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Mild Generation of 5-(2′-Deoxyuridinyl)methyl Radical from a Phenyl Selenide Precursor

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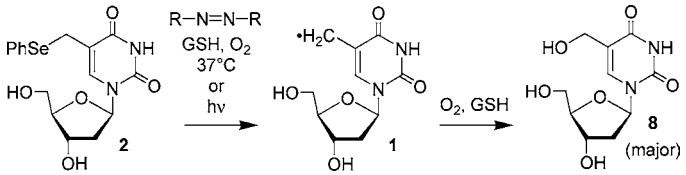
ABSTRACT



5-(2′-Deoxyuridinyl)methyl radical (1) resulting from formal hydrogen atom abstraction from the methyl group of thymidine is produced from the respective phenyl selenide precursor (2) via 350 nm photolysis or mild thermolysis (37 °C in the presence of glutathione) under aerobic or anaerobic conditions. The mild thermal generation of a nucleoside radical provides an alternative to previously reported photochemical methods, which are not always compatible with nucleic acids.

Independent generation of radicals has proven to be a valuable tool for studying nucleic acid oxidation.[1,2] This classical mechanistic approach has proven to be useful for elucidating complex processes, uncovering novel damage pathways, and has contributed to the resolution of related controversies pertaining to DNA damage.[3−8] Many of these studies have utilized UV photolysis to generate the reactive intermediates of interest from alkyl ketones that are designed to undergo Norrish type I photocleavage. This approach is limited sometimes by variable quantum yields for different precursors and possible photodegradation of products.[9] Furthermore, care must be taken when choosing local DNA sequences because of possible deoxyguanosine oxidation by ketone photoexcited states.[10]

Previous reports describe using phenyl sulfide (**3**) or a benzyl ketone (**4**) to generate **1** (and related radicals) via irradiation at ≤300 nm (Figure 1).[9,11,12] Phenyl selenides have been used less frequently in studies on nucleic acids as photolabile radical precursors and on one occasion as a thermal precursor.[13,14] We report using phenyl selenide **2** to

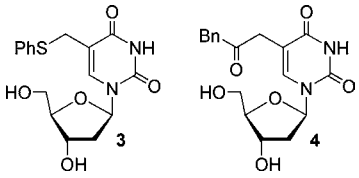


Figure 1. Previously reported photochemical precursors of 5-(2′-deoxyuridinyl)methyl radical (**1**).

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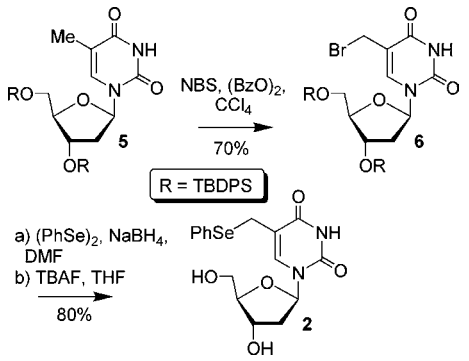
produce 5-(2′-deoxyuridinyl)methyl radical (**1**) under conditions that are compatible with biological molecules.

Phenyl selenide **2** was prepared from allylic bromide **6** (Scheme 1) with the hope that it would be susceptible to

Scheme 1



longer wavelength photolysis than the aforementioned radical precursors. Phenyl selenide **2** exhibits a $\lambda_{max} = 270$ nm ($\epsilon = 1.01 \times 10^4$ M^{-1} cm^{-1}) and its absorption tails into the region between 300 and 350 nm. A 0.1 mM aqueous solution of **2** has a >0.1 absorbance at 325 nm. When **2** (50 μM) was irradiated (5 min) in a Rayonet photoreactor ($\lambda_{max} =$ 350 nm)[15] in the presence of glutathione (GSH, 20 mM) under anaerobic conditions, 73.9 ± 0.8% of it was consumed. Thymidine was the only product formed (42.7 ± 0.6% yield based upon unrecovered starting material (57.7 ± 0.8% mass balance)).

Oxygenated products were observed to the exclusion of thymidine when **2** (50 μM) was photolyzed (5 min) under aerobic conditions over a range of GSH concentrations (Table 1). The extent of conversion of phenyl selenide was

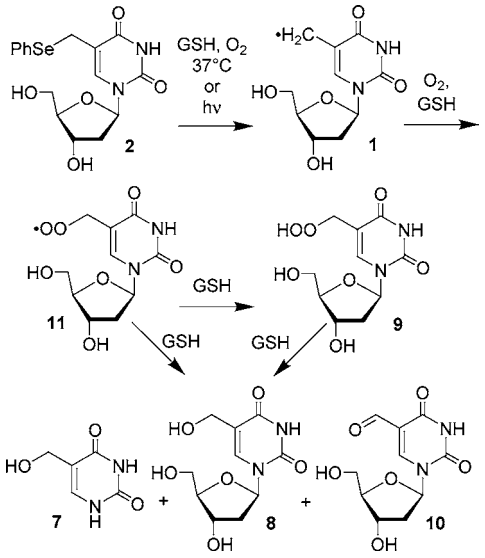
Table 1. Product Yield upon Photolysis of Phenyl Selenide (**2**) under Aerobic Conditions as a Function of GSH Concentration[a]

	% yield[b]			
[GSH] (μM)	**7**	**8**	**9**	**10**
0	1.1 ± 0.4	9.0 ± 0.3	31.6 ± 0.6	8.6 ± 0.2
25	4.5 ± 0.2	10.5 ± 0.9	9.8 ± 0.4	0
50	5.7 ± 0.2	11.8 ± 1.0	5.5 ± 0.4	0
100	8.5 ± 0.7	12.4 ± 0.7	3.5 ± 0.6	0
500	17.9 ± 1.2	13.4 ± 0.4	1.6 ± 0.5	0

[a] [**2**] = 50 μM. [b] Based upon unrecovered starting material. Yields determined using deoxyadenosine as an internal standard.

independent of trap concentration, suggesting that the thiol does not affect formation of the common intermediate, **1**. With the exception of hydroperoxide (**9**), identification of each of the products was verified using authentic samples. The identity of the hydroperoxide was inferred by ESI-MS of the material isolated by HPLC and from its reaction with $NaBH_4$, which produced **8**. The product mixture was dependent upon GSH concentration. Hydroperoxide **9** was the major product in the absence of GSH, but decreases with increasing thiol. We believe that products **7**, **8**, and **10** are derived from the peroxyl radical (Scheme 2) as proposed

Scheme 2



previously and/or reduction of the hydroperoxide.[11] Thymidine is not observed at even the highest GSH concentration (0.5 mM) employed. This is consistent with the approximate bimolecular rate constant ($k_{RSH} \sim 1 \times 10^7$ M^{-1} s^{-1}) expected for the reaction of **1** with a thiol.[16,17] One would not expect thiol trapping to compete with reaction between **1** and O_2 (~0.2 mM, $k_{O_2} \sim 2 \times 10^9$ M^{-1} s^{-1}) under these conditions.

The facile conversion of **2** using lamps that emit maximally at 350 nm bodes well for generating 5-(2′-deoxyuridinyl)-methyl radical (**1**) in DNA. Nonetheless, we were interested in determining if **2** could be used under nonphotochemical conditions. Thiols are typically poor chain carriers, but we rationalized investigating the reaction of **2** under radical conditions because phenyl selenides are excellent participants in radical chain reactions and **1** is resonance stabilized, which should accelerate a radical transfer step. Indeed, reaction of **2** (50 μM) with GSH (5 mM) at 37 °C for 3 h under aerobic conditions produced the expected trapping products of **1** by O_2 (Scheme 2, Table 2). Reactions proceeded to approximately 50% conversion in 3 h. However, the presence of a water-soluble azo initiator (VA-044, $t_{1/2} = 10$ h, 44 °C, 25 μM) had no effect on the reaction.

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(15) Spectral density at 300 nm is less than 1% that at 350 nm. See: www.rayonet.org/spectral-graphs.html for more information.
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Table 2. Reaction of Phenyl Selenide (**2**) with GSH under Aerobic Conditions at 37 °C[a]

	% yield[b]			
VA-044	**7**	**8**	**10**	mass balance
+	6.9 ± 1.2	57.9 ± 0.7	5.9 ± 2.9	85.0 ± 1.5
−	6.3 ± 1.4	60.2 ± 1.3	7.3 ± 2.8	86.8 ± 2.7

[a] [**2**] = 50 μM, [GSH] = 5 mM, [VA-044] = 25 μM, 3 h reaction time. [b] Based upon unrecovered starting material. Yields determined using deoxyadenosine as an internal standard.

The lack of a need for azo initiator to induce decomposition of **2** was attributed to trace metal catalysis, a known pathway for O_2-dependent thiol oxidation.[18] This proposal is supported by the effect of added disodium EDTA (1 mM) on the reaction. Less than 1% reaction is observed over the course of 3 h in the absence of VA-044 when the chelator is added (Figure 2). The overall decomposition rate of **2** is

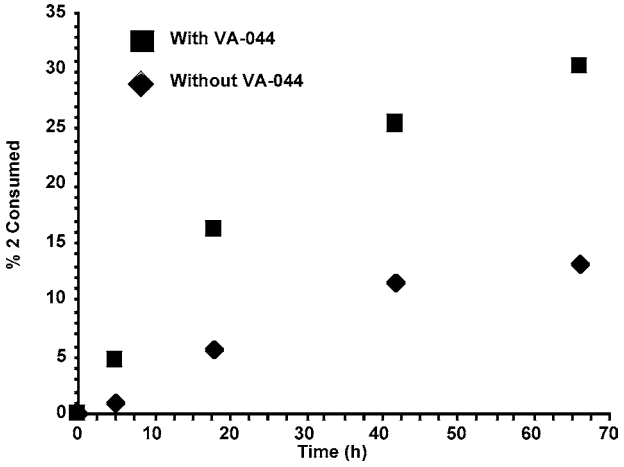
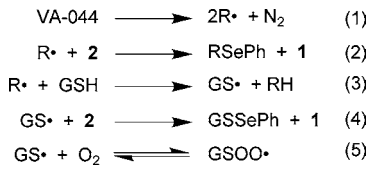


Figure 2. Reaction of phenyl selenide **2** (50 μM) with GSH (5 mM) in the presence of EDTA (1 mM) under aerobic conditions with and without VA-044 (25 μM) at 37 °C.

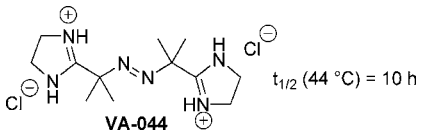
considerably slower in the presence of EDTA, even when VA-044 is added. However, there is a clear increase in phenyl selenide decomposition when the initiator is present.

Regardless of whether EDTA is present, hydroxymethyl-2′-deoxyuridine (**8**) is the major product of the "thermolyses", suggesting that a common intermediate is formed. We believe that the peroxyl radical (**11**) derived from O_2 trapping of 5-(2′-deoxyuridinyl)methyl radical (**1**) is the common intermediate (Scheme 2). Literature precedent suggests that **1** could form under anaerobic conditions by S_H2 reaction between **2** and the alkyl radical resulting from deazatization of VA-044.[19] However, this mechanism is inconsistent with the observed formation by HPLC of the mixed chalcogenide (GSSePh) derived from glutathione and the phenyl selenide (**2**), which was independently prepared.[20] On the basis of literature values for bimolecular rate constants of related reactions, we expect most of the alkyl radicals derived from VA-044 to be scavenged by GSH.[16,19] Although we tentatively suggest that the thiyl radical reacts with **2** to produce **1** and GSSePh (eq 4, Scheme 3), further investigation is

Scheme 3



warranted. A mechanism to explain the formation of **1** under aerobic conditions is also uncertain. In the absence of EDTA, trace metals are expected to produce thiyl radicals, whereas deazatization of VA-044 is the major pathway when EDTA is present. Thiyl radicals are rapidly, but reversibly trapped by O_2.[21] The formation of GSSePh is consistent with the reaction between thiyl radical and phenyl selenide to form **1**. Although the sulfur radicals are typically poor chain propagators, the present reaction conditions for producing **1** do not necessitate a chain mechanism.[22]



In summary, we find that phenyl selenide **2** enables us to generate 5-(2′-deoxyuridinyl)methyl radical (**1**) under milder conditions than **3** or **4**. Furthermore, **1** can be produced from the phenyl selenide under biologically relevant conditions (O_2, GSH, 37 °C) without UV photolysis. The mechanism and scope of this reaction warrants investigation. Regardless, thermolytic formation of **1** should prove to be useful for studying the reactivity of this radical in duplex DNA.

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

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