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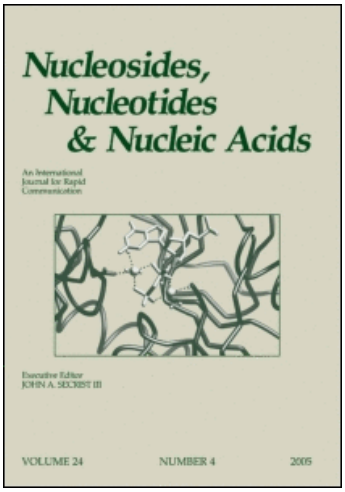

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Conformational Properties of Purine-Like *C*-Nucleosides

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NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 793-807 (1996)

REVIEW

CONFORMATIONAL PROPERTIES OF PURINE-LIKE *C*-NUCLEOSIDES

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ABSTRACT: The multiplicities and chemical shifts of the 5'-hydroxyl resonances in the NMR spectra of a series of purine-like *C*-nucleosides reflect the presence of a hydrogen bond to N(1), and hence afford a method for assessing solution *syn/anti* conformational preferences.

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INTRODUCTION: Early NMR investigations of the conformational properties of the sugar-ring hydroxyl groups in common nucleosides revealed that 5′-hydroxyls rotate freely and rapidly about C5′– O5′ bonds.[1] The coupling constants between the 5′-hydroxyl proton and the two C5′-protons are therefore averaged, which leads to the appearance of the familiar pseudo triplets for the 5′-OH resonances in the NMR spectra of nucleosides in non-aqueous solvents, provided that exchange processes are minimized. In contrast, we have found recently that C5′–O5′ rotation in a variety of purine-like *C*-nucleosides[2] is restricted, apparently by the presence of intramolecular hydrogen bonds between O5′– H and N(1) of the bases. Consequently, the 5′-OH resonances of these compounds appear in the NMR spectra as well-resolved double doublets. Analogous hydrogen bonds involving N(3), which is equivalent to N(1) of the *C*-nucleosides, are frequently observed in the crystal structures of purine nucleosides, but direct evidence for their existence *in solution* has been reported in only a few instances. Examples include the detection of an intramolecular O(5′)-H···N(3) hydrogen bond in N(6)-dimethyl-2′,3′-*O*-isopropylideneadenosine[3] by using NMR, IR and CD spectroscopy;[2] and in 8-bromo-2′,3′-

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This paper is dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.

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O-isopropylideneadenosine[4] by measurement of a long-range H(5")-N(3) spin-spin coupling. The solvent dependent changes in the proton NMR spectra of 2′-deoxyadenosine and its derivatives have been explained recently in terms of intramolecular O(5′)-H···N(3) hydrogen bonds.[5] However, all of these previous examples refer to non-polar solvents such as carbon tetrachloride or chloroform that promote the formation of intramolecular hydrogen bonds. In the present study, the intramolecular hydrogen bond is evident in a much more polar solvent, namely DMSO, and in compounds that retain a full complement of unsubstituted hydroxyl and amino groups. Moreover, we show that the multiplicities and chemical shifts of the 5'-OH resonances of a series of purine-like *C*-nucleosides can be used as a guide for assessing their *syn/anti* preferences.

RESULTS AND DISCUSSION. We first became aware of the unusual multiplicities of the 5′-hydroxyl resonances of purine-like *C*-nucleosides during studies with the free-base form of 9-deazainosine monohydrate. As shown in Figure 1, the 200 MHz proton NMR spectrum of the sugar portion of 9-deazainosine reveals the 5′-hydroxyl resonance as a clear-cut double doublet at 5.24 ppm with coupling constants of 3.9 and 8.4 Hz. If this extra multiplicity indeed reflects the presence of an intramolecular hydrogen bond, it follows that 9-deazainosine must exhibit a number of other conformational features in solution, including:

i) a preponderance of ϕ+ rotamers about the C5′–O5′ bond (Figure 2).
ii) a preponderance of +sc rotamers about the C4′–C5′ bond (Figure 3), and
iii) a marked preference for the *syn* conformation about the glycosyl bond.

These points will be addressed in turn.

(i) **C5′—O5′ Rotamers.** Using the assignments for H5′(S) and H5′(R) shown in Figure 1, which is the sequence found for purine nucleosides in general,[7] and employing the Karplus-type relationships developed for the H–C–O–H coupling path,[8] it is seen that the observed 5'-OH couplings of 9-deazainosine correspond to a ϕ+ population of about 64%.

(ii) **C4′—C5′ Rotamers.** Similarly, using the observed values of $J_{4',5'(S)}$ and $J_{4',5'(R)}$ in the empirical equations developed by Hruska and Sarma[9] produces a value of about 60% for the population of the +sc conformers.

(iii) **Glycosyl Rotation.** In NOE difference spectroscopy (Figure 1c), saturation of H-6 of 9-deazainosine leads to an 8% enhancement at H1′ but less than a 1%

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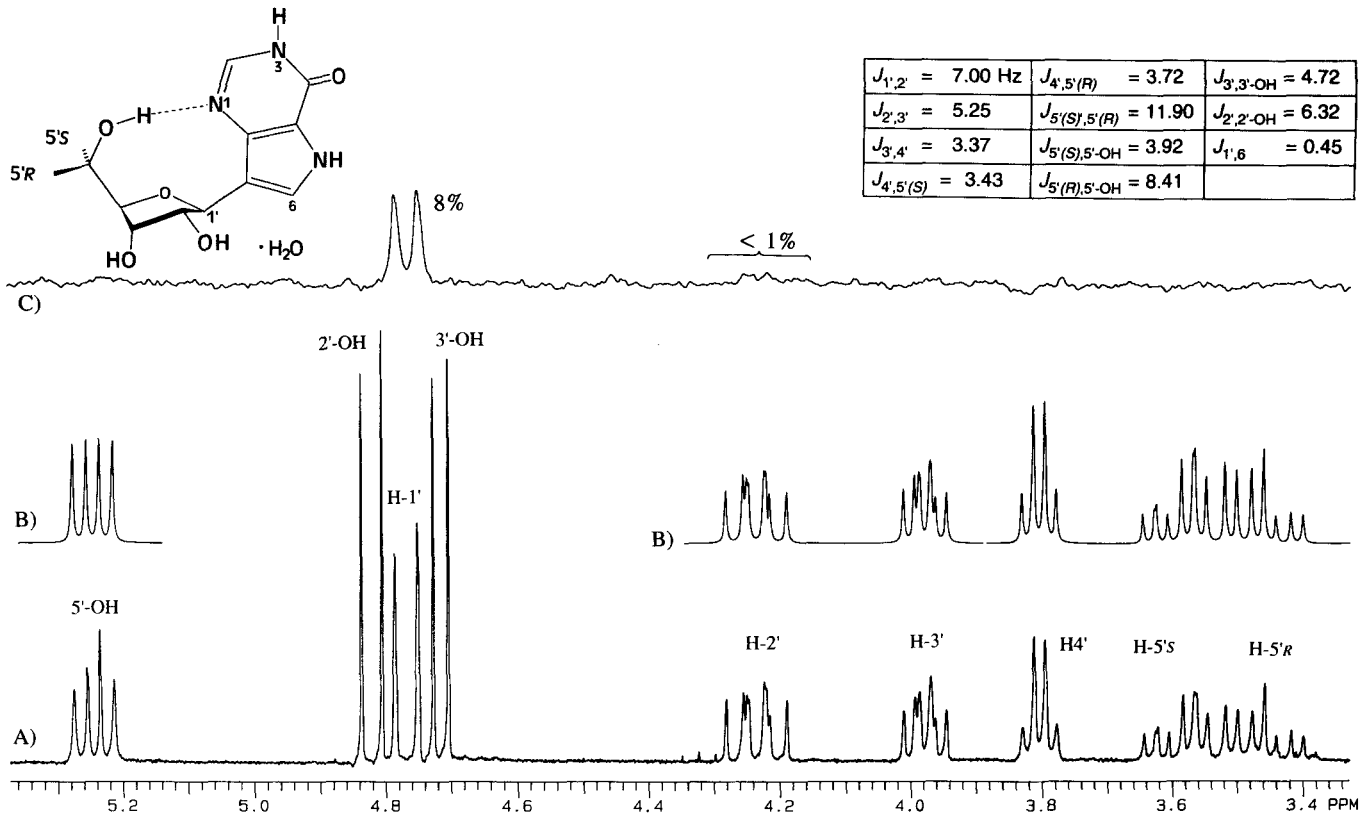

$J_{1',2'}$ = 7.00 Hz	$J_{4',5'(R)}$ = 3.72	$J_{3',3'\text{-OH}}$ = 4.72
$J_{2',3'}$ = 5.25	$J_{5'(S),5'(R)}$ = 11.90	$J_{2',2'\text{-OH}}$ = 6.32
$J_{3',4'}$ = 3.37	$J_{5'(S),5'\text{-OH}}$ = 3.92	$J_{1',6}$ = 0.45
$J_{4',5'(S)}$ = 3.43	$J_{5'(R),5'\text{-OH}}$ = 8.41	

FIGURE 1: Part of the 200 MHz NMR spectrum of 9-deazainosine monohydrate in DMSO-d_6 at 28 °C. A) Observed spectrum. B) Simulated spectrum using parameters shown above obtained by iteration. C) NOE difference spectrum obtained on saturation of H-6 (δ 7.35, 5 sec irradiation, 1 Hz line broadening). In addition to the 8% enhancement of H-1' illustrated, N5-H was enhanced by 7% (data not shown).

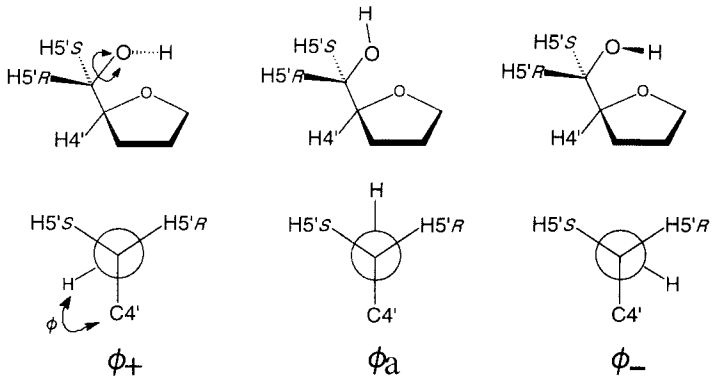

FIGURE 2: Classical Staggered C5′-O5′ Rotamers

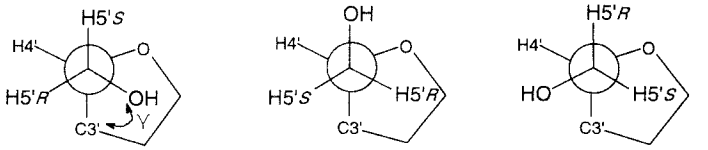

FIGURE 3: Classical Staggered C5′-C4′ Rotamers

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enhancement at H-2′. This result clearly demonstrates a preponderance of *syn* conformers according to the analysis of Rosemeyer *et al.*[10] The same conclusion follows from the phase sensitive NOESY spectrum of 9-deazainosine, which shows that the H-6/H1′ cross peak is much more intense than the H-6/H2′ cross peak. In contrast, similar spectra obtained for inosine, a nucleoside that is known[10] to favor a higher proportion of *anti* conformers, show the opposite situation – that is the H8/H2′ cross peak is much stronger than the H8/H1' cross peak. That 9-deazainosine prefers the *syn* conformation in DMSO is noteworthy in view of the fact that the compound adopts the *anti* form in the crystalline state.[11] The *syn*/+sc combination has previously been correlated with a preference for the S(C2′-*endo*) puckering of the ribose ring;[12] for 9-deazainosine a 70% population of *S* conformers can be estimated from the value of $J_{3',4'}$, using the method of Remin.[13]

From the foregoing analysis, it is clear that 9-deazainosine adopts an overall solution conformation that is consistent with the presence of an intramolecular O(5′)-H···N(1) hydrogen bond. The hydrogen bond is evidently a strong one because the 5′-hydroxy resonance maintains its double doublet character at temperatures as high as 55 °C. At higher temperatures the 5′-hydroxy resonance begins to collapse (reversibly) because of exchange decoupling.

The appearance of the 5′-hydroxyl resonance of 9-deazainosine (**2**) can be compared in figure 4 with those of a number of other purine-like *C*-nucleosides, namely formycin A (**1**), 6-methyl-7-(β-D-ribofuranosyl)-pyrrolo[3,4-*d*]pyrimidin-4(3H)-one[14] (**3**), 4-amino-7-(β-D-ribofuranosyl)-pyrazolo[1,5-*a*]1,3,5-triazine[15] (**4**), 4-methylthio-7-(β-D-ribofuranosyl)-pyrazolo[1,5-*a*]1,3,5-triazine[16] (**5**), 4-amino-7-(β-D-ribofuranosyl)-pyrrolo [2,1-*f*]1,2,4-triazine[17] (**6**) and 7-(β-D-ribofuranosyl)-thieno[3,4-*d*]pyrimidin-4(3H)-one[18] (**7**). These compounds are arranged from left to right with increasing values of $J_{5'OH,H5'(S)}$ and decreasing values of $J_{5'OH,H5'(R)}$. Although the 5′-hydroxyl group of formycin A (**1**) is too broad to show fine splitting, the resonance is clearly not a triplet and the coupling constants can be estimated by simulation of the H5′ resonances. At the other extreme, the 5'-OH of **7** is a conventional pseudo triplet , indicating that rotation about C5'–O5' in that particular case is not restricted. For **6**, the small difference between the two coupling constants leads to the appearance of a shoulder on the central peak of the OH resonance, which is more apparent when viewed at a shallow angle from below. Simulation of the H5' resonances of **6** confirms that the two 5'-OH,H5' coupling constants are indeed slightly different from each other.

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From the data presented in Figure 4, the following trends are discernable:

(i) The 5′-hydroxyl resonances become progressively deshielded as the two 5′-OH coupling constants diverge from each other, which is consistent with the presence of a hydrogen bond. For the compounds on the left (**1** - **3**), 5′-OH resonates *downfield* of 2′-OH and 3′-OH; for the compounds on the right (**4** - **7**), 5′-OH resonates *upfield* of 2′-OH and 3′-OH.

(ii) The populations of the ϕ+ and +sc conformers decrease markedly from left to right, whereas the population of *S* conformers deceases to a smaller extent from left to right.

The NMR spectra of a number of other purine-like C-nucleosides that we have prepared are comparable to those shown in Figure 4. For example, the splitting pattern of the 5'-hydroxyl resonance of 9-deazanebularine[20] (**8**) is virtually identical to that of **2**,

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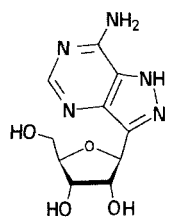
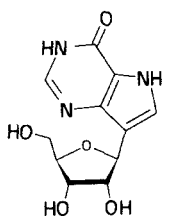
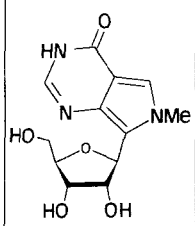
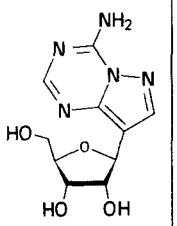
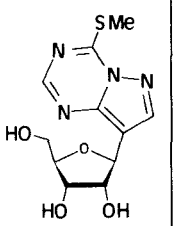
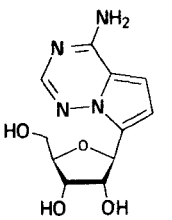
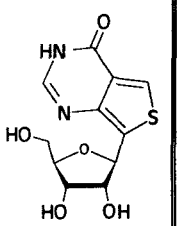
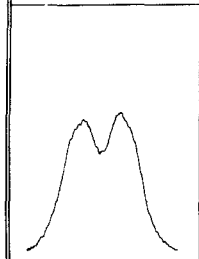
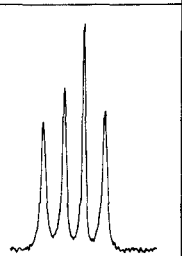
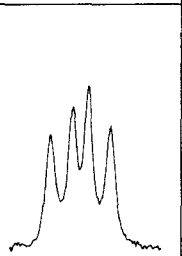
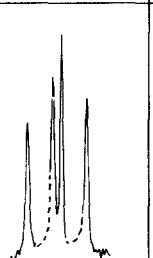
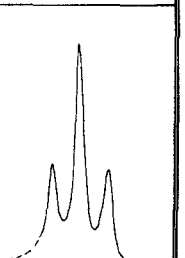
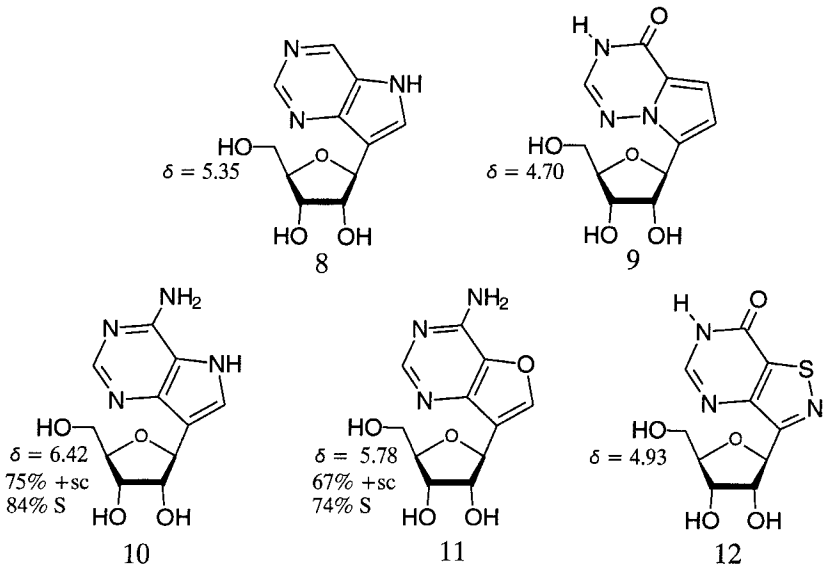
 1	 2	 3	 4	 5	 6	 7
						
δ5.96	δ5.24	δ5.23	δ4.93†	δ4.79	δ4.76	δ4.86‡
J's ≈ 3 and 9 Hz	*J*'s = 3.9 and 8.4	*J*'s = 4.1 and 7.8	*J*'s = 4.9 and 6.9	*J*'s = 5.3 and 6.3	*J*'s = 5.4 and 6.1	both *J*'s ≈ 5.6Hz
70% ϕ_+	64% ϕ_+	56% ϕ_+	49% ϕ_+	43% ϕ_+	41% ϕ_+	36% ϕ_+
63% +sc	59% +sc	52% +sc	46% +sc	42% +sc	41% +sc	44% +sc
74% S	70% S	67% S	63% S	63% S	57% S	62% S

FIGURE 4: 5'-Hydroxyl Resonances and Conformational Properties of Purine-like *C*-Nucleosides in DMSO-d_6. Spectra were obtained at 28 °C. † Chemical shift obtained at 28 °C, but resonance shown was obtained at 52 °C where overlap with 2'-OH was better resolved. ‡ Minor overlap with 3'-OH.

and the conformational populations of these two compounds are clearly alike. Similarly, the 5'-hydroxyl resonance of the 4-oxo analogue[21a] **9** is virtually identical to that of its 4-amino counterpart **6**. However, for other purine-like *C*-nucleosides, such as 9-



8 9 10 11 12

deazaadenosine[22] (**10**) and 4-amino-(7-β-D-ribofuranosyl)-furo[3,2-*d*]pyrimidine[11,23] (**11**), the 5'-OH resonances in DMSO-d_6 appear as broad peaks without discernable multiplicity. In these cases the rate of exchange is such that accurate coupling constants cannot be extracted even from the *5′(S)* and *5′(R)* resonances. Nevertheless, the marked deshielding of the 5′-hydroxyl resonances of **10** and **11**, as well as the fact that only H5′*(R)* shows residual hydroxyl coupling,[24] strongly suggests the presence of intramolecular O(5′)-H···N(1) hydrogen bonds. In addition, compounds **10** and **11** show overwhelming preferences for sugar puckerings of the *S* type and for the +sc C4′–C5′ rotamers, and they clearly belong on the left hand (*syn*) side of Figure 4.

Given the long-standing interest that medicinal chemists have shown in the formycin family of *C*-nucleoside antibiotics and their analogues, it is somewhat surprising that non-triplet 5'-OH resonances have not been observed frequently, especially as much of the relevant NMR data in the literature refers to spectra recorded in DMSO-d_6. However, we are aware of only one other example, namely compound **12**, where the 5'-OH resonance in DMSO-d_6 was reported[25] to be a "multiplet". While it is not clear if this refers to a triplet or a double doublet, the fact that the 5'-OH (δ 4.93) resonates upfield of 2'-OH and 3'-OH would place the compound in about the center of Figure 4.[26]

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We conclude from the above discussion that the compounds in Figure 4 form a graded series where the population of hydrogen-bonded *syn* conformers diminishes on going from left to right. Indeed, the NOESY spectrum of **6** shows a very intense H-6/H-2' cross peak, which is diagnostic of the *anti* conformation, but only a very weak H-6/H-1' cross peak, which arises from the small population of *syn* conformers. This situation is exactly opposite to that seen in the NOESY spectrum of 9-deazainosine (**2**) discussed above. Interestingly, the one compound that shows a triplet for its 5'-OH resonance, namely nucleoside **7,** might be expected to favor the *anti* conformation since there is the possibility of a non-bonded attractive interaction between the sulfur atom and the furanose ring oxygen. Such an interaction is an important determinant of the conformation of the thiazole *C*-nucleoside tiazofurin[19]. On the other hand, why is there such a marked difference in the H-bonded *syn* populations between, for example, **10** and **6**? We reasoned that the strength of such an H-bond would be directly related to the electron density centered at N(1). A measure of the partial negative charge at N(1) for C-nucleosides **1-11** was therefore obtained from Molecular Electrostatic Potential (MEP) computer-mapping of the bases corresponding to compounds **1-11**. A statistical analysis of a scatter plot (Figure 5) of electrostatic potential values calculated at a distance of 1.5Å from N(1) vs. the chemical shifts of 5'-OH for the corresponding nucleosides revealed a direct correlation between the two, with a positive correlation coefficient of $R = 0.887$ ($p < 0.001$). Very similar values were obtained when the 5'-OH chemical shifts were plotted against electrostatic potential values calculated at 2Å from N(1).

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As a group, the purine-like *C*-nucleosides show an extraordinary range of biological activities. However, it is unlikely that these activities can be correlated with particular conformational preferences of the individual nucleosides because of the Curtin-Hammett principle.[10] This principle states, in effect, that if an enzyme is exposed to a population of conformers that are in rapid equilibrium compared with the rate of binding, then the enzyme can seek out the most favorable conformer even if it is present in minute concentrations. On the other hand, if binding is extremely rapid compared with the rate of conformer interconversion, then analogues that are closer to the optimum conformation should be favored relative to those that adopt some other conformation. For the present suite of purine *C*-nucleosides, however, the *syn* and *anti* conformers are in rapid equilibrium, as shown by the fact that cross peaks from both forms are seen in the NOESY spectra. This view is further supported by the fact that the predominantly *syn* nucleoside **2** and the predominantly *anti* nucleoside **7** are equally effective anti-trypanosomal agents.[21b,27] Similarly, **10** (*syn*), **11** (*syn*), and **6** (*anti*) are equipotent inhibitors of the growth of mammalian leukemia cell lines, with ID_{50} values in the *nano*molar range.[17,23] Clearly, these agents are flexible enough to adopt whatever

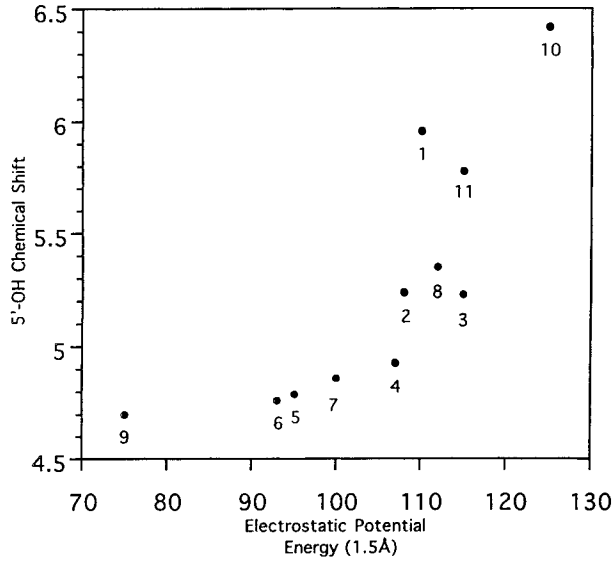

FIGURE 5

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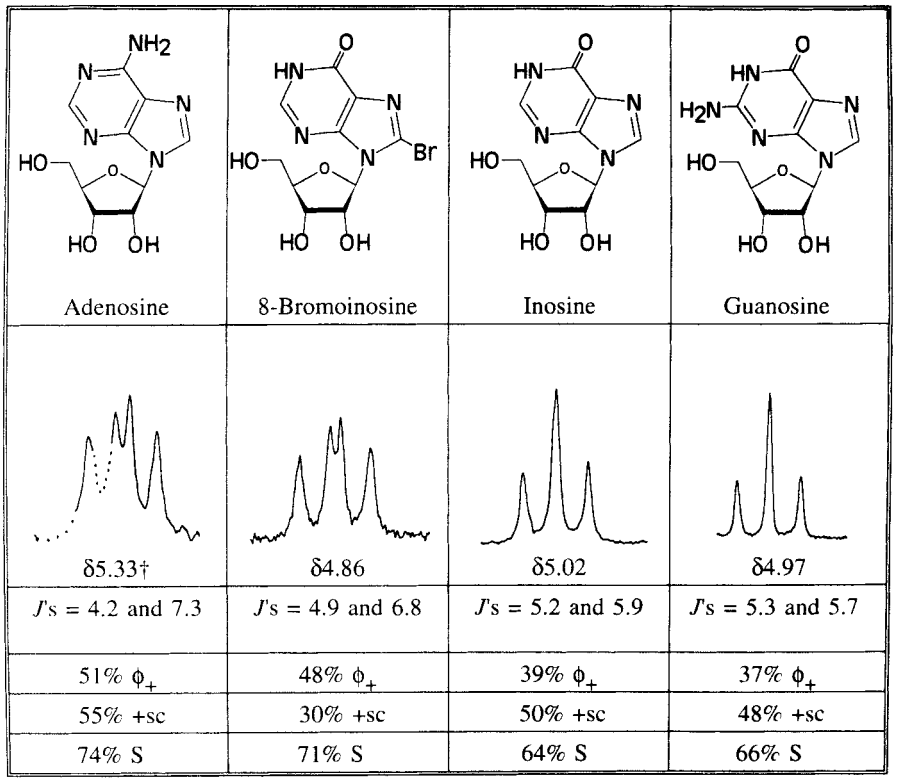
conformation the enzyme (or enzymes) requires. From this viewpoint, it seems to us that the argument[28] that 2',3'-dideoxy-formycin A is ineffective as an anti-HIV agent because it prefers the *syn* conformation is not convincing.

Since the majority of purine-like *C*-nucleosides we have examined do *not* exhibit pseudo triplets for their 5′-OH resonances, we have redetermined the NMR spectra of some of the common purine nucleosides in DMSO-d_6. As seen in Figure 6, the 5′-OH resonances of both inosine and guanosine appear as conventional triplets. Spin simulation of the H5′*(R)* and H5′*(S)* resonances suggests that the coupling constants $J_{5'(S),OH}$ and $J_{5'(R),OH}$ differ slightly from each other for both compounds. However, the observed line widths of these particular 5′-hydroxyl signals would mask such small differences and, for practical purposes, the 5′-hydroxyl groups of inosine and guanosine can be regarded as undergoing free-rotation. For both 8-bromoinosine and adenosine, the 5-hydroxyl resonances appear as double doublets, which suggests the presence of substantial populations of conformers with intramolecular O(5′)-H···N(3) hydrogen bonds.[29] The result with adenosine is interesting because a previously published[1c] spectrum shows the 5′-hydroxyl signal as a triplet. However, that spectrum was recorded in DMSO-d_6 containing 40% benzene-d_6, so the solvent composition clearly affects the various



All spectra were obtained at 37 °C. † Overlapped with 2'-OH.

FIGURE 6: 5'-Hydroxyl Resonances and Conformational Properties of Purine Nucleosides in DMSO-d_6.

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populations of conformers. It is interesting to note that 8-bromoinosine and adenosine, the two compounds that show double doublets for their 5′-OH resonances, are known to favor the *syn* range, either because of the presence of a bulky 8-substituent or from NOE data.[10] On the other hand, inosine and guanosine, which according to NOE studies[10,30] prefer the *anti* range, exhibit pseudo triplets for their 5′-OH resonances.

CONCLUSIONS: For purine and purine-like nucleosides, the appearance of the 5′-hydroxyl resonance as a double doublet is consistent with the presence of an intramolecular hydrogen bond between O(5′)-H and a neighboring nitrogen atom (N3 or N1, respectively) on the base. From this simple NMR observation, it follows that a substantial population of conformers confined within a narrow segment of the *syn* range

TABLE 1: Coupling Constants (Hz). [a] not resolved. [b] $J_{2,N3-H}$ = 3.4 Hz and $J_{6,N5-H}$ = 3.1 Hz. [c] $J_{2,N3-H}$ = 3.5 Hz, [d] $J_{5,6}$ = 4.4 Hz.

Cmpd	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{5',5''}$	$J_{2',OH}$	$J_{3',OH}$
10	7.8	5.1	2.3	2.6	2.9	12.2	6.6	4.0
11	7.0	5.0	3.1	3.1	3.2	12.2	a	a
1	7.2	5.2	3.1	3.2	3.5	12.2	6.8	4.0
8	7.1	5.1	3.3	3.3	3.6	12.0	6.2	4.6
2[b]	7.0	5.3	3.4	3.4	3.7	11.9	6.3	4.5
3[c]	7.2	5.5	3.6	3.6	4.4	11.9	6.6	4.8
4	6.7	5.5	4.0	3.9	4.5	11.8	6.3	4.9
5	6.7	5.3	4.0	4.1	4.6	11.8	6.2	5.1
6[d]	6.4	5.5	4.4	4.0	5.0	11.7	6.3	5.2
9[d]	6.4	5.4	4.6	3.9	5.2	11.7	6.0	5.2
7[c]	6.4	5.2	4.0	3.9	4.7	11.8	6.0	4.9

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must also be present. Conversely, the appearance of the 5′-hydroxyl resonance as a pseudo triplet does not mean that hydrogen-bonded *syn* conformers are not present, but it does suggest that they are not the predominant forms. NOE and CD measurements of nucleosides have shown no gross conformational differences between D_2O and DMSO solutions,[31] so it is likely that our results are relevant to aqueous systems.

EXPERIMENTAL: ^{1}H-NMR spectra of the purine and purine-like *C*-nucleosides in methyl sulfoxide-d_6 were obtained at 28 °C relative to TMS on a Varian XL-200 spectrometer (Tables 1 and 2). All nucleoside samples were recrystallized from water or ethanol and dried in air prior to dissolution (~5 mg/mL) in methyl sulfoxide-d_6 from freshly opened vials. No special precautions were taken to exclude atmospheric moisture, except that the solution of 9-deazainosine in methyl sulfoxide-d_6 was degassed by five cycles of the freeze-pump-thaw method prior to sealing *in vacuo*. The double doublet nature of the 5'-hydroxyl resonance in the NMR spectrum of this particular sample was unchanged for at least three years; more typically, the hydroxyl multiplicities of less carefully prepared

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TABLE 2. Chemical Shifts (δ). [a] H-6, [b] N5-H, [c] H-4, [d] H-5, [e] N3-H, [f] Overlapped with N-Me but in DMSO-D_2O (2:1, 37°C) H4' and N-Me are separated at 3.88 and 3.78 ppm. [g] S-Me at 2.72 ppm.

	Ribosyl Protons								Base Protons			
Cmpd	H1'	H2'	H3'	H4'	H5'	H5"	2'-OH	3'-OH	H2	H4/5/6	N3/5-H	NH_2
10	4.74	4.35	4.01	3.87	3.61	3.47	4.89	4.74	8.04	7.50[a]	10.84[b]	6.84
11	4.77	4.33	4.02	3.89	3.63	3.49	5.07	4.92	8.22	8.21[a]	-	7.46
1	4.92	4.50	4.09	3.93	3.65	3.50	5.00	4.92	8.16	-	12.71[b]	7.42
8	4.90	4.30	4.04	3.86	3.64	3.51	4.86	4.78	8.90	8.78[c] 7.90[d]	11.76[b]	-
2	4.76	4.23	3.97	3.80	3.59	3.46	4.82	4.72	7.82	7.38[a]	11.95[e] 12.01[b]	-
3	4.95	4.61	3.99	3.80[f]	3.57	3.45	4.98	4.89	7.58	7.48[d]	12.02[e]	-
4	4.82	4.19	3.99	3.78	3.57	3.48	4.91	4.84	8.18	8.05[a]	-	8.39
5[g]	4.92	4.19	4.00	3.81	3.58	3.49	4.98	4.90	8.52	8.42[a]	-	-
6	5.11	4.24	3.93	3.79	3.55	3.46	4.94	4.86	7.82	6.84[d] 6.68[a]	-	7.62
9	5.06	4.18	3.95	3.54	3.54	3.46	4.97	4.87	7.81	6.87[d] 6.62[a]	11.60[e]	-
7	5.31	4.05	3.95	3.83	3.57	3.49	5.12	4.93	7.78	8.39[d]	11.64[e]	-

samples gradually collapse on storage of the solution at room temperature. Spectra were also recorded after the addition of D_2O. All values given for coupling constants and chemical shifts that pertain to non first-order portions of the spectra were obtained by spin simulation using Varian software. Iteration was continued until the root-mean-square frequency error between the observed and calculated lines was less than 0.1 Hz. NOESY spectra were generated using a mixing time of 0.5 sec. The contour map plotting of MEPs in the plane of the bases corresponding to compounds **1-11** was carried out using the MNDO semi-empirical calculation method as implemented by Hyperchem™ (Release 3) from Autodesk on a 486 PC compatible. The electrostatic potential values used in the scatter plots were measured along the C4-N1 axis of the appropriate base at either 1.5Å or 2Å away from N1. Prior to all MNDO calculations, the stuctures of the bases were first optimized by molecular mechanics methods using the Polak-Ribière (conjugate gradient) algorithm and MM+ force field (an extension of MM2) as implemented by Hyperchem™.

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