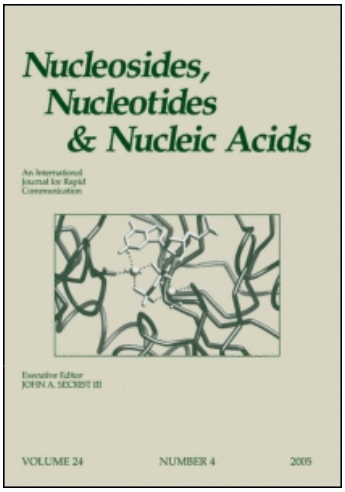


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# Solvent Dependent Changes in the Proton NMR Spectra of 2'-Deoxyadenosine and Its Derivatives

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# SOLVENT DEPENDENT CHANGES IN THE PROTON NMR SPECTRA OF 2'-DEOXYADENOSINE AND ITS DERIVATIVES

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**Abstract:** Intramolecular hydrogen bonding in four purine-2'-deoxyribosides containing free sugar hydroxyls has been observed in $CDCl_3$. This bond results in the appearance of a *double doublet* for H-1' of the C-6 substituted $\beta$-anomers. On increasing the solvent polarity, as in DMSO-$d_6$, the signal appears as triplet, which is typical of previously reported NMR spectra of $\beta$-anomers in $D_2O$ and DMSO-$d_6$. Solvent polarity, therefore plays a significant role in determining the splitting patterns of the anomeric proton in these compounds.



Intramolecular hydrogen bonding between the C-5' hydroxyl group and N-3 of the purine has been reported for purine deoxyribosides in the solid state, and numerous X-ray crystal structures indicating these have been reported in the literature.[1] In the solution state also, such hydrogen bonding has been reported, but for purine ribosides.[2,3,4] This is due to the fact that purine nucleosides can be appropriately derivatized in order to increase their otherwise limited solubility in relatively non-polar solvents. In two such typical examples, the 2',3'-acetonide was synthesized.[3,4] In these cases, both the conformational freedom of the furanose ring and the number of hydrogen bonding sites are reduced. Further, the changes observed in the anomeric H-1' resonance of the purine ribosides on changing solvent polarity are limited to changes in the doublet for this proton.

To our knowledge, $^1H$ NMR data in non-polar solvents for purine-2'-deoxyribosides with free sugar hydroxyl groups have not been reported. Interestingly, the H-1' of these compounds show remarkably different splitting patterns in solvents of differing polarities. The $\alpha$- and $\beta$-anomers of purine-2'-deoxyribosides have commonly been distinguished based upon the appearance of a *double doublet* or a *triplet*, respectively for

H-1'.[5,6,7,8] We report here that H-1' of C-6 substituted β-anomers also appears as a *double doublet* when the NMR solvent is of relatively low polarity ($CDCl_3$) and intramolecular hydrogen bonding between the C-5' hydroxyl group and N-3 of the purine occurs.

In all of the cases examined by NMR spectroscopy in the literature, the solvents used in determining the anomeric configuration were either $D_2O$ or DMSO-$d_6$. In our study, we found that the purine deoxyribosides are sufficiently soluble in $CDCl_3$ to obtain fourier transformed $^1H$ NMR spectra. Spectra (Figure 2) for compounds **1** to **5** (Figure 1) were obtained in both $CDCl_3$ and DMSO-$d_6$.[5] In the latter solvent, compounds **1** to **4** exhibit the expected *triplet*,[6,7,8,9] whereas in $CDCl_3$ they exhibit a *double doublet* for H-1'. In addition, as is explained later, the C-5' protons show a splitting pattern that is consistent with the 5'-OH to N-3 hydrogen bond, in $CDCl_3$. In the absence of any second order couplings, the observed changes in the multiplicity of H-1' can be attributed solely to changes in the sugar ring conformation. Such changes, if any, would also be reflected in the splitting patterns of the other sugar ring protons. In $CDCl_3$ each H-2' appears as a *septet* and a *double doublet*, respectively, whereas in DMSO-$d_6$, these protons appear as a *quintet* and a *doublet of doublet of doublets*. In addition, in $CDCl_3$ H-4' appears as a *singlet* and H-3' appears as a *doublet* and the dihedral angle of one H-2' to H-3' is nearly 90°. On using DMSO-$d_6$, H-4' and H-3' appear as a *quartet* and a broadened signal, respectively. The effects observed on changing solvent polarity are consistent with a change in the sugar conformation, the cause of which is the formation of an intramolecular hydrogen bond between the 5'-OH and N-3. Such a hydrogen bond is also expected to affect each H-5' resonance. As is explained later this is indeed the case. In $CDCl_3$, in order to establish the intramolecular hydrogen bond a *syn*-conformation at C-1' and a 2'-*endo* sugar pucker are essential for close proximity of the hydrogen bonding atoms. Under these circumstances, H-1' has different dihedral angles to each H-2'. In DMSO-$d_6$, on the other hand, the hydroxyl protons can hydrogen bond to solvent, resulting in a predominance of the 3'-*endo* conformation, wherein the dihedral angles of H-1' with each H-2' are nearly equal resulting in the observed splitting patterns (*vide supra*).

Due to the lower solubility of **1** and **2** in $CDCl_3$ small amounts of MeOH-$d_4$ were added to enable the spectra to be observed (50 μL for **1** and 90 μL for **2** in 0.8 mL $CDCl_3$). Under these conditions **1** [Figure 2(a)] and **2** (data not shown) exhibit a *double doublet* for H-1'. The effect of solvent on the H-1' multiplicity of **3** is shown in Figures 2(b), (c) and (d). In $CDCl_3$, H-1'

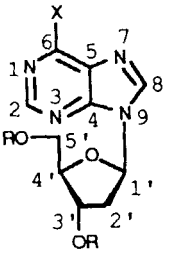


**Figure 1**

| | |
|---|---|
| **1**: X = -$NH_2$ | R = -H |
| **2**: X = -OH (as the amide) | R = -H |
| **3**: X = -Cl | R = -H |
| **4**: X = -F | R = -H |
| **5**: X = -OH (as the amide) | R = -$OCOCF_3$ |
| **6**: X = -$NH_2$ | R = -$OCOCH_3$ |



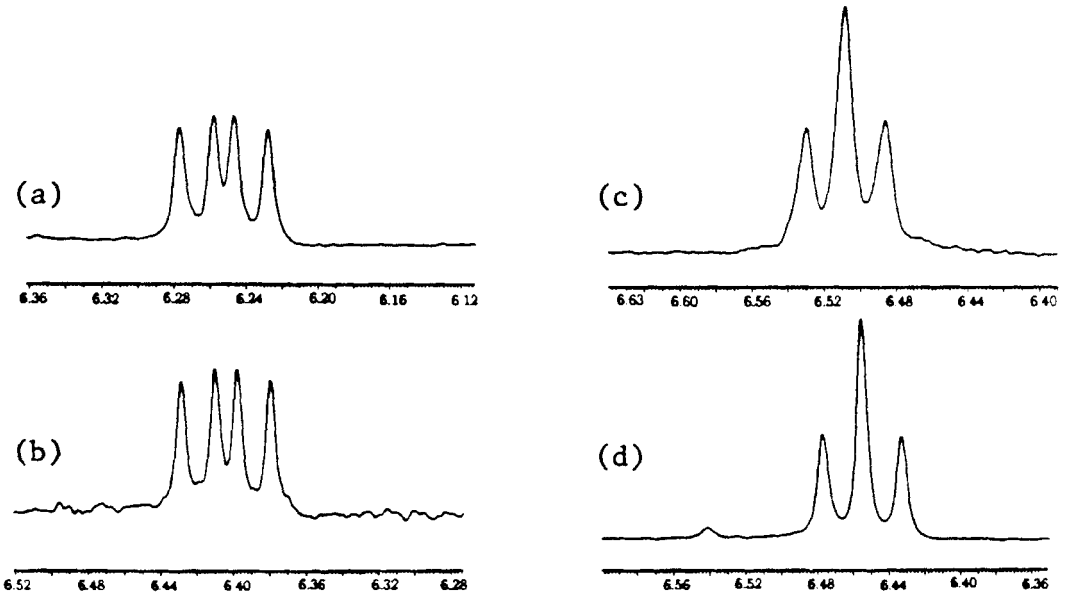


**Figure 2**

*(a) H-1' of* ***1*** *in* $CDCl_3$*; H1' of* ***3*** *in (b)* $CDCl_3$ *(c) MeOH-*$d_4$ *(d) DMSO-*$d_6$

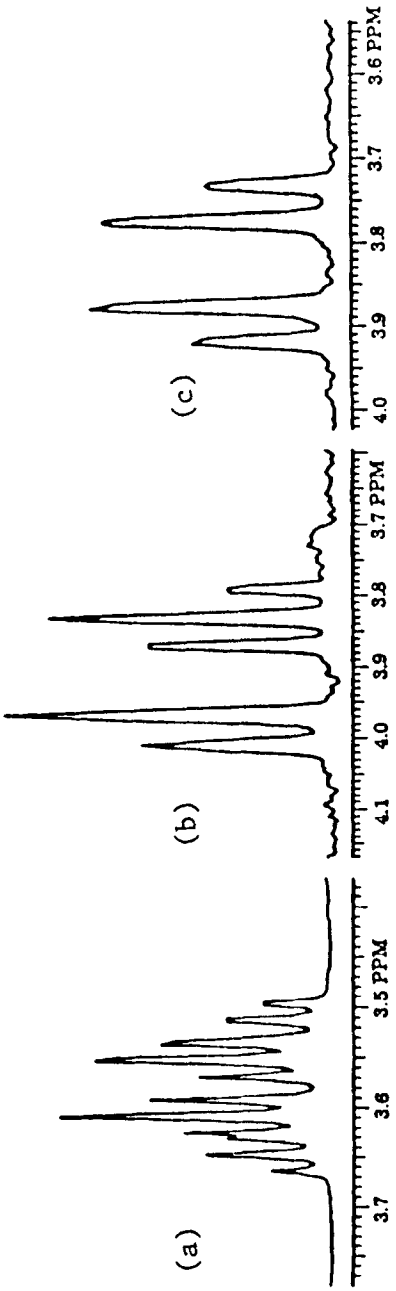


**Figure 3**

*Diastereotopic H-5' of* **3** *in : (a) DMSO-$d_6$ (b) $CDCl_3$ (c) $CDCl_3$+MeOH-$d_4$*

appears as a *double doublet*, in MeOH-$d_4$ the central signals approach coalescence and in DMSO-$d_6$ the signal is a distinct *triplet*.

Figure 3(a) shows the multiplicity of the C-5' protons of **3** in DMSO-$d_6$, whereas Figures 3(b) and (c) show the multiplicities in $CDCl_3$ and $CDCl_3$ with a few drops of MeOH-$d_4$ added, respectively. In $CDCl_3$, the coupling of each H-5' with H-4' is very small (H-4' appears as a *singlet* at $\delta$ 4.22 ppm) and the individual C-5' protons appear as a *doublet* and a *triplet*, respectively. In a rigid framework established by the 5'-OH to N-3 hydrogen bond, one H-5' has a large dihedral angle with the hydroxyl proton (~180°, J = 10.8 Hz), whereas the other has a smaller angle (~40°, and in the case of **4** the smaller H-5' to OH coupling is observed, J = 2.4 Hz). Addition of a few drops of MeOH-$d_4$ results in loss of the hydroxyl proton coupling and the resulting signals are a pair of *doublets* ($J_{gem}$ = 12.7 Hz). Also consistent with this hydrogen bond, in $CDCl_3$ the 5'-OH appears downfield ($\delta$ 5.0 ppm) as a *doublet* ($J_{OH,\ H5'}$ = 10.8 Hz), while the 3'-OH appears at $\delta$ 1.9 ppm. In DMSO-$d_6$, on the other hand, the 5'-OH appears as a *triplet*, indicating its coupling with both C-5' protons. Further, both the 3'- and the 5'-OH appear downfield ($\delta$ 5.4 and 4.88 ppm, respectively)[10], presumably due to hydrogen bonding of both hydroxyl protons to the solvent. Compound **4** shows identical spectral features as **3** in both $CDCl_3$ and DMSO-$d_6$. Table 1 shows the chemical shifts and coupling constants of H-1' in **1** to **6**.

Robins and Robins, in their initial paper describing anomer determination by $^1$H NMR, have mentioned that substitutents on the heterocycle or the sugar could alter the sugar pucker.[6,9] This indeed is the case. Compounds **5** and **6** show a *triplet* for H-1'. Acetylation or silylation of the hydroxyl groups in **4** and silylation of the hydroxyl groups in **2** also results in a *triplet* for H-1' in $CDCl_3$, whereas the parent compounds exhibit a *double doublet*.[10] It is quite possible that inhibition of the intramolecular hydrogen bonding results in a 3'-*endo* conformation in these compounds.

Thus, in a solvent such as chloroform with a low dielectric constant ($\varepsilon$ = 5.62), intramolecular hydrogen bonding causes a predominance of the 2'-*endo* conformation, resulting in the appearance of a *double doublet* for H-1'. On increasing the dielectric constant to that of DMSO ($\varepsilon$ = 46.68), intermolecular hydrogen bonding apparently becomes preferred, and a *triplet* is observed for H-1' in DMSO-$d_6$. In addition to a variety of factors such as anomeric configuration, substitution on the sugar hydroxyls and the purine, attention must be paid to another variable, namely solvent polarity, when using the H-1' splitting patterns for anomer assignment in C-6 substituted purine-2'-deoxyribosides.

**Table 1**: *Chemical shifts and coupling constants for H-1' in 1-6.**

| COMPOUND | SOLVENT | MULTIPLICITY | SHIFT (δ ppm) | J (Hz) |
|---|---|---|---|---|
| **1** | DMSO-$d_6$ | triplet | 6.34 | 7.8 |
| | $CDCl_3$ | double doublet | 6.25 | 9.2, 5.5 |
| **2** | DMSO-$d_6$ | triplet | 6.40 | 6.6 |
| | $CDCl_3$ | double doublet | 6.26 | 8.3, 5.8 |
| **3** | DMSO-$d_6$ | triplet | 6.46 | 6.5 |
| | $CDCl_3$ | double doublet | 6.40 | 9.3, 5.6 |
| **4** | DMSO-$d_6$ | triplet | 6.48 | 6.5 |
| | $CDCl_3$ | double doublet | 6.41 | 9.5, 5.5 |
| **5** | DMSO-$d_6$ | triplet | 6.41 | 6.6 |
| | $CDCl_3$ | triplet | 6.42 | 7.1 |
| **6** | $CDCl_3$ | triplet | 6.40 | 6.8 |

*Spectra were recorded at 300 MHz.



**Note :** Compounds **1-6** were prepared through published procedures.[12,13]
Compound **1** : 2 mg were dissolved in 0.8 mL $CDCl_3$ and 50 μL MeOH-$d_4$, 128 scans were acquired with a line broadening of 0.5.
Compounds **3** and **4** : 1 mg of each compound was dissolved in 0.8 mL $CDCl_3$, 128 scans were acquired with a line broadening of 0.5.
Compound **2** : A saturated solution was prepared in 0.8 mL $CDCl_3$ and 90 μL MeOH-$d_4$. Usually a large number of scans are needed for a good spectrum with a line broadening of 0.5.
Compounds **1** to **4** : 2 mg of each was dissolved in 0.8 mL DMSO-$d_6$ 128 scans were acquired with a line broadening of 0.5.
No resolution enhancement was used while acquiring data for these compounds and all spectra were obtained at room temperature.

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1. This work was supported in part, by grant CA 22985 from the National Cancer Institute, DHHS, to R.E.L.
For some typical examples see:

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5. As typical examples the entire $^1$H NMR spectra of **3** and **4** are:
**3** in DMSO-$d_6$: 2.36 (ddd, 1H$_{2'a}$, J = 13.4, 6.3, 4.0 Hz); 2.75 (quint, 1H$_{2'b}$, J = 6.3 Hz); 3.50-3.70 (m, 2H$_{5'}$); 3.88 (q, 1H$_{4'}$, J = 4.3 Hz); 4.44 (br, 1H$_{3'}$); 4.88 (t, O*H*$_{5'}$, J = 5.3 Hz); 5.40 (d, O*H*$_{3'}$, J = 4.0 Hz); 6.46 (t, 1H$_{1'}$, J = 6.6 Hz); 8.79 (s, 1H$_2$); 8.88 (s, 1H$_8$).
**4** in DMSO-$d_6$: 2.36 (ddd, 1H$_{2'a}$, J = 13.4, 6.2, 4.0 Hz); 2.75 (quint, 1H$_{2'b}$, J = 6.2 Hz); 3.50-3.70 (m, 2H$_{5'}$); 3.88 (q, 1H$_{4'}$, J = 4.4 Hz); 4.44 (br, 1H$_{3'}$); 4.88 (t, O*H*$_{5'}$, J = 5.4 Hz); 5.40 (d, O*H*$_{3'}$, J = 4.0 Hz); 6.48 (t, 1H$_{1'}$, J = 6.7 Hz); 8.79 (s, 1H$_2$); 8.88 (s, 1H$_8$).
**3** in $CDCl_3$: 1.90 (d, O*H*$_{3'}$, J = 3.2); 2.39 (dd, 1H$_{2'a}$, J = 13.5, 5.5 Hz); 3.04 (septet, 1H$_{2'b}$, J = 5.3 Hz); 3.70 (t, 1H$_{5'a}$, J = 10.8 Hz); 3.95 (d, 1H$_{5'b}$, J = 12.7 Hz); 4.22 (s, 1H$_{4'}$); 4.81 (d, 1H$_{3'}$, J = 4.8 Hz); 5.00 (d, O*H*$_{5'}$, J = 10.8 Hz); 6.39 (dd, 1H$_{1'}$, J = 9.3, 5.6 Hz); 8.21 (s, 1H$_2$); 8.74 (s, 1H$_8$).
**4** in $CDCl_3$: 1.90 (d, O*H*$_{3'}$, J = 3.2); 2.40 (dd, 1H$_{2'a}$, J = 13.4, 5.5 Hz); 3.04 (septet, 1H$_{2'b}$, J = 5.3 Hz); 3.78 (t, 1H$_{5'a}$, J = 11.1 Hz); 3.95 (dd, 1H$_{5'b}$, J = 12.6, 2.4 Hz); 4.22 (s, 1H$_{4'}$); 4.81 (br, 1H$_{3'}$); 5.10 (dd, O*H*$_{5'}$, J = 10.9, 2.4 Hz); 6.40 (dd, 1H$_{1'}$, J = 9.5, 5.5 Hz); 8.18 (s, 1H$_2$); 8.63 (s, 1H$_8$).
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9. The only instance where H-1' appeared as a *double doublet* in the $\beta$-anomers, was in the C-8 amino substituted 2'-deoxyadenosine cases. In these cases also, the $^1$H NMR spectra were obtained in $D_2O$/DMSO-$d_6$. Since H-1' of 2'-deoxyadenosine produces only a *triplet* in these solvents, it is possible that the C-8 substituent causes a significant alteration of the sugar pucker as well as the conformation around the glycosidic bond

(*syn* or *anti*), thus resulting in the observed multiplicity. Long, R. A.; Robins, R. K.; Townsend, L. B. *J. Org. Chem.* **1967**, *32*, 2751-2756.

10. Chemical shift (coupling constant) in DMSO-$d_6$ for:

| | 5'-OH | 3'-OH |
|---|---|---|
| **1** | 5.23 (6.4 Hz) | 5.29 (3.6 Hz) |
| **2** | 5.00 (5.7 Hz) | 5.30 (2.2 Hz) |
| **3** | 4.88 (5.3 Hz) | 5.40 (4.0 Hz) |
| **4** | 4.88 (5.7 Hz) | 5.40 (4.0 Hz) |

11. H-1' in the :

3',5'-diacetate derivative of **4** is at $\delta$ 6.50 ppm; $J_{1',2'}$ = 6.3 Hz

3',5'-di-$^t$BuMe$_2$Si derivative of **4** is at $\delta$ 6.51 ppm; $J_{1',2'}$ = 6.4 Hz

3',5'-di-$^t$BuMe$_2$Si derivative of **2** is at $\delta$ 6.40 ppm; $J_{1',2'}$ = 6.4 Hz

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