Comprehensive Conformational Analysis of the Nucleoside Analogue 2‘-β-Deoxy-6-azacytidine by DFT and MP2 Calculations

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JUNE 2007

Impact Factor: 3.3 · DOI: 10.1021/jp066742h · Source: PubMed

Comprehensive Conformational Analysis of the Nucleoside Analogue 2′-β-Deoxy-6-azacytidine by DFT and MP2 Calculations

Yevgen P. Yurenko,[†,‡] Roman O. Zhurakivsky,[§] Mahmoud Ghomi,*[,‡] Svitlana P. Samijlenko,[†] and Dmytro M. Hovorun*[,†]

Department of Molecular and Quantum Biophysics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, vul. Zabolotnoho 150, 03143, Kyiv, Ukraine, UMR CNRS 7033, Laboratoire de Biophysique Moléculaire, Cellulaire et Tissulaire (BioMoCeTi), Université Pierre et Marie Curie, GENOPOLE-Campus 1, 5 rue Henri Desbruères, 91030 Evry Cedex, France and UFR SMBH, Université Paris 13, 74 rue Marcel Cachin, 93017 Bobigny cedex, France, and Department of Quantum Radiophysics, Faculty of Radiophysics, Taras Shevchenko Kyiv National University, pr. Hlushkova 2, korp. 5, 03127, Kyiv, Ukraine

Received: October 13, 2006; In Final Form: February 19, 2007

A comprehensive conformational analysis of isolated 2′-β-deoxy-6-azacytidine (d6AC), an analogue of therapeutically active 6-azacytidine (6AC), has been performed by means of ab initio calculations at the MP2/6-311++G(2df,pd)//DFT B3LYP/6-31G(d,p) level of theory. Among the 81 conformers located within a 7.83 kcal/mol Gibbs energy range at $T = 298.15$ K, 38 contain syn-oriented bases with respect to 2′-deoxyribose; the other conformers include anti-oriented bases. Energetic analysis of these conformers shows that conformational equilibrium of isolated d6AC at $T = 298.15$ K is shifted to syn conformation with a syn/anti ratio estimated as 61.4%:38.6%. As far as the sugar conformation is concerned, 40 conformers contain north (N) (with $0.3° \leq P \leq 40.1°$), and the rest possess south (S) (with $157.1° \leq P \leq 207.0°$) puckers, where P is the pseudorotational angle of the furanose ring. The S/N occupancy ratio is estimated as 80.2%:19.8% ($T = 298.15$ K). The two most stable conformers are energetically quasidegenerate and correspond to both C2′-endo/syn conformers differing only by orientation of the O3′H hydroxyl group. They are both stabilized by means of similar intramolecular H-bonds, i.e., O5′H···O2, C2′H2···O2, and C2′H2···O5′. As examined by AIM criteria, from 1 to 3 H-bonds per conformer were identified among 13 possible interactions: O5′H···O2, O5′H···N6, O3′H···O5′, O5′H···O3′, C1′H···O2, C2′H2···O2, C2′H2···O5′, C3′H···O2, C3′H···N6, C5′H1···O2, C5′H2···O2, C5′H1···N6, and C5′H2···N6. The biological effect of d6AC is conceived as an inhibition of replicative DNA polymerase caused by an unusual orientation of the sugar residue against the base in the only A form DNA-like conformer.

I. Introduction

Conformational adaptability of biological macromolecules appearing through fundamental biological processes originates, particularly, from the conformational flexibility of their building blocks. Considering the important biological functions of nucleic acids,[1−3] the analysis of the structural features of their monomers (nucleosides and nucleotides) is a fundamental task. Nucleosides and especially their chemical analogues can be used as efficient drugs. Among them, we can emphasize the therapeutic activities of chemically modified cytidine, such as 6-azacytidine (6AC, Figure 1), which has manifested a large spectrum of therapeutic properties by its antiviral, antitumoral, and fungicidal effects.[4−7] Particularly, the ability of 6AC to bind to the AIDS virus replication enzyme, i.e., HIV-reverse transcriptase, has been previously evidenced.[8] To enhance therapeutic effects of 6AC, a number of its derivatives obtained by chemical modifications in the bases was synthesized,[9−10] for example, N4 amino derivatives as well as 2′-deoxy analogues of 6AC. Conformational properties, particularly those governed by intramolecular interactions were shown to be a decisive factor in a specific binding of pyrimidine nucleoside analogues, such as 6AC, to enzymes.[11] Crystal structure of 6AC[12] has evidenced a high-anti orientation of the base with respect to the sugar. Further studies on the 6AC structure were performed by experimental[13,14] and theoretical[15,16] methods. Theoretically, it was shown by means of a semiempirical MNDO/H method[15] that the four most energetically favorable conformers with syn, anti, high-anti, or high-syn oriented bases are stabilized with intramolecular H-bonds. Some of the low-energy 6AC conformers were also analyzed in vacuo by ab initio quantum mechanical calculations.[16] It was established that the most energetically favorable conformer of 6AC belongs to the conformational subset including a syn-oriented base associated with a C2′-endo sugar pucker.

In this work, we focus our attention on a 6AC related compound: 2′-β-deoxy-6-azacytidine (d6AC, Figure 1). To our knowledge, no structural or physicochemical data are available in the literature for this modified nucleoside. By quantum chemical investigation of all the possible conformers of isolated d6AC, we aimed to provide deeper insight into conformational properties of this modified nucleoside with a therapeutic effect and to understand the intrinsic structural basis of its biological activity. Vacuum approximation (isolated molecule) used in this work seems to be reasonable, because active centers of most of

* To whom correspondence should be addressed. E-mail: (M.G.) ghomi@smbh.univ-paris13.fr; (D.M.H.) dhovorun@imbg.org.ua.
[†] National Academy of Sciences of Ukraine.
[‡] Université Pierre et Marie Curie and Université Paris 13.
[§] Taras Shevchenko Kyiv National University.

10.1021/jp066742h CCC: $37.00
Published on Web 05/16/2007

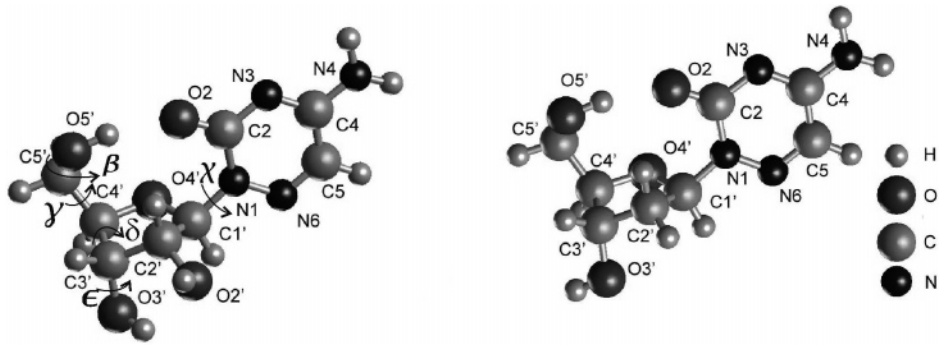

Figure 1. Atom numbering of two related modified nucleosides with therapeutic effect. Left, 6-azacytidine (6AC); right, 2′-deoxy-6-azacytidine (d6AC). Figures shown are relative to the lowest energy conformers of each nucleoside (conformer **1**, see text).

enzymes are rather hydrophobic and characterized by low dielectric constants.[17] An earlier suggestion was made that when nucleotides enter the active site of enzymes, water is displaced.[18,19] For example, in a replicative polymerase active site DNA is in a dehydrated A form.[20]

II. Theoretical Details

Calculation Methods. Density functional theory (DFT) calculations using a nonlocal hybrid B3LYP exchange-correlation functional[21,22] and the 6-31G (d,p) atomic basis set were used in our conformational analysis. Energy minimizations followed by harmonic vibrational calculations were performed at this level of theory. The absence of imaginary frequencies proved that energy-minimized conformers correspond well to the local minima of the energy landscape. Vibrational frequencies were also used in determining the conformers' relative Gibbs energies (ΔG). Harmonic approximation is pertinent in this case because the highest mechanical anharmonicity of high-frequency stretching vibrations of groups OH, NH, and CH[23] contributes negligibly to the Gibbs energy thermal corrections.[24] For low-frequency vibrations of nucleosides, harmonic approximation is commonly accepted (see ref 25 and refs 33−36 and 42 therein). Single-point calculations for all conformers were performed at the MP2 level (Møller−Plessett second-order perturbative method) with a larger set of Gaussian basis functions, that is, 6-311++G(2df,pd). The ΔG values were sums of electronic energies calculated at the MP2/6-311++G(2df,pd) level and the zero-point energies, thermal corrections, and entropy contributions at the B3LYP/6-31G(d,p) level of theory. The four most energetically favorable conformers (two with syn- and other two with anti-oriented bases) were reoptimized at the B3LYP/6-31+G(d,p) and B3LYP/6-31++G(d,p) levels of theory to check the 6-31G(d,p) basis set consistency in determining the conformers' geometry, as well as at the MP2/cc-pVDZ level to consider the electronic correlation effects as accurately as possible. Energy values of transition states between some conformer pairs were determined by a synchronous transit-guided quasi-Newton method (STQN).[26] All the calculations were performed by using the Gaussian03 program[27] running on IBM SP3 workstations.

Conformational Search and Analysis. Chemical structure and atom numbering of d6AC as compared to its relative modified riboside 6AC are presented in Figure 1. The conformational angles of this nucleoside are analogous to those defined previously[1] (Figure 1). Sugar conformation was determined by means of the pseudorotational angle, P.[28] Although the reported calculations correspond to a nucleoside and not to a nucleic acid chain, the orientation of the hydroxyl groups O3′H and O5′H are determined by means of dihedral angles β and ϵ, respectively (Figure 1).[1] Conformational analysis of d6AC is based on the results previously reported on 1′,2′-deoxyribose,[29] a model compound for the sugars involved in 2′-deoxyribonucleosides. Thus, assumption was made that 1′,2′-deoxyribose can adopt, in principle, 27 conformers corresponding to a definite type of sugar puckering.[29] This fact has been considered in the search of the nucleoside optimized structures with anti and syn orientations of the base.

The study of electronic density topology, based on the QTAIM (quantum theory of atoms in molecules) approach,[30] was carried out for all conformers in order to identify possible H-bonds according to the criteria proposed by Koch and Popelier.[31] The presence of a bond critical point (BCP, the so-called (3, −1) type) between H-bond donor and acceptor, as well as a positive value of Laplacian of the density at BCP, were considered as necessary conditions of H-bond formation.

H-bonds were furthermore tested on the basis of geometric and vibrational criteria. The OH···O and OH···N H-bond energies were evaluated by either the empirical Iogansen's formula,[32]

$$-\Delta H = 0.33(\Delta\nu - 40)^{1/2} \quad (1)$$

where ΔH is a bond enthalpy in kcal/mol, $\Delta\nu$ the frequency red shift of a bond-stretch mode ν(X−H) in cm^{-1} (with X = N, O); or according to another Iogansen's formula using a stretching mode intensity change defined as follows,[32]

$$\Delta H = -2.92(I/I_0)^{1/2} \quad (2)$$

where I_0 and I are the band integrated intensity corresponding to a hydroxyl stretching mode in free state (without H-bond) and when involved in an H-bond, respectively. $\Delta\nu = \nu_0 - \nu$ represents a frequency red shift that appeared upon the formation of a given H-bond, where ν is the calculated frequency corresponding to a conformer including the H-bond, and ν_0 represents the average of calculated frequencies relative to the 20 conformers of highest energy not presenting such H-bonds. The average value of the intensity I_0 was determined similarly. It should be noted that the Iogansen's formula 1 produces a more reliable result as compared to that expected from formula 2, since ab initio calculations usually overestimate the intensity increase for a hydroxyl bond stretch, whereas the corresponding calculated frequency shift values agree well with experiment.[33]

Moreover, additional Koch and Popelier criteria, including changes of atomic properties and the mutual penetration of a hydrogen and acceptor atom, tested the weakest H-bonds of each type: such H-bonds were chosen by the least values of density and Laplacian at BCP and by the largest distances between the hydrogen and acceptor atoms, as well. The AIMPAC series of

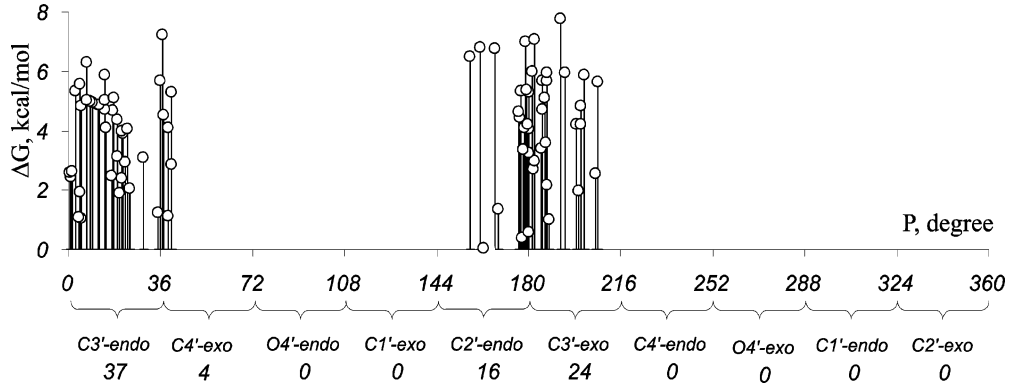

Figure 2. Distribution of 81 conformers of d6AC separated by the relative free Gibbs energy, ΔG (at $T = 298.15°$ K), and the pseudorotational angle, P. Sugar moiety conformational subfamilies and number of conformers they contain are denoted.

TABLE 1: Structural, Energetic and Polar Characteristics of Low-Energy d6AC Conformers Populating at Least 95% of Conformational Equilibrium According to Boltzmann Distribution at Room Temperature According to the MP2/6-311++G(2df,pd)//DFT B3LYP/6-31G(d,p) Calculations

	ΔG^a														
	298 K	0 K	d^b	χ^c	P^c	$\nu_{max}{}^c$	β^c	γ^c	δ^c	ϵ^c	$\tau_1{}^d$	$\tau_2{}^e$	$\tau_3{}^f$	$\tau_4{}^g$	l^h
1	0.00	0.04	5.65	60.9	165.1, C2′-endo	35.5	63.7	43.7	−90.5	−64.2	1.8	0.9	8.9	2.2	1.475
2	0.07	0.00	6.66	60.5	162.4, C2′-endo	35.4	63.2	44.5	−95.8	−179.1	2.4	0.8	10.2	2.1	1.475
3	0.45	0.97	4.98	−126.9	177.1, C2′-endo	32.5	58.7	53.2	−92.2	175.3	2.8	−0.1	14.4	3.8	1.476
4	0.66	1.18	5.17	−122.6	180.1, C3′-exo	32.4	58.4	51.9	−87.2	−63.3	2.9	0.6	13.5	1.8	1.475
5	1.05	1.69	5.57	−121.3	187.8, C3′-exo	32.8	60.9	49.7	−92.2	53.7	3.1	0.4	10.4	1.6	1.475
6	1.09	1.57	4.99	−156.6	5.0, C3′-endo	33.1	45.9	51.5	−147.8	−82.9	1.6	0.1	13.3	4.8	1.499
7	1.14	1.59	4.44	−158.4	4.2, C3′-endo	31.9	46.7	53.6	−149.6	−164.4	1.9	0.0	13.9	4.2	1.500
8	1.19	1.79	7.03	60.9	39.0, C4′-exo	24.8	40.6	44.3	−146.0	−151.6	2.0	0.9	10.2	1.7	1.481
9	1.25	1.67	6.34	59.6	35.0, C3′-endo	26.1	41.7	42.0	−144.7	−87.8	1.9	0.8	8.8	1.6	1.483
10	1.33	1.59	7.80	61.7	167.9, C2′-endo	34.1	65.5	42.2	−98.1	51.1	1.9	0.9	8.1	2.5	1.476
11	1.97	2.39	3.14	−158.1	4.2, C3′-endo	32.1	46.2	54.0	−153.7	64.0	1.4	1.5	10.0	3.6	1.498
12	1.99	3.77	5.56	−116.5	199.3, C3′-exo	35.2	−49.9	167.9	−87.1	48.0	3.3	0.4	10.0	1.1	1.470
13	1.99	3.65	7.43	−110.8	19.7, C3′-endo	33.4	176.3	−58.5	−152.4	−57.6	3.3	0.8	14.8	1.1	1.473

[a] Relative free Gibbs energy (kcal/mol) at $p = 1.00$ atm. [b] Dipole moment (D). [c] For definition of the conformational angles, see Figure 1 and ref 1. [d] Angle between C1′N1 bond and the base plane. [e] Angle between the C4N4 bond and the base plane. [f] Angle between C4N4 bond and NH_2 plane. [g] Maximum of torsion angles in the base. [h] Glycosylic bond length (Å). All angles are given in degrees. For the rest of the conformers, see Table A1 in the Supporting Information.

programs[34] were used for the above-mentioned H-bond identification and topological analysis.

III. Results and Discussion

Energetics and Geometrical Data. Figure 2 displays the energy distribution of 81 d6AC conformers as a function of the pseudorotational angle. Relative free energies ΔG of all conformers lie within the 7.83 and 9.01 kcal/mol intervals for room temperature and 0 K, respectively (Table 1, Table A1 in the Supporting Information). Whatever the temperature is (0 K or room temperature), an energy difference of <0.5 kcal/mol separates two successive calculated conformers (Table 1). To demonstrate that the conformers are well-defined structures, the transition state energies and energetic barriers between conformers differing by only one conformational parameter were evaluated (Table A2 in the Supporting Information). It is noteworthy that the quasidegenerate global minimum on the d6AC hypersurface involves two conformers, **1** and **2** (Table 1, Figure 3). Despite a negligible energy difference between these conformers, they are rather defined structures, with an 0.82 kcal/mol energy barrier between them (Table A2 in the Supporting Information). Both conformers contain a C2′-endo sugar pucker connected to a syn-oriented base differing only by orientation of the O3′H hydroxyl group. These results are similar to those obtained in the case of the 6AC analogue containing a ribose sugar.[16] Both lowest-energy conformers are stabilized with a rather strong intramolecular H-bond, O5′H···O2, whose energy can be estimated by means of formula 1 (3.97 kcal/mol for conformer **1** and 3.93 kcal/mol for conformer **2**). It should be pointed out that the same H-bond also stabilizes the structures of 2′-deoxycytidine (dC)[35,36] and 6AC.[16] Moreover, comparable geometrical parameters are found in the case of the most energetically favorable conformers of other pyrimidine nucleosides, that is, dC,[35] 6AC,[16] and d6AC

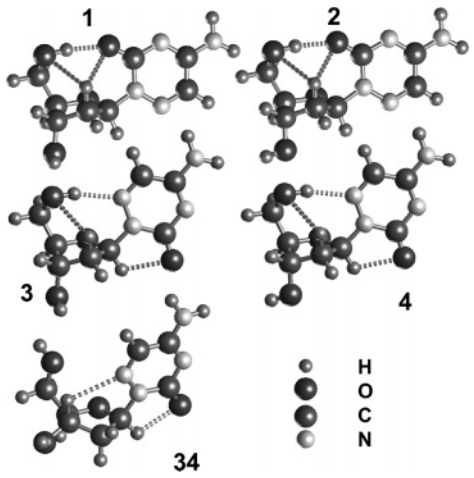

Figure 3. Spatial structures of d6AC relative to conformers **1**, **2**, **3**, **4** (the most energetically favorable conformers). On the bottom, conformer **34**, correspoding to A-form DNA, is also displayed.

(present work). The five most energetically favorable conformers of d6AC form the following order: C2′-endo/syn (**1**, **2**) > C2′-endo/anti (**3**) > C3′-exo/anti (**4**, **5**). These conformers might correspond to those observable in the gas phase or in the inert-gas matrixes at low-temperature. At room temperature, according to the Boltzmann distribution, 70.1% of the total population of isolated d6AC might correspond to conformers **1**−**5** (Table 1, Figure 3). On the other hand, the syn:anti ratio has been estimated as 61.4%:38.6%. Obviously, in essentially a nonequilibrium environment in vivo, other conformers may also become energetically favorable. The optimization of conformers **1**−**4** at the B3LYP/6-31+G(d,p), B3LYP/6-31++G(d,p), and MP2/cc-pVDZ levels of theory shows only slight geometrical changes (Table A3 in the Supporting Information). This result confirms the relevancy of the DFT B3LYP/6-31G(d,p) level in estimating the geometry of d6AC conformers. The most prominent effect appearing at the MP2/cc-pVDZ level is a more discernible nonplanarity of the amino group (CNH_2 fragment), as shown by the values of the τ_2 and τ_3 angles (Table A3 in the Supporting Information).

A bimodal distribution of conformations is obtained as a function of the P angle ($0.3° < P < 40.1°$ and $157.1° < P < 207.0°$, Figure 2). The C3′-endo is the most populated subfamily (38 conformers) and the C3′-exo (24 conformers), the C2′-endo (16 conformers), and C4′-exo (4 conformers) populations occupy the second to the fourth positions, respectively. It should be emphasized that on the basis of X-ray diffraction[1] and NMR[1,37] data and other earlier assumptions,[35,36,38−45] the C3′-endo and C2′-endo subfamilies are traditionally believed to be the most populated in natural nucleosides and nucleotides. In d6AC, the sugar conformations belong to four subfamilies (see above) versus seven subfamilies existing in 1′,2′-deoxyribose.[29] This should be due to the formation of intramolecular H-bonds between the base and sugar. The total number of d6AC conformers is reduced to 81 (instead of 116, as expected from 58 possible conformers of 1′,2′-deoxyribose,[29] multiplied by 2, taking into account both syn and anti orientations of the base). Syn and anti conformers are stabilized by the O5′ H···O2 and O5′ H···N6 H-bonds, respectively.

Variation of bond lengths and valence angles of the sugar ring are presented in Table 2. The C1′O4′ bond and the C1′−C2′−C3′ angle appear to be the most flexible geometrical parameters whatever the sugar conformation. C2′ and C3′ are the most deviated atoms from the C1′−O4′−C4′ plane (Table 2). To examine C2′-endo and C3′-endo sugar puckers appearing in the obtained conformers, minimal (as well as maximal) and mean values of sugar bond lengths were estimated (Table A4 in the Supporting Information).

Figure 4 displays a bimodal distribution for the χ angle: 43 of the conformers possess an anti-oriented ($-160.4° \le \chi \le -98.5°$) base, and the rest of them, a syn-oriented ($55.7° \le \chi \le 76.1°$) one. Values of endocyclic torsional angles, N6−N1−C2−N3 and C5−N6−N1−C2, are located in the [−5.4°, 0.7°] and [−1.3°, 2.7°] intervals, respectively. This fact confirms the nonplanarity and, consequently, the flexibility of the base, as previously reported in dC.[35]

A trimodal distribution for all other conformational angles (β, γ, and ϵ) corresponding to the g^+, t, and g^- regions, respectively (Figure 4).

Intramolecular H-Bonds. QM/AIM analysis proved to be crucial for determining weak H-bonds of CH···O and CH···N types, because in these cases, the values of the frequency shifts appearing in the bond-stretching and torsional modes are insufficient to ensure the presence of such interactions. The 81 analyzed conformers present 13 types of intramolecular H-bonds (Table 3 and Table A5 in the Supporting Information): C1′H···O2 (39 conformers), C3′H···O2 (21 conformers), C2′H2···O2 (17 conformers), C5′H1···O2 (15 conformers), C3′H···N6 (14 conformers), C5′H2···O2 (11 conformers), C2′H2···O5′ (9 conformers), O5′H···O2 (9 conformers), O5′H···N6 (7 conformers), O3′H···O5′ (4 conformers), O5′H···O3′ (4 conformers), C5′H2···N6 (3 conformers), and C5′H1···N6 (2 conformers). This is the first time that H-bonds such as C3′H···N6, C5′H1···N6, C5′H2···N6 and C2′H2···O5′ are evidenced by means of QM/AIM calculations. It should be emphasized that all the above-mentioned H-bonds include those previously evidenced by our calculations on 1′,2′-deoxyribose.[29] The main points concerning these hydrogen bond interactions can be described as follows:

- 12 conformers (5 with anti- and 7 with syn-oriented base) contain 3 H-bonds; 50 of them (22 with anti- and 28 with syn-base) 2 H-bonds, and 19 conformers (16 with anti- and 3 with syn-base) just a single H-bond. Among a total number of 155 H-bonds stabilizing the 81 analyzed conformers, 24 present common interactions of OH···O and OH···N types (17 and 7 conformers, respectively).
- In conformers **1** and **2**, the O5′H···O2 interaction is the strongest one (Table 3). However, on the basis of the ν(O5′H) frequency shifts, we can conclude that the O5′H···O2 interaction is slightly stronger in conformer **1** as compared to conformer **2**. Upon comparison with the geometrical data of 1′,2′-deoxyribose,[29] we have found that the sugar involved in the nucleoside is strongly deformed. This fact might be mainly due to the O5′H···O2 H-bond between the base and the sugar. For 9 conformers containing this H-bond, frequency shifts vary between 83 and 188 cm^{-1}. The average energy of this H-bond (formula 1) is 3.18 kcal/mol.
- Seven anti-base conformers manifest a O5′H···N6 interaction. Among them, conformers **3** and **4** are found to be the most stable. Although conformer **3** is found to be more stable, the O5′H···O2 H-bond appears stronger in conformer **4**. The energy estimates of the O5′H ···N6 interaction (formula 1) in conform-

TABLE 2: Statistical Parameters Corresponding to Sugar Puckers Involved in the 81 Nucleoside Conformers, Obtained by the Calculations at the DFT B3LYP/6-31G(d,p) Level

	C2′	C3′			
h_{min}[a]	0.003	0.002			
h_{max}[b]	0.634	0.769			
h_{mean}[c]	0.193	0.380			
	O4′C1′	C1′C2′	C2′C3′	C3′C4′	C4′C4′
d_{min}[d]	1.401	1.529	1.525	1.530	1.427
d_{max}[e]	1.430	1.546	1.545	1.549	1.451
d_{mean}[f]	1.418	1.537	1.534	1.539	1.438
d_{std}[g]	0.007	0.004	0.005	0.004	0.006
d_{std}/d_{mean}	0.005	0.002	0.003	0.003	0.004
	O4′C1′C2′	C1′C2′C3′	C2′C3′C4′	C3′C4′O4′	C4′O4′O1′
α_{min}[h]	104.8	101.3	101.2	103.5	109.3
α_{max}[i]	107.9	105.8	103.5	108.1	112.3
α_{mean}[j]	106.9	103.4	102.3	106.0	111.0
α_{std}[k]	0.6	1.2	0.6	0.8	0.5
$\alpha_{std}/\alpha_{mean}$	0.005	0.012	0.005	0.008	0.005

[a,b,c] Minimum, maximum, and mean deviations of C2′ and C3′ atoms from C1′O4′C4′ plane. [d,e,f] Minimum, maximum, and mean values of bond lengths. [g] Standard deviations of bond lengths. [h,i,j] Minimum, maximum, and mean values of valence angles. [k] Standard deviations of valence angles. All distances are in angstroms and all angles are in degrees.

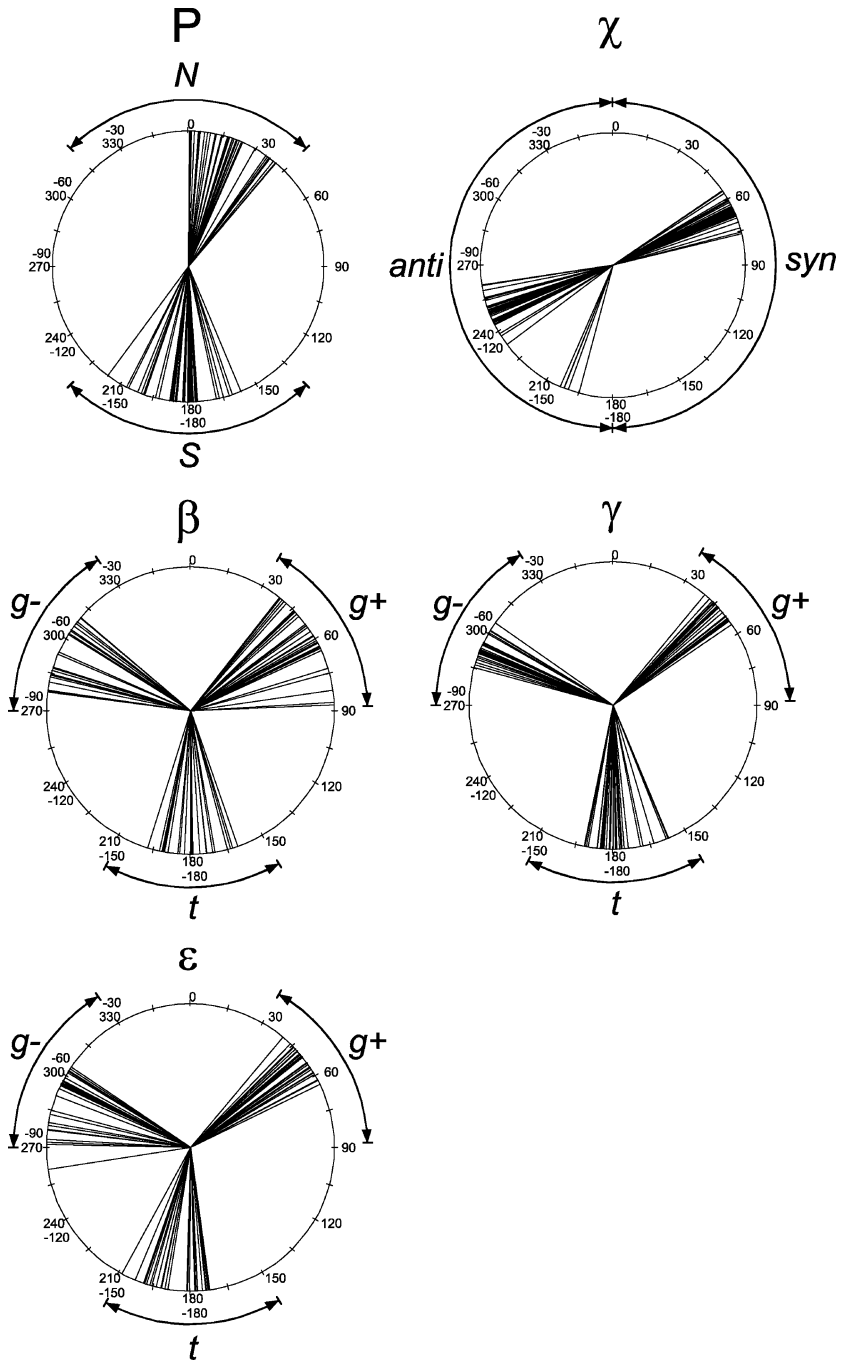

Figure 4. Conformational rings for main structural parameters (P, χ, β, γ, and ϵ) of the d6AC molecule.

ers **3** and **4** are 3.57 and 3.64 kcal/mol, respectively. For seven conformers with the O5′H ···N6 H-bond, frequency shifts vary from 102 to 162 cm^{-1}. The average energy of O5′H ···N6 interaction (formula 1) is 3.18 kcal/mol. As in the case of conformers **1** and **2**, the changes in the sugar geometrical parameters should be noted in conformers **3** and **4** upon comparison with 1′,2′-deoxyribose.

• Two other types of traditional H-bonds, O5′H···O3′ and O3′H···O5′, were observed in two groups of conformers: (**33**, **35**, **37**, **44**) and (**13**, **14**, **25**, **26**), respectively. All these eight conformers contain C3′-endo sugar puckers. The first group of conformers are characterized by the following average values: 138.2° for the O5′H···O3′ angle, an elongation of 0.005 Å in a hydroxyl bond, giving rise to a 77.3 cm^{-1} frequency shift for its stretching mode; the energy estimate of this H-bond is 2.01 kcal/mol. For the second group of conformers, corresponding average values are 127.4° for the O3′H···O5′ angle, 0.0045 Å for the hydroxyl group elongation, and the 57.5 cm^{-1} frequency shift of the O3′H bond stretching mode. A 1.35 kcal/mol value is estimated for the O3′H···O5′ H-bond energy. Interestingly, with similar structures of the sugar residue, the values of the stretching mode frequency shifts are higher for anti conformers (**13** and **25**) as compared to syn conformers (**14** and **26**).

• Each of the most stable conformers, that is, **1**, **2**, **3**, and **4**, corresponding to the conformational landscape of d6AC at room temperature, is stabilized by three intramolecular H-bonds. Conformer **1**: O5′H···O2, C2′H2···O2, and C2′H2···O5′; conformer **2**: O5′H···O2, C2′H2···O2 and C2′H2···O5′; conformer **3**: O5′H···N6, C2′H2···O5′ and C1′H···O2; conformer **4**: O5′H···N6, C2′H2···O5′, and C1′H···O2 (Figure 3).

TABLE 3: Structural and Vibrational Data Relative to the Intramolecular H-Bonds in Which N and O Atoms Are Involved

conformer	AH···B	$d_{A\cdots B}$[a]	$d_{H\cdots B}$[b]	$a_{AH\cdots B}$[c]	Δ_{AH}[d]	$\Delta\nu_{str}$[e]	ν_{vib}[f]	I_{str}/I_{0str}[g]	$-\Delta H$[h]
1	O5′H5′···O2	2.79	1.83	168.0	0.011	185	671	54.5	3.98
2		2.81	1.85	167.0	0.011	182	670	50.8	3.93
8		2.87	1.97	154.3	0.009	128	547	32.4	3.09
9		2.86	1.95	155.6	0.009	143	570	35.4	3.35
10		2.79	1.83	167.9	0.011	170	660	53.5	3.76
22		2.93	2.09	143.3	0.007	84	484	17.6	2.20
24		2.87	1.95	155.0	0.009	129	539	34.1	3.11
29		2.96	2.12	143.1	0.006	83	478	17.5	2.15
31		2.90	2.07	142.9	0.006	86	486	17.4	2.23
3	O5′H5′···N6	3.03	2.09	162.4	0.009	157	642	33.2	3.57
4		3.01	2.06	163.8	0.010	162	642	36.8	3.65
5		2.98	2.03	164.4	0.010	165	644	39.0	3.69
6		2.99	2.07	156.8	0.010	171	601	34.4	3.78
7		2.98	2.07	155.1	0.010	157	582	32.5	3.56
11		2.98	2.07	154.6	0.009	156	580	32.6	3.55
19		3.14	2.30	143.8	0.007	102	490	13.5	2.59
13	O3′H3′···O5′	2.96	2.25	129.9	0.005	64	478	4.7	1.61
14		3.06	2.39	126.0	0.004	50	460	3.5	1.02
25		3.02	2.31	129.2	0.005	66	525	4.2	1.69
26		3.11	2.46	124.2	0.004	50	487	3.2	1.07
33	O5′H5′···O3′	2.99	2.18	140.7	0.005	79	444	7.0	2.05
35		3.02	2.24	136.5	0.005	76	492	5.3	1.98
37		3.00	2.19	140.6	0.005	79	451	7.0	2.06
44		3.04	2.28	134.9	0.005	75	481	4.9	1.95

[a] Distance between A (donor) and B (acceptor) atoms. [b] Distance between H and B atoms. [c] H-bond angle. [d] Difference between AH distances in the presence of H-bond and in nonbonded state. [e] Decrease in AH stretching mode frequency upon H-bonding. [f] Vibrational frequency relative to CAH···B torsion, where C is an atom chemically bonded to A atom. [g] I_{str} and $I_{0\ str}$: AH stretching mode integral intensity upon H-bonding and in nonbonded state, accordingly. [h] H-bond enthalpy calculated by Iogansen (formula 1). Distances are given in angstroms, angles in degrees, frequencies in cm^{-1}, enthalpy values in kcal/mol. Frequencies are calculated at the DFT B3LYP/6-31G(d,p) level and are scaled (scaling factor = 0.9608).[67]

• One hundred and thirty-one intramolecular interactions identified in the 81 analyzed conformers of d6AC correspond to nontraditional weak H-bonds of CH···O and CH···N types (Table A5 in Supporting Information), suggested for the first time in refs 46 and 47, respectively. Despite their *weakness*, these interactions considerably contribute to maintaining the whole nucleoside[48] and nucleic acid structure.[49−52] Among these H-bonds, those belonging to the C3′H···N6 subfamily seem to be stronger, as evidenced by the maximal following characteristic values: $\Delta\nu_{str}$ (C3′−H) = 144 cm^{-1}, $I_{str}/I_{0\ str} \cong 10$, $\Delta d_{CH} \cong 0.010$ Å.

• It was recently supposed[53] that all CH···O interactions, except C6H···O5′, appearing in canonical pyrimidine and purine 2′-deoxyribonucleotides[44,45] should be considered as electrostatic interactions rather than hydrogen bonds. To make sure that the above-mentioned weak interactions in d6AC can be considered as real H-bonds, five "two-molecule" Koch and Popelier[31] criteria were applied additionally to the most questionable representatives of nine types of nontraditional H-bonds: variation in atomic properties of the hydrogen atom upon H-bond formation, namely, positive charge increase Δq; decrease in dipolar polarization ΔM; reduction in atomic volume (Δv); energetic destabilization (ΔE); and mutual penetration (sum $\Delta r_H + \Delta r_B$ of variations in atomic radii of H and an acceptor B). To do this, structurally close conformers without a given H-bond, were considered as molecular references. Here, the results of QM/AIM analysis (Table A6 in the Supporting Information) clearly prove that all weak CH···O and CH···N interactions reported in this work fulfill these H-bonding criteria.[31]

• C1′H···O2 H-bonds seem to be the weakest ones, since the majority of them cannot be identified solely on the basis of spectral and geometric data, such as the identified CH···O and CH···N H-bonds. It should be pointed out that the maximum H-bonding distances are $d_{A\cdots B\ max} = 3.51$ Å and $d_{H\cdots B\ max} = 2.76$ Å, and the minimal CH···B angle observed in the case of the C5′H1···O2 H-bond is 103.3°. No H-bond involving O4′ atom is detected (in contrast to canonical nucleoside dC[36]). It is worth mentioning that only four anti-base conformers—**6**, **7**, **11** and **19**—do not contain the C1′H···O2 hydrogen bond. They are instead stabilized by the O5′H···N6 H-bond alone; the orientation of their base by means of high-syn χ angles (Table 1, Table A1 in the Supporting Information), and the C1′H···O2 angles are found below 100°. The N6 atom does not serve as proton acceptor in three anti base conformers (**75**, **76**, **77**); this explains their high relative Gibbs energies.

• The d6AC conformational family appeared to contain only one DNA-like conformation (conformer **34**; see Figure 3 and Table A1 in the Supporting Information) that can be regarded as A-form-like ($\chi\in$ anti, $P \in$ C3′endo, $\beta \in$t, $\gamma \in g^+$, $\epsilon \in$t) and none of a B-form DNA-like ($\chi \in$anti, $P \in$ C2′endo, $\beta \in$t, $\gamma \in g^+$, $\epsilon \in$t), in contrast to the canonical nucleoside dC, which can adopt both (A and B) DNA-like forms.[39]

It was reported by Hocquet et al.[40] that in dC, the C3′-endo/anti (involved in A-form DNA) conformation is favored because of its lower energy, as compared to the C2′-endo/anti (involved in B form) conformer. The authors have interpreted the mentioned result as a consequence of the stabilizing H-bond C6H···O5′.[36] In d6AC, the lack of hydrogen at the 6 position of cytosine avoids the formation of such a hydrogen bond (necessary for the stabilization of a C2′-endo/anti conformer). To verify this assumption, we have optimized both dC C2′-endo/anti and C3′-endo/anti DNA-like conformers at the MP2/6-311++G(d,p)//DFT B3LYP/6-31G(d,p) level. Corresponding calculated results are compared to those of d6AC in Table 4. The relative Gibbs energy of the dC C2′-endo/anti conformer estimated at the MP2/6-311++G(d,p)//B3LYP/6-31G(d,p) level exceeds that of the C3′-endo/anti conformer by 0.35 kcal/mol. The existence of the stabilizing intermolecular H-bonds, that is, C6H···O5′ and C2′H···O5′ H-bonds in dC C2′-endo/anti and

TABLE 4: Energetic and Geometric Features Corresponding to the DNA-Like Conformers of dAC6 and dC

nucleoside[a]	ΔG^b	χ	P	ν_{max}	β	γ	ϵ	sugar conformation	DNA form
d6AC	4.11	−98.9	14.5	32.3	173.5	51.5	−163.7	C3′-endo	A
dC	1.26	−163.0	12.3	34.4	178.0	54.1	−172.6	C3′-endo	A
	1.61	−145.8	163.7	34.3	178.7	53.9	169.8	C2′-endo	B

[a] d6AC structure and energy are estimated at the MP2/6-311++G(2df, pd)//DFT B3LYP/6-31G(d,p), whereas similar data for dC are obtained at the MP2/6-311++G(d,p)//DFT B3LYP/6-31G(d,p) level of theory. For angular definitions see Table 1 and Figure 1 [b] ΔG: Relative Gibbs energy (kcal/mol) at $T = 298.15$ K (minimum energy conformers are taken as reference).

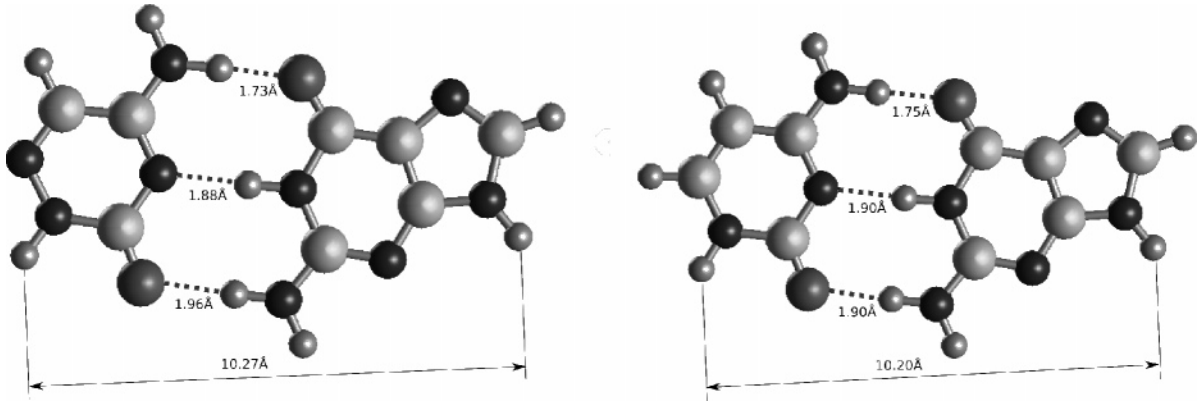

Figure 5. Spatial structure of the 6-aza-cytosine·guanine and cytosine·guanine base pairs according to B3LYP/6-31G(d,p) calculations.

C6H···O5′ and C6H···O4′ H-bonds in dC C3′-endo/anti, is confirmed by applying the QM/AIM approach. The χ angle value in dC C3′-endo/anti is very close to the value reported for the "ideal" A form of DNA.[54,55]

Earlier, Foloppe and MacKerell[39] predicted the formation of A, B, and Z forms of DNA on the basis of intrinsic conformational properties of isolated canonical 2′-deoxyribonucleosides. To check the ability of the conformer **34** to exist in the regular A-form of DNA, its energy minimization with a fixed value of angle $\chi = 201.1°$ as in ideal DNA A-form-like conformation was performed at the B3LYP/6-31G(d,p) level of theory. In such a geometry, d6AC proved to be unstable: after the energy minimization, the 3′OH hydroxyl group turns toward the sugar residue. Based on this result and the fact that in the active site of replicative polymerase DNA adopts A form,[20] we supposed that, unlike canonical nucleoside dC, phosphorylated d6AC cannot be enzymatically incorporated into DNA in the A and, especially, the B form, and this might be one of the reasons for its therapeutic effects.

Biological influence of d6AC is perceived in disturbance of the DNA synthesis fidelity. In spite of quasi-isomorphism of the Gua·6-aza-Cyt and Watson−Crick Gua·Cyt base pairs (Figure 5), a significant difference between the sugar orientations of d6AC and dC might prevent further DNA elongation, since high fidelity replicative DNA polymerases recognize not only the base pair geometry but also the sugar conformation as a consequence of a high level of active site tightness.[56] Similarly, 6AC and its derivatives inhibit DNA-dependent T7 RNA polymerase[57] due to unnatural conformation of the sugar residue.[58]

Base Structural Changes Induced by Nucleoside Formation. The values of the glycosyl bond length lie between 1.461 and 1.500 Å (Table 1, Table A1 in the Supporting Information), with a mean length of $l_{average} = 1.476$ Å and a standard deviation of 0.5%. Both conformers **1** and **2** (global minimum, see above) are characterized by a C1′N1 bond length equal to 1.475 Å. All characteristic conformational angles, together with their deviations, are presented in Table 1. For each conformer, an average base plane was calculated by a least-square fit algorithm. Our calculations of free base 6-aza-Cyt show an overall planarity of its ring. It should be mentioned that the base plane in the nucleoside presents nonplanarity for all the conformers. To show this fact, we mention here that (i) the values of the angle τ_1 between the C1′N1 bond and the base plane fall into the range from −5.4° for conformer **65** to 5.7° for conformer **68** (the deviation of the N1H bond from the base plane is 0.03°), (ii) the τ_2 angle between the C4N4 bond and the base plane varies from −1.1° in conformers **14** and **45** to 1.5° in conformer **11** (the corresponding value for the free base is ±0.9°), (iii) the dispersion of the angle τ_3 between the C4N4 bond and the plane of the NH_2 group covers the values from 8.1° in conformer **10** to 19.3° in conformer **34** (the corresponding value in the free base is 12.9°), (iv) the N6−N1−C2−N3 dihedral angle, τ_4, equal to 0.3° in the free base, takes values ranging from 0.8° (conformer **23**) to 5.4° (conformer **19**). Consideration of these data shows that the glycoside bond is deflected from the base plane in the nucleoside, leading to the N1 atom pyramidalization. The exocyclic CNH_2 fragment also appears to be nonplanar. This result is in agreement with those previously obtained in canonical,[59,60] modified[61] nucleotide bases and related compounds.[62] The pyramidal and asymmetric character of the exocyclic CNH_2 fragment is characterized by opposite deflections of amino protons and the C4N4 bond with respect to the pyrimidine ring plane: the hydrogen atom close to the C5H group is more deflected than the other one (close to N3 atom). Values of the C5C4N4H dihedral angles are the following: C5−C4−N4−$H4_a$ (max. 14.9°, min. 5.9°, average 11.1°), C5−C4−N4−$H4_b$ (max. 7.5°, min. 3.5°, average 5.9°). Corresponding values for these two angles in the free base are 4.8° and 9.5°, respectively.

Concluding Remarks. A number of theoretical reports were previously published on the conformational analysis of nucleosides and nucleotides by means of ab initio calculations.[16,35,36,38−45,53,63−66] In some of them,[39,43] the relation between the energy and the glycosyl angle, χ, has been emphasized. Other investigations provided deeper insight into the conformational properties associated with the sugar puckering.[38,40,42] Vibrational characteristics[16,25,66] and H-bonds[16,36,64] were taken into consideration in nucleosides, and the peculiar

behavior of 2′-deoxycytidine (dC) and cytidine (C) in connection with their energetic schemes was also discussed.[36,40,64]

Here, we have presented a systematic approach for the conformational analysis of a modified nucleoside, 2′-β-deoxy-6-azacytidine (d6AC), based simultaneously on its energetic, vibrational features, and intramolecular interactions. This set of information allows us to understand better the particular structural features of d6AC. Through the comprehensive analysis reported above, the existence of two conformers (differing only by orientation of the O3′H hydroxyl group), both corresponding to the same quasidegenerate global minimum, was evidenced. Moreover, the considerable influence of the sugar conformation on the base−sugar hydrogen bonds was established.

The results obtained pave the way to understanding of the biological influence of modified nucleoside d6AC through inhibition of replicative DNA polymerase as a consequence of unnatural orientation of its sugar residue with respect to the base in the only DNA-like-form conformer.

To develop our notion of the possible molecular mechanism for the biological activity of d6AC, we plan to simulate by molecular mechanics methods the dynamical behavior of incoming d6AC triphosphate to form a complementary H-bond with template guanine nucleotide in a model active center of a replicative DNA polymerase.

Acknowledgment. This work was prepared in the framework of a scientific collaboration between BioMoCeTi (UMR CNRS 7033) and the Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine. Ye. Yu. was granted as a Ph.D. fellow from the French Embassy in Ukraine. The authors also thank the French Calculation Center CINES (Montpellier, France) for the computational facilities on the IBM SP3 workstations. The authors from Kyiv greatly appreciate useful discussions of the results with Inna V. Alexeeva and Larysa H. Palchykivs'ka.

Supporting Information Available: Table A1: Structural, energetic and polar characteristics of the **14**−**81** d6AC conformers calculated at the MP2/6-311++G(2df,pd)//DFT B3LYP/6-31G(d,p) level of theory. Table A2: Energy of transition states and barriers between some conformers differing only by the value of one parameter. Table A3: Geometrical parameters corresponding to conformers **1**, **2**, **3**, and **4** as optimized at the DFT and MP2 levels of theory. Table A4: Statistical estimates of the bond lengths (Å) within the sugar residues belonging to C2′-endo and C3′-endo subfamilies. Table A5: Geometric and vibrational characteristics of the CH···O and CH···N intramolecular H-bonds in the d6AC molecule. Table A6: BCP and "two-molecule" criteria applied to the weakest H-bonds of each type: characteristics of (3, −1) BCPs (LBR is the distance from BCP to the nearest (3, +1) RCP), changes in atomic charges (Δq), polarization moment (ΔM), volume (Δv), energy (ΔE), and radii (H and B denote hydrogen and acceptor atoms involved in H-bonding, respectively). The structurally close conformers were taken as reference molecules in each case. All units are atomic units. The integration over atomic basins was performed in natural coordinates. This material is available free of charge via Internet at http://pubs.acs.org

References and Notes

(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, **1984**.

(2) Dickerson, R. E.; Drew, H. R.; Conner, B. N.; Wing, R. M.; Fratini, A. V.; Kopka, M. L. *Science* **1982**, *216*, 475.

(3) Jones, S.; van Heyningen, P.; Berman, H. M.; Thornton, J. M. *J. Mol. Biol*. **1999**, *287*, 877.

(4) Skoda, J. Mechanism of action and application of azapyrimidines. In *Progress in Nucleic Acid Research*; Davidson, J. N., Cohn, W. E., Eds.; Academic Press: New York, London, 1963; p 197.

(5) Petrusha, N. A. *Farmakol. toksikol.* (Russian), **1987**, *2*, 75.

(6) Bektemirov, T. A.; Linitskaya, G. L.; Chernetsky, V. P.; Galegov, G. A. *Voprosy Med. Khim.* (Russian) **1974**, *20*, 50.

(7) Alexeeva, I. V.; Dyachenko, N. S.; Nosach, N. L.; Zhovnovataya, V.; Rybalko, S.; Lozitskaya, R.; Fedchuk, A.; Lozitsky, V.; Gridina, T.; Shalamay, A.; Palchikovskaja, L.; Povnitsa, O. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1147.

(8) Alexeeva, I. V. Private communication.

(9) Alexeeva, I. V.; Palchikovskaya, L. I.; Shalamay, A. S.; Nosach, N. L.; Zhovnovataya, V. L.; Povnitsa, O.; Dyachenko, N. S. *Acta Biochim. Pol.* **2000**, *47*, 95.

(10) Alexeeva, I. V.; Shalamay, A. S.; Palchykivs'ka, L. I.; Tarnavskiy, S. S.; Nosach, L. N.; Zhovnovataya, V. L.; Dyachenko, N. S. *Biopolim.Kletka* (Ukrainian), **1997**, *13*, 285.

(11) Saran, A. *Int. J. Quant. Chem*. **1981**, *20* (2), 439.

(12) Singh, P.; Hodgson, D. J. *J. Am. Chem. Soc*. **1974**, *96*, 1239.

(13) Samijlenko, S. P.; Alexeeva, I. V.; Palchykivs'ka, L. H.; Kondratyuk, I. V.; Stepanyugin, A. V.; Shalamay, A. S.; Hovorun, D. M. *Spectrochim. Acta A* **1999**, *55*, 1133.

(14) Samijlenko, S. P.; Alexeeva, I. V.; Palchykivs'ka, L. H.; Kondratyuk, I. V.; Stepanyugin, A. V.; Shalamay, A. S.; Hovorun, D. M. *J. Mol. Struct*. **1999**, *484*, 31.

(15) Mishchuk, Ya. R.; Potyagaylo, A. L.; Hovorun, D. M. *J. Mol. Struct*. **2000**, *552*, 283.

(16) Platonov, M. O.; Hovorun, D. M.; Alexeeva, I. V.; Sudakov, O. O.; Boyko, Yu. V.; Palchykivs'ka, L. H. *Dopov. NAN Ukrainy* (Ukrainian), **2004**, *3*, 163.

(17) Mertz, E.; Krishtalik, L. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2081.

(18) Dewar, M. J. S.; Storch, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2225.

(19) Petrushka, J.; Sowers, L. C.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1559.

(20) Kool, E. T. *Annu. Rev. Biophys. Biomol. Struct*. **2001**, *30*, 1.

(21) Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.

(22) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1988**, *37*, 785.

(23) Elyashevich, M. A. *Atomic and Molecular Spectroscopy* (Russian); Veres, L. F., Zhabotinskiy, E. E., Eds.; Fizmatgiz: Moscow, 1962; p 637.

(24) von Schilling, H. *Statistische Physik in Beispielen*; VEB Fachbuchverlag: Leipzig, 1972.

(25) Shishkin, O. V.; Pelmenschikov, A.; Hovorun, D. M.; Leszczynski, J. *Chem. Phys*. **2000**, *260*, 317.

(26) Peng, C.; Schlegel, H. B. *Isr. J. Chem*. **1993**, *33*, 449.

(27) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian03*; Gaussian, Inc.: Pittsburgh, PA, 2003.

(28) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.

(29) Zhurakivsky, R. O.; Yurenko, Ye. P.; Hovorun, D. M. *Dopov. NAN Ukrainy* (Ukrainian) **2006**, *8*, 207.

(30) Bader, R. F. W. *Atoms in Molecules: A Quantum Theory*; Clarendon: Oxford, 1990.

(31) Koch, U.; Popelier, P. L. A. *J. Phys. Chem*. **1995**, *99*, 9747.

(32) Iogansen, A. V. *Hydrogen bond* (Russian); Nauka: Moskow, 1981; p 112.

(33) Hovorun, D. M.; Mishchuk, Ya. R. *Ukr. J. Phys.* (Ukrainian) **1997**, *42*, 933.

(34) Biegler-König, F. W.; Bader, R. F. W.; Tang, Y. H. *J. Comput. Chem.* **1982**, *3*, 317.

(35) Shishkin, O. V.; Pelmenschikov, A.; Hovorun, D. M.; Leszczynski, J. *J. Mol. Struct*. **2000**, *526*, 329.

(36) Hocquet, A. *Phys. Chem. Chem. Phys*. **2001**, *3*, 3192.

(37) Jardetzki, O.; Roberts, G. C. K. *NMR in Molecular Biology*; Academic Press: New York, 1981.
(38) Foloppe, N.; MacKerell, A. D., Jr. *J. Phys. Chem. B* **1998**, *102*, 6669.
(39) Foloppe, N.; MacKerell, A. D., Jr. *Biophys. J.* **1999**, *76*, 3206.
(40) Hocquet, A.; Leulliot, N.; Ghomi, M. J. *Phys. Chem. B* **2000**, *104*, 4560.
(41) Foloppe, N.; Nilsson, L.; MacKerell, A., Jr. *Biopolymers*, **2002**, *61*, 61.
(42) Brameld, K. A.; Goddard, W. A. *J. Am. Chem. Soc*. **1999**, *121*, 985.
(43) Foloppe, N.; Hartmann, B.; Nilsson, L.; MacKerell, A., Jr. *Biophys. J*. **2002**, *82*, 1554.
(44) Shishkin, O. V.; Gorb, L.; Zhikol, O. A.; Leszczynski, J. *J. Biomol. Struct. Dyn*. **2004**, *21* (4), 537.
(45) Shishkin, O. V.; Gorb, L.; Zhikol, O. A.; Leszczynski, J. *J. Biomol. Struct. Dyn*. **2004**, *22* (2), 227.
(46) Sutor, J. *Nature* **1962**, *195*, 68.
(47) Taylor, R.; Kennard, O. *J. Am. Chem. Soc*. **1982**, *104*, 5063.
(48) Wahl, M. C.; Sundaralingam, M. *Trends Biochem. Sci*. **1997**, *22*, 97.
(49) Berger, I.; Egli, M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12116.
(50) Berger, J.; Egli, M. *Chem.—Eur. J.* **1997**, *3*, 1400.
(51) Brandl, M.; Lindauer, K.; Meyer, M.; Sühnel, J. *Theor. Chem. Acc.* **1999**, *101*, 103.
(52) Matta, C. F.; Castillo, N.; Boyd, R. J. *J. Phys. Chem. B* **2006**, *110*, 563.
(53) Shishkin, O. V.; Palamarchuk, G. V.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. B* **2006**, *110*, 4413.
(54) Lavery, R.; Sklenar, H. *J. Biomol. Struct. Dyn*. **1989**, *6*, 655.
(55) Hartmann, B.; Lavery, R. *Q. Rev. Biophys*. **1996**, *29*, 309.
(56) Kool, E. T. *Annu. Rev. Biochem*. **2002**, *71*, 191.
(57) Temiakov, D.; Patlan, V.; Anikin, M.; McAllister, W. T.; Yokoyama, S.; Vassylyev, D. G. *Cell* **2004**, *116*, 381.
(58) Palchykivs'ka, L. H.; Platonov, M. O.; Alexeeva, I. V.; Shved, A. D. *Biopolym. Kletka* (Ukrainian), **2004**, *21*, 559.
(59) Leszczynski, J. *Int. J. Quant. Chem*. **1992**, *44*, 43.
(60) Šponer, J.; Hobza, P. *Int. J. Quant. Chem*. **1996**, *57*, 959.
(61) Podolyan, Ye.; Rubin, Yu. V.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 9964.
(62) Hovorun, D. M.; Gorb, L.; Leszczynski, J. *Int. J. Quant. Chem*. **1999**, *75*, 245.
(63) Hocquet, A.; Ghomi, M. *Phys. Chem. Chem. Phys*. **2000**, *2*, 5351.
(64) Louit, G.; Hocquet, A.; Ghomi, M. *Phys. Chem. Chem. Phys*. **2002**, *4*, 3843.
(65) Leulliot, N.; Ghomi, M.; Scalmani, G.; Berthier, G. *J. Phys. Chem. A* **1999**, *103*, 8716.
(66) Gaigeot, M.-P.; Leulliot, N.; Ghomi, M.; Jobic, H.; Coulombeau, C.; Bouloussa, O. *Chem. Phys*. **2000**, *261*, 217.
(67) http://srdata.nist.gov/cccbdb/vsf.asp.