Tautomeric Equilibrium of Uracil and Thymine in Model Protein−Nucleic Acid Contacts. Spectroscopic and Quantum Chemical Approach

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This work deals with tautomeric transformations of uracil (Ura) and thymine (Thy) in their model complexes with the deprotonated carboxylic group. Essential changes in the UV spectra of the bases upon their interaction with NaAc, vanishing signals of both imino protons in ^{1}H NMR spectra, and a perceptible decrease in intensity of both IR bands, related to the stretching vibrations ν(C═O) of the carbonyl groups, imply involvement of enolic tautomers. Results of quantum chemical calculations of the double complexes of the Ura(Thy) tautomers with CH_3COO^- at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory proved to be incompatible with the spectral features: despite the fact that the complexes of the enolic tautomers are much closer in energy to the diketo ones as compared to isolated tautomers, the energy gap between them is such that in tautomeric equilibrium dominate diketo forms. Calculations of triple complexes of the type **CH_3COO^-:Ura(Thy) tautomer:Na^+**, taking into account the effect of the Na^+ coordination with tautomers, show that three triple complexes formed by enolic tautomers appeared more stable than those formed by diketo ones. This makes the UV and ^{1}H NMR data understandable, but the high residual intensity of the ν(C═O) bands in the IR spectra remains unclear. At that ion, Na^+ itself was not able to disturb the tautomeric equilibrium in the coordination complexes of the type **Ura(Thy) tautomer:Na^+**. To evaluate the DMSO effect, the CPCM solvation model was applied to triple complexes of the Ura tautomers. It appeared that in the solution there is coexistence between the diketo and enolic tautomers in a ratio of 53%:47%. This makes possible reconciliation of our experimental data. The biological significance of high-energy tautomers of nucleotide bases is discussed.

I. Introduction

Uracil (Ura) and thymine (Thy), differing only by a methyl group in Thy instead of a hydrogen atom in Ura at the ring position 5, are pyrimidine nucleotide bases occurring as canonical ones against their complementary base adenine (Ade) in double helices of RNA and DNA, respectively. However, there are a few exceptions. In the case of phage TPBS, deoxyuridine (dU), a deoxynucleoside of Ura, is a normal constituent of DNA instead of thymidine (T).[1] Deoxyuridine was also identified among four canonical DNA nucleosides, isolated from the herring sperm hydrolysate.[2] Generally speaking, the presence of Ura in DNA is harmful for a cell. Deamination of cytosine (Cyt) to Ura, as well as 5-methylcytosine (m^5Cyt) to Thy, is the most often occurring form of the DNA endogenous damage with mutagenic consequences, if the G:T (G: nucleotide of guanine, Gua) resulting mispairing remains unrepaired.[3,4] Such point mutations are frequent in human cancer cells (ref 6 in work 4). The Ura incorporation (under some conditions) in DNA against Ade during replication is not directly mutagenic but cytotoxic.[5] *A propos*, in itself, Ura is believed to be a "fascinating" molecule due to not only its biological significance but also its numerous therapeutic effects and applications in industrial chemistry and agriculture.[6]

Tautomerism is one of the most intriguing features of the nucleic acid bases. Dozens of publications were devoted to this subject. Up to now, tautomerism of the bases was perceived only in the context of DNA spontaneous mutations (see works 7−9 and references therein). Tautomerization of nucleotide bases in vacuum and a water environment was a subject of numerous publications (e.g., see works 10−15). The problem of modeling xanthine tautomers by corresponding methyl derivatives has been considered in the work.[16] The N9 ↔ N7 tautomerism of purine bases (purine, adenine, hypoxanthine, and mercaptopurine) was investigated in methanol and N1N−dimethylformamide solutions by low-temperature ^{1}H and ^{13}C NMR spectroscopy.[17]

The authors came across the problem of the tautomerism of the nucleotide bases in the course of experimental investigations of elementary processes of protein−nucleic acid recognition *via* amino acid carboxylic groups in model systems.[18−22] Highly specific interactions of proteins with nucleic acids, named "recognition" due to their extreme selectivity, is a distinctive attribute of living organisms. Protein−nucleic acid recognition is the very essence of such significant biological processes as storage, replication, transcription, and translation of genetic information,[23] as well as repair of the DNA damage, both exo- and endogenic. According to von Hippel,[24] a search for a physicochemical basis of protein−nucleic acid recognition is a key line of investigations in molecular biophysics and molecular biology. Interactions between the biopolymers proved to depend on the size and relief of their contact surface and, to a large extent, on the affinity between them, determined mainly by hydrogen bonds.[25] At that, certain structural motives of the

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biopolymers, significant for recognition, were distinguished.[26] Processes of specific interactions between proteins and nucleic acids turned out to be accompanied by conformational changes in one or both partners,[27,28] which agrees with the concept of a mutual conformational adaptation of the biopolymers.[29] It is important to point out that the high selectivity of functional protein−nucleic acid complexes is often assured by a few specific point contacts,[30] i.e., between amino acid residues and nucleotide bases. Thus, for the complex of glutaminyl−tRNA with its corresponding ARSase, even a single point contact proved to be crucial.[31]

In some cases, consistent interpretation of UV, IR, and ^{1}H NMR spectroscopic data[18−22] appeared impossible without the suggestion of tautomeric transformations of the bases in their complexes of point protein−nucleic acid recognition in dimethylsulfoxide (DMSO) through the deprotonated carboxylic group of amino acids, modeled by sodium acetate (NaAc, CH_3COO^-:Na^+). Earlier quantum chemical calculations of the Ade,[32−34] isoguanine (isoGua),[35] purine (Pur),[36] and xanthine (Xan)[37] and complexes of their tautomers with the carboxylate ion of acetic acid (CH_3COO^-) evidenced transitions of the bases from the ground-state tautomeric form to high-energy ones: AdeN9H → AdeN7H, isoGuaN9H → isoGuaN7H, PurN9H → PurN7H, and XanN7H → XanN9H, which agrees with experimental data.

This work presents an interpretation of results of UV, ^{1}H NMR, and IR spectroscopic studies of the Ura and Thy complexes with sodium acetate (CH_3COO^-:Na^+) in DMSO, based on quantum chemical calculations of their tautomer complexes with carboxylate ion combined with sodium acetate.

II. Materials and Methods

Experimental Section. In this work, the following chemicals were used: Ura and Thy from "Calbiochem"; solvents DMSO and DMSO-d_6 from "Fluka" that were dried over 5 Å sieves from "Serva". Sodium acetate from "Reakhim" was shown to be a rather good model of the deprotonated carboxylic group of an amino acid in DMSO,[19] decomposing into CH_3COO^- and Na^+. Differential UV absorption spectra were registered on the MPS-2000 spectrophotometer from "Shimadzu" in the 1 mm quartz cells. ^{1}H NMR spectra were obtained by a spectrometer Gemini 200 Varian in the 5 mm tubes against tetramethylsilane (TMS) as an internal standard from "Aldrich". IR spectra were recorded by the Specord M 80 spectrometer in the 0.1 mm CaF_2 cells.

Quantum Chemical Calculations. We followed the strategy, which consists of choosing from all of the possible tautomers of the bases those ones that are capable of forming with the carboxylate ion complexes through two H-bonds, here referred to as double complexes (of the type **CH_3COO^-:Ura(Thy)** tautomer). To test the impact of Na^+ coordination on interactions of the base tautomers with CH_3COO^-, the triple complexes of the type **CH_3COO^-:Ura(Thy)tautomer:Na^+** were calculated, as well as coordinative complexes of the type **Ura(Thy)tautomer:Na^+**.

Molecular geometries and harmonic vibrational frequencies of Ura(Thy) and their complexes were obtained using the Becke3 (B3) exchange[38] and Lee, Yang, and Parr correlation[39] potentials. The orbital basis set 6-311++G(d,p) was used because it proved to show wholly satisfactory accuracy[40,41] and rather low consumption of computer time. Geometry optimizations were performed without any constraints.

Electron correlation was taken into account by using single point calculations, performed by the Møller−Plesset second order perturbation method (MP2) with a larger set of Gaussian basis functions 6-311++G(2df,pd). The relative Gibbs energy values were sums of electron energies calculated at the MP2/6-311++G(2df,pd) level and the zero-point energies, thermal corrections, and entropy contributions at the B3LYP/6-311++G(d,p) level of theory.

To interpret the experimental spectra, we calculated electronic energies of the triple complexes at the MP2/6-311++G(2df,pd) level of theory in dimethylsulfoxide (DMSO) applying the CPCM[42] model.

All of the quantum chemical calculations were performed using the Gaussian 03 program package[43] running on Windows machines.

The hydrogen bonds, stabilizing complexes, were tested on the basis of the "atoms in molecule" (AIM) approach.[44] The presence of a bond critical point (BCP, the so-called (3,−1) point) and a bond path between hydrogen donor and acceptor, as well as the positive value of the Laplacian at the BCP, were considered as necessary conditions for H-bond formation. It is especially important for nontraditional H-bonds such as C−H···O H-bonds. Detailed information on H-bonds will be the subject of a separate publication.

The population analysis of the tautomer double and triple complexes in vacuum and DMSO at room temperature (T = 298.15 K) was performed using the Boltzmann distribution formula:

$$n_i = \frac{e^{-(E_i-E_0)/kT}}{\sum_n e^{-(E_n-E_0)/kT}} \times 100 \qquad (1)$$

where n_i is the population (in %) of the ith complex, E_i the electron or Gibbs energy of the ith complex, E_0 the energy of the most stable complex.

III. Results and Discussion

The experimental and calculated data for Ura and Thy appeared to be rather close, due to similarity of their structures, and will be discussed together.

UV spectroscopic studies of Ura and Thy with sodium acetate, dissociating in DMSO to CH_3COO^- (carboxylate ion) and Na^+, resulted in intensive differential absorption of the complexes as compared to the unperturbed spectra of the bases (Figure 1), which indicates rather strong interactions.

Disappearance of both imino proton signals in the ^{1}H NMR spectra of Ura (N1H, 11.020 ppm; N3H, 10.830 p.p.m) and Thy (N1H, 10.998 ppm; N3H, 10.591 ppm) in the presence of the ligand with a deprotonated carboxylic group in DMSO (Figure 2) gives evidence of their participation in the complex formation.

The IR spectra (Figure 3) demonstrate a perceptible drop in intensities of both stretching vibration bands of the Ura carbonyl groups (1710 and 1683 cm^{-1}) and Thy (1712 and 1675 cm^{-1}) in the presence of sodium acetate (NaAc) in DMSO, which implies their involvement in interactions with ligands. *A propos*, the band at 1580 cm^{-1} present in the spectra is related to the antisymmetric stretching vibration of the deprotonated carboxylic group of the ligand.

In the framework of these data, we cannot propose any consistent schemes of the complexes formed, proceeding from the ground-state diketo tautomers of the bases. The vanishing imino proton signals in ^{1}H NMR spectra of the bases in the complexes points to the involvement of the enolic tautomeric forms. Thus, we have recoursed to quantum chemical calcula-

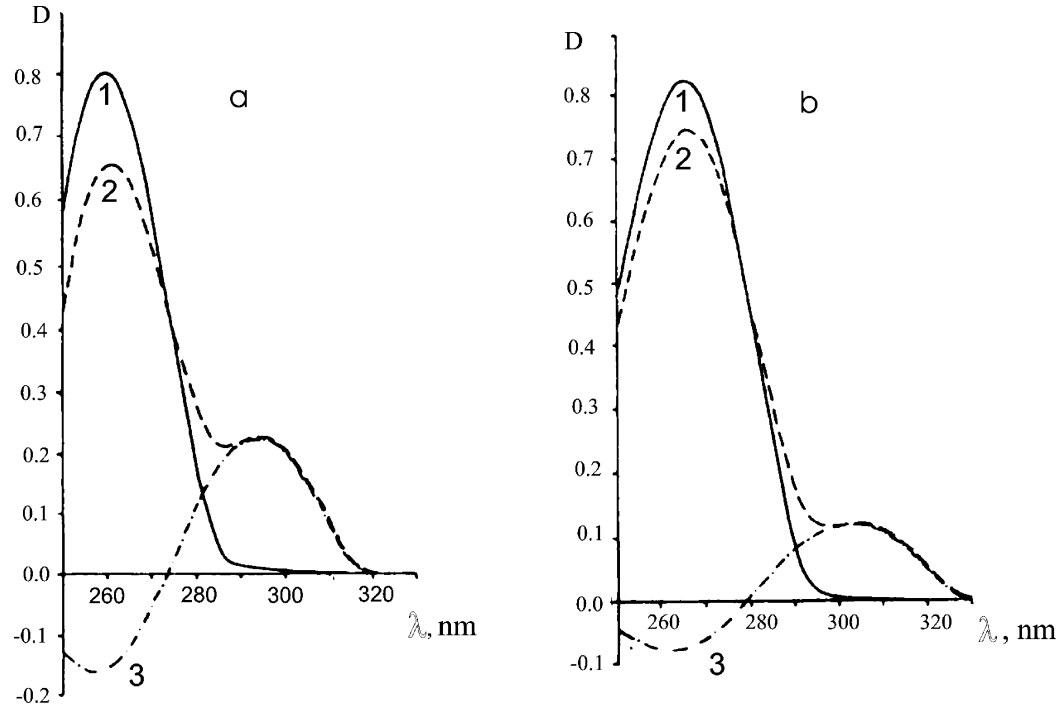

Figure 1. UV absorption spectra in anhydrous DMSO of Ura (a) and Thy (b): 1, a base; 2, its mixture with NaAc; 3, differential spectra.

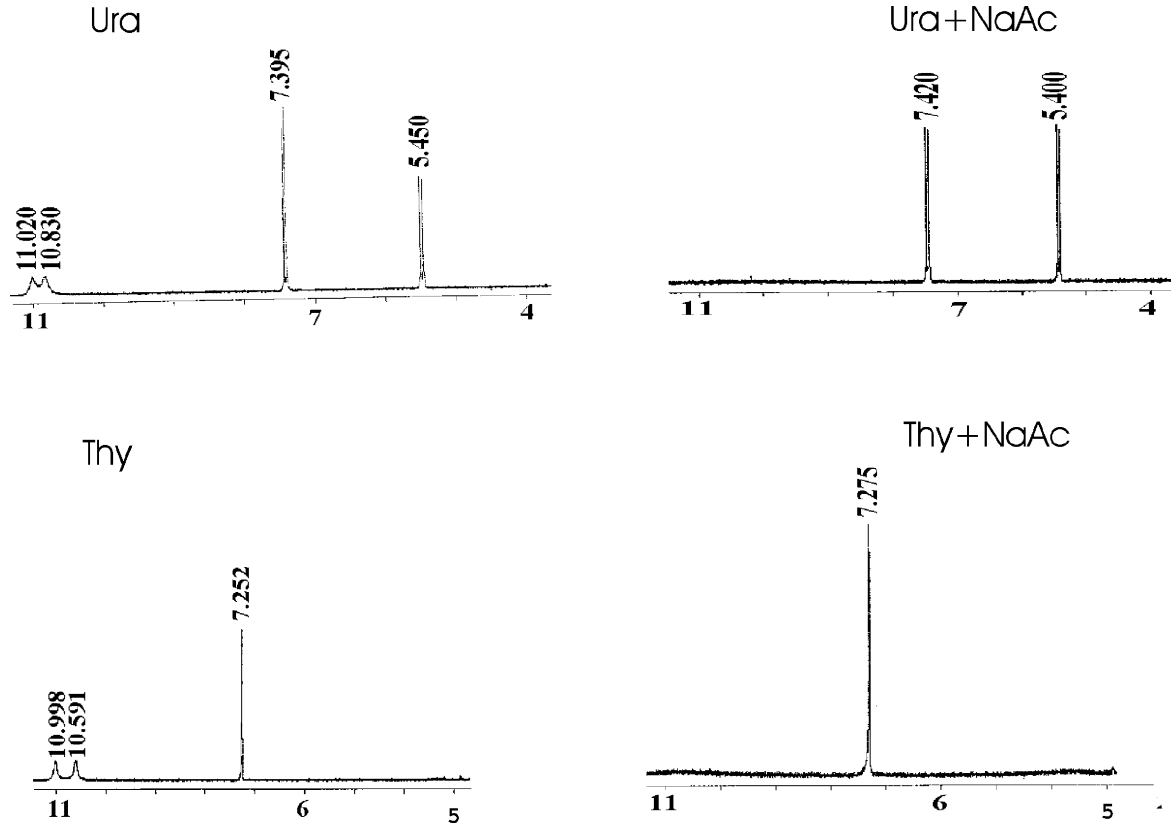

Figure 2. ^{1}H NMR spectra of Ura and Thy and their complexes with NaAc in anhydrous DMSO-d_6. The axis of abscissas presents chemical shifts in ppm.

tions of the energetic features of the Ura and Thy tautomer complexes with acetic acid carboxylate ion.

The calculated values of the relative energy for the Ura and Thy isolated tautomers (Figure 4, Table 1), capable of forming two H-bonds with the carboxylate ion of acetic acid, demonstrate an overwhelming advantage of the ground-state diketo tautomer over the enolic ones (their ΔG values are about 17−26 kcal/mol) and its implicit role in the tautomeric equilibrium of the bases. Noteworthy, discrepancies between the energy values of the corresponding tautomers of Ura and Thy do not exceed 1 kcal/mol.

The results of the quantum chemical calculations of the tautomer double complexes of the **CH_3COO^-:Ura (Thy) tautomer** type (Figure 5, Table 1), modeling the interaction

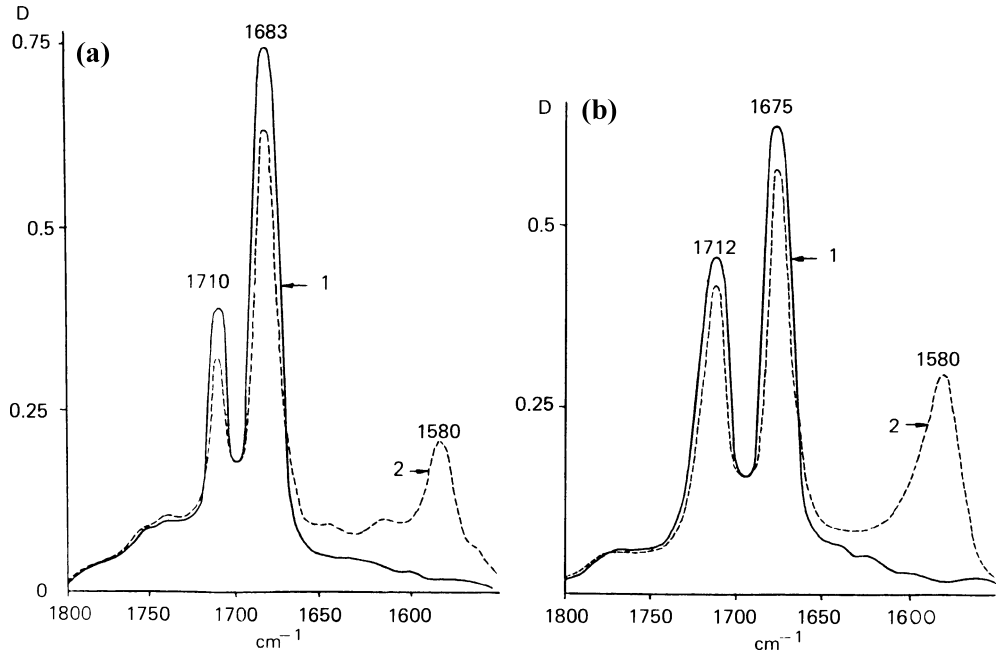

Figure 3. Infrared spectra of: 1 − Ura (a) and Thy (b) and 2 − their complexes with NaAc.

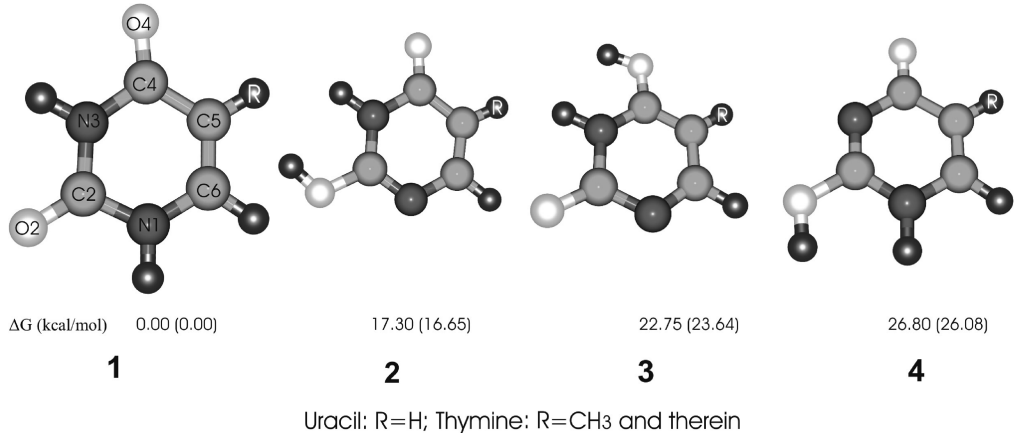

Figure 4. Ura (Thy) tautomers. ΔG: Gibbs energy values from Table 1.

TABLE 1: Calculated Energies of Uracil and Thymine and Their Complexes with CH_3COO^-; Boltzmann Populations of the Complexes at $T = 298.15$ K[a]

double complexes CH_3COO^-:Ura (Thy) tautomers				corresponding tautomers of Ura (Thy)		
numbers of complexes	ΔG_{MP2} (kcal/mol)	μ (D)	Boltzmann population (%)	number of tautomers as in Figure 4	ΔG_{MP2} (kcal/mol)	μ (D)
I	0.00	1.33	98.0	1	0.00	4.58
	(0.00)	(2.98)	(91.5)		(0.00)	(4.53)
II	2.34	0.65	1.9	2	17.30	2.31
	(1.41)	(2.24)	(8.5)		(16.65)	(1.78)
III	4.05	6.59	0.1	3	22.75	5.97
	(5.07)	(6.81)	(0.0)		(23.64)	(6.38)
IV	5.95	6.59	0.0	4	26.80	9.29
	(5.16)	(6.22)	(0.0)		(26.08)	(8.90

[a] ΔG_{MP2}: Gibbs free energy calculated at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. μ: dipole moments calculated at the B3LYP/6-311++G(d,p) level of theory.

between the deprotonated carboxylic group of the amino acids, show that the enolic tautomeric complexes appeared to be much closer in energy to the diketo ones in comparison with the isolated tautomers. In other words, the energy values of these double complexes evidence tendency of their converging: for the enolic tautomers, the ΔG values occupy the range from 2.34 (1.41) to 5.95 (5.16) kcal/mol. Noteworthy, the discrepancies in the energy between the complexes of the Ura and Thy corresponding tautomers are about 1 kcal/mol, just as in the case of the isolated tautomers.

Boltzmann population analysis shows that in the tautomeric equilibrium of the Ura (Thy) double complexes with the carboxylate ion the ground-state diketo forms strongly dominate over the enolic forms, covering about 98% (92%) of the overall tautomeric content (Table 1).

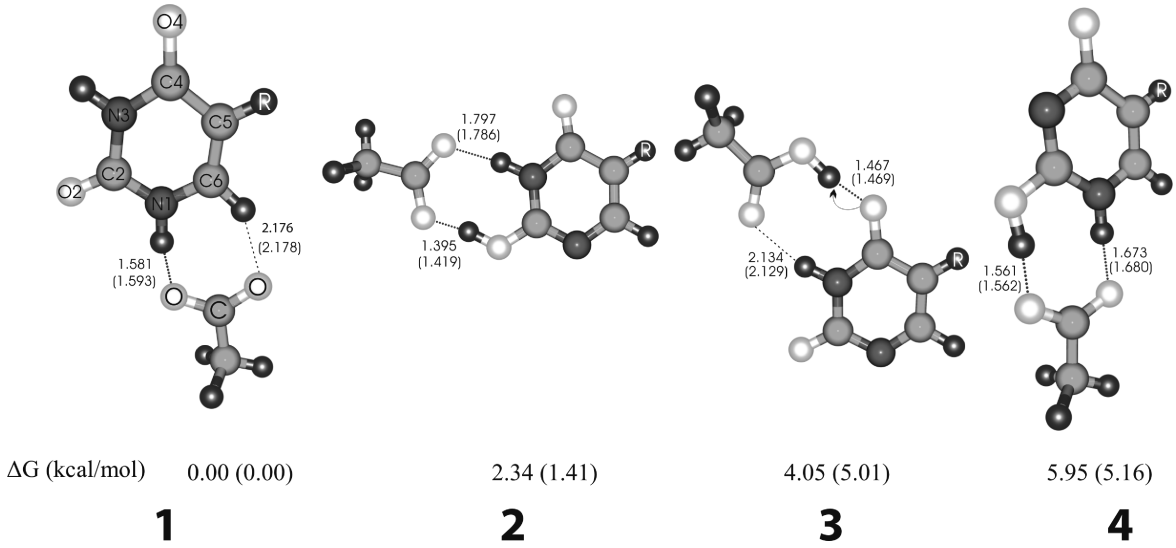

Figure 5. Ura (Thy) tautomer double complexes with CH_3COO^-. ΔG: Gibbs energy values of the complexes from Table 1. Dotted lines show H-bonds, with their lengths presented in Å; the arrow means proton transfer.

Obviously, such a result is not helpful in the interpretation of our experimental data. Therefore, an attempt was made to study the influence of the sodium ion on the interactions of Ura and Thy with carboxylate ion. For this purpose, triple complexes of the type **CH_3COO^-:Ura (Thy) tautomer:Na^+** were calculated, in which CH_3COO^- interacts with the tautomers through two H-bonds (as in the double complexes) and Na^+ coordinates with the oxygen and nitrogen atoms of the bases (Figure 6, Table 2). It turned out that the tautomer that is most unstable in the gas phase, the 2-enol-4-keto-1-imino tautomer **4**, in which the enolic group is formed at the expense of the 3-imino proton transfer to the 2-keto group, forms the most stable triple complex **I** ($\Delta G = 0.00$ kcal/mol). Next in stability order triple complexes **II** are formed by 4-enol-2-keto-3-imino tautomers **3**, resulting from transition of the 1-imino proton to the 4-keto group ($\Delta G = 1.16$ (1.21) kcal/mol). The triple complex **III** of the 2-enol-4-keto-3-imino tautomer **2**, issuing from transfer of the 1-iminoproton to the 2-keto group ($\Delta G = 2.93(2.55)$ kcal/mol), are closely related in energy to the triple complex **IV** of 2-enol-4-keto-1-imino tautomer **4** ($\Delta G = 2.96(2.03)$ kcal/mol), Na^+ coordinating with atoms O2 and N3, unlike the situation in the triple complex **I**, where Na^+ coordinates with atoms O4 and N3.

Hence, the tendency of energy converging, observed in the case of double complexes with CH_3COO^-, is retained also for all triple complexes of the enolic tautomers. However, the energy of the triple complex **V**, formed by the ground-state tautomer **1**, appeared surprisingly high ($\Delta G = 21.02(21.59)$ kcal/mol). It should be noticed that divergence of the energy values for all triple complexes of corresponding tautomers of Ura and Thy is in the limits of 1 kcal/mol.

To distinguish the separate effect of Na^+ on the tautomeric states of Ura and Thy in a vacuum, we have calculated coordination complexes of the base tautomers with Na^+ with coordination types exactly such as in the triple complexes (Figure 7, Table 3). The data obtained give strong evidence that in the gas phase the sodium ion itself is by no means able to upset the tautomeric equilibrium in the bases. In spite of the decrease in energy, the enolic tautomers, coordinating with Na^+, remain separated from the ground-state diketo tautomer, coordinating with Na^+, by a gap not less than 10 kcal/mol. This notwithstanding, in the triple complexes, the sodium ion urges the carboxylate ion to cardinal change in the tautomeric state of the bases.

It should be noted, that the **I**−**IV** double numbering of coordination type in Figure 7 and Table 3 refers to the fact that the Na^+ coordination complex, corresponding the triple complex **IV**, appears unstable and slides to the coordination complex, relative to the triple complex **I.**

Scrutinizing the structures of the calculated complexes of the three types (Figures 5, 6, and 7), some interesting features come out. All H-bonds in the triple complexes are elongated as compared to the corresponding ones in the double complexes, except the significantly shortened H-bond O···H−N3 of the O4-enolic tautomers. And, *vice versa*, in the triple complexes, all distances of Na^+ to the base atoms, which it coordinates, are markedly shorter than in the related coordination complexes. Furthermore, proton transfer from the tautomer to the carboxylate ion characterizes each of the triple complexes: in complexes **I**−**IV**, it is a proton of the enolic group, in complex **V**, the N1H imino proton.

According to Boltzmann population analysis, in a vacuum, the tautomeric equilibrium in Ura (Thy), interacting with CH_3COO^- and Na^+, is determined by two enolic tautomers: 2-enol-4-keto-1-imino (contribution 86.6 (85.1)%) and 4-enol-2-keto-3-imino (contribution 12.2 (11.0)%).

The obtained upset of the tautomeric state of the bases well agrees with the vanishing signal of imino protons in their ^1H NMR spectra in the presence of NaAc but does not completely reflect moderate effects in the UV and much less in the IR spectra.

Therefore, the DMSO solvent effect was evaluated, applying the CPCM solvation model to the triple complexes of the Ura tautomers (Table 2). Unexpectedly, it turned out that under "immersion" in DMSO (with dielectric constant $\varepsilon = 48$) the triple complex of the ground-state diketo tautomer **V** becomes the most stable again. However, the enolic ones only slightly exceed it in energy: their relative energy ΔE values are 0.15, 1.76, 1.89, and 2.18 kcal/mol for complexes **I**, **II**, **III**, and **IV**, respectively. According to Boltzmann population analysis, the most stable diketo tautomer contributes about 53% to the tautomeric equilibrium of the Ura complex with NaAc in DMSO, the next in the stability order the 2-enol-4-keto-1-imino tautomer is responsible for 41% (triple complex **I**) and over

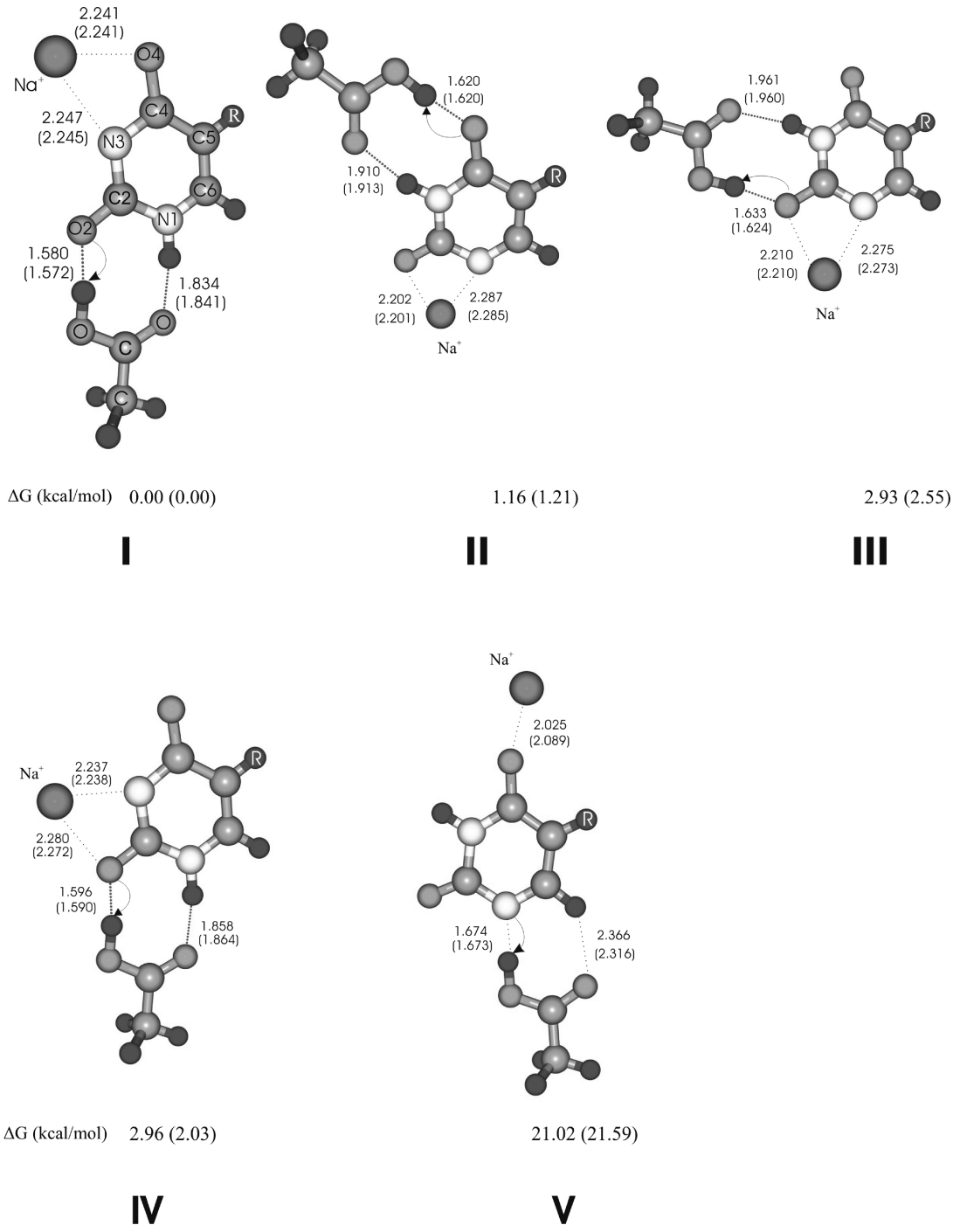

Figure 6. Triple complexes of Ura (Thy) tautomers with CH_3COO^- and Na^+. ΔG: Gibbs energy values of the triple complexes from Table 2. The bold dotted lines show H-bonds, with their lengths presented in Å; the arrows mean proton transfer.

1% (triple complex **IV**) of the equilibrium, i.e., in sum more than 42%. The remaining almost 5% quota of the tautomeric equilibrium is distributed between the Ura tautomers 4-enol-2-keto-3-imino (triple complex **II**) and 2-enol-4-keto-3-imino (triple complex **III**) in such a way: 2.7 and 1.3%, accordingly. Now, it means that in DMSO in the presence of CH_3COO^- and Na^+ there exists an equilibrium between the diketo tautomers and the whole body of enolic ones in almost equal amounts (53 and 47%, correspondingly).

Then, at last, we can reconcile our experimental data. Keeping in mind the statistical nature of the base tautomeric distribution in solution and the fact of proton transfer along the H-bonds in all of the triple complexes (Figure 6), imino protons can be regarded as very mobile. They transit from nitrogen to oxygen atoms under enolic tautomer formation and then are transferred to the carboxylate ion. As in the course of such transformations the bases pass a number of intermediate states, their imino proton signals cannot be detected in the ^{1}H NMR spectra. A much smaller decrease in the IR ν(C═O) band intensities, compared to the effects in the UV and ^{1}H NMR spectra, is determined by those carbonyl groups that became enolic groups. The two ν(C═O) bands of the ground-state triple complex and the remaining one from each enolic complex are responsible for the rather high residual IR absorption of the carbonyl groups.

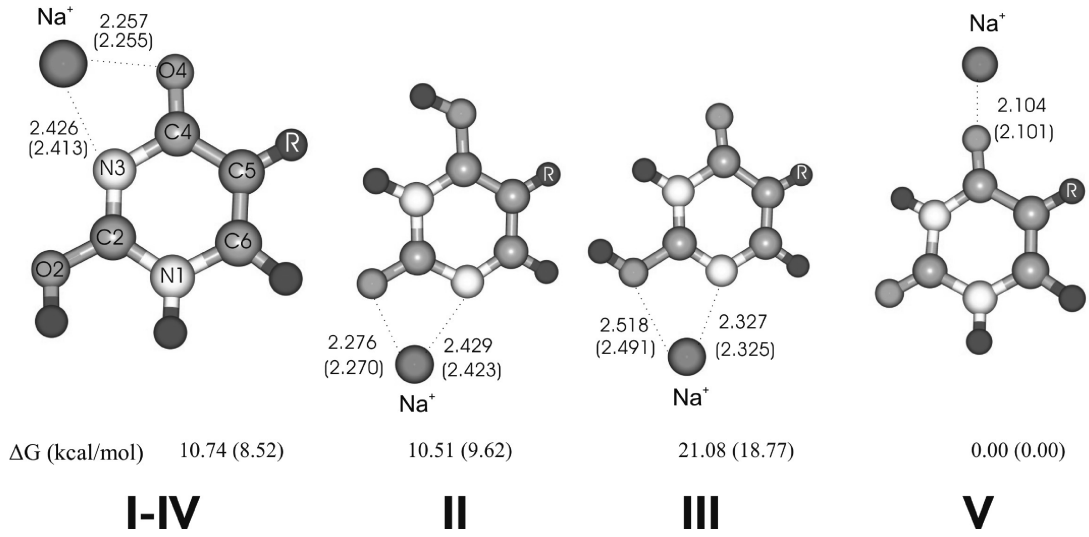

Figure 7. Coordination complexes of Ura (Thy) tautomers with Na^+. ΔG: Gibbs energy values from Table 3. The dotted lines show distances (in Å) of sodium ion from the base atoms, with which it coordinates; Roman numerals denote numbers of corresponding triple complexes.

TABLE 2: Calculated Energies of the Ura and Thy Tautomer Triple Complexes with CH_3COO^- and Na^+; Boltzmann Populations of the Complexes at Room Temperature (298.15 K)[a]

triple complexes CH_3COO^-:Ura (Thy):Na^+ in vacuum					triple complexes CH_3COO^-:Ura:Na^+ in DMSO ($\varepsilon = 48$)			
numbers of complexes	ΔG_{MP2} (kcal/mol)	ΔE_{MP2} (kcal/mol)	μ (D)	Boltzmann population (%)	numbers of complexes as in Figure 1	ΔE_{MP2} (kcal/mol)	μ (D)	Boltzmann population (%)
I	0.00 (0.00)	0.00 (0.00)	4.57 (4.84)	86.6 (85.0)	V	0.00	21.34	52.8
II	1.16 (1.21)	1.26 (1.39)	10.00 (9.61)	12.2 (11.0)	I	0.15	8.08	41.0
III	2.93 (2.55)	3.09 (3.17)	11.76 (11.09)	0.6 (1.2)	II	1.76	12.03	2.7
IV	2.96 (2.05)	2.98 (2.87)	6.30 (5.88)	0.6 (2.8)	III	1.89	15.49	2.2
V	21.02 (21.59)	23.35 (23.49)	18.80 (16.86)	0.0 (0.0)	IV	2.18	8.08	1.3

[a] ΔG_{MP2}: Gibbs free energy calculated at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. ΔE_{MP2}: electronic energy calculated at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. μ: dipole moments calculated at the B3LYP/6-311++G(d,p) level of theory.

TABLE 3: Calculated Energies of Uracil and Thymine Tautomer Coordination with Na^+ [a]

corresponding double complexes Ura (Thy):Na^+		
coordination as in Figure 6 (number of corresponding triple complexes)	ΔG_{MP2} (kcal/mol)	μ (D)
I−IV	10.74 (8.52)	3.80 (4.58)
II	10.51 (9.62)	4.42 (4.97)
III	20.19 (17.78)	12.19 (12.69)
V	0.00 (0.00)	11.54 (11.20)

[a] ΔG_{MP2}: Gibbs free energy calculated at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory. μ: dipole moments calculated at the B3LYP/6-311++G(d,p) level of theory.

IV. Concluding Remarks

Since it is known that the environment of active sites of most enzymes is much less polar (dielectric constant ε equals only a few units) than in DMSO solution ($\varepsilon = 48$) and much closer to vacuum ($\varepsilon = 1$), the conclusion could be made that *in vivo* diketo ↔ enolic equilibrium in Ura (Thy) may be even more shifted to the right in comparison with our model system. Thus, the results of this work together with our previous results[32−37] indicate a possible important role of high-energy tautomers of the nucleotide bases in the processes of protein−nucleic acid recognition.

The biological significance of the results obtained is perceived in the confirmation of the idea of importance of high-energy tautomers of nucleotide base so-called "hidden structures",[45] which cannot be isolated and registered experimentally but may be induced by interaction with ligands of peptide nature and metal ions. Thus, to gain a deeper insight into intimate mechanisms of biochemical reactions with nucleotide bases and nucleic acids, not only the energetically most favorable tautomers of the nucleotide bases should be taken into consideration, especially bearing in mind that a functioning cell is essentially a nonequilibrium system. Moreover, at physiological temperatures, contributions of the high-energy tautomers should increase as compared to room temperature.

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References and Notes

(1) Takahashi, I.; Marmur, J. *Nature* **1963**, *197*, 794.

(2) Dekker, C. A.; Todd, A. R. *Nature* **1950**, *166*, 557.

(3) Hagen, L.; Peñ-Diaz, J.; Kavli, B.; Otterlei, M.; Slupphaug, G.; Krokan, H. E. *Exp. Cell. Res.* **2006**, *312*, 2666.

(4) Liu, P.; Burdzy, A.; Sowers, L. C. *Chem. Res. Toxicol.* **2002**, *15*, 1001.

(5) Kavli, B.; Otterlei, M.; Slupphaug, G.; Krokan, H. E. *DNA Repair* **2007**, *6*, 505.

(6) De Pascuale, R. J. *Ind. Chem. Prod. Res. Dev.* **1978**, *17*, 278.

(7) Danilov, V. I.; Anisimov, V. N.; Kurita, N.; Hovorun, D. M. *Chem. Phys. Lett.* **2005**, *412*, 285.

(8) Jalbout, A. F.; Trzaskowski, B.; Xia, Y.; Li, Y.; Hu, X.; Li, H.; El-Nahas, A.; Adamowicz, L. *Chem. Phys.* **2007**, *332*, 152.

(9) Podolyan, Y.; Gorb, L.; Leszczynski, J. *Int. J. Mol. Sci* **2003**, *4*, 410.

(10) Hanus, M.; Ryjacek, F.; Kabelac, M.; Kubar, T.; Bogdan, T. V.; Trygubenko, S. A.; Hobza, P. *J. Am. Chem. Soc.* **2003**, *125*, 7678.

(11) Trygubenko, S. A.; Bogdan, T. V.; Sponer, J.; Rueda, M.; Orozko, M.; Luque, F. J.; Slaviek, P.; Hobza, P. *Phys. Chem. Chem. Phys.* **2002**, *4*, 4192.

(12) Kosenkov, D.; Kholod, Y.; Gorb, L.; Shishkin, O.; Hovorun, D. M.; Mons, M.; Leszczynski, J. *J. Phys. Chem. B* **2009**, *113*, 6140.

(13) Danilov, V. I.; van Mourik, T.; Kurita, N.; Wakabayashi, H.; Tsukamoto, T.; Hovorun, D. M. *J. Phys. Chem. A* **2009**, *113*, 2233.

(14) Gorb, L.; Leszczynski, J. *Int. J. Quantum Chem.* **1998**, *70*, 855.

(15) Gorb, L.; Podolyan, Y.; Leszczynski, J. *THEOCHEM* **1999**, *487*, 47.

(16) Platonov, M. O.; Samijlenko, S. P.; Sudakov, O. O.; Kondratyuk, I. V.; Hovorun, D. M. *Spectrochim. Acta, Part A* **2005**, *62*, 112.

(17) Bartl, T.; Zacharová, Z.; Sečkářová, P.; Kolehmainen, E.; Marek, R. *Eur. J. Org. Chem.* **2009**, *2009*, 1377–1383.

(18) Zheltovsky, N. V.; Samoilenko, S. A.; Kolomiets, I. N.; Kondratyuk, I. V.; Gubaidullin, M. I. *J. Mol. Struct.* **1989**, *214*, 15.

(19) Kolomiets, I. N.; Kondratyuk, I. V.; Stepanyugin, A. V.; Samoilenko, S. A.; Zheltovsky, N. V. *J. Mol. Struct.* **1991**, *250*, 1.

(20) Zheltovsky, N. V.; Samoilenko, S. A.; Kondratyuk, I. V.; Kolomiets, I. N.; Stepanyugin, A. V. *J. Mol. Struct.* **1995**, *344*, 54.

(21) Samijlenko, S. P.; Kondratyuk, I. V.; Kolomiets, I. M.; Stepanyugin, A. V. *Biopolymers Cell* **1998**, *14*, 47.

(22) Samijlenko, S. P.; Potyahaylo, A. L.; Stepanyugin, A. V.; Hovorun, D. M. *Ukr. Biochem. J.* **2003**, *75*, 42.

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

(24) von Hippel, P. H. *Science* **1994**, *263*, 769.

(25) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin Heidelberg, 1991.

(26) Blumenfeld, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Nucleic Acids. Structure, Properties, and Functions*; University Science Book: Sausalito, CA, 1999.

(27) Travers, A. A. *Curr. Opin. Struct. Biol.* **1992**, *2*, 71.

(28) Gorenstein, D. G. *Chem. Rev.* **1994**, *94*, 1315.

(29) Koshland, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98.

(30) Daune, M. *Molecular Biophysics. Structure in Motion*; Oxford University Press: London, 1999.

(31) Hou, Y. H.; Schimmel, P. R. *Nature* **1988**, *333*, 140.

(32) Samijlenko, S. P.; Bogdan, T. V.; Trygubenko, S. A.; Potyahaylo, A. L.; Hovorun, D. M. *Ukr. Biochem. J.* **2000**, *72*, 92.

(33) Trygubenko, S. A.; Bogdan, T. V.; Samijlenko, S. P.; Hovorun, D. M.; Kabelac, M.; Hobza, P. *Phys. Alive* **2002**, *10*, 39.

(34) Samijlenko, S. P.; Krechkivska, O. M.; Kosach, D. A.; Hovorun, D. M. *J. Mol. Struct.* **2004**, *708*, 97.

(35) Samijlenko, S. P.; Potyahaylo, A. L.; Stepanyugin, A. V.; Dzerzhynskyi, M. E.; Hovorun, D. M. *Ukr. Biochem. J.* **2001**, *73*, 147.

(36) Samijlenko, S. P.; Bogdan, T. V.; Trygubenko, S. A.; Hovorun, D. M. *Biopolymers Cell* **2001**, *17*, 540.

(37) Samijlenko, S. P.; Stepanyugin, A. V.; Krechkivska, O. M.; Potyahaylo, A. L.; Hovorun, D. M. *Dopovidi Nats. Akad. Nauk Ukr.* **2002**, *4*, 187.

(38) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(39) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(40) Wesolowski, S. S.; Leininger, M. L.; Pentchew, P. N.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **2001**, *123*, 4023.

(41) Rienstra-Kiracofe, J. C.; Barden, C. J.; Brown, S. T.; Schaefer, H. F., III. *J. Phys. Chem. A* **2001**, *105*, 524.

(42) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995.

(43) 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. *Gaussian 03*; Gaussian, Inc.: Pittsburgh, PA, 2003.

(44) Bader, R. F. W. *Atoms in Molecules: A Quantum Theory*; Clarendon: Oxford, U.K., 1995.

(45) Kondratyuk, I. V.; Samijlenko, S. P.; Kolomiets, I. M.; Hovorun, D. M. *J. Mol. Struct.* **2000**, *523*, 109.

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