# Solvent Effects on the Conformation of Nucleotides. Part 1. The Conformation of 5'-Adenosine Monophosphate in Water–Dimethyl Sulphoxide using Nuclear Overhauser Effects and Lanthanide Relaxation Probes

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The conformation of 5'-adenosine monophosphate (AMP) in $D_2O$ and in [$^2H_6$]DMSO was studied using nuclear Overhauser effects together with lanthanide-induced relaxation effects and vicinal coupling constants. The conformation of 5'-AMP through the glycosidic bond was studied in detail using a simple two-state model and continuous distributions derived from potential-energy calculations. Either of the two models clearly indicates a base conformation for 5'-AMP in $D_2O$ within the *anti*-region and a flexible *syn–anti* equilibrium in [$^2H_6$]DMSO. These different flexibilities in the two solvents illustrate the importance of solute–solvent interactions in the definition of the conformation of a flexible solute in solution.

The effect of the solvent on the conformation of biological systems has been the subject of considerable discussion. In particular, nuclear magnetic resonance (n.m.r.) studies of nucleosides,[1,2] mononucleotides,[1,3] and dinucleotides [3] in organic solvents such as dimethyl sulphoxide (DMSO) have shown that their conformations and association properties in these solvents are quite distinct from those observed in water. Also optical and n.m.r. studies of the properties of nucleic acids in organic solvents have shown that DNA and RNA dissolved in DMSO have lost essentially all their secondary structure [4] and that *t*-RNA is partially denatured.[5]

The conformation of 5'-mononucleotides in aqueous solution has previously been studied using lanthanide aqueous ions as n.m.r. probes,[6-8] spin-lattice relaxation times,[9,10] and the nuclear Overhauser effect (NOE).[11-13] A preliminary conformational study of 5'-AMP in DMSO using the lanthanide-probe method showed that the preferred conformation of 5'-AMP in DMSO is different from that in water,[14] suggesting that solvent forces play a very important role in the definition of the conformation of the mononucleotide in solution. However, it was later shown that the lanthanide-induced dipolar shifts, upon which this conformational study was based, were incorrectly supposed to have axial symmetry.[15]

In this paper we extend the previous studies using NOE enhancements and proton-distance ratios obtained from relaxation effects induced by $Gd^{III}$ ions in order to obtain more detailed information on the conformation of 5'-AMP in DMSO and in $H_2O$–DMSO mixtures. The solvent-induced conformational changes of 5'-AMP are interpreted in terms of solute–solvent interactions.

## Experimental

The lanthanide(III) chlorides were obtained from Koch-Light and from Research Organic/Inorganic Chemical Corporation, California, and deuteriated DMSO, [$^2H_6$]DMSO, from Ciba. The lanthanide(III) chloride solutions in $D_2O$ were prepared in the usual way.[6] The anhydrous lanthanide(III) chlorides and anhydrous [$^2H_6$]DMSO were prepared from the commercial products as described previously.[14] The 5'-AMP (sodium salt, from Sigma Chemicals) was lyophilized from $H_2O$ at pH 1.8, and then dissolved in $D_2O$, in anhydrous [$^2H_6$]DMSO or in different $D_2O$–[$^2H_6$]DMSO mixtures, in concentrations of 0.03M. The $Ln^{III}$–5'-AMP solutions were prepared by dissolving anhydrous lanthanide chlorides in the 5'-AMP–anhydrous [$^2H_6$]DMSO solution in appropriate proportions. The pH adjustments were made with DCl or NaOD using a Radiometer pHM63 digital pH meter equipped with a Radiometer CK 2321C electrode.

Proton n.m.r. spectra were measured using a Bruker CXP 300 spectrometer, equipped with an Aspect 2000 computer for Fourier transformation, or a Varian XL-200 spectrometer also operating in the Fourier-transform mode. Spin–spin relaxation times ($T_2$ values) were obtained from line-width measurements and spin–lattice relaxation times were measured by application of a 180°–τ–90° pulse sequence repeated at time intervals of longer than $5T_1$. The internal standard was sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSS). The NOE enhancements without decoupling were measured using a gated decoupling technique.[16] Typically, four free-induction decays were acquired with a gated irradiation pulse on resonance. The next four free-induction decays with the same gated irradiation pulse but in an empty region of the spectrum were then acquired. The sequence was repeated in order to get a good signal to noise ratio in both perturbed and unpertubed Fourier-transformed spectra. Signal areas were measured on expanded spectra *via* planimetry, normalized to the same signal of TSS and area differences between the perturbed and the unperturbed spectra were recorded as NOE values, expressed as percentage change. Each value was obtained from at least ten measurements under similar conditions. The experimental precision was 3%. The decoupling power used was the minimum necessary to saturate the spin considered.

## Results and Discussion

For a given molecular geometry defined by internal variables, Ω, and under certain conditions and approximations (all nuclei are under the extreme narrowing condition, relaxation is exclusively dipole–dipole, cross-correlation effects are negligible, and a single correlation time $\tau_c$ can be used for all interaction vectors), which are generally satisfied by small organic systems, we obtained equations (1)—(3),[17,18] where $\gamma_I$

$$f_d(s,\Omega) = \sum_s \frac{\gamma_s \rho_{ds}(\Omega)}{2\gamma_d R_d(\Omega)} - \frac{1}{2\gamma_d R_d(\Omega)} \sum_{n \neq d,s} \gamma_n \rho_{dn}(\Omega) f_n(s,\Omega) \quad (1)$$

$$R_d = \sum_i \rho_{di} + \rho_d^* \quad (2)$$

$$\rho_{ij} = \gamma_i^2\gamma_j^2\tau_c/r_{ij}^6 \quad (3)$$

is the gyromagnetic ratio of nucleus $i$, $r_{ij}$ is the distance between nucleus $i$ and $j$, $\rho_d^*$ is the direct relaxation rate of spin $i$ due to 'other' relaxation paths, and $\tau_c$ is the molecular reorientational correlation time. Comparison of calculated and observed enhancements can give information about molecular geometry because $f_d(s)$ is a function of internuclear distances.

The contributions of bound $Gd^{III}$ ions to the measured spin–lattice ($T_{1M}$) and spin–spin ($T_{2M}$) relaxation times of the nuclei of metal-bound nucleotides, given by the Solomon–Bloembergen equations,[6] can be calculated as a set of ratios, and give directly the relative distances 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,[6] equation (4).

$$\frac{(1/T_{jM})_i}{(1/T_{jM})_o} = \frac{r_i^{-6}}{r_o^{-6}} \; (j = 1,2) \quad (4)$$

Table 1 shows the experimental homonuclear $^1H\{^1H\}$ NOEs for 5′-AMP in $D_2O$ and in [$^2H_6$]DMSO, whereas the experimental ratios of proton relaxation rates induced by $Gd^{III}$ for 5′-AMP in various $D_2O$–[$^2H_6$]DMSO solvent mixtures defined by the [$^2H_6$]DMSO mole fraction $x$[$^2H_6$]DMSO are shown in Table 2. The fact that the $T_{1M}^{-1}$ and $T_{2M}^{-1}$ ratios are very similar for the same proton in the same solvent composition indicates that the scalar part of the relaxation equations can indeed be neglected.

The experimental NOE enhancements and proton relaxation ratios can be used to define and compare the conformation of 5′-AMP in $D_2O$ and in [$^2H_6$]DMSO. This was done by comparison of the experimental data with calculated parameters for different conformational mixtures. The theoretical NOE enhancements, $f_8(s)$, for the H(8) proton due to irradiation of the furanose protons, as a function of the glycosidic torsion angle for the N and S furanose ring geometries, have been calculated elsewhere using equation (1).[13] The NOEs for each conformational mixture were calculated for the slow exchange conditions (case 1, $K \ll R_d$, where the $K$ values are the rate constants for internal motions)[17] and are described by equation (5), a generalization of equation (1) to a discrete

$$f_d(s) = \sum_\Omega P_\Omega f_d(s,\Omega) \quad (5)$$

**Table 1.** Nuclear Overhauser enhancements, $f_d(s)$ of H(8) and H(2) protons of 5′-AMP in different solvents, upon saturation of the sugar protons

| Solvent | Concentration (M) | $f_d(s)$ | Protons irradiated H(1′) | H(2′) | H(3′) | H(5′ + 5″) | H(4′) | Ref. |
|---|---|---|---|---|---|---|---|---|
| $D_2O$ | 0.05 | $f_8(s)$ | 4 | 20.5 | 9 | 6 | 0 | 11 |
| | | $f_2(s)$ [a] | 6.5 | 3 | 2 | 3 | 0 | 11 |
| [$^2H_6$]DMSO | 0.03 | $f_8(s)$ | 18 | 15 | 14 | | 0 | *b* |
| | | $f_2(s)$ | 0 | 5 | 4 | | 0 | *b* |

[a] Data for 5′-AMP deuteriated at the H(8) position. [b] This work.

**Table 2.** $Gd^{III}$-induced proton relaxation ratios ($R_i$) relative to H(5′,″), equivalent to 100 5′-AMP in $D_2O$–[$^2H_6$]DMSO

| $R_i$ [a] | $x$[$^2H_6$]DMSO | $t$/°C | H(8) | H(2) | H(1′) | H(2′) | H(3′) | H(4′) | H(5′,″) |
|---|---|---|---|---|---|---|---|---|---|
| $T_{1M}$ | 0 | 20 | 48 | 4 | 6 | 7 | 14 | 19 | 100 |
| | | 80 | 45 | 4 | 6 | 9 | 15 | 20 | 100 |
| | 1.0 | 20 | 8 | 3 | 6 | 12 | 18 | 23 | 100 |
| | | 80 | 10 | 3 | 7 | 14 | 21 | 26 | 100 |
| $T_{2M}$ | 0 | 20 | 49 | 1 | 6 | *b* | *b* | *b* | 100 |
| | 0.2 | 20 | 28 | 5 | 6 | *b* | *b* | *b* | 100 |
| | 0.5 | 20 | 14 | 4 | 6 | *b* | *b* | *b* | 100 |
| | 0.8 | 20 | 6 | 3 | 6 | *b* | *b* | *b* | 100 |
| | 1.0 | 20 | 6 | 3 | 6 | *b* | *b* | *b* | 100 |

[a] Spin–lattice ($T_{1M}$) or spin–spin ($T_{2M}$) relaxation ratios as indicated. [b] Not observed.

**Table 3.** Calculated proton $f_8(i)$ NOE values and relaxation ratios obtained from various conformational distributions

| Solvent | Distribution [a] | $f_8(1')$ | $f_8(2')$ | $f_8(3')$ | $f_8(5'+5'')$ | $R(8)$ | $R(2)$ | $R(1')$ | $R(5','')$ | $R$ factors [b] | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $D_2O$ | 1 | 3 | 21 | 8 | 11 | 61 | 2 | 10 | 100 | 0.05 (0.02) | 13 |
| [$^2H_6$]DMSO | 2 | 12 | 18 | 11 | 1 | 12 | 6 | 10 | 100 | 0.06 (0.01) | *c* |
| $D_2O$ | 3 | 7 | 15 | 5 | 6 | 65 | 2 | 10 | 100 | 0.10 (0.04) | 23 |
| | 4 | 5 | 19 | 8 | 4 | 55 | 3 | 10 | 100 | 0.02 (0.01) | *c* |
| [$^2H_6$]DMSO | 5 | 23 | 8 | 3 | 3 | 10 | 3 | 10 | 100 | 0.18 (0.01) | 23 |
| | 6 | 17 | 14 | 7 | 2 | 9 | 3 | 10 | 100 | 0.04 (0.04) | *c* |

[a] Distributions 1 and 2 are discrete glycosidic bond distributions ($\chi_N$, $\chi_S$) of (90°, 40°) and (80°, 160°). Distributions 3 (40% C + 60% D), 4 (40% E + 60% F), 5 (40% G + 60% H), and 6 (40% I + 60% J) are continuous distributions defined in Figure 1. [b] $R$ factors[22] for calculated and observed proton NOE and relaxation ratios (in parentheses). [c] This work.

set of conformations, where $P_\Omega$ is the fraction of molecules in conformation $\Omega$, or to a continuous path described by internal variables $\Omega$, equation (6), where $P(\Omega)$ is the distribution function for the variable $\Omega$. The $Gd^{III}$-induced proton relaxation ratios were obtained for the various nucleotide conformations using the BURLESK program [6] and equation (4).

$$f_d(s) = \int f_d(s,\Omega)P(\Omega)d(\Omega) \quad (6)$$

Two conformational models were used. The first one consists of a discrete two-site glycosidic $\chi$ angle distribution ($\chi_N$, $\chi_S$), where $\chi_N$ and $\chi_S$ are associated with the N(C(3'), endo) and S(C(2'), endo) furanose ring conformations.[19] On the basis of an observed correlation of furanose and base conformations,[20] the $\chi_N$ and $\chi_S$ populations, $P_N$ and $P_S$, were assumed to be equal to the populations of the N and S furanose puckered conformations. These populations were derived from analysis of vicinal coupling constants:[8,20] 40% for the N population was used for 5'-AMP in $D_2O$ and in [$^2H_6$]DMSO. The *gauche–gauche* conformation about the C(4')–C(5') bond was chosen as it dominates the equilibrium about this bond.[8,21] This two-state model was preferred to an alternative available in the literature [9] because in previous work [10,15] we have shown that our model defines a conformational average, which, while it interprets spin–lattice relaxation times and NOE values, is in much better agreement with other n.m.r. techniques. Within the approximations of this model, the following glycosidic angle values gave a minimum variance (*R* factor [22]) between experimental and calculated H(8) NOE enhancements and $Gd^{III}$-induced proton relaxation ratios: ($\chi_N$, $\chi_S$) = (90°, 40°) for 5'-AMP in $D_2O$ and ($\chi_N$, $\chi_S$) = (80°, 160°) in [$^2H_6$]-DMSO (distributions 1 and 2 of Table 3). Thus the glycosidic bond conformation of 5'-AMP is restricted within the *anti*-region in $D_2O$ and is in a *syn–anti* equilibrium in [$^2H_6$]DMSO.

As a consequence of residual base stacking, the H(2) NOE enhancements of 5'-AMP in $D_2O$ are dominated by intermolecular relaxation with H(8) of another molecule.[11] This problem is minimized by measurement of H(8) NOE values in 5'-AMP deuteriated at H(8) (Table 1). Large $f_2(1')$ and small $f_2(5' + 5'')$ values indicate a predominant *anti*-conformation of 5'-AMP in $D_2O$. In [$^2H_6$]DMSO, where intramolecular stacking should not occur, the value $f_2(1') = 0$ indicates that the value $\chi$ *ca.* 30° is not highly populated. However, because the intermolecular contributions to H(2) relaxation can be important, the NOE conformational study of 5'-AMP is based on the H(8) enhancements. The H(2) data support the conclusions based on the H(8) data.

The experimental NOE values and relaxation ratios were also interpreted using continuous angular distribution of $\chi_N$ and $\chi_S$ values, derived from published potential-energy curves [23] by Boltzmann's statistics (Figure 1). For instance, the NOE values were calculated using equation (7) where [N] and [S] are the N and S pucker populations and $P_N(\chi_N)$ and $P_S(\chi_S)$ are the glycosidic angle distributions mentioned above.

$$f_8(s) = [N]\int_0^{360} f_8(s,\chi_N)P_N(\chi_N)d\chi_N + [S]\int_0^{360} f_8(s,\chi_S)P_S(\chi_S)d\chi_S \quad (7)$$

Distributions 3—6 were considered (see Table 3 and Figure 1). Distribution 3, derived from potential-energy curves that take both van der Waals and coulombic interactions into account, and distribution 5, based on van der Waals interactions alone, give a reasonably good agreement for 5'-AMP in $D_2O$ and in [$^2H_6$]DMSO, respectively. The fits of theoretical and experimental data can be dramatically improved if angular distributions about $\chi_N = \chi_S$ *ca.* 120° with 30% weight are added to the previous distributions (distributions 4 and 6). This is equivalent to considering that the potential-energy minima at $\chi$ *ca.* 120° are lower than implied by Figure 1A and B. In this method, therefore, the solvent does not affect the general shape of the potential-energy curve for the isolated molecule but only the relative depths of its minima.

The results of potential-energy calculations on 5'-AMP in the gas phase [23] interpret the stabilization of the *anti-gg* conformation in terms of strong coulombic attractions of the negatively charged phosphate group with the positively charged atoms C(8) and H(8) of the base and the destabilization of the *syn-gg* conformation as a consequence of the predominant coulombic repulsion between the oxygen atoms of the phosphate group and the atom N(3) of the base. The observed increase of the population of the *syn*-base conformation of 5'-AMP in [$^2H_6$]DMSO when compared with $D_2O$ cannot be interpreted in terms of bulk solvent effects on those coulombic interactions, because the smaller macroscopic dielectric constant of DMSO relative to $D_2O$ [24] would lead to stronger coulombic interactions in DMSO and to a more rigid base conformation. The opposite situation that is observed can be explained, in part, by the use of microscopic dielectric constants [25] for the solvents, which is justified for ion pairs not separated by the solvent, and which take into account the screening effect of solvent molecules oriented by the negative charge of the phosphate oxygen atoms on the coulombic interactions between the charged centres of 5'-AMP. The more polar DMSO molecules [24] would give a larger microscopic dielectric constant and therefore weaker coulombic interactions.

However, the strong specific solute–solvent interactions must play a very important role in stabilizing certain conformations of a flexible molecule such as 5'-AMP. In this respect the structure of two solvents, which are very different, plays an important part. DMSO molecules certainly interact with the ribose hydroxy groups.[26] If their dipoles are oriented by the phosphate oxygen atom and the N(3) atom of the base [Figure 2, (II)] the interaction of these two molecules will destabilize the *anti- versus* the *syn*-base conformation. Water is a much more structured solvent than DMSO [17] and generally imposes its structure on the solute more, due to the directional nature of its hydrogen bonds, therefore conferring to it a more rigid conformation. In the case of 5'-AMP the stabilization of the *anti*-base conformation by water can be explained by the formation of a bridge between the phosphate group and the base through a water molecule hydrogen bonded to the phosphate oxygen and the base N(3) atom [Figure 2, (I)], or by a better accommodation of 5'-AMP in an *anti*-base conformation in the water structure without the necessity of modification of the hydrogen-bonded solvent structure.[27–29]

The smooth decrease of the H(8) $Gd^{III}$-induced relaxation ratio with the increase in the [$^2H_6$]DMSO mole fraction in $D_2O$–[$^2H_6$]DMSO solvent mixtures (Table 2) favours the explanation based in the existence of a water bridge, which is gradually destroyed in the mixed solvent owing to competition with the [$^2H_6$]DMSO solvent molecules. The second hypothesis would lead to a sharp decrease of the H(8) relaxation ratio to the value in [$^2H_6$]DMSO in small [$^2H_6$]DMSO mole fractions, because a small percentage of [$^2H_6$]DMSO can completely change the water structure, owing to the strong water–[$^2H_6$]DMSO interactions.[30,31] This effect has clearly been seen in a study of the frequency dependence of the near-i.r. overtone band of water at 5 180 $cm^{-1}$ in water–DMSO mixtures.[32] This band shows a frequency decrease up to a 30% DMSO mole fraction, as a result of the disruption of the water-hydrogen-bonded structure by DMSO molecules, followed by a constancy of the frequency of this band for a

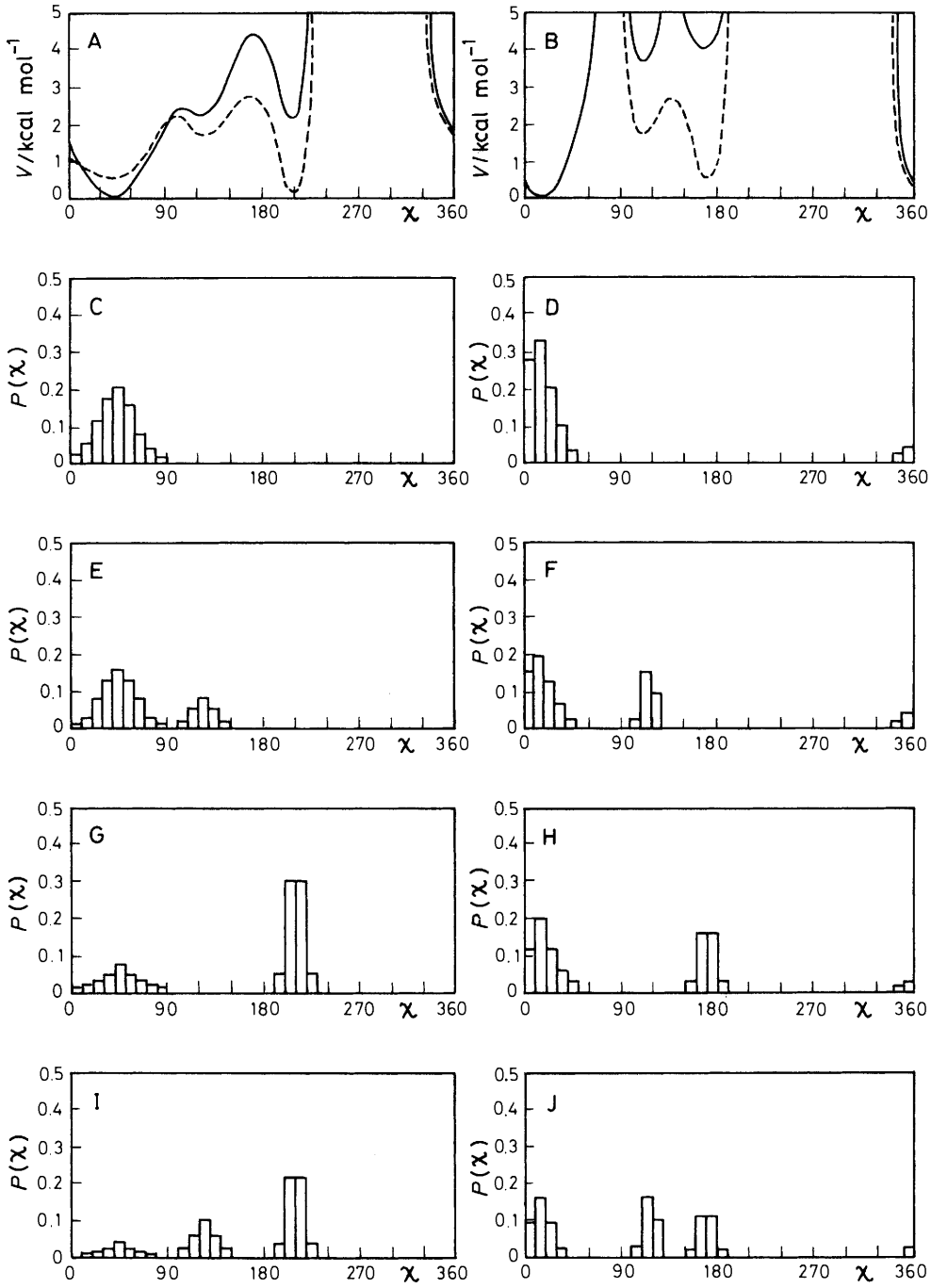


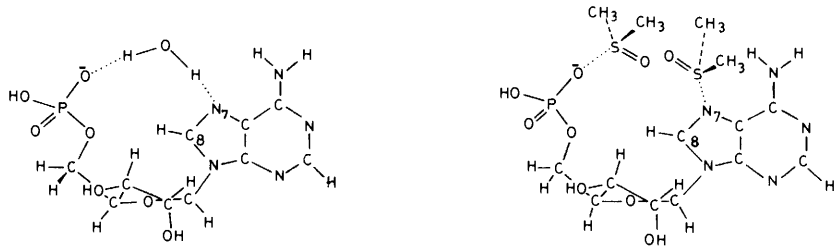

**Figure 1.** Calculated potential-energy curves for 5′-AMP from the literature [23] (A, B) and χ-angle distributions derived from them (C—J); A, C, E, G, and I (N-pucker); B, D, F, H, and J (S-pucker); in A and B the continuous curve (——) represents the variation of non-bonded (van der Waals' + coulombic) energy and the dashed curve (— — —) represents the variation of non-bonded van der Waals' energy alone. $P(\chi)$ is the probability of χ angles in 10° intervals

DMSO mole fraction higher than 30%, characteristic of a new water structure in the mixed solvent.

We must point out that the two solvation schemes of Figure 2 are purely speculative, and that their only value is the capacity to rationalize experimental data.

## Conclusions

This work is another example of the importance of the combined use of different n.m.r. methods in the definition of the solution conformation of a flexible molecule such as 5′-AMP. The combined use of nuclear Overhauser effects and lanthanide relaxation probes allowed the study of the adenine base conformation of 5′-AMP in the solvents $D_2O$ and [$^2H_6$]DMSO to be undertaken in great detail. Comparison of the measured n.m.r. parameters, which are dependent on conformation, with the calculated values for different conformational mixtures derived from or related to potential-energy calculations, showed that the average conformation of 5′-AMP is very solvent dependent: a restricted *anti*-base conformation in



(I) (II)

**Figure 2.** Proposed scheme of interaction of adenine N(7) atom and phosphate negatively charged oxygen of 5'-AMP with solvent water (I) and dimethyl sulphoxide (II) molecules

$D_2O$ and a *syn–anti* equilibrium in [$^2H_6$]DMSO. Of course these conformational mixtures are not unique solutions but are the simplest one compatible with the models used in this work.

The role of the solvent in helping to stabilize a given solute structure is not a simple bulk effect on coulombic interactions between charges in different parts of the solute molecule, but involves specific solute–solvent interactions, which may affect the solute conformational energy in two different ways, the relative importance of which is difficult to estimate. The solvent can modify the coulombic interactions between the charged centres of the solute through a screening effect that arises from the orientation of the solvent molecules by the solute charges. The importance of this effect on the coulombic interactions is evidenced by the absence of a conformational change of adenosine when the solvent is changed from $D_2O$ to [$^2H_6$]DMSO. Alternatively, the solvent, through its donor–acceptor properties, can specifically interact with certain atoms of the solute, contributing to its conformational energy with hydrogen-bonded interactions, steric repulsions, *etc.* In this study two such schemes were proposed.

In the light of the present conclusions on the conformation, the original lanthanide-induced shifts [14] can be reinterpreted. This will be the object of Part 2 of this series of publications.

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