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On the Unusual Stability of Valence Anions of Thymine Based on Very Rare Tautomers: A Computational Study

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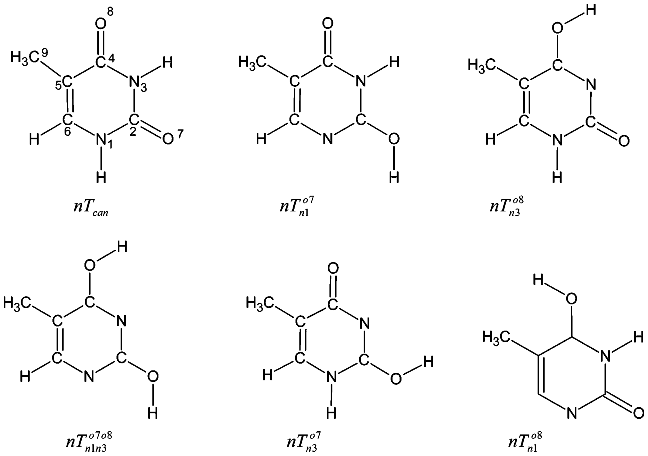
We characterized anionic states of thymine using various electronic structure methods, with the most accurate results obtained at the CCSD(T)/aug-cc-pVDZ level of theory followed by extrapolations to complete basis set limits. We found that the most stable anion in the gas phase is related to an imino-oxo tautomer, in which the N1H proton is transferred to the C5 atom. This valence anion, $aT^{\mathrm{c5}}_{\mathrm{n1}}$, is characterized by an electron vertical detachment energy (VDE) of 1251 meV and it is *adiabatically stable* with respect to the canonical neutral nT_{can} by 2.4 kcal/mol. It is also more stable than the dipole-bound ($aT^{\mathrm{dbs}}_{\mathrm{can}}$) and valence anion ($aT^{\mathrm{val}}_{\mathrm{can}}$) of the canonical tautomer. The VDE values for $aT^{\mathrm{dbs}}_{\mathrm{can}}$ and $aT^{\mathrm{val}}_{\mathrm{can}}$ are 55 and 457 meV, respectively. Another, anionic, low-lying imino-oxo tautomer with a VDE of 2458 meV has a proton transferred from N3H to C5 ($aT^{\mathrm{c5}}_{\mathrm{n3}}$). It is less stable than $aT^{\mathrm{val}}_{\mathrm{can}}$ by 3.3 kcal/mol. The mechanism of formation of anionic tautomers with the carbons C5 or C6 protonated may involve intermolecular proton transfer or dissociative electron attachment to the canonical neutral tautomer followed by a barrier-free attachment of a hydrogen atom to C5. The six-member ring structure of the anionic tautomers with carbon atoms protonated is unstable upon an excess electron detachment. Within the PCM hydration model, the low-lying valence anions become adiabatically bound with respect to the canonical neutral; $aT^{\mathrm{c6}}_{\mathrm{n3}}$ becomes the most stable, being followed by $aT^{\mathrm{c5}}_{\mathrm{n1}}$, $aT^{\mathrm{c5}}_{\mathrm{n3}}$, aT_{can}, and $aT^{\mathrm{c6}}_{\mathrm{n1}}$.

1. Introduction

The principal mutagenic species formed as a result of radiolysis of water are HO$^\bullet$ and H$^\bullet$ radicals as well as secondary low-energy (1−20 eV) electrons.[1] The experiments of Sanche et al.[2,3] indicate that low-energy electrons (at energies well below the ionization potential of nucleic bases: 7.5−10 eV) induce single- and double-strand breaks in DNA, and the damage quantum-yield versus incident electron energy[2] dependence suggests that the process proceeds via specific anionic states.

Proton transfer, a paramount importance reaction proceeding in biological systems, may stabilize valence anions of nucleic acid bases (NABs).[4] The results of our studies on anionic dimers, containing a pyrimidine base and a proton donor molecule,[5−14] confirm that valence-type anions of nucleic bases possess centers with high proton affinities, which enable barrier-free proton transfer to an anionic base. The proton transfer leads to the formation of radicals of hydrogenated pyrimidine bases, which are capable to initiate strand breaks, as demonstrated for gas-phase, water-free DNA.[15]

SCHEME 1: Chemical Structures of Thymine and Its Conventional Rare Tautomers.



For polar molecules, like nucleic acid bases, dipole-bound (DB) anions exist in the gas phase.[16] However, in solution, especially in polar environments, valence anions are more stable than the corresponding DB species.[17] The current view is that valence anions of nucleic acid bases are unbound or weakly bound when isolated,[18] but become dominant in polar solutions as indicated by experimental[4,19] and theoretical studies.[20,21]

Previous experimental[22,23] and computational[24−27] studies on *neutral* thymine were focused on both the canonical and rare tautomers (Scheme 1). The computational data suggest that the diketo (2,4-dioxo) form is by far the most stable. Nevertheless, the fluorescence excitation spectrum of jet-cooled thymine observed in the gas phase at 500 K by Tsuchiya et al.[22] indicates the coexistence of the diketo and one of the keto-enol tautomers (see Scheme 1) and the instability of the latter form with respect to the former was estimated to be at most 9.56 kcal/mol. A smaller instability of a keto-enol tautomer was predicted by

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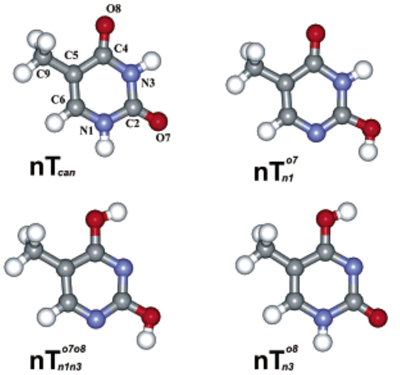

Figure 1. Tautomers of neutral thymine considered in this study at higher than B3LYP levels of theory.

Morsy et al.[23] They suggested that in water ca. 2% of thymine is present in the keto-enol form. This population, however, seems to be significantly overestimated because the very recent computational results of Piacenza and Grimme,[27] obtained at the very accurate QCISD(T)/TZV(2df,2dp) level, show that an energy difference between the isolated diketo form and the second most stable tautomer, 2-hydroxy-4-oxo (see Scheme 1 and Figure 1), is 9.9 kcal/mol, that is, very close to 9.56 kcal/mol found in the fluorescence excitation studies.[22] Moreover, only subtle changes in the relative stability of tautomeric forms were found in calculations that involved hydration effects.[26]

The canonical tautomer of thymine with a dipole moment of 4.13 D[28] supports a DB anion in the gas phase.[29,30] The DB anions of thymine were for the first time observed in the Rydberg electron-transfer spectroscopy (RET) experiments by Schermann et al.[31] and in the photoelectron spectroscopy (PES) experiments by Bowen et al.[32] Their estimates of the adiabatic electron affinity (AEA) of thymine were 68 ± 20 and 69 ± 7 meV, respectively.[33] Two years later, Schiedt et al.[34] published the results of another PES study on the anion of thymine, which yielded the AEA of 62 ± 8 meV.

Two years before the first RET and PES experiments, the DB anion of thymine was studied theoretically by Adamowicz et al., who calculated its AEA to be 88 meV.[29] In 1999, using the same level of theory but better basis sets, Adamowicz et al.[35] predicted the AEA of 32 meV. Finally, the very recent calculations by Jungwirth et al.,[36] carried out at the CCSD(T)//MP2 level with the augmented, correlation-consistent, polarized basis set of double-ζ quality[37] (AVDZ) supplemented with basis functions with small exponents, yielded an AEA of 51 meV.

Many experimental[31−34,38,39] and theoretical[19−20,40−46] studies addressed the stability of valence anion of T in the gas phase. A negative electron vertical attachment energy (VAE) of −290 meV[38] and −300 meV[39] was found. The only direct experimental result suggesting the existence of a marginally stable valence bound (VB) anion of thymine in the gas phase was reported by Desfrancois et al.[39] with the value of AEA very close to zero. The AEA of thymine of 120 ± 120 meV was also estimated by Schiedt et al.,[34] who took advantage of the almost linear relationship between the AEA and the number of water molecules forming thymine−$(H_2O)_n$ clusters.

Theoretical studies on valence anion of the canonical tautomer of T were primarily executed at the density functional level of theory (DFT).[19−20,40−46] The calculated values of AEA cover a broad range, from −640 to 340 meV, which demonstrates that the result is very sensitive to the selection of exchange-correlation functional and/or one electron basis set.

The most recent estimation of AEA for the canonical tautomer of T is only 18 meV and was obtained using highly correlated electronic structure methods (MP2, CCSD(T)) rather than DFT methods.[36] Interestingly, this value is smaller than the AEA for the DB anion based on the same canonical tautomer. This might explain why the DB but not the VB anion was observed in the PES experiments.[32,34] Jungwirth et al.[36] extrapolated the SCF and MP2 components of AEA to the complete basis set (CBS) limit and applied a CCSD(T) correction obtained with a smaller basis set.

In the present report, we have identified and characterized the most stable tautomers of anionic thymine. Our recent hypothesis about valence anionic states of NABs was presented in computational studies on 1-methylcytosine,[47] uracil,[48] and guanine.[49] In these studies, we suggested that the most stable valence anions of NABs might be related to the tautomers that are very unstable as neutral species. We dubbed them "very rare tautomers" to discriminate these tautomers from conventional "rare tautomers" (see Scheme 1). The "very rare tautomers" of thymine that we have in mind are those in which the N1 or N3 atoms are deprotonated and the C5 or C6 atoms are protonated. A hint that the tautomerization involving carbon atoms might be advantageous for anionic NABs came from our earlier studies on *intermolecular* proton transfer in complexes involving valence anions of NABs.[5−14] Recently, we have recognized that the C5 and C6 carbon sites are thermodynamically preferable for intermolecular proton transfer to the anion of a pyrimidinic NAB.[50,51] Indeed, the results of electron spin resonance investigation of γ-irradiated DNA indicate that the valence anion of thymine is protonated at the C6 position.[52] In refs 47−49, we demonstrated that not only *intermolecular* but also *intramolecular* proton transfer, i.e., tautomerization, might favor carbon sites.

The main finding from the current study, which is based on coupled-cluster calculations with single, double, and perturbative triple excitations[53] and AVDZ[37] basis sets, is that an imino-oxo tautomer with the N1H site deprotonated and the C5 atom protonated is the most stable anion of thymine in the gas phase. This valence anion is characterized by an electron vertical detachment energy (VDE) of 1251 meV and it is *adiabatically stable* with respect to the canonical neutral by 2.4 kcal/mol. Kinetic barriers for a unimolecular formation of the anionic tautomers with C5 or C6 protonated exceed 30 kcal/mol. Therefore, the mechanism of formation of anionic tautomers with carbon atoms protonated may involve intermolecular proton transfer or dissociative electron attachment to the canonical neutral tautomer followed by a barrier-free attachment of a hydrogen atom to a carbon atom. The six-member ring structure of very rare tautomers might be unstable upon excess electron detachment. Within the PCM hydration model,[54,55] the low-lying valence anions become adiabatically bound with respect to the canonical neutral and the very rare tautomers remain the most stable. The most stable anionic tautomer results from proton transfer from N3H to C6.

2. Methods

We use the following notation for the neutral and anionic tautomers. The neutral canonical tautomer is named "nT_{can}" and the related valence anion "$aT_{\mathrm{can}}^{\mathrm{val}}$". The anionic dipole-bound state of this tautomer is labeled "$aT_{\mathrm{can}}^{\mathrm{dbs}}$". The names of the remaining tautomers of thymine reflect these intramolecular proton transfers that lead from T_{can} to the tautomer at hand. For example, a neutral imino-hydroxy tautomer with the N1H site deprotonated and the O7 site protonated is labeled $nT_{\mathrm{n1}}^{\mathrm{o7}}$. In addition, prefixes "a" and "na" are used to distinguish properties of the anion and the neutral, respectively, determined at the minimum energy geometry of the anion.

Various tautomers of thymine, with an excess electron on a valence-type orbital, were initially considered at the density functional level of theory with a B3LYP exchange-correlation functional[56−58] and 6-31++G** basis set,[59] see Table S1 and Figure S1 in the Supporting Information. The initial structures were systematically selected using a combinatorial approach. We chose the N1, N3, and C6 atoms as possible proton donors and O7, O8, C2, C4, C5, C6, N1, and N3 centers as possible proton acceptors. Next, we generated initial structures by applying single, double, and triple proton transfers to the canonical tautomer. A structure with M ($M = 1-3$) proton transfers was generated by moving simultaneously M protons from the proton donor sites of the canonical structure to the proton acceptor sites. Thus, the total number of M-type tautomers, N^{M}_{taut}, can be calculated employing the formula:

$$N^{M}_{\text{taut}} = \binom{D}{M}\bullet\binom{A-M}{M} \tag{1}$$

here

$$\binom{X}{Y}$$

is a Newton symbol, and D and A stands for the number of proton donors and proton acceptors, respectively. Hence, the total number of possible tautomers, N_{tot}, can be obtained using the following expression:

$$N_{\text{tot}} = 1 + \sum_{M=1}^{M=3}\binom{D}{M}\bullet\binom{A-M}{M} \tag{2}$$

Because there are three proton donors and eight proton acceptors in thymine, the total number of tautomers considered by us is equal to 77. Most of these structures have two stable rotamers. Moreover, the C2 and C4 centers become chiral after proton attachment, and diastereoisomers develop for the geometries having protons at the C2 and C4 carbons. The results reported in Table S1 and Figure S1 are for the most stable rotamer/isomer of each tautomer.

For further studies, we selected five anionic tautomers, which at the B3LYP/6-31++G** level span a range of 8.0 kcal/mol in terms of the electronic energy corrected for zero-point vibrations (ΔE_{ZPVE}), see Table S1 and Figure 2. In comparison with our previous study on valence anions of uracil (U),[48] the current set includes aT^{c6}_{n3} in addition to aT^{c5}_{n1}, aT^{val}_{can}, aT^{c5}_{n3}, and aT^{c6}_{n1}. Only the latter four uracil analogues were considered in ref 48. The five valence anionic tautomers of thymine were further optimized at the second-order Møller−Plesset (MP2) level with the AVDZ[37] basis set. A typical spin contamination was acceptable for the valence anions with the value of S^2 smaller than 0.8 at the UHF level. The spin contamination was even smaller at the B3LYP/6-31++G** level. The reported MP2 values of AEA and VDE are based on spin-projected MP2 energies (PMP2) of the anions.

Final single-point calculations were performed at the coupled-cluster level of theory with single, double, and noniterative triple excitations[53] (CCSD(T)/AVDZ) at the optimal MP2 geometries. The open-shell CCSD(T) calculations were carried out at the R/UCCSD(T) level. In this approach, a restricted open-shell Hartree−Fock calculation was initially performed to generate the set of molecular orbitals and the spin constraint was relaxed in the coupled-cluster calculation.[60−62] The 1s orbitals of carbon, nitrogen, and oxygen were excluded from the MP2 and coupled-cluster treatments.

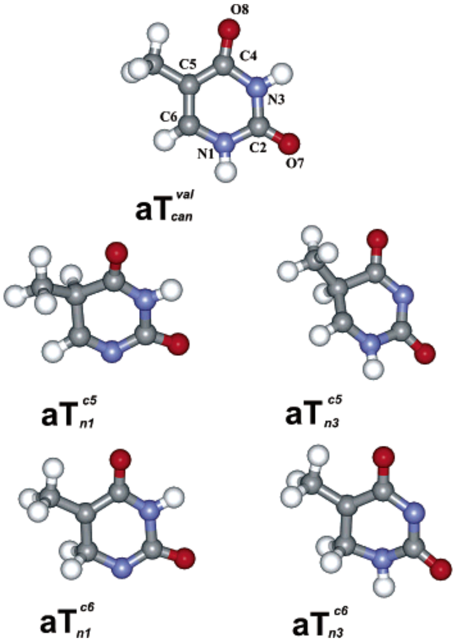

Figure 2. Tautomers of anionic thymine considered in this study at the MP2 and CCSD(T) levels of theory with the AVDZ basis set.

The relative electronic energy of the anion with respect to the neutral was first corrected for unscaled zero-point vibration energies to derive the value of AEA. Next, thermal corrections as well as the entropy terms, calculated at either the B3LYP or MP2 levels for $T = 298$ K and $p = 1$ atm in the harmonic oscillator−rigid rotor approximation, were included to derive the relative stability in terms of Gibbs free energy. The stability of anions measured in terms of Gibbs free energy with respect to nT_{can} will be labeled AEA_{G}.

In view of small adiabatic electron affinities for the three most stable valence anions of thymine, we performed additional calculations in the spirit of extrapolation procedures outlined in ref 36, see also ref 63. These were two-point extrapolation procedures based on an exponential form for the Hartree−Fock energy and a power form for the MP2 correlation energy. Three tautomers were considered: aT^{c5}_{n1}, the most stable anion, aT^{val}_{can}, the anion based on the canonical tautomer that is the most stable among neutral species, and aT^{c5}_{n3}, the anionic tautomer of the highest stability among those in which the N3 center is involved in tautomerization. First, the geometries of these anions and of nT_{can} were optimized at the MP2/AVTZ level (triple-ζ quality basis set). Then the HF (PUHF for the anions) energies and MP2 (PMP2 for the anions) correlation energies were calculated with the AVDZ and AVTZ basis sets for the MP2/AVTZ geometries. These energies were, in turn, employed to estimate the SCF and MP2 CBS limiting energies for the neutrals and the anions. The CCSD(T) correction to AEA was calculated at the CCSD(T)/AVDZ//MP2/AVTZ level. To obtain the final values of AEAs, the extrapolated electronic energies were supplemented with zero-point vibration terms calculated in harmonic approximation at the B3LYP level with the Sadlej's pVTZ basis set[64] and at the MP2/AVTZ level. Scaling factors of 0.9726 and 0.9649 were employed for the B3LYP and MP2 vibrational terms, respectively.[65] Our extrapolations were performed for molecular geometries determined at the MP2/AVTZ level, whereas Svozil et al.[36] used the RI-MP2/AVTZ geom-

TABLE 1: Relative Energies (kcal/mol) of Tautomers of Neutral Thymine Determined at the B3LYP, MP2, and CCSD(T) Levels

	B3LYP/6-31++G**				MP2/AVDZ				CCSD(T)/AVDZ			
tautomer	ΔE	ΔE_{ZPVE}	ΔH	ΔG	ΔE	ΔE_{ZPVE}	ΔH	ΔG	ΔE	ΔE_{ZPVE}	ΔH	ΔG
nT_{can}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nT^{o7}_{n1}	10.91	10.70	10.65	10.83	9.51	9.30	9.25	9.44	9.57	9.36	9.31	9.50
nT^{o8}_{n3}	12.75	12.59	12.52	12.67	11.68	11.50	11.45	11.57	11.75	11.57	11.53	11.64
nT^{o7o8}_{n1n3}	13.29	13.14	13.03	13.34	9.98	9.73	9.65	9.90	10.62	10.37	10.19	10.54

etries. A difference in the AEA for the canonical tautomer that results from using the MP2 vs RI-MP2 geometries is only ca. 1 meV.[36]

Rotation profiles for the methyl group in the neutral and anionic canonical tautomer of thymine were studied at the B3LYP level using a Gaussian basis set specifically optimized for DFT.[66] Namely, a polarized triple-ζ basis set (TZVP)[66] was supplemented with valence diffuse functions (s, p for hydrogen, and s, p, and d for C, N, and O) to better describe the electron charge distribution in the anion (TZVP+). Exponents of the additional diffuse functions were as follows (atom, α_s, α_p, α_d: H, 0.04573, 0.222561; C, 0.04441, 0.02922, 0.196407; N, 0.06035, 0.04073, 0.224692; O, 0.08142, 0.04812, 0.249174). We verified in our study on the valence anion of formic acid dimer that the addition of another set of diffuse functions changes the excess electron binding energy by less than 0.01 kcal/mol.[67]

An error can result if harmonic oscillator approximation is used to calculate a contribution to the molecular partition function from low-frequency modes such as hindered rotation.[68] Thymine contains a methyl group which can, indeed, be treated as an internal rotor. Therefore, we explored the effect of anharmonicity by applying appropriate corrections, calculated according to the protocol of Pitzer and Gwinn.[69] One needs to calculate the V/RT terms for the anion and the neutral (where V is a barrier height for rotation of the methyl group, R is the gas constant, and temperature T is in K) as well as Q_f, a partition function for the free rotation. The corrections were calculated only for the neutral and valence anion of the canonical tautomer, and the corrected value of AEA_G will be reported.

The effects of hydration were studied by performing geometry optimization within the polarized continuum model (PCM)[70−72] at the B3LYP/6-31++G** level of theory. We apply the same value of dielectric constant ($\epsilon = 78.4$) for the neutral and the anion when calculating the values of AEA. The values of VDE and VAE were calculated with $\epsilon = 78$ for the initial state and $\epsilon = 2$ for the final state. This approach relies on the assumption that the solvent geometry remains essentially fixed on the time scale of electron detachment or attachment.[73]

Our study of dipole-bound anionic states is limited to the canonical tautomer, aT^{dbs}_{can}. We applied a standard approach for the analysis of VDE.[74−77] For the definition of VDE determined at the Koopmans's theorem level (D^{KT}), orbital relaxation (ΔD^{SCF}_{ind}), second-order electron correlation terms (ΔD^{MP2}_{disp} and ($\Delta D^{MP2}_{no-disp}$), and higher-order terms (ΔHO^{SD} and ΔHO^{T}), see refs 74−77. We used the AVDZ basis set supplemented with additional s and p symmetry basis functions centered on the H(C6) atom (AVDZ+diff). The exponents form an even-tempered sequence with a geometric progression constant 1/3.23;[78] see Table S2 for the exponent values.

The DFT and MP2 geometry optimizations and frequency calculations were performed with Gaussian03,[59] and the CCSD(T) calculations with the MOLPRO[79] package. The codes were run on clusters of dual Intel/P4Xeon and dual Intel Itanium2 nodes with 1 GB Ethernet interconnect. Molden was used for visualization of singly occupied molecular orbitals.[80]

3. Results

3.1. Tautomers of Neutral Thymine. All tautomers of neutral T presented in Figure 1 and Table 1 have a symmetry plane. The canonical tautomer nT_{can} is the most stable at every level of theory in terms of energy, enthalpy, and Gibbs free energy (Tables 1 and S3). At the CCSD(T)/AVDZ//MP2/AVDZ level, the second most stable tautomer, imino-hydroxy nT^{o7}_{n1}, is less stable by 9.36 and 9.50 kcal/mol in terms of ΔE_{ZPVE} and Gibbs free energy, respectively. This result is consistent with the experimental findings that the nT_{can} tautomer is the most stable in the gas phase, as well as in solution.[22,23] The high stability of nT_{can} in comparison with rare tautomers[22,26,27] might be important for informational fidelity of DNA. Any coexistence of rare tautomers may lead to mismatches of base pairs in comparison to the Watson−Crick scheme and in consequence to a point mutation.

A dihydroxy tautomer nT^{o7o8}_{n1n3} is ca. 1 kcal/mol less stable than nT^{o7}_{n1} and ca. 1 kcal/mol more stable than another mono-hydroxy tautomer, nT^{o8}_{n3}, see Table 1 and Figure 3. The instability of two hydroxy-oxo and the dihydroxy tautomers reported by us, 9.50 kcal/mol < ΔG < 11.64 kcal/mol is very close to the instability of 9.56 kcal/mol of the most abundant minor tautomer determined experimentally by Tsuchiya at al.[22] Our estimates are also in good agreement with the highly correlated results of Piacenza and Grimme.[27] Lower-level methods, such as B3LYP, fail to predict correctly the relative stability of the nT^{o7}_{n1}, nT^{o7o8}_{n1n3}, and nT^{o8}_{n3} tautomers, see Table 1. There is a striking similarity in relative stability among the rare tautomers of uracil and thymine (Figure 3). The ΔE_{ZPVE} values differ by less than 0.7 kcal/mol. Apparently, the methyl group of thymine does not play any significant role in relative stability of the closed-shell neutral tautomers. The neutral nT^{c5}_{n3} and nT^{c6}_{n3} do not support minima for six-member ring structures. The MP2 geometry optimizations result in a linear and a bicyclo structure, respectively, see Figure 4.

A significant dipole moment of nT_{can}, 4.3 D at the MP2/AVDZ level, is sufficient to support a DB state and the resulting anion is adiabatically bound with respect to the neutral. Other neutral tautomers of thymine might be also very polar. For instance, the dipole moment of nT^{o8}_{n3} is larger by ca. 0.9 D than that of nT_{can}, see Table S3. However, the instability of nT^{o8}_{n3} with respect to nT_{can} by ca. 12.6 kcal/mol, see Table 1, is too large to be compensated by the excess electron attachment. Thus in this study, we will discuss only one dipole-bound anion, aT^{dbs}_{can}.

3.2. Anions of Thymine. To put valence anions of thymine and uracil on equal footing, the aU^{c6}_{n3} tautomer, which had been studied in ref 48 only at the B3LYP and MP2 levels of theory, was reconsidered here at the CCSD(T) level and within the PCM solvation model. Table 2 from ref 48, with the results for valence

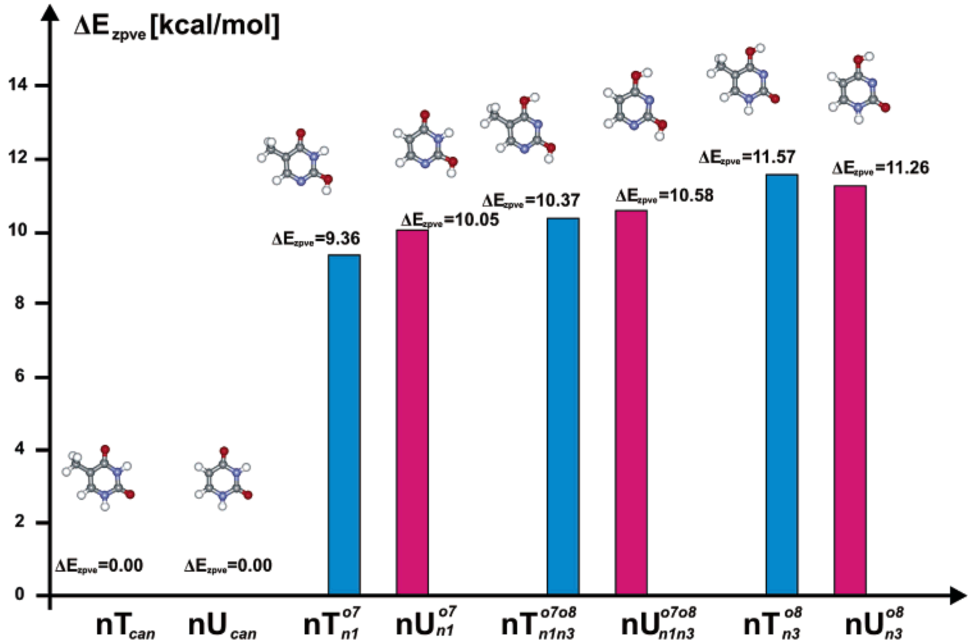

Figure 3. Tautomers of neutral thymine (T) and uracil (U). Relative CCSD(T)/AVDZ electronic energies were corrected for zero-point vibration terms determined at the MP2/AVDZ level. The ΔE_{ZPVE} of the nT_{can} (nU_{can}) tautomer is assumed as zero.

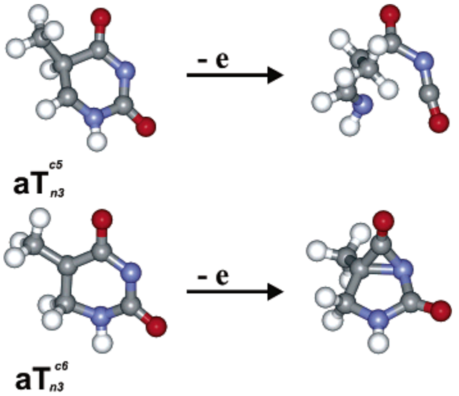

Figure 4. Eexcess electron detachment leads to a decomposition of the six-member ring structure of some anionic tautomers of thymine with carbon atoms protonated (the MP2/AVDZ prediction).

anions of uracil in the gas phase, has been updated with the results for $aU^{\mathrm{c6}}_{\mathrm{n3}}$ and presented in the Supporting Information (Table S4). Our main results for the anions of thymine will be compared with those of uracil.

3.2.1. Valence Anions of Thymine. Our preliminary B3LYP/6-31++G** searches for vertically bound valence anions of thymine in the gas phase ended up with positive results for 73 tautomers, see Table S1. The five most stable structures, $aT^{\mathrm{c5}}_{\mathrm{n1}}$, $aT^{\mathrm{val}}_{\mathrm{can}}$, $aT^{\mathrm{c5}}_{\mathrm{n3}}$, $aT^{\mathrm{c6}}_{\mathrm{n1}}$, and $aT^{\mathrm{c6}}_{\mathrm{n3}}$, have been considered at higher levels of theory. The structures were reoptimized at the MP2/AVDZ level and final energies were calculated at the CCSD(T) level, see Table 2 and Figure 5. All five anions remain vertically bound at the MP2 level, which makes the converged structures reliable, and they maintain their vertical electronic stability at the CCSD(T) level.

Our most important finding is that the most stable valence anion, $aT^{\mathrm{c5}}_{\mathrm{n1}}$, is unrelated to any low-lying tautomer of neutral thymine. The anion $aT^{\mathrm{c5}}_{\mathrm{n1}}$ is characterized by an electron vertical detachment energy of 1251 meV, see Table 2, and it is *adiabatically stable* with respect to the canonical neutral, nT_{can}, by 2.65 kcal/mol, see Figure 5. The CCSD(T)/AVDZ value of AEA is probably larger than the inherent error of the computational model used. One might speculate that the $aT^{\mathrm{c5}}_{\mathrm{n1}}$ anion was observed in the RET spectroscopy experiments by Desfrancois et al.[39] Unfortunately, these authors could not provide estimations of the VDE and AEA values for their valence anion.

The values of AEA and VDE for the tautomers of thymine are presented in Table 2 and those for the same tautomers of uracil in Table S4. The relative stabilities of various tautomers of U^- and T^- are presented in Figure 5. Thermal effects and entropy contributions calculated at standard conditions contribute to the relative stability of the anions by less than 60 meV, see the values of AEA and $\mathrm{AEA_G}$ in Tables 2 and S4. Both for U^-

TABLE 2: Adiabatic Electron Affinities (AEA) and Electron Vertical Detachment Energies (VDE) in meV, Determined at the MP2, CCSD, and CCSD(T) Levels[a]

valence anions	MP2			CCSD			CCSD(T)			
	AEA[b]	AEA[c]	VDE	AEA[b]	AEA[c]	VDE	AEA[b]	AEA[c]	$\mathrm{AEA_G}$[e]	VDE
$aT^{\mathrm{c5}}_{\mathrm{n1}}$	767	4 (−109)	1274	721	76 (−37)	1287	762	115 (2)	146	1251
aT_{can}	−142	−142 (−250)	409	−121	−121 (−229)	512	−87	−87 (−196)	−37	457
$aT^{\mathrm{c5}}_{\mathrm{n3}}$	1101	−265 (−379)	2491	1244	−144 (−258)	2596	1203	−121 (−235)	−87	2458
$aT^{\mathrm{c6}}_{\mathrm{n1}}$ [d]	−363	−363 (−493)	3856	−247	−247 (−377)	3737	−234	−234 (−364)	−172	2816
$aT^{\mathrm{c6}}_{\mathrm{n3}}$	1767	−385 (−499)	3666	1921	−256 (−369)	3694	1907	−248 (−361)	−193	3449

[a] Contributions from electronic energy are given in parentheses. [b] Calculated with respect to the corresponding neutral. [c] Calculated with respect to the nT_{can} tautomer. [d] Geometry optimization of neutral structure at the MP2/AVDZ level converged to nT_{can} form. [e] The values of $\mathrm{AEA_G}$ determine the relative stability of the anion with respect to the nT_{can} tautomer in terms of Gibbs free energy.

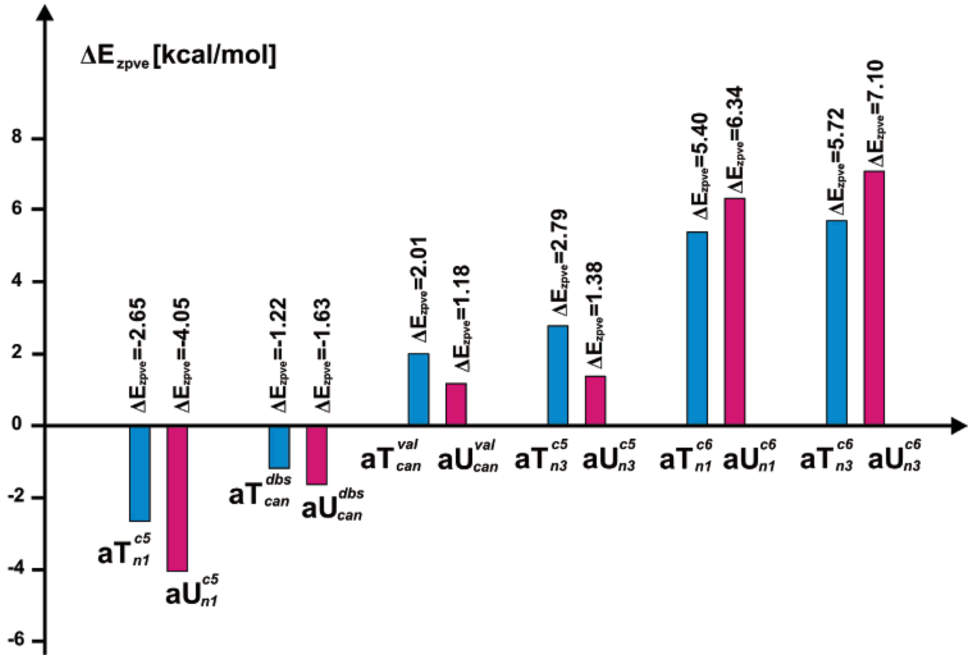

Figure 5. Tautomers of anionic thymine (T) and uracil (U). Electronic energies determined at the CCSD(T) level and zero-point vibrations at the MP2 level. The ΔE_{ZPVE} of the nT_{can} (nU_{can}) tautomer is assumed as zero.

TABLE 3: AEAs (in meV) Calculated with Respect to nT_{can} Obtained by Using Various Extrapolation Procedures

tautomer	$\mathrm{MP2}^{\infty a}$	$\mathrm{MP2}^{\infty}$ + $\mathrm{CCSD(T)}^{b}$	$\mathrm{MP2}^{\infty}$ + CCSD(T) + ZPVE	
			$\mathrm{MP2}^{c}$	$\mathrm{B3LYP}^{d}$
$aT_{\mathrm{n1}}^{\mathrm{c5}}$	−118	−7	103	125
$aT_{\mathrm{can}}^{\mathrm{val}}$	−163	−110	−5	20
$aT_{\mathrm{n3}}^{\mathrm{c5}}$	−399	−256	−146	−119

[a] Calculated as a difference between the MP2 CBS limiting energies for the neutral and the anion; [b] "MP2$^{\infty}$" corrected for higher-order electron correlation terms determined at the CCSD(T) level; [c] "MP2$^{\infty}$ + CCSD(T)" corrected for the zero-point vibration energy contribution calculated at the MP2/AVDZ level. A scaling factor of 0.9649 was employed.[65] [d] "MP2$^{\infty}$ + CCSD(T)" corrected for the zero-point vibration energy contribution calculated at the B3LYP level with the Sadlej's pVTZ basis set. A scaling factor of 0.9726 was employed.[65]

and T^{-}, the imino-oxo tautomers resulting from proton transfer to C5 are more stable than those resulting from proton transfer to C6. Also the imino-oxo tautomers resulting from proton transfer from N1 are more stable than those resulting from proton transfer from N3. The values of VDE are always larger for the anions of U than for T (Tables 2 and S4), which we associate with the electron donating effect of the methyl group. The magnitude of these differences strongly depends on the tautomer and correlates with the amount of excess charge associated with the C5 atom to which the methyl group is connected.

The small values of AEAs prompted us to perform the SCF and MP2 CBS extrapolations. The results, presented in Table 3 for the three most stable valence anions, $aT_{\mathrm{n1}}^{\mathrm{c5}}$, $aT_{\mathrm{can}}^{\mathrm{val}}$, and $aT_{\mathrm{n3}}^{\mathrm{c5}}$, are qualitatively consistent with those presented in Table 2. $aT_{\mathrm{n1}}^{\mathrm{c5}}$ is adiabatically bound with respect to nT_{can} by ca. 2.4 kcal/mol. The stability of $aT_{\mathrm{can}}^{\mathrm{val}}$ is affected more seriously. Its adiabatic *instability* of 2.01 kcal/mol (Figure 2) is drastically reduced, and the sign of AEA depends on whether the B3LYP or MP2 zero-point vibration correction is employed. A result based on the scaled B3LYP correction, AEA = 20 meV, is slightly larger than 18 meV reported by Svozil.[36] Small differences between the RI-MP2 and MP2 geometries are responsible for this discrepancy.[36] On the other hand, the scaled MP2 correction leads to AEA = −5 meV. Clearly, the AEA of thymine is very close to zero, as had been predicted experimentally[34,39] and theoretically.[36]

The effect of anharmonicity on the rotation of methyl group for the neutral and anionic canonical thymine was evaluated using the tables of Pitzer and Gwinn.[69] We found that the differences between the hindered rotor and harmonic oscillator free energies amount to −59.2 and −139.5 cal/mol for $aT_{\mathrm{can}}^{\mathrm{val}}$ and nT_{can}, respectively. As a result, the $\mathrm{AEA_G}$ of $aT_{\mathrm{can}}^{\mathrm{val}}$ decreases by 3.5 meV. We expect that using the hindered rotor model instead of harmonic approximation, the $\mathrm{AEA_G}$ for the remaining tautomers will decrease by a similar amount, i.e., 3−4 meV. Because the effect is so small, the values presented in Tables 1 and 2 were not corrected for the hindered rotor effects.

The hindered rotation of the methyl group of thymine might exert an effect on its propensity to bind an excess electron. The rotation profiles for the neutral and anion of the canonical tautomer are displayed in Figure 6 as a function of the H−C9−C5−C6 angle. The barrier for rotation is ca. 1.3 kcal/mol for the neutral and only ca. 0.4 kcal/mol for the anion. Moreover, the maxima of these curves are shifted by ca. 60°. Thus, the transition state geometry for the anion is a minimum for the neutral and vice versa. The minimum geometry for the anion corresponds to an eclipsed conformation of the C4C5 bond and the CH bond of the methyl group that is closest to the polar C4O8 bond. On the other hand, the minimum energy structure of the neutral has the C4C5 bond staggered between two CH bonds of the methyl group. By selecting a proper wavelength of an external microwave source, one can selectively initiate rotation of the methyl group in the neutral or in the anion. The probability of excess electron hopping to and from thymine might be tuned by rotation of the methyl group. In the case of neighboring thymines, the probability might be tuned by a phase difference in rotating methyl groups. A difference in phases by 60° might be particularly interesting. The spatial and temporal

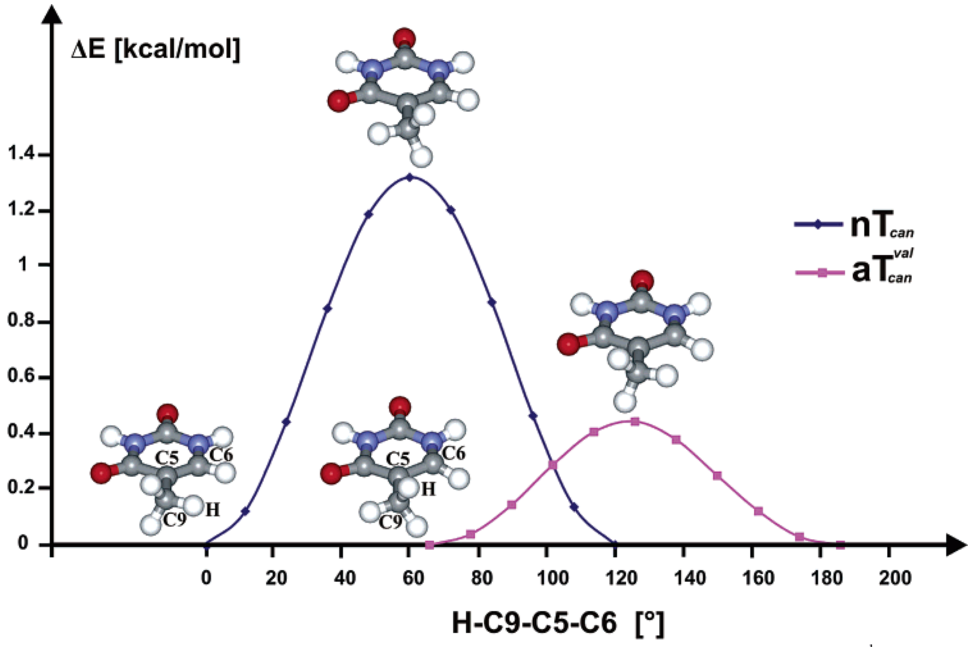

Figure 6. Energy profiles concerning the rotation of methyl group in nT_{can} and $aT_{\mathrm{can}}^{\mathrm{val}}$ calculated at the B3LYP/TZVP+ level.

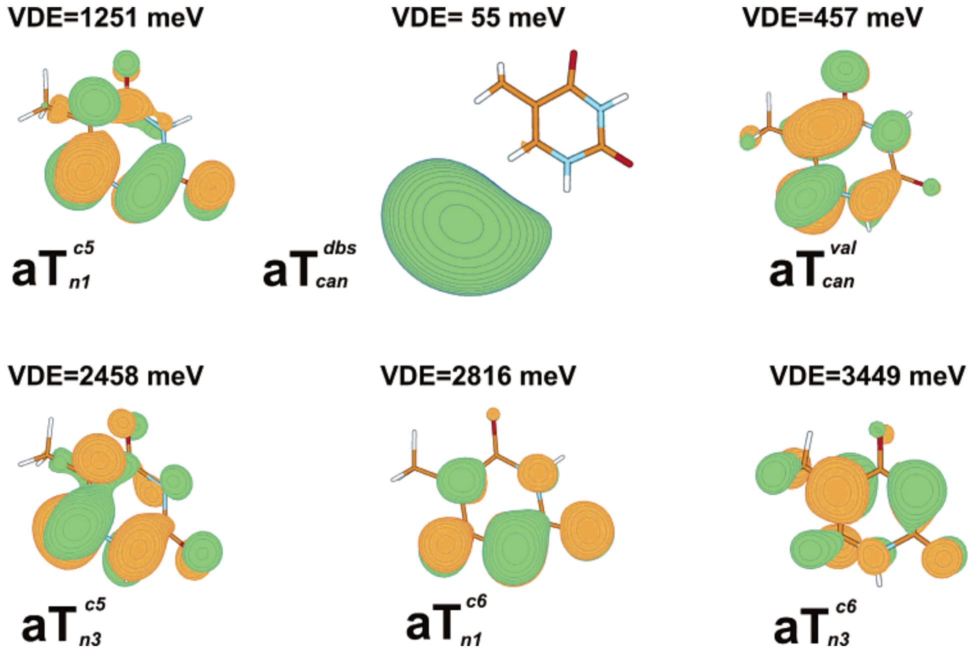

Figure 7. Valence- and dbs-type anionic states of thymine. Singly occupied orbitals plotted with a spacing of 0.03 bohr$^{-3/2}$ for valence states and with 0.0084 bohr$^{-3/2}$ for the dipole-bound state.

control of methyl rotations in thymines might provide a handle to control charge transfer along the DNA double helix.

The vertical electronic stability of valence anions is accompanied by serious geometrical relaxations with respect to the structure of the corresponding neutrals. A serious buckling develops in consequence of localization of the π^* electron in the C4−C5−C6 region, see Figure 7. These geometrical distortions involve both hydrogen and heavy atoms. As measures of nonplanarity, we use deviations from 0° of dihedral angles N1C6C5C4 and N1C2N3C4. Their values are, respectively, −40.9 and −13.6° in $aT_{\mathrm{n1}}^{\mathrm{c5}}$, 13.8 and 0.5° in $aT_{\mathrm{can}}^{\mathrm{val}}$, and −40.7 and −4.8° in $aT_{\mathrm{n3}}^{\mathrm{c5}}$. In consequence of these distortions, the energy of the corresponding neutrals increases by 28.82, 15.07, and 62.13 kcal/mol in comparison with nT_{can}. The resulting values of VDE are only 28.86, 10.54, and 56.70 kcal/mol, which render only the $aT_{\mathrm{n1}}^{\mathrm{c5}}$ tautomer marginally adiabatically bound. Strong distortions of molecular frameworks are characteristic for valence anionic states.

The effect of hydration on the stability of valence anions was studied at the B3LYP/6-31++G** level within the PCM model with fully optimized geometries (Table 4). As expected from the electrostatic model, all five anionic tautomers become adiabatically bound with respect to the neutral nT_{can}, with the stability always exceeding 1.8 eV. What is less expected is that the relative stability of valence anions becomes different from that in the gas phase. $aT_{\mathrm{n3}}^{\mathrm{c6}}$ becomes the most stable anion and is characterized by a VDE of 6.25 eV. $aT_{\mathrm{can}}^{\mathrm{val}}$ is less stable by 2.4 kcal/mol and is characterized by a VDE of 2.98 eV. The values of VAEs for $aT_{\mathrm{n1}}^{\mathrm{c5}}$, $aT_{\mathrm{can}}^{\mathrm{val}}$, and $aT_{\mathrm{n1}}^{\mathrm{c6}}$ are positive but much

TABLE 4: Effects of Hydration on the Stability of Anions of Thymine and Uracil. The Adiabatic Electron Affinity (AEA), Electron Vertical Detachment Energy (VDE), and Electron Vertical Attachment Energy (VAE) Studied at the B3LYP/6-31++G Level within the PCM Model Using Geometries Optimized in the Solvent**

thymine						uracil					
tautomer	relative energy[a] [kcal/mol]	solvation energy [kcal/mol]	VAE [eV]	VDE [eV]	AEA[b] [eV]	tautomer	relative energy[a] [kcal/mol]	solvation energy [kcal/mol]	VAE [eV]	VDE [eV]	AEA[b] [eV]
aT_{n3}^{c6}	0	−76.64	*c*	6.25	1.95	aU_{n3}^{c5}	0	−74.74	*c*	5.16	1.99
aT_{n1}^{c5}	0.47	−61.55	1.06	3.92	1.93	aU_{n1}^{c5}	0.21	−63.57	1.04	3.94	1.98
aT_{n3}^{c5}	0.73	−72.49	*c*	5.12	1.92	aU_{can}	1.09	−65.88	0.09	3.10	1.94
aT_{can}	2.40	−61.12	0.05	2.98	1.85	aU_{n3}^{c6}	2.63	−79.96	*c*	6.61	1.87
aT_{n1}^{c6}	4.21	−65.71	1.06	6.05	1.77	aU_{n1}^{c6}	7.21	−67.73	3.45	6.24	1.68
aT_{Ts1}	52.07	−53.33	*d*	3.69	−0.31	aU_{Ts1}	52.48	−55.17	*d*	3.85	−0.29
aT_{Ts2}	41.36	−59.06	*d*	3.41	0.15	aU_{Ts2}	43.03	−60.74	*d*	3.86	0.12

[a] Calculated with respect to the $aT_{n3}^{c6}(aU_{n3}^{c5})$ tautomer. [b] Calculated with respect to the nT_{can} (nU_{can}) tautomer. [c] The six-member ring structure is unstable for the neutral. [d] Not considered for aT_{Ts1} (aU_{Ts1}) and aT_{Ts2} (aU_{Ts2}) because these structures are anionic *transition states*.

smaller than the AEA's of the respective neutrals. Clearly, the orientation polarization of the solvent and geometrical relaxation of the ring upon an excess electron attachment plays an important role, see Table 4.

The differences and similarities between anionic tautomers of uracil and thymine in the PCM hydration model are also illustrated in Table 4. For each anionic tautomer, the VDE is larger for U^- than for T^-, which is a manifestation of the electron-donating effect of the methyl group. Also, for each anionic tautomer, the solvation energy is larger for uracil than for thymine, which is a reflection of hydrophobicity of the methyl group of thymine. For both bases, the strongest solvation develops for the tautomer with a proton transferred from N3 to C6. This tautomer becomes the most stable for hydrated anionic thymine. For uracil, however, the instability of this tautomer in the gas phase is too large to be compensated by hydration, and the aU_{n3}^{c5} tautomer ends up as the most stable.

3.2.2. Dipole-Bound Anion of the Canonical Tautomer of Thymine. With the most stable valence anion aT_{n1}^{c5} being adiabatically bound with respect to nT_{can} by less than 2.5 kcal/mol (see Figure 5), it is imperative to consider the dipole-bound anion aT_{can}^{dbs}. Electron correlation effects are expected to be very significant for this system because dipole moment of the neutral is seriously overestimated at the Hartree−Fock (HF) level of theory. Indeed, the HF/AVDZ value of the dipole moment of 5.00 D is reduced to 4.34 D at the MP2/AVDZ level of theory, with the experimental value in dioxane at 25 °C being estimated at 4.13 D.[28] In addition to the dipole moment correction, there are usually significant dynamic correlation effects between the excess electron and the electrons of the neutral molecule.[74−76] The two effects have opposite signs for the dipole-bound anion of thymine. Thus a fortuitous cancellation of errors might also develop.

Upon an excess electron attachment to the sp-type diffuse hybrid, see Figure 7, there is only a minor geometrical relaxation and the molecular framework retains the C_s symmetry. The dipole moment of the neutral increases by ca. 0.10 D from the optimal geometry of the neutral to the optimal geometry of the anion. The excess electron is localized on the positive pole of the molecular dipole, with the negative pole being determined by the two carbonyl groups.

The CCSD(T) value of AEA for aT_{can}^{dbs} is 53 meV. Thus, this anionic state is adiabatically bound with respect to nT_{can} by 1.22 kcal/mol, see Figure 5, and is only 1.43 kcal/mol less stable than the valence anion aT_{n1}^{c5}. Our calculated value of AEA for aT_{can}^{dbs}, 53 meV, is in very good agreement with an earlier theoretical result of 51 meV,[36] determined also at at the CCSD(T) level. In addition to aT_{can}^{dbs}, the canonical tautomer supports also a valence bound state, aT_{can}^{val}, see Figure 5. The valence state is, however, vertically unbound at the optimal (planar) geometries of nT_{can} and aT_{can}^{dbs}.

TABLE 5: Electron Vertical Detachment Energies (meV) and Their Components for the Dipole-bound Anions of Thymine (aT_{can}^{dbs}) and Uracil (aU_{can}^{dbs}) Obtained with AVDZ+Diff Basis Sets[a]

anion type	aT_{can}^{dbs}	aU_{can}^{dbs} [b]
D^{KT}	19	30
ΔD_{ind}^{SCF}	4	7
D^{SCF}	23	37
ΔD_{disp}^{MP2}	32	46
$\Delta D_{no-disp}^{MP2}$	−20	−31
ΔD^{MP2}	12	15
D^{MP2}	35	52
ΔHO^{SD}	18	19
D^{CCSD}	53	70
ΔHO^{T}	2	3
$D^{CCSD(T)}$	55	73

[a] For the definitions of components, see refs 74−77. [b] Ref 48.

Next, let us turn to the analysis of VDE for aT_{can}^{dbs}, see Table 5. The Koopmans' theorem contribution of 19 meV is followed by a nonnegligible SCF induction term of 4 meV. The resulting values of D^{SCF} of 23 meV reproduces only 42% of the CCSD(T) value of D. The MP2 dispersion contribution is much larger than the Koopmans' theorem term, and the MP2 nondispersion term is destabilizing because the MP2 dipole moment of the neutral is much smaller than the SCF dipole moment. The resulting values of D^{MP2} of 35 meV reproduce 64% of $D^{CCSD(T)}$. A contribution from higher-than-second-order correlation terms limited to single and double excitations is significant and amounts to 18 meV. A relatively small contributions from triple excitations, 2 meV, implies that the final CCSD(T) values of D of 55 meV might be close to the methodologically converged result. Indeed, the three experimental values of VDE, 69 ± 7,[32] 62 ± 8,[34] and 68 ± 20[31] meV, are in good agreement with our prediction.

In Table 5, we also report the analogous components of D for the dipole-bound anion of uracil, aU_{can}^{dbs}.[48] The MP2/AVDZ value of the dipole moment is larger by only 0.04 D for uracil than for thymine. However, their CCSD(T) values of D differ by as much as 18 meV. The difference develops primarily at

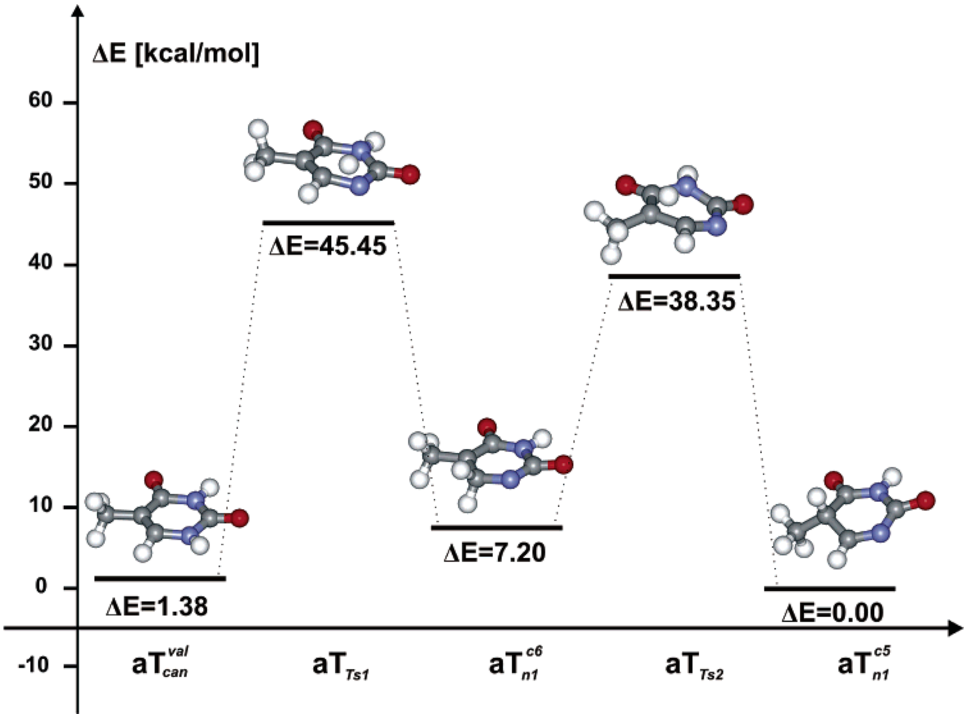

Figure 8. Transition states for the $aT_{\mathrm{can}}^{\mathrm{val}} \rightarrow aT_{\mathrm{n1}}^{\mathrm{c6}} \rightarrow aT_{\mathrm{n1}}^{\mathrm{c5}}$ tautomerizations determined at the B3LYP/6-31++G** level of theory.

low levels of theory: D^{KT} (11 meV), $\Delta D_{\mathrm{ind}}^{\mathrm{SCF}}$ (3 meV), ΔD^{MP2} (3 meV), with the ΔHO^{SD} and ΔHO^{T} differing only by 1 meV. We believe that the differences in the components of D result not only from the difference in dipole moments between uracil and thymine but also from valence repulsion effects exerted by the methyl group of thymine on the excess electron.

IV. Discussion

A question that we addressed in this study is whether unusual tautomers of thymine might support adiabatically bound valence anions. We found that a valence anion, $aT_{\mathrm{n1}}^{\mathrm{c5}}$, which has a proton transferred from N1H to C5, is adiabatically stable with respect to nT_{can} by 103 meV. We believe that this small stability is larger than the inherent error of the computational model used. Another new imino-oxo tautomer, $aT_{\mathrm{n3}}^{\mathrm{c5}}$, is adiabatically unbound with respect to nT_{can}, with the predicted instability of 146 meV. These two anionic tautomers are unrelated to the well-known “rare tautomers” of neutral thymine and are characterized by relatively large values of VDE, 1251 and 2458 meV, respectively. Thus they might be identified in the course of PES experiments.

The tautomers $aT_{\mathrm{n3}}^{\mathrm{c6}}$, $aT_{\mathrm{n1}}^{\mathrm{c5}}$, and, $aT_{\mathrm{n3}}^{\mathrm{c5}}$ are predicted as the most stable in the PCM hydration model, with the same relative stability to within 0.03 eV. The accuracies of the B3LYP method and the PCM model are not sufficient to make a definite prediction of their relative stability in water solution. These tautomers have, however, significantly larger values of the VDE than the $aT_{\mathrm{can}}^{\mathrm{val}}$ tautomer. So far, attention of experimentalists and theorists was focused on the dipole-bound and valence anions of the canonical tautomer. We believe that further explorations of valence anions based on the tautomers with protonated carbon atoms, such as $aT_{\mathrm{n3}}^{\mathrm{c6}}$, $aT_{\mathrm{n1}}^{\mathrm{c5}}$, and, $aT_{\mathrm{n3}}^{\mathrm{c5}}$, would be highly desirable.

A unique feature of tautomers with carbon sites protonated is the high stability of valence anions accompanied by an inherent instability of the corresponding neutral structures. Indeed, the six-member ring structure of $aT_{\mathrm{n3}}^{\mathrm{c5}}$ and $aT_{\mathrm{n3}}^{\mathrm{c6}}$ is unstable upon the detachment of an excess electron, as determined at the MP2 level of theory. The neutral systems resulting from electron detachment from $aT_{\mathrm{n3}}^{\mathrm{c5}}$ and $aT_{\mathrm{n3}}^{\mathrm{c6}}$ evolve along barrier-free decomposition pathways to a linear or a bicyclo structure, respectively. The valence anions might be unstable in solutions with sufficiently strong proton donors, i.e., they may become protonated. One can envision, however, experiments in properly selected solvents, in which oxidation of these anions leads to a spectroscopically detectable destruction of the ring structure.

What might be formation pathways for the $aT_{\mathrm{n1}}^{\mathrm{c5}}$, $aT_{\mathrm{n3}}^{\mathrm{c5}}$, $aT_{\mathrm{n1}}^{\mathrm{c6}}$, and $aT_{\mathrm{n3}}^{\mathrm{c6}}$ anions? We calculated barriers for the unimolecular proton transfer from $aT_{\mathrm{can}}^{\mathrm{val}}$ to $aT_{\mathrm{n1}}^{\mathrm{c6}}$ and from $aT_{\mathrm{n1}}^{\mathrm{c6}}$ to $aT_{\mathrm{n1}}^{\mathrm{c5}}$, and the results are summarized in Figure 8. In view of the high barriers, 44.1 and 31.2 kcal/mol, respectively, the unimolecular tautomerizations are not probable at standard conditions. The barriers are even higher when effects of hydration are taken into account within the PCM model, see Table 4. Formation of the anionic tautomers with carbon atoms protonated might proceed through intermolecular proton transfers when acidic and basic sites interact with $aT_{\mathrm{can}}^{\mathrm{val}}$ through intermolecular proton transfer, but this mechanism is beyond the scope of this project.[81−83] Here, we point out that dissociative electron attachment to nT_{can} followed by a barrier-free attachment of a hydrogen atom to the C5 atom of the deprotonated thymine might be an efficient pathway. Indeed, it was demonstrated in the experiments of Illenberger et al. and Märk et al.[84−86] that nitrogen sites of NABs are the most susceptible to dehydrogenation in the course of dissociative electron attachment:

$$(\mathrm{NAB})_{\mathrm{N-H,C}} + e \rightarrow (\mathrm{NAB})_{\mathrm{N-H,C}}^{*-} \rightarrow (\mathrm{NAB}-\mathrm{H})_{\mathrm{N,C}}^{-} + \mathrm{H}^{\bullet}$$

where $(\mathrm{NAB})_{\mathrm{N-H,C}}$ denotes an intact NAB with a hydrogen atom attached to a nitrogen atom, $(\mathrm{NAB})_{\mathrm{N-H,C}}^{*-}$ denotes a scattering state for an excess electron, and $(\mathrm{NAB}-\mathrm{H})_{\mathrm{N,C}}^{-}$ denotes a deprotonated NAB in the ground electronic state. $\mathrm{H}^{\bullet}$ radicals are common species in irradiated aqueous samples,[1] and they are also produced by dissociative electron attachment to NABs.[84−86] We have found that a hydrogen atom attachment to $(\mathrm{NAB}-\mathrm{H})^-_{\mathrm{N,C}}$

$$(\mathrm{NAB}-\mathrm{H})^-_{\mathrm{N,C}} + H^\bullet \rightarrow (\mathrm{NAB})^-_{\mathrm{N,C-H}}$$

is barrier-free in the case of the C5 position of thymine deprotonated at either the N1 or N3 position. In consequence, the $aT^{\mathrm{c5}}_{\mathrm{n1}}$ and $aT^{\mathrm{c5}}_{\mathrm{n3}}$ anions might be formed.

Are the new tautomers of anionic thymine biologically relevant? It is known from pulse radiolysis and electron spin resonance studies that the anion of thymine becomes protonated in water solution.[4] The rate of this process depends on pH and concentration of the phosphate buffer. The first step is assumed to be reversible as T^- becomes protonated at the O8 site. An estimated rate of this step at pH 7, 1.6×10^3 s^{-1},[87] represents a lifetime of 6×10^{-4} s for T^-. In the second and irreversible step, T^- protonates at C6 with a rate constant of 5×10^3 s^{-1},[88] which implies a lifetime of 2×10^{-4} s. The estimated lifetimes seem to be short in comparison with a typical lifetime for DNA replication, in which about 50 base pairs are copied per second by each replication fork. However, the rate of protonation by water must be dependent on the specifics of molecular environment of T^-. For instance, photoelectron spectra of $(\mathrm{NAB}\cdots\mathrm{H_2O})^-$ were interpreted through the $\mathrm{NAB}^-\cdots\mathrm{H_2O}$ rather than $\mathrm{NABH}\cdots\mathrm{OH}^-$ complexes, i.e., intermolecular proton transfer was not identified.[10] Also, intermolecular proton transfer was not identified from the PES spectra of $(\mathrm{NAB}\cdots(\mathrm{H_2O})_n)^-$ ($n = 0-5$) complexes.[34] Instead, the evolution of VDE and AEA was linear as a function of n and allowed obtaining extrapolated values of AEA for isolated NABs. In conclusion, intermolecular proton transfer was not detected in the time scale of PES experiments, which might be on the order of $10^{-4}-10^{-5}$ s.

The remaining issue is how well is T^- hydrated when embedded in the double helix structure of DNA. Kuwabara et al. reported that six water molecules, located mainly in the minor groove of DNA, are available per base pair.[89] Our inspection of the crystallographic structure of B-DNA[90] revealed that water molecules are primarily localized along the sugar−phosphate backbone rather than spread around the whole available surface of the double helix. The shortest found O(water)−C6(T) distance was 3.57 Å, with the most typical distances being ca. 8 Å. Clearly, hydration of thymine in DNA is restricted in comparison with water solutions of T and its nucleosides and nucleotides. This factor favors lifetimes of T^- larger than 10^{-4} s. On the other hand, we do not know yet how the distribution of water is modified by the excess negative charge when T^- is embedded in DNA. Future MD simulations of hydrated DNA fragments containing negatively charged NABs might provide answers to these important questions.

The canonical tautomer supports both a valence anion, $aT^{\mathrm{val}}_{\mathrm{can}}$, and a dipole-bound anion, $aT^{\mathrm{dbs}}_{\mathrm{can}}$, with the respective VDE of 457 and 55 meV. The dipole-bound anion is adiabatically bound with respect to nT_{can} by 53 meV, whereas the AEA of $aT^{\mathrm{val}}_{\mathrm{can}}$ is very close to zero. On the basis of electronic energies only, i.e., HF and MP2 results extrapolated to the complete basis set limit supplemented with the CCSD(T)/AVDZ correction, $aT^{\mathrm{val}}_{\mathrm{can}}$ is unbound with respect to nT_{can} by 110 meV. The harmonic zero-point vibration contribution favors the anion over the neutral, and the anion is predicted to be bound by 20 meV when the scaled B3LYP vibration correction is included, but unbound by 5 meV when the scaled MP2 vibration correction is applied. How trustworthy are these extrapolated results? According to Halkier et al.,[63] the extrapolations based on AVXZ electronic energies (X = 2 and 3), i.e., an approach employed by us, might be not very reliable. On the other hand, extrapolations for anions of nucleic acid bases based on the X = 3 and 4 results would be computationally very demanding. Finally, accuracy of the zero-point vibration contribution to AEA might be critical for the stability of this anion. So far, the B3LYP and MP2 harmonic estimations differ by 25 meV. Finally, anharmonic effects need to be explored in the future. Our hindered-rotor treatment of the methyl group suggests that these anharmonic effects decrease the anion stability. In our opinion, the accuracy of current results is not sufficient to resolve whether $aT^{\mathrm{val}}_{\mathrm{can}}$ is adiabatically bound. The most recent results of Bachorz et al.[91] for the valence anion of canonical *uracil* shed more light on this problem. When only highly accurate electronic energies are considered, i.e., RI-MP2-R12 results supplemented with the CCSD(T)/AVTZ correction, the anion remains *unbound* by 75 meV. The unscaled, MP2/AVTZ, harmonic, vibration correction leads to a *bound* anion, AEA = 27 meV. The above-mentioned reservations as to the harmonic treatment of vibrations still apply. As was demonstrated in the current study, the valence anion of uracil is more strongly bound than the anion of thymine, which is an effect of the electron donating methyl group on thymine. If so, then the results of Bachorz et al.[91] for U^- imply the AEA for $aT^{\mathrm{val}}_{\mathrm{can}}$ close to zero (positive or negative) and probably smaller than the adiabatic stability of its dipole-bound anion. This is consistent with early experimental results for gas-phase thymine in which only the dipole-bound anion was observed.[31−34]

The conformation of the methyl group is different in the neutral and the valence anion of the canonical tautomer. We suggest that the probability of excess electron hopping to and from thymine might be tuned by rotation of the methyl group. In the case of neighboring thymines, the probability might be tuned by a phase difference in rotating methyl groups. Nowadays the spatial and temporal control of methyl rotations in thymines is not achievable, but in the future, it might provide a handle to control charge transfer along the DNA double helix.

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Supporting Information Available: Relative stability of anionic and neutral tautomers of thymine determined at the B3LYP/6-31++G** level; exponents of diffuse s and p functions used in calculations for $aT^{\mathrm{dbs}}_{\mathrm{can}}$; relative stability of various anionic tautomers of uracil determined at the CCSD(T)/AVDZ level; a figure with structures of 12 valence anions of thymine determined at the B3LYP/6-31++G** level. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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