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# A NUCLEAR OVERHAUSER EFFECT STUDY OF PURINE NUCLEOSIDE GLYCOSYL CONFORMATION IN SOLUTION

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**Abstract**—The glycosyl conformations of adenosine, inosine, guanosine, their 2',3'–O–isopropylidene derivatives, and of xanthosine in dimethylsulfoxide are examined by quantitative application of the nuclear Overhauser effect (NOE). The 2',3'–O–isopropylidene purine nucleoside derivatives in DMSO strongly prefer a single glycosyl conformation in the *syn* range. There is more latitude in the glycosyl torsion angle of the parent nucleosides, which in DMSO solution typically possess a C–2' *endo* sugar. Here the glycosyl conformation appears to be more responsive to nucleobase substituents. Proton chemical shifts are discussed. Relevant circular dichroism spectra are presented.

## INTRODUCTION

THE MAJORITY of purine nucleosides in single crystals and in base-paired co-crystals and also purine nucleotides in DNA–A, DNA–B and RNA have a glycosyl torsion angle, $\Upsilon$ (Fig. 1), from 150° to 240°, the anti range.[1] From detailed consideration of

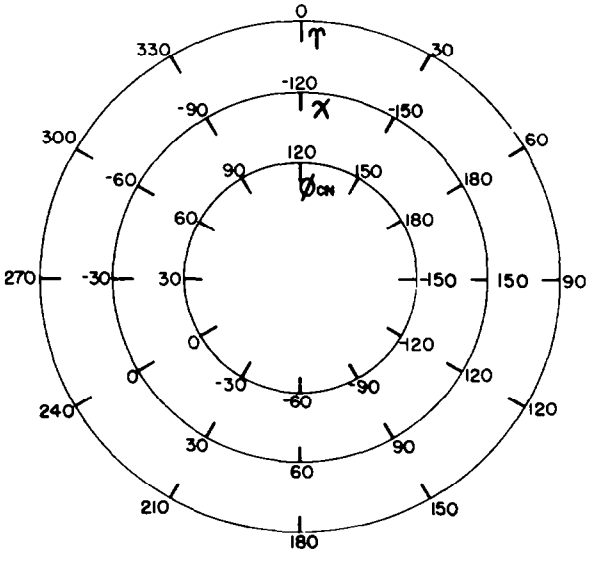



FIG 1. Glycosyl torsion angle conventions.

monomer and polymer X-ray data[1] it is evident that a C-3′ *endo* ribose supports a glycosyl conformation in the range of $\Upsilon = 200$–$240°$ while a C-2′ *endo* ribose is more often associated with $\Upsilon = 150$–$180°$.

Theoretical considerations of purine nucleoside conformation have concluded that energy differences between extreme glycosyl conformations are small.[2] Recent NMR experiments have shown that some purine nucleosides in solution assume a predominant glycosyl conformation in the *syn* range ( $\Upsilon = 330°$ to $30°$),[3,4] nearly diametrically opposed to the commonly observed solid state conformation.[5,6] Further, the cytotoxic nucleoside antibiotic, formycin, is thought to adopt the *syn* glycosyl conformation in solution and under certain conditions[7] polyformycin is thought to manifest the *syn* conformation of its individual nucleotide units.

It has been suggested that the pucker of the furanose ring in DNA may be related to the necessity of distinguishing DNA in its two roles as a template for DNA in replication and for RNA in transcription.[1] It has also been noted that to the extent purine nucleosides or nucleotides can adopt the *syn* conformation, the alternative base pair AG would be possible in double helical nucleic acids.[8]

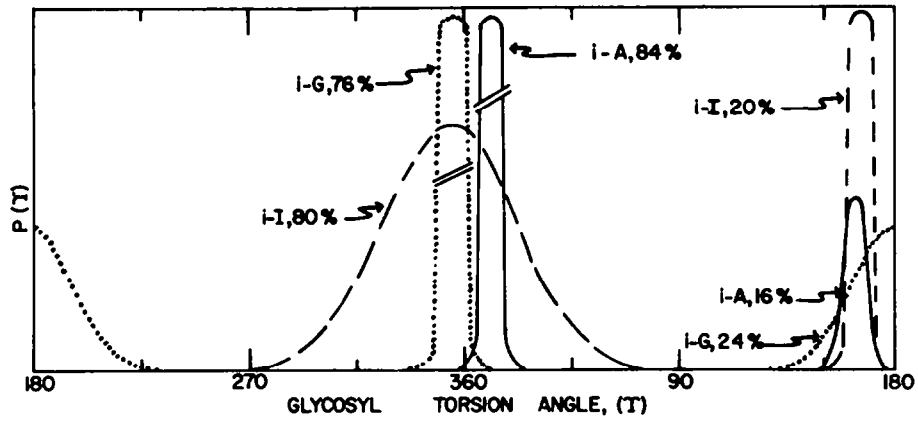
We are working to characterize the determinants of nucleoside and nucleotide glycosyl conformation in solution and report here the effects of sugar conformation.

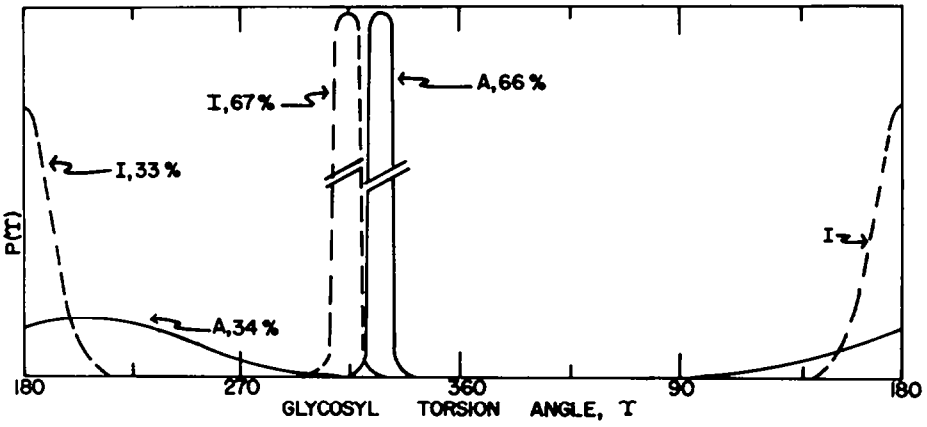
## RESULTS AND DISCUSSION

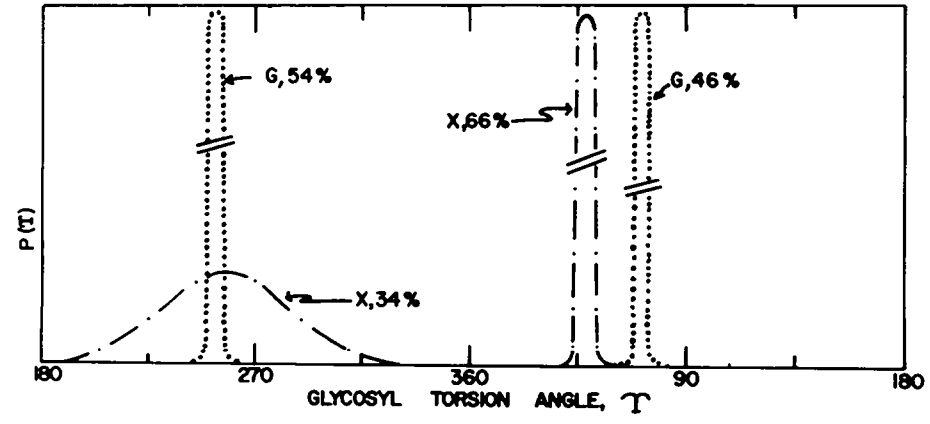
Our approach to relating intramolecular NOE's to glycosyl torsion angle is to establish a reasonable ribose geometry from independent data, principally spin coupling constants, and then to fit the glycosyl conformation to $\gamma$-dependent NOE's calculated using this basic geometry. The large H–1′, H–2′ spin coupling constants of the unblocked nucleosides (Table 4) indicate that these compounds have a H–1′, H–2′ dihedral angle greater than 120° and thus that C–2′ is *endo*. The significantly smaller H–1′, H–2′ coupling constant observed in the blocked compounds, along with arguments reported,[4] suggest that there C–3′ is *endo*. The slightly larger $J_{2'3'}$'s of the blocked compounds in comparison to those of the unblocked compounds indicate a slightly smaller H–2′, H–3′ dihedral angle in the isopropylidene derivatives and hence that C–3′ in that series is less out of plane than C–2′ is the other.

Among the NOE's particularly sensitive to glycosyl conformation are $f_8(1')$, $f_8(2')$, $f_8(3')$, $f_8(5')$ and $f_1(8)$. These interactions are also most convenient to experimentally determine, and thus were measured in A, I, G, X, i-A, i-I, and i-G whose NMR spectra (Fig. 9) are amenable to individual measurement of these interactions

Computer fits of glycosyl torsion angle (Figs 2–4) to experimental NOE's (Table 1) show that the blocked compounds are characterized by a strong preference for a *syn* glycosyl conformation and that the natural nucleosides enjoy a greater conformational freedom, in terms of both position and weighting of the two-fold distribution. The A, i-A and I, i-I pairs follow the rule that predominant ($\sim 80\%$) glycosyl conformer of the blocked compound (C-3′ *endo*) is near $\Upsilon = 0°$ with a minor proportion of the *anti*, while the predominant ($\sim 60\%$) glycosyl conformer of the unblocked compounds (C-2′ *endo*) is at $\Upsilon = 320°$ and the minor one ($\sim 40\%$) at $\Upsilon = 190°$. The positions of X's and G's distributions are noticeably different from those of A and I. The difference is perhaps a function of the C-2 substituent. The conformer distribution of i-G, however, is comparable to that of i-A and iI.




FIGS 2–4. Computer fits of glycosol torsion angle to experimental nuclear Overhauser effects (Table 1) assuming Range II conformer exchange rate. See Experimental. Guanosine-(G), inosine(I), adenosine(A), "i" denotes 2',3'–O–isopropylidene derivative.

Chemical shift data (Fig. 5 and Table 2) are difficult to interpret because the observed trends are the combined effects of electronic changes in the aglycone, variations in the glycosyl conformation, and changes in sugar conformation and structure. The generally increasing values of all the ribose proton chemical shifts on going from the xanthine to the guanine to the hypoxanthine to the adenine nucleosides suggests that the order of all the chemical shifts is due mainly to the electronic effect of the base, which appears to be transmitted *via* the glycosyl bond

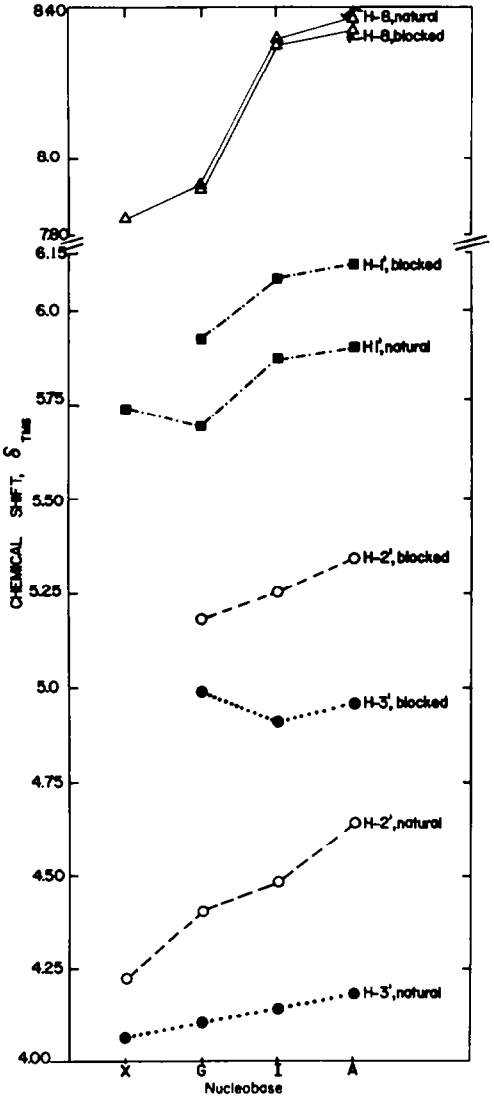



FIG 5. Chemical Shifts of selected purine nucleoside ribose protons. Nucleosides were 0·25 M in DMSO-$d_6$ at 30·C. Xanthosine(X), guanosine(G), inosine(I), adenosine(A). "i" denotes 2',3'–O–isopropylidene derivative.

TABLE 1. INTRAMOLECULAR NUCLEAR OVERHAUSER EFFECTS USED IN FITS[a] OF A PURINE NUCLEOSIDE[b] GLYCOSYL CONFORMATION

| | $f_s(1')$ | $f_s(2')$ | $f_s(3')$ | $f_s(5')$ | $f_s(8')$ |
|---|---|---|---|---|---|
| i—A | 0·22 | 0·12 | 0·03 | 0·02 | 0·20 |
| i—I[c] | 0·18 | 0·14 | 0·04 | 0·04 | 0·16 |
| i—G | 0·16 | 0·16 | 0·07 | 0·03 | 0·19 |
| A | 0·18 | 0·09 | 0·02 | 0·0 | 0·20 |
| I | 0·13 | 0·19 | 0·06 | 0·04 | 0·13 |
| G | 0·10 | 0·205 | 0·08 | 0·025 | 0·0[8] |
| X | 0·24 | 0·10 | $f_1(5')^d = 0·0$ | 0·03 | 0·125 |

[a]See Figs 2–4

[b]0·25 M nucleoside. See Experimental for details.

[c]The best fit of eleven enhancements in ref. 4 including these five is given in Fig. 2.

[d]Though this experiment is not conformationally informative it was used to satisfy the requirement of five enhancements for a two gaussian fit. The other four are the only ones readily measurable.

through several bonds of the ribose, as far as H-3'. H-4' is too distant from the nucleobase to be responsive to the glycosyl conformation; H-5',5' also seems to be insensitive to the relative disposition of the nucleobase and ribose (Table 2). The differences in ribose proton chemical shift (Fig. 5) between the blocked and unblocked nucleosides are due to the electronic effects of the isopropylidene group, with the conformationally modulated nucleobase anisotropy effects superimposed.

The most notable chemical shift correlation with glycosyl conformation is that for H-3' in i-G. In this nucleoside the glycosyl conformation (*syn*-like) is such that H-3' is proximal to the 2-amino function. The larger deshielding of H-3' in i-G relative to the other blocked compounds is consistent with the observed glycosyl conformation and can be rationalized by a "reaction field" effect[12] of the 2-amino group.

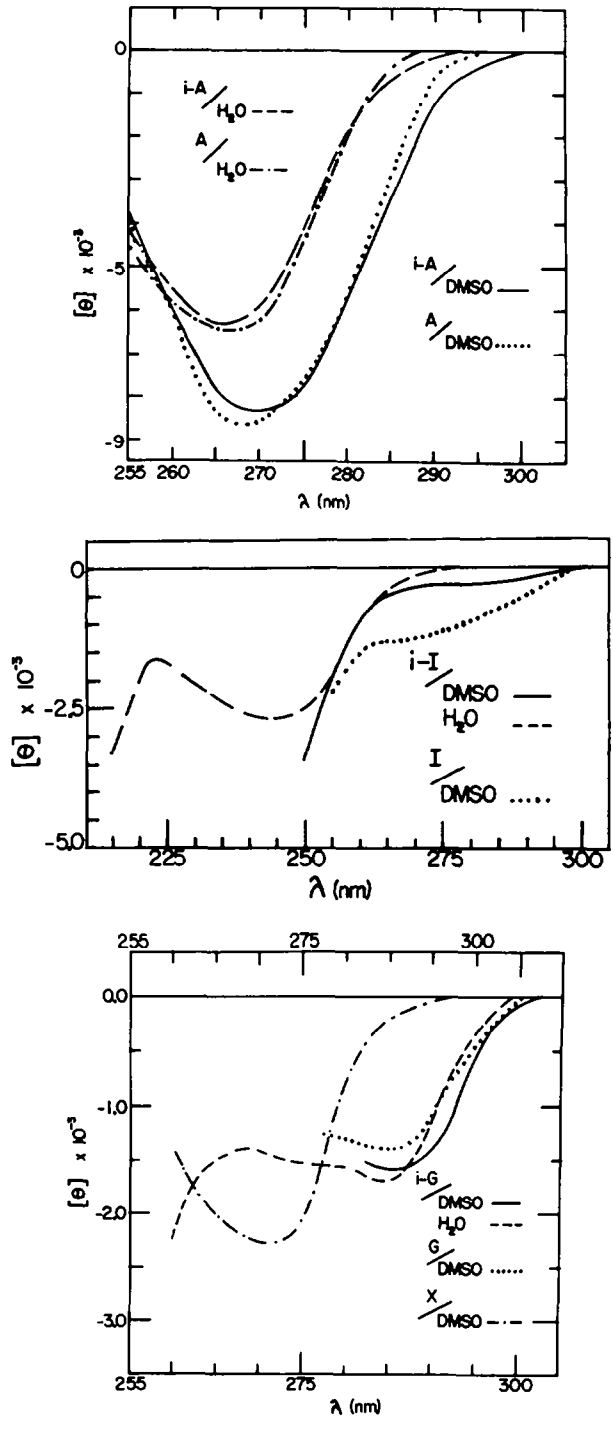
TABLE 2. CHEMICAL SHIFTS[a] OF PURINE NUCLEOSIDE[b] *H*–4' AND *H*–5',5'' PROTONS

| *Nucleoside* | *H*—4' | *H*—5',5'' |
|---|---|---|
| X | 4·06 | 3·67 |
| G | 3·88 | 3·58 |
| I | 3·94 | 3·60 |
| A | 3·99 | 3·60 |
| i—G | 4·12 | 3·54 |
| i—I | 4·23 | 3·56 |
| i—A | 4·24 | 3·54 |

[a]$\delta_{TMS}$ computed from DMSO-$d_5$ internal reference taken as 2·50$\delta$.

[b]0·25 M in DMSO-$d_6$ 30.°C.

The CD spectra of the blocked compounds in DMSO, wherein the NOE data indicate a strongly preferred *syn* conformation, are distinctly negative. The CD spectra of the parent compounds—which have considerably less constrained glycosyl conformations—are also negative and very similar in shape to those of the 2',3'-O-




FIGS 6–8. CD spectra. Guanosine(G), inosine(I), adenosine(A). "i" denotes 2′.3′–O–isopropylidene derivative. See Experimental for details.

isopropylidene derivatives. There is a large concentration difference between the CD and NMR conditions which *a priori* might vitiate CD-NOE correlations. The CD spectrum of 0·5 M A in DMSO was measured in a 0·1 cm cell and was negative down to the absorbance cut off (295 nm) suggesting that such correlations are indeed valid. The consistent similarity of CD spectra in water and DMSO (Table 2 and Figs 6–8) implies that general conformational deductions from NOE data in DMSO hold also in water. The relative insolubility of most purine nucleosides in water renders routine NOE in it difficult.

TABLE 3. ABSORPTION AND CIRCULAR DICHROISM DATA

| *Nucleoside* | $\lambda_{max}$ | $\varepsilon \times 10^{-3}$ | $\lambda$ | $[\theta] \times 10^{-3}$ | $\lambda_{max}$ | $\varepsilon \times 10^{-3}$ | $\lambda$ | $[\theta] \times 10^{-3}$ |
|---|---|---|---|---|---|---|---|---|
| X | 258 | 8·7[a] | 245 | −2·5[a] | 262 | 5·1[b] | 271·5 | −2·3[b] |
| G | 275 | 8·4[a] | 285 | −1·4[a] | 275 | 10·3[b] | 285 | −1·3[b] |
| I | 248 | 12·2[a] | 245 | −3·3[a] | 252·5 | 10·0[b] | $B_{2u}$ | < −2·0[b] |
| A | 260 | 14·4[a] | 266 | −3·2[b] | 262 | 14·0[b] | 268 | −4·4[b] |
| i—G | 275 | 9·25[b] | 285 | −1·7[b] | 275 | 10·4[b] | 285 | −1·6[b] |
| i—I | 249 | 12·2[b] | 244 | −2·7[b] | 252 | 10·5[b] | $B_{2u}$ | < −2·5[b] |
| i—A | 259 | 15·0[b] | 266 | −3·2[b] | 262 | 14·3[b] | 270 | −4·2[b] |

[a]W. Voelter, R. Records, E. Bunnenberg, and C. Djerassi, *J. Am. Chem. Soc.* **90**. 6163 (1968)
[b]This work.

The glycosyl torsion angles reported here for the unblocked (C-2′ *endo*) nucleosides A,G,I, and X are coincident with the two low-energy regions calculated by Berthod and Pullman[13] for C-2′ *endo* purine nucleosides. The population of the *syn* range as well as the *anti* in the liquid phase, in contrast to the solid phase where only the *anti* conformation occurs, cannot be unequivocally rationalized. Solvent effects on the sugar-base dipolar interactions are probably important. Computations of total nucleoside dipole moment in progress[14, 15] show that the dipole moment of individual nucleosides varies considerably with glycosyl conformation. Also, computed dipole moments for different nucleosides in the same conformation are significantly different.

TABLE 4. RIBOSE PROTON SPIN COUPLING CONSTANTS[a] IN TYPICAL PURINE NUCLEOSIDES[b]

| *Nucleoside* | $J_{1',2'}$ | $J_{2',3'}$ |
|---|---|---|
| X | 6·6 | 4·0 |
| G | 6·0 | 5·0 |
| I | 6·0 | 4·8 |
| A | 6·0 | 5·0 |
| i—G | 2·6 | 6·1 |
| i—I | 2·9 | 6·0 |
| i—A | 3·0 | 6·0 |

[a]In Hz ± 0·2
[b]25 M nucleoside in DMSO-$d_6$ 30.°C.

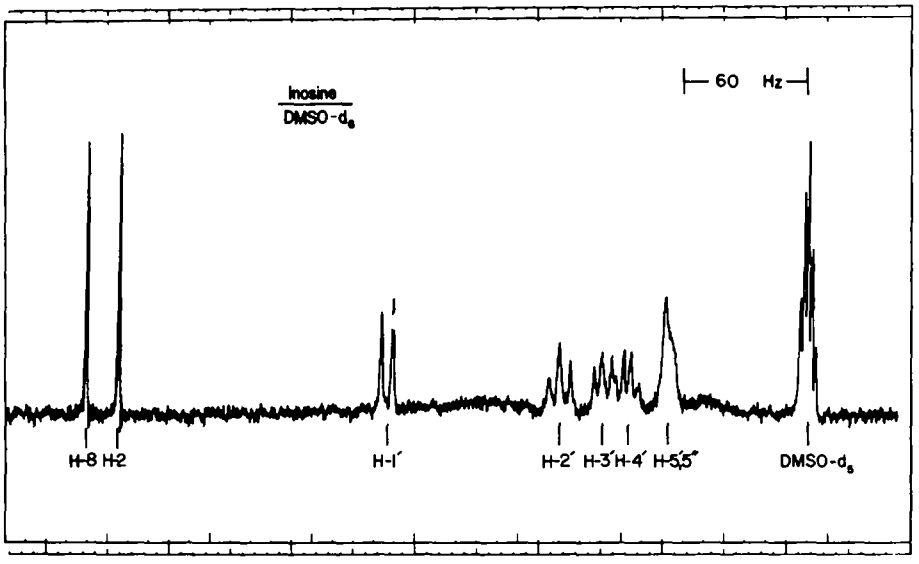



FIG 9. 60 MHz NMR spectrum of a typical natural purine β ribonucleoside. 0·25 M 30°C. See ref. 4 for NMR spectrum typical of a 2',3'–O–isopropylidene purine nucleoside.

The predominance of the *syn* glycosyl conformation in the isopropylidene blocked nucleosides i-A, i-I, and i-G is not consistent with the C-3' *endo* computations of Berthod and Pullman.[13] This disagreement may be ascribed to the more nearly planar or O-4' *endo* sugar conformation in these systems[4] and neither computations nor X-ray results are available on these models.

## CONCLUSION

The glycosyl conformation of the natural purine nucleosides in DMSO (C-2' *endo* sugar) is more variable than the glycosyl conformation of the C-3' *endo* 2',3'-O-isopropylidene derivatives in the same solvent. In the natural series the glycosyl torsion angle appears to be more dependent on nucleobase substituents and in both series sugar conformation is a significant determinant of glycosyl conformation.

If, as suggested above, the glycosyl conformations of the compounds studied here are essentially the same in water and DMSO, nucleic acid structure is subject to subtler influences than have been heretofore explicitly considered. For example, the number of possible structures to be considered in model building analysis of polynucleotide structure would be greatly increased and some of the alternative base pairing schemes proposed by Donohue and Trueblood and others[16] would be plausible.

## EXPERIMENTAL

Adenosine (A), inosine (I), guanosine (G), 2',3'-O-isopropylideneadenosine (i—A), 2',3'-O-isopropylideneinosine (i—I), and 2',3-O-isoproylideneguanosine (i—G) were Sigma grade products of Sigma Chemical Company, St. Louis, Mo. They were lyophilized from $D_2O$ before use for NOE experiments to exchange any bound water and hydroxyl and amino protons.

A and i—I NOE samples were 0·25 M nucleoside in DMSO-$d_6$ (99·5% minimum isotopic purity, Diaprep Incorporated, Atlanta, Georgia) containing 1·8% t-BuOD v/v. They were degassed by multiple

freeze-pump-thaw cycles and sealed under $10^{-5}$ torr before use. X,I,G,i—A, and i—G NOE samples were 0·25 M nucleoside in pure DMSO-$d_6$ in a coaxial, degassed, sealed tube with an external lock sample. NOE experiments were done at 30°C on a Varian HA-100 NMR spectrometer as described.[3] Each experimental NOE is an average of at least four measurements. We specify the glycosyl torsion angle by the convention used in our calculational procedure (Fig. 1).

NOE's as a function of torsion angle were calculated as described by Schirmer *et al.*[4] Bond lengths and angles from the X-ray of adenosine were used.[10] The rate of exchange of individual conformers used in these calculations is $k < 1\ \text{sec}^{-1}$ (Range I). The conformational fitting procedure is valid for a $k$ in the range $1\ \text{sec}^{-1} < k < 10^{8}\ \text{sec}^{-1}$ (Range II).[4] $f_m(n)$ denotes the fractional peak height enhancement of resonance $m$ upon saturation of resonance $n$.

Because the minimum number of experiments (five) was used in fitting the two gaussian distribution (Figs 2–4) the final fit parameters (positions, widths, and weight) are subject to some error, even though they represent best fits by the sum of squares criterion. The widths of the conformer populations are particularly inaccurate, but they can be taken as an indication of relative breadths. The addition of more experiments to the present data (Table 1) will lead to refinements in the distributions shown here (Figs 2–4). The use of two distributions to describe the glycosyl conformation is reasonable since theoretical considerations[11] have typically shown two allowed or preferred ranges of glycosyl conformation.

Chemical shifts (Fig. 5 and Table 2) and Varian HA-100 data measured with respect to DMSO-$d_5$ (2·50 $\delta$) on 0·25 M solutions at 30°C.

CD measurements (Table 3 and Figs 6–8) were made on a Cary 60 recording spectropolarimeter fitted with a Model 6002 CD attachment, with the split programmed for a half-bandwidth of 15 Å. The low wavelength region of the spectrum was inaccessible for the DMSO solutions because of high solvent absorbance below *ca.* 250 nm. The CD is recorded as molecular ellipticity, $[\theta]$, in units of deg.cm$^2$dmol$^{-1}$, and absorbances did not exceed 2. The instrument was calibrated using (+)-camphor sulfonic acid (Aldrich). Nucleoside concentration for the CD experiments done in 0·1 cm cells was 1-2. $\times 10^{-3}$ M. DMSO was dried over molecular seive before use and water was distilled and deionized. UV spectra (Table 3) were recorded on a Cary 14 spectrophotometer.

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Computations were carried out on a Univac 1108 Time Sharing Executive Computer maintained by the University of Wisconsin Computing Center.

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