of the diprotonated $^+ApA^+$, monoprotonated $(ApA)^+$, and unprotonated ApA forms. The macroscopic ionization constants have values of pK_1 3.89 and pK_2 3.02, although it is uncertain whether the 3′- or 5′-base is protonated first. In the experimental conditions used the stacking equilibrium quotients have the values s_0 1.63 ± 0.61, s_1 1.21 ± 0.36, and s_2 *ca.* 0. Therefore the diprotonated form is fully unstacked in solution. Also, as $s_0 \simeq s_1$, the stacked forms of ApA and $(ApA)^+$ have almost the same stability.

Data at pH = 2.2.—At this pH value and 298 K ApA is almost exclusively (92%) in the diprotonated $^+ApA^+$ form, which has an averaged conformation consisting of a large number of unstacked states, whose major degrees of conformational freedom are the ribose rings, usually defined by the pseudorotational model,[31] and the P–O ester bonds which correspond to the torsional angles ω_1' and ω_1 (Figure 1). We will now describe an

analysis of this conformational equilibrium, based on the lanthanide data, coupling constants, and chemical shifts.

Table 5 summarizes the conformer populations for the ribose rings, backbone (ψ_1, ϕ_1', ϕ_2, and ψ_2 torsion angles) and glycosidic bonds (χ_1 and χ_2 torsional angle) of ApA compared to the corresponding mononucleotides at similar temperatures and at pH values such that the ionization states of monomers and dimer are the same, as obtained from the relevant vicinal coupling constants [17,19,21,32,33] and NOE effects.[22]



SCHEME

In the case of $^+$ApA$^+$ the conformational properties of the dimer fragments are approximately the same as the corresponding nucleotides. This observation gives further support to the validity of the applicability of the method suggested by Altona [23] to calculate the fraction of base-stacked forms from $J_{H_1'H_2'}$ or $J_{H_3'H_4'}$ values for dimer and monomers. Such a calculation gives zero percentage of base stacking for $^+$ApA$^+$. The absence of stacked form for $^+$ApA$^+$ is further supported by our observation of only neglegible dimerization shifts for the $^+$ApA$^+$ protons.

We now look at the lanthanide shift and relaxation data at pH 2.2. Tables 2 and 4 show that these parameters are similar to those of the constituent mononucleotides indicating that corresponding moieties ($^+$Ap with $^+$Ap$^-$ and pA$^+$ with $^-$pA$^+$) have similar conformations. In order to interpret quantitatively the lanthanide data on $^+$ApA$^+$, first of all a computer search for a single conformation which fits the experimental data at pH 2.2 and 298 K was undertaken, using the Burlesk program.[26] Different sets of ribose ring puckers for the two ApA moieties were assumed, using published *X*-ray crystal structure co-ordinates, as previously described.[7,27] The best single geometry (which minimizes the *R* factors [34] for the shift and relaxation data) found was the same as in the previous search [27] which did not use the ribose $H_{2'}$, $H_{3'}$, and $H_{4'}$ proton data. However, the calculated shift and relaxation parameters (these in parentheses, see Table 7) show that this extended single geometry does not fit the new data for some of the ribose ring protons (*e.g.*, $H_{2'}^1$, $H_{3'}^1$, $H_{4'}^1$) giving high *R* factors for shift and relaxation data, as it does not fit the coupling constant data, which indicate the existence of conformational equilibria for the sugar rings and backbone of ApA.

The lanthanide data were then fitted, using a method fully described and justified elsewhere,[7] to a conformational mixture using the conformer populations obtained from the coupling constants analysis (Table 5). The main problem of this procedure [7] is to define the rotational state about the phosphodiester bonds (ω_1' and ω_1 torsion angles) for which no coupling constants data can be obtained. Potential energy calculations have shown that the assumption of an ethane-type torsional barrier for these bonds, resulting in nine phosphate conformations, is inappropriate for dimethyl phosphate and nucleoside monophosphates.[35-39] Kim *et al.*[3] have selected seven basic phosphodiester conformations for dinucleoside monophosphates, by comparing crystal structures and potential energy calculations, defined by (ω_1', ω_1) angles given in Table 6. Their selection contains however exclusively ^3E ribose ring conformations, whereas for six out of these seven (P_3 being the obvious exception) there is no reason to postulate a specific preference for ^3E. On the contrary, the temperature dependence of the ribose couplings for ApA [24] and for $^+$ApA$^+$ (Table 5) point to the existence of a $^3E/^2E$ equilibrium in destacked states with K_{eq} very close to the values for the corresponding monomers.

We therefore calculated for each of these seven phosphodiester (ω_1', ω_1) conformations the absolute ribose proton shifts and relaxations (using the Burlesk program) for all the $^+$ApA$^+$ geometries resulting from the $^3E/^2E$ ribose puckers of the two moieties and the rotamers corresponding to the ψ_1, ϕ_1', ϕ_2, and ψ_2 torsion angles. Again for each (ω_1', ω_1) conformer the absolute shifts and relaxations were averaged using as weights the conformer populations given by the coupling constants analysis (Table 5). Then the data for the seven (ω_1', ω_1) values were again averaged, using individual populations which varied by 10% intervals and had a sum of 100%. For each of these averages the calculated proton shifts and relaxations were normalized relative to H_5^2, and *R* factors between calculated and experimental data were calculated. A statistical analysis of the individual (ω_1', ω_1) conformer populations (average and standard deviation) for the conformational mixtures which gave agreement factors $R < 0.06$ is shown in Table 6. As the standard deviations are quite high, these populations have only a qualitative meaning. Nevertheless, we can conclude from the present analysis that for $^+$ApA$^+$ the total population of the *gg* forms is smaller than *gt* + *tg*, possibly because the phosphodiester geometries for which the protonated adenine bases would be close together (P_3 and A_1) are destabilized by their mutual repulsion. We obtained the following relative populations: $S_3 > S_1 > P_1$. Table 7 describes the average conformation of the $^+$ApA$^+$ backbone which gives the lowest *R* factors for the ribose data. This averaged backbone conformation was then used in a search for the conformation of the adenine bases, assuming one glycosidic angle value for each ribose pucker.[7] We found that in the completely unstacked $^+$ApA$^+$ the adenine base ring of the $^-$pA$^+$ unit is in an *anti*-conformation ($\chi_2 \sim 60$—$120°$) and the $^+$Ap$^-$ unit in a *syn* (χ_1 270°) $\rightleftharpoons$ *anti* (χ_1 30°) equilibrium. An increase of temperature to 323 K causes only minor changes to this conformational equilibrium (see Eu^{3+} data, Table 1).

TABLE 5

Temperature and pH dependence of conformer populations for ApA and the corresponding monomers, from n.m.r. parameters [a]

Unit				Ap–						–pA					
						ϕ_1'									
Nucleotide	pH	T/K	% stacked [e]	% ^3E	%gg	^3E (°)	^2E (°)	ω_1'	χ_1	% ^3E	%gg	%$g'g'$	ω_1	χ_2'	Reference
Monomer	5.4	293		31	82	204	276	Free	s/a	40	77	72	Free	*anti*	16
ApA		277	50 ± 4 (55 ± 4)	70	69	202 [c]	278 [c]	*b*	s/a	69	86	87	*b*	s/a	16, 18, 22, 47
		293	42 ± 4 (48 ± 3)	65	69	203	277 [d]	*b*	s/a	65	79	83	*b*	s/a	
		348	22 ± 6 (26 ± 6)	56	63	207	273	*b*	s/a	53	58	73	*b*	s/a	
Monomer	2.0	303		34	74	204	276	Free	s/a	36	66	77	Free	*anti*	32, 33
		343													
ApA	2.2	293	0	33	67	*b*		*b*	*b*	37	72	71	*b*	*b*	*c*

[a] See refs. 16 and 18 and text for details of calculations of conformer populations from vicinal coupling constants. [b] Not measured. [c] This work: Ap–: $J_{1'2'}$ 5.9, $J_{2'3'}$ 5.2, $J_{3'4'}$ 2.9, $J_{4'5'}$ 3.3, $J_{4'5''}$ 4.0, $J_{5'5''}$ −13.2 Hz, $J_{3'P}$ not observed; pA–: $J_{1'2'}$ 4.8, $J_{2'3'}$ 5.3, $J_{3'4'}$ 4.5, $J_{4'5'}$ 2.8, $J_{4'5''}$ 4.0, $J_{5'5''}$ 12.0, $J_{5'P}$ 4.4, $J_{5''P}$ 4.8 Hz. [d] Values from $^3J_{3'P}$ (100% ± 37°); [16] 71%g^- from ^{31}P n.m.r.; [21] 43%g^-, 20%g^+, 37%t from ^{13}C n.m.r.[19] [e] Values from ref. 18 using equation (3a) with $J_{1'2'}$ (stacked 0; values in parentheses from ref. 18 using equation (3a); other values from ref. 16: 38 ± 2 (293 K), 19 ± 4 (348 K) using equation (3b); 43 ± 5 (293 K), 26 ± 1 (348 K) using equation (3a); 38 (293 K), 20 ± 2 (348 K) using equation (3a) with $J_{1'2'}$ (stacked) = 0.

Data at pH 5.8—6.5.—At these pH values ApA is exclusively in the unprotonated form, which has an average conformation consisting of a temperature-dependent equilibrium between a large number of unstacked states and a yet undefined number of stacked states.[24] Dimerization and base-stacking have large effects on the conformation of ApA, as detected by various n.m.r. methods (Table 5), specially the temperature dependence of vicinal coupling constants: an increase of ribose ^3E conformers, of $g'g'$ (ϕ_2 180° of −pA) and of gg(ψ_1,ψ_2 60° for Ap− and −pA), and a decrease of the glycosidic angles χ_1 and χ_2 to zero (*anti*-region). Similar results have been observed for many dimers [16,17,24] and a trimer [40] and have been interpreted in terms of a highly stable conformational unit of monomers being conserved in dimers, which is similar to the rigid nucleotidyl unit suggested by *X*-ray structure determinations [41] and potential energy calculations.[42]

A method of determining relative proportions of base-stacked and unstacked conformations has been suggested by Altona,[23] assuming that base-stacked conformers contain exclusively ^3E-type ribose rings and that conformations of the dimer fragments in the unstacked states are the same as those of the corresponding monomers. Then, the fraction of base-stacked form p(S), is calculated from observed coupling constants for the monomers (M) and dimer (D) by any of the two equations (3a and b) with $J_{1'2'}$ (stacked) ~0.8 Hz [24] and $J_{3'4'}$ (stacked) ~9.5 Hz.[16] By using the two equations for each nucleotide unit p(S) values are obtained (Table 5). These numbers are of course valid within the limitations of the model, but recent work [24] puts this model on a much firmer basis.

$$p(S) = J_{1'2'}{}^{M} - J_{1'2'}{}^{D}/J_{1'2'}{}^{M} - 0.8 \quad (3a)$$

$$p(S) = J_{3'4'}{}^{M} - J_{3'4'}{}^{D}/J_{3'4'}{}^{M} - 9.5 \quad (3b)$$

TABLE 6

Phosphodiester conformers (ω_1', ω_1 values) [a] and corresponding percentage populations for dinucleoside monophosphates obtained by various methods, compared to our analysis of lanthanide data for ApA (where the percentage populations and their standard deviations, in parentheses, are indicated)

Method (reference)	$P_1(g^+g^-)$	$P_2(tg^-)$	$P_3(g^-g^-)$	$S_1(g^+t)$	$S_3(g^-t)$	$A_1(g^+g^+)$	$A_2(tg^+)$	(gg)	$(gt + tg)$	$S_2(tt)$
Theoretical calculations (36) [b]	1	24	1	11	4	9	14	10	53	37
Theoretical calculations (38) [c]	6	6	17	20	11	23	8	46	45	9
Theoretical calculations (36) [d]	24	1	20	4	6	42	1	88	12	0
X-Ray structures (39)	9	0	10	5	0	71	14	81	19	0
^{31}P shifts (43)								80 ± 10	20 ± 10	0
^{31}P shifts (25)								$60_{(s)} + 15_{(u)}$ [f]	25	0
Lanthanide data [e] pH 2.2	18(10)	0(0)	5(5)	26(10)	44(10)	7(7)	0(0)	30(8)	70(8)	0(0)
Lanthanide data [e] pH 5.8	12(10)	0(0)	30(8)	5(4)	17(8)	36(11)	0(0)	77(8)	23(8)	0(0)
Lanthanide data [e] ($T \geqslant 343$K)										

[a] P_1(90°, 270°); P_2(170°, 270°); P_3(290°, 280°); S_1(110°, 200°); S_3(250°, 160°); A_1(80°, 80°); A_2(180°, 80°); S_2(180°, 180°). [b] Classical empirical. [c] Classical semi-empirical. [d] *Ab initio* molecular orbital calculations (PCILO method); in this case the local minimum energy of the conformer marked as S_3 occurs in a region intermediate between S_3, A_3, and A_2. [e] This work. [f] (s) Stacked, (u) unstacked.

In order to analyse the lanthanide data on ApA in a

TABLE 7

Conformational analysis of ApA from observed and calculated shifts and relaxation ratios (these in parentheses) at pH 2.2 and T 298 K; shift ratios relative to $H_{5'}^2 \equiv 100$, relaxation ratios (in parentheses) relative to $H_{5'}^2 \equiv 80$

	Observed		Calculated			
			Single conformations [a]		Average conformations [b]	
Proton	A¹p−	−pA²	A¹p−	−pA²	A¹p−	−pA²
$H_{1'}$	34(10)	12(4)	32(32)	8(9)	33(3)	16(2)
$H_{2'}$	41	20	18	14	41	24
$H_{3'}$	129	30(11)	104	32(13)	130	28(5)
$H_{4'}$	70	31	67	44	67	33
$H_{5'}$	18(14)	100(80)	19(9)	100(80)	18(4)	100(80)
$H_{5''}$	23(14)	103(80)	20(86)	100(80)	20(7)	103(77)
H_8	10(6)	30(21)	7(6)	29(58)	10(6)	31(18)
H_2	0(3)	2(3)	0(3)	1(9)	2(3)	2(4)
R factors			0.17(0.40)		0.04(0.13)	

[a] A¹p−: ^2E, $\phi_{1'}$ 238°, ψ_1 260°, χ_1 50°, $\omega_{1'}$ 105°; −pA²: ^3E, ϕ_2 182°, ψ_2 192°, χ_2 60°, ω_1 330°. [b] A¹p−: 35% ^3E, $\phi_{1'}$(^3E) 210°, ϕ_1(^3E) 270°, 70%gg, χ_1(^3E) 30°, χ_1(^3E) 270°; −pA²: 35% ^3E, 100%$g'g'$, 70%gg, χ_2(^3E) 60°, χ_2(^3E) 120°; S_3 = 40%; $P_2 = A_2$ = 0%; $P_3 = A_1$ = 10%; $P_1 = S_1$ = 20%.

biologically significant way, we must make sure that lanthanide(III) binding does not affect to any great extent the stacked-unstacked nucleotide distribution in solution and its conformations. Although no n.m.r. study of the effect of La^{III} binding on the ApA coupling constants was undertaken we found no major effect of La^{III} binding upon the dimerization shifts of ApA protons at pH *ca.* 5.0. Also an investigation using the c.d. technique [44] found no major effect of the binding of Eu^{III} on the conformation of ApA in solution, at pH 5.7 with [ApA] 60μM and $[Eu^{3+}]$ 10mM and also at pH 1.

We start by analysing the lanthanide data at high temperature (343 K) where ApA is only 20% stacked. The shift data of Table 3 indicate again that the backbone conformations of the Ap– and –pA moieties of ApA and of the corresponding mononucleotides Ap and pA are very similar, but the relaxation data of Table 5 indicate that the conformations of the adenine bases are different.

By performing on the shift and relaxation ratios (extrapolated to high temperatures, see Figure 4 and Table 8) of ApA a conformational analysis similar to the one described for $^{+}ApA^{+}$, we obtained the conformer populations for the phosphodiester (Table 6) and the other bonds (Table 8) of the unstacked forms of ApA:

TABLE 8

Conformational analysis of ApA from observed and calculated shifts and relaxation ratios (shift ratios relative to $H_{5'}^2 \equiv 100$, relaxation ratios relative to $H_{5'}^2 \equiv 80$); $T \geqslant 343$ K; shift ratios extrapolated to high temperatures (see Figure 4); shift data at pH = 5.8, relaxation data at pH 6.5

	Observed		Calculated average conformation [a]	
Proton	A^1p–	–pA^2	A^1p–	–pA^2
$H_{1'}$	30(10)	14(6)	30(4)	14(2)
$H_{2'}$	41	22	40	25
$H_{3'}$	115	36	115	31
$H_{4'}$	65	38	64	34
$H_{5'}$	21	100(80)	22	100(80)
$H_{5''}$	22	103(80)	25	103(71)
H_8	7(10)	34(20)	9(14)	34(25)
H_2	−2(2)	3(4)	2(2)	2(2)
R factor			0.04 (0.11)	

[a] A^1p–: 45% ^3E, $\phi_1(^3E)$ 210°, $\phi_1(^2E)$ 270°, 70%*gg*, $\chi_1(^3E)$ 330°, $\chi_1(^2E)$ 270°; –pA^2: 45% ^3E, 100%*g'g'*, 70%*gg*, $\chi_2(^3E)$ 30°, $\chi_2(^2E)$ 30°; $P_2 = A_2 = 0\%$; $P_1 = S_1 = 10\%$; $P_3 = S_3 = 20\%$; $A_1 = 40\%$

45% ^3E for both ribose rings, *ca.* 70% *gg* conformers, 100% *g'g'* for the –pA residue, and conformations of the $C_{3'}$–$O_{3'}$ bond dependent on sugar ring conformation [ϕ_1' (^3E) 210°, ϕ_1' (^2E) 270°]. The glycosidic bond torsion angle depends on sugar ring conformation, *viz.* –pA *anti* (χ_2 30° for ^3E and ^2E) and Ap– a mixture of *syn*- and *anti*-conformations (χ_1 27° for ^2E and χ_1 330° for ^3E). As far as the phosphodiester bonds are concerned, in the high-temperature unstacked forms of the dimer the *gg* conformations predominate over *gt* + *tg*, and *tt* is very unstable; the populations are $A_1 \gtrsim P_3 > S_3 \sim P_1$ and very small populations of the other conformers.

We now discuss the low-temperature lanthanide data (Figure 4, Tables 1—4). The observed variation of the ApA proton shift ratios (Figure 4, Table 1) when the temperature is lowered reflects the formation of one or more base-stacked conformations in solution. The proton relaxation ratios do not change as clearly with temperature (Table 3). At 277 K the proton shifts of Ap– and –pA moieties of ApA are no longer similar to the same parameters of the mononucleotides Ap and pA (Tables 2 and 4), again a reflection of ApA base stacking (50—60%).

Dimerization shift studies on dinucleoside monophosphates led to contradictory postulations by different authors [15,16,18] of different numbers and geometries of stacked forms for these systems. A more recent study,[24,29] combining c.d. and n.m.r. methods, and which differentiates between intramolecular stacking shifts and bimolecular association shifts, rules out the existence of left-handed stacks and defines the conformational equilibrium of m_2^6ApU as one between a right-handed single-helical stacked conformation and a set of unstacked conformations. In our study we started by performing calculations of shifts and relaxation ratios for the various models proposed as an independent test of their conclusions.

These are summarized in Table 9. Equilibria II [16] and III [18] are ruled out on the basis of their high relaxation *R* factors, in opposition to equilibria I and IV, which give very small shift and relaxation *R* factors. Equilibrium I [15] considers a three-state equilibrium, as it postulates the existence of two stacked conformations (P_3 and A_1), with the additional constraint that both sugar rings in the dimer possess exclusively ^3E-type geometries. The calculated data for the fully stacked forms of ApA, when compared with the experimental values at 277 K (where only 50—60% of ApA is stacked) give low *R* factors, but there are notable disagreements for some of the shift ratios, specially the base protons. The low-temperature shift and relaxation ratios for ApA can also be explained by equilibrium IV, a two-state equilibrium consisting of a mixture of 50% right-handed helix [P_3; ^3E, g^-, *gg*, χ_1 0°; ^3E, *gg*, *g'g'*, χ_2 0°] and a blend of unstacked forms whose populations of ribose puckers and (ω_1', ω_1) angles are obtained from theoretical work: [38] a sum of equal populations of ^3E ^3E ($A_1 + S_1$), ^3E ^2E ($P_3 + S_1$), ^2E ^3E ($A_1 + A_2 + S_3$), and ^2E ^2E ($A_1 + S_1$). The glycosidic angle values for the open forms are χ_1 (^3E) = χ_2 (^3E) = 20° and χ_1 (^2E) = χ_2 (^2E) = 270° indicating a *syn*/*anti* equilibrium for both glycosyl bonds.[22,47]

Conclusions.—The present study shows that the lanthanide probe method, used together with analysis of coupling constants and chemical shifts, can be a powerful conformational method in solution, even for a molecule with many degrees of conformational freedom like a dinucleoside monophosphate. The approach taken here is one of very few that allow some insight into the conformational properties of nucleic acids in the region of

TABLE 9

Comparison of *observed* shift (pH 5.8) and relaxation (pH 6.5) ratios of ApA at low temperature (277 K) with *calculated* data for various conformational equilibria described in the literature (shift ratios relative to $H_{5'}^2 \equiv 100$, relaxation ratios relative to $H_{5'}^2 \equiv 80$). In equilibria I—III A^1p- has in all cases χ_1 0°, ψ_1 60° (*gg*), pA^2 has ϕ_2 180° (*g'g'*), ^3E ribose pucker, ψ_2 60° (*gg*)

	Observed		Calculated							
			I [a]		II [b]		III [c]		IV [d]	
Proton	A^1p-	$-pA^2$	A^1p-	$-pA^2$	A^1p-	$-pA^2$	A^1p-	$-pA^2$		
$H_{1'}$	25(10)	4(7)	20(5)	8(3)	8(10)	11(3)	14(3)	12	29(6)	13(4)
$H_{2'}$	46	9	67	13	25	8	68	12	59	18
$H_{3'}$	142	31	127	34	126	27	129	36	139	30
$H_{4'}$	71	30	45	30	40	27	47	26	63	28
$H_{5'}$	16	100	38	100(80)	27	100(80)	47	100(80)	12	100
$H_{5''}$	32	96	40	98(68)	38	100(140)	34	101(123)	20	106
H_8	−2(8)	20(23)	7(6)	10(40)	−10(7)	22(43)	8(6)	23(15)	4(7)	26(24)
H_2	−11(4)	−10(2)	14(3)	0(2)	9(2)	2(1)	17(2)	6(1)	3(3)	−8(3)
R factors			0.004(0.03)		0.04(0.27)		0.05(0.15)		0.02(0.03)	

[a] Kondo and Danyluk: [15] 50% conformer with $A^1p-(^3E$ ribose), $\phi_{1'} = 210°(g-)$, $-pA^2(\chi_2 = 0°)$, $(\omega_{1'},\omega_1) = (330°,320°)$ (P_3); 50% conformer differing only in $(\omega_{1'},\omega_1) = (50°,80°)$ (A_1). [b] Lee *et al.*: [16] three conformers with same characteristics as in *a*, differing only in $(\omega_{1'},\omega_1)$ values: 30% P_3, $(\omega_{1'},\omega_1) = (280°,285°)$; 30% A_1, $(\omega_1,\omega_1) = (80°,80°)$; 40% P_1, $(\omega_{1'},\omega_1) = (90°,270°)$. [c] Lee and Tinoco: [18] 40% of conformer with $(\omega_{1'},\omega_1) = (300°,290°)$ (P_3), ^3E pucker of A^1p-, $\phi_1 = 210°$, $\chi_2 = 0°$; 40% of conformer with same characteristics differing in $(\omega_{1'},\omega_1) = (30°,100°)$ (A_1); 20% of conformer with $\phi_{1'} = 260°(g^+)$, ^2E ribose pucker for A^1p- $\chi_2 = 100°$, $(\omega_{1'},\omega_1) = (50°,220°)$ (P_1). [d] See text.

the P–O ester bonds and will be very useful in the study of overall conformations of pyridine nucleotidyl units, as such rings have almost no ring current effects.

The conformations of the P–O ester bonds in the unstacked $^+ApA^+$ ($gg < gt + tg$) and in ApA ($gg > gt + tg$) are different, possibly due to repulsion of the adenine bases in $^+ApA^+$. In relation to the low-temperature data at neutral pH, various conformational models were tested, but the method cannot differentiate between the three-state [15] and the two-state model of Altona.[24] However c.d.[45] and recent ^1H n.m.r. data [24] indicate a two- and not a three-state equilibrium for dinucleoside monophosphates. Assuming this later model to be the true one, we analysed the conformations of the open forms on the basis of published theoretical work [38] and concluded that the conformation of the P–O ester bonds can be summarised by $g^-g^-_{(s)} \rightleftharpoons (gg, gt + tg)_{(u)}$ where the populations of the unstacked forms are $(gt + tg) \gtrsim gg$ ($A_1 \simeq S_1 > P_3 \sim A_2 \sim S_3$), in agreement with recent independent work.[25]

We finally point out that the similarity of the shift ratios for the ApA protons at pH 4.3 (where 30% of ApA is monoprotonated) and at pH 5.8 (where all ApA is unprotonated) indicates that the stacked forms (ApA) (unprotonated) and $(ApA)^+$ (monoprotonated) have similar conformations. Further support for the stacked half-protonated conformation can be found in the similar values, $s_0 \simeq s_1$, of the stacking equilibrium quotients for (ApA) and $(ApA)^+$ [30] which show that the stacked forms of ApA and $(ApA)^+$ have almost the same stability, and in the helical ApA^+ fragment demonstrated by the *X*-ray crystallographic analysis of the Ap $^+ApA^+$ crystals.[46]

C. F. G. C. G. acknowledges the support of an I.N.I.C. grant. R. J. P. W. is a member of the Oxford Enzyme Group.

[2/211] *Received, 5th February*, 1982]

REFERENCES

[1] J. Rubin, T. Brennan, and M. Sundaralingam, *Biochemistry*, 1972, **11**, 3112.
[2] J. L. Sussman, N. C. Seeman, S. H. Kim, and H. M. Berman, *J. Mol. Biol.*, 1972, **66**, 403.
[3] S. H. Kim, A. H. Berman, N. C. Seeman, and M. D. Newton, *Acta Crystallogr.*, 1973, **29B**, 703.
[4] R. O. Day, N. C. Seeman, J. M. Rosenberg, and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 849.
[5] J. M. Rosenberg, N. C. Seeman, J. J. P. Kim, F. L. Suddath, H. B. Nicholas, and A. Rich, *Nature (London)*, 1973, **243**, 150.
[6] B. Hingerthy, E. Subramanian, S. D. Stellman, S. B. Broyde, T. Sato, and R. Langridge, *Biopolymers*, 1975, **14**, 227.
[7] C. F. G. C. Geraldes and R. J. P. Williams, *Eur. J. Biochem.*, 1978, **85**, 463.
[8] F. Inagaki, M. Tasumi, and T. Miyazawa, *Biopolymers*, 1978, **17**, 267.
[9] F. Inagaki and T. Miyazawa, *Prog. NMR Spectrosc.*, 1981, **14**, 67.
[10] M. Sundaralingam, B. Pullman, W. Saeger, V. Sasisekharan, and H. R. Wilson, *Jerusalem Symp. Quantum Chem. Biochem.*, 1973, **5**, 815.
[11] D. B. Davies, *Prog. NMR Spectrosc.*, 1978, **12**, 135.
[12] C. Altona, J. H. Van Boom, J. de Jager, H. J. Koeners, and G. Van Binst, *Nature (London)*, 1974, **247**, 558.
[13] F. E. Evans, L. H. Lee, and R. H. Sarma, *Biochem. Biophys. Res. Commun.*, 1975, **63**, 106.
[14] C. H. Lee, F. E. Evans, and R. H. Sarma, *FEBS Lett.*, 1975, **51**, 73.
[15] N. S. Kondo and S. S. Danyluk, *Biochemistry*, 1976, **15**, 756.
[16] C. H. Lee, F. S. Ezra, N. S. Kondo, R. H. Sarma, and S. S. Danyluk, *Biochemistry*, 1976, **15**, 3627.
[17] F. S. Ezra, C. H. Lee, N. S. Kondo, S. S. Danyluk, and R. H. Sarma, *Biochemistry*, 1977, **16**, 1977.
[18] C. H. Lee and I. Tinoco, Jr., *Biochemistry*, 1977, **16**, 5403.
[19] J. L. Alderfer and P. O. P. T'so, *Biochemistry*, 1977, **16**, 2410.
[20] T. Schleicht, B. P. Cross, and I. C. P. Smith, *Nucleic Acids Res.*, 1976, **3**, 355.
[21] P. J. Cozzone and O. Jardetzky, *Biochemistry*, 1976, **15**, 4860.
[22] P. A. Hart, *Biophys. J.*, 1978, **24**, 833.
[23] C. Altona, 'Structure and Conformations of Nucleic Acid and Protein–Nucleic Acid Interactions,' eds. M. Sundaralingam and S. T. Rao, University Park Press, Baltimore, 1975, p. 613.
[24] C. Altona, A. J. Hartel, C. S. M. Olsthoorn, H. P. H. de Leeuw, and C. A. G. Haasnoot, 'Nuclear Magnetic Resonance

Spectroscopy in Molecular Biology,' ed. B. Pullman, Reidel, Dordrecht, 1978, p. 87.
[25] C. A. G. Haasnoot and C. Altona, *Nucleic Acid Res.*, 1979, **6**, 1135.
[26] C. D. Barry, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 471.
[27] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Biochim. Biophys. Acta*, 1972, **262**, 101.
[28] T. Oida, S. Yokoyama, F. Inagaki, M. Tasumi, and T. Miyazawa, *Nucleic Acid Res. Specl. Pub. No. 2*, 1976, s.47—s.50.
[29] J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, 1963, **85**, 193.
[30] N. Ogasawara and Y. Inoue, *J. Am. Chem. Soc.*, 1976, **98**, 7048, 7054.
[31] C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1973, **95**, 2333.
[32] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1975, **14**, 543.
[33] R. H. Sarma, C.-H. Lee, F. E. Evans, N. Yathindra, and M. Sundaralingam, *J. Am. Chem. Soc.*, 1974, **96**, 7337.
[34] M. R. Willcott, R. E. Lenkinski, and R. E. Davis, *J. Am. Chem. Soc.*, 1972, **94**, 1742.
[35] W. K. Olson and P. J. Flory, *Biopolymers*, 1972, **11**, 1, 25, 57.
[36] D. Perahia, B. Pullman, and A. Saran, *Biochim. Biophys. Acta*, 1974, **340**, 299; 1974, **353**, 16.
[37] R. Tewari, R. K. Nanda, and G. Govil, *J. Theor. Biol.*, 1974, **46**, 229.
[38] N. Yathindra and M. Sundaralingam, *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 3325; N. Yathindra and M. Sundaralingam, ref. 23, p. 649.
[39] W. K. Olson, *Biopolymers*, 1975, **14**, 1775.
[40] F. E. Evans and R. H. Sarma, *Nature* (*London*), 1976, **263**, 567.
[41] M. Sundaralingam, *Jerusalem Symp. Quantum Chem. Biochem.*, 1973, **5**, 417.
[42] N. Yathindra and M. Sundaralingam, *Biopolymers*, 1973, **12**, 297.
[43] D. G. Gorenstein, J. B. Findlay, R. K. Mommi, B. A. Luxon, and D. Kar, *Biochemistry*, 1976, **15**, 3796.
[44] P. Bayley and P. Debenham, *Eur. J. Biochem.*, 1974, **43**, 561.
[45] J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, 1967, **25**, 481; J. T. Powell, E. G. Richards, and W. B. Gratzer, *Biopolymers*, 1972, **11**, 235.
[46] D. Suck, P. C. Manor, G. Germain, C. H. Schwalbe, G. Weimann, and W. Saeger, *Nature* (*London*), *New Biol.*, 1973, **246**, 161; W. Saenger, J. Riecke, and D. Suck, *J. Mol. Biol.*, 1975, **93**, 529.
[47] C. Chachaty, B. Perly, A. Forchioni, and G. Langlet, *Biopolymers*, 1980, **19**, 1211.