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*New Concepts*

# Mutagenic Mechanism of the A-T to G-C Transition Induced by 5-Bromouracil: An ab Initio Study†

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ABSTRACT: The tautomerisms of uracil, 5-bromouracil (BrU), G-U, G-BrU, A-U, and A-BrU have been studied theoretically in an effort to investigate the mutagenicity of BrU. The ab initio calculations have been performed using HF and B3LYP methods with various basis sets. The relative stability of all tautomers was established. The intermolecular interactions between U, BrU, U*, BrU* (asterisks denote enol forms), and water have been studied. It shows that the possibility of tautomerism from BrU to BrU* is much more likely than that from U to U*. Further research indicates that BrU* tends to pair with guanine more easily than U*. The proton transfer process has been investigated by potential energy surface (PES) scan and transition state analysis. The results show that the proton transfer between G-U* and G*-U is monodirectional barrier-free proton transfer (BFPT), which terminates the base mismatch induced by U*. On the other hand, the proton transfer between G-BrU* and G*-BrU is bidirectional BFPT, which makes the base mismatch induced by BrU* sustained. On the basis of all of these calculated results, a new mutagenic mechanism for the A-T to G-C transition induced by 5-bromouracil is described in detail for the first time. It might give a new insight into the origin of the mutagenicity of the 5-Br derivative.

Generally, the canonical nucleic acid bases (adenine, guanine, thymine, uracil, and cytosine) exist as the main form in the double helix. The formation of specific purine−pyrimidine Watson−Crick hydrogen bonds is responsible for the maintenance of the genetic code. If a base is replaced with another kind of base, it may lead to the introduction of a wrong genetic code. The ability of nucleic acids to accommodate noncanonical hydrogen bonds has been related to the occurrence of spontaneous mutations in the DNA (*1*−*3*). Recently, a noncanonical hydrogen bond and DNA mismatch have received much attention (*4*−*6*).

Topal and Fresco raised the hypothesis that the existence of enol tautomers of 5-bromouracil is the origin of its mutagenic property (*1*). According to this hypothesis, 5-bromouracil will induce or enable G-C[1] to A-T or A-T to G-C transitions. In the past years, there has been a breakthrough

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[1] Abbreviations: A, adenine; T, thymine; G, guanine; C, cytosine; U, uracil; BrU, 5-bromouracil; U*, enol form tautomers of uracil; BrU*, enol form tautomers of 5-bromouracil; G*, enol form tautomers of guanine; Br, bromine; PES, potential energy surface; BFPT, barrier-free proton transfer; ZPE, zero-point energy; NBO, natural bond orbital; HF, Hartree−Fock; B3LYP, Becke 3 term with Lee, Yang, Parr exchange; TS, transition state.

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Table 1: Free Energy Changes (kilojoules per mole) and Equilibrium Constants of Base Repairing[a]

| | HF/STO-3G | HF/3-21G* | HF/6-31G* | B3LYP/6-31++G(d, p) | references | $K_{eq}$[d] |
|---|---|---|---|---|---|---|
| U* → U | −30.88 | −78.62 | −63.77 | −49.45 | −18.6[b] | $4.61 \times 10^8$ |
| BrU* → BrU | −33.09 | −82.02 | −66.20 | −48.85 | −17.5[c] | $3.61 \times 10^8$ |
| U*-W1 → U-W1 | −12.41 | −57.02 | −52.99 | −37.96 | | $4.47 \times 10^6$ |
| BrU*-W1 → BrU-W1 | −15.27 | −59.73 | −55.35 | −37.42 | | $3.60 \times 10^6$ |
| U*-W2 → U-W2 | −32.83 | −87.26 | −88.00 | −57.50 | | $1.19 \times 10^{10}$ |

[a] All values include ZPE and thermodynamic corrections at 298.15 K and 1.0 atm. [b] Value calculated at the QCISD level of theory (*24*). [c] Value calculated at the MP4(SDTQ) level of theory (*24*). [d] The equilibrium constants were calculated at the B3LYP/6-31++G(d,p) level of theory.

in the experimental study of the mutagenicity of 5-bromouracil, which has been developed in three directions. The first direction concentrated on the structure of the BrU-G mismatch. Three models of BrU-G mispairs (tautomerization, wobble, and ionization) had been reported (*7*). The stability of these models had also been established (*8*). The NMR study showed that the BrU-G mismatch exists in a pH-dependent equilibrium between ionized and wobble structures (*3*, *9*), while BrU-A involves pH-independent Watson−Crick structures (*10*, *11*). The RNA helix G-U mismatch has also been observed in wobble form (*4*, *12*). The second one focused on the basis for the mutagenicity of BrU. BrU binds guanine with great efficiency, and this feature has been suggested to be the basis for its mutagenicity (*3*, *7*). Results of the electrophoretic assay of misincorporation were also consistent with a mispairing mechanism for template BrU wherein the anionic form of the base mispairs with G (*13*). Other research showed that 5-bromouracil is a thymine analogue that can be incorporated into DNA and 5-bromouracil is a well-established mutagen that mispairs with guanine (*14*). The third direction is related to the source of 5-bromouracil and found that HOBr generated by eosinophil peroxidase oxidizes uracil to 5-bromouracil (*15*).

Recently, an ab initio method has become a powerful tool for studying the base pairs (*16*−*18*) and DNA mutations (*19*). The interactions between uracil and water have also been studied extensively using the quantum chemical method with academic interests, including the effect of water molecules located at different sites in the vicinity of uracil (*20*, *21*), various numbers of waters from 1 to 7 (*22*), square and cube models of the interaction (*23*), etc. However, as far as we know, there are no other theoretical works about the mutagenicity of 5-bromouracil except that of Orozco (*24*). Orozco's calculated results showed that the ketone form of BrU was more stable than the enol tautomers in either the gas phase or aqueous solution, so Orozco argued against Topal and Fresco's hypothesis.

However, our calculations provide support for Topal and Fresco's hypothesis. The work presented here focused on the intermolecular interactions between base and water and between base and base as well as the proton transfer processes in the tautomerisms. This study will lead us to (i) compare the possibility of tautomerism from BrU to BrU* with that from U to U*, (ii) compare the ability of BrU* pairing G with that of U* pairing G, and (iii) compare the tautomerisms from G-BrU* to G*-BrU with that from G-U* to G*-U. The calculated results provide a picture on how 5-bromouracil induces the transition from A-T to G-C directly. By comparison with the inducing capability of U*, our results give new insights into the reasons for the mutagenicity of the 5-Br derivative.

## COMPUTATIONAL METHODS

The structures of the nucleic acid bases and their enol tautomers have been optimized at the HF/STO-3G, HF/3-21G*, HF/6-31G*, and B3LYP/6-31++G(d,p) levels of theory. Energy, frequency calculation, and zero-point energy (ZPE) were determined using the same theory. The natural bond orbital (NBO) analysis was used for a better understanding of the nature of corresponding intermolecular interactions (*25*). The potential energy surface (PES) scan was performed to study the potential energy surface of the proton transfer processes.

The computed stationary points were characterized as minima or transition states by diagonalizing the Hessian matrix and analyzing the vibrational normal modes. In this way, the stationary points can be classified as minima if no imaginary frequencies are shown or as transition states if only one imaginary frequency is obtained (*26*). The particular nature of the transition states has been determined by analyzing the motion described by the eigenvector associated with the imaginary frequency. The NBO second-order perturbation stabilization energy $\Delta E_2$ is calculated as

$$\Delta E_2 = \Delta E_{ij}^{(2)} = 2\frac{|\langle \Phi_i|\hat{F}|\Phi_j\rangle|^2}{\epsilon_i - \epsilon_j}$$

where $\hat{F}$ is the Fock operator and $\epsilon_i$ and $\epsilon_j$ correspond to the energy eigenvalues of the donor molecular orbital $\Phi_i$ and the acceptor molecular orbital $\Phi_j$, respectively (*25*). The equilibrium constant $K_{eq}$ is provided by a statistical thermodynamic treatment (*27*) with

$$K_{eq} = \exp(-\Delta_r G/RT)$$

All calculations have been performed with the Gaussian 98 suite of packages (*28*).

## RESULTS AND DISCUSSION

It is useful to remember here that reversion frequencies have been employed to express the levels of mutation (*1*, *29*). Simultaneously, energy calculations have also been adopted to investigate spontaneous DNA mutations (*5*, *19*, *30*). In this paper, three factors are taken into consideration in deciding whether the bromine substituent at position 5 of U leads to the mutation from A-T to G-C.

### *The Tautomeric Reaction from BrU to BrU* Is Easier than That from U to U**

The relative free energies of the isolated base tautomerism from the enol form to the keto form are listed in the top part of Table 1. These results are calculated at the HF/STO-3G,

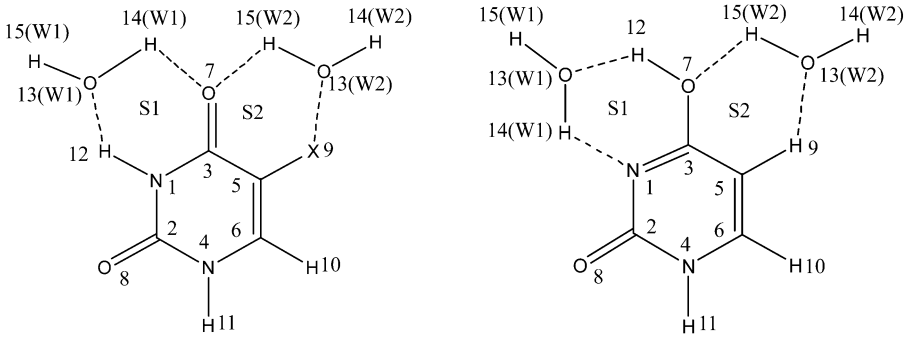


FIGURE 1: Two different sites of water molecules in the vicinities of uracil, 5-bromouracil, and their enol forms. S1 and S2 are the sites for water; W1 and W2 are the water molecules located in the corresponding region.

HF/3-21G*, HF/6-31G*, and B3LYP/6-31++G(d,p) levels of theory, which show clearly that the keto form is the most stable tautomer at all levels of theory. This is in agreement with Orozco's theoretical study (*24*). In fact, many structural features that are necessary for the biological functions of nucleic acids depend on the interactions with surrounding water. For this reason, we also adopted free energy change to investigate the role of water in the tautomerism process. In the vicinities of the uracil and 5-bromouracil, two different sites for water molecules (S1 and S2 in Figure 1) were taken into consideration. All the calculation results are listed in the bottom part of Table 1. It can be concluded that even though the interaction with water is considered, it does not change the main form of uracil and 5-bromouracil. Our results agree qualitatively well with the experimental evidence, which show that the enol forms of uracil derivatives are present in a minority population in water (*31*, *32*).

Calculated results in Table 1 show that the free energy change in the process of isolated U* repaired to U is almost the same as that for the transition from BrU* to BrU at all levels of theory. However, it is worth noticing that water does affect the stability of U* and BrU*. How the water on different sites affects the tautomerism process was studied in detail.

*Single Water in the Region of S1*. W1 was located in the vicinity of S1. It is well-known that water is a highly polar molecule that can be an H-bond acceptor and donor simultaneously. In the tautomerism from U*-W1 to U-W1, water accepts the hydrogen atom of the O7−H12 bond from U*, and at the same time, it donates its hydrogen atom to U* which is accepted by N1. As the results in Table 1 show, water located in S1 decreases the free energy change from U* to U by 11.49 kJ/mol at the B3LYP/6-31++G(d,p) level of theory. That is to say, W1 makes U* more stable than isolated U*. Similarly, in the tautomerism from BrU*-W1 to BrU-W1, W1 plays the same role and decreases the free energy change from BrU* to BrU by 11.43 kJ/mol at the B3LYP/6-31++G(d,p) level of theory. The NBO (natural bond orbital) analyses give us a better understanding of the role of W1 (Table 2). The calculated results in Table 2 show some main stabilization energy between the host molecule and water. For U*-W1 and BrU*-W1, the $n \rightarrow \sigma^*$ interaction between the O13 lone pair and the antiperiplanar O7−H12 antibond is seen to give the strongest stabilization. When U*-W1 with BrU*-W1 are compared, there is almost no difference between the $n(N1) \rightarrow \sigma^*[O13_{(W1)}-H14_{(W1)}]$ and $\sigma[O13_{(W1)}-H14_{(W1)}] \rightarrow \sigma^*(O7-H12)$ transitions. Even though U*-W1 and BrU*-W1 are repaired to U-W1 and BrU-W1, respectively, the water (W1) affects them in the same way.

Table 2: Some Significant Donor−Acceptor Natural Bond Orbital Interactions of U, BrU, U*, BrU*, and W1 and Their Second-Order Perturbation Stabilization Energies (kilojoules per mole)[a]

| donor | acceptor | interactions | $\Delta E_2$ (kJ/mol) |
|---|---|---|---|
| | From U to W1 | | |
| LP(1) O7 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 11.72 |
| LP(2) O7 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 34.31 |
| | From W1 to U | | |
| BD(1) O13−H14 | BD*(1) N1−H12 | $\sigma \rightarrow \sigma^*$ | 3.72 |
| LP(2) O13 | BD*(1) N1−H12 | $n \rightarrow \sigma^*$ | 46.23 |
| | From BrU to W1 | | |
| LP(1) O7 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 11.13 |
| LP(2) O7 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 29.71 |
| | From W1 to BrU | | |
| BD(1) O13−H14 | BD*(1) N1−H12 | $\sigma \rightarrow \sigma^*$ | 3.81 |
| LP(2) O13 | BD*(1) N1−H12 | $n \rightarrow \sigma^*$ | 50.08 |
| | From U* to W1 | | |
| LP(1) N1 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 49.54 |
| | From W1 to U* | | |
| BD(1) O13−H14 | BD*(1) O7−H12 | $\sigma \rightarrow \sigma^*$ | 8.16 |
| LP(2) O13 | BD*(1) O7−H12 | $n \rightarrow \sigma^*$ | 104.3 |
| | From BrU* to W1 | | |
| LP(1) N1 | BD*(1) O13−H14 | $n \rightarrow \sigma^*$ | 40.96 |
| | From W1 to BrU* | | |
| BD(1) O13−H14 | BD*(1) O7−H12 | $\sigma \rightarrow \sigma^*$ | 8.49 |
| LP(2) O13 | BD*(1) O7−H12 | $n \rightarrow \sigma^*$ | 115.4 |

[a] NBO analysis was performed at the B3LYP/6-31++G(d,p) level of theory. BD denotes the occupied bond orbital, and BD* denotes the formally empty antibonding orbital. LP denotes the occupied lone pair.

*Single Water in the Region of S2.* In the region of S2, W2 plays a role completely different from that of W1. In the interaction between U* and W2, water acts as an H-bond donor as well as a weak H-bond acceptor, which is proven by NBO analysis (Table 3). Because the formation of of the $O7\cdots H15_{(W2)}-O13_{(W2)}$ hydrogen bond changes the electron density around O7, the repair process from U*-W2 to U-W2 becomes easier than that from U*-W1 to U-W1. This has been proven by the increment of the free energy change of 19.54 kJ/mol at the B3LYP/6-31++G(d,p) level of theory. The equilibrium constant for the tautomerism from U*-W2 to U-W2 is $2.66 \times 10^3$ times larger than that from U*-W1 to U-W1 (Table 1). Videlicet, the water located in the position of S2 can protect U from tautomerizing to U*.

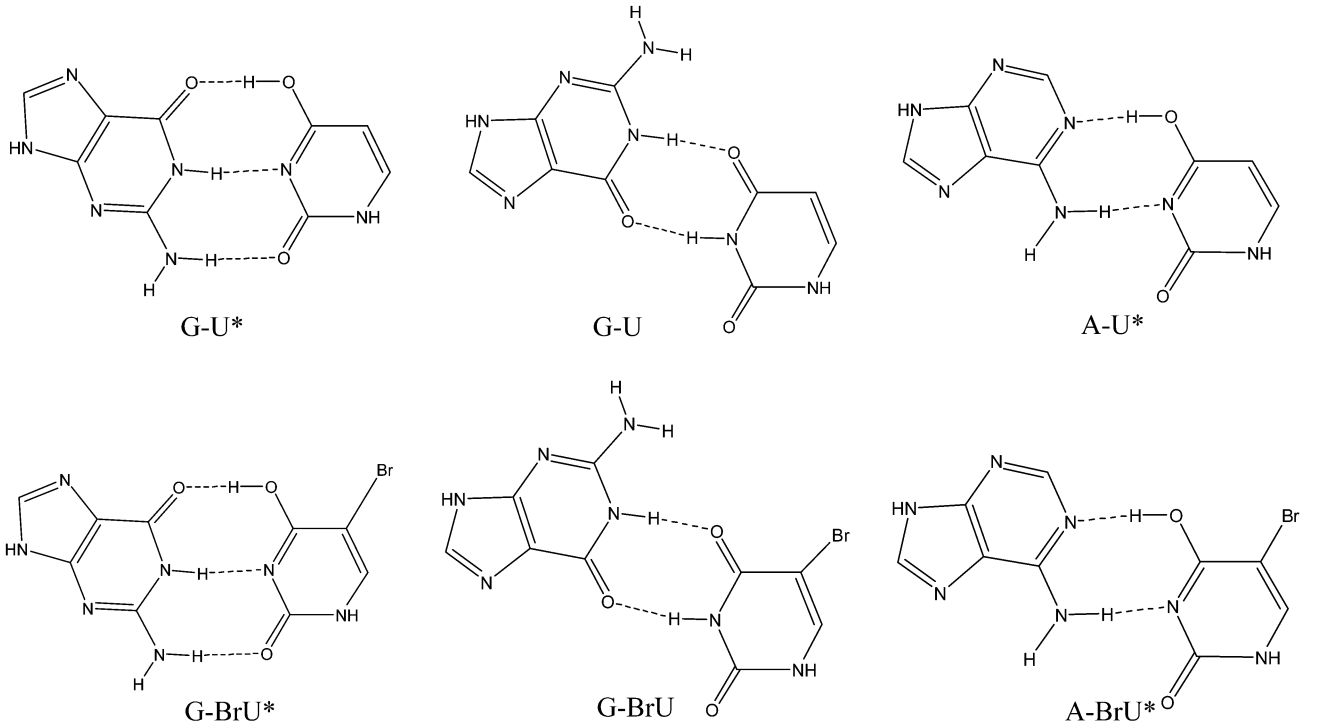


FIGURE 2: Structures of noncanonical base pairs.

Table 3: Some Significant Donor−Acceptor Natural Bond Orbital Interactions of U, BrU, U*, BrU*, and W2 and Their Second-Order Perturbation Stabilization Energies (kilojoules per mole)[a]

| donor | acceptor | interactions | $\Delta E_2$ (kJ/mol) |
|---|---|---|---|
| | From U to W2 | | |
| LP(1) O7 | BD*(1) O13−H15 | n → σ* | 11.84 |
| LP(2) O7 | BD*(1) O13−H15 | n → σ* | 37.74 |
| | From W2 to U | | |
| LP(2) O13 | BD*(1) C5−H9 | n → σ* | 4.94 |
| | From BrU to W2 | | |
| LP(1) O7 | BD*(1) O13−H15 | n → σ* | 16.02 |
| LP(2) O7 | BD*(1) O13−H15 | n → σ* | 16.4 |
| | From W2 to BrU | | |
| LP(1) O13 | BD*(1) C3−O7 | n → σ* | 0.50 |
| | From U* to W2 | | |
| LP(1) O7 | BD*(1) O13−H15 | n → σ* | 16.61 |
| | From W2 to U* | | |
| LP(2) O13 | BD*(1) C5−H9 | n → σ* | 6.61 |
| | From BrU* to W2′ | | |
| LP(1) O7 | BD*(1) O13−H15 | n → σ* | 11.21 |
| | From W2′ to BrU* | | |
| LP(1) O13−H14 | BD*(2) C3−O7 | σ → σ* | 0.67 |

[a] NBO analysis was performed at the B3LYP/6-31++G(d,p) level of theory. BD denotes the occupied bond orbital, and BD* denotes the formally empty antibonding orbital. LP denotes the occupied lone pair.

If position 5 of U is substituted with bromine, there will be an essential difference. In Figure 3, the total charge density surfaces of U, U*, BrU, BrU*, and $H_2O$ are displayed. It is clearly shown that the bromine substitution at position 5 of U introduces a negative charge center around position 5. It can incorporate with the negative charge center around O7 to give birth to an extremely strong negative charge region in BrU and BrU* (Figure 3). Therefore, it is more difficult for a water molecule to enter the region of S2, which can also be attested by geometry optimization (Figure 4) and

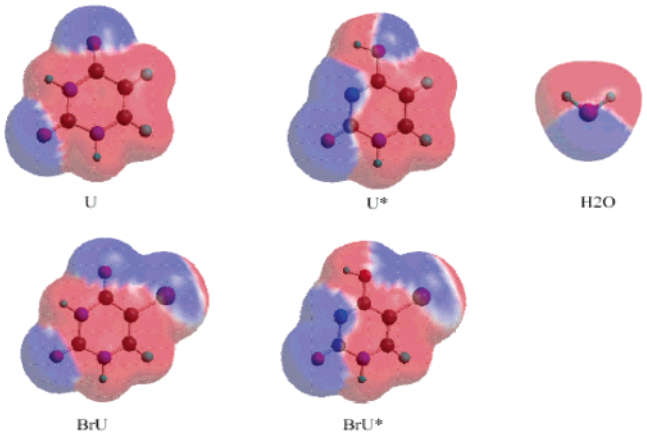


FIGURE 3: Total charge density surfaces of U, U*, BrU, BrU*, and $H_2O$ calculated at the B3LYP/6-31++G(d,p) level of theory. Red means positive and blue negative.

NBO analysis (Table 3) at the B3LYP/6-31++G(d,p) level of theory. When we located a water molecule in the S2 position of BrU and BrU*, it is interesting to find that the water molecule always departs from the region of S2 in the optimization process. It indicates that BrU*-W2 is very unstable. Geometry optimization results show that another spatial position (W2′) near W2 is more appropriate for the water molecule to interact with BrU*; however, the interaction between W2′ and BrU* is very weak (Table 3).

As discussed above, the water located in the region of S1 can catalyze the tautomerism from U to U*, whereas the water located in the region of S2 can protect U from tautomerizing to U*. The bromine substitution at position 5 of U will lead to the loss of the protection induced by water in the region of S2. Hence, the possibility of tautomerism from BrU to BrU* is much more likely than that of U to U*.

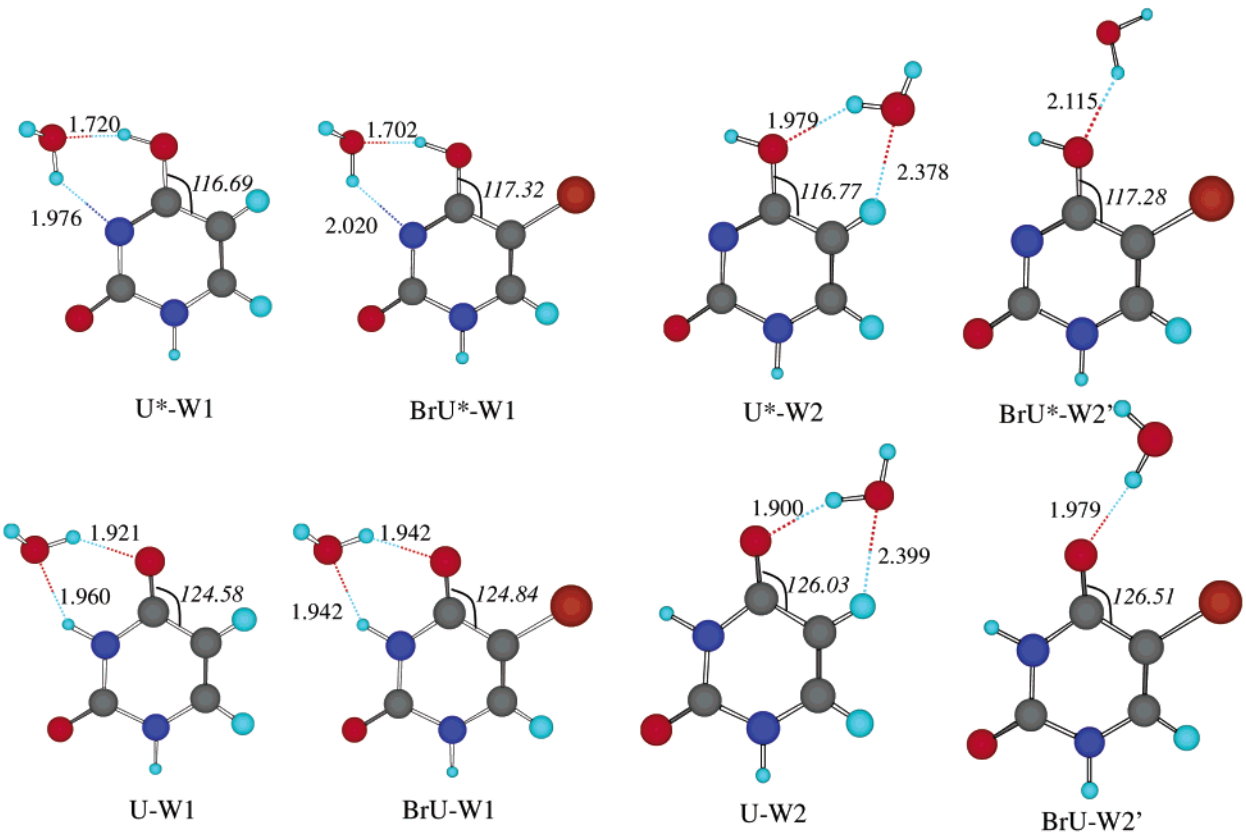


FIGURE 4: Optimized structures of U-W1, U-W2, UBr-W1, UBr-W2′, and their tautomeric forms, calculated at the B3LYP/6-31++G(d,p) level of theory. The numbers refer to computed H-bond distances and intramolecular angles.

Table 4: Energy Changes (kilojoules per mole) of Base Pairing[a]

| | ΔZPE | Δ*H* |
|---|---|---|
| A + U* → A-U* | −63.71 | −63.10 |
| G + U* → G-U* | −111.75 | −113.38 |
| G + U → G-U | −70.84 | −71.42 |
| A + BrU* → A-BrU* | −65.43 | −64.60 |
| G + BrU* → G-BrU* | −133.59 | −136.17 |
| G + BrU → G-BrU | −71.13 | −71.40 |

[a] Calculations were performed at the ab initio level using HF/STO-3G levels of theory. All values include ZPE and thermodynamic corrections at 298.15 K and 1.0 atm.

### *BrU* Is More Favorable for Pairing with G than U**

In Table 1, it is clearly shown that for the uracil and 5-bromouracil systems, calculated results obtained at the HF/STO-3G level of theory are much closer to the results with the higher correlated methods QCISD (*24*) among all the selected theories. Therefore, to analyze the purine−pyrimidine interactions (Figure 2), the HF/STO-3G level of theory is a better compromise between computational cost and reasonable results, especially for qualitative analysis.

The relative energies of base pairing at 298.15 K and 1.0 atm are listed in Table 4. In nature, uracil pairs with adenine (*33*). The binding energy of U* with G is larger than that of U* with A by 50.28 kJ/mol. It suggests that, when uracil tautomerizes to its enol form, its preferred match changes from A to G. G-U* is more stable than G-U, indicating that the tautomerism of uracil to U* increases the stability of the base pair. Similarly, the binding energy of BrU* with G is larger than that of BrU* with A by 71.57 kJ/mol. It shows that the tautomerism from BrU to BrU* changes the corresponding preferred match from A to G. G-BrU* is more stable than G-BrU, indicating that the tautomerism of BrU to BrU* increases the stability of the base pair.

As seen in Table 4, the energy released in the formation of G-BrU is nearly the same as that of G-U; however, for G-BrU*, it is larger than that of G-U* by 22.79 kJ/mol. Videlicet, if U and BrU exist as the keto form, the bromine substituent at position 5 of U will have no effect on their combination with G. However, if U and BrU exist as the enol form, the bromine substituent at position 5 of U makes BrU* pair with G more easily than U*, which will increase the reversion frequencies of A-T to G-C drastically.

### *Comparing the Tautomerisms from G-U* to G*-U and from G-BrU* to G*-BrU*

The tautomerisms from G-BrU* to G*-BrU and from G-U* to G*-U are also studied to shed light on the mutagenic mechanism of the A-T to G−C transition. Figure 5a shows the PES calculation results of G-BrU* to G*-BrU with $R_1$ and $R_2$ as the variables. It can be seen that the minimum energies of all the curves appear in only region I with the lengthening of $R_2$. The $R_1$ of region I varies from 1.500 to 1.600 Å. Both G-BrU* ($R_1$ = 1.557 Å and $R_2$ = 1.070 Å) and G*-BrU ($R_1$ = 1.560 Å and $R_2$ = 1.070 Å) appear in this region. It indicates that G-BrU* and G*-BrU have similar structures. The proton transfer from G-BrU* to G*-BrU occurs via a transition state, TS(G···BrU*) (Figure 6). TS(G···BrU*) also has a structure similar to those of G-BrU* and G*-BrU. The activation energy from G-BrU* to TS(G···BrU*) is very low, and the relative energy between G*-BrU and G-BrU* is nearly zero (Table 5). The hydrogen

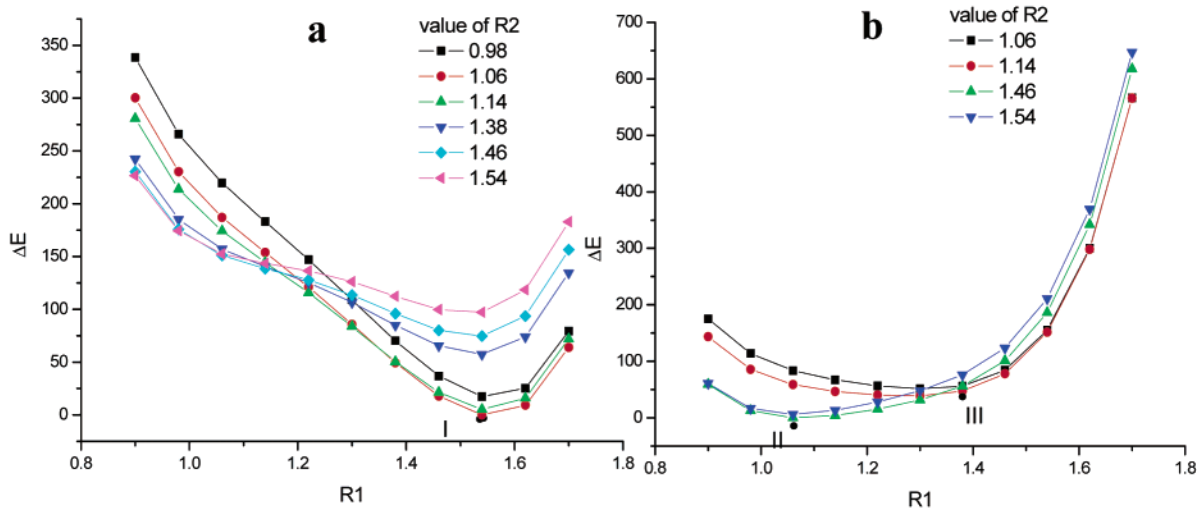


FIGURE 5: Potential energy surface (PES) calculation results for the transition from G-BrU* to G*-BrU (a) and from G-U* to G*-U (b). The PES scan was performed at the ab initio level using HF/STO-3G level of theory. Bond lengths are in angstroms and energies in kilojoules per mole.

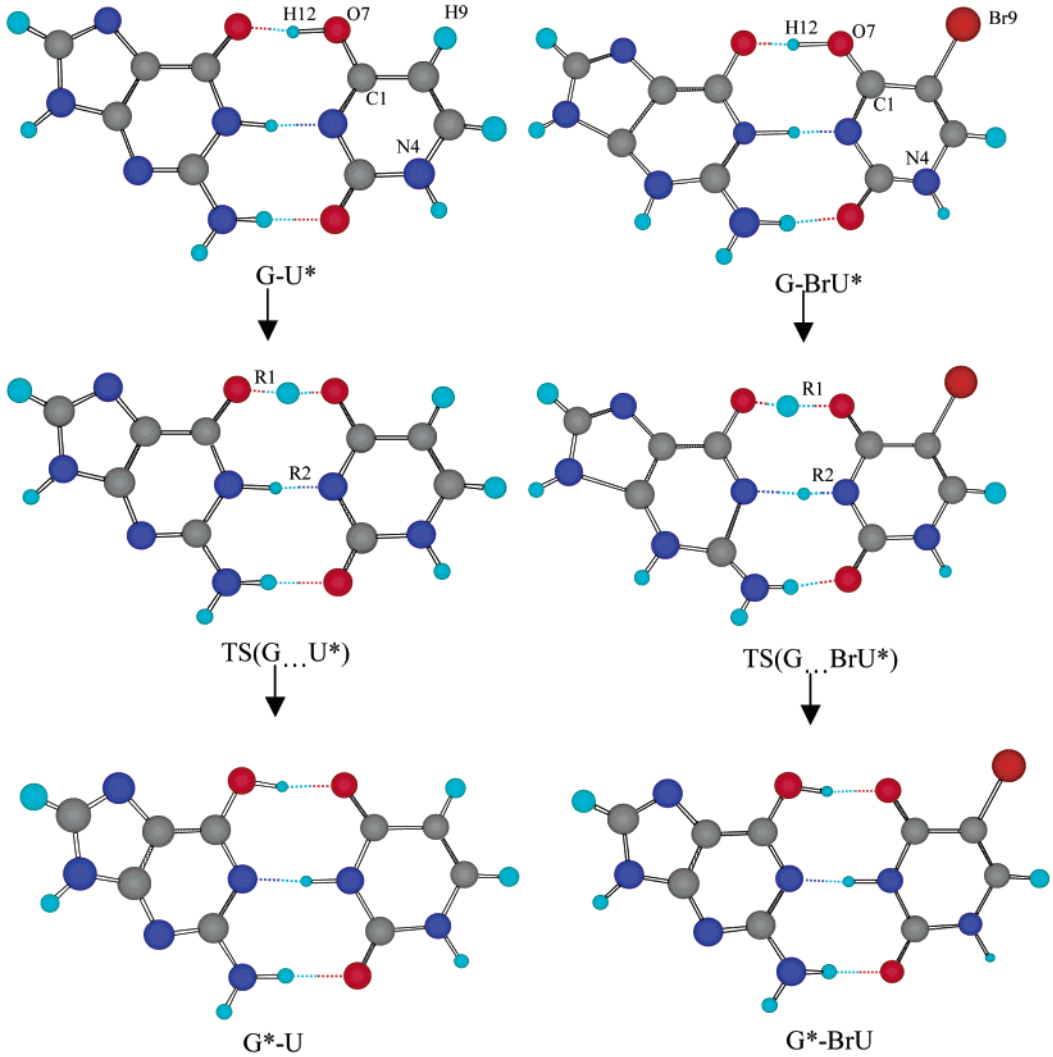


FIGURE 6: Optimized geometries of the relevant stationary points along the G-U* to G*-U and G-BrU* to G*-BrU transitions, calculated at the HF/STO-3G level of theory.

bonds with such a very low barrier lead to proton delocalization in the H-bond, and energy optimization calculations show that the proton transfer from G-BrU* to G*-BrU and the reverse process from G*-BrU to G-BrU* are both barrier-free proton transfers (BFPTs) (*34*, *35*). We defined this process as bidirectional BFPT. More specifically, the equilibrium constant for the tautomeric reaction from G-BrU* to G*-BrU equals 1.01 (Table 5). Thus, G-BrU* and

Table 5: Activation Energies (kilojoules per mole), Free Energy Changes (kilojoules per mole), and Equilibrium Constants of Base Pair Tautomerism[a]

| | activation energy | $\Delta G$ | $K_{eq}$ |
|---|---|---|---|
| G-U* → G*-U | 0.28 | −20.57 | $4.02 \times 10^4$ |
| G-BrU* → G*-BrU | 0.27 | −0.03 | 1.01 |

[a] Calculations were performed at the ab initio level using HF/STO-3G levels of theory. All values include ZPE and thermodynamic corrections at 298.15 K and 1.0 atm.

G*-BrU have the same stability. It indicates that BrU* can introduce G and G* into DNA simultaneously.

The PES of G-U* to G*-U (Figure 5b) is different from that of G-BrU* to G*-BrU. The minimum energies of all the curves appear in two regions (II and III) with the lengthening of $R_2$. The $R_1$ of region II varies from 1.000 to 1.100 Å and for region III from 1.300 to 1.400 Å. G*-U ($R_1$ = 1.010 Å and $R_2$ = 1.060 Å) is in region II, while G-U* ($R_1$ = 1.322 Å and $R_2$ = 1.470 Å) is in region III. The proton transfer from G-U* to G*-U proceeds via a transition state, TS(G···U*) (Figure 6). At the HF/STO-3G level of theory, bond lengths $R_1$ and $R_2$ of TS(G···U*) are 1.225 and 1.380 Å, respectively. The activation energy from G-U* to TS(G···U*) is nearly the same as that from G-BrU* to TS(G···BrU*); however, the relative energy between G*-U and G-U* is 685.7 times as large as that between G*-BrU and G-BrU* (Table 5). Although the proton transfer from G-U* to G*-U is spontaneous, there is a barrier of 20.85 kJ/mol from G*-U to G-U*. Accordingly, the proton transfer from G-U* to G*-U is BFPT, whereas its reverse process from G*-U to G-U* is not BFPT. We defined this process as monodirectional BFPT. The huge value for the equilibrium constant of the tautomeric reaction from G-U* to G*-U (Table 5) indicates that G*-U exists as the main form of the tautomerisms. Hence, U* can introduce only G* into DNA.

*Mutagenic Mechanism of the A-T to G-C Transition*

According to our calculated results, there are essential differences between uracil and 5-bromouracil when they are incorporated into DNA. In the work presented here, we explain how the bromine substitution at position 5 of U induces the mutation from A-T to G-C by comparing U and BrU in DNA (Figure 7).

First, uracil in DNA is considered. As is well-known, uracil can occasionally be incorporated during DNA synthesis and substitute for thymine in DNA (Figure 7, a → b) (*36*). If U tautomerizes to its enol form U*, it will pair with G in the first-generation progeny (Figure 7, b → d). Our theoretical studies indicate that the proton transfer from G-U* to G*-U is spontaneous and G*-U is more stable than G-U* (Figure 7, d → e). In the second-generation progeny, U pairs with A and G* pairs with T (Figure 7, e → f and e → g) (*1*, *30*). In the latter course, the G*-T and A-U mismatches will be repaired to A-T by known modes. As a result, U* will probably not induce the transition from A-T to G-C at all.

When position 5 of U is substituted with bromine, the duplication process of DNA will be quite different from that of DNA containing uracil. 5-Bromouracil is an analogue of thymine that can be mistakenly incorporated into DNA and pair with adenine (Figure 7, a → b1) (*37*). On the basis of

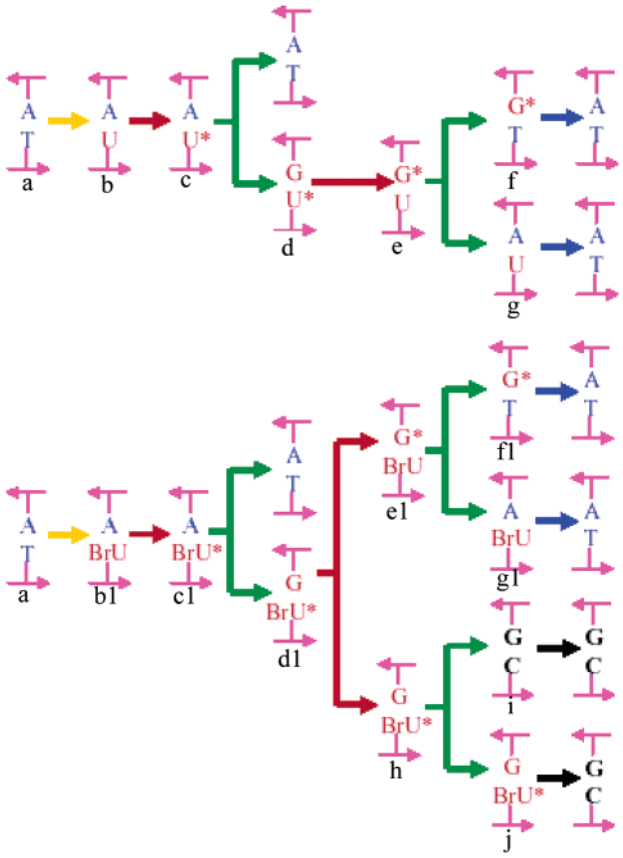


FIGURE 7: Mutagenic mechanism of the transition from A-T to G-C induced by 5-bromouracil. The yellow arrow means base incorporated in DNA. The red arrow represents tautomerism of a base or base pair. The green arrow stands for the duplication of DNA. The blue arrow means the repairing process of the base. The black arrow represents mutation.

our former calculations in The Tautomeric Reaction from BrU to BrU* Is Easier than That from U to U*, BrU is much more likely to form an enol tautomer than U. Thus, the mutagenic effect of BrU in DNA arises because the equilibrium between the two tautomers of BrU is shifted more toward the rarer enol form than U does (Figure 7, b1 → c1). As we concluded in BrU* Is More Favorable for Pairing with G than U*, the BrU* tautomer will pair with G rather than with A, which is in agreement with experimental evidence (*3*). Moreover, BrU* is more likely to pair with G than U* according to our calculated results. Therefore, the mutagenic possibility induced by BrU in DNA is reinforced once more. In addition, our calculated results in Comparing the Tautomerisms from G-U* to G*-U and from G-BrU* to G*-BrU reveal that G-BrU* and G*-BrU have the same stability. Therefore, the probabilities of obtaining G-BrU* and G*-BrU in the first-generation progeny are the same. If G*-BrU is introduced, just like the case induced by U* mentioned above, the transition from A-T to G-C will not happen (Figure 7, d1 → e1). However, if G-BrU* is produced, G pairs with C and BrU* pairs with G in the second-generation progeny. Because G and C are normal bases in DNA, the G-C mismatch is difficult to examine, so the mutation from A-T to G-C induced by 5-bromouracil proceeds (Figure 7, d1 → i).

*Conclusions.* In this work, we describe an ab initio study of tautomer interconversion of uracil and 5-bromouracil bases

and their pairings with guanine and adenine together with the influences of water on all of them. To gain a better understanding of the mutagenicity of the 5-Br derivative, a comparison was made between uracil and 5-bromouracil.

On the basis of the results obtained from our calculations, the following can be stated.

(a) It is broadly accepted that the mutagenic action of BrU is based on enolization and ionization. The presence of the bromine at position 5 significantly alters the distribution of electrons in the base, so BrU can spend part of its existence in the rare enol form. Here we put forward another mechanism for the enolization of BrU: the water located in the region of S1 can catalyze the tautomerism from U to U*, while the water located in the region of S2 can protect U from tautomerizing to U*. The bromine substitution at position 5 of U will lead to the loss of the protection induced by water in the region of S2, so tautomerism from BrU to BrU* is much more likely than that from U to U*.

(b) It is well-known that BrU* pairs with G. Our studies give a deeper comprehension of the rare base pairing ability: the introduction of the 5-Br derivative increases the binding energy of G-BrU* compared with that of G-U*. Therefore, BrU* tends to pair with G more easily than U*.

(c) This work also gives a new perspective on the proton transfer process between the base pairs. The proton transfer between G-BrU* and G*-BrU is bidirectional BFPT; that is, BrU* can introduce G and G* into DNA, which makes the base mismatch induced by BrU* sustained. On the other hand, the proton transfer between G-U* and G*-U is monodirectional BFPT; that is, U* can import only G*, which terminates the base mismatch induced by U*.

All the conclusions became the foundation of the mutation process presented in Figure 7. It is worth noticing that any one of the three factors can increase the frequency of reversion from A-T to G-C. Though a direct experimental proof of the predicted tautomeric equilibrium is missing, we believe that the technique employed is sufficiently established to warrant the rationality of the obtained results. Such a mechanism for mutagenic induction, and conclusions relating to a new concept of the enolization of BrU and reversible versus irreversible proton transfer in the implicated enol−keto states, could provide an incentive for the future development of research on the mutagenicity of the 5-Br derivative.

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## SUPPORTING INFORMATION AVAILABLE

The Z-MATRIX of calculated results, results of second-order perturbation theory analysis of fock matrix on the NBO basis, and a summary of the potential surface scan. This material is available free of charge via the Internet at http://pubs.acs.org.

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