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Formation of Tricyclic [4.3.3.0] Adducts between 8-Oxoguanosine and Tyrosine under Conditions of Oxidative DNA–Protein Cross-Linking

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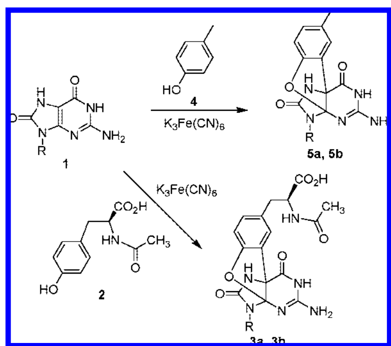
The human genome undergoes thousands of chemical modifications per day, many of which result in a loss of fidelity, compromising the integrity of the genetic blue print.[1] The intimate interaction between DNA and myriad proteins results in DNA–protein cross-links (DPCs) during some of the oxidative damage events.[2] Oxidation of either nucleic acids or proteins generates electrophilic species, which can then be trapped by a nucleophilic group of the partner biopolymer, leading to covalent bond formation between DNA and the bound protein. This class of DNA lesions remains among the least well characterized.

Several groups have explored DPCs by employing either model reaction systems or by investigating fragments obtained from DNA and protein digestion. For example, a covalent adduct formed between C8 of guanine and the ϵ-amino group of lysine during guanosine (dGuo) oxidation has been thoroughly characterized.[3,4] Recently, our laboratory showed that the roles of nucleophilic and electrophilic partners could be reversed, for example, lysine could be oxidized to an ϵ-aminium radical that attacks the guanine base at C8, with the final product structure being dependent on the mechanism of oxidation.[5] The competition of reaction mechanisms arises because of the ambiguity of which partner, DNA or protein, is initially oxidized and which serves as the nucleophile to form the covalent adduct.

A similar question arises with the phenol-containing amino acid tyrosine, particularly when interacting with DNA containing the oxidation-sensitive site 8-oxo-7,8-dihydro-2′-deoxyguanosine (dOG) whose redox potential is slightly lower than that of Tyr. Although some information is available on cross-linking of Tyr and Lys with pyrimidines under photochemical[6,7] and oxidative conditions,[8–10] no adducts between Tyr and dGuo or dOG have been well characterized, although their formation was implicated in studies of DPC formation with single-stranded binding protein (SSB) and dOG-containing oligonucleotides,[11] and other examples suggest that phenol radicals add to C8 of dGuo.[12–14] Tyrosine residues play critical roles in certain DNA-processing enzymes including topoisomerases[15] and are prevalent in the DNA-binding region of high copy number proteins such as SSB.[16] We report here an unusual tricyclic adduct as the major product obtained from oxidation of dOG in the presence of Tyr or its analogue *p*-cresol (Scheme 1).

In the experiment, 3 mM dOG (**1**) was allowed to react with 30 mM *N*-acetyltyrosine (**2**) in the presence of 10 mM $K_3Fe(CN)_6$ at 37 °C for 30 min and then quenched by addition of 10 mM ascorbate. HPLC analysis of the reaction mixture showed two new peaks with identical UV–vis spectra (λ_{max} at 194 and 289 nm), assigned as diastereomers **3a** and **3b** on the basis of the analytical data described herein (Scheme 1). To simplify the structural characterization of the tyrosine adducts, *p*-cresol (**4**) was employed as a model compound. HPLC analysis of an analogous reaction conducted with *p*-cresol again showed two peaks that possessed identical UV–vis spectra (λ_{max} at 196 and 292 nm) similar to the above and consistent with the formation of diastereomers **5a** and **5b**. MS and MS/MS analyses of the adducts formed with Tyr and *p*-cresol reactions also indicate the products from these reactions are very similar.

Scheme 1. Adduct Formation between OG and Tyrosine or *p*-Cresol



NMR data were collected on the diasteromeric products from the *p*-cresol reaction with dOG. Three aromatic protons were observed with H3″ and H5″ showing HMBC correlations with the methyl carbon at 21 ppm. The third, H6″, was a doublet and coupled to H5″. Furthermore H3″ showed an HMBC correlation to C5 of the base, clearly supporting the C–C bond between the ortho position of the phenol and C5 of the base. In the HMBC study, both C4 (111 ppm, coupled to H1′) and C5 (61 ppm, coupled to H3″) showed large upfield shifts (~30 ppm), while C8 (157 ppm, coupled to H1′) moved modestly downfield (5 ppm) compared to dOG.[17] To rationalize these large shifts and the MS data the tricyclic structure **5a/5b** is proposed (Figure 1).

In previous studies, oxidation of dOG in the presence of water or lysine led initially to adducts at C5 that underwent either rearrangement to spiroiminodihydantoin (Sp) or decomposition to guanidinohydantoin (Gh) structures.[11,18–20] In contrast, phenols appear to add to both the C4 and C5 positions of dOG during oxidation, initially without rearrangement. To lend further support to this surprising structure, the ^{13}C NMR resonances and relative energies for the aglycons **5a/5b**, as well as other likely isomers

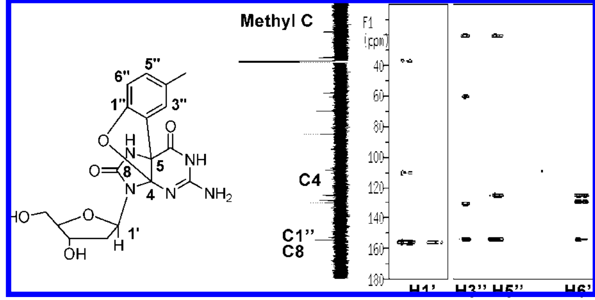


Figure 1. HMBC NMR spectrum of **5a**, **5b**.

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Table 1. Calculated Relative Energies for $C_{13}H_{13}N_5O_3$ Isomers and ^{13}C Chemical Shifts of C4, C5, and C8 for the Corresponding 2′-Deoxyribonucleoside

$C_{13}H_{13}N_5O_3$ Isomer	Relative Energies (kcal/mol)	C4 (ppm)	C5 (ppm)	C8 (ppm)
	11.6	96	191	171
	3.0	188	69	159
	0	119	68	161
Observed:		112	60	156

Scheme 2. Reaction Pathway for *p*-cresol With dOG

with the molecular formula $C_{13}H_{13}N_5O_3$, were calculated using Gaussian 98 with the B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p) method (Table 1).[21,22] The relative energies were obtained at the 6-311+G(2d,p) level and corrected by zero-point vibrational energies from B3LYP/6-31G(d) frequency calculations. The calculated ^{13}C chemical shifts for C-4, C-5, and C-8 in **5a/5b** best fit the experimental values of 112, 60, and 156 ppm, respectively, supporting the assignment as a tricyclo[4.3.3.0] adduct.[23]

The reaction is proposed to be initiated by oxidation of dOG (**1**) to the electrophilic quinoid dOG^{ox} (**6**) which then undergoes nucleophilic attack at C5 by the phenol to form **7**, analogous to the addition of other nucleophiles such as lysine to this position (Scheme 2). In the special case of a phenol, the phenolic oxygen is now well positioned for a second nucleophilic addition to C4, generating **3a/3b** or **5a/5b**. This reaction also parallels the addition of phenol or naphthol to acid-generated cationic species, in which the *o*-carbon reacts with an electrophilic site first, followed by addition of the phenolic oxygen to another electrophilic site.[24]

In aqueous phosphate buffer at pH 7.5, the products **5a/5b** have a half-life of ~20 h, undergoing slow hydration and decomposition to ultimately yield Sp (**10**). However, analogous adducts could be formed in a tetramer d(AGXC) (X = **5a** or **5b**) or an 18-mer (see Supporting Information). Adducts in oligomers were somewhat more stable with ~30% degradation after 24 h incubation at pH 7.5. The predominant degradation process is hydration, yielding compound **9** which could also be characterized by HPLC and MS before slow loss of the phenol to form **10**. Adducts **5a/5b** have longer half-lives at pH 7.0 and 6.0 (~48 and 100 h, respectively) and were stable for weeks in organic solvents such as acetone or DMSO. Therefore, structures such as **5a/5b** could have long lifetimes in the context of a dOG-Tyr cross-link formed in a DNA−protein complex that excludes bulk water.

In summary, we have characterized an unusual tricyclo[4.3.3.0] adduct in oxidative guanine-tyrosine cross-linking. Such "paddlane" structures are uncommon in organic chemistry, and the inherent ring strain may account for the observed slow decomposition of the adduct ultimately to yield the more common spirodihydantoin **10**, a product of 4-e$^-$ oxidation of dGuo or 2-e$^-$ oxidation of dOG. Overall, these explorations of small molecule reactions have important implications in the chemical nature of DNA−protein cross-links derived from oxidative stress.

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Supporting Information Available: Complete ref 21, experimental procedures, HPLC analysis of the reactions of dOG and *N*-AcTyr and *p*-cresol, MS/MS of **3a/3b**, **5a/5b**, and **9**, ^{13}C chemical shifts and NMR spectrum of **5a/5b**, and energies and geometries of calculated structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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