Unexpected Photoproduct Generated via the Acetone-Sensitized Photolysis of 5-Bromo-2′-deoxyuridine in a Water/Isopropanol Solution: Experimental and Computational Studies

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The acetone-sensitized photolysis of 5-bromo-2′-deoxyuridine (5-BrdU) in a water/isopropanol solution with 300 nm photons leads to the formation of 2′-deoxyuridine (dU) and a comparable amount of another photoproduct that has not been reported in the literature so far. The negative and positive mass spectra recorded for this species indicate that they originate from the molecular mass of 286 Da, which corresponds to an adduct of 2′-deoxyuridine and 2-propanol. Quantum chemical calculations carried out at the DFT and TDDFT levels reveal both the structure and the UV spectrum of that adduct. The latter computational characteristic matches well the experimental UV spectrum of the new photoproduct. Our findings indicate that the acetone-sensitized photolysis of 5-BrdU is more complicated than has hitherto been assumed. Nevertheless, since electron transfer is one of the pathways responsible for 5-BrdU decay, acetone-sensitized photolysis of the halogen derivatives of nucleobases could be a convenient tool for studying their radiosensitivity in aqueous solutions.

1. Introduction

Nucleotides containing halogenated nucleobases (Hal-NBs) can be used by a cell for DNA biosynthesis almost as easily as natural ones.[1] On the other hand, while Hal-NBs are dehalogenated by low-energy electrons[2−4] or UV radiation,[5−7] reactive radicals, which may damage DNA, are formed.[8−11] It seems, therefore, that these compounds could be employed in the therapy of cancer diseases. Indeed, it has previously been reported that 5-bromouracil (5-BrU) increases the likelihood of cells being damaged by ionizing radiation.[12] It has also been shown that incorporation of 5-BrdU into DNA increases the biopolymer's susceptibility to single- and double-strand breakage induced by ionizing radiation[13] and diminishes the rate of DNA repair of potentially lethal damage.[14] Moreover, some clinical studies have reported the radiosensitization of malignant brain tumors by bromodeoxyuridine (5-BrdU).[15] Although 5-BrdU has been studied the most extensively, the enhanced response of nonhypotoxic tumor cells to radiation in the presence of iododeoxyuridine (5-IdU) and fluorodeoxyuridine (5-FdU) has been recognized in several clinical trials as well.[16,17]

In spite of the fact that most investigations carried out to date concern the radiosensitizing properties of 5-BrdU, its photosensitizing features have also been tested in a number of model studies.[18] Since UV is much less toxic than ionizing radiation, halogenated nucleobases should, in principle, allow a relatively safe anticancer therapy to be developed, which suggests that further investigations of their photosensitizing characteristics are warranted.

Thus far, photochemical damage to single- and double-stranded DNA labeled with 5-bromouracil has been demonstrated.[19−22] Already in 1990 Saito et al.[23] showed that irradiation of short oligonucleotide sequences containing 5-BrU leads to their efficient photolysis coupled with the release of adenine and formation of a strand containing 2-deoxyribonolactone. The appearance of the latter product prompted Saito et al. to suggest an intramolecular photoinduced single electron transfer (PSET) between photoexcited 5-BrU and the neighbor adenine as being responsible for the observed effect.[24] Motivated by Saito's results, Greenberg's group studied the photochemistry of somewhat longer double-stranded oligomers comprising selected 5′-XBrdU-3′ (where X stands for a native nucleobase) sequences.[25] Using denaturating gel electrophoresis they found that the yield of photoinduced single-strand breaks was 8 times higher for the oligomer containing 5′-ABrdU-3′ than that for the 5′-GBrdU-3′ sequence.[22]

Irrespective of the actual role of PSET in formation of DNA damage, all studies agree that the reactive uracil-5-yl radical is generated in the course of UV irradiation of 5-BrdU-substituted oligonucleotides.[26] Indeed, model studies concerning aqueous solutions of 5-BrU indicate that UV radiation triggers the homolytic dissociation of the C5−Br bond, resulting in the uracil-5-yl radical that is stabilized in a subsequent hydrogen-atom abstraction from the environment, which ultimately leads to formation of deoxyuridine.[5] Although the quantum yield of this process is not especially high (3×10^{-3}),[5] it may also be operative in the substituted DNA. Indeed, a conspicuously higher quantum yield for the formation of 2-deoxyribonolactone, 1.4×10^{-2}, has been measured for a duplex comprising the 5′-ABrdU-3′ sequence.[23] Finally, it was recently demonstrated within the CASPT2/CASSCF study that in the first excited state (S_1) of 5-BrU only a low barrier has to be overcome to access an extended region of degeneracy between the S_1 and the S_0 states: depending on the actual region of the seam the photoreaction can result in bromine elimination or regeneration of the reactant.[27]

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The importance of having knowledge of the photochemical behavior of 5-BrdU cannot be overestimated as far as DNA photosensitization is concerned. In fact, the photoreactivity of substituted DNA should be closely related to the photochemical features of 5-BrdU itself. The most thorough photochemical study on 5-BrdU was published by Görner,[5] who investigated its photodegradation with 254 nm photons in deoxygenated aqueous solutions containing isopropanol and/or acetone. Interestingly, the quantum yield for the formation of 2′-deoxyuridine was ca. 270 times higher in solutions containing acetone besides isopropanol.[5] This huge difference in reactivity is related to the different mechanism operative in the systems compared; when only isopropanol is present in the solution, incident photons lead to the homolytic dissociation of the C5−Br bond in 5-BrdU followed by hydrogen abstraction from isopropanol.[5] On the other hand, the presence of acetone sensitizes the efficient generation of isopropanol radicals, which in the subsequent step transfer their unpaired electrons to the 5-BrdU molecules, leading to 5-BrdU anion radicals that can easily eliminate the bromide anions.[5]

The current paper deals with the photodegradation of 5-BrdU in deoxygenated aqueous solutions including isopropanol and acetone. Instead of the 254 nm photons employed by Görner,[5] we used 300 nm photons. This wavelength seems better suited to the DNA context than the 254 nm light used by Görner. Indeed, when 5-BrdU is incorporated into DNA, only radiation of a wavelength well above the maximum of its absorbance, i.e., > 260 nm, can efficiently damage the substituted biopolymer. The wavelengths of 250−260 nm are effectively absorbed by DNA itself; therefore, if the biopolymer is substituted with 5-BrdU to only a small extent (a usual situation in biological systems), the photochemical process is not triggered at all. Although the absorbance maximum of 5-BrdU amounts to ca. 280 nm, we used photons of 300 nm, which seems to be a good compromise, ensuring significant absorbance of the bromonucleoside but negligible absorbance of DNA itself.

The irradiated solutions of 5-BrdU were analyzed with RP HPLC. We observed, besides 2′-deoxyuridine, a new product that has not been described in the literature so far. This unexpected photoproduct was analyzed with the help of mass spectrometry; it turned out to be an adduct of dU and isopropanol. The molecular structure of the adduct was deciphered with the help of the B3LYP quantum chemical model, and its UV spectrum, predicted with the TDDFT method for the geometry optimized at the B3LYP level, corresponds well to the experimental one.

2. Methods

2.1. Experimental Section. ***2.1.1. Standard Solution.*** 5-Bromo-2′-deoxyuridine (5-BrdU) and 2′-deoxyuridine (dU) were purchased from Sigma-Aldrich (Poland); their purity, determined by high-performance liquid chromatography (HPLC), was over 99%. Acetone (Chempur, Poland) and 2-propanol (Chempur, Poland) were purified by distillation, and deionized water (Hydrolab Polska HLP) was used for the preparation of the standard solution containing 3×10^{-4} M 5-BrdU, 1.4 M isopropanol, and 0.15 M acetone.

2.1.2. Irradiation Conditions. Prior to irradiation the standard solution was freshly prepared and deoxygenated by purging with argon of 99.998% purity for 5 min. We checked with HPLC that the concentration of acetone fell to ca. 0.12 M as a result of the deoxygenation procedure employed. Photolysis was carried out in quartz capillaries (3 × 3 mm) filled with the standard solution in a total volume of 50 μL with a 200 W high-pressure mercury lamp. The 300 nm wavelength of incident light (half-width 2.5 nm) was selected using a prismatic monochromator (SPM-2 Carl Zeiss, Jena). 3,4-Dimethoxynitrobenzene (3,4-DMNB) in aqueous potassium hydroxide (0.5 M KOH) was used as actinometer.[28]

2.1.3. Chromatography Conditions. After UV irradiation the samples were removed from the quartz tubes and analyzed by RP HPLC. The HPLC separation was performed on an HPLC apparatus equipped with a photodiode array detector (Shimadzu, detector PDA SPD-M20A, pumps LC-20AD). The reactants were separated using an analytical Atlantis reverse-phase dC18 column (4.6 mm × 150) maintained at room temperature. Analysis was done using an isocratic mobile phase, a buffer containing deionized water, acetonitrile (Sigma-Aldrich, Poland), and 1% formic acid (POCH S.A., Poland) (pH 2.55; 87.7:2:10.3, v/v/v), as recommended by the column producer, at a flow rate of 1.0 mL/min. All reactants were detected at 260 nm. The yield of dU formation and loss of 5-BrdU were determined by calibration against authentic reference compounds.

2.1.4. Mass Spectrometry. To determine the molecular mass of the unknown photoproduct, 10 samples of the standard 5-BrdU solution were irradiated at 300 nm, one after another, for 1 h each and the photolyte obtained in a total volume of 500 μL was separated with RP HPLC. The longest retention time fraction was collected, lyophilized to dryness, and redissolved in 20 μL of nanopure-grade H_2O. This solution was then analyzed on an MS HCT-ultra mass spectrometer (Bruker Daltonics) equipped with electrospray ionization and an ion trap mass analyzer.

2.2. Theoretical Section. To discover the structure of the adduct in the gas phase, we applied density functional theory with Becke's three-parameter hybrid functional (B3LYP)[29−31] and the 6-31++G(d,p) basis set.[32,33] Additionally, for the solute in an aqueous environment the polarized continuum model (PCM[34]) with the UAHF solvation radii[35] was employed.

All geometries presented here were fully optimized without any geometrical constraints, and analysis of harmonic frequencies proved that all of them are geometrically stable (all the force constants were positive). The relative energies (ΔE) and free enthalpies (ΔG) of the isopropyl radicals and 5-isopropyl-2′-deoxyuridine adduct (isoprop-dU) were defined with respect to their most stable forms.

The absorption spectra of isoprop-dU, dU, and 5-BrdU in aqueous solution were calculated using the TDDFT method[36] with the B3LYP functional and 6-31++G(d,p) basis set. The lowest 20 singlet−singlet excitations were computed and the shapes of the spectra estimated on the basis of the calculated transition energies and oscillator strengths using the GaussView 5 program.[37] The simulated spectra were calculated as a sum of Gaussian functions corresponding to the calculated transitions. Namely, particular Gaussians were positioned at the calculated transition energies, their integrals were proportional to the calculated oscillator strengths, and their half-widths were equal to 0.333 eV.[37]

All quantum chemical calculations were carried out with the GAUSSIAN09[38] code on dual Intel Itanium 2 nodes at the Academic Computer Center in Gdańsk (TASK), and the pictures of the molecules were plotted with the GaussView 5 package.[37]

3. Results and Discussion

3.1. Chromatograms. In Figure 1 a typical chromatogram of the standard solution (for definition of the standard solutions, see Methods) of 5-bromo-2′-deoxyuridine irradiated for 60 min with photons of 300 nm is compared to that of the reference

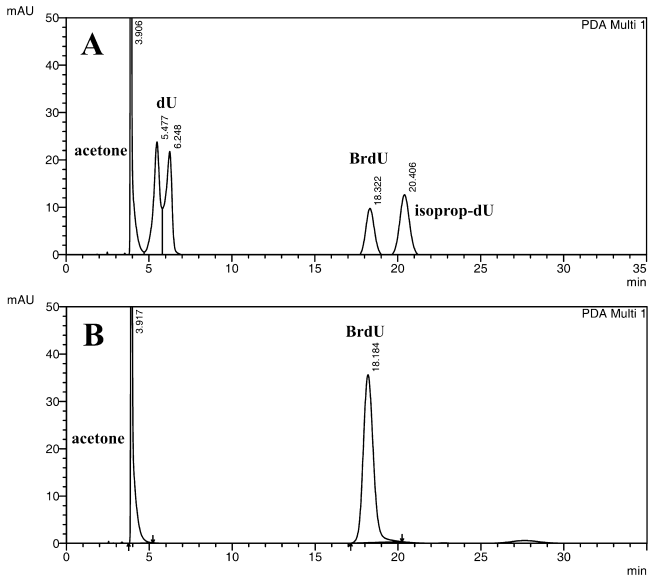

Figure 1. Chromatogram of 5-BrdU (3×10^{-4} M) in 10% isopropanol, 0.12 M acetone, and water (A) after 60 min irradiation with a high-pressure mercury lamp of 200 W at 300 nm and (B) the nonirradiated solution.

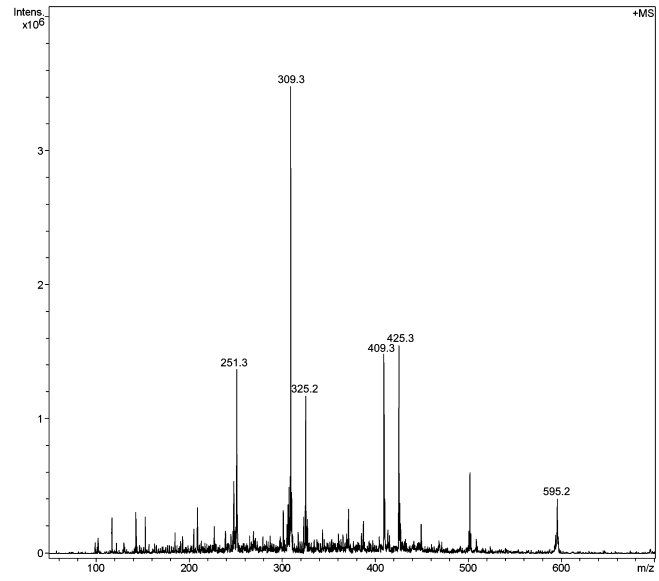

Figure 2. Positive mass spectrum of isoprop-dU.

system, not exposed to UV radiation. The peak with a retention time of ca. 4 min, observed in both chromatograms, is due to acetone (cf. Figure 1A with 1B). The next two poorly separated peaks (see Figure 1A), registered for the irradiated system, are due to 2′-deoxyuridine. The peak at ca. 18 min (both chromatograms, see Figure 1A and 1B) is due to 5-BrdU, and the last one (at ca. 20 min, see Figure 1A) is related to the unknown photoproduct.

At first glance the two peaks corresponding to dU (retention times of 5.5 and 6.2 min, respectively; see Figure 1A) suggest the involvement of more than just a single photoproduct. One should, however, take into account the fact that our standard solution contained a significant amount (10%) of isopropanol, which means that the elution capability of the sample solution itself was greater than that of the mobile phase used for the separation. Moreover, the injection volume of 20 μL is substantial, so one can expect a disturbance in the chromatographic separation that may lead to a change in retention times, broadening of particular peaks, and even their splitting. We carried out a series of HPLC separations for dU dissolved in water, water−acetone (0.12 M), and water−isopropanol (10%). Only in the latter case did we observe the characteristic two poorly separated peaks (as observed for the irradiated standard solution (see Figure 1A)) with respective retention times of 5.4 and 6.2 min (see Figures S1, S2, and S3, Supporting Information). Furthermore, when 50 μL of the irradiated solution was lyophilized to dryness (removal of isopropanol and acetone) and then redissolved in the same amount of pure water, only a single sharp peak corresponding to dU was observed (see Figure S4, Supporting Information). Moreover, when we substituted our Atlantis column, used throughout these studies, with a Kinetex column, the shape of the dU HPLC signal changed dramatically (see Figure S5, Supporting Information). Finally, we confirmed that the UV spectra corresponding to the retention times of 5.5 and 6.2 min in the chromatogram of dU dissolved in water containing 10% of isopropanol are identical (see Figure S6, Supporting Information). All these facts therefore suggest that the double peak observed in the chromatograms of irradiated samples is due to dU and results from a disturbance in the chromatographic process owing to the substantial amount of isopropanol in the standard solution.

Formation of dU as a result of 5-BrdU photodegradation in aqueous solutions containing isopropanol and acetone has already been described by Görner.[5] However, that author used a low-pressure mercury lamp emitting light of 254 nm rather than the 300 nm photons employed in our studies. We decided to carry out photolysis using the latter wavelength since it is still absorbed by 5-BrdU, whereas DNA absorption in this spectral region is negligible. On the other hand, light of 254 nm is strongly absorbed by native DNA, so even if DNA were labeled by 5-BrdU, the increased sensitivity of 5-BrdU toward 254 nm would be masked by the self-absorption of the biopolymer. From a practical point of view, therefore, 5-BrdU sensitivity to 300 nm rather than to 254 nm seems to be of greater interest.

The quantum yield of 5-BrdU degradation, 0.63, measured in our system is in fair agreement with that observed by Görner (0.8).[5] We also verified that irradiation with 254 nm photons leads to the same picture as the photolysis carried out with light of 300 nm, i.e., comparable amounts of dU and the unknown photoproduct are formed. This suggests that the same mechanism is operative under our and Görner's experimental conditions.

3.2. New Photoproduct. The chromatogram (Figure 1A) obtained for the photolyte indicates that formation of dU takes only one of the possible pathways by which the uridine-5-yl radical is stabilized. Indeed, an additional photoproduct with a retention time of ca. 20 min is also formed (see Figure 1A). In order to identify this species we separated ca. 500 μL of the photolyte with RP HPLC and collected the fraction with a retention time of 20 min. This solution was then lyophilized, redissolved in 20 μL of nanopure water, and analyzed by mass spectrometry. The specific mass spectra are shown in Figures 2 and S7, Supporting Information. The main signals, m/z +309.3 (positive ionization, Figure 2) and −285.2 (negative ionization, Figure S7, Supporting Information), correspond to the sum of the mass of the dU and isopropyl radicals (isoprop-dU) and sodium cation ($M_{2\text{-propanol-1}} + M_{dU\text{-}1} + M_{Na}^{+}$) and to the sum of the mass of the dU and isopropyl radicals diminished by one ($M_{2\text{-propanol-1}} + M_{dU\text{-}1} - 1$), respectively. One can also identify the signals of m/z +325.2 and +595.2 (see Figure 2) matching the mass of the unknown product increased by the mass of the

TABLE 1: Relative Energies (Δ*E*) and Free Enthalpies (Δ*G*) Calculated with Respect to II of the Three Types of Isopropyl Radicals As Well As the Expectation Value of S^2 ($\langle S^2 \rangle$) Calculated at the B3LYP/6-31++G Level[a]**

	gas phase			90% water:10% 2-propanol		
radical type (see Figure 3)	Δ*E*	Δ*G*	$\langle S^2 \rangle$	Δ*E*	Δ*G*	$\langle S^2 \rangle$
I	10.3	6.2	0.7538	10.2	8.9	0.7539
II	0.0	0.0	0.7538	0.0	0.0	0.7534
III	10.1	6.8	0.7537	10.4	10.3	0.7539

[a] Energy values are given in kcal/mol.

potassium cation and the doubled mass of isoprop-dU increased by the mass of the sodium cation, respectively. The signal of *m*/*z* + 251.3 may be due to fragmentation of the adduct, i.e., it could have appeared as a result of the detachment of the acetone molecule from the isoprop-dU complex with Na^+. Similarly, the two signals of *m*/*z* +409.3 and +425.3 could have originated from complexes formed by a sodium cation, namely, isoprop-dU and a sugar fragment. Indeed, the latter one, +425.3, corresponds to the sum of the mass of Na^+, isoprop-dU, and a sugar fragment (forming as a result of N-glycosidic bond breakage in isoprop-dU), which stabilizes itself by liberating hydrogen (whereupon a double bond between the C1′ and C2′ atoms in the 2′-deoxyribose moiety is formed), while the former differs from the latter by the mass of oxygen (which could have been released from the sugar fragment). Note that *m*/*z* −399.2 in the negative mass spectrum may have resulted from further dehydrogenation (release of a H_2 molecule) of the same isoprop-dU-sugar fragment complex that is responsible for *m*/*z* +425.2 in the positive mass spectrum. Furthermore, fragmentation of isoprop-dU could explain signals observed in the negative spectrum, namely, *m*/*z* −255.4 and −227.4 (see Figure S7, Supporting Information) could have appeared as a result of the release of CH_2═O and acetone molecule, respectively, from the −285.2 anion. Finally, the signal of *m*/*z* −321.2 (see Figure S7, Supporting Information) corresponds to the sum of the mass of the new photoproduct and two molecules of water diminished by one. Consequently, both the positive and the negative mass spectra suggest that the new photoproduct is an adduct of dU and isopropanol.

3.3. Structure of the 2′-Deoxyuridine−Isopropanol Adduct. To confirm the structure of the new photoproduct, molecular modeling was carried out at the quantum chemistry level. First, we considered the energetics of the isopropyl radicals, then the stability of the probable adduct was characterized at the B3LYP level, and finally the UV absorption spectra were calculated at the TDDFT level for the substrate (5-BrdU) and photoproducts (dU and the adduct).

Table 1 shows the energy characteristics of possible radicals resulting from the abstraction of hydrogen from isopropanol (see Table S1, Supporting Information, for their *xyz* coordinates). Such radicals may be formed during the reaction between isopropanol and triplet acetone.[5] As indicated by the data in Table 1, the most probable radical, **II** (see Figure 3 for its structure), is formed when the hydrogen atom is abstracted from the C2 site of isopropanol. The two other possible radicals, **I** and **III** (see Figure 3), are ca. 10 kcal/mol less stable than **II** both in the gas phase and in the water/isopropanol solution (see Table 1). Therefore, **II** seems to be the species which, when interacting with 5-BrdU, triggers a reaction sequence that leads to both photoproducts.

Both NMR data and molecular dynamics simulations indicate that nucleosides favor the *south* conformation of the sugar ring.[39]

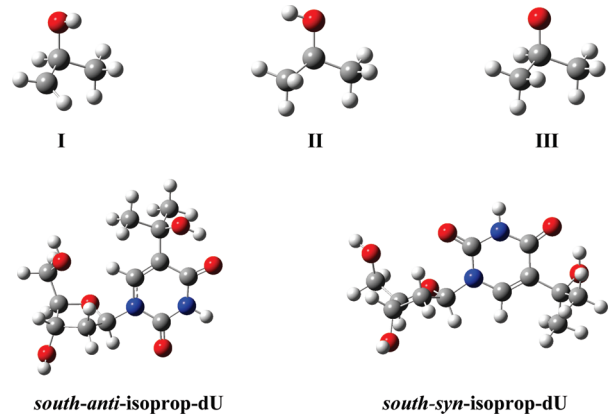

Figure 3. Geometries of possible 2-propanol radicals and the *south-anti* and *south-syn* conformers of isoprop-dU.

TABLE 2: Relative Energies (Δ*E*) and Free Enthalpies (Δ*G*) Calculated with Respect to *south-syn*-isoprop-dU and the Dipole Moments (*μ*) of the Conformers of 5-Isopropyl-2′-Deoxyuridine Calculated at the B3LYP/6-31++G Level[a]**

	gas phase			90% water:10% 2-propanol		
radical type	Δ*E*	Δ*G*	*μ*	Δ*E*	Δ*G*	*μ*
south-anti-isoprop-dU	1.2	0.1	6.8	−4.1	−4.1	9.2
south-syn-isoprop-dU	0.0	0.0	5.2	0.0	0.0	8.3

[a] All energy values are given in kcal/mol, dipole moments in Debyes.

Therefore, only the *south* conformation was considered in the current study. Thus, Figure 3 presents two representative conformations of the adduct (see Table S2, Supporting Information, for their *xyz* coordinates). Although in the gas phase the *south-anti* conformer is destabilized with respect to the *south-syn* one, their stability is reversed in aqueous solution (see Table 2). Indeed, the PCM calculations suggest that in a polar environment the *south-anti* conformation is ca. 4 kcal/mol more stable than the *south-syn* one. Despite the fact that for nucleosides the *anti* conformers are more stable than the *syn* ones both in solution and in the gas phase,[39−41] the steric hindrance present in the *anti* conformer of the adduct explains its smaller stability in vacuum (see Table 2) while the difference in dipole moments of the adduct *anti* and *syn* conformers (see Table 2) supports the larger stability of its *anti* arrangement in a polar environment (see Table 2). Thus, in a water:isopropanol solution the equilibrium mixture of adduct conformers should be completely dominated by the *south-anti* isomer.

Our MS-based assignment of the highest retention time HPLC peak is also confirmed by UV spectroscopy results: since the photolyte was separated with HPLC apparatus equipped with a PDA detector (see Methods), the UV spectra of species contributing to the particular HPLC peaks were measured. Figure 4 compares these experimental spectra to the ones calculated at the TDFT level. The excellent accordance between the measured and the computational characteristics appears to be a strong argument confirming our assignment. The long-wavelength absorption maximum for the adduct is red shifted by ca. 5 nm with respect to that for dU in both the experimental and the calculated spectra. This corresponds with the assumed structure of the adduct, since a similar red shift (+5 nm) in the long-wavelength maximum is observed when the aqueous solution UV spectrum of uridine[42] is compared with that of thymidine.[43]

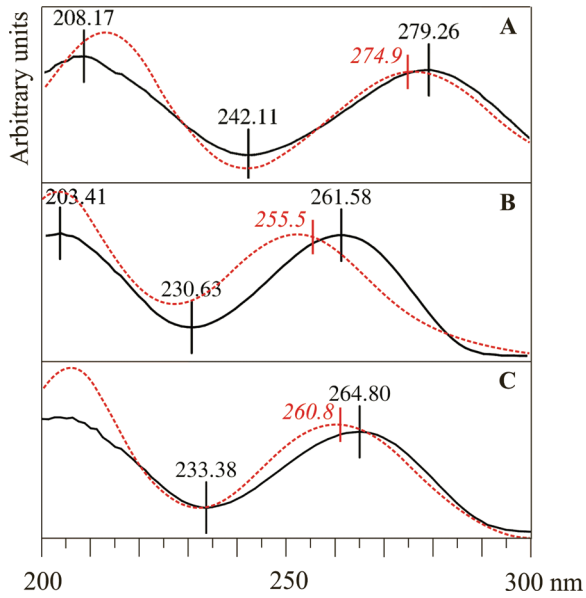

Figure 4. Comparison of the theoretical (red dotted lines) and experimental (black continuous lines) spectra of 5-BrdU (A), dU (B), and isoprop-dU (C). Black vertical lines and numbers indicate the positions and values of bands' extrema, respectively, on the experimental spectra, whereas the red vertical lines and numbers are the positions and values of the first transitions (those of the smallest energy), respectively, on the theoretical spectra.

(i) 5–BrdU + isoprop$^{\bullet}$ $\xrightarrow{ET}$ [5–BrdU]$^{\bullet-}$ + isoprop^{+} $\downarrow$ acetone + H^{+}

(ii) [5–BrdU]$^{\bullet-}$ $\longrightarrow$ Br^{-} + dU$^{\bullet}$

(iii) a) dU$^{\bullet}$ + isoprop $\xrightarrow{k_1}$ dU + isoprop$^{\bullet}$

b) dU$^{\bullet}$ + isoprop$^{\bullet}$ $\xrightarrow{k_2}$ isoprop–dU

Figure 5. Reaction scheme depicting formation of isoprop-dU within the radical−radical process.

3.4. Mechanism. At first glance formation of the *anti*-isoprop-dU adduct in a solution containing acetone and isopropanol seems to be consistent with the photodegradation mechanism of 5-BrdU assumed in the literature.[5] The absorption of 300 nm photons by acetone efficiently produces an acetone triplet, which in the next step abstracts a hydrogen atom from 2-propanol (5-BrdU also absorbs at this wavelength; however, the quantum yield for its decay via direct excitation is ca. 300 times lower than that sensitized by acetone (see above). Therefore, the direct photolysis of 5-BrdU was neglected in the following discussion). The subsequent steps of the photochemically initiated process could proceed as follows (see Figure 5): (i) the isopropanol radical reacts with 5-bromo-2′-deoxyuridine via electron transfer (ET) reaction to yield a closed-shell isopropanol cation (that spontaneously dissociates into acetone and a proton) and the 5-BrdU radical anion; (ii) the latter species easily eliminates the bromide anion, as demonstrated by pulse-radiolysis[44] and DFT studies,[45] leaving the uridine-5-yl radical (dU$^{\bullet}$); (iii) in the next step, the uridine-5-yl radical may be stabilized by removal of a hydrogen atom from isopropanol, which leads to the dU that is detected during the HPLC separation. The latter elemental reaction regenerates the alcohol radical, which can react again with another molecule of 5-BrdU. Steps i−iii thus define a chain reaction. In fact, the quantum yields measured for the photodegradation of 5-BrdU in water:isopropanol:acetone solutions at various pH range from 0.8 to 4.0, which confirms the chain-like nature of this process.

Another stabilization pathway for dU$^{\bullet}$ could be formation of the above-mentioned adduct as a result of a radical−radical reaction involving uridyl and 2-propanol radicals (see Figure 5). It is, however, worth noticing that the amount of adduct produced in the process under investigation is similar to that of dU. If the assumption about the radical−radical route leading to the adduct is correct, then the reaction rates for the elemental steps resulting directly in dU and the adduct should be similar.

In the kinetic model under discussion the rate of formation of dU is governed by $v_{dU} = k_1[dU^{\bullet}][2\text{-propanol}]$ and the rate of formation of the adduct by $v_{adduct} = k_2[dU^{\bullet}][2\text{-propanol radical}]$, where v_i, k_i, and the symbols in square brackets indicate the rate of reaction i, its rate constant, and the concentrations of reactants, respectively. One may further assume that the rate constant for the first reaction, k_1, is of the order of 10^6 M^{-1} s^{-1} (a value typical of a radical−closed-shell molecule bimolecular reaction), whereas k_2 is of the order of 10^9 M^{-1} s^{-1} (a value predicted according to Debye's equation[46] for a diffusion-controlled process in the water(90%):isopropanol(10%) system). Furthermore, taking into account the fact that triplet acetone is produced at the rate of ca. 3×10^{-7} M^{-1} s^{-1} under our experimental conditions, the concentration of 2-propanol radicals should be well below this number during the course of the photochemical decay of 5-BrdU. The above discussion therefore indicates that the concentration of isopropanol radicals is at least 7 orders of magnitude smaller than the concentration of 2-propanol itself during the photochemical degradation of 5-BrdU. Consequently, the v_{adduct}/v_{dU} ratio should not be larger than 10^{-4}, i.e., the adduct should make up only a small fraction (ca. 0.01%) of the dU produced, which stands in obvious contrast with the results of our measurements. Thus, the above discussion leads to the conclusion that the adduct is not formed directly via a radical−radical reaction but via some other process, whose rate is similar to that in which dU is produced. Indeed, we localized, at the B3LYP level, a transition state for the reaction between the isopropyl radical and 5-BrdU leading to the adduct and the bromine atom. The estimated kinetic barrier of this process amounts to only ca. 8 kcal/mol, which translates into a rate constant very similar to that of the reaction responsible for the formation of dU. A mechanistic model, based on the results of quantum chemical studies, which agrees with all the experimental findings reported in the current work, will be the subject of a separate paper.

4. Summary

The photodegradation of 5-BrdU in water/isopropanol solutions containing acetone induced by 300 nm photons was studied. Efficient photolysis (quantum yield of 5-BrdU decay ca. 0.63) leads to formation of dU and a comparable amount of a product not reported in the literature so far. The identity of the latter species was determined with the help of mass spectrometry and quantum chemical modeling. The mass signals demonstrate unequivocally that the unknown product is an adduct of 2′-deoxyuridine and isopropanol. The thermodynamic characteristics of the possible isopropanol radicals as well as the good correspondence between the computed and the experimental spectra of the adduct enabled its probable structure to be confirmed.

UV irradiation of isopropanol/acetone solutions of Hal-NBs may be a convenient method for studying the radiosensitivity of halogen-substituted nucleosides. Indeed, the presence of acetone and 2-propanol besides water triggers electron transfer, leading directly to formation of Hal-NB radical anions. These species should also form during water radiolysis, since low-

energy electrons are the main product in water irradiated with high-energy quanta. Thus, photolysis of Hal-NB water/alcohol solutions containing acetone seems to be a convenient method for studying the reactivity of Hal-NB anions that does not require the use of high-energy radiation.

Photochemical studies of water:isopropanol:acetone solutions containing other Hal-NBs are under way in our laboratory.

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Supporting Information Available: Cartesian coordinates of isopropyl radicals and isoprop-dU conformers; chromatograms of dU in water, in 0.12 M acetone and water, and in 10% isopropanol and water; chromatogram of the photochemical reaction mixture after removal of isopropanol and acetone; chromatogram of dU in 10% isopropanol, 0.12 M acetone, and water obtained on a Kinetex C18 column; UV spectra registered for the double peak of dU dissolved in water containing 10% isopropanol. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Szybalski, W. *Cancer Chemother. Rep.* **1974**, *58*, 539–557.
(2) Abdoul-Carime, H.; Huels, M. A.; Illenberger, E.; Sanche, L. *Int. J. Mass Spectrom.* **2003**, *228*, 703–716.
(3) Abdoul-Carime, H.; Paulo Limão-Vieira, P.; Gohlke, S.; Petrushko, I.; Mason, N. J.; Illenberger, E. *Chem. Phys. Lett.* **2004**, *393*, 442–447.
(4) Wang, C.-R.; Lu, Q.-B. *Angew. Chem., Int. Ed.* **2007**, *46*, 6316–6320.
(5) Görner, H. *J. Photochem. Photobiol. A: Chem.* **1995**, *89*, 147–156.
(6) Jimenez, L. B.; Encinas, S.; Miranda, M. A.; Navacchia, M. L.; Chatgilialoglu, C. *Photochem. Photobiol. Sci.* **2004**, *3*, 1042–1046.
(7) Chatgilialoglu, C.; Bazzanini, R.; Jimenez, L. B.; Miranda, M. A. *Chem. Res. Toxicol.* **2007**, *20*, 1820–1824.
(8) Flyunt, R.; Bazzanini, R.; Chatgilialoglu, C.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2000**, *122*, 4225–4226.
(9) Boussicault, F.; Kaloudis, P.; Caminal, C.; Quinto G. Mulazzani, Q. G.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **2008**, *130*, 8377–8385.
(10) Li, X.; Sanche, L.; Sevilla, M. D. *J. Phys. Chem. A* **2002**, *106*, 11248–11253.
(11) Li, X.; Sevilla, M. D.; Sanche, L. *J. Am. Chem. Soc.* **2003**, *125*, 8916–8920.
(12) Ling, L. L.; Ward, J. F. *Radiat. Res.* **1990**, *121*, 76–83.
(13) Limoli, C. L.; Ward, J. F. *Radiat. Res.* **1993**, *134*, 16–169.
(14) Franken, N. A. P.; Van Bree, C.; Kipp, J. B. A.; Barendsen, G. W. *Int. J. Radiat. Biol.* **1997**, *72*, 101–109.
(15) Matsutani, M.; Kohno, T.; Nagashima, T.; Nagayama, I.; Matsuda, T.; Hoshino, T.; Sano, K. *Radiat Med* **1988**, *6*, 33–39.
(16) Timothy, J.; Kinsella, M. D.; Patricia, P.; Dobson, B. S.; Mitchell, J. B. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1519–1522.
(17) Levin, R. D.; Gordon, J. H. *Cancer* **1993**, *72*, 2895–2901.
(18) Tashiro, R.; Sugiyama, H. In *Radical and Radical Ion Reactivity in Nucleic Acid Chemistry*; John Wiley and Sons, Inc.: New York, 2009; pp 163−189 and references cited therein.
(19) Doddridge, Z. A.; Warner, J. L.; Cullis, P. M.; Jones, G. D. D. *Chem. Commun.* **1998**, *18*, 1997–1998.
(20) Chen, T.; Cook, G. P.; Koppisch, A. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **2000**, *122*, 3861–3866.
(21) Cook, G. P.; Chen, T. Q.; Koppisch, A. T.; Greenberg, M. M. *Chem. Biol.* **1999**, *6*, 451–459.
(22) Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025–10030.
(23) Sugiyama, H.; Tsutsumi, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, *112*, 6720–6721.
(24) Fujimoto, K.; Ikeda, Y.; Saito, I. *Tetrahedron Lett.* **2000**, *41*, 6455–6459.
(25) Chen, T.; Cook, G. P.; Koppisch, T.; Greenberg, M. M. *J. Am. Chem. Soc.* **2000**, *122*, 3861–3866.
(26) Xu, Y.; Sugiyama, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 1354–1362.
(27) Kobyłecka, M.; Migani, A.; Asturiol, D.; Rak, J.; Blancafort, L. *J. Phys. Chem. A* **2009**, *113*, 5489–5495.
(28) Zhang, J.-Y.; Esrom, H.; Boyd, I. W. *Appl. Surf. Sci.* **1999**, *138*−*1399*, 315–319.
(29) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100.
(30) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
(31) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
(32) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724–728.
(33) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257–2261.
(34) Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.* **2005**, *105*, 2999–3093.
(35) Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210–3221.
(36) Dreuw, A.; Head-Gordon, M. *Chem. Rev.* **2005**, *105*, 4009–4037.
(37) Frisch, M. J.; Hratchian, H. P.; Dennington, R. D., II; Todd, A.; Keith, T. A.; Millam, J. *GaussView 5*; Gaussian, Inc.: Wallingford, CT, 2009.
(38) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision A.1; Gaussian, Inc.: Wallingford, CT, 2009.
(39) Wang, F. F.; Gong, L. D.; Dong-Xia Zhao, D. X. *J. Mol. Struct. (THEOCHEM)* **2009**, *909*, 49–56.
(40) Foloppe, N.; Hartmann, B.; Nilsson, L.; MacKerell, A. D., Jr. *Biophys. J.* **2002**, *82*, 1554–1569.
(41) Hocquet, A.; Leulliot, N.; Ghomi, M. *J. Phys. Chem. B* **2000**, *104*, 4560–4568.
(42) Wataya, Y.; Negishi, K.; Hayatsu, H. *Biochemistry* **1973**, *12*, 3992–3998.
(43) Onidas, D.; Markovitsi, D.; Marguet, S.; Sharonov, A.; Gustavsson, T. *J. Phys. Chem. B* **2002**, *106*, 11367–11374.
(44) Rivera, E.; Schuler, R. H. *Z. Phys. Chem.* **1983**, *87*, 3966–3971.
(45) Li, X.; Sanche, L.; Sevilla, M. D. *J. Phys. Chem. A* **2002**, *106*, 11248–11253.
(46) Debye, P. *Trans. Electrochem. Soc.* **1942**, *82*, 265–272.

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