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Copper/H_2O_2-Mediated Oxidation of 2′-Deoxyguanosine in the Presence of 2-Naphthol Leads to the Formation of Two Distinct Isomeric Adducts

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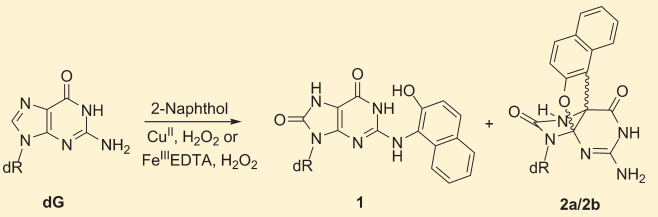
Supporting Information

ABSTRACT: Exposure of cells to phenolic compounds through exogenous and endogenous sources can lead to deleterious effects via nucleobase modifications of DNA occurring under oxidative conditions. 2′-Deoxyguanosine (**dG**) is the most electron rich of the four canonical bases and includes many nucleophilic sites; it is also susceptible to oxidation with numerous reactive oxygen species. In these studies, **dG** was allowed to react with 2-naphthol in the presence of copper or iron salts yielding two principal isomeric products. Spectroscopic analysis and reactions with alkylated nucleosides support the assignment of compound **1a**/**1b** as a pair of atropisomer N^2 adducts and compound **2a**/**2b** as a diastereomeric mixture of tricyclic [4.3.3.0] adducts. Both products are the result of an overall four-electron oxidation process and consequently have the same masses, though drastically different structures, providing mechanistic insight into their formation. Thus, **dG** alkylation by 2-naphthol under oxidative conditions yields products whose structural properties are altered, leading to potentially mutagenic effects in genomic DNA.



INTRODUCTION

Phenols provide two modes of reactivity with the bases of nucleic acids under conditions of oxidation, either as nucleophiles or as radical-generating species. 2′-Deoxyguanosine (**dG**) is the most electron rich of the four canonical nucleosides and provides a reactive heterocyclic platform for coupling to phenols.[1,2] Oxidation of aromatic compounds to either hydroquinones or catechols provides activated phenols that can enter into a stepwise two-electron redox cycle in the presence of a metal catalyst and reductant. Phenolic species known to arylate **dG** include phenoxyl radicals (**PhO**$^{\bullet}$), quinones (**Q**), semiquinone radicals (**SQ**$^{\bullet}$), and quinone methides (**QM**).[2,3] **SQ**$^{\bullet}$ and **Q** electrophiles result from redox-active hydroquinones and catechols that can react with **dG** at N^2, N7 and C8 as exemplified by the products observed from 3,4-catecholic estrones in the presence of **dG** under oxidative conditions (Figure 1).[4−6] In contrast, 4-hydroxyequilenin **SQ**$^{\bullet}$ is proposed to couple initially at the N^2 of **dG**; next, N1 of **dG** acts as a nucleophile to form a new ring between the Watson−Crick face of **dG** and C2 of 4-hydroxyequilenin.[5] 2,3-Catecholic estrones when oxidized diverge from the previous path due to their ability to tautomerize to **QM** that are attacked by N^2 of **dG** yielding stable products.[7] Further studies have shown that **QM**s are predominantly attacked by N^2 of **dG**, but alkylation was also observed at N1 and N7 of **dG**.[8,9] Hydroquinones are oxidized to electrophilic *p*-quinones that are nucleophilically attacked by N^2 of **dG** followed by a second nucleophilic attack by N1 yielding unique tricyclic compounds.[10−13] However, in the case of catechols, they are oxidized to *o*-quinones that undergo conjugate addition by N7 of **dG**; these have all been characterized as the aglycone products.[7,14,15] Lastly, hindered phenols react with C8 of

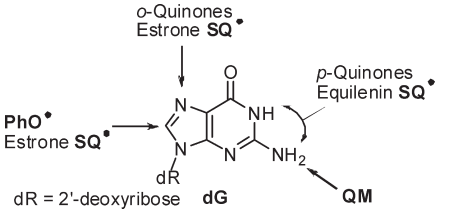


Figure 1. Points of alkylation/arylation on **dG** by electrophilic phenols.

dG under oxidative conditions through their corresponding phenoxyl radicals (Figure 1).[16−18] These studies highlight the fact that phenol electrophiles can specifically arylate many of the possible sites on **dG**. A consequence of **dG** being electron rich is that it has the lowest standard reduction potential (E^0 = 1.29 V vs NHE, pH 7) of the four canonical bases and is prone to oxidation reactions itself as well as alkylation/arylation reactions.[19] Thus, oxidative coupling of purines with phenolic compounds provides diverse products depending on which partner is oxidized first and which serves as the nucleophile or electrophile.

Nucleoside **dG** oxidation with radiolytically formed HO$^{\bullet}$, $SO_4^{\bullet-}$, or photoexcited riboflavin yields many oxidation products.[1,20] Products characterized from chemistry at C5 of **dG** include imidazolone (**Iz**) and its hydrolysis product oxazolone (**Z**), which has been observed in vivo.[21] In the case of **dG** oxidation by Cu^{II}/H_2O_2/reductant,[22] NiCR/$KHSO_5$,[23] Mn-TMPyP/$KHSO_5$,[20] or the epoxidizing agent dimethyldioxirane,[24]

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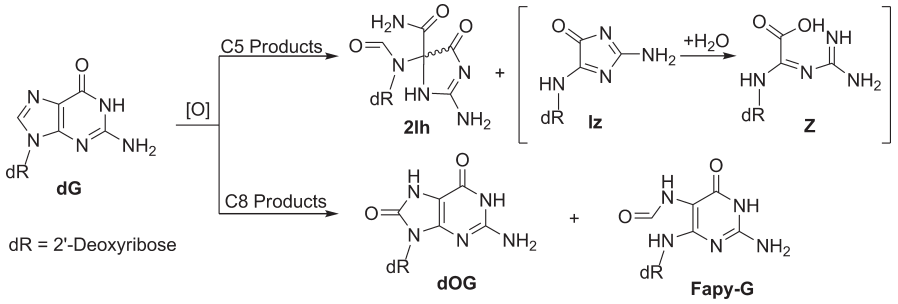
Scheme 1. Products Observed from the Initial Oxidation of dG at C5 or C8

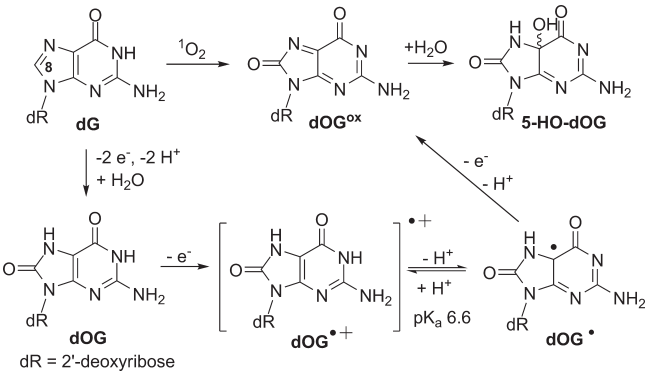


oxidation at C5 was observed to yield 5-carboxamido-5-formamido-2-iminohydantion (**2Ih**, Scheme 1).

Oxidation of **dG** by $HO^{\bullet}$ in DNA yields 8-oxo-7,8-dihydro-2′-deoxyguanosine (**dOG**) as one of the major products under aerobic oxidative conditions (Scheme 1),[25,26] and its in vivo concentration is monitored to determine oxidative stress levels.[27,28] In addition, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**Fapy-G**) is observed upon exposure of **dG** to $HO^{\bullet}$ in the presence of reductants or anaerobic reaction conditions (Scheme 1).[29] A striking feature of **dOG** is its dramatically decreased reduction potential (E^0 = 0.74 V vs NHE, pH 7) relative to **dG**, causing **dOG** oxidation to be much more facile than that of **dG**.[30] The initial one-electron oxidation of **dOG** yields a radical cation/radical pair ($\mathbf{dOG}^{\bullet+}/\mathbf{dOG}^{\bullet}$) with a p$K_a$ of ~6.6 and a lifetime that is dependent on the reaction context and the level of oxidative stress (Scheme 2).[31,32] In the presence of excess oxidant, a second one-electron oxidation of $\mathbf{dOG}^{\bullet+}/\mathbf{dOG}^{\bullet}$ can occur yielding the proposed iminoquinone intermediate $\mathbf{dOG}^{ox}$ that is trapped at C5 by H_2O serving as a nucleophile yielding a second intermediate **5-HO-dOG** (Scheme 2). $\mathbf{dOG}^{ox}$ is the proposed intermediate from the four-electron oxidation of **dG** by $^1O_2$ that yields the intermediate **5-HO-dOG**.[33] **5-HO-dOG** is short-lived and rearranges or decomposes through three possible pathways:[34] (1) a 1,2-acyl migration yielding spiroiminodihydantoin (**Sp**, Figure 2); (2) hydration at C6 followed by decarboxylation to yield guanidinohydantoin (**Gh**, Figure 2); or (3) addition of a second nucleophilic H_2O at C4, followed by decomposition to 4-hydroxy-2,5-dioxo-imidazolidine-4 carboxylic acid (**HICA**, Figure 2). **Sp** is the major nucleoside product observed at pH 7,[35−41] **Gh** is the major product observed under slightly acidic conditions or duplex DNA,[36,42−45] and **HICA** is a minor product at neutral pH that is unstable and readily decomposes.[46] Overall, the oxidation of purines leads to formation of electron-deficient species that are subject to attack by nucleophiles, such as water. Because oxidation reactions do not occur in isolation under in vivo conditions, it has been of interest to see the structural effects that other nucleophiles have on the products derived from **dOG** oxidation.

Several adducts of **dOG** oxidation have already been characterized. Studies with the oxidant $ONOO^-$ at high concentrations suggest that $ONOO^-$ is the nucleophile that adds to C5 of $\mathbf{dOG}^{ox}$ ultimately yielding dehydroguanidinohydantoin (**DGh**) as an intermediate on the pathway to the decomposition product ribosyl-urea (**Ua**, Figure 2).[47] Oxidation of **dOG** by $ONOO^-$ in the presence of 30% MeOH provides a **HICA** analog, **MICA**, bearing a methoxy group at C5. **MICA** is proposed to arise from the coupling of $\mathbf{MeO}^{\bullet}$ to C5 of $\mathbf{dOG}^{\bullet}$ that then undergoes

Scheme 2. Proposed Pathway for Forming the Electrophile $\mathbf{dOG}^{ox}$ from dG and dOG



hydration and decomposition to afford the carboxylic acid end product (Figure 2).[46] Oxidation of **dOG** with Na_2IrCl_6 in the presence of a polyamine, such as spermine (Spm), initially forms the proposed intermediate **5-Spm-dOG** that has limited stability and decomposes to a **Ua** lesion (Figure 2).[48] When **dOG** nucleoside is oxidized by Na_2IrCl_6 in the presence of a primary amine, such as N^{α}-acetyl-lysine (Lys), the final product observed is an analog of **Sp**, with the ε-amino nitrogen of lysine covalently linked to C5 of **dOG** yielding, **5-Lys-Sp** (Figure 2).[49] Both amine adducts are proposed to be the result of the amine nucleophile adding to C5 of $\mathbf{dOG}^{ox}$, in which the primary amine (Lys) can undergo the 1,2-acyl migration to the spirocycle. However, the polyamine (Spm) initially forms a short-lived intermediate that then decomposes through a spiroaminal intermediate to a **Ua** lesion.[48] Finally, when **dOG** is oxidized with $K_3Fe(CN)_6$ in the presence of tyrosine or *p*-cresol, the product characterized was a tricyclic [4.3.3.0] species (collectively named **4,5-PhO-dOG**) with the phenol covalently bound at both C4 and C5 of **dOG** (Figure 2).[50] The tricyclic product is proposed to result from the ambidentate nature of the nucleophilic phenolate anion. This product showed high stability in organic solvents but decomposed in aqueous solutions.[50]

These studies have shown that **dOG** is reactive with radicals at C5, and most of the products are similar in structure to those derived from nucleophilic addition (e.g., **MICA** and **5-Lys-Sp**), while nucleophiles that have more than one reactive site (e.g., Spm and phenols) yield unique products that have limited stability and readily decompose in aqueous conditions. The work reported herein involves the oxidation of **dG** with Cu^{II}/H_2O_2 or $Fe^{III}/EDTA/H_2O_2$/ascorbate, as well as the reaction of **dOG**

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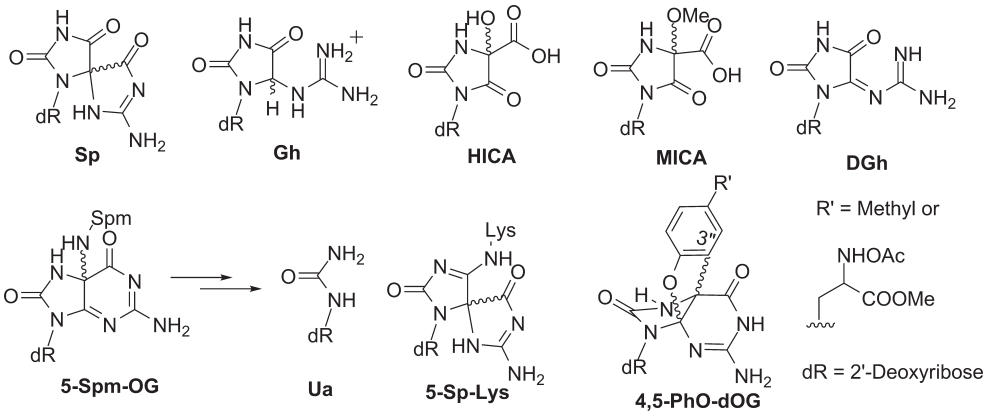

Figure 2. Products characterized from **dOG** oxidation in the presence of various nucleophiles. The products **Sp**, **Gh**, and **HICA** are H_2O adducts; when **dOG** was oxidized in the presence of **MeO$^{\bullet}$**, **MICA** was observed, nucleophilic $ONOO^-$ yielded **DGh**, oxidation in the presence of Spm yielded **5-Spm-OG** and its decomposition product **Ua**, while the presence of Lys yielded **5-Sp-Lys**, and the phenols *p*-cresol and Tyr yielded **4,5-PhO-dOG**.

with Na_2IrCl_6 in the presence of 2-naphthol. 2-Naphthol was selected for study because its redox potential is very similar to **dOG**, posing interesting questions about the interplay of competing pathways. In addition, 2-naphthol mimics certain B-ring aromatized metabolic products of estrogens, such as equilenin. All of the reactions with 2-naphthol yield the same products with the two major products being a newly observed stable adduct to the exocyclic amine of **dOG** (compounds **1a**/**1b**), and the other products are a diastereomeric pair of tricyclic [4.3.3.0] adducts of 2-naphthol to C4 and C5 of **dOG** (compounds **2a**/**2b**). The mass balance is completed by **dG** oxidation products for which 2-naphthol did not participate in the reaction.

RESULTS AND DISCUSSION

When the nucleoside **dG** and 2-naphthol were allowed to react in the presence of Cu^{II} and H_2O_2, two new products were identified by reversed-phase HPLC, in which each elutes as two peaks assumed to represent a pair of stereoisomers. The first pair of peaks, compounds **1a**/**1b**, and the second pair of peaks, compounds **2a**/**2b** each represented a yield of ~40−45%. The mass balance was completed with the **dG** oxidation products **Sp**, **Gh**, **Z**, and **2Ih**, each with yields <5%. For **1a**/**1b**, the ratio of the two peaks was essentially 1:1, while for **2a**/**2b**, the presumed diastereomeric ratio was about 60:40. These compounds were quantified through their extinction coefficients following previously established procedures (Supporting Information).[22] The ESI^+-MS analysis for the new peaks showed that both new products had neutral masses of 425 (**dG** + 2-naphthol +16 - 2) suggesting arylation of **dG** by 2-naphthol plus the formal addition of an oxygen atom had occurred under the oxidative conditions. The additional oxygen atom could be located on the phenol or the nucleobase. An oxygen atom addition to the phenol was ruled out by spectroscopic data discussed later. Furthermore, our experience with the oxidation of **dG** suggested that the oxygen atom could be the result of a two-electron oxidation of **dG** to **dOG** followed by further reaction of **dOG** to yield the adducts.[35,42] To substantiate this hypothesis, a reaction was conducted in which **dOG** and 2-naphthol were allowed to react in the presence of the oxidant Na_2IrCl_6, since this transition metal oxidant has been shown previously to oxidize selectively **dOG** in high yields.[35,42] The Na_2IrCl_6 initiated reaction with **dOG** and 2-naphthol furnished two products that had the same HPLC retention time and masses as those seen in the **dG** reaction with Cu^{II}/H_2O_2 and 2-naphthol. Purification of the two peaks followed by ESI^+-MS/MS analysis of the free base adducts ($(M + H)^+ = 310$) from both reactions supports the observation that the products from the arylation of **dG** and **dOG** by 2-naphthol under oxidative conditions are the same. Additional support for both reactions yielding the same products was established by ^1H NMR spectroscopy. The details of these experiments will be discussed in greater detail while examining the data that led to the proposed structures. Due to the higher yields produced, the reaction with **dOG** was chosen for preparation and purification of sufficient quantities for further spectroscopic analysis. This analysis led to the conclusion that compounds **1a**/**1b** are an N^2 adduct of **dOG** that exists as a pair of atropisomers, while compounds **2a**/**2b** are a diastereomeric pair of the tricyclic [4.3.3.0] adducts **2a** (4*S*,5*S*) and **2b** (4*R*,5*R*) formed between the **dOG** nucleobase and 2-naphthol. The **2a**/**2b** products mirror those recently characterized from the oxidation of **dOG** in the presence of tyrosine or *p*-cresol (**4,5-PhO-dOG**).[50]

Spectroscopic Analysis for 1a/1b. Structural characterization of **1a**/**1b** was achieved through a combination of UV−vis, MS, and NMR analysis after purification, and was aided by mechanistic insight into **dG** alkylation. The UV−vis spectrum of **1a**/**1b** retained the long wavelength absorption of the **dOG** chromophore ($\lambda_{max} = 295$ nm) suggesting that the aromaticity of the nucleobase had remained intact. ESI^+-MS/MS analysis for the free base $(M + H)^+ = 310$) of **1a**/**1b** yielded very little structural information due to the presence of only one fragment that is consistent with the loss of the 2-naphthol ($(M + H)^+ = 166$). The ^1H NMR spectrum indicated the loss of one aromatic proton from 2-naphthol, consistent with one covalent bond being formed between **dOG** and a carbon atom of 2-naphthol (Figure 3). Through COSY, HSQC, and HMBC assignments, it was determined that the missing aromatic proton and point of connectivity to **dOG** was at C1 of 2-naphthol (C1″ in **1a**/**1b**). The anomeric proton of **1a**/**1b** (6.17 ppm) presented as a doublet of doublets in the ^1H NMR spectrum. This observation ruled out adducts that would exist between 2-naphthol and **dOG** at the two carbon atoms C4 and C5; in these cases, formation of an additional stereocenter would present these resonances as a set of two doublets of doublets. Because **1a**/**1b** eluted from the HPLC as two peaks and the anomeric proton was a single doublet of doublets (Figure 3), we concluded that both peaks

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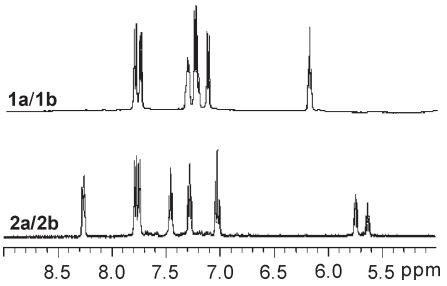


Figure 3. Partial ^{1}H NMR spectra (400 MHz, D_2O, 298 K) for **1a**/**1b**, and **2a**/**2b**. Adducts to **dOG** that form a new stereocenter (**2a**/**2b**) show H1′ as two doublets of doublets. In the aromatic region, both **1a**/**1b** and **2a**/**2b** show only six resonances indicating adduction via a ring carbon of 2-naphthol.

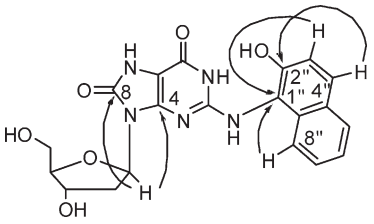


Figure 4. Key HMBC (500 MHz, DMSO-d_6, 298 K) correlations for **1a**/**1b**. The arrows show correlations that establish C1″ as the site of adduction on 2-naphthol. Also, the C4 and C8 resonances were not perturbed compared to **dOG** that were determined through their correlation with H1′, thus establishing that the aromaticity of the purine moiety remains intact.

are atropisomers of the same species, and not two similar products (e.g., constitutional isomers) eluting closely in the HPLC. The HMBC spectrum showed correlations of the anomeric proton to C4 and C8 of **1a**/**1b**, and their chemical shifts were only slightly different from the parent nucleoside **dOG** (Figure 4).[51] The observed ^{13}C chemical shifts corresponded to upfield perturbations of 0.1 ppm (147.1 to 147.0 ppm) for C4 and 1.1 ppm (151.6 to 150.5 ppm) for C8.[51] This provided two critical insights: (1) that the sugar and base are still covalently attached, and (2) that the chemical environments for C4 and C8 have not changed dramatically after arylation by 2-naphthol. The ^{1}H NMR spectrum for **1a**/**1b** in DMSO-d_6 showed conflicting data with respect to the number of exchangeable protons that were present, and the acidic phenol proton overlapped with the alcohol protons on the sugar. To gain an accurate count of these protons, ESI$^+$-MS analysis in deuterated solvents was used to count the six exchangeable protons in the presumptive structure, **1a**/**1b**, in which **dOG** and 2-naphthol have a total of seven exchangeable protons. The fact that one exchangeable proton was missing helped confirm that the covalent linkage between **dOG** and 2-naphthol is positioned at a nitrogen of the purine. Further support for arylation at nitrogen is derived from the reactions discussed below with **N1-methyl-dOG**. When **N1-methyl-dOG** and 2-naphthol were allowed to react in the presence of Na_2IrCl_6, analogous compounds to **1a**/**1b** were observed (discussed below).

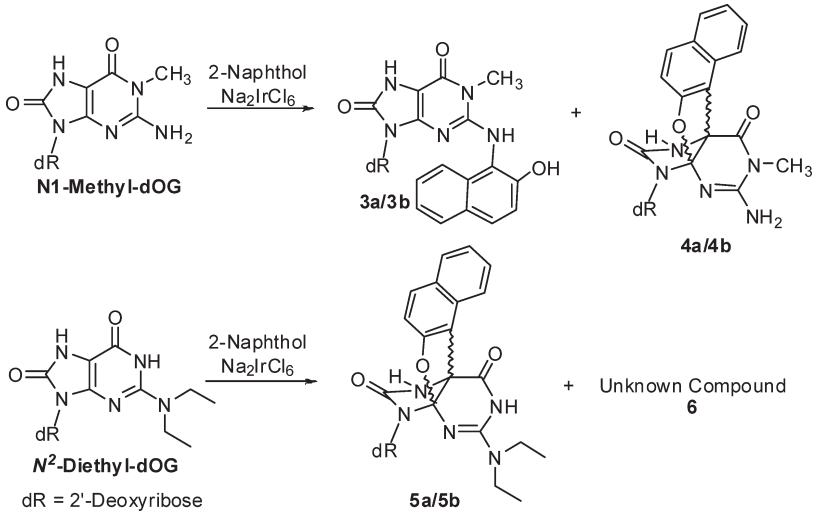
The identity of this heteroatom attachment was determined by observing the ^{13}C NMR shifts for C1″ and C2″ in the phenol moiety of the proposed structure, **1a**/**1b**. HMBC analysis aided in the identification of C1″ (114.6 ppm) through its correlations to H3″ and H8″, and C2″ (152.8 ppm) through its correlation to H4″, where all aromatic protons were identified by COSY and HSQC. Comparison of these shifts to 2-naphthols with aryl amines or aryl ethers covalently linked to C1 suggested that **1a**/**1b** has phenol carbons with chemical shifts that are consistent with an aryl amine attached at C1″ of **1a**/**1b**,[52,53] ruling out arylation of **dOG** at either O^6 or O^8 of the heterocyclic base. The remaining possible points of covalent attachment between C1 of 2-naphthol and **dOG** include the nitrogen atoms N1, N^2, N3, and N7. N3 arylation was ruled out for two reasons: (1) N3 is sterically inaccessible for 2-naphthol addition, and (2) N3 arylation is expected to cause a significant perturbation of the ^{13}C resonance at C4 of the base due to a change in the base aromaticity, which was not observed.[54]

Next, a possible N7 adduct for **1a**/**1b** was ruled out by mechanistic and ^{13}C NMR chemical shift considerations. For a ring nitrogen to act as the nucleophile, it must be unprotonated;[55] however, the pK_a of N7 of **dOG** (11.3) is much higher than that of N1 (8.6), so if arylation occurs through this mechanism, N1 should be the preferred site of reaction.[30,55] Another way to deprotonate N7 is through oxidation of **dOG** to **dOG^{ox}**, but this product is electrophilic and adds nucleophiles at C5, as previously discussed.[30,56] Furthermore, if arylation had occurred at N7, a large ^{13}C chemical shift change (>1.7 ppm) would be expected for C8 and C4 of the base[54] that was not observed.

N1 and N^2 are the other possible points of bond formation between **dOG** and 2-naphthol. Because the ^{1}H NMR spectrum did not provide sufficient data to rule out one adduct over the other, and the ^{13}C NMR data could not be used to uniquely identify C2, C5, and C6 of **1a**/**1b**, *N*-alkyl **dOG** derivatives were synthesized. **N1-Methyl-dOG** and a **N^2-diethyl-dOG** derivative were prepared and allowed to react with 2-naphthol in the presence of Na_2IrCl_6. Under these conditions **N1-methyl-dOG** led to formation of two compounds (**3a**/**3b** and **4a**/**4b**) with 2-naphthol (Scheme 3). Compounds **3a**/**3b** led to an ESI$^+$-MS/MS fragmentation pattern similar to that observed for compounds **1a**/**1b**, suggesting that these two compounds are very similar and that arylation did not occur at N1 of **dOG**. The other compounds, **4a**/**4b**, gave the same ESI$^+$-MS/MS fragmentation pattern as observed with **2a**/**2b** that helped aid in identifying some of the **2a**/**2b** fragments (Supporting Information). When N^2 was blocked by ethylation, the HPLC indicated major products (**5a**/**5b**) that had a ESI$^+$-MS/MS fragmentation patterns consistent with a tricyclic adduct similar to **2a**/**2b**, but there was also an unknown minor product (**6**) observed (Scheme 3 and Supporting Information). Compound **6** was not characterized due to its instability suggesting it is not similar to compounds **1a**/**1b**, because, as will be discussed later, **1a**/**1b** is stable. The only position that would still provide arylation when N1 is blocked and decrease when N^2 is blocked would be N3, but as stated this does not fit the NMR data. We conclude that products **1a**/**1b** are the atropisomers formed from oxidative addition of 2-naphthol to N^2 of **dOG**.

Spectroscopic Analysis for 2a/2b. The structure of compounds **2a**/**2b** was also determined by a combination of UV−vis, ESI$^+$-MS, MS/MS fragmentation and NMR analysis after HPLC purification. The UV−vis spectrum of **2a**/**2b** looked similar to that of 2-naphthol (λ_{max} = 227 nm), and the long-wavelength absorption for the **dOG** chromophore was not observed, suggesting significant perturbation to the aromatic base. The ESI$^+$-MS/MS fragmentation pattern for the free base of **2a**/**2b**, released during facile fragmentation of the glycosidic bond, presented many daughter fragments that aided in the structural

Scheme 3. Products Observed from Oxidation of dOG Alkyl Derivatives in the Presence of 2-Naphthol



analysis ($(M + H)^+$ = 310, 269, 225, 182, and 86, where the alkyl **dOG** derivatives were used to help identify the fragments; see Supporting Information). The ^{1}H NMR spectrum of **2a**/**2b** showed the presence of six aromatic protons suggesting covalent attachment to one of the phenol carbons (Figure 3). The proton assignments were confirmed by COSY, HSQC, and HMBC correlations that supported arylation of **dOG** by C1 of 2-naphthol. The anomeric protons (5.75 and 5.66 ppm), also identified by COSY and HSQC, presented as two doublets of doublets suggesting one or more stereocenters are present in the base. HMBC correlations from the anomeric proton to C8 and C4 of the base show that C8 has shifted downfield by 4.4 ppm (151.6 to 156.2 ppm), while C4 shifted upfield by 35.4 ppm (147.1 to 111.7 ppm) compared to **dOG** (Figure 5).[51] Due to the slow relaxation time for C4, it was not identified in the ^{13}C NMR spectra. Also, C2, C5, and C6 were not unambiguously identifiable from the NMR data. While C5 is important as the point of covalent attachment between **dOG** and 2-naphthol, its chemical shift is proposed by comparison to a previous study in our laboratory.[50] The adduct characterized between Tyr and dOG under related reaction conditions gave a similar product, **4,5-PhO-dOG** (Figure 2), for which C5 was identifiable through an HMBC correlation. HMBC analysis for the previous phenol adduct showed that C5, through its correlation to H3″ had shifted downfield by 37.5 ppm (98.5 to 61.0 ppm) compared to **dOG**.[50,51] Using this as a guide, C5 of **2a**/**2b** is proposed to be the peak at 63.2 ppm that has a comparable downfield shift of 35.3 ppm (98.5 to 63.2 ppm). From the comparison of NMR data between **2a**/**2b** and **4,5-PhO-dOG**, and the observation that **2a**/**2b** and **4,5-PhO-dOG** give similar ESI$^+$-MS/MS fragmentations, the following structure for the tricyclic [4.3.3.0] **2a**/**2b** compounds is proposed to be comprised of the 4*S*,5*S* **2a** and 4*R*,5*R* **2b** diastereomers.

Two-Electron Oxidation that Initiates Adduct Formation. To arrive at arylated products **1a**/**1b** and **2a**/**2b** from the nucleoside **dG**, a four-electron oxidation had to occur. Compounds **1a**/**1b** are a result of a two-electron oxidation on both **dG** and 2-naphthol, yielding a **dOG** aryl adduct. The structure of compounds **2a**/**2b** implies that a four-electron oxidation of **dG** has occurred with 2-naphthol serving as a nucleophile. Standard

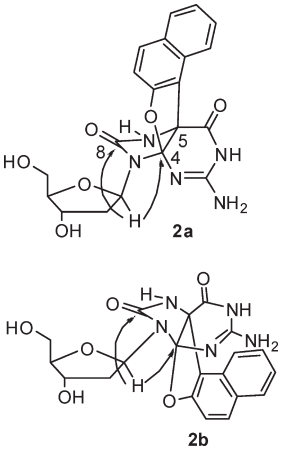


Figure 5. Key HMBC (500 MHz, DMSO-d_6, 298 K) correlations for **2a**/**2b**. The arrows show correlations that establish C1″ as the site of adduction on 2-naphthol. C4 and C8 resonances were assigned that both show large perturbations, suggesting that the purine aromaticity has been disrupted.

reduction potentials of each species indicate that 2-naphthol should be the sole oxidized species in the reaction (Table 1),[30,35,57−60] and one of the main products from this reaction was indeed 2-naphthol dimers and tetramers, as identified by HPLC and ESI$^+$-MS. From LC-ESI$^+$-MS and the mass balance, all guanosine-derived products had a +16 additional mass, indicative of the oxidation of **dG** to **dOG**. The Cu^{II}/H_2O_2 oxidant system is unusual in **dG** oxidation due to the coordination of copper to N7 of **dG**, wherein the bound copper specifically directs oxidation on **dG**.[22,61,62] It is proposed that 2-naphthol reduces the Cu^{II} to Cu^{I}, at which point Cu^{I} and H_2O_2 can mediate the two-electron oxidation of **dG** to **dOG** following a Fenton-like reaction; the details of this reaction are still under debate.[1,63,64] In an attempt to better understand this step, reactions were conducted utilizing the oxidant system Fe^{II}/EDTA/H_2O_2/ascorbate,[65] a known hydroxyl radical generating system, for which compounds **1a**/**1b** and **2a**/**2b** were found, but in lower yields than observed with the Cu^{II}/H_2O_2 system. The decreased

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Table 1. Standard Reduction Potentials for Chemical Species of Interest

Compd	E^{0a}	pK_a	ref
$OH^{\bullet}$,OH^-	1.9		62
dG$^{+\bullet}$,**dG**	1.29	3.3, 9.4	19
$PhO^{\bullet}$,PhO^-	1	10	60
$C_6Cl_5O^{\bullet}$,$C_6Cl_5O^-$	0.99	4.7	60
p-Cresol$^{\bullet}$,*p*-Cresolate	0.9	10.3	60
Na_2IrCl_6 (Ir^{IV},Ir^{III})	0.9		57
2-NpO$^{\bullet}$,2-NpO$^-$	0.82[b]	9.6	58
Na_2IrBr_6 (Ir^{IV},Ir^{III})	0.82		57
dOG$^{+\bullet}$,**dOG**	0.74	8.6, 11.3	30
1-NpO$^{\bullet}$,1-NpO$^-$	0.72[b]	9.3	58
$K_3Fe(CN)_6$ (Fe^{III},Fe^{II})	0.42		57

[a] All values are in V vs NHE, pH 7. [b] The pH adjusted E^0 values for 1- and 2-naphthol were calculated using E^o values found at pH 13 from reference [58] that were corrected for pH 7 utilizing the method outlined in reference.[60] Because the 2-naphthoxyl radical is more resonance stabilized compared to phenol, it is possible that the calculated E^0 values are an overestimate.

yields are due to increased background oxidation leading to many unidentified products according to HPLC analysis. Regardless of the oxidant, **dG** can undergo a two-electron oxidation to **dOG** by these metal oxidant systems,[1,63,66,67] and further studies have shown that DNA damage by these complexes is enhanced in the presence of phenolic compounds.[68,69] Oxidation reactions involving **dG** and 2-naphthol initiated by a weaker oxidant (Na_2IrCl_6) that is incapable of oxidizing **dG** under these conditions did not yield any detectable products, again suggesting that **dOG** is the reactive nucleoside involved in the arylation chemistry.

Interestingly, 2-naphthol in the presence of Cu^{II} does not precipitate out of solution,[70,71] whereas when reactions were conducted with other phenols, such as 1-naphthol and *p*-cresol, under similar conditions, arylation products were not observed in high yields due to precipitation of the corresponding Cu^{II} phenolate complexes.[71] This observation suggests a unique feature associated with 2-naphthols and the ability to arylate **dG** under oxidative conditions.

Possible Reaction Pathways Leading to Compounds 1a/1b from dOG. The mechanistic pathway to yield compounds **1a**/**1b** is proposed to occur initially through the two-electron oxidation of **dG** to the reactive intermediate **dOG**, as previously stated. At this point the reaction can proceed through three possible mechanistic pathways yielding **1a**/**1b**, because of the similarity in reduction potential values for **dOG** and 2-naphthol (Table 1).

Pathway A. In this mechanism, **dOG** proceeds through a two-electron oxidation to the proposed quinonoid imine **dOG^{ox}** (shown as the exocyclic amine tautomer); this species could tautomerize to an exocyclic imine form of **dOG^{ox}** that is attacked by the 2-naphtholate anion (**2-NpO$^-$**) at N^2 yielding a neutral intermediate **7** that enolizes to the stable products **1a**/**1b** (Scheme 4). This mechanism does not explain why the N^2 adduct is phenol specific, as will be discussed later, unless the point of arylation has some degree of control determined by steric properties.[50] Also, there is no literature precedent for N-alkylation of iminoquinones; the preferred site of nucleophilic attack is carbon centered that would yield compounds **2a**/**2b** (see below).[72−81]

Pathway B. It is also possible that **dOG** is oxidized by one-electron to **dOG$^{\bullet}$** (the pK_a for this radical is ∼6.6),[30] while 2-naphthol undergoes a one-electron oxidation to the 2-naphthoxyl radical (**2-NpO$^{\bullet}$**). Next, these two radicals couple yielding a neutral intermediate **7** that enolizes to the more stable final products **1a**/**1b** (Scheme 4). The **2-NpO$^{\bullet}$** has been shown by EPR spectroscopy to have the largest population of unpaired spin density at C1,[82] which is consistent with the spectroscopic data for compounds **1a**/**1b**. A similar mechanism has been proposed by the Tannenbaum laboratory for the generation of **MICA** in which **MeO$^{\bullet}$** couples with **dOG$^{\bullet}$** at C5.[36] DFT calculations conducted on **dOG$^{\bullet}$** suggest that the N^2 centered-aminyl radical represents a smaller population of the unpaired electron spin density when compared to C5 of **dOG$^{\bullet}$**.[56] It is possible that due to the steric bulk of the **2-NpO$^{\bullet}$** and the observation that this radical is longer lived than a single ring phenoxyl radical, that coupling at the exocyclic amine of **dOG$^{\bullet}$** is the preferred point of arylation.[5] Interestingly, it has been proposed that the **dG** aminyl radical couples with the *o*-semiquinone radical of equilenin, a biaryl catechol, yielding the initial covalent attachment between these two compounds.[5] This would suggest a unique feature of naphthyl radicals to couple with the exocyclic aminyl radicals of **dG** and **dOG**.

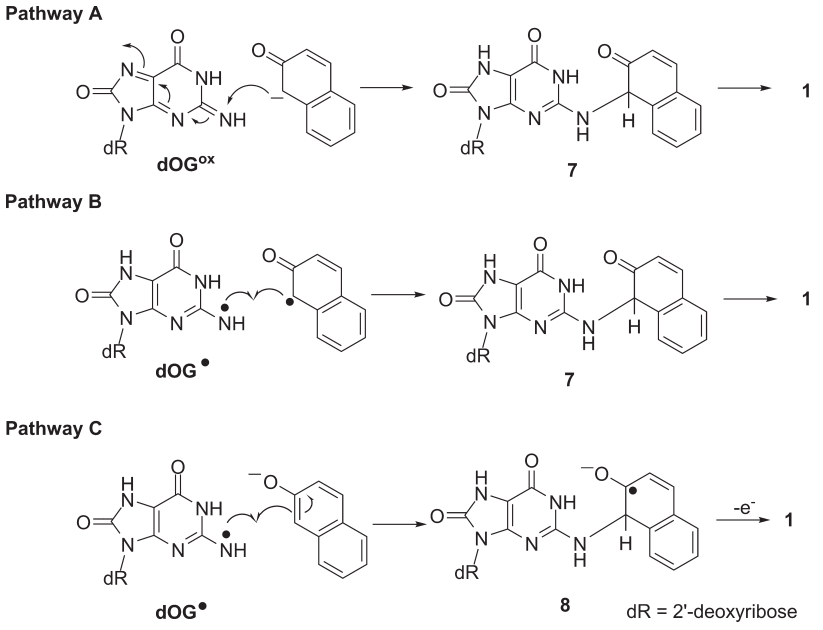
Pathway C. Another possibility is the addition of **dOG$^{\bullet}$** to the **2-NpO$^-$** ring system yielding the radical anion intermediate **8** that goes through a second oxidation and enolizes to the final stable products **1a**/**1b** (Scheme 4). Once again, the N^2 centered **dOG$^{\bullet}$** aminyl radical is not calculated to provide the largest radical population,[56] but sterics could be driving arylation at the exocyclic amine. At first it was thought that radical addition to the **2-NpO$^-$** would not be specific for C1, but studies have shown that the photoinduced phenylation of 2-naphthol occurs specifically at C1 through a possible $S_{RN}1$-like mechanism.[83] In this study it was concluded that sterics of the arylating group and the dominance of the C1-centered **2-NpO$^-$** provide the site specificity,[83] a feature that would also be consistent with the proposed structure of compounds **1a**/**1b**.

The reaction between **dG** and 2-naphthol in the presence of Na_2IrCl_6 does not yield any detectable amount of adducts. Instead the 2-naphthol is preferentially oxidized yielding polymers that rapidly precipitate. This observation suggests that the mechanism has to involve at least a one-electron oxidation of **dOG**, ruling out any possibility of a mechanism not invoking **dOG** oxidation and supporting all three proposed mechanisms. On the basis of the observation that reactions with tyrosine and *p*-cresol, which have higher reduction potentials (Table 1) and did not yield significant amounts of an analogous N^2 product,[50] it is concluded that the possible mechanism should involve both oxidation of the **dOG** and 2-naphthol, supporting **Pathway B**.

Possible Reaction Pathways Leading to 2a/2b from dOG. Compounds **2a**/**2b** are the products observed from the four-electron oxidation product of **dG** in the presence of 2-naphthol; the focus here will be on the events that must occur to arrive at **2a**/**2b**. After the initial two-electron oxidation of the nucleoside **dG** to **dOG**, the species present include **dOG**, 2-naphthol, and oxidant (where the oxidant can be Cu^{II}/H_2O_2 or Na_2IrCl_6). Both **dOG** and 2-naphthol have similar reduction potentials (Table 1); thus, oxidation can be envisioned to occur from either or both species.[19,30,58,60,84,85] From the structure of **2a**/**2b**, there exist two possible pathways for product formation to be initiated.

Pathway D. **dOG** and 2-naphthol each undergo one-electron oxidation to **dOG$^{\bullet}$** and **2-NpO$^{\bullet}$**. These radicals can couple

Scheme 4. Possible Pathways to Form Compound 1a/1b from dOG



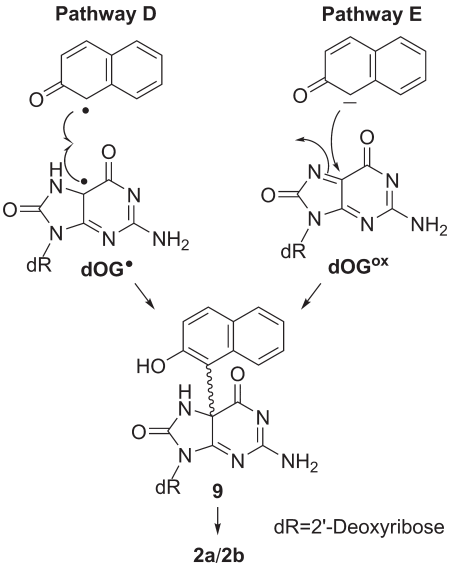
yielding an intermediate species **9** with a covalent bond formed between C1 of 2-naphthol and C5 of **dOG** (Scheme 5).

Pathway E. In this pathway, **dOG** undergoes a two-electron oxidation to the electrophilic quininoid species **dOG^{ox}**, drawn as the exocyclic amine tautomer, or equally likely, the exocyclic imino tautomer, that is attacked at C5 by the C1 centered **2-NpO$^-$**, again giving the proposed intermediate **9** with a newly formed covalent bond between C5 of **dOG** and C1 of 2-naphthol (Scheme 5). Previous studies have shown that the preferred site for the **2-NpO$^-$** to alkylate is at C1 in aqueous solution[86] that is further supported by the spectroscopic data under both possible mechanisms. The analogous intermediate from H_2O attack, **5-HO-dOG**, is short-lived and undergoes further rearrangement/decomposition to the hydantions **Sp** or **Gh**, or a second nucleophilic attack by H_2O can occur at C4 followed by decomposition to **HICA** in which all of these compounds are products under different reaction conditions.[33,35,46] Intermediate **9** has the phenol positioned such that the oxygen centered **2-NpO$^-$** nucleophile can participate in an intramolecular cyclization at C4 yielding the tricyclic [4.3.3.0] products **2a**/**2b** (Scheme 5).

The two diastereomers of **2a**/**2b** can be rationalized through geometric considerations in which the 4*S*,5*S* (**2a**) diastereomer is formed when 2-naphthol is positioned on the *si* face and attacks C4, and the 4*R*,5*R* (**2b**) diastereomer arises when 2-naphthol attacks C4 from the *re* face. This mechanism is similar to that of the two-electron oxidation product of **dOG** to **HICA**, but after the second nucleophilic attack at C4, the **HICA**-like structure is not observed because carbonyl formation is abolished at C4 in **2a**/**2b** preventing further degradation. At this time, it is impossible to determine the most likely pathway for formation of **2a**/**2b**, but **Pathway E** best explains **dOG** oxidation in the presence of H_2O,[36,43] lysine,[49] spermine,[48] and peroxynitrite.[87]

Effect of Oxidant on the Product Distribution. To better understand the oxidation mechanisms leading to bond formation between the reactive **dOG** nucleoside and 2-naphthol, reactions were conducted with the Horse Radish Peroxidase (HRP)/H_2O_2

Scheme 5. Possible Pathways to Form 2a/2b from dOG



oxidant system that was thought to initiate the oxidation reaction on the phenol moiety and not on **dOG**.[12,16] These reactions were conducted with the tri-*O*-acetyl-**OG** nucleoside so that **Sp**, a **dOG** oxidation product, could be retained on a reversed-phase HPLC column allowing a complete mass balance of the reaction in one HPLC run. Control studies with tri-*O*-acetyl-**OG**, HRP, and a 10-fold excess of H_2O_2 led to <5% oxidation of the nucleoside. The HRP/H_2O_2 oxidant system yields of **1a**/**1b**, **2a**/**2b**, and **Sp** with respect to pH (6.4, 7.4, and 8.4) were determined and compared to analogous reactions with Na_2IrCl_6 as the oxidant (Figure 6).

When HRP/H_2O_2 (2.5 mM tri-*O*-acetyl-**OG**, 0.5 mM 2-naphthol, 2.5 U HRP, and 0.5 mM H_2O_2) was utilized to initiate the reaction, regardless of pH, the major product

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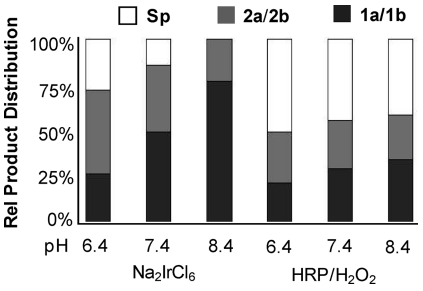

Figure 6. Relative product distributions observed when tri-*O*-acetyl-**OG** was allowed to react with 2-naphthol in the presence of either Na_2IrCl_6 or HRP/H_2O_2. Each reaction was run at 2.5 mM tri-*O*-acetyl-**OG**, 0.5 mM 2-naphthol, and either 2.5 U HRP and 0.5 mM H_2O_2, or 0.5 mM Na_2IrCl_6. Absolute product yields were ~10%; each trial was run in triplicate, giving an average error of 3% (error = one standard deviation).

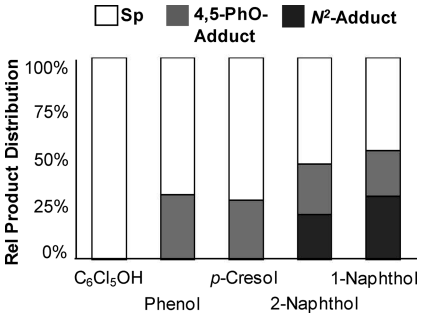

Figure 7. Effect of the phenol on the relative-product distributions while utilizing the HRP/H_2O_2 oxidant system. Each reaction was conducted with 2.5 mM tri-*O*-acetyl-**OG**, 0.5 mM 2-naphthol, and either 2.5 U HRP and 0.5 mM H_2O_2, or 0.5 mM Na_2IrCl_6. Absolute product yields were ~10%; each trial was conducted in triplicate, giving an average error of 3% (error = one standard deviation).

observed was **Sp**; if Na_2IrCl_6 (2.5 mM tri-*O*-acetyl-**OG**, 0.5 mM 2-naphthol, and 0.5 mM Na_2IrCl_6) initiated the oxidation, then compounds **1a**/**1b** and **2a**/**2b** were the major products. The HRP/H_2O_2 oxidant system appears to initiate the reaction through formation of a **2-NpO$^\bullet$** that is capable of oxidizing **dOG**, based on the appearance of **Sp**. Interestingly, the amount of 2-naphthol adducts did not increase, in fact they decreased, suggesting that when the flux of **2-NpO$^\bullet$** is high the radicals couple, outcompeting adduct formation that is supported by increased amounts of 2-naphthol dimers and tetramers observed by HPLC. Another possibility that explains the increased amount of **Sp** when HRP/H_2O_2 was used is the formation of $O_2^{\bullet-}$ from HRP. Because $O_2^{\bullet-}$ reacts with phenoxyl radicals at diffusion controlled rates,[88] 2-naphthol cannot participate in adduct formation. When a nonselective oxidant, such as Na_2IrCl_6 was used, adduct formation increased because the overall flux of **2-NpO$^\bullet$** decreased due to oxidation of the **dOG** base. This result suggests that 2-naphthol adducts to **dOG** require that **dOG** be activated through at least a one-electron oxidation to yield 2-naphthol adducts, therefore ruling out any adduct formation mechanism that has **dOG** acting strictly as a nucleophile. This observation explains why adducts were never observed to **dG** without concomitant oxidation at C8 to **dOG**, since 2-naphthol oxidation will always occur in preference to **dG** oxidation due to its ~500 mV lower standard reduction potential (Table 1).

The next sets of studies were aimed at evaluating the nature of the oxidant with respect to product formation. The weaker one-electron oxidants Na_2IrBr_6 and $K_3Fe(CN)_6$ were utilized to initiate the reaction between **dOG** and 2-naphthol. In these studies it was found that product yields and pH dependence were the same for these oxidants as was observed with the stronger one-electron oxidant, Na_2IrCl_6 (Supporting Information). Since **dOG** and 2-naphthol have similar redox potentials, the nonselective oxidants will likely react with both reactants similarly, providing the same overall relative amounts of radical species.

Effect of pH on the Product Distribution. The reaction product yields were shown to be pH dependent, but the nature of the oxidant did not have much of an effect on the product distributions (Supporting Information). This observation is complicated by the fact that **dOG** and 2-naphthol are both weak acids and show the same pH dependency in their reduction potentials.[30,58] The yield of **1a**/**1b** increases with increasing pH suggesting that 2-naphthol oxidation dominates at higher pH and at lower pH **2a**/**2b** yields increase, and accordingly, **dOG** oxidation dominates at lower pH.

Effect of the Phenol on the Product Distribution. In a previous study conducted in our laboratory the **4,5-PhO-dOG** adduct that is similar in structure to **2a**/**2b** was characterized from the oxidation of **dOG** in the presence of either tyrosine or *p*-cresol using the one-electron oxidant $K_3Fe(CN)_6$.[50] In this study, the phenol was substituted with 2-naphthol, and the product distribution included compounds **1a**/**1b** that were not previously observed for simple phenols. The goals of the following studies were to determine if this new type of adduct (**1a**/**1b**) observed with 2-naphthol was a result of 2-naphthol having a lower standard reduction potential than tyrosine or *p*-cresol, or if it is the naphthalene ring system that is influencing the product distribution (Figure 7). The HRP/H_2O_2 oxidant system was chosen to conduct these studies. When pentachlorophenol (C_6Cl_5OH), a phenol with a high standard reduction potential (Table 1) but resistant to carbon alkylation, was oxidized in the presence of tri-*O*-acetyl-**OG**, the only product observed was **Sp**, providing more evidence that phenoxyl radicals can oxidize the **OG** base. When phenol or *p*-cresol was oxidized by HRP/H_2O_2 in the presence of tri-*O*-acetyl-**OG**, adduct formation was observed but the major product was still **Sp** (Figure 7). In the studies with the phenols that are not blocked at the *ortho* carbon (phenol, *p*-cresol, 2-naphthol, and 1-naphthol) tricyclic adducts were observed. The **4,5-PhO-dOG** adduct is a defining product of phenol arylation of the **OG** base observed through the course of an oxidation reaction. The N^2 adducts were only observed in the case of the naphthalene-based phenols, 1- and 2-naphthol, which is a defining product observed when these phenols arylate the **OG** base under oxidative conditions. The key difference between the simple phenols, phenol and *p*-cresol, and the naphthalene-based phenols, 1- and 2-naphthol, is the stability of the intermediate phenolic radical, that is, the naphthoxyl radical is more resonance stabilized than the phenoxyl radicals.[58] The appearance of the N^2 adducts with naphthalene-based phenols supports a phenolic-radical intermediate as proposed in **Pathway B** of Scheme 4. Comparison of phenol reduction potentials to that of **dOG** suggests that phenoxyl radicals are produced with HRP/H_2O_2 in the presence of **dOG**, the purine base acts as a reductant that quenches the radical species, while being oxidized to **Sp**.

Product Stability Studies. The stabilities of **1a**/**1b** and **2a**/**2b** in aqueous buffer (75 mM NaP_i, pH 7.4, 22 °C) are dramatically different. Under these conditions, **1a**/**1b** was unaltered after 14 days. This supports **1a**/**1b** as an adduct to N^2 because adducts to the exocyclic amine are considered to be relatively stable.[1,89]

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In an attempt to decompose **1a**/**1b**, it was found that this compound is susceptible to further oxidation by Na_2IrCl_6, yielding an early eluting product and many later eluting products in the HPLC (Supporting Information). The early eluting product can arise from two possible oxidation events. The nucleobase of **1a**/**1b** can still undergo a two-electron oxidation to the hydantoins **Sp** or **Gh** in H_2O. Alternatively, the 2-naphthol of **1a**/**1b** is positioned to undergo a two-electron oxidation to an iminoquinone. The iminoquinone is electrophilic and susceptible to nucleophilic attack, in which the nucleophile can be water or an intramolecular cyclization reaction can occur if N1 of the base serves as the nucleophile. This has been observed with *p*-quinones covalently linked to N^2 of **dG**.[5,10,12] This new product gave a mass of 435 (**1a**/**1b** + 9). Attempts to further study this product were not possible due to the instability of this new compound. The later eluting peaks in the HPLC are most likely polymers since oxidation of the phenol portion of **1a**/**1b** yields a radical that can polymerize. These polymers are likely composed of oxidized nucleobases and multiple cross-links between the 2-naphthols, so attempts to characterize these structures where not pursued. This phenomenon has recently been observed for phenols covalently linked to C8 of **dG**.[90]

Compounds **1a**/**1b** elute as two peaks in the HPLC that we assign as atropisomers. In the ^{1}H NMR spectrum, only one anomeric proton was observed indicating that the two isomers are very similar. To determine if the two atropisomers can interconvert, the compounds were purified from one another by HPLC, and then heated at 75 °C for 12 h. The chromatographically enriched samples did not change during this time period; this result was not too surprising because some atropisomers can have interconversion temperatures up to 200 °C.[91] These studies were conducted with nucleoside adducts that retain the 2-deoxyribose moiety. The effect of this group on the interconversion of atropisomers cannot easily be determined. Furthermore, it is anticipated that the free base adduct would not be sufficiently water-soluble enough to allow its study.

Compounds **2a**/**2b** were prone to decomposition under the same conditions (75 mM NaP_i, pH 7.4, 22 °C). By following the loss of starting material, we observed that the two diastereomers gave different stabilities, where one of the diastereomers has a $t_{1/2} \approx 49$ h, and the other was ~69 h (Supporting Information). Also, **2a**/**2b** showed greater stability at lower pH (pH 6.4 the $t_{1/2}$ increased ~46 h) than at higher pH (pH 8.4 the $t_{1/2}$ decreased ~37 h). RP-HPLC analysis of the decomposition of **2a**/**2b** yielded many peaks, and LC-ESI$^+$-MS of this sample gave products with masses of 462 (**2a**/**2b** + 36), 444 (**2a**/**2b** + 18), 416 (**2a**/**2b** - 10), 399 (**2a**/**2b** − 27), and 323 (**2a**/**2b** − 103). Due to low product yields and limited stability, further characterization of these products was not pursued.

CONCLUSIONS

Genomic DNA interacts with many potential reactive species in the cell. Chelated Cu^{II} is observed to be present in micromolar concentrations,[92] H_2O_2 is a metabolic byproduct,[93] and 2-naphthol has been proposed to reach micromolar concentrations in cells that are exposed to high levels of this phenol or its precursor naphthalene.[94] 2′-Deoxyguanosine provides a platform for unique chemical transformations to occur in comparison to the other bases since this nucleobase is particularly electron rich. **dG** and 2-naphthol were shown to react under oxidative conditions to yield stereoisomeric pairs of two major constitutional isomers with unique structures in which both an oxidation and arylation event had occurred in tandem. Compounds **1a**/**1b** are similar to other products previously observed from the arylation of **dG** because N^2 is the site most preferred by resonance-stabilized electrophiles;[1] however, the base has also undergone a two-electron oxidation by Cu^{II}/H_2O_2 yielding **dOG**. Compounds **2a**/**2b**, also base oxidation products of **dG**, are tricyclic compounds that result from the ambidentate nature of 2-naphthol, causing more disruption in the base structure. Both **1a**/**1b** and **2a**/**2b** have the same masses but differ in their RP-HPLC, ^{1}H NMR, and ESI$^+$-MS/MS profiles, providing the means to uniquely identify each. In the cellular context, **1a**/**1b** is expected to be the dominant arylation product of guanine residues under these conditions due to steric considerations. In either case, if **1a**/**1b** or **2a**/**2b** are present in the cell, the consequences would be deleterious, because both would most likely represent polymerase stop points in replication or transcription. The two unique constitutional isomer adducts **1a**/**1b** and **2a**/**2b** observed under oxidative conditions show a stark contrast to the previously identified compounds **Sp**, **Gh**, and **HICA** for which water serves as the nucleophile or when lysine (**5-Lys-Sp**), spermine (**5-Spm-OG**), and peroxynitrite (**DGh**) form adducts to **dG** and **dOG**.

EXPERIMENTAL SECTION

Oxidation of dG and 2-Naphthol in the Presence of a Metal Catalyst and H_2O_2. A 200-μL solution of **dG** (3.0 mM, 0.60 μmoles, 0.16 mg) and 2-naphthol (1.0 mM, 0.20 μmoles, 0.03 mg) was stirred in sodium phosphate buffer (75 mM, pH 7.4). The reaction was initiated by the simultaneous addition of copper(II) acetate (2.0 mM, 0.40 μmoles, 0.07 mg) and H_2O_2 (10.0 mM, 2.0 μmoles, 0.07 mg). The solution was kept at 22 °C for 24 h and then quenched with Na_2EDTA (10.0 mM, 2.0 μmoles, 0.50 mg). Alternatively, a 200-μL solution of **dG** (3.0 mM, 0.60 μmoles, 0.16 mg) and 2-naphthol (1.0 mM, 0.20 μmoles, 0.03 mg) was mixed with Fe^{II}/EDTA (0.50 mM, 0.10 μmoles, 0.04 mg) H_2O_2 (1.0 mM, 0.2 μmoles, 0.05 mg), and ascorbate (1.0 mM, 0.20 μmoles, 0.04 mg) and incubated for 1 h. The Fe^{II}/EDTA complex was made fresh by mixing $Fe(NH_4)_2(SO_4)_2$ and Na_2EDTA in a 1:2 ratio 30 min prior to reaction. The reaction mixtures were analyzed by HPLC (see Supporting Information) giving two product peaks. **1a**/**1b**. HRMS (ESI$^+$-TOF) calcd mass for $C_{20}H_{19}N_5O_6Na$ [M + Na^+] = 448.1233, exp mass = 448.1241. ESI$^+$-MS/MS for the free base [$(M + H)^+$ = 310] = 166. t_R = 18.2 min, and **2a**/**2b**. HRMS (ESI$^+$-TOF) calcd mass for $C_{20}H_{19}N_5O_6Na$ [M + Na^+] = 448.1233, exp mass = 448.1240. ESI$^+$-MS/MS for the free base [$(M + H)^+$ = 310] = 293, 267, 249, 225, 182, 166, and 86. t_R = 21.7 and 22.3 min.

Oxidation of dOG and Base Analogs with 2-Naphthol in the Presence of Na_2IrCl_6. A 200-μL solution of **dOG** (3.0 mM, 0.60 μmoles, 0.17 mg) and 2-naphthol (10 mM, 2.0 μmoles, 0.30 mg) was mixed in sodium phosphate buffer (75.0 mM, pH 6.4, 7.4, or 8.4). The reaction was initiated by the addition of Na_2IrCl_6 (6.0 mM, 1.0 μmoles, 0.67 mg). The solution was kept at 22 °C for 30 min and then quenched with Na_2EDTA (30 mM, 6 μmoles, 1.5 mg). The reaction mixture was purified by HPLC and then concentrated to furnish **1a**/**1b** (5.9 mg, 15% yield) t_R = 18.2 min as a white solid (mp =194.2−196.5, decomposed), and **2a**/**2b** (3.8 mg, 10% yield) t_R = 21.7 and 22.3 as a white solid (mp = 170.8−175.4, decomposed). **1a**/**1b** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (b, 1 H), 8.47 (b, 1 H), 7.84 (d, J = 7.74 Hz, 1 H), 7.80 (d, J = 9.69 Hz, 1 H), 7.38 (t, J = 6.34 Hz, 1 H), 7.30 (t, J = 7.19 Hz, 1 H), 7.25 (d, J = 7.74 Hz, 1 H), 7.23 (d, J = 6.34 Hz, 1 H), 6.90 (b, 1 H), 6.18 (dd, J = 7.16 and 6.63 Hz, 1 H), 5.45 (s, 1 H), 5.19 (b, 2 H), 4.35 (ddd, J = 5.94, 3.31, and 2.93 Hz, 1 H), 3.80 (td, J = 4.57 Hz, 1 H), 3.58 (m, J = 4.57 Hz, 1 H), 3.01 (ddd, J = 6.46 Hz, 1 H), 2.00 (ddd, J = 6.46 Hz, 1 H). ^{13}C NMR (125

MHz, DMSO-d_6) δ 159.7, 155.5, 152.8 (C2″), 150.5 (C8), 147.0 (C4), 132.7, 132.6, 129.2 (C4″), 127.8 (C5″), 126.7 (C7″), 122.6 (C6″), 121.3 (C8″), 119.0 (C3″), 114.6 (C1″), 100.8, 87.6 (C4′), 81.8 (C1′), 71.6 (C3′), 62.6 (C5′), 36.3 (C2′). UV−vis: 220 nm (6.8×10^4 M^{-1} cm^{-1}), 226 nm (7.5×10^4 M^{-1} cm^{-1}), 297 nm (2.5×10^3 M^{-1} cm^{-1}). HRMS (ESI$^+$-TOF): $[M + Na]^+$ calcd for $C_{20}H_{19}N_5O_6Na$, 448.1233, found 448.1229; ESI$^+$-MS/MS for the free base $[(M + H)^+ = 310]$ = 166. **2a**/**2b** ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.97 (s, 1 H), 8.59 (d, J = 8.21 Hz, 1H), 7.89 (s, 1 H), 7.86 (d, J = 8.03 Hz, 1 H), 7.49 (t, J = 8.14 Hz, 1 H), 7.35 (t, 1 H), 7.15 (d, 1 H), 6.64 (b, 2 H), 5.75 (dd, J = 6.33 and 6.01 Hz, 1 H), 5.65 (dd, J = 8.07 and 6.03 Hz, 5.39 (s, 1 H), 5.02(b, 1 H), 4.24 (ddd, J = 5.70, 5.43, and 4.50 Hz, 1 H), 3.70 (td, J = 3.81 Hz, 1 H), 3.51 (m, 2 H), 2.81 (ddd, J = 6.05 Hz, 1 H), 1.85 (ddd, 1 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.7, 156.2 (C8), 155.9 (C2″), 155.7, 132.6 (C4″), 130.0 (C9″), 129.5 (C10″), 128.6 (C5″), 127.2 (C7″), 124.4 (C8″), 123.6 (C6″), 118.1 (C1″), 112.2 (C3″), 86.4 (C4′), 82.5 (C1′), 71.5 (C3′), 63.1, 62.5 (C5′), 36.8 (C2′). UV−vis: 220 nm (3.9×10^3 M^{-1} cm^{-1}), 227 nm (4.6×10^3 M^{-1} cm^{-1}) HRMS (ESI$^+$-TOF) $[M + Na]^+$ calcd for $C_{20}H_{19}N_5O_6Na$, 448.1233, found 448.1229; ESI$^+$-MS/MS for the free base $[(M + H)^+ = 310]$ = 293, 267, 249, 225, 182, 166, and 86. **3a**/**3b** HRMS (ESI$^+$-TOF) $[M + Na]^+$ calcd for $C_{21}H_{21}N_5O_6Na$ 462.1390, found 462.1360; ESI$^+$-MS/MS for the free base $[(M + H)^+ = 324]$ = 180. **4a**/**4b** HRMS (ESI$^+$-TOF) $[M + Na]^+$ calcd for $C_{21}H_{21}N_5O_6Na$ 462.1390, found 462.1391; ESI$^+$-MS/MS for the free base $[(M + H)^+ = 324]$ = 307, 267, 225, 182, 100. **5a**/**5b** HRMS (ESI$^+$-TOF) $[M + Na]^+$ calcd for $C_{24}H_{27}N_5O_6Na$ 504.1859, found 504.1863; ESI$^+$-MS/MS for the free base $[(M + H^+)^+ = 366]$ = 323, 222, 182, 142.

Oxidation with HRP/H_2O_2. Literature protocols were followed to synthesize tri-*O*-acetyl-**OG**.[95] A 200-μL solution of tri-*O*-acetyl-**OG** (3 mM, 0.60 μmoles, 0.17 mg) and each individual phenol (0.5−5 mM, 0.10−0.50 μmoles, 0.014−0.072 mg) was mixed in sodium phosphate buffer (75 mM, pH 6.4, 7.4, 8.4) and H_2O_2 (0.5−5 mM, 0.10−0.50 μmoles, 3−14 μg). The reaction was initiated by the addition of HRP (2.5 U) and kept at 22 °C for 60 min. The reaction mixture was analyzed by HPLC.

Synthesis of N1-methyl-dOG. To a solution of **dOG** (50 mg, 177 μmoles) in 1 mL of DMSO at 22 °C was added K_2CO_3 (2.0 mg, 14 μmoles), followed by addition of four mole equivalents of CH_3I (0.1 g, 708 μmoles), and the mixture was stirred for 48 h. The reaction afforded **N1-methyl-dOG** as a white solid (mp = 225.4−227.0, decomposed), 10 mg (34 μmoles, 20% yield). ^{1}H NMR (DMSO-d_6) δ 10.60 (b, 1 H), 7.05 (b, 2 H), 6.02 (dd, J = 7.03 Hz, 1 H), 5.11 (b, 1 H), 4.71 (t, J = 5.08 Hz, 1H), 4.30 (s, 1 H), 3.70 (s, 1 H), 3.54 (m, J = 4.89 Hz, 1H), 3.29 (s, 3 H), 3.23 (ddd, J = 4.30 Hz, 1 H), 2.96 (ddd, J = 7.81 Hz, 1 H), 1.89 (ddd, J = 6.45 Hz, 1 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.6, 151.7, 150.9, 145.5, 97.9, 87.2, 80.9, 71.3, 62.3, 28.1. HRMS (ESI$^+$-TOF) $[M + H]^+$ calcd for $C_{11}H_{16}N_5O_5$ 298.1151, found 298.1162.

Synthesis of N^2-diethyl-dOG. To a solution of **dOG** (15 mg, 53 μmoles) in 40:60 MeOH/H_2O was added 80 μL acetaldehyde (63.1 mg, 1.4 mmol) and $NaBH_3CN$ (35 mg, 558 μmoles). The resulting mixture was refluxed for 48 h. The reaction afforded **N^2-diethyl-dOG** as a white solid (mp = 182.3−185.1, decomposed), 5 mg (15 μmoles, 33% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.62 (b, 2 H), 6.02 (dd, J = 7.81 Hz, 1 H), 5.39 (s, 1 H), 5.14 (d, J = 3.12 Hz, 1 H), 4.72 (t, J = 4.69 Hz, 1 H), 4.32 (d, J = 3.12 Hz, 1 H), 3.70 (q, J = 4.68 Hz, 1 H), 3.52 (q, J = 7.81 Hz, 1 H), 3.04 (dt, J = 6.25 Hz, 4 H), 1.94 (m, J = 3.12 Hz, 1 H), 1.09 (t, J = 6.25 Hz, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.62, 152.55, 146.38, 87.52, 84.75, 81.22, 71.77, 63.08, 36.19, 42.53, 13.67. HRMS (ESI$^+$-TOF) $[M + H]^+$ calcd for $C_{14}H_{21}N_5O_5Na$ 362.1440, found 362.1418.

■ ASSOCIATED CONTENT

Supporting Information. All UV−vis, HPLC, ESI$^+$-MS, ESI$^+$-MS/MS, NMR spectral data for compounds **1a**/**1b** and **2a**/**2b**, **3a**/**3b**, **4a**/**4b**, and **5a**/**5b**, **N1-methyl-dG**, and **N^2-diethyl-dOG.** HPLC and MS data for the decomposition of **1a**/**1b** and **2a**/**2b** and the pH dependence of the decomposition of **2a**/**2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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