Structure and Properties of $C^8$-Aryl-2'-Deoxyguanosine Adducts: From Mutagenic Lesions to Conformational Probes in Duplex DNA

by

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A significant focus of toxicological research is the identification of electrophiles that covalently modify DNA to form addition products (adducts). These products can be generated when aryl radical species react at the C^8-site of 2'-deoxyguanosine (dG) to form C^8-aryl-dG adducts, which are mutagenic lesions. While this form of DNA modification is detrimental, C^8-aryl-dG adducts also possess intriguing properties that can be exploited for beneficial purposes. This thesis is an investigation of one mechanism believed to contribute to the mutagenicity of C^8-aryl-dG adducts, as well as a study of the photophysical properties of adducts that allow for their application as fluorescent probes.

A common property of C^8-aryl-dG adduction is accompaniment of abasic site formation. To determine how the C^8-aryl moiety contributes to sugar loss, UV-Vis spectroscopy has been employed to determine hydrolysis kinetics, with C^8-aryl-dG adducts found to be more prone than dG to acid-catalyzed hydrolysis. Despite adduct reactivity in acidic media, all adducts are relatively stable at pH 7, suggesting they are unlikely intermediates of abasic site formation at physiological pH. These results have allowed for development of a new rationale for depurination observed upon C^8-aryl-dG.
adduction within duplex DNA.

The determination of photophysical parameters of C^8^-heteroaryl-dG adducts reveals that these nucleosides behave as fluorophores with high fluorescence quantum yields (φ_f). These adducts also exhibit emission sensitivity to their solvent environment and H-bonding interactions. C^8^-Heteroaryl-dG adducts were incorporated in the oligonucleotide 5'-CTCG1G2CG3CCATC, at the G_1 and G_3 sites, that contains the recognition sequence of the NarI Type II restriction enzyme. Hybridization of the modified NarI oligonucleotides to the complementary strand containing either the C or G nucleobase opposite the adduct allowed for characterization of duplex structures by circular dichroism (CD), UV melting temperature analysis and fluorescence spectroscopy. Results suggest that the C^8^-heteroaryl-dG adduct favours an anti conformation with base-paired with C, while a syn conformation is favoured when base-paired to G. Adduct conformation of bulky C^8^-dG adducts is believed to be correlated with their known mutagenic activity. C^8^-Heteroaryl-dG modified nucleosides could therefore be used as fluorescent models of these adducts to aid in elucidation of adduct-induced mutagenesis in biological systems.
In Loving Memory of

Tom & Mary McInnis

(Grampie & Nannie)
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LIST OF ABBREVIATIONS AND SYMBOLS

Å  angstrom
\( \hbar \)  Planck’s constant
\( \Delta G^e \)  Gibbs energy of activation
\( \Delta H^e \)  enthalpy of activation
\( \Delta S^e \)  entropy of activation
\( \Delta \nu \)  Stokes shift
\( \Theta \)  molar ellipticity
\( \delta \)  NMR chemical shift
\( \varepsilon \)  dielectric constant or molar extinction coefficient
\( \eta_s \)  solvent refractive index (standard)
\( \eta_x \)  solvent refractive index (C\(^8\)-heteroaryl-purine adduct)
\( \theta \)  dihedral twist angle (\( \angle(C^{11}C^{10}C^8N^9) \))
\( \lambda \)  wavelength
\( \mu \)  ionic strength
\( \mu_g \)  dipole moment
\( \rho \)  Hammett plot slope; reaction constant
\( \sigma^+ \) or \( \sigma_p \)  Hammett substituent constant
\( \phi_f \)  fluorescence quantum yield
\( \phi_{f_i(s)} \)  fluorescence quantum yield of standard
\( \phi_{f_i(x)} \)  fluorescence quantum yield of C\(^8\)-heteroaryl-purine adduct
10-mer  decanucleotide
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absorbance of adduct at excitation wavelength

AAF  $N$-2-acetylaminofluorene

ABA  3-aminobenzanthrone

Ac  acetyl

Adap  adenosine-1,3-diazaphenoxazine

adduct  addition product

AF  $N$-2-aminofluorene

AP site  apurinic site

Ar-B(OH)$_2$  aryl-boronic acid

aq  aqueous

A. U.  arbitrary unit

B-type  major groove binding conformation

BER  base excision repair

$B_{fur}dA$  8-(2"-benzofuryl)-2'-deoxyadenosine

$B_{fur}dG$  8-(2"-benzofuryl)-2'-deoxyguanosine

BgQ  benzoquinazoline

bissilyl$^{2\text{Pyr}}dG$  8-(2"-pyrrolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine

bissilyl$^{Bth}dG$  8-(2"-benzothienyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine

bissilyldC  3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxycytidine

bissilyldG  3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine

bissilyl$^{\text{Ind}}dG$  8-(2"-indolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine
BP  benzo[a]pyrene
BPDE  benzo[a]pyrene diol epoxide
bs  broad singlet (NMR signal)
Bth dA  8-(2''-benzothiophenyl)-2'-deoxyadenosine
Bth dG  8-(2''-benzothienyl)-2'-deoxyguanosine
C  cytosine
calcd  calculated
CD  circular dichroism
CHOPb dG  8-(4''-formylphenyl)-2'-deoxyguanosine
CNPh dG  8-(4''-cyanophenyl)-2'-deoxyguanosine
CV  cyclic voltammetry
d  doublet (NMR signal)
D-A  donor-acceptor
dA  2'-deoxyadenosine
dC  2'-deoxycytidine
DFT  density functional theory
dG  2'-deoxyguanosine
DMABN  4-(N,N-dimethylamino)-benzonitrile
DMF  N,N-dimethylformamide
DMPx  2,7-dimethylpixyl
DMSO  dimethyl sulfoxide
DMTr  dimethoxytrityl
D<sub>n</sub> leaving group dissociation
DNA deoxyribonucleic acid
dR 2'-deoxyribose
dT 2'-deoxythymidine
dU 2'-deoxyuridine

<math>\ E_a \end{math} activation energy
EDTA ethylenediaminetetraacetic acid
<math>\ E_{p/2} \end{math} half-peak oxidation potential
eq equivalent(s)
ESI electrospray ionization

Et<sub>3</sub>N triethylamine
<math>\ E_T(30) \end{math> electronic transition energy; empirical scale of solvent polarity
EWG electron-withdrawing group

<math>\ F \end{math> fluorescence emission intensity in the presence of natural nucleobase or quencher
<math>\ F_o \end{math> fluorescence emission intensity in the absence of natural nucleobase or quencher
FAF \textit{N}-2-fluoroaminofluorene
Fmoc-Cl 9-fluorenylethoxycarbonyl chloride
FRET fluorescence resonance energy transfer

<math>\ F_s \end{math> integrated area under emission curve of standard
FTIR fourier transform infrared
<math>\ F_x \end{math> integrated area under emission curve of \textit{C}^8\text{-heteroaryl-purine adduct
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>guanine</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>Gh</td>
<td>guanidinohydantoin heterocycle</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>I</td>
<td>fluorescence intensity</td>
</tr>
<tr>
<td>ICD</td>
<td>induced circular dichroism</td>
</tr>
<tr>
<td>( ^{\text{Ind}} \text{dA} )</td>
<td>8-(2''-indolyl)-2'-deoxyadenosine</td>
</tr>
<tr>
<td>( ^{\text{Ind}} \text{dG} )</td>
<td>8-(2''-indolyl)-2'-deoxyguanosine</td>
</tr>
<tr>
<td>( I_{\text{rel}} )</td>
<td>relative emission intensity</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>rate constant for the glycosidic bond cleavage of the monoprotonated species</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>rate constant for the glycosidic bond cleavage of the diprotonated species</td>
</tr>
<tr>
<td>( K_a )</td>
<td>acid dissociation constant or association equilibrium constant</td>
</tr>
<tr>
<td>( k_B )</td>
<td>Boltzmann’s constant</td>
</tr>
<tr>
<td>( k_{\text{obs}} )</td>
<td>observed first-order rate constant</td>
</tr>
<tr>
<td>( K_{\text{sv}} )</td>
<td>Stern-Volmer quenching constant</td>
</tr>
<tr>
<td>( l )</td>
<td>cuvette path length</td>
</tr>
<tr>
<td>L</td>
<td>ligand</td>
</tr>
<tr>
<td>LE state</td>
<td>locally excited state</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LIAD</td>
<td>laser-induced acoustic desorption</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>m</td>
<td>multiplet (NMR signal)</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MePh$_d$G</td>
<td>8-(4''-tolyl)-2'-deoxyguanosine</td>
</tr>
<tr>
<td>mer</td>
<td>denotes length of oligonucleotide</td>
</tr>
<tr>
<td>MOPS</td>
<td>3-(N-morpholino)propanesulfonic acid</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NarI</td>
<td>5'-CTCGGCGCCATC</td>
</tr>
<tr>
<td>NarI(10-mer)</td>
<td>5'-GATGG--CCGAG</td>
</tr>
<tr>
<td>NarI(C)</td>
<td>5'-GATGGCGCCGAG</td>
</tr>
<tr>
<td>NarI(G)</td>
<td>5'-GATGGCGCGAG or 5'-GATGGGGGCGAG</td>
</tr>
<tr>
<td>NarI$_1$</td>
<td>5'-CTCXGCGCCATC</td>
</tr>
<tr>
<td>NarI$_3$</td>
<td>5'-CTCGGCXCCATC</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>$o$-substituent</td>
<td>ortho-substituent</td>
</tr>
<tr>
<td>OAc</td>
<td>acetate</td>
</tr>
<tr>
<td>O$_{OHPh}$dG</td>
<td>8-(4''-hydroxyphenyl)-2'-deoxyguanosine</td>
</tr>
<tr>
<td>$o$_{MePh}dG</td>
<td>8-(2''-tolyl)-2'-deoxyguanosine</td>
</tr>
</tbody>
</table>
OMePh-dG 8-(4''-methoxyphenyl)-2'-deoxyguanosine
oOHPh-dG 8-(2''-hydroxyphenyl)-2'-deoxyguanosine
oOMePh-dG 8-(2''-methoxyphenyl)-2'-deoxyguanosine
OTA ochratoxin-A
p-substituent para-substituent
PA proton affinity
PAH polycyclic aromatic hydrocarbon
PCP pentachlorophenol
PDN or PRODAN 6-propionyl-2-dimethylaminonapthalene
PET photoinduced electron transfer
Ph phenyl
pKa ionization constant
PNA peptide nucleic acid
iPrPac-Cl 4-isopropylphenoxyacetyl chloride
Px 9-phenylxanthen-9-yl
Py pyrenyl
Pyr pyridyl
Q quencher
QdA 8-(8''-quinolyl)-2'-deoxyadenosine
QdG 8-(8''-quinolyl)-2'-deoxyguanosine
QdG(TBDMS)2 8-(8''-quinolyl)-3',5'-tris((tert-butyldimethylsilyl)oxy)-2'-deoxyguanosine
R gas constant
R  ribose
rA  riboadenosine
rG  riboguanosine
rG(TBDMS)₃  2',3',5'-tris-(tert-butylidimethylsilyl)riboguanosine
RNA  ribonucleic acid
ROS  reactive oxygen species
RP  reverse-phase
rT  ribothymidine
rU  ribouridine
s  singlet (NMR signal)
S₀  ground state
Sₙ, n > 1  excited state
S-type  stacked conformation
SCE  saturated calomel electrode
S₅₁  unimolecular nucleophilic substitution
S₅₂  bimolecular nucleophilic substitution
SNP  single nucleotide polymorphism
Sp  spiroiminodihydantoin
St  phenylethenyl
t  triplet (NMR signal)
T  temperature or thymine
½  half-life
TBAF  tetrabutylammonium hexafluorophosphate
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBDMS-Cl</td>
<td>tert-butyldimethylsilyl chloride</td>
</tr>
<tr>
<td>tC</td>
<td>1,3-diaza-2-oxophenothiazine</td>
</tr>
<tr>
<td>tC(^\circ)</td>
<td>1,3-diaza-2-oxophenoxazine</td>
</tr>
<tr>
<td>tC(_{\text{nitro}})</td>
<td>7-nitro-1,3-diaza-2-oxophenothiazine</td>
</tr>
<tr>
<td>TEAA</td>
<td>triethylammonium acetate</td>
</tr>
<tr>
<td>TICT</td>
<td>twisted intramolecular (or internal) charge transfer</td>
</tr>
<tr>
<td>(T_m)</td>
<td>melting temperature</td>
</tr>
<tr>
<td>TMS-Cl</td>
<td>trimethylsilyl chloride</td>
</tr>
<tr>
<td>TPPTS</td>
<td>tris(3-sulfophenyl)phosphine trisodium salt hydrate</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UV-vis</td>
<td>ultraviolet-visible</td>
</tr>
<tr>
<td>V</td>
<td>vinyl</td>
</tr>
<tr>
<td>VPy</td>
<td>vinylpyrenyl</td>
</tr>
<tr>
<td>W-C</td>
<td>Watson-Crick</td>
</tr>
<tr>
<td>W-type</td>
<td>wedged conformation</td>
</tr>
<tr>
<td>X</td>
<td>halide</td>
</tr>
</tbody>
</table>
Chapter 1.

Introduction
1.1 Background Information

1.1.1 Overview of DNA and its Components

Deoxyribonucleic acid (DNA) is the genetic material for all cellular organisms, and most importantly, serves to store and transmit biological information. DNA is comprised of simple units of nucleotides, which are characterized by three main features: a nitrogenous base, a pentose and a phosphate. The molecule lacking the phosphate moiety is known as a nucleoside. The pentose is a 2'-deoxyribose sugar that exists in one of a variety of conformations generally described as ‘puckered’. The nitrogenous bases, which include guanine (G), adenine (A), thymine (T) and cytosine (C), are heterocyclic compounds that are derivatives of two parent compounds, purine and pyrimidine. The purine and pyrimidine bases are hydrophobic and relatively insoluble in water. The purine family of nucleosides includes 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA), while the pyrimidine family includes 2'-deoxythymidine (dT) and 2'-deoxycytidine (dC), as shown in Figure 1-1. An additional pyrimidine nucleoside, known as 2'-deoxyuridine (dU), is typically found only in ribonucleic acid (RNA). Its structure matches that of dT without the 5'-methyl group. The numbering convention for the pentose ring, and purine and pyrimidine bases is also given in Figure 1-1.
The successive nucleotides of DNA are covalently linked through phosphate-group ‘bridges’, in which the 5’-phosphate group of one nucleotide is attached to the 3’-hydroxyl group of the next nucleotide, thereby creating a phosphodiester linkage, as shown in Figure 1-2. This covalent backbone of DNA is hydrophilic. By convention, the structure of a single-strand of DNA is always written in the 5’ to 3’ direction, with the 5’-end lacking a nucleotide at the 5’-position, while the 3’-end lacks a nucleotide at the 3’-position, as shown in Figure 1-2. A polymer of DNA containing ~ 10 – 100 monomer building blocks is commonly referred to as an oligonucleotide, while a longer polymer is known as a polynucleotide. The term ‘mer’ is commonly used to denote the length of a oligonucleotide; a fragment of 15 nucleotides, for example, would be called a 15-mer.
The formation of hydrogen bonds (H-bonds) between the nitrogenous bases allows for a complementary association of two strands of DNA. The most important H-bonding patterns are those defined by Watson and Crick in 1953, in which A bonds to T via two H-bonds and G bonds to C via three H-bonds, that form the two types of Watson-Crick (W-C) base pairs that predominate in double-stranded DNA. The atoms that participate in W-C H-bonding comprise what is commonly referred to as the ‘W-C face’ of the respective base.
In addition to standard W-C type H-bonding, recognition of the complementary base can occur by Hoogsteen-type base pairing. \(^4\) Hoogsteen base-pairing is defined by the participation of the \(N^7\)-, and \(O^6\)- or \(N^6\)-position atoms of the purines in H-bonding with the W-C face of the complementary pyrimidine base, with H-bonding patterns of Hoogsteen base-pairs shown in Figure 1-4. The Hoogsteen base-pair of G with C is most stable at low pH, as a protonated cytosine is required for H-bonding. In the base-pair, the ionization constant (\(pK_a\)) of cytosine is \(> 7.5\), which is considerably higher than its normal value of 4.2.
1.1.2 Structure of DNA

(a) Double Helix

The three-dimensional model of the structure of DNA, largely predicted by Watson and Crick, consists of two strands of nucleic acids wound around the same axis to form a right-handed double helix. The hydrophilic backbone is on the outside of the double helix, facing the surrounding water. The hydrophobic purine and pyrimidine bases are stacked inside the double helix, with the interior of the helix determined to possess a dielectric constant ($\epsilon$) of $3 - 5$. The offset pairing of the two strands facilitates a major groove and a minor groove on the surface for the duplex, as shown in Figure 1-5. The two strands are both antiparallel and complementary to one another. This double helical structure is held together by two important forces; H-bonding between the base-pairs and base-stacking interactions, which include a combination of Van der Waals and dipole-dipole interactions.¹

![Figure 1-5. Double helical structure of DNA, showing the major and minor grooves. Image credit: http://education.technyou.edu.](image_url)
The W-C DNA structure is also known as B-form DNA, and is the most stable structure for DNA under physiological conditions. Variations on this DNA conformation include A-form and Z-form DNA; A-form DNA is also right-handed and favoured in dehydrated solutions, while Z-form DNA possesses a left-handed helical rotation with an almost non-existent major groove and deep minor groove. These three forms of DNA also differ in base pairs per helical turn and rise in the helix per base pair.³

Characterization of the A-, B- or Z-form of DNA can be performed using circular dichroism (CD) spectroscopy.⁶,⁷ This analytical measurement is based on the differential absorption of left and right circularly polarized light, with compounds that exhibit characteristic CD spectra possessing either an asymmetric chromophore or a symmetric chromophore in an asymmetric environment. In the case of DNA, the purine and pyrimidine bases are chromophores with a plane of symmetry, with CD induced by their interactions with the asymmetric environment of the double helix.⁶ The reliance of CD spectroscopy measurements for determination of DNA structure stems from their sensitivity, the requirement for a relatively small amount of material in solution and their non-destructive nature.⁷ On the basis of the measured CD spectra, the structural conformation of a double helix can be determined. The CD spectrum of normal B-form DNA exhibits roughly equal positive (~ 275 nm) and negative (~ 240 nm) bands, with a crossover at ~ 260 nm. The CD spectrum of A-form DNA exhibits large positive (~ 270 nm) and small negative (~ 230 nm) bands, with a crossover at ~ 240 nm, while the CD spectrum of Z-form DNA exhibits small positive (~ 285 nm) and large negative (~ 245 nm) bands, with a crossover at ~ 280 nm.⁷
(b) Triplex and Quadruplex Structures

Although the most abundant form of DNA is the double-stranded helical conformation, it is known that DNA can adopt alternative secondary structures, such as those involving three or four nucleic acid strands.

Three nucleic acid strands wound around each other is known as a triplex. In this structure, a third strand binds to a double-stranded structure through Hoogsteen base-pairing. Triplex formation has been implicated in gene regulation and occurs most readily within long sequences containing only pyrimidines or purines in a given strand. Triplexes can therefore be comprised of two pyrimidine strands and one purine strand, while others contain two purine strands and one pyrimidine strand.

Four-stranded stable secondary structure motifs known as guanine (G)-quadruplexes also exist, and are formed by the folding of one nucleic acid strand or by the association of two or more strands. These structures occur readily for DNA sequences rich in guanine bases and form by cooperative self-association or intermolecular association via Hoogsteen base-pairing. Templating alkali metal cations such as sodium (Na\(^+\)) and potassium (K\(^+\)) are required to stabilize the formation of G-quadruplexes. The base-pairing orientation of a G-quadruplex is shown in Figure 1-6. G-quadruplexes have been implicated in a variety of biological systems and functions of tremendous importance. These higher-ordered structures are found in telomeres, regions of guanine-rich repeating nucleotide sequences located at the ends of chromosomes that play a major structural and regulatory role in chromosomal maintenance. G-quadruplexes may also be involved in recombination and mutation 'hot spot' processes, gene regulation and various human diseases.
1.1.3 Properties of DNA

(a) Oxidative Character

The oxidative properties of the purines and pyrimidines have been extensively studied. Guanine is the most easily oxidized of the nucleobases because it has the lowest oxidation potential; therefore most oxidizing agents attack guanine with a greater frequency than the other nucleobases. Guanine has an oxidation potential of 1.29 V versus normal hydrogen electrode (NHE), while adenine, cytosine and thymine have oxidation potentials of 1.42, 1.6 and 1.7 V/NHE, respectively.\(^9\)

(b) Photophysical Parameters

The purines and pyrimidines are highly conjugated molecules, with resonance among atoms in the ring giving most of the bonds partial double-bond character.\(^2\) As a result of resonance, these DNA molecules are considered chromophores, and absorb ultraviolet (UV) light through excitation of electrons in the ground state (\(\pi\)) orbital to excited state (\(\pi^*\)) orbitals.\(^{20}\) Nucleic acids are characterized by a strong absorption at

![Figure 1-6. H-bonding pattern in a G-quadruplex.](image-url)
wavelengths ($\lambda$) near 260 nm. The specific absorption wavelength maxima, as well as molar extinction coefficients ($\epsilon$), which reflects how strongly a molecule absorbs light at a given wavelength, are provided for each nucleoside in Table 1-1.²¹

<table>
<thead>
<tr>
<th>adduct</th>
<th>$\lambda_{\text{abs/max}}$ (nm)ᵃ</th>
<th>$\epsilon_{\text{max}}$ ($\times 10^3$ M⁻¹ cm⁻¹)ᵃ</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\phi_{\text{fl}}$ ($\times 10^4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dG</td>
<td>253</td>
<td>13.8</td>
<td>334</td>
<td>0.97</td>
</tr>
<tr>
<td>dA</td>
<td>260</td>
<td>14.9</td>
<td>307</td>
<td>0.86</td>
</tr>
<tr>
<td>dT</td>
<td>267</td>
<td>9.86</td>
<td>330</td>
<td>1.32</td>
</tr>
<tr>
<td>dC</td>
<td>271</td>
<td>9.30</td>
<td>328</td>
<td>0.89</td>
</tr>
</tbody>
</table>

ᵃData recorded in aqueous solutions, as taken from Reference no. 21.

It is important to point out that conjugation between two or more chromophores tends to result in shifts in the absorbance maximum to longer wavelengths. This phenomenon is referred to a red-shift, or bathochromic shift, while a shift to a shorter wavelength is known as a blue-shift, or hypsochromic shift.²⁰ The conjugation between two or more chromophores can occur from the attachment of an aryl molecule at a specific purine or pyrimidine site to form a modified nucleoside, such as those discussed in section 1.2.3 (b).

Following absorption of light from the ground state ($S_0$) to an excited state ($S_n$, $n > 1$), the excited molecule will return to the ground state following two successive steps. The molecule at $S_n$ will first return to the lowest excited state ($S_1$) by dissipating a part of its energy in the surrounding environment in a radiationless process known as internal conversion. From the $S_1$ state, the molecule will then reach the ground state via different competitive processes; emission of a photon (fluorescence), dissipation of energy in the form of heat, energy transfer to nearby molecules via collisional quenching or
intersystem crossing to the excited triplet state. In return of the excited molecule to the ground state via emission, the maximum in the resulting emission spectrum will be shifted to longer wavelengths compared to the maximum of the absorption spectrum, with this occurrence known as a Stokes shift.

A molecule’s emissive character can be expressed in terms of fluorescence quantum yield ($\phi_{fl}$), which gives a measure of the efficiency of the fluorescence process. Quantum yield is defined as the ratio of the number of photons emitted to the number of photons absorbed, and can be obtained by comparing the fluorescence intensity of the molecule with that of a reference molecule with a known quantum yield. For highly fluorescent molecules, $\phi_{fl} \approx 1$, while $\phi_{fl} \approx 0$ for weakly fluorescent molecules. The purines and pyrimidines are practically non-emissive, with exceedingly low fluorescence quantum yields, associated with excited state lifetimes on the order of picoseconds. Quantum yields for the nucleosides, along with their emission maximum, are provided in Table 1-1. It is important to note that modifications to the nucleobases can dramatically alter their photophysical characteristics, resulting in a fluorescent molecule, as will be discussed at length in Chapter 3.

(c) Thermal Melting

When DNA is exposed to heat, denaturation, or melting, of the double helix can occur. In denaturation, base stacking and H-bonding between the base-pairs are disrupted resulting in unwinding of the double helix to form two single strands. Renaturation of a DNA molecule is a rapid one-step process, as long as a double-helical segment still unites the two unwound strands. Upon cooling, the unwound segments of the two strands
spontaneously rewind, or anneal, to yield the double helix. Renaturation is a two-step process if the two strands are completely separated. Upon cooling, the two strands first ‘find’ each other by random collisions and form a short double-helical segment. The remaining unpaired bases then register as base pairs, and the two strands ‘zipper’ together to form the double helix.²⁶

When a double helix is denatured, the UV absorption of DNA increases, with this phenomenon known as the hyperchromic effect. By contrast, a decrease in absorbance that occurs upon renaturation is known as the hypochromic effect.² The transition from double-stranded to single-stranded DNA, or vice versa, can therefore be monitored using a UV-Vis spectrophotometer, by recording the absorbance at 260 nm as a function of temperature. The result is a characteristic melting curve, with an example shown in Figure 1-7, that allows for the determination of the melting temperature (\(T_m\)), also known as the temperature of mid-transition, the value of which provides a measure of stability of the DNA sequence.²⁶ Nucleic acids with a higher content of G:C than A:T base pairs have a higher \(T_m\), as G:C base pairs, with three H-bonds, require more heat energy to dissociate than A:T base pairs.²

![Figure 1-7. Thermal melting curve. Image credit: www.els.net/WileyCDA.](image-url)
1.2 DNA Damage

1.2.1. Mutagenesis

The chemistry of DNA damage is diverse and complex. DNA is not indefinitely stable, and numerous sources of DNA-damaging agents of endogenous and exogenous origin can contribute further to its instability. Examples of both exogenous and endogenous species responsible for DNA damage include alcohols, pesticides, certain metals, dietary components and air pollution. When DNA is damaged, a cellular response is induced that includes a wide range of enzymatic systems that catalyze DNA repair. Unrepaired damage to DNA can lead to a change in the nucleic acid sequence, which, if replicated and transmitted to future cell generations, becomes permanent. This heritable change in the DNA sequence is known as a mutation. Much evidence suggests that the accumulation of mutations is strongly linked to the processes of aging and carcinogenesis.

Some types of DNA mutations are described here briefly. A base-substitution mutation involves the replacement of a nucleotide with another. These mutations can be classified as transversions, in which a purine is substituted by a pyrimidine, or vice versa, or transitions, in which a purine is substituted with another purine or a pyrimidine is substituted with another pyrimidine. An insertion mutation involves the addition of one or more nucleotides or base pairs into a DNA sequence, while a deletion mutation involves the loss of one or more nucleotides or base pairs. Both of these events can give rise to frameshift mutations, which results when a given number of nucleotides are not divisible by three. As the reading frame for the genetic code is of a triplet nature, insertion or deletion of nucleotides can change the reading frame, resulting in a different
translation than originally intended. Some of the forms of DNA damage that can give rise to mutagenesis are discussed in the following sections.

1.2.2 Hydrolysis of Nucleotides

Hydrolysis of the C1'-Aβ glycosidic bond between the nucleobase and deoxyribose sugar can occur spontaneously. The resulting site in the DNA sequence that lacks the nitrogenous base is known as an abasic site. Depurination, which refers to the hydrolysis of a purine, is known to occur at a much higher rate than depyrimidination. Approximately one in 10^5 purines is lost from DNA every 24 h under typical cellular conditions. Hydrolysis of DNA will be discussed extensively in Chapter 2.

Nucleotide bases can also undergo spontaneous hydrolysis of their exocyclic amino groups, in a process known as deamination. Deamination of cytosine, for example, occurs in about one of every 10^7 of these residues in 24 h, under normal cellular conditions.

1.2.3 Oxidative Damage

Oxidative metabolism is essential to life, as the oxidation of organic compounds allows for the release of free energy required for many life functions. Oxidative metabolism does, however, also result in the formation of reactive oxygen species (ROS), which cause oxidative stress and have been implicated in cell death (apoptosis), mutations, aging and cancer. The generation of ROS is an endogenous process, often formed as by-products in the mitochondria during respiration. One-electron transfer to molecular oxygen yields the superoxide anion radical (O_2•^-), which in turn is
converted to hydrogen peroxide (H$_2$O$_2$). Reaction of H$_2$O$_2$ with Fe$^{2+}$ yields the highly reactive hydroxyl radical (•OH), which has the ability to abstract hydrogen atoms and add directly to DNA bases. Oxidative DNA damage caused by ROS commonly includes strand scission. Oxidizing reagents can lead to direct strand scission or direct modification to the nucleobase that renders the DNA subject to cleavage in a subsequent step, usually involving deglycosylation and β-elimination of the 3'-phosphate.

The reactive oxygen species •OH is known to react directly with dG to form 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG), shown in Figure 1-8. 8-Oxo-dG is the most common oxidative lesion observed in duplex DNA and subsequently serves as a biomarker for cellular oxidative damage. Several studies have focused on misreading and mutations induced by 8-oxo-dG. It has been shown that while 8-oxo-dG forms a W-C base pair with dC, a Hoogsteen-like base pair can also be formed with dA. The latter base pair has led 8-oxo-dG to be misread during replication as dT, resulting in G to T transversions. As a result of the damage to DNA and potential mutations that can be caused by 8-oxo-dG, a family of DNA repair enzymes has evolved to deal specifically with this lesion.

---

**Figure 1-8.** 8-oxo-dG.
The further oxidation of 8-oxo-dG has also been the focus of much attention, as it is more easily oxidized than any of the normal nucleobases, with an oxidation potential of 0.74 V/NHE.\textsuperscript{41} Electrochemical oxidation of 8-oxo-dG on a glassy carbon electrode revealed oxidation is a reversible process involving a two-electron two-proton charge transfer.\textsuperscript{42,43} Chemical oxidation of 8-oxo-dG has been performed by use of a variety of one-electron oxidants such as IrCl\textsubscript{6}\textsuperscript{2−}, Fe(CN)\textsubscript{6}\textsuperscript{3−} and SO\textsubscript{4}\textsuperscript{−}.\textsuperscript{44,45} The two-electron oxidation and rearrangement\textsuperscript{46} of 8-oxo-dG by these chemical oxidants results in the formation of two major products, the guanidinohydantoin heterocycle (Gh) and spiroiminodihydantoin (Sp),\textsuperscript{44} shown in Figure 1-9. These hydantoin lesions of 8-oxo-dG have been shown to be more detrimental to the genome than 8-oxo-dG itself, as the Gh lesion induces G to C transversions, while the Sp lesion induces both G to T and G to C transversions.\textsuperscript{47,48}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1-9.png}
\caption{The guanidinohydantoin heterocycle (Gh) and spiroiminodihydantoin (Sp) lesions.}
\end{figure}
1.2.4 Adduct Formation

(a) General Overview

Each nucleobase is vulnerable to covalent modification by electrophiles at various positions of the nitrogenous base to form addition products (adducts). Substantial evidence illustrates that these modified nucleobases are biomarkers for carcinogen exposure and if left unrepaired, mutations. The study of such biomarkers may allow for the discovery of the origin of many incidences of human cancer.\textsuperscript{49-52}

The formation of covalent adducts can either occur as a direct process, or require metabolic activation of the carcinogen to an electrophile.\textsuperscript{1,53,54} There are a number of enzymes that work to activate chemical carcinogens, including glutathione transferase, N-acetyltransferase, UDP-glucuronosyl transferase and sulfotransferase.\textsuperscript{55,56} The pattern of DNA adduct formation is not only highly dependent on the reactive intermediate involved, but also on the nucleobase sites available for modification,\textsuperscript{49,57} as electrophiles are site-specific in their reactivity. The target sites for covalent modification are nucleophilic atoms with lone pairs of electrons or a negative charge. ‘Hard’ electrophiles, such as carbonium and nitrenium ions, prefer to react with similarly ‘hard’ nucleophilic centers, such as the lone pairs of electrons on the amino nitrogen and keto oxygen atoms of the nucleobases. The pattern of adduct formation also differs greatly within duplex DNA, due in part to the steric and electronic environment of double-stranded DNA.\textsuperscript{1}

Figure 1-10 depicts the site-specific modification of nucleophilic sites of dG by electrophiles.\textsuperscript{58} Chemical carcinogens that transfer alkyl moieties tend to react with the endocyclic $N^{7}$- and exocyclic $O^{6}$-atoms.\textsuperscript{58,59} Examples of products formed from
electrophilic alkylating agents, such as dimethylnitrosamine, dimethylsulfate, S-adenosylmethionine and nitrogen mustard, include adducts such as $N^7$-methyl-dG or $N^7$-ethyl-dG.\textsuperscript{2} Larger $N^7$-alkyl-dG adducts, such as those generated from reaction with the epoxide of bioactivated aflatoxin, a naturally occurring mycotoxin, can also be formed.\textsuperscript{60} The $N^2$- and $N^1$-sites are both susceptible to modification by Michael acceptors\textsuperscript{58} and quinone methides.\textsuperscript{61} In addition, the $N^2$-atom is commonly targeted by polycyclic aromatic hydrocarbons (PAHs).\textsuperscript{58} PAHs, many of which are potent carcinogens, are ubiquitous environmental pollutants produced in the combustion of organic matter,\textsuperscript{50,62} with sources including furnaces, gasoline and diesel engines, and tobacco smoke.\textsuperscript{50} The $C^8$-site of dG is known to be prone to attack by free aryl radicals,\textsuperscript{1,63} including those derived from aromatic and heterocyclic amines, nitroaromatics, PAHs, and small aryl and phenol moieties.\textsuperscript{58}

![Diagram](image)

**Figure 1-10.** Site-specific modification of dG by chemical carcinogens.

*(b) $C^8$-Aryl-dG Modified Nucleosides*

As the $C^8$-site is prone to attack by aryl radicals, there are numerous known examples of $C^8$-aryl-dG adducts, which can be classified as $O$-linked, $N$-linked or $C$-
linked. The \( O \)-linked \( C^8 \)-aryl-dG adduct (PCP-dG) shown in Figure 1-11 was determined by Manderville and co-workers to form from reaction with the phenolic radical generated following the chemical oxidation of pentachlorophenol (PCP).\[^{58,64,65}\]

![PCP-dG](image)

**Figure 1-11.** \( O \)-linked \( C^8 \)-aryl-dG adduct.

The \( N \)-linked \( C^8 \)-aryl-dG adducts shown in Figure 1-12 include those derived from the potent aromatic amine carcinogens \( N \)-2-aminofluorene (AF-dG),\[^{66,67}\] \( N \)-2-acetylamino-\( \text{fluorene} \) (AAF-dG)\[^{68-70}\] and \( N \)-2-fluoroaminofluorene (FAF-dG),\[^{71,72}\] and are known to induce frameshift mutations.\[^{66,69,70}\] Other examples of \( N \)-linked adducts include those originating from the heterocyclic amine, and known dietary mutagen,\[^{30}\] \( IQ \) (IQ-dG),\[^{73-75}\] aminopyrene, a metabolite of the carcinogen 1-nitropyrene (AP-dG)\[^{76}\] and the carcinogen 4-aminobiphenyl (ABP-dG),\[^{77}\] as shown in Figure 1-12.
Examples of C-linked C^8-aryl-dG adducts, as shown in Figure 1-13, include those derived from phenolic radicals, such as the OTA-dG adduct derived from the mycotoxin ochratoxin-A (OTA), widely found as a contaminant to food products,^{78-80} and the Ni(II)-dG complex derived from a metallopeptide-peptide nucleic acid (PNA) bioconjugate.^{81} The adduct N-Ac-ABA-dG is derived from the N-acetyl derivative of 3-aminobenzanthrone (ABA), a metabolite of the carcinogenic nitroaromatic environmental contaminant 3-nitrobenzanthrone,^{82} while the adduct 6-BP-8-dG is derived from the carcinogenic PAH, benzo[a]pyrene.^{62,83,84} Finally, C-linked C^8-aryl-dG adducts are also formed from reaction with aryl radicals derived from carcinogenic arylhydrazines, with examples including 8-p-CH\textsubscript{3}Ph-dG, 8-p-COOHPh-dG and 8-p-CH\textsubscript{2}OHPh-dG.^{85-87}
Much attention has been given to the formation of C-linked C$^8$-aryl-dG adducts from radical species of arylhydrazines, as arylhydrazine metabolites have been shown to induce skin and gastric cancer in mice.\textsuperscript{88} Arylhydrazines are commonly found in the species \textit{Agaricus bisporus}, the edible mushroom of commerce in the Western hemisphere.\textsuperscript{89,90} Arylhydrazines undergo metabolism to form arenediazonium ions, which then undergo reductive decomposition to form aryl radicals by the removal of N$_2$, as shown in Scheme 1-1.\textsuperscript{86,87}
In order to develop an understanding of the mechanism of carcinogenicity of arylhydrazines, Kohda and co-workers synthesized 8-phenyl-2'-deoxyguanosine (8-Ph-dG) as a model adduct derived from an aryl radical, and following oligonucleotide incorporation and primer extension reactions, discovered that the 8-Ph-dG lesion induced G to T and G to C transversions, in addition to one- and two-base deletions. Gannett and co-workers found that treatment of calf thymus DNA with arenediazonium ions facilitated the formation of C-linked C^8-aryl-dG adducts but also produced a significant amount of depurination. Subsequently, it was postulated that the formation of abasic sites that coincides with C^8-aryl adduction of dG may contribute to the carcinogenicity of arylhydrazines, as will discussed further in Chapter 2. Gannett and colleagues have also examined the effect of such adduct formation on DNA conformation through application of molecular modeling, NMR spectroscopy and thermal denaturation, to learn that the 8-Ph-dG lesion stabilizes Z-form DNA. As this DNA structure is believe to play a role in the regulation of gene expression, the Gannett group proposed that stabilization of the Z-form by 8-Ph-dG may be related to carcinogenicity of aryl radicals.

In general, all C^8-aryl-dG adducts can induce structural changes to DNA. The effect of the C^8-aryl moiety on the conformation of the C1'-N^9 glycosidic bond is of particular interest. The conformation of the glycosyl linkage appears to be an important
factor in adduct persistence in vivo, and furthermore, may influence the toxicological properties of DNA adducts.\textsuperscript{73} For purine bases, the two preferential, sterically allowed, conformations of this linkage are termed \textit{anti} or \textit{syn}.\textsuperscript{73} In normal W-C base pairs, the \textit{anti} conformation is energetically favoured and thus predominates, as shown in Scheme 1-2 for dG.\textsuperscript{93} However, when bulky substituents are attached at the $C^8$-position, the glycosidic bond no longer favours the \textit{anti} conformation, and instead rotates to adopt the \textit{syn} conformation.\textsuperscript{74,93,94} Thermodynamic calculations have estimated that $C^8$-substitution favours the \textit{syn} conformation by $1 - 2$ kcal mol$^{-1}$.\textsuperscript{93,95} The \textit{anti} versus \textit{syn} conformational preference has been analyzed readily for \textit{N}-linked $C^8$-aryl-dG adducts, with $^1$H NMR spectroscopy utilized by the Turesky group to determine the preferred conformation of an IQ-dG adduct,\textsuperscript{73} and crystal structures determined by Ellenberger and co-workers for the AAF-dG adduct.\textsuperscript{96} Structural analyses revealed that the \textit{anti} conformation is strongly unfavourable due to steric hindrance between the acetyl group and the deoxyribose sugar moiety, and therefore, the \textit{syn} conformation predominates, for bulky \textit{N}-linked $C^8$-aryl-dG adducts\textsuperscript{73,96} as shown in Scheme 1-2 for the AAF-dG adduct. The highly distorting \textit{syn} conformation of $C^8$-aryl-dG adducts can interfere with base-pairing, by potentially inducing a base-substitution mutation or posing a strong block to replication. Furthermore, the ability of $C^8$-aryl-dG adducts to halt replication may promote frameshift mutagenesis.\textsuperscript{96}
(c) Manderville Group Research

Research conducted by the Manderville group originates from interest in C-linked $C^8$-aryl-dG adducts derived from phenolic toxins and carcinogenic arylhydrazines. This interest has given rise to the synthesis and study of various $C^8$-phenyl substituted dG nucleoside adducts in order to better understand their biological implications.

Chris McLaughlin investigated the oxidative properties of different C-linked $C^8$-aryl-dG adducts by electrochemical oxidation, as this method has proved successful for the oxidation of arylamine-linked $C^8$-dG adducts.\textsuperscript{46} Oxidation potentials were determined by cyclic voltammetry (CV), with C-linked $C^8$-aryl-dG adducts found to be more readily oxidized than dG. For example, the 8-(4"-hydroxyphenyl)-2'-dG ($^{\text{OHPh}}$dG)
adduct was found to possess a half-peak oxidation potential ($E_{p/2}$) of 0.85 versus saturated calomel electrode (SCE), considerably lower than $E_{p/2}$ determined for dG (1.14 V/SCE).\(^{97}\)

Mark Sun discovered that while the normal nucleobases are weakly fluorescent, phenolic $C^8$-purine adducts act as fluorophores at pH 7 with quantum yields ranging from 0.25 to 0.56. These phenolic nucleoside adducts were also shown to exhibit pH-sensitive fluorescent properties, with fluorescence intensity quenched upon addition of base (pH 11).\(^{98}\)

The glycosidic bond conformation of various dG adducts bearing $C^8$-phenyl rings has also been determined by density functional theory (DFT) calculations carried out by collaborators in the Wetmore group at the University of Lethbridge (Lethbridge, AB), with *anti* structures determined to be less stable than *syn* structures.\(^{99-102}\)

\textit{(d) Synthesis of $C^8$-Aryl-dG Adducts}

The $C$-linked $C^8$-aryl-dG adducts studied by the Manderville group are commonly synthesized by Suzuki-Miyaura coupling reactions. This reaction is defined as a palladium-catalyzed cross-coupling between an organoboron compound and an organic halide for the formation of a carbon-carbon bond.\(^{103,104}\) The mechanism for this reaction involves three major steps in a catalytic cycle, and is shown in Scheme 1-3. A ligand (L), usually triphenylphosphine or derivative thereof, is required to first activate the palladium catalyst. In the first step of the cycle, the palladium catalyst is coupled to the halide (X) via oxidative addition, to form the organopalladium complex, R-Pd(II)L\(_2\)-X. The transmetallation step with the organoboron compound gives the organopalladium complex, R-Pd(II)L\(_2\)-R'. While it is understood that a base is required to activate the
organoboron compound, the exact mechanism of the transmetallation step remains unclear. The final step involves reductive elimination to give the desired product and regenerate the palladium catalyst for re-use in the catalytic cycle.\textsuperscript{103,104}

**Scheme 1-3**

Suzuki-Miyaura cross-couplings for the synthesis of $C^8$-aryl-dG adducts are conducted according to a procedure designed by Shaughnessy and co-workers,\textsuperscript{105} with an aryl-boronic acid (Ar-B(OH)$_2$) as the organoboron and 8-bromo-2'-deoxyguanosine (8-Br-dG) as the organic halide. The starting material 8-Br-dG is first synthesized by bromination at the $C^8$-site of dG by reaction with $N$-bromosuccinimide (NBS), as shown in Scheme 1-4.\textsuperscript{106}
The reaction of 8-Br-dG with the appropriate Ar-B(OH)₂ is carried out using a water-soluble catalyst, palladium (II) acetate (Pd(OAc)₂), in the presence of the phosphine ligand, tris(3-sulfophenyl)phosphine trisodium salt hydrate (TPPTS), as shown in Scheme 1-5.

\[\text{TPPTS} = \text{NaO}_3\text{S-Ph-P-Ph-SO}_3\text{Na}\]
1.3 Incorporation of Modified Nucleosides in DNA

1.3.1 General Overview

In order to determine the fate of a chemical lesion, such as adduct formation, it is important to be able to incorporate the modified nucleobase of interest in DNA. Site-specifically modified oligonucleotides are extremely useful probes for examining the mechanism of mutagenesis and carcinogenesis. There currently exists four major methods for site-specific oligonucleotide incorporation: synthesis of modified phosphoramidites for automated solid-phase oligonucleotide synthesis; postsynthetic modification of DNA bearing a convertible nucleoside; postsynthetic modification of DNA by direct reaction with electrophiles; and incorporation of a modified nucleoside via an enzymatic approach. The predominant approach, and industry standard, for modified nucleobase incorporation is the application of solid-phase DNA synthesis using phosphoramidites, many of which are now commercially available, and the chemistry of which was developed by Beaucage and Caruthers group. This rapid and efficient method allows for the synthesis of oligonucleotides of any sequence up to ~ 200 nucleotides in length. Postsynthetic modification of DNA bearing a convertible nucleoside is also popular, with the Manderville group recently applying this method for the incorporation of C^8-aryl-dG adducts.
1.3.2 Incorporation by the Phosphoramidite Approach

Phosphoramidites are simply monomeric units of nucleotides bearing a phosphoramidite moiety at the 3'-OH of the deoxyribose sugar. Various strategies for the synthesis of C^8-aryl-dG phosphoramidites have been reported, with a typical synthetic protocol shown in Scheme 1-6.

Scheme 1-6

In general, preparation of the phosphoramidite involves first protecting the exocyclic amino group at the \(N^2\)-position of the nucleobase. While Scheme 1-6 shows protection by use of isobutyric anhydride, 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl), acetal derivatives of \(N,N\)-dimethylformamide (DMF) and 4-
isopropylphenoxyacetyl chloride \((\text{PrPac-Cl})\)\textsuperscript{106} have also been employed to protect this position. The 5\textsuperscript{'}-OH of the deoxyribose sugar is typically protected with the 4,4\textsuperscript{'}-dimethoxytrityl group (DMTr).\textsuperscript{121} Alternatives to this protecting group include the 9-phenylxanthen-9-yl (Px) group\textsuperscript{122,123} and the more recently developed 2,7-dimethylpixyl (DMPx) group.\textsuperscript{124} In the final step, the desired phosphoramidite product is generated following reaction with an appropriate phosphitylating agent, typically 2-cyanoethyl-\(N,N\)-diisopropylchlorophosphoramidite.\textsuperscript{116}

The site-specifically incorporated oligonucleotide is then synthesized by an automated process on a DNA synthesizer, a process which involves building the oligonucleotide on a solid support, using nucleotide phosphoramidites, in a repeated series of chemical reactions involving protection, coupling, capping and oxidation,\textsuperscript{2,3,125,126} as shown in Scheme 1-7. Upon synthesis of the desired oligonucleotide, the DNA strand is cleaved from the solid support and all remaining nucleotide protecting groups are removed.
1.3.3 Incorporation by the Postsynthetic Approach

The Manderville group recently developed a unique method for the site-specific oligonucleotide incorporation of $C^8$-aryl-dG adducts involving postsynthetic guanine arylation by Suzuki-Miyaura cross-coupling, by reaction of a brominated oligonucleotide and the appropriate aryl-boronic acid, under conditions similar to those used for the synthesis of $C^8$-aryl-dG adducts, as shown in Scheme 1-8.\textsuperscript{114}
This method was applied using a variety of aryl-boronic acids and DNA sequences of different lengths, including dimers, trimers, decanucleotides (10-mers) and a 15-mer, with good to excellent yields of the desired DNA product. The reaction was also shown to be insensitive to the nature of the nucleobases flanking the convertible 8-Br-G nucleobase. As $C^8$-aryl-dG adducts are sensitive to acids and oxidants commonly applied in solid-phase DNA synthesis, the postsynthetic Suzuki-Miyaura cross-coupling approach is highly advantageous in that it avoids use of these reagents.
1.4 Purpose of Research

The studies presented herein comprise elucidation of the structural and photophysical properties of C-linked C^8^-aryl-dG adducts at both the nucleoside and double helical level. Although biological testing has been performed on adducts derived from the carcinogenic arylhydrazine family, models put forth for the mechanism of mutagenicity have been somewhat limited in their scope. The tendency of C^8^-aryl-dG adducts to undergo depurination leading to abasic site formation will be examined, with a proposal put forth to explain the occurrence of this phenomenon. While adduct formation is ultimately viewed as detrimental and a form of DNA damage, such as in the case of abasic site formation, C^8^-aryl-dG adducts possess properties that can be viewed as beneficial towards the study of DNA structure and conformation. While the native nucleosides are not emissive, modification at the C^8^-position of dG with an aryl moiety gives a strongly fluorescent molecule. The fluorescent properties of a variety of C^8^-heteroaryl-dG nucleoside adducts will be presented, in addition to the effect of oligonucleotide incorporation and duplex formation on emissive parameters. The results presented will demonstrate that fluorescence sensitivity exhibited by adducts towards solvent environment and base-pairing properties allows for determination of anti versus syn conformational heterogeneity of the modified nucleoside within double-stranded DNA. Analysis of the structure and properties of fluorescent C^8^-aryl-dG adducts that are responsive to changes in the DNA environment, and serve as structural representatives of persistent N- and C-linked C^8^-dG adducts formed in vivo from known carcinogens, could assist in furthering the understanding of the means of adduct-induced mutagenesis.
1.5 References


Chapter 2.

Hydrolytic Stability of $C^8$-Aryl-2'-Deoxyguanosine Adducts
Note on Reproduction of Work

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2.1 Introduction

The impetus for the Manderville’s group research on modified nucleosides stems from research on adduction by carcinogens and toxins that undergo metabolic activation to form aryl radical intermediates that can attach covalently to the $C^8$-site of $2'$-deoxyguanosine (dG),$^{1,2,3}$ as was outlined in detail in Chapter 1, section 1.2.4.

A common property of $C^8$-aryl-adducts is their tendency to undergo depurination in studies carried out at physiological pH. Depurination is defined as hydrolysis of the C1’-$N^9$ glycosidic bond of a purine, resulting in the formation of two products, the free purine base, and an abasic, or apurinic (AP), site, as shown in Scheme 2-1 for the hydrolysis of dG.$^4$

Scheme 2-1

![Scheme 2-1](image-url)
At the AP site, the sugar exists initially as a highly unstable cyclic oxocarbenium ion. This ion undergoes rapid hydrolysis resulting in a diastereomeric mixture of 2'-deoxy-α-D-ribose and 2'-deoxy-β-D-ribose, as shown in Scheme 2-2. Interestingly, 2'-deoxyribose anomers formed as a result of depurination may also exist in the open-chain aldehyde configuration, albeit, in a considerably small percentage.

Scheme 2-2

There are numerous examples in the literature of $C^8$-aryl-dG adducts that undergo hydrolysis to form the deglycosylated product, with some of these examples shown in Figure 2-1. Rogan and co-workers discovered that treatment of DNA with the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (BP) in aqueous media with horseradish peroxidase/H$_2$O$_2$ afforded the desired product, the $C^8$-linked BP-dG adduct, 6-BP-8-dG, from the enzymatic digest of the precipitated DNA. The supernatant, however, also
contained roughly the same concentration of the corresponding depurinated 6-BP-8-G adduct.\textsuperscript{7} Reductive activation of 3,4-estrone quinone (3,4-EQ) yields semiquinone radical anion intermediates that can react with dG to give the hydroquinone adduct 8-(3,4-EQ)-dG.\textsuperscript{8-10} Akanni and Abul-Hajj observed that the apurinic adduct 8-(3,4-EQ)-G was formed after a 30 minute incubation of 3,4-EQ in the presence of dG in a 1:1 \textit{N,N}-dimethylformamide (DMF):aqueous buffer.\textsuperscript{8} This observed result led the researchers to propose that 8-(3,4-EQ)-dG is formed as an intermediate prior to the loss of the deoxyribose sugar.\textsuperscript{8} Significant levels of abasic site formation have also been reported for arylhydrazine treatment of DNA.\textsuperscript{11} The Gannett group found that metabolism of carcinogenic arylhydrazines, such as those found in the mushroom species \textit{Agaricus bisporus}, to arenediazonium ions in the presence of calf thymus DNA resulted in a mixture of products. In one case, only 7\% of the product generated was the C\textsuperscript{8}-aryl-dG adduct 8-\textit{p}-CH\textsubscript{2}OHPh-dG, while the deglycosylated adduct 8-\textit{p}-CH\textsubscript{2}OHPh-G was formed in a 93\% yield.\textsuperscript{11} It is important to stress that in these instances of apurinic product formation, adduct hydrolysis was unexpected. Loss of sugar in each example described above occurred under physiological conditions,\textsuperscript{7,8,11} considered to be unusual because dG itself is known to be very stable at pH 7. These results have led to proposals that formation of abasic sites following C\textsuperscript{8}-aryl adduction of dG may contribute to the carcinogenicity of PAHs\textsuperscript{12,13,14} and arylhydrazines.\textsuperscript{11}
Figure 2.1 Examples of depurinating C^8-aryl-dG adducts.

AP sites are repaired on a continuous basis by the apurinic endonuclease activity of the base excision repair (BER) pathway. Abasic sites left unrepaired can be deleterious to cell function, and ultimately lead to genome instability, mutagenesis, carcinogenesis and even cell death. Two of the most commonly observed consequences of unrepaired AP sites include breakage of the DNA strand and the formation of interstrand cross-links. At an unrepaired abasic site, the DNA strand is weakened and can undergo spontaneous cleavage through β-elimination, a process that occurs with a half-life of ~200 hours (8.3 days) under physiological conditions (pH 7.4 buffer at 37 °C). Interstrand cross-links, formed by covalent linkage of opposing DNA strands by a bridging molecule, are particularly problematic, as they are known to block transcription and replication. The Gates group was recently the first to describe the formation of an
interstrand cross-link by reaction of the open-chain aldehyde residue at the AP site with the exocyclic \( N^2 \)-amino group of a guanine residue on the opposite strand of the double helix, with the exocyclic \( N^2 \)-amino group acting as the nucleophile.\(^{19}\) Abasic sites are also commonly oxidized at the C4'-position, which in turn can undergo interstrand cross-link formation with opposing adenine or cytosine bases in double-stranded DNA.\(^{18}\)

For unmodified dG it is well known that development of a positive charge at \( N^7 \), either through protonation\(^{20-24}\) or alkylation,\(^{17}\) accelerates the rate of hydrolysis.\(^{25}\) The \( N^7 \)-site of dG is the most nucleophilic DNA site, as determined by Pullman and coworkers, as it has the highest negative electrostatic potential of all the atoms within the DNA bases, making it prone to attack by both protonating and electrophilic alkylating agents.\(^{25}\)

When DNA is exposed to acidic conditions, protonation of dG occurs at the at \( N^7 \)-position, placing a formal positive charge on the purine residue.\(^{23}\) The acid-catalyzed hydrolysis of dG,\(^{20-22}\) shown in Scheme 2-3, and \( C^8 \)-substituted dG analogues,\(^{26}\) is known to proceed via a stepwise occurring mechanism, involving the aforementioned equilibrium protonation step at \( N^7 \), defined by the acid dissociation constant (\( K_{a1} \)), which precedes the unimolecular rate-limiting cleavage of the glycosidic bond for the monoprotonated species, defined by the rate constant (\( k_1 \)). The p\( K_{a1} \) value for \( N^7 \)-protonated dG was determined by Sigel and co-workers to be 2.34.\(^{27}\) It is important to point out that equilibrium protonation involves both protonation at \( N^7 \) to form the monocation (\( K_{a1} \)), in addition to protonation at \( N^3 \) to form the dication (\( K_{a2} \)), and that both the monoprotonated and diprotonated substrates are subject to glycosidic bond cleavage to form the deglycosylated species.\(^{20-22}\) The presence of a \( C^8 \)-substituent has been shown.
Protonation of $N^7$ of the purine base accelerates the rate of hydrolysis, as the positively charged purine ring becomes a good leaving group for cleavage of the glycosyl bond. Following cleavage, products include the expected free purine residue and the oxocarbenium ion, which quickly undergoes hydrolysis, as shown in Scheme 2-3.

Through the use of pH rate profiles, activation parameters and kinetic solvent isotope effects, acid-catalyzed depurination of dG was classified as an $S_N{1}$ type reaction. According to IUPAC nomenclature, this $S_N{1}$-type mechanism is more specifically referred to as a $D_N*A_N$ reaction, in which leaving group dissociation ($D_N$) of the guanine base is followed by nucleophilic addition ($A_N$) of water, as shown in Figure 2-2. The $^{**}$ sign is representative of the short-lived oxocarbenium ion intermediate formed during the reaction. Theoretical studies involving density functional theory (DFT) calculations, performed by Rios-Font and co-workers, further confirmed that the hydrolysis of $N^7$-protonated-dG follows a $D_N*A_N$ mechanism. This stepwise mechanism is in comparison to the bimolecular $S_N{2}$ type reaction, $A_ND_N$, also shown in Figure 2-2, that involves both nucleophile addition ($A_N$) and dissociation ($D_N$) in the transition state.
Enzymatic depurination, for example, occurs via an $S_N2$ type reaction, in which an activated water molecule, acting as the nucleophile, attacks the anomeric position of the sugar, leading to cleavage of the glycosidic bond.$^{15}$

![Figure 2-2. IUPAC nomenclature for $S_N2$ ($A_ND_N$) and $S_N1$ ($D_N^*A_N$) type reaction mechanisms.](image)

When DNA is exposed to electrophiles, a predominant reaction that occurs is covalent bond formation at the $N^7$-position of guanine.$^{25}$ Alkylation of the $N^7$-position places a formal positive charge on the guanine ring system, greatly increasing the rate of depurination, as guanine becomes a better leaving group.$^{17}$ This mechanism of depurination is an $S_N1$ type reaction, and therefore analogous to that of acid-catalyzed depurination, shown in Scheme 2-3. The half-life for depurination of $N^7$-alkyl-dG in DNA is known to be 144 hours (six days) under physiological conditions (neutral pH at 37 °C).$^5$

The modification of dG by substitution at $C^8$ is known to affect the rate of hydrolysis of the nucleoside. Substitution at the $C^8$-site of dG with electron-withdrawing NO$_2^{28,29}$ or SO$_2$CH$_3^{30}$ moieties greatly accelerates hydrolysis, while electron-donating NH$_2^{31}$ and OCH$_3^{32}$ $C^8$-substituents have been shown to decrease the rate of hydrolysis. Interestingly, bulky arylamino$^{26}$ and dimethylamino$^{31} C^8$-substituents have been found to accelerate the rate of hydrolysis despite their electron-donating character. This effect has
been attributed to release of steric strain that occurs upon removal of the deoxyribose moiety.\textsuperscript{26,33}

Because the loss of sugar from C\textsuperscript{8}-aryl-dG adducts at neutral pH was unexpected,\textsuperscript{8,7,11} it was desirable to determine rates of hydrolysis for a variety of C\textsuperscript{8}-phenyl adducted dG analogues that bear para (p)- and ortho (o)-substituents of varying electronic and steric properties, as shown in Figure 2-3.\textsuperscript{34} These adducts were readily prepared using palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with 8-Br-dG and the appropriate boronic acid,\textsuperscript{35} and include the known arylhydrazine-derived phenyl adduct\textsuperscript{Ph}dG,\textsuperscript{11,36-38} the isomeric C-phenolic adducts\textsuperscript{OHPh}dG and \textsuperscript{oOHPh}dG formed from reactions of DNA with mutagenic diazoquinones,\textsuperscript{39} and other adducts, including those that bear methoxy (\textsuperscript{OMePh}dG and \textsuperscript{oOMePh}dG), methyl (\textsuperscript{MePh}dG and \textsuperscript{oMePh}dG), cyano (\textsuperscript{CNPh}dG) and formyl (\textsuperscript{CHOPh}dG) C\textsuperscript{8}-phenyl substituents, that serve as structural models for C\textsuperscript{8}-aryl-dG adducts in general.\textsuperscript{34}

\textbf{Figure 2-3.} Structure of C\textsuperscript{8}-aryl-dG adducts bearing $p$- and $o$-substituents of varying electronic and steric influences, with atomic numbering and identification of dihedral twist angle $\theta$ ($\angle(C^{11}C^{10}C^{8}N^{6})$).

\begin{align*}
\text{Ph}dG, R^1 = H, R^2 = H \\
\text{OHPh}dG, R^1 = OH, R^2 = H \\
\text{OMePh}dG, R^1 = OCH_3, R^2 = H \\
\text{MePh}dG, R^1 = CH_3, R^2 = H \\
\text{CNPh}dG, R^1 = CN, R^2 = H \\
\text{CHOPh}dG, R^1 = CHO, R^2 = H \\
\text{oOHPh}dG, R^1 = H, R^2 = OH \\
\text{oOMePh}dG, R^1 = H, R^2 = OCH_3 \\
\text{oMePh}dG, R^1 = H, R^2 = CH_3
\end{align*}
To determine how the $C^8$-phenyl moiety contributes to sugar loss for the adducts shown in Figure 2-3, rates for hydrolysis in aqueous solutions of varying acidity were measured and DFT calculations were employed to assist in the interpretation of the kinetic experiments. Incorporation of $C^8$-aryl-dG adducts into trimers was also performed, by either standard phosphoramidite chemistry or Suzuki-Miyaura coupling reactions with 8-Br-G-modified trimers, in order to determine if DNA provides a protective effect with regards to hydrolysis. Observations made in this comprehensive study have led to the development of a new rationale for the noted tendency of apurinic site formation to accompany $C^8$-aryl-dG adduction in duplex DNA at physiological pH.
2.2 Materials and Methods

2.2.1 General Methods

A detailed description of the general methods for experiments pertaining to Chapter 2 can be found in Appendix A.

2.2.2 Synthesis of $C^8$-Aryl-$dG$ Adducts

Synthesis of $C^8$-aryl-$dG$ adducts was conducted according to the literature,\textsuperscript{35} by palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with 8-Br-$dG$ and the appropriate boronic acid. The $C^8$-aryl-$dG$ adducts 8-phenyl-2'-deoxyguanosine ($^\text{Ph}dG$),\textsuperscript{35} 8-(4"-hydroxyphenyl)-2'-deoxyguanosine ($^{\text{OHPh}}dG$),\textsuperscript{2} 8-(4"-methoxyphenyl)-2'-deoxyguanosine ($^{\text{OMePh}}dG$),\textsuperscript{35} 8-(4"-tolyl)-2'-deoxyguanosine ($^{\text{MePh}}dG$),\textsuperscript{35} 8-(4"-cyanophenyl)-2'-deoxyguanosine ($^{\text{CNPh}}dG$),\textsuperscript{3} 8-(4"-formylphenyl)-2'-deoxyguanosine ($^{\text{CHOPh}}dG$),\textsuperscript{3} 8-(2"-hydroxyphenyl)-2'-deoxyguanosine ($^{\text{oOHPh}}dG$)\textsuperscript{1} and 8-(2"-methoxyphenyl)-2'-deoxyguanosine ($^{\text{oOMePh}}dG$)\textsuperscript{1} were synthesized by Ke-wen Mark Sun in the Manderville laboratory at the University of Guelph (Guelph, ON), as previously described. The adduct 8-(2"-tolyl)-2'-deoxyguanosine ($^{\text{oMePh}}dG$) was synthesized by Robert Paugh in the Manderville laboratory at the University of Guelph (Guelph, ON).\textsuperscript{34}

The corresponding guanine analogues of the adducts were prepared by Ke-wen Mark Sun, as previously outlined,\textsuperscript{1,42} by placing the adduct in approximately 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 M NaOH and deglycosylated products were recovered by crystallization and filtering from the aqueous media.
2.2.3 Synthesis of C$_8$-Aryl-G Modified Trimers

(a) Synthesis by Standard Phosphoramidite Chemistry

Synthesis of the C$_8$-aryl-G modified trimer 5'-G(PhG)T was performed by Michael Sproviero in the Manderville laboratory at the University of Guelph (Guelph, ON) on a 1 $\mu$mol scale on a BioAutomation Corporation MerMade 12 automatic synthesizer using standard $\beta$-cyanoethylphosphoramidite chemistry according to published protocols. The modified trimer sample was purified as outlined below in section 2.2.4.

(b) Suzuki-Miyaura Coupling Reactions with 8-Br-G Modified Trimers

Synthesis of C$_8$-aryl-G modified trimers, 5'-A(OMePhG)T, 5'-C(OMePhG)T, 5'-G(OMePhG)T and 5'-G(CNPhG)T was conducted according to the literature and the protocol is briefly described here. Brominated trimers 5'-A(8-Br-G)T, 5'-C(8-Br-G)T and 5'-G(8-Br-G)T were custom-made by Sigma-Aldrich Canada Ltd. (Oakville, ON) on a 1 $\mu$mol scale using standard phosphoramidites, and 8-Br-dG-CE phosphoramidite purchased from Glen Research (Sterling, VA). The brominated trimers were fully deprotected with NH$_4$OH for 24 h, desalted and purified by reverse-phase (RP) chromatography by Sigma-Aldrich Canada Ltd., and the mass spectra of each was obtained at the University of Toronto prior to use in Suzuki-Miyaura coupling in order to confirm identity of the brominated trimer. For Suzuki-Miyaura coupling, the brominated trimer (500 nmol) was initially dissolved in degassed 2:1 H$_2$O:CH$_3$CN. The appropriate aryl-boronic acid (Ar-B(OH)$_2$) and sodium carbonate were added to the solution at the molar ratios Ar-B(OH)$_2$:brominated trimer = 10, and Na$_2$CO$_3$:brominated trimer = 2. The other reaction components were initially prepared as 100× stock solutions in degassed...
water. Through serial dilution, the reagents were added to the reaction mixture at molar ratios brominated trimer:tris(3-sulfophenyl)phosphine trisodium salt hydrate (TPPTS) = 15, and brominated trimer:palladium (II) acetate (Pd(OAc)₂) = 37.5, for a total volume of 700 µL 2:1 H₂O:CH₃CN, and the resulting solution was heated under argon at 80 °C for 24 h. To the reaction mixture was added 1 mL of 5 mM ethylenediaminetetraacetic acid (EDTA) in 50 mM triethylammonium acetate (TEAA), pH 7.2. The resulting solution was added to a Waters Sep-Pak Vac C18 1 cc cartridge and washed with 5 % CH₃CN in 50 mM TEAA, pH 7.2 in order to remove excess reagents. The desired trimer product was eluted with 30 % acetonitrile in 50 mM TEAA, pH 7.2, and the collected solution was further purified by RP high performance liquid chromatography (HPLC), as described below.

2.2.4 C⁸-Aryl-G Modified Trimer Purification and Sample Preparation

Immediately prior to HPLC purification, the C⁸-aryl-G modified trimer solution was filtered using Mandel syringe filters (PVDF 0.20 µm) and concentrated under diminished pressure using a ThermoSavant DNA 120 SpeedVac. Purification was performed using an Agilent 1200 series HPLC instrument equipped with an autosampler, diode array detector (monitored at λₘₐₓ = 258 nm and λₘᵦₙₐ of the incorporated adduct), fluorescence detector (monitored at λₑₓ and λₑₘ of the incorporated adduct), and autocollector. Separation was carried out at 50 °C using a Phenomenox Clarity 3 µm Oligo-RP C18 column (50 × 4.60 mm) with a flow rate of 0.5 mL/min, and gradient elution with buffer B in buffer A (buffer A = 95:5 aqueous 50 mM TEAA (pH 7.2)/acetonitrile; buffer B = 30:70 aqueous 50 mM TEAA (pH 7.2)/acetonitrile). Yields
of C^8^-aryl-G modified trimers synthesized by Suzuki-Miyaura coupling were determined from integration of the HPLC trace. Lyophilization of the isolated product was performed using a Labconco FreeZone 4.5. HPLC analysis of the final isolated product was also performed as a check for purity. Unmodified and C^8^-aryl-G modified trimers were dissolved in 18.2 MΩ water for quantification by UV-vis measurement, with \( \varepsilon_{260} \). Extinction coefficients (\( \varepsilon \)) were obtained from the Integrated DNA Technologies (IDT) internet site (http://www.idtdna.com/analyzer/applications/oligoanalyzer), and C^8^-aryl-G modified trimers were assumed to have the same extinction coefficient at 260 nm as the unmodified trimer.\(^{43,44}\)

### 2.2.5 Mass Spectrometry Analysis of C^8^-Aryl-G Modified Trimers

Methanol used in mass spectrometry (MS) analysis was purchased from EMD Millipore (Billerica, MA), water from OmniSolv (Charlotte, NC) and ammonium acetate (\( \geq 99\% \)) from Fluka (St. Louis, MO). The C^8^-aryl-G modified trimers were analyzed by Keegan Rankin in the Mabury group at the University of Toronto (Toronto, ON), using a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation, Milford, MA). Samples were prepared in a 50:50 MeOH:H\(_2\)O solution with 0.1 mM ammonium acetate. Full scan MS spectra were obtained by direct infusion at a rate of 5 – 10 \( \mu \)L/min into an electrospray ionization (ESI) source operated in negative mode. The capillary and cone voltages were optimized for each analyte and varied from 2.5 to 3.5 kV and 25 to 35 V, respectively. A source offset of 60 V was used for all samples. The desolvation temperature was between 250 and 350 °C. All data was acquired with 36-60 MCA and processed using Waters Mass Lynx V4.1 mass spectrometry software.
2.2.6 Determination of Photophysical Properties

(a) Measurements with C₈-Aryl-dG Adducts

For ultraviolet-visible (UV-Vis) measurements, spectroscopic solutions of adducts were prepared with 25 μL of the adduct stock solution and 1975 μL of 50 mM citrate buffer, pH 4, for a final adduct concentration of 50 μM, with ionic strength (μ) of the buffer maintained using 0.31 M NaCl.

(b) Measurements with C₈-Aryl-G Adducts

For UV-Vis measurements, spectroscopic solutions of deglycosylated adducts were prepared with 25 μL of the adduct stock solution and 1975 μL of 50 mM citrate buffer, pH 4, for a final adduct concentration of 50 μM, with μ of the buffer maintained using 0.31 M NaCl.

(c) Measurements with C₈-Aryl-G Modified Trimers

Excitation and emission spectra were recorded in 0.1 M HCl, with the C₈-aryl-G modified trimer spectroscopic solution again prepared to a final concentration of 1 μM. Spectral measurements were obtained every 1 – 5 min, until no further change was observed in the fluorescence spectrum and a maximum excitation and emission intensity was reached for the deglycosylated species.

2.2.7 pKₐ Determination

The N⁷ pKₐ values of C₈-aryl-dG adducts were determined by UV-Vis spectroscopy at 20 °C, as previously outlined.²⁶ Spectroscopic solutions were prepared
with 25 \( \mu \)L of the adduct stock solution, and 1975 \( \mu \)L of 50 mM phosphate buffer (pH 1 and 1.5), 50 mM citrate buffer (pH 2, 2.5, 3, 3.5, 4, 4.5, 5) or 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 6 and 7), for a final adduct concentration of 50 \( \mu \)M. In all aqueous solutions, \( \mu \) of the buffer was maintained using 0.31 M NaCl. Spectral measurements were obtained at pH values ranging from 1 to 7, and the initial absorbance recorded at 280 nm or 320 nm. \( pK_{al} \) values were obtained with the following equation:

\[
pK_{al} = pH + \log \left( \frac{(A - A_M)}{(A_I - A)} \right)
\]

where \( A \) is initial absorbance, \( A_M \) is initial absorbance of the neutral species (pH 7) and \( A_I \) is initial absorbance of the protonated species (pH 1). \( pK_{al} \) values were obtained at pH values between 1.5 and 6 to allow for determination of mean \( \pm \) standard deviation.

2.2.8 Kinetic Measurements

(a) General Methods for Kinetic Measurements

Hydrolysis of \( C^8 \)-aryl-dG adducts and \( C^8 \)-aryl-dG modified trimers was observed by UV-Vis or fluorescence spectroscopy, by monitoring the appearance of the deglycosylated species at its absorbance maximum, or excitation and emission maxima, respectively. The wavelength maximum of the deglycosylated adduct was determined separately via a scan measurement, as outlined in section 2.2.6 (b), while the excitation and emission maxima of the deglycosylated trimer were determined via scan measurements, as outlined in section 2.2.6 (c). Measurements with \( C^8 \)-aryl-dG adducts were conducted in parallel using the multi-cell changer, with six first-order rate constant values for hydrolysis obtained for each adduct in each set of conditions to allow for
determination of mean ± standard deviation. For all measurements, data points were recorded every 0.1 s until a plateau in the first-order rate curve could be observed. The first-order rate constants were obtained from plots of absorbance versus time, as calculated by the Kinetics program.

(b) Measurements with C8-Aryl-dG Adducts

(i) Hydrolysis in 0.1 M HCl and pH 4 Buffer

Hydrolysis of adducts was observed by UV-Vis spectroscopy. Spectroscopic solutions were prepared using 25 µL of the adduct stock solution, and 1975 µL of 0.1 M HCl or 50 mM citrate buffer, pH 4, for a final adduct concentration of 50 µM, with µ of buffers maintained using 0.31 M NaCl. Kinetic measurements in HCl were carried out at 37.2 °C, while measurements in pH 4 buffer were carried out at 48.4 °C.

(ii) Eyring Plots

Hydrolysis of adducts was observed by UV-Vis spectroscopy. Spectroscopic solutions were prepared using 25 µL of the adduct stock solution and 1975 µL of 0.1 M HCl, for a final adduct concentration of 50 µM. Kinetic measurements were carried out at 25 °C, 30 °C, 37 °C, 45 °C, 55 °C and 60 °C. Eyring plots of ln k_{obs}/T versus 1/T allowed for the determination of activation parameters. Enthalpy of activation was determined from the slope of the Eyring plot:

\[ \text{slope} = -\Delta H^\circ / R \]

where \( \Delta H^\circ \) is the enthalpy of activation and \( R \) is the gas constant, 8.314 J K^{-1} mol^{-1}. 


Entropy of activation was determined from the $y$-intercept of the Eyring plot:

$$y\text{-intercept} (x = 0) = \ln \frac{k_B}{\hbar} + \frac{\Delta S^e}{R}$$

where $k_B$ is Boltzmann’s constant, $1.381 \times 10^{-23}$ J K$^{-1}$, $\hbar$ is Planck’s constant, $6.626 \times 10^{-34}$ J s, $\Delta S^e$ is the entropy of activation and $R$ is as defined previously. Gibbs energy of activation was calculated from the following equation:

$$\Delta G^\# = \Delta H^\# - T\Delta S^e$$

where $\Delta G^\#$ is Gibbs energy of activation, $T$ is temperature (K) and the other parameters are as defined previously.

(iii) pH Rate Profiles

Hydrolysis of adducts was observed by UV-Vis spectroscopy. Spectroscopic solutions were prepared using 25 $\mu$L of the adduct stock solution, and 1975 $\mu$L of 50 mM phosphate buffer (pH 1) or 50 mM citrate buffer (pH 2, 3 and 4), for a final adduct concentration of 50 $\mu$M, with $\mu$ of buffers maintained using 0.31 M NaCl. All kinetic measurements were carried out at 37 °C. pH rate profiles were constructed by plotting log $k$ (min$^{-1}$) versus pH to allow for extrapolation of the rate for hydrolysis at pH 7.

(c) Measurements with $C^8$-Aryl-G Modified Trimers

Hydrolysis of modified trimers was observed by fluorescence spectroscopy. Spectroscopic solutions of modified trimers were prepared in 0.1 M HCl, to a final concentration of 1 $\mu$M. All kinetic measurements were carried out at 37.2 °C, and were conducted in triplicate for each modified trimer to allow for determination of mean ± standard deviation.
2.3 Results and Discussion

2.3.1 Structural Features of C8-Aryl-dG Adducts

(a) Computational Calculated Structural Parameters of C8-Aryl-dG Adducts

Insight into the structural features of the C8-phenyl derivatized dG adducts was obtained from DFT calculations performed by the Wetmore group at the University of Lethbridge (Lethbridge, AB). Structures were fully optimized with B3LYP/6-31G(d) and relative energies were obtained from B3LYP/6-311+G(2df,p) single-point calculations. DFT calculations revealed that the C8-aryl substituent is twisted with respect to the dG nucleoside, where the magnitude of the dihedral twist angle (θ), as defined in Figure 2-3, is dependent on steric considerations and favourable intramolecular interactions. The θ twist angle for the p-substituted adducts is ~ 37°, while the twist increases to angles of 45° and 55° for the o-substituted adducts oMePhG and oOMePhG, respectively. In contrast, this angle in the oOHPHdG adduct is smaller (θ = 27°) due to O−H···N7 H-bonding. Interestingly, DFT calculations for the deglycosylated (deglyco) derivatives of the corresponding C8-aryl-dG adducts show that these structures are planar (θ = 0°), suggesting that the deoxyribose sugar moiety is inducing the twist within the nucleoside. The only exception to this trend was noted for the oMePhdG adduct, which remained significantly twisted (θ = 24°) upon deglycosylation.

(b) Photophysical Spectral Parameters of C8-Aryl-dG Adducts

Absorbance spectra obtained for all C8-aryl-dG adducts revealed further insight into the structural features of these analogues. UV-Vis absorbance spectra for adducts
were initially recorded in aqueous 50 mM citrate buffer, pH 4, at room temperature, conditions under which adducts were relatively stable. Distinct changes were noted in the absorbance spectra for all adducts upon removal of the deoxyribose sugar moiety to form the corresponding deglyco derivative, as shown in Figure 2-4, with spectra of $C^8$-aryl-dG adducts represented as solid lines and spectra of deglyco adducts represented as dashed lines.
Figure 2-4. Absorbance spectra of $C^8$-aryl-dG adducts (solid lines) and their deglyco counterparts (dashed lines) (a) $^{O_{Me}Ph}$dG and $^{O_{Me}Ph}$G, (b) $^{CH_{OPh}}$dG and $^{CH_{OPh}}$G, (c) $^{o_{OHPh}}$dG and $^{o_{OHPh}}$G, and (d) $^{o_{MePh}}$dG and $^{o_{MePh}}$G. Spectra were recorded in 50 mM citrate buffer, pH 4, $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M.

The spectra in Figure 2-4a and 2-4c of the nucleoside adducts $^{O_{Me}Ph}$dG and $^{o_{OHPh}}$dG, respectively, were representative of spectra recorded for the adducts $^{Ph}$dG, $^{O_{HPh}}$dG, $^{O_{MePh}}$dG, $^{Me_{Ph}}$dG and $^{o_{O_{MePh}}}$dG, which all displayed a single broad peak at ~ 280
nm that exhibits a red-shift compared to the absorbance wavelength maximum of dG\textsuperscript{34} at 253 nm.\textsuperscript{45} Of this group of adducts, the deglyco derivatives of the \( p \)-substituted adducts exhibited two absorbance maxima at \( \sim 265 \) nm and \( \sim 310 \) nm, such as those shown for \( \text{OMePhG} \) in Figure 2-4a, while the deglyco derivatives of the \( o \)-substituted adducts exhibited two maxima at \( \sim 290 \) nm and \( \sim 320 \) nm, such as those shown for \( \text{oOHPbG} \) in Figure 2-4c.\textsuperscript{34} In all instances, the red-shifted wavelength maxima are consistent with a planar deglycosylated structure that possesses increased conjugation,\textsuperscript{34} thereby resulting in a shift to a longer, lower energy, wavelength.\textsuperscript{46} The \( p \)-substituted \( C^8 \)-aryl-dG adducts \( \text{CNPh}\text{dG} \) and \( \text{CHOPh}\text{dG} \) adducts showed similar features, with the spectrum for \( \text{CHOPh}\text{dG} \) shown in Figure 2-4b. In pH 4 buffer, these adducts displayed an absorbance maximum at \( \sim 280 \) nm and an additional maximum within the range of \( \sim 310 – 320 \) nm. The corresponding deglyco adducts exhibited a red-shifted absorbance maximum within the range of \( \sim 335 – 350 \) nm, as shown in Figure 2-4b for \( \text{CHOPh}\text{dG} \).\textsuperscript{34} The spectrum of \( \text{oMePh}\text{dG} \) is shown in Figure 2-4d, and features a single peak at 261 nm of weaker intensity and blue-shifted compared to all other adduct absorbance spectra. The deglyco adduct \( \text{oMePh}\text{dG} \) exhibited a broad absorbance with a maximum at \( \sim 285 \) nm, as shown in Figure 2-4d, which was also significantly blue-shifted compared to the absorbance for the other deglycosylated adducts.\textsuperscript{34} The UV-Vis parameters and structural features of all \( C^8 \)-aryl-dG adducts are summarized in Table 2-1.
Table 2-1. Absorbance parameters and structural features of C₈-aryl-dG adducts and their deglyco derivatives.

<table>
<thead>
<tr>
<th>adduct</th>
<th>λ_{abs/ max}</th>
<th>log ε⁺</th>
<th>θ (degrees)ᵃ</th>
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<tbody>
<tr>
<td>Ph² dG</td>
<td>277, 4.33</td>
<td></td>
<td>37.5</td>
</tr>
<tr>
<td>Ph² G (deglyco)</td>
<td>305, 4.28</td>
<td>0.6</td>
<td></td>
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<td>OHPPh² dG</td>
<td>277, 4.36</td>
<td></td>
<td>37.0</td>
</tr>
<tr>
<td>OHPPh² G (deglyco)</td>
<td>309, 4.35</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>OMePh² dG</td>
<td>276, 4.34</td>
<td></td>
<td>37.4</td>
</tr>
<tr>
<td>OMePh² G (deglyco)</td>
<td>310, 4.45</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>MePh² dG</td>
<td>277, 4.38</td>
<td></td>
<td>37.2</td>
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<tr>
<td>MePh² G (deglyco)</td>
<td>308, 4.15</td>
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</tr>
<tr>
<td>CNPh² dG</td>
<td>282, 4.11</td>
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<td>334, 3.92</td>
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<td></td>
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<td>275, 4.10</td>
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<td>55.3</td>
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<td>45.3</td>
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<tr>
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<td>285, 3.70</td>
<td>24.0</td>
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ᵃ Twist angle determined for the neutral molecule by DFT calculations; deglyco adduct determination is with N⁹H. Structures fully optimized with B3LYP/6-31G(d) and relative energies were obtained from B3LYP/6-311+G(2df,p) single-point calculations. ᵇ Spectra were recorded in 50 mM citrate buffer, pH 4, μ = 0.31 M NaCl, using an adduct concentration of 50 μM. For dual absorbance, the less intense absorbance band is in brackets.

2.3.2 Proton Affinity of C₈-Aryl-dG Adducts

Ionization constants (pKₐ values) of C₈-aryl-dG adducts in the low pH region (1 – 4) were determined at 20 °C using the spectroscopic method, ³⁴ as outlined previously for C₈-arylamino-dG adducts by the Novak group. ²⁶ Figure 2-5a shows absorbance overlay spectra for OHPPh² dG as a function of pH at 20 °C, while displayed in Figure 2-5b is a plot of initial absorbance at 320 nm as a function of pH from the absorbance data shown in Figure 2-5a. ³⁴
Figure 2-5. (a) Changes in absorbance spectra (dashed lines) of the $C^8$-aryl-dG adduct $\text{OHPh} \text{dG}$ at 20 °C, upon lowering the pH of the aqueous solution from pH = 7 (solid line) to pH = 1 and (b) the corresponding plot of initial absorbance of $\text{OHPh} \text{dG}$ at 320 nm versus pH. Spectra were recorded in 50 mM phosphate buffer (pH 1), 50 mM citrate buffer (pH 2 – 5) or 50 mM MOPS buffer (pH 6 and 7), with $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M.

The spectroscopically determined $pK_{a1}$ of $\text{OHPh} \text{dG}$ is 2.43 ± 0.12. This value lies in the expected range for the $pK_{a1}$ of an analogue of dG, as recall, the $pK_{a1}$ value for $N^7$-protonated dG is 2.34. The $pK_{a1}$ value for $\text{OHPh} \text{dG}$ was found to be similar to the values determined for the other $p$-substituted adducts $\text{Ph} \text{dG}$, $\text{OMePh} \text{dG}$ and $\text{MePh} \text{dG}$, which all exhibited a red-shift in absorbance wavelength maximum upon protonation and gave a narrow $pK_{a1}$ value range from 2.41 – 2.48, as reported in Table 2-2. Variation in $pK_{a1}$ was observed for the $o$-substituted adducts. Compared to the corresponding $p$-substituted adduct, $^o\text{OHPh} \text{dG}$ possesses a lower $pK_{a1}$ (2.21 ± 0.17), $^o\text{OMePh} \text{dG}$ possesses a higher $pK_{a1}$ (2.47 ± 0.26) and $^o\text{MePh} \text{dG}$ possesses relatively the same $pK_{a1}$ (2.40 ± 0.22).

Gas-phase proton affinity (PA) values were also determined by DFT calculations performed again by the Wetmore group. These values were calculated for the most basic
The $N^7$-PA values for the $C^8$-aryl-dG adducts were found to lie within a narrow range of 222.7 – 234.1 kcal mol$^{-1}$, with CNPhdG possessing the lowest value and $o$OMePhdG possessing the highest value, which recall, also possesses the highest pK$_{a1}$ value. These values are similar to both the calculated $N^7$-PA of dG (232 kcal mol$^{-1}$) and the experimental $N^7$-PA for dG (234.4 kcal mol$^{-1}$). Because pK$_{a1}$ values of CNPhdG and CHOPh dG were unable to be determined experimentally using the spectroscopic method, a plot of $N^7$-PA as a function of spectroscopically determined pK$_{a1}$ values was generated and afforded a straight line, from which pK$_{a1}$ values of CNPhdG and CHOPh dG were estimated. These values are reported in Table 2-2, and were found to be lower than pK$_{a1}$ values for all other $C^8$-aryl-dG adducts. It is important to note that it has been speculated in literature that the presence of $C^8$-substituents may result in the differential stabilization of protonation at the $N^3$-position rather than $N^7$. However, in the present study, this seems unlikely, provided that DFT calculations revealed that protonation of $N^7$ is more energetically favourable than protonation of $N^3$ for dG, as the PA for $N^3$ of dG is 220.1 kcal mol$^{-1}$ lower than the PA for $N^7$ of dG and all $C^8$-aryl-dG adducts. Furthermore, the pK$_{a2}$ for the diprotonated dG substrate is known to be approximately −2.5, while the protonation of 8-oxo-dG occurs at $N^3$ with pK$_{a1}$ determined to be 0.22. These pK$_{a}$ values provide additional evidence as to protonation occurring favourably at the $N^7$-site, as both values are considerably lower than the pK$_{a1}$ values found for dG and all $C^8$-aryl-dG adducts.
Table 2-2. Ionization constants (pK\textsubscript{a1} values) of C\textsuperscript{8}-aryl-dG adducts protonated at N\textsuperscript{7}.

<table>
<thead>
<tr>
<th>adduct</th>
<th>pK\textsubscript{a1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph\textsubscript{d}G</td>
<td>2.41 ± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>OH\textsubscript{Ph}dG</td>
<td>2.43 ± 0.12\textsuperscript{a}</td>
</tr>
<tr>
<td>OMe\textsubscript{Ph}dG</td>
<td>2.48 ± 0.06\textsuperscript{a}</td>
</tr>
<tr>
<td>Me\textsubscript{Ph}dG</td>
<td>2.41 ± 0.12\textsuperscript{a}</td>
</tr>
<tr>
<td>CN\textsubscript{Ph}dG</td>
<td>2.00\textsuperscript{b}</td>
</tr>
<tr>
<td>CH\textsubscript{OPh}dG</td>
<td>2.12\textsuperscript{b}</td>
</tr>
<tr>
<td>O\textsubscript{OHPh}dG</td>
<td>2.21 ± 0.17\textsuperscript{a}</td>
</tr>
<tr>
<td>O\textsubscript{OMePh}dG</td>
<td>2.57 ± 0.26\textsuperscript{a}</td>
</tr>
<tr>
<td>O\textsubscript{MePh}dG</td>
<td>2.40 ± 0.22\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Obtained spectroscopically at 20 °C.
\textsuperscript{b} Estimated from gas-phase N\textsuperscript{7}-proton affinity and spectroscopically determined pK\textsubscript{a} values.

DFT calculations, again determined at the B3LYP/6-31G(d) level, revealed changes in the \( \theta \) twist angle upon protonation at the N\textsuperscript{7}-site. For the \( p \)-substituted C\textsuperscript{8}-aryl-dG adducts, the protonated structures were not significantly different from the neutral structures, with only a slightly greater \( \theta \) angle.\textsuperscript{34} Structures of the \( o \)-substituted C\textsuperscript{8}-aryl-dG adducts, however, can be altered upon protonation of N\textsuperscript{7}. Only a small increase in \( \theta \) is observed for \( o\text{OHPh}dG \), due to H-bonding interactions involving the phenolic group and N\textsuperscript{7}-position that occur with both the neutral and protonated adduct. Conversely, \( o\text{OMePh}dG \) becomes considerably less twisted, by \( \sim 27^\circ \), upon protonation due to N\textsuperscript{7}–H\textbullet\textbullet\textbullet O–CH\textsubscript{3} H-bonding. Preferential stabilization of this adduct is also reflected in the larger pK\textsubscript{a1} value this adduct possesses compared to the other C\textsuperscript{8}-aryl-dG adducts. Finally, the protonated \( o\text{MePh}dG \) adduct was found to be significantly more twisted, by \( \sim 23^\circ \), than the neutral species due to increased steric.\textsuperscript{34} The DFT calculations showing stabilization of adducts upon N\textsuperscript{7}-protonation also support the protonation of C\textsuperscript{8}-aryl-dG adducts at the N\textsuperscript{7}-site.
2.3.3 Rates for Hydrolysis of $C^8$-Aryl-dG Adducts

(a) Hydrolysis in 0.1 M HCl

The hydrolysis of $C^8$-aryl-dG adducts was measured by UV-Vis spectroscopy, by monitoring the appearance of the deglycosylated product at its absorbance maximum, as illustrated in Figure 2-6. First-order rate constants ($k_{obs}$) and half-lives ($t_{1/2}$) were first determined for the hydrolysis of adducts in 0.1 M HCl at 37.2 °C, with results provided in Table 2-3. These particular conditions were chosen in order to draw direct comparison to the rates for hydrolysis of dG, as determined previously by Bruylants and co-workers.\textsuperscript{21}

Figure 2-6. (a) Changes in absorbance spectrum of $^{\text{OMePh}}$dG and (b) plot of absorbance of $^{\text{Ph}}$dG versus time, showing hydrolysis of the respective $C^8$-aryl-dG adducts. Spectrum and plot were recorded in 0.1 M HCl at 37.2 °C, using an adduct concentration of 50 $\mu$M.
Table 2-3. Summary of first-order rate constants ($k$) and half-lives ($t_{1/2}$) for the hydrolysis of $C^8$-aryl-dG adducts in 0.1 M HCl and pH 4 buffer at 37.2 °C and 48.4 °C.

<table>
<thead>
<tr>
<th>adduct</th>
<th>0.1 M HCl</th>
<th>0.1 M HCl</th>
<th>pH 4</th>
<th>pH 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^a$</td>
<td>$k_{obs}/k_{obs}$ (dG)</td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^c$</td>
<td>$k_1$ (min$^{-1})^d$</td>
</tr>
<tr>
<td>Ph dG</td>
<td>0.790 ± 0.008, 0.877</td>
<td>20.2</td>
<td>(2.61 ± 0.08) × 10$^{-2}$, 26.6</td>
<td>1.02</td>
</tr>
<tr>
<td>OMePh dG</td>
<td>0.478 ± 0.009, 1.45</td>
<td>12.2</td>
<td>(3.1 ± 0.1) × 10$^{-2}$, 22</td>
<td>1.15</td>
</tr>
<tr>
<td>MePh dG</td>
<td>0.611 ± 0.006, 1.13</td>
<td>15.6</td>
<td>(2.95 ± 0.09) × 10$^{-2}$, 23.5</td>
<td>0.977</td>
</tr>
<tr>
<td>CNPh dG</td>
<td>0.651 ± 0.009, 1.06</td>
<td>16.6</td>
<td>(2.84 ± 0.08) × 10$^{-2}$, 24.4</td>
<td>1.10</td>
</tr>
<tr>
<td>CHOPh dG</td>
<td>1.78 ± 0.02, 0.389</td>
<td>45.5</td>
<td>(2.3 ± 0.1) × 10$^{-2}$, 30</td>
<td>2.30</td>
</tr>
<tr>
<td>oOMePh dG</td>
<td>0.369 ± 0.008, 1.05</td>
<td>24</td>
<td>(2.6 ± 0.08) × 10$^{-2}$, 22.8</td>
<td>0.977</td>
</tr>
<tr>
<td>oPh dG</td>
<td>0.387 ± 0.001, 2.46</td>
<td>7.21</td>
<td>(1.06 ± 0.02) × 10$^{-2}$, 65.4</td>
<td>0.654</td>
</tr>
<tr>
<td>oOMePh dG</td>
<td>0.291 ± 0.003, 2.38</td>
<td>7.44</td>
<td>(7.4 ± 0.2) × 10$^{-3}$, 94</td>
<td>0.199</td>
</tr>
<tr>
<td>oMePh dG</td>
<td>0.196 ± 0.002, 3.45</td>
<td>5.01</td>
<td>(2.6 ± 0.2) × 10$^{-3}$, 270</td>
<td>0.103</td>
</tr>
<tr>
<td>dG</td>
<td>0.0391, 17.7$^b$</td>
<td></td>
<td>2.44 × 10$^{-4}$, 2840$^b$</td>
<td>0.00110</td>
</tr>
</tbody>
</table>

$^a$ Rates for hydrolysis in 0.1 M HCl were recorded at 37.2 °C, using an adduct concentration of 50 µM. $^b$ Taken from Reference no. 21. $^c$ Rates for hydrolysis were recorded in 50 mM citrate buffer, pH 4, $\mu = 0.31$ M NaCl at 48.4 °C, using an adduct concentration of 50 µM. $^d$ $k_1 \approx k_{obs}K_{a1}/[H^+]$. 
For comparison to dG, the C⁸-aryl-dG adduct PhdG, which bears no phenyl substituent, underwent deglycosylation approximately twenty times faster than the unmodified nucleoside in acidic conditions at 37.2 °C, as determined from comparison of $k_{obs}$ values. Examination of the hydrolytic rate data in Table 2-3 also revealed a trend in the $k_{obs}$ and $t_{1/2}$ values recorded in 0.1 M HCl for the $p$-substituted C⁸-aryl-dG adducts. Adducts with electron-donating $p$-phenyl substituents, including OHPhdG, OMePhdG and MePhdG, with $t_{1/2} = 1.45$, 1.13 and 1.06 minutes, respectively, underwent hydrolysis approximately three times slower than those with electron-withdrawing $p$-phenyl substituents, including CNPhdG and CHOPhdG, with $t_{1/2} = 0.389$ and 0.491 minutes, respectively. The behaviour exhibited by $p$-substituted C⁸-aryl-dG adducts in 0.1 M HCl was contrasted by the $o$-substituted C⁸-aryl-dG adducts, including PhoOHdG, PhoOMecdG and oMePhdG, that underwent hydrolysis at a significantly slower rate than their $p$-substituted counterparts, as shown by the $k_{obs}$ and $t_{1/2}$ values in Table 2-3. In particular, oMePhdG underwent hydrolysis approximately three times slower than MePhdG in 0.1 M HCl and only showed a five-fold increase in rate compared to dG.

In order to better understand the influence of the $p$-phenyl substituent on hydrolysis of C⁸-aryl-dG adducts in 0.1 M HCl, plots were constructed of log $k_{obs}/k_{obs}$ (PhdG) as a function of the Hammett $\sigma_p$ substituent constant, as shown in Figure 2-7.
Figure 2-7. Hammett plot for \( p \)-substituted \( C^8 \)-aryl-dG adducts of \( \log \frac{k_{\text{obs}}}{k_{\text{obs}}(\text{Ph}dG)} \) versus substituent constant \( \sigma_p \). Rates for hydrolysis were recorded in 0.1 M HCl at 37.2 °C, using an adduct concentration of 50 \( \mu \)M.

The resulting Hammett plot shows a strong linear correlation, with a small slope (\( \rho \)) of 0.54 ± 0.02 observed. This reaction constant value, of \( 0 < \rho < 1 \), implies that negative charge build-up occurs during the reaction, and in turn, the rate for hydrolysis of \( C^8 \)-aryl-dG adducts in 0.1 M HCl is increased by the presence of electron-withdrawing \( p \)-substituents. Furthermore, this reaction constant value indicates that the hydrolysis of \( C^8 \)-aryl-dG is less sensitive to the electronic nature of the \( p \)-substituent than benzoic acid.\(^{34}\)

Recall that the mechanism for acid-catalyzed hydrolysis involves equilibrium protonation at \( N^7 \) (\( K_{\text{a1}} \)) followed by rate-limiting cleavage of the glycosidic bond (\( k_1 \)),\(^{20-22, 26}\) as shown in Scheme 2-3 for dG. Hydrolysis is more favourable when the \( p \)-phenyl substituent is electron-withdrawing, evidenced by the faster rates of hydrolysis recorded for the \( \text{CHO}_p \)dG and \( \text{CN}_p \)dG adducts compared to \( \text{OH}_p \)dG, \( \text{O}_{\text{Me}}p \)dG and \( \text{Me}_p \)dG, as the negative charge at the \( N^7 \)-position of dG, that develops as the sugar is removed, is stabilized by the electron-withdrawing moiety.
(b) Hydrolysis in pH 4 Buffer

Hydrolysis of $C^8$-aryl-dG adducts was also evaluated in pH 4 50 mM citrate buffer at 48.4 °C, with results recorded in Table 2-3. These particular conditions were again chosen in order to draw direct comparison to the rates for hydrolysis of dG, as determined previously by Bruylants and co-workers.\textsuperscript{21} The rate for hydrolysis of $C^8$-aryl-dG adducts was found to be considerably slower in pH 4 buffer than in 0.1 M HCl, while comparison of values for $k_{\text{obs}}$ shows that the $C^8$-aryl-dG adduct $^{\text{Ph}dG}$ underwent deglycosylation approximately 100 times faster than dG in pH 4 conditions at 48.4 °C. Recall that $k_{\text{obs}}$($^{\text{Ph}dG}$/$k_{\text{obs}}$(dG) $\approx$ 20 in 0.1 M HCl, considerably lower than the same ratio in pH 4 buffer.\textsuperscript{34} The same phenomenon was observed for the hydrolysis of dG adducts with $C^8$-arylamino substituents, that exhibited significantly higher rates of hydrolysis than dG at pH values of approximately 3 – 7, but became comparable, within a factor of five, at pH < 2.\textsuperscript{26}

Examination of the hydrolytic rate data in Table 2-3 again revealed a trend in the $k_{\text{obs}}$ and $t_{1/2}$ values recorded in pH 4 buffer for the $p$-substituted $C^8$-aryl-dG adducts. Adducts with electron-donating $p$-phenyl substituents, including $^{\text{OHPh}dG}$, $^{\text{OMePh}dG}$ and $^{\text{MePh}dG}$, with $t_{1/2}$ = 26.6, 22 and 23.5 minutes, respectively, underwent hydrolysis approximately 1.2-fold faster than those with electron-withdrawing $p$-phenyl substituents, including $^{\text{CNPh}dG}$ and $^{\text{CHOPh}dG}$, with $t_{1/2}$ = 30 and 29.1 minutes, respectively.\textsuperscript{34} The behaviour exhibited by $p$-substituted $C^8$-aryl-dG adducts in pH 4 buffer was contrasted by the $o$-substituted $C^8$-aryl-dG adducts, including $^{o\text{OHPh}dG}$, $^{o\text{OMePh}dG}$ and $^{o\text{MePh}dG}$, that underwent hydrolysis at a significantly slower rate than their $p$-substituted counterparts, as shown by the $k_{\text{obs}}$ and $t_{1/2}$ values in Table 2-3. In particular, $^{o\text{MePh}dG}$ underwent
hydrolysis approximately eleven times slower than \( ^{\text{MePh}} \text{dG} \) in pH 4 buffer and only showed a ten-fold increase in rate compared to \( \text{dG} \).\(^{34}\)

In order to better understand the influence of the \( p \)-phenyl substituent on hydrolysis of \( C^8 \)-aryl-dG adducts in pH 4 buffer, plots were constructed of \( \log \frac{k_{\text{obs}}}{k_{\text{obs}}(\text{PhdG})} \) as a function of the Hammett \( \sigma^+ \) substituent constant, as shown in Figure 2-8. For hydrolysis in pH 4 buffer, a better Hammett plot correlation was achieved using \( \sigma^+ \) substituent constant values instead of \( \sigma_p \) values, which take into account the generation of positive charge in the transition state of the reaction (at the \( N^7 \)-site) that is stabilized by direct resonance interaction with an electron-donating \( p \)-substituent.

\[ \text{Figure 2-8. Hammett plot for } p \text{-substituted } C^8 \text{-aryl-dG adducts of } \log \frac{k_{\text{obs}}}{k_{\text{obs}}(\text{PhdG})} \text{ versus substituent constant } \sigma^+. \text{ Rates for hydrolysis were recorded in 50 mM citrate buffer, pH 4, } \mu = 0.31 \text{ M NaCl at } 48.4 \, ^\circ \text{C, using an adduct concentration of } 50 \, \mu \text{M.} \]

The resulting Hammett plot shows a strong linear correlation, with a small slope (\( \rho \)) of \(-0.073 \pm 0.006 \) observed. This reaction constant value, of \( \rho < 0 \), implies that positive charge build-up occurs during the reaction, and in turn, the rate for hydrolysis of \( C^8 \)-aryl-dG adducts in pH 4 buffer is increased by the presence of electron-donating \( p \)-
substituents. Recall that with regards to the effect of the electronic nature of the $p$-phenyl substituent on hydrolysis rates, the opposite trend was observed in 0.1 M HCl, with the rate for hydrolysis decreased by the presence of electron-donating $p$-substituents. The difference in sign for the Hammett plot slope for hydrolysis in 0.1 M HCl versus pH 4 buffer is indicative of this difference. Again, as has been reported previously for hydrolysis of dG,$^{20-22}$ as shown in Scheme 2-3, and $C^8$-substituted dG analogues,$^{26}$ the $N^7$-protonated substrate is subject to cleavage ($k_1$) of the glycosidic bond. While the cleavage of the $C1'-N^9$ bond is rate-limiting, the rate of hydrolysis is also influenced by the measure of $K_{a1}$. The equilibrium more strongly favours formation of the protonated species in 0.1 M HCl than in pH 4 buffer. Recall that the logarithmic measure of the acid dissociation constant has been determined for each $C^8$-aryl-dG adduct,$^{34}$ with $pK_{a1}$ values given in Table 2-2. In pH 4 buffer, the presence of electron-donating, as opposed to electron-withdrawing, $p$-substituents, more strongly favours formation of the protonated starting material, as these groups effectively increase the basicity of the $N^7$-position of the adduct through resonance interaction, with evidence provided by the higher $pK_{a1}$ values possessed by $^{\text{OHPH}}\text{dG}$, $^{\text{OMePH}}\text{dG}$ and $^{\text{MePH}}\text{dG}$ compared to $^{\text{CHOPH}}\text{dG}$ and $^{\text{CNPH}}\text{dG}$ adducts.

For the rate data obtained in pH 4 buffer, the $pK_{a1}$ values reported in Table 2-2 were also used to estimate $k_1$ for the rate-limiting glycosyl bond cleavage from the $N^7$-protonated $C^8$-aryl-dG adducts.$^{34}$ It is important to point out that while acid-catalyzed hydrolysis of dG$^{20-22}$ and $C^8$-substituted analogues$^{26}$ involves the formation of both monoprotonated and diprotonated substrates, in pH 4 buffer, involvement of the diprotonated species can be ignored. Therefore, in pH 4 buffer conditions, the rate expression simplifies to $k_{\text{obs}} = k_H a_H$, where $a_H$ is defined as $H^+$ activity and can be
approximated as \([H^+]\), and \(k_1 \approx k_{obs} K_{a1}/[H^+]\). Rearrangement and substitution of the two equations gives \(k_1 \approx k_{obs} K_{a1}/[H^+]\), with values for \(k_1\) given in Table 2-3.\(^{34}\) The attachment of the aryl substituent to the \(C^8\)-position of dG clearly increases the magnitude of \(k_1\) relative to \(k_1\) for dG. Values for \(k_1\) determined for the \(p\)-substituted \(C^8\)-aryl-dG adducts are approximately ninety- to 200-fold larger than \(k_1\) for dG, while values for \(k_1\) determined for the \(o\)-substituted adducts are only about nine to sixty times larger compared to \(k_1\) for dG.\(^{34}\) For comparison, Heinrich and co-workers have shown that \(C^8\)-arylamino substituents increase \(k_1\) by approximately three- to fifteen-fold,\(^{26}\) which is similar to the rate enhancement exerted by the least reactive \(C^8\)-aryl-dG adduct, \(o\text{MePh}dG\).

(c) Influence of Sterics on Hydrolysis

As previously noted, in both 0.1 M HCl and pH 4 buffer conditions, the \(o\)-substituted \(C^8\)-aryl-dG adducts underwent hydrolysis slower than the corresponding \(p\)-substituted adducts.\(^{34}\) This observed decrease in the rate for hydrolysis for the \(o\)-substituted adducts was somewhat surprising given that a bulky substituent at the \(C^8\)-position is expected to increase the rate of hydrolysis due to the relief of strain in the activated complex in an \(D_N^*A_N\) mechanism.\(^{33}\) An explanation for the decrease in rates for hydrolysis for \(o\)-substituted adducts compared to \(p\)-substituted adducts can be provided, however, through the consideration of changes in the twist angle (\(\theta\)) when going from the \(N^7\)-protonated nucleoside adduct to the neutral nucleobase, with \(N^7\)H, lacking the deoxyribose sugar moiety, as shown in Figure 2-9 for \(\text{OHPh}dG\), \(\text{oOMePh}dG\) and \(\text{oMePh}dG\).\(^{34}\) It is important to note that the twist angle for the \(N^7\)H deglycosylated nucleobase is slightly different from that of the \(N^9\)H deglycosylated nucleobase, values of
which were given in Table 2-1. Again, structural properties of the \(N^7\)H deglycosylated nucleobase adducts were determined by DFT calculations, performed by the Wetmore group. Structures were fully optimized with B3LYP/6-31G(d) and relative energies obtained from B3LYP/6-311+G(2df,p) single-point calculations.

![Structural Changes](image)

**Figure 2-9.** Structural changes in the \(C^8\)-aryl-dG adduct upon deglycosylation for \(\text{OHPh}^dG\), \(\text{oOMePh}^dG\) and \(\text{oMePh}^dG\), as determined by DFT calculations.

For all \(p\)-substituted \(C^8\)-aryl-dG adducts, a decrease in twist angle of approximately 40 ° is observed upon removal of the sugar group from the twisted \(N^7\)-protonated species to give the planar nucleobase, as shown in Figure 2-9 for the \(\text{OHPh}^dG\) adduct. A significant relief in steric strain therefore occurs upon hydrolysis of \(p\)-
substituted C\textsuperscript{8}-aryl-dG adducts.\textsuperscript{34}

For the o-substituted adducts \textsuperscript{oOHPh}dG and \textsuperscript{oOMePh}dG the decrease in twist angle upon sugar removal to form the planar nucleobase is only approximately 28 \degree, as the N\textsuperscript{7}-protonated nucleoside adduct is relatively planar due to H-bonding interactions between the o-substituent and N\textsuperscript{7}H\textsuperscript{+}, as shown in Figure 2-9 for \textsuperscript{oOMePh}dG. Therefore, the relief in steric strain for \textsuperscript{oOHPh}dG and \textsuperscript{oOMePh}dG upon sugar removal is not as great as it is for the p-substituted series of C\textsuperscript{8}-aryl-dG adducts, and in turn, the rate of hydrolysis is diminished.\textsuperscript{34} For the other o-substituted adduct \textsuperscript{oMePh}dG, the decrease in twist angle upon formation of the deglycosylated species is approximately 39 \degree, which suggests a substantial relief of strain. However, in this case, unlike all other C\textsuperscript{8}-aryl-dG adducts, the neutral nucleobase is not planar, but instead has a twist angle of 29 \degree, as shown in Figure 2-9. Therefore, because the deglycosylated species is also sterically hindered, the relief of steric strain for \textsuperscript{oMePh}dG upon sugar removal is lowered due to hindrance in the free nucleobase. The lack of steric strain relief implies that deglycosylation of this adduct is not as favourable, thereby resulting in a lower rate of hydrolysis compared to the other C\textsuperscript{8}-aryl-dG adducts.\textsuperscript{34}

\textit{(d) Determination of Activation Parameters}

The effects of the structural changes in the C\textsuperscript{8}-aryl-dG adduct on the rates of deglycosylation can be further analyzed through consideration of experimentally determined activation parameters. To allow for the determination of activation parameters, Eyring plots of ln \textit{k}_{obs}/T as a function of 1/T were generated for the Ph\textsuperscript{dG}, \textsuperscript{OHPh}dG, \textsuperscript{CHOPh}dG and \textsuperscript{oOMePh}dG adducts, with the plot for Ph\textsuperscript{G} shown in Figure 2-10.
Figure 2-10. Eyring plot of \( \ln k_{\text{obs}} / T \) versus \( 1/T \) for the \( C^8 \)-aryl-dG adduct \( \text{Ph}dG \). Rates for hydrolysis were recorded 0.1 M HCl at 25 \(^\circ\)C, 30 \(^\circ\)C, 37 \(^\circ\)C, 45 \(^\circ\)C, 55 \(^\circ\)C and 60 \(^\circ\)C, using an adduct concentration of 50 \( \mu \)M.

The creation of Eyring plots allowed for determination of Gibbs energy of activation (\( \Delta G^\neq \)), enthalpy of activation (\( \Delta H^\neq \)) and entropy of activation (\( \Delta S^\neq \)) parameters for \( \text{Ph}dG,^{34} \text{OHPh}dG, \text{CHOPh}dG \) and \( \text{oOMePh}dG \). Values for these parameters are provided in Table 2-4. Activation parameters have previously been determined for dG in 0.1 M HCl by Bruylants and colleagues,\(^{21}\) and are also provided in Table 2-4 for comparison.

Values of \( \Delta S^\neq \) for the hydrolysis of \( \text{Ph}dG, \text{OHPh}dG, \text{CHOPh}dG \) are positive, which is typical of two-step acid-catalyzed hydrolysis reactions.\(^{20}\) Positive \( \Delta S^\neq \) values are indicative of an increase in entropy, and thus an increase in randomness, of the system. The value of \( \Delta S^\neq \) for the hydrolysis of \( \text{oOMePh}dG \) is negative, which indicates neighbouring group participation and a lower degree of glycosidic bond breakage, resulting in a more ordered transition state structure.\(^{21}\)

Values of \( \Delta G^\neq \) for the hydrolysis of \( C^8 \)-aryl-dG adducts are all lower than the values of the same parameter for dG, by a difference of 2.1 kcal mol\(^{-1}\) (8.8 kJ mol\(^{-1}\)) for \( \text{oOMePh}dG \) to 2.8 kcal mol\(^{-1}\) (12 kJ mol\(^{-1}\)) for \( \text{Ph}dG.^{34} \) This indicates that attachment of the

79
$C^8$-phenyl substituent lowers the barrier for cleavage of the C1'-N° bond, providing further explanation for the increase in rate for hydrolysis observed for $C^8$-aryl-dG adducts compared to dG.\(^{34}\)

### Table 2-4. Activation parameters determined from Eyring plots for the hydrolysis of $C^8$-aryl-dG adducts in 0.1 M HCl.

<table>
<thead>
<tr>
<th>adduct</th>
<th>$\Delta G^\neq$ (kcal mol(^{-1}))(^b)</th>
<th>$\Delta H^\neq$ (kcal mol(^{-1}))(^c)</th>
<th>$\Delta S^\neq$ (cal mol(^{-1}) K(^{-1}))(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhdG</td>
<td>18.4 ± 0.1</td>
<td>19.3 ± 0.2</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>OHPhdG</td>
<td>18.5 ± 0.0</td>
<td>18.9 ± 0.1</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>CHOPhdG</td>
<td>17.9 ± 0.1</td>
<td>18.3 ± 0.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>oMePhdG</td>
<td>19.1 ± 0.0</td>
<td>17.7 ± 0.4</td>
<td>−4.6 ± 1.2</td>
</tr>
<tr>
<td>dG(^d)</td>
<td>21.2</td>
<td>22.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

\(^a\) Taken from Reference no. 21. \(^b\) Calculated from $\Delta G^\neq = \Delta H^\neq - T\Delta S^\neq$ at 25 °C.
\(^c\) Calculated from the slope of the Eyring plot $(-\Delta H^\neq /R)$. \(^d\) Calculated from the $y$-intercept of the Eyring plot ($\ln(k_B/\hbar) + \Delta S^\neq /R$).

(e) Hydrolysis at Physiological pH

Hydrolysis kinetics between pH 1 – 4 for $C^8$-aryl-dG adducts PhdG, OHPhdG, CHOPhdG and oMePhdG were determined at 37 °C, and plots of log $k_{obs}$ as a function of pH yielded straight lines\(^{34}\), as shown in Figure 2-11. Plots of pH rate profiles for dG\(^{20-22,26}\) and other purine nucleosides\(^{50}\) also yielded straight lines. This has been interpreted to mean that $k_1/K_{a1} (k_H) \approx k_2/K_{a2},^{20-22,26,50}$ where $k_2$ is defined as the rate constant for the glycosidic bond cleavage of the diprotonated species (recall that $K_{a2}$ defines protonation to form the dication). Furthermore, the resulting straight lines in pH rate profiles indicates that the rate-limiting step for hydrolysis is unchanging and that hydrolysis shows a continuous first-order dependence on $H^+$ activity.\(^{20-22}\)
Figure 2-11. pH rate profiles of $\log k_{\text{obs}}$ versus pH for the hydrolysis of $C^8$-aryl-dG adducts $\text{Ph}_{\text{dG}}$ (◆), $\text{OHP}_{\text{dG}}$ (■), $\text{CHO}_{\text{dG}}$ (○) and $\text{oMe}_{\text{dG}}$ (×). Hydrolysis was recorded in 50 mM phosphate buffer, $\mu = 0.31$ M NaCl (pH 1) and 50 mM citrate buffer, $\mu = 0.31$ M NaCl (pH 2, 3 and 4) at 37 °C.

Resulting straight lines in pH rate profile plots permitted extrapolation of the rate data to pH 7 for an estimate of hydrolysis rates at physiological pH, as shown in Table 2-5.34
Table 2-5. Summary of first-order rate constants ($k_{obs}$) and half-lives ($t_{1/2}$) for the hydrolysis of C$^{8}$-heteroaryl-dG adducts in pH 1, 2, 3 and 4 buffer at 37 °C.

<table>
<thead>
<tr>
<th>adduct</th>
<th>pH 1</th>
<th>pH 2</th>
<th>pH 3</th>
<th>pH 4</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^{a}$</td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^{b}$</td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^{b}$</td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^{b}$</td>
<td>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (days)$^{c}$</td>
</tr>
<tr>
<td>PhdG</td>
<td>1.36 ± 0.08, 0.510</td>
<td>0.300 ± 0.006, 2.31</td>
<td>(5.91 ± 0.05) $\times$ 10$^{-2}$, 11.7</td>
<td>(4.1 ± 0.1) $\times$ 10$^{-3}$, 170</td>
<td>1.90 $\times$ 10$^{-5}$, 25</td>
</tr>
<tr>
<td>OHPdG</td>
<td>0.95 ± 0.02, 0.73</td>
<td>0.217 ± 0.004, 3.19</td>
<td>(4.24 ± 0.05) $\times$ 10$^{-2}$, 16.3</td>
<td>(5.5 ± 0.1) $\times$ 10$^{-3}$, 125</td>
<td>3.80 $\times$ 10$^{-5}$, 13</td>
</tr>
<tr>
<td>CHOPhD</td>
<td>4.6 ± 0.1, 0.15</td>
<td>0.504 ± 0.007, 1.36</td>
<td>(4.6 ± 0.3) $\times$ 10$^{-2}$, 15</td>
<td>(4.3 ± 0.1) $\times$ 10$^{-3}$, 160</td>
<td>4.06 $\times$ 10$^{-6}$, 118</td>
</tr>
<tr>
<td>oMePhD</td>
<td>0.37 ± 0.03, 1.9</td>
<td>0.047 ± 0.002, 15</td>
<td>(5.4 ± 0.5) $\times$ 10$^{-3}$, 130</td>
<td>(4 ± 1) $\times$ 10$^{-4}$, 1730</td>
<td>5.20 $\times$ 10$^{-7}$, 924</td>
</tr>
</tbody>
</table>

$^{a}$ Determined in 50 mM phosphate buffer, $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M. $^{b}$ Determined in 50 mM citrate buffer, $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M. $^{c}$ Estimated rate data based on first-order dependence on H$^{+}$ activity.
For the Ph\textsubscript{d}G adduct, a first-order rate constant of $1.90 \times 10^{-5}$ min\(^{-1}\), with $t_{1/2} = 25$ days, was determined for hydrolysis at pH 7.\(^{34}\) For comparison, a rate for spontaneous loss of purines from duplex DNA at pH 7.4 at 37 °C is $\sim 3 \times 10^{-11}$ s\(^{-1}\), with $t_{1/2} = 730$ years.\(^{51}\) Lindahl and Karlstrom were the first to investigate the rate of depurination of native DNA in biologically relevant conditions, through the use of double-stranded \textit{Bacillus subtilis} DNA with radioactively labeled purine residues.\(^{51}\) Therefore, while C\(^8\)-aryl-dG adducts are significantly more reactive than dG toward hydrolysis, they are reasonably stable at physiological pH\(^{34}\) and should be even more stable within duplex DNA where purines are more resistant to hydrolysis.\(^{17,52}\)

2.3.4 Kinetic Studies with C\(^8\)-Aryl-G Modified Trimers

\textit{(a) Synthesis of C\(^8\)-Aryl-G Modified Trimers}

As C\(^8\)-aryl-dG adducts are expected to be even more stable within the context of nucleic acids, adducts were incorporated into short three nucleotide sequences (trimers) to facilitate determination of rates for hydrolysis within DNA. Incorporation of \(p\)-phenyl substituted C\(^8\)-aryl-dG adducts Ph\textsubscript{d}G, OMePh\textsubscript{d}G and CNPh\textsubscript{d}G into the same trimer sequence was first performed in order to allow for direct comparison of the effect of the electronic properties of the incorporated adduct on the rate of hydrolysis. Incorporation of the OMePh\textsubscript{d}G adduct into trimer sequences with different 5\(^{\prime}\)-flanking bases was also performed to allow for determination of the effect of sequence on rates for hydrolysis.

The C\(^8\)-aryl-dG adduct Ph\textsubscript{d}G was incorporated into the trimer 5\(^{\prime}\)-GXT at position X using standard $\beta$-cyanoethylphosphoramidite chemistry according to published protocols\(^{40,53}\) to give the modified trimer 5\(^{\prime}\)G(Ph\textsubscript{d}G)T, as carried out by Michael
Sproviero in the Manderville laboratory at the University of Guelph (Guelph, ON). Modified oligonucleotide synthesis by standard phosphoramidite chemistry was previously outlined in Chapter 1, section 1.3.2, with the \textsuperscript{Ph}dG adduct converted into a phosphoramidite, using the synthetic strategy outlined in Scheme 1-6, for trimer incorporation by solid-phase synthesis, as shown in Scheme 1-7. The C\textsuperscript{8}-aryl-dG adducts \textsuperscript{OMePh}dG and \textsuperscript{CNPh}dG were incorporated into the 5'-GXT substrate at position X by application of the postsynthetic method\textsuperscript{41} involving palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with the brominated trimer, 5'-G(8-Br-G)T, and 10 equivalents of 4-methoxyphenyl-boronic acid or 4-cyanophenyl boronic acid, to give the modified trimers 5'-G(\textsuperscript{OMePh}dG)T and 5'-G(\textsuperscript{CNPh}dG)T, respectively. The \textsuperscript{OMePh}dG adduct was also incorporated into the 5'-AXT and 5'-CXT substrates at position X by use of the same reaction with the brominated trimer, 5'-A(8-Br-G)T or 5'-C(8-Br-G)T, and 10 equivalents of 4-methoxyphenyl-boronic acid, to give the modified trimers 5'-A(\textsuperscript{OMePh}dG)T and 5'-C(\textsuperscript{OMePh}dG)T, respectively. This reaction was previously discussed in detail in Chapter 1, section 1.3.3, with the Suzuki-Miyaura synthetic strategy shown in Scheme 1-8.

Immediately following synthesis, all product mixtures of C\textsuperscript{8}-aryl-G modified trimers were purified by RP HPLC using gradient elution with the following buffers: 95:5 aqueous 50 mM TEAA (pH 7.2)/acetonitrile and 30:70 aqueous 50 mM TEAA (pH 7.2)/acetonitrile. The desired product, along with remaining starting material, was identified in the resulting chromatogram using both diode array (monitored at \(\lambda_{\text{abs}} = 258\) nm) and fluorescence (monitored at \(\lambda_{\text{ex}}\) and \(\lambda_{\text{em}}\) of the incorporated adduct) detection. RP HPLC traces of product mixtures following Suzuki-Miyaura coupling reactions are shown in Figures 2-12 to 2-15, with both methods of detection featured.
Figure 2-12. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of 5'-G(8-Br-G)T with 10 eq. of 4-methoxyphenyl-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{ex} = 280$ nm and $\lambda_{em} = 390$ nm.
Figure 2-13. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of 5'-G(8-Br-G)T with 10 eq. of 4-cyanophenyl-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{ex} = 320$ nm and $\lambda_{em} = 390$ nm.
Figure 2-14. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of 5'-A(8-Br-G)T with 10 eq. of 4-methoxyphenyl-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{ex} = 280$ nm and $\lambda_{em} = 390$ nm.
Figure 2-15. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of 5’-C(8-Br-G)T with 10 eq. of 4-methoxyphenyl-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{ex} = 280$ nm and $\lambda_{em} = 390$ nm.

In all cases, a small amount of the brominated trimer starting material was observed to elute at $\sim 13 - 16$ minutes, with a single peak generated for the desired product at $\sim 5$ minutes following the starting material peak. The UV spectral properties of all isolated products can be found in Appendix B. The UV spectra of the products displayed unique characteristics not present in the spectra of the brominated trimers. The UV spectra of brominated trimers exhibited one absorbance band at $\sim 260$ nm; this was expected, as the native DNA bases are known to absorb in the 260 nm wavelength.
Spectra of all C₈-aryl-G modified oligonucleotides showed a predominant absorbance band at ~ 270 nm. In addition, the PhdG and PhOMe dG modified trimers displayed absorbance, in the form of a shoulder, at a red-shifted wavelength in the range of ~ λ = 290 – 300 nm, while the PhCN dG modified trimer displayed an additional well-separated absorbance band at ~ 320 nm. The accompanying absorbance signifies the successful incorporation of the C₈-aryl moiety into the trimer, as it generally matches the absorbance of the corresponding C₈-aryl-dG adduct. Photophysical parameters of C₈-aryl-dG adducts can be reviewed in Table 2-1.

Fluorescence detection also proved advantageous for identification of the product peak. The C₈-aryl-dG modified trimers were expected to show fluorescence, as while the intrinsic fluorescence of the native nucleosides is weak, as previously discussed in Chapter 1, section 1.1.3 (b), modified bases are known to exhibit fluorescence. As shown in Figures 2-12b to 2-15b, fluorescence detection results in a significantly intense peak for the product, and not for any other component of the Suzuki-Miyaura coupled product mixture, allowing for accurate determination of the product peak.

Mass spectrometry (MS) analysis positively confirmed the identity of all C₈-aryl-G modified trimers, synthesized by Suzuki-Miyaura coupling or standard phosphoramidite chemistry. Analysis was performed by Keegan Rankin at the University of Toronto (Toronto, ON), by use of an ESI source operated in negative mode. The ESI spectra showed the expected clusters of multiply charged peaks for the modified trimers, with results of this analysis summarized in Table 2-6. Mass spectra for all C₈-aryl-G modified oligonucleotides, including spectra of the starting brominated trimers, are included in Appendix B. Integration of HPLC traces allowed for determination of yields.
from Suzuki-Miyaura couplings. Yields reflect a significant level of success in the use of this coupling method for the synthesis of $C^8$-aryl-dG modified trimers. Yields ranged from 50 – 94 %, and are given in Table 2-6.
Table 2-6. Yields and ESI\textsuperscript{−} MS analysis of C\textsuperscript{8}-aryl-G modified trimers.

<table>
<thead>
<tr>
<th>trimer</th>
<th>product formula</th>
<th>yield (%)\textsuperscript{c}</th>
<th>calc'd mass\textsuperscript{d}</th>
<th>exptl m/z (ESI\textsuperscript{−})\textsuperscript{e}</th>
<th>exptl mass\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5\textquotesingle-G(PhG)T\textsuperscript{a}</td>
<td>C\textsubscript{36}H\textsubscript{42}N\textsubscript{12}O\textsubscript{17}P\textsubscript{2}</td>
<td>94</td>
<td>976.2</td>
<td>[M − H]\textsuperscript{−} = 975.7</td>
<td>976.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[M − 2H]\textsuperscript{2−} = 487.5</td>
<td></td>
</tr>
<tr>
<td>5\textquotesingle-G(OMePhG)T\textsuperscript{b}</td>
<td>C\textsubscript{37}H\textsubscript{44}N\textsubscript{12}O\textsubscript{18}P\textsubscript{2}</td>
<td>94</td>
<td>1006.2</td>
<td>[M − H]\textsuperscript{−} = 1005.1</td>
<td>1006.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[M − 2H]\textsuperscript{2−} = 502.2</td>
<td></td>
</tr>
<tr>
<td>5\textquotesingle-G(CNPhG)T\textsuperscript{b}</td>
<td>C\textsubscript{37}H\textsubscript{41}N\textsubscript{13}O\textsubscript{17}P\textsubscript{2}</td>
<td>50</td>
<td>1001.2</td>
<td>[M − H]\textsuperscript{−} = 1000.7</td>
<td>1001.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[M − 2H]\textsuperscript{2−} = 499.9</td>
<td></td>
</tr>
<tr>
<td>5\textquotesingle-A(OMePhG)T\textsuperscript{b}</td>
<td>C\textsubscript{37}H\textsubscript{44}N\textsubscript{12}O\textsubscript{17}P\textsubscript{2}</td>
<td>86</td>
<td>990.2</td>
<td>[M − H]\textsuperscript{−} = 989.1</td>
<td>990.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[M − 2H]\textsuperscript{2−} = 494.2</td>
<td></td>
</tr>
<tr>
<td>5\textquotesingle-C(OMePhG)T\textsuperscript{b}</td>
<td>C\textsubscript{36}H\textsubscript{44}N\textsubscript{10}O\textsubscript{18}P\textsubscript{2}</td>
<td>92</td>
<td>966.2</td>
<td>[M − H]\textsuperscript{−} = 965.0</td>
<td>966.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[M − 2H]\textsuperscript{2−} = 482.2</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Synthesized on solid-phase using standard phosphoramidite chemistry. \textsuperscript{b} Synthesized by Suzuki-Miyaura coupling reactions with 8-Br-G-modified trimers. \textsuperscript{c} Yield derived from integration of the HPLC trace. Yield of the modified trimer synthesized by the phosphoramidite approach was not determined. \textsuperscript{d} Monoisotopic mass of most abundant isotopologue. \textsuperscript{e} Measured m/z from mass spectrum.
(b) Rates for Hydrolysis of $C^8$-Aryl-dG Modified Trimers

The hydrolysis of $C^8$-aryl-dG modified trimers was measured by fluorescence spectroscopy, by monitoring the appearance of the deglycosylated product at its excitation and emission maxima. As rates for hydrolysis of $C^8$-aryl-dG modified trimers were recorded using a trimer concentration of 1 µM, fluorescence spectroscopy was employed to measure deglycosylation, as opposed to UV-vis spectroscopy, due to its higher level of sensitivity. First-order rate constants and half-lives were first determined for the hydrolysis of 5'-G($C^8$-aryl-G)T modified trimers in 0.1 M HCl at 37.2 °C, with results provided in Table 2-7.

<table>
<thead>
<tr>
<th>trimer</th>
<th>$k_{obs}$ (min$^{-1}$), $t_{1/2}$ (min)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-G($Ph$G)T</td>
<td>0.037 ± 0.004, 19</td>
</tr>
<tr>
<td>5'-G($OMe$PhG)T</td>
<td>0.0226 ± 0.0003, 30.7</td>
</tr>
<tr>
<td>5'-G($CN$PhG)T</td>
<td>0.063 ± 0.007, 11</td>
</tr>
</tbody>
</table>

$^a$ Rates for hydrolysis were recorded using a trimer concentration of 1 µM.

Examination of the rate data collected for the $C^8$-aryl-dG modified trimers reveals they underwent hydrolysis slower than the modified nucleosides. Comparison to the first-order rate constants and half-lives recorded for the corresponding $C^8$-aryl-dG adducts in Table 2-3 shows that the deoxyribose sugar is lost ~ 25 times slower from the trimer than from the nucleoside adduct. Similar to the rate data collected for the corresponding adducts, the rate for hydrolysis of $C^8$-aryl-dG modified trimers in 0.1 M HCl is increased by the presence of electron-withdrawing $p$-substituents, with $t_{1/2} = 11$.
minutes recorded for the 5'-G(CNPhG)T trimer, while \( t_{1/2} = 30.7 \) minutes was recorded for the 5'-G(OMePhG)T trimer.

First-order rate constants, \( k_{\text{obs}} \), and half-lives, \( t_{1/2} \), were then determined for the hydrolysis of \( \text{OMePh} \)dG modified trimers in 0.1 M HCl at 37.2 °C, with results provided in Table 2-8. Examination of the rate data collected for the \( \text{OMePh} \)dG modified trimers reveals that the 5'-flanking base does not effect the rate of hydrolysis, as for each trimer, rate of hydrolysis was determined to be generally the same, with \( t_{1/2} \approx 30 \) minutes.

<table>
<thead>
<tr>
<th>trimer</th>
<th>( k ) (min(^{-1}))</th>
<th>( t_{1/2} ) (min)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-A(\text{OMePh}G)T</td>
<td>0.0238 ± 0.0002, 29.1</td>
<td></td>
</tr>
<tr>
<td>5'-C(\text{OMePh}G)T</td>
<td>0.0227 ± 0.0005, 30.5</td>
<td></td>
</tr>
<tr>
<td>5'-G(\text{OMePh}G)T</td>
<td>0.0226 ± 0.0003, 30.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Rates for hydrolysis were recorded using a trimer concentration of 1 µM.

These slower rates of hydrolysis recorded for \( C^8\)-aryl-dG incorporated trimers compared to the modified nucleosides were expected, as the rate of depurination is known to become increasingly slower as one moves from nucleosides, to single-stranded DNA, to double-stranded DNA.\(^{17}\) Lindahl and Nyberg determined that purines in single-stranded DNA are released at a rate four-fold greater than that in double-stranded DNA,\(^{52}\) with an understanding of this result provided by Arrhenius plots of \( \ln k \) as a function of \( 1/T \). The activation energy \( (E_a) \) obtained from the slope was similar for each of depurination in single- and double-stranded DNA. The entropy of activation obtained from the \( y \)-intercept, however, revealed that \( \Delta S^\ddagger \) for depurination is significantly more
favourable for the process in single-stranded DNA than in double-stranded DNA. When the glycosyl bond breaks, the purine residue released from the single-stranded DNA will experience an increase in entropic freedom. When this same reaction occurs in double-stranded DNA, the increase in entropic freedom will not be nearly as favourable, as the rotational and translational motion of the purine nucleobase is constrained in the double-strand by stacking and H-bonding interactions. This analysis can also be considered with regards to comparison of rates for hydrolysis of C₈-aryl-dG nucleoside adducts to C₈-aryl-dG modified trimers. The increase in entropic freedom upon hydrolysis of the trimer incorporated adduct would not be as great as the entropic freedom gained upon hydrolysis of the nucleoside, as the nucleobase would be constrained in the short trimer strand by stacking interactions with the flanking bases.

2.3.5 Rationale for Abasic Site Formation

The hydrolysis kinetics obtained for p- and o-phenyl C₈-substituted dG adducts suggests that these adducts are unlikely to be intermediates prior to loss of the deoxyribose sugar and that an alternative explanation accounts for the accompaniment of abasic site formation during C₈-aryl-dG adduct formation at physiological pH, as observed for the adducts shown in Figure 2.1. Insight into the cause of abasic site formation during adduction is provided by recent studies from the Kenttämaa laboratory on reactions of phenyl radicals with nucleic acids in the gas phase. This research involved the use of mass spectrometry coupled with laser-induced acoustic desorption (LIAD) to analyze the reactivity of phenyl radicals with dinucleotides in the gas phase. Both H-atom abstraction from the deoxyribose sugar moiety and direct radical addition to
the $C^8$-position of purine bases was observed,$^{55-57}$ as has been previously noted for the reactivity of phenyl radicals toward DNA in solution.$^{11,36-38,58}$ Interestingly, the radical addition reaction at $C^8$ of purines was found to always be followed by glycosyl bond cleavage, resulting in formation of the deglycosylated nucleobase.$^{55-57}$

**Scheme 2-4**

Scheme 2-4 shows the resonance-stabilized radical nucleoside intermediate that forms upon direct attachment of the phenyl radical at the $C^8$-site of dG. In path A, the radical intermediate undergoes homolytic cleavage of the glycosidic bond to eliminate the free nucleobase, as was observed in the gas phase for addition of the phenyl radical to $C^8$ of purines.$^{55-57}$ Path B illustrates the competitive process to path A, in which H-atom abstraction results in formation of the stable nucleoside adduct. This stable $C^8$-aryl-dG adduct would only be expected to undergo hydrolysis under the appropriate acidic or low pH conditions. In the gas phase, path B cannot compete with path A, and therefore in this state, only homolytic glycosyl bond cleavage is observed.$^{55-57}$ In solution, however, path
B would be expected to compete with path A. A resonance-stabilized cyclohexadienyl radical that undergoes rapid H-atom transfer with molecular oxygen, with a second-order rate constant of $1.64 \times 10^9$ M$^{-1}$ s$^{-1}$ in benzene, can be considered as a model for path B.\textsuperscript{58} This reaction was determined to show essentially no dependence on solvent polarizability, polarity and ability to participate in H-bonding.\textsuperscript{58} Molecular oxygen concentration in air and O$_2$-saturated solutions of 0.24 and 1.2 mM, respectively, have been provided in the literature.\textsuperscript{59} Applying the molecular oxygen concentration for an O$_2$-saturated solution,\textsuperscript{59} a first-order rate constant of $\sim 2 \times 10^6$ s$^{-1}$ can be estimated for path B.\textsuperscript{34} Therefore, oxygen is likely to promote C$^8$-aryl-dG adduct formation in solution by providing a rapid and productive path for aromatization of the nucleoside radical intermediate.\textsuperscript{60} The rate estimated for H-atom abstraction in path B suggests a similar rate for the homolytic C1'-N$^9$ glycosidic bond cleavage in path A, given that significant levels of abasic site formation have been found to accompany C$^8$-aryl-dG adduction,\textsuperscript{7,8,11} with the C1' radical, as shown in Scheme 2-4, expected to form an oxidized abasic site in the presence of molecular oxygen and water.\textsuperscript{61,62}
2.4 Conclusions

This study allowed for conclusions to be drawn with regards to the structural and hydrolytic stability properties of $C^8$-aryl-dG adducts. DFT calculations revealed that removal of the deoxyribose sugar moiety affords a planar nucleobase, that in addition, exhibits red-shifted absorbance compared to the nucleoside.\textsuperscript{34} These adducts undergo protonation at the $N_7$-site of dG, with spectroscopically determined ionization constants similar to that measured for dG.\textsuperscript{34} $C^8$-Aryl-dG adducts are more prone than dG to acid-catalyzed hydrolysis; adducts that bear $p$-phenyl substituents possess $k_1$ values that are approximately ninety to 200-fold larger than $k_1$ for dG, while adducts that bear $o$-phenyl substituents possess $k_1$ values that are approximately nine to sixty-fold larger. Relief of steric strain upon removal of the deoxyribose sugar moiety provides a rationale for this relative reactivity.\textsuperscript{34} Both calculations and experimental data show that in addition to the presence of the $C^8$-substituent, proton affinity of the $N_7$-position and a decrease in Gibbs energy of activation contribute to the increase in the rate of the glycosidic bond cleavage compared to dG.\textsuperscript{34} Despite the enhanced reactivity of $C^8$-aryl-dG adducts in acid compared to dG, they are relatively stable at physiological pH, with $t_{1/2} \approx 25$ days.\textsuperscript{34} Hydrolytic stability of these adducts was further increased upon incorporation into trimers. Given that abasic site formation is known to accompany $C^8$-aryl addition of dG, results suggest these nucleoside adducts are unlikely intermediates to depurination at physiological pH. Instead, a resonance-stabilized, radical nucleoside intermediate that forms upon direct radical attachment at the $C^8$-site of dG, and is a known precursor to $C^8$-aryl-dG adduct formation following H-atom abstraction, is the more likely intermediate to the elimination of the nucleobase by homolytic glycosidic bond cleavage.\textsuperscript{34}
2.5 References


Chapter 3.

$C^8$-Heteroaryl-2'-Deoxyguanosine Adducts as Fluorescent Probes
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3.1 Introduction

Fluorescence spectroscopy is a powerful bioanalytical tool for the study of the structure and dynamics of biological macromolecules. Recall, however, from the Chapter 1, section 1.1.3 (b) discussion on fluorescence spectroscopy that the intrinsic fluorescence emission of the nucleosides, dG, dA, dT and dC, is too weak for consideration of use in biological applications. The purines and pyrimidines are practically non-emissive in neutral aqueous conditions, with fluorescence quantum yield ($\phi_{fl}$), defined as the ratio of photons emitted to photons absorbed, of $\sim 1 \times 10^{-4}$. As fluorescence spectroscopy is one of the most informative and sensitive analytical techniques, the non-emissive character of DNA is a major challenge to biophysical researchers interested in exploring nucleic acids. Interestingly, this limitation in emissive ability can be overcome by replacing a normal base with a modified base that has suitable fluorescent properties, and can successfully report on the surrounding DNA structure. Such modified bases are commonly referred to as fluorescent probes. The search for emissive nucleoside analogues has been extensive, and in particular, has attracted synthetic organic chemists to develop a range of chemical and enzymatic methods to enable the alteration of the natural bases, which, as aromatic heterocycles, are receptive to diverse modifications. Even minimal structural and electronic perturbations to the normal nucleobases can dramatically alter their photophysical characteristics and allow for the generation of a fluorescent molecule. Fluorescent nucleic acid probes have been found to be very useful tools in qualitative and quantitative analyses, including: fluorescence in situ hybridization; fluorescence quenching; emission anisotropy, a phenomenon by which emitted light possesses unequal intensities along different axes of
polarization; and fluorescence resonance energy transfer (FRET).\textsuperscript{5} In FRET, a pair of acceptor/donor fluorophores are covalently attached to specific positions in a nucleic acid strand. Upon excitation of the donor, non-radiative energy is transferred to the acceptor when in close proximity, which is then re-emitted fluorescently at a longer wavelength.\textsuperscript{6} This tool is frequently utilized to detect structural changes in biomacromolecular systems and measure distances in DNA.\textsuperscript{5,7} Interestingly, the first nucleobase analogue FRET-pair was recently designed by Wilhelmsson and co-workers.\textsuperscript{7} Emissive DNA has also found application in biosensing applications, such as aptamer detection. Aptamers are single-stranded DNA molecules with specific ligand binding activities.\textsuperscript{8} Aptamers have been selected for their ability to bind to a variety of small molecules, with examples including organic dyes,\textsuperscript{8} the mycotoxin ochratoxin A (OTA)\textsuperscript{9} and those with therapeutic implications, such as proteins.\textsuperscript{10}

When designing a modified nucleoside for function as a fluorescent probe, a number of factors must be considered. It is ideal for the nucleoside analogue to be highly fluorescent and as structurally similar as possible to the native nucleosides. It is preferable if the probe displays different optical properties, such as excitation and emission maxima, than those of the native nucleosides.\textsuperscript{11} Recall from Table 1-1 that natural DNA absorbs at ~ 260 nm, and typically emits from ~ 300 to 340 nm.\textsuperscript{12} In particular, absorption of the probe at a wavelength different from natural DNA allows for selective excitation of the modified nucleoside. Furthermore, the probe must be stable and soluble in an aqueous environment, and at pH levels tolerated by enzyme activities and DNA interactions.\textsuperscript{11} In a recent review of fluorescent analogues of biomolecular building blocks, Tor suggested the organization of fluorescent modified nucleosides into
five categories; chromophoric base analogs, pteridines, expanded nucleobases, extended nucleobases and isomorphic nucleobases.  

The replacement of the natural nucleobase with an established fluorophore, usually a PAH, constitutes the structure of a chromophoric nucleobase analogue. Many of these fluorescent nucleobases exhibit absorption at a wavelength $\geq 345$ nm, allowing for selective excitation in the presence of natural DNA, and also possess quantum yields nearing unity.  

A vast number of these analogues have been designed and studied by Kool and colleagues, examples of which are shown in Figure 3-1, and include replacement of the natural nucleobase with a pyrene, perylene, benzopyrene, coumarin, terphenyl or terthiophene ring system.

![Figure 3-1. Examples of chromophoric base analogues (dR = 2'-deoxyribose).](image-url)
Pteridines, shown in Figure 3-2, are naturally occurring heterocycles whose structures are related to that of the purines. The use of pteridines as fluorescent probes was developed mainly by Hawkins and co-workers. The most promising probes within the pteridine family are the guanine analogues 3-MI and 6-MI, and the adenine analogues DMAP and 6-MAP. Pteridines display an absorption band above 300 nm, with emission at ~ 430 nm, and are characterized by high quantum yields ranging from $\phi_n = 0.39 – 0.88$.

![Figure 3-2. Examples of pteridines.](image)

Extension of the conjugation of the natural nucleobases via fusion of an additional aromatic ring on to the pyrimidine or purine ring system generates so-called expanded nucleobases. The majority of these expanded nucleobases retain their Watson-Crick (W-C) H-bonding face, but their large surface area may potentially perturb DNA structure upon oligonucleotide incorporation. Expanded nucleobases, examples of which are shown in Figure 3-3, possess favourable photophysical properties, as extending the conjugation of the nucleoside tends to result in red-shifted absorption compared to their natural counterparts, with emission bands in the visible range. One of the first expanded
nucleobases examined was etheno-dA, an expanded dA nucleoside, with Leonard and co-workers investigating its potential application as a fluorescent probe. Etheno-dA has a red-shifted absorption compared to the natural nucleoside, and emits at 415 nm with a large quantum yield.\textsuperscript{16,17} Kool and co-workers synthesized size-expanded purines by inserting a benzene ring in between the two heterocyclic rings of dA or dG, creating nucleosides 2.4 Å larger than the native bases. These analogues were found to absorb light in two bands, at ~ 260 nm and a longer wavelength of ~ 330 nm, emit in a range between ~ 380 – 410 nm and possess high quantum yields.\textsuperscript{18}

A large number of fluorophores in the class of expanded nucleobases are derived from the pyrimidine family. Pyrrolo-dC is a commercially available fluorescent base analogue,\textsuperscript{15} that with a low-energy absorption band at 350 nm, can be selectively excited. This analogue also possesses desirable emission properties, with a wavelength maximum at 460 nm and $\phi_h = 0.20$.\textsuperscript{19} The tricyclic cytosine analogue 1,3-diaza-2-oxophenothiazine (tC), and its oxo-homologue, 1,3-diaza-2-oxophenoxazine (tC$^o$), were originally developed by Matteucci and colleagues for antisense purposes,\textsuperscript{20} with Wilhelmsson later discovering their favourable spectroscopic properties. The tC analogue has an emission maximum at 505 nm, with $\phi_h = 0.20$,\textsuperscript{21} while the tC$^o$ analogue has an emission maximum situated at 455 nm and a similar quantum yield to that of tC.\textsuperscript{15} Recall that the Wilhelmsson group developed the first nucleobase derived FRET-pair; this pair consists of tC$^o$ as the energy donor and another analogue of tC, 7-nitro-1,3-diaza-2-oxophenothiazine (tC$\text{nitro}$), as the energy acceptor.\textsuperscript{7} Also first introduced by Matteucci, and further developed by Sasaki and co-workers,\textsuperscript{3} is the base analogue known as the G-clamp, so-called because of its ability to simultaneously recognize both the W-C and
Hoogsteen faces of guanine through the formation of four H-bonds. The G-clamp displays absorption at ~ 365 nm, with a corresponding emission maximum at 450 nm. The Hudson laboratory has developed its own version of the G-clamp, known as boPhpC, also designed for the purpose of guanine recognition through the formation of four H-bonds. This analogue possesses excellent photophysical properties, with excitation and emission bands centered at 365 nm and 468 nm, respectively, and a quantum yield value of 0.32. Fluorescence was also shown to be sensitive to the solvent environment. The boPhpC analogue was derived from the fluorophore PhpC, also first introduced by the Hudson group. This analogue was generated from substitution of the 6-methyl group in the above-mentioned pyrrolo-dC with a phenyl group. This expanded nucleobase was found to be much more emissive than pyrrolo-dC, although its overall brightness, a measure of the molar extinction coefficient (ε) as a function of quantum yield, was limited by a low extinction coefficient.

The group of Saito and co-workers has developed a range of multi-cyclic fluorescent probes, including the dC-based analogues BPP and NPP. The fluorophore BPP absorbs at ~ 350 nm, well separated from the natural bases, and emits at ~ 390 nm, with a relatively low quantum yield value of 0.04. The structurally related NPP, with an additional benzene ring compared to BPP, absorbs similarly at ~ 350 nm, but emits at ~ 420 nm and displays a substantially higher quantum yield of 0.26. Sekine and colleagues have also explored the use of cyclized dC analogues as fluorescent probes; these analogues extend the heterocycle surface by linking the four and five positions on the pyrimidine core. An early derivative, dC₄pp, was found to emit strong fluorescence at 360 nm upon UV irradiation at 300 nm. The extension of the bicyclic system in dC₄pp to
the tricyclic system in the dC$_{ppp}$ analogue resulted in a large red-shift in both absorption and emission.\textsuperscript{25} The benzoquinazoline fluorophore BgQ, a naphthalene expanded derivative of thymine, exhibits a emission band in the visible range as result of an absorption band at 360 nm, both of which are considerably red-shifted from that of the native nucleobase.\textsuperscript{26} Finally, Tor and co-workers have designed new thymine analogues with a thiophene ring fused to the pyrimidine core. The thieno[3,2]-rT analogue was shown to have absorption and emission bands at 292 and 351 nm, respectively, and a low a quantum yield of $\sim 0.06$.\textsuperscript{15} In contrast, the isomeric [3,4] analogue displays a similar absorption band at 304 nm,\textsuperscript{3} but gives rise to a strong red-shifted visible emission at 412 nm, with $\phi = 0.48$.\textsuperscript{27} The most important property of these fused analogues is their observed sensitivity, with respect to photophysical parameters, to polarity.\textsuperscript{3}
Figure 3-3. Examples of expanded nucleobases.

Nucleosides containing extended nucleobases are distinguished by fluorescent moieties that are linked or conjugated to the natural nucleobase, via either rigid or
flexible linkers. The connection of a known chromophore to a nucleobase typically results in the creation of new fluorophores with unique photophysical characteristics. A drawback to these analogues is that their large size has potential to cause significant structural perturbation upon DNA incorporation. Examples of extended nucleobases with non-conjugating linkers are shown in Figure 3-4. Pyrene was linked directly to dG to give an analogue, Py-dG, with red-shifted absorption and emission, compared to the native nucleoside. Although this analogue is weakly fluorescent, it is responsive to changes in its microenvironment.\textsuperscript{28,29} The Saito group has reported on the synthesis of a pyrenyl-labeled $C^8$-alkylamino-substituted dG derivative (Py\textsubscript{I}dG) that is strongly fluorescent and also possesses the advantage of ease of incorporation into oligonucleotides for future studies in DNA.\textsuperscript{30} Okamato and co-workers designed and synthesized four novel fluorescent nucleosides in which 6-propionyl-2-dimethylaminonapthalene (PRODAN or PDN), a known solvatochromic fluorophore, was attached at position $C^5$ of pyrimidines or $C^8$ of purines to give PDN\textsubscript{X} (X = dU, dC, dA and dG) bases, with the PDN\textsubscript{dG} nucleoside shown in Figure 3-4. These PDN\textsubscript{X} analogues were found to retain the inherent fluorescence of PDN and display a sensitivity in excitation and emission wavelength maxima to solvent polarity.\textsuperscript{31} Another example of an extended nucleobase formed via a non-conjugated linker is the new fluorescent analogue, adenosine-1,3-diazaphenoxazine (Adap), which has shown promise as use for the selective detection of 8-oxo-dG in duplex DNA.\textsuperscript{32}
There also exists a variety of examples of extended nucleobases with conjugating linkers, some of which are shown in Figure 3-5. A structurally simple example of this type of extended nucleobase is the 8-(2)-phenylethenyl-dG (StdG) analogue, which emits at 450 nm as a result of an absorption band at 340 nm, and possesses a high quantum yield of 0.49. Hudson and co-workers found that a dU nucleoside linked at C5-site to a phenylethynyl moiety is fluorescent, while Saito’s group linked the same substituent to the C8-site of dG, and showed that this analogue has strong fluorescence at ~ 475 nm in non-polar solvents, but relatively weak fluorescence in polar solvents. Saito has also designed and synthesized photochromic pyrenyl derivatized nucleobases, amongst which includes a vinylpyrenyl-substituted dG (VPy-dG) analogue, linked to the nucleoside via a double bond, that shows unique ‘on-off’ fluorescence switching owing to its rapid
photoisomerization between the strongly fluorescent $E$-isomer (shown in Figure 3-5), with emission at 420 nm, and the weakly fluorescent $Z$-isomer, with emission at 365 nm.$^{37}$ Furthermore, Saito and co-workers recently reported on the design of novel push-pull type solvatofluorochromic dG derivatives. Push-pull probes are typically composed of three moieties: and electron donor (an electron rich aryl group), an electron acceptor (an electron poor aryl group) and a linker, which can be a single, double or triple bond.$^{38,39}$ In order to construct these donor-accepter systems, an electron-withdrawing (aromatic) group (EWG) was linked to the $C^8$-site of dG via triple bonds separated by a pyrene chromophore, or a short chain of double bonds. The resulting extended nucleobases $^{CN}dG$ and $^{Ac}dG$ emit at ~ 540 nm, with fluorescence showing strong solvent dependency.$^{40}$ The extended nucleobases $^{CB}dG$ and $^{AB}dG$ possessed large brightness factors and also exhibited strong fluorescence sensitivity to solvent polarity.$^{41}$
The final category of fluorescent modified nucleosides as defined by Tor is that of isomorphic nucleobases. These analogues closely resemble the corresponding natural nucleobases with respect to their overall dimensions, H-bonding patterns and ability to form isostructural W-C base pairs. The biggest advantage of these analogues is their strong similarity to the native nucleosides, which results in minimal structural disruption upon oligonucleotide incorporation. However, because favourable photophysical
characteristics for fluorescent modified nucleosides, such as red-shifted absorption and high quantum yields, are normally associated with significant structural changes and extended conjugation, isomorphic nucleobases can be challenging to develop. The most well-known isomorphic nucleobase is 2-aminopurine (2AP), one of the first and most widely used fluorescent nucleoside analogues. Since the initial publication in 1969 highlighting the fluorescent properties of this analogue, 2AP has been reported in more than 1600 contributions.\(^3\) This constitutional isomer of dA has an excitation maximum at \(~305\,\text{nm}\),\(^4\) allowing for selective excitation in the presence of other bases, possesses a quantum yield of 0.68,\(^5\) and its emission undergoes a bathochromic shift with increasing solvent polarity.\(^3\) Moreover, 2AP forms a stable W-C type base pair with thymine and preserves the normal B-form structure of duplex DNA.

Hirao and colleagues have extensively investigated the isomorphic fluorescent nucleosides 2-thienyl-purine and 2-thiazolyl-purine, which can both be viewed as 2AP derivatives. These analogues were designed to form unnatural base pairs with the heterocycles pyridin-2-one and imidazolin-2-one for the purpose of fluorescent labeling of RNA.\(^43-45\) The incorporation of the thiophene or thiazole ring at the 6-position of the purine results in red-shifted absorption (\(~355\,\text{nm}\)) compared to 2AP, with strong emission at \(~450\,\text{nm}\).\(^3\) 8-Aza-dG is a guanosine analogue with \(\text{N}^8\) in place of \(\text{C}^8\), that has a high quantum yield (\(\phi_{\text{fl}} = 0.55\)) when \(\text{N}^8\) is unprotected at high pH, and a much lower fluorescence intensity with \(\text{N}^8\) is protonated at neutral pH.\(^46\) A newly introduced fluorescent mimic VdG, formed from the attachment of a vinyl moiety at the \(\text{C}^8\)-site of dG, possesses a very high quantum yield of 0.72 and displays emission properties sensitive towards changes in the microenvironment.\(^47\)
The attachment of a phenyl moiety at the C⁸-site of a purine nucleobase has resulted in the creation of a number of intriguing fluorophores. Wagner and co-workers have attached a variety of aryl and heteroaryl substituents to C⁸ of guanosine diphosphate (GDP)-mannose to form fluorescent analogues, including 8-phenyl-GDP-mannose, that emit at ~ 400 nm.⁴⁸ The Manderville group has synthesized and examined a variety of fluorescent phenolic purines, including 8-(3''-Cl,4''-OH-Ph)-dA, which emits at 383 nm following excitation at 290 nm, and was determined to have a quantum yield of 0.22.⁴⁹ This phenolic adduct was found to exhibit fluorescent pH-sensing activity in the physiological pH range.⁴⁹ In addition, an analogue with a methoxy-substituted phenyl ring attached to the C⁸-site of dA displayed strong emission at 362 nm, with \( \phi_f = 0.46 \).³⁸

A considerable number of isomeric nucleobases that contain a heteroaryl system have also been developed, with many of the contributions to the preparation and study of these analogues performed by Tor and co-workers. In order to develop emissive nucleosides, they have attached different five-membered heterocycles to both pyrimidines and purines.³ Furan, thiophene, oxazole and thiazole rings have been attached to the C⁵-site of dU pyrimidines. These analogues display maxima in the lowest energy absorption band and fluorescence at ~ 310 nm and ~ 400 – 440 nm, respectively, while their quantum efficiency is relatively low (\( \phi_f = 0.01 – 0.035 \)).³⁵⁰⁻⁵² The 5-(fur-2-yl)-dU analogue shows high responsiveness to the surrounding microenvironment.¹⁵ The furan derivative of dC displays similar photophysical derivatives as the dU derivative, and has been shown to efficiently distinguish between G, 8–oxo-G and T for detection of DNA oxidative damage.⁵³ The incorporation of the furan ring at C⁸ of A and G results in nucleosides with significantly different photophysical properties than the corresponding
pyrimidine analogues. Both 8-(fur-2-yl)-rA and 8-(fur-2-yl)-rG exhibit one absorption band at ~ 300 nm, with emission centered around 375 nm and $\phi_n = 0.69$ and 0.57, respectively.\textsuperscript{51} A microenvironment-sensitive base-modified fluorescent ribonucleoside, 5-(benzothiophen-2-yl)-rU, was also recently presented by Srivatsan and co-workers. Finally, the Luedtke group has synthesized a $C^8$-pyridyl modified deoxyguanosine analogue, 2PyrdG, that exhibits a much higher quantum yield in acetonitrile than water.\textsuperscript{54} This analogue was classified by Luedtke as a push-pull fluorophore, that functions as a highly sensitive internal fluorescent probe of quadruplex folding,\textsuperscript{55} and also exhibits selective binding of metals through a bidentate effect provided by the $N^7$-position of dG and the pyridyl N-atom.\textsuperscript{55} The isomorphous nucleosides described are shown below in Figure 3-6. A number of the fluorescent probes presented in this introduction have been incorporated into oligonucleotides and their applications in single-strand and double-strand DNA are discussed further in the Chapter 4 introduction.
Figure 3-6. Examples of isomorphic nucleosides.
As a contribution to the existing family of fluorescent modified nucleobases, a number of isomorphic nucleosides were synthesized and their photophysical properties extensively investigated and reported here. In the conception of these analogues, design components of previously successful fluorophores were considered. A minimal modification of the nucleoside π-system was targeted, with extension of the purines carried out at the C₈-position by small heteroaryl moieties, including pyrrolyl, furyl, thienyl, indolyl, benzofuryl, benzothienyl and quinolyl substituents.₅₆₋₅₈ Recall from Chapter 1, section 1.2.4, that interest by the Manderville group in C₈-aryl-dG adducts, formed through the intermediacy of aryl free radicals at the C₈-site of dG, originates from the toxicological relevance of these modified nucleosides. The work reported herein this chapter effectively expands on the understanding of structure-activity relationships of C₈-aryl-dG adducts. Both the role of the heteroatom and the size of the C₈-heteroaryl group were examined with regards to changes in photophysical properties. The resulting nucleoside derivatives can be described as push-pull probes, and were found to possess longer excitation and emission wavelengths, in addition to higher quantum yields and intriguing solvatochromic parameters, as compared to the parent compound. These derivatives were also extensively characterized with regards to their structural and redox properties. While the aim of insertion of the heteroatom in the C₈-aryl substituent of the modified nucleoside was to favourably influence photophysical parameters, its presence was also used in part to increase H-bonding functionality, giving these analogues potential to serve as fluorescent probes for Hoogsteen base pairing. A selection of the C₈-heteroaryl-dG adducts studied, including the pyrrolyl- and indolyl-linked derivatives, were determined to be fluorescent reporters of H-bonding specificity, and can be used to
distinguish W-C H-bonding from Hoogsteen H-bonding. A fluorescent probe that can distinguish W-C from Hoogsteen H-bonding would be useful, as Hoogsteen base-pairs play a critical role in both triplex and quadruplex formation. They have also been reported in protein/DNA complexes, in RNA, in mismatches in DNA and may be intermediates in B-to-Z transition. The research presented herein this chapter has provided a deeper understanding of the reporting capabilities of these emissive probes and incentive for their further study in a DNA duplex or triplex environment.
3.2 Materials and Methods

3.2.1 General Methods

A detailed description of the general methods for experiments pertaining to Chapter 3 can be found in Appendix A.

3.2.2 Synthesis of 8-Bromo-2'-Deoxyguanosine

8-Bromo-2'-deoxyguanosine (8-Br-dG) was synthesized according to the literature. To a 1 L Erlenmeyer flask was added dG (8.6782 g, 0.03042 mol), followed by the addition of 400 mL of 4:1 CH$_3$CN:H$_2$O (320 mL:80 mL). N-Bromosuccinimide (NBS) (7.6779 g, 0.04314 mol) was slowly added to the mixture in roughly three eq. portions, while stirring at room temperature. Each addition was made after the yellow colour had almost completely faded from the reaction mixture. The reaction mixture was left to stir for 30 minutes at room temperature, and was then filtered under vacuum and washed with small amounts of acetone. The filtered solid was then re-suspended in approximately 200 mL of acetone (in a 1 L Erlenmeyer flask), stirred for 2 h at room temperature and then cooled on ice overnight. The resulting slurry was then filtered under vacuum and washed with small amounts of cold acetone, and 8-Br-dG was collected as a light pink solid (8.5925 g, 81.6 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 10.80 (s, 1H), 6.49 (s, 2H), 6.16 (t, $J$ = 7.4 Hz, 1H), 5.25 (d, $J$ = 4.5 Hz, 1H), 4.86 (t, $J$ = 6.0 Hz, 1H), 4.40 (bs, 1H), 3.80 (bs, 1H), 3.61 (m, 1H), 3.52 (m, 1H), 3.17 (m, 1H), 2.12 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 155.4, 153.3, 152.0, 120.6, 117.5, 87.9, 85.1, 71.0, 62.1, 36.5. Spectra obtained matched the published $^1$H NMR and $^{13}$C NMR data.
3.2.3 Suzuki-Miyaura Coupling of 8-Br-dG with Boronic Acids

These reactions were conducted according to the literature,\textsuperscript{67} and are briefly described here. Palladium (II) acetate (Pd(OAc)$_2$) (2.2 mg, 0.01 mmol), tris(3-sulfophenyl)phosphine trisodium salt hydrate (TPPTS) (14.8 mg, 0.025 mmol), Na$_2$CO$_3$ (80 mg, 0.75 mmol) and 3:1 boronic acid (1.125 mmol):8-Br-dG (0.375 mmol) was added to degassed 2:1 H$_2$O:CH$_3$CN (3.5 mL), and heated to 80 °C for 4 h under argon balloon. The reaction mixture was diluted with approximately 20 mL of water and the pH adjusted to 6-7 with 1 M HCl (aq). The mixture was allowed to cool to 0 °C for several hours before the product was recovered by vacuum filtration. High resolution mass spectra (HRMS) of all C$_8$-heteroaryl-dG adducts, except for QdG, were recorded at the Biological Mass Spectrometry Facility at the University of Guelph (Guelph, ON) on a Waters Q-Tof, operating in nanospray ionization at 0.5 uL/min detecting positive ions. The HRMS of QdG was recorded at the McMaster Regional Centre for Mass Spectrometry at McMaster University in Hamilton, Ontario, Canada, on a Micromass Global Ultima Quadrupole Time of Flight Spectrometer, operating in electrospray ionization (ESI) detecting positive ions.

8-(2"-pyrrolyl)-2'-deoxyguanosine (2Pyr$dG$). Starting from 8-Br-dG (147.5 mg, 0.4261 mmol), 1-(t-Butoxycarbonyl)-pyrrole-2-boronic acid (178.7 mg, 0.8468 mmol), Pd(OAc)$_2$ (5.1 mg, 0.023 mmol), TPPTS (13.0 mg, 0.0220 mmol), and Na$_2$CO$_3$ (97.9 mg, 0.917 mmol), 2Pyr$dG$ was obtained as a light grey solid (102.1 mg, 72.1 %). \textsuperscript{1}H NMR (DMSO-$d_6$) (300 MHz), $\delta$: 11.57 (s, 1H), 10.67 (s, 1H), 6.89 (s, 1H), 6.47 (s, 1H), 6.32 (m, 3H), 6.18 (d, $J = 2.7$ Hz, 1H), 5.17 (d, $J = 4.2$ Hz, 1H), 5.03 (t, $J = 5.6$ Hz, 1H), 4.39 (bs, 1H), 3.81 (bs, 1H), 3.64 (m, 1H), 3.55 (m, 1H), 3.23 (m, 1H), 2.08 (m, 1H); \textsuperscript{13}C
NMR (DMSO-$d_6$) (75.5 MHz), $\delta$: 156.6, 152.8, 151.8, 141.6, 120.8, 120.8, 116.9, 109.9, 108.9, 87.8, 84.6, 71.2, 62.1, 36.7; HRMS calcd for C$_{14}$H$_{16}$N$_6$O$_4$ [M+H$^+$] 333.1311, found 333.1301.

8-(2''-furyl)-2'-deoxyguanosine ($^{2\text{Fur}}$dG). Starting from 8-Br-dG (130.1 mg, 0.3759 mmol), 2-furan-boronic acid (125.0 mg, 1.117 mmol), Pd(OAc)$_2$ (2.4 mg, 0.011 mmol), TPPTS (15.3 mg, 0.0258 mmol), and Na$_2$CO$_3$ (82.7 mg, 0.775 mmol), $^{2\text{Fur}}$dG was obtained as a white solid (84.1 mg, 67.1 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), $\delta$: 10.75 (bs, 1H), 7.89 (s, 1H), 6.94 (d, $J$ = 3.3 Hz, 1H), 6.78 (m, 1H), 6.42 (s, 2H), 6.35 (t, $J$ = 7.4 Hz, 1H), 5.18 (d, $J$ = 4.2 Hz, 1H), 4.94 (t, $J$ = 5.7 Hz, 1H), 4.37 (bs, 1H), 3.79 (bs, 1H), 3.58 (m, 1H), 3.48 (m, 1H), 3.15 (m, 1H), 2.06 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), $\delta$: 156.4, 153.1, 151.6, 144.4, 144.1, 137.8, 117.4, 112.0, 111.8, 87.9, 84.4, 71.1, 62.1, 37.0; HRMS calcd for C$_{14}$H$_{15}$N$_5$O$_5$ [M+H$^+$] 334.1151, found 334.1142.

8-(2''-thienyl)-2'-deoxyguanosine ($^{2\text{Th}}$dG). Starting from 8-Br-dG (129.6 mg, 0.3744 mmol), thiophene-2-boronic acid (144.0 mg, 1.125 mmol), Pd(OAc)$_2$ (2.1 mg, 0.0095 mmol), TPPTS (14.4 mg, 0.0243 mmol), and Na$_2$CO$_3$ (81.9 mg, 0.768 mmol), $^{2\text{Th}}$dG was obtained as a white solid (53.7 mg, 41.2 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), $\delta$: 10.7 (s, 1H), 7.73 (d, $J$ = 4.5 Hz, 1H), 7.47 (d, $J$ = 3.0 Hz, 1H), 7.20 (t, $J$ = 4.2 Hz, 1H), 6.43 (s, 2H), 6.25 (t, $J$ = 7.4 Hz, 1H), 5.17 (d, $J$ = 4.5 Hz, 1H), 4.92 (t, $J$ = 5.8 Hz, 1H), 4.37 (bs, 1H), 3.80 (bs, 1H), 3.61 (m, 1H), 3.53 (m, 1H), 3.31 (m, 1H), 2.06 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), $\delta$: 156.4, 153.0, 152.1, 141.2, 131.9, 128.6, 128.1, 127.9, 117.1, 87.8, 84.4, 71.1, 62.0, 36.3; HRMS calcd for C$_{14}$H$_{15}$N$_5$O$_4$S [M+H$^+$] 350.0923, found 350.0919.
8-(3'"-pyrrolyl)-2'-deoxyguanosine (3Pyr\textsubscript{d}G). To first remove the NH protecting group of the boronic acid, 1.125 mL of tetrabutylammonium hexafluorophosphate (TBAF) was added to 1-(Triisopropylsilyl)-1H-pyrrole-3-boronic acid (305.3 mg, 1.142 mmol) in distilled THF, and the mixture was stirred until the pyrrole-3-boronic acid precipitated out of solution. Starting from the recovered pyrrole-3-boronic acid, 8-Br-dG (127.4 mg, 0.3681 mmol), Pd(OAc)\textsubscript{2} (2.6 mg, 0.0012 mmol), TPPTS (15.3 mg, 0.0258 mmol), and Na\textsubscript{2}CO\textsubscript{3} (80.6 mg, 0.799 mmol), 3Pyr\textsubscript{d}G was obtained as a white solid (62.0 mg, 50.7 %).

\begin{center}
\textsuperscript{1}H NMR (DMSO-\textsubscript{d}6) (300 MHz), \(\delta\): 11.24 (bs, 1H), 10.61 (s, 1H), 7.16 (s, 1H), 6.89 (d, \(J = 2.1\) Hz, 1H), 6.38 (d, \(J = 1.8\) Hz, 1H), 6.26 (m, 3H), 5.15 (d, \(J = 4.2\) Hz, 1H), 5.03 (t, \(J = 6\) Hz, 1H), 4.38 (bs, 1H), 3.80 (bs, 1H), 3.65 (m, 1H), 3.56 (m, 1H), 3.26 (m, 1H), 2.01 (m, 1H); \textsuperscript{13}C NMR (DMSO-\textsubscript{d}6) (75.5 MHz), \(\delta\): 156.4, 152.3, 151.4, 144.6, 118.8, 118.7, 116.8, 112.6, 108.2, 87.5, 84.5, 71.2, 62.2, 36.4; HRMS calcd for C\textsubscript{14}H\textsubscript{16}N\textsubscript{6}O\textsubscript{4} [M+H\textsuperscript{+}] 333.1311, found 333.1314.
\end{center}

\begin{center}
8-(3"'-furyl)-2'-deoxyguanosine (3Fur\textsubscript{d}G). Starting from 8-Br-dG (129.5 mg, 0.3741 mmol), 3-furan-boronic acid (126.8 mg, 1.133 mmol), Pd(OAc)\textsubscript{2} (2.5 mg, 0.011 mmol), TPPTS (13.7 mg, 0.0231 mmol), and Na\textsubscript{2}CO\textsubscript{3} (82.6 mg, 0.774 mmol), 3Fur\textsubscript{d}G was obtained as a light grey solid (82.3 mg, 66.0 %).

\textsuperscript{1}H NMR (DMSO-\textsubscript{d}6) (300 MHz), \(\delta\): 10.70 (s, 1H), 8.14 (s, 1H), 7.83 (s, 1H), 6.84 (s, 1H), 6.37 (s, 2H), 6.14 (t, \(J = 7.4\) Hz, 1H), 5.15 (bs, 1H), 4.92 (bs, 1H), 4.35 (bs, 1H), 3.77 (bs, 1H), 3.60 (m, 1H), 3.51 (m, 1H), 3.21 (m, 1H), 2.06 (m, 1H). \textsuperscript{13}C NMR (DMSO-\textsubscript{d}6) (75.5 MHz), \(\delta\): 156.4, 152.9, 151.8, 143.9, 142.5, 140.3, 116.9, 116.2, 110.8, 87.8, 84.2, 70.9, 61.7, 36.5; HRMS calcd for C\textsubscript{14}H\textsubscript{15}N\textsubscript{5}O\textsubscript{5} [M+H\textsuperscript{+}] 334.1151, found 334.1158.
\end{center}
**8-(3''-thienyl)-2'-deoxyguanosine (3^{th}dG).** Starting from 8-Br-dG (129.8 mg, 0.3750 mmol), thiophene-3-boronic acid (144.1 mg, 1.126 mmol), Pd(OAc)$_2$ (2.4 mg, 0.011 mmol), TPPTS (14.6 mg, 0.0247 mmol), and Na$_2$CO$_3$ (80.6 mg, 0.755 mmol), 3^{th}dG was obtained as a white solid (89.8 mg, 68.7 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 10.70 (s, 1H), 7.91 (d, $J = 1.8$ Hz, 1H), 7.71 (t, $J = 2.5$ Hz, 1H), 7.41 (d, $J = 5.7$ Hz, 1H), 6.38 (s, 2H), 6.15 (t, $J = 7.3$ Hz, 1H), 5.15 (d, $J = 4.5$ Hz, 1H), 4.95 (t, $J = 5.8$ Hz, 1H) 4.34 (bs, 1H), 3.78 (bs, 1H), 3.61 (m, 1H), 3.53 (m, 1H), 3.24 (m, 1H), 2.07 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.5, 152.9, 151.8, 143.0, 130.9, 128.4, 127.1, 126.6, 116.8, 87.8, 84.4, 71.1, 61.9, 36.4; HRMS calcd for C$_{14}$H$_{15}$N$_5$O$_4$S [M+H$^+$] 350.0923, found 350.0934.

**8-(2''-indolyl)-2'-deoxyguanosine (indG).** Starting from 8-Br-dG (128.0 mg, 0.3698 mmol), 1-N-Boc-indole-2-boronic acid (352.0 mg, 1.348 mmol), Pd(OAc)$_2$ (4.3 mg, 0.020 mmol), TPPTS (16.7 mg, 0.0282 mmol), and Na$_2$CO$_3$ (80.7 mg, 0.756 mmol), indG was recovered as a grey solid, following extraction with CHCl$_3$ (3×) to remove the excess 1-N-Boc-indole-2-boronic acid impurity (88.3 mg, 62.4 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 11.76 (s, 1H), 10.80 (s, 1H), 7.62 (d, $J = 7.8$ Hz, 1H), 7.43 (d, $J = 8.1$ Hz, 1H), 7.15 (t, $J = 7.5$ Hz, 1H), 7.03 (t, $J = 7.2$ Hz, 1H), 6.88 (s, 1H), 6.45 (m, 3H), 5.21 (d, $J = 4.2$ Hz, 1H), 5.04 (t, $J = 5.2$ Hz, 1H), 4.43 (bs, 1H), 3.86 (bs, 1H), 3.68 (m, 1H), 3.57 (m, 1H), 3.26 (m, 1H), 2.12 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.7, 153.1, 152.2, 140.7, 136.6, 127.7, 127.0, 122.6, 120.6, 119.6, 117.3, 111.7, 102.5, 87.8, 84.5, 71.0, 61.9, 36.6; HRMS calcd for C$_{18}$H$_{18}$N$_6$O$_4$ [M+H$^+$] 383.1468, found 383.1432.

**8-(2''-benzofuryl)-2'-deoxyguanosine (BfurG).** Starting from 8-Br-dG (149.2 mg, 0.4310 mmol), benzofuran-2-boronic acid (204.0 mg, 1.260 mmol), Pd(OAc)$_2$ (2.9 mg,
dG was recovered as a grey solid, following extraction with CHCl₃ (3×) to remove the excess benzofuran-2-boronic acid impurity (108.0 mg, 65.2 %). ¹H NMR (DMSO-d₆) (300 MHz), δ: 10.87 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.42 (m, 2H), 7.32 (t, J = 7.5 Hz, 1H), 6.50 (m, 3H), 5.18 (bs, 1H), 4.93 (bs, 1H), 4.41 (bs, 1H), 3.81 (bs, 1H), 3.62 (m, 1H), 3.52 (m, 1H), 3.19 (m, 1H), 2.15 (m, 1H); ¹³C NMR (DMSO-d₆) (75.5 MHz), δ: 156.6, 154.3, 153.5, 152.1, 146.0, 137.5, 127.7, 125.7, 123.7, 121.9, 117.9, 111.4, 108.0, 88.0, 84.4, 71.1, 62.1, 37.3; HRMS calcd for C₁₈H₁₇N₅O₅ [M+H⁺] 384.1264, found 384.1267. Spectra obtained matched the published ¹H NMR and ¹³C NMR data.⁶⁸

8-(2"-benzothienyl)-2'-deoxyguanosine (BthdG). Starting from 8-Br-dG (145.3 mg, 0.4198 mmol), benzothiophene-2-boronic acid (231.3 mg, 1.299 mmol), Pd(OAc)₂ (7.1 mg, 0.0324 mmol), TPPTS (13.4 mg, 0.0226 mmol), and Na₂CO₃ (87.3 mg, 0.818 mmol), BthdG was obtained as a grey solid (133.2 mg, 79.4 %). ¹H NMR (DMSO-d₆) (300 MHz), δ: 10.79 (s, 1H), 8.02 (m, 1H), 7.94 (m, 1H), 7.92 (s, 1H), 7.41 (m, 2H), 6.51 (s, 2H), 6.41 (t, J = 7.2 Hz, 1H), 5.19 (d, J = 4.5 Hz, 1H), 4.97 (t, J = 5.2 Hz, 1H), 4.41 (bs, 1H), 3.83 (bs, 1H), 3.66 (m, 1H), 3.59 (m, 1H), 3.22 (m, 1H), 2.05 (m, 1H); ¹³C NMR (DMSO-d₆) (75.5 MHz), δ: 156.5, 153.3, 152.6, 141.0, 139.7, 139.3, 132.5, 124.9, 124.5, 122.5, 122.3, 117.4, 87.8, 84.2, 70.8, 61.7, 36.7; HRMS calcd for C₁₈H₁₇N₅O₅S [M+H⁺] 400.1080, found 400.1080. Spectra obtained matched the published ¹H NMR and ¹³C NMR data.⁶⁸

8-(8"-quinolyl)-2'-deoxyguanosine (QdG). Starting from 8-Br-dG (133.5 mg, 0.3857 mmol), quinoline-8-boronic acid (213.1 mg, 1.232 mmol), Pd(OAc)₂ (8.8 mg, 0.040
mmol), TPPTS (16.7 mg, 0.0282 mmol), and Na$_2$CO$_3$ (107.6 mg, 1.008 mmol), $^0$dG was obtained in an impure mixture with excess quinoline-8-boronic acid. Purification by flash chromatography on silica was performed, eluting with 9:1 CHCl$_3$:MeOH. Following the removal of solvents under reduced pressure, $^0$dG was obtained as yellow crystals (42.5 mg, 27.9 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 10.88 (bs, 1H), 8.90 (d, $J = 5.7$ Hz, 1H), 8.49 (d, $J = 9.6$ Hz, 1H), 8.18 (d, $J = 9.3$ Hz, 1H), 7.93 (d, $J = 6.9$ Hz, 1H), 7.74 (t, $J = 7.6$ Hz, 1H), 7.62 (m, 1H), 6.38 (s, 1H), 5.53 (bs, 1H), 5.11 (bs, 1H), 4.90 (bs, 1H), 4.22 (bs, 1H), 3.56 (m, 2H), 3.42 (s, 1H), 3.15 (bs, 1H), 2.06 (bs, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.6, 153.0, 151.3, 150.9, 146.0, 136.5, 132.5, 130.4, 129.4, 127.6, 126.3, 122.0, 87.8, 71.1, 62.1, 37.2; HRMS calcd for C$_{19}$H$_{19}$N$_6$O$_4$ [M+H$^+$] 395.1451, found 395.1454.

3.2.4 X-Ray Crystallography of 8-(8"-Quinolyl)-2'-Deoxyguanosine

The crystal structure of 8-(8"-quinolyl)-2'-deoxyguanosine ($^0$dG) was determined at the X-ray crystallography laboratory by Dmitriy Soldatov at the University of Guelph (Guelph, ON). A yellow crystal (thick plate, 0.4 × 0.4 × 0.25 mm) was selected from the crystalline product. The crystal was mounted on a Mitegen probe and studied at 150 K on a SuperNova Agilent single-crystal diffractometer equipped with a microfocus CuK$_\alpha$ ($\lambda = 1.54184$ Å) radiation source and Atlas CCD detector. Diffraction intensity data were collected using ω-scan to the maximum 2θ angle of 148.7° (resolution of 0.8 Å), with the redundancy factor of 16. The unit cell parameters were refined using the entire data set. The data were processed using CrysAlisPro software, as developed by Agilent Technologies (Agilent Technologies, Xcalibur CCD system, CrysAlisPro Software
system, Version 1.171.35.8, 2011). Absorption corrections were applied using the
multiscan method. The structure was solved (direct methods) and refined (full-matrix
least-squares on $F^2$) using SHELXL-97, as developed by G. M. Sheldrick at the
University of Göttingen, Germany (SHELXL-97, Program for refinement of crystal
structures, 2007). Non-hydrogen atoms were refined anisotropically, while hydrogen
atoms were refined isotropically. Geometric calculations were carried out using the
WinGX$^{69}$ and Olex$^{70}$ software packages.

3.2.5 Synthesis of 8-Bromo-2'-Deoxyadenosine

8-Bromo-2'-deoxyadenosine (8-Br-dA) was synthesized according to the
literature.$^{71-73}$ To 500 mL of 1 M NaOAc buffer (adjusted to pH 5 with glacial acetic
acid) was added dA (10.0082 g, 0.03717 mol). Liquid bromine (11.8835 g, 0.07436 mol)
was very slowly added to the mixture, and the reaction mixture was stirred for 4 h at
room temperature. The reaction was quenched by the gradual addition of 5 M NaHSO$_3$,
until the red colour had completely dispersed, resulting in a clear solution. Upon
neutralization of the solution to pH 7 with 4 M NaOH, a precipitate formed. The reaction
mixture was allowed to cool on ice overnight. Following filtration of the reaction
mixture under vacuum, and washing of the collected product with small amounts of water
and acetone, 8-Br-dA was collected as a yellow solid (2.3061 g, 18.8 %). $^1$H NMR
(DMSO-$d_6$) (300 MHz), $\delta$: 8.11 (s, 1H), 7.50 (bs, 2H), 6.29 (t, J = 7.0 Hz, 1H), 5.34 (m,
2H), 4.49 (bs, 1H), 3.89 (bs, 1H), 3.64 (m, 1H), 3.49 (m, 1H), 3.22 (m, 1H), 2.19 (m,
1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), $\delta$: 155.2, 152.4, 149.9, 126.6, 119.8, 88.4, 86.5,
71.2, 62.0, 37.1.
3.2.6 Suzuki-Miyaura Coupling of 8-Br-dA with Boronic Acids

These reactions were conducted according to the literature and are briefly described here. Pd(OAc)$_2$ (2.2 mg, 0.01 mmol), TPPTS (14.8 mg, 0.025 mmol), Na$_2$CO$_3$ (80 mg, 0.75 mmol), 8-Br-dA (0.375 mmol), and the appropriate boronic acid (0.45 mmol) was added to degassed 2:1 H$_2$O:CH$_3$CN (3.5 mL) and heated to 80 °C for 4 h under an argon atmosphere. The reaction was diluted with ca. 20 mL of water and the pH adjusted to 6-7 with 1 M HCl (aq). The mixture was allowed to cool to 0 °C for several hours before the product was recovered by vacuum filtration. HRMS of all C$_8$-heteroaryl-dA adducts were recorded at the McMaster Regional Centre for Mass Spectrometry at McMaster University (Hamilton, ON) on a Micromass Global Ultima Quadrupole Time of Flight Spectrometer, operating in ESI detecting positive ions.

**8-(2''-pyrrolyl)-2'-deoxyadenosine (2Pyr dA).** Starting from 8-Br-dA (145.9 mg, 0.4419 mmol), 1-(t-Butoxycarbonyl)-pyrrole-2-boronic acid (197.6 mg, 0.9364 mmol), Pd(OAc)$_2$ (5.5 mg, 0.025 mmol), TPPTS (69.5 mg, 0.117 mmol), and Na$_2$CO$_3$ (92.4 mg, 0.866 mmol), 2Pyr dA was obtained as a grey solid (93.1 mg, 66.6 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), $\delta$: 11.58 (s, 1H), 8.09 (s, 1H), 7.17 (s, 2H), 7.01 (s, 1H), 6.57 (s, 1H), 6.43 (t, J = 7.0 Hz, 1H), 6.24 (s, 1H), 5.54 (m, 1H), 5.26 (d, J = 3.9 Hz, 1H), 4.47 (s, 1H), 3.88 (s, 1H), 3.69 (m, 1H), 3.65 (m, 1H), 3.51 (m, 1H), 2.19 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), $\delta$: 155.6, 151.4, 149.8, 144.7, 121.8, 119.8, 119.2, 111.4, 109.0, 88.2, 85.4, 71.5, 62.2, 37.4; HRMS calcd for C$_{14}$H$_7$N$_6$O$_3$ [M+H$^+$] 317.1354, found 317.1362.

**8-(2''-furyl)-2'-deoxyadenosine (2Fur dA).** Starting from 8-Br-dA (162.1 mg, 0.4910 mmol), 2-furan-boronic acid (163.6 mg, 1.462 mmol), Pd(OAc)$_2$ (6.8 mg, 0.031 mmol), TPPTS (21.4 mg, 0.0361 mmol), and Na$_2$CO$_3$ (90.2 mg, 0.845 mmol), 2Fur dA was
obtained as a grey solid (20.2 mg, 13.0 %). ¹H NMR (DMSO-δ₆) (300 MHz), δ: 8.12 (s, 1H), 7.99 (s, 1H), 7.47 (bs, 2H), 7.11 (d, J = 3.3 Hz, 1H), 6.76 (m, 1H), 6.52 (t, J = 7.2 Hz, 1H), 5.47 (m, 1H), 5.27 (d, J = 3.3 Hz, 1H), 4.48 (bs, 1H), 3.88 (m, 1H), 3.64 (m, 1H), 3.49 (m, 1H), 3.24 (m, 1H), 2.20 (m, 1H); ¹³C NMR (DMSO-δ₆) (75.5 MHz), δ: 156.0, 152.1, 149.5, 145.3, 143.4, 140.9, 122.8, 119.3, 112.0, 88.2, 85.4, 71.2, 62.1, 37.4; HRMS calcd for C₁₄H₁₆N₅O₄ [M+H⁺] 318.1198, found 318.1202.

8-(2"-indolyl)-2'-deoxyadenosine (InddA). Starting from 8-Br-dA (135.8 mg, 0.4113 mmol), 1-N-Boc-indole-2-boronic acid (321.5 mg, 1.231 mmol), Pd(OAc)₂ (3.1 mg, 0.014 mmol), TPPTS (35.6 mg, 0.0601 mmol), and Na₂CO₃ (80.2 mg, 0.752 mmol), InddA was obtained as a beige solid (80.5 mg, 53.4 %). ¹H NMR (DMSO-δ₆) (300 MHz), δ: 11.73 (s, 1H), 8.15 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.33 (s, 2H), 7.20 (t, J = 7.5 Hz, 1H), 7.07 (t, J = 6.9 Hz, 1H), 6.96 (s, 1H), 6.55 (t, J = 7.2 Hz, 1H), 5.45 (m, 1H), 5.27 (d, J = 4.2 Hz, 1H), 4.50 (bs, 1H), 3.90 (bs, 1H), 3.68 (m, 1H), 3.53 (m, 1H), 3.36 (m, 1H), 2.25 (m, 1H); ¹³C NMR (DMSO-δ₆) (75.5 MHz), δ: 156.0, 152.1, 150.1, 144.1, 136.9, 127.5, 126.1, 123.1, 121.0, 120.2, 119.9, 112.0, 104.4, 88.3, 85.4, 71.3, 62.1, 37.2; HRMS calcd for C₁₈H₁₉N₆O₃ [M+H⁺] 367.1508, found 367.1519.

8-(2"-benzofuryl)-2'-deoxyadenosine (BfurdA). Starting from 8-Br-dA (177.0 mg, 0.5361 mmol), benzofuran-2-boronic acid (288.7 mg, 1.783 mmol), Pd(OAc)₂ (4.2 mg, 0.019 mmol), TPPTS (15.4 mg, 0.0260 mmol), and Na₂CO₃ (95.5 mg, 0.895 mmol), BfurdA was obtained as a white solid (153.6 mg, 78.0 %). ¹H NMR (DMSO-δ₆) (300 MHz), δ: 8.17 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.58 (m, 3H), 7.46 (t, J = 7.9 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 6.61 (t, J = 7.0 Hz, 1H), 5.37 (m, 1H), 5.30 (d, J = 4.5 Hz, 1H), 4.54 (bs, 1H), 3.92 (m, 1H), 3.66 (m, 1H), 3.50 (m, 1H), 3.38
(m, 1H), 2.27 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.4, 154.5, 152.8, 150.0, 145.1, 140.9, 127.5, 126.3, 123.8, 122.3, 119.6, 111.5, 109.6, 88.4, 85.6, 71.3, 62.2, 37.5; HRMS calcd for C$_{18}$H$_{18}$N$_5$O$_4$ [M+H$^+$] 368.1362, found 368.1359.

**8-(2''-benzothiophenyl)-2'--deoxyadenosine (BthdA).** Starting from 8-Br-dA (139.6 mg, 0.4228 mmol), benzothiophene-2-boronic acid (232.7 mg, 1.307 mmol), Pd(OAc)$_2$ (4.9 mg, 0.022 mmol), TPPTS (16.8 mg, 0.0284 mmol), and Na$_2$CO$_3$ (80.4 mg, 0.754 mmol), BthdA was obtained as a grey solid (92.1 mg, 56.8 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 8.17 (s, 1H), 8.06 (m, 1H), 8.01 (m, 1H), 7.98 (s, 1H), 7.50 (m, 4H), 6.53 (t, $J$ = 7.2 Hz, 1H), 5.34 (m, 1H), 5.30 (d, $J$ = 4.2 Hz, 1H), 4.54 (bs, 1H), 3.92 (bs, 1H), 3.68 (m, 1H), 3.53 (m, 1H), 3.42 (m, 1H), 2.26 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.1, 152.5, 150.4, 144.4, 139.8, 139.4, 131.2, 126.1, 125.9, 125.1, 124.9, 122.4, 119.3, 88.4, 85.4, 71.2, 62.0, 37.1; HRMS calcd for C$_{18}$H$_{18}$N$_5$O$_3$S [M+H$^+$] 384.1133, found 384.1130.

**8-(8''-quinoly)-2'--deoxyadenosine (QdA).** Starting from 8-Br-dA (109.7 mg, 0.3323 mmol), quinoline-8-boronic acid (165.2 mg, 0.9550 mmol), Pd(OAc)$_2$ (4.8 mg, 0.022 mmol), TPPTS (17.0 mg, 0.0287 mmol), and Na$_2$CO$_3$ (103.3 mg, 0.9681 mmol), QdA was obtained as a yellow solid (19.8 mg, 15.7 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 9.17 (s, 1H), 8.29 (m, 2H), 7.84 (m, 3H), 7.64 (m, 1H), 7.50 (s, 2H), 5.88 (s, 1H), 5.60 (s, 1H), 5.17 (s, 1H), 4.41 (s, 1H), 3.82 (s, 1H), 3.66 (m, 1H), 3.40 (m, 1H), 3.24 (s, 1H), 2.14 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 156.4, 152.6, 150.0, 149.6, 147.8, 146.3, 135.4, 130.4, 129.6, 127.8, 126.2, 125.5, 123.3, 119.7, 88.6, 85.7, 71.3, 62.2, 37.8; HRMS calcd for C$_{19}$H$_{18}$N$_6$O$_3$Na [M+Na$^+$] 401.1328, found 401.1338.
3.2.7 Silylation of Nucleosides

(a) Silylation by [(iPr)$_2$SiCl]$_2$O

These reactions were conducted according to the literature$^{74,75}$ and are briefly described here. The nucleobase dC, dG, $^{2}$Pyr-dG, $^{Ind}$dG or $^{Bth}$dG and 5 eq. of imidazole were added to 5 mL of anhydrous $N,N$-dimethylformamide (DMF), and the reaction mixture was cooled to 0 °C. The reaction mixture was treated with 2 eq. of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ([((iPr)$_2$SiCl)$_2$O), and then warmed to room temperature and stirred under an argon atmosphere overnight. The product was precipitated upon the addition of approximately 50 mL of ice water, collected by vacuum filtration and purified by flash chromatography on silica, eluting with 9:1 CHCl$_3$:MeOH. Solvents were removed under reduced pressure. HRMS of bissilyl$^{2}$Pyr-dG, bissilyl$^{Ind}$dG and bissilyl$^{Bth}$dG, were recorded at the Biological Mass Spectrometry Facility at the University of Guelph (Guelph, ON) on a Waters Q-Tof, operating in nanospray ionization at 0.5 uL/min detecting positive ions.

3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)- $^{2'}$-deoxycytidine (bissilyldC).

Starting from dC (161.0 mg, 0.6105 mmol), imidazole (228.8 mg, 3.361 mmol), and ([((iPr)$_2$SiCl)$_2$O (0.40 mL, 1.2 mmol), bissilyldC was obtained as a white solid (148.5 mg, 51.8 %). $^1$H NMR (DMSO-$d_6$) (300 MHz), δ: 7.61 (d, $J = 7.5$ Hz, 1H), 7.13 (bs, 2H), 5.97 (dd, $J = 3.3$ Hz, 7.2 Hz, 1H), 5.68 (d, $J = 7.5$ Hz, 1H), 4.46 (m, 1H), 4.02 (dd, $J = 4.8$ Hz, 12.6 Hz, 1H), 3.92 (dd, $J = 3.0$ Hz, 12.6 Hz, 1H), 3.72 (m, 1H), 2.31 (m, 1H), 2.21 (m, 1H), 1.13-0.66 (m, 28H); $^{13}$C NMR (DMSO-$d_6$) (75.5 MHz), δ: 165.5, 154.7, 140.5, 93.6, 84.0, 83.8, 69.4, 61.3, 40.0, 17.3, 17.1, 17.1, 16.9, 16.8, 16.7, 12.6, 12.4 12.2, 11.9. Spectra obtained matched the published $^1$H NMR and $^{13}$C NMR data.$^{76}$
3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyldG).

Starting from dG (142.1 mg, 0.4981 mmol), imidazole (188.8 mg, 2.773 mmol), and ([iPr]2SiCl]2O (0.35 mL, 1.1 mmol), bissilyldG was obtained as a white solid (155.2 mg, 59.4 %). 1H NMR (DMSO-d6) (300 MHz), δ: 10.63 (s, 1H), 7.82 (s, 1H), 6.45 (s, 2H), 6.04 (m, 1H), 4.66 (m, 1H), 3.90 (m, 2H), 3.77 (m, 1H), 2.64 (m, 1H), 2.44 (m, 1H), 0.94 (m, 24H), 0.75 (m, 4H). Spectrum obtained matched the published 1H NMR data.77

8-(2''-pyrrolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyl2Pyr dG). Starting from 2Pyr dG (49.3 mg, 0.148 mmol), imidazole (76.5 mg, 1.12 mmol), and ([iPr]2SiCl]2O (0.10 mL, 0.31 mmol), bissilyl2Pyr dG was obtained as a white solid (21.1 mg, 24.1 %). 1H NMR (DMSO-d6) (300 MHz), δ: 11.61 (bs, 1H), 10.78 (s, 1H), 6.91 (s, 1H), 6.52 (s, 1H), 6.28 (m, 1H), 6.15 (s, 1H), 6.01 (s, 2H), 4.87 (m, 1H), 3.95 (m, 1H), 3.82 (m, 2H), 3.22 (m, 1H), 2.30 (m, 1H), 1.19-0.95 (m, 28H); HRMS calcd for C26H42N6O5Si2 [M+H+] 575.2828, found 575.2823.

8-(2''-indolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilylInd dG). Starting from Ind dG (52.0 mg, 0.136 mmol), imidazole (82.1 mg, 1.21 mmol), and ([iPr]2SiCl]2O (0.10 mL, 0.313 mmol), bissilylInd dG was obtained as a white solid (47.6 mg, 54.8 %). 1H NMR (CD2Cl2) (300 MHz), δ: 12.01 (bs, 1H), 10.53 (bs, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.28 (t, J = 8.1 Hz, 1H), 7.17 (m, 2H), 6.48 (m, 1H), 5.88 (bs, 2H), 5.34 (m, 1H), 4.09 (m, 1H), 4.02 (m, 2H), 3.45 (bs, 1H), 2.48 (m, 1H), 1.19-0.93 (m, 28H); HRMS calcd for C30H44N6O5Si2 [M+H+] 625.2984, found 625.2970.
8-(2'-benzothienyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilylBthdG). Starting from BthdG (131.6 mg, 0.3295 mmol), imidazole (161.1 mg, 2.366 mmol), and [(iPr)2SiCl]2O (0.21 mL, 0.66 mmol), bissilylBthdG was obtained as a white solid (85.6 mg, 39.5%). 1H NMR (DMSO-d6) (300 MHz), δ: 10.9 (bs, 1H), 8.00 (m, 1H), 7.91 (m, 1H), 7.80 (s, 1H), 7.42 (m, 2H), 6.41 (m, 1H), 6.20 (s, 2H), 4.76 (m, 1H), 3.98 (m, 1H), 3.85 (m, 2H), 3.26 (m, 1H), 2.38 (m, 1H), 1.18-0.94 (m, 28H); 13C NMR (DMSO-d6) (75.5 MHz), δ: 156.3, 153.4, 152.1, 140.6, 139.4, 139.2, 132.3, 125.4, 124.7, 124.3, 122.2, 122.1, 117.2, 84.7, 82.4, 73.5, 63.7, 37.5, 17.3, 17.1, 17.0, 16.9, 16.8, 12.6, 12.5, 12.2, 11.9; HRMS caled for C30H43N5O5SSi2 642.2596, found 642.2568.

(b) Silylation by TBDMS-Cl

This reaction was conducted according to the literature78 and is briefly described here. An excess of 2:1 imidazole:tert-butyldimethylsilyl chloride (TBDMS-Cl) was added to G or QdG in 5 mL of anhydrous DMF, and the reaction mixture was stirred at room temperature under an argon atmosphere overnight. The product was precipitated upon the addition of approximately 25 mL of water and collected by vacuum filtration. The products were purified by flash chromatography on silica, eluting with 9:1 CHCl3:MeOH. Solvents were removed under reduced pressure. HRMS of QdG(TBDMS)2 were recorded at the McMaster Regional Centre for Mass Spectrometry at McMaster University (Hamilton, ON) on a Micromass Global Ultima Quadrupole Time of Flight Spectrometer, operating in ESI detecting positive ions.
2',3',5'-tris-(tert-butyldimethylsilyl)riboguanosine (rG(TBDMS)_3). Starting from riboguanosine (173.1 mg, 0.6111 mmol), imidazole (2.004 g, 0.02943 mol), and TBDMS-Cl (2.312 g, 0.01534 mol), rG(TBDMS)_3 was obtained as a white solid (61.6 mg, 16.1 %). ^1H NMR (DMSO-d_6) (300 MHz), δ: 10.59 (s, 1H), 7.88 (s, 1H), 6.43 (s, 2H), 5.73 (d, J = 6.9 Hz, 1H), 4.58 (m, 1H), 4.16 (bs, 1H), 3.94 (bs, 1H), 3.83 (m, 1H), 3.71 (m, 1H), 0.90-0.72 (m, 27H), 0.085-(-0.29) (m, 18H). Spectrum obtained matched the published ^1H NMR data.\(^79\)

8-(8''-quinolyl)-3',5'-tris((tert-butyldimethylsilyl)oxy)-2'-deoxyguanosine (QdG(TBDMS)_2). Starting from QdG (122.4 mg, 0.3104 mmol), imidazole (340.6 mg, 5.003 mmol), and TBDMS-Cl (401.7 mg, 2.665 mmol), QdG(TBDMS)_2 was obtained as a yellow solid (114.4 mg, 59.15 %). ^1H NMR (DMSO-d_6) (300 MHz), δ: 11.71 (s, 1H), 8.88 (d, J = 5.7 Hz, 1H), 8.48 (d, J = 9.9 Hz, 1H), 8.18 (d, J = 9.3 Hz, 1H), 7.93 (d, J = 6.3 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.60 (m, 1H), 6.32 (s, 2H), 5.56 (bs, 1H), 4.36 (bs, 1H), 3.62 (bs, 1H), 3.47 (bs, 3H), 2.02 (bs, 1H), 0.80-0.62 (m, 18H), -0.018-(-0.11) (m, 12H); ^13C NMR (DMSO-d_6) (75.5 MHz), δ: 156.6, 153.0, 152.0, 151.0, 146.0, 136.6, 132.7, 130.4, 127.7, 126.3, 121.9, 86.9, 72.8, 62.9, 35.6, 25.7, 25.3, 17.8, 17.4, -5.0, -5.3; HRMS calcd for C_{31}H_{47}N_{6}O_{4}Si_{2} [M+H^+] 623.3181, found 623.3197.

3.2.8 Determination of Photophysical Properties

(a) Determination of Absorbance and Emission Parameters

For UV-Vis measurements, spectroscopic solutions of C^8-heteroaryl-purine adducts were prepared with 25 µL of the adduct stock solution and 1975 µL of 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer, pH 7, µ = 100 mM NaCl,
for a final adduct concentration of 50 µM. For fluorescence measurements, spectroscopic solutions of C$^8$-heteroaryl-purine adducts were prepared with 10 µL of the adduct stock solution and 1990 µL of 10 mM MOPS buffer, pH 7, µ = 100 mM NaCl, for a final adduct concentration 20 µM.

(b) Extinction Coefficient Determination

Extinction coefficients (ε) of C$^8$-heteroaryl-purine adducts were determined by UV-Vis spectroscopy. Absorbance was recorded for five spectroscopic solutions of increasing adduct concentration (10, 20, 30, 40 and 50 µM) in 10 mM MOPS buffer, pH 7, µ = 100 mM NaCl. The extinction coefficient was determined from the slope of a plot of absorbance versus adduct concentration:

$$\text{slope} = \varepsilon \times l$$

where ε is the extinction coefficient and l is the path length of the cuvette.

(c) Quantum Yield Measurements

Fluorescence quantum yield (φ$^f$) values of C$^8$-heteroaryl-purine adducts were determined in 10 mM MOPS buffer, pH 7, µ = 100 mM NaCl, using the comparative method. Quinine bisulfate (φ$^f$ = 0.55) in 0.5 M H$_2$SO$_4$ was used as the fluorescence quantum yield standard. Absorbance readings were kept below 0.06 to avoid inner-filter and self-absorbance phenomena. The following equation was used to calculate fluorescence quantum yields:

$$\phi_{\text{fi}c} = (A_s/A_x) \left( F_s/F_x \right) \left( \eta_s/\eta_x \right)^2 \phi_{fi}$$

where s is the standard, x is the C$^8$-heteroaryl-purine adduct, A is the absorbance at
the excitation wavelength, $F$ is the integrated area under the emission curve, $\eta$ is the refractive index of the solvent and $\phi_f$ is the fluorescence quantum yield. The refractive index corrective term was not included due to the similar refractive indices of H$_2$O and 0.5 M H$_2$SO$_4$.

(d) Solvatochromic Measurements

For all solvatochromic measurements, spectroscopic solutions of $C^8$-heteroaryl-purine adducts were prepared with 5 $\mu$L of the adduct stock solution and 1995 $\mu$L of the desired solvent, to a final adduct concentration of 10 $\mu$M.

(e) Fluorescence Measurements in Varying Solvent Viscosity

Emission of $C^8$-heteroaryl-dG adducts was first recorded in spectroscopic solutions of increasing solvent viscosity. Measurements were first conducted in 100% 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl, followed by measurements in 80:20, 60:40, 40:60 and 20:80 MOPS:glycerol, before a final measurement in 100% glycerol. For all measurements, 5 $\mu$L of the adduct stock solution was added to 1995 $\mu$L of the spectroscopic solution, for a final adduct concentration 10 $\mu$M. In all cases, emission was recorded at 20 °C. Emission of $C^8$-heteroaryl-dG nucleoside adducts was then recorded in 100% glycerol at increased temperature, in 10 °C increments, to 80 °C. Again, the final adduct concentration in the spectroscopic solution was 10 $\mu$M.
3.2.9 Cyclic Voltammetry

Redox properties of $C^8$-heteroaryl-dG adducts were determined by cyclic voltammetry (CV), as previously outlined,\textsuperscript{49,81,82} using instrumentation of the Houmam group at the University of Guelph (Guelph, ON). Electrochemical oxidation measurements of adducts were conducted using an Autolab PGSTAT30 potentiostat instrument, and electrodes polished with LECO Microid Diamond using the Spectrum System 1000. Measurements were conducted in a three-electrode glass cell under nitrogen in a solution of 0.1 M TBAF in anhydrous DMF. The working electrode used was glassy carbon, with a diameter of 2 mm. The electrode was polished and ultrasonically rinsed with ethanol. A silver wire placed in the 0.1 M DMF/TBAF solution was used as the reference electrode, and was separated from the main solution by a fine porosity frit. The reference electrode potential was calibrated \textit{in situ} against 1 mol eq. of 9,10-anthraquinone (-0.800 V versus saturated calomel electrode (SCE)). The counter electrode used was platinum wrapped in foil. For all CV measurements, the starting potential was 0 V, and the potential was first scanned 1.8 V towards positive potentials, and then scanned 1.8 V towards negative potentials. The scanning rate used was 0.2 V/s. Peak picking was achieved by correlation of values obtained from automatic software methods and manual assignment.

3.2.10 Kinetic Measurements

Hydrolysis of $C^8$-heteroaryl-dG adducts was observed by UV-Vis spectroscopy, using a previously developed method,\textsuperscript{83} as was outlined in Chapter 2, sections 2.2.8 (a), and (b) (i) \textit{and (iii)}. Note that determination of absorbance wavelength maxima of the
deglycosylated adducts was performed differently than outlined in Chapter 2. Here, UV-Vis scan measurements were obtained in acid until no further change was observed in the absorbance spectrum and a maximum absorbance was reached for the deglycosylated species.

3.2.11 Association Equilibrium Constant Determination

Binding constants were determined by fluorescence spectroscopy using only silylated derivatives. Stock solutions of bissilyl\(^{2\text{Pyr}}\)dG, bissilyl\(^{\text{Ind}}\)dG, bissilyl\(^{\text{Bth}}\)dG or \(^{0}\)dG(TBDMS)\(_2\) were prepared in CHCl\(_3\) to a concentration of 4 mM. Stock solutions of bissilyldC or rG(TBDMS)\(_3\) were prepared in CHCl\(_3\) to a concentration of 10 mM. Spectroscopic solutions were prepared with 5 \(\mu\)L of the silylated adduct derivative stock solution and 1993 \(\mu\)L of CHCl\(_3\), to a final adduct concentration of 10 \(\mu\)M. The stock solution of bissilyldC or rG(TBDMS)\(_3\) was then titrated in 2 \(\mu\)L (10 \(\mu\)M) aliquots into the spectroscopic solution until no further change was observed in fluorescence intensity. This procedure was conducted in triplicate to allow for determination of mean ± standard deviation of association equilibrium constant \((K_a)\) values for each dimer system. The \(K_a\) value was determined from the ratio of the \(y\)-intercept to the slope from the double reciprocal plot of \(F_o/(F_o-F)\) versus \(1/[\text{natural nucleobase}]\), where \(F_o\) and \(F\) are the fluorescence emission intensities in the absence and presence of the natural nucleobase, respectively.\(^{84}\)
3.3 Results and Discussion

3.3.1 Synthesis and Characterization of C^8-Heteroaryl-Purine Adducts

(a) C^8-Heteroaryl-dG Adducts

The C^8-heteroaryl-dG adducts, 2Pyr dG, 56 2Fur dG, 58 2Th dG, 58 3Pyr dG, 3Fur dG, 3Th dG, Ind dG, 56 Bfur dG, Bth dG, 57 and Q dG, shown in Figure 3-7, were synthesized using palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with 8-Br-dG and the appropriate boronic acid, using a previously described method. 67 Bromination at the C^8-position of dG was performed by reaction with NBS to afford the starting material 8-Br-dG. 66 These reactions were already discussed extensively in Chapter 1, section 1.2.4 (d), with the synthetic outlines shown in Schemes 1-4 and 1-5.
Rivera and co-workers recently synthesized the $^{\text{Bfur}}dG$ and $^{\text{Bth}}dG$ adducts, but did not investigate their structural, redox or photophysical properties. Adducts $^{2\text{Fur}}dG$, $^{2\text{Th}}dG$ and $^{3\text{Fur}}dG$ have also been synthesized by Rivera, but they were unable to optimize Suzuki-Miyaura coupling to obtain efficient yields of these adducts, and therefore relied on an alternative method of synthesis, involving treatment of 8-Br-dG and the appropriate boronic acid in DMF/CH$_3$CN mixtures under microwave irradiation. Luedtke and co-workers more recently also reported the synthesis of $^{2\text{Fur}}dG$ and $^{2\text{Th}}dG$, but again used an alternative method to Suzuki-Miyaura coupling. The Luedtke group found they were unable to use Suzuki-Miyaura coupling for adduct synthesis in a consistent efficiency, which was attributed to palladium binding to the O$_6$- and N$_7$-positions of dG. To inhibit
coordination of the metal, the $O^6$-position of dG was protected via a Mitsunobu reaction with trimethylsilylethanol, with the desired heteroaryl moiety then attached at $C^8$ under standard Stille conditions.\textsuperscript{55} Despite the problem of inefficient palladium-catalyzed Suzuki-Miyaura cross-coupling in the synthesis of $C^8$-heteroaryl-dG adducts experienced by other researchers, the adducts presented here were all successfully synthesized by Suzuki-Miyaura coupling, in yields of typically $\sim 50\%$, with yields as high as $\sim 70\%$ also reported for the synthesis of $^{2}\text{Fur}dG$, $^{3}\text{Fur}dG$ and $^{3}\text{Th}dG$, as shown in Table 3-1.

**Table 3-1.** Pd-Catalyzed Suzuki-Miyaura coupling yields for synthesis of $C^8$-heteroaryl-dG adducts.

<table>
<thead>
<tr>
<th>adduct</th>
<th>% yield$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{2}\text{Pyr}dG$</td>
<td>48.2</td>
</tr>
<tr>
<td>$^{2}\text{Fur}dG$</td>
<td>67.1</td>
</tr>
<tr>
<td>$^{2}\text{Th}dG$</td>
<td>54.4</td>
</tr>
<tr>
<td>$^{3}\text{Pyr}dG$</td>
<td>50.7</td>
</tr>
<tr>
<td>$^{3}\text{Fur}dG$</td>
<td>66.0</td>
</tr>
<tr>
<td>$^{3}\text{Th}dG$</td>
<td>68.7</td>
</tr>
<tr>
<td>$^{\text{Ind}}dG$</td>
<td>51.1</td>
</tr>
<tr>
<td>$^{\text{Bfur}}dG$</td>
<td>42.7</td>
</tr>
<tr>
<td>$^{\text{Bth}}dG$</td>
<td>56.4</td>
</tr>
<tr>
<td>$^{\text{Q}}dG$</td>
<td>24.8</td>
</tr>
</tbody>
</table>

$^a$ Derived from average yield of successful syntheses.

(b) $C^8$-Heteroaryl-dA Adducts

$C^8$-Heteroaryl-dA adducts, $^{2}\text{Pyr}dA$, $^{2}\text{Fur}dA$, $^{\text{Ind}}dA$, $^{\text{Bfur}}dA$, $^{\text{Bth}}dA$ and $^{\text{Q}}dA$ were also synthesized using palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with 8-Br-dA and the appropriate boronic acid.\textsuperscript{67} Bromination of dA to afford the starting material 8-Br-dA was performed following a different procedure\textsuperscript{71-73} from that used for
the bromination of dG, as shown in Scheme 3-1, involving the reaction of 2:1 Br<sub>2</sub>:dA in sodium acetate buffer.

Scheme 3-1

The C<sup>8</sup>-heteroaryl-dA adducts, shown in Figure 3-8, were synthesized in yields of typically ~ 50 %, with yields as high as ~ 80 % reported for the synthesis of B<sub>fur</sub>dA, as shown in Table 3-2.

**Figure 3-8.** C<sup>8</sup>-Heteroaryl-dA adducts synthesized by Pd-catalyzed Suzuki-Miyaura coupling reactions with 8-Br-dA and the appropriate boronic acid.
Table 3-2. Pd-Catalyzed Suzuki-Miyaura coupling yields for synthesis of $C^8$-heteroaryl-dA adducts.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>% Yield$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Pyr$dA$</td>
<td>51.8</td>
</tr>
<tr>
<td>2Fur$dA$</td>
<td>13.0</td>
</tr>
<tr>
<td>Ind$dA$</td>
<td>50.2</td>
</tr>
<tr>
<td>Bfur$dA$</td>
<td>78.0</td>
</tr>
<tr>
<td>Bth$dA$</td>
<td>52.2</td>
</tr>
<tr>
<td>QdA</td>
<td>15.7</td>
</tr>
</tbody>
</table>

$^a$ Yield derived from average yield of successful syntheses.

Following synthesis, $C^8$-heteroaryl-dG$^{56-58}$ and $C^8$-heteroaryl-dA adducts were characterized by both $^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy, and high resolution mass spectra were collected for each adduct. The $^1$H and $^{13}$C NMR spectra for all adducts can be found in Appendix C.

3.3.2 Structural Features of $C^8$-Heteroaryl-Purine Adducts

(a) Conformational Analysis of $C^8$-Heteroaryl-Purine Adducts

Recall from discussion in Chapter 1, section 1.2.4 (b) that the introduction of bulky substituents at the $C^8$-position of a purine residue is known to shift the conformational preference of the $N^9$-C1' glycosidic bond from anti to syn$^{33,85,86}$ As previously outlined by Luedtke and co-workers, this conformational preference can be evaluated using $^1$H and $^{13}$C NMR spectroscopy.$^{33}$ A conformational shift from anti to syn correlates with a downfield shift of H2', C1', C3' and C4' signals and an upfield shift of the C2' signal, compared to the signals observed for the native dG. The chemical shifts of each of these atoms are provided in Tables 3-3 and 3-4 for each $C^8$-heteroaryl-dG and $C^8$-heteroaryl-dA adduct, respectively.
Table 3-3. Chemical shifts (δ) of H2', C1', C2', C3' and C4' of C8-heteroaryl-dG adducts compared to dG, as recorded in DMSO–d6.

<table>
<thead>
<tr>
<th>compound</th>
<th>δ H2'</th>
<th>δ C1'</th>
<th>δ C2'</th>
<th>δ C3'</th>
<th>δ C4'</th>
</tr>
</thead>
<tbody>
<tr>
<td>dG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.50</td>
<td>82.5</td>
<td>39.5</td>
<td>70.7</td>
<td>87.5</td>
</tr>
<tr>
<td>2Pyr&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.23</td>
<td>84.5</td>
<td>36.7</td>
<td>71.2</td>
<td>87.8</td>
</tr>
<tr>
<td>2Fur&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.15</td>
<td>84.4</td>
<td>37.0</td>
<td>71.1</td>
<td>87.9</td>
</tr>
<tr>
<td>2Th&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.31</td>
<td>84.4</td>
<td>36.3</td>
<td>71.1</td>
<td>87.8</td>
</tr>
<tr>
<td>3Pyr&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.26</td>
<td>84.5</td>
<td>36.4</td>
<td>71.2</td>
<td>87.5</td>
</tr>
<tr>
<td>3Fur&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.21</td>
<td>84.2</td>
<td>36.5</td>
<td>70.9</td>
<td>87.8</td>
</tr>
<tr>
<td>3Th&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.24</td>
<td>84.4</td>
<td>36.4</td>
<td>71.1</td>
<td>87.8</td>
</tr>
<tr>
<td>Ind&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.26</td>
<td>84.5</td>
<td>36.6</td>
<td>71.0</td>
<td>87.8</td>
</tr>
<tr>
<td>Bfu&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.19</td>
<td>84.4</td>
<td>37.2</td>
<td>71.1</td>
<td>88.0</td>
</tr>
<tr>
<td>Bth&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.22</td>
<td>84.2</td>
<td>36.7</td>
<td>70.7</td>
<td>87.8</td>
</tr>
<tr>
<td>Q&lt;sup&gt;a&lt;/sup&gt;dG</td>
<td>3.15</td>
<td>84.6</td>
<td>37.2</td>
<td>71.1</td>
<td>88.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values for dG taken from Reference no. 33. <sup>b</sup> Signal not observed.

Table 3-4. Chemical shifts (δ) of H2', C1', C2', C3' and C4' of C8-heteroaryl-dA adducts compared to dA and 8-Br-dA, as recorded in DMSO–d6.

<table>
<thead>
<tr>
<th>compound</th>
<th>δ H2'</th>
<th>δ C1'</th>
<th>δ C2'</th>
<th>δ C3'</th>
<th>δ C4'</th>
</tr>
</thead>
<tbody>
<tr>
<td>dA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72</td>
<td>83.9</td>
<td>39.4</td>
<td>71.0</td>
<td>88.0</td>
</tr>
<tr>
<td>8-Br-dA</td>
<td>3.22</td>
<td>86.5</td>
<td>37.1</td>
<td>71.2</td>
<td>88.4</td>
</tr>
<tr>
<td>2Pyr&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.51</td>
<td>85.4</td>
<td>37.4</td>
<td>71.5</td>
<td>88.2</td>
</tr>
<tr>
<td>2Fur&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.24</td>
<td>85.4</td>
<td>37.4</td>
<td>71.2</td>
<td>88.2</td>
</tr>
<tr>
<td>Ind&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.36</td>
<td>85.4</td>
<td>37.2</td>
<td>71.3</td>
<td>88.3</td>
</tr>
<tr>
<td>Bfu&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.38</td>
<td>85.6</td>
<td>37.5</td>
<td>71.3</td>
<td>88.4</td>
</tr>
<tr>
<td>Bth&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.68</td>
<td>85.4</td>
<td>37.1</td>
<td>71.2</td>
<td>88.4</td>
</tr>
<tr>
<td>Q&lt;sup&gt;a&lt;/sup&gt;dA</td>
<td>3.24</td>
<td>85.7</td>
<td>37.8</td>
<td>71.3</td>
<td>88.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values for dA taken from Reference no. 87.

According to this analysis, all adducts prefer a syn conformation. The adopted conformation of these adducts might be important in the context of oligonucleotides, where conformational preference is known to play a critical role in the biological activity of bulky N-linked<sup>86,88-90</sup> and C-linked<sup>91-93</sup> C8-dG adducts formed by arylamine and a variety of other chemical carcinogens, respectively.
(b) Crystal Structure Analysis of $^0$dG

The synthesis of $^0$dG yielded the desired product in the form of pale yellow, crystals, allowing for the determination of the crystal structure of this adduct by Dimitriy Soldatov at the University of Guelph (Guelph, ON), as shown in Figure 3-9. Crystals were thick-plate and of a size of $0.40 \times 0.40 \times 0.25$ mm, and the crystal system was determined to be orthorhombic. The crystal structure also shows that the glycosidic bond linkage of the modified nucleoside has adopted the syn conformation. Details of crystal structure determination can be found in Appendix C.

Figure 3-9. Crystal structure of the $^0$dG adduct.
Insight into the structural features and electronic properties of the 2''-substituted C^8-heteroaryl-dG adducts was obtained from computational calculations performed by the Wetmore group at the University of Lethbridge (Lethbridge, AB) by application of density functional theory (DFT) calculations. Structures were fully optimized with B3LYP/6-31G(d) and relative energies were obtained from B3LYP/6-311+G(2df,p) single-point calculations. As predicted from their NMR chemical shifts and crystal structure analysis, DFT calculations confirm that the C^8-heteroaryl-dG adducts adopt the syn conformation.\textsuperscript{56,58} The calculations revealed that the fully optimized global minimum structure for adducts in the syn conformation are $\sim$ 25 kJ mol\textsuperscript{-1} more stable than that for anti, and contain an O5'-H•••N3 H-bond.\textsuperscript{56,58} In addition, the syn preferred global minimum structures of the adducts showed that the heteroatom (NH, O or S) is on the same H-bonding face as N7 of dG.\textsuperscript{56} For a reminder of atom labeling convention of DNA nucleosides, see Figure 3-7. These DFT calculations are in agreement with calculations previously presented for various dG adducts bearing C^8-phenyl rings, for which anti structures were determined to be less stable than syn structures.\textsuperscript{83,94-96}

Further structural information was provided by DFT calculations with regards to the degree of dihedral twist angle ($\theta$), shown in Figure 3-7, between the heteroaryl ring and dG nucleobase, in both the ground ($S_0$) and excited ($S_1$) states. Ground state structures were optimized at the B3LYP/6-31G(d) level, with values corresponding to $S_0$ possessing a relative energy of 0 kJ mol\textsuperscript{-1}. Excited state structures were optimized at the CIS/6-31G(d) level, with $S_1$ state optimization starting from the corresponding $S_0$ state geometry. The ground state $\theta$ angles shown some degree of twist for all C^8-heteroaryl-
dG adducts, ranging from ~15° for 2Pyr\(d\)G, 2Fur\(d\)G, Ind\(d\)G and Bfur\(d\)G adducts, to ~30° for the 2Th\(d\)G and Bth\(d\)G adducts. The larger \(\theta\) twist angle observed for the sulfur-substituted analogues can be ascribed to the larger S-atom, compared to the O and NH atoms in the other analogues. DFT calculations predicted that the twisted \(S_0\) structures relax toward planarity in \(S_1\), which is consistent with the behaviour of unsubstituted biphenyl systems. In numerous publications concerning the conformational structure of biphenyls in solution, it has been determined that in \(S_0\), the interannular twist angle between the two phenyl rings lies between 15 and 40°, whereas in \(S_1\), planar geometry is preferred.

The energy levels of the lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) were also calculated for each 2\("\)-substituted \(C^8\)-heteroaryl-dG adduct. The LUMO and HOMO for 2Pyr\(d\)G, 2Fur\(d\)G, Ind\(d\)G and Bfur\(d\)G adducts consists of delocalized \(\pi\) orbitals with relatively equal density on the heteroaryl ring system and \(d\)G nucleoside. On the other hand, while the HOMO of 2Th\(d\)G and Bth\(d\)G similarly consists of delocalized \(\pi\) orbitals, the LUMO has greater density on the \(C^8\)-thienyl or \(C^8\)-benzothienyl moiety, suggesting these two adducts possess features synonymous with push-pull analogues, as the HOMO-LUMO analysis is consistent with push-pull character from the electron-donating dG to the electron-accepting \(C^8\)-substituent.

Finally, electronic (dipole moment, \(\mu_g\)) properties of these adducts were also predicted by DFT calculations, with ground state electronic properties optimized at the B3LYP/6-31G(d) level, and excited state electronic properties optimized at the CIS/6-31G(d) level. The sulfur-based analogues 2Th\(d\)G and Bth\(d\)G were found to possess the
highest ground state dipole moments, with Debye values of 5.18 and 5.45, respectively, compared to the dipole moments determined for $^{2}$Pyr dG (3.64 Debye), $^{2}$Fur dG (4.27 Debye), $^{\text{Ind}}$dG (4.83 Debye) and $^{\text{Bfur}}$dG (4.37 Debye). In addition, all adducts were found to have higher dipole moments in $S_1$ compared to $S_0$.58

3.3.3 Photophysical Properties of $C^8$-Heteroaryl-Purine Adducts

(a) Spectral Features and Quantum Yields of $C^8$-Heteroaryl-Purine Adducts

(i) $C^8$-Heteroaryl-dG Adducts

Absorption and emission spectra of all $C^8$-heteroaryl-dG adducts were initially recorded in aqueous 10 mM MOPS buffer at pH 7,56-58 as shown in Figures 3-10 and 3-11.

![Figure 3-10. Relative absorption and emission spectra of 2"- (a) and 3"- (b) substituted $C^8$-heteroaryl-dG adducts, with $C^8$-pyrrolyl moiety (solid line), $C^8$-furyl moiety (dashed line) or $C^8$-thienyl moiety (dotted line). Spectra were recorded in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl, using an adduct concentration of 50 $\mu$M (absorbance) or 20 $\mu$M (emission).]
Figure 3-11. Relative absorption and emission spectra of C₈-benzoheteroaryl-dG adducts (a) Ind₈dG (solid line), Bfu₈dG (dashed line) and Bth₈dG (dotted line), and (b) normalized absorption and emission spectra of QdG. Spectra were recorded in 10 mM MOPS buffer, pH 7, μ = 100 mM NaCl, using an adduct concentration of 50 μM (absorbance) or 20 μM (emission).

Table 3-5. Photophysical parameters of C₈-heteroaryl-dG adducts.

<table>
<thead>
<tr>
<th>adduct</th>
<th>λₘₐₓ (nm), log ε²</th>
<th>λₑₜₜ (nm), a φₘ b</th>
<th>Δν (cm⁻¹)c</th>
<th>brightness d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Pyr₈dG</td>
<td>292, 4.36</td>
<td>379, 0.10</td>
<td>7861</td>
<td>2291</td>
</tr>
<tr>
<td>2Fur₈dG</td>
<td>292, 4.31</td>
<td>384, 0.49</td>
<td>8205</td>
<td>10005</td>
</tr>
<tr>
<td>2Th₈dG</td>
<td>284, 4.24</td>
<td>414, 0.79</td>
<td>11057</td>
<td>13729</td>
</tr>
<tr>
<td>3Pyr₈dG</td>
<td>278, 4.36</td>
<td>383, 0.02</td>
<td>9862</td>
<td>458</td>
</tr>
<tr>
<td>3Fur₈dG</td>
<td>274, 4.11</td>
<td>382, 0.04</td>
<td>10318</td>
<td>515</td>
</tr>
<tr>
<td>3Th₈dG</td>
<td>278, 4.29</td>
<td>394, 0.42</td>
<td>10590</td>
<td>8189</td>
</tr>
<tr>
<td>Ind₈dG</td>
<td>321, 4.48</td>
<td>390, 0.78</td>
<td>5512</td>
<td>23556</td>
</tr>
<tr>
<td>Bfu₈dG</td>
<td>323, 4.41</td>
<td>405, 0.76</td>
<td>6268</td>
<td>19535</td>
</tr>
<tr>
<td>Bth₈dG</td>
<td>315, 4.27</td>
<td>419, 0.46</td>
<td>7880</td>
<td>8566</td>
</tr>
<tr>
<td>QdG</td>
<td>313, 3.90</td>
<td>407, 0.03</td>
<td>7379</td>
<td>238</td>
</tr>
</tbody>
</table>

² Determined in 10 mM MOPS buffer, pH 7, μ = 0.1 M NaCl, using an adduct concentration of 50 μM (absorbance) or 20 μM (emission). b Quantum yield determined using the comparative method with quinine bisulfate in 0.5 M H₂SO₄ (φₘ = 0.55).

c Stokes shift (Δν) is calculated as (1/λₘₐₓ - 1/λₑₜₜ). d Brightness factor is calculated as ε × φₘ.
Examination of the data collected from the absorption spectra, as shown in Table 3-5, revealed that all $C^8$-heteroaryl-dG adducts possess absorbance wavelengths red-shifted from that of unmodified dG, which recall absorbs at 253 nm, suggesting that these adducts could be selectively excited in the presence of natural DNA. Note that while $Q_dG$ does display an unusual absorption spectrum, compared to the other $C^8$-benzoheteroaryl-dG adducts, with maximum absorbance at ~ 255 nm, the adduct does also exhibit absorbance at 313 nm for the $C^8$-quinolyl moiety. The adducts containing benzoheteroaryl rings are more red-shifted in absorbance wavelength compared to the adducts containing five-membered heteroaryl rings; $Ind_dG$ is red-shifted by +29 nm, for example, compared to $2Pyr_dG$. This shift is highlighted in Figure 3-12, which directly compares the absorbance spectra of $2''$-substituted $C^8$-heteroaryl-dG adducts to their $C^8$-benzoheteroaryl-dG counterparts. This red-shift in absorbance observed for the $C^8$-benzoheteroaryl-dG adduct series can be attributed to the heightened conjugation these adducts possess from the additional inserted benzene ring, compared to the five-membered $C^8$-substituted series. Increase in conjugation is known to be associated with absorbance at longer, lower energy, wavelengths.
Interestingly, absorbance wavelength of the adducts can be correlated to the computational calculated data. Within each class of 2''-substituted C$^8$-heteroaryl-dG nucleobases (five-membered and benzoheteroaryl rings), the sulfur-substituted analogues have the most blue-shifted absorption spectra, with $\lambda_{abs} = 284$ and 315 nm for $^{2\text{Th}}\text{dG}$ and $^{\text{Bth}}\text{dG}$, respectively. As determined by DFT calculations, these two adducts also possess
the highest degree of twist in angle $\theta$ in the ground state. This twist disfavours conjugation between dG and the $C^8$-moiety, and thus these adducts do not shift as greatly to longer wavelengths as the other $2^n$-substituted $C^8$-heteroaryl-dG nucleobases.$^{58}$

Examination of the data collected from the emission spectra, with wavelengths of fluorescence listed in Table 3-5, revealed that all $C^8$-heteroaryl-dG adducts possess emission wavelengths red-shifted from that of unmodified dG, which recall emits at 334 nm. The adducts containing benzoheteroaryl rings are more red-shifted in emission wavelength compared to the adducts containing five-membered heteroaryl rings; $^{\text{Ind}} \text{dG}$ is red-shifted by +11 nm, for example, compared to $^{2\text{Py}} \text{dG}$. As with the collected absorbance data, emission wavelength of the adducts can be correlated to the computational calculated data. The sulfur-substituted analogues have the most red-shifted emission following relaxation to planarity in $S_1$, with $\lambda_{em} = 414$ and 419 nm for $^{2\text{Th}} \text{dG}$ and $^{\text{Bth}} \text{dG}$, respectively, as these modified nucleosides possess the greatest $S_1$ dipole moments, and thus greater charge separation in the excited state, in comparison to the other $C^8$-heteroaryl-dG adducts. In turn, these adducts are more easily stabilized in the excited state by the solvent (water, in this case), resulting in a shift to longer, lower energy, wavelengths of emission.$^{58}$

It is important to note that the interactions responsible for general solvent effects are best represented by the Lippert equation, which describes stabilization of the excited state, and subsequent shift to lower energy, as dependent on reorientation of the solvent dipoles (relaxation) and redistribution of the electrons in the solvent molecules.$^4$ Upon measurement of emission spectra, Stokes shift ($\Delta \nu$), the difference in wavenumbers between absorbance and emission maximum,$^{31}$ for
each adduct was determined. The sulfur C⁸-substituted analogues exhibit the largest Stokes shifts compared to the other C⁸-heteroaryl-dG adducts, with these analogues possessing the most blue-shifted absorbance and the most red-shifted emission.⁵⁸

Figures 3-10 and 3-11a also showcase the relative emission intensity for the C⁸-heteroaryl-dG adducts (note that emission for QdG shown in Figure 3-11b has been normalized to unity). Quantum yields, along with brightness factors, determined as a function of extinction coefficient by quantum yield (ε × φfl), for all adducts are provided in Table 3-5. Most notably, the C⁸-heteroaryl-dG adducts possess significantly higher quantum yields, on the order of ~ 1000 – 8000 times higher, than the native dG (φfl = 9.7 × 10⁻⁵), and subsequently also possess larger brightness factors. In general, the 2''-substituted C⁸-benzoheteroaryl-dG series of adducts possess higher quantum yields and brightness factors than the corresponding five-membered C⁸-heteroaryl-dG adducts; for example, InddG has a quantum yield of 0.78, compared to that of 0.10 for 2Pyr_dG, and is also ~ ten times brighter.⁵⁶ Interestingly, QdG is considerably less fluorescent (φfl = 0.03) than the C⁸-benzoheteroaryl-dG adducts. The 2''-substituted C⁸-heteroaryl-dG adducts were found to be more fluorescent than the corresponding 3''-substituted series, by factors of five, twelve and two times for the pyrrolyl-, furyl and thienyl-substituted analogues, respectively.

(ii) C⁸-Heteroaryl-dA Adducts

Absorption and emission spectra of all C⁸-heteroaryl-dA adducts were initially recorded in aqueous MOPS buffer at pH 7, as shown in Figures 3-13 and 3-14.
Figure 3-13. Relative absorption and emission spectra of C\textsuperscript{8}-heteroaryl-dA adducts 2Pyr\textsubscript{dA} (solid line) and 2Fur\textsubscript{dA} (dashed line). Spectra were recorded in 10 mM MOPS buffer, pH 7, \( \mu = 100 \) mM NaCl, using an adduct concentration of 50 \( \mu \)M (absorbance) or 20 \( \mu \)M (emission).

Figure 3-14. Relative absorption and emission spectra of C\textsuperscript{8}-benzoheteroaryl-dA adducts (a) Ind\textsubscript{dA} (solid line), Bfur\textsubscript{dA} (dashed line) and Bth\textsubscript{dA} (dotted line), and (b) normalized absorption and emission spectra of Q\textsubscript{dA}. Spectra were recorded in 10 mM MOPS buffer, pH 7, \( \mu = 100 \) mM NaCl, using an adduct concentration of 50 \( \mu \)M (absorbance) or 20 \( \mu \)M (emission).
Table 3-6. Photophysical parameters of C₈-heteroaryl-dA adducts.

<table>
<thead>
<tr>
<th>adduct</th>
<th>λ_{abs} (nm), log ε&lt;sup&gt;a&lt;/sup&gt;</th>
<th>λ_{em} (nm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>φ&lt;sub&gt;fl&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Δν (cm&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>brightness&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Pyr dA</td>
<td>307, 4.26</td>
<td>370, 0.44</td>
<td>5546</td>
<td>8070</td>
<td></td>
</tr>
<tr>
<td>2Fur dA</td>
<td>302, 4.34</td>
<td>373, 0.48</td>
<td>6303</td>
<td>10540</td>
<td></td>
</tr>
<tr>
<td>Ind dA</td>
<td>322, 4.54</td>
<td>385, 0.34</td>
<td>5082</td>
<td>11880</td>
<td></td>
</tr>
<tr>
<td>Bfur dA</td>
<td>318, 4.49</td>
<td>386, 0.59</td>
<td>5540</td>
<td>18231</td>
<td></td>
</tr>
<tr>
<td>Bth dA</td>
<td>311, 4.40</td>
<td>401, 0.68</td>
<td>7217</td>
<td>17000</td>
<td></td>
</tr>
<tr>
<td>Q dA</td>
<td>306, 3.99</td>
<td>381, 0.09</td>
<td>6433</td>
<td>880</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined in 10 mM MOPS buffer, pH 7, µ = 0.1 M NaCl, using an adduct concentration of 50 µM (absorbance) or 20 µM (emission). <sup>b</sup> Quantum yield determined using the comparative method with quinine bisulfate in 0.5 M H₂SO₄ (φ<sub>fl</sub> = 0.55). <sup>c</sup> Stokes shift (Δν) is calculated as (1/λ_{abs} – 1/λ_{em}). <sup>d</sup> Brightness factor is calculated as ε × φ<sub>fl</sub>.

Examination of the data collected from the absorption spectra, as shown in Table 3-6, revealed that all C₈-heteroaryl-dA adducts possess absorbance wavelengths red-shifted from that of unmodified dA, which recall absorbs at 260 nm, suggesting that these adducts could be selectively excited in the presence of natural DNA. Note that while Q dA does display an unusual absorption spectrum, as similarly observed for Q dG, with maximum absorbance at ~ 260 nm, the adduct does also exhibit a distinct band for absorbance at 306 nm for the C₈-quinolyl moiety. The adducts containing benzoheteroaryl rings are more red-shifted in absorbance wavelength compared to the adducts containing five-membered heteroaryl rings; Ind dA is red-shifted by +15 nm, for example, compared to 2Pyr dA. This red-shift in absorbance observed for the C₈-benzoheteroaryl-dA adduct series can again be attributed to the heightened conjugation these adducts possess from the additional inserted benzene ring, compared to the five-membered C₈-substituted series. As already mentioned, increase in conjugation is known to be associated with absorbance at longer, lower energy, wavelengths.

Within the series of 2"-substituted C₈-benzoheteroaryl-dA nucleobases, the sulfur-
substituted analogue has the most blue-shifted absorption spectra, with \( \lambda_{\text{abs}} = 311 \) nm \( \text{Bth} \text{dA} \), compared to \( \lambda_{\text{abs}} = 322 \) and 318, for \( \text{Ind} \text{dA} \) and \( \text{Bfur} \text{dA} \), respectively. Recall that this trend was similarly observed for 2"-substituted \( C^8 \)-heteroaryl-dG and \( C^8 \)-benzoheteroaryl-dG nucleobases. While DFT calculations were not performed for the \( C^8 \)-substituted dA analogues, it can be assumed that \( \text{Bth} \text{dA} \) possesses a larger \( \theta \) twist angle in the ground state compared to \( \text{Ind} \text{dA} \) and \( \text{Bfur} \text{dA} \), ascribed to the larger S-atom. This twist would disfavour conjugation between dA and the \( C^8 \)-moiety, and thus the resulting red-shift in absorbance observed for \( \text{Bth} \text{dA} \), compared to that of dA, is not as great as the shifts observed for \( \text{Ind} \text{dA} \) and \( \text{Bfur} \text{dA} \).

Examination of the data collected from the emission spectra, with wavelengths of fluorescence listed in Table 3-6, revealed that all \( C^8 \)-heteroaryl-dA adducts possess emission wavelengths red-shifted from that of unmodified dA, which recall emits at 307 nm.\(^{12} \) The adducts containing benzoheteroaryl rings are more red-shifted in emission wavelength compared to the adducts containing five-membered heteroaryl rings; \( \text{Ind} \text{dA} \) is red-shifted by +15 nm, for example, compared to \( ^2\text{Pyr} \text{dA} \).

Figures 3-13 and 3-14a also showcase the relative emission intensity for the \( C^8 \)-heteroaryl-dA adducts (note that emission for \( ^0 \text{dA} \) shown in Figure 3-14b has been normalized to unity). Quantum yields, along with brightness factors, for all adducts are provided in Table 3-6. Most notably, the \( C^8 \)-heteroaryl-dA adducts possess significantly higher quantum yields, on the order of \( \sim 4000 \) – \( 8000 \) times higher, than the native dA (\( \phi_n = 8.6 \times 10^{-5} \)), and subsequently also possess larger brightness factors. In general, the 2"-substituted \( C^8 \)-benzoheteroaryl-dA series of adducts possess higher quantum yields and
brightness factors than the corresponding five-membered C\textsuperscript{8}-heteroaryl-dA adducts; for example, \textsuperscript{B}fur\textsuperscript{-}dA has a quantum yield of 0.59, compared to that of 0.48 for \textsuperscript{2}Pyr\textsuperscript{-}dA, and is also \textasciitilde{} two times brighter. Interestingly, \textsuperscript{Q}dA is considerably less fluorescent (\(\phi_\text{fl} = 0.09\)) than the C\textsuperscript{8}-benzoheteroaryl-dA adducts, likewise observed for \textsuperscript{Q}dG.

All subsequent photophysical parameter measurements, and studies involving determination of redox and hydrolytic stability properties, were performed for only C\textsuperscript{8}-heteroaryl-dG adducts, as a deeper understanding of these adducts has been provided by DFT calculations. Furthermore, dG serves as a better electron donor than dA, as it possesses a lower oxidation potential,\textsuperscript{99,100} and therefore, C\textsuperscript{8}-heteroaryl-dG adducts have the potential to serve as more effective push-pull probes than C\textsuperscript{8}-heteroaryl-dA adducts, with dG serving as the electron donor and the C\textsuperscript{8}-substituent behaving as the electron acceptor.

(b) Solvatochromic Properties of C\textsuperscript{8}-Heteroaryl-dG Adducts

In order to determine the sensitivity of C\textsuperscript{8}-heteroaryl-dG adducts to their microenvironment, absorption and emission of all adducts were measured in a variety of solvents, including water, DMSO, acetonitrile, methanol, ethanol, 2-propanol and CHCl\textsubscript{3}. The intriguing results of these measurements are shown in Tables 3-7 to 3-15, with all adducts displaying emission sensitivity, with regards to both wavelength maximum and intensity, to solvent polarity.\textsuperscript{57,58} Note that \textsuperscript{3}Pyr\textsuperscript{-}dG is so weakly fluorescent, detection in most solvents was not possible.
Table 3-7. Solvatochromic spectral properties of $^{2}$Py$r$ dG.

<table>
<thead>
<tr>
<th>solvent (ε)$^a$</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^d$</th>
<th>$I_{rel}$$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>292</td>
<td>379</td>
<td>7861</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>297</td>
<td>370</td>
<td>6643</td>
<td>1.7</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>295</td>
<td>371</td>
<td>6944</td>
<td>0.55</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>294</td>
<td>378</td>
<td>7558</td>
<td>1.1</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>295</td>
<td>377</td>
<td>7373</td>
<td>1.1</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>298</td>
<td>370</td>
<td>6530</td>
<td>1.3</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>304</td>
<td>375</td>
<td>6228</td>
<td>0.12</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.

Table 3-8. Solvatochromic spectral properties of $^{2}$Fur$d$G.

<table>
<thead>
<tr>
<th>solvent (ε)$^a$</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^d$</th>
<th>$I_{rel}$$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>292</td>
<td>384</td>
<td>8205</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>295</td>
<td>381</td>
<td>7652</td>
<td>1.0</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>292</td>
<td>371</td>
<td>7592</td>
<td>0.62</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>294</td>
<td>376</td>
<td>7418</td>
<td>0.52</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>295</td>
<td>372</td>
<td>7016</td>
<td>0.87</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>294</td>
<td>369</td>
<td>6913</td>
<td>0.84</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>298</td>
<td>366</td>
<td>6235</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.

Table 3-9. Solvatochromic spectral properties of $^{2}$Th$d$G.

<table>
<thead>
<tr>
<th>solvent (ε)$^a$</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^d$</th>
<th>$I_{rel}$$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>284</td>
<td>414</td>
<td>11057</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>291</td>
<td>417</td>
<td>10383</td>
<td>0.62</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>287</td>
<td>407</td>
<td>10273</td>
<td>0.81</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>285</td>
<td>405</td>
<td>10396</td>
<td>0.77</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>287</td>
<td>402</td>
<td>9966</td>
<td>0.85</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>288</td>
<td>402</td>
<td>9847</td>
<td>0.82</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>289</td>
<td>400</td>
<td>9602</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.
### Table 3-10. Solvatochromic spectral properties of $^{3}$PyrdG.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{abs}$ (nm)$^d$</th>
<th>$\lambda_{em}$ (nm)$^d$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^e$</th>
<th>$I_{rel}^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>278</td>
<td>383</td>
<td>9862</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>281</td>
<td>404</td>
<td>10835</td>
<td>1.6</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>277</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>279</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>278</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>278</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>280</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Emission not observed. $^d$ Recorded using an adduct concentration of 10 $\mu$M. $^e$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^f$ Relative emission intensities compared to the intensity in MOPS buffer.

### Table 3-11. Solvatochromic spectral properties of $^{3}$FurdG.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^d$</th>
<th>$I_{rel}^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>274</td>
<td>382</td>
<td>10318</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>284</td>
<td>372</td>
<td>8330</td>
<td>1.4</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>280</td>
<td>371</td>
<td>9536</td>
<td>0.80</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>279</td>
<td>372</td>
<td>8960</td>
<td>1.1</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>285</td>
<td>373</td>
<td>8278</td>
<td>1.4</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>279</td>
<td>372</td>
<td>8960</td>
<td>0.5</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>282</td>
<td>364</td>
<td>7988</td>
<td>0.09</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.

### Table 3-12. Solvatochromic spectral properties of $^{3}$ThdG.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta \nu$ (cm$^{-1}$)$^d$</th>
<th>$I_{rel}^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>278</td>
<td>394</td>
<td>10590</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>289</td>
<td>393</td>
<td>9157</td>
<td>1.0</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>283</td>
<td>384</td>
<td>9294</td>
<td>0.50</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>280</td>
<td>383</td>
<td>9605</td>
<td>0.64</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>284</td>
<td>380</td>
<td>8895</td>
<td>0.58</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>282</td>
<td>373</td>
<td>8651</td>
<td>0.53</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>285</td>
<td>377</td>
<td>8562</td>
<td>0.07</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta \nu$) is calculated as $(1/\lambda_{abs} - 1/\lambda_{em})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.
### Table 3-13. Solvatochromic spectral properties of $^{1}\text{Ind}dG$.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\Delta\nu$ (cm$^{-1}$)</th>
<th>$I_{\text{rel}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>321</td>
<td>390</td>
<td>5512</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>333</td>
<td>395</td>
<td>4714</td>
<td>0.89</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>325</td>
<td>381</td>
<td>4522</td>
<td>1.2</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>321</td>
<td>382</td>
<td>4975</td>
<td>1.1</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>324</td>
<td>382</td>
<td>4686</td>
<td>1.2</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>328</td>
<td>381</td>
<td>4241</td>
<td>0.82</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>334</td>
<td>383</td>
<td>3830</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constant. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta\nu$) is calculated as $(1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.

### Table 3-14. Solvatochromic spectral properties of $^{1}\text{Bfur}dG$.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\Delta\nu$ (cm$^{-1}$)</th>
<th>$I_{\text{rel}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>323</td>
<td>405</td>
<td>6268</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>328</td>
<td>413</td>
<td>6275</td>
<td>0.81</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>321</td>
<td>397</td>
<td>5964</td>
<td>1.1</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>322</td>
<td>399</td>
<td>5993</td>
<td>1.1</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>327</td>
<td>395</td>
<td>5264</td>
<td>1.0</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>327</td>
<td>392</td>
<td>5071</td>
<td>0.84</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>324</td>
<td>391</td>
<td>5289</td>
<td>0.78</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta\nu$) is calculated as $(1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.

### Table 3-15. Solvatochromic spectral properties of $^{1}\text{Bth}dG$.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\Delta\nu$ (cm$^{-1}$)</th>
<th>$I_{\text{rel}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>315</td>
<td>419</td>
<td>7880</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>336</td>
<td>424</td>
<td>6177</td>
<td>1.3</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>321</td>
<td>413</td>
<td>6940</td>
<td>1.3</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>315</td>
<td>413</td>
<td>7533</td>
<td>0.98</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>319</td>
<td>412</td>
<td>7076</td>
<td>0.79</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>317</td>
<td>406</td>
<td>6915</td>
<td>0.66</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>318</td>
<td>405</td>
<td>6755</td>
<td>0.55</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ Stokes shift ($\Delta\nu$) is calculated as $(1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}})$. $^e$ Relative emission intensities compared to the intensity in MOPS buffer.
A number of trends are apparent upon review of the collected solvatochromic data. Firstly, it was observed that all adducts show an increase in Stokes shift with increased solvent polarity.\textsuperscript{57,58} This increase in $\Delta \nu$ comes as a direct result of the noticeable blue-shift in emission maximum and red-shift in absorbance maximum observed with decreased solvent polarity. The hypsochromic shift in emission maximum observed with decreased solvent polarity is illustrated in Figure 3-15 for adducts $^{2\text{Th}}$dG and $^{\text{Bth}}$dG.

![Figure 3-15](image)

**Figure 3-15.** Emission spectra of $^8$-heteroaryl-dG adducts (a) $^{2\text{Th}}$dG and (b) $^{\text{Bth}}$dG in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl, acetonitrile and CHCl$_3$ (from right to left, in direction of the arrow), using an adduct concentration of 10 $\mu$M.

An additional method of representation of emission maximum blue-shift with decreased solvent polarity is through creation of plots of wavelength maximum, expressed in terms of energy, as a function of the solvent polarity parameter $E_T(30)$. The $E_T(30)$ scale of solvent polarity was developed by Reichardt. The values are based on the
solvatochromic pyridinium N-phenolate betaine dye as the probe molecule, and are defined as the molar electronic transition energies ($E_T$) of the dissolved dye, measured in solvents of different polarity in kcal mol$^{-1}$ at room temperature.$^{101-103}$ In this UV-vis spectroscopically derived empirical scale of solvent polarity, called $E_T(30)$, an increase in $E_T(30)$ value is representative of an increase in polarity. Plots are shown in Figure 3-16 for adducts $^{2}$Fur$dG$ and $^{2}$Th$dG$, and in Figure 3-17 for adducts $^{B}$fur$dG$ and $^{B}$th$dG$. With decreased solvent polarity, energy increases, consistent with a blue-shift in emission, as high energy light has a shorter wavelength.$^{98}$

![Figure 3-16](image)

**Figure 3-16.** Plots of emission maximum, expressed in terms of energy, versus the corresponding solvent polarity scale ($E_T(30)$) value, for $C^8$-heteroaryl-dG adducts (a) $^{2}$Fur$dG$ and (b) $^{2}$Th$dG$. Emission maxima were recorded in a variety of solvents using an adduct concentration of 10 µM; maxima for water were recorded in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl. $E_T(30)$ values were taken from Reference no. 101.
Figure 3-17. Plots of emission maximum, expressed in terms of energy, versus the corresponding solvent polarity scale ($E_T(30)$) value, for $C^8$-heteroaryl-dG adducts (a) $B^{6d}dG$ and (b) $B^{7d}dG$. Emission maxima were recorded in a variety of solvents using an adduct concentration of 10 $\mu$M; maxima for water were recorded in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl. $E_T(30)$ values were taken from Reference no. 101.

The noted trend of increase in $\Delta \nu$ with increased solvent polarity that these adducts exhibit is consistent with the emitting excited state possessing a dipole moment greater than the ground state,\(^{39,55,104,105}\) which is in line with DFT calculations predicting higher dipole moments in $S_1$ compared to $S_0$ for all $2^\prime$-substituted $C^8$-heteroaryl-dG adducts,\(^{57,58}\) and furthermore, suggests solvent-mediated stabilization of the charge-separated excited state.\(^{55}\) In a polar environment, the excited state fluorophore molecules use some of their energy to reorient the dipole of the solvent. In a non-polar environment, however, the fluorophore in the excited state will induce formation of a dipole. The formation of a new dipole requires more energy than the reorientation of an already existing dipole, and therefore, emission in a non-polar solvent such as CHCl$_3$ yields a spectrum with a blue-shifted maximum, compared to the emission that occurs in a polar solvent.\(^4\) The larger Stokes shift in polar solvents also suggests the existence of specific solute-solvent interactions, such as H-bonding,\(^{31}\) and furthermore, that large molecular motion is involved in the excited state relaxation processes.\(^{106}\)
Not only were solvatochromic trends observed in the maximum wavelength of emission, but they were also in the observed intensity of emission for the adducts. The emission consistently decreased in intensity with decreasing solvent polarity, as displayed by the $I_{\text{rel}}$ values given in Tables 3-7 to 3-15. The largest difference in emission intensity was therefore observed in polar protic water, for which $I_{\text{rel}} = 1$ for all adducts, versus non-polar CHCl$_3$, for which $I_{\text{rel}}$ values in a range of 0.06 – 0.78 were recorded. The fluorescence quenching observed in CHCl$_3$ compared to water is featured in Figures 3-18 and 3-19 for 2"-substituted $C^8$-heteroaryl-dG adducts. The significant decrease in emission intensity observed in CHCl$_3$ as compared to other solvents could be attributed to external conversion, a radiationless process that involves deactivation of the excited state following interaction and energy transfer between the excited molecule and the solvent, or could be the result of a photoinduced electron transfer (PET) quenching process, involving electron transfer between the adduct (donor) and CHCl$_3$ (acceptor). The PET process will be discussed further in section 3.3.6 (b).

![Relative emission spectra of $C^8$-heteroaryl-dG adducts (a) $2\text{Pyr}$dG, (b) $2\text{Fur}$dG and (c) $2\text{Th}$dG in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl (solid line) and CHCl$_3$ (dotted line), using an adduct concentration of 10 $\mu$M.](image-url)

**Figure 3-18.** Relative emission spectra of $C^8$-heteroaryl-dG adducts (a) $2\text{Pyr}$dG, (b) $2\text{Fur}$dG and (c) $2\text{Th}$dG in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl (solid line) and CHCl$_3$ (dotted line), using an adduct concentration of 10 $\mu$M.
Figure 3-19. Relative emission spectra of $C^8$-benzoheteroaryl-dG adducts (a) $\text{Ind}^\text{dG}$, (b) $\text{Bfur}^\text{dG}$ and (c) $\text{Bth}^\text{dG}$ in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl (solid line) and CHCl$_3$ (dashed line), using an adduct concentration of 10 $\mu$M.

As illustrated in the two figures above, fluorescence quenching in the non-polar solvent is most prominent for $2\text{Pyr}^\text{dG}$, $2\text{Fur}^\text{dG}$ and $\text{Ind}^\text{dG}$. Interestingly, emission spectra are structureless in water, while structured spectra, possessing a feature known as a vibronic progression, are produced in CHCl$_3$, an effect seen more prominently for the $C^8$-benzoheteroaryl-dG adducts as compared to their $C^8$-heteroaryl-dG counterparts. Chen and co-workers state this feature is typical of a molecule possessing torsional disorder in the ground state, but a planar excited state,\cite{107} which is in agreement with the predicted $\theta$ twist angle in $S_0$ and $S_1$ as determined by DFT calculations.\cite{57,58} The fluorescence quenching observed in CHCl$_3$ compared to water provides indication that $C^8$-heteroaryl-dG adducts have the ability to serve as ‘on-off’ probes of the exterior versus interior environment of the DNA double helix, as water resembles the polar, aqueous environment that surrounds the double helix, and the dielectric constant of CHCl$_3$ ($\varepsilon = 4.9$) is similar to that in the DNA double helix ($\varepsilon = 3-5$).\cite{108}

Emission exhibited by $C^8$-heteroaryl-dG adducts can be related to that exhibited
by biphenyls; these adducts were first likened to biphenyls with regards to ground and excited state structure, in section 3.3.2 (c). Emission spectra of biphenyl systems show only one band that originates from the $\pi,\pi^*$ locally excited state (LE),$^{97,109}$ and thus the emission exhibited by C$_8$-heteroaryl-dG adducts can similarly be attributed to decay from the LE state.

The solvatochromic spectral properties of $^Q$dG, as given in Table 3-16, are presented separately, as the trends in emission maximum and intensity noted for all other C$_8$-heteroaryl-dG adducts were not observed here.

<table>
<thead>
<tr>
<th>solvent ($\varepsilon$)$^a$</th>
<th>$\lambda_{abs}$ (nm)$^c$</th>
<th>$\lambda_{em}$ (nm)$^c$</th>
<th>$\Delta\nu$ (cm$^{-1}$)$^c$</th>
<th>$I_{rel}$$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>water$^b$ (78.5)</td>
<td>313</td>
<td>407</td>
<td>7379</td>
<td>1</td>
</tr>
<tr>
<td>DMSO (48.9)</td>
<td>316</td>
<td>363</td>
<td>4097</td>
<td>27</td>
</tr>
<tr>
<td>acetonitrile (37.5)</td>
<td>315</td>
<td>510 (384)$^d$</td>
<td>12138 (5704)</td>
<td>9.1</td>
</tr>
<tr>
<td>methanol (32.6)</td>
<td>315</td>
<td>378</td>
<td>5291</td>
<td>4.4</td>
</tr>
<tr>
<td>ethanol (24.3)</td>
<td>313</td>
<td>382</td>
<td>5771</td>
<td>4.6</td>
</tr>
<tr>
<td>2-propanol (8.3)</td>
<td>316</td>
<td>379 (493)$^d$</td>
<td>5260 (11362)</td>
<td>4.7</td>
</tr>
<tr>
<td>CHCl$_3$ (4.70)</td>
<td>318</td>
<td>468</td>
<td>10079</td>
<td>18</td>
</tr>
</tbody>
</table>

$^a$ Dielectric constants at 25 °C, taken from Reference no. 102. $^b$ 10 mM MOPS buffer, pH 7, $\mu$ = 100 mM NaCl. $^c$ Recorded using an adduct concentration of 10 $\mu$M. $^d$ For dual emission in acetonitrile and 2-propanol, less intense emission band is in brackets. $^e$ Stokes shift ($\Delta\nu$) is calculated as (1/$\lambda_{abs}$ – 1/$\lambda_{em\max}$). $^f$ Relative emission intensities are compared to the intensity in MOPS buffer and are calculated using intensity at wavelength of maximum emission.

The solvatochromic spectral properties of the $^Q$dG adduct are considerably different from those exhibited by the other C$_8$-heteroaryl-dG adducts. The $^Q$dG adduct intriguingly displayed dual fluorescence in both acetonitrile and 2-propanol. Emission at CHCl$_3$, at 468 nm, was observed at approximately the same wavelength as the second
emission band exhibited in acetonitrile and 2-propanol, at 510 and 493 nm, respectively. Emission spectra of $^O\text{dG}$ in water, acetonitrile and CHCl$_3$ are illustrated in Figure 3-20.

![Relative emission spectra of the C$_8$-heteroaryl-dG adduct $^O\text{dG}$ in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl (solid line), acetonitrile (dashed line) and CHCl$_3$ (dotted line), using an adduct concentration of 10 $\mu$M.](image)

**Figure 3-20.** Relative emission spectra of the C$_8$-heteroaryl-dG adduct $^O\text{dG}$ in 10 mM MOPS buffer, pH 7, $\mu = 100$ mM NaCl (solid line), acetonitrile (dashed line) and CHCl$_3$ (dotted line), using an adduct concentration of 10 $\mu$M.

The emissive behaviour exhibited by $^O\text{dG}$ can be related to that commonly exhibited by classic donor-acceptor biaryl systems.$^{97,110-112}$ These biaryl molecules are characterized by the possession of a push-pull $\pi$-electron system, with donor (D) and acceptor (A) groups, a common example of which includes a $p,p'$-disubstituted biphenyl, with dimethylamino (D) and cyano (A) substituents.$^{97}$ Donor-acceptor biaryls belong to an extensive class of twisted intramolecular (or internal) charge transfer (TICT) molecules, a model of which is shown in Figure 3-21.$^{113}$ These molecules are already twisted, or in an orthogonal conformation, in the ground state.$^{114}$ In the TICT mechanism, rotation about the bond connecting the D and A groups leads to decoupling
of the orbitals of the two groups, with greater electron density placed on the donor. Orbital decoupling, and subsequent full charge separation, provides a means by which nearly complete electron transfer from the donor to the acceptor can occur, and a strongly twisted excited state biradicaloid species results. This highly polar, and charge separated, twisted excited state can be preferentially stabilized, under appropriate solvent conditions, with respect to a planar LE state. Subsequently, emission from the TICT state occurs at a red-shifted, and thus lower energy, wavelength than emission from the LE state. As the twisted form of the D-A molecule is energetically destabilized in the ground state, TICT state formation brings the excited and ground states closer, permitting more rapid internal conversion. The idea of TICT states came from the discovery by Lippert and co-workers that rotamerism accompanied the intramolecular separation of charges in the D-A molecule 4-(\(N,N\)-dimethylamino)-benzonitrile (DMABN), and was truly defined by Grabowski and co-workers, upon the further study of DMABN and other model pre-twisted compounds.

![Figure 3-21. Model of LE and TICT excited states for a D-A molecule.](image)

The Q\(^d\)G adduct is a biaryl molecule with both donor (dG) and acceptor (\(C^8\)-quinolyl) groups, and possesses TICT character as evidenced by its emissive properties. TICT molecules are well known to exhibit dual fluorescence in solvents of different
polarity, as was observed for QdG in both acetonitrile and 2-propanol (Table 3-16 and Figure 3-20). In both of these solvents, emission observed at ~ 380 nm can be attributed to an LE excited state, shown in Figure 3-21, while emission at ~ 500 nm can be attributed to a TICT state that results following electron transfer from the donor to acceptor group to form a highly twisted biradicaloid QdG adduct. In acetonitrile, emission from the TICT state is more intense than emission from the LE state, indicating that the TICT excited and ground states are further separated than the LE excited and ground states, while the opposite is true in 2-propanol.

In the other solvents measured, only one emission band was observed. In CHCl$_3$, emission at 468 nm can be ascribed to the TICT state, with the wavelength of emission from this excited state blue-shifted compared to that in acetonitrile and 2-propanol. Emission in CHCl$_3$ occurs at a lower energy wavelength as this non-polar is unable to stabilize the polar excited state of the QdG molecule. In water, DMSO, methanol and ethanol, emission is observed from only the LE excited state. Electron transfer preceding formation of the TICT excited state still occurs, but decay from the TICT state in these particular solvents is radiationless. As with the other C$^8$-heteroaryl-dG adducts, an overall increase in $\Delta \nu$ of emission from the LE state was observed with increased solvent polarity, indicative of the emitting excited state possessing a dipole moment greater than the ground state and solvent-mediated stabilization of the charge-separated excited state. It is also important to note that the emission intensity of QdG in water ($I_{rel} = 1$) compared to CHCl$_3$ ($I_{rel} = 18$), as shown in Figure 3-20, also opposed the trend observed for all other C$^8$-heteroaryl-dG adducts, in which fluorescence was quenched in the non-
polar solvent. This result prompted determination of the quantum yield of $^0dG$ in CHCl$_3$, and was found to be 0.21, seven times higher than in water (Table 3-5). The fluorescence quenching observed in water compared to CHCl$_3$ provides indication that $^0dG$ has the ability to serve as an ‘off-on’ probe of the exterior versus interior environment of the DNA double helix.

The solvatochromic properties of $^0dG$ assisted in providing a better understanding of the difference between this analogue and the other $C^8$-heteroaryl-dG adducts, showing that while $^0dG$ resembles a classic D-A molecule and exhibits dual fluorescence from both LE and TICT states, the other $C^8$-heteroaryl-dG adducts exhibit non-competitive fluorescence, with emission from only the LE state. Furthermore, while $^0dG$ has potential to behave as an ‘off-on’ probe in polar versus non-polar environments, recall that the remaining $C^8$-heteroaryl-dG adducts show ‘on-off’ behaviour under the same conditions.

(c) Influence of Solvent Viscosity on Fluorescence of $C^8$-Heteroaryl-dG Adducts

The changes of emission with increased solvent viscosity and temperature were measured for the 2"-substituted $C^8$-benzoheteroaryl-dG series of adducts and one five-membered 2"-substituted $C^8$-heteroaryl-dG adduct, $^{2\text{Fur}}dG$. Figures 3-22 to 3-25 show changes for these adducts in glycerol-water mixtures at 20 °C (Figures 3-22a to 3-25a) and with increased temperature in 100 % glycerol (Figures 3-22b to 3-25b), with the data collected from these measurements summarized in Table 3-17.
Figure 3-22. Changes in fluorescence emission spectrum (dashed lines) of the $C^8$-heteroaryl-dG adduct $2$Fur$dG$ (10 µM) upon (a) increasing the glycerol content from 0 % (solid line, 10 mM MOPS, pH 7, $\mu = 100$ mM NaCl) up to 100 % (added in 20 % increments), recorded at 20 °C and upon (b) increasing the temperature in 10 °C increments from 20 °C (solid line) to 80 °C in 100 % glycerol.

Figure 3-23. Changes in fluorescence emission spectrum (dashed lines) of the $C^8$-heteroaryl-dG adduct $Ind$ dG (10 µM) upon (a) increasing the glycerol content from 0 % (solid line, 10 mM MOPS, pH 7, $\mu = 100$ mM NaCl) up to 100 % (added in 20 % increments), recorded at 20 °C and upon (b) increasing the temperature in 10 °C increments from 20 °C (solid line) to 80 °C in 100 % glycerol.
Figure 3-24. Changes in fluorescence emission spectrum (dashed lines) of the C₈-heteroaryl-dG adduct (10 µM) upon (a) increasing the glycerol content from 0 % (solid line, 10 mM MOPS, pH 7, υ = 100 mM NaCl) up to 100 % (added in 20 % increments), recorded at 20 ºC and upon (b) increasing the temperature in 10 ºC increments from 20 ºC (solid line) to 80 ºC in 100 % glycerol.

Figure 3-25. Changes in fluorescence emission spectrum (dashed lines) of the C₈-heteroaryl-dG adduct (10 µM) upon (a) increasing the glycerol content from 0 % (solid line, 10 mM MOPS, pH 7, υ = 100 mM NaCl) up to 100 % (added in 20 % increments), recorded at 20 ºC and upon (b) increasing the temperature in 10 ºC increments from 20 ºC (solid line) to 80 ºC in 100 % glycerol.
Table 3-17. Photophysical properties of C8-heteroaryl-dG adducts in the viscous solvent glycerol compared to water.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>λ_{em} (nm)\textsuperscript{a}</th>
<th>λ_{em} (nm)\textsuperscript{a}</th>
<th>Δ λ_{em} (nm)\textsuperscript{c}</th>
<th>I_{rel}\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Fur dG</td>
<td>384 (100 % MOPS\textsuperscript{b})</td>
<td>378 (100 % glycerol)</td>
<td>−6</td>
<td>0.40</td>
</tr>
<tr>
<td>Ind dG</td>
<td>390 (100 % MOPS\textsuperscript{b})</td>
<td>388 (100 % glycerol)</td>
<td>−2</td>
<td>0.30</td>
</tr>
<tr>
<td>Bfu dG</td>
<td>405 (100 % MOPS\textsuperscript{b})</td>
<td>400 (100 % glycerol)</td>
<td>−4</td>
<td>0.39</td>
</tr>
<tr>
<td>Bth dG</td>
<td>419 (100 % MOPS\textsuperscript{b})</td>
<td>413 (100 % glycerol)</td>
<td>−6</td>
<td>0.25</td>
</tr>
<tr>
<td>Q dG</td>
<td>407 (100 % MOPS\textsuperscript{b})</td>
<td>460, 430 (shoulder) \textsuperscript{e}</td>
<td>+53 (new band)</td>
<td>2.90</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Recorded using an adduct concentration of 10 μM. \textsuperscript{b}10 mM MOPS buffer, pH 7, µ = 100 mM NaCl. \textsuperscript{c}Change in emission maximum is for the adduct in glycerol versus MOPS buffer. \textsuperscript{d}Relative emission intensity is determined as \frac{I_{adduct in glycerol}}{I_{adduct in MOPS}}.

The spectral changes show an approximate three- to four-fold decrease in emission intensity from buffered water to 100 % glycerol, accompanied by a blue-shift in emission wavelength maximum of −2 to −6 nm. Increasing the temperature from 20 °C to 80 °C in 100 % glycerol resulted in an approximate two-fold increase in the emission intensity.\textsuperscript{58} With increased viscosity, excited states have geometries that strongly resemble that of the ground state,\textsuperscript{118} because increased solvent rigidity increases barriers to rotation following excitation, thereby reducing the probability and amplitude of the twisting about the angle of interest.\textsuperscript{118,119} Birbaum and co-workers have previously shown that emission from the originally excited twisted conformation of a class of substituted triazines occurs at shorter wavelengths in more viscous solvents, while in solutions of decreased viscosity, an energetically more favourable conformation can be reached before emission, which appears at a longer wavelength. Furthermore, these researchers found that fluorescence quantum yield decreased with increasing solvent viscosity.\textsuperscript{119} Therefore, a possible explanation for the emissive behaviour of these C8-heteroaryl-dG adducts with increased viscosity could stem from the generation of a twisted S1 structure, which would possess little difference in terms of twist angle (θ) from
the twisted $S_0$ structure. The barrier to rotation caused by increased solvent viscosity prevents the adducts from achieving a more energetically favoured conformation in the excited state, and thus this higher energy excited state twisted structure results in a blue-shift in emission wavelength maximum and decrease in intensity. Relaxation of the solvent with respect to the excited molecule will also become less effective with increasing viscosity, thus providing a second contribution to the blue-shift of the emission. Increased temperature in 100 % glycerol decreases the barrier to relaxation and emissive planar structures can once again be generated.

The changes of emission with increased solvent viscosity and temperature were also measured for $^{\text{QdG}}$. Spectral changes for this adduct in glycerol-water mixtures at 20 °C are shown in Figure 3-26a, with changes upon increased temperature in 100 % glycerol shown in Figure 3-26b. The data collected from these measurements is summarized in Table 3-17.
Figure 3-26. Changes in fluorescence emission spectrum (dashed lines) of the C8-heteroaryl-dG adduct 5dG (10 µM) upon (a) increasing the glycerol content from 0 % (solid line, 10 mM MOPS, pH 7, µ = 100 mM NaCl) up to 100 % (added in 20 % increments), recorded at 20 °C and upon (b) increasing the temperature in 10 °C increments from 20 °C (solid line) to 80 °C in 100 % glycerol.

The spectra reveal changes in emission different from those observed for 2Fur-dG, Ind-dG, Bfur-dG and Bth-dG adducts. Spectral changes show an increase in emission intensity from buffered water to 100 % glycerol, accompanied by the presence of a new emission band at 460 nm. Increasing the temperature from 20 °C to 80 °C in 100 % glycerol resulted in a decrease in the emission intensity, accompanied by disappearance of the 460 nm emission band, with emission observed only at 407 nm. Recall that increased solvent viscosity decreases the barrier to rotation. Therefore, the band observed at 460 nm in 100 % glycerol is likely due to emission from the TICT excited state, as the reduced probability of rotation about the θ angle facilitates formation of a highly twisted excited state molecule. The increased rigidity of the system provided by the viscous solvent
would also contribute to the overall increase in emission intensity.\textsuperscript{98} With increased temperature in 100\% glycerol, the barrier to rotation about the $\theta$ twist angle decreases and emission from the LE state would predominate.

3.3.4 Redox Parameters of $C^8$-Heteroaryl-dG Adducts

The electron donor properties of the $C^8$-heteroaryl-dG adducts were determined by cyclic voltammetry (CV) performed in anhydrous DMF, as outlined previously by the Manderville group for various $C^8$-aryl-dG analogues.\textsuperscript{49,81,82} All adducts showed irreversible one-electron oxidation peaks,\textsuperscript{56,58} with examples of cyclic voltammograms, of current as a function of potential, shown in Figure 3-27 for $2Fur$ dG and $Bfur$ dG.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{voltammograms.png}
\caption{Cyclic voltammograms, of current versus potential, of the $C^8$-heteroaryl-dG adducts $2Fur$ dG and $Bfur$ dG, recorded in a solution of 0.1 M TBAF in anhydrous DMF using a glassy carbon working electrode.}
\end{figure}

From the cyclic voltammograms, half-peak oxidation potentials ($E_{p/2}$) versus saturated calomel electrode (SCE) were determined for each adduct, and are given in
Table 3-18. Potentials of adducts were found to be in the range of ~ 0.78 to 1.1 V/SCE. Interestingly, all potentials were lower than that of dG, which under the same experimental conditions gave $E_{p/2} = 1.14$ V/SCE.\textsuperscript{81} This indicates that attachment of the heteroaryl moiety at the $C^8$ position of dG enhances the electron-rich characteristics of the purine nucleoside, making $C^8$-heteroaryl-dG adducts better donors than dG.\textsuperscript{56,58} The pyrrolyl-linked $2\text{Pyr}dG$ and $3\text{Pyr}dG$, and indolyl-linked $\text{Ind}dG$, analogues possessed the lowest $E_{p/2}$ values of 0.78, 0.86 and 0.88, respectively, followed closely by the oxygen-containing analogues $2\text{Fur}dG$, $3\text{Fur}dG$ and $\text{Bfur}dG$ with $E_{p/2}$ values of 0.91, 0.94 and 0.92 V/SCE, respectively, and followed finally by the sulfur-containing analogues $2\text{Th}dG$, $3\text{Th}dG$ and $\text{Bth}dG$, with $E_{p/2}$ values of 1.05, 0.99 and 1.06 V/SCE, respectively. These results indicate that pyrrolyl and indolyl $C^8$-substituents are the strongest electron-donating groups, while the thienyl and benzothienyl $C^8$-substituents are the strongest electron-withdrawing groups.\textsuperscript{56,58} Therefore, of the family of $2''$- and $3''$-substituted $C^8$-heteroaryl-dG adducts, the sulfur-substituted analogues are those that best fit the description of push-pull probes, with the dG component serving as the electron donor and the thienyl or benzothienyl moiety serving as the electron acceptor, as predicted by the HOMO-LUMO analysis of these adducts outlined in section 3.3.2 (c). It is important to note however, that of all $C^8$-heteroaryl-dG adducts analyzed by CV for their respective electron donor properties, the $QdG$ adduct possesses the lowest oxidation potential, with $E_{p/2} = 1.10$ V/SCE. Therefore, from comparison of all adducts, it can be said that $QdG$ best serves as a push-pull probe, with an electron-donating component (dG) and strong electron-withdrawing substituent ($C^8$-quinolyl).
Table 3-18. Oxidation potentials of \(C^8\)-heteroaryl-dG adducts as determined by cyclic voltammetry.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>(E_{p/2}) vs. SCE (V)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Pyr(^a)dG</td>
<td>0.78</td>
</tr>
<tr>
<td>2Fur(^a)dG</td>
<td>0.91</td>
</tr>
<tr>
<td>2Th(^a)dG</td>
<td>1.05</td>
</tr>
<tr>
<td>3Pyr(^a)dG</td>
<td>0.86</td>
</tr>
<tr>
<td>3Fur(^a)dG</td>
<td>0.94</td>
</tr>
<tr>
<td>3Th(^a)dG</td>
<td>0.99</td>
</tr>
<tr>
<td>Ind(^a)dG</td>
<td>0.88</td>
</tr>
<tr>
<td>Bfu(^a)dG</td>
<td>0.92</td>
</tr>
<tr>
<td>Bth(^a)dG</td>
<td>1.06</td>
</tr>
<tr>
<td>Q(^a)dG</td>
<td>1.10</td>
</tr>
</tbody>
</table>

\(^a\) Half-peak potentials in volts versus SCE recorded in a solution of 0.1 M TBAF in anhydrous DMF using a glassy carbon working electrode.

Interestingly, a clear trend is apparent between \(C^8\)-heteroaryl-dG adduct half-peak oxidation potential and emission wavelength, where an increase in \(E_{p/2}\), indicative of stronger electron-withdrawing character of the \(C^8\)-substituent, correlates with a shift to a longer wavelength of emission. Emission is observed at a lower energy wavelength, as the charge-separated excited state, that forms as a direct result of the dual electron-donating (dG) and electron-withdrawing (\(C^8\)-moiety) nature of the adduct, can be solvent-stabilized. Conversely, a decrease in \(E_{p/2}\), indicative of stronger electron-donating character of the \(C^8\)-substituent, correlates with a shift to a shorter wavelength of emission. Here, emission is observed at a higher energy wavelength as charge separation in the excited state will not be as great, due to the electron-donating nature of the \(C^8\)-moiety, and subsequently, solvent-mediated stabilization will be less efficient.\(^58\)
3.3.5 Hydrolytic Stability of C^8-Heteroaryl-dG Adducts

It was important to develop an understanding of the hydrolytic stability of these adducts if they are to be considered useful as fluorescent probes. As was similarly determined for C^8-aryl-dG adducts in Chapter 2, the first-order rate constants and half-lives for hydrolysis of C^8-heteroaryl-dG adducts were measured by UV-Vis spectroscopy, by monitoring the appearance of the deglycosylated product at its absorbance maximum. Hydrolysis was initially measured in both 0.1 M HCl at 37.2 °C and 50 mM citrate buffer, pH 4, at 48.4 °C, with ionic strength maintained at 0.31 M NaCl. Results are provided in Table 3-19. Note that in pH 4 buffer, the rate of hydrolysis was unable to be determined for BthdG, due to insolubility of the deglycosylated intermediate formed following hydrolysis.

| Adduct | \( k_{\text{obs}} \) (HCl) (min\(^{-1}\)), \( t_{1/2} \) | \( k_{\text{obs}} \) (pH 4) (min\(^{-1}\)), \( t_{1/2} \)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2Pyr(_dG)</td>
<td>0.61 ± 0.02, 1.1</td>
<td>(1.28 ± 0.02) ( \times 10^{-2} ), 54.1</td>
</tr>
<tr>
<td>2Fur(_dG)</td>
<td>2.16 ± 0.08, 0.321</td>
<td>(1.99 ± 0.04) ( \times 10^{-2} ), 34.8</td>
</tr>
<tr>
<td>2Th(_dG)</td>
<td>1.37 ± 0.02, 0.506</td>
<td>(2.12 ± 0.08) ( \times 10^{-2} ), 32.7</td>
</tr>
<tr>
<td>3Pyr(_dG)</td>
<td>0.128 ± 0.002, 5.41</td>
<td>(1.43 ± 0.09) ( \times 10^{-2} ), 48.5</td>
</tr>
<tr>
<td>3Fur(_dG)</td>
<td>0.47 ± 0.01, 1.5</td>
<td>(0.32 ± 0.06) ( \times 10^{-2} ), 220</td>
</tr>
<tr>
<td>3Th(_dG)</td>
<td>0.73 ± 0.01, 0.95</td>
<td>(2.09 ± 0.05) ( \times 10^{-2} ), 33.2</td>
</tr>
<tr>
<td>Ind(_dG)</td>
<td>1.58 ± 0.05, 0.438</td>
<td>(1.11 ± 0.08) ( \times 10^{-2} ), 62.4</td>
</tr>
<tr>
<td>Bfur(_dG)</td>
<td>3.8 ± 0.2, 0.18</td>
<td>(1.2 ± 0.2) ( \times 10^{-2} ), 56</td>
</tr>
<tr>
<td>Bth(_dG)</td>
<td>1.92 ± 0.02, 0.362</td>
<td>-</td>
</tr>
<tr>
<td>Q(_dG)</td>
<td>0.219 ± 0.002, 3.16</td>
<td>(0.15 ± 0.01) ( \times 10^{-2} ), 460</td>
</tr>
<tr>
<td>dG(^d)</td>
<td>0.0391, 17.7</td>
<td>2.44 ( \times 10^{-4} ), 2840</td>
</tr>
</tbody>
</table>

\(^a\) Taken from Reference no. 120. \(^b\) Determined in 0.1 M HCl at 37.2 °C, using an adduct concentration of 50 \( \mu \)M. \(^c\) Determined in 50 mM citrate buffer, pH 4, \( \mu = 0.31 \) M NaCl at 48.4 °C, using an adduct concentration of 50 \( \mu \)M. \(^d\) Rate for hydrolysis indeterminable.
As was observed for $C^8$-aryl-dG adducts, the $C^8$-heteroaryl-dG adducts all undergo hydrolysis considerably faster than dG in acidic conditions, in a range from three times for the $3\text{Pyr}$dG adduct, to almost one hundred times for the $B\text{fur}$dG adduct. The $C^8$-heteroaryl-dG adducts also undergo hydrolysis considerably faster than dG at pH 4, in a range from six times for the $Q$dG adduct, to almost ninety times for the $2\text{Th}$dG adduct.

In addition, hydrolysis kinetics between pH 1 – 4 for $C^8$-heteroaryl-dG adducts were determined at 37 °C and plots of log $k_{obs}$ (min$^{-1}$) versus pH yielded straight lines, showing a continuous first-order dependence on $H^+$ activity. Note that in pH 3 and 4 buffer, the rate of hydrolysis was again unable to be determined for $B\text{th}$dG, and therefore subsequently at pH 7, due to insolubility of the deglycosylated intermediate formed following hydrolysis. In addition, rates of hydrolysis could not be determined in pH 1 buffer for both $B\text{fur}$dG and $B\text{th}$dG, as hydrolysis was too fast to allow for accurate determination of a rate constant by UV-Vis spectroscopy. Resulting straight lines in pH rate profile plots permitted extrapolation of the rate data to pH 7 for an estimate of hydrolysis rates at physiological pH, as shown in Table 3-20.
Table 3-20. Summary of first-order rate constants ($k$) and half-lives ($t_{1/2}$) for the hydrolysis of C<sup>8</sup>-heteroaryl-dG adducts at 37 °C.

<table>
<thead>
<tr>
<th>adduct</th>
<th>pH 1</th>
<th></th>
<th>pH 2</th>
<th></th>
<th>pH 3</th>
<th></th>
<th>pH 4</th>
<th></th>
<th>pH 7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{obs}$ (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$t_{1/2}$ (min)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>$k_{obs}$ (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$t_{1/2}$ (min)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>$k_{obs}$ (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$t_{1/2}$ (min)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>$k_{obs}$ (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$t_{1/2}$ (min)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>$k_{obs}$ (min&lt;sup&gt;-1&lt;/sup&gt;), $t_{1/2}$ (days)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2PyrdG</td>
<td>0.69 ± 0.01</td>
<td>1.0</td>
<td>0.114 ± 0.002</td>
<td>6.08</td>
<td>(3.27 ± 0.04) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>21.2</td>
<td>(5 ± 8) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>139</td>
<td>4.45 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td>2Fur</td>
<td>2.35 ± 0.09</td>
<td>0.295</td>
<td>0.32 ± 0.01</td>
<td>2.2</td>
<td>(4.61 ± 0.07) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>15.0</td>
<td>(5.3 ± 0.3) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>131</td>
<td>1.18 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>41</td>
</tr>
<tr>
<td>2Th</td>
<td>1.18 ± 0.02</td>
<td>0.587</td>
<td>0.306 ± 0.006</td>
<td>2.26</td>
<td>(2.66 ± 0.08) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>26.0</td>
<td>(5.8 ± 0.1) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>120</td>
<td>2.50 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>19</td>
</tr>
<tr>
<td>3PyrdG</td>
<td>0.119 ± 0.003</td>
<td>5.82</td>
<td>0.0232 ± 0.0003</td>
<td>29.9</td>
<td>(1.3 ± 0.2) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>53</td>
<td>(4.4 ± 0.4) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>160</td>
<td>1.88 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>3Fur</td>
<td>0.52 ± 0.01</td>
<td>1.3</td>
<td>0.076 ± 0.001</td>
<td>9.1</td>
<td>(1.45 ± 0.05) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>47.8</td>
<td>(1.2 ± 0.1) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>580</td>
<td>3.70 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>130</td>
</tr>
<tr>
<td>3Th</td>
<td>0.597 ± 0.007</td>
<td>1.16</td>
<td>0.100 ± 0.004</td>
<td>6.93</td>
<td>(1.83 ± 0.03) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>37.9</td>
<td>(5.3 ± 0.1) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>130</td>
<td>3.54 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>Ind</td>
<td>7.2 ± 0.2</td>
<td>0.096</td>
<td>0.231 ± 0.003</td>
<td>3.00</td>
<td>(3.5 ± 0.3) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>20</td>
<td>(3.2 ± 0.5) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>216</td>
<td>1.51 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>319</td>
</tr>
<tr>
<td>BFsrdG</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.46 ± 0.02</td>
<td>1.5</td>
<td>(5.2 ± 0.4) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>13</td>
<td>(3.3 ± 0.4) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>210</td>
<td>2.19 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>220</td>
</tr>
<tr>
<td>BthdG</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.340 ± 0.007</td>
<td>2.04</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>QdG</td>
<td>0.90 ± 0.04</td>
<td>0.77</td>
<td>0.0304 ± 0.0005</td>
<td>22.1</td>
<td>(0.40 ± 0.03) × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>170</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>6.20 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>7760</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined in 50 mM phosphate buffer, $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M. <sup>b</sup> Rate for hydrolysis indeterminable. <sup>c</sup> Determined in 50 mM citrate buffer, $\mu = 0.31$ M NaCl, using an adduct concentration of 50 $\mu$M. <sup>d</sup> Estimated rate data based on first-order dependence on $H^+$ activity.
For the five-membered $C^8$-substituted adducts, the half-life was found to be, on average, $\sim 39$ days, for hydrolysis at physiological pH. This is comparable to the half-life of 25 days determined at pH 7 for the $C^8$-phenyl substituted dG analogue. For the $C^8$-benzoheteroaryl-dG adducts, half-lives for hydrolysis at pH 7 were longer, with $t_{1/2} = 319$ days for $\text{Ind}dG$ and $t_{1/2} = 220$ days for $\text{Bfur}dG$. The half-life for the hydrolysis of $QdG$ was found to be substantially longer, with a value of 21 years. The $C^8$-quinolyl moiety provides a competitive nitrogen site for protonation, thereby effectively increasing the hydrolytic stability of the adduct.

The rate for spontaneous loss of purines from duplex DNA at pH 7.4 at $37 \, ^\circ C$ is $\sim 3 \times 10^{-11}$ s$^{-1}$ ($t_{1/2} = 730$ years), as reported by Karlstrom and co-workers. Thus, while $C^8$-heteroaryl-dG adducts are significantly more reactive than dG toward hydrolysis, they are reasonably stable at physiological pH, and should be even more stable within duplex DNA where purines are more resistant to hydrolysis. For a comprehensive discussion and further understanding of the kinetics of adduct hydrolysis, refer to Chapter 2.

### 3.3.6 Hydrogen Bonding Properties of $C^8$-Heteroaryl-dG Adducts

#### a) Synthesis of Silylated Nucleoside Derivatives

The intriguing emissive properties of a number of $C^8$-heteroaryl-dG adducts prompted their testing as fluorescent reporters of H-bonding specificity. As the establishment of association equilibria for base pairing was to be conducted in CHCl$_3$, adducts and natural nucleobases first required modification at the OH positions of the deoxyribose sugar moiety to afford silylated derivatives soluble in this non-polar solvent. As shown in Scheme 3-2, $2\text{Pyr}dG$, $\text{Ind}dG$ and $\text{Bth}dG$ were treated with [(iPr)$_2$SiCl]$_2$O to
afford bissilyl analogues that were soluble in CHCl$_3$.\textsuperscript{56,57} As shown in Scheme 3-3, the O$_2$dG adduct was reacted with TBDMS-Cl to afford a CHCl$_3$ soluble derivative, O$_2$dG(TBDMS)$_2$.

The natural nucleobases were also modified to allow for solubility in the non-polar solvent, with the synthesis of bissilyldC shown in Scheme 3-2.\textsuperscript{56} While the bissilyldG nucleobase was also initially synthesized, it was found to be insufficiently soluble in CHCl$_3$. The poor solubility of the 3',5'-silylated derivative of dG was previously reported by Temps and co-workers.\textsuperscript{125} These researchers instead found that riboguanosine (rG) bearing three TBDMS groups was sufficiently soluble,\textsuperscript{125,126} and thus this analogue was synthesized here, as shown in Scheme 3-3.\textsuperscript{56}

Following synthesis, the silylated natural nucleobases and adducts were characterized by both $^1$H and $^{13}$C NMR spectroscopy, and high resolution mass spectra were also collected.\textsuperscript{56,57} The $^1$H and $^{13}$C NMR spectra can be found in Appendix C.
Scheme 3-2

\[
\text{2Py}^\text{dG} \xrightarrow{\text{imidazole, anhydrous DMF, 24 h}} \text{bissily}^\text{2Py}^\text{dG}
\]

\[
\text{Ind}^\text{dG}, R = \text{NH} \xrightarrow{\text{imidazole, anhydrous DMF, 24 h}} \text{bissily}^\text{Ind}^\text{dG}, R = \text{NH}
\]

\[
\text{Bth}^\text{dG}, R = \text{S} \xrightarrow{\text{imidazole, anhydrous DMF, 24 h}} \text{bissily}^\text{Bth}^\text{dG}, R = \text{S}
\]

\[
\text{dC} \xrightarrow{\text{imidazole, anhydrous DMF, 24 h}} \text{bissily}^\text{dC}
\]
(b) Association Constant Determination

Association equilibria for W-C and Hoogsteen H-bonding were established by mixing the silyl-protected C\(^8\)-heteroaryl-dG adduct in CHCl\(_3\) with the corresponding bissilyldC and rG(TBDMS)\(_3\). The formation of equilibria was monitored by fluorescence spectroscopy. These experiments were conducted in CHCl\(_3\),\(^{56}\) as this aprotic solvent is well-known to enforce the formation H-bonded heterodimers.\(^\text{127}\) In water, the H-bonding donor and acceptor sites of the nucleobases would be virtually saturated by H\(_2\)O molecules, thereby preventing the formation of H-bonded base pairs.\(^\text{125}\) Furthermore, the dielectric constant of CHCl\(_3\) (\(\varepsilon = 4.9\)) is similar to that of the DNA double helix interior (\(\varepsilon = 3 – 5\)),\(^\text{108}\) and thus serves as a reasonable model to study specific H-bonding effects in duplex DNA. Shown in Figures 3-28 to 3-31 are changes in fluorescence of the silyl-protected C\(^8\)-heteroaryl-dG adduct upon addition of bissilyldC and rG(TBDMS)\(_3\).\(^{56,58}\)
Figure 3-28. Emission spectra of bissilyl2Pyr-dG upon addition of (a) bissilyldC or (b) rG(TBDMS)₃, with (c) and (d) corresponding double reciprocal plots of $F_0(F_0/F)$ versus $1/[\text{natural nucleobase}]$. Spectra were recorded in CHCl₃, using an adduct concentration of 10 µM. The native nucleoside was added to the adduct sample as 10 µM aliquots.
Figure 3-29. Emission spectra of bissilyl$^{\text{Ind}}$dG upon addition of (a) bissilyl$dC$ or (b) rG(TBDMS)$_3$, with (c) and (d) corresponding double reciprocal plots of $F_o(F_o/F)$ versus $1/\text{[natural nucleobase]}$. Spectra were recorded in CHCl$_3$, using an adduct concentration of 10 µM. The native nucleoside was added to the adduct sample as 10 µM aliquots.
Figure 3-30. Emission spectra of bissilylBrh dG upon addition of (a) bissilyldC or (b) rG(TBDMS)_3, with (c) and (d) corresponding double reciprocal plots of $F_0(F_0/F)$ versus $1/[\text{natural nucleobase}]$. Spectra were recorded in CHCl$_3$, using an adduct concentration of 10 $\mu$M. The native nucleoside was added to the adduct sample as 10 $\mu$M aliquots.
Figure 3-31. Emission spectra of $^Q$dG(TBDMS)$_2$ upon addition of (a) bissilyldC or (b) rG(TBDMS)$_3$, with (c) and (d) corresponding double reciprocal plots of $F_o/(F_o-F)$ versus 1/[natural nucleobase]. Spectra were recorded in CHCl$_3$, using an adduct concentration of 10 $\mu$M. The native nucleoside was added to the adduct sample as 10 $\mu$M aliquots.

Interestingly, in all cases, addition of bissilyldC caused a decrease in the fluorescent intensity of the adduct, while in contrast, addition of rG(TBDMS)$_3$ caused an increase in fluorescence intensity, illustrating the emissive sensitivity of these adducts to H-bonding. The addition of bissilyldC to the silyl-protected $^{2\text{Pyr}}$dG, $^{56\text{Ind}}$dG, $^{56\text{Bth}}$dG or $^Q$dG modified nucleosides resulted in a 4.1-, 3.5-, 1.4- or 1.5-fold decrease, respectively, in emission intensity of the adduct. On the other hand, addition of rG(TBDMS)$_3$ to the silyl-protected $^{2\text{Pyr}}$dG, $^{56\text{Ind}}$dG, $^{56\text{Bth}}$dG or $^Q$dG adduct resulted in an approximate one-fold increase in emission intensity.
The decrease in fluorescence intensity of the $C^8$-heteroaryl-dG adduct observed with titration with dC can be attributed to a PET process, shown in Scheme 3-4. Upon photo-excitation of the dimer, a redox reaction occurs between a chromophore in the excited state, in this case the $C^8$-heteroaryl-dG adduct, and another functionality within the same complex, in this case the dC nucleoside. In this process, an electron is transferred from the electron donating adduct to the electron accepting pyrimidine. As pyrimidines are better electron acceptors than purines, indicated by higher oxidation potentials, and $C^8$-heteroaryl-dG adducts possess even lower oxidation potentials than that of natural dG (Table 3-18), the modified nucleosides act as good electron donors in this redox reaction. As PET is typically fast and fully reversible, it essentially constitutes a fluorescence quenching process.

Scheme 3-4

It is of interest to note that in titrations of $^{^{18}}$dG and $^{^{129}}$dG with dC (Figures 3-29a and 3-30a, respectively), the emission intensity of the adduct was not quenched as greatly as in the titrations of $^{^{2Pyr}}$dG and $^{^{Ind}}$dG with dC (Figures 3-27a and 3-28a, respectively).
This is likely because while the oxidation potentials of $^{\text{Bth}}dG$ and $^{\text{Q}}dG$ are lower than that of $dG$, they are higher than the oxidation potentials of $^{\text{2Pyr}}dG$ and $^{\text{Ind}}dG$ (Table 3-18). Subsequently, both $^{\text{2Pyr}}dG$ and $^{\text{Ind}}dG$ serve as better electron donors than $^{\text{Bth}}dG$ and $^{\text{Q}}dG$ to the electron accepting dC base in the PET process, resulting in a less efficient quenching of fluorescence intensity for the latter adducts.

The increase in fluorescence intensity of the $C^8$-heteroaryl-dG adducts observed upon titration with G may be the result of increased rigidity that occurs as the adduct is complexed with the natural purine.\textsuperscript{56} Fluorescence is favoured in molecules with rigid structures that have a lower collisional probability, and subsequent higher fluorescent potential, while molecules with a high degree of flexibility tend to exhibit a low fluorescence intensity due to a high collisional probability resulting in an enhanced internal conversion and consequent increase in likelihood for radiationless decay.\textsuperscript{98}

Double reciprocal plots of $F_o/(F_o-F)$ as a function of 1/natural base (or titrant) concentration were generated,\textsuperscript{56} with examples shown in Figures 3-28 to 3-31. These plots afforded straight lines, consistent with a 1:1 binding interaction.\textsuperscript{56} The association equilibrium constants ($K_a$) provided in Table 3-21 were determined from the ratio of the $y$-intercept to the slope.\textsuperscript{84} For the interaction of the silylated $C^8$-heteroaryl-dG adducts $^{\text{2Pyr}}dG$, $^{\text{Ind}}dG$, $^{\text{Bth}}dG$ and $^{\text{Q}}dG$ with bissilyldC, the corresponding $K_a$ values were determined to be in the range of $(2.40 \pm 0.40) \times 10^4$ M$^{-1}$ to $(3.80 \pm 0.40) \times 10^4$ M$^{-1}$. Binding of $^{\text{2Pyr}}dG$, $^{\text{Ind}}dG$, $^{\text{Bth}}dG$ and $^{\text{Q}}dG$ with rG(TBDMS)$_3$ afforded lower $K_a$ values in the range of $(0.73 \pm 0.01) \times 10^4$ M$^{-1}$ to $(1.42 \pm 0.40) \times 10^4$ M$^{-1}$. 

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Recently, Schwalb and co-workers examined the formation of H-bonded complexes between natural nucleosides in CHCl₃. Structures and association equilibria values of the base pairs reflected the apparent stabilities of the complexes as characterized by fourier transform infrared (FTIR) spectra. Interpretation of the FTIR spectra was accompanied by computational analyses, with DFT calculations of the binding energies and vibrational frequencies at the PW91/6-311++G(d,p) and TPSS/cc-pVTZ levels, and G3B3 calculations used to determine the most stable tautomers.¹²⁵ For rG:dC formation, a $K_a$ value of $(3.4 \pm 0.8) \times 10^4$ M⁻¹ was determined for the W-C H-bonded pair.¹²⁵ Comparison to the $K_a$ values for dimers of $C^8$-heteroaryl-dG adducts with dissilyldC, obtained by fluorescence spectroscopy, indicates that W-C binding strength is unaffected by presence of the $C^8$-heteroaryl moiety, as the $K_a$ values for $^{2\text{Pyr}}$dG:dC, $^{\text{Ind}}$dG:dC, $^{\text{Bth}}$dG:dC and $^{\text{Q}}$dG:dC complexes are within experimental error of $K_a$ determined for G:C by Schwalb and co-workers.⁵⁶,⁵⁸

### Table 3-21. Association equilibrium constant ($K_a$) values of dimers involving silylated derivatives of the natural nucleobases, $^{2\text{Pyr}}$dG, $^{\text{Ind}}$dG, $^{\text{Bth}}$dG and $^{\text{Q}}$dG.

<table>
<thead>
<tr>
<th>dimer</th>
<th>$K_a \times 10^4$ M⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>rG:dC</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>rG:rG</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>$^{2\text{Pyr}}$dG:dC</td>
<td>2.40 ± 0.40</td>
</tr>
<tr>
<td>$^{2\text{Pyr}}$dG:rG</td>
<td>1.15 ± 0.97</td>
</tr>
<tr>
<td>$^{\text{Ind}}$dG:dC</td>
<td>3.16 ± 0.20</td>
</tr>
<tr>
<td>$^{\text{Ind}}$dG:rG</td>
<td>1.38 ± 0.20</td>
</tr>
<tr>
<td>$^{\text{Bth}}$dG:dC</td>
<td>3.80 ± 0.98</td>
</tr>
<tr>
<td>$^{\text{Bth}}$dG:rG</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>$^{\text{Q}}$dG:dC</td>
<td>3.06 ± 0.12</td>
</tr>
<tr>
<td>$^{\text{Q}}$dG:rG</td>
<td>1.42 ± 0.40</td>
</tr>
</tbody>
</table>

⁺ Data taken from Reference no. 125.

⁵ Recorded in CHCl₃ at room temperature.
For the H-bonded bridged dimer of rG (rG:rG), Gaussian and FTIR data obtained by Schwalb favoured a two-point H-bonded reverse-Hoogsteen structure with a $K_a$ value of $(0.10 \pm 0.02) \times 10^4$ M$^{-1}$. Comparison to the $K_a$ values for dimers of $C^8$-heteroaryl-dG adducts with rG(TBDMS)$_3$ obtained by fluorescence spectroscopy, indicates that while the H-bond strength in the complexes is less than that observed for W-C rG:dC, the $C^8$-heteroaryl moiety does strengthens the Hoogsteen binding interaction of the base pair$^{56,58}$ as $K_a$ values for $2$PyrdG:rG, $\text{Ind}dG$:rG, $\text{Bth}dG$:rG and $QdG$:rG complexes are all higher than $K_a$ determined for rG:rG by Schwalb and co-workers. It is predicted that in the case of the $2$PyrdG:rG, $\text{Ind}dG$:rG and $QdG$:rG dimers, association is strengthened by the formation of three H-bonds in the Hoogsteen interaction between the adduct and rG, resulting in an overall increase in stability as compared to the two-point Hoogsteen structure of rG:rG, shown in Figure 3-32$^{56}$ While the $C^8$-benzothienyl moiety does also strengthen the Hoogsteen binding interaction as compared to H-bonding in the rG:rG dimer, the $K_a$ values for $2$PyrdG:rG, $\text{Ind}dG$:rG and $QdG$:rG are approximately double that of $\text{Bth}dG$:rG, indicating the $\text{Bth}dG$ is only capable of forming a two-point, as opposed to a three-point, H-bonding interaction with rG$^{58}$.

![Figure 3-32. Two-point reverse-Hoogsteen H-bonded pair of rG:rG.](image-url)
Sessler and co-workers recently synthesized an intriguing pyrrolo-containing purine ribonucleoside, and similarly concluded that the additional H-bonding functionality provided by the pyrrole NH results in a donor-acceptor-acceptor (D-A-A) motif, capable of forming a three-point extended Hoogsteen-type interaction with guanosine, as shown in Figure 3-32.130

![Pyrrole-containing purine](image)

**Figure 3-33.** Three-point extended reverse-Hoogsteen interaction with riboguanosine and a pyrrole-containing purine.

Support for the experimental $K_a$ values determined by fluorescence spectroscopy was provided by DFT calculations performed by the Wetmore group. Structures of the H-bonded pairs were optimized at the B3LYP/6-31G(d,p) level, while relative energies of H-bonding strengths were calculated at the B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d,p) level of theory, and include ZPVE and BSSE corrections. The calculations predicted little influence of the $C^8$-pyrrolyl,156 indolyl156 or benzothienyl group157 on W-C H-bonding in adduct complexation with dC, with both the adduct and natural base determined to possess anti glycosyl bond linkage conformations. The structures of these H-bonded pairs are shown in Figure 3-33. While association of $Q_d$G with dC was not
confirmed by computational calculations, its W-C H-bonded structure can be assumed to be the same as those for the other dimers with $C^8$-heteroaryl-dG adducts, due to similarity in experimentally determined $K_a$ values.

**Figure 3-34.** Watson-Crick H-bonded pairs of (a) $2\text{pyr}dG:dC$, (b) $\text{Ind}dG:dC$ and (c) $\text{Bth}dG:dC$, as optimized by DFT calculations.

For H-bonding of $2\text{pyr}dG$ and $\text{Ind}dG$ to rG, structures of the base pairs were modeled after the three-point Hoogsteen H-bonding ensemble proposed by Sessler and co-workers involving the pyrrolyl-linked derivative. DFT calculations by the Wetmore group confirmed that the most stable base pair for $2\text{pyr}dG:rG$ and $\text{Ind}dG:rG$ is a three-point reverse-Hoogsteen interaction involving an H-bond from the pyrrole or indole NH atom to the $O^6$-position of G, shown in Figure 3-34. In these complexes, the glycosyl bond linkage of the adduct was determined to exist in the syn conformation, while the rG nucleobase retains the anti conformation.
Three-point reverse-Hoogsteen H-bonded pairs of (a) 2PyrdG:rG and (b) InddG:rG, as optimized by DFT calculations.

For H-bonding of BthdG to rG, DFT calculations confirmed two possible structures for the dimer; a two-point reverse-Hoogsteen or two-point Hoogsteen interaction, as shown in Figure 3-35. In both of these interactions, glycosyl bond linkage of the adduct was determined to exist in the syn conformation, while the rG nucleobase remains in the anti conformation. Calculations also revealed these complexes to be less stable by ~ 20 kJ mol$^{-1}$ compared to the three-point 2PyrdG:rG and InddG:rG base pairs.

(a) Two-point reverse-Hoogsteen and (b) two-point Hoogsteen H-bonded pairs of BthdG:rG, as optimized by DFT calculations.
3.4 Conclusions

The $C^8$-heteroaryl-dG adducts described herein all possess a number of properties that highlight their applicability to serve as fluorescent probes in DNA. These adducts display absorbance separated from that of normal dG, allowing for selective excitation ($\sim 280 - 320$ nm) within oligonucleotides. Adducts are strongly fluorescent, with high quantum yields, and also display intriguing solvatochromic properties. Their solvent dependency with regards to maximum emission wavelength and intensity revealed their potential to probe the DNA microenvironment. These adducts also enhance the electron-donor characteristics of dG, with all analogues possessing lower oxidation potentials than that of the purine nucleoside. The structural and electronic analyses provided by DFT calculations assisted in the understanding of the photophysical and redox properties of the $C^8$-heteroaryl-dG adducts. The 2"'-substituted sulfur-substituted analogues possess the largest ground state and excited state dipole moments, with the emitting excited state possessing a dipole moment greater than the ground state, and furthermore, the largest change in $\theta$ twist angle from the ground state to excited state, contributing to the increase in Stokes shift with increased solvent polarity and strong electron withdrawing character of the thienyl and benzothienyl groups. While these analogues therefore possess features synonymous with push-pull probes, of the $C^8$-heteroaryl-dG adducts studied, the $Q$ dG adduct can best be described as a push-pull probe, owing to its donor-acceptor like structure and its TICT excited state character.

The $C^8$-heteroaryl-dG adducts also exhibit fluorescence that is responsive to H-bonding interactions with dC and rG. The fluorescence is quenched upon Watson-Crick H-bonding to dC, attributed to a photoinduced electron transfer process, while H-bonding
to rG enhances fluorescence intensity.\textsuperscript{56,57} Fluorescence was most prominently enhanced for the C\textsuperscript{8}-pyrrolyl- and C\textsuperscript{8}-indolyl-linked analogues that form a rigid three-point Hoogsteen complex with rG.\textsuperscript{56} Adducts were determined to adopt an \textit{anti} glycosyl bond conformation upon W-C H-bonding with dC, and a \textit{syn} conformation upon Hoogsteen binding to rG. These adducts therefore have the potential to fluorometrically differentiate between W-C and Hoogsteen base-pairing, and subsequently, \textit{anti} and \textit{syn} adduct conformation, and to probe for mismatch formation, providing impetus for further study in a double helical environment.\textsuperscript{56,57}
3.5 References


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Chapter 4.

$C^8$-Heteroaryl-2'-Deoxyguanosine Adducts as Conformational Fluorescent Probes in the NarI Recognition Sequence
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4.1 Introduction

The number of fluorescent nucleosides has increased vastly over the last decade, with the search for new probes with improved photophysical and structural features still continuing.\(^1\) Numerous examples of such bases and their myriad of potential applications were presented in the Chapter 3 Introduction. While a nucleoside may possess the qualities of an ideal fluorescent probe, a new set of parameters must be considered if this nucleoside is to still function as designed within the confines of DNA. The development of fluorescent probes for studying reactions and interactions in DNA-containing systems can be challenging, as a result of the strict parameters set by the helical-stacking environment of DNA. A number of critical properties, including size, shape, charge, polarity and hydrogen-bonding motif, need to be thoughtfully pondered when designing a fluorescent probe for DNA.\(^1\) There exists two major classes of molecules developed for fluorescent labeling of nucleic acids; molecules that interact non-covalently with DNA, and those that interact covalently.\(^2\) Examples of molecules that interact non-covalently include DNA intercalators, such as ethidium bromide and thiazole orange.\(^3\) Molecules that interact covalently with DNA have been developed to enable either external or internal modifications. An external modification involves covalently tethering fluorophores to the backbone of an oligonucleotide strand, but outside of the actual base-stack. Common fluorophores used to modify DNA in this manner include fluorescein, rhodamines, and Alexa and Cy dyes.\(^2\) A covalent internal modification involves replacement of the normal base with a modified base, or fluorescent base analogue, within the base-stack.\(^4\) These base analogues have become increasingly popular in use, as they have major advantages compared to intercalators or external modifiers in that
they cause little perturbation, and thus preserve the native structure of DNA. Fluorescent base analogues are similar in shape to the natural nucleobases and are able to form one or more hydrogen bonds to a natural nucleobase in the complement. In addition, base analogues are located rigidly in the base-stack, resulting in a predictable orientation within DNA.\(^5\)

An expansive collection of fluorescent base analogues have been developed and incorporated into oligonucleotides with a wide variety of purposes. Tor classified the main areas where DNA-incorporated analogues have found applicability: single nucleotide polymorphism (SNP) detection, involving detection of single base substitutions; nucleic acid microenvironment sensitivity, allowing for investigation of nucleobase damage, depurination or depyrimidination, or base flipping; signalers in DNA and RNA assays, particularly for monitoring ligand binding; and reporters of nucleic acid structure and function,\(^6\) which is of particular interest in the work here presented. Appropriately selected fluorescent base analogues, imperatively incorporated within the oligonucleotide, can photophysically report hybridization, folding and conformational changes, examples of which are outlined below.\(^6\)

The widely used fluorescent adenine analogue 2AP, previously shown in Figure 3-6, causes little perturbation to the structure of DNA, as it forms a stable W-C type base pair with T and preserves the normal B-form structure of the duplex.\(^4\) This analogue provides high sensitivity for monitoring subtle changes in nucleic acid structure, with the fluorescence of 2AP strongly quenched when incorporated into single-strand oligonucleotides, and quenched further still when hybridized to a complementary strand.\(^7\) The decrease in fluorescence quantum yield (\(\phi_f\)) also varies with oligonucleotide
sequence, temperature and helical conformation. These characteristics have been exploited to understand structural dynamics, base-stacking interactions, protein-DNA interactions and charge transfer within duplex and hybrid (i.e. RNA:DNA) helical structures. Other examples of hybridization probes include: members of the pteridine family, previously shown in Figure 3-2, that are highly quenched, the degree of which is dependent on the nature of the surrounding base pairs, when incorporated into DNA; the expanded nucleobases pyrrolo-dC and the benzoquinazoline derivative (BqQ), structures of which can be found in Figure 3-3, that show a significant drop in quantum yield after incorporation into an oligonucleotide and further reduction of quantum yield following duplex formation; and the isomorphic nucleobase 8-aza-dG, originally shown in Figure 3-6, which also displays a decrease in emission in single-strand oligonucleotides and an even more pronounced decrease in double-stranded DNA. The class of hybridization probes can also include base-discriminating probes, named for their capacity to recognize a complementary base. Examples of these analogues include the expanded dC-derivatized nucleobases BPP and dChpp, shown previously in Figure 3-3, and the extended PRODAN-linked nucleobase PDN-dG, as in Figure 3-4. The analogue BPP has been applied to discriminate between purines, using the change in quantum yield when base paired with G (\( \phi_n = 0.002 \)) or A (\( \phi_n = 0.035 \)). The fluorescence of the dC_HPP-incorporated oligonucleotide was greatly suppressed when a dC_HPP-dG base pair was formed, while maintained when a dC_HPP-dA base pair was formed. Finally, PDN-dG incorporated into DNA also exhibits unique photochemical behaviour sensitive to the nature of the base complementary to the modified nucleoside.

Generally speaking, fluorescent base analogues, such as 2AP, are highly
quenched inside DNA, but probes that retain high quantum yields upon oligonucleotide incorporation have also been developed.\textsuperscript{2} The quantum yield of the fluorescent tricyclic cytosine analogue tC, is unaffected by the single- and double-stranded nature of DNA.\textsuperscript{19} This expanded nucleobase, shown prior in Figure 3-3, has a quantum yield of 0.22 in a peptide nucleic acid (PNA) single-strand and of 0.21 in a PNA-DNA hybrid duplex, roughly the same quantum yield (0.20) as in the monomeric form.\textsuperscript{20} The oxo-homologue of tC, known as tC\textsuperscript{O}, also shown in Figure 3-3, has been shown to exhibit unprecedented brightness when incorporated into DNA.\textsuperscript{1} Furthermore, Hudson’s version of the G-clamp, the expanded nucleobase known as boPhpC and shown in Figure 3-3, notably exhibited a quantum yield in a PNA single-strand greater than the free fluorophores, although the quantum yield was found to decrease by approximately 50% upon duplex formation.\textsuperscript{21} Another Hudson designed nucleoside analogue, the extended nucleobase 5-phenylethynyl-dU, shown in Figure 3-5, was found to be an excellent fluorimetric reporter of hybridization. This derivative possessed little fluorescence as a single-strand incorporated species, whereas in the presence of complementary DNA, a six-fold increase in fluorescence was observed.\textsuperscript{16}

Fluorophore-linked nucleobases can include those with π-systems attached to the natural bases. These analogues are also known to retain high quantum yields upon DNA incorporation. Within this division of analogues, modification of the C\textsuperscript{8}-position of purines is attractive, as this site is not directly involved in base-pairing interactions within the duplex, and thus will not interfere with hydrogen bonding to the complement. Recent examples include: the pyrene-modified extended nucleobase Py-dG (Figure 3-4), which acts as a duplex sensitive probe, with increased fluorescence intensity upon DNA
hybridization as compared to that of the corresponding single-strand oligonucleotide;\(^{22}\) the pyrene-labeled alkylamino-linked extended nucleobase \(^{\text{Py}}d\text{G}\) (Figure 3-4), found to be highly emissive in single-strand DNA while exhibiting weak fluorescence once incorporated into the duplex, thus possessing applicability as a sensor to discriminate between single- and double-stranded DNA structures; and the isomorphic pyridine-modified fluorophore \(^{2}\text{PyrdG}\) (Figure 3-6) that displayed increased quantum yields upon their incorporation into single-stranded or duplex DNA.\(^{23}\) Finally, while the vinyl-linked derivative \(Vd\text{G}\), depicted in Figure 3-6, does show a decrease in emission in single- and double-stranded DNA, its emission properties are very sensitive towards changes within the surrounding DNA microenvironment.\(^{24}\)

In addition to reporting hybridization and folding, nucleobase analogues can be used to detect conformational changes. As discussed in Chapter 1, section 1.2.4 (b) and shown in Scheme 1-2, modification of the \(d\text{G}\) \(C^8\)-position can shift the conformational equilibrium of the glycosidic bond from \(\text{anti}\) to \(\text{syn}\).\(^{23,25,26}\) When the \(C^8\)-modified base adopts the \(\text{anti}\) conformation, the \(C^8\)-substituent is located in the major groove, and three W-C hydrogen bonds are intact between the modified guanine and its natural pyrimidine base-pairing partner. This minimal disruption to H-bonding and stacking interactions results in adoption of a B-form helix with similar stability to that of the unmodified strands.\(^{27}\) When the \(C^8\)-modified nucleobase adopts the \(\text{syn}\) conformation, a much greater disruption of the helical structure is noted. When the adduct is paired with its natural pyrimidine partner, the \(C^8\)-substituent is located in the narrow minor groove,\(^{28}\) forcing the opposing base to flip out of its natural intrahelical position to the solvent-exposed extrahelical environment.\(^{29}\) There is greater distortion of the base-pairing as compared
with the corresponding *anti* base-pairs, as the modified adduct undergoes H-bonding with
the complementary C by utilizing the Hoogsteen edges of the respective paired
nucleobases.\textsuperscript{28} Conversely, if the \( C^8 \)-modified base is mismatched with a purine, the \( C^8 \)-
substituent is located in the minor groove, with \( \pi \)-stacking interactions with neighbouring
nucleobases increased. In addition, base-pairing stability is significantly increased. The
Hoogsteen face of the G component of the \( C^8 \)-modified base H-bonds with the W-C face
of the opposing purine, resulting in mismatch stabilization due to additional interactions
with the \( C^8 \)-moiety.\textsuperscript{27,29} It should be noted that in this conformational scenario, the
opposing purine nucleobase is not flipped out of the intrahelical environment.

Recall that the Manderville group interest in \( C^8 \)-modified bases, and in particular,
\( C^8 \)-aryl-dG adducts, stems from their toxicological relevance, as discussed thoroughly in
Chapter 1, section 1.2.4. A number of chemical mutants generate radical species that
undergo direct addition reactions at the \( C^8 \)-site of dG to afford these adducts.\textsuperscript{30,31} These
adducts are structurally related to \( N \)-linked \( C^8 \)-dG adducts derived from arylamine
carcinogens, for which the different \textit{anti} versus \textit{syn} induced conformational likelihoods
play a crucial role in their biological activity. Common examples of these lesions include
adducts of \( N \)-2-aminofluorene (AF-dG),\textsuperscript{32,33} \( N \)-2-acetylaminofluorene (AAF-dG),\textsuperscript{34-36} \( N \)-
2-fluoroaminofluorene (FAF-dG),\textsuperscript{28,37} the heterocyclic amine IQ (IQ-dG),\textsuperscript{26,38,39}
aminopyrene (AP-dG)\textsuperscript{40} and 4-aminobiphenyl (ABP-dG).\textsuperscript{41} Structures of these adducts
were shown previously in Figure 1-12. While the conformational heterogeneity of \( C \)-
linked \( C^8 \)-dG adducts generated by various chemical carcinogens is not as well
understood as for \( N \)-linked adducts, it is still thought to play an important role in their
biological activity. Examples of such adducts include those generated from
nitroaromatics, such as 3-nitrobenzanthrone (N-Ac-ABA-dG),\textsuperscript{42} PAHs, such as benzo[a]pyrene (6-BP-8-dG)\textsuperscript{43-45} and arylhydrazines.\textsuperscript{46-48} Structures of these adducts were also shown previously in Figure 1-12.

Extensive research has been conducted that shows \textit{N}-linked \textit{C}\textsuperscript{8}-dG adducts adopt three distinct duplex structural motifs depending upon the location, and \textit{anti/syn} conformational preference, of the adduct: the major groove binding B-type conformation; the base-displaced ‘stacked’ S-type conformation; and the minor groove binding ‘wedged’ W-type conformation.\textsuperscript{33} In the B-type conformation, the \textit{N}-linked \textit{C}\textsuperscript{8}-dG modified base adopts an \textit{anti} glycosidyl configuration, while in the S- and W-type conformations, the adduct adopts a \textit{syn} glycosidyl configuration,\textsuperscript{33} with the \textit{syn} conformers considered to be pro-mutagenic.\textsuperscript{34} When base-paired with its normal pyrimidine partner C, these adducts assume either B- or S-type duplex conformations,\textsuperscript{33} while the W-type conformation was found to predominate in duplexes in which the adduct was mismatched with dA.\textsuperscript{49} It is of upmost importance to note that these lesions are non-fluorescent, and therefore fluorescent spectroscopy cannot be employed as a method of conformational analysis. Instead, less sensitive analytical tools have been employed to distinguish conformation of \textit{N}-linked \textit{C}\textsuperscript{8}-dG adducts, including circular dichroism (CD),\textsuperscript{33} UV absorption,\textsuperscript{50} and \textsuperscript{19}F and \textsuperscript{1}H nuclear magnetic resonance (NMR)\textsuperscript{51,52} spectrosopies. Fluorescent \textit{C}\textsuperscript{8}-modified-dG nucleobases therefore possess an attractive alternative for probing conformational preference.

The Manderville group recently applied thermal melting (\textit{T}_m) studies, CD, molecular dynamics (MD) and notably, fluorescence spectroscopy, to the study of the conformational preference of phenolic \textit{C}\textsuperscript{8}-dG adducts in duplex DNA, with results
illustrating a *syn* conformation preference for the adduct, with the C⁸-linked phenolic group in a W-type conformation. The use of fluorescence spectroscopy in defining adduct conformation did present limitations, as the excitation maxima of the adducts (∼280 nm) overlapped with that of natural DNA. Recall that the novel fluorescent C⁸-heteroaryl-dG adducts presented in Chapter 3, as shown in Figure 3-7, displayed absorption wavelengths, given in Table 3-5, well separated from that of natural DNA. Furthermore, ²Pyr-dG, ¹ªdG, Bth-dG and Q-dG adducts were all shown to act as emission sensitive reporters of W-C versus Hoogsteen H-bonding (Chapter 3, section 3.3.6), and were found to strengthen the H-bonding interaction to the unnatural base-pairing partner G, compared to the unmodified G:G mismatch. These findings highlighted the potential use of these C⁸-heteroaryl-dG adducts as fluorescent reporters of *syn* versus *anti* conformation in duplex DNA. The benzothienyl derivative, Bth-dG, has also previously been incorporated in two decanucleotide (10-mer) sequences and was found to act as an emissive conformational probe. In this case the reporting ability of Bth-dG was ascribed to changes in π-stacking and charge transfer character between the benzothienyl moiety and the native DNA nucleobases in the duplex.

On the basis of these intriguing photophysical properties, the C⁸-heteroaryl-dG adducts ²Fur-dG, ¹ªdG, Bfur-dG, Bth-dG and Q-dG were chosen for incorporation into oligonucleotides in order to test their performance as probes in a DNA environment. The adducts were incorporated by one of two methods; utility of a postsynthetic Suzuki-Miyaura cross-coupling strategy or by standard phosphoramidite chemistry. The sequence of incorporation was thoughtfully considered, with C⁸-heteroaryl-dG adducts incorporated into G₁ or G₃ of a 12-mer oligonucleotide that contains the recognition
sequence (shown in bold) of the NarI Type II restriction endonuclease, 5'-CTCG1G2CG3CCATC. A strain of aerobic bacteria carries the gene for this enzyme; the name NarI is derived from the name of the bacteria, Nocardia argentinae. This bacteria produces an antibiotic effective against Gram-positive bacteria, particularly Staphylococcus and Clostridia. Type II restriction enzymes constitute an important defense mechanism against viral attacks, as they catalyze double-strand DNA breakage at specific recognition sites.

The NarI recognition sequence contains a GC-repeat that is prone to spontaneous and induced frameshift mutations; these mutations may be important contributors to human cancers. Recall from Chapter 1, section 1.2.1 that frameshift mutations involve a shift in the reading frame of a polymerase enzyme during extension of a DNA sequence as part of the replication process, and can ultimately lead to errors in protein translation. In particular, the NarI recognition sequence is considered a ‘hot spot’ for high-frequency −2 frameshift mutations induced by binding of bulky N-linked C8-aryl moieties to the G3 site in the recognition sequence. The net result of this mutagenic event is the loss of two nucleotides (G3C) within the GC-repeat of the recognition sequence. Rizzo and co-workers proposed a two-base slippage mechanism for frameshift mutation induced by the attachment of the heterocyclic amine IQ at the G3 site in the dinucleotide repeat of the NarI sequence, with reproduction of this mechanism shown below in Scheme 4-1.
The scheme shows that replication by the prokaryotic DNA polymerase pol II resulted in two-base deletions when the IQ-dG adduct was placed at the G₃ site. Replication by pol II proceeds until opposite the adduction site, with the −2 frameshift event resulting from the elongation of a misaligned polymerase. Adduction at the G₃ site by N-linked C₈-aryl moieties induces loss of G₃C at a frequency that is ~10⁷-fold higher than the spontaneous frequency. Interestingly, adduction at the G₁ site of the NarI recognition sequence does not induce frameshift mutation. The C₈-heteroaryl-dG adducts were thus incorporated at the G₃ site, with the dG adduct also incorporated at the G₁ site. While modification at this position does not stimulate mutagenesis, incorporation was performed at this site in order to compare overall stability, and general photophysical and structural properties, of the NarI recognition sequence modified at different positions.
The work reported herein this chapter effectively demonstrates that $C^8$-heteroaryl-dG adducts retain their interesting photophysical properties upon DNA incorporation, and exhibit thermal stability and fluorescence spectroscopy sensitivity towards H-bonding properties and $syn$ versus $anti$ conformation. $C^8$-heteroaryl-dG fluorescent probes that are responsive to changes in the DNA environment, and serve as structural representatives of persistent $N$- and $C$-linked $C^8$-dG adducts formed $in$ $vivo$, could assist in the understanding of the means of the adduct-induced mutagenesis through use of luminescence-based assays.
4.2 Materials and Methods

4.2.1 General Methods

A detailed description of all general methods for experiments pertaining to Chapter 4 can be found in Appendix A.

Note that the $C^8$-heteroaryl-dG nucleoside adducts $2\text{Fur}dG$, $\text{Ind}dG$, $\text{Bfur}dG$, $\text{Bth}dG$ and $QdG$ used in fluorescence measurements as outlined below in sections 4.2.7 and 4.2.8, were synthesized by Suzuki-Miyaura coupling, with the synthetic protocol and details previously provided in Chapter 3, section 3.2.3.

4.2.2 Synthesis of $C^8$-Heteroaryl-G Modified NarI Oligonucleotides

(a) Synthesis by Standard Phosphoramidite Chemistry

Synthesis of the $C^8$-heteroaryl-G modified NarI oligonucleotide, 5'-CTCXGCGCCATC, with $X = QG$, and $C^8$-heteroaryl-G modified NarI oligonucleotides, 5'-CTCGGCCCATC, with $X = 2\text{Fur}G$ or $X = QG$, was performed by Michael Sproviero in the Manderville laboratory at the University of Guelph (Guelph, ON) on a 1 $\mu$mol scale on a BioAutomation Corporation MerMade 12 automatic synthesizer using standard $\beta$-cyanoethylphosphoramidite chemistry according to published protocols. Modified NarI oligonucleotide samples were purified as outlined below in section 4.2.3.

(b) Suzuki-Miyaura Coupling Reactions with 8-Br-G Modified Oligonucleotides

Synthesis of $C^8$-heteroaryl-G modified NarI oligonucleotides, 5'-CTCGGCXCCCATC, with $X = \text{Ind}G$, $\text{Bfur}G$ or $\text{Bth}G$, was conducted according to the literature. Brominated NarI oligonucleotides, 5'-CTCGGCXCCCATC, with $X = 8\text{-Br}G$,
were custom-made by Sigma-Aldrich Canada Ltd. (Oakville, ON) on a 1 µmol scale using standard phosphoramidites, and 8-Br-dG-CE phosphoramidite purchased from Glen Research (Sterling, VA). The brominated oligonucleotides were fully deprotected with NH₄OH for 24 h, desalted and purified by reverse-phase (RP) chromatography by Sigma-Aldrich Canada Ltd., and the mass spectrum of NarI(X = 8-Br-G) was obtained at the University of Toronto prior to use in Suzuki-Miyaura coupling in order to confirm identity of the brominated oligonucleotide. The subsequent synthesis of C⁸-heteroaryl-G modified NarI oligonucleotides follows the same protocol as previously outlined for C⁸-aryl-G trimers in Chapter 2, section 2.2.5 (b). Note that the term ‘brominated trimer’ as it appears in section 2.2.5 is to be substituted here for ‘NarI(X = 8-Br-G)’. The resulting C⁸-heteroaryl-G modified NarI oligonucleotide solutions were further purified by RP high-performance liquid chromatography (HPLC), as described below.

4.2.3 Oligonucleotide Purification and Sample Preparation

Purification, preparation and quantification of C⁸-heteroaryl-G modified NarI oligonucleotide samples were performed following the same procedure as previously outlined for C⁸-aryl-G trimers in Chapter 2, section 2.2.6. C⁸-heteroaryl-G modified NarI oligonucleotides were also assumed to have the same extinction coefficient (ε) as the unmodified NarI oligonucleotide.⁶⁰,⁶¹ In all cases of hybridization, oligonucleotides were annealed by heating at 80 °C for 10 min, cooling to room temperature and refrigerating until analysis.
4.2.4 Mass Spectrometry Analysis of Oligonucleotides

Mass spectrometry (MS) analysis was performed by Keegan Rankin in the Mabury group at the University of Toronto (Toronto, ON), by one of two methods, as outlined below. Methanol used was purchased from EMD Millipore (Billerica, MA), water from OmniSolv (Charlotte, NC) and ammonium acetate (≥99%) from Fluka (St. Louis, MO).

(a) Mass Spectrometry Analysis by API4000

The C₈-heteroaryl-G modified NarI oligonucleotide, 5'-CTCGGCXCCATC, with X = IndG, was analyzed with an API4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON). The sample was prepared in a 50:50 MeOH:H₂O solution with 0.1 mM ammonium acetate. Full scan MS spectra were obtained by direct infusion at a rate of 5 — 10 µL/min into an electrospray (ESI) source operated in negative mode. The entrance and declustering voltages were optimized for each analyte and varied from -5 to -10 V and -20 to -35 V, respectively. All data was acquired with 20 MCA and processed using Analyst 1.51 mass spectrometry software.

(b) Mass Spectrometry Analysis by Waters Xevo TQ-S

The C₈-heteroaryl-G modified NarI oligonucleotide, 5'-CTXGCXCCATC, with X = QG, and brominated or C₈-heteroaryl-G modified NarI oligonucleotides, 5'-CTCGGCXCCATC, with X = 8-Br-G, X = 2Fur-G, X = Bfur-G, X = Bth-G or X = QG were analyzed using a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation, Milford, MA), with the protocol for analysis previously outlined in Chapter 2, section 2.2.7.
4.2.5 Circular Dichroism Measurements

Circular dichroism (CD) measurements were performed using instrumentation of the Merrill group at the University of Guelph (Guelph, ON). Spectra were obtained on a Jasco J-815 CD Spectrophotometer, equipped with a 1 × 6 Multicell Block Peltier, thermal controller and Julabo AWC 100 water circulator unit. Spectroscopic solutions were prepared in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using 1.25 µM of the unmodified or C\textsuperscript{8}-heteroaryl-G modified NarI oligonucleotide and 1 eq. of its complementary strand. Spectra were collected at 10 °C from 400 to 200 nm, with a bandwidth of 1 nm and scanning speed at 100 nm/min. Each oligonucleotide sample was scanned nine times and background corrected.

4.2.6 UV Thermal Melting Measurements

All melting temperatures (T\textsubscript{m}’s) of oligonucleotides were measured by ultraviolet-visible (UV-Vis) spectroscopy. Spectroscopic solutions were prepared in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using 1.25 µM of the unmodified or C\textsuperscript{8}-heteroaryl-G modified NarI oligonucleotide and 1 eq. of its complementary strand. The UV absorption at 260 nm was monitored as a function of temperature. The temperature was increased from 10 °C to 80 °C, or decreased from 80 °C to 10 °C, at a heating rate of 1 °C/min. The T\textsubscript{m}’s of the duplexes were measured in triplicate to allow for determination of mean ± standard deviation, and calculated by determining the first derivative of the melting curve or by using Hyperchromicity (Vant Hoff) calculations.
4.2.7 Fluorescence Measurements

All excitation and emission spectra of \( C^8 \)-heteroaryl-G modified NarI oligonucleotides were recorded using spectroscopic solutions prepared in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl. All single-strand oligonucleotide samples were prepared to a final concentration at 1.25 \( \mu \)M, and duplex samples prepared using 1.25 mM of the modified NarI oligonucleotide and 1 eq. of its complementary strand. In addition, to allow for comparison, spectra were recorded for the corresponding \( C^8 \)-heteroaryl-dG adduct, prepared in the same solution as above, to a final concentration of 1.25 mM. For the purposes of this comparison, spectra of \( C^8 \)-heteroaryl-dG adducts were recorded using the same excitation and emission slit widths setting of 5 nm. Spectra were initially recorded at 10 °C, and then at increasing 10 °C intervals to a maximum of 80 °C. Samples were held at each temperature for 5 min prior to recording spectra.

4.2.8 Collisional Fluorescence Quenching Studies

All quenching studies were carried out using KI as the quencher, following a previously described method.\(^{62}\) A 5 M stock solution of KI was prepared in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, and 0.1 mM Na$_2$S$_2$O$_3$ was added to the stock solution to prevent I$_3^-$ formation. Spectroscopic solutions were prepared in 50 mM sodium phosphate, pH 7, with 100 mM NaCl, using 1.25 \( \mu \)M of the modified NarI oligonucleotide and 1 eq. of its complementary strand. In addition, to allow for comparison, spectra were recorded for the corresponding \( C^8 \)-heteroaryl-dG adduct, prepared in the same solution as above to a final concentration of 1.25 \( \mu \)M. KI was added to the adduct or duplex sample as 0.025, 0.05, 0.1, 0.5 or 1 M aliquots.
Fluorescence emission intensity was measured at the emission wavelength maximum for the corresponding $C^8$-heteroaryl-dG adduct. This procedure was conducted in triplicate to allow for determination of mean ± standard deviation of Stern-Volmer quenching constant ($K_{sv}$) values. Quenching data for the homogeneous single fluorophores system were analyzed using the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{sv}[Q]$$

where $F_0$ and $F$ are the fluorescence emission intensities in the absence and presence of the quencher, respectively, and [Q] is the concentration of the quencher, KI. Values for $K_{sv}$ were determined from the slope of $F_0/F$ versus [Q] plots.
4.3 Results and Discussion

4.3.1 Synthesis and Characterization of C\textsuperscript{8}-Heteroaryl-G Modified Narl Oligonucleotides

To test the emissive properties of C\textsuperscript{8}-heteroaryl-dG probes in a helical environment, the benzoheterocyclic series of adducts Ind\textsubscript{d}G, Bfur\textsubscript{d}G, Bth\textsubscript{d}G and Q\textsubscript{d}G, along with the 5-membered aryl ring linked derivative 2Fur\textsubscript{d}G, were incorporated into the 12-mer Narl substrate at two different G sites. The benzoheterocyclic series of adducts all exhibit excitation maxima distinct from DNA and permit the role of the heteroatom to be determined, while incorporation of the 2Fur\textsubscript{d}G nucleobase into the 12-mer substrate provides a direct comparison to Bfur\textsubscript{d}G in terms of heteroaryl ring size.\textsuperscript{54} Recall that the choice of Narl as the oligonucleotide substrate stems from its extensive use as the substrate for related N-linked C\textsuperscript{8}-dG adducts derived from arylamine carcinogens,\textsuperscript{39,50,58,63} for which adduct conformation correlates with biological activity.\textsuperscript{58,63}

The C\textsuperscript{8}-heteroaryl-dG adduct 2Fur\textsubscript{d}G was incorporated into the 12-mer Narl\textsuperscript{3} substrate at the G\textsubscript{3} position, and Q\textsubscript{d}G was incorporated into both Narl\textsuperscript{1} at the G\textsubscript{1} position and Narl\textsuperscript{3} at the G\textsubscript{3} position using standard β-cyanoethylphosphoramidite chemistry according to published protocols\textsuperscript{59,64} as carried out by Michael Sproviero in the Manderville laboratory at the University of Guelph (Guelph, ON).\textsuperscript{54} Modified oligonucleotide synthesis by standard phosphoramidite chemistry was previously outlined in Chapter 1, section 1.3.2, with the 2Fur\textsubscript{d}G and Q\textsubscript{d}G nucleoside adducts converted into a phosphoramidite, using the synthetic strategy outlined in Scheme 1-6, for oligonucleotide incorporation by solid-phase synthesis, as shown in Scheme 1-7. The remaining C\textsuperscript{8}-heteroaryl-dG adducts Ind\textsubscript{d}G, Bfur\textsubscript{d}G and Bth\textsubscript{d}G were incorporated into the 12-mer Narl\textsuperscript{3} substrate at the G\textsubscript{3} position by application of the postsynthetic method\textsuperscript{55} involving
palladium-catalyzed Suzuki-Miyaura cross-coupling reactions with brominated NarI₃ oligonucleotides and ten equivalents of 1-N-Boc-indole-2-boronic acid, benzofuran-2-boronic acid or benzothiophene-2-boronic acid, respectively.⁵⁴ This reaction was previously discussed in detail in Chapter 1, section 1.3.3 with the Suzuki-Miyaura synthetic strategy shown in Scheme 1-8. The brominated starting material and C⁸-heteroaryl-G modified oligonucleotide sequences, along with their complementary oligonucleotide sequences, are illustrated in Figure 4-2. NarI'(C) refers to the normal complementary strand to both the NarI¹ and NarI³ modified oligonucleotides. NarI'(G), both sequences of which are shown in Figure 4-2, also refers to a complementary strand in which a G nucleotide has been inserted at the appropriate site to allow for positioning opposite the site of C⁸-heteroaryl-G modification upon duplex formation (shown in bold). In all instances in this work, NarI'(G) refers to the complementary strand in which a G has been inserted at the appropriate site to allow for positioning opposite the site of C⁸-heteroaryl-G modification upon duplex formation. The NarI'(G) sequence with G at the ⁹th position from the 5'-end is complementary to NarI¹, while the NarI'(G) sequence with a G at the ⁶th position from the 5'-end is complementary to NarI³. The NarI'(10-mer) is a truncated NarI³ modified oligonucleotides, with a two nucleotide (CG) deletion compared to the NarI'(C) oligonucleotide.
Immediately following synthesis, all product mixtures of $C^8$-heteroaryl-G modified Nar$^1$ and Nar$^3$ oligonucleotides were purified by RP HPLC using gradient elution with the following buffers: 95:5 aqueous 50 mM triethylammonium acetate (TEAA) (pH 7.2)/acetonitrile and 30:70 aqueous 50 mM TEAA (pH 7.2)/acetonitrile. The desired product, along with remaining starting material, was identified in the resulting chromatogram using both diode array (monitored at $\lambda_{\text{abs}} = 258$ nm) and fluorescence (monitored at $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ of the incorporated adduct) detection.\(^{54}\) RP HPLC traces of product mixtures following Suzuki-Miyaura coupling reactions are shown in Figures 4-2 to 4-4, with both methods of detection featured.
Figure 4-2. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of Nar1\(^1\)(X = 8-Br-G) with 10 eq. of 1-N-Boc-indole-2-boronic acid, detected by (a) diode array at \(\lambda = 258\) nm and (b) fluorescence at \(\lambda_{\text{ex}} = 321\) nm and \(\lambda_{\text{em}} = 390\) nm.
Figure 4-3. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of $\text{Nar}1^3(X = 8\text{-Br-G})$ with 10 eq. of benzofuran-2-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{\text{ex}} = 323$ nm and $\lambda_{\text{em}} = 405$ nm.
Figure 4-4. RP HPLC traces of product mixtures following Pd-catalyzed Suzuki-Miyaura coupling reactions of Nar1$^3$(X = 8-Br-G) with 10 eq. of benzothiophene-2-boronic acid, detected by (a) diode array at $\lambda = 258$ nm and (b) fluorescence at $\lambda_{ex} = 315$ nm and $\lambda_{em} = 420$ nm.

In all cases, a large amount of the Nar1$^3$(X = 8-Br-G) starting material was observed to elute at ~ 15 minutes, with a single peak generated for the desired product at ~ 2 – 3 minutes following the starting material peak. The UV spectral properties of all isolated products, in addition to that of the unmodified Nar1 oligonucleotide, can be found in Appendix D. The UV spectra of the products displayed unique characteristics not present in the spectra of unmodified Nar1 or Nar1$^3$(X = 8-Br-G). The UV spectrum of unmodified Nar1 or Nar1$^3$(X = 8-Br-G) exhibited one absorbance band at ~ 260 nm; this
was expected, as the native DNA bases are known to absorb in the 260 nm wavelength region. \(^{65}\) Spectra of all \(C^8\)-heteroaryl-G modified oligonucleotides showed a predominant absorbance band between \(\lambda = 260 - 265\) nm, as well as an additional absorbance at a red-shifted wavelength in the range of \(\sim \lambda = 315 - 325\) nm. This additional absorbance signifies the successful incorporation of the \(C^8\)-heteroaryl moiety into the oligonucleotide, as it closely matches that of the absorbance of the corresponding \(C^8\)-heteroaryl-dG adduct. Photophysical parameters of \(C^8\)-heteroaryl-dG adducts can be reviewed in Table 3-5. One exception is important to note; while the \(^{2}\text{Fur}dG\) adduct absorbs at \(\lambda = 292\), the spectrum of the \(^{2}\text{Fur}G\) modified \(NarI^3\) displays an absorbance for the modified nucleoside at \(\lambda = 318\) nm. As discussed previously in Chapter 2, section 2.3.1 (a) and Chapter 3, section 3.3.2 (c), \(C^8\)-aryl-dG\(^{66}\) and \(C^8\)-heteroaryl-dG adducts\(^{54}\) respectively, adopt a ground state twisted structure. Data presented in Chapter 2, section 2.3.1 (a) also shows that the corresponding nucleoside lacking the deoxyribose moiety is planar.\(^{66}\) This suggests that \(^{2}\text{Fur}dG\) may have adopted a planar structure within the \(NarI\) oligonucleotide.

Fluorescence detection also proved advantageous for identification of the product peak. \(NarI^3\) oligonucleotides with \(^{2}\text{Fur}G, \text{Ind}G, \text{Bfur}G\) or \(^{Bth}G\) modifications were expected to show fluorescence, as the corresponding \(C^8\)-heteroaryl-dG adducts all possess high quantum yields, as originally outlined in Table 3-5. As shown in Figures 4-2b, 4-3b and 4-4 (b), fluorescence detection results in a significantly intense peak for the product, and not for any other component of the Suzuki-Miyaura coupled product mixture, allowing for accurate determination of the product peak.

Mass spectrometry analysis positively confirmed the identity of all \(C^8\)-heteroaryl-
G modified oligonucleotides, synthesized by Suzuki-Miyaura coupling or standard phosphoramidite chemistry. Analysis was performed by Keegan Rankin in the Mabury group at the University of Toronto (Toronto, ON), by use of an ESI source operated in negative mode. The ESI spectra showed the expected clusters of multiply charged peaks for the modified oligonucleotides, with results of this analysis summarized in Table 4-1. Mass spectra for all $C^8$-heteroaryl-G modified oligonucleotides, including spectra of the starting brominated $NarI^3$ oligonucleotide, are included in Appendix D. Integration of HPLC traces allowed for determination of yields from Suzuki-Miyaura couplings. Yields ranged from 10 – 21 % and are given in Table 4-1. $^{54}$
Table 4-1. Yields and ESI-MS analysis of C\textsuperscript{8}-heteroaryl-G modified oligonucleotides.

<table>
<thead>
<tr>
<th>oligonucleotide ( X )</th>
<th>product formula</th>
<th>yield (%)( ^{c} )</th>
<th>calcd mass( ^{d} )</th>
<th>exptl ( m/z ) (ESI)( ^{e} )</th>
<th>exptl mass( ^{d} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nar\textsuperscript{1}(X = 2Fur-G)( ^{a} )</td>
<td>( C_{118}H_{149}N_{42}O_{72}P_{11} )</td>
<td>–</td>
<td>3647.6</td>
<td>([M - 5H + Na]^{4+} = 916.5)</td>
<td>3648.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 6H + Na]^{5+} = 932.9)</td>
<td>3647.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 6H]^{6+} = 607.0)</td>
<td>3648.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H]^{7+} = 520.2)</td>
<td>3648.4</td>
</tr>
<tr>
<td>Nar\textsuperscript{1}(X = Ind\textsuperscript{G})( ^{b} )</td>
<td>( C_{122}H_{152}N_{43}O_{71}P_{11} )</td>
<td>17</td>
<td>3696.7</td>
<td>([M - 4H]^{+} = 923.1)</td>
<td>3696.4</td>
</tr>
<tr>
<td>Nar\textsuperscript{1}(X = Bfur\textsuperscript{G})( ^{b} )</td>
<td>( C_{122}H_{151}N_{42}O_{72}P_{11} )</td>
<td>21</td>
<td>3697.7</td>
<td>([M - 6H + 2Na]^{4+} = 934.5)</td>
<td>3698.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 6H + Na]^{5+} = 743.0)</td>
<td>3698.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H + Na]^{6+} = 619.0)</td>
<td>3698.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H]^{7+} = 527.4)</td>
<td>3698.8</td>
</tr>
<tr>
<td>Nar\textsuperscript{1}(X = Bth\textsuperscript{G})( ^{b} )</td>
<td>( C_{122}H_{151}N_{42}O_{71}P_{11}S )</td>
<td>10</td>
<td>3713.6</td>
<td>([M - 9H + 5Na]^{4+} = 954.9)</td>
<td>3713.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 9H + 4Na]^{5+} = 759.4)</td>
<td>3713.6</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>([M - 10H + 4Na]^{6+} = 632.8)</td>
<td>3714.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 10H + 3Na]^{7+} = 539.0)</td>
<td>3714.8</td>
</tr>
<tr>
<td>Nar\textsuperscript{1}(X = QG)( ^{a} )</td>
<td>( C_{123}H_{152}N_{43}O_{71}P_{11} )</td>
<td>–</td>
<td>3708.7</td>
<td>([M - 7H + 3Na]^{4+} = 942.7)</td>
<td>3708.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H + 2Na]^{5+} = 749.5)</td>
<td>3708.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H + Na]^{6+} = 620.8)</td>
<td>3708.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 9H + 2Na]^{7+} = 535.1)</td>
<td>3708.7</td>
</tr>
<tr>
<td>Nar\textsuperscript{1}(X = QG)( ^{a} )</td>
<td>( C_{123}H_{152}N_{43}O_{71}P_{11} )</td>
<td>–</td>
<td>3708.7</td>
<td>([M - 4H]^{+} = 926.3)</td>
<td>3709.2</td>
</tr>
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<td></td>
<td></td>
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<td></td>
<td>([M - 5H]^{+} = 740.7)</td>
<td>3708.5</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 6H]^{+} = 617.1)</td>
<td>3708.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>([M - 7H]^{+} = 528.9)</td>
<td>3709.3</td>
</tr>
</tbody>
</table>

\( ^{a} \) Synthesized on solid-phase using standard phosphoramidite chemistry. 
\( ^{b} \) Synthesized by Suzuki-Miyaura coupling reactions with 8-Br-G-modified oligonucleotides. 
\( ^{c} \) Yield derived from integration of the HPLC trace. Yields of the modified Nar\textsuperscript{1} oligonucleotides synthesized by the phosphoramidite approach were not determined. 
\( ^{d} \) Monoisotopic mass of most abundant isotopologue; assumes one \(^{13}\)C isotope. 
\( ^{e} \) Measured \( m/z \) from mass spectrum.
4.3.2 Features of Circular Dichroism Spectra of C\(^8\)-Heteroaryl-G Modified NarI Oligonucleotides

Circular dichroism (CD) spectra of C\(^8\)-heteroaryl-G modified NarI\(^3\) oligonucleotides, with X = \(^{2}\text{Fur}\)G, \(^{3}\text{Bfur}\)G or \(^{4}\text{Q}\)G, hybridized to the complementary strand NarI(C) or NarI(G), were obtained and shown in Figures 4-5 to 4-7. CD spectra for the unmodified NarI oligonucleotide hybridized to complementary strands NarI(C) or NarI(G) were also obtained for comparison and are shown in Appendix D.

**Figure 4-5.** CD spectra of the C\(^8\)-heteroaryl-G modified oligonucleotide NarI\(^3\)(X = \(^{2}\text{Fur}\)G) hybridized to its complementary strand (a) NarI(C) or (b) NarI(G). Spectra were recorded at 10 °C in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI\(^3\) and its complementary strand.
Figure 4-6. CD spectra of the $C^8$-benzoheteroary-G modified oligonucleotide $NarI^3(X = BfuG)$ hybridized to its complementary strand (a) $NarI'(C)$ or (b) $NarI'(G)$. Spectra were recorded at 10 °C in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $NarI^3$ and its complementary strand.

Figure 4-7. CD spectra of the $C^8$-benzoheteroary-G modified oligonucleotide $NarI^3(X = QG)$ hybridized to its complementary strand (a) $NarI'(C)$ or (b) $NarI'(G)$. Spectra were recorded at 10 °C in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $NarI^3$ and its complementary strand.

For all duplexes with $C^8$-heteroary-G modified $NarI^3$ hybridized to $NarI'(C)$, the CD spectra displayed characteristics of normal B-form DNA with roughly equal positive (~ 275 nm) and negative (~ 240 nm) bands, and a crossover at ~ 260 nm. The same CD spectral features were also observed for $C^8$-heteroary-G modified $NarI$ hybridized to $NarI'(G)$. The spectra for modified $NarI^3$ hybridized to either $NarI'(C)$ or $NarI'(G)$ did
not exhibit resolved molar ellipticities (Θ) for an induced CD (ICD) signal for the C₈-furyl, benzofuryl or quinolyl moiety. For related N-linked C₈-dG adducts, ICD has been applied as a diagnostic tool for probing syn versus anti conformation, with a positive ICD signal indicative of an S- or W-type duplex conformation with the adduct residing in the chiral environment in the syn conformation. The Manderville group has previously observed a strong positive ICD signal at ~ 320 nm for the C₈-benzothienyl moiety incorporated in a decanucleotide (10-mer), hybridized to its complementary strand with the nucleotide G opposite the adduct. Conversely, the CD spectrum of the same 10-mer hybridized to its complementary strand with the nucleotide C opposite the adduct did not exhibit an ICD signal for the C₈-benzothienyl moiety, suggesting it did not reside in the chiral environment of the DNA helix. It is important to note that the absence of an ICD signal is not necessarily conclusive evidence for anti versus syn adduct preference; as the sensitivity of CD is low, it is possible a resolved ICD band was unable to be detected. As ICD bands were not evident in Figures 4-5 to 4-7, CD cannot be used as a marker for conformation of C₈-heteroaryl-G adducts. Therefore, other methods of analysis must be applied in order to confirm the glycosidic bond conformation of the adduct.

Finally, CD spectra of C₈-heteroaryl-G modified Nar₁³ oligonucleotides, with X = ²FurG, BfurG or QG, hybridized to the complementary strand Nar¹'(10-mer) were obtained and shown in Figure 4-8.
Figure 4-8. CD spectra of the $C^8$-heteroaryl-G modified oligonucleotides (a) $Nar\overline{I}^3(X = 2\text{Fur}\overline{G})$ and (b) $Nar\overline{I}(X = \text{Bfur}\overline{G})$, hybridized to the complementary strand $Nar\overline{I}$(10-mer). Spectra were recorded at 10 °C in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $Nar\overline{I}^3$ and its complementary strand.

Because the $Nar\overline{I}$ recognition sequence is a hotspot for −2 frameshift mutation, $C^8$-heteroaryl-G modified $Nar\overline{I}^3$ oligonucleotides were hybridized to a 10-mer complement in order to simulate the formation of the so-called slippage product, in which the CG nucleotides opposite the adduct were deleted, thus forming a two-nucleotide bulge that involves the $C^8$-heteroaryl-G derivative. For both modified duplex oligonucleotides, the CD spectra displayed characteristics of normal B-form DNA with roughly equal positive (≈ 275 nm) and negative (≈ 240 nm) bands, and a crossover at ≈ 260 nm. Again, no ICD signals were observed for the $C^8$-furyl or benzofuryl moiety, and thus CD was unable to be applied in order to definitively distinguish the conformational preference of the adduct within this 12-mer:10-mer duplex.
4.3.3 Thermal Stability of C⁸-Heteroaryl-G Modified NarI Oligonucleotides

(a) Thermal Stability Upon Hybridization to NarI’(C) and NarI’(G)

The C⁸-heteroaryl-G modified oligonucleotides NarI³(X = ²FurG), NarI³(X = IndG), NarI³(X = BfurG) and NarI³(X = BthG) were hybridized to the complementary strand NarI’(C) or NarI’(G), and the effect of the adduct on the thermal stability of the DNA duplex was measured. Melting temperatures ($T_m$'s) were determined by UV-Vis spectroscopy by monitoring absorbance at 260 nm as a function of temperature. Melting curves are shown in Figures 4-9 to 4-12, and results summarized in Table 4-2.

![Figure 4-9. Thermal melting spectra, of absorbance versus temperature, of the C⁸-heteroaryl-G modified oligonucleotide NarI³(X = ²FurG), hybridized to its complementary strand (a) NarI’(C) or (b) NarI’(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI³ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.](image)
Figure 4-10. Thermal melting spectra, of absorbance versus temperature, of the C₈-benzoheteroaryl-G modified oligonucleotide NarI³(X = \textsuperscript{Ind}G), hybridized to its complementary strand (a) NarI(C) or (b) NarI(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI³ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.

Figure 4-11. Thermal melting spectra of, absorbance versus temperature, of the C₈-benzoheteroaryl-G modified oligonucleotide NarI³(X = \textsuperscript{Bfur}G), hybridized to its complementary strand (a) NarI(C) or (b) NarI(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI³ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.
Figure 4-12. Thermal melting spectra of, absorbance versus temperature, of the C8-benzoheteroaryl-G modified oligonucleotide NarI3(X = BthG), hybridized to its complementary strand (a) NarI(C) or (b) NarI(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI3 and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.

Table 4-2. Tm values of C8-heteroaryl-G modified NarI3 oligonucleotides hybridized to NarI(C) or NarI(G).

<table>
<thead>
<tr>
<th>duplex</th>
<th>Tm (°C)ᵃ</th>
<th>ΔTm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NarI(X = G):NarI(C)</td>
<td>60.5 ± 1.3</td>
<td>–</td>
</tr>
<tr>
<td>NarI(X = G):NarI(G)</td>
<td>49.6 ± 1.0</td>
<td>–</td>
</tr>
<tr>
<td>NarI3(X = 2FurG):NarI(C)</td>
<td>51.0 ± 0.9</td>
<td>–9.5</td>
</tr>
<tr>
<td>NarI3(X = 2FurG):NarI(G)</td>
<td>56.8 ± 1.6</td>
<td>+7.2</td>
</tr>
<tr>
<td>NarI3(X = IndG):NarI(C)</td>
<td>50.4 ± 2.6</td>
<td>–10.1</td>
</tr>
<tr>
<td>NarI3(X = IndG):NarI(G)</td>
<td>55.3 ± 0.4</td>
<td>+5.7</td>
</tr>
<tr>
<td>NarI3(X = BfurG):NarI(C)</td>
<td>48.7 ± 0.5</td>
<td>–11.8</td>
</tr>
<tr>
<td>NarI3(X = BfurG):NarI(G)</td>
<td>57.5 ± 1.0</td>
<td>+7.9</td>
</tr>
<tr>
<td>NarI3(X = BthG):NarI(C)</td>
<td>54.5 ± 1.0</td>
<td>–6.0</td>
</tr>
<tr>
<td>NarI3(X = BthG):NarI(G)</td>
<td>55.2 ± 0.9</td>
<td>+5.6</td>
</tr>
</tbody>
</table>

ᵃ Thermal melting spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI or NarI3 and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹.

In each instance, when the adduct was paired with its normal pyrimidine partner C, the duplex was considerably destabilized, in a range from –6.0 to –11.8 °C, compared to the unmodified duplex. These probes are clearly perturbing and therefore are not good
emissive models for unmodified dG. The $T_m$ result for $Nar^3(X = ^2\text{Fur} \text{G})$ was particularly surprising, as the small $C^8$-furyl modification was not expected to have such a destabilizing influence on overall duplex stability ($\Delta T_m = -9.5 \degree C$). These findings, however, are in agreement with previous studies showing that $C$-linked $C^8$-aryl-G ($\Delta T_m = -6$ to $-17 \degree C$), $C^8$-pyrenyl-G ($\Delta T_m \sim -10 \degree C$) and $N$-linked $C^8$-arylamine-G ($\Delta T_m = -7$ to $-18 \degree C$) lesions significantly decrease duplex stability when paired to C. Furthermore, $C^8$-benzothienyl-G modified 10-mers, hybridized to the complement with C opposite the adduct, were also previously found to destabilize the duplex by $\sim 10 \degree C$. This therefore suggests that the $C^8$-heteroaryl-dG adducts are reasonable fluorescent models of bulky adducts generated by chemical mutants.

Recall that for $N$-linked $C^8$-dG adducts base-paired with C, an equilibrium exists between the B- and S-type conformational motifs, with an exchange time in the millisecond range. Both of these conformations are destabilizing. Because duplex destabilization was observed for double helical structures in which $C^8$-heteroaryl-dG adducts were paired with their natural pyrimidine partner, it can be postulated that these adducts similarly exist in a B- or S-type duplex structural motif, with an anti or syn conformation preference, respectively.

In a B-type motif, it is expected that the $C^8$-heteroaryl-dG adducts $^2\text{Fur} \text{dG}$, $^\text{Ind} \text{dG}$, $^\text{Bfur} \text{dG}$ and $^\text{Bth} \text{dG}$ adopt the anti conformation within $Nar^3$ upon base-pairing to their natural pyrimidine partner, placing the $C^8$-heteroaryl moiety in the major groove. Recall that the anti conformation is not energetically favoured for $C^8$-aryl-dG adducts, and thus in turn, adoption of this conformation could be a contributing factor to the destabilization of $C^8$-aryl-dG modified duplexes. Furthermore, the location of the $C^8$-heteroaryl moiety
in the major groove results in exposure of the lipophilic C^8-furyl, indolyl, benzofuryl or benzothienyl moiety to the aqueous extrahelical environment, and subsequently, duplex destabilization and a decrease in T\textsubscript{m}, as compared to the unmodified duplex. The degree of duplex destabilization can be correlated to the relative polarity of the adducts.

The ground state dipole moments (\(\mu_g\)) of the C^8-heteroaryl-dG adducts, as determined by DFT calculations, were previously noted in Chapter 3, section 3.3.2. The Bth-dG adduct was found to have the largest ground state dipole moment of the nucleoside adducts, implying that the C^8-benzothienyl moiety would be easier to stabilize in a polar environment as compared to the other C^8-heteroaryl substituents, as supported by \(\Delta T\textsubscript{m}\) values shown in Table 4-2. The NarI(X = BthG):NarI(C) duplex has the smallest \(\Delta T\textsubscript{m}\) of \(-6\) °C, as compared to the unmodified duplex, while C^8-furyl, indolyl and benzofuryl modified duplexes show decreases of \(9.5\) °C, \(10.1\) °C and \(11.8\) °C, respectively, as compared to the unmodified duplex. Additionally, steric clash between the major groove located C^8-heteroaryl substituent, and phosphate backbone and sugar moieties, may be a factor in duplex destabilization.\(^5\)

By contrast, duplex destabilization could be the result of syn conformer adaptation of the C^8-heteroaryl-dG adduct in an S-type duplex motif, when base-paired with C. In this structure, the C^8-heteroaryl moiety is intercalated between its flanking nucleobases, in turn flipping the opposing pyrimidine out of the helix. The resulting helical distortion and loss of W-C H-bonding would account for the decreased duplex stability, and decrease in \(T\textsubscript{m}\), compared to the unmodified duplex.\(^{29,50}\)

The observed B/S-type heterogeneity in bulky N-linked C^8-arylamine-dG adducts base-paired with C is known to be sequence-dependent, with the flanking bases of the
adduct influencing the preferred conformation.\textsuperscript{33} For the AF-dG adduct, % S-type values were obtained by the Cho group for a central trimer portion of a 12-mer duplex using \textsuperscript{19}F NMR, in which the modified base (X = AF-dG) was flanked by purines, pyrimidines or one of each type, in differing sequences.\textsuperscript{69} The Manderville group also recently demonstrated the sequence dependency of B- versus S-type in 10-mer \textsuperscript{Bth}dG-modified duplexes. In a duplex with the adduct flanked on the 5'-side by T and on the 3'-side by C, \textsuperscript{Bth}dG was determined to predominantly adopt a \textit{syn} conformation and S-type structure. On the other hand, in a duplex with the adduct flanked on the 5'-side by G and on the 3'-side by A, \textsuperscript{Bth}dG was determined to adopt an \textit{anti} conformation and B-type structure.\textsuperscript{29} These conformations were confirmed by both experimental CD and MD analysis, and were also supported by the % S-type data from Cho and co-workers. Of particular interest to the study presented here is the % S-type value determined by the Cho group for a 5'-CXC trimer, as the \textit{C}\textsuperscript{8}-heteroaryl-dG adduct is also flanked on both the 5' and 3' sides by C in the \textit{NarI} sequence. The 5'-CXC trimer was calculated to exist as 47 % S-type conformer,\textsuperscript{69} suggesting that the \textit{anti} conformation, with B-type structure, is slightly more preferable for \textit{NarI}\textsuperscript{3} incorporated \textit{C}\textsuperscript{8}-heteroaryl-dG adducts base-paired with C.

Interestingly, when the modified \textit{NarI}\textsuperscript{3} oligonucleotides were hybridized to the \textit{NarI}(G) complement, thereby introducing a mismatch, the \textit{C}\textsuperscript{8}-heteroaryl-dG adduct was observed to considerably increase duplex stability, in the range of +5.6 to +7.2 °C, compared to that of the unmodified duplex.\textsuperscript{54} This stabilization effect was previously observed to occur with both phenolic\textsuperscript{27} and benzothienyl\textsuperscript{29} \textit{C}\textsuperscript{8}-dG modified 10-mers, hybridized to the complement with G opposing the adduct, with observed increases in \(T_m\) of \(\sim +10\) °C.
These C-linked phenolic\textsuperscript{27} and benzothienyl\textsuperscript{29} C\textsuperscript{8}-dG modified duplexes, in addition to N-linked C\textsuperscript{8}-arylamine-dG modified duplexes,\textsuperscript{49} are known to favour a W-type motif, with the adduct in the \textit{syn}-conformation, when the adduct is mismatched with a purine. With duplex stabilization observed for double helices in which C\textsuperscript{8}-heteroaryl-dG adducts were mismatched to G, it can be postulated that these adducts similarly exist as the \textit{syn}-conformer, with a W-type duplex structural preference.\textsuperscript{54} Recall that the \textit{syn} conformation is energetically favoured for C\textsuperscript{8}-aryl-dG adducts, and thus in turn, adoption of this conformation could be a contributing factor to the stabilization of C\textsuperscript{8}-aryl-dG modified duplexes. Furthermore, in this conformation, the lipophilic C\textsuperscript{8}-heteroaryl moiety is located in the minor groove in a non-polar environment, and is thus favourably protected from the aqueous environment surrounding the duplex. The degree of duplex stabilization can be correlated to the relative polarity of the adducts. The adducts with the lowest ground state dipole moments, 2Fur\textsubscript{dG} and Bfur\textsubscript{dG}, have the highest $\Delta T_m$ values of +7.2 °C and +7.9 °C, respectively, upon mismatch formation, as the less polar adducts would prefer the non-polar interior of the helix, while Ind\textsubscript{G} and Bth\textsubscript{dG}, with higher dipole moments, have $\Delta T_m$ values of +5.7 and +5.6 °C, respectively.\textsuperscript{54} Duplex stabilization also comes as a result of an increase in $\pi$-stacking due to interaction of the modified base with its flanking bases, and increase in H-bonding stability as a result of additional interactions present in the Hoogsteen base-pairing between the C\textsuperscript{8}-substituent and its complementary nucleobase.\textsuperscript{27,29}

The C\textsuperscript{8}-heteroaryl-G modified oligonucleotides Nar1\textsubscript{1}(X = ^{0}\text{G}) and Nar1\textsubscript{3}(X = ^{0}\text{G}) were hybridized to the complementary strand Nar1\textsubscript{1}(C) or the appropriate Nar1\textsubscript{1}(G), and
the effect of the adduct on the thermal stability of the DNA duplex was measured.

Melting curves are shown in Figures 4-13 and 4-14, and results are summarized in Table 4-3.

**Figure 4-13.** Thermal melting spectra, of absorbance versus temperature, of the C^8^-benzoheteroaryl-G modified oligonucleotide NarI^1(X = QG), hybridized to its complementary strand (a) NarI'(C) or (b) NarI'(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI^1 and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.

**Figure 4-14.** Thermal melting spectra, of absorbance versus temperature, of the C^8^-benzoheteroaryl-G modified oligonucleotide NarI^3(X = QG), hybridized to its complementary strand (a) NarI'(C) or (b) NarI'(G). Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI^3 and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min⁻¹. The corresponding unmodified duplex is shown as a dashed line.
Table 4-3. $T_m$ values of $C^8$-quinolyl-G modified $NarI^1$ and $NarI^3$ oligonucleotides hybridized to their respective complementary strands.

<table>
<thead>
<tr>
<th>duplex</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta T_m$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$NarI(X = G):NarI'(C)$</td>
<td>60.5 ± 1.3</td>
<td>−</td>
</tr>
<tr>
<td>$NarI(X = G):NarI'(G)^a$</td>
<td>47.7 ± 1.5</td>
<td>−</td>
</tr>
<tr>
<td>$NarI(X = G):NarI'(C)^b$</td>
<td>49.6 ± 1.0</td>
<td>−</td>
</tr>
<tr>
<td>$NarI(X = Q^G):NarI'(C)$</td>
<td>32.5 ± 0.7</td>
<td>−28</td>
</tr>
<tr>
<td>$NarI^1(X = Q^G):NarI'(G)$</td>
<td>45.0 ± 0.2</td>
<td>−2.7</td>
</tr>
<tr>
<td>$NarI^3(X = Q^G):NarI'(C)$</td>
<td>45.5 ± 0.7</td>
<td>−15</td>
</tr>
<tr>
<td>$NarI^3(X = Q^G):NarI'(G)$</td>
<td>43.9 ± 0.2</td>
<td>−5.7</td>
</tr>
</tbody>
</table>

$^a$ Complement to $NarI^1$.  $^b$ Complement to $NarI^3$.  $^c$ Thermal melting spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $NarI$, $NarI^1$, $NarI^3$ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 $^\circ$C min$^{-1}$.

In each instance, when the $Q^dG$ adduct was paired with its normal pyrimidine partner C, the duplex was substantially destabilized, with the $NarI^1(X = Q^G):NarI'(C)$ duplex showing a decrease in $T_m$ of −28 $^\circ$C and the $NarI^3(X = Q^G):NarI'(C)$ duplex showing a decrease of −15 $^\circ$C, compared to the unmodified duplex. It is expected that $Q^dG$ would adopt the anti conformation with a B-type structural motif, with the $C^8$-quinolyl moiety located in the major groove, as predicted for the structurally related $2Fur^dG$, $Ind^dG$, $Bfur^dG$ and $Bth^dG$ adducts paired with C. Destabilization of the $C^8$-quinolyl modified duplexes upon base-pairing to C can therefore be similarly attributed to exposure to the lipophilic $C^8$-quinolyl to the polar extrahelical environment. It should be noted that $C^8$-quinolyl modification results in a marked increase in duplex destabilization as compared to the destabilization caused by $2Fur^dG$, $Ind^dG$, $Bfur^dG$ and $Bth^dG$. The larger lipophilic ring size of the $C^8$-quinolyl substituent, as compared to the other heteroaryl ring sizes, would make solvation of the quinoline ring in the aqueous extrahelical environment more difficult.
The Nar\(1\)(X = \(^\text{O}\)G):Nar\(1\)'(C) duplex decreases the stability of the duplex by an additional −13 °C, as compared with the destabilization induced by the Nar\(3\)(X = \(^\text{O}\)G):Nar\(1\)'(C). Recall that the difference in the modified Nar\(1\) versus Nar\(3\) oligonucleotide is the site of adduction, with \(^\text{O}\)dG located at the fourth nucleobase from the 5'-end in Nar\(1\), and the adduct located at the seventh, and more central position, from the 5'-end in Nar\(3\). Modification at the ‘end’ of the oligonucleotide versus the ‘middle’ of the oligonucleotide results in enhanced duplex distortion, and subsequently enhanced instability, as this promotes fraying at the end of duplex,\(^70\) and thus this result was not unexpected.

In each instance, when the \(^\text{O}\)dG adduct was mismatched with G, the duplex was observed to slightly destabilize, with the Nar\(1\)(X = \(^\text{O}\)G):Nar\(1\)'(G) duplex showing a decrease in \(T_m\) of −2.7 °C, and the Nar\(3\)(X = \(^\text{O}\)G):Nar\(1\)'(G) duplex showing a decrease of −5.7 °C, compared to the unmodified duplex. These results differ from melting analysis performed for Nar\(3\) oligonucleotides modified by \(2\text{Fur}dG\), \(\text{Ind}dG\), \(\text{Bfur}dG\) or \(\text{Bth}dG\), in which \(T_m\) values were found to instead increase upon mismatch formation. It is still expected that \(^\text{O}\)dG would adopt the \(\text{syn}\) conformation with a \(\text{W}\)-type structural motif, with the \(\text{C}^8\)-quinolyl moiety located in the major groove, as this is the predicted orientation for the structurally related \(2\text{Fur}dG\), \(\text{Ind}dG\), \(\text{Bfur}dG\) and \(\text{Bth}dG\) adducts paired with G, and also the structural arrangement confirmed for \(\text{C}\)-linked phenolic\(^{27}\) and benzothienyl\(^{29}\) \(\text{C}^8\)-dG modified duplexes, in addition to \(\text{N}\)-linked \(\text{C}^8\)-arylamine-dG modified duplexes,\(^{49}\) with purine mismatches.

Recall that in this mismatch formation, the \(\text{C}^8\)-substituent is located in the minor groove, and typically, in this arrangement, \(\pi\)-stacking interactions with neighbouring
nucleobases are enhanced, with base-pairing stability also significantly augmented as a result of additional interactions with the \( C^8 \)-substituent in H-bonding of the Hoogsteen face of the G component of the \( C^8 \)-modified base with the W-C face of the opposing G.\(^{27,29} \) Due to its large aryl ring size, it can be conjectured that while the \( C^8 \)-quinolyl moiety may still be located in the minor groove, it is not as ideally located to enable enhanced stability, and as a result additional \( \pi \)-stacking and base-pairing interactions may be limited.

(b) Thermal Stability Upon Hybridization to Narl'(10-mer)

Finally, the \( C^8 \)-heteroaryl-G modified oligonucleotides Narl(\( X = 2\text{Fur} \)G), Narl(\( X = B\text{fur} \)G) and Narl(\( X = Q \)G) were hybridized to the complementary strand Narl'(10-mer) and the effect of the adduct on the thermal stability of the DNA duplex was measured. Melting curves are shown in Figure 4-15, and results are summarized in Table 4-4.
Figure 4-15. Thermal melting spectra, of absorbance versus temperature, of $C^8$-heteroaryl-G modified oligonucleotides hybridized to the complementary strand NarI'(10-mer), with (a) NarI$^3(X = \text{2Fur G})$, (b) NarI$^3(X = \text{Bfur G})$ or (c) NarI$^3(X = \text{Q G})$. Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI$^3$ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min$^{-1}$. The corresponding unmodified duplex is shown as a dashed line.
Table 4-4. $T_m$ values of $C^8$-heteroaryl-G modified NarI$^3$ oligonucleotides hybridized to NarI'(10-mer).

<table>
<thead>
<tr>
<th>duplex</th>
<th>$T_m$ (°C)$^a$</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NarI(X = G):NarI'(10-mer)</td>
<td>29.5 ± 1.5</td>
<td>–</td>
</tr>
<tr>
<td>NarI$^3$(X = 2FurG):NarI'(10-mer)</td>
<td>28.6 ± 0.5</td>
<td>−0.9</td>
</tr>
<tr>
<td>NarI$^3$(X = BfurG):NarI'(10-mer)</td>
<td>37.5 ± 0.8</td>
<td>+8.0</td>
</tr>
<tr>
<td>NarI$^3$(X = QG):NarI'(10-mer)</td>
<td>37.4 ± 0.8</td>
<td>+7.9</td>
</tr>
</tbody>
</table>

$^a$ Thermal melting spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI or NarI$^3$ and its complementary strand. Absorbance was monitored at 260 nm, with a heating rate of 1 °C min$^{-1}$.

As was previously outlined in section 4.3.2 above $C^8$-heteroaryl-G modified NarI$^3$ oligonucleotides were hybridized to a 10-mer complement in order to simulate a −2 frameshift, in which the CG nucleotides opposite the adduct were deleted, thus forming a two-nucleotide bulge$^{50}$ that involves the $C^8$-heteroaryl-G derivative. Upon hybridization of the modified oligonucleotide NarI$^3$(X = 2FurG) to the truncated strand, a very slight, almost negligible, duplex destabilization, as compared to the unmodified duplex, was observed. Interestingly, upon hybridization of the modified oligonucleotide NarI$^3$(X = BfurG) or NarI$^3$(X = QG) to the truncated strand, a very sizable increase in duplex stabilization was noted, as compared to the unmodified duplex, with $\Delta T_m$ values of +8.0 and +7.9 °C recorded, respectively.

Slipped-mutagenic duplex intermediates have previously been measured for stability. The Rizzo group reported that hybridization of an IQ-dG adducted NarI oligonucleotide to the complementary 10-mer resulted in an increased $T_m$ of +10 °C, as compared to the unmodified duplex.$^{26}$ It should be noted that adduction by IQ was at the same G$_3$ position in the NarI sequence as modification by the $C^8$-heteroaryl-dG adducts discussed presently. In addition, the Lefèvre group incorporated the AAF-dG adduct into a central site within a GC-rich 12-mer, and found that upon hybridization to a
complementary 10-mer, the duplex was stabilized, with an increase in $T_m$ of $+15\,^\circ\text{C}$.\textsuperscript{36}

This data correlates positively with the stabilization observed for hybridization of $\text{Bfur}^\text{dG}$ and $\text{Q}^\text{dG}$ adducted $\text{NarI}$ with the 10-mer complement. These findings appear to suggest that a larger aryl ring size is required to stabilize the bulge formed by duplex slippage.

The $\text{Bfur}^\text{dG}$ and $\text{Q}^\text{dG}$ adducts stabilized the modified $\text{NarI}^3:\text{NarI}'(10\text{-mer})$ duplex by approximately the same degree in $\Delta T_m$ as found for modified $\text{NarI}^3:\text{NarI}'(G)$ duplex stabilization by $\text{2Fur}^\text{dG}$, $\text{Ind}^\text{dG}$, $\text{Bfur}^\text{dG}$ and $\text{Bth}^\text{dG}$ adducts, suggesting that the $\text{Bfur}^\text{dG}$ and $\text{Q}^\text{dG}$ adducts also reside in a syn conformation with a W-type structural motif upon incorporation within the modified $\text{NarI}^3:\text{NarI}'(10\text{-mer})$ duplex. The bulge in the duplex created by the deleted nucleotides in the 10-mer complement provides ample space for intercalation of the $\text{C}^8$-heteroaryl moiety into the minor groove. It is thought that $\text{C}^8$-furyl ring does not significantly impact duplex stabilization as the small ring would not be able to strongly participate in $\pi$-stacking with the flanking nucleobases. The large biaryl ring size of the $\text{C}^8$-benzofuryl and quinolyl moieties however, would be favourable for interaction with the $\pi$-stack, thereby increasing overall duplex stability. It is important to call attention to the fact that while the $\text{Q}^\text{dG}$ adduct was found to destabilize the helix upon mismatch formation, as hypothesized by its large ring size, here, its large size is advantageous, with the bulge in the slippage product allowing room for $\text{Q}^\text{dG}$ intercalation and strong $\pi$-stacking interactions.
4.3.4 Photophysical Properties of C₈-Heteroaryl-G Modified NarI Oligonucleotides

(a) Emission and Excitation Spectra of C₈-Heteroaryl-G Modified NarI Oligonucleotides

In order to definitively classify these C₈-heteroaryl-dG adducts as probes in the NarI recognition sequence, their photophysical properties upon oligonucleotide incorporation needed to be examined. Emission and excitation spectra of the ²Fur dG, ïnd dG, Bfur dG and Bth dG adducted NarI₃ oligonucleotides in the single-strand state, and upon hybridization to the complementary strands NarI'(C) and NarI'(G) were obtained, and are shown in Figure 4-16, with tabulation of photophysical parameters in Table 4-5.54 Furthermore, temperature dependent emission and excitation spectra were obtained for the oligonucleotides and duplexes and are shown in Figure 4-17. Fluorescence spectra of the free C₈-heteroaryl-dG modified nucleosides, for comparison, can be reviewed in Figures 3-10 and 3-11.
Figure 4-16. Excitation and emission spectra of \( C^8\)-heteroaryl-G modified oligonucleotides, in the single-strand state (solid line), or hybridized to its complementary strand, \( NarI(C)\) (dashed line) or \( NarI(G)\) (dotted line), with (a) \( NarI^3(X = 2\text{fur}G)\), (b) \( NarI^3(X = \text{Ind}G)\), (c) \( NarI^3(X = \text{Bfur}G)\) or (d) \( NarI^3(X = \text{Bth}G)\). All spectra of single-strand oligonucleotides (1.25 \( \mu \)M) and duplexes (equivalent amounts (1.25 \( \mu \)M) of \( NarI^3\) and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl at 10 °C.
Table 4-5. Photophysical parameters of $C^8$-heteroaryl-G modified $NarI^3$ oligonucleotides in the single-strand state, or hybridized to $NarI'(C)$ or $NarI'(G)$.

<table>
<thead>
<tr>
<th>oligonucleotide or duplex</th>
<th>$\lambda_{ex}$(nm)$^a$</th>
<th>$\Delta\lambda_{ex}$(nm)$^b$</th>
<th>$\lambda_{em}$(nm)$^c$ ($I_{rel}$)$^c$</th>
<th>$\Delta\lambda_{em}$(nm)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$NarI^3(X = 2Fur\ G)$</td>
<td>306</td>
<td>+2</td>
<td>386 (0.045)</td>
<td>+2</td>
</tr>
<tr>
<td>$NarI^3(X = 2Fur\ G):NarI'(C)$</td>
<td>305</td>
<td>−1</td>
<td>385 (0.63)</td>
<td>−1</td>
</tr>
<tr>
<td>$NarI^3(X = 2Fur\ G):NarI'(G)$</td>
<td>306</td>
<td>−</td>
<td>387 (0.18)</td>
<td>+1</td>
</tr>
<tr>
<td>$NarI^3(X = Ind\ G)$</td>
<td>308</td>
<td>−15</td>
<td>389 (0.057)</td>
<td>−1</td>
</tr>
<tr>
<td>$NarI^3(X = Ind\ G):NarI'(C)$</td>
<td>308</td>
<td>−</td>
<td>391 (1.0)</td>
<td>+2</td>
</tr>
<tr>
<td>$NarI^3(X = Ind\ G):NarI'(G)$</td>
<td>307</td>
<td>−1</td>
<td>387 (0.17)</td>
<td>−2</td>
</tr>
<tr>
<td>$NarI^3(X = Bfur\ G)$</td>
<td>320</td>
<td>−3</td>
<td>403 (0.060)</td>
<td>−</td>
</tr>
<tr>
<td>$NarI^3(X = Bfur\ G):NarI'(C)$</td>
<td>335</td>
<td>+15</td>
<td>411 (0.67)</td>
<td>+8</td>
</tr>
<tr>
<td>$NarI^3(X = Bfur\ G):NarI'(G)$</td>
<td>325</td>
<td>+5</td>
<td>395 (0.62)</td>
<td>−8</td>
</tr>
<tr>
<td>$NarI^3(X = Bth\ G)$</td>
<td>317</td>
<td>−</td>
<td>413 (0.062)</td>
<td>−4</td>
</tr>
<tr>
<td>$NarI^3(X = Bth\ G):NarI'(C)$</td>
<td>328</td>
<td>+11</td>
<td>419 (1.4)</td>
<td>+6</td>
</tr>
<tr>
<td>$NarI^3(X = Bth\ G):NarI'(G)$</td>
<td>319</td>
<td>+2</td>
<td>408 (0.65)</td>
<td>−5</td>
</tr>
</tbody>
</table>

$^a$ All spectra of single-strand oligonucleotides (1.25 µM) and duplexes (equivalent amounts (1.25 µM) of $NarI^3$ and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl at 10 °C. $^b$ Change in excitation or emission maximum for single-strand $NarI^3$ is versus the free $C^8$-heteroaryl-dG adduct, while change for the duplex is versus single-strand $NarI^3$. $^c$ Relative emission intensity for single-strand $NarI^3$ is determined as $I_{single-strand}/I_{adduct}$. $I_{adduct}$ was determined at a concentration of 1.25 µM. Relative emission intensity for the duplex is determined as $I_{duplex}/I_{single-strand}$. All intensity values for determination of $I_{rel}$ were measured at the same wavelength.
Figure 4-17. Excitation and emission spectra of $C^8$-heteroaryl-G modified oligonucleotides in the (a) single-strand state, or hybridized to its complementary strand (b) NarI'(C) or (c) NarI'(G), with (i) NarI$^3$(X = $2\text{Fur}$G), (ii) NarI$^3$(X = $\text{Ind}$G), (iii) NarI$^3$(X = $\text{Bfur}$G) or (iv) NarI$^3$(X = $\text{Bth}$G). All spectra of single-strand oligonucleotides (1.25 µM) and duplexes (equivalent amounts (1.25 µM) of NarI$^3$ and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 0.1 M NaCl at 10 °C (solid line) and 80 °C (dashed line).
While still fluorescent, all modified \( \text{Narl}^3 \) oligonucleotides exhibited significantly quenched emission intensity compared to the free modified nucleosides, with \( I_{rel} \) values of 0.045, 0.057, 0.060 and 0.062 for \( \text{Narl}^3(X = 2\text{Fur}\text{G}) \), \( \text{Narl}^3(X = \text{Ind}\text{G}) \), \( \text{Narl}^3(X = 2\text{fur}\text{G}) \) and \( \text{Narl}^3(X = \text{Bth}\text{G}) \) respectively. This large decrease in fluorescence intensity upon oligonucleotide incorporation is similarly observed for many fluorescent nucleobase analogues, including 2AP, which recall, exhibits quenched fluorescence when incorporated into DNA. Several mechanisms have been proposed for fluorescence of 2AP, including base-stacking effects, photoinduced electron transfer and the presence of dark states. Recent theoretical calculations reveal two possible pathways that can lead to fluorescence quenching; the first pathway involves the conversion of the bright state \( (\pi,\pi^*) \) into a dark minimum \( (n,\pi^*) \) involving lone pair orbitals on 2AP, while the second pathway involves charge transfer (CT) between the bases, leading to radiationless decay. Barton and co-workers have also provided direct chemical evidence that charge transfer between photoexcited 2AP and DNA bases is in part responsible for quenching.

The wavelengths of emission for the modified oligonucleotides do not distinctly differ from those of the free adducts, with only very subtle shifts, or no change, in wavelength observed, and no discernible pattern in the shift present. The excitation spectra of the modified \( \text{Narl}^3 \) oligonucleotides did display other features distinct from the spectra of the free modified nucleosides. In all spectra, a new excitation band was present between ~ 270 and 280 nm. In particular, this new band was very clearly visible in spectra of \( \text{Narl}^3(X = \text{Ind}\text{G}) \) and \( \text{Narl}^3(X = 2\text{fur}\text{G}) \). This peak occurs at the red edge of DNA absorbance and can be ascribed to charge transfer from the natural DNA nucleobases to the adduct. Similar bands have been observed in the emission spectra of
2AP modified oligonucleotides, in the 260 – 270 nm region. The wavelengths of excitation for the modified oligonucleotides did not differ greatly from those of the free adducts, with one exception; the excitation of $\text{NarI}^3(X = \text{IndG})$ is considerably blue-shifted, by 15 nm, as compared to the $\text{InddG}$ adduct, suggesting a more twisted $\text{IndG}$ structure within $\text{NarI}^3$. This observed blue-shift may also be the direct result of the redox character of $C^8$-heteroaryl-dG adducts. As discussed previously in Chapter 3, section 3.3.4, a relationship exists between half-peak oxidation potentials and emission wavelength, where decrease in $E_{p/2}$, indicative of stronger electron-donating character, correlates with a shift in emission to shorter wavelengths. As can be reviewed in Table 3-18, of the $C^8$-heteroaryl-dG adducts incorporated into oligonucleotides, $\text{InddG}$ possesses the lowest oxidation potential, and subsequently does exhibit a blue-shifted emission compared to the other adducts, as is similarly observed in the emission spectrum of the $\text{NarI}^3(X = \text{IndG})$ compared to the other modified oligonucleotides. Within the oligonucleotide, the electron-donating character of $\text{InddG}$ would be even further enhanced due to electron transfer from the other DNA bases to the adduct, contributing to the blue-shift in emission.

When spectral measurements were obtained following heating of the modified $\text{NarI}^3$ strand from 10 to 80 °C, it was noted for all oligonucleotides that the temperature increase resulted in a decrease in emission and excitation intensity. This decrease in fluorescence intensity can be attributed to the loss in overall rigidity of the oligonucleotide structure. Molecules with a high degree of flexibility tend to have low fluorescence intensity due to a high collisional probability, while by contrast, more rigid structures have a lower collisional probability, and therefore higher fluorescent potential.
The oligonucleotide would be more rigid at lower temperatures, and in turn more flexible at higher temperatures, thereby accounting for the decrease in fluorescence intensity observed upon heating of the molecule. All other fluorescent characteristics previously apparent for the modified oligonucleotides remained intact following the rise in temperature.

The spectral changes upon hybridization are markedly different depending on the nature of the base opposing the adduct in the duplex. In particular, base-pairing differences were reflected in differences in fluorescence intensity; it is interesting to recall that Figures 3-28 to 3-31 illustrated that dimer formation of the C-heteroaryl-dG adduct with dC or rG elicited different emission intensity responses dependent upon the nature of the nucleobase complexed with the adduct.

For all modified NarI oligonucleotides upon hybridization to NarI'(C), an additional excitation band was observed at ~ 275 nm, as was observed in the excitation spectra of the single-strand oligonucleotides. Little change was observed in emission intensity, with $I_{rel}$ values close to ~ 1, as compared to the single-strand oligonucleotide. The wavelength of emission for NarI(X = IndG), NarI(X = BfurG) and NarI(X = BthG) was notably red-shifted upon base-pairing to C, by increments of 2, 8 and 6 nm, respectively, as compared to the single-strand oligonucleotides. Substantial red-shifts in wavelength were also observed in the excitation spectra of NarI(X = BfurG):NarI'(C) and NarI(X = BthG):NarI'(C), of 15 and 11 nm, respectively. Heating of the duplexes from 10 to 80 °C generally resulted in a decrease in fluorescence intensity. An obvious shift in wavelength parameters was also observed, most prominently for the NarI(X = BfurG):NarI'(C) and NarI(X = BthG):NarI'(C) duplexes. The spectra recorded at 80 °C
displays wavelength blue-shifts for both emission and excitation, as compared to spectra recorded at 10 °C. For example, emission of \( NarI^3(X = B\text{fur}G):NarI'(C) \) blue shifts by 8 nm, from 411 to 403 nm, and excitation blue-shifts by 13 nm from 335 to 322 nm, upon heating of the sample. As evident from the values listed in Table 4-5, these blue-shifted wavelengths match those of the single-strand oligonucleotide. Heating of the duplex induces denaturation, and therefore it is not a surprise that the spectral properties of the DNA molecule at 80 °C resemble those of the single-strand oligonucleotide. Negligible shifts in emission and excitation wavelengths were observed for the \( NarI^3(X = 2\text{Fur}G):NarI'(C) \) duplex, which may be indicative of a bulky \( C^8 \)-moiety as a requirement of notable changes in fluorescence wavelength parameters, such as those observed for the \( NarI^3 \) oligonucleotides with \( \text{Ind}dG, B\text{fur}dG \) or \( B\text{th}dG \) modifications.\(^5\)

Photophysical parameters obtained for the \( C^8\)-heteroaryl-dG modified \( NarI^3 \) oligonucleotides hybridized to the complement \( NarI'(C) \) point to an \textit{anti} preferred conformation for the adduct, with a B-type duplex structural motif. The \textit{anti}-conformer was assumed to be preferred due to the lack of significant difference observed in the fluorescent properties, including intensity, of the duplex compared to the corresponding single-strand oligonucleotide.\(^5\) This assertion is supported by previous research conducted by the Manderville group showing that the general spectral features of a \( B\text{th}dG \) modified 10-mer oligonucleotide remained unchanged upon hybridization to a complementary strand with C opposing the adduct.\(^29\) As already discussed, in this duplex, the \( B\text{th}dG \) adduct was determined to be in the \textit{anti} conformation, with supporting evidence from thermal melting, CD and MD analysis. In the \textit{anti} conformation, the \( C^8 \)-heteroaryl moiety would be located in the major groove and exposed to the polar,
aqueous environment surrounding the helical structure, while the syn-conformer is located in a non-polar environment in the minor groove. Recall from Figures 3-18 and 3-19, that C₈-heteroaryl-dG adducts are fluorescent in water, but typically quenched in the non-polar CHCl₃. It is therefore reasonable to suggest that in order for the adduct to fluoresce, it must be located in an aqueous-accessible environment, and thus in the anti conformation.

By contrast, hybridization to the NarI(G) complement, and thereby induction of mismatch formation, resulted in different changes to the emission and excitation properties. The energy transfer assigned excitation band previously viewed for the single-strand oligonucleotides and NarI(C) hybridized duplexes was not observed, or was relatively indiscernible, in NarI(G) hybridized duplexes. Furthermore, mismatch to the opposing G resulted in a decrease in emission intensity, as compared to the corresponding single-strand oligonucleotide with the largest decrease in intensity observed for the NarI³(²FurG):NarI'(G) and NarI³(IndG):NarI'(G) duplexes, with Iᵣₑᵢ = 0.18 and 0.17, respectively. It is important to recall that the majority of the quench, as compared to the free C₈-heteroaryl-dG adduct, occurs upon initial incorporation into the single-strand oligonucleotide, with a less severe additional quench upon annealing with pairing to G, suggesting that quenching originates mainly in base-stacking interactions rather than base-pairing. It is of interest to note the degree of emission intensity decrease correlates with decrease in intensity observed for the corresponding C₈-heteroaryl-dG adduct in CHCl₃ versus water, as while all adducts were less emissive in the non-polar solvent, both ²FurDG and IndG were significantly quenched in CHCl₃ versus water. A negligible shift in wavelength of fluorescence was again observed for the
The collection of photophysical data for the $C^8$-heteroaryl-dG modified $NarI^3$ oligonucleotides hybridized to the complement $NarI'(G)$ point to a syn preferred conformation for the adduct, with a W-type duplex structural motif. The syn-conformer was the assumed preference due to the blue-shift observed in emission for the modified $NarI^3: NarI'(G)$ duplexes as compared to the $NarI^3: NarI'(C)$ duplexes. The Manderville group has shown in prior research that a syn-preferred $Bth$-dG modified 10-mer oligonucleotide mismatched to G in the duplex is blue-shifted in emission by 33 nm, as compared to the duplex in which the adduct is paired with C and in an anti conformation. Upon base-pairing to G, the $Ind$-dG, $Bfur$-dG and $Bth$-dG modified duplexes show blue-shifted emission, by 4, 16 and 11 nm, respectively, as compared to hybridization involving base-pairing with C. In the syn conformation, the $C^8$-heteroaryl
moiety would be located in the interior of the helix, which is non-polar in nature, with a
dielectric constant similar to \( \text{CHCl}_3 \).\(^{76}\) Again, recall from Figures 3-18 and 3-19 that \( \text{C}^8 \)-heteroaryl-dG adducts are fluorescent in water, while quenched \( \text{CHCl}_3 \), and furthermore, that adduct emission was observed at blue-shifted wavelengths in \( \text{CHCl}_3 \) compared to water. As discussed in section 3.3.3 (b), emission is blue-shifted due to lack of solvent-mediated stabilization of the excited state in the non-polar environment. From data collected in Tables 3-13 to 3-15, the \( \text{Ind} \)_dG, \( \text{Bfur} \)_dG and \( \text{Bth} \)_dG adducts were determined to exhibit blue-shifts in \( \text{CHCl}_3 \) of 7, 14 and 14 nm, respectively, as compared to emission in water, very similar to the size of shifts observed for the corresponding adduct-incorporated oligonucleotides upon mismatch formation with G. Additionally, recall from Figures 3-22a to 3-25a that the emission wavelength maximum of \( \text{2Fur} \)_dG, \( \text{Ind} \)_dG, \( \text{Bfur} \)_dG and \( \text{Bth} \)_dG adducts is blue-shifted, and the intensity is significantly quenched, in the viscous solvent glycerol compared to water. In glycerol, the adducts would have restricted movement, similar to the lack of free rotation the \( \text{C}^8 \)-heteroaryl moiety would possess when in the \( \text{syn} \) conformation, due to the rigid stacking the double helix.\(^{54}\) The solvatochromic, including viscosity-related, properties of the \( \text{C}^8 \)-heteroaryl-dG adducts can therefore account for the dramatic blue-shift and decrease in emission intensity observed upon change in base-pairing, and subsequently conversion of glycosyl bond conformation.\(^{54}\)

Emission and excitation spectra of the \( \text{2Fur} \)_dG and \( \text{Bfur} \)_dG adducted \( \text{NarI} \)\(^3\) oligonucleotides upon hybridization to the complementary strand \( \text{NarI'}(10\text{-mer}) \) were also obtained, and are shown in Figure 4-18, with tabulation of photophysical parameters provided in Table 4-6.
Figure 4-18. Excitation and emission spectra of $C^8$-heteroaryl-G modified oligonucleotides in the single-strand state (solid line) or hybridized to its complementary strand NarI'(10-mer) (dotted line), with (a) NarI$^3$(X = $^2$FurG) or (b) NarI$^3$(X = $^8$furG). All spectra of single-strand oligonucleotides (1.25 $\mu$M) and duplexes (equivalent amounts (1.25 $\mu$M) of NarI and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 0.1 M NaCl at 10 °C.

Table 4-6. Photophysical parameters of $C^8$-heteroaryl-G modified NarI$^3$ oligonucleotides hybridized to NarI'(10-mer).

<table>
<thead>
<tr>
<th>duplex</th>
<th>$\lambda_{ex}$ (nm)$^a$</th>
<th>$\Delta \lambda_{ex}$ (nm)$^b$</th>
<th>$\lambda_{em}$ (nm)$^a$ ($I_{rel}$)$^c$</th>
<th>$\Delta \lambda_{em}$ (nm)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NarI$^3$(X = $^2$FurG):NarI'(10-mer)</td>
<td>307</td>
<td>–</td>
<td>386 (1.24)</td>
<td>–</td>
</tr>
<tr>
<td>NarI$^3$(X = $^8$furG):NarI'(10-mer)</td>
<td>318</td>
<td>–2</td>
<td>401 (0.89)</td>
<td>–2</td>
</tr>
</tbody>
</table>

$^a$ All spectra of single-strand oligonucleotides (1.25 $\mu$M) and duplexes (equivalent amounts (1.25 $\mu$M) of NarI$^3$ and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 0.1 M NaCl at 10 °C. $^b$ Change in excitation or emission maximum for single-strand NarI$^3$ is versus the free $C^8$-heteroaryl-dG adduct, while change for duplex is versus modified NarI$^3$. $^c$ Relative emission intensity for single-strand NarI$^3$ is determined as $I_{single-strand}/I_{adduct}$. $I_{adduct}$ was determined at a concentration of 1.25 $\mu$M. Relative emission intensity for the duplex is determined as $I_{duplex}/I_{single-strand}$. All intensity values for determination of $I_{rel}$ were measured at the same wavelength.

Although not as distinct as observed upon hybridization NarI'(G), hybridization to NarI'(10-mer) resulted in a decrease in emission intensity, as compared to the corresponding single-strand oligonucleotide. No change in emission or excitation wavelength was observed for NarI$^3$($^2$FurG) upon base-pairing to the truncated strand, while the wavelength of emission and excitation for NarI$^3$($^8$furG) was blue-shifted upon
formation of this slippage product, as compared to the single-strand oligonucleotides. A blue-shift in emission of 10 nm was also noted for the NarI<sup>3</sup>(BfurG):NarI'(10-mer) duplex compared to the NarI<sup>3</sup>:NarI'(C) duplex, and can again be ascribed to the solvatochromic properties of the C<sup>8</sup>-heteroaryl-dG adduct. As the spectral properties mimic those of the modified NarI<sup>3</sup>:NarI'(G) duplexes, the conformation of the adduct in the slipped duplex can be confirmed as syn, with the duplex adopting a W-type structural arrangement.

(b) Collisional Fluorescence Quenching of C<sup>8</sup>-Heteroaryl-G Modified NarI Oligonucleotides

Collisional fluorescence quenching studies provided further confirmation as to the conformer preference of the C<sup>8</sup>-heteroaryl-dG adduct in the duplex. Collisional quenching, known also as dynamic quenching, involves a diffusion controlled, collisional interaction between a fluorophore, in this case the C<sup>8</sup>-heteroaryl-dG adduct, and a quencher. The collision induces a loss of fluorophore energy, with the fluorescence lifetime of the fluorophore lowered, and in turn, a decrease in fluorescence intensity and quantum yield are observed. Collisional quenching has proved to be useful for the study of aqueous accessibility of fluorescent groups, with changes in sensitivity, exposure and accessibility following ligand binding indicative of a change in conformation of the fluorophore. Collisional quenching, using quenchers such as acrylamide and dU (neutral), iodide ion (negatively charged) and cesium ion (positively charged) has been used to provide insight on the conformation of proteins, benzopyrene diol epoxide (BPDE)-DNA adducts and 2AP.

Collisional quenching experiments were first conducted with the free C<sup>8</sup>-heteroaryl-dG adducts, using KI as the quencher. Examples of emission spectra and
plots of $F_o/F$ versus [KI] of the fluorescence quenching process of the $^{2}\text{Fur}dG$ and $^{B}dG$ adducts are shown below in Figures 4-19 and 4-20, respectively. Slopes of the plots derived from KI quenching experiments provide the Stern-Volmer quenching constant ($K_{sv}$) values, which are listed in Table 4-7. Quenching constant values reflect the extent of adduct accessibility toward the quencher.

**Figure 4-19.** (a) Emission spectrum and (b) plot of $F_o/F$ versus [KI] for the collisional fluorescence quenching of $^{2}\text{Fur}dG$. Spectra were recorded in 50 mM sodium phosphate, pH 7, with 100 mM NaCl, using 1.25 µM of the adduct. KI was added to the adduct sample as 0.05 M aliquots.
Figure 4-20. (a) Emission spectrum and (b) plot of $F_0/F$ versus [KI] for the collisional fluorescence quenching of $^{Bth}$dG. Spectra were recorded in 50 mM sodium phosphate, pH 7, with 100 mM NaCl, using 1.25 µM of the adduct. KI was added to the adduct sample as 0.05 M aliquots.

Table 4-7. Stern-Volmer quenching constants ($K_{sv}$) for collisional fluorescence quenching of $^C^8$-heteroaryl-dG modified nucleosides.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>$K_{sv}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{2Fur}$dG</td>
<td>10.2 ± 1.2</td>
</tr>
<tr>
<td>$^{Bth}$dG</td>
<td>11.8 ± 1.1</td>
</tr>
</tbody>
</table>

$^a$ $K_{sv}$ values were determined from the slope of $F_0/F$ versus [KI] plots.

The $K_{sv}$ values determined for the quenching of $^{2Fur}$dG and $^{Bth}$dG by KI are large, and reflect a high accessibility of the fluorophore to the quencher, and in turn, the aqueous environment. Of more interest are the $K_{sv}$ values from the quenching of $^{2Fur}$dG and $^{Bth}$dG modified duplexes, as this data would provide desired insight as to the *anti-* versus *syn-*conformer preference of the adduct. Collisional quenching experiments were conducted with the $NarI^3(X = ^{2Fur}G)$ and $NarI^3(X = ^{Bth}G)$ oligonucleotides hybridized to $NarI'(C)$ or $NarI'(G)$, $^{54}$ and the $NarI^3(X = ^{Bfur}G)$ oligonucleotide hybridized to $NarI'(10$-mer), using KI as the quencher. Examples of emission spectra and plots of $F_0/F$ versus
[KI] of the fluorescence quenching process of these duplexes are shown below in Figures 4-21 to and 4-23 respectively, with corresponding $K_{sv}$ values listed in Table 4-8.

**Figure 4-21.** (a) Emission spectrum and (b) plot of $F_0/F$ versus [KI] for the collisional fluorescence quenching of the $C^8$-heteroaryl-G modified oligonucleotide $\text{NarI}^3(X = \text{2Fur})_G$ hybridized to its complementary strand (i) $\text{NarI}^3(\text{C})$ or (ii) $\text{NarI}^3(\text{G})$. Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $\text{NarI}$ and its complementary strand. KI was added to the duplex sample as (i) 0.025 or 0.05 M aliquots, or (ii) 0.5 or 1 M aliquots.
Figure 4-22. (a) Emission spectrum and (b) plot of $F_o/F$ versus [KI] for the collisional fluorescence quenching of the $C^8$-benzoheteroaryl-G modified oligonucleotide $NarI^3(X = \text{Bth}G)$ hybridized to its complementary strand (i) $NarI'(C)$ or (ii) $NarI'(G)$. Spectra were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $NarI$ and its complementary strand. KI was added to the duplex sample as (i) 0.025 or 0.05 M aliquots, or (ii) 0.05, 0.1 or 0.5 M aliquots.
Figure 4-23. (a) Emission spectrum and (b) plot of $F_o/F$ versus [KI], for the collisional fluorescence quenching of the C$^8$-benzoheteroaryl-G modified oligonucleotide $NarI^3(X = ^{Bfur}G)$ hybridized to its complementary strand $NarI'(10$-mer). The spectrum was recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of $NarI$ and its complementary strand. KI was added to the duplex sample as 0.025 or 0.05 M aliquots.

Table 4-8. Stern-Volmer quenching constants ($K_{sv}$) for collisional fluorescence quenching of $C^8$-heteroaryl-G modified $NarI^3$ oligonucleotides hybridized to $NarI'(C)$, $NarI'(G)$ or $NarI'(10$-mer).

<table>
<thead>
<tr>
<th>duplex</th>
<th>$K_{sv}$ (M$^{-1}$)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$NarI^3(X = ^{2Fur}G):NarI'(C)$</td>
<td>2.51 ± 0.08</td>
</tr>
<tr>
<td>$NarI^3(X = ^{2Fur}G):NarI'(G)$</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>$NarI^3(X = ^{Bth}G):NarI'(C)$</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>$NarI^3(X = ^{Bth}G):NarI'(G)$</td>
<td>0.64 ± 0.03</td>
</tr>
<tr>
<td>$NarI^3(X = ^{Bfur}G):NarI'(10$-mer)</td>
<td>1.75 ± 0.09</td>
</tr>
</tbody>
</table>

$^a$ $K_{sv}$ values were determined from the slope of the plot of $F_o/F$ versus [KI] (quencher concentration), expressed as values ± fitting error.

An examination of the $K_{sv}$ values determined for the quenching of modified duplexes by KI first revealed that the values are much smaller than those determined for the corresponding adducts, reflecting a lower accessibility of the fluorophores to the quencher when located in the helical environment. The $K_{sv}$ values also show that when base-paired to C, the fluorophore is more accessible to the aqueous environment than when mismatched to G, as larger $K_{sv}$ values were derived for the modified
oligonucleotides hybridized to Narl'(C) than when hybridized to Narl'(G) or Narl'(10-mer).\textsuperscript{54}

Recall that the $T_m$ data for $C^8$-heteroaryl-dG modified Narl oligonucleotides hybridized to Narl'(C) pointed towards two possible destabilizing adduct conformations; an *anti*-preferred conformation, with a B-type duplex motif, or a *syn*-preferred conformation with a S-type duplex motif. The $K_{sv}$ values obtained following collisional quenching of Narl'(C) annealed duplexes allows for a defining classification of the adduct conformation.\textsuperscript{54} As already noted, the larger quenching constants for fluorescence quenching of Narl$^3(X = 2\text{Fur}G)$:Narl'(C) and Narl$^3(X = \text{Bth}G)$:Narl'(C) duplexes, compared to Narl$^3(X = 2\text{Fur}G)$:Narl'(G), Narl$^3(X = \text{Bth}G)$:Narl'(G) and Narl$^3(X = \text{Bfur}G)$:Narl'(10-mer) duplexes, can be equated to a more readily accessible fluorophore to the aqueous environment surrounding the double helix, and thus the quencher. In order for the fluorophores to be solvent exposed, the adduct must be in an *anti* conformation, as in this conformation, the $C^8$-heteroaryl is located outside of the helix and in the major groove.\textsuperscript{54} From the corroboration of thermal melting, emission and excitation spectra, and finally collisional fluorescence quenching studies, the conclusion can be drawn that when base-paired to C, the $C^8$-heteroaryl-dG adduct exists as an *anti*-conformer, with a B-type duplex structure.\textsuperscript{54}

Conversely, the smaller slopes from plots of $F_o/F$ versus [KI] determined from the quenching of Narl$^3(X = 2\text{Fur}G)$:Narl'(G), Narl$^3(X = \text{Bth}G)$:Narl'(G) and Narl$^3(X = \text{Bfur}G)$:Narl'(10-mer) suggest that the fluorophore has become more shielded and is likely stacked within the helix in the minor groove.\textsuperscript{37} This implies a *syn*-preferred conformation for the $C^8$-heteroaryl-dG adduct upon mismatch formation, or upon formation of the
slippage product following hybridization to the complementary strand NarI'(10-mer). Collectively, thermal melting, emission and excitation spectra, and finally collisional fluorescence studies, allow for one to conclude that in NarI'(G) and NarI'(10-mer) hybridized duplexes, the C\textsuperscript{8}-heteroaryl-dG adduct adopts the \textit{syn} conformation, with a W-type duplex structural motif.\textsuperscript{54}

\textit{(c) Emission and Excitation Spectra of C\textsuperscript{8}-Quinolyl-G Modified NarI Oligonucleotides}

The photophysical properties of the \textsuperscript{Q}dG adduct upon oligonucleotide incorporation were also examined. Emission and excitation spectra of the \textsuperscript{Q}dG adducted NarI\textsuperscript{3} oligonucleotide in the single-strand state, and upon hybridization to the complementary strands NarI'(C), NarI'(G) and NarI'(10-mer) were obtained, and are shown in Figure 4-24, with tabulation of photophysical parameters in Table 4-9. Furthermore, temperature dependent emission and excitation spectra were obtained for the oligonucleotide and duplexes and are shown in Figure 4-25. The emission spectrum of the free \textsuperscript{Q}dG modified nucleoside, for comparison, can be reviewed in Figure 3-11b.
Figure 4-24. Excitation and emission spectra of the $C^8$-benzoheteroaryl-G modified oligonucleotide $Narl^3(X = Q\text{G})$, in the single-strand state (solid line), or hybridized to its complementary strand, $Narl'(C)$ (dashed line), $Narl'(G)$ (dotted line) or $Narl'(10\text{-mer})$ (circled line). Spectra of the single-strand oligonucleotide (1.25 $\mu$M) and duplexes (equivalent amounts (1.25 $\mu$M) of $Narl^3$ and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl at 10 °C.

Table 4-9. Photophysical parameters of $C^8$-quinolyl-G modified $Narl^3$ oligonucleotides in the single-strand state, or hybridized to $Narl'(C)$, $Narl'(G)$ or $Narl'(10\text{-mer})$.

<table>
<thead>
<tr>
<th>oligonucleotide or duplex</th>
<th>$\lambda_{ex}$ (nm)$^a$</th>
<th>$\Delta \lambda_{ex}$ (nm)$^b$</th>
<th>$\lambda_{em}$ (nm)$^a$</th>
<th>$(I_{rel})^c$</th>
<th>$\Delta \lambda_{em}$ (nm)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Narl^3(X = Q\text{G})$</td>
<td>307</td>
<td>-22</td>
<td>387 (1.09)</td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>$Narl^3(X = Q\text{G});Narl'(C)$</td>
<td>302</td>
<td>-5</td>
<td>389 (0.86)</td>
<td></td>
<td>+2</td>
</tr>
<tr>
<td>$Narl^3(X = Q\text{G});Narl'(G)$</td>
<td>301</td>
<td>-6</td>
<td>388 (max), 450 (0.80)</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>$Narl^3(X = Q\text{G});Narl'(10\text{-mer})$</td>
<td>302</td>
<td>-5</td>
<td>388 (max), 450 (0.94)</td>
<td>+1</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All spectra of single-strand oligonucleotides (1.25 $\mu$M) and duplexes (equivalent amounts (1.25 $\mu$M) of $Narl^3$ and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl at 10 °C. $^b$ Change in excitation or emission maximum for modified $Narl^3$ is versus the free $C^8$-heteroaryl-dG adduct, while change for duplex is versus modified $Narl^3$. $^c$ Relative emission intensity for single-strand $Narl^3$ is determined as $I_{single-strand}/I_{adduct}$. $I_{adduct}$ was determined at a concentration of 1.25 $\mu$M. Relative emission intensity for the duplex is determined as $I_{duplex}/I_{single-strand}$. All intensity values for determination of $I_{rel}$ were measured at the same wavelength.
Figure 4-25. Excitation and emission spectra of the C^8-benzoheteroaryl-G modified oligonucleotide, NarI^2(X = Q,G), in the (a) single-strand state, or hybridized to its complementary strand (b) NarI(C), (c) NarI(G) or (d) NarI(10-mer). All spectra of single-strand oligonucleotides (1.25 μM) and duplexes (equivalent amounts (1.25 μM) of NarI and its complementary strand) were recorded in 50 mM sodium phosphate buffer, pH 7, with 100 mM NaCl at 10 °C (solid line) and 80 °C (dashed line).
The $^{0}\text{dG}$ adduct retained its same degree of fluorescing ability upon NarI$^{3}$ incorporation with an $I_{\text{rel}}$ value of 1.09 for NarI$^{3}(X = ^{0}\text{G})$ as compared to the free modified nucleoside. The wavelength of emission for the modified oligonucleotide does not differ from that of $^{0}\text{dG}$, while the excitation spectra does display features distinct from the spectra of the free base, with a large blue shift of 22 nm observed for NarI$(X = ^{0}\text{G})$ compared to $^{0}\text{dG}$. When spectral measurements were obtained following heating of the modified NarI$^{3}$ strand from 10 to 80 °C, it was noted that the temperature increase resulted in a decrease in emission and excitation intensity. This decrease in fluorescence intensity can again be attributed to the loss in overall rigidity of the oligonucleotide structure.$^{75}$ No change was observed in the emission and excitation wavelength parameters at 80 °C compared to 10 °C.

Hybridization to NarI'(C) caused little change to the spectral properties as compared to the single-strand. The emission of NarI$^{3}(X = ^{0}\text{G}):$NarI'(C) is similar in intensity to that of the single-strand, with $I_{\text{rel}} = 0.86$. The wavelength of emission for the duplex was not significantly different from that of the single-strand, while the excitation was blue-shifted compared to the single-strand by 5 nm. Heating of the duplex from 10 to 80 °C resulted in a decrease in fluorescence intensity, just as was observed for heating of the single-strand oligonucleotide. No change in wavelength parameters was observed upon heating. Comparison of emission and excitation spectra of the heated duplex to that of the heated oligonucleotide in Figure 4-25 reveals a nearly identical resemblance. Heating of the duplex induces denaturation, and therefore it is not a surprise that the spectral properties of the DNA molecule at 80 °C resemble those of the single-strand oligonucleotide.
Fluorescence spectral properties amassed for the $Narl^3(X = QG):Narl'(C)$ duplex indicates an anti-preferred glycosyl bond conformation of the adduct, with a B-type duplex structural motif. The anti-conformer was the assumed preference due to the similarity in spectral properties for the duplex compared to the single-strand, and in addition, to the $QdG$ adduct. Recall from the fluorescence spectral properties of the adduct in Figure 3-20 that $QdG$ is relatively non-fluorescent in water. Adoption of the anti conformation correlates to location of the $C^8$-quinolyl moiety in the aqueous, major groove. The fluorescent nature of $QdG$ in water can therefore account for the anti glycosyl bond conformation of the adduct in the $Narl'(C)$ annealed duplex.

In addition, mismatch to the opposing G or formation of the slippage product did not result in significant changes to emission intensity, with $I_{rel}$ values of 0.80 and 0.94, respectively, compared to the single-strand. At this junction, it is worthwhile to note that while dimer formation of the $QdG$ adduct with dC or rG elicited different emission intensity responses dependent upon the nature of the nucleobase complexed with the adduct, as shown previously in Figure 3-31, no such differences were reflected in fluorescence intensity upon changes to base-pairing within the duplex. The wavelength of emission for the $Narl^3(X = QG):Narl'(G)$ to $Narl^3(X = QG):Narl'(10-mer)$ duplexes was not significantly different from that of the single-strand, while the excitation was blue-shifted compared to the single-strand, by 6 and 5 nm, respectively. Hybridization of $Narl^3(X = QG)$ to $Narl'(G)$ or $Narl'(10-mer)$ did result in an intriguing change to the emission spectra compared to the spectra of the single strand and $Narl'(C)$ hybridized oligonucleotides, with a new emission band present at 450 nm. This band was even more prominent in the emission spectra of the slippage product compared to the mismatched
duplex. Heating of the duplexes from 10 to 80 °C resulted in a decrease in fluorescence intensity, just as was observed upon heating of the single-strand and Narl'(C) hybridized oligonucleotide, and a loss of the 450 nm emission band. Comparison of emission and excitation spectra of the heated duplexes to that of the heated oligonucleotide in Figure 4-25 reveals a nearly identical resemblance. Heating of the duplex induces denaturation, and therefore it is not a surprise that the spectral properties of the DNA molecule at 80 °C resemble those of the single-strand oligonucleotide.

The collection of photophysical data for the C8-quinolyl-dG modified Narl3 oligonucleotide hybridized to the complement Narl'(G) or Narl'(10-mer) points to a syn preferred conformation upon duplex formation, with a W-type duplex structural motif. The syn-conformer was the assumed preference due to the appearance of the emission band at 450 nm upon hybridization to Narl'(G) or Narl'(10-mer). In the syn conformation, the C8-quinolyl moiety would be intercalated in the non-polar minor groove of the helix. Refer to the solvatochromic fluorescence spectral properties of the QdG adduct highlighted in both in Table 3-16 and Figure 3-20. As stated above, the QdG adduct is minimally fluorescent (φf = 0.03) in water. In the non-polar solvent CHCl3, the adduct is strongly fluorescent (φf = 0.21) at 486 nm, attributed to emission from the TICT excited state, as discussed at length in section 3.3.3 (b). Additionally, recall from Figure 3-26a that the emission intensity of the QdG adduct is significantly different in the viscous solvent glycerol compared to water, with a new emission band present at 460 nm in glycerol not observed in water, again attributed to emission from the TICT excited state, as discussed in 3.3.3 (c). In glycerol, adducts have restricted movement, similar to the lack of free rotation the C8-quinolyl moiety would possess when in the syn
conformation, due to the stacking within the double helix. The presence of the 450 nm emission band upon duplex formation is thus indicative of the presence of an intercalated $C^8$-quinolyl moiety in a non-polar, rigid environment. It can be postulated that this emission band is more pronounced in the slippage product, as the bulge in the duplex allows more room for intercalation of the $C^8$-quinolyl moiety. The solvatochromic, including viscosity-related, properties of the $QdG$ adduct therefore account for the dramatic changes to the emission spectra observed upon hybridization to NarI'(G) or NarI'(10-mer), and subsequent conversion to syn glycosyl bond conformation.
4.4 Conclusions

Incorporation of $C^8$-heteroaryl-dG adducts in the recognition sequence of the NarI restriction enzyme was performed in order to determine the viability of such modification as probes of the DNA microenvironment and structural conformation. In summary, these adducts can all be selectively excited ($\lambda_{ex} = 306 – 320$) within the NarI oligonucleotide, and evidence has been provided that shows thermal melting, emission and excitation spectra and collisional fluorescence quenching analysis can be used to distinguish anti- from syn- conformers of the bulky $C$-linked fluorophores.\textsuperscript{54} In addition, these adducts can be used as probes to differentiate the nature of H-bonding interactions, W-C versus Hoogsteen base-pair formation, between the fluorophore and its opposing nucleobase in DNA.\textsuperscript{54} Upon base-pairing to their natural pyrimidine partner, and subsequent conversion to the anti conformation, $2^Fur$dG, $^{1}Ind$dG, $^{Bfu}r$dG and $^{Bth}$dG adducts impress a duplex destabilization, and decrease in $T_m$ value as compared to the unmodified duplex. This hybridization event results in a red-shift in emission wavelength of the $^{Ind}$dG, $^{Bfu}r$dG and $^{Bth}$dG modified duplexes, compared to the corresponding single-strand oligonucleotide. Here it is proposed that the duplex adopts the B-type conformation, with the $C^8$-heteroaryl substituent located in the major groove.\textsuperscript{54} Inversely, base-pairing to the unnatural purine partner G allows the adduct to adopt the syn conformation, resulting in duplex stabilization and subsequent increase in $T_m$ values as compared to the unmodified duplex. The observed stabilization of the mismatch may in part explain the occurrence of G to C transversions as induced by the formation of $C^8$-aryl-dG adducts, such as 8-Ph-dG, noted previously in Chapter 1, section 1.2.4 (b). This mismatch formation also results in a blue-shift in the emission wavelength of the $^{Ind}$dG, $^{Bfu}r$dG and $^{Bth}$dG modified.
duplexes, compared to the corresponding single-strand oligonucleotide. Here it is proposed that the duplex adopts the W-type conformation, with the $C^8$-heteroaryl substituent located in the minor groove.$^{54}$ It is also highly important to note that marked differences in fluorescence intensity and Stern-Volmer quenching constants are observed based upon the nature of the opposing base in the duplex, with base-pairing to G causing a larger decrease in fluorescence intensity, with smaller $K_{sv}$ values, compared to base-pairing to C.$^{54}$ The QdG adduct responds to base-pairing differences with changes in thermal melting and emission spectra. Base-pairing to C causes a substantial decrease in $T_m$ compared to the unmodified duplex, but little change in the emission spectrum compared to the single-strand oligonucleotide. Mismatch formation only slightly destabilizes the duplex, and results in the appearance of a new emission band at 450 nm.

The specific photophysical parameters of each $C^8$-heteroaryl-G modified NarI$^3$ oligonucleotide provide insight as to the best nucleoside probe for application in future studies.$^{54}$ The emission of the $C^8$-benzothienyl-linked derivative $B$dhG proved highly sensitive to conformation within the NarI duplex structure. When base-paired with C (NarI$^3(X = B$dh$)$:NarI'(C)), the $B$dhG probe exhibited a red-shift of 6 nm and an increase in emission intensity 1.4-fold compared to the emission of the probe in the single-strand oligonucleotide. In contrast, when base-paired with G (NarI(X = $B$dhG):NarI'(G)) the probe exhibited a blue-shift of 5 nm and exhibited quenched fluorescence compared to the probe emission in the single-strand. Overall, base-pairing with C provided a 2-fold increase in emission intensity and an 11 nm red-shift compared to the probe emission when base-paired with G, with this change in emission characteristics attributed to a change in conformation from anti to syn. DFT calculations have also indicated that $B$dhG
possesses the largest ground-state and excited-state dipole moment. The emission of the $C^{8}$-benzofuryl-linked derivative $^{\text{Bth}}$dG was also sensitive to conformation on the basis of wavelength (16 nm difference), but not with regards to emission intensity. In contrast, the emission of the $C^{8}$-indolyl-linked derivative $^{\text{Ind}}$dG was strongly quenched in the syn-conformation ($I_{\text{rel}} = 0.17$, compared to the single-strand), but it was not possible to distinguish base-pairing of $^{\text{Ind}}$G with C from the single-strand Narl$^3$ on the basis of emission intensity or wavelength. Furthermore, the probe failed to show changes in emission wavelength for hybridization to Narl'(C) versus Narl'(G). The $C^{8}$-quinolyl-linked derivative $^{\text{Q}}$dG displayed almost no difference in emission intensity for the single-strand incorporated oligonucleotide versus duplex formation with either complementary strand. Base-pairing to C also resulted in little change to the emission wavelength compared to the single-strand oligonucleotide, while mismatch formation did result in the appearance of a new emission band at 450 nm. The smaller $C^{8}$-furyl-linked derivative $^{\text{Fur}}$dG exhibited quenched emission upon formation of both possible duplex structures and the emission wavelength lacked sensitivity to base-pairing. Thus, $^{\text{Bth}}$dG was the only probe to show differences in both emission intensity and wavelength upon change in conformation within the Narl$^3$ duplex, making it the most sensitive probe for monitoring conformation.$^{54}$

The different duplex conformations, B- and W-type, formed as a result of $C^{8}$-heteroaryl-dG oligonucleotide modification, are known to play a critical role in the biological activity of bulky $N$-linked $C^{8}$-dG adducts formed by arylamine carcinogens$^{26,35,40,41}$ and C-linked $C^{8}$-dG adducts generated by various chemical carcinogens.$^{42,43,47}$ The $C^{8}$-heteroaryl-dG adducts presented here are similar to these $N$-
and C-linked $C^8$-dG adducts in bulkiness and their impact of duplex structure, but are advantageous in their fluorescent nature, which $N$- and C-linked $C^8$-dG adducts generally do not possess. Study of these adducts in the recognition sequence of the NarI restriction enzyme holds further toxicological relevance. Because $N$-linked $C^8$-dG adducts are known to induce −2 frameshift mutations in the NarI sequence,\textsuperscript{26,34,36,39,58} it was of particular importance to examine the consequence of hybridization to a truncated 10-mer with a two-base deletion, in order to form a duplex that mimics strand slippage that would normally occur during frameshift mutation. Both $NarI^3(X = Bfur\textsuperscript{G})$ and $NarI^3(X = Q\textsuperscript{G})$ were annealed to $NarI'(10mer)$, with fluorescence spectra and collisional quenching studies in agreement with assignment of a syn adduct conformation, and a W-type duplex structural motif, for these duplexes. Substantial stabilization ($\Delta T_m \sim +8^\circ C$) of the bulge in the slippage product duplex by both of these adducts is also notable. It is thus important to note that while the $Bfur\textsuperscript{dG}$ and $Q\textsuperscript{dG}$ adducts do not behave as the most emissive sensitive probes for monitoring conformation, they can instead as serve as models to assist in the understanding of induction, and subsequent consequences of, frameshift mutation by bulky adduct formation in DNA.

Adduct conformation of $N$-linked $C^8$-dG adducts is believed to be directly related to their mutagenic activity. The differences in the accumulation and rates of removal of these adducts may be ascribed to the differences in conformation about the glycosyl bond. As syn-conformer adducts are known to create greater distortions of the duplex arrangement, they are thought to be more easily recognized and excised. Adducts in the anti conformation, meanwhile, are less structurally disruptive and are thus proposed to be more refractory towards repair.\textsuperscript{39,41} $C^8$-Heteroaryl-dG adducts could therefore be used as
fluorescent models of bulky $C^8$-dG adducts, and their usage in biological systems could help in the elucidation of the mechanism of mutagenesis generated by these adducts.$^{54}$
4.5 References


(56) Fuchs, R. P.; Fujii, S. DNA Repair 2007, 6, 1032–1041.


(64) Vongsutilers, V.; Daft, J. R.; Shaughnessy, K. H.; Gannett, P. M. Molecules 2009, 14, 3339–3352.


Appendix A.

General Experimental Methods
Unless otherwise noted, commercial compounds were used as received, and in general, were purchased from Sigma-Aldrich (St. Louis, MO). All nucleosides, including 2'-deoxyadenosine (dA) monohydrate, 2'-deoxycytidine (dC) monohydrochloride, 2'-deoxyguanosine (dG) monohydrate and riboguanosine (rG) were purchased from ChemGenes (Wilmington, MA), boronic acids from Frontier Scientific (Logan, UT), and trisphenylphosphine-3,3',3''-trisulfonic acid trisodium salt hydrate (TPPTS) from Alfa Aesar (Ward Hill, MA). All normal (unmodified) oligonucleotides were synthesized and purified by Sigma-Aldrich Ltd. (Oakville, ON), including Nar1, 5'-CTCGGCAGCCATC, and its complementary strands Nar1'(C), 5'-GATGGCGCCGAG, Nar1'(G), 5'-GATGGGGCCGAG and Nar1'(10mer), 5'-GATGGGCGCCGAG. Triethylammonium acetate (TEAA) buffer was prepared from glacial acetic acid and triethylamine (Et$_3$N), and buffer pH adjusted using glacial acetic acid or Et$_3$N. Water used for buffers and spectroscopic solutions was obtained from a Milli-Q filtration system (18.2 MΩ).

$^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 DPX at 300.1 MHz and 75.5 MHz, respectively. Unless specified all NMR experiments were carried out at room temperature. All NMR spectra were referenced to the residual proton solvent signal of the deuterated solvent and processed using TopSpin 2.1 software. pH measurements were taken at room temperature with an Accumet 910 pH meter with an Accumet pH Combination Electrode with stirring. UV-Vis measurements were recorded on a Cary 300-Bio UV-Visible Spectrophotometer equipped with a 6 × 6 Multicell Block Peltier, stirrer and temperature controller with Probe Series II. Fluorescence measurements were recorded on a Cary Eclipse Fluorescence Spectrophotometer equipped with a 1 × 4 Multicell Block Peltier, stirrer
and temperature controller with Probe Series II.

Unless otherwise noted, stock solutions of nucleoside adducts for use in spectroscopic measurements were prepared in DMSO, due to sparing solubility in other solvents, to a concentration of 4 mM. Spectroscopic solutions were prepared directly in the cuvette. Quartz glass cells from Hellma Analytics were used for all spectroscopic measurements. For analysis of all nucleosides, UV-Vis spectra were recorded using 100-QS cells with a light path of 10 mm, and fluorescence spectra recorded using 101-QS cells with a light path of $10 \times 10$ mm. For analysis of all oligonucleotides, UV-Vis spectra were recorded using 108.002-QS cells with a light path of 10 mm, and fluorescence spectra recorded using 108.002F-QS cells with a light path of $10 \times 2$ mm. All CD spectra were recorded using 110-QS cells with a light path of 1 mm. All UV-Vis and fluorescence measurements were recorded at room temperature, unless otherwise noted, with stirring and baseline correction. Unless otherwise noted, UV-Vis spectra were recorded from 400 to 220 nm. Fluorescence excitation spectra were recorded at the emission wavelength (maximum) of the adduct, from 200 nm to 10 nm below the emission wavelength, while fluorescence emission spectra were recorded at the excitation wavelength (maximum), from 10 nm above the excitation wavelength to 600 nm. Unless otherwise noted, for all fluorescence measurements with nucleosides, excitation and emission slit widths were kept constant at 2.5 nm, while for all fluorescence measurements with oligonucleotides, excitation and emission slit widths were kept constant at 5 nm.
Appendix B.

Characterization of $C^8$-Br-G and $C^8$-Aryl-G Modified Trimers by ESI$^-$ Mass Spectrometry and UV Spectroscopy
Figure B-1. Mass spectrum of the brominated trimer 5'-A(8-Br-G)T, obtained with an ESI source operated in negative mode.

Figure B-2. Mass spectrum of the brominated trimer 5'-C(8-Br-G)T, obtained with an ESI source operated in negative mode.
Figure B-3. Mass spectrum of the brominated trimer 5'-G(8-Br-G)T, obtained with an ESI source operated in negative mode.

Table B1. ESI− MS analysis of brominated trimers.

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<th>trimer</th>
<th>product formula</th>
<th>calcd mass&lt;sup&gt;b&lt;/sup&gt;</th>
<th>exptl m/z (ESI)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>exptl mass&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
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<tr>
<td>5'-A(8-Br-G)T</td>
<td>C&lt;sub&gt;30&lt;/sub&gt;H&lt;sub&gt;37&lt;/sub&gt;BrN&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;16&lt;/sub&gt;P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>962.1</td>
<td>([M - H]^- = 961.0)</td>
<td>962.0</td>
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<tr>
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<td></td>
<td></td>
<td>([M - 2H]^2^- = 480.2)</td>
<td></td>
</tr>
<tr>
<td>5'-C(8-Br-G)T</td>
<td>C&lt;sub&gt;29&lt;/sub&gt;H&lt;sub&gt;37&lt;/sub&gt;BrN&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;17&lt;/sub&gt;P&lt;sub&gt;2&lt;/sub&gt;</td>
<td>938.1</td>
<td>([M - H]^- = 936.9)</td>
<td>937.9</td>
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<td></td>
<td>([M - 2H]^2^- = 468.1)</td>
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</tr>
<tr>
<td>5'-G(8-Br-G)T</td>
<td>C&lt;sub&gt;37&lt;/sub&gt;H&lt;sub&gt;44&lt;/sub&gt;N&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;18&lt;/sub&gt;P&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>([M - 2H]^2^- = 488.2)</td>
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<sup>a</sup> Provided by Sigma-Aldrich Ltd.  
<sup>b</sup> Monoisotopic mass of most abundant isotopologue.  
<sup>c</sup> Measured m/z from mass spectrum.
Figure B-4. Spectral properties of the C8-aryl-G modified trimer 5'-G(P3G)T: (a) UV spectrum ($\lambda_{\text{max}} = 270$ and 298 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure B-5. Spectral properties of the C\textsuperscript{8}-aryl-G modified trimer 5'-G(\text{PhOMe}G)T: (a) UV spectrum (\(\lambda_{\text{max}} = 272\) and 296 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure B-6. Spectral properties of the $C^8$-aryl-G modified trimer 5'-G(PhCN-G)T: (a) UV spectrum ($\lambda_{\text{max}} = 272$ and 324 nm (weaker absorbance)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure B-7. Spectral properties of the $C^8$-aryl-G modified trimer $5'-A^{(\text{PhOMe})G}T$: (a) UV spectrum ($\lambda_{\text{max}} = 266$ and 292 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure B-8. Spectral properties of the C<sup>5</sup>-aryl-G modified trimer 5'-C<sub>PhOMe</sub>G)T: (a) UV spectrum ($\lambda_{\text{max}} = 272$ and 300 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Appendix C.

Characterization of 8-Br-dG, 8-Br-dA and C^8-Heteroaryl-Purine Adducts by \(^1\text{H}\) and \(^{13}\text{C}\) NMR Spectroscopy, and X-Ray Crystallography
Figure C-1. $^1$H NMR spectrum of 8-bromo-2'-deoxyguanosine (8-Br-dG) in DMSO-$d_6$. 
Figure C-2. $^{13}$C NMR spectrum of 8-bromo-2'-deoxyguanosine (8-Br-dG) in DMSO-$d_6$. 
Figure C-3. $^1$H NMR spectrum of 8-(2''-pyrrolyl)-2'-deoxyguanosine ($^{2\text{pyr}}$dG) in DMSO-$d_6$. 
Figure C-4. $^{13}$C NMR spectrum of 8-(2''-pyrrolyl)-2'-deoxyguanosine ($^{2Pyr}$dG) in DMSO-$d_6$. 
Figure C-5. $^1$H NMR spectrum of 8-(2''-furyl)-2'-deoxyguanosine ($^{2Fur}dG$) in DMSO-$d_6$. 
Figure C-6. $^{13}$C NMR spectrum of 8-(2"-furyl)-2'-deoxyguanosine (2Fur dG) in DMSO-$d_6$. 
Figure C-7. $^1$H NMR spectrum of 8-(2"-thienyl)-2'-deoxyguanosine ($^{2\text{Th}}$dG) in DMSO-$d_6$. 
Figure C-8. $^{13}$C NMR spectrum of 8-(2"-thienyl)-2'-deoxyguanosine ($^{2\text{Th}}$dG) in DMSO-$d_6$. 
Figure C-9. $^1$H NMR spectrum of 8-(3''-pyrrolyl)-2'-deoxyguanosine (3Pyr dG) in DMSO-$d_6$. 
Figure C-10. $^{13}$C NMR spectrum of 8-(3''-pyrrolyl)-2'-deoxyguanosine ($^{3\text{Py}}$dG) in DMSO-$d_{6}$. 
Figure C-11. $^1$H NMR spectrum of 8-(3"-furyl)-2'-deoxyguanosine ($^{2\text{Fur}}dG$) in DMSO-$d_6$. 
Figure C-12. $^{13}$C NMR spectrum of 8-(3''-furyl)-2'-deoxyguanosine ($^{3\text{Fur}}$dG) in DMSO-$d_6$. 
Figure C-13. $^1$H NMR spectrum of 8-(3''-thienyl)-2'-deoxyguanosine ($^{3\text{Th}}$dG) in DMSO-$d_6$. 

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Figure C-14. $^{13}$C NMR spectrum of $8'$-(3''-thienyl)-2'-deoxyguanosine (3ThdG) in DMSO-$d_6$. 
Figure C-15. $^1$H NMR spectrum of 8-(2''-indolyl)-2'-deoxyguanosine (IndG) in DMSO-$d_6$. 
Figure C-16. $^{13}$C NMR spectrum of 8-(2'-indolyl)-2'-deoxyguanosine ($^{\text{Ind}}$dG) in DMSO-$d_6$. 
Figure C-17. $^1$H NMR spectrum of 8-(2''-benzofuryl)-2'-deoxyguanosine ($^{Bfur}$dG) in DMSO-$d_6$. 
Figure C-18. $^{13}$C NMR spectrum of 8-(2'-benzofuryl)-2'-deoxyguanosine ($^{Bfur}$dG) in DMSO-$d_6$. 
Figure C-19. $^1$H NMR spectrum of 8-(2''-benzothienyl)-2'-deoxyguanosine ($^{Bth}$dG) in DMSO-$d_6$. 
Figure C-20. $^{13}$C NMR spectrum of 8-(2''-benzothienyl)-2'-deoxyguanosine ($^{Bth}$dG) in DMSO-$d_6$. 
Figure C-21. $^1$H NMR spectrum of 8-(8''-quinolyl)-2'-deoxyguanosine ($^Q$dG) in DMSO-$d_6$. 
Figure C-22. $^{13}$C NMR spectrum of 8-(8"-quinolyl)-2'-deoxyguanosine ($^{0}$dG) in DMSO-$d_6$. 
Figure C-23. $^1$H NMR spectrum of 8-bromo-2'-deoxyadenosine (8-Br-dA) in DMSO-$d_6$. 
**Figure C-24.** $^{13}$C NMR spectrum of 8-bromo-2'-deoxyadenosine (8-Br-dA) in DMSO-$d_6$. 
**Figure C-25.** $^1$H NMR spectrum of 8-(2"-pyrrolyl)-2'-deoxyadenosine ($^{2\text{Pyr}}$dA) in DMSO-$_d_6$. 
**Figure C-26.** $^{13}$C NMR spectrum of 8-(2'-pyrrolyl)-2'-deoxyadenosine ($^{2\text{Pyr}}$dA) in DMSO-$d_6$. 
Figure C-27. $^1$H NMR spectrum of 8-(2''-furyl)-2'-deoxyadenosine (2Fur dA) in DMSO-$d_6$. 
Figure C-28. $^{13}$C NMR spectrum of 8-(2"-furyl)-2'-deoxyadenosine (2Fur dA) in DMSO-$d_6$. 
Figure C-29. $^1$H NMR spectrum of 8-(2"-indolyl)-2'-deoxyadenosine ($^{\text{Ind}}$dA) in DMSO-$d_6$. 
Figure C-30. $^{13}$C NMR spectrum of 8-(2''-indolyl)-2'-deoxyadenosine (InddA) in DMSO-$d_6$. 
Figure C-31. $^1$H NMR spectrum of 8-(2”-benzofuryl)-2'-deoxyadenosine ($^{Bfur}$dA) in DMSO-$d_6$. 
Figure C-32. $^{13}$C NMR spectrum of 8-(2"-benzofuryl)-2'-deoxyadenosine ($^{\text{Bfur}}$dA) in DMSO-$d_6$. 
Figure C-33. $^1$H NMR spectrum of 8-(2''-benzothienyl)-2'-deoxyadenosine ($^{Bth}$dA) in DMSO-$d_6$. 
Figure C-34. $^{13}$C NMR spectrum of 8-(2"-benzothienyl)-2'-deoxyadenosine ($^{\text{Bth}}$dA) in DMSO-$d_6$. 
Figure C-35. $^1$H NMR spectrum of 8-($8''$-quinolyl)-2'-deoxyadenosine ($Q$dA) in DMSO-$d_6$. 
Figure C-36. $^{13}$C NMR spectrum of 8-$(8''$-quinolyl)-2$'$-deoxyadenosine ($^0$dA) in DMSO-$d_6$. 
Figure C-37. $^1$H NMR spectrum of 3',5'-O-(1,1,3,3-tetraisopropylsiloxy-1,3-diyl)- 2'-deoxycytidine (bissilyldC) in DMSO-$d_6$. 
Figure C-38. $^{13}$C NMR spectrum of 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)- 2'-deoxycytidine (bissilyldC) in DMSO-$d_6$. 
Figure C-39. $^1$H NMR spectrum of 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)- 2'-deoxyguanosine (bissilyldG) in DMSO-$d_6$. 
Figure C-40. $^1$H NMR spectrum of 8-(2"-pyrrolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyl$^{2Py}dG$) in DMSO-$d_6$. 
Figure C-41. $^1$H NMR spectrum of 8-(2''-indolyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyl$^\text{Ind}$dG) in CD$_2$Cl$_2$. 
Figure C-42. $^1$H NMR spectrum of 8-(2"-benzothienyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyl$^{Bth}$dG) in DMSO-$d_6$. 
Figure C-43. $^{13}$C NMR spectrum of 8-(2$''$-benzothienyl)-3',5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diyl)-2'-deoxyguanosine (bissilyl$^{Bth}$dG) in DMSO-$d_6$. 
Figure C-44. $^1$H NMR spectrum of 2',3',5'-tris-(tert-butyldimethylsilyl)riboguanosine (rG(TBDMS)$_3$) in DMSO-$d_6$. 
Figure C-45. $^1$H NMR spectrum of 8-(8''-quinolyl)-3',5'-tris((tert-butyldimethylsilyl)oxy)-2'-deoxyguanosine ($^0$dG(TBDMS)$_2$) in DMSO-$d_6$. 
Figure C-46. $^{13}$C NMR spectrum of 8-(8'-quinolyl)-3',5'-tris((tert-butyldimethylsilyl)oxy)-2'-deoxyguanosine ($^{\text{Q}}\text{dG(TBDMs)}_2$) in DMSO-$d_6$. 
X-Ray Crystallography of 8-(8''-Quinolyl)- 2'-Deoxyguanosine (QdG)

Crystal data and structure refinement for final crystal structure:

Empirical formula                C19 H20 N6 O5
Formula weight                    412.41
Temperature                       150 K
Wavelength                        1.54184 A
Crystal system, space group       Orthorhombic,  P 21 21 21
Unit cell dimensions             a = 6.68426(5) A  
b = 9.28108(8) A  
c = 29.6696(2) A
Volume                            1840.62(2) A^3
Z, Calculated density             4,  1.488 Mg/m^3
Absorption coefficient           0.930 mm^-1
F(000)                            864
Crystal size                      0.40 x 0.40 x 0.25 mm
Theta range for data collection  4.99 to 75.84 deg.
Limiting indices                  -8<=h<=8, -11<=k<=11, -37<=l<=37
Reflections collected / unique    36075 / 3837 [R(int) = 0.0221]
Completeness to theta = 75.84     99.6 %
Absorption correction            Semi-empirical from equivalents
Max. and min. transmission        0.8008 and 0.7074
Refinement method                 Full-matrix least-squares on F^2
Data / restraints / parameters    3837 / 0 / 331
Goodness-of-fit on F^2            1.105
Final R indices [I>2sigma(I)]    R1 = 0.0251, wR2 = 0.0647
R indices (all data)              R1 = 0.0251, wR2 = 0.0647
Absolute structure parameter      0.06(11)
Largest diff. peak and hole       0.198 and -0.196 e.A^-3

Bond lengths [Å] and angles [deg] for final crystal structure:

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C(1)-H(1)                         0.957(15)
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C(2)-H(2A)                       0.984(16)
C(2)-H(2B)                       0.982(16)
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C(2G)-N(2G)                     1.3416(14)
C(2G)-N(1G)                     1.3743(13)
C(2Q)-N(1Q)                     1.3191(16)
C(2Q)-C(3Q)                     1.4179(17)
C(2Q)-H(2Q)                     0.974(17)
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C(3)-H(3)                        1.021(15)
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C(3Q)-H(3Q)                     0.989(18)
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C(4)-C(5)                        1.5093(14)
C(4)-H(4)                        0.998(15)
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Appendix D.

Characterization of NarI and C₈-Br-G and C₈-Heteroaryl-G Modified NarI Oligonucleotides by ESI Mass Spectrometry, and UV and CD Spectroscopy
Figure D-1. UV spectrum of the NarI oligonucleotide, 5’-CTCGCGCCATC ($\lambda_{\text{max}}$ = 263 nm).
Figure D-2. Mass spectrum of the brominated oligonucleotide, NarI(X = 8-Br-G), obtained with an ESI source operated in negative mode.

Table D-1. Yields and ESI⁻ MS analysis of brominated NarI oligonucleotides.

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<th>exptl m/z (ESI⁻) c</th>
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a Provided by Sigma-Aldrich Ltd. b Monoisotopic mass of most abundant isotopologue; assumes one ¹³C isotope. c Measured m/z from mass spectrum.
Figure D-3. Spectral properties of the $C^8$-heteroaryl-G modified oligonucleotide, Nar1$^1 (X = \text{2Fur})$: (a) UV spectrum ($\lambda_{\text{max}} = 266$ and 318 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure D-4. Spectral properties of the $C^8$-heteroaryl-G modified oligonucleotide $Nar1^\dagger(X = \text{IndG})$: (a) UV spectrum ($\lambda_{\text{max}} = 262$ and 322 nm (weaker absorbance)) and (b) (i) and (ii) mass spectra obtained with an ESI source operated in negative mode.
Figure D-5. Spectral properties of the $C^8$-heteroaryl-G modified oligonucleotide Narl$^1$($X = \text{Bfur}G$): (a) UV spectrum ($\lambda_{\text{max}} = 266$ and 324 nm (weaker absorbance)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure D-6. Spectral properties of the $C^8$-heteroaryl-G modified oligonucleotide $NarI^1(X = \text{Bth}G)$: (a) UV spectrum ($\lambda_{\text{max}} = 266$ and $318$ nm (weaker absorbance)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure D-7. Spectral properties of the C₈-heteroaryl-G modified oligonucleotide NarI(X = QG): (a) UV spectrum ($\lambda_{\text{max}} = 262$ and 314 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure D-8. Spectral properties of the $C^8$-heteroaryl-G modified oligonucleotide NarI$(X = \text{G})$: (a) UV spectrum ($\lambda_{\text{max}} = 262$ and 314 nm (shoulder)) and (b) mass spectrum obtained with an ESI source operated in negative mode.
Figure D-9. CD spectra of the NarI oligonucleotide, hybridized to its complementary strand (a) NarI'(C) or (b) NarI'(G). Spectra were recorded at 10 °C in 50 mM phosphate buffer, pH 7, with 100 mM NaCl, using equivalent amounts (1.25 µM) of NarI and its complementary strand.