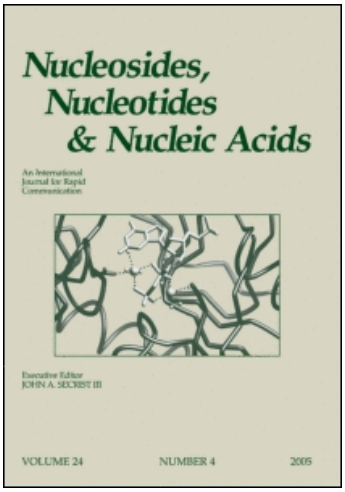

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María T. Baumgartner[a]; Marisa I. Motura[b]; Rubén H. Contreras[c]; Adriana B. Pierini[a]; Margarita C. Briñón[b]
[a] Departamento de Química Orgánica-INFIQC, Universidad Nacional de Córdoba, Córdoba, Argentina [b] Departamento de Farmacia, Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina [c] Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

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Conformational Studies of Novel Antiretroviral Analogs of Zidovudine

María T. Baumgartner,[1] Marisa I. Motura,[2] Rubén H. Contreras,[3] Adriana B. Pierini,[1] and Margarita C. Briñón[2,*]

[1]Departamento de Química Orgánica-INFIQC
[2]Departamento de Farmacia, Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina
[3]Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

ABSTRACT

Conformational properties of three novel zidovudine analogs, namely 3′-azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso, **2**), (−)-*trans*-(5S,6S)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (**3**) and (+)-*trans*-(5R,6R)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (**4**), have been investigated by AM1 calculations and NMR studies, and compared with those of the parent nucleoside (AZT, **1**). Based on the results obtained the following correlation may be established, a) AZT and AZT-Iso exhibit a conformational behavior analog to other pyrimidinic nucleosides, displaying a dynamic equilibrium in solution where the two conformers (North and South) undergo a constant transformation. b) Compounds **3** and **4** show a different conformational profile. The estimate of the pseudorotation phase angle reveals the rigid structures of the latter compounds, which do not evidence conformational equilibrium in solution;

*Correspondence: Margarita C. Briñón, Departamento de Farmacia, Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina; Fax: 54-351-4334127; E-mail: macribri@dqo.fcq.unc.edu.ar.

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the azide group being the only group free to rotate. c) Diastereoisomers **3** and **4** exhibit an extra conformational parameter compared with other pyrimidinic nucleosides: the *chair* or *boat* conformation in the third ring formed between the sugar and the base. In all cases, a reasonable correlation was obtained between theoretical and NMR spectroscopic data.

Key Words: Antiretroviral analogs; Novel AZT derivatives; Conformational analysis; AM1 calculations; NMR studies.

3′-Azido-3′-deoxythymidine (zidovudine, AZT), the first clinically successful nucleoside analog for the treatment of acquired immunodeficiency syndrome (AIDS), is a potent reverse transcriptase inhibitor (NRTI) of the human immunodeficiency virus (HIV-1) replication,[1] particularly in combination with other drugs, such as other NRTI, non-nucleoside transcriptase reverse and protease inhibitors.[2] Soon after the first successful clinical uses of AZT, many scientists were prompted to correlate its conformational features with its biological activity for the development of other active antiretroviral nucleosides.[3–8]

The conformation of a pyrimidinic nucleoside usually involves the determination of three main dihedral angles χ_{CN}, $\gamma_{C4'\text{-}C5'}$ and $\varphi_{N\alpha\text{-}C3'}$,[4–6] which are presented in Fig. 1 for AZT.

The glycosyl torsion angle χ_{CN}, determines the *syn* or *anti* disposition of the base in relation to the sugar moiety; *syn* when the C_2 carbonyl of pyrimidine lies over the sugar ring and *anti* when this group is oriented in the opposite direction (Fig. 1).[6]

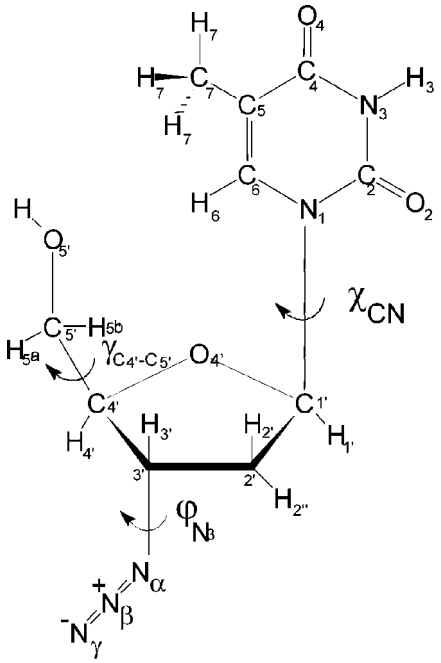

Figure 1. Atoms numbering and dihedral angles for zidovudine (AZT, **1**).

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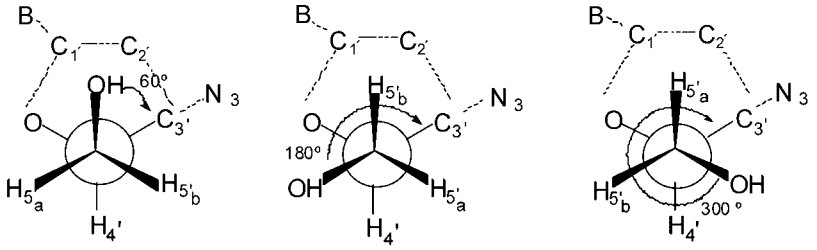

gauche-gauche, γ+ $\gamma_{C4'\text{-}C5'} = 60^{o}$

gauche-trans, γ^{t} $\gamma_{C4'\text{-}C5'} = 180^{o}$

trans-gauche, γ^{-} $\gamma_{C4'\text{-}C5'} = 300^{o}$

Figure 2. Torsion angle $\gamma_{C4'\text{-}C5'}$($O_{5'}$-$C_{5'}$-$C_{4'}$-$C_{3'}$) of the three main rotamers around the exocyclic $C_{4'}$-$C_{5'}$ bond.

The torsion angle $\gamma_{C4'\text{-}C5'}$($O_{5'}$-$C_{5'}$-$C_{4'}$-$C_{3'}$) (Fig. 1), determines the orientation of the 5′-OH group represented by the three main rotamers namely, γ^{+}, γ^{t}, γ^{-} (Fig. 2).[9,10]

The puckering of the furanose ring and its deviation from planarity, which is described by the phase angle of pseudorotation P (0–360°), is derived from an expression that depends on the five endocyclic sugar torsion angles (Eq. 1, Fig. 1).[11,12]

$$\tan P = \frac{(C_{3'}\text{-}C_{4'}\text{-}O_{4'}\text{-}C_{1'} + O_{4'}\text{-}C_{1'}\text{-}C_{2'}\text{-}C_{3'}) - (C_{2'}\text{-}C_{3'}\text{-}C_{4'}\text{-}O_{4'} + C_{4'}\text{-}O_{4'}\text{-}C_{1'}\text{-}C_{2'})}{2C_{1'}\text{-}C_{2'}\text{-}C_{3'}\text{-}C_{4'}(\sin 36^{\circ} + \sin 72^{\circ})} \quad (1)$$

When $C_{1'}$-$C_{2'}$-$C_{3'}$-$C_{4'}$ is negative, 180° is added to the calculated value of P. When $-90^{\circ} \leq P \leq 90^{\circ}$, the conformation is described as type North (N); for $90^{\circ} \leq P \leq 270^{\circ}$, it is denoted as type South (S) (Fig. 3). A phase angle P = 0° corresponds to an absolute north conformation possessing a symmetrical twisted form $^{3}T_{2}$ ($C_{3'}$-endo, $C_{2'}$-exo), whereas its south antipode, $^{2}T_{3}$, is represented by P = 180° ($C_{2'}$-endo, $C_{3'}$-exo). The superscripts and subscripts represent, respectively, the carbon atoms displaced above and below the plane relative to other atoms of the five-member ring (Fig. 3).[4,13] The maximum out-of-plane pucker value attained by any dihedral ring angle in a complete cycle of pseudorotation, is given by τ_{max} ($\tau_{max} = C_{1'}\text{-}C_{2'}\text{-}C_{3'}\text{-}C_{4'}/\cos P$).[11]

Since one of the proposed inhibitory actions of the 2′,3′-deoxynucleosides may be related to the preferred conformation of the modified furanose ring,[10] and taking into consideration that we have been involved in a research program aimed at exploring more convenient novel anti HIV-1 analogs of AZT,[14–16] the purpose of this paper was to perform the structural and conformational analyses of 3′-azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso, **2**), **(−)**-*trans*-(5S,6S)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine [**(−)**-*trans*-(5S,6S), **3**] and **(+)**-*trans*-(5R,6R)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine [**(+)**-*trans*-(5R,6R), **4**], three novel derivatives of **1** (Fig. 4) with proved anti HIV activity,[14–16] and its comparison with the prototype.

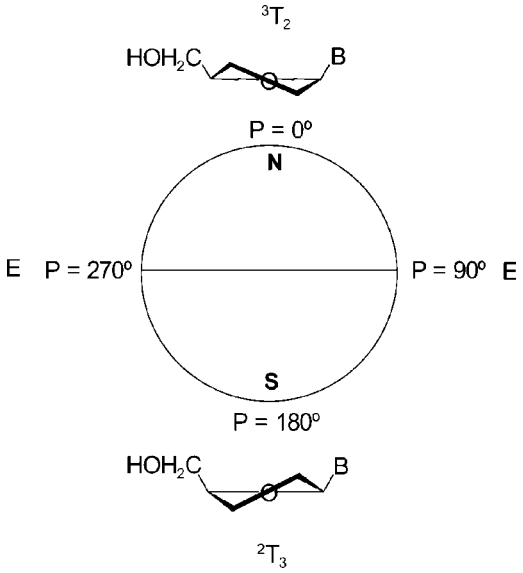

Figure 3. Schematic pseudorotational cycle of the furanose ring in nucleosides. E = enveloped and T = twisted.

Our main interest was focused over solution conformations due to their similarity with the biological media, taking into account that the knowledge of the conformational properties of these AZT analogs in this medium, could contribute to our understanding of the structure-activity relationship of nucleoside pyrimidinic derivatives, therefore being a guidance in the design of more active compounds.

EXPERIMENTAL SECTION

NMR Spectroscopy

^1H NMR and ^{13}C NMR spectra were recorded on a Brüker AMX500 spectrometer (500.138 MHz) at room temperature, using DMSO-d_6 (99%, SIGMA) as solvent and HMDS as internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. Accurate coupling constants (J) and chemical shifts were obtained by spectral simulation procedures using the LAOCOON PC, a modified version of the original LAOCN3.[17] Sugar conformations were estimated on the basis of the NMR coupling constants using a PC (DOS) version of the program PSEUROT V6.2.[18]

Theoretical Calculations

The initial geometry for these molecules was obtained from crystallographic data inputs of AZT[19] by molecular MMX calculations using the Hyperchem

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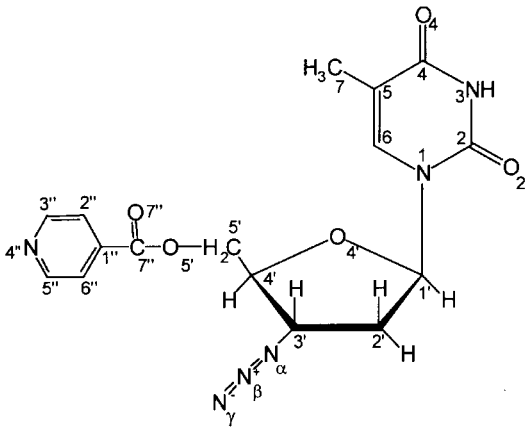
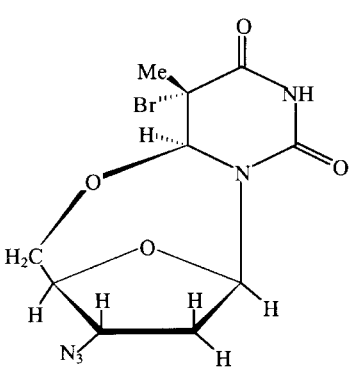
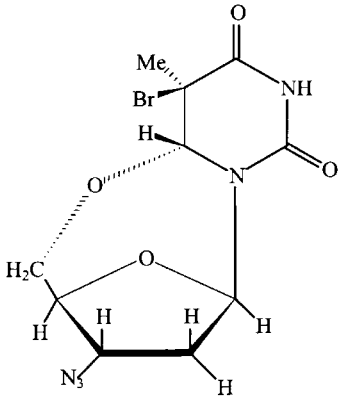


AZT-Iso, **2**



(-)-*trans*-(5S,6S), **3**



(+)-*trans*-(5R,6R), **4**

Figure 4. Molecular structures of 3′-azido-3′-deoxy-5′-O-isonicotinoylthymidine (AZT-Iso, **2**), (−)-*trans*-(5S,6S)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine [(−)-*trans*-(5S,6S), **3**] and (+)-*trans*-(5R,6R)-5-bromo-6,5′-epoxy-5,6-dihydro-3′-azido-3′-deoxythymidine [(+)-*trans*-(5R,6R), **4**].

package.[20] All subsequent calculations were carried out with the semiempirical AM1 method.[21] This procedure has proved to be adequate for this type of molecules,[3,6] and was chosen due to its ability to deal with hydrogen bonds.[21]

We studied the conformational potential surface of the compounds varying the principal angles of torsion, with a total rotation of 360° for each one. Geometries were fully optimized with the keywords PRECISE and GNORM = 0.1 to obtain the lowest gradient residue as possible, which was necessary due to quite small energy differences among the several conformers. Force constant calculations were carried out to verify minimal local energy for all the stable conformers located. Then, the pseudorotational angle P for each resulting isomer was determined using Eq. (1).

The AM1/^{1}H NMR coupling constants were calculated from its output with the PCMODEL 4.0 program.[22]

RESULTS

NMR Studies

The ^{1}H NMR assignments of compounds **1-4** and their confirmation by spectral simulation with the LAOCOON PC program are presented in Table 1. These assignments, which compares well with those determined with a 200.13 MHz spectrometer,[14,16] are based on chemical shifts and observed multiplicities as well as on comparison with those of related compounds.[23–27]

The ^{13}C NMR assignments of compounds **1-4** are presented in Table 2 and were confirmed by using 135° and 90° DEPT techniques, as well as COSY homonuclear and heteronuclear (^{1}H-^{13}C) spectra. These chemical shifts are in accordance with the values calculated with the ACD program.[28]

As can be seen from the tables, the ^{1}H NMR and ^{13}C NMR of **2** and **1** are similar. In compounds **3** and **4** the chemical shift of the pyrimidinic-H_6 is shifted toward the aliphatic region as expected for a C_5-C_6 single bond.

The *trans* configuration of **3** and **4** (hydrogen and bromine atoms on the same side of the pyrimidinic plane) has been determined by comparison of their ^{13}C NMR spectra with that of 6-azide-5-bromo-5,6-dihydrozidovudine.[29] In this compound the chemical shift of C_5 constitutes the most significant difference between the *cis* and *trans* bromine isomers. The C_5 signal appears at $\delta \cong 52$ and $\delta \cong 62$ for the *trans* and *cis* isomers, respectively. On the other hand, the $C_{3'}$ signal of both isomers remains unchangeable at $\delta \cong 62$.[29] Thus, the upfield shifting of the C_5 signals of both compounds (**3**, $C_5 = 56.656$; **4**, $C_5 = 52.317$) with respect to that of $C_{3'}$ (**3**, $C_{3'} = 61.917$; **4**, $C_{3'} = 66.597$) was taken as one of the main evidences for the *trans* assignment of both compounds.[14]

Theoretical Calculations

AZT-Iso, **2** – In this compound the main torsion angles are, χ_{CN} (C_2-N_1-$C_{1'}$-$O_{4'}$), $\gamma_{C4'\text{-}C5'}$ ($O_{5'}$-$C_{5'}$-$C_{4'}$-$C_{3'}$), $\phi_{C5'\text{-}O5'}$ ($C_{7''}$-$O_{5'}$-$C_{5'}$-$C_{4'}$), $\phi_{C7''\text{-}O5'}$ ($O_{7''}$-$C_{7''}$-$O_{5'}$-$C_{5'}$) and $\phi_{C1''\text{-}C7''}$ ($C_{2''}$-$C_{1''}$-$C_{7''}$-$O_{7''}$) (Fig. 4). On the basis of crystallographic data and previous calculations for AZT, only three values for $\varphi_{N\alpha\text{-}C3'}$ (N_β-N_α-$C_{3'}$-$C_{2'}$) $= -60.0°$,[3] 170°[20] and 60° were used. This study allowed us to find different minima on the potential energy surface which are summarized in Table 3 and presented in Fig. 5.

From the analysis of the theoretical results it emerged that, a) The conformers of lower energy are *anti* with respect to the glycoside ring with a torsion angle $\chi_{CN} \cong -120$. b) The most stable conformations have a dihedral angle φ_{N3} of approximately $-60°$. Variations in this angle do not produce changes in the conformation adopted by the sugar or the rest of the molecule (Table 3, compare D-F with G-I). c) Pyridine ring rotations around the $C_{1''}$-$C_{7''}$ bond, depicted by variations of

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Table 1. ^{1}H NMR chemical shifts and coupling constants of compounds **1-4**.

	Compounds							
	AZT, **1**		AZT-Iso, **2**		(−)-*trans*-(5S,6S), **3**		(+)-*trans*-(5R,6R), 4	
Groups	Exp	Calc[a]	Exp	Calc[a]	Exp	Calc[a]	Exp	Calc[a]
Chemical shifts (*ppm*)								
CH_3	1.731	–	1.584	–	1.744	–	1.800	–
OH	(*)	–		–	–	–	–	–
NH	(*)	–	11.3	–	10.932	–	11.037	–
H_6	7.623	–	7.361	–	5.228	–	4.938	–
$H_{1'}$	6.047	6.045	6.114	6.114	6.043	6.041	6.374	6.372
$H_{2'a}$	2.331	2.328	2.470	2.473	2.534	2.533	2.362	2.362
$H_{2'b}$	2.222	2.223	2.359	2.360	2.232	2.232	2.317	2.317
$H_{3'}$	4.345	4.344	4.604	4.604	4.237	4.235	4.770	4.769
$H_{4'}$	3.773	3.772	4.077	4.078	4.420	4.419	4.394	4.393
$H_{5'a}$	3.605	3.602	4.581	4.579	4.017	4.015	3.885	3.878
$H_{5'b}$	3.553	3.554	4.481	4.482	3.760	3.759	3.871	3.869
$H_{2''}$, $H_{6''}$	–	–	7.820	–	–	–	–	–
$H_{3''}$, $H_{5''}$	–	–	8.766	–	–	–	–	–
Coupling constants (*Hz*)								
$^{2}J(H_{2'a}\ H_{2'b})$	13.77	13.774	14.00	13.995	14.60	14.600	15.26	15.260
$^{2}J(H_{5'a}\ H_{5'b})$	11.97	11.965	12.14	12.144	12.76	12.629	12.29	12.337
$^{3}J(H_{1'}\ H_{2'a})$	6.38	6.380	5.88	5.856	1.98	1.988	1.54	1.284
$^{3}J(H_{1'}\ H_{2'b})$	6.67	6.651	7.29	7.264	7.05	7.014	7.44	7.768
$^{3}J(H_{2'a}\ H_{3'})$	7.40	7.435	7.80	7.994	8.00	8.010	7.80	7.800
$^{3}J(H_{2'b}\ H_{3'})$	5.15	5.112	6.07	6.046	3.68	3.868	4.44	4.440
$^{3}J(H_{3'}\ H_{4'})$	4.85	5.000	5.95	6.121	1.48	1.501	1.25	1.251
$^{3}J(H_{4'}\ H_{5'a})$	4.03	3.929	3.71	3.651	< 0.5	−0.004	4.71	6.026
$^{3}J(H_{4'}\ H_{5'b})$	3.77	3.865	4.84	4.875	2.44	1.880	1.81	1.526

[a]Parameters obtained by LAOCOON PC program.
(*) NH and OH are not observed with precision due to a rapid chemical exchange with the solvent.

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Table 2. ^{13}C NMR chemical shifts (δ) of compounds **1–4** in DMSO-d_6.

Groups	Compounds			
	AZT, **1**	AZT-Iso, **2**	(−)-*trans*-(5S,6S), **3**	(+)-*trans*-(5R,6R), **4**
CH_3	10.243	9.897	21.719	21.932
C_2	148.481	148.383	149.912	151.787
C_4	161.778	161.617	167.186	167.066
C_5	107.590	107.996	56.656	52.317
C_6	134.099	133.935	89.313	83.101
$C_{1'}$	82.109	81.711	86.295	85.486
$C_{2'}$	34.326	33.712	40.529	35.206
$C_{3'}$	58.268	57.786	61.917	66.595
$C_{4'}$	81.559	78.426	85.859	84.929
$C_{5'}$	58.903	62.307	73.069	71.025
$C_{1''}$	–	134.534	–	–
$C_{2''}$ ($C_{6''}$)	–	120.628	–	–
$C_{3''}$ ($C_{5''}$)	–	148.838	–	–
$C_{7''}$(=O)O	–	162.493	–	–

the torsion angle $\phi_{C1''\text{-}C7''}$, present a significant minimum in energy, leading to a predominant form with torsion angles between −4° and 2°, but with a rotational barrier energy of about 7 kcal/mol, allowing for $C_{1''}$-$C_{7''}$ free rotation. d) The rotation around the $C_{7''}$-$O_{5'}$ bond, ($\phi_{C7''\text{-}O5'}$), presents a rotational barrier of 50 kcal/mol, indicating that this angle has a practically fixed position with values between −6° and 0°; the angle $C_{1''}$-$C_{7''}$-$O_{5'}$-$C_{5'}$ being around 175–180°. e) The variations of the $\gamma_{C4'\text{-}C5'}$ angle have a similar behavior to that determined for **1**.[9,10] f) The conformers close in energy show both conformational groups, North (Table 3, entry A-C) and South (Table 3, entry D-F).

Table 3. AM1 relative energies, main geometrical parameters and phase angle of pseudorotation (P), for AZT-Iso, **2**.

Conf.	ΔH_f (kcal/mol)	χ_{CN}	$\gamma_{C4'\text{-}C5'}$	φ_{N3}	$\phi_{C1''\text{-}C7''}$	$\phi_{C7''\text{-}O5'}$	$\phi_{C5'\text{-}O5'}$	P[a]
A	−50.28	−115.09	−38.90	−58.6	0.3	−5.8	102.3	359.5, North
B	−49.61	−108.38	−157.56	−58.8	−3.7	−1.8	−176.6	340.1, North
C	−49.53	−118.65	32.38	−61.9	−2.6	−3.3	−170.2	359.3, North
D	−50.26	−124.22	−176.48	−62.1	−3.3	−4.3	−113.4	180.2, South
E	−49.43	−118.74	37.19	−59.5	−0.7	−0.4	−166.2	186.2, South
F	−49.13	−119.57	−81.46	−62.1	1.7	−1.6	177.6	187.1, South
G	−46.86	−124.80	−176.73	179.1	−3.2	−5.7	−112.3	175.6, South
H	−46.72	−120.25	−80.7	177.6	−0.3	−2.1	177.8	177.7, South
I	−46.29	−117.06	38.06	174.3	−1.2	0.2	−156.4	181.0, South

[a]Calculated from Eq. (1).

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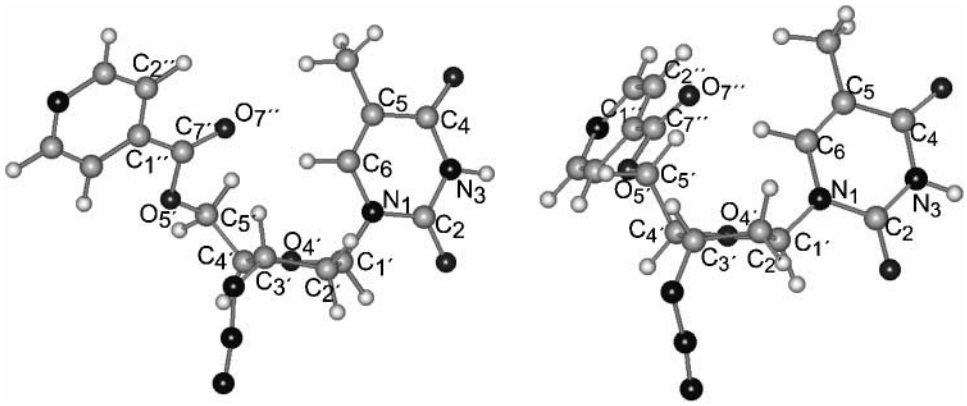

Figure 5. AM1 most stable conformation (A and D) of AZT-Iso, **2**.

(−)-*trans*-(5S,6S), 3 and **(+)-*trans*-(5R,6R), 4** – Bromine derivatives can adopt eight possible structures, among them *cis* or *trans* configurations around the C_5-C_6 bond, as well as *chair* or *boat* conformations in the novel third ring. From all these possible structures, the most stable are *trans* with preferred *boat* and *chair* conformation for the third ring of the *R,R* and the *S,S* diastereomers respectively (Table 4, Fig 6).

On an AM1 basis, the following conclusions were reached. a) The most stable conformation of azide group is the same as in AZT and AZT-Iso. b) Only North conformations are observed for the sugar moiety. The deoxyribose ring has a P value between 250 and 280°, depending on the conformer taken into consideration. c) In general $\gamma_{C4'\text{-}C5'}$ is not greater than 100° (around and lower than 90° for conformers J and K respectively, Table 4), presenting mainly γ^+ and γ^t conformers (Fig 2). This behavior is ascribed to the intramolecular bond between the base and the sugar, and for this reason the γ^- conformation of the sugar is not possible.

Table 4. AM1 relative energies, main geometrical parameters (χ_{CN}, $\gamma_{C4'\text{-}C5'}$ and φ_{N3}) and phase angle of pseudorotation (P), for bromine conformers.

Conf.		ΔH_f (Kcal/mol)	χ_{CN}	$\gamma_{C4'\text{-}C5'}$	φ_{N3}	$\phi_{Br\text{-}C5\text{-}C6\text{-}H6}$	P[a]
J	(5R,6R) boat	−42.68	−87.27	93.0	− 62.0	50.0	282.2 North
K	(5S,6S) chair	−39.64	−122.12	54.91	−60.4	39.9	259.7 North
L	(5R,6R) chair	−38.47	−69.04	27.52	−62.4	45.9	278.8 North
M	(5S,6S) boat	−32.91	−164.18	134.9	−60.2	37.7	260.2 North
N	(5R,6S) chair	−38.63	−121.97	51.6	−59.5	176.9	264.5 North
O	(5S,6R) boat	−38.38	−82.76	94.9	−61.9	−66.8	277.2 North
P	(5R,6S) boat	−36.69	−155.45	131.7	−60.4	175.2	263.1 North
Q	(5S,6R) boat	−33.25	−62.46	29.8	−62.7	−87.15	270.4 North

[a]Calculated from Eq. (1).

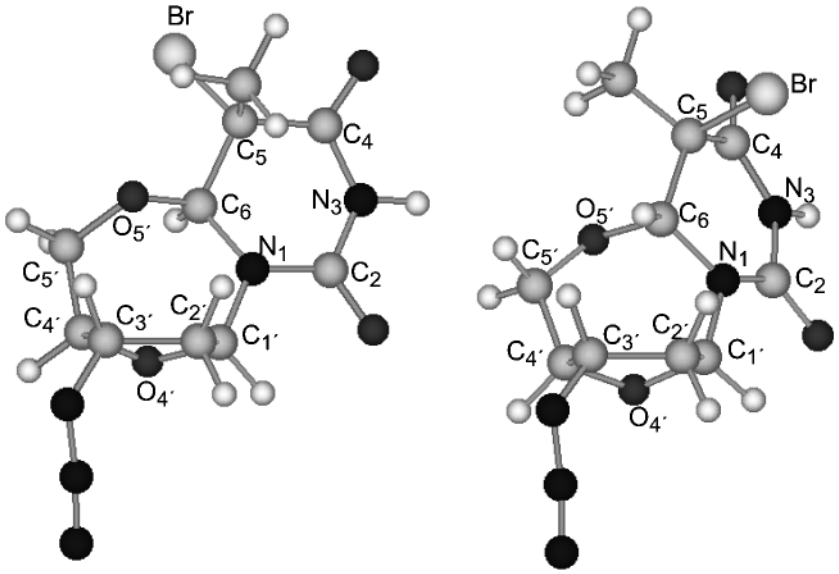

Figure 6. AM1 most stable conformation of **(−)**-*trans*-(5S,6S), **3** and **(+)**-trans-(5R,6R), **4**.

DISCUSSION

The correlation between the AM1 and NMR structural and conformational properties of compounds **1-4** is presented here. The preferred conformations can be obtained from the NMR data with the generalized Karplus equation, which relates coupling constants to torsion angles.[30]

The phase angle of pseudorotation (P) and the degree of ring puckering (τ_{max}) can be evaluated from the ^{1}H NMR coupling constants using the relationships derived by Altona and Sundaralingam.[11,12] From these data the furanose endocyclic torsion angles can be computed.

Glycosyl–Pyrimidyl Rings Conformation

Based on Karplus equation, $^3J_{C2\ H1'}$ coupling constants values of $\cong$3 Hz and $\cong$7 Hz have been predicted for the *anti* and *syn* conformers respectively, with values of the C_2-N_1-$C_{1'}$-$H_{1'}$ angle between $0° \pm 90°$ and $180° \pm 90°$ for the *anti* and *syn* regions, respectively. The Karplus relationship adjusted for this type of coupling constant is presented in Eq. (2).[31]

$$^3J_{C2\,H1'} = 5.0\cos^2\phi - 2.1\cos\phi + 0.1 \quad (2)$$

By means of the previous equation and the $^3J_{C2\ H1'}$ obtained from ^{13}C NMR spectra the C_2-N_1-$C_{1'}$-$H_{1'}$ angles were evaluated. These values and the AM1 calculated ones are presented in Table 5 showing that all the studied compounds have *anti*

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Table 5. Glycosyl-pyrimidyl rings conformation ($\phi_{C2\text{-}N1\text{-}C1'\text{-}H1'}$).

	$^3J_{C2\ H1'}$ (Hz)		$\phi_{C2\text{-}N1\text{-}C1'\text{-}H1'}$		
Compounds	Exp	Calc[a]	AM1	Calc[b]	Conf.
AZT, **1**	2.25	–	–	25	*anti*
AZT-Iso, **2**	2.71	2.88–2.95	−9.69–6.25	15	*anti*
(−)-*trans*-(5S,6S), **3**	1.93	2.97	−4.57	32	*anti*
(+)-*trans*-(5R,6R), **4**	0.85	2.08	29.02	49	*anti*

[a]Calculated from Eq. (2) using AM1 dihedral angles.
[b]Calculated from Eq. (2) using experimental $^3J_{C2\ H1'}$.

conformations. Generally, this is the normal conformation of the pyrimidinic nucleoside compounds. Moreover, for compounds **3** and **4** the *anti* conformation around the glycoside bond is the only one possible due to the bridge between $C_{5'}$ and C_6 which prevents rotation around this bond.

Pseudorotational analysis

The predicted variations of the furanose coupling constants $J_{H1'\ H2'}$ and $J_{H3'\ H4'}$ as well as $J_{H2'\ H3'}$ and $J_{H3'\ H4'}$ with P are represented in Figs. 7a and 7b, respectively.[10,32] Closed curves are obtained as P varies from 0° (North region) via 180° (South region), to 360° (North region).[10] The two curves in both figures correspond to the puckering amplitudes $\tau_m = 35°$ (solid curve) and $\tau_m = 40°$ (dashed curve), typical values for a ribose or deoxyribose ring.

The conformations of the furanose ring in diluted solutions can be elucidated by analyzing these vicinal coupling constants, $^3J_{H\ H}$, in terms of the pseudorotation parameters.[11,12] The experimental coupling constants for **1-4** are indicated on the diagram by numbered dots. As it can be seen, the studied structures can be clustered around two different groups. The values for AZT (dot 1) and AZT-Iso (dot 2) are in the central region of the pseudorotational pathway of Figs. 7a,b. This situation holds when the conformers, belonging to the N and to the S region of the pseudorotation pathway, are in equilibrium. As it is known, the conformation of the furanose appears to be a blend of puckered states and no single state on the pseudorotation pathway can account for the observed three-bond proton NMR coupling constants ($^3J_{H\ H}$)[32] which are time-averaged couplings of the two conformers (J_N, J_S) and their relative populations (X_N, X_S) in the equilibrium as indicated in Eq. (3).[12,33]

$$J_{obs} = X_N J_N + (1 - X_N) J_S \tag{3}$$

Thus, the values obtained indicate that the coupling constant corresponds to a combination of different conformers and that AZT-Iso and AZT have very similar conformational preferences (equilibrium between N and S). On the other hand, the experimental values determined for **3** and **4** (Figs. 7a,b) indicate that they have rigid structures without conformational equilibrium in diluted solutions.

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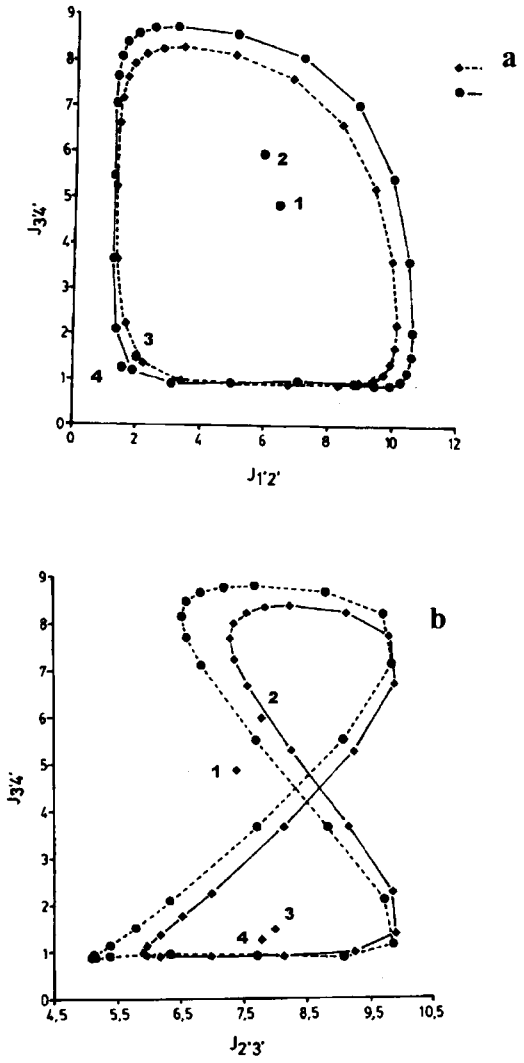

Figure 7. Representations of a) $J_{H1'\ H2'}$ vs. $J_{H3'\ H4'}$ and b) $J_{H2'H3'}$ vs. $J_{H3'H4'}$ according to Plaveck et al. methodology[10] using Karplus equation as developed by Altona et al.[11,12] The phase angle of pseudorotation (P) varies from 0–360° counterclockwise at fixed puckering amplitude (τ_m) of 35° (♦—♦) and 40° (●---●). Data points **1-4** are the experimental values.

The ^{1}H NMR coupling constants were also analyzed using the computer program PSEUROT[18,34] in order to determine P (Table 6).

The P values obtained from experimental results (NMR-PSEUROT) or from AM1 calculations (Tables 3 and 4) lead to concomitant results, inferring two different groups of compounds; AZT-Iso, which in diluted solutions display two conformers (N and S) in equilibrium (similar to AZT) and the bromine molecules which

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Table 6. Conformational parameters of furanose ring.[a]

Compounds	P[b] (North)	τ_m^c (North)	P[b] (South)	τ_m^c (South)	X[d] (South)	rms[e] (Hz)
AZT, **1**	12	26*	142	36*	0.48	0.25
AZT-Iso, **2**	39	30	149	33*	0.34	0.32
(−)-*trans*-(5S,6S), **3**	273	32	–	–	0	0.60
(+)-*trans*-(5R,6R), **4**	277	37	–	–	0	0.48

[a]Evaluated with PSEUROT assuming an equilibrium mixture between N and S conformers for compound **2**.
[b]P (phase angle of pseudorotation in grades);
[c]τ_m: The maximum out-of-plane pucker value (in grades);
[d]X: molar fractions of type south conformers;
[e]rms: mean square root;
*average values.

have one conformation with rigid structure. As can be seen from Tables 4 and 6, the furanosic rings of the bromine derivatives exist in an abnormally high region of the pseudorotational cycle with P values near to 270°, in a conformation neighboring $O_{4'}$-exo, the oxygen of the ring being downwards with respect to the ring plane and $C_{5'}$ (Fig. 6). In the most common nucleosides, the latter conformation is sterically highly unfavorable as P = 271 represent a high energetic barrier region; but in the case of **(−)**-*trans*-(5S,6S), **3** and **(+)**-*trans*-(5R,6R), **4** it is forced by the $C_{5'}$(sugar)-C_6(base) bond.

Dihedral Angles of the Sugar Ring

The dihedral angles of **2** were calculated from the P values, puckering amplitudes (τ_m) and molar fraction obtained with the PSEUROT method, as indicated in Table 7.[10,32,33]

Since only one conformer was detected for each bromine derivative, it was also possible to calculate dihedral angles not only from P and τ_m (PSEUROT) but also from the Karplus equation[30] by using experimental $J_{H\ H}$ coupling constants[11,12] (Table 8).

In Tables 7 and 8 the dihedral angles calculated by AM1 method are also included. As it can be seen, a reasonable agreement is observed among the PSEUROT, Karplus and AM1 determinations.

The Exocyclic $C_{4'}$-$C_{5'}$ Bond ($\gamma_{C4'\text{-}C5'}$)

Information about the exocyclic $C_{4'}$-$C_{5'}$ bond conformation in diluted solutions may be obtained from examination of the vicinal H H coupling constants $^3J_{H4'\ H5'a}$ and $^3J_{H4'\ H5'b}$, which can be interpreted in terms of γ^+, γ^t and γ^- rotamers[10] (Fig. 2, Table 9).

The results obtained show that for AZT and AZT-Iso the γ^+ conformation is favored (near to 60% and 50% in AZT and AZT-Iso, respectively), accompanied

Table 7. Dihedral angles of AZT-Iso, **2** calculated from AM1 and from P, τ_m and molar fractions obtained with PSEUROT.

	North		South	
	Calc[a]	AM1	Calc[a]	AM1
$\phi_{H1'\ H2'a}$	113	109.14	155	141.99
$\phi_{H1'\ H2'b}$	−7	−9.92	34	22.52
$\phi_{H2'a\ H3'}$	27	23.13	−28	−16.13
$\phi_{H2'b\ H3'}$	91	143.65	137	103.7
$\phi_{H3'\ H4'}$	−157	−142.70	−110	−110.53
$\phi_0 = C_{2'}\text{-}C_{1'}\text{-}O_{4'}\text{-}C_{4'}$	−11	5.74	−25	−6.05
$\phi_1 = O_{4'}\text{-}C_{1'}\text{-}C_{2'}\text{-}C_{3'}$	−8	−14.56	33	15.83
$\phi_2 = C_{1'}\text{-}C_{2'}\text{-}C_{3'}\text{-}C_{4'}$	23	17.18	−28	−18.77
$\phi_3 = C_{2'}\text{-}C_{3'}\text{-}C_{4'}\text{-}O_{4'}$	−30	−14.33	13	15.83
$\phi_4 = C_{3'}\text{-}C_{4'}\text{-}O_{4'}\text{-}C_{1'}$	25	5.52	7	−6.29

[a]The corresponding values of ϕ were calculated with data obtained from PSEUROT by using the following equations:[9b]
$\phi_i = \tau_m \cos\,[P + 144\ (i-2)]$; $\phi_{H1'H2'a} = 121.4 + 1.03\ \phi_1$; $\phi_{H1'H2'b} = 0.9 + 1.02\ \phi_1$; $\phi_{H2'a\ H3'} = 2.4 + 1.06\ \phi_2$; $\phi_{H2'b\ H3'} = 122.9 + 1.06\ \phi_3$; $\phi_{H3'H4'} = 124.0 + 1.09\ \phi_3$

by a minor proportion of γ^t (20% in AZT, 10% in AZT-Iso) and γ^- (20% in AZT, 40% in AZT-Iso). Coupling constants of **3** and **4** are not within the general values of the γ^+, γ^t and γ^- rotamers. These results are in accordance with those calculated from the AM1 structures.

Table 8. Dihedral angles of **(−)**-*trans*-(5S,6S), **3** and **(+)**-*trans*-(5R,6R), **4**, calculated from P and $\tau_m$values (PSEUROT), experimental coupling constants (Karplus), and AM1.

	(−)-*trans*-(5S,6S), **3**			**(+)**-*trans*-(5R,6R), **4**		
	Calc[a]	Karplus[b]	AM1	Calc[a]	Karplus[b]	AM1
$\phi_{H1'\ H2'a}$	101	±104	113.2	95	±100	102.1
$\phi_{H1'\ H2'b}$	−20	±35	−6.0	−25	±32	−16.6
$\phi_{H2'a\ H3'}$	4	±27	−1.7	7	±28	9.2
$\phi_{H2'b\ H3'}$	140	±118	118.5	142	±122	129.5
$\phi_{H3'\ H4'}$	−105	±109	−103.7	−104	±107	−112.1
$\phi_0 = C_{2'}\text{-}C_{1'}\text{-}O_{4'}\text{-}C_{4'}$	31		29.7	36		34.6
$\phi_1 = O_{4'}\text{-}C_{1'}\text{-}C_{2'}\text{-}C_{3'}$	−20		−13.4	−25		−22.7
$\phi_2 = C_{1'}\text{-}C_{2'}\text{-}C_{3'}\text{-}C_{4'}$	2		−6.0	5		4.2
$\phi_3 = C_{2'}\text{-}C_{3'}\text{-}C_{4'}\text{-}O_{4'}$	17		23.6	18		15.9
$\phi_4 = C_{3'}\text{-}C_{4'}\text{-}O_{4'}\text{-}C_{1'}$	−30		33.8	−34		−31.9

[a]Data obtained as indicated in Table 7.
[b]Coupling constants calculated from the following equation $^3J_{HH} = 10.5\ \cos^2\phi - 1.2\ \cos\phi + C$, where $C = 1$ for $^3J_{H1'H2'}$ and $C = 0$ for the other coupling constants.

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Table 9. Conformation of exocyclic $C_{4'}$-$C_{5'}$ bond (γ).

Compounds	$^3J_{H4'H5'a}$ (Hz)			$^3J_{H4'H5'b}$ (Hz)		
	Exp	Calc[a]	AM1	Exp	Calc[a]	AM1
AZT, **1**	4.03	3.92	–	3.77	3.87	–
AZT-Iso, **2**	3.71	3.65	4.98–4.09[b]	4.84	4.87	6.02–3.66[b]
(−)-*trans*-(5S,6S), **3**	< 0.5	− 0.04	0.52	2.44	1.88	3.55
(+)-*trans*-(5R,6R), **4**	4.71	6.03	7.1	1.81	1.53	2.02

[a]Data obtained by theoretical LAOOCON 3 program.
[b]Obtained from Eq. (3), using as J_S and J_N the values arising from:[9b] $J_{N/S} = X\gamma^+J\gamma^+ + X\gamma^tJ\gamma^t + X\gamma^-J\gamma^-$ with $X\gamma^+ = 50\%$, $X\gamma^t = 10\%$ and $X\gamma^- = 40\%$ and $J\gamma^+$, $J\gamma^t$ and $J\gamma^-$ obtained from the PCMODEL 2 program using the A-C and D-F conformers of Table 3.

CONCLUSIONS

These studies demonstrate a reasonable agreement between ^{1}H NMR data of **1–4** and semiempirical AM1 calculations. Thus, the results of the present investigation indicate that conformational preferences of AZT-Iso are very similar to those of the parent compound (AZT). The 5′-substituent in AZT-Iso does not affect either the sugar conformation or the pyridine ring. This fact is supported by a similar behavior to that shown by other pyrimidinic nucleosides that exhibit a dynamic equilibrium in solution, where both conformers North and South are in equilibrium.

In addition, **(−)-*trans*-(5S,6S), 3** and **(+)-*trans*-(5R,6R), 4** present a different behavior. They exhibit an extra conformational parameter with respect to standard pyrimidinic nucleosides due to the *chair* or *boat* conformation in the third ring between the sugar and the base. Besides, this extra ring is responsible of a higher molecular rigidity, with practically no changes in the sugar conformation, factor that is also shown by the pseudorotation phase angle calculations.

Despite their structural rigidity, compounds **3** and **4** have been determined to have an in vitro activity similar to that of AZT and AZT-Iso, which could be indicating the importance of the glycosyl – pyrimidyl rings *anti* conformation in the activity of the compounds.

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