Kinetics of Hydrolysis of 8-(Arylamino)-2′-deoxyguanosines

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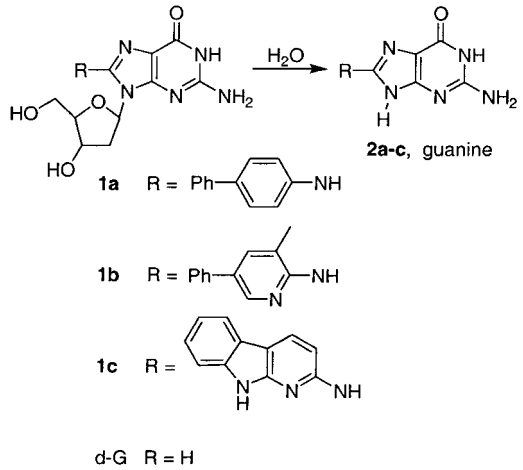
The 8-(arylamino)-2′-deoxyguanosines, or C-8 adducts, are the major adducts formed by reaction of *N*-arylnitrenium ions derived from carcinogenic and mutagenic amines with 2′-deoxyguanosine (d-G) and guanosine residues of DNA. The hydrolysis kinetics of three C-8 adducts **1a**−**c** were determined by UV and HPLC methods at 20 °C under acidic, neutral, and mildly alkaline conditions. At pH < 2 the dominant hydrolysis process is spontaneous cleavage of the C−N bond of the doubly protonated substrate, $\mathbf{1H}_2^{+2}$ (Scheme 2). The C-8 adducts are 2- to 5-fold more reactive than d-G under these conditions. At 3 < pH < 6 the hydrolysis kinetics are dominated by cleavage of the C−N bond of the monoprotonated nucleoside $\mathbf{1H}^+$. Under these conditions the hydrolysis kinetics are accelerated by 40- to 1300-fold over that of d-G. The rate increase appears to be caused by a combination of steric acceleration of C−N bond cleavage and a decrease in the ionization constant of $\mathbf{1H}^+$, K_{a1}, due to the electron-donating properties of the arylamino C-8 substituent. Under neutral pH conditions a slow ($k_{obs} \approx 10^{-8}$ s^{-1} to 5×10^{-7} s^{-1}) spontaneous cleavage of the C−N bond of the neutral nucleoside, **1**, occurs that has not been previously reported for simple purine nucleosides. Finally, under mildly alkaline conditions a process consistent with spontaneous decomposition of the anion $\mathbf{1}^-$ or OH^--induced decomposition of **1** is observed. The latter process has been observed for other purine nucleosides, including the closely related **1d**, and involves nucleophilic attack of OH^- on C-8 to cleave the imidazole ring of the purine.

Introduction

During our studies of the trapping of *N*-arylnitrenium ions by 2′-deoxyguanosine (d-G) we have isolated and characterized a number of 8-(arylamino)-2′-deoxyguanosines commonly referred to as C-8 adducts (Scheme 1).[1] These are the most common adducts isolated from the reactions of mutagenic and carcinogenic metabolites of carbocyclic and heterocyclic aromatic amines with d-G, DNA oligomers, and DNA both in vitro and in vivo.[1,2] We noted that several of these C-8 adducts underwent hydrolysis of the C−N glycosidic bond at appreciable rates in moderately acidic to neutral aqueous buffers (3 ≤ pH ≤ 7 at 20 °C), conditions under which d-G shows very little reactivity ($k_{obs} < 10^{-6}$ s^{-1}).[1]

In DNA this hydrolysis would result in depurination of guanine sites of the DNA polymer. It has been noted that DNA modified with these C-8 adducts is subject to relatively rapid spontaneous depurination of the modified purinic sites.[3] Spontaneous depurination is thought to contribute to some of the mutagenic effects of several classes of mutagens and to cellular death.[4] Although the hydrolysis reaction may be of some physiological importance and is also interesting from a mechanistic point of view, it has never been investigated quantitatively.

Scheme 1



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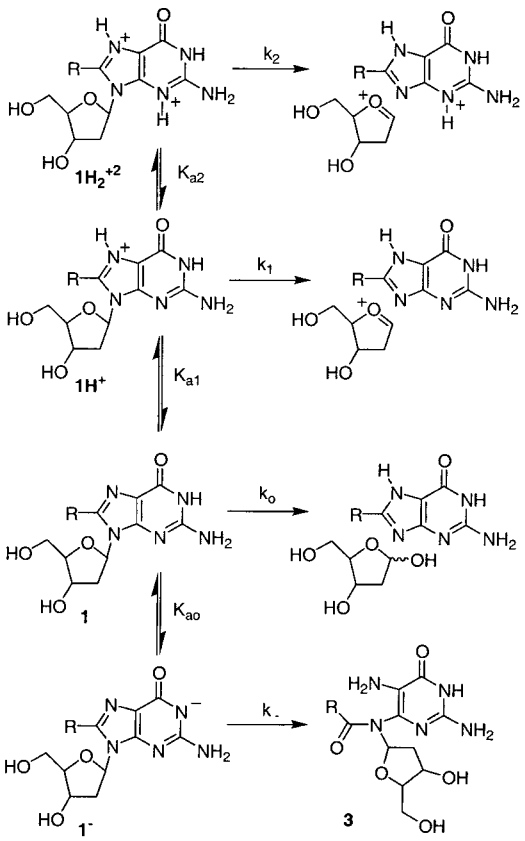
Scheme 2



Acid-catalyzed hydrolysis of monomeric purine nucleosides has received a considerable amount of attention and is a well understood reaction.[5−8] A general mechanism for the acid-catalyzed reaction that is consistent with kinetic data, lack of buffer effects, isotope effects, entropy of activation data, and the lack of anomerization of unreacted substrate is shown in Scheme 2. Both monoprotonated (**1H**$^+$) and diprotonated (**1H**$_2^{+2}$) substrate formed via preequilibrium protonation appear to be subject to rate-limiting C−N bond cleavage. On the basis of data from the 2-deoxyglucosyl oxocarbenium ion and similar species, it is likely that the 2-deoxyribosyl oxocarbenium ion has a short but finite lifetime of ca. 10^{-12} to 10^{-11} s that requires all reactions of the cation to occur in a complex in which the leaving group is present.[9]

Depurination of single and double stranded DNA occurs via a similar acid-catalyzed pathway that predominates from moderately acidic conditions to at least the physiologically relevant pH 7.4.[10,11] Loss of guanine and adenine occurs at similar rates, and the extrapolated rate constant for depurination of double stranded DNA at 37 °C and pH 7.4 is 3×10^{-11} s^{-1}.[11]

In contrast to their lability under acidic conditions simple purine nucleosides such as adenosine (A), 2′-deoxyadenosine (d-A), guanosine (G), and d-G are relatively inert under neutral to weakly basic conditions ($7 \leq$ pH ≤ 9).[5−8,12] Under more strongly alkaline conditions purine nucleosides are subject to a base-catalyzed hydrolysis that involves OH^- attack on C-8 with cleavage of the imidazole ring of the nucleoside.[12] Products include pyrimidine and purine bases as well as some materials that are not UV active.[12] Some 8-(arylamino)-2′-deoxyguanosines are also subject to this alkaline decomposition reaction.[13]

In this paper we present a kinetic study of the decomposition of three representative C-8 adducts, **1a**−**c**, (Scheme 1) in the acidity range $H_o \approx -1$ to pH 9. The kinetics of hydrolysis of **1a**−**c** have been compared and contrasted with those of d-G. Kinetic analysis shows that four different pathways contribute to the hydrolysis of **1a**−**c** under these conditions. The *N*-arylamino-C-8 adducts **1a**−**c** do exhibit significantly accelerated hydrolysis compared to d-G at pH > 2, although under more strongly acidic conditions they undergo hydrolysis at rates comparable to that of d-G. The reasons for the accelerated hydrolysis of **1a**−**c** at pH > 2 are described herein.

Results and Discussion

Hydrolysis kinetics for **1a**−**c** and d-G were monitored in 20 vol % CH_3CN-H_2O at 20 °C for all compounds and at 60 °C for d-G. In the pH range ionic strength was maintained at 0.5 ($NaClO_4$), but no attempt was made to maintain constant ionic strength at $[H^+] > 0.5$ M. $HClO_4$ was used to maintain pH or H_o at pH $\leq$ 2.5. Buffers of HCO_2H/HCO_2Na, AcOH/AcONa, NaH_2PO_4/Na_2HPO_4, and trisH^+/tris were used to maintain pH in the range from ca. 2.5 to 9.5. Repetitive wavelength scans showed that isobestic points held during the decomposition of all compounds at $H_o \geq -1$. Pseudo-first-order rate constants were obtained by fitting UV absorbance vs time data ($k_{obs} > 2 \times 10^{-5}$ s^{-1}) or HPLC peak area vs time data (2×10^{-6} s^{-1} < k_{obs} < 2×10^{-5} s^{-1}) to the standard first-order rate equation. For $k_{obs} < 2 \times 10^{-6}$ s^{-1}, rate constants were obtained by initial rates methods from HPLC peak area vs time data for the starting nucleosides during the first 5% of the hydrolysis reaction. Figure 1 provides representative examples of these data for **1a**.

Rate constants did depend on pH or H_o but were independent of buffer concentrations in all buffers except tris. In those solutions buffer-independent rate constants were obtained by extrapolation to zero buffer concentration. In other buffers rate constants were typically measured in solutions with total buffer concentration of 0.02 M. Tables of all buffer-independent rate constants obtained in this study are provided in Supporting Infor-

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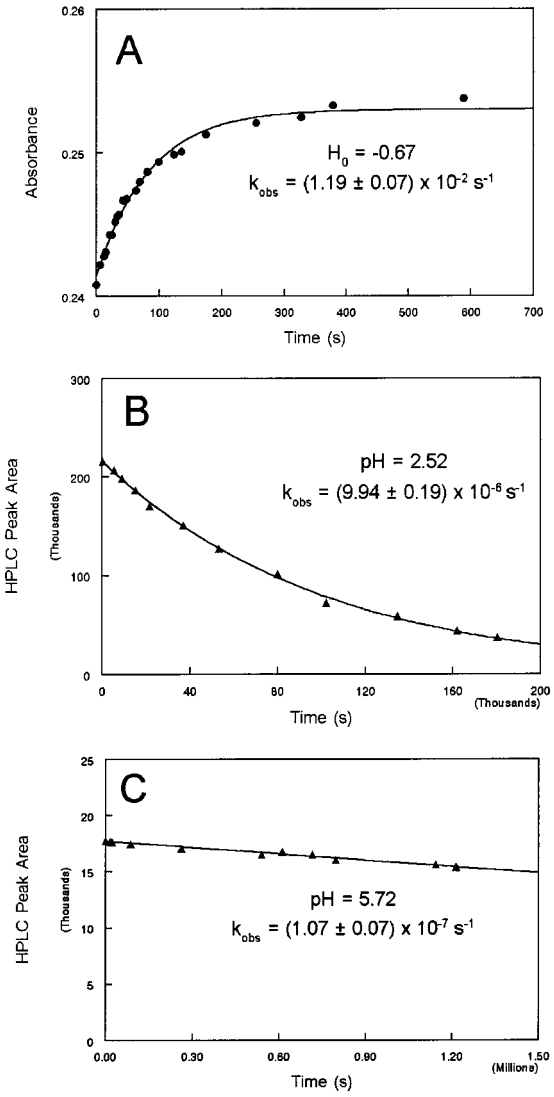

Figure 1. Kinetics of the decomposition of **1a**. (A) Absorbance at 308 nm vs time at $H_o = -0.67$. (B) and (C) HPLC peak area for **1a** vs time at pH 2.52 (B) and 5.72 (C).

mation. For **1a**, **1b**, and **1c** it was not possible to obtain reliable values of k_{obs} at $H_o < -1$ because absorbance vs time data no longer fit the first-order rate equation or consecutive first-order rate equation well. No attempt was made to examine d-G hydrolysis kinetics beyond $H_o \approx -1$.

Product isolation and characterization, combined with HPLC monitoring of reaction mixtures, confirmed that the major initial hydrolysis products observed throughout the H_o and pH range up to ca. pH 7 were the expected bases **2a**−**c** and guanine (Scheme 1). In all solutions at pH < 6 these products are formed quantitatively. In the more basic buffers the yields of **2a** and **2b** are not quantitative and decrease with increasing basicity, becoming negligible at pH ≥ 9, although no other products were isolated under these conditions. No attempt was made to isolate or identify the sugar product generated from the hydrolysis reactions. Under strongly acidic conditions some of the initially formed bases, notably **2c**, did exhibit slow decomposition, but these reactions were not examined further.

Plots of log k_{obs} vs pH or H_o are provided in Figure 2. The H_o acidity scale was chosen because previous studies of purine nucleoside hydrolysis have shown a linear

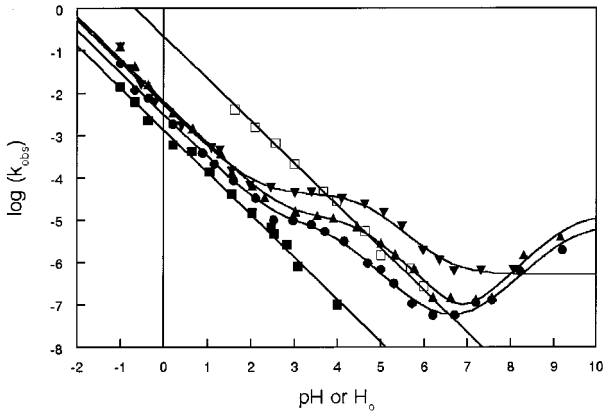

Figure 2. Log (k_{obs}) vs pH or H_o for **1a** (●), 1b (▲), 1c (▼), and d-G (■ at 20 °C, □ at 60 °C). Data were fit as described in the text to obtain the kinetic parameters summarized in Table 1.

dependence of log k_{obs} on H_o.[5b,7a] Figure 2 shows that the C-8 adducts **1a**−**c** hydrolyze at 20 °C at considerably larger rates than does d-G in moderately acidic to neutral pH solutions, although rate constants for hydrolysis at pH < 2 are comparable (within a factor of 5) for all four compounds. For all four compounds the plots show a linear dependence of log k_{obs} on pH or H_o at pH < 2 ($k_{obs} \approx k_2 a_H/K_{a2}$, see Scheme 2). For d-G this linear dependence continues at 20 °C to pH 4.0, beyond which the reaction kinetics were not monitored because $k_{obs} < 10^{-7}$ s^{-1}. At 60 °C the linear dependence of d-G hydrolysis rate constants on pH can be shown to extend to at least pH 6.0 (Figure 2). Hydrolysis rate constants for d-G in acidic solutions at 60 °C are 1.7×10^2 larger than at 20 °C. Between pH 3.0 and 6.0, k_{obs} for **1a**−**c** appears to depend on the concentration of the acid form of a substrate species with a pK_a in the range of 3.5−4.5 ($k_{obs} \approx k_1 a_H/(K_{a1} + a_H)$). At pH > 6.0 a pH-independent term is observed ($k_{obs} \approx k_o$), and at pH > 8.0 a term dependent on the concentration of the basic form of a substrate species with pK_a ~9.0 dominates the kinetics for **1a** and **1b** ($k_{obs} \approx k_- K_{ao}/(K_{ao} + a_H)$). Hydrolysis kinetics for **1c** were not followed beyond pH 8.

The kinetic behavior was analyzed in terms of the mechanism of Scheme 2 that, in part, has been used to describe the pH-dependent behavior of the hydrolysis kinetics of other nucleosides.[5−8,12,13] The lack of buffer effects in formate, acetate, and phosphate buffers is consistent with hydrolysis via rate-limiting unimolecular decomposition of $\mathbf{1H_2}^{+2}$, $\mathbf{1H}^+$, and **1**. Buffer effects in tris buffers indicate the reaction is more complicated in this pH range, but the buffer-independent rate constants in tris buffers do appear to follow this scheme. The expression for k_{obs} derived from this Scheme (eq 1) can be simplified to eq 2 if $a_H/K_{a2} \ll 1$. Under our conditions there is no evidence for an ionization corresponding to K_{a2} in solutions as acidic as $H_o = -1.0$, and pK_{a2} measured for other guanosine nucleosides is generally in the range of −2.5.[7b,14] The assumption used to derive eq 2 appears to be valid for **1a**−**c** in the acidity range of this study. The kinetic data for **1a**−**b** were fit to eq 2 to produce the lines shown in Figure 2A. It was assumed in performing these fits that p$K_{ao} = 9.4$, the known value for d-G under these conditions.[15] This assumption was

(14) The ribose unit does lower pK_{a2}: Benoit, R. L.; Frechette, M. *Can J. Chem.* **1984**, *62*, 995−1000; **1985**, *63*, 3053−3056.
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$$k_{obs} = \frac{k_o k_{a1}/a_H + k_1 + k_2 a_H/K_{a2} + k_- K_{ao} K_{a1}/a_H^2}{1 + K_{a1}/a_H + a_H/K_{a2} + K_{ao} K_{a1}/a_H^2} \quad (1)$$

$$k_{obs} = \frac{k_o K_{a1} + k_1 a_H + k_2 a_H^2/K_{a2} + k_- K_{ao} K_{a1}/a_H}{a_H + K_{a1} + K_{ao} K_{a1}/a_H} \quad (2)$$

made because pK_{ao} is not likely to be strongly affected by C-8 substituents and because we have insufficient kinetic data to determine pK_{ao} with good certainty. The kinetics in this pH region were not pursued further because extrapolating the rate constants for such slow reactions to zero tris buffer concentration is very time-consuming and because the hydrolysis kinetics in this pH range are not the main focus of this paper. The kinetics of hydrolysis of **1c** were fit to eq 2 with the last term of both the denominator and numerator removed because there is no evidence for reaction through $\mathbf{1}^-$ (Scheme 2) for this compound in the pH range examined. The rate constants and pK_a's derived from the fits are summarized in Table 1.

Hydrolysis kinetics for d-G at both 20 and 60 °C confirmed the previously reported observation that this nucleoside does not exhibit a break in its pH-rate profile around its known pK_{a1} of ca. 2.5.[5a] This has been interpreted to mean that $k_1/K_{a1} \approx k_2/K_{a2}$ for this, and several other purine nucleosides, that exhibit this behavior.[5a,7b] The kinetic data for d-G were fit to eq 3, where $k_H \approx k_1/K_{a1} \approx k_2/K_{a2}$:

$$k_{obs} = k_H a_H \quad (3)$$

Spectrophotometric pK_{a1}'s were measured for **1a**−**c** and d-G at 20 °C to confirm the magnitudes of the kinetically determined pK_{a1}'s and to provide pK_{a1} for d-G so that k_1 could be estimated for this compound. A plot of initial absorbance vs pH for **1a** (Figure 3) is typical. The spectrophotometrically determined pK_{a1}'s are reported in Table 1. For **1a**−**c** they are identical, within experimental error, to the kinetically determined values. The pK_{a1} of 2.7 ± 0.3 for d-G is in good agreement with the pK_{a1} of 2.3−2.5 for d-G measured previously in similar aqueous solvent systems.[5a,16]

The 8-(arylamino) substituents of the C-8 adducts **1a**−**c** are electron-donating groups that are expected to increase the pK_a of the N-7 protonated nucleosides. A comparison of pK_{a1} for d-G and **1a-c** (Table 1) shows that this is the case. The stronger acid weakening effects of the heterocyclic substituents of **1b** and **1c** may be caused by an intramolecular H-bonding interaction (Scheme 3). The electron-donating properties of the substituents would be expected to decrease the magnitude of k_1 as has been observed in the hydrolysis reaction of other C-8 substituted nucleosides.[7c,8a] This is not the case. The effects are not large, but k_1 for **1a**−**c** are ca. 3.0- to 15.0-fold larger than k_1 for d-G (Table 1). The overall substituent effect on k_1/K_{a1} ranges from a 40-fold increase for **1a** to a 1.3×10^3-fold increase for **1c** compared to d-G. The combined substituent effects on K_{a1} and k_1 are responsible for the rate accelerations of the hydrolysis of **1a**−**c** that are evident in Figure 2 in the pH range from ca. 2.0 to 6.0.

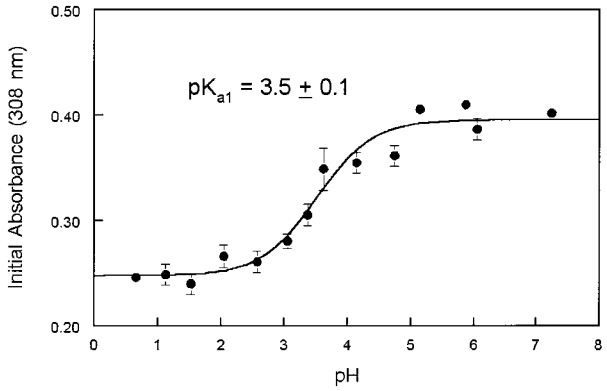
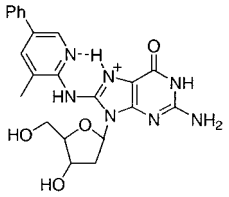


Figure 3. Initial absorbance at 308 nm vs pH for **1a**. Data were fit to a standard titration curve to obtain pK_{a1}.

Scheme 3



A similar result has been obtained for the hydrolysis of 8-dimethylaminoguanosine compared to the parent nucleoside G.[8a] The 8-dimethylamino substituent increases pK_{a1} and k_1 compared to G even though the 8-amino and 8-methylamino substituents decrease k_1.[8a] The increase in k_1 for the 8-dimethylamino substituent was attributed to release of steric strain in the 8-dimethylamino compound that was presumably locked into a syn glycosyl conformation by the bulk of the C-8 substituent.[8a] The importance of the conformational preference in establishing this rate acceleration has been disputed, but the bulk of the substituent does appear to be critical to the observed acceleration.[8b]

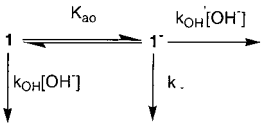
An alternative explanation for the rate accelerations caused by the 8-(arylamino) substituents could be that they differentially stabilize protonation at N-3 rather

Table 1. Summary of Rate Constants and Ionization Constants for 1a−c and d-G at 20 and 60 °C

	1a[a]	**1b**[a]	**1c**[b]	d-G[c]
k_1 (s^{-1})	$(8.4 \pm 2.9) \times 10^{-6}$	$(1.2 \pm 0.3) \times 10^{-5}$	$(4.2 \pm 1.1) \times 10^{-5}$	$2.8 \pm 1.4 \times 10^{-6}$
k_1/K_{a1} (M^{-1} s^{-1})	$(5.6 \pm 3.3) \times 10^{-2}$	$(3.6 \pm 1.5) \times 10^{-1}$	1.8 ± 1.0	$(1.4 \pm 0.1) \times 10^{-3}$ $(2.2 \pm 0.2) \times 10^{-1}$[f]
k_2/K_{a2} (M^{-1} s^{-1})	$(3.1 \pm 0.4) \times 10^{-3}$	$(6.5 \pm 0.8) \times 10^{-3}$	$(5.3 \pm 0.9) \times 10^{-3}$	$(1.4 \pm 0.1) \times 10^{-3}$ $(2.2 \pm 0.2) \times 10^{-1}$[f]
k_o (s^{-1})	$(3.3 \pm 1.6) \times 10^{-8}$	$(1.2 \pm 1.0) \times 10^{-8}$	$(5.2 \pm 1.6) \times 10^{-7}$	
k_- (s^{-1})	$(7.2 \pm 1.7) \times 10^{-6}$	$(1.3 \pm 0.3) \times 10^{-5}$		
pK_{a1}[d]	3.8 ± 0.2	4.5 ± 0.2	4.6 ± 0.2	
pK_{a1}[e]	3.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	2.7 ± 0.3

[a] Derived from fits of log k_{obs} to the logarithmic version of eq 2 with the assumption that p$K_{ao} = 9.4$. [b] Derived from fits of log k_{obs} to the logarithmic version of eq 2 with last terms in both the numerator and denominator removed. [c] Derived from fits of log k_{obs} to the logarithmic version of eq 3. [d] Obtained from the kinetic fits. [e] Obtained from spectrophotometric titration. [f] Measured at 60 °C.

Scheme 4



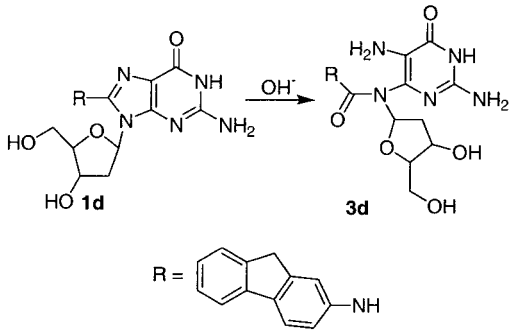
than N-7, and the N-3 monoprotonated nucleosides are subject to more rapid decomposition than N-7 monoprotonated nucleosides.[17] This possibility cannot be entirely ruled out, but ^{15}N chemical shift data for protonation of 8-dimethylaminoguanosine, 8-methylaminoguanosine, 8-aminoguanosine, and G by TFA in DMSO are consistent with exclusive monoprotonation at N-7 in all cases.[8b] It is unlikely that the 8-(arylamino) substituents of this study would have a substantially different effect on the protonation site.

The 8-(arylamino) substituent effect on k_2/K_{a2} is severely attenuated compared to k_1/K_{a1}. It ranges from 2.2 for **1a** to 4.6 for **1b** compared to d-G. The 8-(arylamino) substituents are expected to have little effect on K_{a2}. It has been shown previously that this pK_a is ca. −2.5 for guanosine derivatives with quite different structures.[7b,14] The steric accelerations previously observed for k_1 appear to also occur for k_2.

The C-8 adducts **1a**−**c** are subject to a unique hydrolysis pathway that is observed under neutral pH conditions: the uncatalyzed hydrolysis of the neutral nucleoside **1** governed by k_o. Significant neutral pH hydrolysis of simple nucleosides such as d-G or d-A has not previously been reported.[5−8] At 60 °C we can place an upper limit on k_o for d-G of ca. 2×10^{-7} s^{-1} based on the lack of observable uncatalyzed hydrolysis for d-G at the highest pH examined (6.0). If the rate constant ratio of 1.7×10^2 observed for acid-catalyzed hydrolysis of d-G at 60 and 20 °C applies to k_o, this rate constant is no larger than ca. 1.2×10^{-9} s^{-1} for d-G at 20 °C and is probably considerably smaller.[11] This suggests that k_o for **1a**−**c** is accelerated by a minimum of between 10 (**1b**)- and 400 (**1c**)-fold over k_o for d-G. Although we have insufficient data to write a detailed mechanism for the uncatalyzed reaction, we do know from product studies that the bases **2a**−**c** appear to be the products of these reactions. This is particularly true for **1c** because the alkaline hydrolysis (below) does not compete with the neutral hydrolysis of **1c** in the pH range examined. Release of steric strain may be an important factor in the acceleration of the neutral hydrolysis of **1a**−**c**. We currently do not understand the reason for the accelerated neutral hydrolysis of **1c** compared to that of **1a** and **1b**.

The apparent spontaneous hydrolysis of **1**$^-$ for **1a** and **1b** is kinetically equivalent to OH$^-$-induced decomposition of **1** (Scheme 4). There is precedent for OH$^-$ attack on C-8 of closely related nucleosides.[12,13] If the assumed rate constant for such a process is designated k_{OH} and it accounts for the entire reaction under these mildly basic conditions, its value would be given by $K_{ao}k_-/K_w$, where k_- is the value shown in Table 1, K_{ao} is assumed to be the value for d-G,[15] and K_w has its usual definition. For **1a**, k_{OH} would be ca. 3×10^{-1} M^{-1} s^{-1}, whereas for **1b** it would be ca. 5×10^{-1} M^{-1} s^{-1}. The magnitudes of k_- and k_{OH} cannot be used to eliminate either of the two possible processes, although the OH$^-$-induced decomposition of **1** is more chemically reasonable than spontaneous decomposition of **1**$^-$.[12,13]

Scheme 5



There is a direct precedent for the OH$^-$ pathway in a closely related C-8 adduct.[13] The product and kinetics of the alkaline hydrolysis of **1d** (Scheme 5) have been reported in the pH range from 9.5 to 12.5.[13] The product is the pyrimidine derivative **3d** formed by OH$^-$ attack on C-8 of the nucleoside.[13] Analysis of the kinetic data at 37 °C according to the mechanism of Scheme 4 provides a value of k_{OH} of ca. 1 M^{-1} s^{-1} and k_{OH}' of ca. 2×10^{-3} M^{-1} s^{-1} for **1d** if all of the observed hydrolysis occurs via these two paths. The ratio k_{OH}/k_{OH}' of 5×10^2 is consistent with the expected deceleration of attack of OH$^-$ on **1d**$^-$ caused by a combination of electrostatic effects and increased electron density at C-8 of **1d**$^-$.

Although the products of hydrolysis of **1a** and **1b** under alkaline conditions were not isolated, it is very likely that the same reaction occurs for these compounds as for **1d**. The reaction kinetics are consistent with this interpretation, and the magnitudes of k_{OH} for **1a** and **1b** are comparable to that for **1d** if the temperature difference is taken into account. The decreased yields of **2a** and **2b**, the normal products of C−N glycosidic bond cleavage, observed under moderately alkaline conditions are also consistent with the reaction of Scheme 5.

In conclusion, the *N*-arylamino C-8 adducts **1a**−**c** undergo hydrolysis under acidic and weakly basic conditions via processes that have been described for other purine nucleosides.[5−8,12,13] The rate accelerations compared to that of d-G that are apparent in moderately acidic solutions in which the observed rate constant is k_1/K_{a1} are caused by an apparent steric increase in k_1 and a concurrent decrease in K_{a1} caused by the electron-donating effect of the *N*-arylamino substituent. A unique uncatalyzed hydrolysis of the neutral nucleosides also causes these compounds to be quite labile compared to simple nucleosides under neutral pH conditions.

Experimental Section

General. All salts used in the preparation of buffers were reagent grade. The purifications of CH_3CN and DMF have been described elsewhere.[18] Water for kinetic studies and product studies was distilled, deionized, and distilled again. All other reagents and solvents were reagent grade and distilled. All pH measurements were made at 20 °C with an Orion model 701 pH meter equipped with a Radiometer GK-2402C electrode. UV kinetics and titration experiments were performed on a Cary 3 UV−visible spectrophotometer equipped with thermostated cell holders. Some of the kinetics were performed on an Applied Photophysics stopped-flow spectro-

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photometer (SX.18MW). LC-MS experiments were carried out on a Bruker ESQUIRE-LC-MS instrument with either EI or ACPI. Deoxyguanosine was purchased from Fluka.

Synthesis. The syntheses and characterization of **1a**−**c** are described in the literature.[1,2a]

***N*-(Guanin-8-yl)-4-aminobiphenyl (2a).** This material was obtained by allowing 25 mg (0.058 mmol) of **1a** to decompose in 250 mL of $HClO_4$ solution (μ = 0.5 M ($NaClO_4$), 20% CH_3CN-H_2O, 0.316 M $HClO_4$, 20 °C). Compound **1a** was dissolved in 10 mL of water and added in 0.9 mL aliquots to the $HClO_4$ solution at 10 min intervals. The reaction mixture was allowed to sit for 100 min after the last addition. The solution was neutralized with 5% $NaHCO_3$ to pH 6.5 and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried over Na_2SO_4, and the solvent was evaporated to dryness. The impurities were removed by triturating the precipitate with anhydrous ether to give **2a:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (2H, d, J = 8.4 Hz), 7.70 (4H, m), 7.44 (3H, m); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 153.8 (C), 151.8 (C), 148.4 (C), 140.0 (C), 139.6 (C), 138.0 (C), 134.9 (C), 129.0 (CH), 127.4 (CH), 127.1 (CH), 126.2 (CH), 120.1 (C), 118.1 (CH); LC-MS (ESI, positive ion mode), $C_{17}H_{14}N_6OK$ (M + K) requires *m/e* 357.08, found 357.14; LC-MS (ESI, negative ion mode), $C_{17}H_{13}N_6O$ (M − H) requires *m/e* 317.12, found 317.35; High-resolution MS (ES, positive), $C_{17}H_{15}N_6O$ (M + H) requires *m/e* 319.1307, found 319.1325.

***N*-(Guanosin-8-yl)-2-amino-3-methyl-5-phenylpyridine (2b).** Compound **2b** was obtained by allowing 50 mg of **1b** to decompose in 250 mL of $HClO_4$ solution (μ = 0.5 ($NaClO_4$)), 20% CH_3CN-H_2O, 0.316 M $HClO_4$, 20 °C) as described above for **2a**. The CH_2Cl_2 extracts were dried over Na_2SO_4, and the solvent was evaporated to dryness. The impurities were removed by triturating the precipitate with anhydrous ether to give **2b**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 13.48 (1H, bs), 8.24 (1H, s), 8.14 (1H, s), 7.80 (2H, bs), 7.67 (1H, d, J = 6 Hz), 7.44 (4H, m); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 152.2 (C), 141.5 (CH), 134.4 (C), 130.4 (CH), 129.1 (CH), 128.2 (CH), 127.1 (C), 126.7 (C), 126.0 (CH), 124.8 (C), 122.7 (C), 16.5 (CH_3); LC-MS (ESI, positive ion mode), $C_{17}H_{16}N_7OH$ (M + H) requires *m/e* 334.14, found 334.15; LC-MS (ESI, negative ion mode), $C_{17}H_{14}N_7OH$ (M − H) requires *m/e* 332.13, found 332.45.

***N*^{2}-(Guanin-8-yl)-2-amino-9*H*-pyrido[2,3-*b*]indole (2c).** This compound was synthesized by a literature procedure:[19] ^{1}H NMR (300 MHz, DMSO-d_6) δ (1H, s, exchangeable), 11.05 (1H, s, br, exchangeable), 10.73 (1H, s, br, exchangeable), 8.39 (1H, d, J = 8.4 Hz), 7.99 (1H, d, J = 7.7 Hz), 7.46 (1H, d, J = 8.0 Hz), 7.33 (1H, t, J = 8.1 Hz), 7.17 (1H, t, J = 7.5 Hz), 6.90 (1H, d, J = 8.4 Hz), 6.33 (2H, s, br, exchangeable); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 153.0 (3 x C), 152.0 (C), 150.0 (C), 137.6 (C), 131.0 (CH), 124.8 (CH), 121.1 (C), 119.7 (CH), 119.7 (C), 119.6 (CH), 111.1 (CH), 108.6 (C), 103.0 (CH); MALDI-TOF-MS, $C_{16}H_{13}N_8O$ (M + H) requires *m/e* 333.12, found 333.00.

Kinetics and Titrations. Reactions were performed in 20 vol % CH_3CN-H_2O solutions, μ = 0.5 ($NaClO_4$) at 20 or 60 °C. At pH ≤ 2.5, $HClO_4$ was used to maintain pH. For all other solutions buffers of HCO_2Na/HCO_2H, NaOAc/AcOH, Na_2HPO_4/NaH_2PO_4, and tris/trisH^+ were used to maintain pH. Ionic strength was not maintained for $HClO_4$ solutions in the H_o range. These solutions were prepared as described in the literature.[20] Initial concentrations of **1a**−**c** or d-G of $(1-2) \times 10^{-5}$ M were obtained by injecting 15 μL of ca. $(2-4) \times 10^{-3}$ M DMF stock solutions into 3.0 mL of the reaction solution that had been incubated at the appropriate temperature for at least 15 min.

Kinetics were monitored by UV or HPLC methods that have been described.[21] HPLC was performed using C-8 reverse phase analytical columns with $MeOH/H_2O$ eluents buffered with 0.05 M 1/1 KOAc/HOAc. HPLC peaks were monitored by UV spectroscopy. Absorbance or HPLC peak area vs time data were fit to the standard first-order rate equation for $k_{obs} > 2 \times 10^{-6}$ s^{-1}. For slower reactions initial rates were determined from the slopes of HPLC peak area vs time data for **1a**−**c** and d-G for the first 5% of the decomposition. Initial absorbances for spectrophotometric titrations were determined by extrapolation to t = 0 using either a linear extrapolation or the first-order rate equation.

Wavelengths used in these studies are as follows:

1a	UV; 308 nm, HPLC; 280 nm
1b	UV; 351 or 280 nm, HPLC; 280 nm
1c	UV; 380 nm, HPLC; 340 nm
d-G	UV; 257 nm, HPLC; 280 nm

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Supporting Information Available: Table of rate constants obtained for the hydrolysis of **1a**−**c** and d-G. This material is available free of charge via the Internet at http://pubs.acs.org.

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