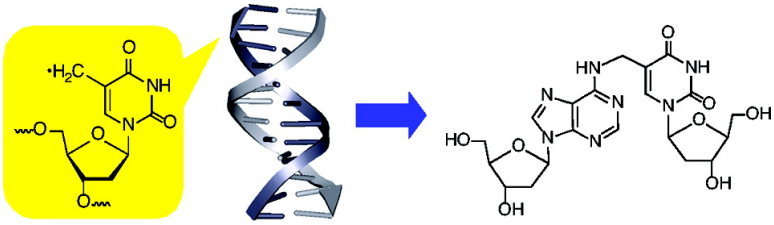
Communication

# Efficient DNA Interstrand Cross-Link Formation from a Nucleotide Radical

In Seok Hong, and Marc M. Greenberg

*J. Am. Chem. Soc.*, **2005**, 127 (11), 3692-3693• DOI: 10.1021/ja042434q • Publication Date (Web): 26 February 2005





## More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 20 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 02/26/2005

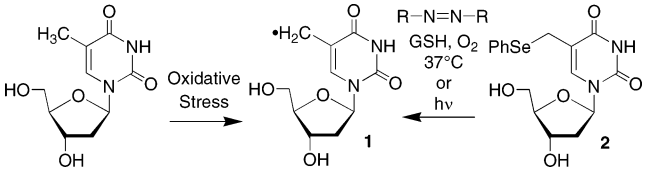
# Efficient DNA Interstrand Cross-Link Formation from a Nucleotide Radical

In Seok Hong and Marc M. Greenberg*

*Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218*

Received December 16, 2004; E-mail: mgreenberg@jhu.edu

Interstrand DNA cross-links exert significant biological effects. For instance, DNA cross-links are believed to be the source of the antitumor agent mitomycin C's cytotoxicity.[1] Other natural products and alkylating agents form interstrand cross-links (ISC).[2,3] More recently, antitumor agents that damage DNA through radical processes (e.g., C-1027, neocarzinostatin) have also been observed to produce ISCs.[4] In each instance, DNA ISC formation is mediated by a molecule, which forms a covalent bond to each strand. We describe efficient DNA ISC formation by the radical resulting from formal hydrogen atom abstraction from the thymine methyl group, 5-(2′-deoxyuridinyl)methyl (**1**). This is the first example in which formation of a DNA radical results in an interstrand cross-link directly.




Product studies indicate that 5-(2′-deoxyuridinyl)methyl radical (**1**) is produced during $\gamma$-irradiation of DNA and other methods of oxidative stress.[5,6] The reactivity of **1** has been examined as a monomer and as a component in single-stranded oligonucleotides by independently generating it from photochemical precursors.[7,8] The analogous radical derived from 5-methyl-2′-deoxycytidine has also been studied.[9] These radicals form tandem lesions (intrastrand cross-links) via addition to adjacent guanines. However, the reactivity of these radicals in duplex DNA has not been described.

5′-d(AGA TGG AC**2** CAG GTA C)
3′-d(TCT ACC TGA GTC CAT G)
**3**

We recently reported using phenyl selenide **2** as a mild thermal and photochemical (350 nm) precursor for 5-(2′-deoxyuridinyl)-methyl radical (**1**).[10] This precursor was introduced at defined sites in oligonucleotides using standard oligonucleotide synthesis methods.[11] ESI-MS analysis indicates that photolysis of **3** for 30 min under degassed or aerobic conditions completely consumes **2**. Denaturing polyacrylamide gel electrophoresis analysis reveals formation of a product in 25% yield whose migration is severely retarded relative to unreacted oligonucleotide, indicative of interstrand cross-linked material (Figure 1A). Direct strand breaks and alkali labile lesions are formed in much smaller quantities.[11] Cross-linked product is observed in even higher yield (60%) when decomposition is induced thermally using commercially available VA-044 in the presence of $O_2$ and glutathione (GSH) (Figure 1B).[10] Hydroxyl radical cleavage of gel-purified cross-linked DNA in which each oligonucleotide was 5′-$^{32}$P-labeled in separate experiments was used to determine which nucleotides are covalently bonded to one another.[12] Cross-linking occurred exclusively between the thymidine at which **1** was generated and the opposing

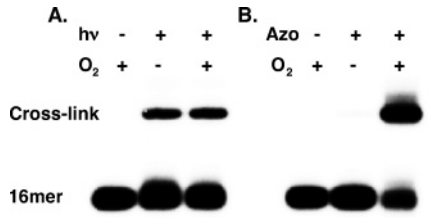



***Figure 1.*** Phosphorimage autoradiogram of denaturing PAGE analysis of the decomposition of **3** (20 nM). (A) Photolysis (350 nm, 20 min). (B) Thermolysis (VA-044 (0.5 mM), GSH (0.1 mM), 12 h, 37 °C).

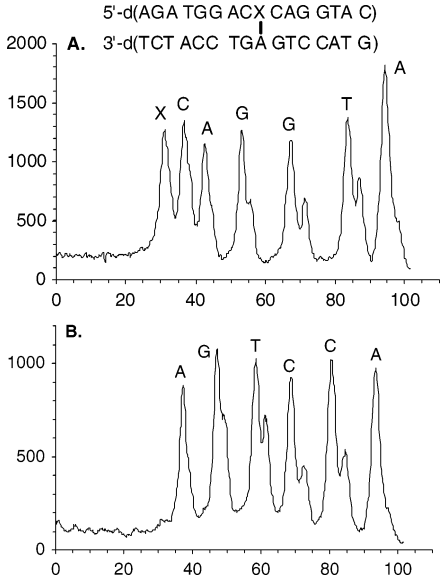
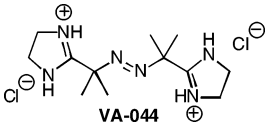



***Figure 2.*** Histogram showing Fe·EDTA cleavage of cross-linked product formed from **3**. Reduction in intensity indicates position of cross-linking on the appropriately labeled strand. (A) Strand containing **2**, X = position where **2** is incorporated. (B) Complementary strand.

deoxyadenosine (Figure 2). In contrast, DNA ISCs produced by small molecules typically do not involve nucleotides base paired to one another prior to DNA damage.[1−4]




Initial structural characterization of the cross-linked product was obtained by ESI-MS of material isolated from the denaturing gel.[11] The observed molecular weight (observed $m/z$ = 9760.1, calculated $m/z$ = 9760.4) is consistent with that of the reaction between **1** and the opposing strand, followed by formal one-electron oxidation and deprotonation. The observed molecular weight is also consistent with the observation that $O_2$ is unnecessary for interstrand cross-link formation. This effect is surprising given the near diffusion


**10.1021/ja042434q CCC: $30.25** 

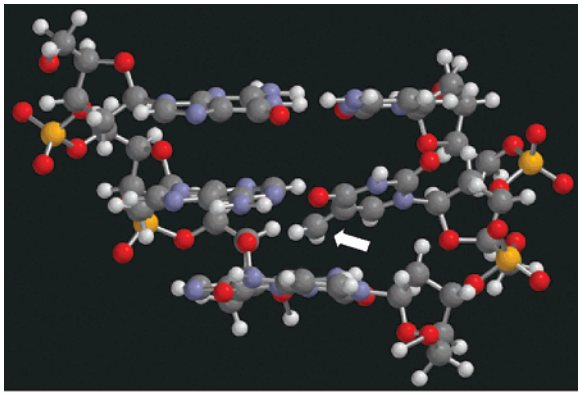
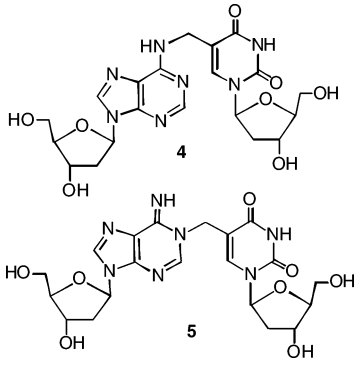


***Figure 3.*** Molecular modeling of *syn*-**1** in 5′-d(C**1**C·GAG).[19] Arrow indicates radical center. The distance between the radical center and N1-dA is 2.289 Å.

controlled trapping of alkyl radicals by $O_2$. It is possible that formation of the peroxyl radical from benzylic radical-like **1** is reversible, but further investigations are required to determine this.[13−15] The specific structure of the cross-linked product obtained following enzyme digestion and isolation by reverse-phase HPLC was determined by $^1$H NMR, absorption spectroscopy, and ESI-MS. These data are identical to that for **4**, which was obtained from the reaction of monomeric **1** (generated at 90 °C from **2**) with deoxyadenosine in solution. $^1$H NMR indicated the presence of three vinyl protons and two exchangeable protons.[11] These data are consistent with covalent linkage between N6 of deoxyadenosine and the methylene carbon derived from the radical center (**4**). In addition, ESI-MS/MS analysis, which gave rise to glycosidic bond cleavage, is also consistent with N6 adduct (**4**). The absence of purine fragmentation in the mass spectrum and the $\lambda_{max}$ ($H_2O$) = 266 nm argue against **5** or an adduct resulting from addition to C2 of deoxyadenosine.[11,16,17] One possibility is that **4** is produced via Dimroth rearrangement of the originally formed N1 adduct (**5**) during the isolation procedure.[18] Rearrangement of an initially formed adduct (**5**) is consistent with the slight change in gel mobility of the ISC that is observed upon piperidine treatment.[11]




The reaction conditions and previous experiments using **2** are consistent with cross-linking occurring through **1**.[10,20] Alkyl radicals add to the nitrogen of carbon−nitrogen $\pi$-bonds under appropriate thermodynamic conditions and conformational constraints.[21] Molecular modeling suggests that *syn*-**1** is well positioned to add to N1 of the opposing purine (Figure 3). Cross-link formation requires *syn*-**1**, which could form via conformational isomerization within the duplex or if phenyl selenide **2** adopts this isomer because it destabilizes the duplex in its anti form. We consider the latter explanation to be unlikely because there are numerous examples of C5-substituted pyrimidines that form stable base pairs with purines. Indeed, comparison of the UV melting temperature ($T_M$, Table 1) of **3** and a duplex containing thymidine shows the phenyl selenide only slightly thermally destabilizes the duplex ($\Delta T_M$ = 2.7 °C). The $T_M$s of mismatched duplexes exhibit almost identical decreases, providing further support for Watson−Crick base pairing between **2** and dA. These results imply that DNA ISC from **1** is not due to an artificially populated structure resulting from the presence of **2** in a duplex.

***Table 1.*** UV Melting Temperatures of Duplexes Containing **2** or Thymidine

5′-d(AGA TGG AC**X** CAG GTA C)
3′-d(TCT ACC TG**Y** GTC CAT G)

| X | Y | $T_M$ (°C) |
|---|---|---|
| **2** | A | 59.7 ± 0.4 |
| **2** | G | 54.7 ± 0.4 |
| T | A | 62.4 ± 0.4 |
| T | G | 57.0 ± 0.1 |

In conclusion, we have discovered an efficient DNA interstrand cross-linking process that emanates from a nucleotide radical (**1**). This process should provide the impetus for the design of novel DNA cross-linking agents.

**Acknowledgment.** We are grateful for support from the National Institute of General Medical Sciences (GM-054996). Dedicated to Professor David I. Schuster on the occasion of his 70th birthday.

**Supporting Information Available:** ESI-MS of oligonucleotides, autoradiograms of Fe·EDTA, and piperidine treatment of cross-linked product, synthetic procedures for the phosphoramidite used to prepare the oligonucleotide containing **2**, preparation, and characterization of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Tomasz, M.; Palom, Y. *Pharmacol. Ther.* **1997**, *76*, 73−87.
(2) Gates, K. S. In *Comprehensive Natural Products Chemistry, DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999.
(3) Richter, S. N.; Maggi, S.; Mels, S. C.; Palumbo, M.; Freccero, M. *J. Am. Chem. Soc.* **2004**, *126*, 13973−13979.
(4) Xu, Y.-j.; Xi, Z.; Zhen, Y.; Goldberg, I. H. *Biochemistry* **1997**, *36*, 14975−14984.
(5) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: London, 1987.
(6) Wagner, J. R.; van Lier, J. E.; Johnston, L. J. *Photochem. Photobiol.* **1990**, *52*, 333−343.
(7) Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett.* **2000**, *2*, 1085−1088.
(8) Anderson, A. S.; Hwang, J. T.; Greenberg, M. M. *J. Org. Chem.* **2000**, *65*, 4648−4654.
(9) Zhang, Q.; Wang, Y. *J. Am. Chem. Soc.* **2003**, *125*, 12795−12802.
(10) Hong, I. S.; Greenberg, M. M. *Org. Lett.* **2004**, *6*, 5011−5013.
(11) See Supporting Information.
(12) Paz, M. M.; Hopkins, P. B. *J. Am. Chem. Soc.* **1997**, *119*, 5999−6005.
(13) Tallman, K. A.; Pratt, D. A.; Porter, N. A. *J. Am. Chem. Soc.* **2001**, *123*, 11827−11828.
(14) Dussy, A.; Meggers, E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 7399−7403.
(15) Nangia, P. S.; Benson, S. W. *Int. J. Chem. Kinet.* **1980**, *12*, 29−42.
(16) Singer, B.; Sun, L.; Fraenkel-Conrat, H. *Biochemistry* **1974**, *13*, 1913−1920.
(17) Selzer, R. R.; Elfarra, A. A. *Chem. Res. Toxicol.* **1996**, *9*, 875−881.
(18) Fujii, T.; Itaya, T. *Heterocycles* **1998**, *48*, 359−390.
(19) Modeling was carried out using Spartan ’02.
(20) Cross-link formation can also be explained via formation of the carbocation derived from **1**.
(21) Friestad, G. *Tetrahedron* **2001**, *57*, 5461−5496.

JA042434Q