Conformational Properties of a Phototautomerizable Nucleoside Biomarker for Phenolic Carcinogen Exposure

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We have characterized the conformational properties of the C8-deoxyguanosine (C8-dG) nucleoside adduct, 8-(2"-hydroxyphenyl)-2′-dG (**1**), which is a potential biomarker for exposure to phenolic carcinogens. Adduct **1** possesses the unique ability to phototautomerize, through an excited-state intramolecular proton transfer (ESIPT) process, to generate its keto form. This tautomerization depends on the presence of an intramolecular hydrogen (H)-bond between the phenolic OH and the imine nitrogen (N7) and has permitted insight into the equilibrium ground states of adduct **1**. The results of our studies demonstrate that adduct **1** undergoes an ESIPT despite preferring a nonplanar "twisted" conformation that is imposed by the deoxyribose (dR) sugar moiety. Interestingly, a planar conformation of adduct **1** is also formed in certain aprotic solvents due to the anchoring effect of the intramolecular H-bond. Our results provide a basis for future studies aimed at determining the conformations of adduct **1** within DNA that will aid in our understanding of phenol-mediated carcinogenesis.

Introduction

Modified nucleosides are used as therapeutics,[1,2] bioprobes for drug design,[3,4] designer molecules,[5] or as biomarkers for exposure to chemical carcinogens that form covalent DNA adducts.[6,7] Their use as biomarkers has stimulated our interest from work on phenolic carcinogens that form C8-deoxyguanosine (C8-dG) nucleoside adducts through the intermediacy of radical species.[8−10] Covalent attachment of the phenoxyl radical to dG may form oxygen (O)-bonded adducts, as well as carbon (C)-bonded adducts due to the ambident nature of phenoxy radicals (Scheme 1).[11] The phenolic C-adducts shown in Scheme 1 are also produced from phenol in the presence of excess nitrite,[12] which generates ortho- and para-substituted diazonium intermediates. Other aryl C-bonded C8-dG adducts are also derived from carcinogenic aryl hydrazines that are found in the mushroom, *Agaricus bisporus*.[13−15] Our research program is focused on the synthesis and characterization of C8-dG adducts derived from phenolic toxins that are expected to cause mutations and provide a basis for phenol-mediated carcinogenesis.

Currently, we focus on the properties of the ortho-substituted C-adduct, 8-(2"-hydroxyphenyl)-2′-dG (**1**), that is structurally related to 2-(2′-hydroxyphenyl)benzimidazole (HBI) and the corresponding benzoxazole (HBO), benzothiazole (HBT) series of compounds (HBX, X = NH, O, S).[16−18] As outlined in Scheme 2, the HBX derivatives exist in a conformational equilibrium between the syn- ("closed"), anti-, and "open-" enols in the ground state. The planar "closed" syn-enol configuration that possesses an intramolecular hydrogen (H)-bond between the phenolic OH and the imine nitrogen (N) undergoes an ESIPT process, in which the ground-state syn-enol phototautomerizes to the keto form, which generates a large Stokes-shifted fluorescence. The structural and dynamic properties of this ESIPT process observed in the HBX series[16−18] have been utilized to model DNA base pair tautomerization[19−21] and to generate nonlinear optical materials.[22] These reports prompted our current study, as the interplay between ESIPT and conformational flexibility exhibited by the ortho C-adduct (Scheme 1) was expected to provide a measure for probing the ground-state structural properties of the nucleoside that would help determine biological activity. The results of our studies demonstrate that the ortho C-adduct undergoes ESIPT in certain aprotic solvents despite preferring a nonplanar "twisted" conformation that is imposed by the deoxyribose (dR) sugar moiety. Interestingly, a planar conformation of the nucleoside adduct is also formed due to the anchoring effect of the intramolecular H-bond between the phenolic OH and N7. Our results expand on the "normal" ESIPT behavior established for the planar HBX series and demonstrate this process for a modified nucleoside with biological implications.

Experimental Section

Materials and Methods. Unless otherwise noted, commercial compounds were used as received. Pyridine was distilled from KOH and stored over 3 Å molecular sieves. Anhydrous DMF was obtained from Caledon Chemicals and used without further drying. Boronic acids were purchased from Frontier Scientific. $Pd(PPh_3)_4$ was purchased from Strem Chemical and 2′-deoxyguanosine (dG) was purchased from ChemGenes. Formic acid and 3-morpholinopropanesulfonic acid (MOPS) were purchased from Sigma. Quinine bisulfate·H_2SO_4 was obtained from Aldrich and used without purification. Water used for buffers and spectroscopic solutions was obtained from a MilliQ filtration system (18.2 MΩ). Solvents not of spectroscopic grade were analyzed and corrected for spurious absorbance and emission intensities. Due to the limited solubility of nucleoside adducts, all stock solutions were made in DMSO, requiring that

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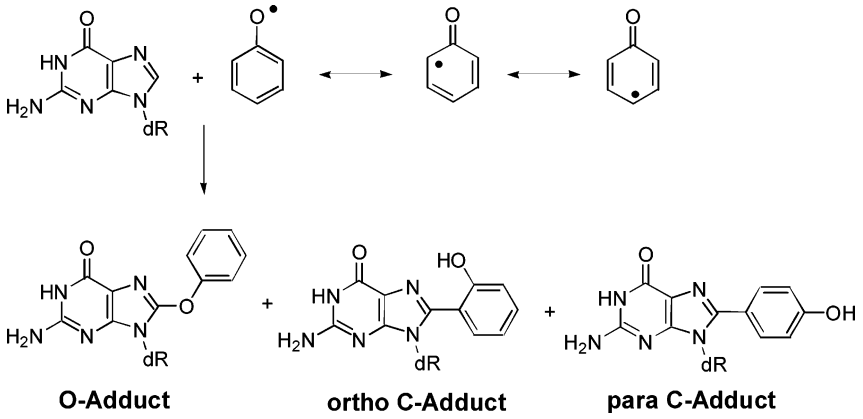
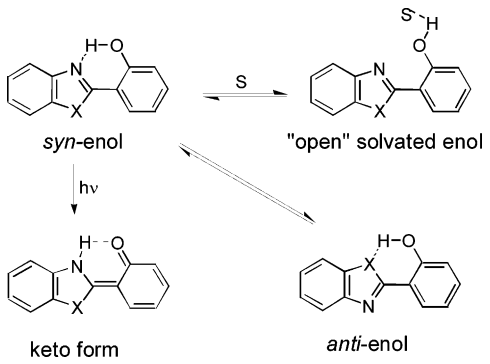
SCHEME 1



SCHEME 2



experimental spectroscopic solutions contained ≤1% DMSO by volume. The syntheses of 8-Br-dG,[23] N1-Me-dG,[24] and N1-Me-8-Br-dG[25] were performed according to the literature. Moisture-sensitive reactions were performed under an argon atmosphere using oven-dried (120 °C) glassware, syringes, and needles. Compounds were dried under vacuum and over P_2O_5 (12−24 h) prior to use in water-sensitive reactions.

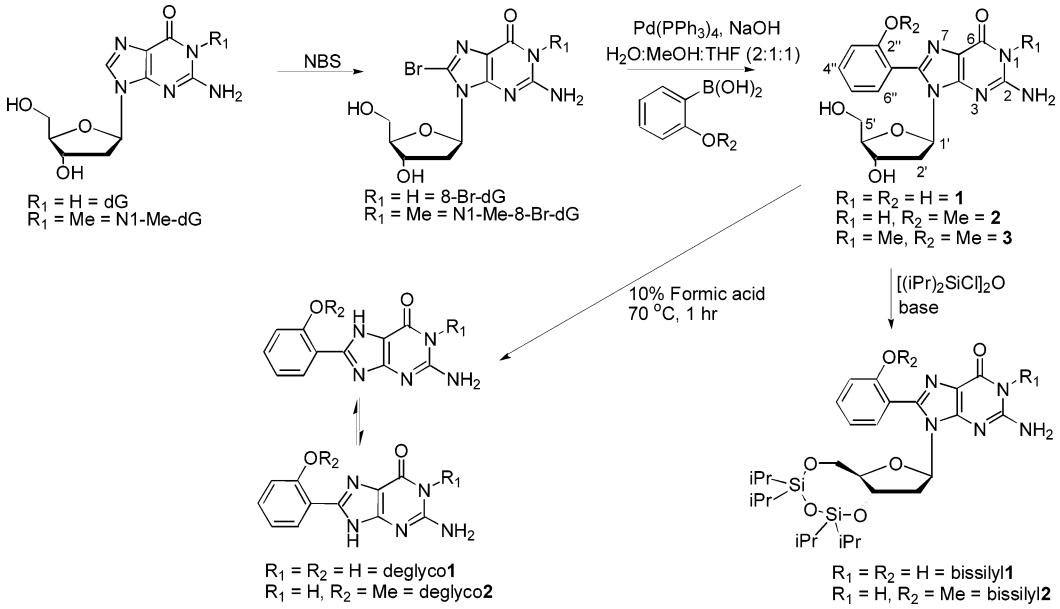
NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer (^{1}H, 300.1 MHz; ^{13}C, 75.5 MHz) in DMSO-d_6, $CDCl_3$, or CD_2Cl_2. ^{1}H NMR spectra were referenced to the residual proton solvent signal, and ^{13}C NMR spectra were referenced to the ^{13}C NMR resonance of the deuterated solvent. All ^{13}C NMR spectra were acquired using the *J*-modulated (JMOD) pulse sequence[26] with C, CH_2 carbon atoms pointing up and CH, CH_3 carbon atoms pointing down. Assignment of the ^{13}C resonances were carried out with gradient-selected ^{1}H−^{13}C long-range heteronuclear correlation (HMBC) spectra. The ^{1}H and ^{13}C sweep widths were set to 14 and 250 ppm, with 2048 points in the F2 dimension and 512 points the F1 dimension with 64 and 256 scans per time increment, respectively. Processing ^{1}H−^{13}C 2-D spectra consisted of making both F2 and F1 dimensions 1024 points. UV−vis spectra were recorded on a Cary 300-Bio UV−vis spectrophotometer equipped with a Peltier block-heating unit and temperature controller. Fluorescence emission and excitation spectra were performed on a Cary Eclipse fluorescence spectrophotometer. Standard 10 mm light path quartz glass cells from Hellma GmbH & Co. were used. All UV−vis spectra were recorded at 25 °C with baseline/background correction. Quantum yield values for nucleoside adducts were determined in MOPS buffer (10 mM, pH 7.2, 0.1 M NaCl) using the comparative method.[27−29] The pH measurements were carried out using an Accumet portable AP63 pH meter equipped with an Accumet combination microelectrode with a calomel reference. Calibration was done using commercial buffers (Fisher Scientific, pH 4.00, 7.00, and 10.00, all ±0.01). High-resolution mass spectra (HRMS) were conducted at the Mass Spectrometry Facility of Duke University or at the McMaster Regional Centre for Mass Spectrometry. Melting points are reported uncorrected.

Synthesis. General Method of Suzuki Coupling. These reactions were conducted according to the literature[30] and are briefly described here. 8-Br-dG (372 mg, 1.10 mmol) was dissolved in 60 mL of a 1:1 (THF/H_2O) solution in a 250 mL two-neck round-bottom flask. An aqueous NaOH (862 mg, 22.0 mmol) solution (20 mL) was added to the 8-Br-dG solution followed by the addition of MeOH (30 mL). The appropriate boronic acid (1.10 mmol) was weighed into a vial and taken into a glovebox where $Pd(PPh_3)_4$ (124 mg, 0.11 mmol) was added. The vial was capped, removed from the glovebox, and dissolved in THF (15 mL) to yield a yellow solution that was quickly added to the solution containing 8-Br-dG. The slightly cloudy yellow solution was refluxed overnight while kept under a N_2 atmosphere. The reaction was then allowed to cool to room temperature, and then the pH was neutralized with 1.5 M HCl. The organics were extracted with EtOAc, and the combined extracts were dried with $MgSO_4$ and filtered. The solution was concentrated and the resulting solid was chromatographed on a silica gel column using 4:1 CH_2Cl_2/acetone until a bright yellow band eluted. The solvent system was then changed to 9:1 EtOAc/MeOH, and the collected fractions were concentrated to dryness by reduced pressure.

(a) 8-(2"-Hydroxyphenyl)-2′-deoxyguanosine (1). Starting from 8-Br-dG (0.448 g, 4.18 mmol) and 2-hydroxyphenyl boronic acid (0.625 g, 4.53 mmol), adduct **1** was obtained as a white solid (1.16 g, 78%). Mp = 156−158 °C (dec); UV−vis (DMSO) λ_{max} 282 nm, ϵ_{282} 17 814 cm^{-1} M^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 1H, N1H), 10.20 (s, 1H, C2″OH), 7.34 (m, 2H, H6″, H4″), 6.98 (d, J = 8.4 Hz, 1H, H3″), 6.93 (t, J = 7.5 Hz, 1H, H5″), 6.35 (s, 2H, NH_2), 5.84 (t, J = 7.3 Hz, 1H, H1′), 5.13 (d, J = 4.1 Hz, 1H, 3′OH), 5.08 (t, J = 5.7 Hz, 1H, 5′OH), 4.33 (s, 1H, H3′), 3.72 (s, 1H, H4′), 3.62, 3.50 (m, 2H, H5′), 3.07, 2.09 (m, 2H, H2′); ^{13}C NMR (DMSO-d_6) δ 156.4, 155.8 (C2″), 152.9, 151.2 (C4), 145.3 (C8), 131.5, 131.1, 119.1, 117.24, 117.18, 115.8, 87.9 (C4′), 85.5 (C1′), 71.3 (C3′), 62.3 (C5′), 37.3 (C2′); HRMS [M + H]$^+$ calcd for $C_{16}H_{17}N_5O_5$, 360.1308; found, 360.1323.

(b) 8-(2″−Methoxyphenyl)-2′-deoxyguanosine (2). Adduct **2** was synthesized from 8-Br-dG (0.19 g, 0.55 mmol) and 2-methoxyphenyl boronic acid (0.084 g, 0.55 mmol) as a white solid (0.117 g, 57%). Mp = 159−161 °C (dec); UV−vis (DMSO) λ_{max} 283 nm, ϵ_{283} 18 800 cm^{-1} M^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.74 (s, 1H, N1H), 7.53 (t, J = 7.2 Hz, 1H, H4″), 7.39

SCHEME 3



(d, $J = 7.4$ Hz, 1H, H6″), 7.18 (d, $J = 7.4$ Hz, 1H, H3"), 7.09 (t, $J = 7.4$ Hz, 1H, H5″), 6.32 (s, 2H, NH_2), 5.66 (t, $J = 7.2$ Hz, 1H, H1′), 5.12 (d, $J = 4.2$ Hz, 1H, 3′OH), 5.05 (t, $J = 5.7$ Hz, 1H, 5′OH), 4.32 (s, 1H, H3′), 3.78 (s, 3H, OCH_3), 3.69 (s, 1H, H4′), 3.56, 3.50 (m, 2H, H5′), 3.02, 2.05 (m, 2H, H2′); ^{13}C NMR (DMSO-d_6) δ 157.5, 156.8 (C2″), 153.3, 151.5, 144.9 (C8), 132.3, 131.9, 121.0, 119.5, 117.9, 111.8, 88.2 (C4′), 85.7 (C1′), 71.6 (C3′), 62.6 (C5′), 55.7 (OCH_3), 37.8 (C2′); HRMS $[M + H]^+$ calcd for $C_{17}H_{19}N_5O_5$, 374.1465; found, 374.1461.

(c) N1-Methyl-8-(2"-hydroxyphenyl)-2′-deoxyguanosine (3). Adduct **3** was prepared from N1-Me-8-Br-dG (0.100 g, 0.28 mmol) and 2-hydroxyphenyl boronic acid (0.039 g, 0.28 mmol) as a gray solid (0.017 g, 16%). UV−vis (DMSO) λ_{max} 277 nm, ϵ_{277} 17 722 cm^{-1} M^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.2 (s, 1, C2″-OH), 7.34 (m, 2, H6″,H4″), 6.95 (m, 2, H3″, H5″), 6.87 (br s, 2, NH_2), 5.84 (t, $J = 7.2$ Hz, 1H, H1′), 5.10 (d, $J = 3.9$ Hz, 1H, 3′OH), 4.94 (t, $J = 5.7$ Hz, 1H, 5′OH), 4.34 (s, 1H, H3′), 3.70 (s, 1H, H4′), 3.58−3.65 (m, 2H, H5′, H5″), 3.34 (s, 3H, masked by residual H_2O peak N−CH_3), 2.07 (m, 1H, H2′); ^{13}C NMR (DMSO-d_6) δ 156.6, 156.3, 153.9, 150.0, 146.0, 131.9, 131.9, 131.6, 119.6, 117.6, 116.6, 116.3, 88.3, 85.7, 71.7, 62.7, 37.4, 31.2, 28.5.

Deglycosylation of 1 and 2. Deglycosylation of adducts **1** and **2** to afford guanine analogues was achieved by placing nucleosides **1** and **2** in ca. 20 mL of 10% formic acid under heat (75 °C) for 1 h. After cooling, the reaction mixtures were brought to pH 6 with 1 N NaOH and adduct-nucleobases were recovered by crystallization and filtering from the aqueous media. NMR analyses showed that this procedure of deglycosylation generated two adduct nucleobases that were ascribed to two enol tautomers shown in Scheme 3. NMR analyses did not permit unambiguous assignment of the guanine adduct structures. Peak assignments for the major tautomers ($\sim$ 80%) are given.

(d) 8-(2′-Hydroxyphenyl)-guanine (deglyco1). Treatment of adduct **1** (0.215 g, 0.60 mmol) with acid afforded deglyco**1** (0.115 g, 80%) as a white solid. UV−vis (DMSO) λ_{max} 327 nm, ϵ_{327} 20 874 cm^{-1} M^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.16 (s, 1H, NH), 12.68 (s, 1H, C2″OH), 10.76 (s, 1H, N1H), 7.87 (d, $J = 7.7$ Hz, 1H, H6″), 7.27 (t, $J = 7.8$ Hz, 1H, H4″), 6.94 (m, 2H, H3″, H5″), 6.52 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ 157.1, 155.8, 154.0, 152.2, 145.7, 130.5, 125.2, 118.9, 116.8, 112.6.

(e) 8-(2′-Methoxyphenyl)-guanine (deglyco2). Treatment of adduct **2** (0.129 g, 0.35 mmol) with acid afforded deglyco**2** (0.072 g, 80%) as a white solid. UV−vis (DMSO) λ_{max} 325 nm, ϵ_{325} 18 221 cm^{-1} M^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.96 (br s, 1H, NH), 10.61 (br s, 1H, N1H), 7.98 (d, $J = 7.5$ Hz, 1H, H6″), 7.37 (m, 1H, H4″), 7.13 (d, $J = 8.2$ Hz, 1H, H3″), 7.03 (m, 1H, H5″), 6.30 (br s, 2H, NH_2), 3.89 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 156.7, 156.1, 153.2, 152.6, 142.5, 130.3, 128.8, 120.7, 120.6, 118.7, 111.7, 55.4.

Bissilyation of Adducts 1 and 2. For bissilyation of adduct **1**, dry pyridine was used as solvent, while for the bissilyation of **2**, the reaction was carried out in dry DMF using imidazole as the base. In each case, excess 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane ($[(^iPr)_2SiCl]_2O$) was added to the cooled reaction mixtures under argon in three aliquots with stirring. The reaction mixtures were then warmed to room temperature and allowed to stir overnight. The resulting mixtures were poured into 40 mL of water and extracted with CH_2Cl_2 (3 $\times$ 50 mL). The combined organic extracts were washed with water (3 $\times$ 25 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The products were purified by flash chromatography on silica, eluting with EtOAc/hexane (5:1).

(f) 8-(2″-Hydroxyphenyl)-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl) Guanosine (bissilyl1). Adduct **1** (0.111 g, 0.309 mmol) was treated with excess $[(^iPr)_2SiCl]_2O$ (0.109 mL, 0.107 g, 0.340 mmol) to afford bissilyl**1** (0.153 g, 50% yield) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.75 (s, 1H, N1H), 10.14 (s, 1H, C2″OH), 7.30 (m, 2H, H6″, H4″), 6.95 (d, $J = 8.4$ Hz, 1H, H3"), 6.90 (t, 1H, $J = 7.6$ Hz, H5″), 6.18 (br s, 1H, NH_2), 5.94 (dd, 1H, $J = 7.9$, 5.4 Hz, H1′), 4.34 (m, 1H, H3′), 3.85 (m, 1H, H4′), 3.72−3.60 (m, 2H, H5′), 3.06, 2.28 (m, 2H, H2′), 1.09−0.90 (m, 28H); ^{1}H NMR ($CDCl_3$) δ 11.66 (br s, 1H, N1H), 10.55 (br s, 1H, C2"OH), 7.74 (d, $J = 7.4$ Hz, 1H, H6″), 7.33 (m, 1H, H5″), 7.08 (d, $J = 8.1$ Hz, 1H, H3″), 6.96 (m, 1H, H5″), 6.16 (dd, $J = 7.6$, 5.2 Hz, 1H, H1′), 5.94 (br s, 2H, NH_2), 5.24 (m, 1H, H3′), 4.08 (m, 1H), 4.00 (m, 1H), 3.90 (m, 1H), 3.27, 2.36 (m, 2H, H2′), 1.11−1.01 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 157.2, 156.1, 151.8, 151.1, 146.5,

130.4, 127.4, 118.4, 116.5, 115.1, 112.2, 84.2, 82.2, 72.1, 62.4, 60.7, 36.9, 16.5, 16.5, 16.3, 16.2, 16.0, 12.4, 12.2, 11.9, 11.5; HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{28}H_{44}N_5O_6Si_2$, 602.2830; found, 602.2828.

(g) 8-(2″-Methoxyphenyl)-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl) Guanosine (bissilyl2). Adduct **2** (0.215 g, 0.58 mmol) was treated with excess $[(^iPr)_2SiCl]_2O$ (0.24 mL, 0.236 g, 0.75 mmol) in DMF containing excess imidazole (0.196 g, 2.88 mmol) to afford bissilyl**2** (0.087 g, 25% yield) as a white solid. ^1H NMR (CD_2Cl_2) δ 12.39 (br s, 1H), 7.49 (m, 2H), 7.08 (m, 2H), 6.78 (s, overlapped with previous, 2H), 5.71 (m, 1H), 5.04 (br s, 1H), 3.90 (m, 2H), 3.83 (s, 3H, OCH_3), 3.67 (m, 1H), 3.10 (br s, 1H), 2.34 (s, 1H), 1.01 (m, 28H); ^{13}C NMR (CD_2Cl_2) δ 159.1, 157.5, 153.4, 151.7, 145.6, 132.0, 131.8, 121.0, 118.9, 117.2, 111.0, 84.7, 83.1, 73.6, 64.2, 39.0, 17.3, 17.2, 17.1, 16.8, 13.3, 13.2, 12.9, 12.7.

Determination of pK_a Values. Acidity constants (pK_a) were determined spectrophotometrically by monitoring absorbance changes in the UV−vis spectra after additions of dilute HCl or NaOH solutions under constant temperature conditions (25 °C). Ionic strength (μ) was kept constant at a concentration of 0.1 M using NaCl. After temperature equilibration, solutions were blank corrected and pH measurements were taken directly in the quartz cuvettes. Following each addition of acid or base, the pH of the solution was measured and UV−vis spectra were recorded by the overlay method in the wavelength range of 250−400 nm. The pK_a values were determined from a plot of the log(ionization ratio) vs pH, as described.[31]

Results and Discussion

Synthesis. Adduct **1** and the methoxy analogue **2**, which served as a non- proton-transfer model, were synthesized from 8-Br-dG and the appropriate boronic acids using a palladium-catalyzed Suzuki cross-coupling reaction (Scheme 3).[30] A sample of the N1-Me analogue **3** was also synthesized by the Suzuki cross-coupling reaction starting from the known N1-Me-8-Br[25] that is prepared by treating N1-Me-dG[24] with NBS. The sample of **3** was utilized to unambiguously determine a phenolic pK_a value. Nucleoside adducts **1** and **2** were also treated with (i) $[(^iPr)_2SiCl]_2O$ to modify the dR moiety and afford bissilyl adducts with solubility in $CHCl_3$ suitable for NMR analyses and (ii) formic acid to facilitate deglycosylation and generate guanine analogues (deglyco**1**, deglyco**2**). This procedure of deglycosylation generated two adduct nucleobases in a 4:1 ratio that were ascribed to the two enol tautomers shown in Scheme 3. The bissilyl and deglyco analogues helped provide insight into the conformational equilibria of adduct **1**.

Spectral Properties in Aqueous Media. The spectral properties of the nucleoside adducts **1**−**3** were initially examined in aqueous MOPS buffer, pH 7.0 and in water under basic (pH 11) and acidic (pH 2.0) conditions by setting the pH to 11 or 2 using NaOH or HCl (Figure 1). At neutral pH, adducts **1**−**3** showed a single absorption at ∼280 nm, $\epsilon \sim 18\,000$ cm^{-1} M^{-1}. Unlike the normal nucleosides that show nominal fluorescence with quantum yields for dG $\sim 9.7 \times 10^{-5}$,[32] the C8-dG adducts act as fluorophores and show emission at ∼395 nm with quantum yields ranging from 0.44 to 0.78 at pH 7. That the emission maxima at pH 7 were almost identical for the three adducts indicates that the phenolic adducts **1** and **2** do not undergo ESIPT in aqueous media and that the emission at ∼395 nm is due to the enol tautomer. Increasing the pH to 11 generated a new absorption with reduced intensity at 305 nm for adducts **1** (Figure 1a) and **3** (Figure 1c) but not for the methoxy adduct **2** (Figure 1b), suggesting that the phenolate of

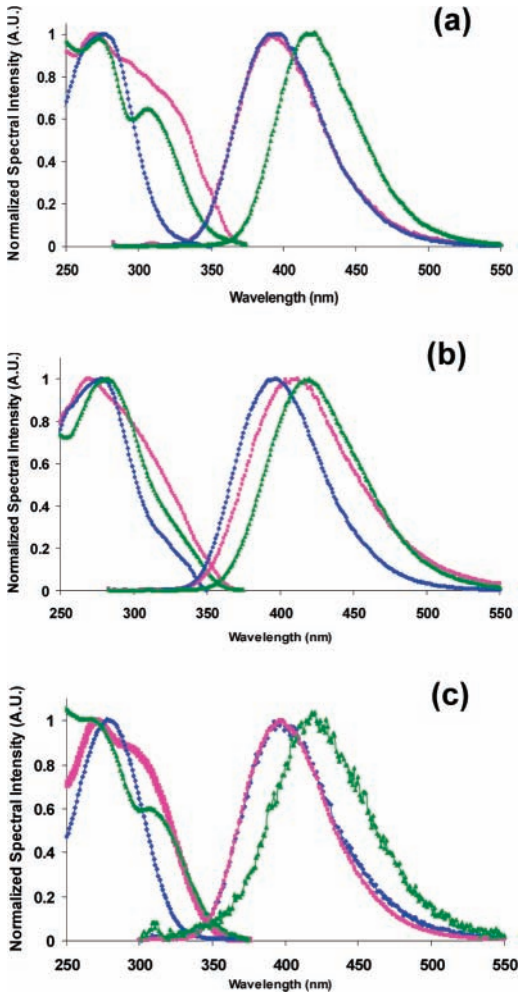


Figure 1. Normalized absorbance and emission spectra of (a) **1**, (b) **2**, and (c) **3** in buffered water (3-morpholinopropanesulfonic acid (MOPS), 10 mM, pH 7, 0.1 M NaCl) (◆, blue), water at pH 2.0 (■, pink), and water at pH 11 (▲, green). Emission spectra recorded with excitation at 280 nm.

1 and **3** has an absorbance at 305 nm. The emission spectra of **1**−**3** at pH 11 exhibited maxima at 420 nm. Compared with the emission at pH 7, the emission at pH 11 was considerably quenched for adduct **3** but not for **1** and **2**. In general, lowering the pH to 2 caused a broadening of the absorbance bands but did not dramatically affect the emission spectra that resembled the spectra recorded at pH 7 (Figure 1).

The spectrophotometric procedure was utilized in an effort to measure the phenolic and N7 pK_a values of adduct **1**. For the HBX series (Scheme 2), a water-soluble HBI analogue possesses pK_a values of 5.90 for protonation of the benzimidazole nitrogen and 8.63 for deprotonation of the phenolic group,[18] while the N7 pK_a of dG is 2.34.[33] In the case of **1**, increasing or decreasing the pH failed to generate isosbestic points and pK_a values could not be extracted from the titrations. In fact, for all three nucleoside adducts, N7 pK_a values could not be obtained due to adduct instability at pH < 2, which was ascribed to deglycosylation. In the basic region, adducts **2** and **3** showed clean isosbestic points (Figure 2) that permitted determination of pK_a values. For the methoxy adduct **2**, the addition of NaOH caused a decrease in the absorbance at 276 nm that was accompanied by a slight bathochromic shift; isosbestic points at 296 and 240 nm were observed (Figure 2a). A pK_a value of 9.21 was measured that was attributed to deprotonation of N1; the corresponding value for dG is 9.25.[33] The N1-Me adduct **3** showed two isosbestic points at 257 and

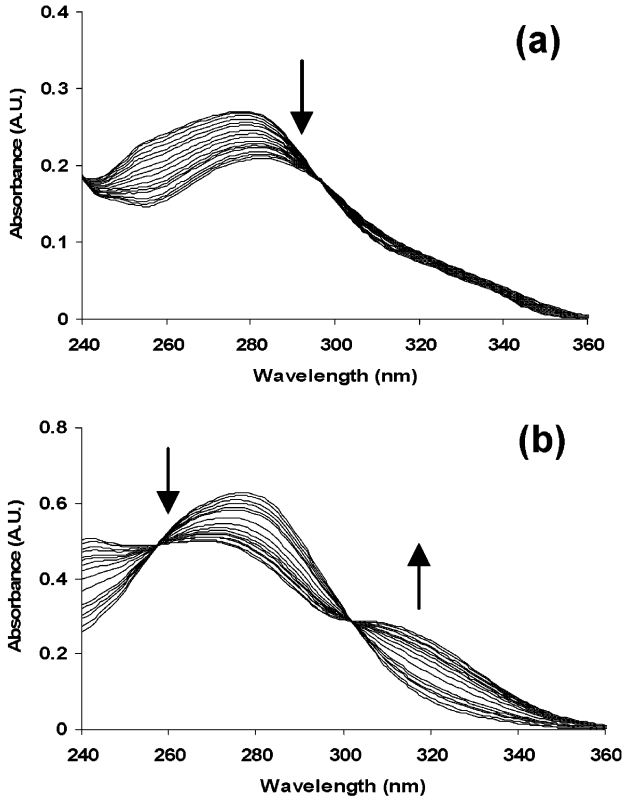


Figure 2. UV−vis absorbance of (a) adduct **2** and (b) adduct **3** as a function of pH ranging between 7 and 11, μ = 0.1 M NaCl, 25 °C.

TABLE 1: Photophysical Data and Protonation Constants of Adducts 1, 2, and 3

adduct	absorption λ_{max} (nm), ϵ (cm^{-1} M^{-1})[a]	emission λ_{max} (nm), Φ_{Fluor}[a]	pK_a (N1)[b]	pK_a (PhOH)[b]
1	276, 17932	395, 0.44		
2	278, 18595	397, 0.78	9.21 ± 0.03	
3	277, 17722	396, 0.57		9.05 ± 0.02

[a] 10 mM MOPS buffer, pH 7.0, μ = 0.1 M NaCl. [b] Determined from UV−vis titrations at 25 °C, μ = 0.1 M NaCl.

300 nm (Figure 2b) and yielded a pK_a of 9.05 for deprotonation of the phenolic moiety. These results confirmed that adduct **1** exists as a dianion at pH 11 due to deprotonation of both N1 and the phenolic group. The results of these studies are summarized in Table 1.

Solvent Effects and Conformational Equilibria. The photophysical properties recorded for **1** and **3** in aqueous media showed only enol emission at ~395 nm, suggesting that in water the intramolecular H-bond required for ESIPT is interrupted. The spectral properties of **1**, and the control **2**, were examined in aprotic solvents to determine whether **1** can in fact undergo ESIPT, as observed for the HBX series.[16−18] Figure 3a shows absorption and emission spectra of **1** in buffered water and hexane. In hexane, excitation at 280 nm gave an emission that was considerably red shifted (476 nm, Stokes shift 14 834 cm^{-1}) and was ascribed to the ESIPT emission of the keto tautomer, highlighting the ability of **1** to undergo ESIPT in a nonpolar environment.

The absorption spectrum of **1** in hexane also displayed a shoulder at ~320 nm that was not present in water (Figure 3a). Figure 3b shows the absorption spectra of **1** in a wider range of aprotic solvents where the absorbances at 320 and 280 nm are of almost equal intensity in chloroform ($CHCl_3$) and acetonitrile (MeCN), while the 320 nm absorbance is completely absent in DMSO. Excitation-dependent emission spectra shown in Figure

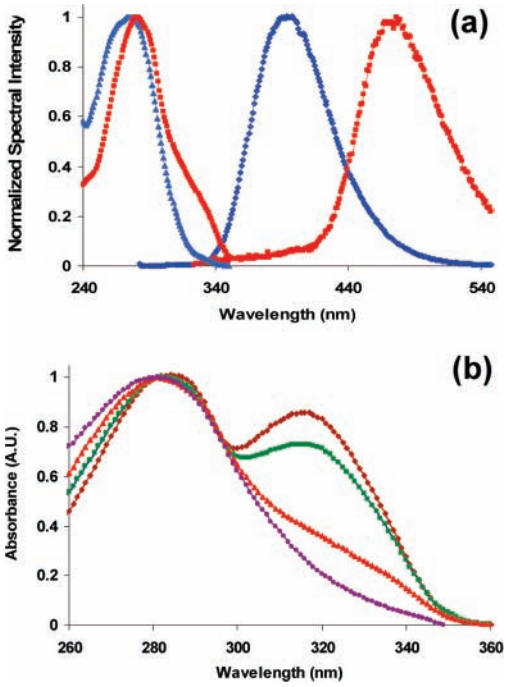


Figure 3. (a) Normalized absorbance and emission spectra of **1** in buffered water (MOPS), 10 mM, pH 7, 0.1 M NaCl (◆, blue) and hexane (■, pink). Emission spectra recorded with excitation at 280 nm. (b) Absorbance spectra of **1** (22 μM) in $CHCl_3$ (◆, dark red), MeCN (■, green), hexane (▲, bright red), and DMSO (●, purple).

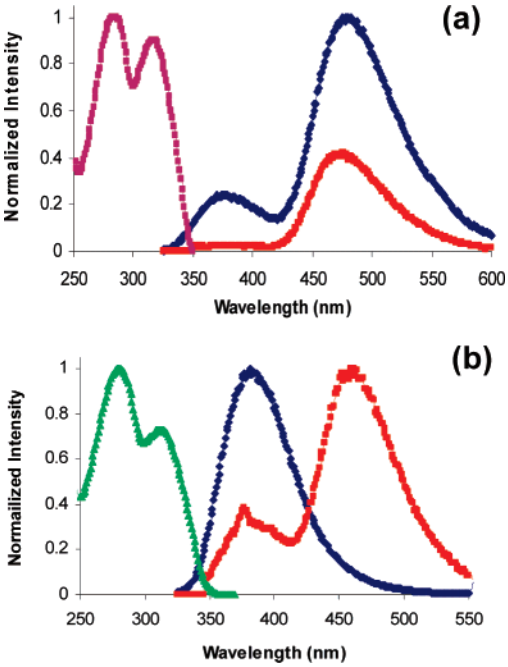


Figure 4. Absorbance and excitation-dependent emission spectra of **1** obtained at excitation wavelengths of 280 nm (◆, blue) and 340 nm (■, red) in (a) $CHCl_3$ and (b) MeCN where the original intensity of emission from excitation at 340 nm was much less than that for excitation at 280 nm.

4 demonstrated that excitation at 280 nm in $CHCl_3$ gives rise to both enol (383 nm) and keto (481 nm) emission (Figure 4a), while only the enol emission was found in MeCN (Figure 4b). In contrast, excitation at 340 nm in both solvents generated the keto emission preferentially. The methoxy adduct **2** failed to generate the low-energy absorbance at 320 nm and the keto emission in aprotic solvents (Figure S1, Supporting Information). These results suggested the possibility that the 320 nm absorp-

TABLE 2: Photophysical Data and Protonation Constants of deglyco1 and deglyco2

adduct	absorption λ_{max} (nm), ϵ (cm^{-1} M^{-1})[a]	emission λ_{max} (nm), Φ_{Fluor}[a]	pK_a (N7)[b]
deglyco**1**	319, 18887	393, 0.20	3.19 ± 0.02
deglyco**2**	320, 14910	398, 0.96	3.12 ± 0.09

[a] 10 mM MOPS buffer, pH 7.0, μ = 0.1 M NaCl. [b] Determined from UV−vis titrations at 25 °C, μ = 0.1 M NaCl.

tion, which exhibits a red shift of 40 nm from the enol absorption at 280 nm and preferentially gives rise to the keto emission, represents a ground-state keto absorbance of adduct **1**.

In an effort to determine the structure of **1** responsible for the 320 nm absorbance, **1** and **2** were treated with (i) $[(^iPr)_2SiCl]_2O$ to modify the dR moiety and afford bissilyl adducts with solubility in $CHCl_3$ suitable for NMR analyses and (ii) formic acid to facilitate deglycosylation and generate guanine analogues (deglyco**1** and deglyco**2**) to probe the role of the sugar moiety (Scheme 3). The absorbance spectrum of bissilyl**1** in $CHCl_3$ (Figure S2, Supporting Information) was identical to the UV characteristics recorded for **1** in the same solvent (Figure 3b) and showed the double maxima at 280 and 320 nm. The $^1H-^{13}C$ long-range heteronuclear correlation (HMBC) spectrum of bissilyl**1** in $CDCl_3$ (Supporting Information) showed that C2″ resonated at ∼155 ppm, as noted for **1** in DMSO-d_6 where it exists as the enol tautomer. This observation ruled out ground-state tautomerization of **1** to the keto form, as suggested for HBI in water,[16,18] because a downfield shift of C2″ toward 180 ppm for the conjugated carbonyl in the keto form would have been expected.[34,35]

The spectral properties of the guanine analogues, deglyco**1** and deglyco**2**, in MOPS buffer, pH 7.0, along with N7 pK_a values are given in Table 2. The absorption spectra for both guanine adducts were structured with λ_{max} ∼ 320 nm, features similar to those noted for the planar HBX series[16−18] and occurring at the same red-shifted maxima noted for the nucleoside adduct **1** in $CHCl_3$ and MeCN (Figure 3b and Figure 4). In buffered water at pH 7.0, deglyco**1** only gives rise to enol emission at 393 nm, suggesting solvation and disruption of the intramolecular H-bond needed for ESIPT to the keto form. This observation contrasts with a benzimidazole (HBI) derivative that does undergo ESIPT to form the keto tautomer in buffered water at pH 7.2.[18] However, for deglyco**1**, a pK_a value of 3.19 was determined for protonation of N7 (Table 2), which is 2.7 pK_a units below the pK_a value for protonation of the benzimidazole nitrogen of the HBI derivative.[18] Thus, the HBI derivative forms a stronger intramolecular H-bond and must exist in the syn-enol conformation shown in Scheme 2 in buffered water. In contrast, the weaker intramolecular H-bond predicted for deglyco**1** must favor the "open" solvated enol in water that cannot undergo ESIPT.

Figure 5a shows absorption and emission spectra of deglyco**1** in buffered water and DMSO. In DMSO, excitation at 325 nm yields the Stokes-shifted keto fluorescence band at 460 nm. Figure 5b highlights the absorption and emission spectra of deglyco**1** vs nucleoside **1** in DMSO. Clearly, the dR moiety in **1** causes a 40 nm blue shift in absorption and inhibits ESIPT to form the keto emission. The phenolic OH proton of **1** resonates at δ 10.20 ppm in DMSO-d_6, while the corresponding proton of deglyco**1** resonates at δ 12.68 ppm (Supporting Information). This downfield shift in the phenolic OH proton resonance coupled with the 40 nm red shift in the absorbance spectra are consistent with the planar H-bonded "closed" syn-enol configuration for deglyco**1** in DMSO and a nonplanar "twisted" conformation for nucleoside **1** that lacks the intramolecular

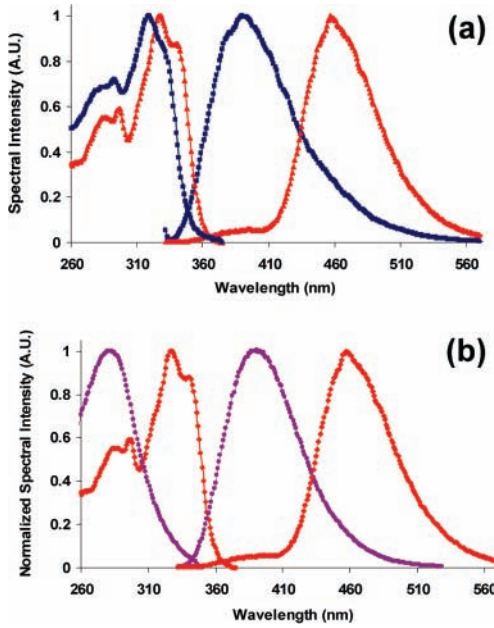
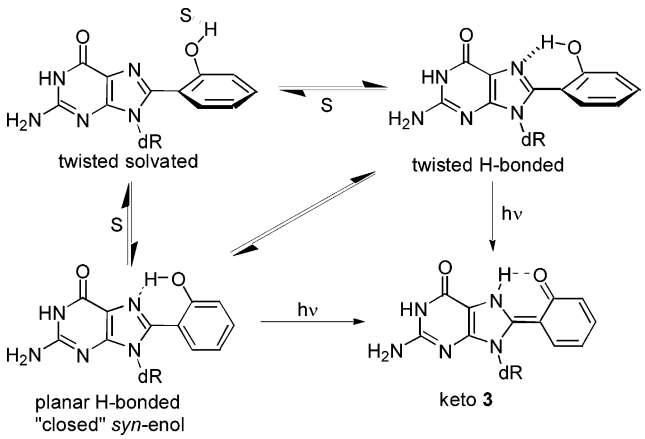


Figure 5. (a) Absorbance and emission spectra of deglyco**1** in buffered water (MOPS, 10 mM, pH 7, 0.1 M NaCl) (■, blue) and DMSO (◆, red); emission spectra recorded with excitation at 325 nm. (b) Absorbance and emission spectra for **1** (●, purple) and deglyco**1** (◆, red) in DMSO. Excitation wavelengths for **1** and deglyco**1** were 280 and 325 nm, respectively.

SCHEME 4



H-bond. Since the enol emission of deglyco**1** and adduct **1** in water occurs at essentially the same maxima also implies that the nonplanar "twisted" adduct **1** achieves planarity in the excited state, as noted for biphenyls.[36] The results also suggest that **1** fluctuates between planar (λ_{max} ∼ 320 nm) and nonplanar "twisted" (λ_{max} ∼ 280 nm) conformations in $CHCl_3$, MeCN, and hexane solvents (Figure 3b). That the methoxy adduct **2** fails to generate the 320 nm absorbance even though deglyco**2**, like HBI, favors a planar structure (Table 2) suggests strongly that the intramolecular H-bond in the nucleoside **1** acts as an anchor to stabilize the planar structure that generates the keto emission preferentially (Figure 4).

The results of our studies have provided insights into the conformational equilibria of adduct **1**, which are summarized in Scheme 4. In water and DMSO, **1** exists in a solvated (S) nonplanar "twisted" conformation that cannot undergo ESIPT. In hexane, **1** undergoes ESIPT despite absorbing strongly at ∼280 nm for a nonplanar "twisted" conformation, suggesting the predominance of a twisted H-bonded species. In $CHCl_3$ and MeCN, the equilibrium shifts more in favor of the planar

H-bonded conformation, as the absorbance at ~320 nm grows in intensity (Figure 3b). These results highlight the solvent dependence on the equilibrium ground states of adduct **1** and show how the dR moiety often leads to a favored nonplanar orientation, as noted for fleximer nucleoside bioprobes.[3,4]

Conclusions

The ortho-substituted C-adduct, 8-(2"-hydroxyphenyl)-2′-dG (**1**), is a potential biomarker for exposure to phenolic toxins. The spectral properties of adduct **1** have been determined to provide a basis for understanding its structural and biological impact within duplex DNA. Adduct **1** is structurally related to 2-(2′-hydroxyphenyl)benzimidazole (HBI) and the corresponding benzoxazole (HBO), benzothiazole (HBT) series of compounds (HBX, X = NH, O, S) that undergo an excited-state intramolecular proton transfer (ESIPT) process, in which the ground-state syn-enol phototautomerizes to the keto form, which generates a large Stokes-shifted fluorescence. Our results demonstrate that adduct **1** exists in a nonplanar "twisted" conformation ($\lambda_{max} \sim 280$ nm) in aqueous media and DMSO and fails to undergo ESIPT to generate the keto form. However, in hexane, $CHCl_3$, and MeCN, the nucleoside adduct fluctuates between nonplanar and planar ($\lambda_{max} \sim 320$ nm) conformations and undergoes ESIPT to generate the keto tautomer. These features should provide a basis for understanding the local solvent environment of adduct **1** within duplex DNA. If the adduct forms normal Watson−Crick H-bonding base-pairs with deoxycytosine and the phenolic moiety is exposed to the aqueous media, then a nonplanar adduct structure that does not undergo ESIPT may be expected. However, given the tendency of C8-dG adducts to adopt a syn orientation, the phenolic moiety of **1** may be turned into the duplex and point away from the aqueous solvent. Such an orientation may favor a planar structure that undergoes ESIPT to generate the keto form preferentially. Work on the conversion of **1** into a phosphoramidite suitable for solid-phase DNA synthesis is currently underway to determine the sequence-dependent behavior of this adduct whose biological properties are predicted to aid our understanding of phenol-mediated carcinogenesis.

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Supporting Information Available: Figures S1 and S2 described in the text and NMR characterization of synthetic products. This material is available free of charge via the Internet at http://pubs.acs.org.

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