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Trapping a Labile Adduct Formed between an *ortho*-Quinone Methide and 2′-Deoxycytidine

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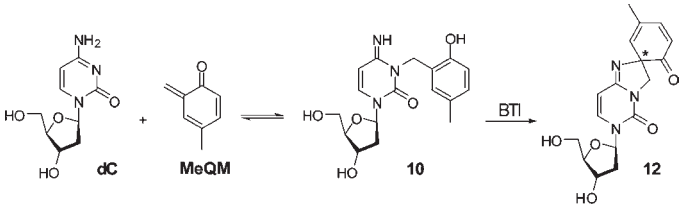
ABSTRACT



Selective oxidation by bis[(trifluoroacetoxy)iodo]benzene (BTI) provides an effective trap for quenching adducts formed reversibly between dC and an *ortho*-quinone methide (QM) under physiological conditions. A model adduct generated by 4-methyl-*o*-QM and 2′-deoxycytidine is rapidly converted by intramolecular cyclization and loss of aromaticity to a characteristic product for quantifying QM alkylation. However, BTI induces a surprising rearrangement driven by overoxidation of a derivative lacking an alkyl substituent at the 4-position of the QM.

The reversibility of DNA alkylation by certain quinone methide intermediates (QM) is advantageous for the design of cross-linking reagents,[1] target-promoted reactions,[2,3] covalent combinatorial systems,[4,5] and protocols for chiral resolution.[6] Conversely, such reversibility is detrimental to the detection of QM adducts formed in DNA since only the most stable derivatives may persist through standard enzymatic digestion and chromatographic separation.[7−9] Identifying the full profile and yield of QM capture by DNA is a prerequisite for understanding the genotoxicity of QMs generated by metabolism of food additives, natural products, and synthetic drugs.[10−14] Satisfactory analysis awaits development of a method to suppress QM release

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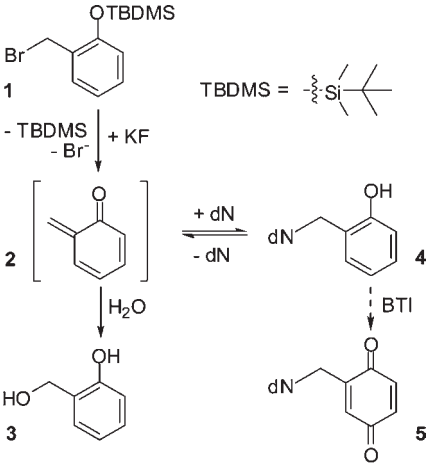
from its most labile adducts. Model studies based on deoxynucleosides have previously indicated that strong nitrogen nucleophiles of DNA react with the parent *ortho*-QM most efficiently, but reversibly.[9,15] Lifetimes of these adducts (>2 h) are sufficient to elicit a variety of biological responses although not adequate for easy detection. Reaction with weak nitrogen nucleophiles of DNA is significantly less efficient but irreversible and amenable to routine detection.[7,8]

Most techniques for quantifying alkylation are predicated on the formation of stable adducts. For example, polymerase stop assays have detected reaction of tamoxifen metabolites with the weakly nucleophilic *exo*-amino group of dG.[16] However, no comparable information is available on possible adduct formation with the more nucleophilic N7 of dG. Labile adducts generated by other potential carcinogens can often be stabilized for detection by an acid or alkaline quench prior to enzymatic analysis,[17] but neither treatment is appropriate for QM adducts.[15,18,19] Alternatively, mass spectrometry offers an excellent method for simultaneously observing a range of DNA products,[20] yet certain QM adducts and benzyl substituted phenols in general (e.g., **1**, below) often lack sufficient stability for detecting their parent ions. Still, adducts formed between a QM-like intermediate derived from the natural product lucidin and the weakly nucleophilic *exo*-amino groups of dA and dG have been observed by electrospray mass spectrometry, but again no equivalent data are available on potential reaction with the strongly nucleophilic sites of dG N7 and dA N1.[21]

Acylation, alkylation, silylation, or reduction of the resulting phenolic product of QM alkylation was not considered for quenching the reversible reaction since these approaches are not likely to exhibit sufficient selectivity for this product when studies involve genomic DNA. In contrast, oxidative dearomatization has the potential to target the phenolic products uniquely. Singlet oxygen is often used for such dearomatization but is not suitable for this application based on its oxidation of guanine.[22,23] Potassium nitrosodisulfonate (Fremy's salt) was very selective for oxidation of the QM adducts, but the reaction could not be driven to completion, a necessary criterion for quantifying the adducts formed.[24b] Hypervalent iodine reagents in general and bis[(trifluoroacetoxy)iodo]benzene (BTI) in particular effect equivalent dearomatization and function well under aqueous conditions.[25−27] Most importantly, a control study indicated that BTI did not react with deoxynucleotides under the conditions described below.[28]

Scheme 1. Quinone Methide Generation, Reversible Alkylation, and Trapping



The goal of this study was to test whether oxidation of an *o*-QM-deoxynucleoside (QM-dN) adduct forms a stable and identifiable compound under physiological conditions. 2′-Deoxycytidine (dC) was chosen as the initial deoxynucleoside of interest since it forms only a single QM adduct (Scheme 1).[29] Similarly, 6-methylene-cyclohexa-2,4-dienone (**2**) was chosen as the first model QM since its dC adduct exhibits only modest reversibility for challenging the oxidative trapping by BTI. In the absence of such a trap, the dC adduct decomposes over days by releasing the QM for irreversible addition by water.[9]

o-(*tert*-Butyldimethylsilyl)-2-(bromomethyl)phenol (**1**) was prepared as the source of QM **2** according to a literature procedure,[9,29] and the dC adduct **6** was generated *in situ* under standard conditions (37 °C).[29] After 20 min, a 4-fold excess of BTI in acetonitrile was added and the mixture was cooled to room temperature. Reversed-phase chromatography indicated complete consumption of the dC adduct and formation of a new compound (**8**) within 20 min.[28] This product was isolated under equivalent conditions and remained stable (90%) over 6 days in aqueous acetonitrile. Initial characterization by ^{1}H and ^{13}C NMR spectroscopy indicated that the expected product (**7**, Scheme 2) had not formed based on the lack of $^{1}H-^{1}H$ coupling anticipated for the benzoquinone protons and the absence of signals for two quinone carbons.

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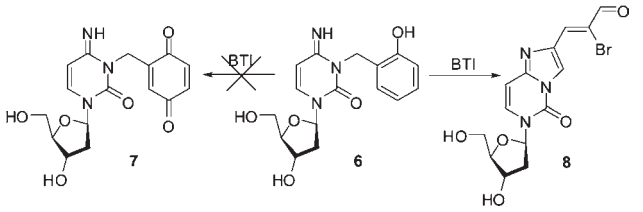
Scheme 2. Extensive Oxidation of an Unsubstituted Quinone Methide Adduct



Loss of the two carbons was confirmed by electrospray mass spectrometry (ESI-MS), and surprisingly the parent ion revealed the presence of one bromine by its distinct isotope ratio.[28] MS/MS experiments generated the characteristic deglycosylation and debromination products to support the structural assignment based on extensive NMR data. Signals (^{1}H and ^{13}C) based on literature values[30] for both the pyrimidine and ribose groups were observed, and their assignments were confirmed by ^{1}H−^{13}C HSQC and ^{1}H−^{13}C HMBC analysis.[28]

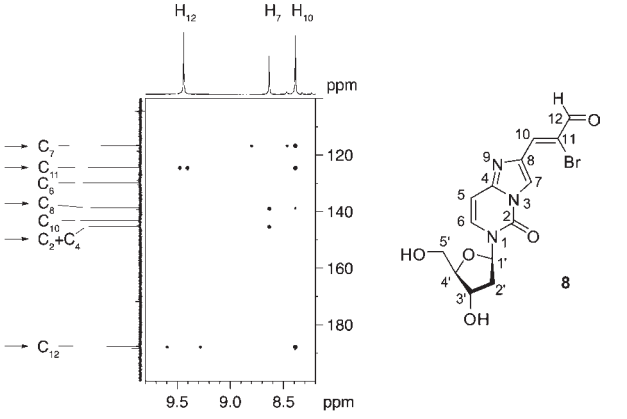
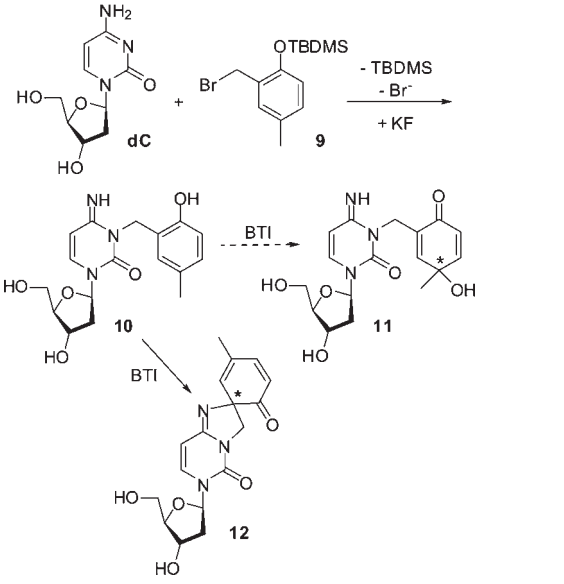


Figure 1. ^{1}H−^{13}C HMBC of **8** in DMSO-d_6 at 600 MHz.

The unsaturated nature of the *o*-QM remnant was apparent from the remaining ^{13}C signals that all ranged between 116.6 and 187.6 ppm (Figure 1). The large 2-bond coupling ($^2J_{CH}$ = 43 Hz) observed for the cross peak between C11 and H12 in the HMBC spectrum is unique to aldehydes (Figure 1).[31,32] Connectivities between C7 through C12 were established by a combination of ^{1}H−^{13}C HSQC, ^{1}H−^{13}C HMBC, and ^{1}H−^{15}N HMBC analysis.[28] Finally, the bromine was placed on C11 to satisfy its valence and lack of attached proton. The entire conjugated system is illustrated in a favorable thermodynamic configuration although the stereochemistry has not yet been confirmed experimentally. A mechanism by which this product forms and incorporates the bromide released from the QM precursor **1** is far from obvious. Hence, its use for quantifying QM alkylation is dubious.

Overoxidation and rearrangement of the dC adduct was subsequently prevented by use of an alternative QM that generated products containing an alkyl group *para* to the phenolic −OH.[25,26] The necessary precursor 2-bromomethyl-4-methyl-*O*-(*tert*-butyldimethylsilyl)phenol (**9**) was prepared from 5-methylsalicylaldehyde using standard procedures.[28] The presence of the methyl substituent was not expected to alter the profile of DNA adducts formed by 4-methyl-6-methylene-cyclohexa-2,4-dienone (MeQM) after deprotection of **9** since a related set of substituents had not significantly altered the profiles of equivalent QM adducts.[24] However, the electron-donating properties of the methyl group stabilize the electron-deficient QM intermediate, leading to faster generation and increased lability of the resulting adducts formed by MeQM vs those of QM (**2**).[24] Thus, the methyl-substituted model may block overoxidation but poses a greater challenge for the trapping system due to the increased reversibility of the MeQM adduct.

Scheme 3. Oxidative Trapping of a Methyl-Substituted Quinone Methide Adduct



The MeQM-dC adduct (**10**) was prepared *in situ* by deprotection of **9** with aqueous KF in the presence of dC under conditions similar to those used to prepare the dC adduct **6** (Scheme 3). The structure of **10** was confirmed by ^{1}H NMR and UV−vis data based on previous analysis of the parent adduct **6** (Scheme 2).[29] Most diagnostic are the benzylic protons that vary by less than 0.1 ppm (4.96 ppm for **6**,[29] 4.89 ppm for **10**) and the λ_{max} values that vary by only 1 nm (278 nm for **6**,[29] 279 nm for **10**). The absorption data

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are also most similar to those of N3-ethyl dC (λ_{max} = 280 nm) rather than those of N^4-ethyl dC (λ_{max} = 270 nm).[33]

Treatment of the alkylation mixture above with 4 equiv of BTI transformed the dC adduct **10** into a new compound within 20 min as detected by reversed phase chromatography.[28] This compound also persisted unchanged (>85%, 24 h) in solution (9 mM ammonium formate pH 6.8, 12% CH_3CN) for convenient analysis and future application with DNA. Initial characterization by ^{1}H NMR immediately confirmed the ability of the *para*-methyl substituent to block the overoxidation that had plagued the reaction of its unsubstituted parent **6**. Coupling between adjacent vinyl protons was now evident for the oxidized product of **10**, and none of its ^{1}H signals were observed downfield of 8 ppm.[28] ESI-MS confirmed that oxidative trapping of adduct **10** did not cause loss of carbon atoms. Instead, the $(M + H)^+$ of m/z 346.18 provided the first suggestion that the oxidized product **12** (calculated m/z 346.14 $(M + H)^+$) had formed rather than the alternative product **11** (calculated m/z 364.15 $(M + H)^+$) (Scheme 3).

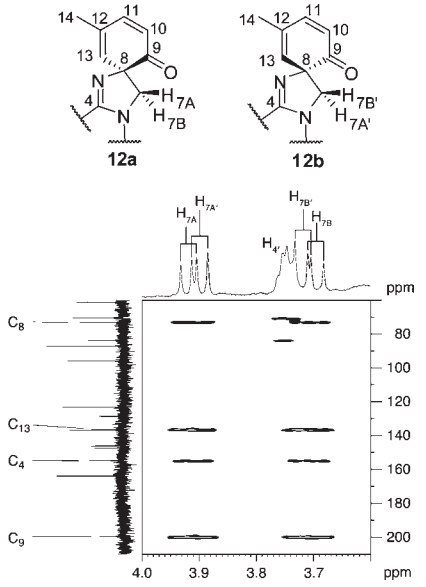

Figure 2. ^{1}H−^{13}C HMBC of **12** in DMSO-d_6 at 400 MHz.

NMR signals (^{1}H and ^{13}C) corresponding to the pyrimidine and ribose moieties of **12** were again assigned from literature values[30] and confirmed by ^{1}H−^{13}C HSQC and ^{1}H−^{13}C HMBC spectra.[28] These spectra also provided the necessary data to establish the connectivities for the oxidized QM adduct. For example, C8 was identified by its proximity to the protons of C7, and the ^{13}C chemical shift of C8 (73.1 ppm) was most consistent with the sp^3 hybridization of the spiro carbon (Figure 2). This chemical shift is quite distinct from that predicted for the corresponding sp^2 carbon in **11** (ca. 133 ppm) based on the model compound 4-hydroxy-2,4-dimethyl-2,5-cyclohexadien-1-one (**13**).[22] Additionally, a correlation between the *para*-methyl protons (H14) and the adjacent vinyl proton (H13) was observed in a 2D-COSY experiment as expected for **12**. Such a correlation would not be likely for **11**[28] and was not previously detected for the model **13**.[22] Finally, restricted rotation of the former benzylic carbon (C7) is apparent from the diastereotopic relationship of the attached protons (H7A and H7B) and their proximity to the carbonyl oxygen of C9 that alternatively extends in front or back of C8. This configuration creates a pair of doublets in the ^{1}H NMR spectrum, but these signals are further complicated by the diastereomeric mixture of **12** (**12a** and **12b**) formed by oxidation of the dC adduct by BTI (Figure 2).

The general ability of BTI to promote intramolecular cyclization and dearomatization was reported earlier and has since been integrated into a number of synthetic strategies.[27,34,35] This process obviously dominates the oxidation of **10** and may even begin to explain the origins of the imidazole ring in the overoxidized product **8**. In both cases, intramolecular addition of the *exo*-imine to a position *ortho* to the phenolic oxygen is favored over the intermolecular addition of water at the *para*-position. At least for 4-alkyl QMs such as that formed by the precursor **9**, BTI is an effective quench for preventing the reversible reactions of QM. Oxidation proceeds rapidly under physiological conditions and is selective for the phenolic product formed by QM alkylation of dC. Use of this reagent should provide an opportunity to study the intrinsic susceptibility of dC toward alkylation in DNA without worry of spontaneous QM release from its adducts or adventitious oxidation of the parent nucleosides. This approach of oxidative quenching should also be applicable to adducts of the other deoxynucleosides in DNA and ultimately should allow for quantifying the kinetic products of reaction between QMs and genomic DNA for the first time.

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Supporting Information Available. Synthesis and characterization of all compounds including 2D NMR and MS spectra of **8**, **10**, and **12**; HPLC chromatograms of dC adducts and their oxidized derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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