DOI: 10.1002/chem.200800783

Synthesis and Oxidation-Induced DNA Cross-Linking Capabilities of Bis(catechol) Quaternary Ammonium Derivatives

Zhibin Song,[a] Xiaocheng Weng,[a] Liwei Weng,[a] Jing Huang,[a] Xiaolin Wang,[a] Minghui Bai,[a] Yangyang Zhou,[a] Guangfu Yang,[b] and Xiang Zhou*[a, c]

Many antitumor agents such as cisplatin and nitrogen mustard work through a DNA cross-linking mechanism, in which interstrand cross-linking is especially important.[1] These agents covalently bind to the DNA duplex and inhibit both replication and transcription in tumor cells eventually killing them.[2] However, the application of these drugs has been limited by their toxic side effects in normal cells. The possibility of selectively destroying the DNA of tumor cells by induction has been given much attention in medicinal research. Inducible DNA cross-linking agents have provided a promising method for specifically damaging tumor cells.[3] The inducible formation of *o*-quinone methide (*o*-QM) as a precursor of antitumor agents has been reported by many groups because *o*-QM is highly reactive with DNA.[4] Our group has reported several efficient DNA cross-linking agents derived from an *o*-QM intermediate induced by a biphenol quaternary ammonium structure[5] and a biphenol selenide structure.[6] Recently, Kodadek's group reported that biotinylated catechol derivatives could cross-link proteins via an *o*-quinone intermediate induced by $NaIO_4$.[7] The quinone intermediate induced by the oxidation of a catechol appears to be a promising agent for studying the properties of complicated biological complexes,[8] and moreover, this oxidative activity can be carried out by a ubiquitous enzyme, that is, tyrosinase.[9] Encouraged by their reports, we designed and synthesized a new family of DNA cross-linking agents: bis(catechol) quaternary ammonium derivatives (Figure 1). We found that they could cross-link DNA potently via an *o*-quinone intermediate induced by oxidation.

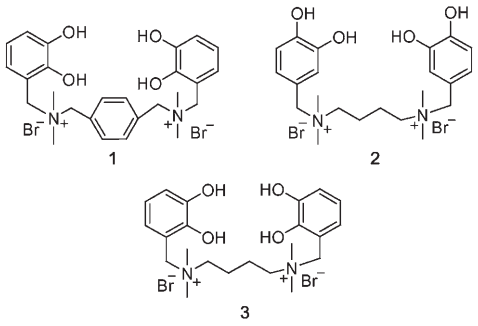


Figure 1. Structures of bis(catechol) derivatives.

Compounds **1–3** are composed of two catechol monomers acting as DNA cross-linking units, and are joined by different linkers that act as DNA binding units. In order to achieve high affinity to DNA, positive charged linkers and quaternary ammonium were considered for use. Furthermore, aliphatic and aromatic chains were investigated to determine which of them resulted in favorable cross-linking reactions, and to determine the relationship between agent flexibility and DNA cross-linking abilities. These compounds were prepared as shown in the supporting information. All new compounds were fully characterized by NMR spectroscopy and HRMS analysis.

The DNA–DNA cross-linking abilities of compounds **1–3** were firstly studied using tyrosinase as oxidative agent. Tyrosinase is a binuclear copper enzyme occurs in all organisms, which can catalyze the oxidation of catechols to qui-

[a] Z. Song,[+] X. Weng,[+] L. Weng,[+] J. Huang, X. Wang, M. Bai, Y. Zhou, Prof. X. Zhou
College of Chemistry and Molecular Sciences
Wuhan University
Hubei, Wuhan 430072 (P.R. of China)
Fax: (+86) 27-87336380
E-mail: xzhou@whu.edu.cn

[b] Prof. G. Yang
College of Chemistry
Hua Zhong Normal University
Hubei, Wuhan 430072 (P. R. of China)

[c] Prof. X. Zhou
The State Key Laboratory of Natural and Biomimetic Drugs and
State Key Laboratory of Applied Organic Chemistry

[+] Three authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200800783.

nones.[10] The experiments were performed by denaturing alkaline agarose gel electrophoresis, as previously reported.[11] In the presence of tyrosinase, each compound exhibited potential cross-linking abilities (Figure 2). It is not surprising that compound **2** obtains the best cross-linking result, because it has the similar aromatic units with DOPA, which is the natural oxidation substrate of tyrosinase.[9,10]

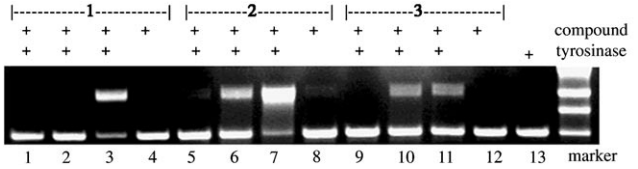


Figure 2. Concentration dependence of compounds **1–3** for DNA cross-linking (tyrosinase oxidation). The concentration of tyrosinase was fixed as 28 μM. lane 4, 8, 12 are the control lane without tyrosinase oxidation. Lane 13 is the control lane only with tyrosinase and without the compounds. lane 1, 0.7 μg pBR322 + 10 μM **1** + tyrosinase; lane 2, 0.7 μg pBR322 + 20 μM **1** + tyrosinase; lane 3, 0.7 μg pBR322 + 50 μM **1** + tyrosinase; lane 4, 0.7 μg pBR322 + 50 μM **1**; lane 5, 0.7 μg pBR322 + 10 μM **2** + tyrosinase; lane 6, 0.7 μg pBR322 + 20 μM **2** + tyrosinase; lane 7, 0.7 μg pBR322 + 50 μM **2** + tyrosinase; lane 8, 0.7 μg pBR322 + 50 μM **2**; lane 9, 0.7 μg pBR322 + 10 μM **3** + tyrosinase; lane 10, 0.7 μg pBR322 + 20 μM **3** + tyrosinase; lane 11, 0.7 μg pBR322 + 50 μM **3** + tyrosinase; lane 12, 0.7 μg pBR322 + 50 μM **3**; lane 13, 0.7 μg pBR322 + tyrosinase; Marker lane, 1.5 μg lambda DNA/HindIII (molecular weight standard).

Upon different oxidative activations, these compounds also exhibit fine DNA cross-linking ability (Figure 3). Sodium periodate, which is inert to common proteins and nucleotides, is a suitable reagent to induce oxidation at physiological pH. One interesting phenomenon is that the results of compounds **2** in two inductive methods were widely divergent. Similar conclusion from previous study that experimental results could be quite different by using $NaIO_4$ and tyrosinase encourages us to look for more reasonable explanations.[12]

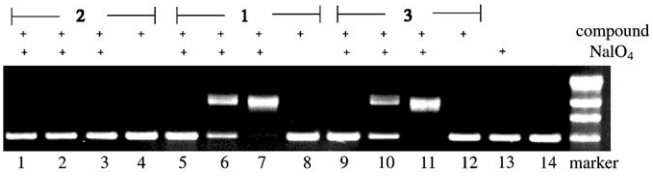
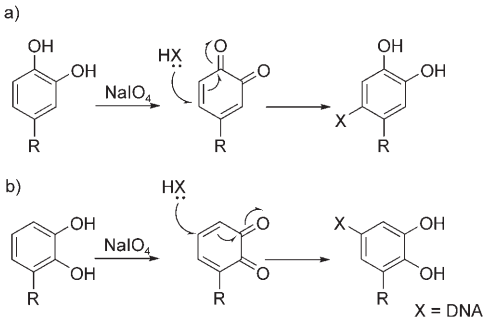


Figure 3. Concentration dependence of compounds **1–3** for DNA cross-linking ($NaIO_4$ oxidation). The concentration of $NaIO_4$ was fixed as 200 μM. lane 4, 8, 12 are the control lane without the $NaIO_4$ oxidation. Lane 13 is the control lane only with $NaIO_4$ and without the compounds. lane 1, 0.7 μg pBR322 + 1 μM **2** + $NaIO_4$; lane 2, 0.7 μg pBR322 + 20 μM **2** + $NaIO_4$; lane 3, 0.7 μg pBR322 + 50 μM **2** + $NaIO_4$; lane 4, 0.7 μg pBR322 + 50 μM **2**; lane 5, 0.7 μg pBR322 + 1 μM **1** + $NaIO_4$; lane 6, 0.7 μg pBR322 + 20 μM **1** + $NaIO_4$; lane 7, 0.7 μg pBR322 + 50 μM **1** + $NaIO_4$; lane 8, 0.7 μg pBR322 + 50 μM **1**; lane 9, 0.7 μg pBR322 + 1 μM **3** + $NaIO_4$; lane 10, 0.7 μg pBR322 + 20 μM **3** + $NaIO_4$; lane 11, 0.7 μg pBR322 + 50 μM **3** + $NaIO_4$; lane 12, 0.7 μg pBR322 + 50 μM **3**; lane 13, 0.7 μg pBR322 + $NaIO_4$; lane 14, 0.7 μg pBR322 (control); Marker lane, 1.5 μg lambda DNA/HindIII (molecular weight standard).

No visible cross-linking band in lane 1–3 (Figure 3) shows that the cross-linking ability of compound **2** has disappeared under the oxidation by $NaIO_4$. We proposed that the position of the dihydroxy groups plays an important role in its different cross-linking ability in two ways. In the mechanism reported by Kodadek,[7,8] the oxidized product of catechol **a** or **b** has only one main position for the nucleophilic reaction. The mechanism of nucleophilic addition is similar with the addition reactions of unsaturated aldehydes or ketones (Scheme 1). The product formation step for substrate **a** might be more disturbed by the steric hindrance effect than for substrate **b**. For compound **2**, the steric hindrance might prevent the electron deficiency region of the quinone to be the reaction position with DNA.



Scheme 1. Proposed mechanism of oxidative addition reaction between DNA and catechol.

The different stability of these kinds of substrates to oxidation might be another effective influence factor in the different cross-linking results. Previous research in the catechols analogues reported that the diphenol such as compound **2** with a 3,4-dihydroxy substitution in the benzene ring is more sensitive to the oxidation than a 2,3-dihydroxy substitution such as in compound **3**.[13] The unstable property of compound **2** might induces more side reactions and decreases its cross-linking capacity.

The oxidative reactions were monitored by UV/Vis spectroscopy by following the formation of quinone intermediate.[14] As shown in Figure 4, the increment of an absorption band at 380 nm and opposite change of the band at 280 nm reflected the consumption of catechol units and the subsequent oxidation to *ortho*-quinones. The difference between the titration experiments of compound **2** and **3** was the appearance of one additional band around 310 nm within the oxidative process of compound **2** (Figure 4b). This new band indicated the generation of side products. This could also explain the assumption that the instability of **2** might decrease the cross-linking yield, especially under the intense oxidative condition.

Obviously, interstrand cross-linking is the most efficacious modification for thoroughly blocking strand opening, and has the potential for application in tumor chemotherapy. To identify the cross-linking mode, we used linearized pUC 19 together with pBR322 in denaturing alkaline agarose gel

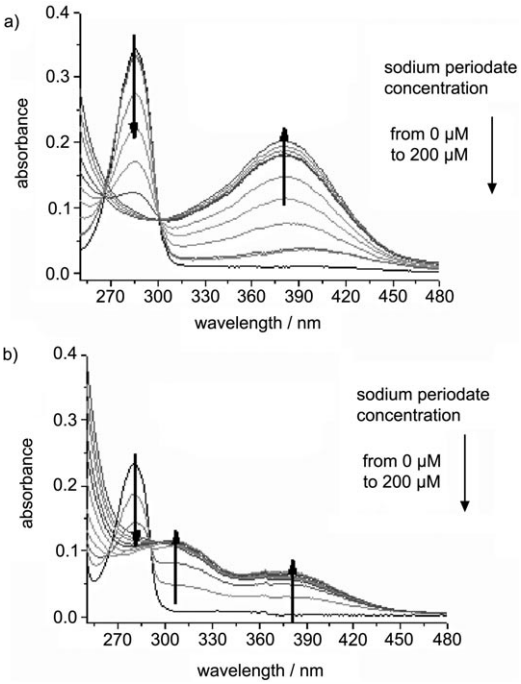


Figure 4. UV/Vis spectra obtained from titration of compound **2**, **3** with $NaIO_4$ solution in phosphate buffer (pH 7.7). Arrows indicate trends in absorbance with the addition of $NaIO_4$ solution; the oxidation of compound a) **3** and b) **2**.

electrophoresis (Figure 5, Figures S1, S2 in Supporting Information). The absence of a band for interhelix DNA cross-linking clearly demonstrated that interstrand cross-linking was definitely the main type of DNA cross-linking. The lack of the band near the one stands for single strand plasmid also indicated that intrastrand cross-linking rarely occurred.[15]

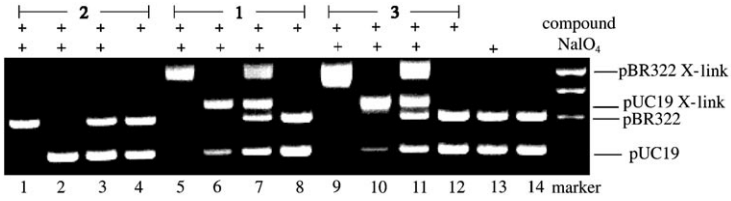


Figure 5. Denaturing alkaline agarose gel for linearized plasmid DNA pBR322 and pUC19 by compounds **1**, **2**, **3** under $NaIO_4$ oxidation. The concentration of $NaIO_4$ was fixed as 200 μM. lane 4, 8, 12 are the control lane without the $NaIO_4$ oxidation. Lane 13 is the control lane only with $NaIO_4$ and without the compounds. lane 1, 0.7 μg pBR322 + 50 μM **2** + $NaIO_4$; lane 2, 0.7 μg pUC19 + 50 μM **2** + $NaIO_4$; lane 3, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **2** + $NaIO_4$; lane 4, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **2**; lane 5, 0.7 μg pBR322 + 50 μM **1** + $NaIO_4$; lane 6, 0.7 μg pUC19 + 50 μM **1** + $NaIO_4$; lane 7, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **1** + $NaIO_4$; lane 8, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **1**; lane 9, 0.7 μg pBR322 + 50 μM **3** + $NaIO_4$; lane 10, 0.7 μg pUC19 + 50 μM **3** + $NaIO_4$; lane 11, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **3** + $NaIO_4$; lane 12, 0.7 μg pBR322 + 0.7 μg pUC19 + 50 μM **3**; lane 13, 0.7 μg pBR322 + 0.7 μg pUC19 + $NaIO_4$; lane 14, 0.7 μg pBR322 + 0.7 μg pUC19 (control); marker lane, 1.5 μg lambda DNA/HindIII (molecular weight standard).

To further investigate the DNA cross-linking capabilities of these agents, plasmid DNA was substituted with oligonucleotide OD1 (Figure 6, Figures S3, S4 in the Supporting Information). After annealing, the non-cross-linked duplex was mixed with excess bis(catechol) derivatives. Then, a $NaIO_4$ or tyrosinase solution was added in excess. The reaction mixtures were analyzed by 20% denaturing PAGE, with the 39-mer oligonucleotide serving as the marker. The similar position of the 39-mer oligonucleotide and the high-molecular-weight products generated by three compounds indicated the formation of cross-linked products. The intensity order of interstrand cross-linking using oligonucletide by different oxidative methods was consistent with the results using linearized plasmid DNA. This observation confirm our assumption that tyrosinase attacked compound **2** with dihydroxy groups in *meta*- and *para*-positions, but under $NaIO_4$ oxidation conditions, compound **3** the electron-rich region of which has less steric hindrance acts as a more efficient cross-linking agent. We are currently further investigating this effect as well as the structures generated.

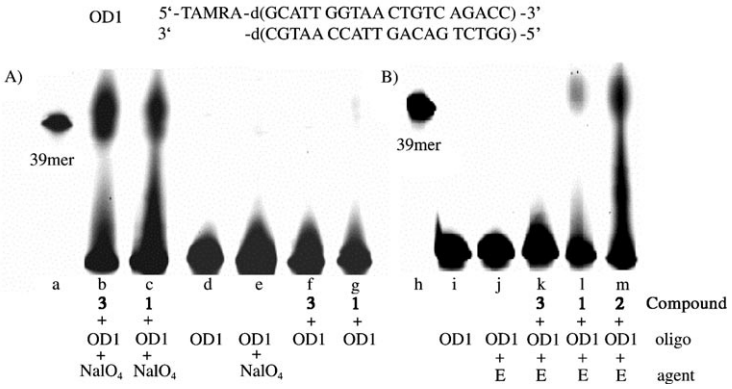


Figure 6. Cross-linking results of compound **1**, **2** and **3** with duplex OD1 under oxidation. (A) $NaIO_4$ as oxidative agent. The concentration of $NaIO_4$ was fixed as 50 mM. lane b, compound **3** (4 mM) + OD1 (4 μM) + $NaIO_4$ (50 mM) (cross-linking yield 9.3%); lane c, compound 1 (4 mM) + OD1 (4 μM) + $NaIO_4$ (50 mM) (cross-linking yield 7.2%) (B) tyrosinase as oxidative oxidation, E = tyrosinase. The concentration of tyrosinase. was fixed as 28 μM. lane k, compound **3** (10 mM) + OD1 (4 μM) + tyrosinase (28 μM); lane l, compound **1** (10 mM) + OD1 (4 μM) + tyrosinase (28 μM) (cross-linking yield 8.7%); lane m, compound **2** (1.25 mM) + OD1 (4 μM) + tyrosinase (28 μM) (cross-linking yield 16.6%).

In addition we calculated the structure of the three compounds on the basis of DFT geometry optimization to understand the binding properties of these compounds with DNA. From the energy-minimized structure, we found that the linker chains spread in the greatest degree of relaxation. In this state, the positive charges in the chains might be exposed in maximum extent. Thus the static interaction between the compounds and DNA could immobilize the complexes tight which allows an easy quinone nucleophilic attack to DNA after the binding and oxidation process.

In conclusion, a series of bis(catechol) compounds was designed and synthesized, and their abilities to cross-link plas-

mid DNA and oligonucleotide were investigated by $NaIO_4$ and enzyme oxidative inducement. The site at which the compounds reacted with oligonucleotide and their potency provide potential avenues of research or the development of new anticancer drugs.

Experimental Section

Full experimental details for the preparation of the compounds, experimental procedures for the DNA cross-linking experiments, UV/Vis spectra analysis, and calculation experiments are included as Supporting Information.

Acknowledgements

X.Z. thanks the NSFC (National Science Fund of China (No. 20272046, 20672084), National Science Fund for Distinguished Young Scholars (No. 20425206), the Cultivation Fund of the Key Scientific and Technical Innovation Project, the Ministry of Education of China (No. 706040) and the State Key Laboratory of Natural and Biomimetic Drugs and State Key Laboratory of Applied Organic Chemistry.

Keywords: catechol · DNA damage · enzymes · oxidation · quinones

[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] M. Gniazdowski, C. Cera, *Chem. Rev.* **1996**, *96*, 619–634.
[3] a) S. Park, C. Anderson, R. Loeber, M. Seetharaman, R. Jones, N. Tretyakova, *J. Am. Chem. Soc.* **2005**, *127*, 14355–14365; b) I. S. Hong, H. Ding, M. M. Greenberg, *J. Am. Chem. Soc.* **2006**, *128*, 485–491; c) I. S. Hong, H. Ding, M. M. Greenberg, *J. Am. Chem. Soc.* **2006**, *128*, 2230–2231.
[4] a) H. Wang, M. S. Wahi, S. E. Rokita, *Angew. Chem.* **2008**, *120*, 1311–1313; *Angew. Chem. Int. Ed.* **2008**, *47*, 1291–1293; b) F. Doria, S. N. Richter, M. Nadai, S. Colloredo-Mels, M. Mella, M. Palumbo, M. Freccero, *J. Med. Chem.* **2007**, *50*, 6570–6579; 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] 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.
[6] X. C. Weng, L. G. Ren, L. W. Weng, J. Huang, S. G. Zhu, X. Zhou, L. H. Weng, *Angew. Chem.* **2007**, *119*, 8166–8169; *Angew. Chem. Int. Ed.* **2007**, *46*, 8020–8023.
[7] B. Liu, L. Burdine, T. Kodadek, *J. Am. Chem. Soc.* **2006**, *128*, 15228–15235.
[8] H. S. Lim, D. Cai, C. T. Archer, T. Kodadek, *J. Am. Chem. Soc.* **2007**, *129*, 12936–12937.
[9] a) H. Decker, T. Schweikardt, F. Tuczek, *Angew. Chem.* **2006**, *118*, 4658–4663; *Angew. Chem. Int. Ed.* **2006**, *45*, 4546–4550; b) M. Garaud, D. Pei, *J. Am. Chem. Soc.* **2007**, *129*, 5366–5367.
[10] a) L. M. Mirica, M. Vance, D. J. Rudd, B. Hedman, K. O. Hodgson, E. I. Solomon, T. D. P. Stack, *Science* **2005**, *308*, 1890–1892; b) E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev.* **1996**, *96*, 2563–2605.
[11] a) T. R. Cech, *Biochemistry* **1981**, *20*, 1431–1437; b) J. J. Tepe, R. M. Williams, *J. Am. Chem. Soc.* **1999**, *121*, 2951–2955.
[12] P. A. Suci, G. G. Geesey, *J. Colloid Interface Sci.* **2000**, *230*, 340–348.
[13] P. J. Patel, W. S. Messer, R. A. Hudson, *J. Med. Chem.* **1993**, *36*, 1893–1901.
[14] a) M. G. Gichinga, S. Striegler, *J. Am. Chem. Soc.* **2008**, *130*, 5150–5156; b) J. Munoz, F. Garcia-Molina, R. Varon, J. N. Rodriguez-Lopez, P. A. Garcia-Ruiz, F. Garcia-Canovas, J. Tudela, *J. Agric. Food Chem.* **2007**, *55*, 920–928.
[15] Y. Song, P. Wang, J. J. Wu, X. Zhou, X. L. Zhang, L. H. Weng, X. P. Cao, F. Liang, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1660–1664.

Received: April 24, 2008
Published online: May 19, 2008

