# Tandem Quinone Methide Generation for Cross-Linking DNA

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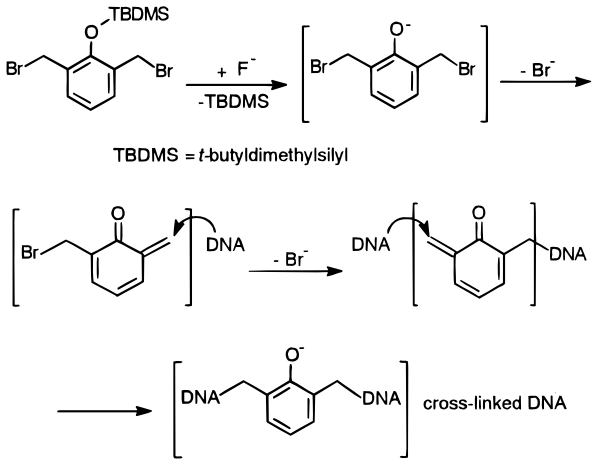
Alkylation and cross-linking provide an important method for determining the structure and function of nucleic acids as well as an effective mechanism for anticancer antibiotics. The ultimate utility of such reactions is governed in part by our ability to promote their specific and beneficial activity while limiting their nonspecific and undesirable side reactions. This paper describes the use of a readily prepared reagent for cross-linking DNA that is controlled through an inducible process initiated by fluoride ion and not subject to spontaneous reaction. Its mechanism of action is consistent with transient and sequential formation of two quinone methide intermediates. Equivalent intermediates have been previously implicated in a range of biologically significant processes including antibiotic activation,[1] xenobiotic metabolism,[2] enzyme inhibition,[3] and sclerotization of arthropod cuticle.[4] Quinone methides have also been used in organic synthesis and have recently been generated under aqueous conditions by irradiating (254 nm) solutions of hydroxy-substituted benzyl alcohols.[5] The simplicity and hence versatility of the parent derivative described here and the mild conditions necessary for its transformation should support a wide range of future investigations.

Our laboratory has previously developed a series of site-directed reagents that were designed to alkylate selected sequences of DNA via quinone methides generated by irradiation (>335 nm), reduction, fluoride, and target-probe association.[6] Further modification of the target might likely have been accomplished by (i) attaching a second reactive equivalent to the site-directing component as illustrated with N-mustards[7] or (ii) simply coupling the two equivalents directly together as illustrated with CC-1065[8] and tomaymycin.[9] Alternatively, a derivative designed to yield a single quinone methide could be elaborated for a series of tandem reactions as reported here for *O*-(*tert*-butyldimethylsilyl)-2,6-bis(bromomethyl)phenol (**1**). The intrinsic specificity of this reagent is now presented to aid subsequent construction of new site-directed reagents.




Incubation of **1**[10] in the presence of duplex oligodeoxynucleotides **OD1**/**OD2** and **OD1**/**OD3** produced high molecular weight species with electrophoretic mobilities that are expected for cross-linking (Figure 1).[6] Reaction was induced only by addition of a fluoride source (in this example, 10 mM KF, lanes 4 and 5). Inclusion of other salts such as NaCl neither promoted (lanes 1 and 8) nor inhibited reaction (lanes 2 vs 3 and 6 vs 7). Interestingly, the duplex **OD1**/**OD3** containing an extended unpaired sequence provided a greater yield of cross-linked product (6.2%, lane 5)[10] than **OD1**/**OD2** (2.2%, lane 4). Yields also increased slightly when the concentration of fluoride was raised from 10 to 200 mM (7.5%, lane 6, and 3.0%, lane 3) and are within the range of yields reported for cross-linking by mitomycin.[11]

The cross-linked products were isolated by electrophoresis and subjected to various treatments to induce strand scission at the site of alkylation. The linkage between 5′-[$^{32}$P]**OD3**/**OD1** was not stable to heat (90 °C) or alkaline conditions (0.2 M piperidine, 90 °C) and primarily formed a radiolabeled species that comigrated with the initial sequence 5′-[$^{32}$P]**OD3**. Strand fragmentation of the cross-link was also observed and is represented here by a densitometric trace of its electrophoretic separation (Figure 2A). DNA cross-links formed by both mitomycin[12] and CC-1065[13] are similarly reversible and, in the case of CC-1065, generate strand fragmentation at sites of cross-linking as well.[14] The major site of cross-

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(10) A detailed description of the synthesis, characterization and application of **1**−**3** is included in the Supporting Information. Product yields were determined by excising the cross-linked product and starting material from the gel and measuring their radioactivity by scintillation counting. The yields reported were calculated relative to the total material recovered.

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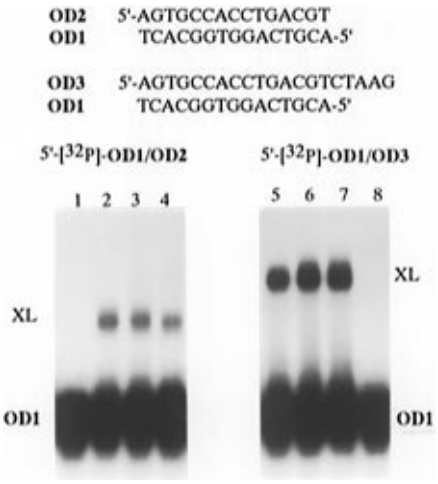


**Figure 1.** Audioradiograms of denaturing polyacrylamide (20%, 7 M urea) gels used to monitor cross-linking of duplex DNA (XL). 5'-[$^{32}$P]-**OD1**/**OD2** (3 $\mu$M, 17 nCi) (lanes 1−4) and 5′-[$^{32}$P]-**OD1**/**OD3** (3 $\mu$M, 17 nCi) (lanes 5−8) were incubated for 24 h (20 °C) in buffer 2-(*N*-morpholino)ethansulfonate (MES, 2 mM pH 7), 30% acetonitrile, and **1** (450 $\mu$M). Reaction was alternatively initiated by addition of 1 M NaCl (lanes 1 and 8), 800 mM NaCl and 200 mM KF (lanes 2 and 7), 200 mM KF (lanes 3 and 6), and 10 mM KF (lanes 4 and 5). Samples were then dialyzed, dried under high vacuum, and analyzed by electrophoresis.

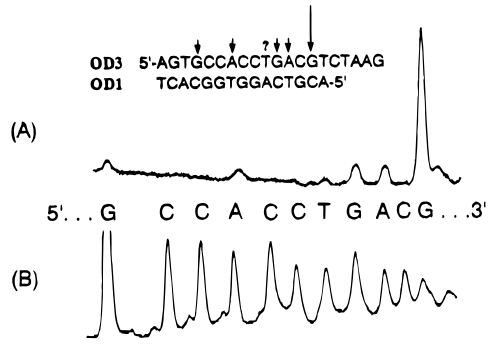


**Figure 2.** Densitometric scans representing diagnostic fragmentation of cross-linked DNA. (A) The high molecular weight product formed by **1**, **OD1**, and 5′-[$^{32}$P]-**OD3** was isolated after gel electrophoresis, incubated with piperidine (0.2 M, 90 °C, 30 min), and analyzed by denaturing gel electrophoresis (20%). (B) A standard set of all possible fragments was generated by hydroxyl radical footprinting.[17]

linking in the duplex region of **OD3** occurred at G14 (69%), and minor sites included G11 (10%), A12 (9%), G4 (5%), A7 (4%), and even perhaps T10 (3%). The selectivity for 5′...CG...3′ is analogous to that of mitomycin and a series of related pyrroles and may reflect an optimum geometry or environment for reaction.[11]

Since benzyl bromide derivatives often exhibit significant intrinsic reactivity, direct substitution and cross-linking via an associative ($S_N2$) pathway was initially considered as a reasonable alternative to the dissociative mechanism involving a quinone methide. However, this alternative was not consistent with the structural requirements for the cross-linking agent. An associative pathway would have been equally probable for derivatives containing substituents such as *o*-hydroxy, methoxy, or siloxy groups that have comparable inductive effects.[15]

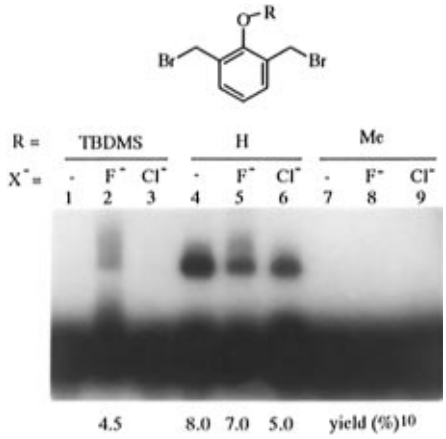


**Figure 3.** Autoradiogram of a denaturing polyacrylamide gel (20%, 7 M urea) used to compare DNA cross-linking. The duplex formed by **OD1** and 5′-[$^{32}$P]-**OD3** (3 $\mu$M, 6 nCi) was incubated for 24 h (20 °C) in 3-(*N*-morpholino)propanesulfonate (MOPS, 10 mM, pH 7.5), 30% acetonitrile, and 450 $\mu$M of the phenolic derivatives. NaCl and KF (200 mM) were added as indicated.

Neither the siloxy nor methoxy derivatives (**1** and *O*-methyl-2,6-bis(bromomethyl)phenol **3**, respectively) produced detectable cross-linking in the absence of fluoride (Figure 3, lanes 1, 3, 7, and 9), and only the siloxy derivative expressed activity after its conversion to the hydroxyl derivative upon fluoride addition (lanes 2 vs 8).[16] The methoxy derivative remained inert in the presence of fluoride. Similarly, chloride did not mimic the activity of fluoride (lane 3), thus suggesting that lysis of the O−Si bond initiated DNA modification. Finally, the hydroxyl derivative **2** reacted spontaneously and independently of fluoride (lanes 4−6) as expected for a species capable of deprotonation and quinone methide formation.[16]

The ability of **1** to act as an inducible cross-linking agent demonstrates the facility by which a quinone methide precursor can be elaborated for tandem and multiple application in biological chemistry. The geometry and reactivity of the leaving groups may now be adapted for alternative targets, and the *O*-substituent may be designed to induce reaction upon exposure to a wide range of selected conditions including heat, light, pH, or enzymes such as hydrolyases and hydroxylases.

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**Supporting Information Available:** Preparation and characterization of **1**−**3**; DNA modification and analysis (4 pages).

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(16) The mixed water−acetonitrile medium appeared by ultraviolet spectroscopy to dissolve the methoxy derivative completely, thus suggesting that its inability to modify DNA was not due to a potential problem in solubility. The expected pH dependence for reaction of the hydroxyl derivative has been confirmed with a model system (manuscript in preparation).

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