DNA Cross-Linking

DOI: 10.1002/anie.200700844

Synthesis and Biological Studies of Inducible DNA Cross-Linking Agents**

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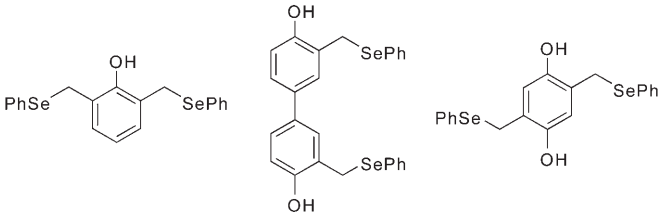
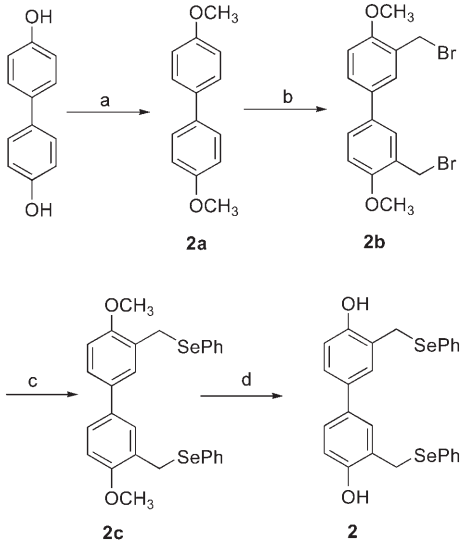
Some antitumor drugs targeting DNA can induce DNA interstrand cross-links (ISCs) or alkylation. Many bifunctional alkylating agents (cross-linking agents), such as nitrogen mustard, cisplatin, and mitomycin C, can form covalent bonds with DNA. This prevents the separation of DNA double strands, disrupts DNA replication and transcription, and finally leads to cell death.[1] One strategy to reduce the toxicity of these alkylation agents for normal cells and enhance potential medical application is to trigger the prodrug in the tumor cells. Along these lines plenty of novel inducible DNA cross-linking or alkylating agents have been designed and studied because of their high intrinsic reactivities.[2] Quinone methides (QMs) have been found to be very important intermediates in the process of DNA alkylation and DNA cross-linkage.[3] They can be generated by many methods, such as photo-irradiation,[4] oxidation,[5] and fluoride-induced activation.[6] Our group has reported an efficient DNA ISC agent based on a QM which triggers bisalkylation; the biphenyl structure is suitable for effective interaction with a DNA helix.[7] The biphenyl pharmacophore might be a good starting point for the development of further modified and decorated DNA recognition agents. Recently, Greenberg's laboratory developed a new method to effect efficient DNA interstrand cross-linking by a nucleotide radical or methide from a phenyl selenide precursor.[8,9] Meanwhile, selenium is reported to be one of the most important elements used in tumor therapy.[10] All of this information encouraged us to design and investigate the new inducible reactivities of the phenyl selenide compounds **1**–**3**.



1 **2** **3**

Compounds **1**–**3** were prepared from corresponding commercially available phenol derivatives through successive protection, bromination, substitution, and deprotection steps (Scheme 1).[11] All new compounds have been fully characterized by NMR spectroscopy and HRMS or elemental analysis.[11]



Scheme 1. Synthesis of compound **2**. a) CH_3I, acetone, RT, 89%; b) HBr, $(CH_2O)_n$, CH_3COOH, 30%; c) $(PhSe)_2$, $NaBH_4$, DMF, RT, 62%; d) BBr_3, CH_2Cl_2, 0°C, 47%.

The DNA cross-linking abilities of compounds **1**–**3** were studied using linearized plasmid DNA by denaturing alkaline agarose gel electrophoresis (Figure 1, see Figure S1 in the Supporting Information).[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).

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[**] X.Z. thanks the National Science Fund of China (no. 20672084), the National Science Fund for Distinguished Young Scholars (no. 20425206), the Ministry of Education of China, the State Key Laboratory of Natural and Biomimetic Drugs, and the State Key Laboratory of Applied Organic Chemistry. We thank two referees for their critical and helpful comments as well as Professor Dehua Pei, Ohio State University, for insightful comments on this paper.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

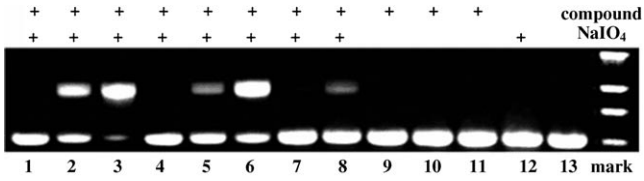


Figure 1. Concentration dependence of compounds **1**–**3** in DNA cross-linking ($NaIO_4$ oxidation). Lanes 1–8 with $NaIO_4$ (5 mM): lane 1: 0.7 μg pBR322 + 10 μM **1**; lane 2: 0.7 μg pBR322 + 50 μM **1** (cross-linking yield 50%); lane 3: 0.7 μg pBR322 + 100 μM **1** (84%); lane 4: 0.7 μg pBR322 + 1 μM **2**; lane 5: 0.7 μg pBR322 + 5 μM **2** (43%); lane 6: 0.7 μg pBR322 + 10 μM **2** (72%); lane 7: 0.7 μg pBR322 + 100 μM **3**; lane 8: 0.7 μg pBR322 + 1000 μM **3** (30%). Lanes 9–11 without $NaIO_4$: lane 9: 0.7 μg pBR322 + 100 μM **1**; lane 10: 0.7 μg pBR322 + 10 μM **2**; lane 11: 0.7 μg pBR322 + 1000 μM **3**; lane 12: 0.7 μg pBR322 + $NaIO_4$; lane 13, 0.7 μg pBR322 (control). Mark lane: 1.5 μg lambda DNA/HindIII (molecular weight standard).

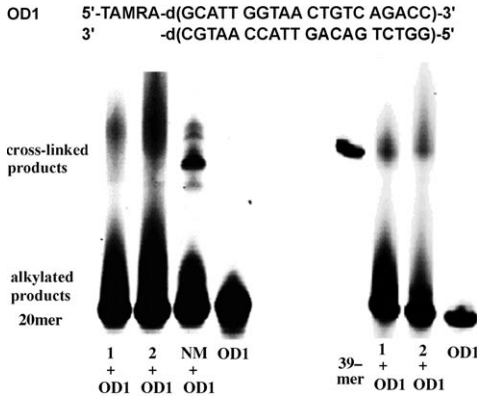


Figure 2. The duplex consisting of sequence OD1 and its complement, and the cross-linking of compounds **1** and **2** with OD1 (oxidation with 50 mM $NaIO_4$). **1**+OD1: **1** (10 mM) + OD1(4 μM) + $NaIO_4$; **2** + OD1: **2** (10 mM) + OD1(4 μM) + $NaIO_4$; NM + OD1: nitrogen mustard (10 mM) + OD1 (4 μM). A 39-mer oligonucleotide served as a molecular weight marker. TAMRA = 6-carboxytetramethylrhodamine, succinimidyl ester.

The DNA cross-linking abilities of the three compounds in the presence of sodium periodate, an oxidant which does not react with common proteins and nucleotides, were studied (Figure 1).[13] Compound **2** produced nearly 80% DNA cross-linking at a concentration as low as 10 μM and was found to be about 10 times more efficient than compound **1** as a DNA cross-linking agent. The biphenol compound **2** displays very potent ISC properties; this is consistent with our previously published result that structural conformation might be a key factor for this kind of ISC.[7] However, compound **3**, which is synthesized from the unstable hydroquinone, produced only slight DNA cross-linking even at high concentration (Figure 1, lane 8). This may be explained by its instability, especially upon oxidation by $NaIO_4$. We propose that the rapid and complete oxidation of the hydroquinone framework prevented ISCs between DNA and compound **3**.

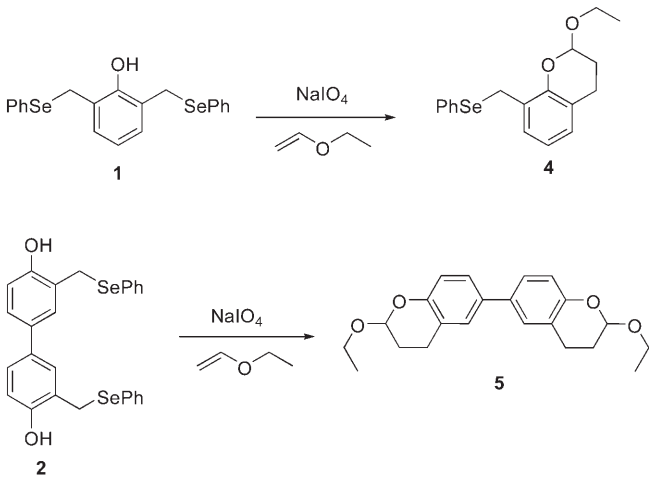
Upon photochemical activation (50-W high-pressure mercury lamp), with Rose bengal as the singlet oxygen sensitizer,[9] these compounds also exhibit fine DNA cross-linking abilities (see Figure S1 in the Supporting Information). The phenomenon of interstrand cross-linking was the most remarkable for compound **2**, followed by compound **1**, and compound **3**, which was consistent with the results with $NaIO_4$ as the oxidant.[11] These observations suggest that the designed compound **2** could find potential use as an auxiliary drug in photodynamic therapy.

To study the site specificity of the DNA cross-linking, we chose the 5′-TAMRA-labeled 20-mer oligonucleotide OD1 and its complementary strand as substrates to study the reaction on short oligonucleotide duplexes (Figure 2). After annealing, the non-cross-linked duplex was mixed with the phenyl selenide derivatives. Then $NaIO_4$ solution was added in excess to this mixture. The reaction mixture was analyzed by 20% denaturing PAGE. As it is a well-known DNA ISC reagent, nitrogen mustard served as a control; it induces interstrand DNA cross-linking, which was evident from the slowly migrating band in the gel.[14] The samples of oligonucleotide with compounds **1** and **2** were also observed to form this kind of band (Figure 2, see Figure S2 in the Supporting Information).[11] Since the molecular weight of the 39-mer oligonucleotide is similar to that of the cross-linked products, it served as a molecular-weight marker in DPAGE. The similar velocity of the bands arising from the 39-mer oligonucleotide and the high-molecular-weight product produced by oligonucleotide and compound **1** or **2** indicates the formation of cross-linked products (Figure 2, see Figure S3 in the Supporting Information).[11]

The gel-purified cross-linked DNAs were isolated by electrophoresis and treated by several methods to research their alkylation and cross-linking sites. Under alkaline conditions (1M piperidine, 90 °C), direct strand breaks at guanine, cytosine, and adenine residues were observed, and these suggested that the alkylation occurred at these three residues (see Figures S4 and S5 in the Supporting Information).[11] Owing to the high reactivity of the cross-linking agent, the product mixture is too complex to determine the interstrand cross-linked site. In a separate experiment the exact cross-linking site was determined by hydroxyl radical cleavage with each fluorescence-labeled strand. The decreased intensity of fragments from C15 to C20 in OD1 and the absence of cleavage product in OD2 defined that CAGACC from C15 to C20 in OD1 as the cross-linked region (see Figures S6 and S7 in the Supporting Information).[11] An interesting phenomenon of the appearance of a strong band at C15 is currently under investigation.

In keeping with the mechanism reported by Greenberg et al.,[8,9] we propose that this reaction might proceed via an *o*-quinone methide intermediate (see Scheme S1 in the Supporting Information).[11] When the phenyl selenide compounds were oxidized in the presence of a large excess of ethyl vinyl ether (EVE) as a trapping agent for *o*-QM, we obtained the expected QM–EVE adducts (Scheme 2).[15]

Therefore, the ISC properties of phenyl selenide derivatives might be affected by two factors. First, compound **1** reacts to form interstrand cross-linking in two steps, while compound **2** probably generates a bis(quinone methide) intermediate.[7] This conclusion could explain the obvious difference between compounds **1** and **2** concerning cross-linking properties.



Scheme 2. Trapping reactions in the presence of EVE.

The crystal data and structure of compound **2c** suggest that the configuration might be another important factor for the mode of action of **2** (Figure 3).[11] The dihedral angle

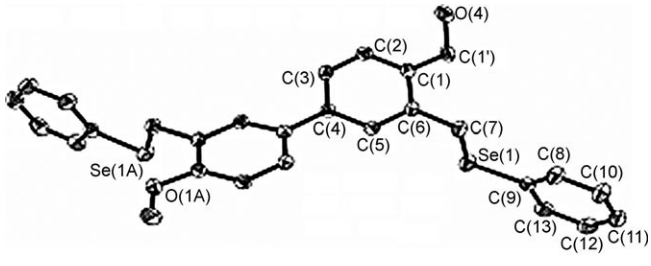


Figure 3. X-ray crystal structure of **2c.** ORTEP representation of **2c** with hydrogen atoms omitted for clarity.

between the planes of the two phenyl rings of the biphenyl core is around 29°, and the two phenyl selenide groups are on the same side like two arms; the structure of the deprotection product, compound **2**, should be analogous. This twist structure probably makes the molecule suitable to interact with the backbone of double-stranded DNA, and with the appropriate configuration, the attached functional groups of **2** might bind DNA effectively.

In conclusion, we have synthesized a series of phenyl selenide compounds and have shown their capability of generating DNA cross-linking through the formation of *o*-QM induced by periodate oxidation or light. We are currently testing related compounds and testing their potency as DNA cross-linking agents in biological and medicinal applications.

Experimental Section

Synthesis of compound **2**: **2b**: Paraformaldehyde (1.332 g, 44.4 mmol) and **2a** (1.90 g, 8.88 mmol) were suspended in 50 mL of acetic acid and 5 mL of 40% HBr in 30 mL of acetic acid was added to the mixture in one portion at 80 °C. The suspension cleared immediately. The reaction was complete after 3 h at this temperature. The reaction mixture was allowed to cool and was stored for 24 h at 4 °C. The crystallized product was filtered, washed with water, and dissolved in dichloromethane. The organic phase was washed with saturated sodium bicarbonate solution several times, dried over anhydrous Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 10:1) to yield the desired product as a white solid (0.92 g, 30% yield). ^{1}H NMR ($CDCl_3$): $\delta = 3.94$ (s, 6H), 4.62 (s, 4H), 6.93 (d, $J = 8.4$ Hz, 2H), 7.45 (dd, $J = 2.4$, 8.4 Hz, 2H), 7.51 ppm (d, $J = 2.40$ Hz, 2H); ^{13}C NMR ($CDCl_3$): $\delta = 29.19$, 56.02, 111.56, 126.65, 128.52, 129.44, 133.17, 156.92 ppm. FAB-MS [M^+] 400.

2c: Under N_2 atmosphere, diphenyl diselenide (0.622 g, 2 mmol) and sodium borodydride (0.149 g, 4 mmol) were dissolved in anhydrous DMF (5 mL) at room temperature for 10 min. A solution of compound **2b** (0.400 g, 1 mmol) in DMF (10 mL) was added dropwise by syringe and stirred overnight. The mixture was diluted with ethyl acetate, washed with brine several times, dried with Na_2SO_4, and concentrated to dryness. The product was purified by silica gel column chromatography (chloroform/hexane, 2:3) to give the product as a white solid (0.343 g, 62% yield). ^{1}H NMR ($CDCl_3$): $\delta = 3.84$ (s, 6H), 4.12 (s, 4H), 6.83 (d, $J = 8.4$ Hz, 2H), 6.92 (d, $J = 2.1$ Hz, 2H), 7.21–7.25 (m, 8H), 7.48–7.52 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 27.31$, 55.80, 110.98, 126.42, 127.51, 127.72, 128.74, 129.07, 130.93, 132.90, 134.52, 156.33 ppm; ^{77}Se NMR ($CDCl_3$): $\delta = 374.44$ ppm. Elemental analysis calcd for $C_{28}H_{26}O_2Se_2$: C 60.88, H 4.74; found: C 60.50, H 4.60.

2: Under N_2 atmosphere, BBr_3 (neat, 0.26 mL, 2.88 mmol) was added gradually to a solution of **2c** (0.2 g, 0.36 mmol) in freshly distilled CH_2Cl_2 (20 mL) at 0 °C. After the mixture had been stirred for 2 h at room temperature, the solution was poured into a large amount of ice water and extracted with $CHCl_3$. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 3:1) to give the desired product as a white solid (0.089 g, 46.7% yield). ^{1}H NMR ($CDCl_3$): $\delta = 4.13$ (s, 4H), 5.53 (s, 2H), 6.81–6.85 (m, 4H), 7.12 (dd, $J = 2.4$, 8.4 Hz, 2H), 7.23–7.27 (m, 6H), 7.46–7.49 ppm (m, 4H). ^{13}C NMR ($CDCl_3$): $\delta = 28.08$, 116.96, 124.67, 127.13, 128.04, 129.08, 129.30, 129.50, 133.54, 134.60, 153.25 ppm; ^{77}Se NMR ($CDCl_3$): $\delta = 333.83$ ppm. Elemental analysis calcd for $C_{26}H_{22}O_2Se_2$: C 59.55, H 4.23; found: C 59.68, H 4.50.

Received: February 25, 2007
Revised: August 1, 2007
Published online: September 13, 2007

Keywords: antitumor agents · DNA alkylation · DNA cross-linking · quinone methide

[1] a) S. R. Rajski, R. M. Williams, *Chem. Rev.* **1998**, *98*, 2723–2796; b) D. M. Noll, T. M. Mason, P. S. Miller, *Chem. Rev.* **2006**, *106*, 277–301.

[2] a) Y. Na, V. S. Li, Y. Nakanishi, K. F. Bastow, H. Kohn, *J. Med. Chem.* **2001**, *44*, 3453–3462; b) K. Mitra, J. C. Marquis, S. M. Hillier, P. T. Rye, R. Zayas, A. S. Lee, J. M. Essigmann, R. G. Croy, *J. Am. Chem. Soc.* **2002**, *124*, 1862–1863; c) S. Park, C. Anderson, R. Loeber, M. Seetharaman, R. Jones, N. Tretyakova, *J. Am. Chem. Soc.* **2005**, *127*, 14355–14365.

[3] a) P. Wang, Y. Song, L. X. Zhang, H. P. He, X. Zhou, *Curr. Med. Chem.* **2005**, *12*, 2893–2931; b) M. Freccero, C. Di Valentin, M. Sarzi-Amade', *J. Am. Chem. Soc.* **2003**, *125*, 3544–3553.

[4] a) E. Modica, R. Zanaletti, M. Freccero, M. Mella, *J. Org. Chem.* **2001**, *66*, 41–52; b) S. N. Richter, S. Maggi, S. C. Mels, M. Palumbo, M. Freccero, *J. Am. Chem. Soc.* **2004**, *126*, 13973–13979; c) E. E. Weinert, R. Dondi, S. Colloredo, K. N. Frankenfield, C. H. Mitchell, M. Freccero, S. E. Rokita, *J. Am. Chem. Soc.* **2006**, *128*, 11940–11947.

[5] a) J. Liu, H. Liu, R. B. van Breemen, G. R. J. Thatcher, J. L. Bolton, *Chem. Res. Toxicol.* **2005**, *18*, 174 – 182; b) S. R. Angle, W.-J. Yang, *J. Org. Chem.* **1992**, *57*, 1092 – 1097; c) D. C. Thompson, K. Perera, R. London, *Chem. Res. Toxicol.* **1995**, *8*, 55 – 60.
[6] a) Q. P. Zeng, S. E. Rokita, *J. Org. Chem.* **1996**, *61*, 9080 – 9081; b) P. Pande, J. Shearer, J. Yang, W. A. Greenberg, S. E. Rokita, *J. Am. Chem. Soc.* **1999**, *121*, 6773 – 6779; c) W. F. Veldhuyzen, Y.-F. Lam, S. E. Rokita, *Chem. Res. Toxicol.* **2001**, *14*, 1345 – 1351.
[7] P. Wang, R. P. Liu, X. J. Wu, H. J. Ma, X. P. Cao, P. Zhou, J. Y. Zhang, X. C. Weng, X. L. Zhang, J. Qi, X. Zhou, L. H. Weng, *J. Am. Chem. Soc.* **2003**, *125*, 1116 – 1117.
[8] a) I. S. Hong, M. M. Greenberg, *Org. Lett.* **2004**, *6*, 5011 – 5013; b) I. S. Hong, M. M. Greenberg, *J. Am. Chem. Soc.* **2005**, *127*, 3692 – 3693; c) I. S. Hong, H. Ding, M. M. Greenberg, *J. Am. Chem. Soc.* **2006**, *128*, 485 – 491; d) I. S. Hong, H. Ding, M. M. Greenberg, *J. Am. Chem. Soc.* **2006**, *128*, 2230 – 2231.
[9] I. S. Hong, M. M. Greenberg, *J. Am. Chem. Soc.* **2005**, *127*, 10510 – 10511.
[10] L. Bjoerkhem-Bergman, U. Torndal, S. Eken, C. Nystroem, A. Capitanio, E. H. Larsen, M. Bjoernstedt, L. C. Eriksson, *Carcinogenesis* **2004**, *26*, 125 – 131.
[11] See Supporting information.
[12] a) T. R. Cech, *Biochemistry* **1981**, *20*, 1431 – 1437; b) J. J. Tepe, R. M. Williams, *J. Am. Chem. Soc.* **1999**, *121*, 2951 – 2955.
[13] a) L. Burdine, T. G. Gillette, H. J. Lin, T. Kodadek, *J. Am. Chem. Soc.* **2004**, *126*, 11442 – 11443; b) B. Liu, L. Burdine, T. Kodadek, *J. Am. Chem. Soc.* **2006**, *128*, 15228 – 15235.
[14] a) J. T. Millard, S. Raucher, P. B. Hopkins, *J. Am. Chem. Soc.* **1990**, *112*, 2459 – 2460; b) S. M. Rink, M. S. Solomon, M. J. Taylor, S. B. Rajur, L. W. McLaughlin, P. B. Hopkins, *J. Am. Chem. Soc.* **1993**, *115*, 2551 – 2557; c) S. M. Rink, P. B. Hopkins, *Biochemistry* **1995**, *34*, 1439 – 1445.
[15] a) K. Nakatani, N. Higashida, I. Saito, *Tetrahedron Lett.* **1997**, *38*, 5005 – 5008; b) D. Brousmiche, P. Wan, *Chem. Commun.* **1998**, 491 – 492.

