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Biomarkers for Phenol Carcinogen Exposure Act as pH-Sensing Fluorescent Probes

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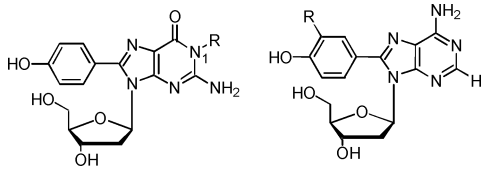
Modified nucleobases serve as biomarkers for exposure to chemical carcinogens that form DNA adducts.[1] They also serve as therapeutics,[2] bioprobes for drug design,[3] and sensors to detect light[4] or metal ions.[5] Furthermore, fluorescent nucleobases, such as 2-aminopurine, have been used to monitor mismatch dynamics,[6] to probe protein−DNA interactions,[7] and to study electron-transfer processes within duplex DNA.[8]



Our interest in modified nucleobases stems from research on DNA adduction by phenolic carcinogens. These toxins form C8-deoxyguanosine (C8-dG) nucleoside adducts via radical intermediates.[9] A representative member is the para C-adduct **1**. While this adduct is a biomarker for phenol exposure[10] and its biological properties may aid our understanding of phenol-mediated carcinogenesis, it soon became apparent that **1** could serve a dual purpose and act as a bioprobe for sensing the electronic properties of the attached purine base. Indeed, para-substituted phenols have been utilized historically to determine substituent (σ^-) constants.[11] These adducts also act as fluorophores,[9d] and phenolic groups attached to BODIPY dyes have shown a protonation/deprotonation on/off fluorescence switching mechanism that can be used in aqueous solution to sense pH.[12] The unexpected finding that **2** and **3** act similarly as pH-sensitive fluorophores prompted the synthesis of the dA analogue **4** as the first nucleobase analogue with fluorescent pH-sensing properties in the physiological pH region.

Adducts **1** and **3** were synthesized from 4-hydroxyphenylboronic acid and the appropriate 8-Br-nucleoside using a palladium-catalyzed Suzuki cross-coupling reaction (see Supporting Information for details). The spectrophotometric procedure was utilized to measure phenolic pK_a values. However, a phenolic pK_a value could not be determined for adduct **1** by titration due to overlap with N1 deprotonation (N1 pK_a of dG is 9.25[13]). For the N1-Me-dG adduct **2**, a phenolic pK_a of 8.90 was determined from basic pH titrations that showed clean isosbestic points and a shift from 279 to 292 nm with increased intensity for formation of the phenolate (Figure S1, Supporting Information). For the dA adduct **3**, a phenolic pK_a of 8.70 was determined from the titration shown in Figure 1, in which the phenolate absorbs at λ_{max} 308 nm with increased intensity compared to the neutral adduct with λ_{max} at 282 nm.

The phenolic pK_a values of **2** and **3** show that purine nucleosides are electron-withdrawing and stabilize the negative charge of the phenolate. That the dA adduct **3** is a stronger acid than **2** is consistent with the one-electron oxidation potentials (E^o) for purine nucleosides (1.42 V/NHE for A vs 1.29 V/NHE for G in neutral water[14]) that establish dG as the most electron-rich DNA base. Calculation of Hammett substituent constants for **2** and **3** provides a means to gauge the magnitude of the electron-withdrawing ability of dG and dA and draw comparison to other para substituents (Table 1). Since consistency with literature values is critical, the calculations were performed using the equation pK_a = 9.92 − 2.23($\sum\sigma$) derived by Biggs and Robinson for substituted phenols[15] that incorporates the phenol reference pK_a as 9.92 and uses a ρ value of 2.23. This equation has been used to calculate numerous σ^- values[16] and afforded a σ^- of 0.55 for dA and 0.46 for N1-Me-dG (Table 1).

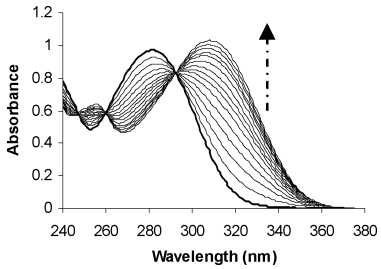


Figure 1. UV−vis pH titration of adduct **3** with pH ranging between 7 (bold line) and up to 11 (indicated by the arrow); μ = 0.1 M NaCl, 25 °C.

While the normal nucleosides are weakly fluorescent, the phenolic adducts **1**−**3** act as fluorophores and show emission at ∼390 nm with quantum yields (Φ_{fluor}) ranging from 0.25 to 0.56 at pH 7. Interestingly, their fluorescence intensity quenched upon addition of base (pH 11, Figure S2, Supporting Information). For adduct **1** at pH 11, the dianion showed λ_{em} at 408 nm with ∼50% fluorescent intensity compared to that of the neutral adduct at pH 7. In contrast, adducts **2** and **3** that form the phenolate at pH 11 and contain neutral purine bases were ∼98% quenched. These results suggested the possible utility of phenolic dA adducts as fluorescent pH indicators. However, to be of practical value for this purpose, the probe should have a ground-state pK_a ∼ 7. This prompted the synthesis of the dA analogue **4** (see Supporting Information for details) that has a calculated pK_a ∼ 7.2 (dA, σ^- = 0.55, Cl, σ_{ortho} = 0.68[17]). Using the spectrophotometric procedure, a phenolic pK_a of 7.29 was determined for **4**. Figure 2 shows the pH dependence on the emission spectrum of **4** that now displays fluorescent pH-sensing activity in the physiological pH range. The optical properties of adducts **1**−**4** are summarized in Table S1.

For other phenolic-substituted fluorophores that exhibit protonation/deprotonation on/off switching,[12] photoinduced electron transfer (PET) from the phenolate to the excited-state fluorophore acceptor has been proposed.[12] However, for the phenolic purine adducts, a similar mechanism is difficult to envision because purines are poor electron acceptors with very negative reduction potentials

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Table 1. Hammett σ^- Values for dA, N1-Me-dG, and Other Electron-Withdrawing para Substituents[a]

para substituent	σ^-	para substituent	σ^-
dA	0.55	N1-Me-dG	0.46
2-pyridyl	0.55	N=NC_6H_5	0.45
$CONH_2$	0.61	C_6F_5	0.43
$N(CF_3)_2$	0.53	SC=O(CH_3)	0.46

[a] Literature σ^- values are taken from ref 16.

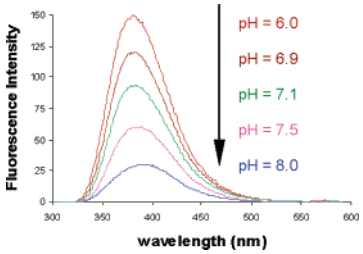


Figure 2. Emission spectra of **4** (4 μM) in water as a function of pH. Spectra were recorded with excitation at the absorbance maxima of **4** (280−308 nm) at the different pH values.

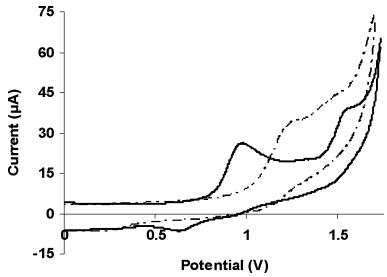
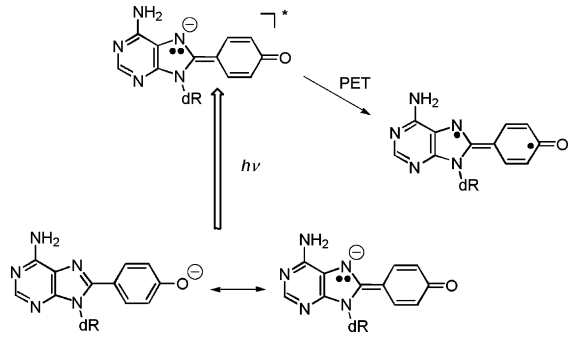


Figure 3. Cyclic voltammograms of 0.2 M **1** (solid line) and **3** (dotted line) in anhydrous DMF containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAF) using a glassy carbon (diameter 2 mm) working electrode; ν = 0.2 V/s. Potentials are versus SCE.

(dG < −2.76 V/NHE, dA = −2.5 V/NHE[18]). To gain insight into the redox properties of the parent adducts **1** and **3**, cyclic voltammetry in anhydrous DMF was employed (Figure 3). The adducts showed irreversible one-electron oxidation peaks with half-peak potentials ($E_{p/2}$) at 0.85 V/SCE for **1** and 1.08 V/SCE for **3**. Both adducts are oxidized more readily than dG, which gave $E_{p/2}$ = 1.14 V/SCE for $dG^{\bullet+}$ formation under our experimental conditions. Thus, attachment of the phenolic moiety stabilizes radical cation formation and enhances the one-electron donor characteristics of the purine nucleoside. Hence, for PET quenching, we propose that the purine base is the donor and the phenolate the electron acceptor. As shown in Scheme 1 for the phenolate of **3**, the resonance structure with negative charge at N7 has a quinone-like moiety, which is a well-known electron acceptor in PET processes.[19] For PET quenching in **2**−**4**, delocalization of the phenolate negative charge by the purine base plays a key role in generating the acceptor moiety. PET quenching is not as effective for adduct **1** (Figure S2a) since at basic pH it is a dianion and delocalization of the phenolate negative charge is not as favorable.

In summary, the C8 phenolic purine nucleoside adducts **2**−**4** are the first nucleobases to exhibit pH-sensitive fluorescent properties. The phenolic ionization constants of **2** and **3** have also permitted the first ever determination of substituent constants (σ^-) for purine nucleosides. In addition to utility as pH sensors within nucleic acids and electronic probes of purines, these adducts may

Scheme 1



help establish a basis for phenol-mediated carcinogenesis. Work to establish their pH-dependent redox properties and transient absorption spectra in aqueous media is underway, as is their conversion into phosphoramidites suitable for solid-phase DNA synthesis.

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Supporting Information Available: Experimental procedures, NMR spectra of **1**−**4**, Table S1, and Figures S1−S3 described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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