Further Evidence for a S-Syn Correlation in the Purine(β)ribosides: The Solution Conformation of Two Tricyclic Analogs of Adenosine and Guanosine

G. Klimke, H.-D. Lüdemann,

Institut für Biophysik und Physikalische Biochemie, Universität Regensburg,
Postfach 397, D-8400 Regensburg

and L. B. Townsend

Division of Medicinal Chemistry College of Pharmacy, University of Michigan,
Ann Arbor, Michigan, 48109, USA

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Tricyclic Nucleosides, Conformation, NMR

From the analysis of the HRNMR spectra of two tricyclic analogues of adenosine and guanosine, 4,5-diamino-9-(β -D-ribofuranosyl) pyrimido[5,4-f]pyrrolo[2,3-d]pyrimidine (adenosine-adenosine, AA) and 4,7-diamino-9-(β -D-ribofuranosyl) pyrimido[5,4-f]pyrrolo-[2,3-d]pyrimidin-5-one (adenosine-guanosine, AG), dissolved in liquid ND₃ the preferred conformations of the ribose moiety are derived in the temperature range between +40 and –60 °C. The analysis is based on the two state N $\rightleftharpoons$ S model of the furanoside ring proposed by Altona and Sundaralingam. Both compounds show a pronounced stabilization of the S-conformer of the sugar ring ([S] $\sim$ 0.8). The van't Hoff enthalpy for the S $\rightleftharpoons$ N equilibrium is –3 kJ mol^{–1}. The syn $\rightleftharpoons$ anti equilibrium is even at –60 °C fast compared to the HRNMR time scale.

Introduction

From the analysis of the common purine(β)ribosides by HRNMR and NMR relaxation techniques a correlation between the conformation of the furanoside ring and the glycosyl torsion angle could be derived [1, 2]. In the N-form (C 3'-endo) of the ribose the glycosyl torsion angle of the base is found in the *anti* region ($180^\circ \leq Y \leq 260^\circ$), while in the S conformation of the sugar (C 2'-endo) the base reveals a pronounced preference for the *syn* range of the glycosyl torsion angle ($0^\circ \leq Y \leq 60^\circ$). The common purine(β)ribosides, however, only show a slight preference for the S-syn conformation, N-anti is with a mole fraction around 0.4 almost equally strong populated. Subsequent studies on 2'- and 3'-deoxyamino-adenosine [3], two compounds showing a pronounced preference for the S and N ribose, respectively, confirmed the correlations derived for the natural purine(β)ribosides.

The two tricyclic analogues of adenosine and guanosine studied here, represent compounds where the base is free to interconvert by $\pm 180^\circ$ around the glycosidic bond without changing the interaction with the sugar moiety. The sterical relationships between sugar and base are at all allowed glycosyl torsion angles equal to the geometrical demands of the syn conformation in the purine(β)ribosides.

Experimental

The synthesis of the tricyclic nucleosides adenosine-adenosine and adenosine-guanosine has been published previously [4]. 5 mg of each substance were dissolved in 0.5 ml liquid trideuteroammonia. Details of the sample preparation have been given elsewhere [1, 2].

The spectra were obtained at 100.1 MHz on a Varian XL-100-15 FT spectrometer equipped with a 16 k 6201-100 computer and disk accessory. The digital resolution of the spectra was 0.1 Hz. Temperatures were measured before and after each experiment with a miniature thermocouple. They are accurate to $\pm 0.5^\circ\text{C}$. The analysis of the proton spectra of the ribose moiety was performed by application of the computer program LAME (QCPE no. 111). The simulations were considered successful, if the

Abbreviations: A, adenosine; 8 BrA, 8-bromoadenosine; AA, 4,5-diamino-9-(β -D-ribofuranosyl) pyrimido[5,4-f]pyrrolo[2,3-d]pyrimidine (adenosine-adenosine); G, guanosine; 8 BrG, 8-bromoguanosine; AG, 4,7-diamino-9-(β -D-ribofuranosyl) pyrimido[5,4-f]pyrrolo[2,3-d]pyrimidin-5-one.

Reprint requests to Dr. H.-D. Lüdemann.

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deviation between all resolved lines in the experimental and simulated spectra was ≤ 0.1 Hz.

Chemical shifts given in Table III are referenced to an external standard of 1% TMS dissolved in CS_2 . No attempts to correct for bulk magnetic susceptibility effects were made. The proton longitudinal relaxation measurements were performed with a $180^\circ\text{-}\tau\text{-}90^\circ\text{-}5 \cdot T_1$ pulse sequence of the SYMON program of the disk accessory. Details of the experi-

mental procedures have been described elsewhere [1, 2]. The time dependent amplitudes did not show any deviations from a single exponential function for times $\tau \leq 2 T_1$.

Theoretical Section

The sterical flexibility of the nucleoside framework is given by three equilibria (Fig. 1):

1. The rotation around the glycosidic bond: the *syn* $\leftrightarrow$ *anti* equilibrium of the base.
2. The conformational equilibrium of the furanose ribose ring: The pseudorotation of the ring with the two states of minimum energy *N* and *S* [5, 6].
3. The rotation of the exocyclic 5'CH₂OD-group around C 4'–C 5' described by the three rotamers *g*⁺, *t*, and *g*[−] [7, 8].

The equilibria 2. and 3. concern the ribose moiety only and the positions of these equilibria can be derived from an analysis of the vicinal proton proton coupling constants of the ribose protons [5–8].

Details of the procedure employed for the ND_3 solutions have been published earlier [9].

For the purine(β)ribosides the quantitative description of the *syn* $\leftrightarrow$ *anti* equilibrium could be obtained from the analysis of the proton longitudinal relaxation rates and the intra-molecular NOE factors [10, 1, 2]. The complete longitudinal relaxation rate of a specific proton in a molecule of the size of a nucleoside is given by [10, 11]:

$$R_i = \sum_{j \neq i} \rho_{ij} + \rho_i^* \quad (1)$$

with ρ_{ij} the direct dipolar relaxation rate given by

$$\rho_{ij} = \gamma_H^4 \hbar^2 \tau_c r_{ij}^{-6} \quad (2)$$

γ_H being the gyromagnetic ratio of the proton, r_{ij} the distance between proton *i* and *j* and τ_c the rotational correlation time. The term ρ_i^* includes all other intra-molecular relaxation paths of spin *i*.

However, because of the r^{-6} dependence, relaxation studies are only applicable, if at least one proton at the base moiety approaches some of the ribose protons to a distance between 0.40 and 0.25 nm. At greater distances the badly defined term ρ_i^* becomes the dominating term in Eqn (1) and one cannot extract any geometrical information from the R_i studies.

Fig. 1. Structural formulas and possible internal motions of the nucleoside analogs studied: A) chemical structure of A, AA, G and AG; B) rotation around the glycosidic bond; C) pseudorotation of the ribose ring; D) Newmann projection along the C 4'–C 5' bond showing the three classical rotamers.

Results and Discussion

Relaxation rate studies, position of the *syn* $\rightleftharpoons$ *anti* equilibrium

Table I contains the relaxation rates obtained for the single protons of AA and AG at -60°C compared with the previously published data for A [1]. In the purine(β)ribosides the base proton H 8 is ideally suited for an analysis of the *syn* $\rightleftharpoons$ *anti* equilibrium [10, 1, 2], while the experimental relaxation rate of H 2 is almost an order of magnitude smaller than the rates of the remaining protons, thus the relaxation of H 2 is dominated by the g_2^* term. The same holds for the corresponding protons (2 and 7) in AA and AG,

Table I. Longitudinal relaxation rates of the single protons of A, AA, and AG in ND_3 at -60°C .

$1/T_1$ [sec^{-1}]	A	AA	AG
H 1'	0.26	0.34	0.32
H 2'	0.84	0.80	0.91
H 3'	0.91	0.87	0.71
H 4'	0.63	0.91	1.0
H 5' _{A+B}	2.86	3.3	3.3
H 2	0.0059	0.10	0.081
H 8	0.44	—	—

the only base protons remaining in the tricyclic analogs. As can be seen from the interproton distances given in Fig. 2 these protons can approach only H 2' in a very small region of the glycosyl torsion angle to distances below 0.4 nm and these ranges of the glycosyl torsion angles are energetically unfavourable because of close contacts between H 2' and N 3, respectively N 1 or N 7 in the tricyclic analogs.

It is thus impossible to derive from the relaxation studies the position of the *syn* $\rightleftharpoons$ *anti* equilibrium.

The base proton signals in AA and AG remain sharp down to the lowest temperature, and also the sugar moiety reveals, compared to A sharp signals down to the lowest temperatures reached. The chemical shifts (Table III) of H 2 (H 7), H 1' and H 2' in AA and AG differ at the lowest temperature by up to 0.15 ppm and one can consequently expect, that these signals should become broadened and finally separated, if the rate of the *syn* $\rightleftharpoons$ *anti* conversion becomes comparable to the chemical shift difference. This result is in marked contrast to the behaviour of the pteridinenucleosides [12], in these compounds the sugar signals broaden at temperatures below 0°C and at -60°C two resolved multiplets are seen for H 1' and H 2', indicating that the *syn* $\rightleftharpoons$ *anti*

inversion in these compounds is slow on the time scale of a HRNMR experiment. The sharp signals found in the tricyclic analogs and especially the fact that only one signal is found for H 2 and H 7 in AA, can thus tentatively be taken as evidence that the activation energy for the *syn* $\rightleftharpoons$ *anti* inversion for these compounds is considerably lower than the activation energy of $\sim 40\text{ kJ mol}^{-1}$ found for the furanoside pteridinenucleosides [13].

HRNMR studies, conformation of the ribose moiety

The results of the analysis of the proton spectra are contained in Tables II and III. In both substances $J_{1'2'}$ increases and $J_{3'4'}$ decreases with falling temperature. The only significant difference between AA and AG is found in $J_{4'5'A+B}$. In AG these coupling constants are significantly smaller than the corresponding results observed in AA.

Fig. 2. a) Distances between the base proton H 2 and the four ribose ring protons H 1' to H 4' as a function of the glycosyl torsion angle χ ($^\circ$). b) Distances between the ribose ring proton H 2' and some base atoms as function of the glycosyl torsion angle χ ($^\circ$). The dotted line indicates the van-der-Waals' contact distances of N...H and C...H. Both figures show the case of the symmetric AA. The data are derived from the interatomic distances calculated for adenosine. In the tricyclic analogs N 3 of the purine rings corresponds to N 1 and N 8 while C 2 corresponds to C 2 and C 7.

Fig. 3. Experimental proton high resolution spectra of solutions of AA and AG in ND_3 at -60°C .

Table II. Vicinal proton proton coupling constants of the ribose protons in Hz of the two tricyclic compounds dissolved in ND_3 at $+40^\circ\text{C}$ and -60°C .

J_{ij} [Hz]	AA		AG	
	$+40$	-60	$+40$	-60
$J_{1'2'}$	6.8	7.7	7.1	7.7
$J_{2'3'}$	5.4	5.1	5.4	5.0
$J_{3'4'}$	2.9	1.8	2.4	1.7
$J_{4'5'A}$	3.5	3.6	2.8	3.2
$J_{4'5'B}$	4.6	3.9	3.7	3.5

Table III. Chemical shifts of the single protons of AA, AG, A and G dissolved in ND_3 at $+40^\circ\text{C}$ and -60°C .

δ [ppm]	A		AA		G		AG	
	$+40$	-60	$+40$	-60	$+40$	-60	$+40$	-60
H 1'	5.63	5.67	6.143	6.147	5.36	5.44	5.935	6.020
H 2'	4.15	4.14	4.802	4.864	4.08	4.05	4.705	4.803
H 3'	3.86	3.87	3.949	3.954	3.78	3.82	3.905	3.945
H 4'	3.67	3.71	3.666	3.774	3.58	3.64	3.638	3.758
H 5' A	3.39	3.40	3.396	3.485	3.31	3.37	3.387	3.490
H 5' B	3.28	3.29	3.257	3.327	3.20	3.25	3.247	3.346
H 2	7.88	7.84						
H 8	8.08	8.27			7.37	7.57		

Table IV. Results of the conformational analysis for the two tricyclic compounds compared to A, 8 BrA, G and 8 BrG.

T [$^\circ\text{C}$]	A		8 BrA		AA		G		8 BrG		AG	
	$+40$	-60	$+40$	-60	$+40$	-60	$+40$	-60	$+40$	-60	$+40$	-60
[N]	0.45	0.43	0.28	0.17	0.27	0.18	0.40	0.37	0.21	0.12	0.25	0.18
[g ⁺]	0.68	0.70	0.45	0.22	0.55	0.62	0.73	0.75	0.70	0.72	0.73	0.71
[t]	0.15	0.20	0.26	0.47	0.16	0.17	0.13	0.14	0.14	0.14	0.08	0.13

From the coupling constants the populations of the different conformers are derived. These data are shown together with previously published results [1, 9] for A, G, 8 BrA and 8 BrG in Table IV. In both AA and AG the S-conformer is dominating. It can be seen that the two compounds behave very similar to the 8-bromo substituted purine(β)nucleosides. The position of the $\text{N} \leftrightarrow \text{S}$ equilibrium is within the accuracy of the analysis identical in 8 BrA and AA, while the stabilization of the S-ribose is marginally more pronounced in 8 BrG as compared to AG. In our opinion this latter difference is an additional hint that both conformers of AG, with the adenine part respectively the guanine in the *syn* range are populated with comparable mole fractions.

The g^+ conformer, with the 5'-hydroxyl group above the furanoside ring in greatest proximity to the base moiety, is under all conditions the dominant conformer.

Compared to the purine(β)ribosides the tricyclic compounds AA and AG have to be regarded as pure *syn* nucleosides, since in all possible ranges of the glycosyl torsion angle they have one of the two pyrimidine rings above the ribose ring. These compounds thus confirm a correlation between the *syn* position of the base and the S state of the ribose previously derived for the purine(β)ribosides [1–3]. The same correlation appears to hold also for the

Fig. 4. Temperature dependence of the mole fraction in the S and N state of AA and AG, compared to A, 8 BrA, G and 8 BrG.

5'-adenosinemonophosphatemonomethylester, a natural 5'-purine(β)nucleotide analogue [14, 15].

It must, however, be stated, that this correlation was only derived and thoroughly tested for purine(β)nucleosides and analogues with similar geometrical constraints. In these substances a correlation between S-*syn* and N-*anti* could be derived. It is not to be expected that these correlations should also hold for the pyrimidine(β)nucleosides and -nucleotides including their analogues [16].

In Fig. 4 the temperature dependence of the position of the N $\leftrightarrow$ S equilibrium is shown in a van't

Hoff plot. For comparison also the previously published data on A, G, 8 BrA, and 8 BrG are given. The minute differences in the [S]/[N] ratios for AA and AG as compared to the more pronounced changes in 8 BrA and 8 BrG could possibly be explained by a slight preference of AG to adopt the "syn-adenosine" conformation, with the 7-amino group completely exposed to the polar environment of the solvent molecules. A van't Hoff enthalpy of -3 kJ mol^{-1} is derived for the stabilization of the S-conformer for all purine(β)nucleoside analogues constrained to the *syn*-position of the base.

The line through the results for 8 BrG as well as the line through the data for 8 BrA, AA and AG intercept for $T \rightarrow \infty$ the origin of the diagram. This is a further strong evidence for the validity of the description of the conformational flexibility of the furanoside ring in the two state N $\leftrightarrow$ S model.

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