THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Nucleic Acid Bases in Anionic 2′-Deoxyribonucleotides: A DFT/B3LYP Study of Structures, Relative Stability, and Proton Affinities

Gennady V. Palamarchuk,[†] Oleg V. Shishkin,*[,†,‡] Leonid Gorb,[§,∥] and Jerzy Leszczynski[∥]

[†]STC Institute for Single Crystals, National Academy of Sciences of Ukraine, 60 Lenina ave., Kharkiv 61001, Ukraine

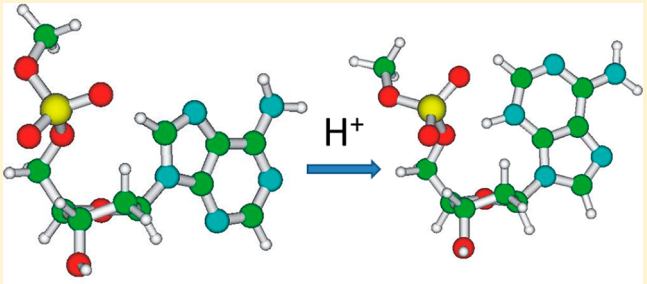
[‡]V.N.Karazin Kharkiv National University, 4 Svobody sq., Kharkiv 61077, Ukraine

[§]Laboratory of Computational Structural Biology, Department of Molecular Biophysics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Key State Laboratory in Molecular and Cell Biology, 150 Vul. Zabolotnogo, Kyiv 03143, Ukraine

[∥]Interdisciplinary Center for Nanotoxicity, Department of Chemistry and Biochemistry, Jackson State University, P.O. Box 17910, 1325 Lynch Street, Jackson, Mississippi 39217, United States

Supporting Information

ABSTRACT: Protonation of nucleobases in anions of canonical 2′-deoxyribonucleotides has been investigated by the DFT computational study at the B3LYP/aug-cc-pvdz level of theory. It is demonstrated that the protonation leads to a significant decrease of conformational space of purine nucleotides while almost all conformers found for non-protonated molecules correspond to minima of the potential energy surface for protonated mdTMP and mdCMP. However, in all nucleotides, only one conformer is populated. This applies to all tautomers of protonated molecules except the mdTMP and mdCMP with the proton attached to the carbonyl group where a minor population of second conformer is observed. Protonation of nucleobase leads to significant elongation of the N-glycosidic bond. These findings agree well with suggestions that protonation of nucleobase is a first step in cleavage of the glycosidic bond. The oxygen atoms of both carbonyl groups of thymine and the N3 atom of the pyrimidine ring of cytosine, guanine, and adenine represent the most preferable sites for protonation of anions of 2′-deoxyrobonucleotides. The highest proton affinity is observed for the base in mdGMP and the lowest for the thymine moiety in mdTMP. It should be noted that calculated values of the proton affinities in anionic nucleotides are significantly higher (by 2−3 eV) than for nucleosides and neutral nucleotides. This allows assuming that the proton affinity of the base in DNA macromolecule may be tuned by changing the extent of shielding or neutralization of negative charge of the phosphate group.



1. INTRODUCTION

Protonation, in some sense, is one of the simplest acid−base chemical reactions that are observed in both living systems and inorganic species. In the case of DNA, the protonation of nucleobases significantly influences the structure and function of this type of biopolymers. In particular, the protonated cytosine makes significant contribution to the stabilization of DNA triplexes.[1−3] The protonation can also cause mutations in the DNA via mispairing of complementary bases.[4−7] It was suggested[8] that the structures of so-called rare tautomers stabilized by transition metals could also be presented as complexes between protonated bases and metal. Protonation is considered as a catalytic factor for the hydrolytic cleavage of the N-glycosidic bond[9−12] high reactivity of the C8 atom in purine bases,[13−15] and it is closely related to the conformational dynamics of nucleotides.[16]

Being so important, acid−base equilibrium involving nucleic acid bases has been widely studied by experimental and theoretical methods both in gas and condensed phases. There are several fundamental questions for these studies to address, namely, comparative proton affinity of different nucleobases, preferable sites of protonation within each nucleic acid base, and changes of the molecular structure and conformational characteristics of DNA constituents induced by a protonation of nucleobases.

Experimental[17−24] and theoretical[24−28] studies of proton affinities (PAs) of nucleobases in the gas phase demonstrated clear differences in PA values of DNA bases. In particular, proton affinities of thymine were found to be considerably lower (8.9−9.2 eV) as compared to guanine, cytosine, and adenine (9.6−9.9 eV). It should also be noted that there was good agreement between experimental and theoretical data. Appearance of a sugar fragment in molecules of 2′-deoxyribonucleosides results in an increase of proton affinities

Received: November 17, 2012
Revised: February 20, 2013

   dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

of all nucleobases.[20−32] Nevertheless, the PA value for 2′-deoxythymidine (9.8 eV) remains lower than that for other nucleosides (10.1−10.3 eV). According to experimental data,[31,33] the presence of a neutral phosphate group in 2′-deoxyribonculeotides does not influence the proton affinities of the nucleobases. For instance, the value of PA for 2′-deoxythimidine 5′-monophosphate (dTMP) is 9.7−9.8 eV and it is 10.1−10.3 eV for 2′deoxycytidine (dCMP), 2′-deoxyguanosine (dGMP), and 2′-deoxyadenosine (dAMP) 5′-monophosphates. In general, these data agree well with results of semiempirical quantum-chemical calculations by the AM1 method.[31] Further increase of the proton affinities of the nucleobases was found in anions of 2′-deoxyribonucleotides.[34] As expected, appearance of negative charge due to deprotonation of the phosphate group results in an increase of the PA values by 2.7−2.9 eV. However, similar to results for isolated bases, the base in the dTMP anion has the lowest proton affinity. Besides that, the PA value for dCMP also becomes slightly lower than that for dAMP and dGMP ($\Delta PA \approx 0.3$ eV).

A structure of protonated DNA bases was studied in detail only for the case of isolated DNA bases. The most comprehensive investigation of protonation of nucleobases demonstrated[28] that the most preferable sites for protonation are the oxygen atom of the C4=O carbonyl group in thymine, the oxygen atom of the carbonyl group and the N3 atom in cytosine, and the N7 atom in guanine and the N1 atom in adenine. The structure of protonated 2′-deoxyribunucleotides containing a neutral phosphate group was investigated only by the semiempirical AM1 method.[31,33] These studies were focused on calculations of values of the proton affinities of nucleobases, without analysis of the conformation of protonated molecules. It was concluded that appearance of a phosphate group in 2′-deoxyribonucleotides results in a change of preferable protonation sites. In the case of neutral dCMP, dGMP, and dAMP molecules, the highest proton affinities were found for the N3 atom, while in dTMP the oxygen atom of the C4=O carbonyl group remains the most preferable site for protonation. In the case of anionic 2′-deoxyribonucleotides containing a deprotonated phosphate group, it was concluded on the basis of calculations by the AM1 method that the N7 atom has the highest PA value for dGMP.[34] Other anionic nucleotides have the same preferable sites of protonation as molecules with a neutral phosphate group. In the case of anionic dAMP, the highest stability of tautomer with the proton located at the N3 atom was also confirmed by calculations using DFT methods.[35] However, contrary to AM1 data, it was found that the N7 atom of adenine is the most preferable site for protonation of a molecule with a neutral phosphate group.

It should be noted that analysis of the molecular structure of protonated 2′-deoxyribonucleotides represents a considerably more complex task as compared to nucleobases. It is well-known that the nucleotides can adopt several stable conformations differing in geometrical parameters and energy.[36−38] Moreover, the presence of negative charge on the phosphate group significantly influences the relative stability and geometry of molecules. Protonation of such molecules may lead to significant changes in their conformations and energetics. For example, investigation of protonated dAMP indicated[35] that attachment of a proton to the N3 atom results in switching of the base orientation from anti to syn because of the formation of a strong intramolecular hydrogen bond. Also, a significant increase of strength of usually weak C−H···O hydrogen bonds was found in protonated dAMP due to electrostatic attraction between the negatively charged phosphate group and protonated adenine. Taking into account these data, it is possible to assume that each tautomer of protonated nucleotide can exist in several stable conformations characterized by different energy. Therefore, comprehensive evaluation of proton affinity requires careful consideration of the population of conformers of non-protonated anionic 2′-deoxyribonucleotides and each tautomer of protonated molecules.

In this paper, we present results of the first comprehensive study of the molecular structure of protonated anions of methyl ethers of canonical 2′-deoxyribonucleotides and proton affinities of nucleobases in these molecules calculated on the basis of the analysis of the population of the stable conformers. Taking into account that in real DNA macromolecules nucleotides exist in anionic form, in our opinion, it is more important to consider protonation of anions instead of neutral molecules. Presence of the $POCH_3$ fragment in our models, instead of the POH, allows avoiding the formation of artificial intramolecular hydrogen bonds which are absent in DNA.[39]

2. METHOD OF CALCULATION

The molecular structures of methyl ethers of 2′-deoxyribonucleotides, namely, thymidine-5′-phosphate (mdTMP), 2′-deoxycytidine-5′-phosphate (mdCMP), 2-deoxyadenosine-5′-phosphate (mdAMP), and 2′-deoxyguanosine-5′-phosphate (mdGMP), and their protonated forms were optimized using the density functional theory with the Becke's three-parameter exchange functional, the gradient-corrected functional of Lee, Yang, and Parr,[40−44] and the standard aug-cc-pvdz basis set. Geometries of protonated nucleotides were obtained by addition of a proton to a suitable site of protonation (the O and N atoms within a nucleobase) for each optimized conformer of corresponding non-protonated molecule and optimization of all geometrical parameters without any constrains. Local minima were verified by establishing that the matrix of energy second derivatives (Hessian) has only positive eigenvalues. The energy of zero point vibrations was calculated at the same level of theory (B3LYP/aug-cc-pvdz) within the harmonic approximation. All calculations were performed using the Gaussian 03 program.[45]

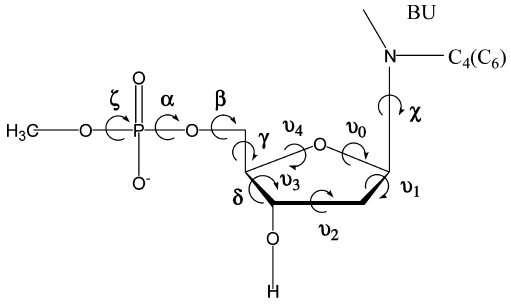
An analysis of the electron density distribution was carried out within Bader's "Atoms in Molecules" (AIM) approach[46] using the B3LYP/aug-cc-pvdz wave function. The existence of intramolecular hydrogen bonds was established on the basis of the presence of (3, −1) bond critical points (BCP) between two nonbonded atoms. The energy of intramolecular hydrogen bonds was estimated using Espinosa's equation[47] based on properties of the bond critical point. An AIM analysis has been performed using the AIM2000 program[48] with all default options.

The most important considered torsion angles in nucleotides were denoted in accordance with ref 49 (Scheme 1). The puckering of the furanose ring was described by pseudorotation angle (P) and the degree of puckering (ν_{max}). Intervals of the value of the P angle corresponding to the south (S) and north (N) regions are 144−180 and 0−36°, respectively. These regions correspond to the C2′-endo and C3′-endo deviation of corresponding carbon atom from the average plane of remaining atoms of the sugar ring.

Conformations of 2′-deoxyribonucleotides also may be classified according to the syn or anti orientation of a base with respect to the furanose ring. Therefore, all conformations

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

Scheme 1



of the 2′-deoxyribonucleotides may be designated by the sugar conformation and orientation of base (for example, the S/anti conformation means that the conformation of the furanose ring belongs to the south region of the pseudorotation angle and the base has the anti orientation with respect to the sugar). If the conformation of furanose ring is out of the south or north regions of the pesudorotation cycle, they may be denoted in terms of deviation (endo or exo) of a corresponding carbon atom (for example, C3′-exo/syn).

Proton affinity (PA) values have been calculated as was suggested earlier[28] using eq 1:

$$\mathrm{PA} = -[(E_{\mathrm{tot}}(\mathrm{mXMP{-}H}) + E_{\mathrm{corr}}(\mathrm{mXMP{-}H})) - (E_{\mathrm{tot}}(\mathrm{mXMP}) + E_{\mathrm{corr}}(\mathrm{mXMP}))] + 5/2RT \quad (1)$$

where E_{tot} is the total energy of protonated (mXMP−H) and non-protonated (mXMP) nucleotide obtained from DFT calculations, E_{corr} is the thermal correction to enthalpy, and the term of $5/2RT$ includes ΔnRT for acid−base reaction and translational energy of proton.

Taking into account that each tautomer exists in several conformations, the observed proton affinity represents an average value. Therefore, a proton affinity for every protonation site was calculated using eq 2:

$$\mathrm{PA} = -[\sum_{i=1}^{M} (E_{\mathrm{tot}}(\mathrm{mXMP{-}H})_i + E_{\mathrm{corr}}(\mathrm{mXMP{-}H})_i)P_i - \sum_{j=1}^{N} (E_{\mathrm{tot}}(\mathrm{mXMP})_j + E_{\mathrm{corr}}(\mathrm{mXMP})_j)P_j] + 5/2RT \quad (2)$$

where M is the number of conformers of a protonated tautomer of nucleotide, N is the number of conformers of non-protonated nucleotide, and P is the population of each conformer calculated using a Boltzmann distribution function ($0 \le P_i \le 1$).

The proton affinity of nucleobases in 2′-deoxyribonucleotides was calculated in the same way taking into account the population of all tautomers of protonated nucleotide using the following equation:

$$\mathrm{PA} = -[\sum_{l=1}^{K} P_l(\sum_{i=1}^{M} (E_{\mathrm{tot}}(\mathrm{mXMP{-}H})_i + E_{\mathrm{corr}}(\mathrm{mXMP{-}H})_i)P_i - \sum_{j=1}^{N} (E_{\mathrm{tot}}(\mathrm{mXMP})_j + E_{\mathrm{corr}}(\mathrm{mXMP})_j)P_j)] + 5/2RT \quad (3)$$

where K is the number of tautomers for protonated nucleotide and P_l is the population of each tautomer.

The relative stability of tautomers was calculated as the difference in average Gibbs free energies as compared to the most stable tautomer. Average Gibbs free energies were calculated on the basis of the population of conformers of tautomers using eq 4:

$$G_{\mathrm{av}} = \sum_{i=1}^{M} G_i P_i \quad (4)$$

where G_i is the calculated Gibbs free energy of the ith conformer and P_i is the population of this conformer ($0 \le P_i \le 1$). All proton affinities and the relative stability of conformers and tautomers were calculated at 298 K.

3. RESULTS AND DISCUSSION

Structure of Protonated 2′-Deoxorybonucleotides and Relative Stability of Conformers. Before starting an analysis of the influence of a protonation on the molecular structure and relative stability of conformers of protonated molecules, it is necessary to consider conformational characteristics of non-protonated nucleotides. It is well-known[49] that, despite the high conformational flexibility of nucleotides, they adopt only four conformational states, being incorporated into DNA macromolecules. These states are characterized by the conformation of a furanose ring belonging to the south or north region of a pseudorotation cycle and by the syn or anti orientation of base. Therefore, only these conformations are considered usually for 2′-deoxyribonucletides as related to DNA. Earlier,[38,39] it was found that conformers with a syn orientation of base are absent in pyrimidine nucleotides as well as in dAMP. However, in the case of dCMP and dAMP, it was found that minima on the potential energy surface correspond to conformers with an almost orthogonal orientation of base with respect to the C1′−H bond and geometry of the furanose ring belonging to the north region of the pseudorotation cycle which may be designated as N/ort conformers. Thus, non-protonated 2′-deoxyribonucleotides contain two (mdTMP), three (mdCMP, mdAMP), or four (mdGMP) stable conformers.[39] On the basis of the relative Gibbs energy of these conformers, it is possible to conclude that for every nucleotide only one of them dominates in the gas phase state (Table 1). There is an S/anti conformer in mdTMP, mdCMP, and mdAMP and an S/syn conformer in mdGMP. The latter conformer is stabilized by a strong intramolecular N−H···O hydrogen bond between the amino and phosphate groups.[38,39]

Starting geometries of protonated nucleotides were generated from each stable conformer of non-protonated molecule by addition of a proton to the heteroatom. Therefore, it is possible to expect that every tautomer of protonated nucleotides will have two to four stable conformers. However, results of calculations demonstrated (Table 1) that protonation results in significant changes of the number of stable conformers. As follows from obtained results, up to six conformers are observed for tautomers of protonated pyrimidine nucleotides and only one to two conformers for tautomers of mdGMP and mdAMP.

The mdTMP anion has only two sites for protonation, namely, the oxygen atoms of carbonyl groups. However, each of these protonated tautomers possesses an additional degree of freedom caused by rotation around the C−O bond, leading to existence of conformers with cis and trans orientation of the

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

Table 1. Relative Gibbs Free Energy (ΔG) at 298 K and Population (P) of Conformers of Non-Protonated 2′-Deoxyribonucleotides and Their Protonated Tautomers[a]

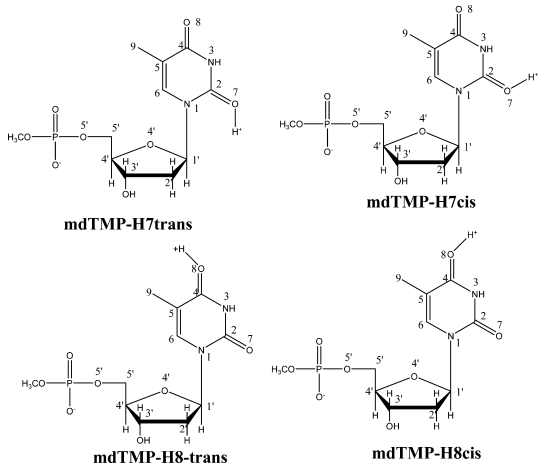
nucleotide	tautomer	conformer	ΔG (kcal/mol)	P (%)
mdTMP		S/anti	0	100
		N/anti	4.85	0
mdCMP		S/anti	0	99.96
		N/anti	3.22	0.04
		N/ort	8.22	0
mdAMP		S/anti	0	100
		N/anti	6.95	0
		N/ort	12.00	0
mdGMP		S/anti	8.11	0
		N/anti	16.28	0
		S/syn	0	100
		N/syn	4.18	0
mdTMP−H	H7cis	S/ort	11.67	0
	H7cis	N/anti	19.61	0
	H7trans	C4′-endo/syn	0	100
	H8cis	S/ort	5.65	0
	H8cis	S/anti	6.11	0
	H8trans	S/anti	0	86.2
	H8trans	N/anti	1.09	13.8
mdCMP−H	H3	S/anti	4.06	0.1
	H3	N/anti	5.35	0
	H3	C2′-exo/ort	0	99.9
	H7cis	S/anti	1.04	14.3
	H7cis	N/anti	3.55	0.2
	H7cis	C2′-exo/ort	2.06	2.6
	H7trans	N/anti	10.36	0.00
	H7trans	C4′-endo/syn	0	82.9
	H7trans	C2′-exo/ort	9.37	0.00
mdGMP−H	H3	S/syn	0	100
	H7	C3′-exo/ort	0	97.5
	H7	S/syn	2.50	2.5
	H10	C3′-exo/ort	0	100
	H11	C3′-exo/ort	16.13	0
	H11	S/syn	0	100
mdAMP−H	H1	S/syn	9.24	0
	H1	S/ort	0.00	100
	H3	C3′-exo/ort	0.00	100
	H7	C3′-exo/ort	0.00	100
	H10	C3′-exo/ort	0.00	100

[a]Energies for non-protonated molecules are taken from ref 39.

hydrogen atom of the protonated carbonyl group with respect to the N3 atom (Scheme 2).

Results of calculations reveal that the most stable conformers of both tautomers of mdTMP (mdTMP−H7 and mTMP−H8 in Scheme 2) have the hydrogen atom of the protonated carbonyl group being oriented out of the N3 atom of the pyrimidine ring (Table 1). However, the geometry of molecules in these conformers differs significantly. The furanose ring adopts only slightly different conformations: south for mdTMP−H8trans and C4′-endo for mdTMP−H7trans. The C4′-endo conformation is close to the south region of the pseudorotation cycle. The thymine moiety has an opposite orientation: syn in mdTMP−H7trans and anti in mdTMP−H8trans. In both cases, the orientation of the base is stabilized by intramolecular hydrogen bonds (Table 2), namely, strong O−H···O in the C4′-endo/syn conformer of mdTMP−H7trans

Scheme 2. Tautomers of Protonated mdTMP



and considerably weaker C−H···O bonds in the S/anti conformer of TMP−H8trans. It should be noted that only the last type of the hydrogen bonds is observed in all other conformers of mdTMP forms (see Table S1, Supporting Information). Some of such hydrogen bonds are quite strong because of opposite charge assistance, as was mentioned earlier.[35] Nevertheless, the influence of relatively strong C−H···O interactions on the relative energy of conformers is considerably smaller as compared to the strong conventional O−H···O hydrogen bond. Thus, it is possible to conclude that the mdTMP−H7 tautomer exists exclusively as the C4′-endo/syn conformer, while the conformational state of the mdTMP−H8 tautomer may be described as S/anti with a minor supplement of N/anti conformer (Table 1).

In the case of mdCMP, it is possible to suggest existence of three tautomers with a protonated ring nitrogen atom, carbonyl and amino group (Scheme 3). However, results of calculations demonstrated that the mdCMP−H8 tautomer does not correspond to a minimum on the potential energy surface because of proton transfer from the protonated amino group to the phosphate group during optimization of geometry. Therefore, only tautomers with a protonated ring nitrogen and carbonyl group should be considered.

Taking into account two possible orientations of the OH bond of a protonated carbonyl group, it is possible to conclude that tautomers of protonated mdCMP have three stable conformers similar to a non-protonated molecule (Table 1, Scheme 3). Only in the case of the mCMP−H7trans tautomer, protonation leads to disappearance of the S/anti conformer because of the transition of the base from anti to syn orientation accompanied by deformation of the furanose ring (Table 1).

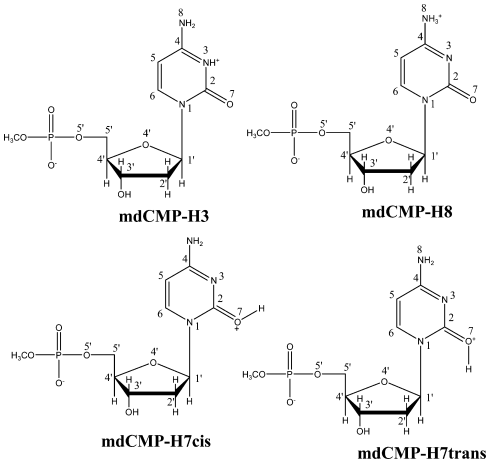
Results of calculations show that protonation of the mdCMP results in significant stabilization of conformers with orthogonal orientation of cytosine with respect to the C1′−H bond. This conformer possesses the lowest energy among the mdCMP−H3 tautomers, and it has only slightly higher energy for the mdCMP−H7 tautomer. In the last tautomer, the most stable conformer is the C4′-endo/syn with trans orientation of the hydrogen atom of a protonated carbonyl group with respect to the N3 atom of the pyrimidine ring (Table 1, Scheme 3). It should be noted the conformers with the lowest energy in both tautomers are stabilized by the N−H···O (mdCMP−H3) and O−H···O (mdCMP−H7) hydrogen bonds (Table 2). The N−

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

Table 2. Geometrical Parameters (Å, deg), Values of Electron Density (ρ, e/au^3), Laplacian of Electron Density (Lap, e/au^5), and Estimated Energy (E_{HB}, kcal/mol) of Intramolecular Hydrogen Bonds in the Most Stable Conformers of Tautomers of Protonated 2′-Deoxyribonucleotides

tautomer	conform.	D−H···A	H···A	D−H···A	ρ	Lap	E_{HB}
mdTMP−H7trans	C4′-endo/syn	O7−H···O4′	1.61	148.2	0.0579	0.1762	17.5
		C2′−H···O−P	2.13	137.3	0.0208	0.0591	4.6
mdTMP−H8trans	S/anti	C3′−H···O(1)−P	2.29	126.5	0.0147	0.0464	3.3
		C9−H···O(2)−P	2.16	140.8	0.0181	0.0541	4.0
		C6−H···O5′	1.93	167.6	0.0284	0.0915	6.4
mdCMP−H3	C2′-exo/ort	C2′−H···O7	2.25	120.9	0.0170	0.0556	3.9
		C3′−H···O(2)−P	2.14	155.4	0.0196	0.0555	4.3
		N8−H···O(1)−P	1.83	145.2	0.0357	0.1090	8.2
mdCMP−H7trans	C4′-endo/syn	O7−H···O4′	1.74	143.6	0.0401	0.1439	10.7
		C3′−H···O(1)−P	2.25	137.2	0.0164	0.0461	3.6
		C6−H···O(2)−P	1.90	145.9	0.0313	0.1003	7.1
mdGMP−H3	S/syn	N10−H···O−P	1.47	172.2	0.0808	0.0394	28.6
		C3′−H···O−P	2.26	145.8	0.0152	0.0431	3.3
		N1−H···O5′	1.73	173.8	0.0418	0.1331	10.2
mdGMP−H7	C3′-exo/ort	C8−H···O−P	1.65	171.1	0.0538	0.1583	14.5
		C3′−H···O−P	2.29	133.7	0.0147	0.0432	3.2
mdGMP−H10	C3′-exo/ort	C2′−H···O−P	2.36	161.6	0.0139	0.0363	2.9
		C8−H···O−P	1.90	173.8	0.0302	0.0950	6.6
		C3′−H···O−P	2.28	137.8	0.0156	0.0432	3.4
mdGMP−H11	S/syn	C3′−H···O(1)−P	2.21	132.3	0.0173	0.0520	3.9
		N10−H···O(2)−P	1.33	174.2	0.1160	0.0125	49.0
		C2′−H···N3	2.45	125.2	0.0119	0.0369	2.3
mdAMP−H1	S/ort	N10−H···O(1)−P	2.01	129.2	0.0236	0.0778	5.6
		C3′−H···O(2)−P	2.71	149.9	0.0062	0.0204	1.2
		C3′−H···N3	2.45	118.7	0.0120	0.0388	2.3
mdAMP−H3	C3′-exo/ort	N3−H···O5′	1.60	176.6	0.0574	0.1629	16.6
		C3′−H···O−P	2.23	140.7	0.0163	0.0471	3.6
		C2−H···O−P	2.34	117.7	0.0131	0.0442	2.9
mdAMP−H7	C3′-exo/ort	C8−H···O−P	1.64	168.9	0.0555	0.1607	15.2
		C3′−H···O−P	2.30	133.5	0.0144	0.0424	3.1
mdAMP−H10	C3′-exo/ort	C8−H···O−P	1.81	176.3	0.0366	0.1166	8.4
		C3′−H···O−P	2.29	136.4	0.0151	0.0428	3.3

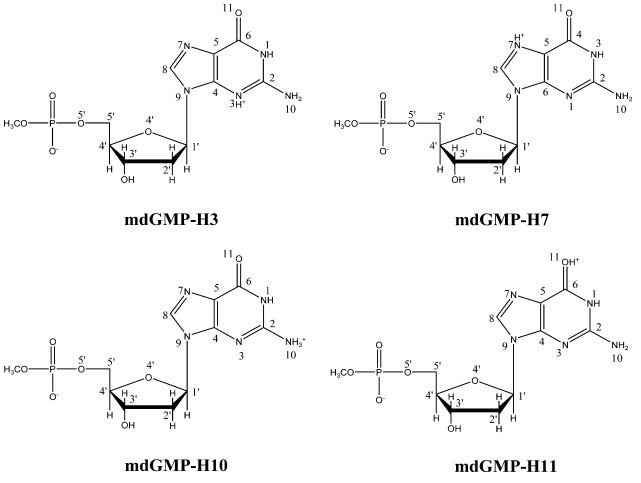
Scheme 3. Tautomers of Protonated mdCMP



H···O bonds also are found in the C2′-exo/ort conformers of the mdCMP−H7 tautomer. However, its energy is considerably smaller as compared to the C4′-endo/syn conformer of mCMP−H7trans (Table S2, Supporting Information). This allows suggesting that the strength of the N−H···O or O−H···O hydrogen bonds plays a very important role in stabilization of conformers of protonated mdCMP.

The most drastic changes of conformational space are observed for purine nucleotides. In the case of mdGMP, protonation leads to complete disappearance of conformers with anti orientation of the guanine moiety (Table 1, Scheme 4). Minima on the potential energy surface are found only for conformers with syn and orthogonal orientation of the base.

Scheme 4. Tautomers of Protonated mdGMP



dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

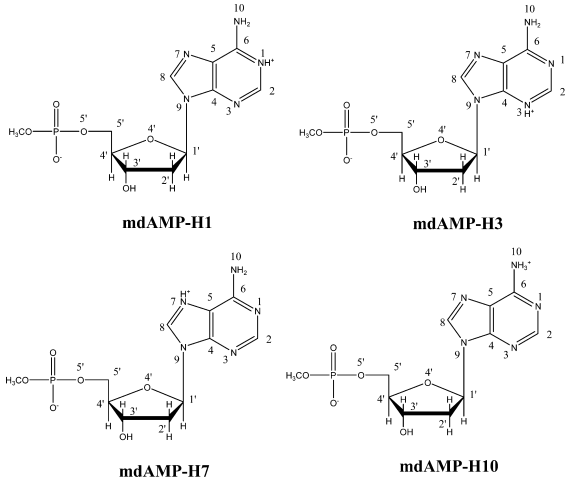
Besides that, all stable conformers of protonated mdGMP have an S or C3′-exo conformation of the furanose ring in contrast to a non-protonated nucleotide (Table 1). The number of stable conformers is limited to one (H3 and H10 tautomers) or two (H7 and H11 tautomers).

One may suppose that the significant decrease of conformational space of protonated mdGMP is caused by the formation of strong intramolecular hydrogen bonds (Table 2 and Table S3, Supporting Information). Especially strong hydrogen bonding is observed between the amino and phosphate group in mdGMP−H3 and mdGMP−H11 tautomers. Geometrical parameters and energy of the N−H···O bonds in these conformers (Table 2) allows suggesting almost free transition of the hydrogen atom between interacting heteroatoms. However, only one minimum on the potential energy surface corresponding to the location of the hydrogen atom at the nitrogen of the amino group was found.

The C3′-exo/ort conformers of the mdGMP−H7 and mdGMP−H10 tautomers are stabilized only by the C−H···O hydrogen bonds (Table 2). However, their strength is increased significantly by electrostatic interactions between the nucleobase and phosphate group possessing opposite charges. Earlier, it was demonstrated[16,35] that an opposite charge assistance in hydrogen bonding may lead to transformation of usually weak C−H···X hydrogen bonds into very strong bonds. Such a situation is observed for the C8−H···O−P hydrogen bond in the C3′-exo/ort conformer of the mdGMP−H7 tautomer (Table 2). Geometrical parameters and estimated energy of bonding indicate that this C−H···O bond is stronger than the quite strong conventional N1−H···O5′ hydrogen bond in the S/syn conformer of the mdGMP−H3.

An even more significant decrease of conformational space is found for protonated mdAMP (Table 1, Scheme 5). Only one stable conformer was located for all tautomers, except the mdAMP−H1. All the most stable conformers have the S or C3′-exo conformation of the furanose ring with orthogonal orientation of base with respect to the C1′−H bond (Table 1). They are stabilized by strong intramolecular N−H···O or C−H···O hydrogen bonds (Table 2 and Table S4, Supporting Information), as was described earlier.[35] The second conformer of the mdAMP−H1 tautomer has a syn orientation of base with respect to sugar and considerably higher energy as compared with the S/ort conformer (Table 1).

Scheme 5. Tautomers of Protonated mdAMP



More detailed analysis of geometrical parameters of protonated nucleotides demonstrates that, besides the conformation of the molecules, the protonation also results in significant changes of bond lengths within the C4′−O4′−C1′−N fragment (Table S9, Supporting Information). In agreement with previous findings,[36−39] the length of the N-glycosidic bond C1′−N in non-protonated purine nucleotides is smaller than that for pyrimidine ones (average values are 1.464 and 1.482 Å, respectively). This is caused by the nature of the base.[50] The same situation is observed in protonated molecules. The average length of the C1′−N bond in the purine nucleotides (1.481 Å) remains shorter than that in the pyrimidine nucleotides (1.519 Å). However, in all cases, the protonation results in significant elongation of this bond, causing its weakening. This effect is more pronounced in the pyrimidine nucleotides than in the mdAMP and mdGMP (differences between average values of the C1′−N bond lengths are Δl = 0.037 Å for pyrimidine and Δl = 0.017 Å for purine nucleotides).

Recently,[51] it was concluded that the length of the C1′−O4′ bond is closely related to the length of the N-glycosidic bond. Elongation of the C1′−N bond results in shortening of the C1′−O4′ bond due to increase of the contribution of oxocarbenium ion in the structure of the furanose ring. However, analysis of geometrical parameters of protonated nucleotides revealed a much more complex situation. The correlation is observed between values of bond lengths within the N−C1′−O4−C4′ fragment (Figure 1A and B) only for the purine nucleotides. The elongation of the N-glycosidic bond results in shortening of the C1′−O4′ bond and elongation of the O4′−C4′ bond (Tables S5−S9, Supporting Information). No such correlations have been observed in the case of the pyrimidine nucleotides (Figure 1C and D). For example, the S/anti conformer of the mdTMP−H8trans has the shortest of all the C−O bonds in contrast to the purine nucleotides (Table S9, Supporting Information). Therefore, it is possible to presume that close relations between bond lengths within the N−C1′−O4′−C4′ fragment represent a specific feature of the purine nucleotides.

However, it should be noted that these correlations include not only protonated nucleotides but also non-protonated molecules (Table S9, Supporting Information). Therefore, these effects, probably, are caused by stereoelectronic interactions within the C−O−C−N fragment rather than a specific influence of positive charge of a nucleobase, as was suggested before.[51]

At the end of this section, we would like to mention the following. It is well-known that protonation is a first stage of hydrolytic cleavage of the N-glycosidic bond.[11,12,51] The transition state of this process is highly dissociative with substantial elongation of the C1′−N bond.[51−53] Therefore, increase of the N-glycosidic bond length due to protonation creates favorable conditions for disruption of this bond. It should be noted that this process has been investigated mainly for the purine nucleotides because it represents the first step in the base excision repair pathway.[54,55] However, results of the present calculations demonstrate that weakening of the N-glycosidic bond is more pronounced in the pyrimidine nucleotides than in the mdAMP and mdGMP. This means that cleavage of the N-glycosidic bond in the pyrimidine nucleotides should be even easier than in the purine ones, especially taking into account longer the C1′−N bond.

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

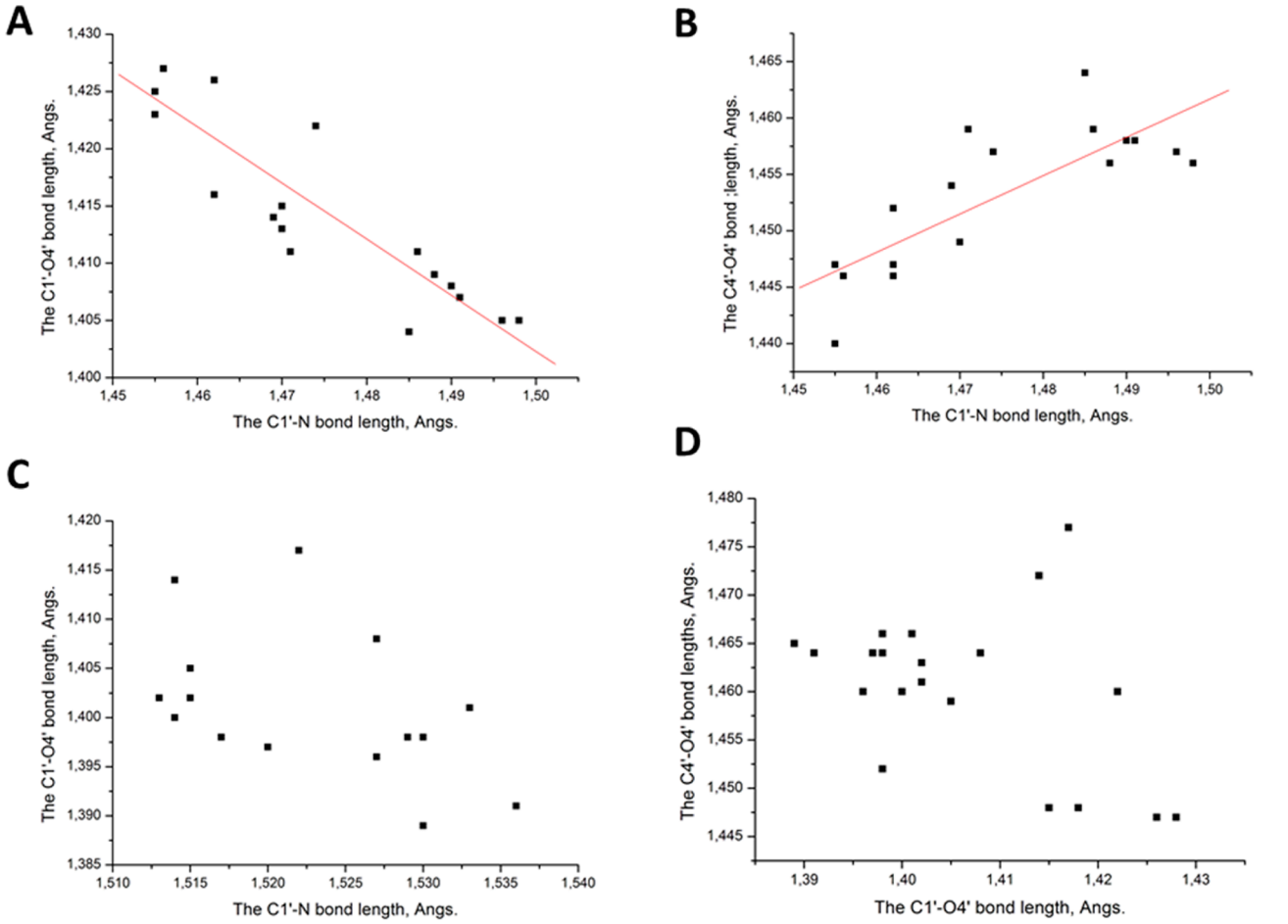


Figure 1. Relationship between lengths of bonds within the N−C1′−O4′−C4′ fragment of purine (A, correlation coefficient is 0.89; B, correlation coefficient is 0.79) and pyrimidine (C, D) nucleotides. All protonated and non-protonated molecules are included.

Relative Stability of Tautomers. Calculations of the relative stability of tautomers in molecules with multiple stable conformers represent a complex task because at room temperature each tautomer may exist as a mixture of several conformers. Therefore, accurate estimation of the relative energy of a tautomer requires taking into account the presence of conformational equilibrium for each tautomer. In other words, the energy of a tautomer should be calculated as the sum of energies of its conformers multiplied by their populations.

Results of calculations of the relative stability of tautomers in protonated 2′-deoxyribonucleotides demonstrate an existence of the tautomeric equilibrium only in protonated mdTMP molecule (Table 3). Other protonated nucleotides have only one stable tautomer. This especially concerns the purine nucleotides where differences in energy between the most stable H3 tautomer and other tautomers are higher than 10 kcal/mol (Table 3). The analogous tautomer is also the most stable for the mdCMP. However, in the case of this nucleotide, the difference in energy between the H3 and H7 tautomers is considerably smaller.

It should be noted that the most stable tautomers of all protonated nucleotides do not contain the strongest hydrogen bonds (Tables 2 and 3). For example, in the mdGMP, the strongest N−H···O bond is observed for the H11 tautomer (Table 2) which has significantly higher energy than the H3 containing a weaker N−H···O bond. This indicates that the strength of hydrogen bonds strongly influences the geometry and relative stability of conformers but provides only a minor contribution to the relative stability of tautomers.

Table 3. Relative Energy of Tautomers (Δ*E*, kcal/mol), Their Populations (*P*, %), and Proton Affinities (PA, kcal/mol) for 2′-Dexyrobonucleotides and Relative Energy of Tautomers for Isolated Bases Calculated by the MP4(SDTQ)/6-31+G(d,p)//MP2/6-31+G(d,p) Method Taken from ref 28

		nucleotides			bases
molecule	tautomer	Δ*E*	P	PA	Δ*E*
mdTMP	H7	0	65.92	12.07	6.7
	H8	0.39	34.07	12.06	0
mdCMP	H3	0	99.98	13.33	0.1
	H7	5.0	0.02	12.77	0
mdGMP	H3	0	100.00	13.59	15.4
	H7	12.93	0.00	13.19	0
	H10	74.83	0.00	10.51	37.4
	H11	19.03	0.00	12.58	6.2
mdAMP	H1	14.84	0.00	13.18	0
	H3	0	100.00	13.30	2.0
	H7	11.14	0.00	12.82	8.1
	H10	31.96	0.00	11.29	17.5

Comparison of the relative stability of tautomers in protonated nucleotides and nucleobases (Table 3) demonstrates drastic differences. Only in the mdCMP, the most stable

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

Table 4. Proton Affinities (eV) of Nucleic Acid Bases, Nucleosides, Neutral, and Anionic Nucleotides Obtained from Experimental and Theoretical Studies

	thymine	cytosine	guanine	adenine
	Nucleobases			
experiment, ref 20	9.09	9.79	9.86	9.72
experiment, ref 21	9.05	9.70	9.67	9.69
MP4(SDTQ)/6-31+G(d,p)//MP2/6-31+G(d,p), ref 28	8.94	9.91	9.86	9.75
	Nucleosides			
experiment, ref 20	9.75	10.11	10.16	10.13
	Neutral Nucleotides			
experiment, ref 31	9.72	10.27	10.28	10.29
	Anionic Nucleotides			
B3LYP/aug-cc-pvdz	12.07	13.33	13.59	13.30

H3 tautomer of nucleotide has almost the same relative stability in protonated cytosine. In other molecules, the most stable tautomers of protonated bases are significantly destabilized in protonated nucleotides (Table 3). However, they reveal the same pattern as experimental data and the data of accurate quantum-chemical calculations for isolated DNA bases. Moreover, the relative difference in proton affinity is considerably higher for nucleotides. This indicates that at least in some cases properties of isolated nucleobases cannot relate directly to properties of bases in DNA macromolecules.

Proton Affinities. Results of calculations demonstrate that the N3 atom of mdCMP, mdGMP, and mdAMP possesses the highest proton affinity, while the PA values for the oxygen atoms of the carbonyl groups of the mdTMP have almost equal PA values (Table 3). Thus, the most preferable protonation sites of anionic nucleotides are the same as for neutral nucleotides.[31,33] In contrast to previous conclusions based on AM1 data,[34] the N7 atom of the mdGMP has a significantly smaller PA value as compared to the N3 site (Table 3).

On the basis of proton affinities and populations of the individual tautomer of each protonated molecule, it is possible to calculate the PA for anionic nucleotides in whole. According to these values, the mdGMP possesses the highest proton affinity and the mdTMP has the lowest PA value (Table 4). Proton affinities of the mdCMP and mdGMP are almost the same. Comparison of the PA values of nucleic acid bases in different molecules (Table 4) indicates that the proton affinities depend on the presence of the substituent and its charge. Appearance of the sugar moiety in nucleosides or the sugar−phosphate substituent in neutral nucleotides results in an increase of the PA values for all bases. Further increase of the proton affinities is observed for anionic nucleotides (Table 4). Therefore, one may suggest that the variation of the degree of neutralization of negative charge of the phosphate group of DNA nucleotides may be used as a tool for tuning of the proton affinity of base.

CONCLUSIONS

Results of calculations of protonated anions of canonical 2′-deoxyribonucleotides by the B3LYP/aug-cc-pvdz approach demonstrated that the protonation leads to a significant decrease of the conformational space of purine nucleotides, while almost all conformers of non-protonated molecules are observed in protonated mdTMP and mdCMP. However, only one conformer is populated in all tautomers of protonated nucleotides, except the mdTMP and mdCMP with the proton attached to the carbonyl group where minor population of the second conformer is also observed. The most stable conformers of protonated molecules are stabilized by strong intramolecular hydrogen bonds between the base and phosphate group. Besides conventional N−H···O and O−H···O hydrogen bonds, an unusually strong C−H···O bond is found with geometrical parameters being close to conventional strong H bonds and which energy is higher than 10 kcal/mol according to estimation based on the AIM properties of the bond.

It should be noted that a significant decrease of the number of stable conformers and an appearance of unusually strong intramolecular hydrogen bonds may be explained by the presence of non-neutralized negative charge of a phosphate group. Earlier,[36,37] it was demonstrated that similar effects are observed in dianions of canonical 2′-deoxyribonucleotides. Taking into account that in DNA macromolecule nucleotides are surrounded by water, proteins, hydrated counterions, etc., it is possible to expect that the effect of protonation of bases should be less pronounced.

It is found that lengths of the N-glycosidic bond in the purine nucleotides correlate well with lengths of the C−O bonds of the furanose ring. Elongation of the C1′−N bond results in shortening of the C1′−O4′ bond and elongation of the O4′−C4′ bond. However, no such correlation is observed for the pyrimidine nucleotides. These data agree well with suggestions that protonation of the nucleobase is a first step in cleavage of the glycosidic bond.

The oxygen atoms of both carbonyl groups of thymine and the N3 atom of the pyrimidine ring of cytosine, guanine, and adenine represent the most preferable sites for protonation in anions of 2′-deoxyrobonucleotides. The highest proton affinity is observed for the base in mdGMP and the lowest for the thymine moiety in mdTMP. It should be noted that calculated values of the proton affinities in anionic nucleotides are significantly higher (by 2−3 eV) than for nucleosides and neutral nucleotides. This allows assuming that the proton affinity of the base in DNA macromolecule may be tuned by change of extent of shielding or neutralization of negative charge of the phosphate group. Partial desolvation of backbone and transfer counterions away from the nucleosides provides a local increase of the proton affinity of a specific nucleobase, making protonation easier and faster.

ASSOCIATED CONTENT

Supporting Information

Geometrical parameters, values of electron density, Laplacian of electron density, ellipticity, distance to nearest ring critical point for (3, −1) bond critical points and energy of intramolecular hydrogen bonds of hydrogen bonds in protonated nucleotides (Tables S1−S4), selected geometrical parameters of protonated

dx.doi.org/10.1021/jp311363c | J. Phys. Chem. B XXXX, XXX, XXX−XXX

nucleotides (Tables S5−S8), and selected bond lengths in conformers of non-protonated 2′-deoxyribonucleotides and the most stable conformers of their protonated tautomers (Table S9). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: shishkin@xray.isc.kharkov.com.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the National Science Foundation (NSF/CREST HRD-0833178) for financial support. This work was performed using computational facilities of joint computer cluster of SSI "Institute for Single Crystals" and Institute for Scintillation Materials of National Academy of Science of Ukraine incorporated into Ukrainian national Grid.

REFERENCES

(1) Frank-Kamenetskii, M. D.; Mirkin, S. *Annu. Rev. Biochem.* **1995**, *64*, 65−95.
(2) Sinden, R. R. *DNA Structure and Function*; Academic Press: San Diego, CA, 1994.
(3) Lee, H.-T.; Khutsishvili, I.; Marky, L. A. *J. Phys. Chem. B* **2010**, *114*, 541−548.
(4) Florian, J.; Leszczynski, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010−3017.
(5) Lowdin, P. O. *Adv. Quantum Chem.* **1965**, *2*, 213−354.
(6) Hunter, W. N.; Brown, T.; Anand, N. N.; Kennard, O. *Nature* **1986**, *320*, 552−555.
(7) Jissy, A. K.; Datta, A. *J. Phys. Chem. B* **2010**, *114*, 15311−15318.
(8) Sponer, J.; Sponer, J. E.; Gorb, L.; Leszczynski, J.; Lippert, B. *J. Phys. Chem. A* **1999**, *103*, 11406−11413.
(9) O'Brien, P. J.; Ellenberger, T. *Biochemistry* **2003**, *42*, 12418−12429.
(10) Rios-Font, R.; Rodriguez-Santiago, L.; Bertran, J.; Sodupe, M. *J. Phys. Chem. B* **2007**, *111*, 6071−6077.
(11) Chen, X.-Y.; Berti, P. J.; Schramm, V. L. *J. Am. Chem. Soc.* **2000**, *122*, 1609−1617.
(12) Francis, A. W.; Helquist, S. A.; Kool, E. T.; David, S. S. *J. Am. Chem. Soc.* **2003**, *125*, 16235−16242.
(13) Kennedy, S. A.; Novak, M.; Kolb, B. A. *J. Am. Chem. Soc.* **1997**, *119*, 7654−7664.
(14) McClelland, R. A.; Dicks, A. A.; Licence, V. E. *J. Am. Chem. Soc.* **1999**, *121*, 3303−3310.
(15) Qi, S.-F.; Wang, X.-N.; Yang, Z.-Z.; Xu, X.-H. *J. Phys. Chem. B* **2009**, *113* (16), 5645−5652.
(16) Shishkin, O. V.; Dopieralski, P.; Palamarchuk, G. V.; Latajka, Z. *Chem. Phys. Lett.* **2010**, *490*, 221−225.
(17) Gonella, N. C.; Nakanishi, H.; Holtwick, J. B.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 2050−2055.
(18) Brown, R. D.; Godfrey, P. D.; McNaughton, D.; Pierlot, P. *Chem. Phys. Lett.* **1989**, *156*, 61−63.
(19) Lias, S. G.; Liebmann, J. F.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*, 695−731.
(20) Greco, F.; Liguori, A.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1990**, *112*, 9092−9096.
(21) Meot-Ner (Mautner), M. *J. Am. Chem. Soc.* **1979**, *101*, 2396−2403.
(22) Kurinovich, M. A.; Lee, J. K. *J. Am. Chem. Soc.* **2000**, *122*, 6258−6262.
(23) Kurinovich, M. A.; Lee, J. K. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 985−995.
(24) Liu, M.; Li, T. F.; Amegayibor, S.; Cardoso, D. S.; Fu, Y.; Lee, J. K. *J. Org. Chem.* **2008**, *73*, 9283−9291.
(25) Bonaccorsi, R.; Pullman, A.; Scrocco, E.; Tomasi, J. *Theor. Chim. Acta* **1972**, *24*, 51−68.
(26) Russo, N.; Toscano, M.; Grand, A.; Jolibois, F. *J. Comput. Chem.* **1998**, *19*, 989−1000.
(27) Colominas, C.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1996**, *118*, 6811−6821.
(28) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 7346−7352.
(29) Liguori, A.; Napoli, A.; Sindona, G. *J. Mass Spectrom.* **2000**, *35*, 139−144.
(30) Di Donna, L.; Napoli, A.; Sindona, G.; Athanassopoulos, C. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1080−1086.
(31) Green-Church, K. B.; Limbach, P. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 24−32.
(32) Wilson, M. S.; McCloskey, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 3436−3444.
(33) Green-Church, K. B.; Freitas, M. A.; Marshall, A. G.; Limbach, P. A. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 268−277.
(34) Pan, S.; Verhoeven, K.; Lee, J. K. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1853−1865.
(35) Shishkin, O. V.; Palamarchuk, G. V.; Gorb, L.; Leszczynski, J. *Chem. Phys. Lett.* **2008**, *452*, 198−205.
(36) Shishkin, O. V.; Gorb, L.; Zhikol, O. A.; Leszczynski, J. *J. Biomol. Struct. Dyn.* **2004**, *21*, 537−553.
(37) Shishkin, O. V.; Gorb, L.; Zhikol, O. A.; Leszczynski, J. *J. Biomol. Struct. Dyn.* **2005**, *22*, 227−243.
(38) Shishkin, O. V.; Palamarchuk, G. V.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. B* **2006**, *110*, 4413−4422.
(39) Palamarchuk, G. V.; Shishkin, O. V.; Gorb, L.; Leszczynski, J. *J. Biomol. Struct. Dyn.* **2009**, *26*, 653−661.
(40) Hohenberg, P.; Kohn, W. *Phys. Rev. B.* **1964**, *136*, 864−871.
(41) Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.
(42) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098−3103.
(43) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(44) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789.
(45) 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.; et al. *Gaussian 03*, revision C.01; Gaussian, Inc.: Wallingford, CT, 2004.
(46) Bader, R. F. W. *Atoms in Molecules. A Quantum Theory*; Clarendon: Oxford, U.K., 1990.
(47) Espinosa, E.; Molins, E.; Lecomte, C. *Chem. Phys. Lett.* **1998**, *285*, 170−173.
(48) Biegler-Konig, F.; Schonbohm, J.; Bayles, D. *J. Comput. Chem.* **2001**, *22*, 545−559.
(49) Saenger, W. *Principles of Nucleic Acid Structures*; Springer: New York, 1988.
(50) Shishkin, O. V.; Pelmenschikov, A.; Hovorun, D. M.; Leszczynski, J. *J. Mol. Struct.* **2000**, *526*, 329−341.
(51) Ebrahimi, A.; Habibi-Khorassani, M.; Bazzi, A. *Phys. Chem. Chem. Phys.* **2011**, *13*, 3334−3338.
(52) Berti, P. J.; Tanaka, K. S. E. *Adv. Phys. Org. Chem.* **2002**, *37*, 239−314.
(53) Loverix, S.; Geerlings, P.; McNaughton, M.; Augustyns, K.; Vandemeulebroucke, A.; Steyaert, J.; Versees, W. *J. Biol. Chem.* **2005**, *280*, 14799−14802.
(54) Mol, C. D.; Parikh, S. S.; Putnam, C. D.; Lo, T. P.; Tainer, J. A. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 101−128.
(55) Cao, C.; Kwon, K.; Lin Jiang, Y.; Drohat, A. C.; Stivers, J. T. *J. Biol. Chem.* **2003**, *278*, 48012−48020.