Probing the Interrelation between the Glycosyl Torsion, Sugar Pucker, and the Backbone Conformation in C(8) Substituted Adenine Nucleotides by ¹H and ¹H-{³¹P} Fast Fourier Transform Nuclear Magnetic Resonance Methods and Conformational Energy Calculations

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Abstract: The conformations of the C-8 substituted 5'-nucleotides, 8-Br-5'-AMP and 8-CH₃S-5'-AMP, have been investigated by ¹H and ¹H-{³¹P} fast Fourier transform nuclear magnetic resonance methods and semiempirical conformational energy calculations. The results are compared with the nucleosides adenosine and 8-bromoadenosine and also with the nucleotide 5'-AMP at different pH's. It is found that the bulky substituents at C(8) destabilize the normal anti orientation of the base in favor of the syn. This change about the glycosyl C(1')–N(9) bond orientation is accompanied by a distortion in the sugar–phosphate backbone conformation about the C(4')–C(5') bond from the normal gauche–gauche (gg) to the gauche-trans (gt) or trans-gauche (tg). In poly(8-Br-A) the syn-tg conformation is destabilized by the electrostatic repulsion between the adjacent phosphate groups favoring the syn-gt. These results contrast sharply with the preferred anti-gg conformation found in 5'-AMP indicating a striking correlation between the orientation of the base and the backbone conformation. The two major conformational combinations for purine nucleotides are (i) anti-(²E ⇌ ³E)-gg-g'g' and (ii) syn-(²E ⇌ ³E)-gt-g'g'. In i the sugar pucker shows a 2:1 preference for the ²E while in ii there is only a slight bias toward ²E. (The g'g' refers to the conformation about the C(5')–O(5') bond in relation to the C(5') methylene protons and it is synonymous with the trans conformation for the backbone C(4')–C(5')–O(5')–P.) The conformational equilibrium is slightly affected by the base protonation and the phosphate ionization. For the nucleoside adenosine at pH 8.0, the anti conformation is favored, while the syn conformation is favored for 8-bromoadenosine. In both cases the C(4')–(C5') bond conformation shows about 75% preference for the gauche–gauche indicating the presence of an intramolecular hydrogen bond between O(5')–H and the ring nitrogen N(3) in 8-bromoadenosine. Protonation of N(1) of the base at pH 2.0 tends to weaken the hydrogen bond with a consequent decrease in the gg population. These results suggest that there is a mutual interrelation between the sugar pucker and the torsions about the glycosyl and the exocylic C(4')–C(5') bonds.

In recent years considerable information has been gained on the stereochemical aspects of nucleotides, the building blocks of nucleic acids. Mutual interactions between the base and the sugar–phosphate backbone restrict the number of preferred conformations for the nucleotide to essentially two basic conformations differing mainly in the sugar ring pucker.[2,3] Two parameters which show pronounced conformational constraint in common 5'-nucleotides, in sharp contrast to nucleosides, are the glycosyl and the exocyclic C(4')–C(5') bond conformations. X-Ray studies on 5'-nucleotides have shown that the anti orientation for the base and the gauche–gauche[4] conformation[5] about the backbone C(4')–C(5') bond are strongly favored.[2a] On the other hand, the corresponding nucleosides show greater flexibility,[2b,3] and exhibit the syn conformation in addition to anti for the base, particularly for the purine nucleosides, and also the three staggered conformations about the C(4')–C(5') bond. It has also been recognized[6] that in going from the anti to the syn conformation in nucleotides, the backbone conformation about the C(4')–C(5') bond would change from the normal gg to either gt or tg, further suggesting a correlation (syn-gt or syn-tg) between the orientations of the base and the backbone. These deductions have been substantiated by mapping the conformational energies as a function of the rotations about the side chain glycosyl and the backbone C(4')–C(5') bonds in 5'-AMP.[7] More recently this procedure of mapping the base-phosphate interactions has been extended to pyrimidine[8] and other purine nucleotides.[9,10] Nmr studies[11-16] carried out on 5'-nucleotides have also shown that the anti-gg confor-

mational combination is strongly favored in solution as well.

The syn conformation for the base in a nucleotide may be induced by introducing a bulky substitution at the C(8) position of a purine base[17] like Br or CH₃S. It is anticipated that unfavorable steric and electrostatic interactions between the substituents and the ribose unit would constrain the base orientation to the syn range which in turn may affect the sugar–phosphate conformation. In order to gain information on the conformational distortions produced by the C(8) substituents, we have carried out both nuclear magnetic resonance investigation and semiempirical potential energy calculations on 8-Br-5'-AMP and 8-CH₃S-5'-AMP (Figure 1). Investigations have also been extended to 5'-AMP and the nucleosides adenosine and 8-Br-A to compare them with the results obtained on the corresponding substituted nucleotides.

Methods

Experimental. The ¹H nmr spectra were obtained at 100 MHz on a Varian HA 100D spectrometer interfaced to a Digilab FTS-3 Fourier transform data system. The frequencies for the observing channel (¹H, ¹³C, ³¹P, etc.) and lock channel (¹⁹F) were derived from a Digilab 10-94 frequency synthesizer and a Digilab 400-2 pulser. Hexaflurobenzene in a 1-mm capillary provided the signal for the ¹⁹F lock. The internal reference was tetramethylammonium chloride. The sample temperature was 30.5°. The spectra in which the phosphorus nuclei were decoupled were recorded with irradiation derived from a Digilab 50-80 PD plug-in amplifier.
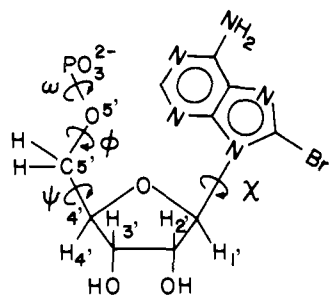
Figure 1. Schematic representation of the chemical structure of 8-Br-5'-AMP. Replacing bromine by CH₃S- would result in 8-CH₃S-5'-AMP. The definition and notations of the torsion angles are the same as given in ref 2a.

The nucleotides, 5'-AMP, 8-Br-5'-AMP, 8-CH₃S-5'-AMP, and the nucleosides, 8-Br-A and adenosine, were commercial preparations. The concentrations employed were such that no significant intermolecular interaction existed at that concentration. The various samples were lyophilized two times from 99.8% D_2O and the spectra were taken in commercial "100%" D_2O at pH values 8.0, 5.0, and 2.0 for nucleotides. At pH 8.0, the phosphate group is a dianion; at 5.0 it is a monoanion; at pH 2.0, the N(1) of the purine moiety will carry a formal positive charge. The ^1H nmr data at the various pH values should enable the determination of the effect of phosphate ionization and N(1) protonation on the conformation of this class of molecules. In the case of the nucleosides adenosine and 8-Br-A, the pH employed was 8.0 and 2.0.

The spectra of nucleosides and nucleotides obtained at different pH values were analyzed by a UNIVAC 1108 computer using a LACOON III program. The line shapes of the spectra were simulated from the derived data using a program developed by one of us (C.-H. L.). Usually, the ^1H-{^{31}P} spectra of a particular nucleotide were simulated first. Once excellent agreement is reached between the observed and simulated ^1H-{^{31}P} spectra, then the undecoupled spectra were simulated.

Theoretical. The spatial relationship of the base with respect to the sugar-phosphate moiety can be elegantly described by the (χ, ψ) energy surface as detailed elsewhere.[7] Consequently, approximate potential energies have been computed by simultaneous variation of the rotations χ and ψ for the above nucleotide analogs taking into account the contributions from van der Waals, electrostatic, and torsional interactions. As in previous cases,[7] the torsion angle ϕ about the backbone C(5')-O(5') bond has been kept fixed at the energetically preferred trans conformation (ϕ = 180°) and the phosphate oxygens are staggered with respect to the C(5') atom. The nature of the potential functions and the parameters used here are the same as reported earlier.[7] The partial electronic charges for the various atoms in these substituted bases have been taken to be the sum of the σ and π charges. The σ charges have been computed by the Del Re[18] method while the π charges have been computed following the HMO approach using the parameters given by Berthod and Pullman[19] and Renugopalakrishnan, et al.[20] These values computed for the substituted bases are given in Table I. Partial charges for the sugar-phosphate chain and for the N(1) protonated and unprotonated forms of adenine have been taken from the literature.[20]

The two primary modes of sugar puckering, namely, the C(2')-endo and the C(3')-endo, have been considered. The structural parameters that are adopted for the sugar and the base are the same as in the earlier work.[7,21,22] The structural parameters involving the bromine atom and the CH₃S- group have been taken from the crystal structures of 8-Br-A[23] and N^6-(Δ^2-isopentenyl)-2-methylthioadenos-

ine,[24] respectively. Calculations have been performed for both the N(1) protonated and unprotonated adenine rings and with the phosphate in the monoanionic and dianionic states. The computations have also been extended to 5'-AMP since no such data are available to assess the effects of N(1) protonation or the ionization of the phosphate group. In order to compare these results with the corresponding nucleosides, calculations have also been carried out on adenosine and 8-Br-A.

Results

The experimental and computer simulated ^1H and ^1H-{^{31}P} spectra of 8-Br-5'-AMP and 8-CH₃S-5'-AMP at pH 8.0 are illustrated in Figures 2 and 3. It can be seen that excellent agreement exists between the observed and simulated spectra. Similar agreement has been achieved in all the other cases between the simulated and observed spectra obtained at different pH values. The nmr parameters computed for the various compounds are given in Table II.

(a) Estimation of Conformer Population about the C(5')-O(5') Bond from $\Sigma'(^3J_{P-5'} + ^3J_{P-5''})$. X-Ray studies on 5'-nucleotides and dinucleosides have shown[4] that the preferred conformation (ϕ) about the C(5')-O(5') bond is in the trans range (C(4')-C(5')-O(5')-P). Potential energy calculations on 5'-nucleotides have also indicated that the gauche conformations (±60°) are energetically unlikely and that the trans conformation is the most stable conformer.[7] Since the system H-C-O-P in 5'-nucleotides shows an angular dependence of spin-spin coupling, information in this regard can be obtained from the magnitude of the coupling constants due to coupling between ^{31}P and H(5') and H(5'').[25] The coupling constant corresponding to the gauche (g'g')[26] (Figure 4a) conformation (ϕ = 180°) is in the range of 3 ± 2 Hz and that corresponding to the trans (g't')[26] (ϕ = 60°) is in the range of 21 ± 3 Hz.[27-32] Unfortunately, an absolute distinction between the two methylene 5' and 5'' hydrogens cannot be made. Consequently, one cannot distinguish by nmr between g't' (Figure 4b) (ϕ = +60°) and t'g' (Figure 4c) (ϕ = −60°) populations, as has been pointed out earlier by Hruska and coworkers.[33-36] It has further been shown by Hruska, et al.,[13] that one could discuss the C(5')-O(5') conformer populations in terms of the sum $\Sigma'(^3J_{P-5'} + ^3J_{P-5''})$ without any consideration of the assignments of the C(5') hydrogens because Σ' reflects the relative contribution of the g'g' form and the combined contribution from g't' and t'g' populations hereafter referred to as (g'/t'). Any perturbation resulting in an increase in the g'g' conformer population should cause a reduction in the magnitude of Σ' whereas an increase in the g't' population, at the expense of g'g', should result in an increase in the value of Σ'. It has been shown elsewhere[11-13] that the population of g'g' and the combined population of g't' + t'g' (g'/t') conformers can be computed from expressions 1 and 2, respectively. From the Σ' values compiled in

$$\text{percentage population of } g'g' = (24 - \Sigma')100/18 \tag{1}$$

$$\text{percentage population of } g'/t' = 100 - \% \, g'g' \tag{2}$$

Table II we have computed the percentage populations of g'g' and g't' conformers for 5'-AMP, 8-Br-5'-AMP, and 8-CH₃-S-5'-AMP at pH values 8.0, 5.0, and 2.0, and the data are presented in Table III. The results indicate that in all the 5'-nucleotides examined the g'g' conformer strongly predominates over the g'/t'. This observation is in agreement with the solid state[2,3] and the theoretical studies on 5'-mononucleotides.[7] These results are also compatible with the recent nmr findings in pyridine coenzymes[35-37] and related systems.[38] The data in Table III also show that the

Table I. The σ and π Charges for the Modified Adenine Bases

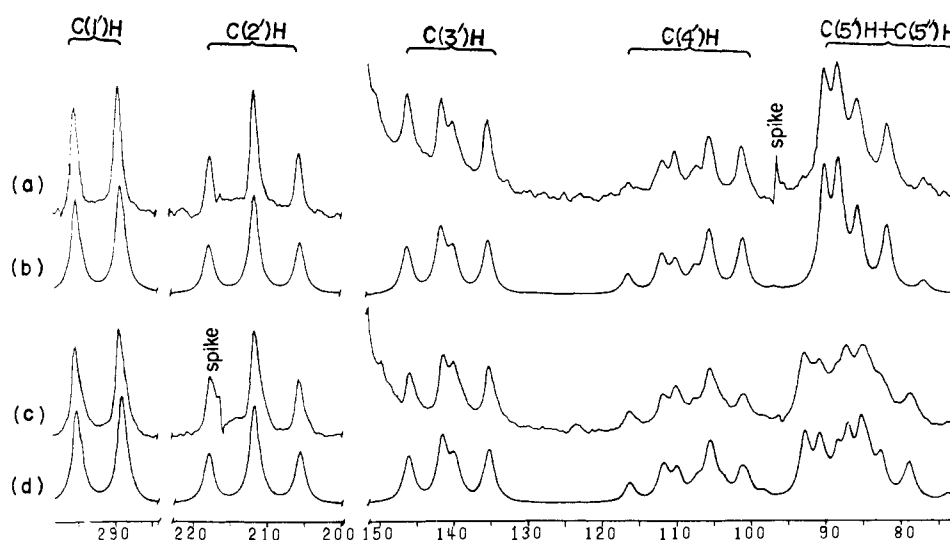| —————8-Bromoadenine—————			N(1) protonated 8-bromoadenine			————8-CH₃S-Ad[a]————			N(1) protonated 8-CH₃S-Ad[a]		
Atom	σ	π	Atom	σ	π	Atom	σ	π	Atom	σ	π
N(1)	−0.283	−0.244	N(1)	−0.453	−0.670	N(1)	−0.283	−0.243	N(1)	−0.453	−0.669
C(2)	0.202	0.107	C(2)	0.191	0.246	C(2)	0.202	0.108	C(2)	0.191	0.248
N(3)	−0.281	−0.233	N(3)	−0.283	−0.224	N(3)	−0.281	−0.233	N(3)	−0.281	−0.223
C(4)	0.241	0.072	C(4)	0.241	0.133	C(4)	0.242	0.073	C(4)	0.242	0.134
C(5)	0.157	−0.063	C(5)	0.157	0.067	C(5)	0.167	−0.063	C(5)	0.167	−0.067
C(6)	−0.204	0.145	C(6)	0.196	0.306	C(6)	0.204	0.147	C(6)	0.196	0.308
N(6)	−0.511	0.105	N(6)	−0.511	0.138	N(6)	−0.511	0.105	N(6)	−0.511	0.138
N(7)	−0.242	−0.277	N(7)	−0.242	−0.268	N(7)	−0.280	−0.262	N(7)	−0.280	−0.253
C(8)	0.342	0.130	C(8)	0.342	0.131	C(8)	0.207	0.122	C(8)	0.207	0.122
N(9)	−0.239	0.337	N(9)	−0.239	0.347	N(9)	−0.275	0.339	N(9)	−0.275	0.349
Br	−0.219	0.016	Br	−0.219	0.016	C(8′)	−0.128		C(8′)	−0.128	
H(2)	0.070		H(1)	0.197		S(8)	−0.035		S(8)	−0.035	
H(6)	0.224		H(2)	0.070		H(8)	0.055		H(8)	0.055	
H(6′)	0.224		H(6)	0.224		H(8′)	0.055		H(8′)	0.055	
			H(6′)	0.224		H(8″)	0.055		H(8″)	0.055	
						H(2)	0.070		H(1)	0.197	
						H(6)	0.224		H(2)	0.070	
						H(6′)	0.224		H(6)	0.224	
									H(6′)	0.224	

[a] Ad = adenine.

Figure 2. (a) The observed ^{1}H-$\{^{31}$P$\}$ 100 MHz fast Fourier transformed nmr spectrum of 8-Br-5′-AMP (0.01 M, pH 8.0, temperature 30.5°). The spikes identified in the figure are data system artifacts. (b) The computer simulated spectrum of 8-Br-5′-AMP under conditions in (a). The agreement between spectra (a) and (b) is excellent. (c) The observed ^{1}H 100 MHz fast Fourier transformed nmr spectrum of 8-Br-5′-AMP under the same conditions as in (a). (d) The computer simulated spectrum of 8-Br-5′-AMP under conditions as in (c). The agreement between spectra (c) and (d) is excellent.

conformer population about C(5′)-O(5′) is not significantly altered whether the phosphate is a dianion (pH 8.0) or a monoanion (pH 5.0) and whether the N(1) of the base is protonated or not. The data further indicate that substituents such as Br and CH₃S in the 8 position of purine slightly reduce the g′g′ conformer population. Theoretical potential energy calculations also indicate that the g′t′ and t′g′ become slightly more important only when the base is in syn orientation and that the g′g′ is overwhelmingly favored for the anti base.

(b) **Estimation of the Conformer Population about the C(4′)-C(5′) Bond from** $\Sigma(^3J_{4'5'} + {}^3J_{4'5''})$. The Newman projections shown in Figure 5 illustrate the three possible conformations about the C(4′)-C(5′) bond in nucleosides and nucleotides. As discussed in section a, while ^{1}H nmr methods can evaluate the contribution from the gauche-gauche (gg) conformer, they can only give the combined contribution from the gauche-trans (gt) and trans-gauche (tg) conformers since an unambiguous assignment of the two C(5′) protons of a 5′-mononucleotide cannot be made at present. As shown elsewhere,[11-13] expressions 3 and 4 yield the per-

percentage populations of gg $= (13 - \Sigma)100/10$ (3)

percentage population of g/t $= 100 - \%$ gg (4)

centage populations of the gg and the combined contribution (g/t) from gt and tg conformers. In both expressions, Σ stands for $^3J_{4'5'} + {}^3J_{4'5''}$. From the Σ values compiled in Table II, the calculated percentage populations of gg and g/t rotamers in the nucleosides adenosine and 8-bromoadenosine at pH values 8.0 and 2.0 are compiled in Table III along with such data for the nucleotides 5′-AMP, 8-Br-5′-AMP, and 8-CH₃S-5′-AMP computed at pH values 8.0, 5.0, and 2.0. It can be seen that the nucleosides, adenosine and 8-bromoadenosine, and the nucleotide, 5′AMP, at pH 8.0 show pronounced preference for the gg conformation.

On the other hand, in the case of 8-Br-5′-AMP and 8-CH₃S-5′-AMP, on a time-average basis, nearly 80-85% of the conformer population is associated with the g/t conformer in dramatic contrast to those found in all the known purine 5′-nucleotides.[11] Interestingly, the data in Table III also show that both 8-Br-5′-AMP and 8-CH₃S-5′-AMP

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Table II. Nmr Parameters for Adenosine, 8-Bromoadenosine, 5'-AMP, 8-Br-5'-AMP, and 8-CH₃S-5'-AMP[a]

	Adenosine		8-Br-A		5'-AMP		8-Br-5'-AMP				8-CH₃S-5'-AMP		
pH	8.0	2.0	8.0	2.0	8.0	5.5	2.0	8.0	5.0	2.0	8.0	5.0	2.0
Concn, M	0.02	0.02	0.01	0.01	0.005	0.015	0.01	0.01	0.01	0.01	0.05	0.05	0.05
$\delta_{1'}$	2.868	2.976	2.960	2.987	2.950	2.940	3.027	2.922	2.924	2.984	2.837	2.824	2.831
$\delta_{2'}$	1.600	1.597	1.876	1.929	1.621	1.564	1.602	2.119	2.009	2.089	1.924	1.886	1.861
$\delta_{3'}$	1.240	1.263	1.307	1.363	1.327	1.316	1.344	1.409	1.431	1.522	1.332	1.339	1.377
$\delta_{4'}$	1.105	1.098	1.936	1.078	1.179	1.200	1.225	1.083	1.102	1.135	1.076	1.092	1.115
$\delta_{5'}$	0.728	0.736	0.749	0.738	0.836	0.943	0.976	0.91	1.012	1.011	0.911	1.014	1.032
$\delta_{5''}$	0.649	0.667	0.673	0.688	0.806	0.943	0.976	0.828	0.950	0.977	0.845	0.973	0.993
δ_2	4.98	5.26	5.01	5.21	5.08	5.05	5.28	5.03	5.00	5.24	4.92	4.92	5.17
δ_8	5.10	5.36			5.44	5.29	5.46						
δ_{CH_3}											−0.47	−0.48	−0.43
$J_{1'-2'}$	6.2	5.4	7.1	6.0	6.0	5.7	5.2	6.1	6.0	5.1	6.25	6.2	5.8
$J_{2'-3'}$	5.2	5.3	5.4	5.7	5.1	5.2	5.2	6.3	6.1	5.7	6.25	6.2	5.8
$J_{3'-4'}$	3.5	4.3	2.4	3.8	3.5	3.7	3.8	4.8	4.5	4.7	4.6	4.5	4.5
$J_{4'-5'}$	2.8	3.1	2.5	3.2	3.9	3.1	3.2	4.8	4.7	4.8	5.1	5.0	5.0
$J_{4'-5''}$	3.6	4.4	3.1	4.5	2.6	3.1	3.2	6.3	5.3	4.8	6.2	5.4	4.5
$J_{4'-5'} + J_{4'-5''}$	6.4	7.5	5.6	7.7	6.5	6.2	6.4	11.1	10.0	9.6	11.3	10.4	9.5
$J_{4'-P}$					1.7	2.0	2.1	0.2	0.8	0.6	0.0	0.0	0.0
$J_{5'-5''}$		−12.6	−12.6	−12.6	−11.8	−12.0	−12.0	−11.4	−15.0	−11.4	−11.5	−14.0	−14.0
$J_{5''-P}$					4.6	5.0	5.1	6.0	6.2	6.0	6.2	5.8	5.8
$J_{5'-P} +$					4.6	5.0	5.1	5.6	6.2	5.6	6.0	5.8	5.8
$J_{5''-P}$					9.2	10.0	10.2	11.6	12.4	11.6	12.2	11.6	11.6

[a] Temperature 30.5°. Chemical shifts in ppm upfield from tetramethylammonium chloride. The coupling constants are in hertz.
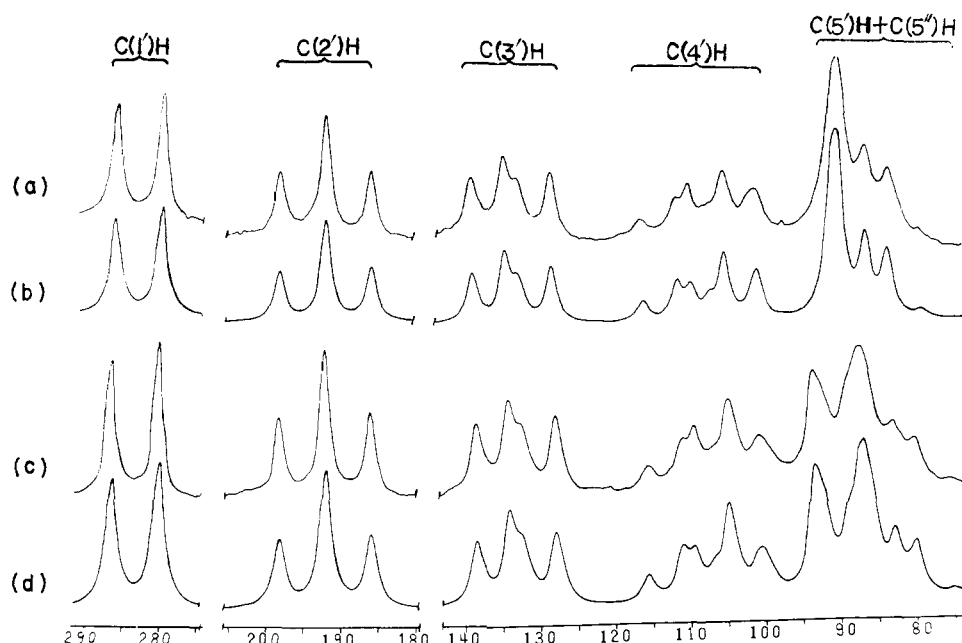

Figure 3. (a) The observed ^{1}H-{^{31}P} 100 MHz fast Fourier transformed nmr spectrum of 8-CH₃S-5'-AMP (0.05 M; pH 8.0; temperature 30.5°). (b) The computer simulated spectrum of 8-CH₃S-5'-AMP under conditions in (a). The agreement between spectra (a) and (b) is excellent. (c) The observed ^{1}H 100 MHz fast Fourier transformed nmr spectrum of 8-CH₃S-5'-AMP under the same conditions as in (a). (d) The computer simulated spectrum of 8-CH₃S-5'-AMP under the same conditions as in (c). The agreement between spectra (c) and (d) is excellent.

show a progressive increase in the gg population as the phosphate becomes a monoanion and the base gets protonated in contrast to 5'-AMP where the population distribution about the C(4')–C(5') bond is not sensitive to protonation of the N(1) or to the ionization of the phosphate. However, the nucleosides adenosine and 8-bromoadenosine show the opposite behavior; i.e., gg population undergoes a drastic reduction from 70–75 to 55% as the adenine moiety becomes protonated.

(c) Analysis of the Interdependence of C(4')–C(5') and C(5')–O(5') Bond Conformers from the Four Bond ^{31}P–^{1}H "W" Coupling and the ϕ,ψ Map. Conformational energies

computed as a function of the rotation angles ϕ and ψ in 5'-AMP for the C(3')-endo and C(2')-endo sugars are shown in Figure 6. It is seen that there are three low energy minima associated with the three possible conformers about the C(4')–C(5') bond for the g'g' range about the C(5')–O(5') bond. There are no minima corresponding to the g'/t' conformations (±60°) about the C(5')–O(5') bond indicating that they are energetically unlikely in 5'-nucleotides as pointed out earlier.[7] Interestingly, it is found that more than 95% of the probability is associated with the global minimum at $(\phi,\psi) = (180°,60°)$ for both the C(2')-endo and C(3')-endo sugars. Calculations carried out previously

Table III. Conformational Parameters for Adenosine, 8-Br-A, 5'-AMP, 8-Br-5'-AMP, and 8-CH₃S-5'-AMP

Compd	pH	Conformational preferences about various bonds				Preferred sugar–base torsion	Magnetic nonequivalence shift, Hz
		C(5')–O(5') Percentage[a]		C(4')–C(5') Percentage[a]			
		g'g'	g'/t'	gg	gt		
Adenosine	8.0			70	30	Anti	7.9
	2.0			55	45		6.9
8-Bromoadenosine	8.0			75	25	Syn	7.6
	2.0			55	45		5.0
5'-AMP	8.0	85	15	65	35	Anti	3.0
	5.0	80	20	68	32		small to detect
	2.0	77	23	66	34		small to detect
8-Br-5'-AMP	8.0	70	30	20	80	Syn	8.2
	5.0	65	35	30	70		6.2
	2.0	70	30	35	65		3.4
8-CH₃S-5'-AMP	8.0	65	35	15	85	Syn	6.6
	5.0	70	30	25	75		4.1
	2.0	70	30	35	65		3.9

[a] A difference in percentage less than 5 is insignificant.
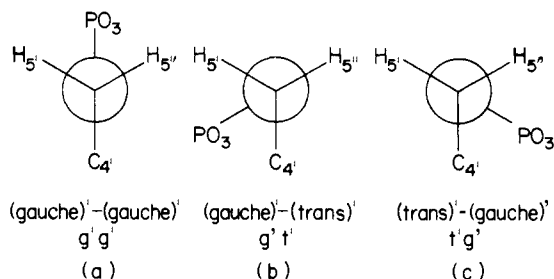
Figure 4. Newman projections showing the three conformers about the backbone C(5')–O(5') bond.
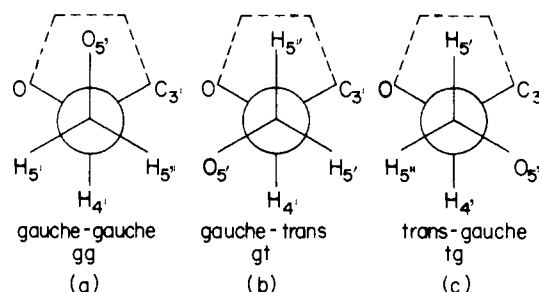

Figure 5. Newman projections showing the three staggered conformations about the exocyclic C(4')–C(5') bond.

by other authors[39,40] without taking into account the base or the phosphate oxygens failed to indicate the strong energetic preference for this conformational combination. It is also found that values of ϕ smaller than 180° (within the broad potential well in the trans range) would further increase the gg conformer population about the C(4')–C(5') bond due to increased phosphate–base attractive interactions.[7] Our present results are in complete conformity with the experimental data obtained from X-ray studies on 5'-nucleotides and dinucleosides.[4] Thus, it is clear that the alternative conformations are less likely in common 5'-nucleotides when the base is in the anti orientation. Potential energy calculations similar to those above carried out for the syn conformation for the base also indicate that the g'g' conformation corresponding to $\phi = 180°$ about the C(5')–O(5') bond is strongly favored. Although there are no quantum mechanical calculations on 5'-AMP, studies on 5'-IMP by Saran, et al.,[41] with a C(2')-endo sugar and the anti base gave results similar to those above. However, the results of Olson and Flory[42] differ from both the experimental[2a,3] and the above theoretical results particularly for the C(3')-endo sugars.[43]

It has been shown by Sarma, et al.,[44] that $^4J_{P4'}$ is a simultaneous measure of the conformational distribution about C(4')–C(5') and C(5')–O(5') bonds for 5'-β-nucleotides and that if $^4J_{P4'}$ is >1.0 Hz the predominant backbone conformations about the C(4')–C(5') and C(5')–O(5') bonds are gg and g'g', respectively. Inspection of Table II indicates that only 5'AMP has $^4J_{P4'}$ values greater than 1.0 indicating that the preferred conformation of the main chain in 5'-AMP is gg-g'g' in agreement with the above theoretical deductions. The percentage population distributions of the C(4')–C(5') and C(5')–O(5') conformers in 5'-AMP (Table III), computed from three bond coupling data,
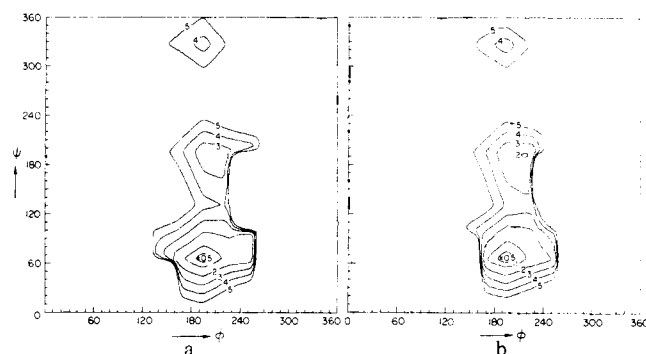

Figure 6. Variation of the total potential energy as a function of ϕ and ψ in (a) C(3')-endo 5'-AMP with $\chi = 10°$ and (b) C(2')-endo 5'-AMP with $\chi = 40°$. In this and the contour diagrams that follow, the numbers on the contours indicate the energy values in kcal/mol drawn relative to the global minimum marked X. Energy contours higher than 5 kcal/mol are not shown since they do not contribute to the statistical weight.

also agree well with the prediction from the four bond ^{31}P–^{1}H coupling data. It has been further noted[44] that if the magnitude of $^4J_{P4'}$ is smaller[45] it is an indication that the backbone will preferentially exist in g/t-g'g' conformation. Examination of the data in Table II indicates the $^4J_{P4}$ to be smaller for 8-Br-5'-AMP and 8-CH₃S-5'-AMP suggesting that in these nucleotides the preferred conformation of the backbone is g/t-g'g'. This prediction is again corroborated by the data in Table III derived from three bond coupling constants.

(d) Analysis of the Preferred Sugar–Base Torsion Angle. The torsion angle about the C(1')–N(9) bond denoted by χ has a value of zero when the N(9)–C(8) bond in the adenine moiety is cis planar to the O(1')–C(1') bond.[2a] The
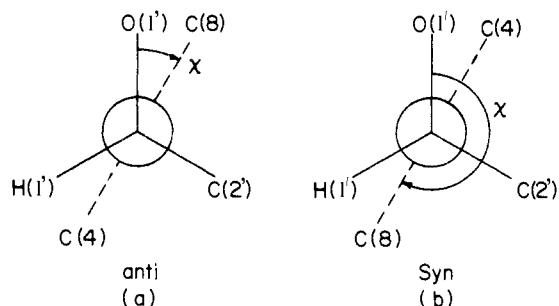
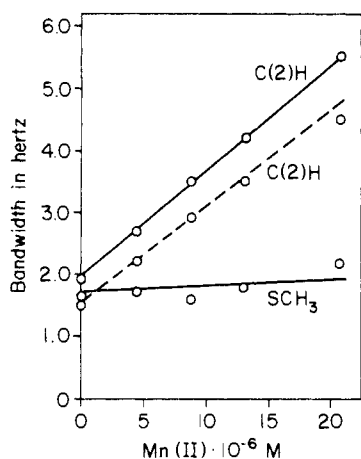Figure 7. Newman projections showing the anti and syn conformations for the purine base.

Figure 8. Effect of Mn(II) ion concentration on the line width of C(2)H of 8-Br-5'-AMP (- - -) and 8-CH₃S-5'-AMP (———). Also shown is the effect on the line width of the SCH₃ group of 7.7 and the concentration of both nucleotides was 0.003 M. Mn(II) ion concentration has no effect on the line width of the C(2)H of 5'-AMP; a detailed study of the effect of Mn(II) ion on 5'-AMP line width is presented elsewhere.[49] It may be noted that the effect of Mn(II) ion on the line width of the SCH₃ group is not any more than experimental error coupled with nonspecific broadening. Hence, the data on 8-CH₃S-5'-AMP may be interpreted to mean that this analog exists exclusively in the syn conformation. However, one is not sure that the slight effect on SCH₃ is due to the presence of small populations of anti conformers. The situation becomes difficult to rationalize on the basis of experimental errors at very high concentrations of Mn(II) ions (65 μM) when the SCH₃ resonance showed a broadening over 2 Hz (see text). Hence, it is unreasonable to conclude that the molecule is exclusively syn.

Newman projections of a typical anti and syn conformations are shown in Figure 7.

It has been demonstrated that 5'-AMP preferentially exists in the anti conformation from Mn(II) ion binding studies of Chan and Nelson[46] and pH ionization studies of Schweizer, et al.,[47] and Danyluk and Hruska.[48] We have carried out Mn(II) ion binding studies similar to those described earlier[46,37] and our results on 5'-AMP are published elsewhere.[49] The data on 8-Br-5'-AMP and 8-CH₃S-5'-AMP are shown in Figure 8. The data on 5'-AMP show that the line width of C(2)H is unaffected by Mn(II) ions; from this and other reasonings it is concluded[49] that 5'-AMP exists almost entirely in the anti conformation. It is seen from Figure 8 that in the case of 8-CH₃S-5'-AMP the C(2)H undergoes clear broadening while the effect on the SCH₃ group is not any more than beyond experimental error and/or nonspecific broadening, at the depicted Mn(II) ion concentration.

The mutually reinforcing observation of the broadening of C(2)H and the lack of significant effect on the SCH₃ line
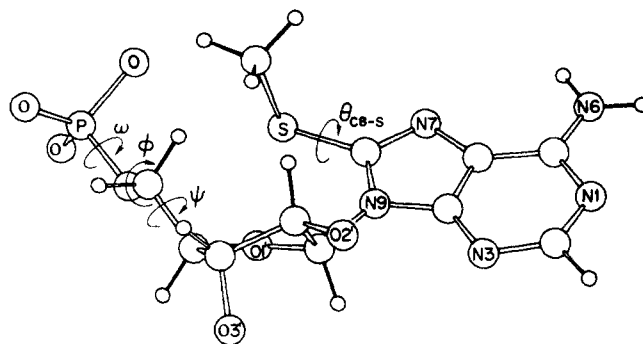

Figure 9. Perspective views showing the anti-gt conformation in 8-CH₃S-5'-AMP, the magnitude of θ (i.e, the torsion about C(8)–S(8)) being 90°. Examination of stereomodels designed by Dr. Christine Jardetzky from X-ray data and marketed by Metalo Alass, Boston, Mass., shows that the distance between the SCH₃ group and the ³¹P atom is the largest in an anti conformation when θ is 90° and the backbone is gt. The measured average distance for above is ≃4.5 Å which is undoubtly within the line broadening range of phosphate bound Mn(II) ions.

width is best rationalized on the ground that 8-CH₃S-5'-AMP shows an overwhelming preference, if not exclusive, for the syn conformation. When the Mn(II) ion concentration is increased to 65 μM, while the concentration of 8-CH₃S-5'-AMP is maintained at 0.003 M, the SCH₃ resonance underwent a broadening of 2.7 Hz (proper corrections were made for general broadening at such high levels of Mn(II) ions from the change in line width of internal standard) and the C(2)H resonance is broadened beyond recognition. Such observation may indicate that at the employed high levels of Mn(II) concentrations, the anti conformations may become accessible even though the system is predominantly syn oriented. However, it should be emphasized that recording of nmr spectra at high Mn(II) concentrations is very difficult because of the necessity of employing several thousand pulses to obtain a meaningful spectrum with fair signal to noise ratio. Computer line broadening as well as difficulty in maintaining good homogeneity could artificially broaden a resonance line. While the data presented so far support a predominantly syn model for 8-CH₃S-5'-AMP, it may also be pointed out that the same data strongly argue against a predominantly anti conformation of the type shown in Figure 9 in which the backbone is g/t-g'g' and the torsion about C(8)–S(8) is such that the distance between the methyl protons and the phosphate group is the *longest* possible, for the following reasons.

(i) If 8-CH₃S-5'-AMP predominantly existed in anti conformation, one would not expect the C(2)H to undergo the observed Mn(II) ion induced broadening.[46,37]

(ii) The measured average distance between the ³¹P atom and the three methyl hydrogens in Figure 9 is 4.5 ± 0.5 Å. At such a distance one indeed would expect the phosphate bound Mn(II) to broaden considerably the CH₃ resonance. For example, in syn purine systems where the distance between C(2)H and ³¹P is 4 ± 0.5 Å, the phosphate bound Mn(II) ion indeed broadens the C(2)H resonance.

(iii) Consideration of molecular models and theoretical calculations to be discussed later clearly show that conformations such as the ones in Figure 9 are forbidden.

We have been able to show that the nucleotide 8-CH₃S-5'-AMP predominantly exists in the syn conformation because of the availability of proton probes on both ends of the molecule. In this connection 8-Br-5'-AMP presents a problem.

The observation that in the case of 8-Br-5'-AMP Mn(II)

ions affect the line width of C(2)H indicates that syn conformations are accessible for this compound. However, the arguments presented below strongly support a model with considerable preference for the syn conformation.

(i) Inspection of Figure 8 shows that the line width of C(2)H of 8-Br-5'-AMP and that of 8-CH₃S-5'-AMP are affected by Mn(II) ions *in an identical way* (The slight displacement between the two profiles in Figure 8 is, among other things, due to the difference in inherent line width at the starting point when [Mn(II)] = 0). The data indicate that the time average geometrical disposition of C(2)H in both nucleotides with respect to the backbone phosphate is most likely the same; *i.e.*, 8-Br-5'-AMP would most likely exhibit an overwhelming preference, like its thiomethyl analog, for the syn conformation.

(ii) Theoretical computations on 8-Br-5'-AMP presented later indicate a clear preference for the syn range.

(iii) X-Ray and H¹ nmr data on the nucleoside 8-bromoadenosine show that the molecule is syn.[23,50a]

(iv) We have shown elsewhere[49] that a comparison of the chemical shifts of C(2)H and C(8)H of the base with that in the nucleoside or nucleotide enables one to distinguish between the preference for syn and anti conformations. In Table IV we have reproduced part of the data from ref 49.

Table IV. Chemical Shifts of C(2)H and C(8)H in Adenine Related Compounds at Concentrations at Which the Chemical Shifts Are Independent of Concentration (pH 8.0, Temperature 30.5°, Shifts Are Expressed in Hertz at 100 MHz Upfield from Tetramethylammonium Chloride)[a]

Compd	C(2)H	C(8)H
Adenine	508	503
Adenosine	508	516
5'-AMP	508	544
8-Br-Ad[b]	491	
8-Br-A	502	
8-Br-5'-AMP	506	

[a] Data for this table are from ref 49. [b] Ad = adenine.

It is seen that the C(2)H has identical chemical shift in the base (adenine), in the nucleoside (adenosine), and the nucleotide (5'-AMP), an observation that is best rationalized on the ground that the C(2)H in adenosine and in 5'-AMP resides in an environment away from the ribofuranose ring as in the anti conformation, as has been shown earlier.[46-49] The observation that the C(2)H of 8-bromoadenosine and that of 8-Br-5'-AMP appears 10 and 15 Hz downfield from that of 8-bromoadenine clearly shows that the ribofuranose and the ribofuranophosphate systems influence the chemical shift of C(2)H, and this is possible only if C(2)H in 8-bromoadenosine and 8-Br-5'-AMP spent considerable amount of time over the ribose moiety as in the syn conformation.

Thus, the results presented above indicate that the preferred conformation of 8-Br-5'-AMP and 8-CH₃S-5'-AMP is syn-g/t. Unfortunately, it is not possible at this stage to estimate the approximate magnitude of χ. In this section, we propose a method which should, in a relative sense, enable us to say whether the syn range which a group of purine nucleotides occupies is the same or different. This is based on the fact that in the syn conformer the purine base is in the immediate neighborhood of the ribose protons which are geometrically positioned as to experience the strong ring current field of the heterocyclic system. Provided no significant difference exists in the isoshielding contours of the purine bases of the nucleotides which are being compared, and if the base on a time average basis has the same values for χ, the ribose protons would be expected to

experience the ring current shifts[50a] of the same magnitude and direction. The compounds 8-Br-5'-AMP and 8-CH₃S-5'-AMP both of which exist preferentially in the syn conformation provide a case in point. As a first approximation, it is reasonable to assume that there exists no wide variation in the isoshielding patterns of the bases in these molecules.[50b] Table V gives the computed difference in chemical

Table V. Difference in Chemical Shifts in Hertz of the Ribose Protons between 5'-AMP and the *syn*-5'-β'-Purine Nucleotides, 8-Br-5'-AMP and 8-CH₃S-5'-AMP

Protons	Δ (8-Br-5'-AMP) − (5'-AMP)	Δ (8-CH₃S-5'-AMP) − (5'-AMP)
1'	+3	+11
2'	−50	−30
3'	−8	−1
4'	+10	+10
5'	−7	−8
5''	−2	−4

shifts for the ribose protons between 5'-AMP and the two syn nucleotides reported. The data in Table V are an indirect measure (however crude it may be) of the change in chemical shift the ribose protons will experience, if the purine moiety is rotated from the anti to the syn conformational range. It is remarkable to see that the direction of the shift of all the six ribose protons in 8-Br-5'-AMP and 8-CH₃S-5'-AMP is identical, indicating that in all likelihood the purine moiety in these two compounds occupies most likely the same narrow syn range, if not identical, as can be seen from the effect on 1', 2', and 3' chemical shifts (Table V). Such a conclusion is compatible with the observed identical effect on C(2)H line width of 8-CH₃S-5'-AMP and 8-Br-5'-AMP by Mn(II) ions discussed earlier (Figure 8). However, it should be pointed out that if significant differences existed between the ribose conformations of the species under comparison, the ribose protons would experience different anisotropic shielding from the base and caution should be applied in deducing any conclusions of the above type from ribose chemical shifts. In the present case, data discussed below show that the various nucleotides under comparison have *in general* almost the same time average distribution of ribose conformers; the slight differences that exist in their computed populations (Table VI) cannot in any way significantly perturb the magnitude of chemical shifts.

Table VI. Conformation of the Ribose Ring

Compd	pH	Time averaged ribose conformations (the populations in % are in parentheses)[a]
Adenosine	8.0	S(67) ⇌ N(33)
	2.0	S(58) ⇌ N(42)
8-Br-A	8.0	S(78) ⇌ N(22)
	2.0	S(64) ⇌ N(36)
5'-AMP	8.0	S(67) ⇌ N(33)
	5.0	S(65) ⇌ N(35)
	2.0	S(64) ⇌ N(36)
8-Br-5'-AMP	8.0	S(54) ⇌ N(46)
	5.0	S(57) ⇌ N(43)
	2.0	S(55) ⇌ N(45)
8-CH₃S-5'-AMP	8.0	S(56) ⇌ N(44)
	5.0	S(57) ⇌ N(43)
	2.0	S(57) ⇌ N(43)

[a] The calculated conformer populations are subject to an error of 5%.

(e) Determination of the Conformation of the D-Ribofuranose Moiety. Sugar ring conformation plays a pivotal role in deciding the stereochemistry of nucleic acids, and in particular, it exercises a profound influence on the conformations of its exocyclic bonds. Comprehension of the dynamics of the sugar ring is therefore of central importance in understanding the overall conformation of nucleic acids and their structural products. Recently Altona and Sundaralingam[51] described the sugar pucker in terms of the pseudorotation parameters P and τ_m. P is the phase angle of pseudorotation, which in theory can take up any value from 0 to 360°, and τ_m is the amplitude or degree of pucker. The majority of the observed sugar conformations in normal nucleosides and nucleotides are confined to two narrow ranges of phase angles separated by a barrier of about 2 to 4 kcal/mol.[51-53] The two ranges of phase angles are: (a) $P = 0$–36° referred to as N type which includes ^3_2T, $^3T_2$, ^3E, $^3T_4$, and ^3_4T sugar puckerings and (b) $P = 144$ to 180° referred to as S type which comprises ^2_1T, $^2T_1$, ^2E, ^2_3T, and $^2T_3$ forms of puckering. It may be noted that the P values other than these two ranges are found to occur preferentially in certain derivatives, e.g., cyclic nucleotides and cyclonucleosides.[54,55]

Altona and Sundaralingam[56] have suggested a method for estimating the percentage population of $N[C(3')$-endo] and $S[C(2')$-endo] type conformers of the sugar from the ribose coupling constants $J_{2'3'}$ and $J_{1'2'} + J_{3'4'}$. They obtained[56] average values of 5.1 Hz (range 5.0 to 5.5 Hz) for $J_{2'3'}$ and 10.1 Hz (range 9.5 to 10.8 Hz) for $J_{1'2'} + J_{3'4'}$ from available data on anti purine and pyrimidine sstems. Table VI shows the population distribution of N and S conformation computed using the magnitude of $J_{3'4'}$. Inspection of the data in Table VI shows that adenosine, 8-bromoadenosine, and 5'-AMP at pH 8.0 show nearly 2:1 preference for the S-type conformation. On the other hand, the C(8) substituted analogs show only a slight bias for the S-type conformation. The protonation of the phosphate or the base does not perturb the population distribution of the sugar conformations to any significant extent in the case of the nucleotides, while in the case of the nucelosides adenosine and 8-bromoadenosine, protonation of N(1) of the base tends to reduce the population of the S-type conformations.

The observation that the $J_{2'3'}$ values for 8-Br-5'-AMP and 8-CH₃S-5'-AMP are over 6 Hz compared to about 5 Hz for 5'-AMP (Table II) is probably due to the predominance of the syn conformer population in C(8) substituted analogs. This suggests that the 2' and 3' hydrogens are more eclipsed in the syn conformation indicating that the ribose rings in the C(8) substituted analogs are flatter than in 5'-AMP. Similar interpretation[56] was also made in the case of orotidine and β-cyanuric acid both of which favored the syn glycosyl conformation and showed a higher value for $J_{2'3'}$ (6.3–6.4 Hz). Thus, the magnitude of the coupling constant seems to show some dependence on whether the preferred orientation of the base is syn or anti.

(χ,ψ) Conformational Energy Maps. Interrelation between the Glycosyl and Sugar–Phosphate Backbone Conformations

Two-dimensional conformation maps have been effectively used in correlating the conformational dependence of the neighboring bond rotations in nucleosides and nucleotides.[2] The (χ,ψ) conformation maps[7] which involve the simultaneous rotations about the glycosyl bond and the exocyclic C(4')-C(5') bond of the sugar–phosphate backbone possess unique features. This map which represents the mutual interactions of the base and the sugar–phosphate chain reveals information not only on the effect of the base type

Table VII. (χ,ψ) Conformer Population Distribution in Adenosine and 5'-AMP

Molecule		—C(2')-Endo—			—C(3')-Endo—		
		gg	gt	tg	gg	gt	tg
5'-AMP (as in poly(A))	Anti	80	4	10	84	7	9
	Syn	1	1	4	0	0	0
5'-AMP (N1 protonated)	Anti	88	3	9	87	5	8
	Syn	0	0	0	0	0	0
5'-AMP (dianionic phosphate)	Anti	98	0	2	96	2	2
	Syn	0	0	0	0	0	0
Adenosine	Anti	22	17	22	38	30	30
	Syn	1	14	24	0	1	1
Adenosine (N1 protonated)	Anti	22	17	22	39	29	30
	Syn	1	13	25	0	1	1

on its conformation relative to the sugar but also on the possible influence of the orientation of the base (syn or anti) on the sugar–phosphate backbone conformation. Hence conformational energy surfaces have been constructed for the substituted nucleotides 8-Br-5'-AMP and 8-CH₃S-5'-AMP and also for the nucleosides adenosine and 8-Br-A. Further, in order to assess the relative importance of the different minima in the (χ,ψ) energy surface, we have computed the percentage probability of occurrence of the conformers associated with the various minima using the expression

$$P(\chi,\psi) = \frac{\exp[-V(\chi,\psi)/RT]}{\Sigma_\chi\Sigma_\psi \exp[-V(\chi,\psi)/RT]}100$$

These values computed for different molecules considered in the present study are given in Tables VII and VIII. Since the present theoretical calculations give a simultaneous evaluation of the preferred glycosyl as well as the exocyclic C(4')-C(5') bond conformations these results are presented together. It may be noted that these analyses can distinguish between the gt and tg conformers in addition to giving the quantitative estimates of the various dihedral angles.

(χ,ψ) Energy Surface for 5'-AMP. The calculations have been carried out first on 5'-AMP with the N(1) atom of adenine protonated and the phosphate group in the monoanionic and dianionic states. The (χ,ψ) energy surfaces thus obtained fro 5'-AMP for the C(2')-endo and C(3')-endo sugar puckerings are shown in Figure 10. It is clear from Figure 10 as well as from Table VII that the present results show essentially the same conformational features as obtained earlier[7] with the base unprotonated and the phosphate group in the monanionic form. It is noteworthy (Table VII) that the preference for the anti-gg conformational combination is further enhanced when the 5'-phosphate group is a dianion and also when the N(1) atom of the base is protonated. Increased electrostatic attractive interactions between the base and the dianionic phosphate in the former situation are responsible for further stabilization of the anti-gg conformer. These results again demonstrate that the above conformational combination is strongly favored for 5'-AMP irrespective of whether the phosphate is a monoanion or a dianion or whether the base is protonated or unprotonated. This is also true for both the C(2')-endo and C(3')-endo sugars.

(χ,ψ) Energy Surface for 8-Br-5'-AMP. It is well established that the syn conformation for the base would in general favor the C(2')-endo class of puckers covering the phase angle P from 144 to 180°.[51] Further, the syn base seems to show a bias for the $^2T_1$ puckering.[57] Hence, detailed calculations have been made only for C(2')-endo sugars in substituted nucleotides. Preliminary calculations with

Table VIII. (χ,ψ) Conformer Population Distribution in 8-Br-A, 8-Br-5'-AMP, and 8-CH₃S-5'-AMP

Molecule	χ	As in polymers			N(1) protonated			Dianionic phosphate		
						ψ				
		gg	gt	tg	gg	gt	tg	gg	gt	tg
8-Br-A	Anti	0	0	2	0	0	2			
	Syn	3	34	61	3	34	61			
8-Br-5'-AMP	Anti	0	0	2	0	0	3	0	0	3
	Syn	26	26	46	30	18	49	22	11	64
8-CH₃S-5'-AMP,	Anti	0	0	1	0	0	1	0	0	3
$\theta = 90°$	Syn	18	30	51	12	32	55	11	19	67
8-CH₃S-5'-AMP,	Anti	19	29	35	24	30	37	44	17	35
$\theta = 180°$	Syn	4	5	8	2	3	6	0	1	3
8-CH₃S-5'-AMP,	Anti	24	21	34						
$\theta = -90°$	Syn	4	6	11						
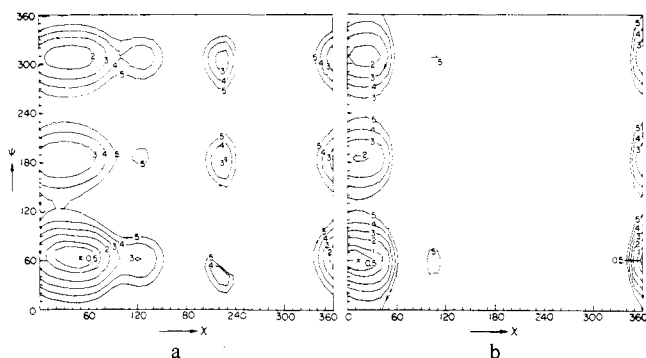
Figure 10. Variation of the total potential energy as a function of χ and ψ in N(1) protonated 5'-AMP for (a) C(2')-endo and (b) C(3')-endo sugar puckerings with $\phi = 180°$.

Figure 11. Variation of the total potential energy as a function of χ and ψ in N(1) protonated 8-Br-5°-AMP for C(2')-endo sugar puckering with $\phi = 180°$.

C(3')-endo sugars $(P = 0-18°)$ indicated that the nucleotide system is quite constrained and it is less likely that a syn conformation in a nucleotide would favor a C(3')-endo sugar pucker $(P = 0-18°)$. However, it might be possible that a sugar pucker having a phase angle much higher than a C(3')-endo pucker $(P > 18°)$ might allow syn conformation for the base.[54] The equienergy contour diagram obtained by simultaneous rotations of χ and ψ for C(2')-endo 8-Br-5'-AMP is displayed in Figure 11. It is seen that there appears three minima corresponding to the three familiar staggered conformers about the C(4')-C(5') bond only in the syn range $(\chi = 200-240°)$ in sharp contrast to 5'-AMP (Figure 9). Strong van der Waals repulsive interactions between the bromine and the atoms of the ribofuranose do not energetically allow the normal anti range of conformations. Consequently, there are no low energy minima in the anti region for any of the staggered conformations about the C(4')-C(5') bond as might be anticipated. It is found from Table VIII that more than 97% of the statistical weight is associated with the normal syn conformation. The global minimum in the (χ,ψ) energy surface occurs at $(210°,300°)$ corresponding to the syn-tg conformational combination. The population distributions among the three staggered conformations about the C(4')-C(5') bond indicate that the tg conformer is energetically more favorable than either gg or gt. The gt and tg conformers combined together contribute more than 75% of the statistical weight indicating excellent agreement with the nmr data. Similar results[10] obtained recently by semiempirical energy calculations are in good agreement with the present data. It may be mentioned that in poly(8-Br-rA) the presence of the 3'-phosphate group would destabilize the tg conformer owing to electrostatic repulsions between the adjacent phosphate groups.[8,10,42] Thus, it is clear that when the base is constrained to the syn conformation, the favored conformation about the C(4')-C(5') bond of the sugar-phosphate backbone in a poly(8-Br-rA) is no longer gg (as is the case for
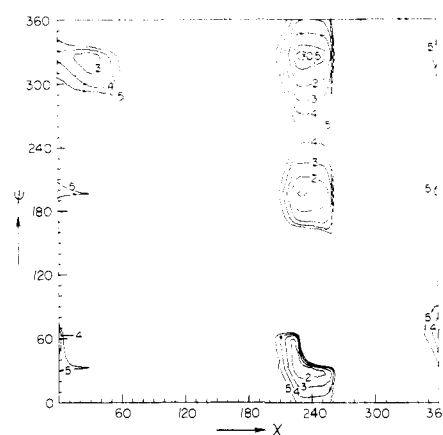
the anti base) but is gt. This distortion in the backbone conformation clearly suggests that there is a striking correlation between the backbone and the side chain conformations.[6-10] The perspective views of the two possible conformations, syn-gt and syn-tg, are shown in Figure 12.

Calculations similar to those above performed with the dianionic phosphate group at the 5' position essentially yield similar results. The only noticeable observation is that the gg conformer population is slightly decreased compared to the situation where the 5'-phosphate is a monoanion and N(1) of the base is protonated. This is in qualitative agreement with the above nmr data. The results obtained on the nucleoside 8-Br-A show similar conformational features.

(χ,ψ) **Energy Surface for 8-CH₃S-AMP.** In 8-CH₃S-5'-AMP there is an additional conformational parameter involving the thiomethyl group. The (χ,ψ) energy surface in 8-CH₃S-AMP is therefore dependent on the orientation of the thiomethyl group relative to the adenine base. Calculations have therefore been made first to determine the preferred orientation of the thiomethyl group about the C(8)-S bond. The results indicate that there is a broad range of conformation angles from 90 to 280° (N(9)-C(8)-S-C(8')) which are energetically feasible for the methyl group with the region around 100° being energetically slightly more favored. Hence, in computing the (χ,ψ) energy surface, the torsion angle θ (N(9)-C(8)-S-C(8')) has been kept fixed at 90° as found from energetic criterion. The equienergy (χ,ψ) contour surface so obtained for C(2')-endo 8-CH₃S-5'-AMP (Figure 13) exhibits three distinct minima which are associated with the syn-tg, syn-gt, and syn-gg conformations. As in the case of 8-Br-5'-AMP, there are no energy minima in the anti range of conformation of the base for any of the staggered conformations about the C(4')-
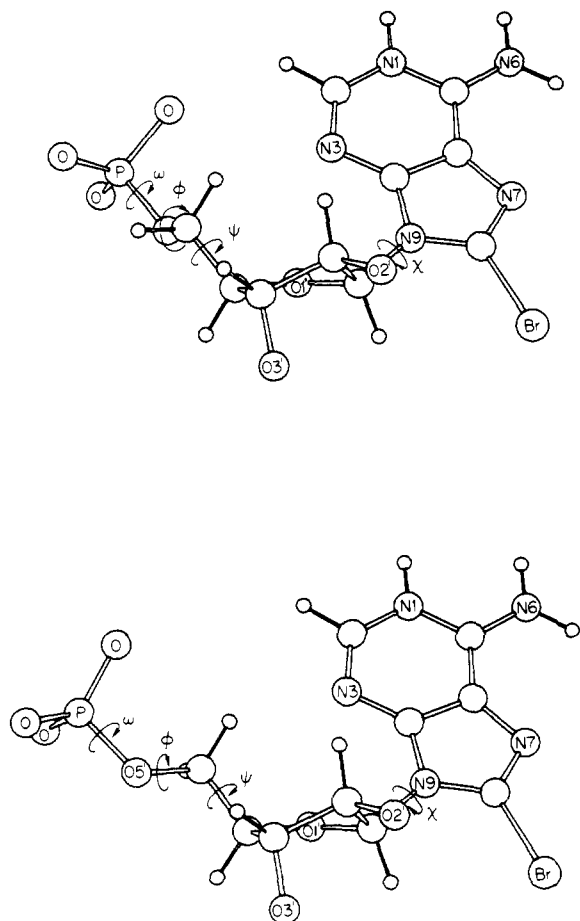
Figure 12. Perspective views showing the syn-gt (220°,180°) and syn-tg (220°,300°) conformations in 8-Br-5′-AMP.

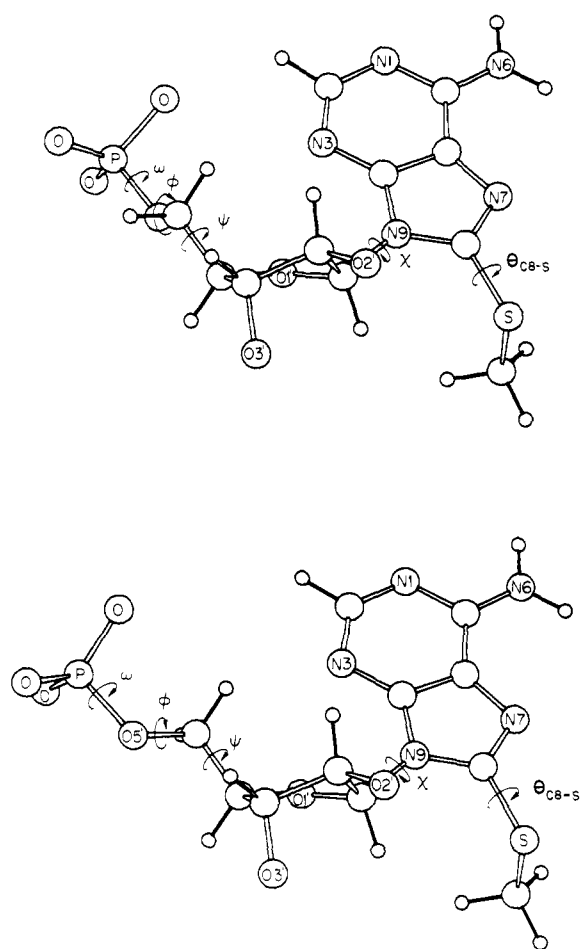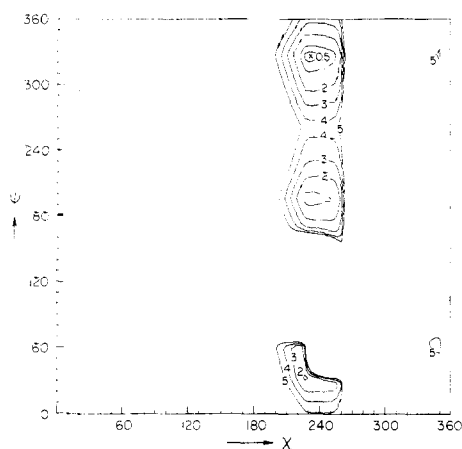Figure 13. Variation of the total potential energy as a function of χ and ψ in N(1)-protonated 8-CH$_3$S-5′-AMP for C(2′)-endo sugar puckering with $\phi = 180°$.

Figure 14. Perspective views showing the syn-gt (220°,180°) and the syn-tg (220°,300°) conformation in 8-CH$_3$S-5′-AMP.

C(5′) bond. It is found that strong van der Waals repulsive interactions due to the close proximity of the thiomethyl group with the ribose do not permit the anti conformation. Further, the possible range of syn angle is quite restricted ($\chi = 210$–$240°$) similar to that found in 8-Br-5′-AMP. The syn-tg and syn-gt conformers (Figure 14) combined together contribute more than 80% of the statistical weight (Table VIII) while the syn-gg conformation comprises only 16% of the population. As in 8-Br-5′-AMP, the syn-tg conformer is found to be slightly more favored than the syn-gt. The effect of the dianionic phosphate group does not alter the es-

sential conformational features observed in the (χ,ψ) surface obtained with the monanionic phosphate group with N(1) protonated. There is a slight increase in the syn-tg conformer population with a corresponding decrease in the syn-gt and syn-gg conformer population as also observed in 8-Br-5′-AMP. These results are in good qualitative agreement with the nmr data presented above.

As noted earlier, there are two other possible conformations for the 8-thiomethyl group in the range corresponding to $\theta(C8-S) = -90°$ and $\theta(C8-S) = 180°$. Consequently, it is of interest to assess the influence of the orientation of this substituent on the (χ,ψ) energy surface. Calculations similar to above have therefore been performed for C(2′)-endo-8-CH$_3$S-5′AMP with the torsion angle $\theta(C8-S)$ kept fixed at -90 and $180°$. The results obtained are summarized in Table VIII. It is seen that for both these orientations of the methyl group, the anti conformation for the glycosyl conformation is favored owing to the increased van der Waals and electrostatic interactions between the phosphate group and the thiomethyl group. More than 80% of the statistical weight is associated with the anti range of conformations. Further, the anti-tg conformation is found to be more favored than either anti-gt or anti-gg conformational combination. The fact that the syn conformer is preferred when $\theta(C8-S) = 90°$ in conformity with the nmr data suggests that the 8-thiomethyl group is most likely oriented in a direction normal to the base plane in solution.

Discussion

The results presented in previous sections show that the preferred conformation of adenosine at biological pH is anti

and that of 8-bromoadenosine is syn; however, in both cases at pH 8.0, the C(4')–C(5') shows a 70–75% preference for the gg conformer. *Prima facie* it may appear that in the case of nucleo*sides*, the backbone conformation is independent of the conformation about the glycosyl linkage. It is difficult to conceive that repulsive interactions between the gg backbone and the pyrimidine part of the adenine will not take place in a syn conformation; it appears more likely that *syn*-8-bromoadenosine is engaged in an intramolecular hydrogen bonding O(5')–H · · · N(3) and that such hydrogen bonding increases the gg population. In fact, it has been shown by Rao and Sundaralingam[6] that a gg conformation is essential for the formation of the above hydrogen bond.[58] It can further be seen that (Table III) protonation of N(1) causes a reduction of about 20% in the gg population suggesting a weakening of the hydrogen bond O(5')–H · · · N(3). It is interesting to note that the reduction in the gg population also perturbs the population distribution of the sugar conformers (Table VI); *i.e.*, as the gg population decreases and becomes increasingly g/t, the pucker of the sugar tends to become less S and more N. Similar trends in nucleosides have already been observed by Hruska.[33] It may also be noted that the difference in chemical shifts between 5' and 5″ protons (the magnetic nonequivalence shift, Table III) decreases from 7.6 Hz at pH 8.0 to 5.0 at pH 2.0 indicating that the conformational perturbation on the ribose and the exocyclic linkage produced by protonation of N(1) affect the degree of chirality of the C(5') region. The preferred solution conformation for 8-bromoadenosine is essentially the same as found in the crystal.[23]

The observation that adenosine exists predominantly in the anti-gg conformational combination at pH 8.0 is in good agreement with the theoretical results (Table VII). The interrelation between the conformations about the glycosyl and the C(4')–C(5') bonds is dependent on the sugar pucker. It is seen from Table VII that C(3')-endo adenosine shows an exclusive preference for the anti conformation while C(2')-endo adenosine shows only 60% preference for the anti. However, in the latter case the presence of the intramolecular hydrogen bond would strongly favor the syn-gg.[59] In the absence of a hydrogen bond, the g/t conformers become predominant.[60] This in fact has been observed by nmr when N(1) is protonated. It is found that protonation causes a reduction of the gg population by 15% from 70% at pH 8.0 to 56% at pH 2.0 with about 10% reduction in the population of S sugar conformations. The observed preferred solution conformation of adenosine anti-S ⇌ N-gg is not the same as found in the crystal,[61] anti-³T₂-gt. It is interesting, however, that the crystal conformation of adenosine[62] in its complex with 5-bromouridine is anti-³T₂-gg which is in general agreement with the solution conformation.

The common nucleotide 5'-AMP at biological pH exists almost entirely in the anti conformation,[49] the preferred conformation for the backbone being g'g' (85%) and gg (65%), that of the sugar S. When the conformation about the C(4')–C(5') bond becomes increasingly gg the g'g' population about the C(5')–O(5') bond also increases. The preferred solution conformation of 5'-AMP is essentially the one preferred by the molecule in crystals,[63] except for the sugar pucker. The theoretical calculations of Yathindra and Sundaralingam[7] and Olson[10] also predict the observed anti-gg conformation of 5'-AMP to be the preferred one irrespective of whether the sugar pucker is C(2')-endo or C(3')-endo. It is instructive to note that the nmr data in Tables III and IV indicate that protonation of the phosphate or for that matter protonation of N(1) has no significant effect on the conformational preference of 5'-AMP while the

theoretical results predict increased stabilization of the anti-gg conformer (Table VII). The observed change of less than 5% in g'g', gg or sugar conformer population is not significant because the calculation employed has an error margin within 5% as well as no reasonable estimates of the effect of phosphate protonation on the magnitude of $J_{5'P}$ + $J_{5''P}$ or $J_{4'5'}$ + $J_{4'5''}$ are known.

The conformations of the C(8) substituted nucleotides, 8-Br-5'-AMP and 8-CH₃S-5'-AMP, present a dramatic contrast to that of 5'-AMP. A comparative study of the conformation of 5'-AMP *vis-à-vis* that of 8-Br-5'-AMP and 8-CH₃S-5'-AMP should give important insight into the factors which determine the stereochemistry of nucleic acid components. The preferred conformation of both 8-Br-5'-AMP and 8-CH₃S-5'-AMP at biological pH is syn-N ⇌ S-g/t-g'g'. The population of g'g' in 5'-AMP is 85% which has undergone a reduction of 15–20% in the 8-bromo and 8-thiomethyl analogs; the reduction observed in the gg population is an overwhelming 45–50%. The reduction in the gg population is so much that the preferred conformation is g/t. The interrelation between the *syn*-glycosyl and the backbone C(4')–C(5') bonds in 8-Br-5'-AMP and 8-CH₃S-5'-AMP is exemplified by the conformational energy calculations also (Table VIII).

The single factor which produces these conformational distortions is the torsional variation about the glycosyl linkage, which is necessitated by the steric requirements in the C(8) substituted analogs. Repulsive steric interactions between the bulky bromo or thiomethyl groups and the ribofuranose–phosphate system in the anti conformation force the molecule to adopt a sterically favorable syn conformation. The bulky and charged phosphate group in the g'g' and gg orientation still cannot accommodate the *syn*-purine moiety. Hence, the C(4')–C(5') undergoes torsional variation to the more comfortable syn-g/t orientations indicating a correlation between the side chain and backbone conformations. The torsional variation about the glycosyl bond and the consequent backbone conformational change slightly affects the equilibrium distribution of the sugar conformations. The S pucker is preferred in 5'-AMP whereas the C(8) substituted analogs show a very slight bias for the S type.

Data in Tables III and IV further indicate that the conformations of 8-Br-5'-AMP and 8-CH₃S-5'-AMP are sensitive to protonation of the phosphate and the base N(1). This is again a contrast to the behavior of 5'-AMP. As one goes from a monophosphate dianion to a monophosphate monoanion bearing a protonated N(1), the gg population *increases* by 15–20% (Table III). The increase in the syn-gg population may be due to the reduction in the electrostatic repulsive interactions produced by the changes in the electronic charge distribution when N(1) of the base is protonated. Data in Table III also show that the populations of g'g' conformers remain unaffected by change in pH values for 8-Br-5'-AMP and 8-CH₃S-5'-AMP. In 5'-AMP, the observed magnetic nonequivalence shift (Table III) is 3 Hz at pH 8.0; however, at lower pH values, no such shift is detectable. In 8-Br-5'-AMP and 8-CH₃S-5'-AMP, the magnetic nonequivalence shift starts at 7–8 Hz at pH 8.0 and progressively decreases to 3–4 Hz as the pH is lowered. The data indicate that the C(5') region of *syn*-β-5'-purine nucleotides with a predominant g/t population resides in an environment which is more asymmetric than the corresponding environment in the anti-gg torsional isomers.

Conclusion

These investigations have permitted our comprehension of the solution conformational dynamics of 5'-β-purine nucleotides. The following guidelines will aid in the solution

conformational analysis of other 5'-β-purine nucleotides and related compounds.

(i) The preferred conformation of the C(5')-O(5') bond is g'g' (trans) irrespective of whether the base is anti or syn.

(ii) Glycosyl torsion has a profound impact on the conformational preferences about the C(4')-C(5') bond. The preferred conformational combination is anti-gg in normal purine nucleotides. The presence of a bulky group at C(8) of the base would destabilize the anti conformation, favoring the syn. The onset of syn orientation about the glycosyl linkage will be followed by rotation about C(4')-C(5') into the g/t conformations.

(iii) Introducing a formal positive charge in the base increases the time average preferred population of the gg conformer because of electrostatic attractive interactions between the base and the phosphate.

(iv) Even though the conformational preference about the C(5')-O(5') bond is always g'g', the preferred time average g'g' population will be strongly influenced by rotational preference about C(4')-C(5'). The trend will be that as C(4')-C(5') becomes increasingly gg, the C(5')-O(5') g'g' population will also show an increase.

(v) The ribose moiety of 5'-β-purine nucleotides exhibits a fairly uniform predictable conformational dynamics in solution. It can be safely predicted that the ribose moiety of 5'-β-purine nucleotides (whether syn-g/t or anti-gg) will exist as equilibrium mixtures of S and N conformations with the anti-gg nucleotides showing a slight bias toward the S conformer.

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(5) Modifications in the base are expected to alter the preferred anti-gg conformational combination in nucleotides. For instance, replacement of the C(6)-H by an electronegative atom N(6) as in 6-aza-5'-UMP or interchange of N(9) and C(8) as in 5'-FMP would produce distortion from the normal anti-gg because of electronegative repulsive interactions. Consequently conformational features of modified nucleotides should not be treated on par with the normal nucleotides.
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(26) The gauche (g'g') conformation about the C(5')-O(5') bond referred to here by nmr terminology is with respect to the C(5') methylene hydrogens and is synonymous with the trans conformation (ϕ = 180°) with respect to the backbone atom C(3') generally used.²ᵃ Therefore, g't' corresponds to ϕ = +60° and t'g' corresponds to ϕ = −60° (see Figures 1 and 4) with respect to the backbone atom C(3').
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