Independent Generation and Reactivity of 2′-Deoxy-5-methyleneuridin-5-yl, a Significant Reactive Intermediate Produced from Thymidine as a Result of Oxidative Stress

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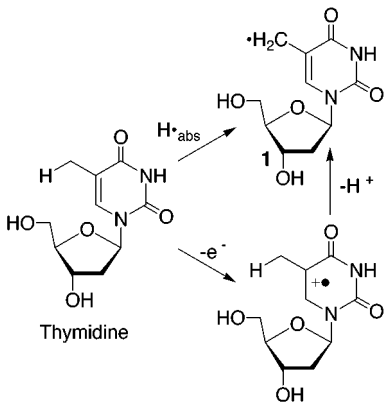
2′-Deoxy-5-methyleneuridin-5-yl (**1**) is produced in a variety of DNA damage processes and is believed to result in the formation of lesions that are mutagenic and refractory to enzymatic repair. 2′-Deoxy-5-methyleneuridin-5-yl (**1**) was independently generated under anaerobic conditions via Norrish Type I photocleavage during Pyrex filtered photolysis of the benzyl ketone **7**. The radical (**1**) exhibits behavior consistent with that of a resonance-stabilized radical. The KIE for hydrogen atom transfer from *t*-BuSH was found to be 7.3 ± 1.7. Competition studies between radical recombination and hydrogen atom donors (2,5-dimethyltetrahydrofuran, $k_{Trap} = 46.1 \pm 15.4$ M^{-1} s^{-1}; propan-2-ol, $k_{Trap} = 13.6 \pm 3.5$ M^{-1} s^{-1}) chosen to mimic the carbohydrate components of 2′-deoxyribonucleotides suggest that 2′-deoxy-5-methyleneuridin-5-yl (**1**) may be able to transfer damage from the nucleobase to the deoxyribose of an adjacent nucleotide in DNA under hypoxic conditions.

Ionizing radiation (γ-radiolysis, UV-irradiation) is a general and effective means for inducing nucleic acid damage.[1] The carbohydrate centered radicals produced by many of the well-studied antitumor antibiotics (e.g., bleomycin) are included among the abundant group of reactive intermediates resulting from the interaction of nucleic acids with ionizing radiation.[2,3] Nucleobase radicals and radical ions constitute a significant portion of species produced via the interaction of ionizing radiation with nucleic acids and represent a group of reactive intermediates that are unique to this method of inducing nucleic acid damage and related radiomimetic systems.[2,4−6] Pyrimidine radicals resulting from direct and/or formal radical addition to the 5,6-double bond have been studied under ionizing radiation conditions and by using chemical precursors designed to generate specific radicals.[1,2,5] The latter method has facilitated examination of the reactivity of these nucleobase radicals and resulted in the characterization of an unusual mechanism for the formation of tandem lesions in DNA.[7] The nucleobase radical **1** resulting from formal hydrogen atom abstraction from the methyl group of thymidine has garnered increasing attention. In addition to being formed by direct hydrogen atom abstraction, 2′-deoxy-5-methyleneuridin-5-yl (**1**) is produced from the pyrimidine cation radical via deprotonation (Scheme 1).[8−10] It has also been suggested to result from initial intramolecular hydrogen atom abstraction, following photoionization.[11] We describe herein initial experiments in our group on the independent generation and study of the reactivity of 2′-deoxy-5-methyleneuridin-5-yl (**1**).[12]

Scheme 1



2′-Deoxy-5-methyleneuridin-5-yl (**1**) has been implicated in a number of DNA damage processes. Under

(1) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: London, 1987.

(2) Greenberg, M. M. In *Comprehensive Natural Products Chemistry, Vol. VII, DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Pergammon Press: Oxford, 1999; p 371.

(3) (a) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089. (b) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746.

(4) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.

(5) Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1235.

(6) (a) Aruoma, O. I.; Halliwell, B.; Gajewski, E.; Dizdaroglu, M. *J. Biol. Chem.* **1989**, *264*, 20509. (b) Dizdaroglu, M.; Aruoma, O. I.; Halliwell, B. *Biochemistry* **1990**, *29*, 8447.

(7) (a) Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Goodman, B. K.; Matray, T. J.; Tronche, C.; Venkatesan, H. *J. Am. Chem. Soc.* **1997**, *119*, 1828. (b) Tallman, K. A.; Greenberg, M. M. Submitted for publication.

(8) (a) Martini, M.; Termini, J. *Chem. Res. Toxicol.* **1997**, *10*, 234. (b) Wang, W.; Razskazovskii, Y.; Sevilla, M. D. *Int. J. Radiat. Biol.* **1997**, *71*, 387.

(9) (a) Wagner, J. R.; Van Lier, J. E.; Johnston, L. J. *Photochem. Photobiol.* **1990**, *52*, 333. (b) Decarroz, C.; Wagner, J. R.; Van Lier, J. E.; Krishnal, C. M.; Riesz, P.; Cadet, J. *Int. J. Radiat. Biol.* **1986**, *50*, 491.

(10) (a) Catterall, H.; Davies, M. J.; Gilbert, B. C. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1379. (b) Shaw, A. A.; Voituriez, L.; Cadet, J. *J. Chem. Soc., Perkin Trans. 2* **1988**, 1303. (c) Lange, L.; Weiland, B.; Hüttermann, J. *Int. J. Radiat. Biol.* **1995**, *68*, 475.

(11) Malone, M. E.; Symons, M. C. R.; Parker, A. W. *J. Chem. Soc., Perkin Trans. 2* **1993**, 2067.

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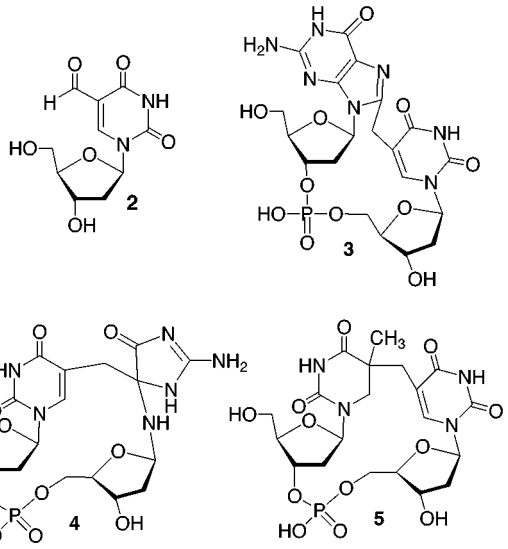
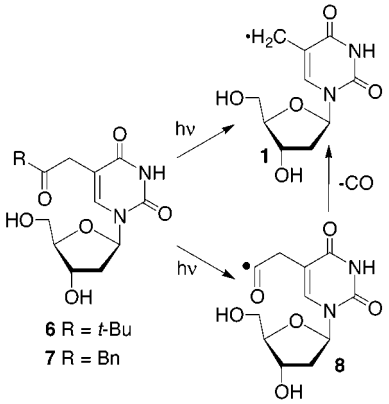
aerobic conditions, formation of **1** is believed to give rise to thymidine derivatives that are oxidatively modified at the C5-methyl group.[13] One of these oxidatively modified thymidines, the C5-formyl nucleotide **2** has been shown to be a premutagenic lesion and inhibits restriction enzyme activity.[14] 2′-Deoxy-5-methyleneuridin-5-yl (**1**) is believed to result in the formation of tandem lesions **3** and **4** under anaerobic and aerobic conditions, respectively.[15,16] Tandem lesion **3** is the major lesion identified



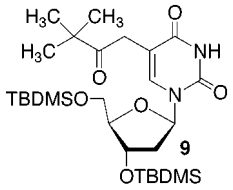
in a tetranucleotide following γ-radiolysis under anaerobic conditions. Photoproduct **5**, a tandem lesion putatively involving an adjacent thymidine and **1**, is also formed in significant amounts in bacterial spores and under other conditions of low humidity.[17] Thermodynamic considerations and experimental evidence suggest that **1** is inefficient at effecting hydrogen atom abstraction from carbon−hydrogen bonds.[8b] However, this pathway is difficult to investigate in experiments involving generation of **1** from thymidine because the product of the radical reaction is the substrate. Whether **1** abstracts a hydrogen atom from the carbohydrate component of a deoxyribonucleotide in DNA is important, as such a process is required for the formation of direct strand breaks from this nucleobase radical under anaerobic conditions. We anticipated that this issue and others concerning the reactivity of **1** could be addressed by independently generating the nucleobase radical from a chemically synthesized precursor.

Scheme 2



Results and Discussion

Design and Synthesis of a Photochemical Precursor for 2′-Deoxy-5-methyleneuridin-5-yl (1). The Norrish Type I photochemical reaction has been successfully employed in the generation of several radicals that are involved in DNA damage processes.[2,7a,18] We anticipated that either the *tert*-butyl (**6**) or benzyl (**7**) ketones would be reasonable precursors for **1**. Competition between which carbon−carbon bond would cleave initially was not a concern (Scheme 2). We expected that the resonance stabilization of **1** would induce a sufficiently high driving force such that bimolecular reactions (e.g., thiol trapping) would not compete with decarbonylation from **8**.[19] The bis-silyl *tert*-butyl ketone **9** was prepared by a direct but low yielding route via alkylation of the carbanion.[20] The major product was the result of *N*-alkylation. Synthesis of the benzyl ketone was not amenable to this approach. Instead, **7** was synthesized by condensing the lithiated dithiane of phenylacetaldeyde with the allylic bromide **10**, followed by cleavage of the dithiane protecting group (Scheme 3).[21] The deprotected ketones **6** and **7** were obtained following fluoride mediated desilylation.



Photochemical Characterization of 6 and 7 and Independent Synthesis of Potential Photoproducts. Ketones **6** and **7** proved to be stable to extended irradiation at $\lambda_{max} = 350$ nm. Similarly, the *tert*-butyl ketone was also relatively inert to irradiation at $\lambda_{max} = 300$ nm. However, evidence for the formation of **1** from the benzyl ketone (**7**, 5 mM) in the presence of 2-mercaptoethanol (BME, 50 mM) was gleaned from the formation of thymidine. The yield of thymidine was 40% (based upon unrecovered starting material) and was found to decrease slightly with increasing conversion of ketone. While

(12) While this manuscript was under review an independent report concerning the independent generation and study of **1** appeared. Please see: Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett*. **2000**, *2*, 1085.

(13) Bjelland, S.; Eide, L.; Time, R. W.; Stote, R.; Eftedal, I.; Volden, G.; Seeberg, E. *Biochemistry* **1995**, *34*, 14758.

(14) (a) Zhang, Q.-M.; Sugiyama, H.; Miyabe, I.; Matsuda, S.; Kino, K.; Saito, I.; Yonei, S. *Int. J. Radiat. Biol*. **1999**, *75*, 59. (b) Zhang, Q.-M.; Sugiyama, H.; Miyabe, I.; Matsuda, S.; Saito, I.; Yonei, S. *Nucleic Acids Res*. **1997**, *25*, 3969.

(15) (a) Box, H. C.; Budzinski, E. E.; Dawidzik, J. D.; Wallace, J. C.; Evans, M. S.; Gobey, J. S. *Radiat. Res*. **1996**, *145*, 641. (b) Budzinski, E. E.; Dawidzik, J. D.; Rajecki, M. J.; Wallace, J. C.; Schroder, E. A.; Box, H. C. *Int. J. Radiat. Biol*. **1997**, *71*, 327.

(16) Delatour, T.; Douki, T.; Gasparutto, D.; Brochier, M.-C.; Cadet, J. *Chem. Res. Toxicol*. **1998**, *11*, 1005.

(17) (a) Begley, T. P. In *Comprehensive Natural Products Chemistry, Vol. V, Enzymes, Enzyme Mechanisms, Proteins, and Aspects of NO Chemistry*; Poulter, C. D., Ed.; Pergammon Press: Oxford, 1999; p 371. (b) Mehl, R. A.; Begley, T. P. *Org. Lett*. **1999**, *1*, 1065.

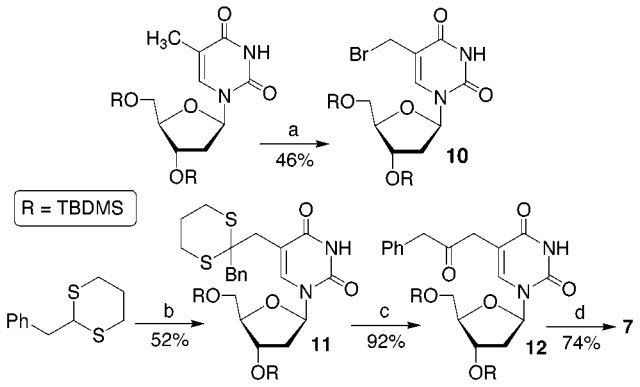
(18) (a) Hwang, J. T.; Greenberg, M. M. *J. Am. Chem. Soc*. **1999**, *121*, 1, 4311. (b) Goodman, B. K.; Greenberg, M. M. *J. Org. Chem*. **1996**, *61*, 2.

(19) (a) Lunazzi, L.; Ingold, K. U.; Scaiano, J. C. *J. Phys. Chem*. **1983**, *87*, 529. (b) Hany, R.; Fischer, H. *J. Chem. Phys*. **1993**, *172*, 131.

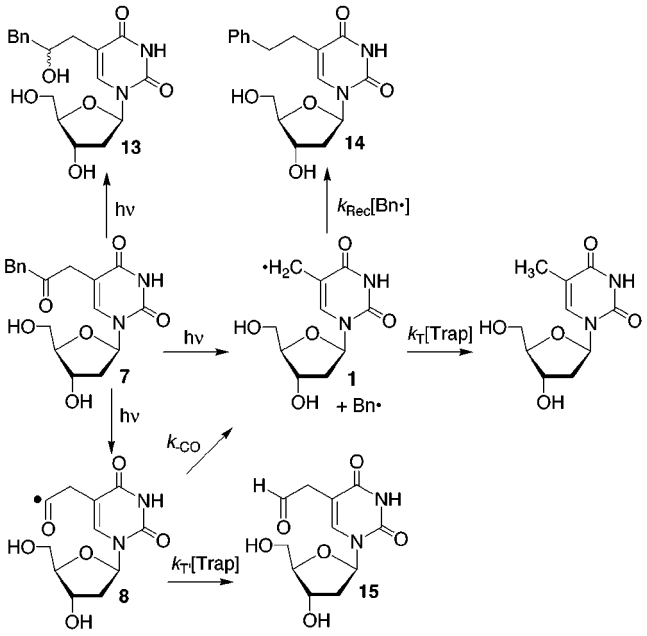
(20) Armstrong, R. W.; Gupta, S.; Whelihan, F. *Tetrahedron Lett*. **1989**, *30*, 2057.

(21) Seebach, D.; Corey, E. J. *J. Org. Chem*. **1975**, *40*, 231.

Scheme 3



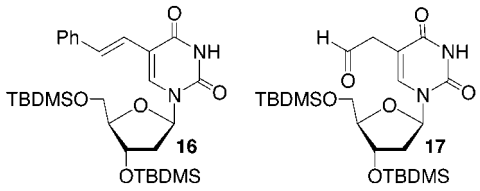
Scheme 4



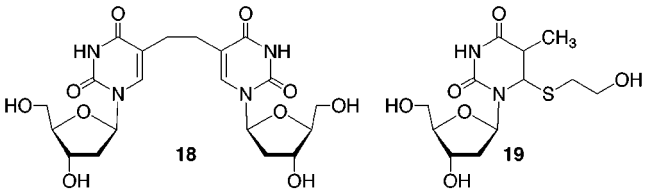
thymidine was the product of the desired photochemical formation of **1** in the presence of hydrogen atom donors, the syntheses of several other potential products were carried out in order to facilitate a more complete characterization of the photochemical behavior of **7** (Scheme 4).

The product resulting from the photoreduction of the ketone group (**13**) was prepared as a mixture of diastereomers from **12**. The recombination product **14** was obtained following hydrogenation of **16**, which was prepared from bis-silylated 5-iodo-2′-deoxyuridine via a Heck coupling.[22] Finally, the bis-silyl precursor (**17**) of the product resulting from trapping of the acyl radical formed upon Type I cleavage (**15**) was prepared using the method of Glick.[23]

Irradiation of **7** in the absence of BME gave rise to recombination product **14** in 73% yield based upon the amount of unrecovered ketone. In addition, small amounts of a product which had a molecular weight equal to twice that of **1** were isolated by HPLC and characterized by mass spectrometry. This product is tentatively believed



to be the symmetrical dimer **18**. Formation of this product was eliminated upon addition of 50 mM BME. In addition, inclusion of BME in the photolysis samples also resulted in the formation of small amounts of the photoreduction product **13** (2−9% based upon the amount of **7** consumed). In contrast, the acyl radical trapping product **15** was not detected in the presence of BME ranging from 10 to 200 mM. However, the detection of small amounts of **15** (<10%) was compromised due to equilibration of the aldehyde in protic solvents to yield a mixture of the free carbonyl, hydrate, hemiacetal, and acetal. The formation of thymidine and **13**−**15** did not account for 100% of the ketone **7** consumed. Consequently, the possibility that thymidine was unstable under the photolysis conditions was explored.[24] Extended (12 h) irradiation of thymidine (5 mM) in the presence of BME (50 mM) resulted in isolation of a diastereomeric mixture of adduct **19**. However, HPLC analysis revealed



that this adduct was not produced under the analytical photolysis conditions. In principle, the benzyl ketone **7** can undergo an analogous photochemical reaction with BME. While no evidence was obtained for such an adduct, or any subsequent decomposition product(s), this photochemical pathway cannot be discounted.[25] Despite this limitation, mass balances of >65% were consistently obtained by reducing the number of equivalents of thiol relative to **7** to 5, and curtailing irradiation times so as to maintain photoconversion of **7** to <50%.

Hydrogen Atom Abstraction by 2′-deoxy-5-methyleneuridin-5-yl (1). The proclivity with which **1** abstracts hydrogen atoms from the deoxyribose ring in DNA determines its ability to participate in direct strand break formation. Insight into this possibility was sought by examining the ability of **1** to abstract hydrogen atoms from model compounds of the carbohydrate component of nucleotides. 2,5-Dimethyltetrahydrofuran, propan-2-ol, and ethanol were chosen to mimic the C1′, C4′, and C5′ positions of the sugars, respectively. These molecules were also chosen as model substrates, because they could be employed in high concentration in order to mimic the potential effective molarity of a deoxyribonucleotide adjacent to **1** in DNA. Additionally, their volatility

(22) (a) Selvidge, S. D., III; Jang, J.; Ray, P. S. *Nucleosides Nucleotides* **1997**, *16*, 2019. (b) Bigge, C. F.; Kalaritis, P.; Deck, J. R.; Mertes, M. P. *J. Am. Chem. Soc.* **1980**, *102*, 2033.

(23) Osborne, S. E.; Cain, R. J.; Glick, G. D. *J. Am. Chem. Soc.* **1997**, *119*, 1171.

(24) (a) Shetlar, M. D.; Hom, K. *Photochem. Photobiol.* **1987**, *45*, 703. (b) Jellinek, T.; Johns, R. B. *Photochem. Photobiol.* **1970**, *11*, 349.

(25) In principle, the analogous BME addition product to **7** could undergo subsequent Type I cleavage to yield **18**. This adduct could also undergo intramolecular hydrogen atom transfer to yield Norrish Type II and Yang type products. For reviews of this subject, see: (a) Wagner, P.; Park, B.-S. *Org. Photochem.* **1991**, *11*, 227. (b) Descotes, G. L. In *CRC Handbook of Organic Photochemistry and Photobiology*; Horspool, W. M., Song, P.-S., Eds.; CRC Press: Boca Raton, FL, 1995.

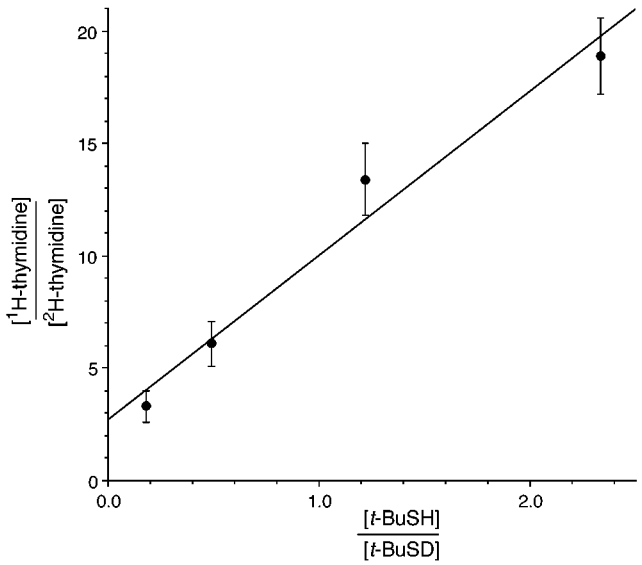


Figure 1. Kinetic isotope effect for the reaction of **1** with *tert*-butylthiol.

facilitates sample analysis. Attempts to approximate the rate constants for reaction of **1** with these hydrogen atom donors were made by using formation of recombination product **14** and reaction with deuterated thiols as competing processes, or "clock" reactions.

Estimates of rate constants for hydrogen atom abstraction from the model substrates could potentially be determined by competition with thiols which trap alkyl radicals at known rate constants. Since in the current experiment deuterated thiols are required in order to distinguish between reaction with the model substates and the clock reaction thiol, it was necessary to measure the KIE for the reaction between **1** and *t*-BuSH. The KIE (7.3 ± 1.7) for the transfer from *t*-BuSH was determined from the slope of a plot of [^{1}H]thymidine:[^{2}H]thymidine over a range of *t*-BuSH:*t*-BuSD ratios in order to remove any contribution of background processes to the formation of [^{1}H]thymidine (Figure 1). The ratio of *t*-BuSH:*t*-BuSD was determined directly by ^{1}H NMR in order to take into account the equilibrium isotope effect of the thiol fractionation in a mixture of H_2O and D_2O.[26] The ratio of [^{1}H]thymidine:[^{2}H]thymidine was determined by analysis of the respective M − 15 ions of bis-trimethylsilylthymine obtained following formic acid cleavage of crude photolyzates and accounting for the natural abundance of the heavier (M + 1) isotopomer. As expected, control experiments showed that no protons were exchanged from the C6 position of thymine under these conditions. The magnitude of the KIE is within range of analogous measurements reported for the reaction of other resonance-stabilized alkyl radicals with thiols.[27] The sizable KIE is also reflected by the observation that a small but increasing amount of recombination product **14** was observed with increasing D_2O content of the solvent. Furthermore, in 100% D_2O small amounts of **18** were also believed to be formed.[28]

$$\frac{[\text{Thymidine}]}{[\mathbf{14}]} = \frac{k_T\,[\text{Trap}]\,[\mathbf{1}]}{k_{Rec}\,[\text{Bn}^\bullet]\,[\mathbf{1}]} = \frac{k_T\,[\text{Trap}]}{k_{Rec}\,[\text{Bn}^\bullet]} \qquad (1)$$

The ability of the recombination product (**14**) of **4** and benzyl radical to compete with thiol trapping suggested that this process may also be used as a clock reaction for estimating the rate of hydrogen atom abstaction from models of the carbohydrate component of deoxyribonucleotides. This is fortunate, because employing the reaction of **1** with *t*-BuSD as a clock reaction proved difficult due to the formation of significant (attributable to the large KIE) and variable amounts of [^{1}H]thymidine, presumably resulting from trace amounts of a hydrogen atom source. Consequently, the rate constants for hydrogen atom abstraction from the above-mentioned model compounds by **1** were estimated by measuring the ratio of thymidine: recombination product **14** as a function of trap concentration (eq 1). On the basis of mechanistic studies of the Norrish Type I photochemical reaction, we believe that **14** is formed via freely diffusing radicals.[29] The rate constant for recombination (k_{Rec}) employed was 2×10^9 M^{-1} s^{-1}.[30] This value for k_{Rec} should be considered to be a lower limit for this process. Any increase in k_{Rec} would result in a proportional increase in the estimate of k_T (Scheme 4, eq 1). The steady-state concentration of benzyl radical was estimated by assuming the that velocity of the Type I process was constant at the low conversions of ketone employed. Hence, the steady-state concentration of benzyl radical was determined on the basis of the concentration of ketone converted (measured by HPLC for each sample) over the time of irradiation (720 s). No thymidine was observed when **7** was photolyzed in the presence of ethanol (maximum [EtOH] = 11.4 M). However, varying amounts of thymidine were formed when **7** was irradiated in the presence of propan-2-ol (3.9−6.5 M) or 2,5-dimethyltetrahydrofuran (2.5−4.2 M). In addition to the formation of thymidine and **14**, small amounts (<10%) of photoreduction product **13** were also observed under these conditions, particularly when **7** was irradiated in the presence of increasing amounts of 2,5-dimethyltetrahydrofuran. The average rate constants determined for trapping of **4** by 2,5-dimethyltetrahydrofuran and propan-2-ol were found to be 46.1 ± 15.4 and 13.6 ± 3.5 M^{-1} s^{-1}.

Summary. Pyrex filtered photolysis of benzyl ketone **7** provides an independent source of 2′-deoxy-5-methyleneuridin-5-yl (**1**). Generation of **1** under anaerobic conditions in the presence of hydrogen atom donors produces thymidine. 2′-Deoxy-5-methyleneuridin-5-yl (**1**) is also capable of abstracting hydrogen atoms from molecules employed as mimics of the deoxyribose component of nucleotides. The estimated bimolecular rate constant derived from competition experiments involving **1** and 2,5-dimethyltetrahydrofuran is sufficiently high that hydrogen atom abstraction from deoxyribonucleotides in DNA may be able to compete to some extent with

(26) (a) Szawelski, R. J.; Wharton, C. W.; White, S. *Biochem. Soc. Trans.* **1982**, *10*, 232. (b) Hibbert, F.; Thomas, G. A. I. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1761.

(27) (a) Pryor, W. A.; Knipep, K. G. *J. Am. Chem. Soc.* **1971**, *93*, 5584. (b) Work by Franz et al. suggests that the KIE for hydrogen atom abstraction from thiophenol by benzyl radical may be significantly smaller than those reported by Pryor and Knipep. See: Franz, J. A.; Suleman, N. K.; Alnajjar, M. S. *J. Org. Chem.* **1986**, *51*, 19.

(28) Small amounts of the dimer were isolated by HPLC and analyzed by LRMS FAB (*m/z* obsd 482.1, calcd 482.5). GC/MS analysis showed that the dimer did not decompose to thymine upon formic acid treatment. Hence, formation of this product does not affect the analysis of the reactions of **1** with hydrogen atom donors.

(29) Turro, N. J.; Chow, M. F.; Chung, C. J.; Tanimoto, Y.; Weed, G. C. *J. Am. Chem. Soc.* **1981**, *103*, 4574.

(30) (a) Fischer, H.; Paul, H. *Acc. Chem. Res.* **1987**, *20*, 200. (b) Claridge, R. F. C.; Fischer, H. *J. Phys. Chem.* **1983**, *87*, 1960. (c) Arends, I. W. C. E.; Mulder, P.; Clark, K. B.; Wayner, D. D. M. *J. Phys. Chem.* **1995**, *99*, 8182.

reduction by thiols (in vivo concentration ≈5 mM, $k_{RSH} \approx 10^6$ M^{-1} s^{-1}), provided the effective molarity of the adjacent deoxyribonucleotides is sufficiently high (>10 M). It should also be noted that the current experiments do not address the competition between hydrogen atom abstraction and addition to the double bond of an adjacent nucleobase. Moreover, given the nature of the above model experiments, and the number of approximations made during the estimation of the biomolecular trapping rate constants, this issue should be addressed by directly studying the reactivity of 2′-deoxy-5-methyleneuridin-5-yl (**1**) within DNA.

Experimental Section

All reactions were carried out in oven-dried glassware under an atmosphere of argon or nitrogen unless otherwise noted. THF was freshly distilled from sodium benzophenone ketyl. Chloroform, methanol, ethanol, propan-2-ol, 2,5-dimethyltetrahydrofuran, tetramethylethylenediamine (TMEDA), and carbon tetrachloride were distilled from CaH_2. Phenylacetaldehyde, 1,3-propanedithiol, boron trifluoride−diethyl etherate, 2-mercaptoethanol, and *tert*-butylthiol were distilled from themselves. Acetonitrile was purified by filtration through anhydrous $CuSO_4$, followed by distillation from CaH_2. *N*-Bromosuccinimide was either recrystallized from water or sublimed. Bis-trimethylsilyl trifluoroacetamide (BSTFA) was used as received (Sigma). HPLC analysis was carried out using a Waters gradient, multiwavelength detector, system equipped with either Baseline or MacIntegrator software and a Rainin autosampler. Separations were carried out using a Rainin Microsorb-MV 100 Å C-18 reverse phase column using the following gradient: solvent A, H_2O:MeOH (95:5); solvent B. MeOH; t = 0−10 min, 100% A; t = 10−35 min, 0−100% B linearly; t = 35−40 min, 0−100% A linearly. GC/MS was performed using a Hewlett-Packard 5890 GC equipped with a HP 5970 mass selective detector. The column used was a 50 m DB-1. Oven conditions were the following: initial temperature 150 °C, rate 10 °C/min, final temperature 200 °C. Data were collected using selected ion monitoring (*m*/*z* 255.1, 256.1).

General Photolysis and Analysis Procedures. The following procedure was used for all anaerobic photolyses. Reactant, trap, and solvent were added to Pyrex tubes. These solutions were vortexed and then centrifuged in an IEC clinical centrifuge. The tubes were degassed (freeze, pump 3 min, thaw; 3 cycles), sealed in vacuo, and irradiated at the appropriate wavelength (λ_{max} = 300, 350 nm) in a Rayonet photoreactor (12 lamps). After irradiation, the tubes were vortexed and centrifuged prior to cracking. The appropriate amount of internal standard (2′-deoxyuridine) was added, and the solutions were transferred to Kontes glass conical vials and evaporated to dryness in a Savant speed-vac. The contents of the conical vials were redissolved in an appropriate solvent (methanol or 1:1 $CH_3OH/NH_4^+HCOO^-$, 25 mM, pH 6.2) and transferred to HPLC vials for HPLC analysis.

For GC/MS analysis, the samples were prepared as above for HPLC, until just after the evaporation of the reaction mixture. The dry reaction mixture was dissolved in an appropriate solvent and a portion (usually 50 μL from 100 μL of solution) was removed for HPLC analysis. To the remaining solution was added formic acid (∼450 μL to 50 μL of solution), and the solution was heated to 100 °C for 1 h. The solutions were lyophilized and then dried azeotropically from EtOH (3 × 50 μL). BSTFA (25−50 μL) was added to the residue, followed by heating at 100 °C for 2 h. GC/MS analysis was performed by injecting approximately 1 μL of each silylated solution.

Preparative-Scale Thiol Photoaddition to Thymidine. Thymidine (26.1 mg, 5 mM) was photolyzed at 300 nm under degassed conditions (freeze, pump 4 min, thaw; 4 cycles) for 12 h in the presence of either 2-mercaptoethanol (50 mM) in 3:1 $CH_3CN:H_2O$ (20 mL). The solvent and thiol were removed in vacuo. Flash chromatography on silica gel eluting with 10% MeOH:CH_2Cl_2 afforded thymidine and adduct **19** (6.6 mg, 19% yield) as a mixture of diastereomers. ^{1}H NMR (CD_3OD) δ: 6.30 (dd, J = 7.8, 6.3 Hz, 1H), 6.20 (dd, J = 8.4, 6 Hz, 0.4H), 4.28 (m, 1.4H), 3.74−3.83 (m, 2.2H), 3.60−3.72 (m, 5.6H), 3.50 (m, 1.4H), 3.04 (ddd, J = 18, 12, 3 Hz, 0.4H), 2.88−2.69 (m, 3.4H), 2.32−2.15 (m, 2H), 2.01 (ddd, J = 10.2, 3.6, 3 Hz, 0.4H), 1.88 (d, J = 1.2 Hz, 1.2 H), 1.52 (d, J = 3.3 Hz, 3H). LRMS (FAB): calcd (M^+ + H) 321.4, found 321.1.

Kinetic Isotope Effects. Pyrex tubes were prepared so as to contain the following: **7** (2 mM), *tert*-butylthiol (25 mM), and the appropriate solvent. The tubes contained various volume ratios of $H_2O:D_2O$ dissolved in CH_3CN. The samples were degassed, photolyzed (300 nm), and analyzed by HPLC and GC/MS as above.

Competition between Hydrogen Atom Donors and Benzyl Radical for Trapping of 1. Ketone 5 (2 mM), hydrogen atom donor (2,5-DMTHF, *i*-PrOD, or EtOD; 30−60 vol %), and 3:1 $CH_3CN:D_2O$ were added to Pyrex tubes. The samples were degassed, photolyzed, and analyzed by HPLC and GC/MS as above.

1,3-Dithiane of Phenylacetaldehyde. Chloroform (83 mL), phenylacetaldehyde (2.00 g, 16.6 mmol), and 1,3-propanedithiol (1.80 g, 16.6 mmol) were added to a 250 mL round-bottomed flask. The reaction was cooled to −20 °C, and BF_3•OEt_2 (210 μL, 236 mg, 1.66 mmol) was added. The solution was heated at reflux for 45 min and then cooled to room temperature. Distilled water was added to the reaction mixture. The solution was transferred to a separatory funnel, and the organic layer was washed with water, 10% aqueous KOH, and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash column chromatography on silica gel eluting with 25% EtOAc:hexanes afforded the dithioacetal as a viscous oil that became a white solid upon freezing (3.11 g, 91%). ^{1}H NMR ($CDCl_3$) δ = 7.27 (m, 5H), 4.24 (t, J = 7.2 Hz, 1H), 3.11 (d, J = 7.2 Hz, 2H), 2.85 (m, 4H), 2.19 (m, 1H), 1.95 (m, 1H).

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-(bromomethyl)-2′-deoxyuridine (10). 3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-thymidine (5.80 g, 12.32 mmol), *N*-bromosuccinimide (4.60 g, 25.9 mmol), and benzoyl peroxide (89 mg, 0.37 mmol) were placed in a 250-mL round-bottom flask. Carbon tetrachloride (123 mL) was added via syringe. The reaction was heated at reflux for 1 h, during which time the solution changed from colorless to orange. The reaction was allowed to cool and was vacuum filtered through a fritted funnel to remove excess NBS and succinimide. The solvent was removed from the filtrate in vacuo by rotary evaporation to yield a crude yellow solid. Flash column chromatography on silica gel eluting with 4:1 hexanes:EtOAc afforded the bromide (**10**) as an off-white solid (3.08 g, 46%): mp 59−62 °C. ^{1}H NMR ($CDCl_3$) δ: 8.10 (s, 1H), 7.91 (s, 1H), 6.31 (dd, J = 6, 1.8 Hz, 1H), 4.41 (m, 1H), 4.31 (d, J = 10.8 Hz, 1H), 4.23 (d, J = 10.8 Hz, 1H), 3.99 (t, J = 2.4 Hz, 1H), 3.91 (dd, J = 2.4, 11.1 Hz, 1H) 3.79 (dd, J = 2.4, 11.1 Hz, 1H), 2.33 (m, 1H), 2.01 (m, 1H), 0.98 (s, 9H), 0.92 (s, 9H), 0.17 (s, 6H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 161.7, 149.8, 139.2, 111.7, 88.1, 85.5, 72.2, 63.0, 41.8, 25.9, 25.7, 25.1, 18.4, 17.9, −4.7, −4.9, −5.4. IR (film): 2930, 1711, 1463, 1254, 1098, 835 cm^{-1}. HRMS (FAB): $C_{22}H_{41}BrN_2O_5Si_2$ calcd (M^+ + H) 549.1816, found 549.1802.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-(3-phenyl-2,2-(1,3-dithio))propyl-2′-deoxyuridine (11). The dithioacetal prepared from phenylacetaldehyde (702 mg, 3.34 mmol) and THF (13.3 mL) were placed in a 100-mL round-bottomed flask and cooled to −40 °C. *n*-Butyllithium (1.9 M solution in cyclohexane, 1.8 mL, 3.5 mmol) was then added dropwise. The solution was stirred for 12 h, at which time the reaction was cooled to −78 °C. A solution of **10** (873 mg, 1.59 mmol) in THF (1.6 mL) was then added. The reaction was stirred for an additional 12 h at −78 °C. Saturated NH_4Cl was added to quench the reaction, and the mixture was diluted with ethyl acetate. The solution was washed with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a crude yellow oil. Flash column chromatography on silica gel eluting with 15−25% EtOAc:hexanes afforded the dithioketal **11** as an off-white solid (566 mg, 52.5%): mp 83−85 °C. ^{1}H

NMR ($CDCl_3$) δ: 8.49 (s, 1H), 7.49 (s, 1H), 7.33 (m, 5H), 6.36 (t, J = 6.8 Hz, 1H), 4.44 (m, 1H), 3.93 (m, 1H), 3.74 (t, J = 3 Hz, 2H), 3.31 (d, J = 3.9 Hz, 2H), 2.91 (m, 6H), 2.34 (m, 1H), 2.08 (m, 1H), 0.93 (s, 9H), 0.89 (s, 9H), 0.114 (s, 3H), 0.107 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 163.5, 149.8, 139.0, 135.9, 131.3, 127.7, 126.9, 109.4, 87.4, 84.7, 71.8, 62.8, 54.7, 46.2, 0.7, 35.6, 26.4, 25.9, 25.7, 24.1, 18.3, 18.0, −4.7, −4.9, −5.3, −5.5. IR (film): 2953, 2929, 2857, 682, 1463, 1254, 1103, 837 cm^{-1}. Anal. Calcd for $C_{33}H_{54}N_2O_5S_2Si_2$: C, 58.34; H, 6.54; N, 4.13. Found: C, 58.23; H, 6.22; N, 4.13.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-(3-phenyl-2-oxopropyl)-2′-deoxyuridine (12). Mercuric chloride (544 mg, 2.0 mmol), $CaCO_3$ (216 mg, 2.17 mmol), and 4:1 CH_3CN:H_2O (8.3 mL) were added to a 50-mL round-bottom flask containing **11** (566 mg, 0.83 mmol). The white slurry was heated at reflux for 8 h. The solution was allowed to cool to room temperature, and the condenser was rinsed with CH_2Cl_2. The reaction mixture was then filtered through Celite and rinsed with CH_2Cl_2 to remove mercury salts and $CaCO_3$. The filtrate was washed with H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a crude white solid. Flash column chromatography on silica gel eluting with 10−20% EtOAc:CH_2Cl_2 afforded **12** as a white foam (365 mg, 92%): mp 62−63 °C (shrinks), 65−67 °C (glass). ^{1}H NMR ($CDCl_3$) δ: 9.79 (s, 1H), 7.50 (s, 1H), 7.30 (m, 5H), 6.36 (dd, J = 6, 1.8 Hz, 1H), 4.39 (m, 1H), 3.93 (m, 1H), 3.85 (s, 2H), 3.82 (dd, J = 3, 11.4 Hz), 3.74 (dd, J = 3, 11.4 Hz), 3.52 (d, J = 17.1 Hz, 1H), 3.32 (d, J = 17.1 Hz, 1H), 2.28 (m, 1H), 2.03 (m, 1H), 0.91 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H), 0.06 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 204.2, 163.1, 150.2, 138.4, 134.0, 129.7, 128.9, 127.3, 108.6, 88.0, 85.3, 72.4, 63.1, 50.2, 41.5, 39.4, 26.1, 26.1, 25.9, 18.5, 18.2, −4.5, −4.7, −5.3. IR (film): 2954, 2857, 1688, 1471, 1255, 1097, 1031, 837, 778 cm^{-1}. HRMS (FAB): $C_{30}H_{48}N_2O_6Si_2$ calcd (M^+ + H) 589.3129, found 589.3134.

5-(1-(3-Phenyl-2-oxopropyl))-2′-deoxyuridine (7). To a 50-mL round-bottom flask containing **12** (360 mg, 0.76 mmol) were added THF (7.6 mL) and Et_3N·3HF (1.24 mL, 1.23 g, 7.6 mmol). The reaction was stirred for 10 h at room temperature. The solvents were removed in vacuo by rotary evaporation. Flash chromatography on silica gel eluting with 10−20% MeOH:EtOAc afforded deprotected ketone as a white solid (204 mg, 74%): mp 163−165 °C. ^{1}H NMR (CD_3OD) δ: 7.82 (s, 1H), 7.25 (m, 5H), 6.26 (t, J = 6.8, 1H), 4.38 (m, 1H), 3.89 (m, 1H), 3.86 (s, 2H), 3.73 (dd, J = 3.3, 9.6 Hz, 2H), 3.48 (d, J = 6.6 Hz, 2H), 2.21 (m, 2H). ^{13}C NMR (CD_3OD) δ: 207.6, 152.3, 140.7, 135.8, 130.9, 129.7, 128.1, 110.0, 89.0, 86.6, 72.2, 62.9, 50.3, 50.0, 41.5, 40.8. IR (film): 3366, 1682, 1470, 1275, 1091, 1056. HRMS (FAB): $C_{18}H_{20}N_2O_6$ calcd (M + 1) 361.1400, found 361.1406.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-(1-(3-phenyl-2-hydroxypropyl))-2′-deoxyuridine. Ethanol (500 μL) was added to a 10-mL pear-shaped flask containing **12** (21.3 mg, 36.2 μmol). The solution was cooled to 0 °C, and $NaBH_4$ (1.4 mg, 36.2 μmol) was then added. The reaction was allowed to stir for 12 h while warming to room temperature. The reaction was quenched with H_2O and washed with CH_2Cl_2 and H_2O. The aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a clear glass. Flash column chromatography on silica gel eluting with 4:1 CH_2Cl_2:EtOAc (75 mL) afforded the bis-TBDMS-protected alcohol (21.3 mg, 99.5%) as a 2:1 mixture of diastereomers: mp 50−53 °C. ^{1}H NMR ($CDCl_3$) δ: 8.54 (bs, 1H), 7.50 (s, 0.6H) 7.44 (s, 0.4H), 7.28 (m, 5H), 6.30 (m, 1H), 4.39 (m, 1H), 4.03 (bs, 1H), 3.93 (m, 1H), 3.81 (dd, J = 3, 11.4 Hz, 1H), 3.73 (dd, J = 3, 11.4 Hz, 1H), 2.80−2.62 (m, 4H), 2.43 (m, 1H), 2.25 (m, 1H), 2.00 (m, 1H), 0.90 (s, 18H), 0.08 (s, 12H). ^{13}C NMR ($CDCl_3$) δ: 164.7, 164.4, 150.1, 150.0, 138.4, 138.4, 138.1, 138.0, 129.7, 128.7, 126.7, 112.0, 111.9, 88.2, 88.0, 85.5, 85.2, 72.7, 72.4, 71.8, 71.5, 63.3, 63.2, 44.2, 44.1, 41.5, 41.3, 35.3, 35.0, 26.1, 26.0, 18.6, 18.2, −4.4, −4.6, −5.1, −5.2. IR (film): 3185, 2953, 1711, 1471, 1116, 1062, 836 cm^{-1}. HRMS (FAB): $C_{30}H_{50}N_2O_6Si_2$ calcd (M^+ + H) 591.3286, found 591.3284.

5-(1-(3-Phenyl-2-hydroxypropyl))-2′-deoxyuridine (13). The bis-*O*-TBDMS-protected alcohol (33 mg, 55.9 μmol), THF (500 μL), and Et_3N·3HF (91 μL, 90.1 mg) were placed in a 10-mL round-bottom flask and stirred for 24 h. After the solvents were removed in vacuo, flash chromatography on silica gel eluting with 5−10% MeOH/EtOAc afforded **13** as a white solid (18.8 mg, 96%): mp 164−166 °C. ^{1}H NMR (CD_3OD) δ: 7.79 (s, 0.6H), 7.76 (s, 0.4H), 7.23 (m, 5H), 6.26 (m, 1H), 4.39 (m, 1H), 4.02 (m, 1H), 3.90 (m, 1H), 3.77 (dd, J = 2.7, 9.3 Hz, 1H), 3.72 (J = 2.7, 9.3 Hz, 1H), 2.77 (m, 2H), 2.60 (m, 1H), 2.32 (m, 1H), 2.26 (m, 2H). ^{13}C NMR (CD_3OD) δ: 166.3, 152.4, 140.4, 140.1, 140.0, 130.7, 129.4, 127.3, 112.7, 89.0, 86.5, 72.3, 71.8, 71.7, 63.0, 45.0, 41.4, 41.3, 36.0. IR (film): 3389, 2925, 1685, 1471, 1276, 1053 cm^{-1}. HRMS (FAB): $C_{18}H_{22}N_2O_6$ calcd (M^+ + H) 363.1556, found 363.1563.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-(2-phenylethyl)-2′-deoxyuridine. 3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-5-styrenyl-2′-deoxyuridine (**16**)[22a] (75 mg, 0.14 mmol), 10% palladium on carbon (39.7 mg), and MeOH (16.9 mL) were placed in a 100-mL hydrogenation chamber. The chamber was pressurized with hydrogen gas (40 psi) and allowed to stir overnight. The reaction mixture was filtered through Celite, and the solvent was removed in vacuo. Flash chromatography on silica gel eluting with 33−100% EtOAc:hexanes afforded the reduced compound as a light yellow oil (44.7 mg, 59.2%). ^{1}H NMR ($CDCl_3$) δ: 8.83 (bs, 1H), 7.24 (m, 5H), 6.30 (dd, J = 5.7, 2.1 Hz, 1H), 4.34 (m, 1H), 3.89 (m, 1H), 3.73 (t, J = 3.6, 2H), 2.85 (m, 2H), 2.63 (m, 2H), 2.19 (m, 1H), 1.82 (m, 1H), 0.93 (s, 9H), 0.91 (s, 9H), 0.11 (s, 6H), 0.09 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 163.4, 150.4, 141.4, 136.2, 128.9, 128.6, 126.2, 114.2, 87.8, 84.8, 72.3, 63.1, 41.2, 35.0, 29.8, 26.2, 26.1, 26.0, 18.6, 18.2, −4.4, −4.6, −5.1, −5.2. IR (film): 2953, 2929, 2857, 1696, 1463, 1254, 1101, 1030, 835 cm^{-1}. HRMS (FAB): $C_{29}H_{48}N_2O_5Si_2$ calcd (M^+ + H) 561.3180, found 561.3188.

5-(2-Phenylethyl)-2′-deoxyuridine (14).[22b] 3′,5′-Bis-*O*-(tert-butyldimethylsilyl)-5-(2-phenylethyl)-2′-deoxyuridine (44.7 mg, 80 μmol), Et_3N·3HF (13 μL, 13 mg, 0.8 mmol), and THF (0.8 mL) were placed in a 25-mL pear-shaped flask. The reaction was allowed to stir for 24 h, at which time the solvents were removed in vacuo. Flash column chromatography on silica gel eluting with 19:1 EtOAc:MeOH (50 mL) afforded **14** as a white solid (23 mg, 90%). ^{1}H NMR ($CDCl_3$) δ: 7.51 (s, 1H), 7.17 (m, 5H), 6.2 (t, J = 6.9, 1H), 4.24 (m, 1H), 3.82 (dd, J = 3.3, 6.6 Hz, 1H), 3.63 (qd, J = 12, 3.3 Hz, 2H), 2.73 (m, 3H), 2.50 (m, 1H), 2.11 (m, 1H), 1.92 (m, 1H).

5-Ethanal-2′-deoxyuridine (15). THF (800 μL) and Et_3N·3HF (134 μL, 133 mg, 0.82 mmol) were added to a 25-mL round-bottom flask containing **17**[23] (41 mg, 82 μmol). The reaction was allowed to stir for 10 h at room temperature, at which time the solvents were removed in vacuo. Flash chromatography on silica gel eluting with 10−20% MeOH:EtOAc afforded **15** as a clear glass (16.4 mg, 74%). ^{1}H NMR (D_2O) δ: 9.60 (s, 0.3 H), 7.68 (s, 1H), 6.23 (t, J = 7 Hz, 1H), 5.18 (t, J = 5 Hz, 1H), 4.41 (m, 1H), 3.98 (m, 1H), 3.77 (m, 2H), 2.57 (d, 1H), 2.38 (m, 2H). ^{1}H NMR (CD_3OD) δ: 7.80 (d, 1H), 6.27 (t, J = 7 Hz, 1H), 4.74 (t, J = 5 Hz, 1H), 4.39 (m, 1H), 3.91 (m, 1H), 3.75 (dd, 2H), 3.36 (s, 3H), 2.55 (m, 2H), 2.23 (m, 2H). IR (film): 3374, 2928, 1682, 1471, 1276, 1094, 1052 cm^{-1}. LRMS (FAB): $C_{11}H_{14}N_2O_6$ calcd 289.3 (M^+ + H), found 289.1.

3′,5′-Di-(*tert*-butyldimethylsilyl)-5-(3,3′-dimethyl-2-oxobutyl)-2′-deoxyuridine (9). To a solution of the protected 2′-deoxyuridine (0.25 g, 0.55 mmol) in THF (20 mL) was added TMEDA (0.16 g, 1.37 mmol), followed by *s*-BuLi (1.05 mL of 1.3 M in cyclohexane, 1.37 mmol) at −78 °C. After 1 h of stirring at −78 °C, 1-bromopinacolone (0.49 g, 2.74 mmol) in THF (3 mL) was added to the reaction mixture which was allowed to warm to room temperature. The reaction mixture was quenched with saturated NH_4Cl solution, followed by H_2O. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water, brine, dried over Na_2SO_4, and then evaporated under reduced pressure to give a brown oil. Flash chromatography (eluent; hexanes:ether, 3:1) yielded **9** (60 mg, 20%) as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ: 8.08 (s, 1H), 6.26 (dd, J = 5.7, 7.5 Hz, 1H), 4.91 (s, 2H), 4.36 (m, 1H), 3.95 (q, J = 2.2 Hz, 1H), 3.87 (dd, J = 2.8, 11.6 Hz, 1H), 3.74 (dd, J = 2.2, 11.6 Hz, 1H), 2.29 (ddd, J = 2.6, 5.7, 13.1 Hz, 1H), 2.00 (m, 1H), 1.23 (s, 9H), 0.91 (s, 9H), 0.86

(s, 9H), 0.12 (d, $J = 2.9$ Hz, 6H), 0.05 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 206.8, 158.7, 150.0, 138.0, 96.4, 88.6, 86.7, 72.5, 63.2, 46.8, 43.6, 42.3, 26.5, 26.3, 26.0, 18.8, 18.3, −4.30, −4.6, −5.0, −5.1. IR (film): 2955, 2930, 2858, 1714, 1669, 1447, 1257, 1112, 1071, 836 cm^{-1}. HRMS (FAB): calcd for $C_{27}H_{51}N_2O_6Si_2$ ($M^+ + H$) 555.3286; found 555.3296.

5-(3,3′-Dimethyl-2-oxobutyl)-2′-deoxyuridine (6). To a solution of **9** (50 mg, 0.08 mmol) in anhydrous methanol (10 mL) was added NH_4F (26 mg, 0.73 mmol). The reaction mixture was heated at 55−60 °C for 27 h. After the solvent was removed under reduced pressure, the residue was purified by flash chromatography (eluent; CH_2Cl_2:MeOH, 20:1 to 10:1) to yield **6** (24 mg, 85%) as a white foam. ^{1}H NMR (CD_3OD) δ: 8.59 (s, 1H), 6.21 (t, $J = 6.6$ Hz, 1H), 4.97 (s, 2H), 4.40 (m, 1H), 3.94 (q, $J = 3.0$ Hz, 1H), 3.83 (dd, $J = 3.0$, 12.3 Hz, 1H), 3.73 (dd, $J = 3.0$, 12.0 Hz, 1H), 2.37−2.19 (m, 2H), 1.24 (s, 9H). ^{13}C NMR (CD_3OD) δ: 209.6, 160.7, 151.4, 141.0, 136.1, 96.2, 89.4, 88.2,71.8, 62.4, 48.1, 44.5, 42.1, 26.7 (3C). IR (film): 3450 (br), 2954, 1708, 1658, 1449, 1267, 1213, 1096, 1073 cm^{-1}.

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Supporting Information Available: HPLC retention times and response factors versus 2′-deoxyuridine for thymidine, **7**, and **13**−**15**. Ratio of protio versus deuterio *tert*-butylthiol as a function of H_2O:D_2O ratio. Tabulated data for competition for **1** between recombination and hydrogen atom donation from 2,5-dimethyltetrahydrofuran and propan-2-ol. Plot of recombination product **14** yield versus extent deuteration of *tert*-butylthiol. This material is available free of charge via the Internet at http://pubs.acs.org.

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