Selective Trapping of *N*-Acetyl-*N*-(4-biphenylyl)nitrenium and *N*-Acetyl-*N*-(2-fluorenyl)nitrenium Ions by 2′-Deoxyguanosine in Aqueous Solution

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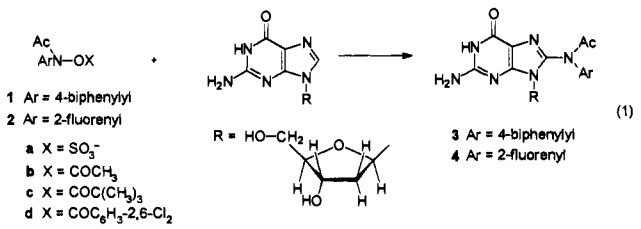
Esters of carcinogenic *N*-arylhydroxamic acids, such as **1a**−**d** and **2a**−**c**, react with DNA predominantly at the guanosine base.[1] The major product of this reaction is a C-8 adduct.[1] A similar reaction (eq 1) occurs with guanosine or 2′-deoxyguanosine (d-G) to generate **3** and **4** in low yield in mixed organic/aqueous solvents.[1,2] The mechanism of this reaction has never



(1)

been investigated, but it is widely assumed to involve a nitrenium ion.[2,3] Although **1a** and **2a**−**c** generate nitrenium ions in H_2O, previous studies have concentrated on the lability of the ions in H_2O, not on their reactions with biologically relevant nucleophiles.[4,5] These ions are attacked by most nucleophiles at the aromatic ring, not at N.[4,5] Some reactions of similar compounds have been shown to be S_N2 in nature,[6] and C-8 of guanosine is not normally nucleophilic to electrophiles.[7] For these reasons, it was not clear how this reaction occurred. We report herein the first evidence that this reaction occurs in homogeneous aqueous solution by a $D_N + A_N$ (S_N1) mechanism involving a remarkably selective trapping of a nitrenium ion generated by rate-limiting N−O bond heterolysis.

In 5% CH_3CN-H_2O (μ = 0.5 ($NaClO_4$), pH 7.5 (0.02 M NaH_2PO_4/Na_2HPO_4), T = 20 °C), the rate constant, k_0, for disappearance of **1a** is independent of [d-G] up to 10 mM (Table 1), at which about 75% of **1a** is converted into **3**.[2a,8] A plot of the inverse of the fraction of products obtained from trapping by solvent (**5** and **6**),[4] $1/f_s$, vs [d-G] is linear, with an intercept of 1.0 (Figure 1A). This shows that solvent and d-G compete for a common intermediate.[9] The slope, 290 ± 10 M^{-1}, is $k_{d\text{-}G}/k_s$, the ratio of the second-order rate constant for trapping of **7** by d-G, $k_{d\text{-}G}$, and the pseudo-first-order rate constant for trapping by solvent, k_s (eq 2). At [d-G] = 3.4 mM, 50% of **7** is trapped by d-G. Under identical conditions, except for buffer

Table 1. Rate Constants for the Decomposition of **1a** and **2c** in the Presence of d-G[a]

[d-G], mM	10^4k_o, s^{-1} **1a**[b]	**2c**[c]
0	4.0 ± 0.5	2.0 ± 0.3
2.5		2.4 ± 0.1
4.0	4.1 ± 0.2	
5.0		2.0 ± 0.1
10.0	4.6 ± 0.2	

[a] Determined by UV (at [d-G] = 0 mM) or HPLC methods at pH 7.5 (NaH_2PO_4/Na_2HPO_4), μ = 0.5 ($NaClO_4$), and T = 20 °C. [b] Initial concentration of **1a**, 1.0 × 10^{-4} M. [c] Initial concentration of **2c**, 4.0 × 10^{-5} M.

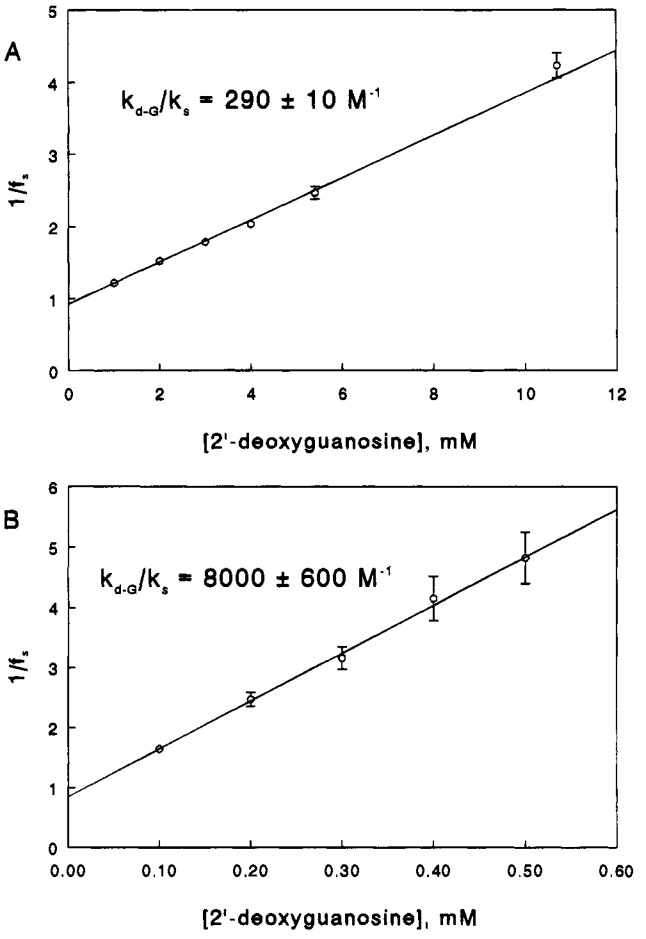


Figure 1. Plots of $1/f_s$, the inverse of the fraction of products obtained from trapping of the solvent, vs [d-G] for **7** (A) and **8** (B). The lines are calculated from a weighted least-squares fit. (A) **1a**, 1.0 × 10^{-4} M. (B) **2a**, 2.0 × 10^{-5} M.

(1) Kriek, E. *Chem.−Biol. Interact.* **1969**, *1*, 3−17. Kriek, E. *Chem.−Biol. Interact.* **1971**, *3*, 19−28. Meerman, J. H. N.; Beland, F. A.; Mulder, G. J. *Carcinogenesis* **1981**, *2*, 413−416. Fuchs, R. P. P. *Anal. Biochem.* **1978**, *91*, 663−673. Beland, F. A.; Dooley, K. L.; Jackson, C. D. *Cancer Res.* **1982**, *42*, 1348−1354. Tamura, N.; King, C. M. *Carcinogenesis* **1990**, *11*, 535−540.

(2) (a) Underwood, G. R.; Price, M. F.; Shapiro, R. *Carcinogenesis* **1988**, *9*, 1817−1821. (b) Kriek, E.; Miller, J. A.; Juhl, U.; Miller, E. C. *Biochemistry* **1967**, *6*, 177−182. (c) Lee, M.-S.; King, C. M. *Chem.−Biol. Interact.* **1981**, *34*, 239−248. (d) Nelson, J. H.; Grunberger, D.; Cantor, C. R.; Weinstein, I. B. *J. Mol. Biol.* **1971**, *62*, 331−346. (e) Evans, F. E.; Miller, D. W.; Levine, R. A. *J. Am. Chem. Soc.* **1984**, *106*, 396−401.

(3) Miller, J. A.; Miller, E. C. *Environ. Health Perspect.* **1983**, *49*, 3−12. Garner, R. C.; Martin, C. N.; Clayson, D. B. In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society; Washington, DC, 1984; Vol. 1, pp 175−276. Beland, F. A.; Kadlubar, F. F. *Environ. Health Perspect.* **1985**, *62*, 19−30.

(4) Novak, M.; Kahley, M. J.; Eiger, E.; Helmick, J. S.; Peters, H. E. *J. Am. Chem. Soc.* **1993**, *115*, 9453−9460.

(5) (a) Panda, M.; Novak, M.; Magonski, J. *J. Am. Chem. Soc.* **1989**, *111*, 4524−4525. (b) Underwood, G. R.; Kirsch, R. B. *J. Chem. Soc., Chem. Commun.* **1985**, 136−138. (c) Novak, M.; Kahley, M. J.; Lin, J.; Kennedy, S. A. *J. Am. Chem. Soc.*, in press.

(6) Novak, M.; Martin, K. A.; Heinrich, J. L. *J. Org. Chem.* **1989**, *54*, 4530−4531. Ulbrich, R.; Famulok, M.; Bosold, F.; Boche, G. *Tetrahedron Lett.* **1990**, *31*, 1689−1692.

(7) Methylating reagents alkylate guanosine at N-7 or O-6: Saffhil, R.; Margison, G. P.; O'Connor, P. J. *Biochim. Biophys. Acta* **1985**, *823*, 111−145. Beranek, D. T. *Mutat. Res.* **1990**, *231*, 11−30. Benzylic carbocations react at N-2 in low yield: Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1986**, *51*, 4180−4185.

(8) In both cases, one adduct was detected by HPLC. Low yields (≤2%) of other adducts could have escaped detection. **3** was identified by comparison with an authentic sample (ref 2a); **4** was identified from published NMR data (ref 2b,d,e).

(9) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 8238−8248.

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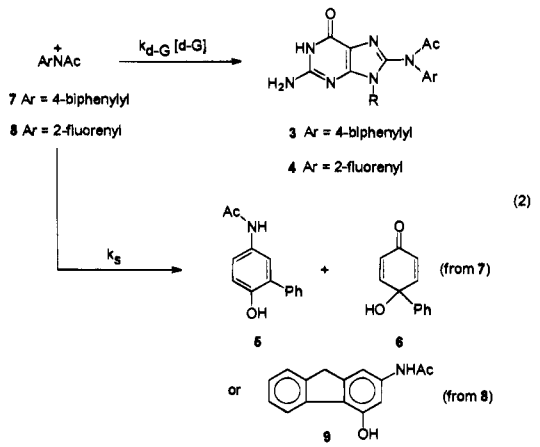
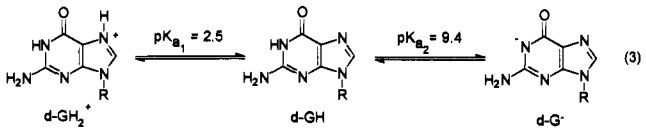


concentrations of 0.005 M, k_0 for **2c**[10] is independent of [d-G] up to 5 mM (Table 1), at which the yield of **4** is greater than 95%.[2b,8] At this pH, the product of solvent trapping is **9**.[5a] A plot of $1/f_s$ vs [d-G] (Figure 1B), determined for **2a**, provides $k_{d\text{-}G}/k_s$ for **8** of 8000 ± 600 M^{-1}. At [d-G] = 0.13 mM, **8** is trapped to the extent of 50% to form **4**.

The magnitude of $k_{d\text{-}G}$ can be determined for **7** and **8** since k_s is known for both ions under these conditions from LFP measurements.[11] The calculated values of $k_{d\text{-}G}$ for **7** and **8** are 1.7×10^9 and 6.2×10^8 M^{-1} s^{-1}, respectively. These rate constants are within an order of magnitude of the diffusion-controlled limit, and they show that **7** and **8** can react selectively with d-G in vivo. The reaction with guanosine in DNA may be facilitated by electrostatic attraction between the cation and the anionic phosphate backbone of DNA.

Since $k_{d\text{-}G}/k_s$ for **7** is pH independent from pH 3.5 to 7.5, the reactive form of d-G in this pH range must be the neutral form d-GH (eq 3).[12] The anion d-G^- may also be reactive but could



(3)

only contribute ca. 5% to the trapping reaction at pH 7.5, even if the rate constant for the reaction with d-G^- was diffusion limited at ca. 5×10^9 M^{-1} s^{-1}. We cannot rule out the possibility that this reaction occurs by initial attack of N-7 on the ortho carbon of **7** or **8** followed by a Cope-type rearrangement, or by attack of N-7 on N of **7** or **8** followed by deprotonation of C-8 and a Stevens-type rearrangement,[13] instead of by direct nucleophilic attack of C-8. No intermediates were detected, however, and **3** and **4** are generated in a clean first-order fashion. Carbocations react preferentially with N-2 rather than N-7,[7] but no evidence of an N-2 adduct was found in either reaction mixture.

The selective reaction of **7** and **8** with d-G could not occur if trapping by H_2O was not relatively slow. Hard nucleophiles, including H_2O, attack nitrenium ions at the aromatic ring.[4,5] This process is accompanied by loss of aromaticity that contributes to the barrier to the reaction. It has been suggested that the remarkably long lifetime of nitrenium ions in H_2O compared to similar carbenium ions is due to this barrier.[11,14] This long lifetime is the critical factor which allows **7** and **8** to react selectively with the relatively weakly nucleophilic d-G.

Low yields reported for the reaction of eq 1 are not caused by low efficiency of trapping of the nitrenium ion by d-G or its derivatives. Acetic acid esters such as **1a** are known to undergo ester hydrolysis in preference to N−O bond cleavage.[5b,15] Some of these esters acetylate nucleosides rather than forming adducts such as **3** or **4**.[16] Since most of the reactants are only slightly water soluble, these reactions have often been performed under nonhomogeneous conditions in mixed solvents.[2] These conditions suppress N−O bond heterolysis and lead to side reactions, including acyl transfer. The hydrolytic instability of the sulfuric acid esters **1a** and **2a** is also not appreciated. These compounds undergo extensive decomposition if they are not handled under dry conditions. Good yields of **3** and **4** can be obtained in homogeneous aqueous solution if the proper procedure is followed.[17]

The carcinogenic potential of **1a** and **2a** appears to be related to the very high selectivity of **7** and **8** in aqueous solution for C-8 of d-G. We are continuing this investigation to determine the detailed mechanism of the trapping reaction and whether this is a general phenomenon for nitrenium ions derived from other esters of *N*-arylhydroxamic acids and *N*-arylhydroxylamines.

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(10) **2a** decomposes too rapidly in H_2O to be monitored by HPLC methods (ref 5a).

(11) Davidse, P. A.; Kahley, M. J.; McClelland, R. A.; Novak, M. *J. Am. Chem. Soc.* **1994**, *116*, 4513−4514.

(12) p*K* data in eq 3 were obtained from the following references: Rawitscher M.; Sturtevant, J. M. *J. Am. Chem. Soc.* **1960**, *82*, 3739−3740 (pK_{a_1}, $\mu = 0.1$, $T = 25$ °C). Clauwaert, J.; Stockx, J. *Z. Naturforsch.* **1968**, *23b*, 25−30 (pK_{a_2}, $\mu = 0.5$, $T = 20$ °C).

(13) There is some evidence that this may occur for deacetylated analogues of **1a** and **2a**: Humphreys, W. G.; Kadlubar, F. F.; Guengerich, F. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8278−8282.

(14) Bolton, J. L.; McClelland, R. A. *J. Am. Chem. Soc.* **1989**, *111*, 8172−8181.

(15) Underwood, G. R.; Kirsch, R. *Tetrahedron Lett.* **1985**, *36*, 147−150. Underwood, G. R.; Callaban, R. J. *Tetrahedron Lett.* **1987**, *28*, 5427−5430.

(16) Scribner, J. D.; Scribner, N. K.; Smith, D. L.; Jenkins, E.; McCloskey, J. A. *J. Org. Chem.* **1982**, *47*, 3143−3145.

(17) A 25 mL saturated solution of d-G (ca 0.02 M) in 5% CH_3CN−H_2O ($\mu = 0.5$ ($NaClO_4$), 0.02 M NaH_2PO_4/Na_2HPO_4, pH 7.5, 20 °C) was stirred as 48 mg of the K^+ salt of **2a** (0.134 mM) in 1 mL of dry DMF was added in 200 μL portions at 10 min intervals. About 30 min after the last addition, the solution was cooled in an ice bath and filtered to recover precipitated **4**. This material was washed with ice-cold H_2O and dried under vacuum to yield 26 mg of **4** (40% yield, >98% pure by HPLC). HPLC of the aqueous solution and washings shows that d-G and much of **4** (ca. 32% yield) remain in solution. This was extracted with EtOAc and purified as described elsewhere.[2] No other significant (>2−3% yield) products could be detected.