Conformations of 3-Deazaadenosine, 3-Deaza-8-azaadenosine, and Benzimidazole-1-β-D-riboside in Solution: HRNMR-, Proton-Relaxation-Time-, and Nuclear-Overhauser-Effect Studies

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The solution conformations of 3-deazaadenosine, 3-deaza-8-azaadenosine, and benzimidazole-1-β-D-riboside have been determined by nuclear magnetic resonance methods in aqueous and ammonia solutions. The two-state S ⇄ N model of Altona and Sundaralingam is used to analyse the ribose moiety. In order to characterize the orientation of the base relative to the ribose, longitudinal proton relaxation time and nuclear Overhauser enhancement measurements have been carried out. It is shown that 3-deazaadenosine and benzimidazole-1-β-D-riboside exist preferentially in the S-*syn*-g^+/t (70%) and the N-*anti*-g^+/t (30%) conformation families. In the case of 3-deaza-8-aza-adenosine, some destabilization of the g^+ rotamer occurs. In this case, the pulsed methods seem to indicate a preference of the base for the anti range.

Introduction

The interchange of carbon and nitrogen in the purine ring of purine(β) nucleosides leads to nucleoside analogs with cytotoxic activity (8-azaadenosine, 7-deazaadenosine, or formycin) or without any biological activity (3-deazaadenosine) [1, 2]. These nucleoside analogs vary also in their ability to act as a substrate or inhibitor for certain enzymes. Thus, 3-deazaadenosine and 7-deazaadenosine are neither substrate nor inhibitor of adenosine deaminase [3], while 8-azaadenosine or formycin are both substrate and inhibitor of the same enzyme [4]. On the other hand, 7-deazaadenosine and formycin are good substrates for the enzyme adenosine kinase [1]. Conformational differences are often invoked to explain such observations. Therefore, in continuation of our previous studies on the preferred conformations of purine(β) nucleosides and analogs in solution [5–9], we have now analysed the conformations of 3-deazaadenosine (c^3Ado), 3-deaza-8-azaadenosine (c^3z^8Ado), and benzimidazole-1-β-D-riboside (Bza) in solution. These substances are also interesting because the replacement of the nitrogen N(3) by a C-H group prevents the formation of a hydrogen bond between the 5′-hydroxymethyl group and the base. It has been previously suggested that the *syn*-g^+-S conformation adopted by some nucleosides is stabilized by an intramolecular hydrogen bond between O(5′) and N(3) in solution [5, 9, 10] as well as in the solid state [28].

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Experimental

3-deazaadenosine (c^3Ado), benzimidazole-1-β-D-ribofuranoside (Bza), and 3-deaza-8-azaadenosine (c^3z^8Ado) were synthesized as published in previous papers [11, 12]. The deuterated solvents were purchased from Sharp and Dohme Ltd, München, BRD. Exchangeable protons were removed from the compounds by two lyophylisations from deuterium oxide. The oxygen free ammonia solutions, containing 5 mg of the nucleosides per 0.4 ml of liquid ND_3 were prepared as described previously [13].

1. *NMR spectra*

The PMR spectra were obtained on a Varian XL-100-15 spectrometer (16 k, 620-l-100 computer) in the FT mode. The digital resolution of the spectra was 0.1 Hz. The temperatures given were measured with a calibrated thermocouple. They are accurate to ±0.5 °C. Chemical shifts given in the data tables were referenced to internal TMS (for the pyridine solutions) and DSS (for the deuterium oxide solutions). The shifts given for the ND_3 solutions were

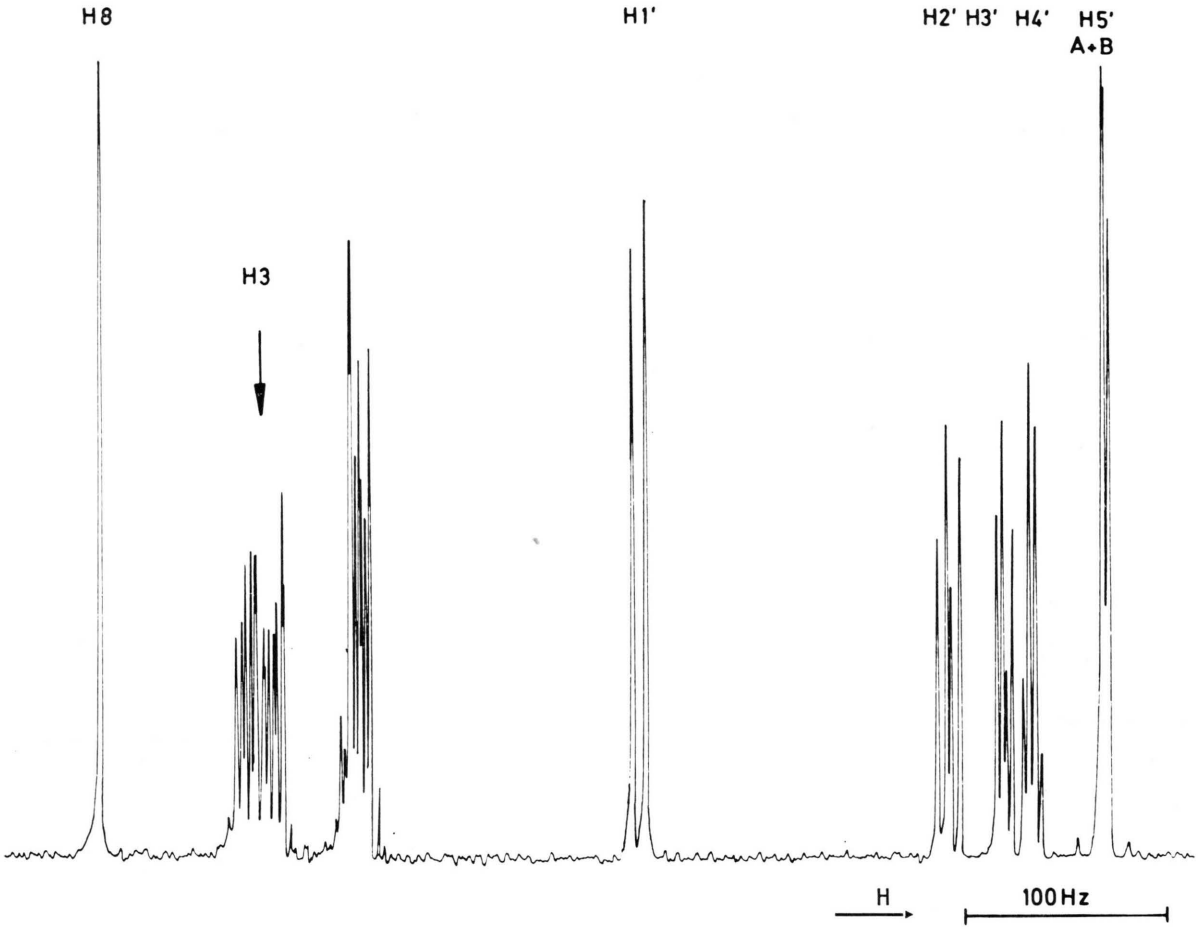

Fig. 1. Experimental proton high resolution spectrum of a olution of Bza in ND_3 at -60 °C. The arrow indicates the frequency of the H_2-RF-field applied in the NOE studies.

referenced to an external solution of 2% TMS in CS_2. No corrections for bulk magnetic susceptibility effects were attempted in the latter solutions, since we are only interested in the relative frequency differences of the proton signals of the ribose moiety.

The spectra of the ribose protons were analysed with the computer program LAME (QCPE n° 111). The simulations were considered successful if the deviations between experimental and simulated spectra were $\leqq \pm 0.1$ Hz.

The longitudinal relaxation times T_1 were determined with a $t-90°$-t-$180°$-t_1-$90°$ pulse sequence by application of a preliminary Varian program. t_1 was varied between 0.01 T_1 and 1.5 T_1. Within this range no significant deviation from linearity in the log amplitude versus t_1 plots was observed. Numerous repetitions of the experiments gave a reproducibility of $\pm 5\%$.

The nuclear Overhauser enhancements (NOE) were taken from completely relaxed FT spectra. The data given are the results of approximately 30 determinations for each $f_d(s)$. They are judged reliable to ± 0.02.

Fig. 1 shows an experimental spectrum of Bza, the arrow gives the frequency of the RF-field applied in the determination of $f_d(3)$; $d = 1', 2', 3'$.

Conformational Analysis

Purine nucleosides are flexible molecules where the activation energies between the various conformers are 3 to 8 kcal·mol^{-1} in solution [14, 15]. Thus, nuclear magnetic resonance methods yield, at room temperature measurements resulting from

dynamic equilibria, which are weighted averages over the diverse conformers. In order to characterize the conformations of purine nucleosides in solution, it is therefore necessary to determine simultaneously the geometrical parameters and the populations of the conformers adopted. Also, several dynamic equilibria are operating and correlations between them are expected.

Theoretically, HRNMR spectra allow the characterization of the furanose ring puckering with the equilibrium constant and the determination of the classical rotamer populations of the exocyclic group. The conclusions attained are based on an analysis of the vicinal proton-proton coupling constants of the ribose, which is made possible by the use of a proper Karplus equation. For the determination of the ribose puckering extensive solid-state data had to be incorporated in the treatment [16, 17]. According to the concept of pseudorotation, each conformation of the furanose ring is determined by two parameters, the phase angle of pseudorotation, P, and the degree of puckering, τ_m. This leads to two types of ribose conformations: type N and type S [16, 17]. As for the rotation of the hydroxymethyl group around the C(4′) – C(5′) bond, the main assumption is that it adopts only the three classical staggered rotamers: *gauche*$^+$, *trans*, *gauche*$^-$. The equations we have used are given in our previous papers [5 – 7].

With regard to the third major mode of internal motion of purine nucleosides, the rotation about the glycosyl bond [18, 19], vicinal proton coupling constants are not of very much help and relaxation studies of the base and sugar protons, proton relaxation rates and nuclear Overhauser enhancements, have to be applied [8, 13].

For the furanose ring, the observed vicinal proton coupling constants, are weighted averages of the coupling constants in the pure N and S states:

$$J^{ij}_{obs} = [N]J^{ij}_N + [S]J^{ij}_S . \qquad (1)$$

Since the relaxation rates of the protons, R_d, are much smaller than the rate constants for the internal motions, they are given by [20 – 22]:

$$\begin{aligned} R_d = C_1\,\tau_c \{ &[N]\sum_{\text{sugar}} r^{-6}_{dn_N} + [S]\sum_{\text{sugar}} r^{-6}_{dn_S} \\ &+ [N]\sum_i [Y_{i_N}] r^{-6}_{d8Y_{i_N}} + [S]\sum_i [Y_{i_S}] r^{-6}_{d8Y_{i_S}} \\ &+ [N]\sum_i [Y_{i_N}] r^{-6}_{d3Y_{i_N}} + [S]\sum_i [Y_{i_S}] r^{-6}_{d3Y_{i_S}} \} \\ d = 1', 2', 3', 4' \quad & C_1 = \gamma_H{}^4 \hbar^2 . \end{aligned} \qquad (2)$$

With R_d in s^{-1}, τ_c in 10^{-10} s, and r in Å $C_1 = 56.9$ Å^6 or

$$\begin{aligned} R_d &= C_1\,\tau_c \{[N]K_N + [S]K_S + \langle r^{-6}_{d8}\rangle + \langle r^{-6}_{d3}\rangle\} \\ d &= 1', 2', 3', 4' \end{aligned} \qquad (3)$$

where K_N and K_S are constants depending only on the interribose proton distances in the states N and S. In the previous paper [8], we have discussed and shown that Eqn (2) is valid at $-60\,^\circ$C. The first two terms of Eqn (2) are easily computed on the basis of single crystal data. However, without further assumption, it is not possible to calculate the last four terms. The same problem is encountered with the treatment of the nuclear Overhauser enhancement. When saturating either the base proton H(8) or H(3), the nuclear Overhauser enhancements are given by Eqn (4) where $s = 3$ or 8 and $d = 1', 2', 3'$.

$$\frac{2\,R_d}{C_1\,\tau_c} f_d(s) + \sum_{n=d,s} \langle r^{-6}_{dn}\rangle f_n(s) = \langle r^{-6}_{ds}\rangle . \qquad (4)$$

Insertion of the experimental values for $f_d(s)$ in Eqn (4) yields $\langle r^{-6}_{ds}\rangle$, which are the weighted averages over the glycosyl torsion angles covered by the base of the inverse sixth power of the distance between H(8) or H(3) and the ribose protons d. Since the probabilities of the various glycosyl torsion angles possible are not determined experimentally, further assumptions are needed in the treatment.

There is another method which yields $\langle r^{-6}_{ds}\rangle$ with $s = 8$ [23, 24]. In this method, the proton relaxation rates are measured before and after the deuteration of the H(8) proton. From Eqn (2), one obtains by substraction of both relaxation rates:

$$\begin{aligned} R_d - R_c(\text{C8 deut}) = C_1\,\tau_c \{ &[N]\sum_i [Y_{i_N}] r^{-6}_{d8Y_{i_N}} \\ &+ [S]\sum_i [Y_{i_S}] r^{-6}_{d8Y_{i_S}} \}, \; d = 1', 2' \end{aligned} \qquad (5)$$

or

$$\Delta_d = C_1\,\tau_c \langle r^{-6}_{d8}\rangle, \quad d = 1', 2' . \qquad (6)$$

In these two equations, d covers only the ribose protons 1′ and 2′ because of the large error which results for $\Delta_{3'}$.

Results

High-resolution spectra

The chemical shifts and coupling constants of 3-deazaadenosine, 3-deaza-8-azaadenosine, and benz-

Table I. Chemical shifts in ppm of c^3Ado, Bza, and c^3z^8Ado dissolved in ND_3, D_2O, and pyridine at several temperatures.

ND_3	c^3Ado		c^3z^8Ado		Bza	
δ	°C +40	−60	+40	−60	+40	−60
1′	5.28	5.33	5.80	5.95	5.56	5.45
2′	3.82	3.86	4.39	4.48	4.04	3.93
3′	3.64	3.63	3.90	3.98	3.82	3.65
4′	3.48	3.50	3.68	3.80	3.65	3.52
$5'_A$	3.17	3.16	3.25	3.34	3.34	3.18
$5'_B$	3.11	3.12	3.18	3.29	3.28	3.13
3	6.38	6.52	6.64	6.92	—	—
2	7.18	7.20	7.45	7.59	—	—
8	7.74	7.96	—	—	8.05	8.11

Pyr	c^3Ado		c^3z^8Ado	
δ	°C +60	+10	+60	+40
1′	6.34	6.52	6.78	6.88
2′	4.99	5.15	5.49	5.56
3′	4.88	5.01	5.06	5.14
4′	4.64	4.76	4.74	4.81
$5'_A$	4.22	4.29	4.21	4.26
$5'_B$	4.13	4.21	4.13	4.17
3	7.14	7.17	7.17	7.23
2	8.03	8.10	8.05	8.08
8	8.62	8.86	—	—

D_2O	c^3Ado		c^3z^8Ado		Bza
δ	°C +60	+10	+60	+10	+40
1′	5.86	5.75	6.19	6.09	5.84
2′	4.46	4.38	4.84	4.78	4.42
3′	4.23	4.13	4.41	4.40	4.15
4′	4.10	4.00	4.14	4.05	4.00
$5'_A$	3.76	3.66	3.70	3.62	3.67
$5'_B$	3.70	3.61	3.59	3.51	3.61
3	6.95	6.80	6.93	6.75	—
2	7.68	7.49	7.75	7.53	—
8	8.18	8.08	—	—	6.18

imidazole-1-β-D-riboside dissolved in various solvents and measured at several temperatures are contained in Tables I and II. Some typical experimental and simulated spectra are shown in Figs 2 and 3. The simulations were done with the values of the chemical shifts and of the coupling constants contained in Tables I and II. The mole fractions of the two states of the ribose and of the three rotamers of the exocyclic group have been calculated using the methods mentioned above and are contained in Table III. The outstanding features of Table III are the following for c^3Ado and Bza:

i) In all solvents, the S state of the ribose dominates with a mol fraction of .60 and especially in ND_3 at −60 °C where it reaches .70. In ND_3, the temperature dependence of the mol fractions of the ribose states is stronger than that of the common nucleosides but less marked than that of the 8-bromoderivatives or 3′-deoxyadenosine [5, 7, 9].

ii) In ND_3, the exocyclic group is to a large extent in the g^+ and t conformation. In D_2O, however, the preference for g^+ is much less marked and the populations are more or less equally distributed between g^+ and t. As previously noted [6], the pyridine solutions provoke an increase of the g^+ population.

Table II. Vicinal proton coupling constants of the ribose protons in c^3Ado, Bza, and c^3z^8Ado dissolved in ND_3, in D_2O, and in pyridine at several temperatures.

ND_3	c^3Ado		c^3z^8Ado		Bza	
J °C	+40	−60	+40	−60	+40	−60
1′2′	5.9	6.5	5.4	6.6	5.8	5.9
2′3′	5.2	4.8	5.1	4.8	5.1	5.6
3′4′	3.6	3.0	4.2	3.0	3.7	2.8
$4'5'_A$	3.7	3.3	4.2	3.9	3.0	2.6
$4'5'_B$	3.5	2.9	4.9	4.6	3.7	3.5
$5'_A5'_B$	−12.0	−12.4	−12.0	−12.1	−11.9	−11.9

Pyr	c^3Ado		c^3z^8Ado	
J °C	+60	+10	+60	+40
1′2′	5.5	5.6	4.9	5.0
2′3′	5.4	5.2	5.4	5.2
3′4′	4.2	3.8	4.4	4.4
$4'5'_A$	2.8	2.8	3.4	3.0
$4'5'_B$	3.1	3.3	4.9	4.5
$5'_A5'_B$	−11.7	−11.7	−11.7	−11.7

D_2O	c^3Ado		c^3z^8Ado		Bza
J °C	+60	+10	+60	+10	+40
1′2′	5.8	5.9	4.3	4.0	5.9
2′3′	5.7	5.4	5.2	5.1	5.6
3′4′	4.2	4.1	5.2	5.4	4.2
$4'5'_A$	2.4	2.0	3.1	2.9	2.3
$4'5'_B$	5.8	5.4	5.8	5.8	5.8
$5'_A5'_B$	−12.3	−12.3	−12.4	−12.6	−12.3

The effects of the 8-aza substitution on 3-deazaadenosine are very similar to those observed on adenosin compared to 8-azaadenosine [6]. Thus, a strong destabilization of the g^+ conformer is noted in all solvents. However, in ND_3, the population of the g^+ conformer increases with decreasing tempera-

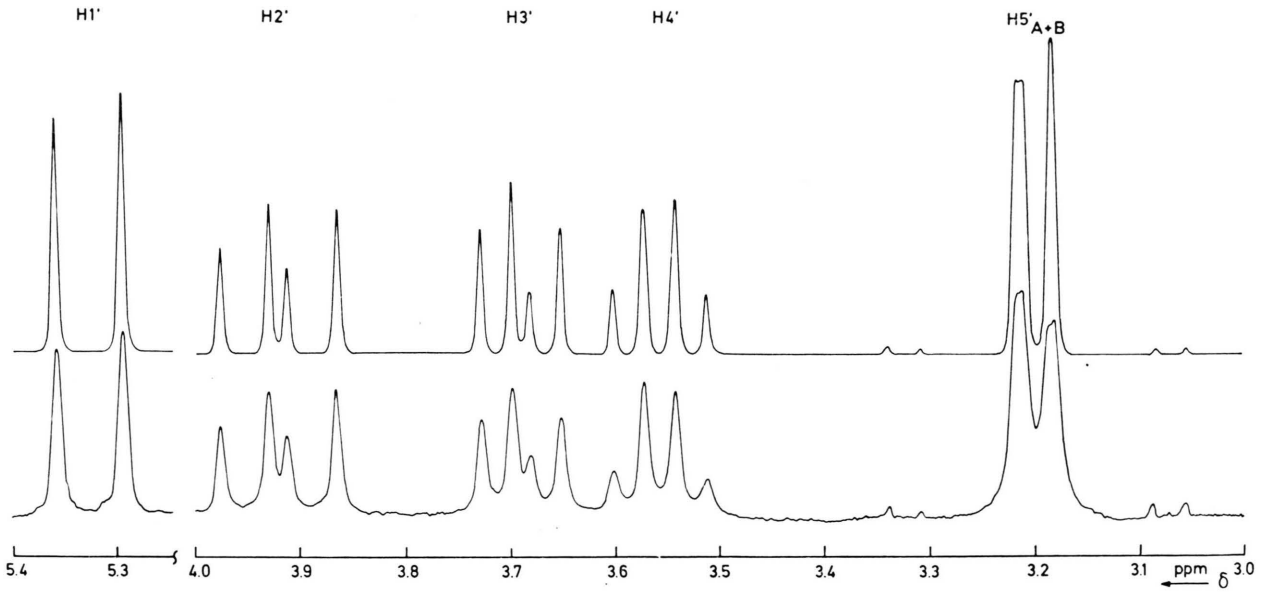

Fig. 2. Experimental proton high resolution spectrum (bottom) of a solution of c^3Ado in ND_3 at −60 °C compared with the simulated spectrum (top).

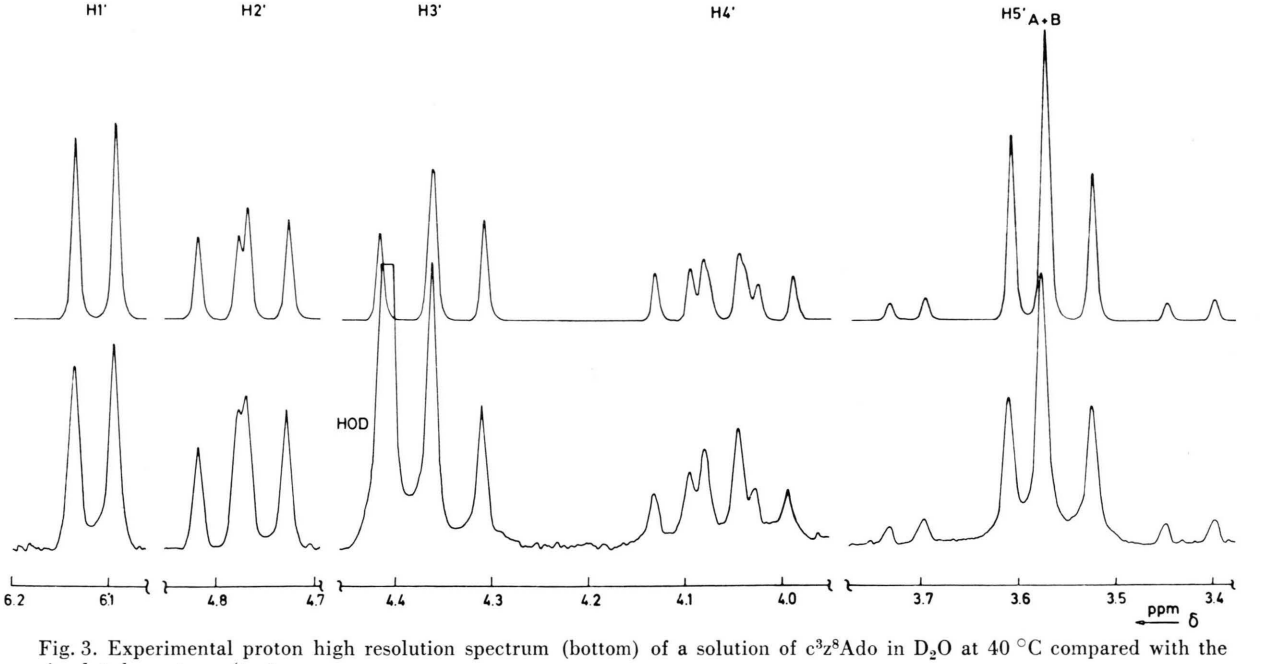

Fig. 3. Experimental proton high resolution spectrum (bottom) of a solution of c^3z^8Ado in D_2O at 40 °C compared with the simulated spectrum (top).

ture while the opposite occurs for adenosine. Also, in D_2O, there is a slight preference for the N state of the ribose as observed in the case of 8-azaadenosine, 8-azaguanosine, and 8-azainosine. Again, the pyridine solutions provoke an increase in both the populations of the S state and of the g^+ rotamer [5]. From the analysis of the high resolution spectra taken in various solvents, we draw therefore the following conclusions. In all solvents studied, the unmodified ribose moiety of purine(β)ribosides

Table III. Results of the conformational analysis of the ribose moiety for the compounds studied.

ND_3	T (°C)	P_N	N	P_S	S	g^+	t	g^-
c^3Ado	+40	3	.38	161	.62	.65	.19	.16
	−60	3	.32	161	.68	.76	.14	.10
c^3z^8Ado	+40	3	.43	161	.57	.44	.24	.32
	−60	3	.31	161	.69	.50	.21	.29
Bza	+40	3	.39	161	.61	.66	.11	.23
	−60	3	.31	161	.69	.77	.06	.17
D_2O								
c^3Ado	+60	3	.40	145	.60	.54	.42	.04
	+10	3	.39	145	.61	.63	.37	.00
c^3z^8Ado	+60	25	.53	175	.47	.46	.42	.12
	+10	25	.55	175	.45	.48	.42	.10
Bza	+40	3	.40	145	.60	.55	.42	.03
Pyr								
c^3Ado	+60	3	.43	145	.57	.80	.08	.12
	+10	3	.40	161	.60	.77	.09	.14
c^3z^8Ado	+60	10	.45	175	.55	.53	.15	.32
	+40	3	.47	161	.53	.62	.11	.27

and analogs shows a clear preference for the conformation S-g^+. The only exceptions being the 8-azanucleosides, which reveal a destabilization of S and g^+ [6]. The C-nucleosides and the 8-bromonucleosides present even a stronger preference for the conformation S-g^+ [7]. Substitution of the three hydroxyl groups of the ribose moiety by other groups or atoms leads to a variety of conformational preferences: 2′deoxyadenosine [9] and 2′amino-2′-deoxyadenosine [8] show at the same time a preference for S and a destabilization of g^+, 3′deoxyadenosine [9] and 3′deoxy-3′-aminoadenosine [8] are almost conformationally pure N g^+ compounds, while 5′-deoxyadenosine neither the N nor S state is preferred.

Longitudinal relaxation rates of the protons

The experimental relaxation rates for the protons of the three nucleosides studied are collected in Table IV. The calculated relaxation rates for the ribose protons, the H(2) proton, the H(3) proton, and the H(8) proton in the pure N and S states are shown in Fig. 4. These calculations are applicable to the cases of c^3Ado and Bza. The mol fractions of the rotamers of the exocyclic group are 0.70, 0.15, 0.15 for g^+, t, and g^- respectively. Similar curves can be calculated for c^3Ado (C8-deut), Bza (C8-deut), and c^3z^8Ado with the appropriate mol fractions of the rotamers of the exocyclic group.

The first problem concerns the determination of τ_c. In the cases of c^3Ado and c^3z^8Ado, we determined τ_c from the relaxation rate of the H(C2) proton assuming that it interacts only with the H(C3) proton. This yields $\tau_c(c^3\text{Ado}) = 128$ ps and $\tau_c(c^3z^8\text{Ado}) = 148$ ps. The curves of Fig. 4 indicate that this approximation is valid, once the possibility of stacking has been excluded. This method is not applicable to Bza, since the protons of the benzene ring form a complex ABCD spectrum (Fig. 1). However, assuming similar conformations for c^3Ado and Bza, one can estimate τ_c(Bza) from the ratios of the relaxation rates of these two compounds. This gives $\tau_c(\text{Bza}) = 100$ ps. The reduced relaxation rates ($R_1/56.9\,\tau_c$) obtained with the given τ_c, are presented in Table V. It is apparent that c^3z^8Ado adopts conformations similar to those of c^3Ado and Bza, for the relaxation rates of c^3z^8Ado and c^3Ado (C8-deut) and Bza (C8-deut) are identical within experimental errors. The difference observed for the H(4′) proton arises most probably from the different mol fractions of the exocyclic C-5′ group in c^3z^8Ado and c^3Ado and Bza. First, consider c^3Ado (C8-deut) [or Bza (C8-deut)] and c^3z^8Ado. At any glycosyl angle, we calculate $R_{4'}$ to be around 0.014, which is much higher than observed. The same situation occurs with $R_{3'}$. This relaxation rate is independent of the glycosyl torsion angle between 60° and 270° and is equal to 0.016. This dis-

Table IV. Longitudinal relaxation rates of the single protons of c^3Ado, Bza, and c^3z^8Ado dissolved in ND_3 as well as of c^3Ado and Bza where the H(C8) proton has been exchanged against a deuteron.

Nucleoside	c^3Ado			c^3Ado c^8 deut		
Temperature °C	+40	0	−60	+40	0	−60
1′	0.083	0.15	0.48	0.052	0.098	0.32
2′	0.123	0.24	0.83	0.096	0.18	0.63
3′	0.113	0.21	0.62	0.095	0.17	0.57
4′	0.089	0.17	0.54	0.070	0.12	0.52
5′	0.351	0.33	2.50	0.213	0.38	2.56
2	0.048	0.090	0.32	0.046	0.080	0.33
3	0.110	0.20	0.76	0.090	0.16	0.72
8	0.062	0.109	0.43	—	—	—

Nucleoside	c^3z^8Ado		
Temperature °C	+40	0	−60
1′	0.055	0.10	0.43
2′	0.112	0.20	0.88
3′	0.095	0.19	0.72
4′	0.070	0.14	0.38
5′	0.188	0.33	2.38
2	0.053	0.10	0.37
3	0.115	0.21	0.75

Nucleoside	Bza			Bza c^8 deut		
Temperature °C	+40	0	−60	+40	0	−60
1′	0.053	0.099	0.34	0.052	0.094	0.27
2′	0.062	0.130	0.71	0.088	0.17	0.55
3′	0.060	0.120	0.56	0.086	0.16	0.49
4′	0.056	0.110	0.47	0.071	0.13	0.42
5′	0.152	0.300	2.27	0.162	0.30	2.04
8	0.038	0.076	0.31	—	—	—

Table V. Experimental reduced relaxation rates of the ribose protons of the compounds studied.

Compound	$R_{1'}$	$R_{2'}$	$R_{3'}$	$R_{4'}$
c^3Ado (τ_c=128 ps)	0.0066	0.0114	0.0085	0.0074
Bza (τ_c=100 ps)	0.0060	0.0125	0.0098	0.0083
c^3Ado (c^8 deut)	0.0044	0.0086	0.0078	0.0071
Bza (c^8 deut)	0.0047	0.0097	0.0086	0.0074
c^3z^8Ado (τ_c=148 ps)	0.0051	0.0105	0.0085	0.0046

crepancy might have several origins (*in proper* account of the exocyclic group rotation, paramagnetic impurities, etc....). Therefore, in the following we will concentrate on $R_{1'}$ and $R_{2'}$. The calculated relaxation rates in absence of interaction with H(C8) for H(1′) and H(2′) are not strongly dependent on the ribose state. This prevents to make conclusions about eventual preference of the ribose states for some glycosyl torsion angles. In which case, two regions of the glycosyl torsion angle give agreement between experimental and calculated relaxation rates: $Y = 120°$ in the high-anti region and $Y = 240°$ in the anti region. For $Y = 120°$, one obtains $R_{1'} = 0.0043$ and $R_{2'} = 0.0084$ and, for $Y = 240°$, $R_{1'} = 0.0043$ and $R_{2'} = 0.0110$. Apparently, it would seem that the first angle applies to c^3Ado (C8-deut) and the second one to c^3z^8Ado. However, since we consider the experimental relaxation rates of these two compounds to be identical within experimental errors, we would suggest that the base is flexible about the glycosyl bond and adopts these two glycosyl torsion angles. It should be added that, because of sterical hindrance, most other angles are forbidden. his can be seen in Figs 5 and 6 where the distances between the ribose protons and H(3) of the base are plotted against the glycosyl torsion angle. Essentially, the same conclusion is reached at which c^3Ado. When, in addition to the ribose protons, one considers also R_3 and R_8 it appears that the first glycosyl torsion angle region has to be extended to the *syn* range down to 60°. But again, it is not possible to assign preferential base orientation to the two ribose states.

In conclusion, the proton relaxation studies indicate that in these compounds the base occupies mainly two glycosyl torsion regions: one, fairly large, extending from the *syn* range to the so-called high-*anti* region and a narrow one at the usual *anti* angle around 240°.

Nuclear Overhauser enhancements

The experimental NOE are contained in Table VI. From these, together with the experimental relaxation rates and deduced τ_c, one obtains the conformationally averaged distances between the ribose protons H(1′) to H(3′) and the base protons H(8) or H(3). These distances are contained in Table VII. In this table, the distances obtained from the third method mentioned in the theoretical section (DESERT) are also given. They agree within ex-

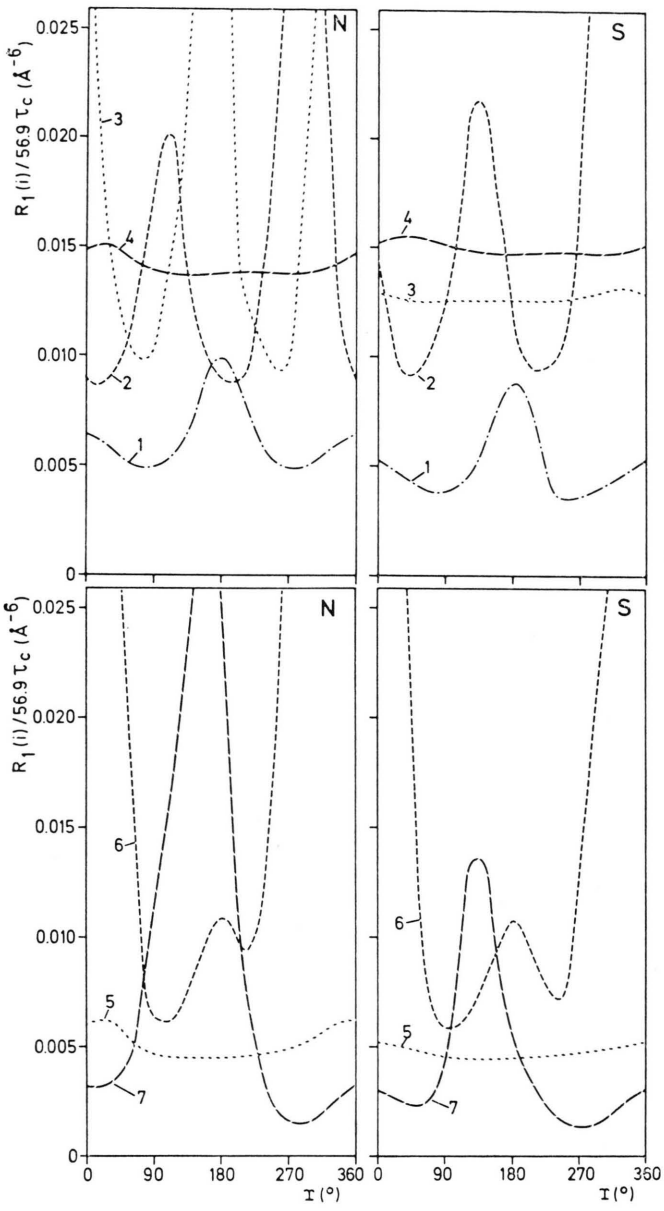

Fig. 4. Calculated reduced relaxation rates for the ribose protons (top) and the base protons (bottom) in the pure N and S states as a function of the glycosyl torsion angle, Y. The definition of the glycosyl torsion angle, Y, follows Hart and Davis [27]. (1) = H(1′); (2) = H(2′); (3) = H(3′); (4) = H(4′); (5) = H(C2); (6) = H(C3); (7) = H(C8).

Table VI. Nuclear Overhauser enhancements between H(8) and the ribose protons and between H(3) and the ribose protons of c^3Ado and Bza and between H(3) and the ribose protons of c^3z^8Ado. The compounds were dissolved in ND_3 and measured at −60 °C.

Compound	NOE					
	$f_{1'}(3)$	$f_{2'}(3)$	$f_{3'}(3)$	$f_{1'}(8)$	$f_{2'}(8)$	$f_{3'}(8)$
c^3Ado	0.114	0.043	0.012	0.150	0.061	0.019
Bza	0.132	0.064	0.0	0.112	0.068	0.022
c^3z^8Ado	0.053	0.054	0.0	—	—	—

perimental errors. The similarity between c^3Ado and Bza is again noticeable. Also, the only difference between these two compounds and c^3z^8Ado occurs in $\langle r_{1'3}^{-6} \rangle$. We first consider c^3z^8Ado. With only one glycosyl torsion angle, it is possible to reproduce the experimental distances, namely in the *anti* region $Y = 240° - 270°$. However, it is also possible to reproduce the distances assuming that the S state is present in the *syn* range $Y_S = 30° - 90°$ and the N state is in the *anti* range $Y_N = 240°$. In

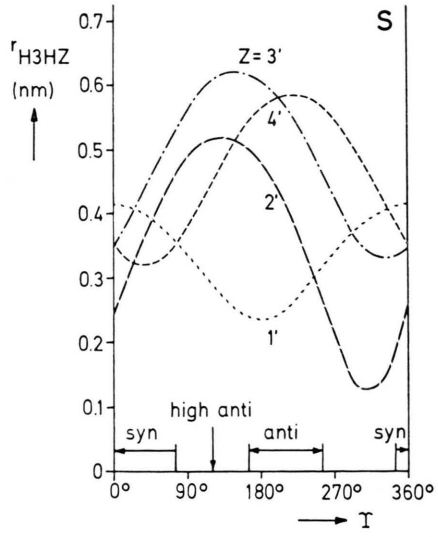

Fig. 5. Distances rH3-HZ between H(3) and the ribose protons H(1') to H(4') in the S state as a function of the glycosyl torsion angle Y.

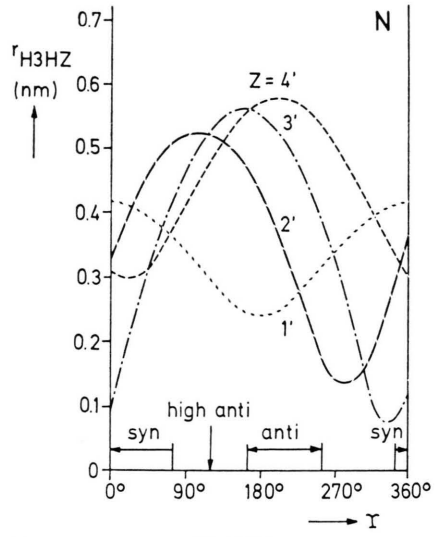

Fig. 6. Distances rH3-HZ between H(3) and the ribose protons H(1') to H(4') in the N state as a function of the glycosyl torsion angle Y.

Table VII. Experimentally determined averaged distances between the ribose and the base protons for the compounds studied.

Compound	Method	$\langle r^{-6}_{1'8}\rangle$	$\langle r^{-6}_{2'8}\rangle$	$\langle r^{-6}_{3'8}\rangle$	$\langle r^{-6}_{1'3}\rangle$	$\langle r^{-6}_{2'3}\rangle$	$\langle r^{-6}_{3'3}\rangle$
c^3Ado	NOE	0.0021	0.0017	0.0007	0.0016	0.0012	0.0005
$\tau_c = 1.28$	DESERT	0.0022	0.0028	—	—	—	—
		±0.0006	±0.0015	—	—	—	—
Bza	NOE	0.0015	0.0020	0.0008	0.0017	0.0018	0.0004
$\tau_c = 1.0$	DESERT	0.0012	0.0028	—	—	—	—
		±0.0006	±0.0015	—	—	—	—
c^3z^8Ado	NOE	—	—	—	0.0006	0.0012	0.0003
$\tau_c = 1.48$							

Table VIII. Experimental and calculated averaged distances between the ribose and the base protons for c^3z^8Ado and c^3Ado. The glycosyl torsion angle is represented by Y when it is not specifically assigned to any state of the ribose, otherwise, it is represented by Y_S or Y_N for the S and N state respectively.

Compound	Proton d	$\langle r^{-6}_{d3}\rangle_{exp}$	$\langle r^{-6}_{d3}\rangle_{theor}$	$\langle r^{-6}_{d3}\rangle_{theor}$	$\langle r^{-6}_{d3}\rangle_{theor}$
			$Y = 240° - 270°$	$Y_S = 75°$; $Y_N = 240°$	$.35\,(75°)_S + .35\,(240° - 270°)_S + .30\,(240° - 270°)_N$
c^3z^8Ado	1'	0.0006	0.0007	0.0009	0.0006
	2'	0.0012	0.0026	0.0021	0.0023
	3'	0.0003	0.0004	0.0001	0.0003
	d	$\langle r^{-6}_{d8}\rangle_{exp}$	$\langle r^{-6}_{d8}\rangle_{theor}$	$\langle r^{-6}_{d3}\rangle_{exp}$	$\langle r^{-6}_{d3}\rangle_{theor}$
c^3Ado	1'	0.0021	$Y_S = 0° + Y_N = 240°$ 0.0021	0.0016	$Y_S = 120° + Y_N = 240°$ 0.0017
	2'	0.0017	$75° \leqq Y_S \leqq 90° + Y_N = 210°$ 0.0019	0.0012	$Y_S = 75° + 210° \leqq Y_N \leqq 240°$ 0.0016
	3'	0.0007	$75° \leqq Y_S \leqq 90° + Y_N = 240°$ 0.0006	0.0005	$Y_S = 75° + Y_N = 240°$ 0.0005

this case, the distances are given by:

$$\langle r_{d3}^{-6} \rangle = [N] (r_{d3}^{-6})_N + [S] (r_{d3}^{-6})_S . \quad (7)$$

This is shown in Table VIII. Even, when assuming that 50% of the ribose in the S state is in the *anti* range at the angle $Y = 240^\circ - 270^\circ$ it is possible to obtain agreement. It should be added that we write $Y = 240^\circ - 270^\circ$ because the distances between H(3) and the protons H(1′) and H(3′) are best reproduced at $Y = 270^\circ$ and that with H(2′) at $Y = 240^\circ$. It is therefore difficult to give clear-cut conclusions. The presence of the base in the *anti* range up to 65% could explain the destabilization of the g^+ rotamer observed, since at this orientation electrostatic repulsion between O(5′) and N(8) would occur. Another argument in favour of a less flexible glycosyl bond in c^3z^8Ado comes from the temperature dependence of the S⇄N equilibrium. We have previously noted [5] that, when the base is constrained to a region of the glycosyl torsion angle, a strong temperature dependence of the S⇄N equilibrium is observed. It is therefore interesting to remark that the temperature dependence of the S⇄N equilibrium in c^3z^8Ado is the same as that of formycin or the 8-bromonucleosides [7] which are known to be constrained to the *syn* region. The most pronounced temperature dependence of the S⇄N equilibrium was observed for 3′-deoxyadenosine and 3′-amino-3′-deoxyadenosine, the latter was shown to exist exclusively in the *anti* range [8]. In the solid state [29, 30], 8-azapurinenucleosides exist in the so-called high-*anti* region ($Y \approx 140^\circ$) with the S state of the ribose. We calculated the following averaged distance assuming that both states of the ribose are in the high-*anti* region at $Y = 140^\circ$: $\langle r_{1'3}^{-6} \rangle = 0.0025$ and $\langle r_{2'3}^{-6} \rangle = \langle r_{3'3}^{-6} \rangle$ smaller than 0.0001. These values are sufficiently different from the experimentally deduced averaged distances to exclude this possibility. Also, assuming that only the S state of ribose exists in the high-*anti* region with the N state in the usual *anti* range ($240^\circ - 270^\circ$), one obtains too large values for $\langle r_{1'3}^{-6} \rangle$. The S state has to be in the usual *syn* region in order to reduce $\langle r_{1'3}^{-6} \rangle$ to the small value deduced experimentally.

We next consider c^3Ado and Bza. Since the experimental averaged distances are equal within experimental errors, we will restrict the discussion to c^3Ado. In this case, it is not possibe to reproduce the distances between both protons H(3) and H(8) and the ribose protons with only one glycosyl torsion angle. However, with the S state constrained to the *syn* region ($Y_S = 0^\circ - 120^\circ$) and the N state to the *anti* region ($Y_N = 210^\circ - 240^\circ$), relatively good agreement is obtained. As can be seen from Table VIII, regions of glycosyl torsion angles have to be considered to reproduce all distances. Thus, according to the correlation between the S state and the *syn* region, c^3Ado and Bza are up to 70% constrained to the *syn* region. In the case of c^3z^8Ado it cannot be excluded that some or even all molecules with the ribose in the S state have the base fixed in the *anti* range. These conclusions are in agreement with those based on T_1-measurements. For clarity, it should be added that the treatment of the experimental results relies on the assumption of a correlation between the ribose states and the orientation of the base. Such a correlation has received recently support from studies of 3′-amino-3′-deoxyadenosine and 2′-amino-2′-deoxyadenosine [8]. In this paper [8], we could show that with the sugar in the N state the base is restricted to the *anti* range, whereas with the sugar in the S state the base is mainly in the *syn* range but occurs also partly in the *anti* range (≈ one-third). Also, the glycosyl torsion angle was found to be not variable in the *anti* range (210° to 225°) and to vary between 30° and 97.5° in the *syn* range. In the solid state [25], c^3Ado exists in the family conformations *anti*-N-g^+. Empirical calculations, however, indicate that the lowest energy conformation of the isolated molecule occurs in the *syn* range. The crystal structures of two benzimidazole derivatives have been published: 2-Cl-1-(β-D-ribofuranosyl)benzimidazole [31] and 2-S-1-(β-D-ribofuranosyl)-3H-benzimidazole [32]. These compounds, due to the substituent at position 2 (equivalent to position 8 in purine(β)-nucleosides), exist in the *syn* conformation ($Y = 10^\circ$) with the ribose in the S state. In the first structure, there is a weak attractive C−H...O interaction between H(7) [or H(3) in purine nomenclature] and O(1′). As shown in Fig. 7 such an interaction can occur at $Y = 75^\circ - 90^\circ$ also and could add to the stabilization of the *syn*-g^+ conformation in the S state. However, in the second structure, there is no such C−H...O interaction and the C(4′)−C(5′) bond exists in the *t* conformation. It should be remembered that, in D_2O solutions, the populations of the g^+ and *t* rotamers are nearly equal for the 3-deazapurineribosides. From

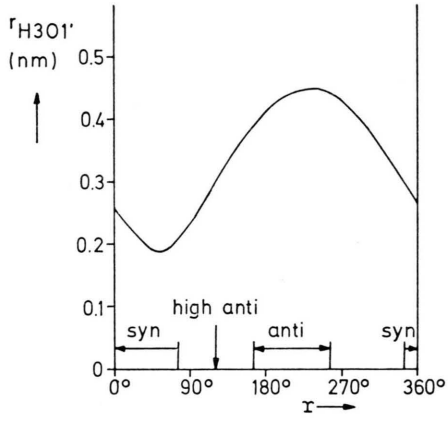

Fig. 7. Distances rH3-01′ as function of the glycosyl torsion angle Y. (Distances in N and S state are equal.)

a CD study of the conformations of 3-deazapurine-nucleosides it was also concluded that these compounds favour the *syn* conformation [26].

Conclusions

Most of the purine nucleoside analogs present essentially the same conformations as the common purine(β)nucleosides with the ribose in the S-g^+ conformer and roughly equal populations in the *syn* and *anti* ranges. This was thought to indicate the existence of an intramolecular H-bond between O(5′) and N(3) which would stabilize the *syn* orientation.

Previous results on 5′deoxyadenosine (5′Ado) and 5′deoxy8bromoadenosine (5′d8BrAdo) give support to this hypothesis [9]. In both substances the replacement of the 5′hydroxylgroup by a hydrogen leads to the reduction of the S-mole fraction. The absolute decrease being more pronounced for 5′d8BrAdo. In these compounds the geometry of the base and all other interactions between the furanose ring and the base moiety remain unchanged and the differences observed monitor directly the influence of the 5′hydroxylgroup on the conformational equilibria. The data obtained with c^3Ado and Bza show that in these substances, even in the absence of a hydrogen bond between O(3′) and N(3), the S-state and the g^+ rotamer are slightly favoured compared to the common purine(β)-ribosides [5]. The introduction of an unpolar C – H group in position (3) of the purine instead of a polar nitrogen does not only exclude the formation of the postulated hydrogen bond, or change the other favourable interactions between the purine- and the ribose moiety, but alters simultaneously the sterical interference of the base with the furanose ring. Considering the small changes observed in the S and N mol fractions, it is obvious that only minor energy differences are involved, and these could be found in weak hydrogen bonds between H(3) and the ribose.

Some interaction between O(5′), O(1′) and the base leading to a stabilisation of the S-g^+ conformer could occur in c^3Ado and Bza. Such sugar-base interactions promote the *syn* orientation of the base since they can only occur in the S-*syn* conformation. For the 8-bromonucleosides and the C-nucleosides, the interactions may consist of an intramolecular H-bond between O(5′) and N(3). In the case of the 3-deazapurine(β)nucleosides, favourable interactions between O(5′), O(1′), and H(3) could contribute to the stabilisation. On the other hand, the N-state of the ribose stabilizes the g^+ rotamer, which in turn promotes the *anti* orientation of the base for two reasons. First, due to steric hindrance, the N-state occupies reluctantly the *syn* range *. Secondly, the N-g^+-*anti* conformation allows the formation of an attractive C – H . . . O interaction between O(5′) and H(8).

Concerning the orientation of the base relatively to the glycosyl bond, the results presented here are in agreement with previous ones concerning purine-(β)nucleosides [8, 13]. Thus, the correlation between the S state of the ribose and the *syn* range and between the N state and the *anti* range allows to reproduce theoretically the experimental relaxation times and the averaged distances obtained from NOE measurements. In this treatment, the population of the *syn* range reaches 70% in c^3Ado and Bza. It has been argued [26] that the inactivity of the 3-deaza purine analogs against bacteria, tumor cells, and viruses springs from their preference for the *syn* orientation of the base. The conformational preferences of c^3z^8Ado are different from those adopted by c^3Ado and Bza. The experimental data could be explained by an exclusive preference for the *anti* range. However, while the high-*anti* range could be excluded, the possibility of some molecules in the *syn* orientation had to be retained. It should not be concluded from this that the 8-azapurine-

* Only two crystal structures of purine(β)ribosides are known to exist with the N state of the ribose in the *syn* range: 2-methylformycin [33] and 6-chloropurineriboside [34].

nucleosides do not exist in the high-*anti* range in solutions. Even in the case of 8-azaadenosine, theoretical calculations [29] indicate the *syn* orientation to be the most stable one with the high-*anti* orientation 0.5 kcal·mol^{-1} above. For c^3z^8Ado, this difference could be amplified. Also the behaviour of the 8-azapurinenucleosides in solution is not similar to that of the 8-bromo or the C-purinenucleosides. Thus, the 8-azapurinenucleosides may not exist in the same *syn* range as the other two compounds, which could mean either high-*anti* or *anti*.

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