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A Potent, Water-Soluble and Photoinducible DNA Cross-Linking Agent

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Induced DNA interstrand cross-links (ISC) by chemical agents or photoactivation play very important roles for cancer therapy.[1] Several important clinical drugs (e.g., cisplatin, psoralens, and mitomycin C) are known to induce DNA ISC formation which can disrupt cell maintenance and replication.[2] The design of novel dimeric agents targeting DNA has been investigated by several groups.[3] Among these antitumor agents, one mechanism was involved in *o*-quinone methide (*o*-QM, Figure 1a) intermediate. *o*-QM is a quinone methide derivative which has played important roles in organic syntheses as well as in chemical and biological processes.[4,5] *o*-QM has been found to have significance for their nucleic acid bases and DNA alkylation.[6] More recently, the Wan, Freccero, and Kresge groups[7−9] have significantly reported the generation of *o*-QMs upon photochemical and thermal activation in aqueous solution. According to Freccero's report, phenol possessing a quaternary ammonium group can form *o*-quinone methide by photoactivation in aqueous solution (Figure 1a).[8] Meanwhile, photo-cross-links of DNA by irradiation with light under mild conditions and without any additives such as metals, oxidative agents, and reducing agents will offer considerable potential in medicine.[1] These encouraged us to design and synthesize the water-soluble phenol biquaternary ammonium **3** and biphenol biquaternary ammonium **6** derivatives which may have potential applications for DNA ISC under photochemical activation.

To our surprise, compound **6** demonstrated very potent ISC properties upon photoactivation using visible light. Furthermore, we found that the structural conformation might be the key factor for this mode of action. Herein, we report our preliminary results.

Two phenol and biphenol quaternary ammonium derivatives have been synthesized according to the procedure shown in Figure 2. Compound **2** was obtained by a Mannich reaction. Quaternarization was carried out by reaction with methyl iodide. Compound **5** was obtained by a reported procedure,[10] and its quaternarization was performed as above. Purification of the two derivatives was performed by crystallization with methanol and ethyl ether. Both compounds were fully characterized by NMR, HRMS, and elemental analysis (see Supporting Information).

The DNA−DNA cross-linking ability of compounds **3** and **6** was investigated using linearized plasmid DNA by denaturing alkaline agarose gel electrophoresis as reported by Cech[11] and Williams.[12] The duplex DNA was linearized using the *Eco*RI restriction endonuclease digestion. DNA cross-linking experiments were carried out in phosphate buffer (pH = 7.7). Samples were exposed to a 50-W high mercury lamp (wavelength > 400 nm) placed 20 cm away at 25 °C. The crude reaction mixtures were loaded onto a denaturing 0.9% alkaline agarose gel. Lambda *Hin*dIII DNA was used as a molecular weight marker.

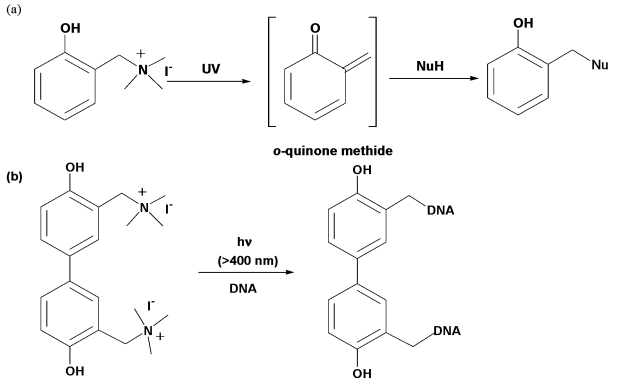

Figure 1. *o*-Quinone methide formation by photoactivation.

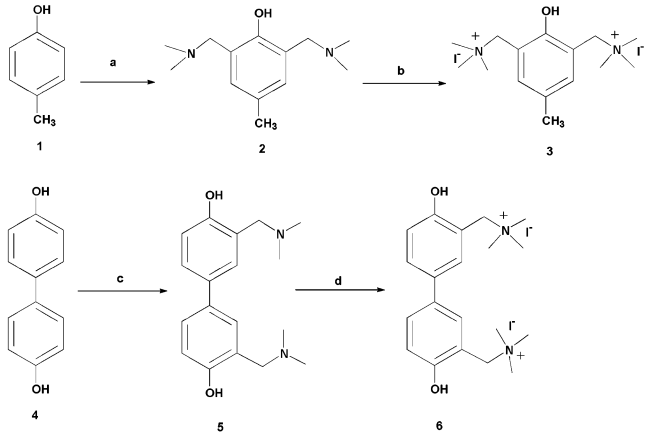

Figure 2. Synthesis of compounds **3** and **6**. Reagents and conditions: (a) 33% aqueous $(CH_3)_2NH$, 37% formaldehyde aqueous solution, 30 °C for 30 min, then 90−95 °C 2 h, 39.0%. (b) CH_3I, CH_3CN, 61.0%. (c) $(CH_2O)_n$, 33% aqueous $(CH_3)_2NH$, C_2H_5OH, overnight, 13.2%. (d) CH_3I, CH_3CN, 62.4%.

Results of concentration dependences for compounds **3** and **6** are illustrated in Figures 3 and 4. DNA cross-linking by compound **6** was observed at a concentration as low as 1.0 μM. At 10 μM concentration, compound **6** afforded predominantly cross-linked DNA. Upon comparison with compound **3**, compound **6** was found to be around 100-fold more potent as a DNA cross-linking agent. It suggests that compound **3** might not be involved in spontaneous reaction and that its mechanism of action is transient and consists

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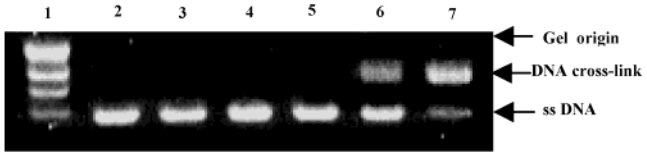

Figure 3. Concentration dependence of **6** for DNA cross-linking. Lane 1, 1.5 μg lambda DNA/*Hin*dIII (molecular weight standard); lane 2, 0.7 μg pBR322 (control); lane 3, 0.7 μg pBR322 + 50 μM **6** ; lane 4, 0.7 μg pBR322 + $h\nu$ (30 min); lane 5, 0.7 μg pBR322 + 0.1 μM **6** + $h\nu$ (30 min); lane 6, 0.7 μg pBR322 + 1 μM **6** + $h\nu$ (30min); lane 7, 0.7 μg pBR322 + 10 μM **6** + $h\nu$ (30 min).

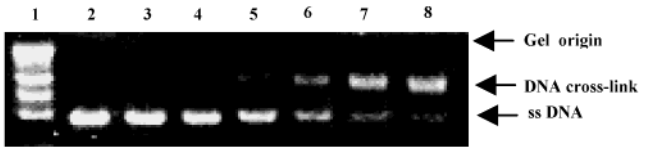

Figure 4. Concentration dependence of **3** for DNA cross-linking. Lane 1, 1.5 μg lambda DNA/*Hin*dIII (molecular weight standard); lane 2, 0.7 μg pBR322 (control); lane 3, 0.7 μg pBR322 + 1000 μM **3**; lane 4, 0.7 μg pBR322 + $h\nu$ (30 min); lane 5, 0.7 μg pBR322 + 10 μM **3** + $h\nu$ (30 min); lane 6, 0.7 μg pBR322 + 100 μM **3** + $h\nu$ (30 min); lane 7, 0.7 μg pBR322 + 500 μM **3** + $h\nu$ (30 min); lane 8, 0.7 μg pBR322 + 1000 μM **3** + $h\nu$ (30 min).

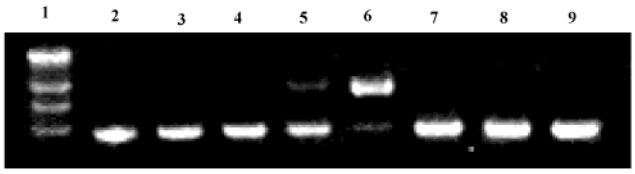

Figure 5. pH dependence of DNA cross-link by compound **6.** Lane 1, 1.5 μg lambda DNA/*Hin*dIII (molecular weight standard); lane 2, 0.7 μg pBR322 (control); lane 3, 0.7 μg pBR322 + 10 μM **6** (pH = 5.0); lane 4, 0.7 μg pBR322 + $h\nu$ (30 min, pH = 5.0); lane 5, 0.7 μg pBR322 + 10 μM **6** (pH = 5.0) + $h\nu$ (30 min); lane 6, 0.7 μg pBR322 + 10 μM **6** (pH = 7.7) + $h\nu$ (30 min); lane 7, 0.7 μg pBR322 + 10 μM **6** (pH = 10.0); lane 8, 0.7 μg pBR322 + $h\nu$ (30 min, pH = 10.0); lane 9, 0.7 μg pBR322 + 10 μM **6** (pH = 10.0) + $h\nu$ (30 min).

of sequential formation of two *o*-quinone methide intermediates[13] after photoactivation. When time-courses of cross-link formation by compounds **3** and **6** at the same concentration (10 μM) were performed (Figures 6 and 8, see Supporting Information), compound **6** was obviously found to be very sensitive to time increase, whereas compound **3** was inert. When the concentration of compound **3** was increased to 500 μM, no significant increase of DNA cross-linking was observed (Figure 7, see Supporting Information). Meanwhile, we found that DNA cross-linking by compound **6** was affected by pH variation (Figure 5). These results show that the most intense cross-linking occurred at pH 7.7 and the weaker cross-linking was at pH 5.0. On the contrary, no DNA cross-linking occurred at all at pH 10.0, implying that the decay of *o*-quinone methide intermediate was accelerated in basic buffer[9] and that the elimination to form quinone methide occurred with difficulty because of deprotonation of phenol in acid buffer.

We predicted the structure of **6** on the basis of DFT geometry optimization and then from their ^{13}C chemical shift calculations.[14,15]

We found that the dihedral angle between the two phenyl rings is about 35° and that the two quaternary ammonium groups were on the same side like two anchors, thus forming a twist structure (see Supporting Information for calculation details).

The significant difference between compounds **3** and **6** concerning cross-linking properties probably involves two factors. First, the formation of cross-link by **3** requires two steps, whereas cross-link by **6** is probably by formation of a bisquinone methide intermediate[18] or by nonspontaneous quinone methide formation. Second, on comparison with planar **3**, compound **6**, like many DNA-specific binders,[16] apparently has good shape orientation to adopt a helical conformation (twist $\approx$ 35°).[17] Therefore, compound **6** might naturally exhibit the strongest DNA binding and cross-linking abilities upon photoactivation.

In summary, we prepared a potent, water-soluble and photoinducible DNA cross-linking agent. This finding will encourage us to modify its structure for multiple applications in biological and medicinal chemistry. Further tethering to DNA binding agents for potential drug applications is currently under investigation.

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Supporting Information Available: Synthesis and characterization of **3** and **6**, DNA experimental data and detailed calculation for **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kohn, K. W. In *Topics in Molecular and Structural Biology 3. Molecular Aspects of Anti-cancer Drug Action*; Neidle, S., Waring, M., Eds.; Verlag Chemie Gmbh: D-6940, Weinheim, 1994; p 315. (b) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723. (c) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.
(2) (a) Cimino, G. D.; Gamper, H. B.; Isaacs, S. T.; Hearst, T. E. *Annu. Rev. Biochem.* **1985**, *54*, 1151. (b) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153. (c) Li, V.-S.; Choi, D.; Tang, M.-S.; Kohn, H. *J. Am. Chem. Soc*. **1996**, *118*, 3765.
(3) (a) Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardine, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939. (b) Hartley, J. A.; Hazrati, A.; Hodgkinson, T. J.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F. *Chem. Commun.* **2000**, 2325. (c) Na, Y.; Li, V.-S.; Nakanishi, Y.; Bastow, K. F.; Kohn, H. *J. Med. Chem.* **2001**, *44*, 3453. (d) Nagatsugi, F.; Tokuda, N.; Maeda, M.; Sasaki, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2577.
(4) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55. (b) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.
(5) (a) Peter, M. G. *Angew. Chem., Int. Ed. Engl*. **1989**, *28*, 555. (b) Gaikwad, N. W.; Bodell, W. J. *Chem.-Biol. Interact.* **2001**, *138*, 217. (c) Amouri, H.; Le Bras, A. J. *Acc. Chem. Res.* **2002**, *35*, 501.
(6) (a) Pande, P.; Shearer, J.; Yang, J.; Greenberg, W. A.; Rokita, S. E. *J. Am. Chem. Soc.* **1999**, *121*, 6773. (b) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **2000**, *65*, 2022.
(7) Brousmiche, D.; Wan, P. *Chem. Commun.* **1998**, 491.
(8) Modica, E.; Zanaletti, R.; Freccero, M.; Mella, M. *J. Org. Chem.* **2001**, *66*, 41
(9) Chiang, Y.; Kresge, A. J.; Zhu, Y. *J. Am. Chem. Soc.* **2001**, *123*, 8089.
(10) Mathai, K. P. *J. Indian Chem. Soc.* **1966**, *43*, 422.
(11) Cech, T. R. *Biochemistry* **1981**, *20*, 1431.
(12) Tepe, J. J.; Williams, R. M. *J. Am. Chem. Soc.* **1999**, *121*, 2951.
(13) Zeng, Q.-P.; Rokita, S. E. *J. Org. Chem.* **1996**, *61*, 9080.
(14) Zhang, R. Q.; Wong, N. B.; Lee, S. T.; Zhu, R. S.; Han, K. L. *Chem. Phys. Lett.* **2000**, *319*, 213.
(15) de Dios, A. C.; Pearson, J. G.; Oldfield, E. *Science* **1993**, *260*, 1491.
(16) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Hurley, L. H. *J. Med. Chem.* **1989**, *32*, 2027. (c) Qu, X.-G.; Trent, J. O.; Fokt, I.; Priebe, W.; Chaires, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12032. (d) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215. (e) Satz, A. L.; Bruice, T. C. *Acc. Chem. Res.* **2002**, *35*, 86.
(17) Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.* **1999**, *32*, 1043.
(18) Nakatani, K.; Higshida, N.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 5005.

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