# Determination of Glycosidic Bond Conformations of Pyrimidine Nucleosides and Nucleotides using Vicinal Carbon–Proton Coupling Constants

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Measurements have been made of $^3J$(C-6,H-1′) and $^3J$(C-2,H-1′) magnitudes of pyrimidine nucleosides and nucleotides in solution by natural abundance, $^{13}$C n.m.r. spectroscopy and the results have been interpreted in terms of the glycosidic bond conformations of the nucleic acid derivatives. Based on *X*-ray crystal structure results a four-state conformational model for the glycosidic bond is introduced consisting of an equilibrium between two *syn* and two *anti* conformations; each *syn* or *anti* conformation is symmetrically related to the C-1′–H-1′ bond direction and appropriate *syn* and *anti* conformations are symmetrically related by 180°. It is shown that a quantitative estimate of the glycosidic bond conformation may be determined from the sum of observed coupling constants [*i.e.*, $\Sigma J$ = $J$(C-6,H-1′) + $J$(C-2,H-1′)] and that the equilibrium composition of *syn* and *anti* conformers may be determined from the difference in observed coupling constants, *i.e.*, $\Delta J$ = $J$(C-6,H-1′) − $J$(C-2,H-1′). Results for uridine and cytidine derivatives are discussed.

The glycosidic bond *syn* ⇌ *anti* conformer equilibrium of base with respect to sugar ring is of fundamental importance in determining the conformations of nucleic acid derivatives in solution. Representative *syn* and *anti* conformations for pyrimidine derivatives are shown diagrammatically for uridine in Figure 1. The majority of *X*-ray structure determinations of nucleosides, nucleotides, oligonucleotides, and polynucleotides show that purine and pyrimidine base rings exist in the *anti* conformation.[1–4] The preference for the *anti* conformation has been rationalised by semi-empirical potential-energy calculations of 5′-nucleotides which indicated a stabilising interaction between exocyclic phosphate group and the base ring.[5] The *syn* conformation is generally observed when the base ring contains a bulky substituent (at C-8 for purine and C-6 for pyrimidine) or by stabilisation by N-3 ··· OH-5′ intramolecular hydrogen bonding in purine derivatives.[6] These results indicate that the *anti* conformers of both purine and pyrimidine nucleosides are intrinsically more stable than the *syn* conformers; this conclusion is confirmed by potential-energy calculations.[7]

Many n.m.r. methods have been used to determine the glycosidic bond *syn* ⇌ *anti* conformational equilibrium using chemical-shift changes,[8–11] long-range proton spin coupling in pyrimidine nucleosides[12] and nucleotides,[13,14] lanthanide ion probe techniques,[15–17] nuclear Overhauser enhancements,[18–22] proton spin-lattice relaxation times,[23–26] and $^3J$($^{13}$C,H-1′) measurements.[27–30] The two methods based on relaxation phenomena (n.O.e. and $T_1$) have provided quantitative descriptions of glycosidic bond conformations, especially for purine derivatives, where it was concluded that significant proportions of the *syn* conformer exist in equilibrium with the *anti* conformer. By using n.O.e. measurements on pyrimidine nucleosides it was suggested that, although uridine (U) exists in a predominant *anti* conformation, i-U has a predominant *syn* conformation and increasing proportions of the *syn* conformer are found for cytidine (C) and i-C.[31] On the other hand, analysis of proton relaxation times of purine and pyrimidine 5′-nucleotides were interpreted[26] in terms of preferred *syn* conformations with χ *ca.* 70 ± 10°. In general, n.O.e. and $T_1$ measurements indicate greater proportions of the *syn* conformer compared with conclusions based on δ and *J* criteria where predominant *anti* conformations are indicated. As glycosidic bond conformations are important for determining the over-all conformations of nucleosides and nucleotides, a study of the glycosidic bond conformations of pyrimidine derivatives has been made in this work based on analysis of vicinal carbon–proton coupling constants $^3J$(C-6,H-1′) and $^3J$(C-2,H-1′). The method is independent of previous determinations based on δ and *J* criteria and relaxation phenomena and should provide a way to compare results from different methods.

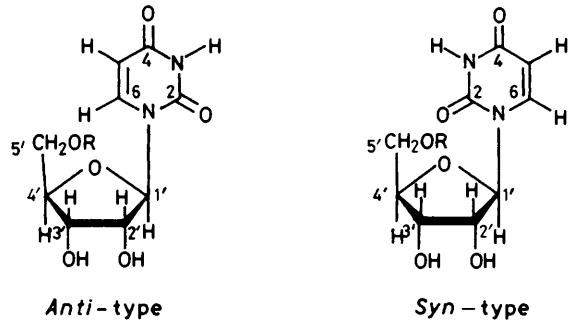


**Figure 1.** Diagrammatic representation of glycosidic bond *syn* and *anti* conformations of pyrimidine nucleosides (uridine)

Using uracil derivatives $^{13}$C-enriched at C-2, Lemieux and co-workers[27,28] showed that C-2,H-1′ vicinal carbon–proton coupling between the base and sugar of pyrimidine nucleosides follows an approximate Karplus-type dependence. It was suggested[30] that both C-2,H-1′ and C-6,H-1′ are needed to determine the glycosidic bond conformation because one $^3J$ magnitude cannot be used to differentiate between *syn* and *anti* conformers for $^3J \leqslant 5$ Hz. Until recently a quantitative analysis of the glycosidic bond conformation was hampered because there was no information on the appropriate Karplus relations to be used for coupling in both molecular fragments. By using $^{13}$C n.m.r. measurements of a number of cyclonucleosides, Karplus relations were determined[32] for vicinal coupling in the C-2,H-1′ and C-6,H-1′ molecular fragments of uridine derivatives, permitting a quantitative analysis of the glycosidic bond conformation to be developed.

Two conformational models for analysis of proton–carbon vicinal coupling constants are investigated. Both models, based on *X*-ray crystal structure results, assume a four-state conformational equilibrium between *syn* and two *anti* conformations. One model utilises average conformational angles derived from crystal structures but suffers because only two of

the four conformer populations can be determined from the two observed proton–carbon coupling constants. The second conformational model assumes that the *syn* and *anti* conformations are symmetrically related which enables both the conformational angles and equilibrium compositions to be determined. From $^{13}C$ n.m.r. measurements on a number of pyrimidine nucleosides and nucleotides it is found that most exhibit a preference for the *anti* conformation, although differences in conformational equilibria are observed between ribo- and deoxyribo-derivatives, or as a result of 2′-, 3′-, or 5′-phosphorylation of ribo-derivatives. Substitution of ribose rings by 2′,3′-*O*-isopropylidene rings or 2′,3′-cyclic phosphate groups causes flattening of the sugar ring which in turn results in approximately equal proportions of *syn* and *anti* conformers. As expected, substitution at C-6 of the base ring by a bulky group causes the *syn* conformer to predominate. Although these results are not expected, they show that the $^{13}C$ method is consistent with previous work and has the advantage that it leads to quantitative results that can be applied to both uridine and cytidine derivatives.

## Experimental

*Materials and Methods.*—Pyrimidine nucleosides and nucleotides were purchased from Sigma Chemical Company. The 50 MHz $^{13}C$ n.m.r. spectra were observed with a JEOL FX-200 Fourier transform spectrometer locked on $D_2O$ (or [$^2H_6$]DMSO) used as the solvent. Proton-coupled spectra were observed under gated decoupling conditions over 2 000 Hz sweep width using 8K data points and the resulting interferogram was calculated with zero-filling and resolution enhancement[33] to a data resolution of 0.12 Hz per point. Proton coupling on quaternary carbon atoms (C-2, C-4) was observed with short pulses (30°), long delays (*ca.* 4 s) accumulating *ca.* 10K transients (overnight) for 0.2M solutions and up to 60K transients (weekend) for the more dilute solutions (*ca.* 0.05M). Magnitudes of $^1J$(C-1′,H-1′), $^3J$(C-2,H-1′), and $^3J$(C-6,H-1′) for the pyrimidine nucleosides and nucleotides are summarised in Table 1.

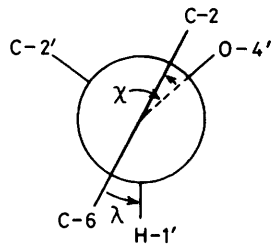


**Figure 2.** Definition of torsion angle λ (C-6–N-1–C-1–H-1′) in terms of glycosidic bond conformational angle χ (C-2–N-1–C-1′–O-4′)

## Discussion

(1) *Notation.*—A number of nomenclature systems have been devised for the glycosidic bond conformation[3] but the definition in which the *syn* conformation has the range 0 ± 90° and the *anti* conformation has the range 180 ± 90° recommended by IUPAC[34] has been used in this work. For convenience of analysis of $^3J(^{13}C,H\text{-}1')$ magnitudes the angle between sugar ring C-1′–H-1′ and pyrimidine ring C-2–N-1 and C-6–N-1 bonds is important so an angle λ is defined as the angle between the C-6–N-1 and C-1′–H-1′ bonds as shown in Figure 2. The relationship between λ and χ is given by equation (1).

$$\lambda \simeq (60 - \chi) \quad (1)$$

The glycosidic bond conformation of pyrimidine derivatives has previously been defined for the convenience of $^1H$ n.O.e. measurements using an angle τ between C-6–N-1 and C-1′–H-1′ bonds.[20] The angles λ and τ are equal in magnitude but opposite in sign.

(2) *Crystal Structures.*—Analysis of many crystal structures shows that the *anti* conformation is overwhelmingly preferred for nucleosides, nucleotides, and polynucleotides,[1–4] although some examples of *syn* conformations are known.[5, 35–38] All 76 base rings of yeast $tRNA^{Phe}$ also exhibit the *anti* conformation.[4] The results for pyrimidine derivatives are summarised in Table

**Table 1.** $^{13}C$–$^1H$ Coupling constants of pyrimidine nucleosides and nucleotides[a]

| | C-2 | | C-4 | | C-5 | | C-6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H-6 | H-1′ | H-6 | H-5 | H-5 | H-6 | H-6 | H-5 | H-1′ | C-1′,H-1′ |
| 5-Bromouridine | 8.2 | 2.1 | 8.7 | | | 3.1 | 186.8 | | 3.4 | |
| 5-Iodouridine | 8.0 | 2.0 | 9.5 | | | 2.7 | 185.6 | | 3.9 | 172.4 |
| Uridine | 8.1 | 2.4 | 10.7 | 1.7 | 177.8 | 2.8 | 184.1 | 4.5 | 3.6 | 168.5 |
| 2′-UMP | 7.3 | 3.8 | 10.6 | 1.5 | 178.2 | 2.5 | 184.2 | 4.4 | 3.9 | 170.0 |
| 3′-UMP | 8.2 | 2.3 | 10.7 | 1.7 | 178.2 | 2.4 | 184.3 | 4.3 | 3.8 | 169.1 |
| 5′-UMP | 8.3 | 2.2 | 10.7 | 1.6 | 178.1 | 2.5 | 184.2 | 4.4 | 3.7 | 169.2 |
| 2′,3′-UMP | 8.0 | 4.2 | 10.9 | 1.5 | 177.7 | 2.7 | 184.8 | 4.3 | 3.8 | 170.0 |
| 2′,3′-i-U[b] | 8.0 | 3.2 | 10.7 | 1.7 | 175.8 | 2.6 | 182.5 | 4.6 | 4.2 | 164.8 |
| Deoxyuridine | 8.1 | 2.1 | 10.8 | 1.6 | 177.9 | 2.8 | 183.6 | 4.4 | 3.6 | 174.5 |
| Thymidine | 7.9 | 2.2 | 9.9 | 4.2[c] | | 2.5 | 181.3 | 6.1[d] | 3.8 | 173.5 |
| 5′-dUMP | 8.0 | 1.8 | 10.6 | 1.7 | 178.2 | 2.5 | 184.2 | 4.3 | 3.6 | 168.5 |
| Orotidine | | 5.5 | | 1.2 | 177.0 | | | 4.4 | 3.6 | 164.1 |
| Cytidine | 6.2 | 2.0 | 9.2 | 1.7 | 174.9 | 3.3 | 182.8 | 4.4 | 3.4 | 169.9 |
| 2′-CMP | 6.0 | 3.2 | 9.3 | 1.5 | 173.8 | 3.0 | 182.2 | 4.3 | 3.9 | 168.9 |
| 3′-CMP | 6.1 | 1.9 | 9.1 | 1.5 | 177.6 | 3.0 | 183.1 | 4.6 | 3.5 | 169.6 |
| 5′-CMP | 6.2 | 1.7 | 9.2 | 1.8 | 175.8 | 2.9 | 183.7 | 4.4 | 3.5 | 170.9 |
| 5′-dCMP | 6.4 | 1.7 | 9.3 | 2.2 | 174.8 | 2.9 | 184.2 | 4.0 | 3.5 | 167.2 |
| 2′,3′-CMP | 7.9 | 4.1 | 10.7 | 1.5 | 176.1 | 3.0 | 184.9 | 4.3 | 3.7 | 167.2 |
| 2′,3′-i-C[b] | 6.0 | 2.8 | 9.1 | 1.2 | 175.8 | 3.0 | 181.3 | 5.0 | 3.6 | 169.1 |
| Deoxycytidine | 6.2 | 1.8 | 9.1 | 1.8 | 174.7 | 3.4 | 182.6 | 4.3 | 3.4 | 171.8 |

[a] 50 MHz $^{13}C$ n.m.r. measurements in $D_2O$ solutions (pD 7—8) with data resolution 0.12 Hz per point except for coupling on C-5 and C-1′ signals which were taken from ref. 30 (15 MHz $^{13}C$ measurements, data resolution 0.3 Hz per point). [b] [$^2H_6$]DMSO solutions. [c] $^3J$(C-4,$CH_3$). [d] $^3J$(C-6,$CH_3$).

**Table 2.** Glycosidic bond conformations of pyrimidine nucleosides and nucleotides from *X*-ray crystal structures[a]

| Derivative | Sugar ring | No. of analyses | $\chi$[b] |
|---|---|---|---|
| A *Anti*-type | | | |
| tRNA[c] | C-2′-*endo*, S | 6 | 252(±28) |
| | C-3′*endo*, N | 30 | 195(±8) |
| Nucleosides[d] | C-2′-*endo*, S | 19 | 233(±8) |
| | C-3′-*endo*, N | 13 | 198(±4) |
| B *Syn*-type | | | |
| Nucleosides | C-2′-*endo*, S | 2 | 78[e], 69.5[f] |
| | C-3′-*endo*, N | 2 | 68.5[g], 76.8[h] |

[a] Definition of *syn* ($\chi$ 0 ± 90) and *anti* ($\chi$ 180 ± 90) follow IUPAC recommendations, ref. 34. [b] Average $\chi$ together with average variation from mean. [c] Ref. 4. [d] Ref. 3. [e] 6,7-Dimethyl-lumazine-N1-β-D-ribofuranoside, ref. 35. Approximate value derived from angle C-2′–C-1′–N-1–C-8a of 138°. [f] 6-Methyluridine, ref. 36. Average of values (69.6, 69.4°) for two molecules in the unit cell. [g] 3′,5′-Diacetyl-2′-deoxy-2′-fluorouridine, ref. 37. [h] 4-Thiouridine, ref. 38.

2 in terms of the number of analyses of each type, the average value of $\chi$, and the average variation from the mean. The results are differentiated as a function of sugar-ring pucker and show that there is a preferred *anti* conformational state ($\chi$ *ca.* 195°) for nucleic acid derivatives with N-type sugar ring conformations and that this conformation varies on average by only 8° for 30 nucleotidyl units in yeast tRNA$^{Phe}$ and by less than 5° for 13 crystal structures of nucleosides and nucleotides. The *anti* conformational state for sugar ring S conformers appears to have a broad potential well, because variations in average $\chi$ from 230—250° are observed with larger variations from the mean than for N conformations. There are a few examples of pyrimidine nucleosides with *syn* conformations in which $\chi$ is in the range 70—80° for the sugar ring in both the S ($\chi_{78}$ 6,7-dimethyl-lumazine-N1-β-D-ribofuranoside,[35] $\chi_{69.4}$ and $\chi_{69.6}$ for two molecules in the unit cell of 6-methyluridine[36]) and N ($\chi_{68.5}$ for 3′,5′-diacetyl-2′-deoxy-2′-fluorouridine[37] and $\chi_{76.8}$ for 4-thiouridine[38]) conformation. *Syn* conformations with $\chi$ *ca.* 45° have only been observed for purine nucleosides in the solid state where stabilisation of this conformer usually occurs by N-3 • • • • HO-5′ intramolecular hydrogen bonding.[6] The conformational models investigated in this work utilise the conclusion from analysis of *X*-ray crystal structures that pyrimidine nucleosides and nucleotides may adopt two ranges of *syn* and *anti* conformations in solution. Although the analysis of crystal structure results support this assumption, it is necessarily a condition of the $\cos^2\theta$ dependence of the Karplus relation between vicinal coupling and dihedral angle.

(3) *Conformational Model based on Crystal Structures.*—For nucleosides and nucleotides in solution n.O.e. measurements have shown that both *syn* and *anti* conformations exist in equilibrium.[18–22] Observed n.m.r. parameters are weighted averages of the magnitudes in different conformations, and measurement of two vicinal proton spin-coupling constants of pyrimidine derivatives enables only two conformational parameters to be determined. If one assumes that the four conformations of the glycosidic bond of pyrimidine derivatives in solution are the same as the average values in the crystal state (*i.e.* $\chi_{45}$, $\chi_{75}$, $\chi_{195}$, and $\chi_{240}$) only two conformer populations may be determined unless other independent information is available. It was shown recently that $^1J$(C-1′,H-1′) magnitudes of a number of cyclo-uridine compounds vary quantitatively with the conformation of the glycosidic bond, though some dependence on the sugar ring conformation is also expected.[39] It is possible that the one-bond carbon–proton coupling constants may also be used to determine glycosidic bond

**Table 3.** N.m.r. parameters derived from average crystal structures[a]

| Conformation | $^1J$(C-1′,H-1′)/Hz | $^3J$(C-6,H-1′)/Hz | $^3J$(C-2,H-1′)/Hz |
|---|---|---|---|
| *Syn* | | | |
| $\chi_{45}$[b] | 174 | 3.5 | 6.7 |
| $\chi_{75}$ | 174 | 3.5 | 6.7 |
| *Anti* | | | |
| $\chi_{195}$ | 181 | 4.8 | 0.8 |
| $\chi_{240}$ | 168 | 8.7 | 2.8 |

[a] Crystal structures taken from Table 2 and n.m.r. parameters from Figure 3. [b] Conformation observed in crystal structures of purine but not pyrimidine derivatives, ref. 6.

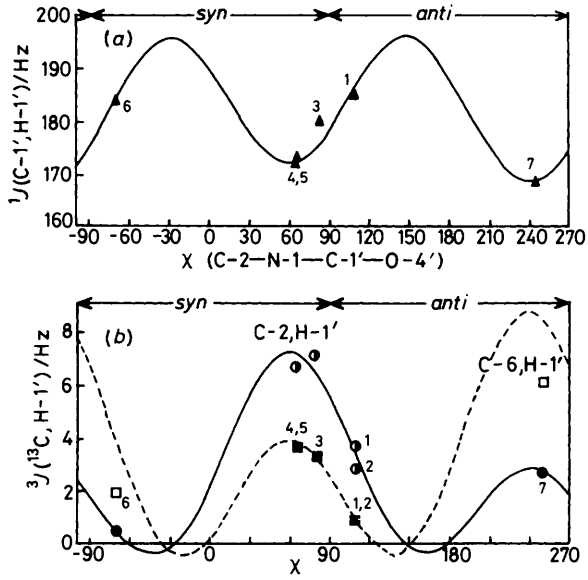


**Figure 3.** Variation of one-bond (*a*) and three-bond (*b*) carbon–proton coupling constants with glycosidic bond angle $\chi$ of cyclouridine derivatives. (**1**) 2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil; (**2**) 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine; (**3**) 2,3′-anhydro-1-(β-D-xylofuranosyl)uracil; (**4**) 2,5′-anhydro-2′,3′-*O*-isopropylideneuridine; (**5**) 2,5′-anhydro-1-(β-D-ribofuranosyl)uracil; (**6**) 2′,6-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil; (**7**) 5′,6-anhydro-2′,3′-*O*-isopropylideneuridine

conformer populations in conjunction with the three-bond coupling constants.

Measurements of one-bond [$^1J$(C-1′,H-1′)] and three-bond [$^3J$(C-6,H-1′) and $^3J$(C-2,H-1′)] carbon–proton spin[32,39] coupling constants of a number of cyclouridine nucleosides are plotted in Figure 3 against glycosidic bond conformation ($\chi$) together with the derived Karplus curves appropriate to each coupling constant. The key to cyclo-compounds (**1**)—(**7**) is given in the legend to Figure 3. Any set of three coupling constants for one compound may be satisfied by two conformational angles ($\chi$) because in the angular range $\chi$(0—180°) the curves are symmetrical about $\chi_{60}$ and in the range $\chi$(180—360°) the curves are symmetrical about $\chi_{240}$. None of the sets of the three coupling constants for pyrimidine nucleosides and nucleotides summarised in Table 1 correspond to a particular single conformation. The n.m.r. parameters derived for conformational angles corresponding to the average crystal structure determinations are summarised in Table 3. Because of the similar magnitudes of both the $J$(C-6,H-1′) *ca.* 3.5 Hz and $J$(C-2,H-1′) *ca.* 6.7 for the $\chi_{45}$ and $\chi_{75}$ conformations (being symmetrical about $\chi_{60}$), it is possible to determine the populations of the two *anti* conformers ($\chi_{195}$, $\chi_{240}$) from the two observed vicinal

carbon–proton spin coupling constants but only the sum of populations of the *syn* conformers ($\chi_{45}$, $\chi_{75}$). Unfortunately, recourse to $^1J$(C-1′,H-1′) magnitudes does not solve the problem because the magnitudes determined for the constituent conformers in Table 3 predict somewhat larger values than those observed for the pyrimidine derivatives in Table 1.

A possible origin of the discrepancy between observed and calculated $^1J$(C-1′,H-1′) magnitudes may be that the relation between $^1J$(C-1′,H-1′) and conformational angle $\chi$ summarised in Figure 3 was determined for a number of cyclonucleosides with fixed conformations in which the sugar ring is constrained in particular conformations whereas the sugar ring exists in an N $\rightleftharpoons$ S conformational equilibrium for the pyrimidine nucleosides and nucleotides measured in this work. An explanation of the variation in $^1J$(C-1′,H-1′) with glycosidic bond conformational angle was given in terms of the relation between the C-1′–H-1′ bond direction and the direction of the lone pair on the nitrogen atom involved in the glycosidic bond.[39] It is likely that the lone pairs of electrons on the furanose ring oxygen atom (O-4′) also affect the magnitude of $^1J$(C-1′,H-1′), especially when the conformation of the sugar ring can take up both the N(C-3′-*endo*) and S(C-2′-*endo*) conformations in flexible molecules. The method of conformational analysis outlined in the present section is fine in principle but not very fruitful in practice because of the limitations in our knowledge of $^1J$(C-1′,H-1′) magnitudes as well as the assumption that average structures observed in the solid state necessarily define the preferred conformation of the glycosidic bond in solution. An alternative approach accepts these limitations and, by making some assumptions about the symmetry of permitted conformers, enables the conformational angles and equilibrium compositions to be determined.

(4) *Symmetrical Conformational Model.*—In this model it is assumed that the *syn* and *anti* conformations correspond to particular angular regions (similar to those observed by *X*-ray crystallography), that these conformational regions are symmetrically related with respect to the C-1′–H-1′ vector (again, approximately in line with crystal structure data except for the broad *anti*-S region) and that each region can be characterised by one value of angle $\lambda$. A consequence of these conditions is that one value of $\lambda$ ($0 < \lambda < 90°$) generates four conformations characterised by $\pm\lambda$ and $180 \pm \lambda$ as shown in Figure 4 which, in turn, corresponds to four conformations characterised by $\chi = 60 \pm \lambda$ and $\chi = 240 \pm \lambda$, respectively. The four conformations consist of two *syn* and two *anti* conformations symmetrically related with respect to $\chi_{60}$ and $\chi_{240}$, which is a condition of the $\cos^2\lambda$ dependence of the Karplus relation in equation (2), where A, B, and C are constants. Because the same form of the Karplus relation was used to describe the variation of one-bond[39] and three-bond[32] proton–carbon coupling constants with torsion angle $\lambda$, the curves are symmetrical with respect to $\chi_{60}$ and $\chi_{240}$, as shown in Figure 3.

$$J = A\cos^2\lambda + B\cos\lambda + C \qquad (2)$$

Magnitudes of $^3J(^{13}$C,H-1′) are observed between H-1′ and both C-2 and C-6 of pyrimidine rings. For planar base rings the C-6 and C-2 couplings to H-1′ are 180° out of phase. For any conformational state the magnitudes of these vicinal coupling constants are related to the torsion angle $\lambda_i$ according to equations (3) and (4) where the constants $A_6$, $B_6$, $C_6$ and $A_2$, $B_2$, $C_2$ are the Karplus parameters for C-6–N-1–C-1′–H-1′ and C-2–N-1–C-1′–H-1′ molecular fragments, respectively.

$$J_6 = A_6\cos^2\lambda_i + B_6\cos\lambda_i + C_6 \qquad (3)$$

$$J_2 = A_2\cos^2(\lambda_i + 180) + B_2\cos(\lambda_i + 180) + C_2 = A_2\cos^2\lambda_i - B_2\cos\lambda_i + C_2 \qquad (4)$$

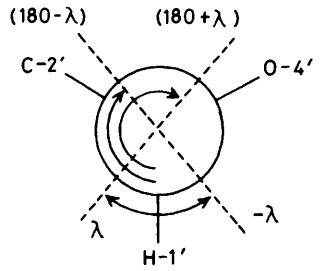


**Figure 4.** Four-state glycosidic bond conformational model showing the symmetrical relationships between the two *syn* ($\pm\lambda$) and two *anti* ($180 \pm \lambda$) conformations

The usefulness of equations (3) and (4) depends on a knowledge of the Karplus parameters for each molecular fragment, such as those determined recently for uridine derivatives.[32] For an equilibrium between the two *syn* ($\pm\lambda_i$) and two *anti* ($180 \pm \lambda_i$) conformations that is rapid on the n.m.r. time-scale, the observed $^3J(^{13}$C,H-1′) magnitudes are related to the relative populations of the *syn* ($p_s$) and *anti* ($p_a$) conformers by equations (5) and (6), where the subscripts of $J$ correspond to the appropriate carbon atom, C-2 or C-6, and the superscripts of $J$ refer to the *syn* or *anti* conformer.

$$J_6\,(\text{obs.}) = J_6^{\circ} = p_a J_6^{a} + p_s J_6^{s} \qquad (5)$$

$$J_2\,(\text{obs.}) = J_2^{\circ} = p_a J_2^{a} + p_s J_2^{s} \qquad (6)$$

$$1 = p_a + p_s \qquad (7)$$

(5) *Conformational Angles.*—The sum of observed coupling constants ($\Sigma J$) may be calculated from the sum of equations (5) and (6) and substituting for the results in equations (3), (4), and (7), as shown in equation (8).

$$\begin{aligned}\Sigma J &= (J_6^{\circ} + J_2^{\circ}) \\ &= (A_2 + A_6)\cos^2\lambda_i + (B_6 - B_2)(p_a - p_s)\cos\lambda_i + (C_2 + C_6) \\ &\approx (A_2 + A_6)\cos^2\lambda_i + (C_2 + C_6)\end{aligned} \qquad (8)$$

It is found that for all calculated values of $\lambda$ (25—45°, see below) and ($p_a - p_s$) (range between 0.6 and −0.6, see below), the cos $\lambda$ term can be neglected in relation to the $\cos^2\lambda$ term because of the small magnitude of $(B_6 - B_2) = 0.3$ Hz, compared with the sum of $(A_6 + A_2) = 11.2$ Hz. Hence it is found that the sum of coupling constants is independent of the *syn* $\rightleftharpoons$ *anti* conformer equilibrium according to equation (8), from which the magnitude of $\lambda_i$ and the four conformers $\pm\lambda_i$ and $(180 \pm \lambda_i)$ may be calculated from the observed magnitude of $\Sigma J$. Some support for the assumptions on which this analysis is based is given by the fact that the observed magnitudes of $\Sigma J$ are approximately constant for uridine ($5.8 \pm 0.2$ Hz) and cytidine ($5.2 \pm 0.2$ Hz) derivatives where the sugar ring is not constrained, whereas different magnitudes of $\Sigma J$ are observed for ribose rings constrained by 2′,3′-cyclic phosphate or 2′,3′-*O*-isopropylidene groups (*i.e.* $\Sigma J$ 7.6 $\pm$ 0.2 Hz) or nucleosides that are *syn*-type because of substitution at C-6, *e.g.*, orotidine, $\Sigma J = 9.1 \pm 0.2$ Hz. Using Karplus parameters determined for uridine derivatives ($A_6 = 6.2$, $B_6 = -2.4$, $C_6 = 0.1$ Hz and $A_2 = 5.0$, $B_2 = -2.1$, $C_2 = 0.1$ Hz)[32] the magnitude of $\lambda$ has been calculated from observed $\Sigma J$ of each uridine nucleoside and nucleotide and the results are summarised in Table 4. Similar calculations have been performed for cytidine derivatives using, to a first approximation, the same Karplus parameters for C-6,H-1′ and C-2,H-1′ coupling as in uridine. An error of $\Sigma J$ of $\pm 0.2$ Hz leads to an error in $\lambda$ of $\pm 1°$. It is found

**Table 4.** Glycosidic bond conformational angles (λ) and *anti* conformer populations ($p_a$) for pyrimidine nucleosides and nucleotides[a,b]

| | Compound | $\Sigma J$[c] | $\Delta J$[d] | λ/° | $p_a$ |
|---|---|---|---|---|---|
| 5-Bromouridine | (**8**) | 5.5 | 1.3 | 47 | 0.62 |
| 5-Iodouridine | (**9**) | 5.9 | 1.9 | 45 | 0.70 |
| Uridine | (**10**) | 6.0 | 1.2 | 44 | 0.59 |
| 3′-UMP | (**11**) | 6.1 | 1.5 | 44 | 0.63 |
| 5′-UMP | (**12**) | 5.9 | 1.5 | 45 | 0.64 |
| Deoxyuridine | (**13**) | 5.7 | 1.5 | 46 | 0.64 |
| Thymidine | (**14**) | 6.6 | 1.4 | 41 | 0.61 |
| 5′-dUMP | (**15**) | 5.4 | 1.8 | 47 | 0.70 |
| 2′-UMP | (**16**) | 7.7 | 0.1 | 35 | 0.40 |
| 2′,3′-i-U | (**17**) | 7.4 | 1.0 | 37 | 0.53 |
| 2′,3′-UMP | (**18**) | 8.0 | −0.4 | 33 | 0.34 |
| Orotidine | (**19**) | 9.1 | −1.9 | 27 | 0.14 |
| Cytidine | (**20**) | 5.4 | 1.4 | 47 | 0.64 |
| 3′-CMP | (**21**) | 5.4 | 1.6 | 47 | 0.67 |
| 5′-CMP | (**22**) | 5.2 | 1.8 | 48 | 0.71 |
| 5′-dCMP | (**23**) | 5.2 | 1.8 | 48 | 0.71 |
| Deoxycytidine | (**24**) | 5.2 | 1.6 | 49 | 0.80 |
| 2′-CMP | (**25**) | 7.1 | 0.7 | 38 | 0.49 |
| 2′,3′-i-C | (**26**) | 6.4 | 0.8 | 42 | 0.52 |
| 2′,3′-CMP | (**27**) | 7.8 | −0.4 | 35 | 0.34 |

[a] Conformer angles (λ) and *anti* populations ($p_a$) were calculated from $\Sigma J$ and $\Delta J$ results, respectively, according to equations (8) and (10), respectively. [b] For observed error in $\Sigma J$ and $\Delta J$ ±0.2 Hz the calculated errors are λ ±1° and $p_a$ ±0.02. [c] $\Sigma J = (J_6 + J_2)$ Hz. [d] $\Delta J = (J_6 - J_2)$ Hz.

that the behaviour of both sets of pyrimidine derivatives is essentially the same, *i.e.*, the conformational angle λ is about 43—47° for pyrimidine nucleosides and nucleotides, about 33—37° for their 2′,3′-*O*-isopropylidene and 2′,3′-cyclophosphate derivatives, and about 27° for the *syn*-type nucleoside orotidine. The relevance of the different conformations will be discussed after an estimate of the equilibrium populations of *syn* and *anti* conformers is made.

(6) *Conformer Equilibrium.*—The conformer equilibrium may be monitored by either observed coupling constant ($J_6°$ or $J_2°$) but the most convenient method is by the difference in observed $J$ values, $\Delta J = J_6° - J_2°$. An expression relating $\Delta J$ to the *syn* and *anti* conformer population can be derived from equations (5) and (6) making appropriate substitutions from equations (3) and (4), as shown in equation (9).

$$\Delta J = (J_6° - J_2°) = (A_6 - A_2)\cos^2\lambda + (p_a - p_s)(B_6 + B_2)\cos\lambda + (C_6 - C_2) \quad (9)$$

Given magnitudes of the Karplus parameters for uridine derivatives[32] and approximate conformational angles (λ, 25—45°) determined in the previous section, no term can be neglected in equation (9). Calculations of conformer populations are conveniently made by equation (10), which is derived by rearrangement of equation (9) and substitution according to equation (7).

$$p_a = 0.5 - \frac{(\Delta J - \Delta C)}{2\Sigma B \cos\lambda} + \frac{\Delta A \cos\lambda}{2\Sigma B} \quad (10)$$

For the uridine and cytidine derivatives magnitudes of $p_a$ have been calculated using the appropriate Karplus parameters for each nucleoside and the appropriate cos λ term from the sum of observed coupling constants. The results summarised in Table 4 indicate a variation in $p_a$ from 0.6—0.8 for uridine and

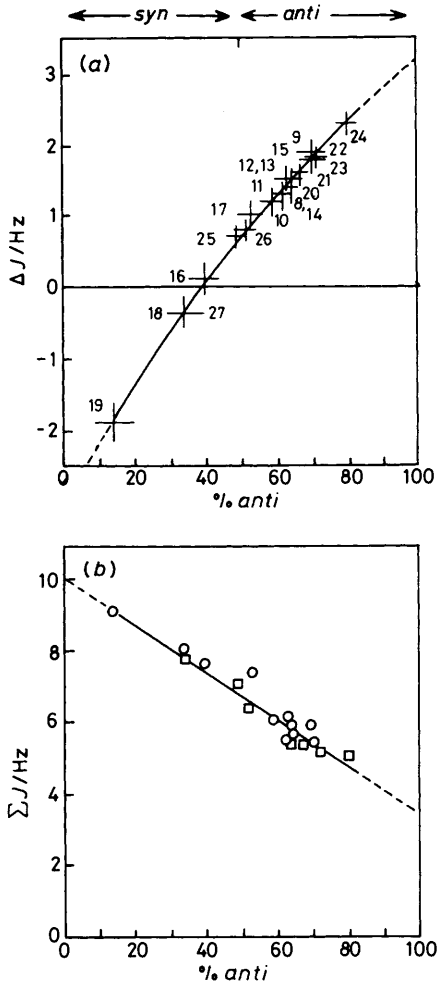


**Figure 5.** Dependence of glycosidic bond *anti* conformer population ($p_a$) with magnitudes of (*a*) $\Delta J$ (including error limits) and (*b*) $\Sigma J$ observed for uridine (○) and cytidine (□) derivatives

cytidine nucleosides and nucleotides, approximately equal *syn* and *anti* conformer populations for ribose nucleosides with sugar rings constrained by an isopropylidene or cyclic phosphate ring and a definite preference for the *syn* conformation of orotidine ($p_a = 0.14$, *i.e.* $p_s = 0.86$) in line with previous qualitative determinations of the glycosidic bond conformation. An observed error of $\Delta J$ (±0.2 Hz) leads to an error in calculated population of $p_a$ (±0.02) so that differences in $p_a \geqslant 0.04$ between molecules are significant.

The variation of glycosidic bond conformer population ($p_a$) with $\Delta J$ magnitudes is shown in Figure 5(*a*) together with the observed error limits in $\Delta J$ and calculated error in $p_a$. The dependence of $p_a$ and $\Delta J$ exhibits a slight curvature according to equation (10) where two terms depend on cos λ, which is found to vary for different molecules (Table 4). However, the term $\Delta A \cos\lambda/2\Sigma B$ is approximately constant only varying slowly (0.12—0.09) with λ in the range 25—45°, whereas, for the range of compounds studied, the variation in the $\Delta J$ term is much larger (−0.23 to 0.36) thereby dominating the calculation of $p_a$ and leading to the approximately linear dependence of $p_a$ with $\Delta J$ according to equation (10).

A seemingly surprising feature of the results in the present work is that magnitudes of observed $\Sigma J$ also vary with glycosidic bond conformation ($p_a$) as shown in Figure 5(*b*). In this case the approximately linear dependence of $\Sigma J$ with $p_a$ may be used to determine the glycosidic bond conformer popu-

lation from equation (11), where $\Sigma J_{anti}$ and $\Sigma J_{syn}$ correspond to $\Sigma J$ magnitudes of *anti* and *syn* conformers derived by extrapolation of the linear dependence of Figure 5(*b*).

$$p_a = \frac{(\Sigma J_{obs.} - \Sigma J_{syn})}{(\Sigma J_{anti} - \Sigma J_{syn})} \approx \frac{(\Sigma J_{obs.} - 3.6)}{6.4} \qquad (11)$$

The extrapolated magnitude of $\Sigma J_{syn}$ for the 100% *syn* conformer (10 Hz) corresponds to λ *ca.* 21° calculated from equation (8), which, in turn, corresponds to glycosidic bond conformers $\chi_{39}$ or $\chi_{81}$. The *syn* conformations observed in the solid state (Table 1) range from 68.5—78°, somewhat similar to the 81° determined for molecules in solution. The extrapolated magnitude of $\Sigma J_{anti}$ (=3.6 Hz) corresponds to λ *ca.* 56° and glycosidic bond conformers $\chi_{184}$ or $\chi_{296}$ of which the conformer $\chi_{184}$ is somewhat similar to the range observed for C-3′-*endo* sugar ring conformations (χ *ca.* 195° ± 8) in the solid state (Table 2) but the other possible *anti* conformer, $\chi_{296}$, is substantially different from the range observed in the solid state (χ 230—250°).

## Conclusions

From a knowledge of $^3J$(C-2,H-1′) and $^3J$(C-6,H-1′) magnitudes for uridine and cytidine derivatives, the populations of the *anti* conformer were determined by equation (10) and the angles ($\lambda_i$) that specify the conformations of the four-state model were determined by equation (8). The results listed in Table 4 give the first systematic quantitative analysis of the glycosidic bond conformations of pyrimidine nucleosides and nucleotides.

(*i*) The $^{13}$C n.m.r. method gives a range of values of $p_a$ from 20% to 80% (*i.e.*, no measurement outside the 0—100% range) and, depending on molecular type, the results are consistent with the qualitative trends determined by other methods, *e.g.*, pyrimidine nucleosides and nucleotides exhibit predominant *anti* conformations in solution (60—80%), substitution of the base ring by a bulky group in the C-6 position gives a predominant *syn* conformation (*ca.* 14% for orotidine) and substitution of the ribose ring by the 2′,3′-isopropylidine or 2′,3′-cyclic phosphate group alters the conformation of the sugar ring such that about equal proportions of the *syn* and *anti* conformers exist for the glycosidic bond conformation.

(*ii*) The changes in conformational equilibrium for different molecules are accompanied by changes in the conformational angles, *i.e.*, *anti*-type molecules have λ *ca.* 45 ± 2°, *syn*-type molecules have λ *ca.* 25 ± 2°, and λ tends to 35 ± 2° for approximately equal populations of *syn* and *anti* conformers.

(*iii*) The changes in conformational angles mirror the behaviour observed in the solid state by *X*-ray crystallography and the behaviour expected by consideration of non-bonded interactions. The *anti*-type conformer (λ *ca.* 45°) corresponds to a glycosidic bond conformation characterised by χ *ca.* 195° which is consistent with crystal structure data (Table 1); the alternative *anti*-type conformer ($\chi_{285}$) is somewhat different from that observed in the solid state. The *syn*-type conformer (χ *ca.* 25°) corresponds to a glycosidic conformation in which a compromise is achieved between the interactions of the bulky group at C-6 with the C-1′–H-1′ bond and the interactions of the C-2 carbonyl group with either the C-1′–O-4′ or C-1′–C-2′ bonds. Of the two possible *syn* conformers ($\chi_{85}$ or $\chi_{35}$) only the conformer characterised by $\chi_{85}$ has been observed in the crystal state (Table 1).

The four-state symmetrical conformational model for the glycosidic bond of pyrimidine nucleosides and nucleotides enables the *syn* and *anti* conformations and their equilibrium compositions to be determined from measurements of $J$(C-6,H-1′) and $J$(C-2,H-1′) magnitudes and a knowledge of the appropriate Karplus relations. The conformational model should also be applicable to purine derivatives by analysis of $J$(C-8,H-1′) and $J$(C-4,H-1′) magnitudes and a knowledge of the appropriate Karplus relations. At present the analysis of glycosidic bond conformations of purine derivatives is hampered by the lack of the Karplus relations for C-8,H-1′ and C-4,H-1′ coupling with dihedral angle though it is expected that, once determined, the same relationship will be applicable to all purine derivatives (adenosine, guanosine, xanthosine, and inosine) because the C-8 and C-4 atoms are part of the imidazole ring system common to all of them.

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