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Photochemical Generation and Reactivity of the Major Hydroxyl Radical Adduct of Thymidine

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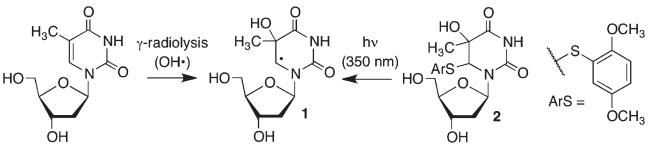
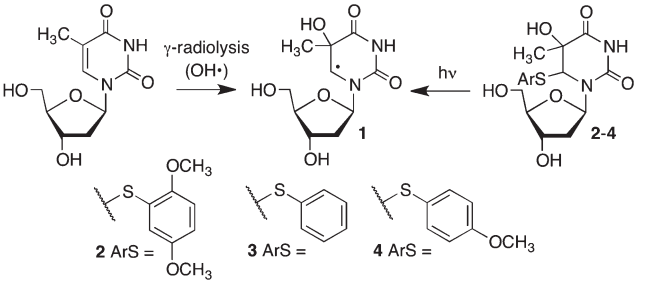
ABSTRACT



5,6-Dihydro-5-hydroxythymidin-6-yl radical (1), the major reactive intermediate resulting from hydroxyl radical addition to C5 of the pyrimidine, is produced via 350 nm photolysis of a 2,5-dimethoxyphenylsulfide precursor (2). Competition between O_2 and thiol for 1 suggests that the radical reacts relatively slowly with β-mercaptoethanol compared to other alkyl radicals. Overall, aryl sulfide 2 should be an effective precursor for the major hydroxyl radical adduct of thymidine in DNA.

Hydroxyl radical abstracts hydrogen atoms and adds to double bonds with rate constants that can approach the diffusion controlled limit.[1] The reactivity of the hydroxyl radical is particularly important in nucleic acid damage by γ-radiolysis and radiomimetic systems, such as Fe•EDTA.[1,2] Nucleobase radicals resulting from π-bond addition are the major family of reactive intermediates formed when nucleic acids are exposed to the hydroxyl radical. Because the hydroxyl radical reacts approximately equally with each nucleotide and without any sequence selectivity in nucleic acids,[2,3] methods have been developed for independently generating the corresponding reactive intermediates in DNA and RNA.[4−7] We describe herein photochemical generation of the major hydroxyl radical adduct of thymidine (5,6-dihydro-5-hydroxythymidin-6-yl, **1**) from **2** using 350 nm irradiation and examination of the reactivity of **1** (Scheme 1).

Scheme 1



The purpose of independently generating reactive intermediates is to simplify studying their reactivity. Previously, nucleobase radicals have largely been generated via Norrish

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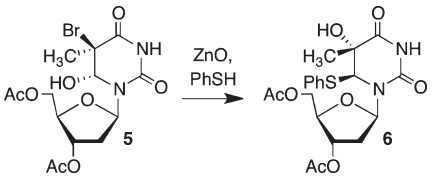
Scheme 2

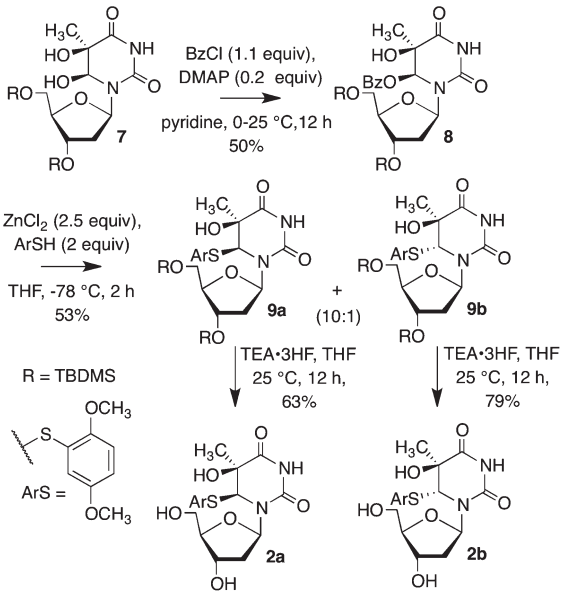


Type I photocleavage of ketones.[4−6,8−10] Ketones are potentially limited in their use with respect to sequence due to potential excited state quenching by dG.[11] Monomeric 5,6-dihydro-5-hydroxythymidin-6-yl (**1**) was independently generated from a *m*-trifluoromethyl benzoate via photoinduced single electron transfer, but this method was incompatible with forming **1** at defined sites in oligonucleotides.[12] More recently, an aryl sulfide (**3**) was used to produce **1**, but this molecule required 254 nm irradiation.[13,14] Products attributable to **1** were formed in low yield, and it is likely that irradiation of DNA containing this molecule at 254 nm would damage the biopolymer.

Aryl sulfide **3** was previously synthesized from **6** following treatment of the bromohydrin (**5**) with ZnO in the presence of thiophenol (Scheme 2).[13] We attempted using this strategy to prepare **2**, but the sulfurization reaction was inconsistent in our hands. Consequently, we pursued a slightly different approach using the protected thymidine glycol (**7**, Scheme 3) in which the C6-hydroxyl was activated for substitution by benzoylation. Secondary benzoate **8** was obtained from the major diastereomer of the glycol (**7**).[16] After significant experimentation, the aryl sulfide was reproducibly obtained by treating **8** with $ZnCl_2$ (2.5 equiv) in the presence of the appropriate thiol (2.0 equiv) at −78 °C.[15] Similar yields were obtained when either 4-methoxythiophenol or 2,5-dimethoxythiophenol were used as nucleophiles. In each instance two diastereomers were obtained, albeit in approximately a 10:1 ratio. NOE experiments carried out on the bis-silyl ethers (**9a**,**b**) indicated that the cis isomer (**9a**) was the major product.[17] In order to enhance UV-detection of the anticipated products from **1** following HPLC separation, the 5′-benzoyl derivative of **2** (**10**), as well as **13**−**15**, were independently synthesized (Figure 1).

Irradiation (30 min) using lamps with maximum output at 350 nm under degassed conditions in the presence of 0−350 mM *β*-mercaptoethanol (BME) consumed more than 97% of **10**. The major product formed was the thymidine C5-hydrate (**14**), even in the absence of BME (Table 1). Significant amounts of thymidine glycol (**13**, as a

Scheme 3



mixture of epimers at C6) were also formed, and the overall mass balance was >85%. The ratio of diastereomeric glycols (∼1:1), their yield relative to **14** (∼1.5), and their overall

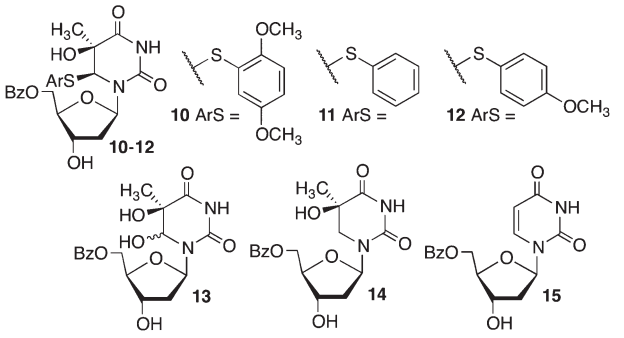


Figure 1. Structures of 5′-benzoylated photochemical substrates (**10**−**12**), anticipated photoproducts (**13**, **14**), and internal standard (**15**).

yield showed little dependence on BME concentration (Table 1). The formation of glycols (**13**) and **14** in the absence of O_2 and BME, respectively, warranted closer examination. Glycol formation under anaerobic conditions suggested that the carbocation (**16**, Scheme 4) was an intermediate. The observation that the yield of **13** in the presence of NaN_3 (0.6 M) is half as much as in the absence of azide is consistent with the intermediacy of **16**. The carbocation (**16**) could form by direct heterolysis upon excitation and/or electron transfer between the radical pair (Scheme 4).[18] The lack of an effect of BME concentration on the yield of **13** indicates that the glycols arise from

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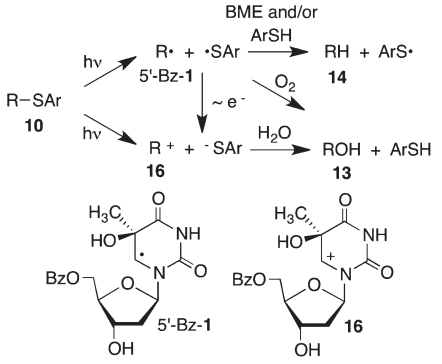
Table 1. Product Yields upon Photolysis of Aryl Sulfide (**10**) under Anaerobic Conditions As Function of BME Concentration[a]

[BME] (mM)	% yield[b] **13**	% yield[b] **14**	mass balance
0	36.4 ± 2.0	52.3 ± 4.7	89.7 ± 6.1
1	33.8 ± 5.0	51.5 ± 6.2	86.0 ± 8.0
20	41.6 ± 1.6	58.5 ± 3.0	100.1 ± 4.4
150	42.3 ± 1.3	58.3 ± 2.9	100.6 ± 4.4
350	43.5 ± 2.4	57.7 ± 0.9	101.1 ± 2.9

[a] Initial [**10**] = 30 μM. [b] Based upon unrecovered starting material. Yields are the average ± std. dev. of three experiments determined by HPLC.

heterolysis and/or electron transfer between the neutral intermediates within the initially formed radical cage. The aryl thiol produced in conjunction with **16** (and ultimately **13**) was suspected as the source of the hydrogen atom in the absence of BME. Taking advantage of the relatively low pK_a of the thiol supported this hypothesis. No thymidine hydrate (**14**) was observed when **10** was photolyzed in the presence of NaOH (1 mM) and no BME, presumably due to deprotonation of any aryl thiol that formed in situ.[19]

Scheme 4



For comparison, we analyzed the photolyzates of **11** and **12** in order to gauge their suitability as precursors for the nucleobase radical (**1**). Although aryl sulfide **12** was not susceptible to irradiation at 350 nm under anaerobic conditions, photolysis using lamps that emit maximally at 300 nm produced **13** and **14**. Irradiation of **12** for 30 min resulted in ≥70% conversion. The yields of glycols (**13**) and hydrate (**14**) from **12** were independent of BME concentration, but the ratio of **14:13** was greater (~2.0) than that obtained from photolysis of **10** (~1.5). The 5′-benzoyl derivative (**11**) of the previously reported phenyl sulfide required irradiation in quartz tubes using 254 nm lamps. Irradiation of **11** under these conditions resulted in its rapid consumption and the formation of a complex mixture of products. However, we

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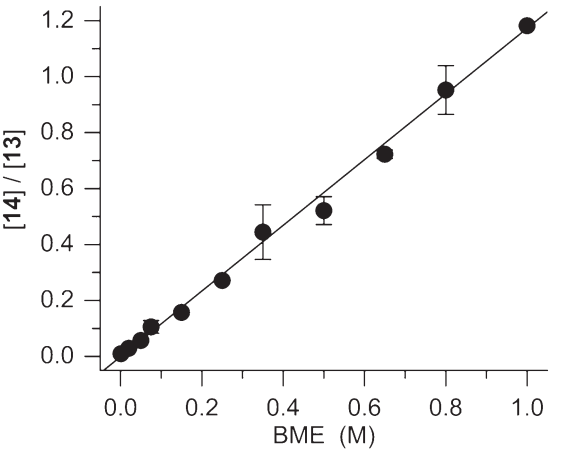

Figure 2. Relative yields of thymidine C5-hydrate (**14**) and glycols (**13**) from photolysis of **10** as a function of thiol (BME) concentration. (Yields of **13** are corrected for nonradical pathways. See text.)

were unable to detect **13** or **14** in

$$\frac{[\mathbf{14}]}{[\mathbf{13}]} = \frac{k_{BME}[5'\text{-Bz-}\mathbf{1}][BME]}{k_{O2}[5'\text{-Bz-}\mathbf{1}][O_2]} \quad (1)$$

the photolyzates by HPLC. These data suggested that overall the dimethoxyphenyl sulfide (**10**) was the best photochemical radical precursor, and all subsequent experiments were carried out using it.

Photolysis of **10** under aerobic conditions resulted in considerably higher glycol (**13**) yields. For instance, **13** was formed in 98.9 ± 0.5% under conditions where BME (1 mM) would not be expected to effectively compete with O_2 for 5′-Bz-**1**. The amount of glycol (**13**) attributable to O_2 trapping of the radical (5′-Bz-**1**) was determined by subtracting the yield of this product detected under anaerobic conditions from the total yield. A range for the rate constant describing BME trapping of 5′-Bz-**1** (k_{BME}) was determined by subtracting either the low or high yield of **13** detected under anaerobic conditions (Table 1). The rate constant for hydrogen atom abstraction from BME by 5′-Bz-**1** was estimated by using O_2 as a competitor. The ratio of **14:13** varied linearly with respect to the concentration of BME (Figure 2). An approximate rate constant for BME trapping of 5′-benzoylated **1** (k_{BME}) was obtained from the slope of this line by assuming that $k_{O_2} = 2 \times 10^9$ $M^{-1}s^{-1}$ and $[O_2]$ = 0.2 mM (eq 1). The rate constant for BME trapping ($k_{BME} = (4.7 \pm 0.1) \times 10^5$ to $(5.7 \pm 0.1) \times 10^5$ $M^{-1}s^{-1}$) is considerably lower than that for typical alkyl radical reactions with thiols.[20] The estimated rate constant (k_{BME}) is only ~5-fold slower than the rate constant for the reaction of the related radical (**17**) with BME ($k_{BME} = (2.6 \pm 0.5) \times 10^6$ M^{-1} s^{-1}).[21] Computational experiments support the relatively slow reactivity of **17** (and by inference **1**).[5] The even

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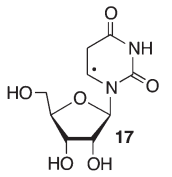
greater recalcitrance of 5′-Bz-**1** to react with BME may be due to increased steric hindrance by the C5-substituents. Moreover, the reactivity difference between **1** and **17** in hydrogen atom abstraction reactions may carry over to studies in DNA. However, the higher barriers encountered when abstracting hydrogen atoms from carbon-hydrogen bonds should result in an even greater difference in reactivity between these two nucleobase radicals.



This possibility and the reactivity of 5,6-dihydro-5-hydroxythymidin-6-yl (**1**) in general will be addressed using the 2,5-dimethoxyaryl sulfide (**2**, **10**) as a photochemical precursor in DNA. The experiments described above suggest that the sulfide will be useful for generating **1** under conditions that will not randomly damage DNA.

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Supporting Information Available. Experimental procedures for the synthesis of **2**. Spectroscopic characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.