Tautomerism of 1-Methyl Derivatives of Uracil, Thymine, and 5-Bromouracil. Is Tautomerism the Basis for the Mutagenicity of 5-Bromouridine?

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The tautomerism of the *N*-1-methylated derivatives of uracil, thymine, and 5-bromouracil has been studied in order to analyze its implications in the mutagenicity of 5-bromouridine. The tautomeric preference in the gas phase was determined by means of state-of-the-art ab initio quantum mechanical calculations. The influence of solvation in water on the tautomerism was examined by using ab initio self-consistent reaction field and Monte Carlo free energy perturbation techniques. Finally, the effect of the DNA environment on the relative stability between tautomers was estimated from Poisson−Boltzmann calculations. The theoretical results indicate that there are no relevant differences in the intrinsic tautomeric preference of the three pyrimidine bases. The canonical oxo form is the main, if not the exclusive, form in the gas phase. Indeed, neither solvation in water nor solvation in the duplex DNA changes sensibly the relative stability between tautomers. Therefore, our results provide a basis for ruling out the involvement of noncanonical enol tautomers as the origin of the mutagenic properties of 5-bromouridine.

Introduction

Nucleic acids are formed with five nucleic acid bases: two purines (adenine, A; guanine, G) and three pyrimidines (thymine, T; uracil, U; cytosine, C). The formation of specific purine−pyrimidine Watson−Crick hydrogen bonds is responsible for the maintenance of the genetic code (see Figure 1). The Watson−Crick pairings A−T and G−C are strong and lead to steps of similar size, which largely stabilizes the double helix. However, despite the stability of canonical AT and GC steps, other recognition patterns are possible and can even lead to stable helical structures. Thus, nucleic acid fragments with pairings other than Watson−Crick ones, including hydrogen bonding between purines or between pyrimidines, have been found.[1] A much more complex pattern of hydrogen bonds appear if noncanonical tautomeric forms of the bases are considered.

The ability of nucleic acids to accommodate noncanonical hydrogen bonds has been related to the occurrence of spontaneous mutations in the DNA.[1−4] Furthermore, it suggests the possibility of "expanding" the genetic code using nonstandard bases or forcing the formation of anomalous tautomers.[1,2,5,6] In fact, inspection of some nucleic acid structures such as the transfer-RNA demonstrate that nonstandard bases can be incorporated without large structural alterations.[7,8] Numerous studies have shown the possibility of forming stable DNA structures with nonstandard bases.[1,6,9,10] It has been also shown that at least some of these nonstandard bases can be incorporated in vivo into nucleic acids during their synthesis by the polymerases.[5,9,11] On the other hand, noncanonical tautomeric or ionized forms were supposed to be very unstable, and their role in physiological DNA structures was assumed to be negligible for years. However, an increasing number of results support the importance of noncanonical tautomers or ionized bases in the stabilization of certain nucleic acid structures.[1c,2,4]

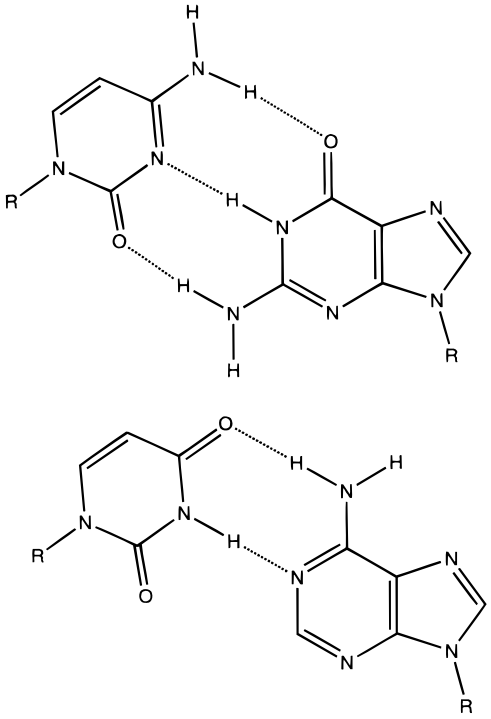

Figure 1. Representation of the Watson−Crick hydrogen-bonded pairings between adenine−thymine and guanine−cytosine

Mutagenicity is associated with some of the nonstandard nucleic acid bases, like 5-bromouracil (Br-U),[1−4,12] which is a mimic of thymine. This base binds adenine in the DNA duplexes without dramatic structural alterations.[13] In addition, it binds guanine with great efficiency, and this feature has been

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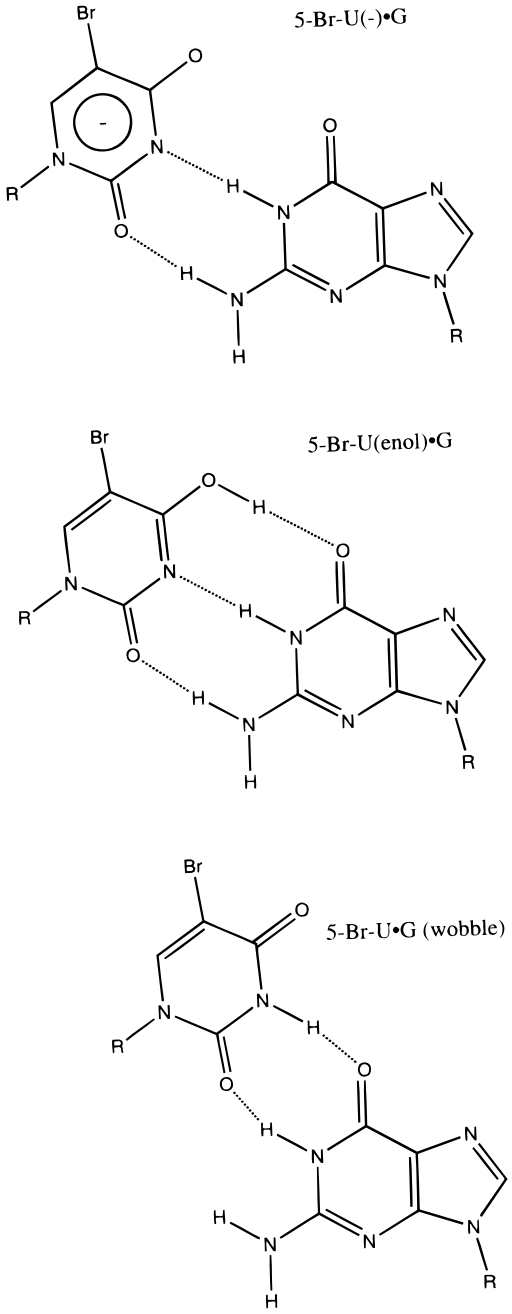

Figure 2. Proposed molecular basis of the origin of the mutagenic properties of 5-bromouracil: ionization of the base, formation of enol tautomers, and change in the hydrogen-bond pattern mediated by a "wobble" mechanism.

suggested to be the basis for its mutagenicity.[4,12] The recognition between Br-U and G was first explained assuming the formation of the enol form of Br-U (Figure 2). Other authors suggested that the recognition is mediated by ionization of Br-U (Figure 2). Finally, a third possibility is the involvement of "wobble" hydrogen bonds (Figure 2). It is worth noting that this latter possibility does not seem to depend intrinsically on the presence of bromine in position 5. Therefore, the mutagenic properties of Br-U should stem from (a) a larger percentage of enol tautomer with respect to the parent compound (uracil and thymine) or (b) an increase in the acidity, which would facilitate loss of the proton at N3 and formation of the 5-Br-U^{-}···G complex.

In this paper we examine the possible involvement of tautomerism as the molecular basis for the mutagenic properties of 5-bromouridine. For this purpose, we have studied the tautomerism of the 1-methyl derivatives of uracil, 5-bromouracil, and thymine in the gas phase, in aqueous solution, and in the duplex DNA. Calculations provide a complete picture of the reactivity of the three bases and give new insights into the reasons for the mutagenicity of haloderivatives of uracil, and particularly of the 5-Br-derivative.

Methods

The tautomerization energies for the 1-methyl derivatives of uracil, 5-bromouracil, and thymine in the gas phase were determined by using high-level ab initio techniques. Three tautomers (the oxo N3 and the two enol forms O4c and O2c; Figure 3) were considered. In addition, the enol form O4t (Figure 3) was also examined in the case of 5-bromouracil. Low level ab initio calculations showed that other tautomers were very unstable, and they were not considered in the study.

Geometries were optimized at the HF/6-311+G(d,p) level,[14] and the minimum-energy nature of the stationary points was verified by frequency analysis. Single-point energy calculations were performed at the SCF and MP2[15] levels using the 6-311+G(d,p) basis set. The MP4(SDTQ), QCISD, and QCISD(T) estimates were obtained by adding the MP4-MP2, QCISD-MP2, or QCISD(T)-MP2 energy difference determined with the 6-31G(d) basis set[16] to the energy calculated at the MP2/6-311+G(d,p) level. This technique is expected to reproduce very closely the MP4, QCISD, or QCISD(T) results determined with the large 6-311+G(d,p) basis set.[17] To determine the enthalpies and free energy differences between tautomers at 298 K and 1 atm, zero-point energy and thermal and entropic corrections were added to the tautomerization energies. These terms were computed using the HF/6-311+G(d,p) frequencies and the standard procedures in Gaussian.[18]

Solvent effects on tautomerism were typically introduced using standard themodynamic cycles (eq 1). Absolute free energies of solvation (ΔG^{sol}) were determined using self-consistent reaction field (SCRF) methods, while relative free energies of solvation ($\Delta\Delta G^{sol}$) were determined using both SCRF and Monte Carlo free energy perturbation techniques (MC-FEP).

$$\Delta G_{A\rightarrow B}^{solution} = \Delta G_{A\rightarrow B}^{gas\ phase} + \Delta G_{B}^{sol} - \Delta G_{A}^{sol} = \Delta G_{A\rightarrow B}^{gas\ phase} + \Delta\Delta G_{A\rightarrow B}^{sol} \quad (1)$$

The SCRF values were determined using our 6-31G(d)-optimized version[19] of the Miertus, Scrocco, and Tomasi (MST) method.[20] Standard cavities and van der Waals parameters were used.[19] Parameters for bromine, which are not available in the standard set, were taken from a current parametrization study (radius = 1.95 Å, $\xi = -0.0767$ kcal mol^{-1} Å^{-2}; REF). The gas-phase HF/6-311+G(d,p) optimized geometries were used in calculations. However, to examine the influence of geometry relaxation in water on the results, the free energy of hydration of O2c and O4c tautomers of uracil was computed using the full optimized MST/6-31G(d) geometry. The extra contribution to ΔG_{sol} due to geometry relaxation was around 1 kcal/mol in both cases, and the change in $\Delta\Delta G_{sol}$ was only 0.2 kcal/mol. It is then clear that the solvent-induced relaxation of the solute geometry does not lead to dramatic effects and that the use of gas-phase optimized geometries is reasonable.[17,21]

The complex mutation scheme used in MC-FEP calculations is shown in Figure 4. To obtain an additional measure of the consistency in FEP calculations, closure thermodynamic cycles

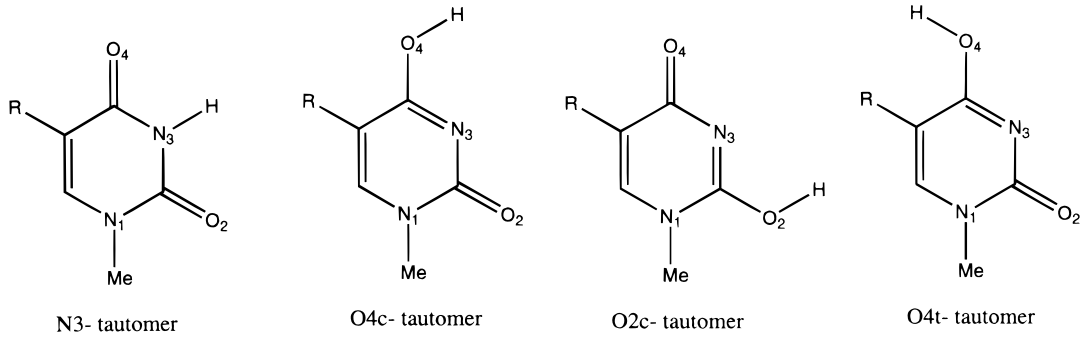

Figure 3. Representation of the oxo and enol tautomers considered in the study.

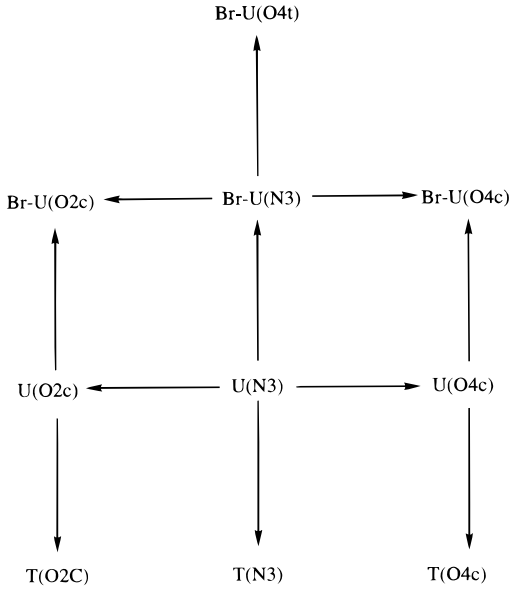

Figure 4. Representation of the mutations performed in MC-FEP calculations.

were also considered. Free energy changes were computed using Zwanzig's theory.[22] The solute was placed in a cubic box (around 15 600 Å^3) containing 503 TIP4P water molecules.[23] Periodic boundary conditions were applied in conjunction with a residue-based 10 Å cutoff for solute−solvent and 9 Å for solvent−solvent nonbonded interactions. Simulations were performed in the isothermic−isobaric ensemble (*NPT*; 1 atm, 298 K). Solute rotations and translations were adjusted to obtain around 40% acceptance.

Mutations were performed using 21 double-wide sampling windows, which allowed us to determie the hysteresis in the calculations. Each window consisted of 2×10^6 configurations for equilibration and 3×10^6 configurations for averaging. The average part of each window was divided into five blocks to estimate the standard errors in the free energy differences. Gas-phase geometries were used in all cases, and they were not explicitly sampled during the MC runs. Atomic charges were determined by fitting quantum mechanical ESP charges.[24] The van der Waals parameters for most atom types were taken from the OPLS force field.[25] The parameters for the bromine atom (not available in the OPLS force field) were determined by fitting HF/6-31G(d) and classical energies for different configurations of the CH_3Br:OH_2 system.[26]

Poisson−Boltzmann calculations[27] were used to obtain a qualitative measure of the work necessary to generate a thymidine, uridine, or 5-bromouridine in a piece of standard DNA compared with the work necessary to generate the same molecule in water. The three bases were considered in their oxo (N3) and enol (O4c) forms. The oxo forms were paired with adenine, while the enol forms were paired with guanine. In all the cases the same structure (a decamer of standard B-DNA[28] with sequence d(A_4XA_5), with X = A or G) was used. The same set of charges and van der Waals parameters used in MC calculations was considered here to make calculations more comparable. The dielectric response of DNA and water was defined by dielectric constants of 2 and 80 relative to that of the gas phase. This implies that a dielectric response of 2 is also assumed for the nucleic acid bases. The solution of Poisson−Boltzmann equations was performed using the lineal approach,[27] assuming an ionic strength in the solvent of 1 M for DNA simulations. To reduce problems due to the limited size of the grid, we used a three-step focusing process, leading to a final grid density of around 34 points × Å^3. The same grid is used for the aqueous and DNA calculations to reduce numerical errors in calculations.

Gas-phase calculations were performed with Gaussian-94.[18] SCRF calculations were carried out with a locally modified version of MonsterGauss[29] and HONDO-8 [30] programs. ESP charges were determined using MOPETE/MOPFIT.[31] MC-FEP calculations were done using BOSS-3.4.[32] Finally, Poisson−Boltzmann calculations were carried out using DelPhi.[27] All calculations were performed on the SP2 computer of the Centre de Supercomputació de Catalunya (CESCA), as well as on workstations in our laboratory.

Results and Discussion

The HF/6-311+G(d,p) optimized geometries of the most stable tautomers of 1-methyluracil (U), 1-methylthymine (T), and 1-methy-l,5-bromouracil (Br-U) are shown in Figure 5. The structural parameters for U and T are found to be almost identical, and they are also very similar to those of Br-U. This suggests that the presence of the methyl and bromine substituents in position 5 does not change sensibly the electronic distribution in the pyrimidine ring, which allows us to expect small differences in the chemical properties of the three bases.

The tautomerization energies, enthalpies, and free energies for U, T, and Br-U are given in Table 1. The results show clearly that the oxo form is the most stable tautomer in the gas phase in all cases. The difference in stability with regard to the other tautomers is so large as to guarantee that the oxo tautomer N3 is the only important tautomer in the gas phase, as previously suggested both experimentally[33] and theoretically[34] for similar systems. It is also clear that entropic effects play a minor role in the oxo−enol tautomerism. The results also reveal the importance of electron correlation effects for a quantitative description of the oxo−enol tautomerism.[17,21,34] Thus, electron correlation stabilizes by 2−3 kcal/mol the enol forms for the three molecules. This finding mimics the trends found in other systems (see, for example, ref 17a). However, comparison of

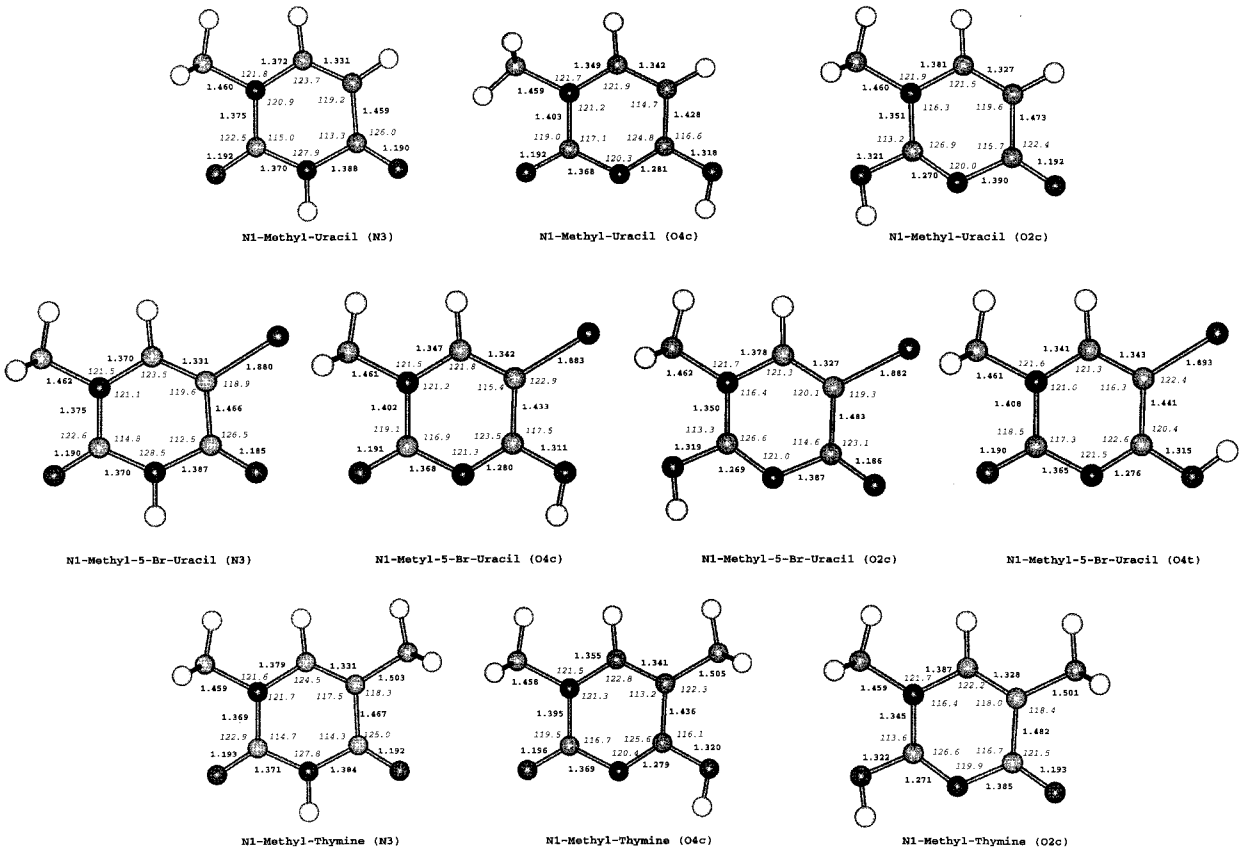

Figure 5. Optimized structures of the different species considered in this study. Bond lengths are in angstroms, and angles are in degrees.

TABLE 1: Themodynamic Quantities (kcal/mol) for Selected Tautomers of 1-Methyl Derivatives of Uracil, 5-Bromouracil, and Thymine in the Gas Phase[a]

		uracil			5-bromouracil			thymine		
tautomer	level	ΔE	ΔH	ΔG	ΔE	ΔH	ΔG	ΔE	ΔH	ΔG
O4c	SCF	13.2	13.1	13.7	13.8	13.7	14.3	14.2	14.1	14.9
	MP2	10.0	9.9	10.5	10.8	10.6	11.2	11.0	10.9	11.7
	MP4(SDTQ)	10.7	10.6	11.2	11.5	11.3	11.8	11.8	11.6	12.5
	QCISD	11.0	10.9	11.5						
	QCISD(T)	10.2	10.3	10.9						
O2c	SCF	20.7	20.6	20.7	19.4	19.3	19.4	22.2	22.1	22.3
	MP2	18.2	18.1	18.2	17.0	16.9	17.0	17.8	17.6	17.8
	MP4(SDTQ)	18.7	18.6	18.7	17.5	17.4	17.5	18.3	18.1	18.4
	QCISD	18.6	18.5	18.6						
	QCISD(T)	18.1	18.0	18.1						
O4t	SCF				19.4	19.1	19.6			
	MP2				15.6	15.3	15.8			
	MP4(SDTQ)				16.1	15.8	16.3			

[a] Calculations were performed at the ab initio level using a 6-311+G(d,p) basis set and the HF/6-311+G(d,p) optimized geometry (see Methods). All the values given relative to the N3 tautomer.

MP2, MP3 (data not shown), MP4, QCISD, and QCISD(T) results demonstrate that the energy values are reasonably well converged even at the MP2 level, in disagreement with the situation found for other tautomeric processes, where large levels of correlation are necessary to obtain converged results (see, for example, ref 17c).

The tautomerization free energies in the gas phase are similar for the three molecules and are also similar to the results for related molecules.[34] In all cases the tautomer O4c is the most stable enol form, whereas the species O2c is disfavored by 6−7 kcal/mol. It is worth noting that theoretical estimates of tautomerization enthalpy are clearly smaller than those suggested by calorimetric measures,[33c] which is probably due to the very large range of error of such experimental estimates.[33c,34b] The substituents at position 5 of U leads to changes in the free energy of tautomerization of around 1 kcal/mol or even less. In particular, attachment of bromine and methyl reduces the stability of tautomer O4c by 0.6 and 1.3 kcal/mol and increases that of O2c by 1.2 and 0.3 kcal/mol. Therefore, the intrinsic tautomeric preferences of U and T are not greatly affected upon inclusion of bromine at position 5. Indeed, the changes in relative stability due to the substituent does not change at all the preference of the oxo form in the gas phase.

The solvent effect on the tautomerism of U, T, and Br-U was analyzed by means of the use of standard thermodynamic cycles (see Methods) from SCRF-MST and MC-FEP calculations. The MC-FEP estimates of the relative hydration free energies were determined from the mutations shown in Figure 4. The results in Table 2 demonstrate the statistical goodness of the simulations, as noted in the hysteresis and standard errors

TABLE 2: Differences in Free Energy of Hydration (kcal/mol) between Tautomers of 1-Methyl Derivatives of Uracil, 5-Bromouracil, and Thymine[a]

mutation	ΔG	SE	HST
U(N3) → U(O4c)	−1.15	0.16	0.10
U(N3) → U(O2c)	−2.14	0.17	0.14
Br-U(N3) → Br-U(O4c)	−1.21	0.15	0.07
Br-U(N3) → Br-U(O2c)	−3.31	0.16	0.14
Br-U(N3) → Br-U(O4t)	−1.59	0.18	0.15
U(N3) → Br-U(N3)	+1.10	0.12	0.08
U(O4c) → Br-U(O4c)	+1.47	0.11	0.01
U(O2c) → Br-U(O2c)	+0.26	0.10	0.06
U(N3) → T(N3)	+1.45	0.07	0.05
U(O4c) → T(O4c)	+1.92	0.06	0.06
U(O2C)→ T(O2C)	+2.19	0.07	0.02

[a] Mutations were performed between tautomers of the same compound, as well as between different compounds in the same tautomeric form. SE: statistical error in the free energy difference. HST: hysteresis in the free energy difference.

TABLE 3: Absolute (ΔG_{hyd}) and Relative ($\Delta\Delta G_{hyd}$) Free Energy of Hydration and Tautomerization Free Energies (ΔG_t) in Water (kcal/mol) of Tautomers of 1-Methyl Derivatives of Uracil, 5-Bromouracil, and Thymine[a]

compd	tautomer	ΔG_{hyd}	$\Delta\Delta G_{hyd}$	$\Delta\Delta G_{hyd}$	ΔG_t
uracil	N3	−11.7	0.0	*0.0*	0.0
	O4c	−13.3	−1.6	*−1.2*	9.8
	O2c	−15.2	−3.5	*−2.1*	15.9
5-Br-U	N3	−12.1	0.0	*0.0*	0.0
	O4c	−12.5	−0.4	*−1.2*	11.0
	O2c	−14.9	−2.8	*−3.3*	14.5
	O4t	−15.5	−3.4	*−1.6*	13.8
thymine	N3	−13.0	0.0	*0.0*	0.0
	O4c	−14.3	−1.3	*−0.7*	11.5
	O2c	−16.1	−3.1	*−1.4*	16.2

[a] SCRF calculations were performed at the MST/6-31G(d) level. The $\Delta\Delta G_{hyd}$ values determined from MC-FEP results in Table 2 are given in italics. The ΔG_t values were computed by adding the MP4 results in the gas phase to the average solvent effect determined from MC-FEP and MST/6-31G(d) calculations.

in the free energy values (less than 0.2 kcal/mol in all cases). An additional test of the quality of the simulations is provided by closure thermodynamic cycles (Figure 4). Thus, the cycle Br-U(O2c) → U(O2c) → U(N3) → Br-U(N3) → Br-U(O2c) is closed with an error of 0.3 kcal/mol, and the cycle U(N3) → Br-U(N3) → Br-U(O4c) → U(O4c) → U(N3) is closed with an error of 0.4 kcal/mol. Finally, the most challenging cycle U(O4c) → Br-U(O4c) → Br-U(N3) → Br-U(O2c) → U(O2c) → U(N3) → U(O 4c) is closed with a negligible error of 0.1 kcal/mol.

The values of $\Delta\Delta G_{hyd}$ are given in Table 3. There is general agreement between SCRF-MST and MC-FEP results, which is remarkable considering the methodological differences between the two techniques. Thus, the rms and the average deviation between MST and MC-FEP estimates of $\Delta\Delta G_{hyd}$ are only 1.1 and 1.0 kcal/mol, respectively. Enol forms are better hydrated than the corresponding oxo tautomers, even though the differences are not very large. The tautomer O2c is more stabilized by water than O4c by 1−2 kcal/mol in all cases. The presence of substituents (methyl, bromine) at position 5 does not affect greatly the relative hydration of the different tautomers, the largest changes being around 1 kcal/mol.

The free energy of tautomerization in water was determined by adding the average value of MST-SCRF and MC-FEP relative free energies of hydration to the differences in free energy of tautomerization in the gas phase (computed at the MP4 level). The results in Table 3 indicate that solvation does not change sensibly the gas-phase preference between tautomers and that the oxo form is the only relevant species in water. Our results agree qualitatively well with the experimental evidence, which show that the enol forms of uracil derivatives are very minor in water.[35] However, the free energy differences found here are larger (in absolute values) than the values derived from acidity measurements[35b,35c] or Hammet constants.[35b]

The results in Table 3 also indicate that the presence of substituents at position 5 does not increase the stability of enol forms in water. On the contrary, the free energy difference between oxo and enol forms in water is larger for Br-U and T than for U. This finding does not support early suggestions derived from basicity measurements, which suggested that enol tautomers of Br-U were around 1−2 kcal/mol more stable than those of U,[35b] but agree well with recent experiments that show no evidence of enol species for the brominated derivates of uridine.[4,13] In summary, the results point out that the mutagenic properties of Br-U do not seem to obey to a greater population of enol forms in aqueous solution.

TABLE 4: Free Energy Changes (kcal/mol) for Replacement of the Oxo and Enol Tautomers of 1-Methyluracil by the Corresponding Forms of 1-Methylthymine and 1-Methyl-5-bromouracil in Two DNA Sequences

mutation	$d(A_{10})$	$d(A_5GA_4)$
U(N3) → T(N3)	1.1	
U(N3) → Br-U(N3)	1.1	
U(O4c) → T(O4c)		1.0
U(O4c) → Br-U(O4c)		1.1

The influence of the DNA environment on the tautomerism of U, T, and Br-U was explored using Poisson−Boltzmann calculations, which allowed us to estimate the difference in solvation free energy between water and the DNA.[36] Only the oxo N3 and O4c enol tautomers were considered, and the DNA fragments $d(A_{10})$ and $d(A_5GA_4)$ were used for the oxo and enol tautomers, respectively (see Methods).

The mutation of the oxo tautomer N3 of U to the corresponding species of either T or Br-U leads to a free energy change of 1.1 kcal/mol. These results show that the effect of the duplex DNA environment on the three molecules is very similar and suggest that a piece of DNA of sequence $d(A_{10})$ should be equally stable irrespective of the base (U, T, or 5-Br-U) hydrogen-bonded to the sixth adenine. The corresponding mutations for the enol tautomer O4c gives free energy differences of 1.0 kcal/mol in d(A5GA4) (U → T) and 1.1 kcal/mol in d(A5GA4) (U → Br-U). This indicates that the shift in the stability of the enol tautomer on going from water to the duplex DNA is similar for the three compounds and allows us to envisage that a fragment of duplex DNA made with the enol forms of thymidine, uridine, or 5-bromouridine paired with guanine would have similar stability. Overall, the results in Table 4 point out that attachment of methyl or bromine at position 5 of uracil has a small effect on the ratio between oxo and enol tautomers in the DNA environment.

In summary, the results indicate that the enol tautomers of uracil, thymine, and 5-bromouracil are intrinsically unstable in the gas phase and that neither solvation in water nor solvation in the duplex DNA environment justifies large changes in the ordering of stability between tautomers. Moreover, the results point out that the differences in the tautomeric preference of uracil, thymine, and 5-bromouracil are small.

The whole of the results strongly argues against the hypothesis that the existence of enol tautomers of 5-bromouracil is the origin of its mutagenic properties.[2] This finding confirms recent results from NMR analysis, which were not able to indicate

the presence of the enol tautomer for the 5-bromoracil bound to guanine.[4] On the contrary, our results indirectly favor the alternative hypothesis, which implies that the formation of complexes between guanidine and 5-bromouridine occurs after ionization of this latter compound.[3,4] At this point, it is worth noting that the experimental pK_a (in water) of 5-bromouracil is suggested to be around 8,[13b,35b] i.e., around 1.5 units below the pK_a of uracil.[1,13a,35a] The larger acidity of 5-bromoracil compared with that of the unsubstituted compound is also observed in the gas phase, since calculations (data not shown) at the MP4/6-311+G(d,p) level indicate that the ionization of 5-bromouracil is 7 kcal/mol easier than that of uracil, and preliminary PB calculations suggest that the same situation might occur in the DNA.[37] The preceding discussion provides a basis to suggest that the mutagenic properties of 5-bromouridine stems from its ability to lose a proton at N3 rather than from its tendency to form enol tautomers.

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References and Notes

(1) (a) Blackburn, G. M. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. N., Gait, M. J., Eds.; IRL Press: Oxford, 1990; Chapter 2. (b) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1988. (c) Sinden, R. R. In *DNA Structure and Function*; Academic Press: San Diego, 1994.

(2) Topal, M. D.; Fresco, J. R. *Nature* **1976**, *263*, 285.

(3) Sowers, L. C.; Fazakerley, G. C.; Eritja, R.; Kaplan, B. E.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5434.

(4) Sowers, L. C.; Goodman, M. F.; Eritja, R.; Kaplan, B. E.; Fazakerley, G. C. *J. Mol. Biol.* **1989**, *205*, 437.

(5) Goodman, N. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10493.

(6) (a) Piccirilli, J. A.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1991**, *30*, 10350. (b) Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33. (c) Benner, S. A.; Ellington, A. D.; Tauer, A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7054.

(7) Söll, D.; Rajbhandary, U. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. N., Gait, M. J., Eds.; IRL Press: Oxford, 1990; Chapter 6.

(8) McCloskey, J. A.; Nishimura, S. *Acc. Chem. Res.* **1977**, *10*, 403.

(9) Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863.

(10) Danenberg, P. V.; Heidelberger, C.; Mulkins, M. A.; Peterson, A. R. *Biochem. Biophys. Res., Commun.* **1981**, *102*, 617.

(11) Moran, S.; Ren, R.; Kool, E. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10506.

(12) Yu, H.; Eritja, R.; Bloom, L. B.; Goodman, M. F. *J. Biol. Chem.* **1993**, *268*, 15935.

(13) (a) Sowers, L. C.; Eritja, R.; Kaplan, B. E.; Goodman, M. F.; Fazakerley, G. V. *J. Biol. Chem.* **1987**, *262*, 15436. (b) Fazakerley, G. V.; Sowers, L. C.; Eritja, R.; Kaplan, B. E.; Goodman, M. F. *J. Biomol. Struct. Dyn.* **1987**, *5*, 639.

(14) McLean, A. D.; Chandler, G. S. *J. Chem. Phys.* **1980**, *72*, 5639.

(15) Möller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.

(16) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1978**, *28*, 213.

(17) (a) Alhambra, C.; Luque, F. J.; Estelrich, J.; Orozco, M. *J. Org. Chem.* **1995**, *60*, 969. (b) Colominas, C.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **1996**, *118*, 6811. (c) Luque, F. J.; Lopez-Bes, J. M.; Cemeli, J.; Aroztegui, M.; Orozco, M. *Theor. Chem. Acc.* **1997**, *96*, 105.

(18) Frisch, M. J.; Trucks, G. W.; Schelgel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, G. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.,; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian-94*, Revision D.3; Gaussian Inc.: Pittsburgh, PA, 1995.

(19) (a) Bachs, M.; Luque, F. J.; Orozco, M. *J. Comput. Chem.* **1994**, *15*, 446. (b) Luque, F. J.; Bachs, M.; Orozco, M. *J. Comput. Chem.* **1994**, *15*, 847. (c) Orozco, M.; Bachs, M.; Luque, F. J. *J. Comput. Chem.* **1995**, *16*, 563.

(20) (a) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117. (b) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, 65, 239.

(21) (a) Orozco, M.; Luque, F. J. *J. Am. Chem. Soc.* **1995**, *117*, 1378. (b) Hernandez, B.; Luque, F. J.; Orozco, M. *J. Org. Chem.* **1996**, *61*, 5964. (c) Hernandez, B.; Orozco, M.; Luque, F. J. *J. Comput. Aided Mol. Des.* **1996**, *10*, 535. (d) Hernandez, B.; Orozco, M.; Luque, F. J. *J. Comput. Aided Mol. Des.* **1997**, *11*, 153.

(22) Zwanzig, R. W. *J. Chem. Phys.* **1954**, *22*, 1420.

(23) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.

(24) (a) Bonaccorsi, R.; Scrocco, E.; Petrongolo, C.; Tomasi, J. *Theor. Chim. Acta* **1971**, *20*, 331. (b) Momany, F. A. *J. Phys. Chem.* **1978**, *82*, 592. (c) Orozco, M.; Luque, F. J. *J. Comput. Aided Mol. Des.* **1990**, *4*, 31.

(25) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810.

(26) Determination of Br van der Waals parameter was done by fitting HF/6-31G(d) and classical energy profiles for the approach of a water molecule to the CH_3Br molecule. SCF values were corrected for basis set superposition error using the counterpoise method. A total of 15 configurations for the dimer, corresponding to different Br−O distances, were selected. The error in the location of the minima was 0.0 Å (in Br−O distance), the error at the minimum was 0.1 kcal/mol, and the rms error in the fitting was 0.5 kcal/mol.

(27) (a) Gilson, M. K.; Honig, P. H. *Nature* **1987**, *330*, 84. (b) Gilson, M. K.; Honig, P. H. *Proteins* **1988**, *4*, 7. (c) *DELPHI* (computer program); Biosym Co.: San Diego, 1994.

(28) Arnott, S.; Hukins, D. W. L. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 1504.

(29) Peterson, M.; Poirier, R. *MONSTERGAUSS*; Department of Chemistry, University of Toronto: Canda (version modified by Cammi, R.; Bonaccorsi, R.; Tomasi, J. in 1987 and by Luque, F. J.; Orozco, N. in 1995).

(30) Dupuis, M.; Farazdel, A.; Karma, S. A.; Maluendes, S. *HONDO-8, IBM Corporation Scientific and Engineering Computations*; IBM Corporation: Kingston, NY (modified by Cossi, M.; Cammi, R.; Tomasi, J. in 1994).

(31) Luque, F. J.; Orozco, M. *MOPETE/MOPFIT* (computer programs); University of Barcelona: Spain, 1998.

(32) Jorgensen, W. L. *BOSS 3.4* (computer program); Yale University: New Haven, CT, 1993.

(33) (a) Green, D. W.; Mathews, F. S.; Rich, A. *J. Biol. Chem.* **1962**, *237*, 3573. (b) Shugar, D.; Szczepaniak, J. *Int. J. Quantum Chem.* **1981**, *20*, 573. (c) Beak, P.; White, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 7073. (d) Fujii, M.; Tamura, T.; Mikami, N.; Ito, M. *Chem. Phys. Lett.* **1986**, *126*, 583. (e) Brady, B. H.; Peteanu, L. A.; Levy, D. H. *Chem. Phys. Lett.* **1986**, *126*, 583. (f) Tsuchiya, Y.; Tamura, T.; Fujii, M.; Ito, M. *J. Phys. Chem.* **1988**, *92*, 1760. (g) Zwierzchowska, Z.; Dobrosz-Teperek, K.; Lewandowski, W.; Kolos, R.; Bajdor, K.; Dobrowolski, J. C.; Mazurek, A. P. *J. Mol. Struct.* **1997**, *410*, 415.

(34) (a) Leszcynski, J. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1991**, *18*, 9. (b) Leszcynski, J. *J. Phys. Chem.* **1992**, *96*, 1649 and references therein. (c) Boughton, J. W.; Pulay, P. *Int. J. Quantum Chem.* **1993**, *47*, 49. (d) Gould, I. R.; Burton, N. A.; Hall, R. J.; Hillier, I. H. *J. Mol. Struct.: THEOCHEM* **1995**, *331*, 147. (e) Marino, T.; Russo, N.; Toscano, M. *Int. J. Quantum Chem.* **1997**, *62*, 459.

(35) (a) Marshall, J. R.; Walker, J. *J. Chem. Soc.* **1951**, 1005. (b) Katritzky, A. R.; Waring, A. J. *J. Chem. Soc.* **1962**, 1540. (c) Poulter, C. D.; Frederick, G. D. *Tetrahedron Lett.* **1975**, 2171.

(36) Poisson Boltzman calculations give only the electrostatic contribution to the solvation free energy. PB is probably less precise than MC-FEP or MST calculations owing to the use of a rigid geometry and a rigid and oversimplified expression of the solute charge distribution. It is not simple to compare PB with MC-FEP or MST calculations. But as a reference, the electrostatic contribution to the free energy of solvation computed from PB calculations for the N3 forms considered here ranges from −22 to −24 kcal/mol ($\epsilon = 1$ for solute) and from −12 to −13 kcal/mol ($\epsilon = 2$ for the solute). The equivalent MST estimates range between −15 and −17 kcal/mol.

(37) Orozco, M.; Hernandez, B.; Luque, F. J. To be published.