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5-Thiocyanato-2′-Deoxyuridine as a Possible Radiosensitizer: Electron-Induced Formation of Uracil-C5-Thiyl Radical and Its Dimerization

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Abstract:

In this work, we have synthesized the 5-thiocyanato-2′-deoxyuridine (SCNdU) along with the C6-deuterated nucleobase 5-thiocyanatouracil (6-D-SCNU) and studied their reaction with radiation-produced electrons. ESR spectra in γ-irradiated nitrogen-saturated frozen homogeneous solutions (7.5 M LiCl in H_2O or D_2O) of these compounds show that electron-induced S-CN bond cleavage occurs to form a thiyl radical (dU-5-S• or 6-D-U-5-S•) and CN^- via the initial π-anion radical ($SCNdU^{\bullet-}$) intermediate in which the excess electron is on uracil base. HPLC and LC-MS/MS studies of γ-irradiated N_2-saturated aqueous solutions of SCNdU in presence of sodium formate as a OH-radical scavenger at ambient temperature show formation of the dU-5S-5S-dU dimer in preference to dU by about 10 to 1 ratio. This shows both possible routes of electron-induced bond cleavage (dUC5-SCN and S-CN) in $SCNdU^{\bullet-}$ and dU-5-S• formation is preferred to the σ-type uracilyl radical (dU•) production by 10 fold. DFT/M06-2x/6-31++G(d,p) calculations employing the polarizable continuum model (PCM) for aqueous solution show that dU-5-S• and CN^- formation was thermodynamically favored by over 15 kcal/mol (ΔG) to dU• and SCN^- production. The activation barriers for C5-S and S-CN bond cleavage in $SCNdU^{\bullet-}$ amount to 8.7 and 4.0 kcal/mol respectively favoring the dU-5-S• and CN^- formation. These results support the experimental observation of the S-CN bond cleavage by electron addition to SCNdU that results in dU-5-S• and the subsequent dU-5S-5S-dU dimer formation. This establishes SCNdU as a potential radiosensitizer that could cause intra- and interstrand crosslinking as well as DNA-protein crosslinking via S-S dimer formation.

Keywords: radiosensitizers, thiocyanatouridine, dimerization, electron spin resonance, DFT calculations

1. Introduction

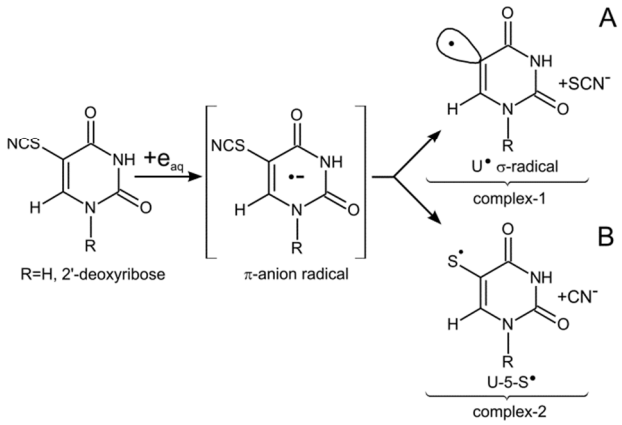
5-Bromo-2′-deoxyuridine (5-BrdU) and its nucleobase, 5-bromouracil (5-BrU), belong to the class of DNA radiosensitizers that are incorporated into DNA during replication and repair.[1-5] Owing to the very similar van-der-Waals' radii of $-CH_3$ and Br, 5-BrdU easily substitutes thymine in DNA without affecting its function.[1] Various investigations have demonstrated a correlation between the extent of 5-BrdU incorporation and radiosensitization.[4,5] The major mechanism for 5-BrdU-induced radiosensitization has been proposed to be the fast irreversible electron attachment to 5-BrdU incorporated into DNA leading to the formation of the halouracil π-anion radical; subsequently, halouracil π-anion radical very rapidly dissociates into the uracil-5-yl π-radical (U•) along with Br^- loss via dissociative electron attachment (DEA).[1] U•, being a vinyl-type radical, is highly reactive and can undergo rapid addition to the double bonds leading to crosslinking (e.g., addition to the parent C5=C6 double bond of 5-BrU)[1,6] and H-atom abstraction reaction[1,7] as competitive reactions. U• can abstract a hydrogen atom from adjacent[8-10] sugar residue leading to a single strand break (SSB). Quite recently, it is demonstrated that U• is sufficiently reactive to trigger the release of a OH-radical from a proximate water molecule.[11] We note here that the OH-radicals are well-known genotoxic agents which, if produced in sufficient proximity to the double helix, readily lead to DNA strand breaks by H-atom abstraction.[1,12] Furthermore, U• - induced H-atom abstraction from the sugar has been employed as a probe to find various conformations (A, B, Z, and G-quadruplex) of DNA-oligomers.[12,13]

Under hypoxia, commonly observed in the solid tumor cells,[14] water radiolysis releases a large number of solvated electrons[15] which are, however, not able to damage native DNA[16]

unless a radiosensitizer such as 5-BrdU is present. Therefore, the employment of some type of radiosensitization seems to be indispensable for an efficient ionizing radiation treatment.

Although in *in vitro* experiments BrdU turned out to be a potent radiosensitizer,[4,5] *in vivo* tests were much less optimistic. One of the most extended clinical trial on brain cancer patients[17] did not show any advantages of the radiotherapy with 5-BrdU as an adjuvant agent over the irradiation treatment alone. Thus, such a situation calls for new sensitizers that would be efficient not only *in vitro* but also in patients.

Very recently, we investigated the efficiency of electron-induced U• formation on various C5-H substituted uracils.[18] Therefore, we carried out a DFT modelling of DEA process in 5-substituted uracils. We assumed two premises: (i) a 5-substituent has to increase electron affinity of the studied derivative compare to that of uracil and (ii) the C5-X (where X stands for a substituent) bond should be weak enough to assure an efficient dissociation (irreversible reaction) in the π-anion radical formed due to electron attachment. The free energy profiles for DEA process calculated for only 10 derivatives resulted in two compounds, 5-thiocyanatouracil (SCNU) and 5-oxocyanatouracil (OCNU), which should be comparable or even better radiosensitizers than 5-BrU.[18]



Scheme 1. Formation of U• (ca. 10% Yield) and Uracil-5-thiyl Radical (U-5-S•, ca. 90% Yield) by Electron Addition to 5-thiocyanatouracil (SCNU) or 5-thiocyanato-2′-deoxyuridine (SCNdU) Resulting in Dissociative Electron Attachment (DEA).

In the current work, the sensitivity of SCNdU (Scheme 1) to electron attachment is investigated through the use of electron spin resonance (ESR) studies at low temperatures, steady-state radiolysis at ambient temperature, and DFT modeling. We demonstrate that π-anion radical formed in the nucleoside (SCNdU) or in the C6-deuterated nucleobase, 5-thiocyanatouracil (6-D-SCNU), owing to electron attachment leads to two types of radicals via competitive reactions (Scheme 1). Formation of dU in low abundance as a stable product points to the U• production along with SCN^- loss that was postulated in our previous studies.[18] This originated from the dissociation of the C5-S bond in the SCNdU π-anion radical, $SCNdU^{\bullet-}$ (Scheme 1). However, in this work, ESR studies at low temperature and product analyses at ambient temperature show that the predominant radical species is the 2′-deoxyuridine-5-thiyl radical (dU-5-S•). dU-5-S• is formed by S-CN bond cleavage of the thiocyanate substituent in SCNdU. The work of Houmam on aryl and benzyl thiocyanates[19,20] shows that S-CN bond cleavage via DEA is feasible in the π-anion radical; especially in aryl thiocyanate π-anion radical, S-CN bond cleavage is the favored process. Thus, our observation of predominant formation of dU-5-S• from $SCNdU^{\bullet-}$ is in accord with these results and in fact, is the first report of the base-thiyl radical formation via DEA in a DNA model system. Furthermore, our results show that dU-5-S• dimerizes to form dU-5S-5S-dU. This result allows us to propose that dU-5-S• when formed in DNA will lead to both inter and intrastrand crosslinks as well as DNA-protein crosslinks. In addition, the second product of this DEA process, CN^-, is highly cytotoxic as it

irreversibly binds to cytochrome oxidase which effectively halts cellular respiration[21] thereby proposing that SCNdU or SCNU may be an effective radiosensitizer.

2. Experimental section

Materials. Cyclohexene (≥98%), acetonitrile and methanol (HPLC grade), 2′-deoxyuridine (≥99%), potassium thiocyanate (≥99%) was purchased from Sigma-Aldrich (Poznań, Poland). Formic acid (≥99%) was purchased from POCH (Gliwice, Poland). 6-Deuterouracil (uracil-6-d_1) was purchased from CDN isotopes Inc. (Quebec, Canada) in a 0.1 vial and stored at room temperature (15-23ºC) away from light and moisture. Chlorine gas (Cl_2) was purchased from Linde AG (Munich, Germany). Lithium chloride (LiCl) (ultra dry, 99.995% (metals basis)) was obtained from Alfa Aesar (Ward Hill, MA, USA). Potassium ferrocyanide ($K_4[Fe(CN)_6]$) and deuterium oxide (D_2O) (99.9 atom % D) were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA). Chromasolv® HPLC grade H_2O was purchased from Sigma-Aldrich (St Louis, MO, USA). All these compounds were used without further purification.

Synthesis of 5-thiocyanato-2′-deoxyuridine. A 500 mL three-necked, round-bottomed flask equipped with a mechanical stirrer and drying tube was prepared. The flask was filled with an ice–cold solution of Cl_2 (1.55 g, 21.80 mmol) in dry acetic acid (300 mL) prepared by passing Cl_2 gas through a $CaCl_2$ trap connected with flask. Dried KSCN (2.34 g, 24.08 mmol) was then added to the mixture for preparation of ClSCN. The solution was then stirred for 1.5 hours at room temperature. 2′-deoxyuridine (0.5 g, 2.19 mmol) was added in one portion to the solution of ClSCN in acetic acid and stirring was continued for 2.5 hours in room temperature. Half an hour before the end of reaction, 15 mL of cyclohexene was added. After filtration, the solution was evaporated under vacuum. The residue was kept during 12 hours in 50% methanol. MeOH

was subsequently evaporated and the residue was extracted with hot water. The aqueous phase was purified with preparative HPLC (LC-20AP) with UV detector (SPD-20A) set at 260 nm. Gemini (Phenomenex®) reverse-phase C18 column (10 mm x 250 mm, 5 µm in particle size and 110 Å in pore size) with a mobile phase consisting of water, acetonitrile, and 1% formic acid (pH 2.55; 87.7:2:10.3, v/v/v) was used. The flow rate was set at 4 mL/min (see ESI, Figure S8). The resulting product (130 mg) was obtained as white powder, with a yield of 40%. IR (KBr) 3416 (NH), 2163 (CN), 1726 (CO), 1673 (CO), 1614 (CN), 1297 (CN), 1163 (CH), 1094 (CO), 766 (CH), 665 (CS), 553 (CC); Anal. ($C_{10}H_{11}N_3O_5S$): calculated C, 42.10; H, 3.89; N, 14.73; S, 11.23 found C, 41.81; H, 4.00; N, 14.43; S, 11.47; Purity HPLC: 99% tR=21.043 min; ESI-MS $[M-H]^-$ = 284.06 (MW = 285.27)

Synthesis of 5-thiocyanato-6-d_1-uracil. Following the above-mentioned protocol of SCNdU synthesis, here also a 500 mL three-necked, round-bottomed flask equipped with a mechanical stirrer and drying tube was prepared. Subsequently, the flask was filled an ice-cold solution of Cl_2 (0.53 g, 7.5 mmol) in dry acetic acid (200 mL) prepared by passing Cl_2 gas through a $CaCl_2$ trap connected with flask. For ClSCN preparation, dried KSCN (0.80 g, 8.23 mmol) was added to the mixture. The solution was then stirred for 1.5 hour at room temperature. Uracil-6-d_1 (0.1 g, 0.88 mmol) was added in one portion to the solution of ClSCN in acetic acid and stirring was continued for 2.5 hours while maintaining the room temperature. 10 mL of cyclohexene was added to the reaction mixture just at half an hour before the completion of reaction (i.e., substitution of C5-H atom in the uracil base of 6-D-U by the SCN group). After filtration, the solution was evaporated under vacuum. The residue was extracted with hot water. The aqueous phase was purified with preparative HPLC (LC-20AP) with UV detector (SPD-20A) set at 260 nm. Gemini (Phenomenex®) reverse-phase C18 column (10 mm x 250 mm, 5 µm in particle size

and 110 Å in pore size) with a mobile phase consisting of water, acetonitrile and 1% formic acid (87.7:2:10.3, v/v/v pH 2.55) was used (see ESI, Figure S7). The flow rate was set at 4 mL/min. The resulting product was white powder (25 mg, 35% yield). IR (KBr) 3432 (NH), 2279 (CD), 2160 (CN), 1705 (CO), 1666 (CO), 1188 (CH), 734 (CH), 666 (CS); Anal. ($C_5H_3DN_3O_2S$): calculated C, 35.29; H, 1.78; N, 24.69; S, 18.84 found C, 35.61; H, 1.49; N, 25.01; S, 19.14; Purity HPLC: 99% tR= 5.563 min; ESI-MS $[M-H]^-$ = 169.16 (MW = 170.18).

ESR measurements: (A) Preparation of homogeneous solutions: Following methods from our previous studies on various model systems of DNA and RNA,[22-27] homogeneous solutions of SCNdU and 6-D-SCNU were prepared by dissolving 2 mg/mL in either 7.5 M LiCl as well as in 7.5 M LiBr in D_2O or in H_2O in the presence of $K_4[Fe(CN)_6]$ (6 mg/mL) which acts as a scavenger of radiation produced holes. The purpose of addition of a hole scavenger is to follow directly and only the formation of the one-electron reduced species and its subsequent reactions via ESR spectroscopy.

(B) pH adjustments: The homogeneous solutions have high ionic strength (7.5 M LiCl or LiBr); therefore, the pH meters would not provide accurate pH measurements of these solutions. pH values reported in this work were obtained using pH papers and are approximate measurements and as described in our previous efforts.[22] The pH of SCNdU in either 7.5 M LiCl as well as in 7.5 M LiBr in D_2O or in H_2O was adjusted to the range of ca. 5 (native pH of the 7.5 M LiCl or 7.5 M LiBr) to ca. 11 depending on the experiment. These pH adjustments were performed by quickly adding μL amounts 1 M HCl or 1 M NaOH under ice-cold conditions.

(C) Preparation of glassy samples and their storage: Following our works,[22] to remove the dissolved oxygen in these pH-adjusted homogenous solutions, they were bubbled thoroughly with nitrogen. Subsequently, these solutions were immediately drawn into 4 mm Suprasil quartz

tubes (Catalog no. 734-PQ-8, WILMAD Glass Co., Inc., Buena, NJ, USA) and were rapidly cooled in liquid nitrogen (77 K). Transparent homogeneous glassy solutions were formed as a result of the rapid cooling of these degassed homogeneous liquid solutions at 77 K. These glassy solutions were later used for γ-irradiation and subsequent progressive annealing experiments. All glassy samples were stored at 77 K in Teflon containers in the dark.

(D) Irradiation and storage of γ-irradiated glassy samples for ESR studies: Following our previous efforts,[22] these glassy solutions were γ (^{60}Co)-irradiated using a model 109-GR 9 that utilizes a shielded ^{60}Co irradiator (J.L. Sheppard and Associates, Inc.) (absorbed dose = 400 to 600 Gy) at 77 K. For LC-MS and LC-MS/MS studies (vide infra), N_2-bubbled and sealed solutions of SCNdU (0.01 mg/mL in Chromasolv® HPLC grade H_2O) were γ-irradiated at varying doses between 50 to 200 Gy at room temperature in the presence of sodium formate (1 mg/mL) as the OH-radical scavenger. During each experiment, one unirradiated sample of SCNdU was left as the control.

(E) Annealing of glassy samples for ESR studies: Following our earlier works,[22-27] a variable temperature assembly was employed which passed liquid nitrogen-cooled nitrogen gas past a thermistor and over the sample. The glassy samples have been annealed anywhere from (140 – 170) K for 15 min. Annealing leads to the softening of the glass and as a result, the π-anion radical of SCNdU or 6-D-SCNU held in a homogeneous rigid glass at 77 K becomes mobile and undergoes subsequent reactions. Thus, via progressive annealing, the subsequent reactions of only the π-anion radical of the solute, e.g., SCNdU or 6-D-SCNU, have been followed directly by ESR studies in this work.

(F) Electron Spin Resonance: Following our earlier studies,[22-27] immediately after γ-irradiation of the glassy sample at 77 K, the ESR spectrum was recorded at 77 K. Also, immediately after each

annealing step, the sample was cooled to 77 K by immersing it immediately in liquid nitrogen (77 K) and the ESR spectrum was recorded at 77 K which maximizes signal height and allows for comparison of signal intensities.[27] A Varian Century Series X-band (9.3 GHz) ESR spectrometer with an E-4531 dual cavity, 9-inch magnet, and a 200 mW Klystron was used and Fremy's salt (g_{center} = 2.0056, A(N) = 13.09 G) was employed for the field calibration. All ESR spectra have been recorded at 77 K and at 40 dB (20 μW).

Anisotropic simulations of ESR spectra have been performed using the WIN-EPR and SimFonia programs of Bruker as per our previous works.[22-27] The simulated spectra thus obtained, were compared to experimental spectra and ESR parameters were adjusted for the best fit.[22-27]

HPLC Conditions. The HPLC separation was performed on a Dionex UltiMate 3000 System with a Diode Array Detector, which was set at 260 nm for monitoring the effluents. The analytes were separated on a Wakopak® Handy ODS column (4.6 mm × 150 mm; 5 μm in particle size and 100 Å in pore size). The mobile phase A consisted of 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) and mobile phase B was 80% acetonitrile. The gradient started from 100% A to 95% A in 20 min, than to 90% A in next 10 min. The flow rate was set at 1 mL/min.

LC-MS and LC-MS/MS Conditions. LC analyses were performed on an Eksingent Micro LC system (AB SCIEX). The analytes were separated on Eksingent 5C18-EP-120 column (0.5x100 mm, 5 μm, 120 Å). The mobile phase A consisted of 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) and mobile phase B was 80% acetonitrile. The gradient was from 100% A to 90% A in 5 min. The flow rate was 50 μL/min and the injected volume was 2 μL of sample. The micro LC system was

coupled directly to a QTRAP quadrupole time-of-flight (QTOF) mass spectrometer (AB SCIEX) equipped with duo-electrospray interface operated in negative ionization mode. MS and MS/MS operation parameters were the same for both types of scan and were as follows: the spray voltage was -4.0 kV and nebulizer gas (N_2) pressure 25 psi, flow rate 11 L/min and source temperature 200 ^{o}C. Each spectrum was obtained by averaging 3 scans, and the time of each scan was 0.2 s.

Computations. All calculations were performed with density functional theory (DFT), using the M06-2x[28] hybrid meta-exchange correlation functional and the 6-31++G(d,p) basis set.[29] The unconstrained geometry optimizations for stationary points (minima and transition states) on the potential energy surface of SCNdU were carried out in an aqueous solution, employing the Polarizable Continuum Model (PCM)[30] of water in order to account for the effect of polar environment.

The Gibbs free energy changes (ΔGs) calculated for particular reaction steps are the differences between the electronic energies of products and substrates, corrected for zero-point energies, thermal contributions to energies, the pV and entropy terms. These terms were computed in the rigid rotor-harmonic oscillator approximation[31] at T = 298 K and p = 1 atm.

The Gaussian09[32] code was used for all computations, while the molecule structures were visualized with the GaussView package.[33]

3. Results

ESR studies. Owing to ionization events in irradiated LiCl aqueous glasses, the electrons formed are predominantly trapped in shallow wells.[34] Although some traps can be as deep as 2.6 eV, but generally, the most abundant trapped electron species has been observed at traps ca. 0.5 eV below the continuum.[35-37] Prior to its complete solvation, these partially solvated electrons react

with the solutes. As a result, the reactions of electrons in the irradiated glasses are primarily due to these partially solvated electrons which are known as presolvated (prehydrated) electrons (e_{pre}^-).[34-39]

In Figure 1(A), we show the spectrum obtained after subtraction of the $Cl_2•^-$ ESR spectrum[23(b)] from the experimentally recorded (77 K) 400 G wide ESR spectrum (black) of one-electron reduced SCNdU by e_{pre}^- formed on π-irradiation at 77 K at pH (pD) ca. 5 in a homogeneous glassy 7.5 M LiCl/D_2O solution in the presence of the hole scavenger $K_4[Fe(CN)_6]$. Superimposition of the 77 K spectrum (blue) obtained from a matched sample of 6-D-SCNU at pD ca. 5 on top black spectrum in Figure 1(A) shows that the blue spectrum from one-electron reduction of 6-D-SCNU is identical to the black spectrum except the collapse of the central doublet of ca. 16 G in the black spectrum to a singlet in the blue spectrum. This collapse of the central doublet to a singlet on deuteration at C6 in 6-D-SCNU shows that the doublet splitting arises from the C6H proton coupling which is is only in accord with the formation of the π-anion radicals of SCNdU and 6-D-SCNU (SCNdU•$^-$ and 6-D-SCNU•$^-$) upon e_{pre}^- addition to the parent molecules (SCNdU or 6-D-SCNU, Scheme 1). In addition to this central doublet, both spectra in Figure 1(A) show a line component at g_{zz} = 2.085. This *g*-value is much higher than the observed C-centered and N-centered radicals in DNA[22-27] and based on the reported *g*-values of thiyl radicals,[40-43] the line components due to g_{zz} = 2.085 has been assigned to U-5-S•. Another small line component at g = 2.027 (indicated by *) is unassigned. It is not observed in 7.5 M LiBr glasses (see ESI, Figures S2, S4, S5).

Upon annealing from 155 to 160 K for 15 min, SCNdU•$^-$ and 6-D-SCNU•$^-$ resulted in spectra shown in Figures 1(B) and 1(C) respectively. Employing an anisotropic simulation by using *g*-tensor principal values (2.0110, 2.0000, 2.0700), anisotropic linewidth (8, 10, 25) G, and

mixed Lorentzian/Gaussian lineshape = 1, a simulated spectrum (red, Figure 1(D)) is obtained and this spectrum matches the spectra in Figures 1 (B) and 1(C) quite well. Based on the *g*-tensor principal values of S-centered radicals in the literature,[40-43] both spectra (B) and (C) are assigned to U-5-S•. Comparison of spectrum 1 (B or C) with the spectra in Figure 1(A) clearly show that the central doublet from SCNdU•$^-$ and 6-D-SCNU•$^-$ in Figure 1(A) is lost on annealing with the increase in the signal intensity of U-5-S•. The small line component at g_{zz} = 2.027 (indicated by *) is also lost. Moreover, the apparent g_{zz} values = 2.085 due to U-5-S• decreases to 2.070 upon annealing from 77 K to 160 K. In the frozen matrix, increased interaction of U-5-S• with the surrounding solvent on annealing to 160 K somewhat sharpens the low field signal and decreases the apparent g_{zz} value to 2.070. This behavior is consistent for the near orbitally degenerate radicals, such as thiyl radicals.[40-43]

Thus, in our ESR studies, we have unequivocally identified the π-anion radical of SCNdU and its deuterated analog 6-D-SCNU which undergoes cleavage of the S-CN bond forming U-5-S• (see Scheme 1). It is apparent from the Figure 1 that U-5-S• is the only radical species found at 160 K. These results clearly establish that the formation of U-5-S• via S-CN bond cleavage in SCNdU•$^-$ and in 6-D-SCNU•$^-$ is a lower energy path than U• production by C5-S bond breaking along with SCN^- loss (Scheme 1).

Our results show that formation of U-5-S• is not affected by the solvent (D_2O vs. H_2O), the glassy solution (7.5 M LiCl vs. 7.5 M LiBr), its ionic strength (7.5 M vs. 15 M), the pH range of ca. 3 to 11, and with SCNdU concentrations; these results support the U-5-S• assignment (see ESI, Figures S1 to S4, S9, and S10). The lack of SCNdU concentration dependence for U-5-S• formation from SCNdU•$^-$ support the unimolecular mechanism presented in Scheme 1.

Moreover, unlike the aliphatic thiyl radicals,[40-43] U-5-S• does not react with molecular oxygen at low temperature (see ESI, Figure S5).

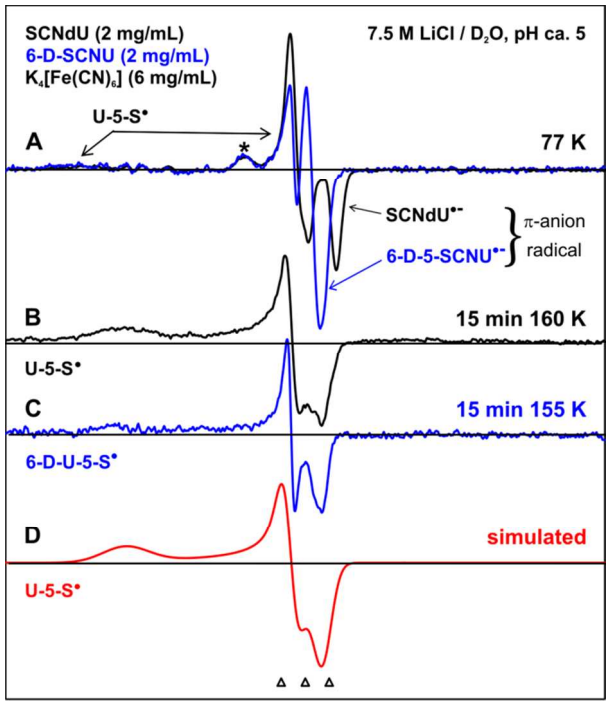


Figure 1. ESR spectra recorded at 77 K of γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/D_2O solutions of SCNdU and 6-D-SCNU with a hole scavenger $K_4[Fe(CN)_6]$. (A) The π-anion radical of SCNdU and 6-D-SCNU upon e_{pre}^- addition. Both spectra show the line components from U-5-S• as well (indicated by arrows). (B) U-5-S• spectrum after annealing at 160 K for 15 min. (C) 6-D-U-5-S• spectrum from the 6-D-5-SCNU sample obtained after annealing at 155 K for 15 min. (D) An anisotropic simulation of the spectra (B) and (C) of U-5-S• using *g*-tensor principal values. The three reference markers (open triangles) show the position of Fremy's salt resonance with the central marker at g = 2.0056 and 13.09 G marker spacing.

Radiolysis at Ambient Temperature. In order to verify stable products due to U-5-S• arise at ambient temperature, where water radiolysis leads to solvated electrons, we carried out a room temperature steady-state radiolysis of SCNdU solution saturated with N_2 and containing sodium

formate as a hydroxyl radical scavenger. HPLC traces before and after radiolysis are depicted in the middle panel of Figure 2. The energy delivered via ionizing radiation leads to the substantial decay of the substrate. Comparing the chromatograms before and after radiolysis, one can observe two products: a major product with the retention time of 22.41 min and a minor one with the retention time of 6.17 min (Figure 2). The comparison of the radiolyte chromatogram, shown in Figure 2, with the HPLC traces of 2′-deoxyuridine (dU) measured under the same conditions (not shown) proves that the signal at 6.17 min is from dU. The identity of this product is also confirmed by the LC-MS and LC-MS/MS analyses (Figure 2). The parent anion of dU with the m/z ratio equal to 227.0697 (see Table 1 for comparison between the actual and theoretical mass) corresponds to the most intensive MS signal (see the upper part of Figure 2). The fragmentation of this anion (see the bottom part of Figure 2) shows the loss of water, HCNO or sugar fragment. A similar analysis allows the signal at 22.41 min (Figure 2) to be identified. The parent anion with the m/z ratio of 517.0766 (see Table 1) corresponds to the dimer, dU-5S-5S-dU that arises due to dimerization of U-5-S• and its structure is shown in Figure 2. The appropriate fragmentation spectrum (see Figure 2, the lower panel) confirms this proposal since signals corresponding to the loss of HNCO, deoxyribose and water, and cleavage of the S-S bond (formation of dU-S(-H) anion) are observed. The presence of two additional small HPLC peaks at 3.99 min, dU-SO_2H, and 10.46 min, dU-S-O-SO_2H, (see Figure 2) confirms the involvement of U-5-S• and its dimerization product in the studied process. Formation of dU-SO_2H and dU-S-O-SO_2H in small extent points towards the fact that the residual oxygen produced during radiolysis may react with U-5-S• that accounts for dU-SO_2^- observed in the LC-MS analysis (see ESI, Figure S6). Comparison of these results obtained at ambient temperature with those obtained via ESR spectra at low temperatures suggest that the reaction of molecular oxygen with

U-5-S• has a thermal activation barrier because U-5-S• reacts with molecular oxygen at ambient temperatures but not at temperatures below 170 K (compare Figure 2 with Figure S5 in the ESI). The small amount of these reactants remains in line with the fact that only residual oxygen is present in the studied system.

Table 1. Comparison of the Theoretical (Calculated) and Actual (Measured) m/z Values for the $[M-H]^-$ Ions Identified in the MS Analyses.

Ion	Calculated mass [Da]	Measured mass [Da]
$[dU\text{-}5S\text{-}5S\text{-}dU(-H)]^-$	517.0705	517.0766
$[SCNdU(-H)]^-$	284.0347	284.0384
$[dU\text{-}S\text{-}O\text{-}SO_2]^-$	338.9962	339.0005
$[dU\text{-}SO_2]^-$	291.0292	291.0294
$[dU(-H)]^-$	227.0673	227.0697

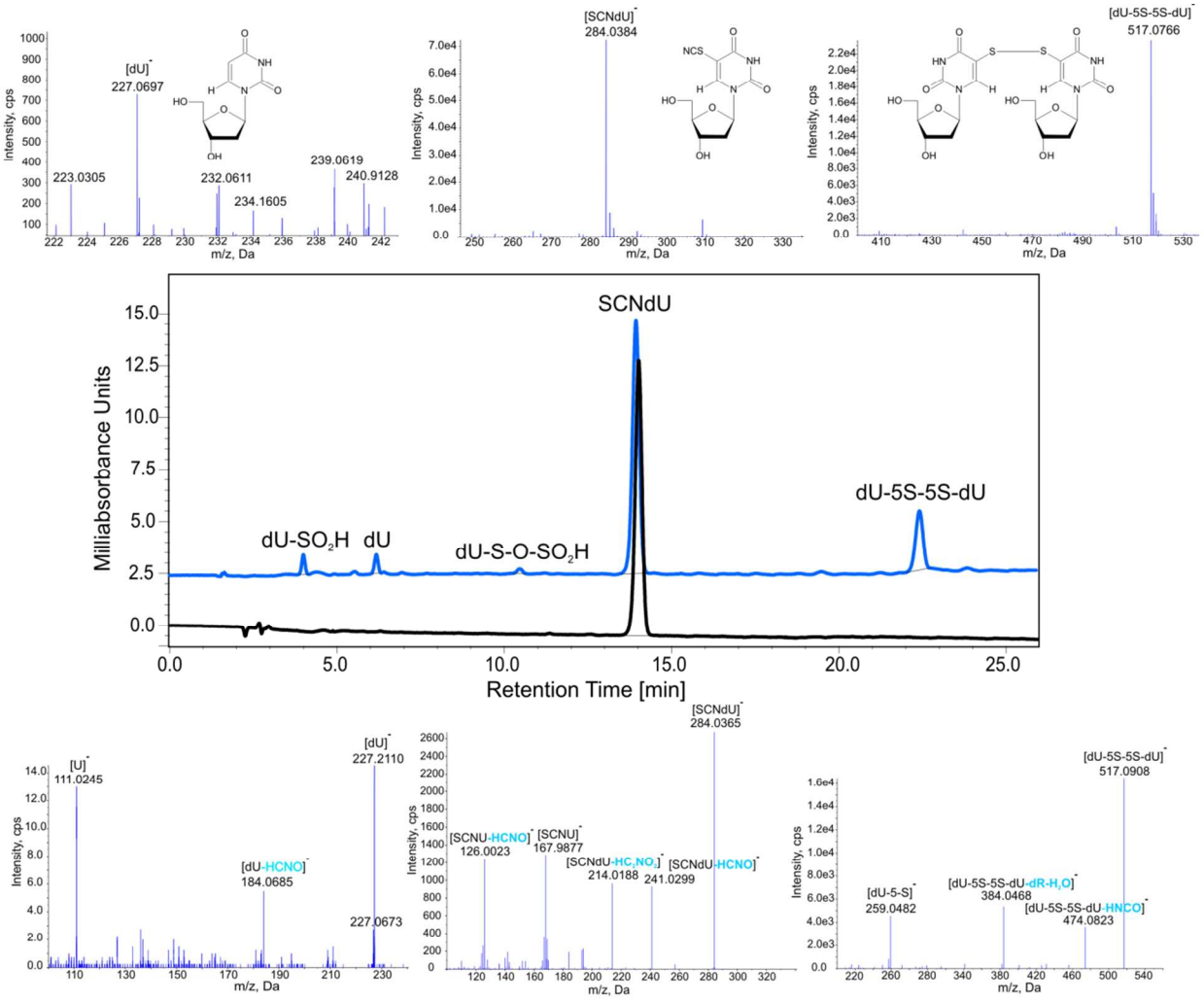


Figure 2. HPLC and MS analysis of the solution of SCNdU. Middle panel – the HPLC traces of SCNdU before (black) and after (blue) γ-irradiation with the dose of 50 Gy; upper part the MS spectra (in the negative ionization mode) of the selected HPLC signals; lower panel – the MS/MS spectra of the selected HPLC signals along with ion identities.

The data presented above unequivocally show that the SCNdU π-anion radical leads to two parallel reactions producing quite different products. In one path, the C5-S bond is broken in SCNdU•$^{-}$ and the secondary U• reacts with the formate anion resulting in dU as a stable product. On the other path, the S-CN bond cleavage in the thiocyanate substituent produces the U-5-S•

that ultimately forms a stable dimer (dU-5S-5S-dU). Moreover, neither the ESR spectral results nor the product analyses studies present any observable evidence of perthyl i.e., R-S-S• intermediates.[44]

Computational Results. The mechanisms already suggested by the above-described ESR results and steady-state radiolysis (Scheme 1) can be confirmed using computational chemistry. In order to do that, one of the most accurate and effective DFT methods has been used, namely the M06-2x functional which estimates reaction thermodynamics and kinetics with high accuracy.[28] In Figure 3, the optimized geometries of substrate are displayed. Two types of conformational freedom were taken into consideration: sugar ring puckering (C3′-exo/C3′-endo) and rotation of the -SCN substituent around the C5-S bond (front/back). These resulted in the four conformers of SCNdU, with the C3′-exo-front being the most stable thermodynamically (see Figure 3).

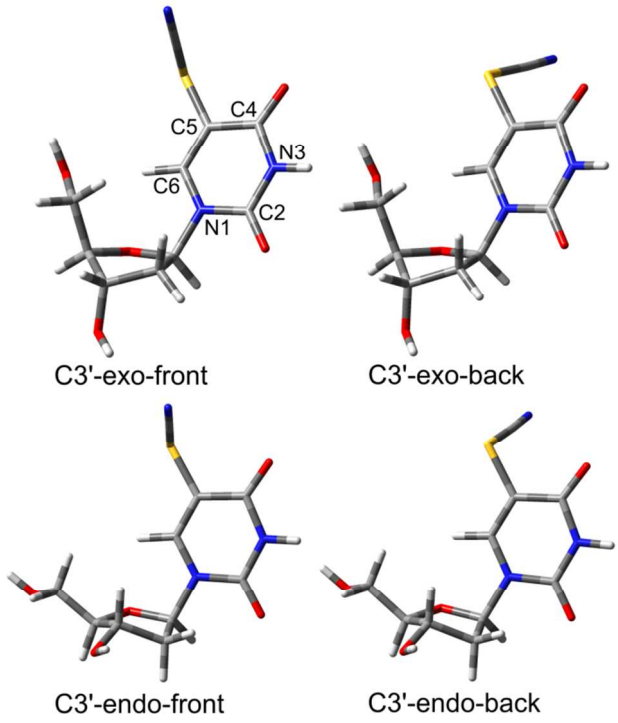


Figure 3. 3D visualization of the optimized geometries of four neutral SCNdU conformers.

The excess electron localizes on the pyrimidine ring of SCNdU that leads to its folding (the N1-C2-N3-C4 dihedral angle (for atom numbering see Figure 3) amounts to 13 degrees while it is equal to 0 in the neutral molecule). The driving force of ring folding is related to diminishing of antibonding interactions in the π^* orbital to which the excess electron is attached. On the other hand, Mulliken population analyses indicate that the electron localizes mainly on the C6 atom since it amount to -0.862 in the anion radical.

Adiabatic electron affinity (AEA_G), calculated as the differences in free enthalpy of the neutral and corresponding anion radicals in their optimal geometries, were found to be significantly positive. These results and spin density analysis point out that all the considered structures form electronically stable π-anion radicals (see SCNdU•$^-$ at Figure 4 and the AEA_G values in Table 2). These radical anions may basically transform via two competitive degradation paths: one leading to the detachment of the anionic SCN^- substituent via complex-1 (see path A, Scheme 1 and Figure 4) and another one resulting in the CN^- detachment via complex-2 (path B, Scheme 1 and Figure 4).

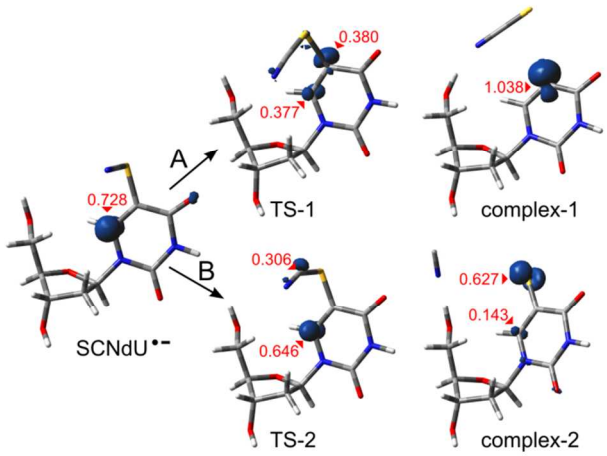


Figure 4. SCNdU•$^-$ degradation path A – leading via transition state TS-1 to C5-SCN bond break (complex-1) and B – via TS-2 to S-CN bond break (complex-2). 3D geometry

visualization along with spin density surfaces (for the density value of 0.02 e/A^3). The highest Mulliken spin values marked with red.

The first of the considered path was found to be thermodynamically allowed only in the case of C3′-exo-front conformation. Indeed, the C5-S bond in the C3′-exo-front radical is likely to be broken, as the kinetic barrier of this process is relatively low (see ΔG* = 8.69 kcal/mol in Table 2) and the thermodynamic stimulus is favorable too (ΔG = -1.60 kcal/mol). The degradation of the remaining radical conformers is unfavorable due to the positive thermodynamic stimuli (from 2.3 to 4.9 kcal/mol, see Table 2). A complete separation of monomers forming complex-1 is slightly unfavorable for each conformer considered (ΔG = 0.88 to 1.72 kcal/mol, see Table 2).

On the other hand, breaking the S-C bond inside the SCN substituent occurs quite efficiently. The activation free energy for this cleavage is about 4-6 kcal/mol (see Table 2), while thermodynamic stimuli are highly negative (ΔG lower than -11 kcal/mol). The complete separation of reaction products is thermodynamically favorable (see Table 2).

Table 2. Adiabatic Electron Affinity (AEA_G) and Thermodynamic (ΔG) and Activation (ΔG*) Barriers, Calculated for Electron-Induced Degradation of SCNdU. All Values Given in kcal/mol, Free Enthalpy Scale.

Sugar ring conformation	SCNdU → SCNdU•$^-$	SCNdU•$^-$ → complex-1		SCNdU•$^-$ → dU•+ SCN^-	SCNdU•$^-$ → complex-2		SCNdU•$^-$ → dU-5-S•+ CN^-
	AEA_G	ΔG*	ΔG	ΔG	ΔG*	ΔG	ΔG

C3'-exo-front	60.8	8.69	-1.60	0.88	4.07	-16.21	-16.57
C3'-exo-back	61.2	10.69	4.32	1.08	4.66	-12.12	-16.37
C3'-endo-front	62.2	8.85	2.33	1.65	4.04	-15.70	-15.44
C3'-endo-back	62.2	9.46	4.94	1.72	5.72	-11.16	-15.36

These results show that the excess electron readily attaches to SCNdU owing its large electron affinity (above 60 kcal/mol, see Table 2), which is comparable to the AEA of 5-BrU (57 kcal/mol at B3LYP/6-31++G(d,p) and the PCM model of water[45]). However, the further fate of the SCNdU π-anion radical differs substantially from that of 5-BrdU•$^-$. Indeed, the path which leads to the release of SCN^- and the U• formation which remains in full analogy to the release of Br^- from 5-BrdU•$^-$ and has been confirmed by the combined DFT and photoelectron spectroscopy studies,[18] is shown to be a minor decomposition channel of SCNdU•$^-$ both in glassy solution at low temperature as well as in aqueous solution at ambient temperature.

4. Disscussion

The dominant reaction path found in both the low-temperature ESR experiments and steady-state radiolysis at ambient temperature is the dissociation of the S-CN bond in the π-anion radical of SCNdU. This reaction leads to two products of significant cytotoxicity, the cyanide anion, CN^-, and the uracil-5-thiyl radical, U-5-S•. The former species is a well-known poison whose cytotoxicity is attributed to the cessation of aerobic metabolism in the cell;[21] CN^- binds irreversibly to cytochrome oxidase in mitochondria.[21] The radical product of this DEA process, the U-5-S•, has potential to be quite cytotoxic. U-5-S• should show comparable chemistry to aryl thiyl radicals which are not good H atom abstracting agents.[19,20,44] Formation of stable dimer

products, i.e., dU-5S-5S-dU, suggests that U-5-S• should be able to form intra- and interstrand cross-links as well as DNA-protein crosslinks. These crosslinks are especially cytotoxic since they are hardly repairable and clusters of several inter-strand crosslinks are sufficient to terminate the cell.[46] Finally, since under cellular environment DNA interacts permanently with proteins like histones, replication and repair enzymes[47,48] there is high probability that U-5-S• formed in double-helix would lead to DNA-protein cross-links via the formation of disulfide bond in the reaction with cysteine side chains.

In addition to the electron-induced dominant reaction path by loss of CN^- resulting in U-5-S• formation (Scheme 1 path B), a minor path by loss of SCN^- (Scheme 1 path A) from SCNdU•$^-$, leads, at ambient temperature, to the formation of a highly cytotoxic species, i.e. to U•. Several recent radiation-chemical studies by Hunting on synthetic DNA with 5-BrdU incorporated in the place of thymidine have demonstrated that 5-BrdU sensitizes DNA to the aqueous electrons formed in water radiolysis.[9] The electron-induced radiosenstization observed in 5-BrdU incorporated DNA results from the production of U• that is formed via attachment of electron to 5-BrdU via an irreversible release of the bromide anion by DEA. Depending on DNA conformation[9,45,46] and its local sequence,[47] U• abstracts a hydrogen from the adjacent sugar residue (B-DNA, single stranded DNA), forming ultimately a strand break, or leads, in a sequence of secondary processes, to interstrand cross-links (mismatched B-DNA) or to alkali labile sites (A-DNA). Thus, our radiation-chemical study predicts that electron addition to SCNdU is likely to be an effective radiosensitizer since electron addition to SCNdU leads to the formation three highly cytotoxic species - CN^-, U-5-S•, and U•.

5. Conclusions

In the current work, degradation of 5-thiocyanato-2'-deoxyuridine (SCNdU) induced by excess electron attachment has been studied using low-temperature ESR, steady-state radiolysis at ambient temperature, and molecular modeling at the DFT level. The title compound was synthesized via the reaction of chlorothiocyanate with 2'-deoxyuridine in dry acetic acid. Then, the γ-irradiated N_2-saturated frozen homogeneous solutions (7.5 M LiCl in H_2O or in D_2O) containing SCNdU resulted in an ESR spectrum having contributions from two radical species. Our ESR studies unambiguously establish the pre-hydrated electron-induced cleavage of the S-CN bond to form the uracil base-thiyl radical (U-5-S•) intermediate along with CN^- by dissociative electron attachment (DEA). Moreover, comparison of the ESR spectrum for totally protiated SCNdU with that obtained from a matched sample of the nucleobase SCNU having deuteration at the C6 position of the uracil base unequivocally demonstrates that the primary anion radical formed in the studied system is a typical π^*-anion radical in which the unpaired spin is mainly localized in the uracil ring at C6.

The HPLC and LC-MS/MS analyzes carried out for γ-irradiated and N_2-saturated aqueous solutions of SCNdU, containing sodium formate as the OH-radical scavenger, show that hydrated electrons produced the dU-5S-5S-dU dimer by recombination of the U-5-S• in preference to the anticipated formation of the σ-type uracilyl radical via removal of thiocynate anion to form the dU by about 10 to 1.

Applying the M06-2x/6-31++G(d,p) method and polarizable continuum model (PCM) of water, complete separation of U-5-S• and CN^- anion was found to be thermodynamically more favorable by ca. -15.4 kcal/mol (ΔG) than breaking the C5-SCN bond. Similarly, activation barrier for cleavage of the C5-S bond in the 5-thiocyanate-2′-deoxyuridine radical anion is more than 2-fold larger than that related to breakage of the S-CN bond in the thiocyanate substituent.

Thus, this radiation chemical study of electron addition to SCNdU establishes SCNdU as a potential radiosensitizer. SCNdU•$^{-}$ leads to U• formation similar to 5-BrdU•$^{-}$ in small yield (10%). However, SCNdU•$^{-}$ results in predominant (90%) formation of U-5-S•. The latter species will likely lead to both intra- and interstrand DNA crosslinking as well as DNA-protein crosslinking, via the formation of S-S dimers. Since cross-links belong to the most cytotoxic damage,[1,9] such dimers would significantly impede DNA damage repair activity. which might enhance, in turn, the extent of DNA damage in the cancer cells.

Acknowledgments

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Electronic Supplementary Information:

Electronic Supplementary Information (ESI) available: (i) Figure S1 - ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/D_2O and 7.5 M LiCl/H_2O solutions of SCNdU with a hole scavenger $K_4[Fe(CN)_6]$. (ii) Figure S2 - ESR spectra recorded at 77 K of

matched γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/H_2O and 7.5 M LiBr/H_2O solutions of SCNdU with a hole scavenger $K_4[Fe(CN)_6]$. (iii) Figure S3 - ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/H_2O and 15 M LiCl/H_2O solutions solutions of SCNdU with a hole scavenger $K_4[Fe(CN)_6]$. (iv) Figure S4 - ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiBr/H_2O solutions with pHs ca. 5 and ca. 11 of SCNdU with a hole scavenger $K_4[Fe(CN)_6]$. (v) Figure S5 - ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiBr/H_2O solutions with pH ca. 11 and in the presence and in absence of oxygen of SCNdU with a hole scavenger $K_4[Fe(CN)_6]$. (vi) Figure S6- MS and MS/MS spectra (in negative ionization mode) of the additional products of the γ-radiolysed solution of SCNdU (the dose of 50 Gy) and ion identities. (vii) Figures S7 and S8 - HPLC chromatogram and MS spectra (in negative ionization mode) of synthesized and purified 6-deutero-5-thiocyanatouracil (6-D-5-SCNU) and SCNdU respectively. (viii) Figure S9- ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/D_2O solutions with two different concentrations of SCNdU. (ix) ESR spectra recorded at 77 K of matched γ-irradiated (77 K) N_2-saturated 7.5 M LiCl/D_2O solutions with pHs ca. 3, 5, and ca. 9 of SCNdU. (x) Stationary points geometries – Cartesian coordinates and absolute values of electronic energy and free enthalpy.

References

(1) C. von Sonntag in *The Chemical Basis of Radiation Biology*, Taylor and Francis, London U.K., 1987.

(2) T. S. Lawrence, M. A. Davis, J. Maybaum, P. L. Stetson, W. D. Ensminger, *Radiat. Res.,* 1990, **123**, 192.

(3) R. Watanabe, H. Nikjoo, *Int. J. Radiat. Biol.,* 2002, **78**, 953.

(4) T. J. Kinsella, P. A. Dobson, J. B. Mitchell, A. J. Fornance, *Int. J. Radiat. Oncol., Biol., Phys.,* 1987, **13**, 733.

(5) G. Iliakis, S. Kurtzman, G. Pantelias, R. Okayasu, *Radiat. Res.,* 1989, **119**, 286.

(6) E. Rivera, R. H. Schuler, *J. Phys. Chem.,* 1983, **87**, 3966.

(7) R. Mertens, C. von Sonntag, *Angew. Chem. Int. Ed.,* 1994, **33**, 1262.

(8) J. D. Zimbrick, J. F. Ward, L. S. Myers, Jr. *Int. J. Radiat. Biol.,* 1969, **16**, 525.

(9) S. Cecchini, S. Girouard, M. A. Huels, L. Sanche, D. J. Hunting, *Radiat. Res.,* 2004, **162**, 604.

(10) Y. Park, K. Polska, J. Rak, J. R. Wagner, L. Sanche, *J. Phys. Chem. B,* 2012, **116**, 9676.

(11) L. Chomicz, A. Petrovici, I. Archbold, A. Adhikary, A. Kumar, M. D. Sevilla, L. Rak, *Chem. Commun.,* 2014, **50***,* 14605.

(12) Y. Xu, R. Tashiro, H. Sugiyama, *Nature Prot.,* 2007, **2**, 78.

(13) H. Sugiyama, *Bull. Chem. Soc. Jpn.,* 2007, **80**, 823.

(14) Oronsky, B. T.; Knox, S. J.; Scicinski, J. *Trans. Oncol.* 2011, **4**, 256.

(15) von Sonntag, C. Formation of Reactive Free Radicals in an Aqueous Environment. *Free-radical-induced DNA damage and its repair;* Springer-Verlag: Heidelberg, Germany, 2006.

(16) Rezaee, M.; Sanche, L.; Hunting, D. *Radiat. Res.*, 2013, **179**, 323.

(17) Prados, M. D.; Scott, C.; Sandler, H. Buckner, J. C.; Phillips, T.; Schultz, C.; Urtasun, R.; Davis, R.; Gutin, P.; Cascino, T. L.; Greenberg, H. S.; Curran, W. J. Jr *Int. J. Radiat. Oncol. Biol. Phys.* **1999**, *45*, 1109.

(18) L. Chomicz, M. Zdrowowicz, F. Kasprzykowski, J. Rak, A. Buonaugurio, Y. Wang, K. H. Bowen, *J. Phys. Chem. Lett.,* 2013, **4**, 2853.

(19) A. Houmam, E. M. Ahmed, I. W. J. Still, *J. Am. Chem. Soc.,* 2003, **125**, 7258.

(20) E. M. Ahmed, H. Doai, C. K. McLaughlin, A. Houmam, *J. Am. Chem. Soc.,* 2006, **128**, 6595.

(21) A. H. Hall, R. Dart, G. Bogdan, *Ann. Emerg. Med.,* 2007, **49**, 806.

(22) (a) A. Adhikary, D. Khanduri, M. D. Sevilla, *J. Am. Chem. Soc.,* 2009, **131**, 8614. (b) A. Adhikary, M. D. Sevilla, *J. Phys. Chem. B,* 2011, **115**, 8947.

(23) (a) A. Adhikary, A. Kumar, A. N. Heizer, B. J. Palmer, V. Pottiboyina, Y. Liang, S. F. Wnuk, M. D. Sevilla, *J. Am. Chem. Soc.,* 2013, **135**, 3121. (b) D. Khanduri, A. Adhikary, M. D. Sevilla, *J. Am. Chem. Soc.*, 2011, **133**, 4527.

(24) A. Adhikary, A. Kumar, B. J. Palmer, A. D. Todd, M. D. Sevilla, *J. Am. Chem. Soc.,* 2013, **135**, 12827.

(25) A. Adhikary, A. Kumar, B. J. Palmer, A. D. Todd, A. N. Heizer, M. D. Sevilla, *Int. J. Radiat. Biol.,* 2014, **90**, 433.

(26) A. Adhikary, D. Khanduri, V. Pottiboyina, C. T. Rice, M. D. Sevilla, *J. Phys. Chem. B,* 2010, **114**, 9289.

(27) A. Adhikary, A. Kumar, R. Rayala, R. M. Hindi, S. F. Wnuk, M. D. Sevilla, *J. Am. Chem. Soc.,* 2014, **136**, 15646.

(28) Y. Zhao, D. G. Truhlar, *Chem. Theory Comput.,* 2011, **7**, 669.

(29) (a) R. Ditchfield, W. J. Hehre, J. A. Pople, *J. Chem. Phys.,* 1971, **54**, 724. (b) W. J. Hehre, R. Ditchfield, J. A. Pople, *J. Chem. Phys.,* 1972, **56**, 2257.

(30) (a) S. Miertuš, E. Scrocco, J. Tomasi, *Chem. Phys.,* 1981, **55**, 117. (b) S. Miertuš, J. Tomasi, *Chem. Phys*., 1982, **65**, 239. (c) M. Cossi, V. Barone, R. Cammi, J. Tomasi, *Chem. Phys. Lett.,* 1996, **255**, 327.

(31) D. A. McQuarrie, J. D. Simon, *Molecular Thermodynamics*; University Science Books: Sausalito, CA, USA, 1999.

(32) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. Montgomery, J. A., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O¨ . Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09, Rev. C.01, Wallingford, CT, 2009.

(33) R. Dennington, T. Keith, J. Millam, GaussView, Version 5, *Semichem Inc*., Shawnee Mission KS, 2009.

(34) H. A. Gillis, G. G. Teather, G. V. Buxton, *Can. J. Chem*., 1978, **56,** 1889.

(35) M. D. Sevilla, S. Swarts, R. Bearden, K. M. Morehouse, T. Vartanian, *J. Phys. Chem.,* 1981, **85,** 923.

(36) J. F. Kheir, L. Chomicz, A. M. Engle, J. Rak, M. D. Sevilla, *J. Phys. Chem. B,* 2013, **117**, 2872.

(37) A. Petrovici, A. Adhikary, A. Kumar, M. D. Sevilla, *Molecules,* 2014, **19,** 13486.

(38) R. K. Wolff, J. E. Aldrich, T. L. Penner, J. W. Hunt, *J. Phys. Chem*., 1975, **79**, 210.

(39) D. M. Bartels, D. Gosztola, C. D. Jonah, *J. Phys. Chem*. *A,* 2001, **105**, 8069.

(40) Q. B. Lu, J. S. Baskin, A. H. Zewail, *J. Phys. Chem. B,* 2004**, 108***,* 10509.

(41) D. Becker, S. G. Swarts, M. Champagne, M. D. Sevilla, *Int. J. Radiat. Biol.,* 1988, **53**, 767.

(42) M. D. Sevilla, M. Yan, D. Becker, *Biochem. Biophys. Res. Commun.,* 1988, **155**, 405.

(43) J. Covès, L. Le Hir de Fallois, L. Le Pape, J. L. Décout, M. Fontecave, *Biochemistry,* 1996, **35**, 8595.

(44) Z. B. Alfassi (Ed), *S-Centered Radicals;* John Wiley & Sons, Inc.: New York, 1999.

(45) L. Chomicz, J. Rak, P. Storoniak, *J. Phys. Chem. B,* 2012, **116**, 5612.

(46) N. Magana-Schwencke, J. A. Henriques, R. Chanet, E. Moustacchi, *Proc. Natl. Acad. Sci. U.S.A.,* 1982, **79**, 1722.

(47) Y. Mandel-Gutfreund, O. Schueler, H. Margalit, *J. Mol. Biol.,* 1995, **253**, 370.

(48) M. M. Hoffman, M. A. Khrapov, J. C. Cox, J. Yao, L. Tong, A. D. Ellington, *Nucleic Acids Res.,* 2004, **32**, D174.