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Independent Generation and Study of 5,6-Dihydro-2′-deoxyuridin-6-yl, a Member of the Major Family of Reactive Intermediates Formed in DNA from the Effects of γ-Radiolysis

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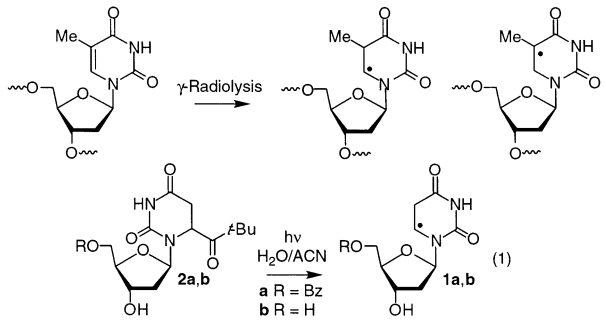
Nucleobase radicals are the major family of reactive intermediates formed when nucleic acids are exposed to γ-radiolysis. Elucidation of their reactivity is complicated by the formation of multiple species randomly throughout the biopolymers. 5,6-Dihydro-2′-deoxyuridin-6-yl (**1**) was generated upon photolysis (350 nm) of the respective *tert*-butyl ketone (**2**). The radical abstracts hydrogen atoms from β-mercaptoethanol ($k = 8.8 \pm 0.5 \times 10^6$ M^{-1} s^{-1}) and 2,5-dimethyltetrahydrofuran ($k = 31 \pm 2.5$ M^{-1} s^{-1}). The latter was used as a model for the 2-deoxyribose component of DNA. The major product formed in the presence of O_2 was 6-hydroxy-5,6-dihydro-2′-deoxyuridine (**11**), which is believed to be formed directly from the peroxy precursor and not via elimination of superoxide. Small amounts of 2-deoxyribonolactone (**13**) were also formed under aerobic conditions. This product is believed to result from intramolecular hydrogen atom abstraction by the C6-peroxyl radical (**14**) and suggests that γ-radiolysis may indirectly result in oxidation of the C1′-position of nucleotides, despite the inaccessibility of this hydrogen in duplex DNA.

DNA damage plays a significant role in the etiology and treatment of diseases and has been implicated in aging. Many of these processes involve radical intermediates.[1−4] Nucleobase radicals constitute the largest family of reactive intermediates produced when DNA is exposed to γ-radiolysis, which is the most common nonsurgical treatment of cancer. Nucleobase radicals account for as many as 90% of the reactions between pyrimidines and hydroxyl radical, which is produced by γ-radiolysis and Fe·EDTA (Scheme 1). The significant effects of DNA damage on human health and the prevalence of nucleobase radicals in these processes make understanding their reactivity an important endeavor. Studies on radical-mediated DNA damage are complicated by the randomness with which agents such as γ-radiolysis attack the biopolymer. Our research group and others have elucidated the reactivity of DNA radicals by independently generating them from photolabile precursors.[4−11] These studies have clarified reaction mechanisms, revealed complexities in damage pathways, and uncovered previously unrecognized DNA damage pathways. For instance, studies on 5,6-dihydrothymidin-5-yl (Scheme 1) revealed O_2-dependent formation of tandem lesions.[9] Tandem lesions involve damage at contiguous nucleotides in DNA and are of growing interest because of their possible mutagenicity and inhibition of DNA repair.[8,12,13]

SCHEME 1



* Corresponding author.

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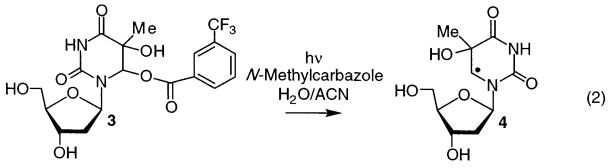
We wish to report on the generation and initial examination of 5,6-dihydro-2′-deoxyuridin-6-yl (**1**, eq 1), which is a member of a major family of DNA radicals.[1]

γ-Radiolysis studies indicate that hydrogen atom and hydroxyl radical addition to pyrimidine double bonds is the major pathway for reaction of these species, which makes the resulting 5,6-dihydropyrimidin-6-yl and 5,6-dihydropyrimidin-5-yl species the major reactive intermediates. Hydroxyl radical shows an approximately 4:1 preference for addition to the C5-position, whereas the less electrophilic hydrogen atom shows a modest preference for the C6-position. The roles of 5,6-dihydropyrimidin-6-yl species in DNA damage have been inferred from product studies, but this class of radical has not been independently generated in the nucleic acids. 5-Hydroxy-5,6-dihydrothymidin-6-yl (**4**) was generated from the *m*-trifluoromethyl benzoate (**3**, eq 2).[14] However, the photoinduced single electron-transfer conditions used to produce **4** limited these studies to anaerobic conditions and prohibited its generation at defined sites in oligonucleotides. Consequently, many important questions regarding the reactivity of **4** and 5,6-dihydropyrimidin-6-yl radicals in general are unanswered. The Norrish Type I photochemical reaction of ketones has proven to be very useful for generating DNA radicals at the monomeric level and within chemically synthesized biopolymers. We used this photochemical reaction to generate 5,6-dihydro-2′-deoxyuridin-6-yl (**1**, eq 1) under aerobic conditions, which has allowed us to address issues concerning the reactivity of this family of radicals.

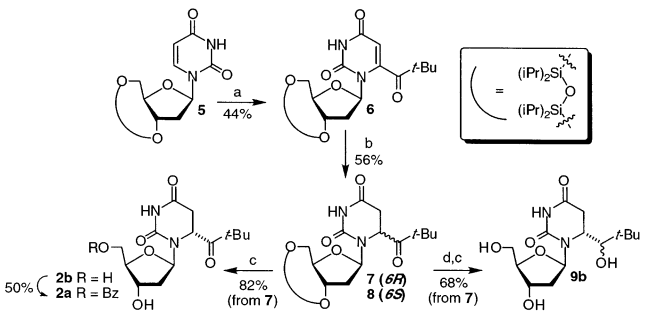


(2)

Results and Discussion

5,6-Dihydro-2′-deoxyuridin-6-yl (**1**) is a less hindered and less electrophilic model of **4**. However, due to the ease of synthesizing the *tert*-butyl ketone precursor (**2**) and the modest anticipated differences in reactivity between the two radicals, we believed that 5,6-dihydro-2′-deoxyuridin-6-yl (**1**) was a good model of **4**. Ketone **2** was synthesized using the method for functionalizing the C6-position of pyrimidines developed by the Showa group (**6**, Scheme 2).[15] Hydrogenation afforded two diastereomers (**7**, **8**), which were separable by column chromatography in an 8:1 ratio. The stereochemistry at C6 in these molecules was determined using NOE experiments in conjunction with molecular modeling. The anti conformation was predicted to be considerably lower in energy (8−10 kcal/mol) than the respective syn isomer for each diastereomer.[16] The verity of this surprising result was confirmed by carrying out similar calculations on **6** and the analogous C6-*tert*-butyl nucleoside. Rotation of the acyl group in the anti conformational isomers of **6**−**8** enables the *tert*-butyl group to avoid clashing with the C5′-position.[17] The simple C6-substituted *tert*-butyl nucleoside cannot avoid this, and the syn isomer is favored by >3 kcal/mol. The predicted ^{1}H−^{1}H distances in the minimized structures of *anti*-**7** and *anti*-**8** correlate well with the relative NOEs measured for the major and minor diastereomers, respectively (Table 1). Assigning the (6*R*)-diastereomer as the major product is also consistent with the differences in the chemical shifts of the C2′-protons in the two products. The C2′-protons in the major isomer (**7**) are well resolved (δ 2.12, 1.87 ppm). This is consistent with greater chemical shift dispersion attributed to interaction between the pro-*S* proton (δ 2.12 ppm) and the carbonyl group, whose oxygen is within 2.62 Å of this proton in *anti*-**7**. In *anti*-**8**, the pro-*S* C2′-proton is distant (5.33 Å) from the carbonyl group and the C2′-methylene group appears as a multiplet over a narrow range in chemical shift (δ 2.29−2.35 ppm).

SCHEME 2[a]



[a] Key: (a) LDA, pivaloyl chloride, THF, −78 °C; (b) 5% Rh/Al_2O_3, H_2, MeOH; (c) $Et_3N{\cdot}3HF$, THF; (d) $NaBH_4$, MeOH; (e) BzCN, Et_3N, DMF, −40 °C.

TABLE 1. Correlation between ^{1}H NOEs and Calculated Distances in 7 and 8

proton		percent enhancement		calcd distance (Å)	
irradiated	observed	**7**	**8**	**7**	**8**
C6	C2′	1.5	1.0	3.25	3.36
C6	C3′	1.0	1.8	3.78	3.34
C6	C5′		0.4	2.31	1.73
C3′	*t*-Bu	0.7	0.1	2.31	4.32

Initial photolysis experiments were carried out in order to determine whether **2b** generates 5,6-dihydro-2′-deoxyuridin-6-yl (**1b**) cleanly. Photolysis of **2b** under anaerobic conditions in the presence of even a modest excess of β-mercaptoethanol (BME) produces high yields (≥85% based upon **2b** converted, ≥95% mass balance) of 5,6-dihydro-2′-deoxyuridine (**10b**). 2′-Deoxyuridine and **10b** were produced as the sole products (dU:**10b**, 1.3:1) in the absence of thiol, presumably via radical−radical reactions. However, the mass balances of these photolyses were considerably lower (73%). Photolysis of the (6*S*)-diastereomer of **2b** in the presence of BME afforded >95% yield of **10b** (based upon ketone consumed, ≥95% mass balance). However, for synthetic convenience, the

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(16) Calculations were carried out using Spartan '02. Energies of the syn and anti isomers of all compounds were minimized at the PM3 level.
(17) See Supporting Information.

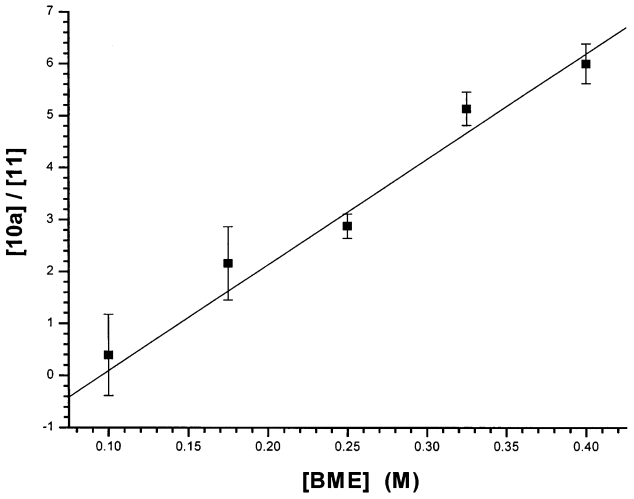
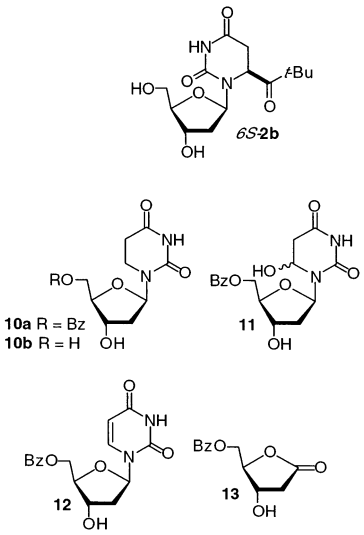


FIGURE 1. Ratio of 5,6-dihydro-2′-deoxyuridine (**10a**) to 6-hydroxy-5,6-dihydro-2′-deoxyuridine (**11**) as a function of BME concentration.

(6*R*)-diastereomer (**2a**,**b**) was used in all subsequent experiments. The photoreduction product (**9b**) is not observed even when **2b** is photolyzed in the presence of 100 mM (100 equiv) BME. The 5′-benzoate esters of the radical precursor (**2a**) and anticipated products of **1b** were prepared in order to enhance the sensitivity of UV absorbance detection during HPLC analysis of photolysates. High yields of **10a** were obtained upon anaerobic photolysis of the benzoylated ketone in the presence of excess BME, indicating that the integrity of the photochemical reaction is not compromised. 5,6-Dihydro-6-hydroxy-2′-deoxyuridine (**11**) was not observed under anaerobic conditions in the presence of thiol.

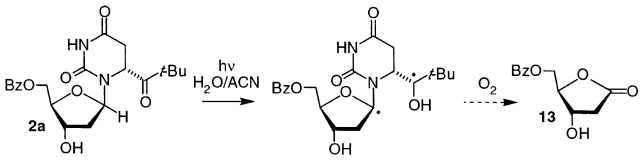


Reactivity of 5′-Benzoylated 5,6-Dihydro-2′-deoxyuridin-6-yl (1a) in the Presence of O_2. Photolysis of **2a** in aerated solution in the presence of varying concentrations of BME produced **10a** and **11** as the major products. The requirement of O_2 for the formation of **10a** indicates that it is produced from the peroxyl radical and is not formed as a result of one-electron oxidation of **1a** by other species. Minor amounts of 5′-benzoyl-2′-deoxyuridine (**12**) and the protected 2-deoxyribonolactone (**13**) were also observed. The rate constant for trapping of **1a** by BME was estimated by measuring the yields of **10a** and **11** as a function of BME concentration and assuming a rate constant for $k_{O_2} = 2 \times 10^9$ $M^{-1}s^{-1}$ and $[O_2] = 0.2$ mM (eq 3, Figure 1). The trapping rate constant was found to be $8.8 \pm 0.5 \times 10^6$ M^{-1} s^{-1}, which is fairly typical for a reaction between a thiol and alkyl radical.[18]

$$\frac{[\mathbf{10a}]}{[\mathbf{11}]} = \frac{k_{RSH}[\mathbf{1a}][BME]}{k_{O_2}[\mathbf{1a}][O_2]} \qquad (3)$$

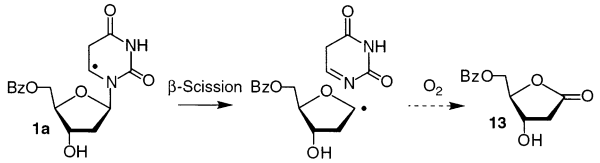
The formation of **13** from photolysis of **2a** was very surprising. 2-Deoxyribonolactone formation has been detected from the cation radicals of nucleosides, but not from a nucleobase radical formed via radical addition to a pyrimidine.[19] The yield of **13** was inversely dependent upon the BME concentration, but in contrast to **11**, very low concentrations of thiol efficiently quenched its formation. We considered three mechanisms for the formation of 2-deoxyribonolactone (**13**). The possibility that **13** was not formed from **1a**, but was instead derived from a Norrish Type II photoreaction of **2a** (Scheme 3), was

SCHEME 3



dismissed on the basis of the strong thiol effect on lactone formation. Physiologically relevant concentrations of thiol (5 mM) quenched lactone formation, but photoreduction product was not observed even in the presence of 0.1 M BME. Hence, the BME cannot be competing with the C1′-hydrogen for the excited state of the ketone (Scheme 3). β-Fragmentation of **1a** can also be eliminated from consideration on the basis of the ability of low concentrations of BME to quench 2-deoxyribonolactone (**13**) formation (Scheme 4). If β-scission were responsible for **13**, it

SCHEME 4



would have to occur with a rate constant less than 10^4 s^{-1} in order for low concentrations of BME (5 mM) to compete, assuming that the thiol reacts with **1a** at $\sim 9 \times 10^6$ $M^{-1}s^{-1}$ (see above). However, if this were so, β-scission would not be able to compete with O_2 ($\sim$0.2 mM) trapping of **1a** ($\sim 2 \times 10^9$ M^{-1} s^{-1}) and 2-deoxyribonolactone would not be observed.

The pathway that is most compatible with the effect of thiol on lactone formation involves intramolecular

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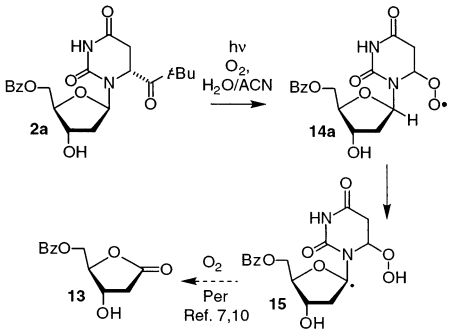
hydrogen atom abstraction by peroxyl radical **14a** (Scheme 5). Comparable reactions involving other peroxyl radicals

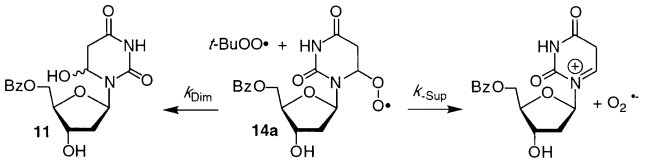
SCHEME 5



have been observed, and the rate constants for such reactions are on the order of 1 s^{-1}.[20] NMR analysis of **11** showed that this molecule exists as a mixture of anti and syn conformational isomers.[21] We believe that the peroxyl radical (**14a**) will also exist as a mixture of conformational isomers, facilitating the proposed intramolecular hydrogen atom abstraction. Assuming that BME reacts with **14a** with a rate constant $\leq 5 \times 10^3$ M^{-1} s^{-1}, a rate constant for intramolecular reaction in the range noted would be expected to be quenched by the concentrations of BME used.[22] The C1′-radical (**15**) formed upon intramolecular hydrogen atom abstraction is expected to produce the 2-deoxyribonolactone (**13**) via the respective peroxyl radical. Previous studies on a closely related C1′-radical showed that 2-deoxyribonolactone is indeed formed via superoxide elimination from the respective peroxyl radical.[10] The rate constant for this heterolytic fragmentation is expected to be too fast ($k > 10^4$ s^{-1}) for thiol trapping to compete.[7]

The possibility that superoxide is released from **14** en route to the major product, 2′-deoxyuridine C6-hydrate (**11**), was also considered (Scheme 6). Superoxide release

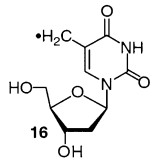
SCHEME 6



$$\frac{[O_2^{\bullet -}]}{[\mathbf{11}]} \approx \frac{k_{-\mathrm{Sup}}[\mathbf{14a}]}{2k_{\mathrm{Dim}}[\mathbf{14a}]^2} \approx \frac{k_{-\mathrm{Sup}}}{2k_{\mathrm{Dim}}[\mathbf{14a}]} \qquad (4)$$

from **14** would be important because it can react further to generate reactive species capable of damaging DNA (e.g., hydroxyl radical, peroxynitrite). Transformation of superoxide into these more reactive species in the vicinity of DNA due to its release from a DNA lesion could result in formation of clustered lesions from a single initial chemical event. Recent evidence suggests that some clustered lesions are more difficult to repair than an isolated one, indicating that such a process could be biologically significant.[12] Superoxide elimination has been detected from the peroxyl radical analogous to **14** derived from uracil and from the C1′-peroxyl radical of 2′-deoxyuridine (*vide infra*).[7,10,23] However, the rate constant for elimination from the uracil peroxyl radical is highly dependent upon pH. Superoxide elimination is very slow under conditions where the nucleobase is protonated, which is more closely related to the system studied here. Assuming that superoxide elimination from **14b** must compete with radical−radical reactions, we carried out photolyses of **2b** at 50 μM in order to maximize the possibility that slow unimolecular elimination of superoxide could compete with these fast reactions (Scheme 6, eq 4). Nonetheless, no superoxide was detected using cyctochrome *c* as a probe when **2b** was photolyzed in the presence of O_2.

An upper limit can be placed on the rate constant for superoxide elimination from **14a** ($k_{-\mathrm{Sup}}$) by comparing the yields of **11** and 2-deoxyribonolactone (**13**) from **2a** under the conditions in which the cytochrome *c* experiments were carried out (eq 4). The rate constant for superoxide elimination from **14a** ($k_{-\mathrm{Sup}}$) may be estimated using the yield of 2-deoxyribonolactone as a limiting factor. Assuming **13** is formed by the proposed mechanism (Scheme 5), an equal amount of superoxide will be formed. Estimating values for the rate constant of radical−radical reactions (k_{Dim}) and the steady-state peroxyl radical (**14a**) concentration, we predict that $k_{-\mathrm{Sup}}$ is $< 10^{-3}$ s^{-1}.



Reactivity of 5′-Benzoylated 5,6-Dihydro-2′-deoxyuridin-6-yl (1a) with a Model Deoxyribose. Alkyl radicals typically abstract hydrogen atoms slowly from carbon−hydrogen bonds. However, a secondary structure such as that found in duplex DNA brings the carbon−hydrogen bonds of adjacent nucleotides within close proximity to nucleobase radicals. This increases the effective molarity of the reactants, and such reactions may be viable under anaerobic conditions. Recently, we reported on the ability of a nucleoside radical (**16**) generated via the Norrish Type I process to abstract hydrogen atoms from volatile models of deoxyribose by using radical−radical reactions as a clock.[24] Similar experiments involving **2a** were carried out using 2,5-dimethyltetrahydrofuran (MTHF). The rate constant for hydrogen atom abstraction from MTHF was estimated by measuring the

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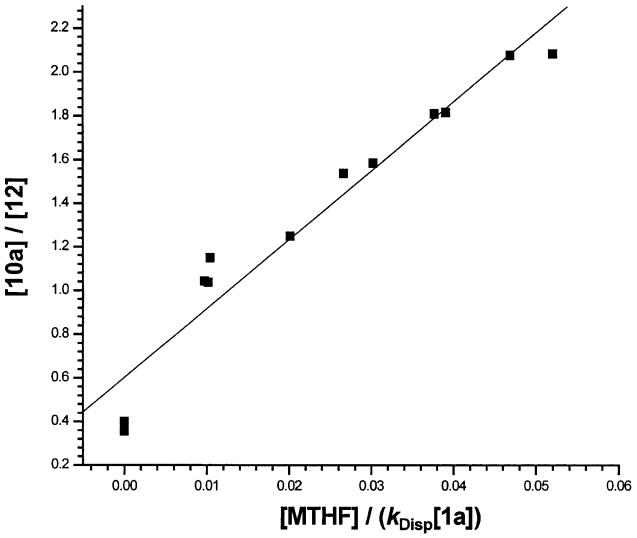

FIGURE 2. Ratio of 5,6-dihydro-2′-deoxyuridine (**10a**) to 2′-deoxyuridine (**12**) as a function of 2,5-dimethyltetrahydrofuran (MTHF) concentration.

yields of 2′-deoxyuridine and 5,6-dihydro-2′-deoxyuridine as a function of trap concentration. Both products are formed via reactions between radicals, but **10a** is also formed by MTHF trapping of **1a**. Extraction of k_{Trap} required us to assume the rate constant for radical−radical reactions which form **12** ($k_{Disp} = 2 \times 10^9$ M^{-1} s^{-1}). The concentration of radicals in solution was assumed to be constant and estimated by dividing the amount of ketone (**2a**) converted by photolysis time. Extraction of k_{Trap} from the product distribution as a function of MTHF concentration was different for **2a** than for **16** because both products are formed in reactions between radicals. Hence, a plot of [**10a**]/[**12**] versus [MTHF]/(k_{Disp}[**1a**]) was a straight line with a nonzero intercept (eq 5, Figure 2, Scheme 7). The fact that the y-intercept is not 1 is consistent with the observation that the rate constants for radical−radical reactions that yield **10a** and **12** are unequal. The product analysis and above assumptions yield an estimate for $k_{Trap} = 31 \pm 2.5$ M^{-1} s^{-1}, which is within the experimental error of the comparable process involving **16**.[24]

$$\frac{[\mathbf{10a}]}{[\mathbf{12}]} = \frac{k_{Rec}}{k_{Disp}} + \frac{k_{Trap}[\mathrm{MTHF}]}{k_{Disp}[\mathbf{1a}]} \qquad (5)$$

Summary. These experiments indicate that the Norrish Type I photoreaction is useful for generating a 5,6-dihydropyrimidin-6-yl in solution. Studies on the radical indicate that it reacts with hydrogen atom donors with rate constants comparable to those exhibited by similar radicals. The nucleoside peroxyl radical reveals a minor, unexpected, and interesting pathway that results in formation of 2-deoxyribonolactone. The lactone is an alkali-labile lesion, which has recently been shown to cross-link the *Escherichia coli* base excision repair enzyme, endonuclease III. 2-Deoxyribonolactone is also produced via γ-radiolysis, despite the fact that the C1′-hydrogen atom has poor accessibility.[25] The process reported here provides a possible alternative pathway for 2-deoxyribonolactone generation in DNA by γ-radiolysis. These studies provide a foundation for the independent generation of **1** in DNA, which will enable investigation of the role that this member of an important family of reactive intermediates plays in nucleic acid damage.

Experimental Section

General Methods. Benzoyl cyanide, BME, and pivaloyl chloride were distilled prior to use. Diisopropylamine was distilled from NaOH. THF was distilled from Na^0/benzophenone. Et_3N, DMF, MeOH, and 2,5-dimethyltetrahydrofuran were distilled from CaH_2. CH_3CN was passed through anhydrous $CuSO_4$ prior to distillation from CaH_2. All photolyses were carried out in Pyrex tubes using a Rayonet photoreactor fitted with an appropriate number of lamps having an output maximum at 350 nm. All anaerobic photolyses were carried out in sealed Pyrex tubes, which were degassed and sealed using standard freeze−pump−thaw degassing techniques (three cycles, 3 min each). 5′-Benzoyl-6-hydroxy-5,6-dihydro-2′-deoxyuridine (**11**) was prepared as previously described.[21]

HPLC samples were analyzed with a Microsorb-MV C18 5 μm column (4.6 × 25 mm). Samples were detected at 230 nm using one of the following three gradients. Gradient A: 95/5 10 mM ammonium formate, pH 6.2/CH_3CN for 5 min, ramped to 35% CH_3CN linearly over 40 min, and then held for 10 min. Gradient B: same as gradient A except that water was substituted in place of ammonium formate. Gradient C: 95/5 10 mM ammonium formate, pH 6.2/CH_3CN for 5 min, ramped to 35% CH_3CN linearly over 50 min, and then held for 10 min. Gradient D: 95/5 H_2O/MeOH for 5 min, ramped to 100% B over 10 min, and then held for 10 min. Steady-state radical concentrations ([R•]) were calculated as follows: [R•] = [X]-(fraction X converted)/(photolysis time in seconds), where [X] is the starting concentration of the photosubstrate (e.g., **2a**). The fraction of X converted was determined by HPLC.

Computer calculations were carried out using Spartan '02 (Wavefunction, Inc.; Irvine, CA). Equilibrium geometries were determined using molecular mechanics, and energies were minimized using semiempirical calculations (PM3).

Disiloxane-Protected C-6 *t*-Butyl Ketone (6). To a solution of diisopropylamine (16.7 mmol, 1.69 g) in THF (14.7 mL) at −78 °C was added 12.2 mL (5 equiv) of a 1.3 M solution of *s*-BuLi in cyclohexane. The solution was allowed to warm to room temperature and then cooled to −78 °C. A solution of **5** (1.50 g, 3.19 mmol) in THF (4 mL) was added, and the reaction was stirred for 1 h at −78 °C. A solution of pivaloyl chloride (603 mg, 4.79 mmol) in THF (4 mL) was added slowly. After 0.5 h, the reaction was quenched with glacial acetic acid, taken up in Et_2O, washed with H_2O and then brine, dried over Na_2SO_4, and concentrated in vacuo. The crude reaction mixture was purified via silica gel flash chromatography (5−40% EtOAc/hexanes) to afford **7** (534 mg, 30%) as a white solid (mp 66−68 °C): ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1 H), 5.48 (s, 1 H), 5.30 (dd, J = 8.7, 4.8 Hz, 1 H), 4.87 (dd, J = 6.3, 2.4 Hz, 1 H), 4.14−3.98 (m, 2 H), 3.81−3.77 (m, 1 H), 2.90−2.75 (m, 1 H), 2.39−2.35 (m, 1 H), 1.24 (s, 1 H), 1.12−0.96 (m, 28 H); ^{13}C NMR ($CDCl_3$) δ 201.1, 158.0, 148.2, 144.3, 94.4, 83.3, 82.0, 68.8, 59.4, 40.8, 35.9, 22.5, 13.1, 13.0, 12.95, 12.90, 12.7, 12.6, 8.81, 8.67, 8.28, 8.15; IR (film) 2945, 2867, 1703, 1463, 1388, 1362, 1249, 1091, 885 cm^{-1}; HRMS (FAB) calcd 555.2921 (M + H), found 555.2903.

Hydrogenated, Disiloxane-Protected C-6 *t*-Butyl Ketone (7 and 8). To a solution of **6** (534 mg, 0.96 mmol) in MeOH (9.6 mL) was added 5% Rh/Al_2O_3 (54 mg). The reaction was pressurized with H_2 and stirred for 2 h. The crude reaction mixture was filtered through Celite and concentrated in vacuo. The crude product was purified via silica gel flash chromatography (10−40% EtOAc/hexanes) to afford **7** and **8** (440 mg, 82%) as an 8.2:1 mixture. **Compound 7**: ^{1}H NMR ($CDCl_3$) δ 7.39 (s, 1 H), 5.97 (t, J = 6.0 Hz), 4.79 (d, J = 8.1 Hz, 1 H),

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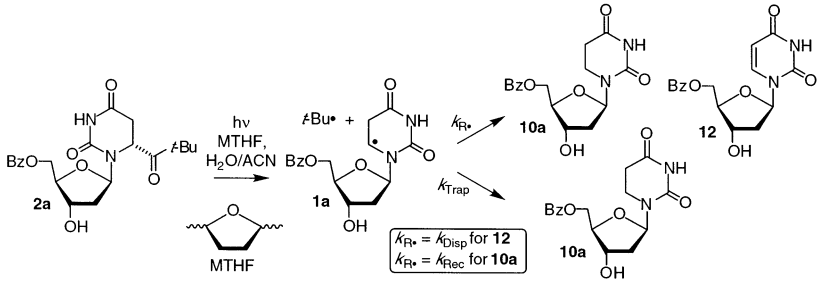

4.42−4.39 (m, 1 H), 4.08−4.03 (m, 1 H), 3.84−3.70 (m, 2 H), 3.03 (dd, J = 16.9, 8.1 Hz, 1 H), 2.79 (d, J = 17.1 Hz, 1 H), 2.18−2.05 (m, 1 H), 1.95−1.80 (m, 1 H), 1.25 (s, 9 H), 1.17−0.89 (m, 28 H); ^{13}C NMR ($CDCl_3$) δ 207.5, 163.9, 148.5, 80.9, 79.7, 68.4, 59.07, 49.6, 38.8, 32.9, 29.1, 21.6, 12.2, 12.1, 12.0, 11.8, 11.7, 8.8, 8.6, 8.4, 8.0; IR (film) 3214, 3088, 2945, 2893, 2868, 1718, 1464, 1387, 1119, 1087, 1038, 972, 920 cm^{-1}; HRMS (FAB) calcd 557.3078 (M + H), found 557.3058. **Compound 8**: ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1 H), 5.66 (dd, J = 6.0, 2.7 Hz), 5.10 (dd, J = 7.2, 2.4 Hz, 1 H), 4.38 (q, J = 8.7 Hz, 1 H), 3.96 (d, J = 3.0 Hz, 1 H), 3.66−3.62 (m, 1 H), 2.88−2.84 (m, 2 H), 2.35−2.29 (m, 2 H), 1.17 (s, 9 H), 1.05−0.91 (m, 28 H); ^{13}C NMR ($CDCl_3$) δ 211.2, 166.8, 153.2, 84.8, 84.4, 68.1, 60.5, 52.9, 43.2, 40.7, 33.6, 27.6, 17.7, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.6, 13.3, 13.1, 12.5; IR (film) 3211, 2945, 2867, 1716, 1463, 1039 cm^{-1}; HRMS (FAB) calcd 557.3078 (M + H) 557.3087 found.

C-6 *t*-Butyl Ketone (2b). To a solution of **7** (241 mg, 0.433 mmol) in THF (4 mL) was added $Et_3N{\cdot}3HF$ (697 mg, 4.33 mmol). The reaction was stirred overnight and concentrated in vacuo. The crude product was purified twice via silica gel flash chromatography (10% MeOH/$CHCl_3$) to afford **2b** (112 mg, 82%) as a white solid (mp 199−200 °C); ^{1}H NMR (MeOH-d_4) δ 6.23 (dd, J = 8.4, 4.2 Hz, 1 H), 5.30 (d, J = 7.8 Hz, 1 H) 4.25−4.23 (m, 1 H), 3.77−3.67 (m, 3 H), 3.14 (dd, J = 17.1, 8.1 Hz, 1 H), 2.84 (d, J = 17.2 Hz, 1 H), 1.79−1.71 (m, 1 H), 1.24 (s, 9 H); ^{13}C NMR (MeOH-d_4) δ 207.7, 163.9, 149.0, 81.3, 79.9, 66.8, 57.2, 48.2, 38.4, 32.2, 29.1, 22.1; IR (Film) 3261, 2965, 1711, 1465, 1371, 1289, 1226, 1092, 1049, 968, 873, 759 cm^{-1}; HRMS (FAB) calcd 315.1556 (M + H), found 315.1556.

5′-Benzoyl C-6 *t*-Butyl Ketone (2a). To a solution of C-6 *tert*-butyl ketone **2b** (30 mg, 0.095 mmol) and Et_3N (12 mg, 0.12 mmol) in DMF (1.0 mL) at −40 °C was added benzoyl cyanide (15 mg, 0.11 mmol). The reaction was stirred overnight and allowed to warm to ambient temperature. The reaction was quenched with H_2O and diluted with EtOAc. The crude reaction mixture was washed with H_2O and then brine, dried over Na_2SO_4, and concentrated. The crude product was purified via silica gel flash chromatography (15−25% EtOAc/CH_2Cl_2) to afford **2a** (20 mg, 50%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 8.06−8.04 (m, 2 H), 7.81 (s, 1H), 7.62−7.57 (m, 1 H), 7.49−7.44 (m, 2 H), 6.20 (t, J = 6.0 Hz, 1 H),4.86 (d, J = 6.3 Hz, 1 H), 4.56−4.41 (m, 2 H), 4.33−4.31 (m, 1 H), 4.11−4.09 (m, 1 H), 2.99 (dd, J = 17.4, 6.3 Hz, 1 H), 2.82 (d, J = 17.4 Hz, 1 H), 2.71 (d, J = 4.2 Hz, 1 H), 2.13−2.05 (m, 1 H), 1.77−1.70 (m, 1 H), 1.17 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 211.1, 166.8, 166.7, 152.5, 133.7, 129.9, 129.8, 128.8, 84.8, 82.8, 71.8, 64.4, 52.5, 43.7, 37.0, 34.3, 27.6; IR (Film) 3227, 2965, 2360, 1716, 1457, 1370, 1276, 1090, 1026 cm^{-1}; HRMS (FAB) calcd 419.1818 (M + H), found 419.1804.

Photolysis of 2b under Anaerobic Conditions. Photolyses were carried out in 9:1 H_2O/MeOH under degassed conditions for 1.5 h in a Rayonet Photoreactor containing the full complement of 16 lamps. The concentration of **2b** was 1.0 mM and did not vary depending upon whether BME was present (in the concentrations noted in the above text). After photolysis, the tubes were rinsed with H_2O, and the combined photolysates were lyophilized. The photolysates were resuspended in H_2O and analyzed by HPLC (gradient D).

Competitive Kinetic Studies Using 2,5-Dimethyltetrahydrofuran (MTHF). Photolyses were carried out for 2 h using 4 lamps in 9:1 CH_3CN/H_2O under degassed conditions. The concentration of **2a** was 0.1 mM, and the concentration of 2,5-dimethyltetrahydrofuran (MTHF) ranged from 0 to 1.0 M. Duplicate samples were prepared for each trap concentration. After photolysis, the 5′-benzoate ester of thymidine was added as an internal standard and the photolysates were transferred to Eppendorf tubes. The Pyrex tubes were rinsed with 1:1 CH_3CN/H_2O, and this was then combined with the photolysates. The photolysates were lyophilized, resuspended in 1:1 CH_3CN/H_2O, and analyzed by HPLC (Gradient B).

Competitive Kinetic Studies Using BME. Photolyses were carried out in 1:1 CH_3CN/20 mM HCO_2NH_4, pH 6.2, for 3 h under aerobic conditions using 2 lamps. The concentration of **2a** was 8.3 μM, and the concentration of BME ranged from 100 to 400 mM. Low concentrations of **2a** were employed in order to maintain O_2 under pseudo-first-order conditions. Duplicate samples were prepared for each concentration. After photolysis, the 5′-benzoate ester of thymidine was added as an internal standard and the photolysates were transferred to Eppendorf tubes. The tubes were rinsed with 1:1 CH_3CN/20 mM HCO_2NH_4, pH 6.2, and this was then combined with the photolysates. BME was added to bring all samples to equal thiol concentrations, and the samples were maintained at ambient temperature for 1 h. The photolysates were lyophilized, resuspended in 1:1 CH_3CN/20 mM HCO_2NH_4, pH 6.2, and analyzed by HPLC (Gradient A). The rate of BME trapping was estimated by assuming $k_{O_2}[O_2] = 4 \times 10^5$ s^{-1}.

Measurement of 2-Deoxyribonolactone in Photolysates. Photolyses were carried out in 4:1 H_2O/CH_3CN for 8 h using 2 lamps. The concentration of **2a** was 25 μM, and the concentration of BME ranged from 0 to 5 mM. After photolysis, the tubes were rinsed with 1:1 CH_3CN/20 mM HCO_2NH_4, pH 6.2, and this was then combined with the photolysates, which were then lyophilized. The photolysates were resuspended in 1:1 CH_3CN/H_2O mM HCO_2NH_4, pH 6.2, and analyzed by HPLC (Gradient C).

General Procedure for Cytochrome *c* Superoxide Assay. A solution of **2b** (50 μM), cytochrome *c* (50 μM), and catalase (1 μg) in 50 mM phosphate buffer (pH 8.0) and 0.1 mM EDTA was prepared in a UV cuvette. A control sample lacking **2b** was prepared in the same way. The samples were photolyzed at 350 nm (2 lamps). The absorbance of each solution was checked by UV−vis at 550 nm at appropriate time intervals up to 90 min.

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Supporting Information Available: Experimental procedures for the syntheses of **9b**, **10a**, and **13**, a table of HPLC response factors, and results of calculations on **6**−**8** and the disiloxane of 6-*tert*-butyl-2′-deoxyuridine. This material is available free of charge via the Internet at http://pubs.acs.org.

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