

## On the Origin of the Ultrafast Internal Conversion of Electronically Excited Pyrimidine Bases

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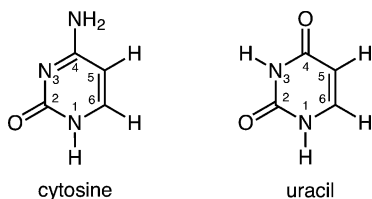
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The ultrafast radiationless decay of photoexcited uracil and cytosine has been investigated by ab initio quantum chemical methods based on CIS and CR-EOM-CCSD(T) electronic energy calculations at optimized CIS geometries. The calculated potential energy profiles indicate that the  $S_1 \rightarrow S_0$  internal conversion of the pyrimidine bases occurs through a barrierless state switch from the initially excited  ${}^1\pi\pi^*$  state to the out-of-plane deformed excited state of biradical character, which intersects the ground state at a lower energy. This three-state nonradiative decay mechanism predicts that replacement of the C5 hydrogen by fluorine introduces an energy barrier for the initial state switch, whereas replacement of the C6 hydrogen by fluorine does not. These predictions are borne out by the very different fluorescence yields of 5-fluorinated bases relative to the corresponding 6-fluorinated bases. It is concluded from these results that the origin of the ultrafast radiationless decay is the same for the two pyrimidine bases.

The hallmarks of the photophysical properties of DNA bases are the ultrafast radiationless decay to the ground state, leading to the very short excited-state lifetime (subpicosecond in solution),<sup>1</sup> and the dramatic lifetime lengthening that results from



simple chemical modifications.<sup>1</sup> In cytosine, for which the substituent dependence of lifetime has been most extensively studied, the  $\sim 720$  fs lifetime of the unmodified base increases to  $\sim 88$  ps by replacement of the C5 hydrogen by fluorine (to yield 5-fluorocytosine) and to  $\sim 280$  ps by acetylation of a hydrogen atom in the amino group attached to the C4 carbon (to produce  $N^4$ -acetylcytosine).<sup>1,2</sup> A similar effect of fluorination on the excited-state lifetime has also been observed in uracil,<sup>3</sup> which possesses the  $S_1$  state of  ${}^1n\pi^*$  character at the optimized ground-state geometry. These observations are key to understanding the ultrafast  $S_1$  radiationless decay of the unmodified pyrimidine bases. Although there are a number of theoretical studies that address the ultrafast nonradiative decay of nucleic acid components,<sup>4–7</sup> none of the existing models are able to provide a rational explanation of the observed substituent effects on  $S_1$  lifetime.

Very recently, we have presented a theoretical model for the ultrafast internal conversion of cytosine, in which a state switch from the initially prepared  ${}^1\pi\pi^*$  state to the out-of-plane deformed excited state of biradical character controls the rate of the  $S_1$  ( ${}^1\pi\pi^*$ ) decay.<sup>8</sup> This mechanism, based on configuration interaction singles (CIS) and completely renormalized equation of motion coupled-cluster (CR-EOM-CCSD(T)) calculations, successfully accounts for the dramatically longer  $S_1$  lifetimes of 5-fluorocytosine and  $N$ -acetylcytosine relative to cytosine. Replacement of the C5 hydrogen atom by a methyl group is predicted to lead to a substantial, but not dramatic, increase in the  $S_1$  lifetime, also consistent with experiment. An attempt has been made by Blancafort et al. to explain the increase of an excited-state lifetime in 5-fluorocytosine,<sup>9</sup> but no firm conclusion has been reached.

The question of considerable interest is whether a mechanism similar to that proposed for cytosine also accounts for the dramatic differences between the excited-state lifetimes of uracil and 5-fluorouracil. In this letter, we show that the calculated decay path of uracil is essentially the same as that of cytosine. The dramatically different excited-state lifetimes of 5-fluoro and 6-fluoro compounds predicted by the theoretical model, and confirmed by experiment, provides a strong, if not compelling, support to the proposed mechanism for the ultrafast radiationless decay of the two pyrimidine bases.

The ab initio study of the excited-state potential energy surfaces of uracil, cytosine, and their fluorinated derivatives involved the application of a simple CIS/cc-pVDZ method to find constrained<sup>10</sup> optimized geometries and the use of these geometries to perform CR-EOM-CCSD(T) and CIS energy

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**TABLE 1: Vertical Excitation Energies (eV) of Uracil at the Optimized CIS/cc-pVDZ Planar Geometries of the  $S_0$ ,  ${}^1\pi\pi^*$ , and  ${}^1n\pi^*$  States**

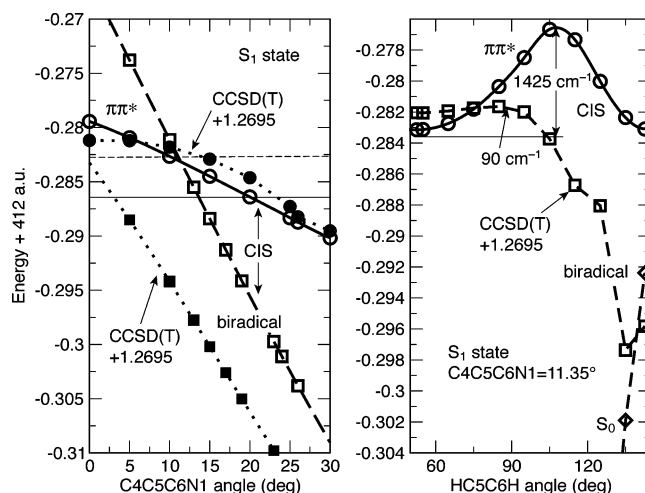
geometry	state	CIS	CCSD	CCSD(T)	QDPT2
$S_0$	${}^1n\pi^*$	6.486	5.428	5.743	4.988
	${}^1\pi\pi^*$	6.816	6.015	6.291	5.883
${}^1\pi\pi^*$	${}^1n\pi^*$	6.325	5.041	5.324	5.149
	${}^1\pi\pi^*$	5.726	5.001	5.256	5.291
${}^1n\pi^*$	${}^1n\pi^*$	4.661	4.609	4.925	4.556
	${}^1\pi\pi^*$	6.567	5.725	5.995	6.134

calculations to probe energetics of the low-lying electronic states. The CIS calculations were performed with the use of the *Gaussian 98* program.<sup>11</sup> The equation of motion coupled-cluster doubles calculations with type III perturbative corrections for connected triples<sup>12,13</sup> were done with the GAMESS program.<sup>14</sup> The completely renormalized single-reference coupled-cluster approach allows treatment of states with significant double excitation character. It also provides qualitatively correct potential energy surfaces for bond-breaking reactions.<sup>15</sup> We also used state-averaged CASSCF(8,8)<sup>16</sup>/cc-pVTZ and the corresponding quasi-degenerate second-order perturbation theory, QDPT2<sup>17,18</sup>/cc-pVTZ to calculate vertical excitation energies at optimized planar geometries of uracil in its  $S_0$ ,  ${}^1\pi\pi^*$ , and  ${}^1n\pi^*$  states. In CASSCF orbital optimization, the ground state and the three lowest excited states were included with equal weights.

It should be mentioned that neither CR-EOM-CCSD(T) nor QDPT2 calculations presented here aim for benchmark-quality results. Instead, our purpose is to examine the effect of electron correlation on the qualitative features of the potential energy surfaces. Nondynamical correlations, associated with the multiconfigurational nature of the excited states, are examined through QDPT2 calculations, based on minimal (8,8) active space. As is well-documented,<sup>19</sup> this active space is too small to represent the lowest  ${}^1\pi\pi^*$  and  ${}^1n\pi^*$  excitations quantitatively. Such quantitative description requires (10,12) and (10,10) active spaces for the separate treatment of  ${}^1\pi\pi^*$  and  ${}^1n\pi^*$  excitations, respectively.<sup>19</sup> At nonplanar geometries, the combined active space and difficulty in maintaining a consistent active space at different geometries would make such calculations prohibitively expensive. The QDPT2 calculations capture dynamical correlation effects only partially, through the second order of perturbation theory. Higher-order dynamical correlation effects are qualitatively captured by the CR-EOM-CCSD(T) calculations. However, because of the relatively small basis set (double- $\zeta$  quality) and single-reference treatment, the results of these calculations should not be expected to show quantitative agreement with experiment. Since we are after changes of energies with geometrical deformations, we expect that the approach reproduces well the relative energy changes caused by molecular deformations.

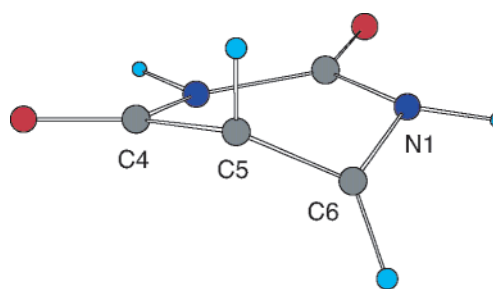
Table 1 collects the calculated vertical excitation energies for the  ${}^1\pi\pi^*$  and  ${}^1n\pi^*$  states at the planar-ring stable geometry of the ground state and unstable planar-ring geometries of the  ${}^1\pi\pi^*$  and  ${}^1n\pi^*$  states. It is seen that, for the planar-ring  ${}^1\pi\pi^*$  geometry, CIS, CCSD, and CCSD(T) predict the  ${}^1\pi\pi^*$  energy below the  ${}^1n\pi^*$  state, but QDPT2 places the  ${}^1n\pi^*$  slightly below the  ${}^1\pi\pi^*$  state. As we show below, the nonplanar deformation of the ring in the initially excited  ${}^1\pi\pi^*$  state quickly leaves the  ${}^1n\pi^*$  state at higher energies, while the  ${}^1\pi\pi^*$  state switches to the biradical state which intersects the ground state.

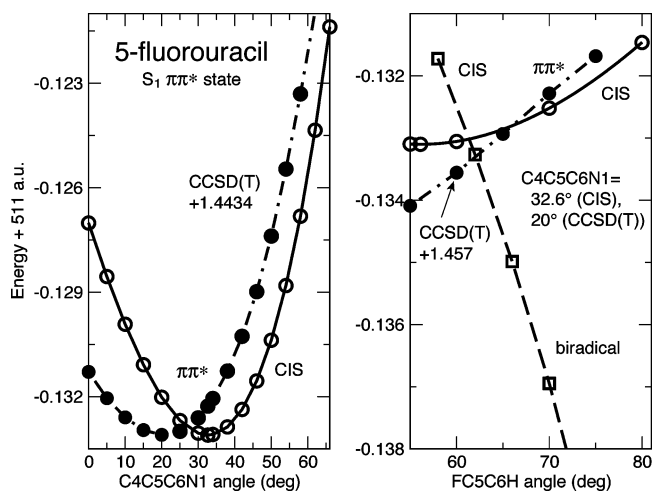
The results of the computation for uracil are shown in Figure 1, which presents the potential energy profiles as a function of the C4C5C6N1 dihedral angle (left) and of the HC5C6H angle (right). While the  $S_1$  state is of  ${}^1n\pi^*$  character at the optimized ground-state geometry, the  ${}^1\pi\pi^*$  state is lower (CIS, CR-EOM-



**Figure 1.** The calculated CIS and CCSD(T) energies for the partially optimized CIS geometries of the  ${}^1\pi\pi^*$  and biradical states of uracil. The CCSD(T) energies are shifted uniformly up by 1.2695 au. The left-hand panel shows the instability of the  ${}^1\pi\pi^*$  state against the out-of-plane C4C5C6N1 deformation angle. The horizontal lines indicate the range of energies for the  ${}^1n\pi^*$  state. The right-hand panel shows the energies for the HC5C6H dihedral angle at the crossing-point C4C5C6N1 angle of 11.35° between the  ${}^1\pi\pi^*$  and biradical states. The closed-shell ground-state energy and its crossing with the biradical state potential surface is also indicated.

CCSD(T) or quasi-degenerate (QDPT2) in energy with the  ${}^1n\pi^*$  state at the optimized  ${}^1\pi\pi^*$  planar geometry (Table 1). The  ${}^1n\pi^*$  state therefore plays no direct role in the nonradiative decay of the optically prepared  ${}^1\pi\pi^*$  state. There are two imaginary frequency out-of-plane vibrations ( $428i\text{ cm}^{-1}$  and  $272i\text{ cm}^{-1}$ ) deforming the C4C5C6N1 part of the ring. Thus, the  ${}^1\pi\pi^*$  state generated by optical excitation of the ground-state molecule is unstable with respect to out-of-plane deformation, leading to the twist of the C5C6 double bond. This tendency of the  $\pi\pi^*$  state to relax into a twisted configuration was noted by Sobolewski and Domcke<sup>20</sup> for cytosine in the guanine–cytosine base pair and by Shukla and Mishra<sup>21</sup> for uracil. Matsika<sup>22</sup> found stabilization of the  ${}^1\pi\pi^*$  state of uracil by out-of-plane displacement of the C5 hydrogen (leaving the C6 hydrogen in the initial molecular plane) and located the conical intersection of its surface with the ground state. Our calculation shows that the  ${}^1\pi\pi^*$  state evolves down the energy slope along the C4C5C6N1 out-of-plane deformation until it intersects the biradical-like state in which there is a strong rehybridization of the C5 and C6 orbitals. In this new state, the C5 and C6 hydrogen atoms are almost perpendicular to the average ring plane and displaced in opposite directions as shown below. As a result, the  $p_z$  orbitals of the C5 and C6 carbon atoms are decoupled from the  $\pi$ -electron system and singly occupied, giving the state a biradical character. The CCSD(T) calculation places the biradical state below the  ${}^1\pi\pi^*$  state for all C4C5C6N1 angles, whereas the CIS calculation places it below the  ${}^1\pi\pi^*$  state for angles



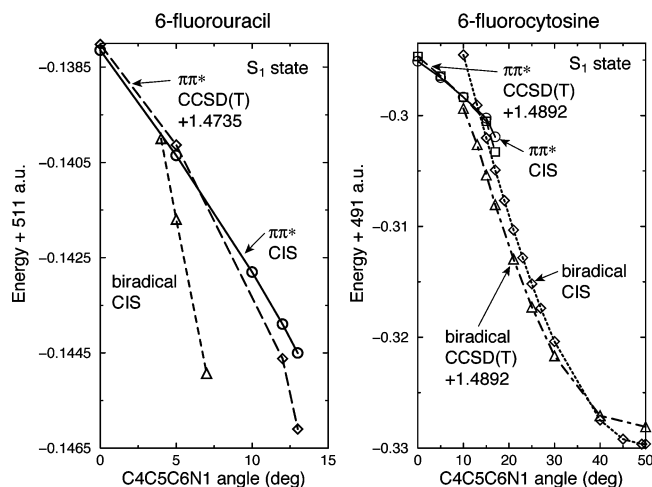


**Figure 2.** The same as Figure 1, but for 5-fluorouracil. The energy shifts of the CCSD(T) results are indicated in the panels. The right-hand panel shows the energies for the HC5C6H dihedral angle at the minimum-point C4C5C6N1 angles of 32.6° (CIS) and 20° (CCSD(T)).

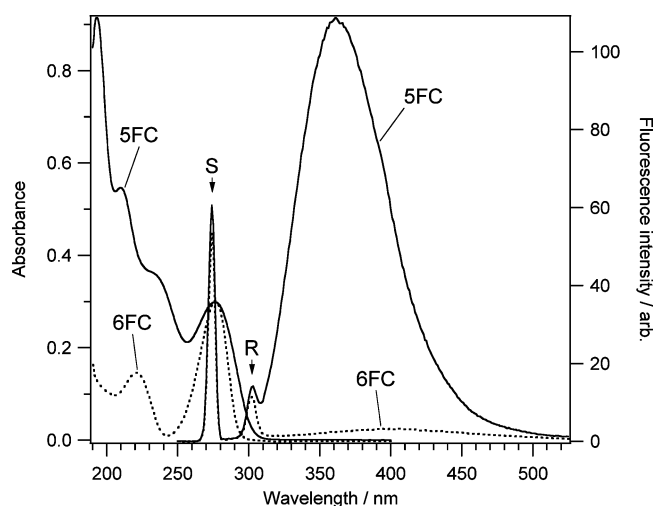
greater than about 12°. In both cases, the barrierless state switch from the  $^1\pi\pi^*$  state to the biradical state, which intersects the ground state at lower energy,<sup>8</sup> provides a very efficient nonradiative decay channel for the initially excited  $^1\pi\pi^*$  state. The energy of the  $^1\pi\pi^*$  state, as a function of the HC5C6H dihedral angle, is given in the right-hand panel. The barrier along this route is about 4 kcal/mol (CIS) and about 0.25 kcal/mol (CCSD(T)). These results are very similar to those for cytosine,<sup>8</sup> and they suggest that uracil and cytosine decay by the same radiationless decay mechanism. The only significant difference between the two bases is that the state switch from the initially excited  $^1\pi\pi^*$  state to the biradical state is expected to be more efficient for uracil because of the smaller stability of the  $^1\pi\pi^*$  state with respect to the out-of-plane deformation, consistent with the shorter excited-state lifetime of uracil relative to cytosine (~1 ps for cytosine and ~0.2 ps for uracil).<sup>1</sup> The dashed and solid horizontal lines indicate CIS energies of the  $^1\pi\pi^*$  state for its optimized planar-ring and nonplanar-ring geometries, respectively. We see that as soon as the C4C5C6N1 nonplanarity of the ring develops in the doorway  $^1\pi\pi^*$  state, its energy gets below even the optimized energy of the  $^1n\pi^*$  state, thus rendering the decay through the  $^1n\pi^*$  state of secondary importance.

As in the case of cytosine,<sup>8</sup> replacement of the C5 hydrogen atom by a fluorine atom stabilizes the initially prepared  $^1\pi\pi^*$  state by introducing an energy barrier before conical intersection with the biradical state is reached. Thus, a distinct minimum develops on the  $S_1$  surface for an out-of-plane C4C5C6N1 deformation of 20° (CCSD(T)) and 33° (CIS), as shown in the left-hand panel of Figure 2. The stabilization of the  $^1\pi\pi^*$  state explains the dramatically longer excited-state lifetime of 5-fluorouracil relative to uracil. The switch from the  $^1\pi\pi^*$  state to the biradical state occurs at FC5C6H angle of about 62° with an energy barrier (right panel). The barrier height is significantly smaller in 5-fluorouracil than in 5-fluorocytosine,<sup>8</sup> consistent with a shorter  $S_1$  lifetime of the former relative to the latter.

In sharp contrast to the case of 5-fluorouracil or 5-fluorocytosine, the CIS and EOM-CCSD(T) calculations indicate that replacement of the C6 hydrogen by fluorine does not create an energy barrier for the state switch from the initially excited  $^1\pi\pi^*$  state to the biradical state. The left panel of Figure 3 presents the potential energy profiles of the two low-lying excited states and the ground state of 6-fluorouracil as a function of the



**Figure 3.** The crossing of the  $^1\pi\pi^*$  and biradical states in 6-fluorouracil (left) and 6-fluorocytosine (right). For the uracil, the circles and the triangles represent the CIS energies of the  $^1\pi\pi^*$  state and biradical state, respectively, and the diamonds denote the CCSD(T)  $^1\pi\pi^*$  energies shifted by 1.4735 au. The CCSD(T) energies of the biradical state are significantly lower, and they are not shown. For the cytosine, the circles (CIS) and the squares (CCSD(T)) are the  $^1\pi\pi^*$  state energies, whereas the diamonds (CIS) and the triangles (CCSD(T)) are the energies of the biradical state. The CCSD(T) energies are shifted by 1.4892 au.



**Figure 4.** The absorption and emission spectra of 5-fluorocytosine (solid) and 6-fluorocytosine (dashed) in aqueous solutions, which have absorbance of 0.3 in 1 cm path length cuvette at the excitation wavelength of 265 nm. The very weak emission of 6-fluorocytosine with intensity maximum at about 400 nm is due to an impurity in the aqueous solvent. The peaks at 265 nm (S) and at about 303 nm (R) are due to scattered exciting light and Raman scattering of the solvent, respectively.

C4C5C6N1 dihedral angle. It is evident from the result of the CIS calculation that the intersection of the  $^1\pi\pi^*$  state with the biradical state in 6-fluorouracil occurs at a smaller C4C5C6N1 angle than in uracil, leading to a prediction that the lifetime of the initially excited  $^1\pi\pi^*$  state of the fluorinated base may be significantly shorter than that of unmodified uracil. Similar behavior is expected by fluorine substitution on the C6 carbon atom in cytosine, as illustrated in the right panel of Figure 3.

The very different nonradiative decay rates of 5-fluorinated and 6-fluorinated pyrimidine bases, predicted by the ab initio calculation, are confirmed by the steady-state fluorescence measurements. Figure 4 presents the fluorescence spectra of 5-fluorocytosine (5FC) and 6-fluorocytosine (6FC), recorded using aqueous solutions that have identical absorbance (0.3 in

1 cm path length cuvette) at the same excitation wavelength (265 nm). It is evident that the fluorescence is much weaker for 6FC as compared to 5FC. In fact, the emission from 6FC is too weak, so that only the emission from a minute quantity of fluorescent impurity is apparent in the measured spectrum. A lower limit of about 150 can be estimated for the 5FC/6FC intensity ratio from the spectra. Since the radiative lifetime, calculated from the absorption and emission spectra using the Strickler–Berg equation,<sup>23</sup> is very long ( $\sim 13$  ns for 5FC and  $\sim 7$  ns for 6FC), the vastly weaker emission intensity of 6FC must be due to much greater nonradiative decay rate of the compound. On the basis of the measured relative fluorescence intensities ( $> 150$ ) and measured fluorescence lifetime ( $\sim 73$  ps)<sup>9</sup> of 5FC, we estimate an upper-limit lifetime of about 250 fs for 6FC. An excited-state lifetime of very similar magnitude (210 fs) is also obtained from the estimated quantum yield of fluorescence ( $< 3 \times 10^{-5}$ ) and the radiative lifetime of 7 ns. Thus, the  $S_1$  nonradiative decay rate of 6FC is more than 2 orders of magnitude greater than that of 5FC. The nonradiative decay rate is also much greater for 6-fluorouracil than for 5-fluorouracil.<sup>24</sup>

In summary, the three-state nonradiative decay mechanism, involving the intermediacy of a biradical state, satisfactorily accounts for the ultrafast radiationless decay of unmodified pyrimidine bases and very different nonradiative decay rates of 5-fluorinated and 6-fluorinated bases. The details of the calculations and experiment and the relevance of the proposed mechanism to the photophysics of thymine as well as guanine–cytosine and adenine–thymine base pairs will be presented in future publications.

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