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Deconvoluting the Reactivity of Two Intermediates Formed from Modified Pyrimidines

Liwei Weng,[†] Sonia M. Horvat,[‡,§] Carl H. Schiesser,[‡,§] and Marc M. Greenberg*[,†]

Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore Maryland 21218, United States, Australian Research Council Centre of Excellence for Free Radical Chemistry and Biotechnology, Australia, and School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, 3010, Australia

mgreenberg@jhu.edu

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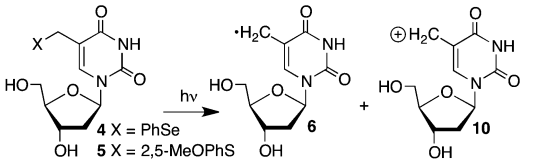
ABSTRACT



Generation of the 5-(2′-deoxyuridinyl)methyl radical (6) was reexamined. Trapping by 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl confirms that 6 is generated. However, trapping by methoxyamine reveals that the respective carbocation (10) is also produced. Examining the effects of these traps on products in DNA reveals that the carbocation and not 6 yields interstrand cross-links. Cross-link formation from the carbocation is consistent with DFT calculations that predict that addition at the N1 position of dA is essentially barrierless.

Aryl sulfides and phenyl selenides are useful as photochemical precursors to alkyl radicals. Substituting the aryl ring with electron-donating substituents enables one to photolyze sulfides at > 300 nm, which is useful for employing such precursors in the presence of molecules that absorb in the ultraviolet region, such as nucleic acids.[1,2] Our and other groups have used these types of precursors to independently generate radicals in nucleosides and oligonucleotides.[3−8] For instance, we recently reported using **1** to produce the major hydroxyl radical adduct of thymidine, 5-hydroxy-5,6-dihydrothymidin-6-yl radical (**2**, Scheme 1).[9] While studying the photochemistry of **1** we determined that it yields **2** and the respective carbocation, **3**. We were unable to distinguish between forming **3** directly from **1** upon photolysis or via electron transfer within the caged radical pair (Scheme 1). The formation of **3** led us to reinvestigate the photochemical generation of the 5-(2′-deoxyuridinyl)methyl radical (**6**) from similar precursors (**4**, **5**).[10−13] The results of these studies are the subject of this report.

The 5-(2′-deoxyuridinyl)methyl radical (**6**) was previously generated by 350 nm photolysis of the phenyl

[†] Johns Hopkins University.
[‡] Australian Research Council Centre of Excellence for Free Radical Chemistry and Biotechnology.
[§] The University of Melbourne.

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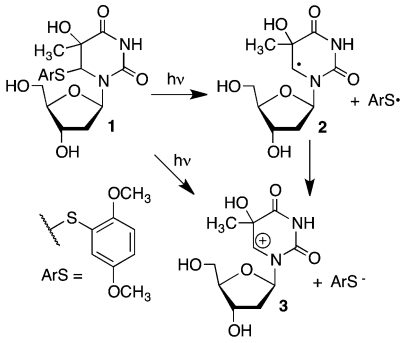
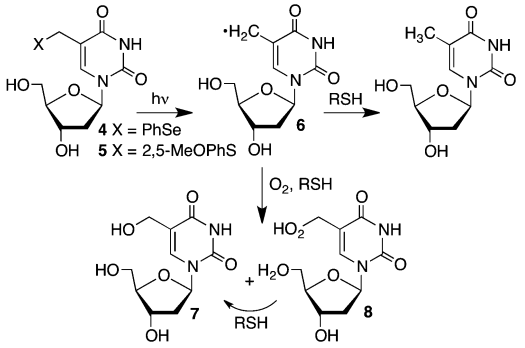
Scheme 1



Scheme 2



selenide (**4**) and aryl sulfide (**5**, Scheme 2).[10,11,13] Irradiating either nucleoside yields thymidine in the presence of thiol under anaerobic conditions, and the expected oxygenated products (**7** and **8**) under aerobic conditions. Competition studies between thiol and oxygen also indicated that peroxyl radical formation is reversible.[11] However, the mass balances for these reactions were less than 75%. Photolyzing duplex DNA containing **4** (or **5**) yielded interstrand cross-links (ICLs) with the opposing 2′-deoxyadenosine (Scheme 3). Identical ICLs were produced when DNA containing **4** (but not **5**) was exposed to oxidants such as $NaIO_4$ or $^1O_2$.[10,14] Cross-link formation was ascribed to the intermediacy of **6** upon photolysis and the quinone methide type species (**9**) under oxidizing conditions (Scheme 3). Thiol quenching of ICL formation was also consistent with the involvement of **6**. However, a thiol could also trap a carbocation, such as **10** (Scheme 4).

Indeed, photolysis of phenyl selenide **4** in the presence of *tert*-butyl thiol (50 mM) produces the expected radical trapping products (thymidine, **7**, and **8**) previously described. However, sulfide **11**, which was independently synthesized from previously reported **12**, was also produced. The yields of **11** under aerobic (5.1 ± 0.1%) and anaerobic (6.2 ± 1%) conditions were within experimental

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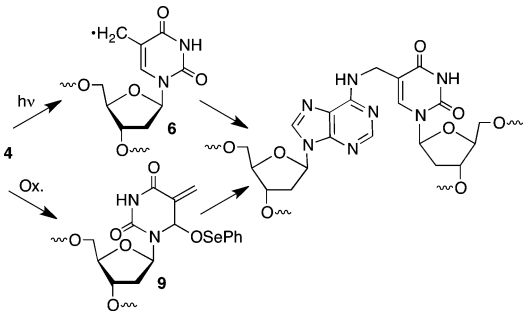
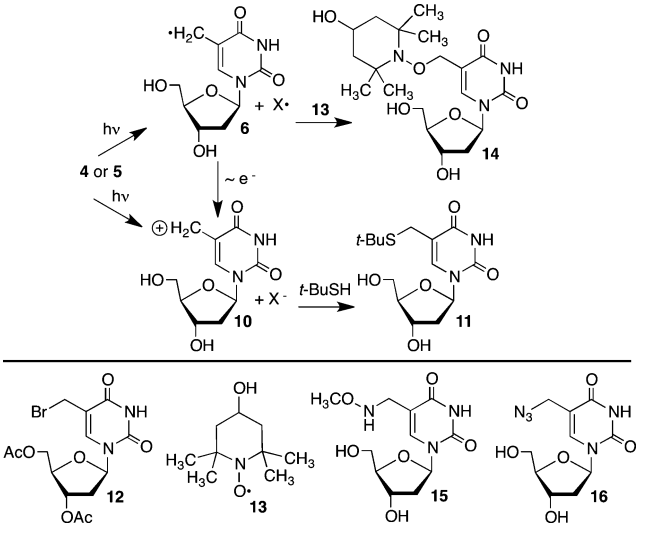
Scheme 3



error of one another and provide unequivocal support for carbocation **10** as its source. Having confirmed that irradiation of **4** yields **10** in addition to the 5-(2′-deoxyuridinyl)-methyl radical (**6**), we sought traps that react with only one of the reactive intermediates to determine the species responsible for ICL formation in DNA.

4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (HO-TEMPO, **13**) was chosen as a trap for **6**, and **14** (Scheme 4) was obtained (and fully characterized) following photolysis of **4**.[15] The yield of **14** (8 ± 0.2%) from the phenyl selenide (**4**) under anaerobic conditions varied by less than 2% between 0.5 and 20 mM HO-TEMPO (**13**). Higher concentrations (up to 200 mM) of **13** were required under aerobic conditions to reach a maximum yield of **14** (10.1 ± 0.7%), due to competition with O_2. Photolysis of aryl sulfide **5** also produced essentially identical yields of **14** under anaerobic (16.7 ± 0.3%) and aerobic (16.6 ± 0.4%) conditions. The absolute yields of **14** were greater from **5** than from phenyl selenide **4**, suggesting that the former yields a higher proportion of **6**.

Scheme 4



(15) See Supporting Information.

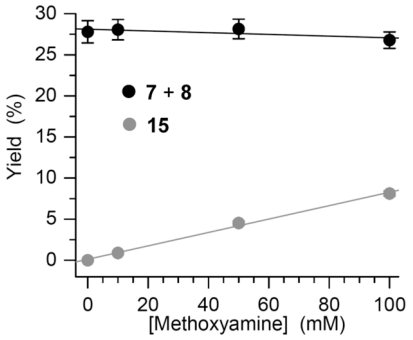


Figure 1. Effect of methoxyamine on product distribution from **5** under aerobic conditions.

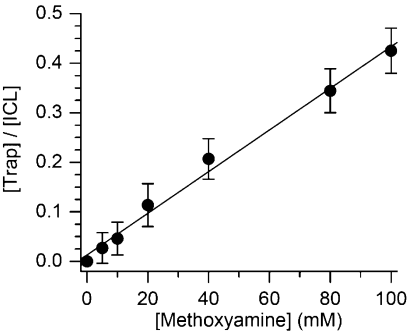


Figure 2. Effect of methoxyamine on interstrand cross-link formation from **5** (**17**) under aerobic conditions.

Sodium azide was examined as a potential trap for the carbocation (**10**), as it was successfully employed to probe for **9**. However, the photochemistry of **16** complicated product analysis. (The azide was partially converted to the aldehyde under the aqueous conditions, presumably through the nitrene.) Consequently, we utilized methoxyamine to trap **10**. Adduct **15** was independently prepared from **12** and was produced upon irradiation of **4** and **5** under aerobic and anaerobic conditions. Importantly, while the yield of **15** from **5** increased in the presence of greater methoxyamine concentration, the amounts of radical trapping products were unaffected. Under aerobic conditions the yield of oxygenated products (**7**, **8**) was unchanged (Figure 1), as was that of thymidine under anaerobic conditions.[15] Similar to the situation involving **1** (Scheme 1), we are unable to distinguish between direct heterolysis and formation of **10** via electron transfer within the initially formed radical cage (Scheme 4).

5'-^{32}P-d(AGA TGG AC**5** CAG GTA C)
3'-d(TCT ACC TGA GTC CAT G)

17

$$\frac{[\text{Trap}]}{[\text{ICL}]} = \frac{[\text{ICL}_0] - [\text{ICL}_x]}{[\text{ICL}_x]} = \frac{k_T}{k_{ICL}}[\text{Methoxyamine}] \quad (1)$$

The orthogonal traps, methoxyamine, and **13** were then tested separately as competitors for ICL formation upon irradiation of **5** in **17**. In the absence of competitor, the average cross-link yield was independent of O_2, as previously reported and ranged from 21.8 to 23.2% in multiple experiments, each consisting of three replicates.[11] Addition of **13** up to 100 mM had no effect on ICL formation when **17** was photolyzed under aerobic or anaerobic conditions, indicating that the 5-(2′-deoxyuridinyl)-methyl radical (**6**) is not responsible for cross-link formation.[15] However, **14** was detected by UPLC following enzyme digestion of the photolysate, confirming formation of **6**.[15] In contrast, methoxyamine competed with ICL formation, and **15** was detected in digested samples by UPLC.[15] In a typical experiment the cross-link yield decreased from 22.6 ± 0.7% to 15.8 ± 0.2% from 0 to 100 mM of methoxyamine. The amount of **10** trapped by methoxyamine was determined by subtracting the cross-link yield in the presence trap from the ICL formed in the absence of methoxyamine (eq 1). The ratio of trapped carbocation (**10**) to ICL varied linearly with respect to methoxyamine concentration (Figure 2). The slope of this line corresponds to the ratio of the rate constant for trapping of **10** by the nucleophile (k_T) to that for cross-linking (k_{ICL}). The average slope of this line obtained from experiments carried out under aerobic conditions (4.5 ± 0.2 M^{-1}, 5 experiments) was within experimental error of that obtained under anaerobic conditions (3.2 ± 1.2 M^{-1}, four experiments).[15] Extracting a rate constant for k_{ICL} from these data requires approximating k_T. Hydrazines and alkoxyamines have similar nucleophilicities. Rate constants for reactions between benzyhydrilium ions and hydrazine range from ~2×10^2 to 5×10^3 M^{-1} s^{-1}.[16] We expect less conjugated **10** to react more rapidly than benzyhydrilium ions. However, we do not know how much faster, making it difficult to estimate k_{ICL}. Carbocations can react orders of magnitude faster with nucleophiles than the examples cited. Regardless of the magnitude of k_{ICL}, these data clearly indicate that cross-linking is due to the carbocation (**10**), which is formed either upon direct photolysis and/or via electron transfer within the radical pair, as was previously proposed for **1** (Scheme 1).

In order to shed further light on this intriguing reaction, we examined the fundamental radical reaction between adenine **17** and the uridinylmethyl radical **18** to afford adduct **19** using computational techniques (Scheme 5). This study showed that the energy barrier ($\Delta E^{\ddagger}$) exceeds 50 kJ mol^{-1} at all levels of theory employed, with the M06-2X/6-311G (d,p) level providing a value of 58.8 kJ mol^{-1}. Several other levels of theory provided similar data.[15] It is interesting to note the M06-2X/6-311G (d,p) optimized transition state (**21**) for the formation of **19** is assisted by hydrogen bonding (Figure 3). However, given that the energy barrier is in excess of 50 kJ·mol^{-1}, it is unlikely that the cross-linking reaction that occurs when DNA containing **4** or **5** is photolyzed is radical in nature.

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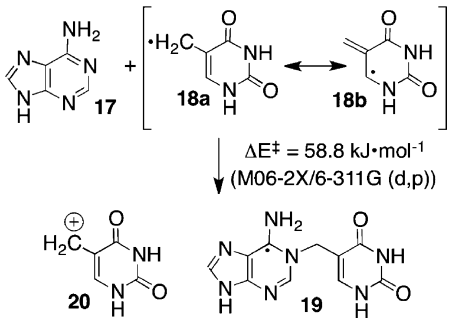
Scheme 5



Natural bond orbital analysis at the BHandHLYP/6-311G (d,p) level of theory on **21** reveals significant involvement of the lone pair on N1.[15,21] Indeed the $LP_N \rightarrow$ SOMO interaction is calculated to be worth 1584 kJ mol^{-1}, while the SOMO $\rightarrow \pi^*_{NC}$ is worth 1325 kJ mol^{-1}. This indicates that resonance structure **18b** is a significant contributor to this chemistry and that the key step is one in which the radical predominately acts as a Michael acceptor for the N1 nitrogen of **17**.

With this in mind, it came as no surprise that we were unable to locate a transition state for the reaction of the analogous cation **20** with **17**. This reaction is predicted to be barrierless in the gas phase, with an exothermicity of 190.8 kJ mol^{-1} at the B3LYP/6-311G(d,p) level of theory, consistent with the above experiments indicating that the observed cross-links are produced by the carbocation.

Determination of the fact that carbocation **10** is responsible for cross-linking when DNA containing **4** or **5** is photolyzed clarifies and reconciles a number of observations in the literature. Cross-linking from **10** provides a simple explanation for why ICL formation is independent of O_2 since the ionic intermediate (**10**) does not react with O_2. The formation of identical products from photolysis or oxidation of DNA containing **4** is also consistent with the intermediacy of **10**, which the above computational studies indicate should face a significantly lower barrier to adding to N1 of dA than radical **6**. The formation of ICLs from **10** also reconciles observations in the literature in which the

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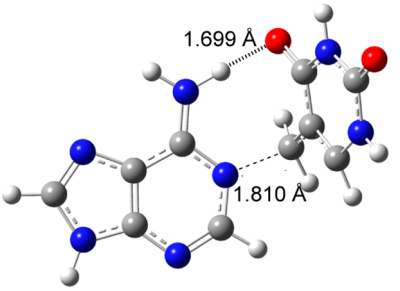

Figure 3. M06-2X/6-311G (d,p) optimized structure of the transition state **21** involved in the reaction of **17** with **18**.

5-(2′-deoxyuridinyl)methyl radical (**6**) is generated via hole migration in A·T sequences, but cross-links are not observed.[17,18] These experiments also suggest that other purported precursors to the 5-(2′-deoxyuridinyl)methyl radical (**6**) and related molecules that produce ICLs may also generate **10** and that other phenyl selenides used to generate radicals in DNA produce the corresponding carbocations.[3,4,6,19] Finally, the recent report by Li showing that **6** can be oxidized raises the possibility that the radical may indirectly lead to interstrand cross-links.[20]

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Supporting Information Available. All experimental procedures. Spectroscopic characterization of all new compounds. Effect of methoxyamine on products from **5** under anaerobic conditions, and effect **13** and methoxyamine (under anaerobic conditions) on ICL formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.