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# Ultrafast Intersystem Crossing of 4-Thiothymidine in Aqueous Solution

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ABSTRACT Ultrafast intersystem crossing of UVA-sensitive 4-thiothymidine in aqueous solution was investigated by pump−probe transient absorption. 4-Thiothymidine in the excited singlet state exhibited a distinctively different photophysical pathway from that of thymidine, with intersystem crossing to the triplet manifold being dominant and the triplet formation being completed within approximately 10 ps. This was further supported by quantum chemical calculations. We propose that the unique behavior of photoexcited 4-thiothymidine results from its distinctive molecular and electronic structures and that the triplet form is the initial toxic source to DNA and other biomolecules.

SECTION Dynamics, Clusters, Excited States
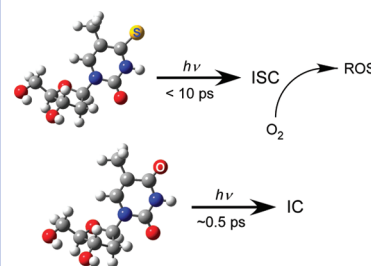
D NA is the most fundamental molecule. Although generally stable, DNA is susceptible to internal and external factors, including chemical and physical forces. It is well-known that DNA can be damaged by its exposure to UVB (280−320 nm) and UVC (<280 nm) radiation.[1−4] The damaged DNA, if not repaired, will induce injurious processes, including cell mutagenesis, carcinogenesis, and death. To elucidate the photoinduced damage of the DNA, numerous studies have been performed on the photophysics and photochemistry of the DNA bases, nucleosides, and nucleotides. An advanced technique, ultrafast absorption and emission spectroscopy, was recently exploited to study DNA constituents and to offer some useful insights to DNA photochemistry. For instance, the excited singlet states (the apparent initial stage for any photochemical pathway) of the DNA bases were reported to exhibit remarkably rapid decay to the ground state.[5] This finding strongly indicates that the DNA itself is a highly photostable material.

Photodynamic therapy (PDT) has been applied to cancer treatment. In the therapy, a drug (photosensitizer) and a particular wavelength of visible light are employed together. When photosensitizers are exposed to the light, reactive oxygen species are produced, and nearby cells are damaged.[6] However, such damages are not specifically targeted to the cellular DNA. A more specific approach would be to introduce a chromophore into a target DNA and then excite the chromophore by irradiation with the light that does not harm normal DNA. As a result, only the DNA containing the chromophore would be selectively destroyed.

4-Thiothymidine (S[4]-TdR) is such a chromophore. It is an analogue of the naturally occurring nucleoside thymidine (TdR) and has a strong absorption in the UVA (320−400 nm) region.[7,8] Massey et al. reported that S[4]-TdR could be readily incorporated into the cellular DNA and that the incorporated 4-thiothymine, like its parent base (thymine), can form a base pair very well with adenine. On the other hand, low doses of UVA light can easily inflict lethal damage on the DNA containing S[4]-TdR, causing cell death.[9] Recently, we reported that photoexcitation of S[4]-TdR gave rise to its triplet with the quantum yield of unity and effectively generated reactive singlet oxygen $O_2(^1\Delta_g)$ that is highly cytotoxic.[10] These results clearly indicates that S[4]-TdR can be used as a promising agent for PDT. However, an understanding of the dynamic behavior of the excited state of S[4]-TdR would be required to further the exploitation of S[4]-TdR-related PDT.

In this Letter, we report our findings on ultrafast excited-state dynamics of S[4]-TdR by use of pump−probe transient absorption measurements and quantum chemical calculations.

Figure 1a shows a time profile of transient absorption of thymidine (TdR) in aqueous solution (pH 7.4) monitored at 570 nm after femtosecond laser excitation (at 263 nm). The transient absorption decayed quickly to the initial level. The time profile can be well fitted by a Gaussian function with a time constant of 0.50 ps. As the time constant is equal to the present instrumental response time, the lifetime of the transient would be equal to or shorter than 0.50 ps. This result is in good agreement with the lifetime of the bright $^1\pi\pi*$ state reported by Pecourt et al. (540 fs).[11] It has been proposed that the $S_2(^1\pi\pi*)−S_1(^1n\pi*)$ internal conversion (in the cases of uracil and thymine) is followed by remarkably rapid $S_1-(^1n\pi*)−S_0$ relaxation.[12,13] Therefore, it is highly likely that TdR in the initially excited $S_2(^1\pi\pi*)$ state would relax into the
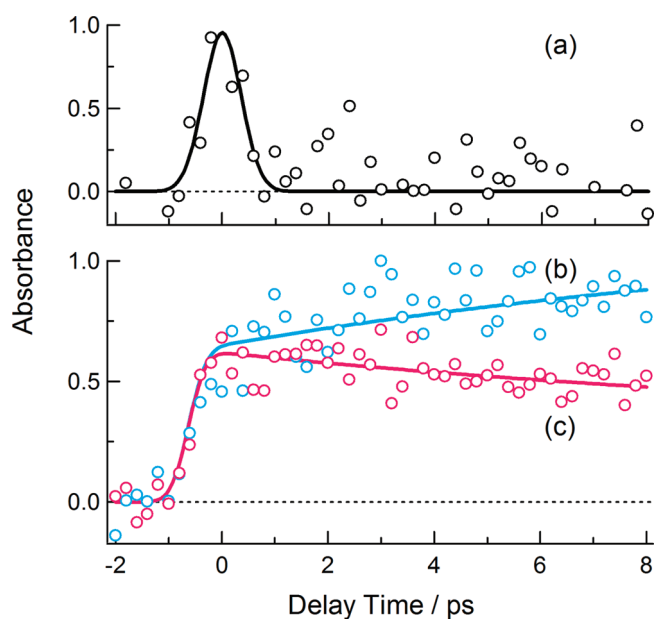
Figure 1. Time profiles of the transient absorption of (a) TdR (at 570 nm), (b) S^4-TdR (at 500 nm), and (c) S^4-TdR (at 570 nm) in phosphate buffer solution (pH 7.4).

ground state within 0.5 ps through ultrafast internal conversion.

Time profiles of the transient absorption of S^4-TdR monitored at 500 and 570 nm are shown in Figure 1b and c, respectively. The transient absorption at 570 nm emerged within the instrumental response time (< 0.50 ps) and then decayed very slowly, indicating that the excited state of S^4-TdR can have a longer lifetime than that of TdR. The time profiles were fitted by a sum of exponentials convoluted with a Gaussian instrument response. At 500 nm, the transient absorption rose up partially within 0.50 ps and grew up with a time constant which corresponds to the decay at 570 nm (Figure 1c). After 10 ps, the transient absorption remained almost flat. Figure 2a shows the transient absorption spectrum of S^4-TdR at 285 ps after the laser excitation. No change regarding the spectral feature was observed after the first 10 ps, suggesting that almost all of the relaxation process was completed within the 10 ps. Because this spectrum well agrees with the triplet–triplet (T–T) absorption spectrum obtained with the nanosecond laser (Figure 2b), we can confidently assign the spectrum (at 285 ps) to the T–T absorption and conclude that the triplet formation was completed within 10 ps. Considering the exceedingly high quantum yield (near unity) for intersystem crossing (ISC) to the triplet manifold of S^4-TdR in acetonitrile,[10] the ISC rate would be equal to or larger than 10^{11} s^{-1} (see below).

The optimized structure and the molecular orbitals (MOs) of S^4-TdR are shown in Figure 3. The base in S^4-TdR, like that of TdR, can be regarded as planar.[14,15] The MOs involved in the S$_1$ and the S$_2$ electronic transitions are localized on the base moiety. In the Franck–Condon region of S^4-TdR, the S$_1$ state originate mainly from the HOMO–LUMO transition with an nπ^* character, whereas the S$_2$ state results predominantly from the HOMO-1 to LUMO transition with a $\pi\pi^*$ character.

Figure 4 shows the vertical transition energies from the S$_0$ state for S^4-TdR and TdR. The HOMO-1 of S^4-TdR is mainly localized on the C=C and the C=S double bonds, while the corresponding π orbitals of TdR (and thymine) are mainly localized on the C=C double bond.[14,16] Since the lone pair orbital on the S atom contributes to HOMO and the π-type p atomic orbital on the S atom largely contributes to HOMO-1, the ^{1}n$\pi^* \rightarrow ^3\pi\pi^*$ or $^1\pi\pi^* \rightarrow ^3n\pi^*$ transition corresponding to a one-electron transition between the π electron (HOMO-1) and the n orbital (HOMO) could occur around the S atomic nucleus. This one-center ISC is commonly known as a mechanism for the ISC process.[17] The n and π orbitals on the S atom of S^4-TdR have an overlap larger than that on the corresponding oxygen atom of TdR, and the spin–orbit coupling of the S atom is also larger than that of the oxygen atom (as the coupling constant increases with the biquadrate of the atomic number); therefore, these orbitals are likely to contribute to the ultrafast ISC process for the S-containing molecule.

Table 1 summarizes the spin–orbit matrix elements of 4-thiothymine (S^4–thymine) calculated at the optimized structure. The results clearly show that ^{1}nπ^*/$^3\pi\pi^*$ ISC take place more efficiently than $^1\pi\pi^*$/^{3}nπ^* ISC. Therefore, we conclude that S^4–thymine excited to the vibronic level of the S$_2$($^1\pi\pi^*$) state should be destined for the T$_1$($^3\pi\pi^*$) state, following internal conversion to the S$_1$(^{1}nπ^*) state.

Two candidates are proposed here for the relaxation process appearing as the decaying component in Figure 1c. The first candidate is the vibrationally hot triplet of S^4-TdR, produced through ultrafast ISC from the $^1\pi\pi^*$ or ^{1}nπ^* state. In this case, the decay time of 10 ps corresponds to vibrational cooling in water. We have noted some similar cases in the literature. For instance, vibrational deactivation times of azulene in various solvents such as hydrocarbons and alcohols were reported to be ∼10 ps;[18] the time constants for adenosine in water obtained by the pump–probe transient absorption were reported to be varied from 0.1 to 2.0 ps, depending on the probing wavelength.[11] In the latter case, solute–solvent H-bonds were proposed to be responsible for the fast rate of vibrational cooling in water.

The second candidate is the bright S$_2$($^1\pi\pi^*$) state. S^4-TdR has a large energy gap between the S$_2$ and S$_1$ states; the S$_1$ state is situated at ∼10000 cm^{-1} below the S$_2$ state according to the theoretical calculation (see Figure 4). In general, the rate of the nonradiative transition (IC and ISC) decreases with increasing energy gap.[19] For thymine, the lifetime of the S$_1$ state was reported to be 6.4 ps in the gas phase[20] and 1.2 ps in the liquid phase.[21] The ultrafast decay of the excited singlet state of thymine in the solution phase was attributed to the solvation that would reduce the barrier for the conical intersection between the excited and the ground states.[12] Efficient ISC prior to vibrational cooling was also reported to take place in the ^{1}nπ^* state for 1-cyclohexyluracil in protic and aprotic solvents.[22] Our recent work[23,24] with aza-substituted nucleobases showed that the dark lower-lying ^{1}nπ^* state rather than the bright higher-lying $^1\pi\pi^*$ would play an important role in the ISC process. Since the S$_2$($^1\pi\pi^*$) $\rightarrow$ S$_1$(^{1}nπ^*) internal conversion is believed to be a rate-determining step and ISC from S$_1$(^{1}nπ^*) is significantly fast, the lifetime of the S$_2$($^1\pi\pi^*$) state should be reflected in the observable time constant of 10 ps.

In conclusion, the singlet−triplet ISC for excited S^4-TdR was found to take place within a time of approximately 10 ps. The ultrafast ISC process is believed to result from its distinctive molecular and electronic structures. This is because S^4-TdR has a larger spin−orbit coupling moment and smaller S_1−T_1 energy gap obtained by the TD-DFT calculation relative to TdR. The resulting reactive triplet S^4-TdR is considered to have a longer lifetime and play a crucial role as the cytotoxic agent. The current study presented the first photochemical analysis of the initial stage of excited S^4-TdR and explained how the molecule becomes reactive when photoexcited. The findings in the work should be useful for improving photodynamic therapy where S^4-dT is used to target DNA in cancerous cells.

EXPERIMENTAL SECTION

The femtosecond transient absorption measurement[25] was carried out with pump−probe method by using a Ti:
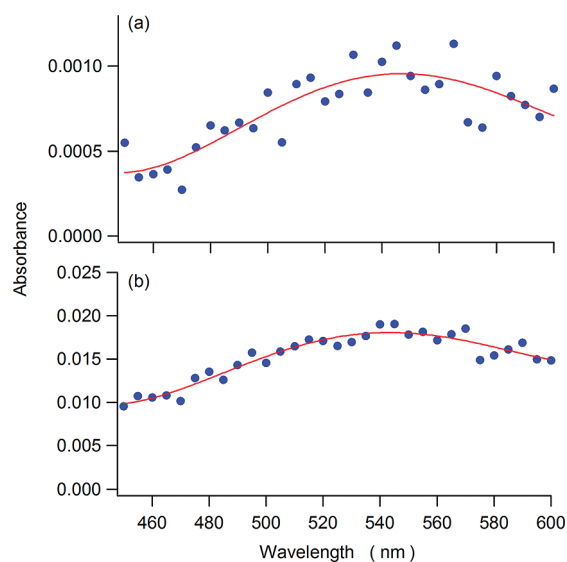

Figure 2. (a) Transient absorption spectrum of S^4-TdR at 285 ps after the laser and (b) the triplet−triplet absorption spectrum obtained with the nanosecond laser system in phosphate buffer solution (pH 7.4).

Sapphire regenerative amplifier system (Spectral Physics, Spitfire, wavelength 790 nm, ∼1 mJ/pulse, pulse width 230 fs, repetition rate 1 kHz). An output of the regenerative amplifier was introduced to the second and third harmonic generators, and the third harmonic (263 nm, < 40 μJ) was used for pumping. The fundamental dropped out of the harmonic generator was focused into a cell containing water to generate a femtosecond white light continuum. The time delay between the pump and probe pulses was regulated by an optical delay circuit with a computer-controlled stepping motor. The probe pulse was split into two parts, one for monitoring of transient absorption and the other for a reference. The pump and probe pulses were overlapped at a sample cell, with their polarization set to the magic angle (54.7°) to ensure the observed kinetics were free from the effects of molecular rotations. For the spectral measurement, the probe beam passing through a sample cell (optical path length 1 mm) was detected by a CCD camera cooled with liquid nitrogen. For obtaining the time profile of the transient absorption at a certain wavelength, the probe and reference intensities were passed through a band-pass filter set before the cell and then detected by photomultiplier tubes, and time-resolved intensities were obtained by a boxcar integrator where the pump pulse was chopped every other shot.

4-Thiothymidine was synthesized as previously reported.[26] The sample solution flew into the cell to avoid the influence of a photoproduct. Becke's three-parameter hybrid functional and Lee−Yang−Parr correlation functions (B3LYP) were adopted. A 6-31G(d,p) basis set was used for the geometry optimization calculations. Vertical transition energies and oscillator strengths of the excited singlet and the triplet states of S^4-TdR and TdR were calculated by time-dependent density functional theory (TD-DFT), using the optimized geometry of the S_0 state. The GAUSSIAN 03 suite of programs[27] was used for the calculations. The molecular orbitals were viewed using GAUSSVIEW.[28]

Spin−orbit matrix elements (SOMEs) for S^4−thymine were computed using the MOLPRO (revision 2006.1)[29] code. The SOMEs at the equilibrium structure on the respective state optimized using the state-averaged (four singlet and four triplet state were considered) complete active space self-consistent field (CASSCF) energy gradient without symmetry
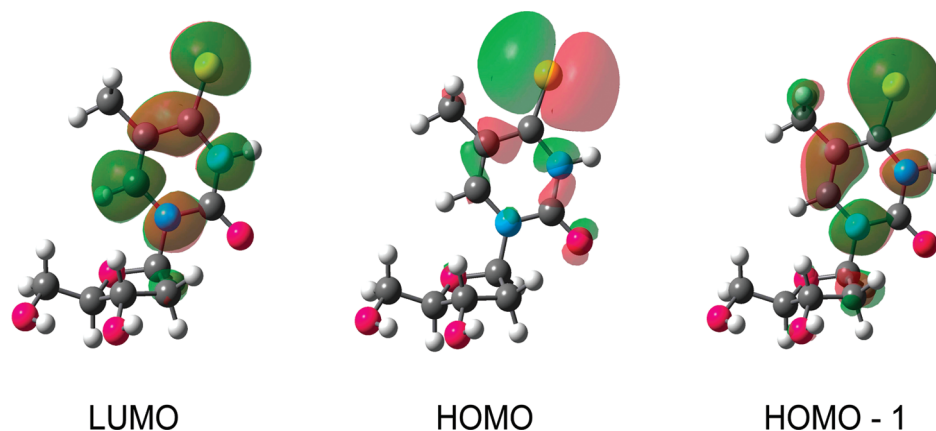

LUMO   HOMO   HOMO - 1

Figure 3. Description of molecular orbitals involved in the S_1 and the S_2 electronic transitions for S^4-TdR.
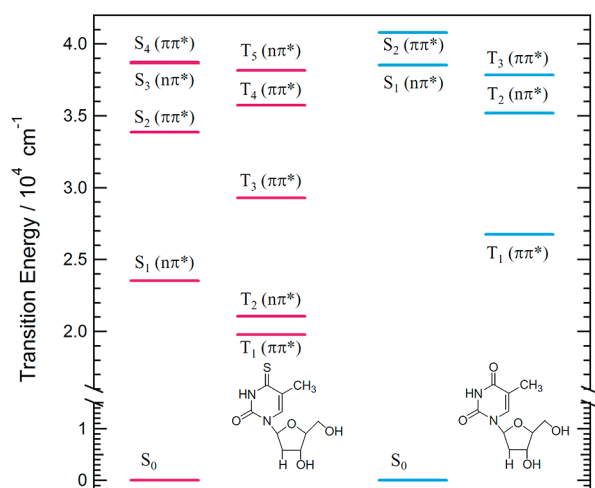
Figure 4. Energy diagrams of the excited singlet and triplet states of (left) S^4-TdR and (right) TdR. The horizontal bars represent each energy level for vertical transition from the ground state calculated by the TD-DFT method.

Table 1. Spin−Orbit Matrix Elements $\langle S_n|\hat{H}_{SO}|T_n\rangle$ (cm^{-1}) for S^4-Thymine

component	$^1n\pi^*/^3\pi\pi^*$	$^1\pi\pi^*/^3n\pi^*$
	S_0 minimum	
$\hat{H}_{SO,x}$	0.00	−0.00
$\hat{H}_{SO,y}$	−58.88	35.24
$\hat{H}_{SO,z}$	130.94	−80.19
sum of squares	20612	7672
	S_1 minimum	
$\hat{H}_{SO,x}$	0.02	−0.02
$\hat{H}_{SO,y}$	−66.64	37.08
$\hat{H}_{SO,z}$	136.97	−77.72
sum of squares	23201	7415
	S_2 minimum	
$\hat{H}_{SO,x}$	0.00	−0.00
$\hat{H}_{SO,y}$	−74.29	44.80
$\hat{H}_{SO,z}$	129.56	−79.75
sum of squares	22304	8367

constraint were evaluated by the multireference configuration interaction method using the CASSCF wave function. The double-ζ plus polarization (cc-pVDZ) Gaussian basis sets of Dunning were used for all atoms. The active space was comprised of 11 active orbitals and 16 active electrons [CASSCF(16,11)]; the active orbitals were composed of 9 π and 2 lone pair orbitals from both thionyl S and carbonyl O atoms.

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REFERENCES

(1) Morrison, H. *Photochemistry and the Nucleic Acid*; Wiley-Interscience: New York, 1990.

(2) Davis, R. J. H. Ultraviolet Radiation Damage in DNA. *Biochem. Soc. Trans.* **1995**, *23*, 407–418.

(3) Pfeifer, G. P. Formation and Processing of UV Photoproducts: Effects of DNA Sequence and Chromatin Environment. *Photochem. Photobiol.* **1997,** *65*, 270–283.

(4) Cadet, J.; Sage, E.; Douki, T. Ultraviolet Radiation-Mediated Damage to Cellular DNA. *Mutat. Res.* **2005,** *571*, 3–17.

(5) Crespo-Hernandez, C. E.; Cohen, B.; Hare, P. M.; Kohler, B. Ultrafast Excited-State Dynamics in Nucleic Acids. *Chem. Rev.* **2004,** *104*, 1977–2020.

(6) Dolmans, E. J. G. J.; Fukumura, D.; Jain, R. K. Photodynamic Therapy for Cancer. *Nat. Rev. Cancer* **2003,** *3*, 380–387.

(7) Massey, A.; Xu, Y.-Z.; Karran, P. Photoactivation of DNA Thiobases as a Potential Novel Therapeutic Option. *Curr. Biol.* **2001,** *11*, 1142–1146.

(8) Xu, Y.-Z.; Zhang, X.; Wu, H.-C.; Massey, A.; Karran, P. 4-Thio-5-bromo-2′-deoxyuridine: Chemical Synthesis and Therapeutic Potential of UVA-Induced DNA Damage. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 995–997.

(9) Massey, A.; Xu, Y.-Z.; Karran, P. Ambiguous Coding is Required for the Lethal Interaction between Methylated DNA Bases and DNA Mismatch Repair. *DNA Repair* **2002,** *1*, 275–286.

(10) Harada, Y.; Suzuki, T.; Ichimura, T.; Xu, Y.-Z. Triplet Formation of 4-Thiothymidine and Its Photosensitization to Oxygen Studied by Time-Resolved Thermal Lensing Technique. *J. Phys. Chem. B* **2007,** *111*, 5518–5524.

(11) Pecourt, J.-M. L.; Peon, J.; Kohler, B. DNA Excited-State Dynamics: Ultrafast Internal Conversion and Vibrational Cooling in a Series of Nucleosides. *J. Am. Chem. Soc.* **2001,** *123*, 10370–10378.

(12) He, Y.; Wu, C.; Kong, W. Photophysics of Methyl-Substituted Uracils and Thymines and Their Water Complexes in the Gas Phase. *J. Phys. Chem. A* **2004,** *108*, 943–949.

(13) The notation of "S_1" by Pecourt et al. means the first "optically allowed" $^1\pi\pi^*$ excited state. It has been known that TdR should have the dark nπ^* state at a lower energy region. Thus, the "S_1" state noted by Pecourt et al. was rewritten to the "S_2" state.

(14) Zhu, X.-M.; Wang, H.-G.; Zheng, X.; Phillips, D. L. Role of Ribose in the Initial Excited State Structural Dynamics of Thymidine in Water Solution: A Resonance Raman and Density Functional Theory Investigation. *J. Phys. Chem. B* **2008,** *112*, 15828–15836.

(15) So, R.; Alavi, S. Vertical Excitation Energies for Ribose and Deoxyribose Nucleosides. *J. Comput. Chem.* **2007,** *28*, 1776–1782.

(16) Gustavsson, T.; Banyasz, A.; Lazzarotto, E.; Markovitsi, D.; Scalmani, G.; Frisch, M. J.; Barone, V. Singlet Excited-State Behavior of Uracil and Thymine in Aqueous Solution: A Combined Experimental and Computational Study of 11 Uracil Derivatives. *J. Am. Chem. Soc.* **2006,** *128*, 607–619.

(17) Turro, N. J. *Modern Molecular Photochemistry.*; Benjamin Cummings Publishing: San Francisco, CA, 1991.

(18) Schwarzer, D.; Troe, J.; Votsmeier, M.; Zerezke, M. Collisional Deactivation of Vibrationally Highly Excited Azulene in Compressed Liquids and Supercritical Fluids. *J. Chem. Phys.* **1996,** *105*, 3121–3131.

(19) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley-Interscience: New York, 1970.

(20) Kang, H.; Lee, K. T.; Jung, B.; Ko, Y. J.; Kim, S. K. Intrinsic Lifetimes of the Excited State of DNA and RNA Bases. *J. Am. Chem. Soc.* **2002,** *124*, 12958–12959.

(21) Reuther, A.; Iglev, H.; Laenen, R.; Laubereau, A. Femtosecond Photo-Ionization of Nucleic Acid Bases: Electronic Lifetimes and Electron Yields. *Chem. Phys. Lett.* **2000,** *325*, 360–368.

(22) Hare, P. M.; Crespo-Hernandez, C. E.; Kohler, B. Solvent-Dependent Photophysics of 1-Cyclohexyluracil: Ultrafast Branching in the Initial Bright State Leads Nonradiatively to the Electronic Ground State and a Long-Lived $^1n\pi^*$ State. *J. Phys. Chem. B* **2006,** *110*, 18641–18650.

(23) Kobayashi, T.; Harada, Y.; Suzuki, T.; Ichimura, T. Excited State Characteristics of 6-Azauracil in Acetonitrile: Drastically Different Relaxation Mechanism from Uracil. *J. Phys. Chem. A* **2008,** *112*, 13308–13315.

(24) Kobayashi, T.; Kuramochi, H.; Harada, Y.; Suzuki, T.; Ichimura, T. Intersystem Crossing to Excited Triplet State of Aza Analogues of Nucleic Acid Bases in Acetonitrile. *J. Phys. Chem. A* **2009,** *113*, 12088–12093.

(25) Nakabayashi, T.; Kamo, S.; Watanabe, K.; Sakuragi, H.; Nishi, N. Observation of Formation Dynamics of Solvated Aromatic Cation Radicals Following Photoionization. *Chem. Phys. Lett.* **2002,** *355*, 241–248.

(26) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. Simple Synthesis of 4-Thiothymidine, 4-Thiouridine and 6-Thio-2′-deoxyguanosine. *Tetrahedron Lett.* **1991,** *32*, 2817–2820.

(27) Frisch, M. J.; et al. *Gaussian 03*, revision D.02; Gaussian, Inc.: Wallingford, CT, 2004.

(28) Dennington, R.; Keith, T.; Millam, J.; Eppinnett, K.; Hovell, W. L.; Gilliland, R. *GaussView*, Version 3.09; Semichem, Inc.: Shawnee Mission, KS, 2003.

(29) Werner, H.-J.; Knowles, P. J.; Lindh, R.; Manby, F. R.; Schütz, M.; Celani, P.; Korona, T.; Rauhut, G.; Amos, R. D.; Bernhardsson, A.; Berning, A.; Cooper, D. L.; Deegan, M. J. O.; Eckert, A. J. D. F.; Hampel, C.; Hetzer, G.; Lloyd, A. W.; McNicholas, S. J.; Meyer, W.; Mura, M. E.; Nicklass, A.; Palmieri, P.; Pitzer, R.; Schumann, U.; Stoll, H.; Stone, A. J.; Tarroni, R.; Thorsteinsson, T. *MOLPRO*, version 2006.1, a package of ab initio programs; 2006; see http://www.molpro.net.