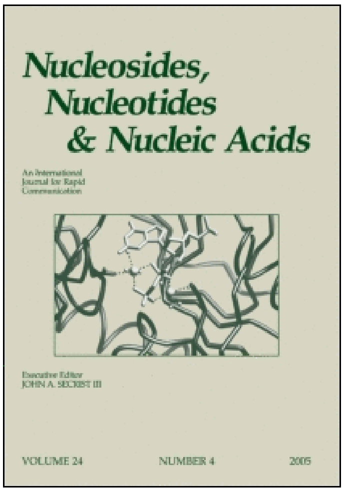

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NMR STUDIES OF N^1- AND N^3-(D-2'-DEOXYRIBOFURANOSYL) NUCLEOSIDES FROM ETHYL 5-AMINOIMIDAZOLE-4-CARBOXYLATE

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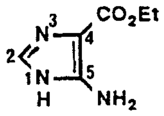
Abstract The conformation of deoxyribosides derived from N^1 and N^3 substitution of the aglycone ethyl 5-aminoimidazole-4-carboxylate (AICE) has been determined by ^{1}H and ^{13}C NMR spectroscopy. The deoxyribose ring shows considerable variation in the form and population of the contributing N and S conformers but the *syn/anti* ratio is similar to that in the purine analogues.

Introduction

Imidazoles of type **1** are related to intermediates involved in the biosynthesis of purine nucleotides. We have been investigating[1] various nucleoside derivatives of the imidazoles as inhibitors of enzymes involved in the biosynthetic pathway. Specificity in inhibitory activity must be related to the structure and conformation of these compounds and hence we have initiated a detailed study of the conformation of a series of four deoxyribosides of **1** using ^{1}H and ^{13}C NMR spectroscopy. Preliminary study[2] of the deoxyribosylation of **1** using a blocked sugar halide indicated that reaction can occur at both N^1 and N^3 sites. Using this synthetic procedure and a procedure involving biotransformation on encapsulated cells the α and β anomers of N^1- and N^3-deoxyribosides of **1** were obtained. This aglycone is designated AICE (*cf* AICA for **2**) and the nucleoside ethyl 5-amino-1-(β-D-2'-deoxyribofuranosyl)-imidazole-4-carboxylate **3β** is referred to as 1-βdAICE. The other isomers **4β**, **3α** and **4α** are respectively 3-βdAICE, 1-αdAICE and 3-αdAICE.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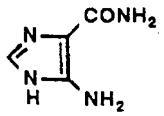
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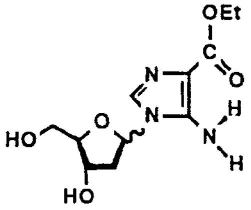
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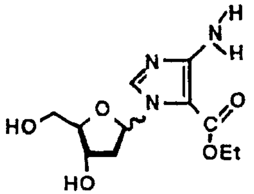
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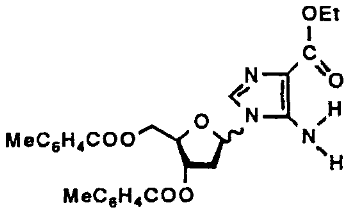
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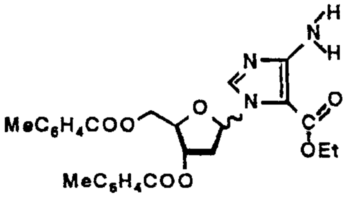
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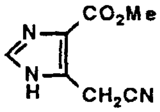
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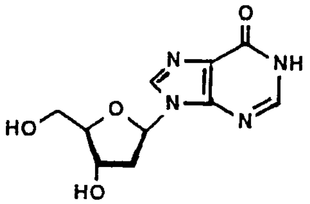
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8

Results and Discussion

The ^1H and ^{13}C chemical shifts are given in Table 1 for the α and β forms of the unprotected nucleosides **3** and **4** and for the corresponding O-protected species **5** and **6**. The protecting group is *p*-toluoyl ($CH_3C_6H_4CO$). These data were obtained for solutions in DMSO-d_6 [(**3**) and (**4**)] and $CDCl_3$ [(**5**) and (**6**)] and this solvent difference accounts for some of the evident chemical shift differences. In the case of the unprotected nucleosides **3** and **4** only 3-βdAICE had clearly separated peaks for H-5' and H-5" and for the protected species this was the case only for the β-N^1 form **5**β. This degeneracy in the H-5' and H-5" shifts means that only the sum ($J_{4',5'} + J_{4',5''}$) is properly accessible and the entries in Table 1 are mean values in most cases. Protons H-2' and H-2" are distinguishable in all cases and in line with work on other deoxyribosides[3] the lowfield multiplet was assigned to H-2" except as discussed below.

The site of glycosylation of AICE (N^1 or N^3) can be determined from several features of the ^1H and ^{13}C spectra. The sugar proton H-1' is deshielded by 0.4-0.6 ppm in the N^3-isomers **4** and **6**. This downfield shift results from the close proximity of the ester group to H-1' in these compounds for the *anti* conformation of the base. Similar but smaller shifts are observed[4] for the 2'-deoxyribosides obtained from N^1 and N^3 glycosylation of the analogous imidazole **7**. The anisotropic effect of the ester group has been implicated in this and other examples[4] but the deshielding is greater in derivatives of AICE because an intramolecular hydrogen bond between the 4-COOEt and 5-NH_2 fixes the orientation of the ester group. An X-ray diffraction study of the riboside of **2** confirms the existence of this type of hydrogen bond.[5]

A further consequence of the close approach of the OEt group to the chiral environment of the deoxyribose ring in the N^3-isomers is the nonequivalence of the prochiral protons in the OCH_2 group. This nonequivalence (up to 0.04 ppm) is unequivocally diagnostic for N^3-glycosylation, since it is independent of anomeric configuration (*cf* data in Table 1 for **4**α and **4**β). The notable deshielding (0.3 - 0.7 ppm) of the protons of the 5-NH_2 group in the N^1 isomers by the sugar ring is another convenient means of distinguishing N^1 and N^3 isomers.

The ^{13}C chemical shifts show the usual effect of glycosylation on the carbon atoms alpha and beta to the substituted nitrogen *ie* upfield shift for the alpha site and downfield shift for the beta site. Typically these glycosylation shifts have been measured relative to the anion of the aglycone.[4,6] This is not easy for complex imidazoles such as AICE and it is sufficient to note the *ca* 10 ppm change in C-4 and C-5 chemical shifts on going from an alpha to a beta relationship to the site of N-substitution. Thus the C-4 and C-5 chemical shifts are also diagnostic of the site of glycosylation in this particular case since these shifts are well separated in the aglycone moiety.

TABLE 1 N.m.r. data[a] for imidazole nucleosides **3**, **4**, **5** and **6**[b]

	3α	**3β**	**4α**	**4β**	**5α**	**5β**	**6α**	**6β**
H-1'	5.93	5.96	6.35	6.36	6.00	5.98	6.60	6.62
H-2'	2.65	2.39	2.56	2.16	2.95	3.02	2.95	2.4
H-2"	2.12	2.14	2.03	2.28	2.89	2.60	2.57	2.91
H-3'	4.3	4.32	4.2	4.2	5.68	5.65	5.62	5.58
H-4'	4.02	3.82	4.1	3.78	4.6	4.54	4.87	4.60
H-5'	3.4	3.55	3.4	3.58	4.6	4.71	4.56	4.67
H-5"	3.4	3.55	3.4	3.52	4.6	4.62	4.56	4.67
H-2	7.42	7.35	7.67	7.83	7.36	7.12	7.60	7.68
NH_2	6.14	6.16	5.66	5.7	5.27	5.29	4.86	4.89
OCH_2	4.16	4.16	4.18 4.22	4.18 4.21	4.34	4.32	4.28 4.32	4.31 4.31
C-2	129.5	129.5	137.9	137.3	128.8	128.8	136.4	135.9
C-4	109.1	109.5	156.4	156.5	112.4	113.0	156.1	156 0
C-5	145.9	145.9	99.3	99.4	145.9	145.7	100.8	101.0
C-1'	88.0	87.4	89.1	87.2	84.9	85.4	89.2	87.5
C-2'	*c*	*c*	*c*	41.9	36.4	36.2	41.0	40.7
C-3'	70.6	70.6	70.7	69.2	74.5	74.4	74.9	74.7
C-4'	83.4	83.7	87.1	85.8	83.8	83.0	85.4	82.9
C-5'	61.5	61.3	61.7	60.8	63.8	63.9	64.3	64.0
CO	163.9	163.9	160.5	160.4	164.8	164.8	161.1	160.9
OCH_2	58.4	58.3	58.9	59.0	60.0	60.0	60.0	60.0
$J_{1',2'}$	7.7	7.8	7.1	5.9	6.6	9.0	6.6	7.0
$J_{1',2''}$	3.4	6.1	1.9	6.1	2.5	5.2	<1.0	5.8
$J_{2',2''}$	−14.2	−13.2	−14.2	−13.3	−15.5	−14.1	−15.4	−14.1
$J_{2',3'}$	7.0	6.2	6.6	6.0	6.7	6.7	6.6	6.6
$J_{2'',3'}$	3.0	2.8	1.9	5.0	2.4	1.8	<1.0	2.8
$J_{3',4'}$	3.0	2.8	2.6	3.8	2.3	2.6	1.0	3.3
$J_{4',5'}$	4.5[d]	3.9[d]	*c*	4.0	*c*	3.6	4.2	3.8[d]
$J_{4',5''}$	4.5[d]	3.9[d]	*c*	4.4	*c*	3.0	4.2	3.8[d]
$J_{5',5''}$	*c*	*c*	*c*	−13.9	*c*	−12.3	*c*	*c*

[a] Anomers of **3** and **4** were examined in DMSO-d_6 and anomers of **4** and **5** in $CDCl_3$.

[b] Data for dI : H-1' 6.31, H-2' 2.61, H-2" 2.31, H-3' 4.39, H-4' 3.87, H-5' 3.00, H-5" 3.54, C-1' 87.9, C-2' obscured, C-3' 70.6, C-4' 83.5, C-5' 61.6 ppm, $J_{1',2'}$ 7.3, $J_{1',2''}$ 6.3, $J_{2',2''}$ −13.2, $J_{2',3'}$ 6.0, $J_{2'',3'}$ 3.4, $J_{3',4'}$ 3.1, $J_{4',5'}$ 4.5, $J_{4',5''}$ 4.6, $J_{5',5''}$ −11.7. [c] Data inacessible. [d] Average values.

Assignment of anomeric configuration in nucleosides derived from AICE or AICA is difficult since there are no clearly diagnostic chemical shift differences in either the ^{1}H or ^{13}C spectra which are applicable to all glycosides. The protected (isopropylidene)-ribosides 1-αrAICE and 1-βrAICE and analogous derivatives of AICA, show only a 0.1 ppm difference in H-1' shifts[7] but there is some evidence[8] that 2-substituted-AICE forms N^1 ribosides in which H-1' is consistently deshielded (0.2-0.4 ppm) in the α-anomer. Unfortunately exactly the opposite trend is apparent[4] in N^1- and N^3-deoxyribosides of imidazole **7**. The splitting pattern for H-1' was the best indication of anomeric configuration since the coupling $J_{1',2''}$ is 2 to 3 Hz in the α-anomer and 6 to 7 Hz in the β-anomer. A larger value (1 Hz more negative) of $J_{2',2''}$ is also observed in the α-forms. In mixtures of α- and β-anomers the ratio of these species can be obtained from the H-2 singlets which are distinguishable for each set of four isomers **3α**, **3β**, **4α**, **4β** and **5α**, **5β**, **6α**, **6β**.

Glycosidic bond conformation. Generally nucleosides can adopt *syn* (χ 90°) and *anti* (χ 270°) conformations.[9] Purine ribonucleosides adenosine (A), guanosine (G) and inosine (I) are thought to exist in both *syn* and *anti* forms with the *syn* predominant, although evidence is conflicting.[9,10] The analogous deoxyribosides dA, dG and dI have also been investigated[3,11] but the glycosyl conformation is not well established. Davies[9] appears to classify dA and dG as *anti* type. For nucleosides from AICE (and AICA) the difference in energy between the *anti* form (as shown in **3** - **6**) and the *syn* form is certain to be less than in the case of the purine derivatives such as dI **8** due to reduced interaction of the base with the CH_2OH group in the *syn* conformation. A hydrogen bond between purine N-3 and OH-5' can stabilise the *syn* form of dA but such a bond is less likely in AICE nucleosides since the equivalent N atom (in the NH_2 group) is not basic (*ie* pyridine type). An X-ray study[5] of 1-βrAICE indicates an anti conformation (χ 243°) in the solid state.

Comparison of compounds **3** and **4** with **8** gives the chemical shift differences shown in Table 2. For the β-anomers there are only small effects at H-4', H-5' and H-5" suggesting a common average conformation for the base.

For 1-βdAICE proton H-2 produces equal NOE at H-1' and H-3' (*ca* 3%) and the NH_2 group has a 4% NOE at H-1' and 2% NOE at H-3'as expected for approximately equal contributions from *syn* and *anti* conformations. In contrast 3-βdAICE appears to be largely in the *anti* conformation. This is established by the H-2 NOE at H-2' (2.5%) compared to H-2"(0%) and by positive enhancements at H-5', H-5" and OH-5'. Confirmation comes from the observation of a 2.5% NOE at the ester methyl group from H-1'.

The α-anomer of the N^1 deoxyriboside appears to be similar to the β-anomer but with a greater proportion of the *anti* conformer. The ratio of NOE from NH_2 and H-2 is 3:1 at H-1' and 1:2 at H-4' suggesting a *anti*/*syn* ratio of *ca* 2:1. For 3-αdAICE the heterocyclic ring

TABLE 2 Comparison of N^1- and N^3-AICE nucleosides with Deoxyinosine (dI)[a]

	H-1'	H-2'	H-2"	H-3'	H-4'	H-5'	H-5"
8 – 3β	0.35	0.22	0.17	0.07	0.05	0.05	−0.01
8 – 4β	−0.05	0.33	0.15	0.19	0.09	0.02	0.02
8 – 3α	0.38	−0.04	0.19	0.09	−0.15	0.20	0.14
8 – 4α	−0.04	0.05	0.28	0.29	−0.23	0.20	0.14

[a] Tabulated values are [$\delta(H_i)$ in dI] – [$\delta(H_i)$ in AICE deriv.]

proton produces approximately equal enhancements at H-2' and H-2" which clearly suggests a *syn* conformation as does the 9% enhancement of H-2 by H-1'. This conformation may be stabilised by hydrogen bonding between the ester group and OH-3'. Thus the imidazole β-nucleosides are similar to purine-type nucleosides with the population of the *anti* conformation being 50% or more. However the α-anomers show a more structure dependent variation in the glycosidic bond conformation.

Deoxyribose ring conformation. Pseudorotational analysis of the riboside has been developed in detail,[9,12-14] although almost entirely for β-anomers. Generally the pentose ring in these nucleosides is in conformational equilibrium between two forms, an S-type (*endo* C-2') and an N-type (*endo* C-3'). The precise position in the pseudorotational circuit is not easily inferred from the coupling constants in any particular case since these observable parameters are a function of the fractional populations (X_N, X_S) as well as the specific phase angle (P) and pucker angle (τ) of each form.[15] A necessary consequence of restricting the pentose ring to these two conformations is that the values of $J_{2',3'}$ and the sum $\Sigma = J_{1',2'} + J_{3',4'}$ should be approximately constant for all compounds in a particular class, these values being almost independent of the population distribution between S and N forms. These parameters are given in Table 3 for six purine-type β-deoxyribonucleosides including the new derivatives of AICE. The data for 1-βdAICE show close agreement with the other values suggesting that this deoxyriboside is populated by normal N and S forms. This is further supported by comparison with the calculated values (Table 3) based on the average pseudorotational parameters evaluated by Hasnoot *et alia*[14] for dA and related nucleosides.

Starting with the average parameters established for dA and its derivatives ($P_N = 9°$, $P_S = 167°$, $\tau_N = \tau_S = 35°$) and the available calculated J values[12] we have explored the

TABLE 3 Characteristic *J* values for 2'-deoxyribosides

	3β	4β	dI	dG[a]	dA[b]	pdA[c]	Calc.[d]	3α	4α
$J_{2',3'}$	6.2	6.0	6.0	5.7	6.2	6.3	6.2	7.0	7.1
$J_{1',2'} + J_{3',4'}$	10.6	9.8	10.4	10.4	10.5	10.3	10.7	*e*	*e*

[a] Reference 9. [b] Reference 2. [c] 5'-Methylphosphatyl-dA (reference 13). [d] Calculated for a β-deoxyriboside with $P_N = 9°$, $P_S = 167°$, $\tau = 35°$, and $X_N = 0.29$ (reference 13). [e] Inapplicable to α-anomers.

TABLE 4 Optimised pseudorotational parameters and calculated coupling constants for **3** and **4**

	P_N	P_S	τ_N	τ_S	X_S	$J_{1',2'}$	$J_{1',2''}$	$J_{2',3'}$	$J_{2'',3'}$	$J_{3',4'}$	Σ^a	e^b
3β	–9	162	30	37	0.75	8.04	6.17	6.24	3.06	2.70	10.83	0.18
4β	–18	171	40	35	0.58	6.20	6.29	6.10	4.95	3.70	9.90	0.18
3α	9	171	30	30	0.73	7.69	3.22	6.95	3.10	2.96		0.10
4α	9	153	32	33	0.90	7.06	1.88	6.75	1.92	2.41		0.11

[a] $\Sigma = J_{1',2'} + J_{3',4'}$. [b] RMS error with respect to experimental coupling constants.

surrounding conformational space to find a good fit to the five experimental pentose couplings (and Σ in the case of the β-anomers) in the new imidazole nucleosides. The results are given in Table 4. The conformational equilibrium found for 1-βdAICE has a population distribution which has shifted slightly in favour of an S form, relative to adenosine. This S form has the ^2E conformation (*endo*-C-2') with a larger ring pucker. The N form is close to the E_2 conformation (*exo*-C-2') with a decreased ring pucker. Thus 1-βdAICE is essentially in equilibrium between the two envelope forms corresponding to the flipping of C-2'. The value of $K_{eq} = X_S/X_N = 3.0$ indicates a free energy difference of -2.7 kJ mol^{-1}. For purine-type β-deoxyribosides Davies[9] finds an average value of $K_{eq} = 2.13$, and $\Delta G = -1.8$ kJ mol^{-1}.

In 3-βdAICE coupling of protons H-2' and H-2" to both protons H-1' and H-3' is of similar magnitude and the assignment was not obvious. In an NOE experiment the largest enhancement resulting from the irradiation of H-1' was to the peak at δ 2.28 which is assigned to H-2". This peak also received an enhancement from H-4'. Furthermore a positive enhancement of the peak at δ 2.16 (H-2') by irradiation of H-2 on the imidazole ring confirms the reversal of the chemical shifts of H-2' and H-2" in this compound. This reversal is also observed in the corresponding blocked nucleoside **6β** (in which the couplings are diagnostic). Pseudorotational analysis gave a set of parameters showing excellent agreement with experimental couplings (Table 4). The N form has the E_2 conformation with decreased ring pucker and the S form is close to the ^2E conformation with normal pucker. Thus 3-βdAICE also has C-2' as the principal out-of-plane atom in the deoxyribose ring and there is close similarity between **3β** and **4β** although the population distribution is shifted significantly in favour of the N domain in the latter case ($K_{eq} = 1.38$). This is probably a result of increased interaction of the base with the C-2' methylene group when the ester group is closer to the pentose ring.

The blocked β-anomers **5β** and **6β** were not analysed in detail. Both species have an increased value for $J_{2',3'}$ suggesting a decreased ring pucker in both cases. Using the close parallel between $J_{1',2'}$ and X_S observed in the unblocked β-isomers the estimated X_S values are 0.9 for **5β** and 0.7 for **6β**. Thus the vicinal interaction of the large groups at C-3' and C-4' forces a shift in population of the ring conformations towards the S domain.

For the determination of the conformation of the α-anomers we required calculated data for $J_{1',2'}$ and $J_{1',2''}$ as a function of P and τ (Altona[12] does not deal with α-deoxyribose). Assuming that H-1' in the α-form is phase shifted by 120° with respect to the β-form then the torsion angles in the α-anomers are given by equations (1) and (2) which are derived from the corresponding relations for β-deoxyribose.[14]

$$\theta_{1',2'} = 1.4^\circ + 1.03\tau\cos(P-144^\circ) \qquad (1)$$

$$\theta_{1',2''} = -119.1^\circ + 1.02\tau\cos(P-144^\circ) \qquad (2)$$

TABLE 5 Conformational distribution at C-5' in imidazole nucleosides

	4β	3β	3α	dI	dA[a]	dG[a]
p_+	0.43	0.49	0.37	0.36	0.54	0.47
p_a	0.26	0.51	0.63	0.64	0.29	0.32
p_-	0.31				0.31	0.21
Σ[b]	8.4	7.8	9.0	7.4	8.0	
Δδ[c]	0.06	0	0	0	0.06	0.05

[a] Reference 8. [b] $\Sigma = J_{4',5'} + J_{4',5''}$ (Hz). [c] Separation of H-5 and H-5' (ppm).

A range of appropriate values of $J_{1',2'}(\alpha)$ was obtained by inspection from the available data for $J_{1',2''}(\beta)$ provided due allowance was made for the changed orientation of the electronegative substituents; careful analysis shows that for a given torsion angle θ, the effect of inversion at C-1 is such that $J(\theta)\alpha = J(-\theta)\beta$. In a similar fashion a range of values of $J_{1',2''}(\alpha)$ was obtained for the appropriate torsion angles; no electronegativity corrections are necessary in this case since the net effect is the same for both anomers. Our tabulations of values of $J_{1',2'}$ and $J_{1',2''}$ are fully compatible with those for the β series and pseudorotational analysis was carried out in the usual way, giving the results shown in Table 4. The population distribution in the N^1 species is similar to that in the corresponding β-anomer with $K_{eq} = 2.7$, $\Delta G = -2.4$ kJ mol^{-1}. Both N and S forms are intermediate between envelope and twist conformations but are close to the average forms for purine deoxyribosides. However a slight flattening of the ring is evident in both forms (a reduction of τ from 35° to 30°) compared to the β-anomer. The N^3 α-anomer is significantly different from the corresponding β-anomer. Not only is the S form highly populated with $K_{eq} = 9$, $\Delta G = -5.4$ kJ mol^{-1}, but it adopts a conformation close to a $^2T_1$ twist structure (*exo*-C-1', *endo*-C-2'). This is in keeping with a preponderance of the *syn* conformation as indicated above.

The blocked α-anomers were not analysed fully but in the N^1 species the population of the S form increases to *ca* 85% since $J_{1',2''}$ and $J_{2'',3'}$ have reduced values and the N^3 species is probably almost entirely in the S conformation since $J_{1',2''}$ and $J_{2'',3'}$ are both less than 1 Hz. This change in conformation towards the S domain occurs as a result of the vicinal interaction of the groups at C-3' and C-4'. This interaction must predominate over that between the C-3' group and the heterocyclic ring which is now located on the *exo* (lower) face of the deoxyribose ring.

Conformation of C-5'. The coincident chemical shifts of H-5' and H-5" in most of the unblocked nucleosides precludes a full analysis of the population distribution among the C-5' conformers. Exceptionally, for 3-βdAICE H-5' and H-5" are separated by 0.06 ppm and the conformer populations were calculated using the standard values[9] of $J_t = 11.0$ Hz and $J_g = 1.5$ Hz. Assuming that H-5' is the downfield proton the calculated populations (Table 5) show the expected predominance of the ϕ_+ conformer and the usual order of the population of the minor conformers, $p_a > p_-$. The reverse assignment of H-5' and H-5" would make $p_- > p_a$ which is unlikely. The population of the ϕ_+ form in **3β** is similar to that in **4β**, dA, and dG. The β-anomers of the imidazole nucleosides adopt a conformational distribution at C-5' which indicates a strong similarity with purine nucleosides, particularly with respect to cross-ring interactions between C-1' and C-5' substituents. Data for only one α-anomer was accessible and that indicates a shift towards equal populations for the conformers at C-5'.

EXPERIMENTAL

The glycosylation of ethyl 5-aminoimidazole-4-carboxylate (AICE) with 2-deoxyribofuranosyl chloride was achieved under a variety of conditions. In most cases a mixture of the four isomers **3α**,**3β**,**4α** and **4β** or **5α**,**5β**,**6α** and **6β** was obtained. Details of these synthetic procedures and of the chromatographic separation of the isomers will be given elsewhere.[15] The NMR spectra were obtained from samples which were pure isomers or were predominantly one species. Spectra were measured on a JEOL GX270 spectrometer using standard conditions with a data point resolution of *ca* 0.1 Hz with sample concentrations in the range 1-5 mg cm^{-3}. Chemical shifts are relative to TMS for ^1H and relative to the solvent signal for ^{13}C spectra. Assignments were made by examination of coupling patterns and by comparison with literature data.[16] NOE measurments were obtained by analysis of multiple integration data normalised to the solvent or TMS signal in each case, and compared with off-resonance irradiation as standard.

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