

# **<sup>1</sup>H NMR STUDY OF THE CONFORMATION OF THE RIBOSE PHOSPHATE MOIETY OF 6-AZAURIDINE-5'-MONOPHOSPHATE – A NUCLEOTIDE WITH AN UNUSUAL CONFORMATION**

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## **1. Introduction**

6-Azaauridine-5'-monophosphate (5'-azaUMP, I) is known to be a potent antileukemic agent [1] and to determine whether this activity may be correlated with an unusual conformation, we have examined its molecular geometry in solution using <sup>1</sup>H NMR spectroscopy. Comparison of the <sup>1</sup>H NMR data on U (uridine), 5'-UMP, 6-azauridine (azaU) and 5'-azaUMP, show that the presence of the 6-aza base has a marked destabilizing influence upon the gauche-gauche (II) conformation constrained to the C(4') – C(5') bond of 5'-azaUMP.

Table 1

Coupling constants in Hz for U, 6-azaU, 5'-UMP and 5'-azaUMP, D<sub>2</sub>O solutions, 30°.

Nuclei	U	aza U	5'-UMP	5'-azaUMP
J <sub>1'2'</sub>	4.4	3.6	5.3	4.2
J <sub>2'3'</sub>	5.3	5.2	5.0	5.4
J <sub>3'4'</sub>	5.5	5.8	3.9	5.4
J <sub>4'5'</sub>	3.0	3.2	2.5	4.6
J <sub>4'5''</sub>	4.4	5.6	3.0	6.1
J <sub>5'5''</sub>	-12.7	-12.4	-13.5	-11.3
J <sub>1'5</sub>	0.35	0.61	<0.2	0.55
J <sub>P4'</sub>	—	—	1.8	<0.2
J <sub>P5'</sub>	—	—	3.2	5.6
J <sub>P5''</sub>	—	—	5.1	6.1
J <sub>4'5'</sub> + J <sub>4'5''</sub>	7.4	8.8	5.5	10.7
J <sub>P5'</sub> + J <sub>P5''</sub>	—	—	8.3	11.7

## **2. Materials and methods**

Spectra of U, azaU, 5'-UMP and 5'-azaUMP (commercial products), 0.1 M, pD 8.0, 30°, in D<sub>2</sub>O, were obtained using either a 220 MHz, continuous wave or a 100 MHz, fast Fourier transform system. Spectra were recorded both with coupling to the <sup>31</sup>P of the phosphate and with <sup>31</sup>P decoupling and were analyzed using the computer program LAME. In fig. 1 we have illustrated the experimental and computer simulated spectrum (only the ribose region) of 5'-azaUMP. The data are compiled in the table.

## **3. Results and discussion**

Population distribution of the rotamers constrained to the C(4') – C(5') bond (II–IV) can be evaluated from the experimental value of J<sub>4'5'</sub> + J<sub>4'5''</sub> (Σ) [2, 3]. Any perturbations resulting in an *increase* in the g–g rotamer populations should be manifested by a *decrease* in the experimental *sum* J<sub>4'5'</sub> + J<sub>4'5''</sub>, whereas an increase in the g–t and/or t–g populations, at the expense of the g–g conformer, should result in an *increase* in the observed sum [2, 3]. Populations distribution of the rotamers constrained to the C(5') – O(5') bond (V–VII) can be monitored in an analogous way.

The experimental sum J<sub>4'5'</sub> + J<sub>4'5''</sub> (7.4 Hz for U; 8.8 Hz for azaU), shows that the presence of the 6-azauracil base has a destabilizing influence, relative

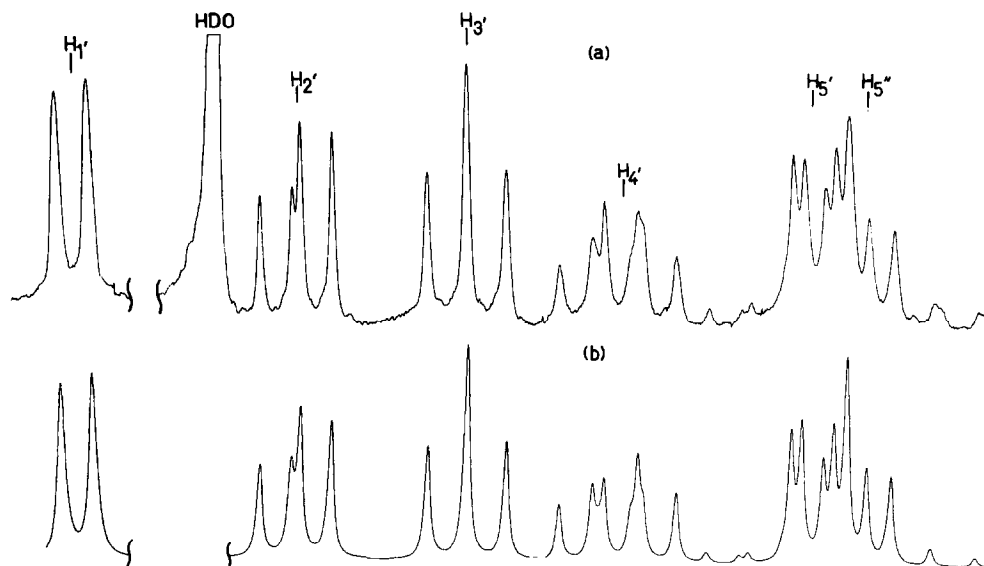


Fig. 1. a) Experimental spectrum of the ribose region of 6-aza-5'-UMP taken in a 100 MHz NMR system, using a 16K fast Fourier transform system. b) Computer simulated spectrum of the ribose region of 6-aza-5'-UMP.

to uracil, upon the *g-g* orientations (II) about the C(4') – C(5') bond of a nucleoside. This destabilizing effect is more conspicuous for the *monophosphate derivative* for which an increase of 5.2 Hz is observed when the uracil is substituted by the 6-azabase. A simple relationship (1) between  $P_{gg}$  (the fractional population of the *g-g* rotamer) and  $\Sigma$  (the observed sum of  $J_{4'5'} + J_{4'5''}$ ) can be derived by manipulating equations 1–3 in reference 2. This equation provides

$$P_{gg} \approx \frac{12 - \Sigma}{8} \quad (1)$$

rough estimates of *g-g* populations. Substitutions of the data into (1) leads to  $P_{gg}$  values: U(60%); azaU (40%); 5'-UMP (80%); 5'-azaUMP (15%). We have derived a similar expression (2) for the C(5') – C(5') bond of the monophosphates

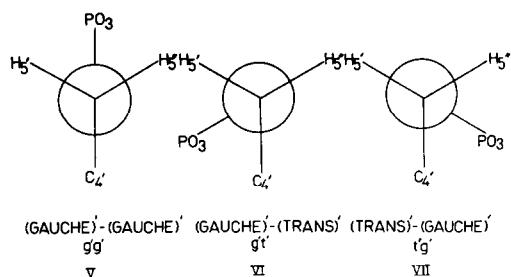
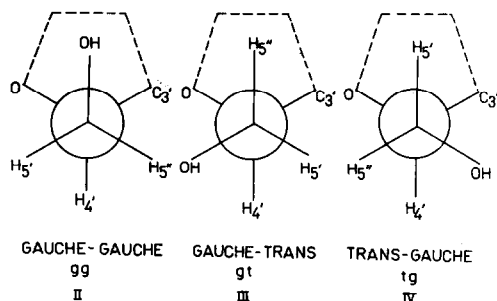
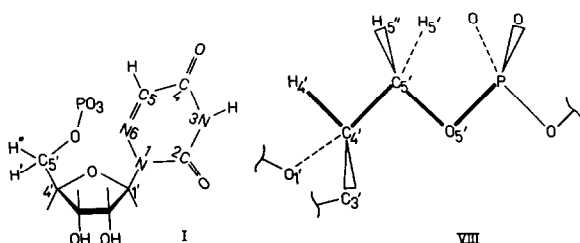
$$P_{g'g'} \approx \frac{24 - \Sigma}{18} \quad (2)$$

where  $P_{g'g'}$  and  $\Sigma$  are, respectively, the populations of the *g'-g'* rotamer (V) and the observed sum  $J_{P-H(5')} + J_{P-H(5'')}$ . The coupling constants used are:  $J_{PH(gauche)} \approx 3$  Hz and  $J_{PH(trans)} = 21$  Hz [4, 5]. The derived  $P_{g'g'}$  values – 5'-UMP (90%), 5'-aza-

UMP (70%) – suggest a slight destabilizing influence of the 6-aza base upon the *g'g'* rotamer of a 5'-nucleotide.

In 5'-UMP, but *not* in 5'-azaUMP, we have observed a four bond  $J_{P4'}$ , long-range coupling. Four bond H–H coupling constants are known to be strongly dependent upon the stereochemical arrangement of the intervening bonds and largest for the all *trans* (W) conformation [6]. Hall and coworkers [4, 7] have presented clear evidence that H–P coupling in H–C–O–P fragments do have a similar stereorelationship. The “W” relationship between the phosphorus and 4' hydrogen of a 5' mononucleotide is encountered when the molecule is simultaneously oriented *g-g* and *g'-g'* (VIII), but is destroyed by rotations about either of the C(4') – C(5') or C(5') – O(5') bonds into one of the *trans* conformations. We believe that the observed  $^4J_{P4'}$  values for 5'-UMP (1.8 Hz) and 5'-azaUMP ( $\approx 0.1$  Hz) provide further evidence for the suggested destabilizing influence of a 6-azauracil base upon the *g-g* and *g'-g'* conformer of a 5' nucleotide.

The limitations of the applications of Karplus equation to decipher ribose conformation from  $J_{1'2'}$ ,  $J_{2'3'}$  and  $J_{3'4'}$  have been discussed elsewhere [8] and it has been suggested that the coupling constant data are best treated in terms of two favored modes viz C(2')



*endo* and C(3') *endo*. The J values in the table indicate that the ribofuranose ring in the nucleosides and nucleotides is not conformationally pure and it may exist as an equilibrium mixture of C(2') *endo* and C(3') *endo* modes undergoing interconversion via pseudorotation [9]. If the small differences in the magnitudes of the J values is anything of value, one may indicate that 5'-UMP displays a slight preference for the C(2') *endo* mode and 5'-azaUMP, a slight preference for the C(3') *endo* mode.

Comparison of the X-ray data, obtained by Saenger and Suck [10] with the present NMR data, show that 5-azaUMP retains its unusual solution conformation, in the solid state as well, and this is the first time one has encountered a 5' nucleotide whose exocyclic  $\text{CH}_2\text{OPO}_3^{2-}$  side chain does not follow the general

pattern [9a] of g-g, g'-g' conformation; and it is likely that the antileukemic property of 5'-azaUMP is related to this unusual conformation.

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